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Owoce rokitnika pospolitego (*Hippophaë rhamnoides*) w projektowaniu żywności o ukierunkowanym potencjale prozdrowotnym

Sea buckthorn (*Hippophaë rhamnoides*) fruits in designing foods with specific health-promoting potential

Rozprawa doktorska

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Spis treści

CYKL PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

Publikacja 1: **Tkacz K.**, Wojdyło A., Turkiewicz I.P., Bobak Ł., Nowicka P. 2019. Anti-oxidant and anti-enzymatic activities of sea buckthorn (*Hippophaë rhamnoides* L.) fruits modulated by chemical components. *Antioxidants*, 8, 618. doi:10.3390/antiox8120618.
IF 6,313; 100 punktów MEiN, cyt.: 19

Publikacja 2: **Tkacz K.**, Wojdyło A., Turkiewicz I.P., Ferreres F., Moren, D. A., Nowicka P. 2020. UPLC-PDA-Q/TOF-MS profiling of phenolic compounds and carotenoids and their influence on anticholinergic potential for AchE and BuChE inhibition and on-line antioxidant activity of selected *Hippophaë rhamnoides* L. cultivars. *Food Chemistry*, 309: 125766. doi: 10.1016/j.foodchem.2019.125766.
IF 7,514; 200 punktów MEiN; cyt.: 22

Publikacja 3: **Tkacz K.**, Wojdyło A., Turkiewicz I.P., Nowicka P. 2021. Triterpenoids, phenolic compounds, macro- and microelements in anatomical parts of sea buckthorn (*Hippophaë rhamnoides* L.) berries, branches and leaves. *Journal of Food Composition and Analysis,* 103, 104107. doi: 10.1016/j.jfca.2021.104107.
IF 4,556; 100 punktów MEiN; cyt.: 2

Publikacja 4: **Tkacz K.**, Gil-Izquierdo Á., Medina S., Turkiewicz I.P., Domínguez-Perles R., Nowicka P., Wojdyło, A. 2021. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 11345. doi: 10.1002/jsfa.11345.
IF 3,639; 100 punktów MEiN; cyt.: 1

Publikacja 5: **Tkacz K.,** Chmielewska J., Turkiewicz I.P., Nowicka P., Wojdyło A. 2020. Dynamics of changes in organic acids, sugars and phenolic compounds and antioxidant activity of sea buckthorn and sea buckthorn-apple juices during malolactic fermentation. *Food Chemistry*, 332: 127382. doi: 10.1016/j.foodchem.2020.127382.
IF 7,514; 200 punktów MEiN; cyt.: 18

Publikacja 6: **Tkacz K.,** Wojdyło A., Turkiewicz I.P., Nowicka P. 2021. Anti-diabetic, anti-cholinesterase, and antioxidant potential, chemical composition and sensory evaluation of novel sea buckthorn-based smoothies. *Food Chemistry*, 338, 128105. doi: 10.1016/j.foodchem.2020.128105.
IF 7,514; 200 punktów MEiN; cyt.: 13

Publikacja 7: **Tkacz K.,** Wojdyło A, Michalska-Ciechanowska A., Turkiewicz I.P., Lech K., Nowicka P. 2020. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules*, 25(17), 3801. doi: 10.3390/molecules25173801.
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STRESZCZENIE

Obecne trendy żywieniowe i świadomość konsumentów zorientowane są na relację między spożywaną żywnością i stosowaniem naturalnej diety a wpływem na stan zdrowia i prewencją chorób. Konsekwentnie, rosnące zagrożenia zdrowotne związane z nieodpowiednim odżywianiem zwiększają presję rozwoju innowacji i produktów funkcjonalnych ukierunkowanych na surowce roślinne o udowodnionym działaniu prozdrowotnym. W tym kontekście, owoce rokitnika pospolitego (*Hippophaë rhamnoides* L.) wpisują się w strategię poszukiwania naturalnych czynników profilaktyki chorób niezakaźnych, jednak ich walory sensoryczne i złożoność fazy hydrofilowo-lipidowej stanowią wyzwanie nie tylko dla konsumentów, ale także dla przemysłu spożywczego.

Celem pracy było określenie profilu prozdrowotnego owoców rokitnika pospolitego i opracowanie technologii otrzymywania funkcjonalnych i atrakcyjnych sensorycznie produktów o ukierunkowanych właściwościach prozdrowotnych na bazie owoców tej rośliny. Osiągnięcie celu oparto o realizację 4 etapów badawczych: (1) analiza frakcji biologicznie aktywnej rokitnika pospolitego; (2) optymalizacja procesu fermentacji jabłkowo-mlekowej soków na bazie owoców rokitnika pospolitego; (3) opracowanie recepturowe i optymalizacja technologii otrzymywania produktów funkcjonalnych z sokiem z owoców rokitnika pospolitego; (4) analiza właściwości fizykochemicznych i potencjału prozdrowotnego mikrokapsułek otrzymanych z soku z owoców rokitnika pospolitego.

Wyniki uzyskane w pierwszym etapie pozwoliły na stwierdzenie, że jagody rokitnika pospolitego mają unikatową kompozycję lipofilowych i hydrofilowych związków bioaktywnych istotnie modulujących aktywność przeciwcukrzycową, przeciw otyłości, przeciwstarzeniową, przeciwzapalną i przeciwutleniającą. Określono różnorodność anatomicznych części rokitnika pospolitego (skórka, miąższ, nasiona, endokarp, pędy i liście) pod względem związków fenolowych, triterpenów i składników mineralnych. Analiza soków, powszechnej komercyjnie formy przetworzenia owoców rokitnika pospolitego, potwierdziła działanie przeciwcukrzycowe, przeciw otyłości i potencjał zwiększenia funkcjonalności soków w ograniczaniu zmian neurodegeneracyjnych z uwagi na obecność fitoprostanów, fitofuranów, tokoferoli, tokotrienoli i aminokwasów.

W dalszej kolejności rozważono potencjał jakim owoce rokitnika pospolitego się charakteryzują przez aspekt technologiczny związany z projektowaniem żywności o ukierunkowanym potencjale prozdrowotnym. Określono dynamikę zmian zawartości kwasów organicznych, cukrów, związków fenolowych oraz aktywności przeciwutleniającej podczas fermentacji jabłkowo-mlekowej soku z owoców rokitnika pospolitego i soku mieszanego rokitnik – jabłko. Wybrane szczepy *Lactobacillus plantarum* i *L. plantarum* subsp. *argentoratensis* charakteryzowała wysoka aktywność metaboliczna, sprzyjały akumulacji flawonoli i wzrostowi aktywności przeciwutleniającej, stąd fermentację jabłkowo-mlekową z ich zastosowaniem uznano za obiecującą metodę biologicznego odkwaszania soków na bazie owoców rokitnika pospolitego.

Zaprojektowano produkty typu smoothie zawierające wysoki udział soku z owoców rokitnika pospolitego (25% i 50%), jednocześnie o wysokim potencjale prozdrowotnym i akceptacji konsumenckiej. Nowatorskie formuły produktowe należy traktować jako uzupełnienie diety o potencjalnych właściwościach przeciwutleniających, przeciwstarzeniowych i przeciwcukrzycowych. Komponowanie soku z owoców

rokitnika pospolitego z innymi owocami i warzywami, powszechnie dostępnymi dla przemysłu przetwórczego w Polsce, wpłynęło także na poprawę atrakcyjności smaku, barwy i aromatu produktów.

Badania nad optymalizacją procesu mikroenkapsulacji soku z owoców rokitnika pospolitego pozwoliły na wskazanie maltodekstryny jako substancji powlekającej konkurencyjnej w porównaniu do inuliny, ze względu na wyższe stężenie związków fenolowych, aktywność przeciwutleniającą, niesprzyjanie brązowieniu nieenzymatycznemu i akumulacji HMF w mikrokapsułkach. W pracy przedstawiono wpływ nośników polisacharydowych i rodzajów suszenia w kontekście stabilności wybranych związków chemicznych i aktywności przeciwutleniającej soku z owoców rokitnika pospolitego po procesie suszenia i przechowywania.

Na podstawie przeprowadzonych badań wskazano, że rokitnik pospolity stanowi wartościowy surowiec do produkcji jednocześnie funkcjonalnych i atrakcyjnych sensorycznie produktów. Stwierdzono wysoki potencjał owoców rokitnika pospolitego jako składnika żywności bogatej w związki bioaktywne o potencjale przeciwutleniającym, przeciwcukrzycowym, przeciw otyłości, przeciwzapalnym i przeciwstarzeniowym.

Słowa kluczowe: rokitnik pospolity, części anatomiczne, soki, smoothie, fermentacja jabłkowo-mlekowa, mikrokapsułki, związki bioaktywne, przeciwutleniający, przeciwstarzeniowy, przeciwcukrzycowy, przeciw otyłości, przeciwzapalny

ABSTRACT

Current dietary trends and consumer awareness focus on the relationship between the consumed food, a natural diet and its impact on health and disease prevention. Therefore, growing health risks related to inadequate nutrition increase the pressure to develop innovative and functional products based on plant raw materials with proven health-promoting effects. In this context, sea buckthorn (*Hippophaë rhamnoides* L.) berries fit in the strategy of searching for natural agents that may help prevent non-communicable diseases. However, their sensory qualities and the complexity of their hydrophilic-lipid phase pose a challenge not only for consumers but also for the food industry.

The aim of the study was to determine the health-promoting profile of sea buckthorn berries and to develop a technology for the production of functional and tasteful products with targeted health-promoting properties based on sea buckthorn fruits. This objective was implemented in four research steps: (1) analysis of the biologically active fraction of sea buckthorn; (2) optimization of the malolactic fermentation of juices based on sea buckthorn fruits; (3) development of recipes and optimization of technology for obtaining functional products with sea buckthorn fruit juice; (4) analysis of physicochemical properties and health-promoting potential of microcapsules obtained from sea buckthorn fruit juice.

The results obtained in the first step demonstrated a unique composition of lipophilic and hydrophilic bioactive compounds of sea buckthorn berries that significantly modulate anti-diabetic, anti-obesity, anti-aging, anti-inflammatory, and antioxidant activities. The diversity of the anatomical parts of sea buckthorn (skin, pulp, seeds, endocarp, shoots, and leaves) was determined in terms of their content of phenolic compounds, triterpenes, and mineral nutrients. Analysis of juices, a commercially common form of processing sea buckthorn fruits, confirmed their anti-diabetic and anti-obesity properties. It also indicated the potential to increase the functionality of juices in reducing neurodegenerative changes due to their content of phytoprostanes, phytofurans, tocopherols, tocotrienols, and amino acids.

Further, the potential of sea buckthorn fruits was considered in the technological aspect related to the design of foods with targeted health-promoting properties. The dynamics of changes in the content of organic acids, sugars, phenolic compounds, and antioxidant activity during malolactic fermentation of sea buckthorn fruit juice and mixed sea buckthorn-apple juice was determined. Selected strains of *Lactobacillus plantarum* and *L. plantarum* subsp. *argentoratensis* were characterized by high metabolic activity. They promoted accumulation of flavonols and increased the antioxidant activity of the juices. Malolactic fermentation was therefore considered a promising method of biological deacidification of sea buckthorn fruit-based juices.

The designed smoothie products, containing high proportion of sea buckthorn fruit juice (25% and 50%), achieved both high health-promoting potential and consumer acceptance. Novel product formulations should be considered as dietary supplements with potential antioxidant, anti-aging, and anti-diabetic properties. Mixing sea buckthorn fruit juice with other fruits and vegetables, commonly available for processing industry in Poland, improved the taste, color, and aroma of the final products.

Studies on the optimization of microencapsulation of sea buckthorn fruit juice identified maltodextrin as a coating agent more valuable than inulin due to a higher content of phenolic compounds, greater antioxidant activity, lower non-enzymatic browning, and HMF accumulation in the microcapsules. This paper presents the

effects of polysaccharide carriers and drying method type on the stability of selected chemical compounds and antioxidant activity of sea buckthorn fruit juice after drying and storage.

The study confirmed that sea buckthorn is a valuable raw material for the production of both functional and palatable products. It corroborated a high potential of sea buckthorn berries as a food component rich in bioactive compounds with antioxidant, antidiabetic, anti-obesity, anti-inflammatory, and anti-aging properties.

Key words: sea buckthorn, anatomical parts, juices, smoothies, malolactic fermentation, microcapsules, bioactive compounds, antioxidant, anti-aging, anti-diabetic, anti-obesity, anti-inflammatory

1. WSTĘP

Rokitnik pospolity (*Hippophaë rhamnoides* L.) jest liściastym i owocującym krzewem z rodziny oliwnikowatych *Elaeagnaceae*. Obecnie rozpoznanych jest sześć gatunków *Hippophaë* i 12 podgatunków, w tym najważniejsze o znaczeniu gospodarczym, ekonomicznym i odżywczym są ssp. *sinensis*, ssp. *mongolica* i ssp. *rhamnoides*. Rokitnik pospolity naturalnie występuje w wielu regionach półkuli północnej. Globalna powierzchnia dzikiej i uprawianej rośliny szacowana jest na 3,0 mln ha, z czego około 85% obejmują tereny Chin, następnie Mongolii, Indii i Pakistanu. W niektórych krajach Europy, w Chinach, Japonii, Rosji, Chile i Kanadzie rokitnik pospolity wykorzystywany jest na skalę przemysłową (Rafalska i in., 2017; Ruan i in., 2013).

Kuliste lub owalne, błyszczące jagody rokitnika pospolitego przybierają barwę skórki i miąższu w odcieniach od żółtej do czerwonej. To charakterystyczne zabarwienie spowodowane jest wysoką zawartością związków karotenoidowych z co najmniej siedmioma sprzężonymi wiązaniami podwójnymi (Pop i in., 2014). Jagody są kilkakrotnie zasobniejsze w witaminę C niż popularne owoce, takie jak truskawki, cytryny czy jeżyny, i wyróżnia je wysoka zawartość tłuszczu, średnio 10% świeżej masy owoców, bogatego w kwasy tłuszczowe omega-3, omega-6, omega-7 i omega-9 (**Publikacja 1**; Teleszko i in., 2015).

Zastosowanie rokitnika pospolitego do celów terapeutycznych wywodzi się z medycyny tybetańskiej, mongolskiej i starożytnej Grecji, a badania współcześnie prowadzone potwierdzają, że związki biologicznie aktywne wpływają na szereg właściwości prozdrowotnych rośliny (Piłat i in., 2015; Rafalska i in., 2017). Testy przeprowadzone z udziałem pacjentów kardiologicznych wskazały, że flawonoidy z rokitnika pospolitego przyczyniły się do obniżenia poziomu cholesterolu całkowitego, triacyloglicerydów i frakcji lipoprotein o niskiej gęstości (LDL) oraz zwiększenia frakcji lipoprotein o dużej gęstości (HDL) (Rafalska i in., 2017). Guo i in. (2017b) dowiedli, że kardioprotekcyjne działanie rokitnika pospolitego można przypisać zawartości flawonoidów i β-sitosterolu. Ponadto, olej z rokitnika pospolitego o wysokim stężeniu karotenoidów może być korzystny w leczeniu i profilaktyce miażdżycowych chorób tętnic, ponieważ skutecznie hamuje agregację płytek krwi (Xu i in., 2011). Enkhtaivan i in. (2017) dowiedli, że aktywność w kierunku infekcji wywołanej zakażeniem wirusem grypy silnie korelowała z zawartością aglikonów i monoglikozydów flawonoli, podczas gdy di- i triglikozydy flawonoli wskazały silną korelację z działaniem cytotoksycznym wobec komórek prawidłowych i nowotworowych. W przemyśle kosmetycznym roślina wykorzystywana jest w prewencji i terapii chorób dermatologicznych, pielęgnacji włosów, rewitalizacji ran i oparzeń skóry oraz jako forma naturalnej ochrony przed promieniowaniem UV-B (Rafalska i in., 2017). Zainteresowanie rokitnikiem pospolitym stale wzrasta wraz z powiększającą się pulą wyników badań *in vitro* i *in vivo* potwierdzających możliwe korzystne implikacje dla zdrowia człowieka, w tym skuteczność ekstraktów w zapobieganiu hiperglikemii, hiperinsulinemii i hiperlipidemii, aktywność hepatoprotekcyjną, kardioprotekcyjną, neuroprotekcyjną, cytoprotekcyjną, radioprotekcyjną, immunomodulującą, przeciwnowotworową, przeciwutleniającą, przeciwdrobnoustrojową, a także pozytywne funkcjonowanie układu pokarmowego i wzroku (Arimboor i in, 2008; Kim i in., 2010; Suryakumar i Gupta, 2011; Dulf i in., 2012; Patel i in., 2012; Guo i in., 2017b; Rafalska i in., 2017).

Obecnie w centrum uwagi znajdują się tradycyjnie uprawiane rośliny, a zapotrzebowanie na ich produkcję wzrasta dzięki strategii hamowania lub opóźniania chorób za pomocą naturalnej diety (Ciesarová

i in., 2020). Rosnąca tendencja zagrożeniami zdrowotnymi związanymi z nieodpowiednim odżywianiem zwiększa presję na poszukiwanie autentyczności, czystych etykiet i zatwierdzonych oświadczeń żywnościowych. Konsekwentnie trendy konsumenckie zorientowane są na produkty roślinne i prozdrowotne, a przemysł spożywczy rozwija innowacyjne i alternatywne receptury z dodatkami funkcjonalnymi, ciekawymi połączeniami smakowymi, zapomnianymi roślinami oraz superowocami i warzywami (Baiano i in., 2012; Di Cagno i in., 2011). W tym kontekście, prowadzono badania nad owocami rokitnika pospolitego pochodzącymi z różnych regionów świata, jednak wiedza o związkach biologicznie aktywnych i potencjale prozdrowotnym odmian uprawianych w Polsce jest ograniczona. Obecnie, Polska jest jednym z największych krajów w Europie zainteresowanych uprawą rokitnika pospolitego na skalę przemysłową. Mając na uwadze powyższe, ważne było scharakteryzowanie wybranych odmian rokitnika pospolitego jako bogatego źródła składników odżywczych i metabolitów wtórnych o ukierunkowanym działaniu prozdrowotnym.

Badania przeprowadzone nad wybranymi odmianami uprawianymi w Polsce zaprezentowane w **Publikacji 1** pozwoliły na stwierdzenie, że jagody rokitnika pospolitego są zasobne w kwasy organiczne, związki fenolowe, karotenoidy, tokoferole i kwasy tłuszczowe, które związane są z aktywnością przeciwcukrzycową, przeciw otyłości, przeciwzapalną i przeciwutleniającą. Następnie, w ramach badań zreferowanych w **Publikacji 2**, wskazano wysoki potencjał hamowania cholinoesteraz przez owoce rokitnika pospolitego, które mogą stanowić składnik nowych funkcjonalnych i wartościowych produktów bogatych we flawonole i karotenoidy o działaniu przeciwstarzeniowym. Co więcej, zgodnie z ideą zrównoważonego rozwoju i technologii bezodpadowej dostrzeżono potencjał zagospodarowania pozostałości po zbiorze rokitnika pospolitego i procesach przetwórczych z jego udziałem. Zawansowanie badań własnych i równoległy niedobór doniesień literaturowych na temat składu całej rośliny zaowocowały badaniami, w ramach których stwierdzono różnorodność triterpenoidów, związków fenolowych oraz makro- i mikroelementów w zależności od anatomicznych części rokitnika pospolitego, skórki, miąższu, nasion, endokarpu, pędów i liści, wysokoplonujących odmian uprawianych w Europie Środkowo-Wschodniej (**Publikacja 3**). Stąd, dobór surowca, jego wstępna obróbka, zastosowane parametry i rodzaje procesów technologicznych będą istotne w uzyskaniu produktów o zachowanych i ukierunkowanych walorach prozdrowotnych. Potwierdziły to badania własne nad sokami stanowiącymi komercyjnie powszechną formą przetworzonego rokitnika pospolitego (**Publikacja 4**). Wyniki badań zamieszczone w **Publikacji 4** dowiodły także, że soki z owoców rokitnika pospolitego to propozycja produktów żywnościowych o działaniu przeciwcukrzycowym, przeciw otyłości i przeciwstarzeniowym wynikającym z zasobności w fitoprostany, fitofurany, tokoferole, tokotrienole i aminokwasy.

Jednakże, pomimo szerokiego występowania rokitnika pospolitego, niewielkich wymagań co do warunków uprawy oraz niepowtarzalnego składu chemicznego i działania prozdrowotnego, wciąż odnotowuje się niskie spożycie jagód oraz nikłe zainteresowanie od strony przemysłu. Według Międzynarodowego Stowarzyszenia Rokitnika (ISA) z owoców rokitnika pospolitego produkowana jest żywność głównie w skali lokalnej, w tym soki, napoje bezalkoholowe, herbaty, dżemy, przekąski i likiery. Olej pozyskiwany z nasion i miazgi stosowany jest jako kosmetyk i suplement diety, zaś pozostałości produkcyjne mogą być funkcjonalnym składnikiem pasz (Bal i in., 2011, Rafalska i in., 2017). Chociaż roślina ta jest cennym surowcem w przemyśle kosmetycznym i farmaceutycznym, to jej potencjał jako składnik żywności wciąż pozostaje niewykorzystany. Stanowi wyzwanie zarówno dla przemysłu spożywczego,

jak i konsumentów ze względu na utrudniony zbiór i obróbkę technologiczną, a przede wszystkim postrzeganą jakość sensoryczną (Ciesarová i in., 2020).

W smaku jagód dominuje intensywna kwasowość wynikająca ze skrajnie niskiego stosunku cukrów do kwasów organicznych, a także cierpkość skorelowana z niektórymi glikozydami flawonoli, proantocyjanidynami, etylo-β-D-glukopiranozydem i kwasem jabłkowym oraz gorycz związana ze stosunkiem kwasów organicznych i związków fenolowych. Aromat jest opisywany jako intensywnie ostry z nutami fermentacyjnymi. Z kolei konsystencja czystego soku jest niejednorodna ze względu na podatność fazy tłuszczowej i osadu na separację (Laaksonen i in., 2016; Ma i in., 2020; Tiitinen i in., 2006; **Publikacja 1**). Wciąż brak w literaturze naukowej doniesień o produktach z dużym udziałem jagód rokitnika pospolitego, które byłyby atrakcyjne dla potencjalnego konsumenta jednocześnie pod względem właściwości prozdrowotnych i sensorycznych. Rozwiązaniem dla zwiększenia spożycia i zastosowania rokitnika pospolitego w przemyśle spożywczym może być skorygowanie kwaśnego smaku soku poprzez fermentację jabłkowo-mlekową (malolaktyczną; MLF) (**Publikacja 5**). Proces ten obejmuje dekarboksylację kwasu jabłkowego do kwasu mlekowego i dwutlenku węgla oraz jest szeroko stosowany w celu obniżenia kwasowości, zwiększenia stabilności mikrobiologicznej oraz modyfikacji aromatu, smaku i tekstury win czerwonych i niektórych win białych (Wojdyło i in., 2020). Potencjalne zastosowanie MLF badano również w sokach i miazgach owocowych i warzywnych. Wyniki sugerowały, że fermentacja z użyciem *Lactobacillus plantarum* może mieć korzystny wpływ na właściwości fizykochemiczne, zawartość i profil związków bioaktywnych, potencjał przeciwutleniający i ocenę sensoryczną m.in. oliwek (Kachouri i in., 2015), kiwi (Zhou i in., 2020), owoców granatu (Mousavi i in., 2013) i morwy (Kwaw i in., 2018).

Owoce rokitnika pospolitego stosowane są jako kilkuprocentowy dodatek wzbogacający walory prozdrowotne i zwiększający kwasowość przetworów owocowo-warzywnych. Jednak niwelacji problemu braku atrakcyjności sensorycznej można upatrywać w komponowaniu soku z owoców rokitnika pospolitego z wyselekcjonowanymi surowcami powszechnie stosowanymi w przetwórstwie owocowo-warzywnym (**Publikacja 6**). Selvamuthukumaran i in. (2007) opracowali galaretki na bazie jagód rokitnika pospolitego i winogron (1:1), które uzyskały wysoką aprobatę w ocenie sensorycznej w porównaniu do produktów z rokitnika pospolitego z papają i arbuzem. Poprzednio, w badaniu przeprowadzonym przez Hartvig i in. (2014), tylko dzieci wśród konsumentów z Danii aprobowały słodko-kwaśny sok z owoców rokitnika pospolitego, który jest powszechnym składnikiem diety nordyckiej.

Alternatywą wykorzystania owoców rokitnika pospolitego może być proces mikroenkapsulacji soku z nośnikami polisacharydowymi w oparciu o proces suszenia (**Publikacja 7**). Dotychczas z sukcesem otrzymano mikrokapsułki z soków, ekstraktów i wytłoków owocowych, m.in. z mango, jabłek, pomarańczy, ekstraktów fenolowych z czarnej porzeczki i skórki winogron, metodami suszenia rozpyłowego i sublimacyjnego z zastosowaniem maltodekstryn, inuliny, gumy arabskiej, gumy karagenowej, karboksymetylocelulozy, skrobi, pektyn i białka serwatki (Caparino i in., 2012; Barbosa i in., 2015; Kuck i Noreña, 2016; Michalska i Lech, 2018). Proces mikroenkapsulacji zapewnia ochronę cennych, wrażliwych lub docelowych składników w materiale powłokowym, dlatego w jego zastosowaniu upatruje się wydłużenia okresu przydatności do spożycia, poprawy właściwości fizycznych, wartości odżywczych i prozdrowotnych, jednocześnie zapewniając niższy koszt transportu i przechowywania innowacyjnych mikrokapsułek (Çam i in., 2014; Michalska i Lech, 2018).

2. CEL I HIPOTEZA BADAŃ

Celem niniejszej pracy było określenie profilu prozdrowotnego owoców rokitnika pospolitego (*Hippophaë rhamnoides* L.) i opracowanie technologii otrzymywania funkcjonalnych i atrakcyjnych sensorycznie produktów o ukierunkowanych właściwościach prozdrowotnych na bazie owoców tej rośliny.

Cel główny pracy realizowano w oparciu o **cele szczegółowe** wynikające bezpośrednio z monotematycznego cyklu publikacji wchodzącego w skład niniejszej rozprawy doktorskiej:

1. Analiza aktywności biologicznej w odniesieniu do wybranych składników bioaktywnych (flawonole, kwasy fenolowe, ksantofile, karoteny, estryfikowane karotenoidy, tokoferole, tokotrienole, kwasy tłuszczowe) i podstawowego składu chemicznego (cukry, kwasy organiczne, sucha masa, ekstrakt ogólny, pH, kwasowość, popiół, pektyny, witamina C) owoców sześciu powszechnie uprawianych w Polsce odmian rokitnika pospolitego (**Publikacja 1**).
2. Szczegółowa identyfikacja i oznaczanie ilościowe związków fenolowych i karotenoidów oraz ocena aktywności przeciwstarzeniowej owoców wybranych odmian rokitnika pospolitego (**Publikacja 2**).
3. Jakościowe i ilościowe oznaczenie triterpenów, związków fenolowych, makro- i mikroelementów części anatomicznych jagód (skóra, miąższ, endokarp, nasiona), pędów i liści wybranych odmian rokitnika pospolitego (**Publikacja 3**).
4. Jakościowe i ilościowe oznaczenie fitoprostanów, fitofuranów, tokoferoli, tokotrienoli, karotenoidów i wolnych aminokwasów oraz ocena potencjału przeciwstarzeniowego, przeciwcukrzycowego, przeciw otyłości, przeciwzapalnego i przeciwutleniającego komercyjnie dostępnych soków z owoców rokitnika pospolitego (**Publikacja 4**).
5. Określenie aktywności metabolicznej wybranych szczepów bakterii wraz z dynamiką zmiany zawartości kwasów organicznych, cukrów, związków fenolowych i aktywności przeciwutleniającej podczas fermentacji jabłkowo-mlekowej soku z owoców rokitnika pospolitego i soku mieszanego rokitnik – jabłko (1:1) (**Publikacja 5**).
6. Ocena potencjału przeciwstarzeniowego, przeciwcukrzycowego, przeciw otyłości i przeciwutleniającego, analiza związków fenolowych, podstawowego składu chemicznego i jakości sensorycznej nowatorskich smoothies na bazie owoców rokitnika pospolitego (**Publikacja 6**).
7. Ocena wpływu metod suszenia i nośników polisacharydowych na właściwości fizyczne, składniki chemiczne i aktywność przeciwutleniającą mikrokapsułek z soku z owoców rokitnika pospolitego (**Publikacja 7**).

Weryfikacji poddano **hipotezę badawczą** zakładającą, że owoce rokitnika pospolitego charakteryzuje unikatowy profil związków bioaktywnych o określonym potencjale prozdrowotnym przez co mogą one stanowić wartościowy surowiec do produkcji jednocześnie funkcjonalnych i atrakcyjnych sensorycznie produktów.

3. ORGANIZACJA BADAŃ

3.1. Materiał i zakres badań

Przedmiotem badań niniejszej pracy były owoce rokitnika pospolitego (*Hippophaë rhamnoides* L.). W pierwszym etapie doświadczeń analizowano jagody sześciu odmian: ‘Aromatnaja’, ‘Botaniczeskaja-Lubitelskaja’, ‘Józef’, ‘Luczistaja’, ‘Moskwiczka’ i ‘Podarok Sadu’, pozyskane na przełomie lipca i sierpnia 2018 roku z Sadu Doświadczalnego Dąbrowice Instytutu Ogrodnictwa w Skierniewicach (51°56′N 20°06′E) (**Publikacje 1** i **2**).

Zakres badań pierwszego etapu poszerzono o analizy części anatomicznych jagody, w tym skórki, miąższu, nasion i endokarpu, oraz liści, srebrzysto-zielonych pędów jednorocznych i owocujących pędów dwuletnich pokrytych srebrzystą korowiną (dalej określanych jako: „pędy”) rokitnika pospolitego. Materiał badawczy frakcjonowano z krzewów siedmiu odmian: ‘Botaniczeskaja-Lubitelskaja’, ‘Golden Rain’, ‘Luczistaja’, ‘Maryja’, ‘Podarok Sadu’, ‘Prozrocznaja’ i ‘Tatiana’, w połowie września 2020 roku z plantacji Gospodarstwa Ogrodniczego Stanisław Trzonkowski w Sokółce (**Publikacja 3**).

W pierwszym etapie badań przebadano soki z owoców rokitnika pospolitego ogólnodostępne na polskim rynku detalicznym w porównaniu z sokiem wykonanym w skali laboratoryjnej z owoców odmiany ‘Józef” (**Publikacja 4**).

W drugim etapie badań przeprowadzono fermentację jabłkowo-mlekową soku z owoców rokitnika pospolitego odmiany ‘Józef” i soku mieszanego (1:1) z owoców rokitnika pospolitego i jabłek odmiany ‘Champion’ (Stacja Doświadczalna Oceny Odmian w Zybiszowie, k. Wrocławia). Zastosowano liofilizowane kultury szczepów *Lactobacillus plantarum* (DSM 100813, DSM 13273, DSM 20174, DSM 10492 i DSM 6872), *Lactobacillus plantarum* subsp. *argentoratensis* (DSM 16365) i *Oenococcus oeni* (DSM 20255) pozyskane z the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (Brunszwik, Niemcy) (**Publikacja 5**).

Etap trzeci obejmował analizę produktów typu smoothies wytworzonych w skali laboratoryjnej z soku z owoców rokitnika pospolitego odmiany ‘Józef’ oraz z przecieru otrzymanego z owoców: gruszy (*Pyrus communis*), jabłoni (*Malus domestica*), moreli (*Prunus armeniaca*), brzoskwini (*Prunus persica*), pomarańczy (*Citrus sinensis*) i zielonych winogron (*Vitis vinifera*), oraz korzeni warzyw: selera (*Apium graveolens*), marchwi (*Daucus carota*), pietruszki (*Petroselinum crispum*). Surowce zakupiono w pierwszej połowie września 2020 roku na rynku detalicznym (**Publikacja 6**).

W czwartym etapie badano mikrokapsułki utrwalone poprzez proces suszenia rozpyłowego, sublimacyjnego i próżniowego, składające się z soku z owoców rokitnika pospolitego odmiany „Józef” z 20%-owym dodatkiem wagowym inuliny, maltodekstryny i mieszanek inuliny z maltodekstryną (BENEO-Orafti S.A., Oreye, Belgia) w proporcjach 2:1 i 1:2 (**Publikacja 7**).

3.2. Procesy technologiczne

Soki z owoców rokitnika pospolitego i jabłek przygotowano odpowiednio za pomocą prasy hydraulicznej (SRSE, Warszawa, Polska) i wyciskarki wolnoobrotowej (Hurom HG 2G, Puregreen S.C., Sławno, Polska) (**Publikacje 4**, **5** i **7**).

Proces fermentacji jabłkowo-mlekowej (**Publikacja 5**) prowadzono zgodnie z procedurą przygotowania zawiesin bakteryjnych, zaszczepiania i 72-godzinnej fermentacji z pobieraniem próbek według Tkacz i in. (2020a).

Produkcja smoothies (**Publikacja 6**) składała się z czterech etapów:

(1) przygotowanie soku z owoców rokitnika pospolitego,

(2) przetwarzania owoców i warzyw na przecier,

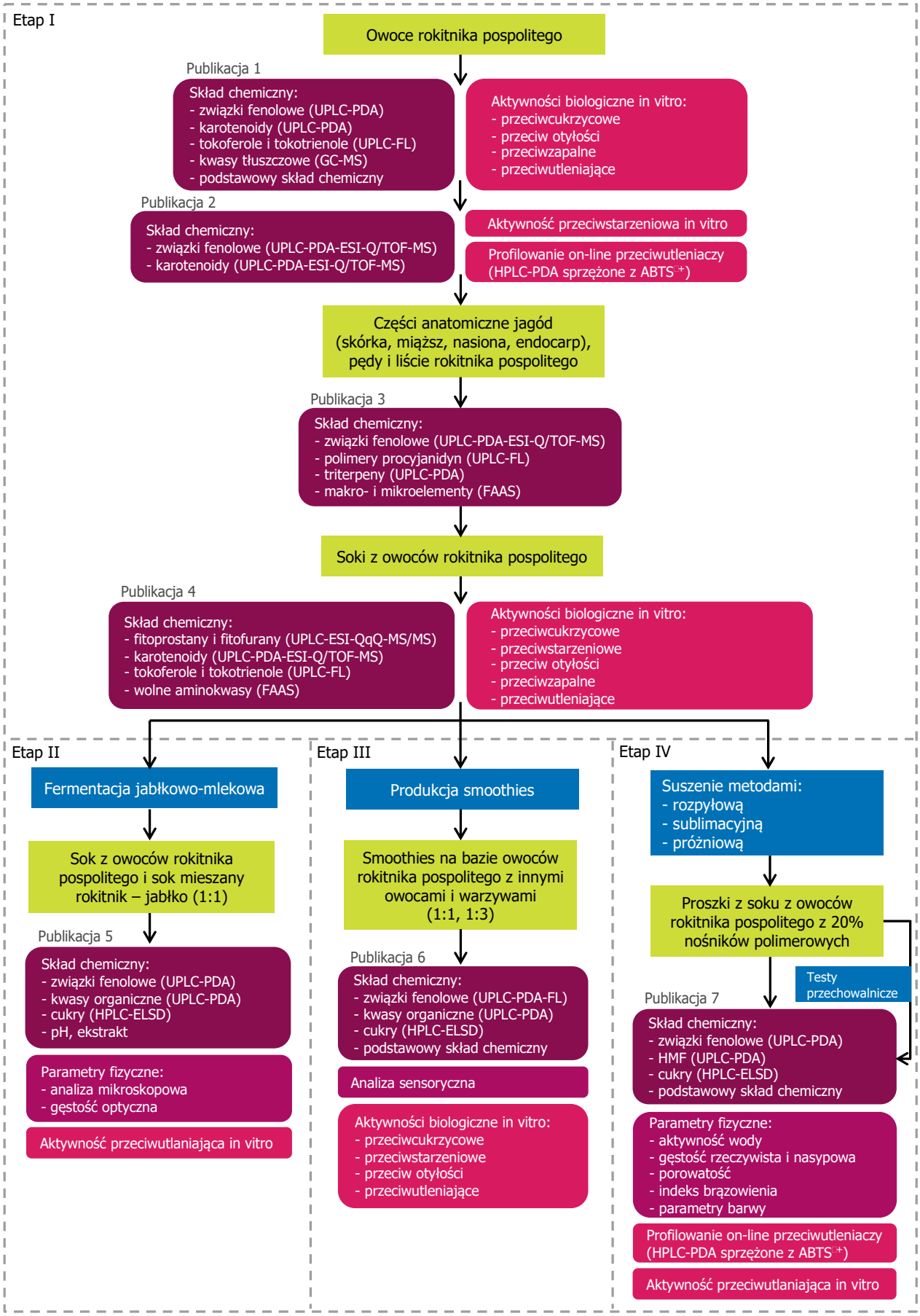
(3) połączenia składników w smoothies,

(4) obróbka termiczna, zgodnie z protokołem według Tkacz i in. (2021b).

Proces produkcji mikrokapsułek z soku z owoców rokitnika pospolitego z dodatkiem nośników polisacharydowych (**Publikacja 7**) wykonano metodami suszenia rozpyłowego, sublimacyjnego i próżniowego, zgodnie z procedurą według Tkacz i in. (2020b). Testy przechowalnicze mikrokapsułek (**Publikacja 7**) prowadzono przez okres sześciu miesięcy w warunkach podanych przez Tkacz i in (2020b).

3.3. Etapy badań

Cele pracy realizowano w oparciu o **IV główne etapy badań zaprezentowane na Schemacie 1**, gdzie przedstawiono organizację badań z uwzględnieniem analiz składu chemicznego, parametrów fizycznych, aktywności biologicznej i analizy sensorycznej.



Schemat 1. Organizacja badań nad owocami rokitnika pospolitego w projektowaniu żywności o ukierunkowanym potencjale prozdrowotnym

3.4. Metody badań

Analizy składu chemicznego

Materiał badawczy (surowiec i produkty), w zależności od etapu badań (**Publikacje 1-7**), poddano następującym analizom składu chemicznego:

- oznaczenie ilościowe i identyfikacja związków fenolowych metodą chromatografii cieczowej z układem fotodiodowym sprzężonej z tandemowym spektrometrem mas typu kwadrupol-analizator czasu przelotu (UPLC-PDA-ESI-Q/TOF-MS) zgodnie z Wojdyło i in. (2018) z modyfikacjami według Tkacz i in. (2020c);
- oznaczenie ilościowe polimerów procyjanidyn metodą bezpośredniej floroglucynolizy z użyciem ultrasprawnej chromatografii cieczowej (UPLC) sprzężonej z detekcją fluorescencyjną (FL) według Teleszko i Wojdyło i in. (2015);
- oznaczenie ilościowe i identyfikacja karotenoidów metodą UPLC-PDA-ESI-Q/TOF-MS według Wojdyło i in. (2018);
- oznaczenie ilościowe i identyfikacja tokoferoli i tokotrienoli metodą UPLC-FL według Tkacz i in. (2019b);
- oznaczenie ilościowe i identyfikacja wolnych aminokwasów metodą UPLC-PDA-ESI-Q/TOF-MS według Turkiewicza i in. (2020);
- oznaczenie ilościowe i identyfikacja kwasów tłuszczowych metodą chromatografii gazowej sprzężonej ze spektrometrią mas (GC-MS) według Nowackiego i in. (2017);
- oznaczenie ilościowe i identyfikacja triterpenów metodą UPLC-PDA-ESI-Q/TOF-MS według Zhang i in. (2013);
- oznaczenie ilościowe i identyfikacja fitoprostanów i fitofuranów metodą ultrawysokosprawnej chromatografii cieczowej sprzężonej z potrójną kwadrupolową spektrometrią mas (UHPLC-ESI-QqQ-MS/MS) według Collado-González i in. (2015)
- oznaczenie ilościowe i identyfikacja kwasów organicznych metodą ultrasprawnej chromatografii cieczowej (UPLC) sprzężonej z detekcją fotodiodową (PDA) według Wojdyło i in. (2018);
- oznaczenie ilościowe i identyfikacja cukrów metodą wysokosprawnej chromatografii cieczowej (HPLC) sprzężonej z detektorem rozproszenia światła (ELSD) według Wojdyło i in. (2018);
- oznaczenie ilościowe i identyfikacja hydroksymetylofurfuralu (HMF) metodą UPLC-PDA według Tkacz i in. (2020b);
- oznaczenie ilościowe i identyfikacja makroelementów i mikroelementów metodą atomowej spektrometrii absorpcyjnej z atomizacją w płomieniu (FAAS) według Tkacz i in. (2021a);
- podstawowe analizy chemiczne:
  - zawartość ekstraktu ogólnego metodą refraktometryczną według PN-90/A-75101/02,
  - zawartość suchej masy metodą wagową według PN-90/A-75101/03,
  - kwasowość ogólna metodą miareczkową według PN-90/A-75101/04,
  - pH metodą potencjometryczną według PN-90/A-75101/06,
  - zawartość popiołu ogólnego metodą wagową według PN-90/A-75101/08;
  - zawartość witaminy C metodą miareczkową według PN-90/A-75101/11;

- zawartość pektyn metodą Morrisa według Wojdyło i in. (2017).

Analizy parametrów fizycznych

Analizy mikroskopowe zawiesin szczepów bakterii użytych w procesie fermentacji jabłkowo-mlekowej soków na bazie owoców rokitnika pospolitego (**Publikacja 5**) wykonano z użyciem imersji olejowej (100-krotne powiększenie obiektywu) zgodnie z protokołem Tkacz i in. (2020a).

Gęstość optyczną (OD_{560}) określającą wzrost komórek bakteryjnych (**Publikacja 5**) mierzono metodą spektrofotometryczną przy 560 nm według Tkacz i in. (2020a).

Mikrokapsułki z soku z owoców rokitnika pospolitego (**Publikacja 7**) analizowano pod kątem parametrów fizycznych, tj. aktywność wody, gęstość rzeczywista, gęstość nasypowa, porowatość, indeks brązowienia, analizowano według Michalskiej i Lecha (2018). Parametry barwy w przestrzenie CIE L*a*b*, parametr nasycenia C (Chroma), kąt barwy (h°) i całkowitą różnicę barwy (dE) analizowano według Kuck i Noreña (2016) i Šumić i in. (2013).

Analizy aktywności biologicznej

Analizy aktywności przeciwutleniającej wykonano posługując się następującymi metodami:

- oznaczenie aktywności przeciwutleniającej z kationorodnikiem $ABTS^{\bullet+}$ według Re i in. (1999);
- oznaczania zdolności redukowania jonów Fe^{3+} (FRAP) według Benzie i Strain (1996);
- oznaczania zdolności absorpcji rodników tlenowych (ORAC) według Ou i in. (2001);
- profilowanie on-line przeciwutleniaczy metodą HPLC-PDA sprzężoną z derywatyzacją pokolumnową z użyciem odczynnika $ABTS^{\bullet+}$ zgodnie z Kusznierewicz i in. (2011) z modyfikacjami według Tkacz i in. (2019a).

Aktywności biologiczne związane z regulacją aktywności enzymów wyznaczono w oparciu o metody *in vitro*:

- oznaczenie aktywności przeciwstarzeniowej jako zdolności inhibicji enzymów acetylocholinoesterazy (AChE) i butylocholinoesterazy (BuChE) według Gironés-Vilaplana i in. (2015);
- oznaczenie aktywności przeciwcukrzycowej jako zdolności inhibicji enzymów α-amylazy i α-glukozydazy według Nowicka i in. (2018);
- oznaczenie aktywności przeciw otyłości jako zdolności inhibicji enzymu lipazy trzustkowej według Podsędek i in. (2014);
- oznaczenie aktywności przeciwzapalnej jako zdolności inhibicji enzymu 15-lipooksygenazy (15-LOX) według Chung i in. (2009).

Analiza sensoryczna

Analizę sensoryczną produktów na bazie owoców rokitnika pospolitego (**Publikacja 6**) przeprowadzono wśród 23 przeszkolonych panelistów oceniających atrybuty: barwę, aromat, konsystencję, smak, posmak kwaśny, posmak obcy i ogólną akceptację w 5-stopniowej skali hedonicznej, z zachowaniem standardów procesu według ISO 13299:2016 (Tkacz i in., 2021b).

3.5. Analiza statystyczna

Wyniki oznaczeń składu chemicznego i parametrów fizycznych, aktywności biologicznej oraz analizy sensorycznej poddano analizie statystycznej. Celem testowania istotności różnic między średnimi, dane poddano analizie wariancji (ANOVA) i testowi rozsądnej istotnej różnicy (RIR, ang. HSD) Tukeya (**Publikacje 1-3, 5-6**) lub testowi wielozakresowemu Duncana (**Publikacja 7**). Dane dotyczące soków komercyjnych z owoców rokitnika (**Publikacja 4**) poddano nieparametrycznej analizie wariancji stosując test Kruskala-Wallisa, a następnie test wielokrotnych porównań Dunna. Różnice statystyczne na poziomie $p < 0,05$ oznaczono w tabelach i na rysunkach kolejnymi literami (a, b, c, …). Wszystkie pomiary przeprowadzono w trzech powtórzeniach, a wyniki przedstawiono jako wartość średnią ($n = 3$) ± odchylenie standardowe (SD). Dodatkowo, przedstawiono współczynniki korelacji Pearsona (r), analizę głównych składowych (PCA) w oparciu o macierz korelacji i z zastosowaniem autoskalowania oraz aglomeracyjne klastrowanie hierarchiczne (AHC) uzyskane na podstawie odmiennych odległości euklidesowych, stosując aglomeracyjną metodę grupowania Warda. Analizy statystyczne i opracowanie graficzne wyników wykonano przy użyciu oprogramowań Statistica 13.1 (StatSoft, Kraków, Polska) oraz XLSTAT Statistical Software w wersji 2016.4 (Addinsoft Inc, New York, NY, USA) zintegrowanego z Microsoft Excel 2010/2017/2019 (Microsoft Corp., Redmond, WA, USA).

4. OMÓWIENIE I DYSKUSJA WYNIKÓW

4.1. Analiza frakcji biologicznie aktywnej rokitnika pospolitego

W pierwszym etapie pracy analizom poddano owoce sześciu, powszechnie uprawianych w Polsce, odmian rokitnika pospolitego (*Hippophaë rhamnoides* L.): 'Aromatnaja', 'Botaniczeskaja-Lubitelskaja', 'Józef', 'Luczistaja', 'Moskwiczka' i 'Podarok Sadu'. Celem tego badania było określenie aktywności biologicznej *in vitro* jako potencjału przeciwutleniającego i zdolności inhibicji α-amylazy, α-glukozydazy, lipazy trzustkowej i 15-lipooksygenazy, w stosunku do składników bioaktywnych (kwasów fenolowych, flawonoli, ksantofili, karotenów, zestryfikowanych karotenoidów, tokoferoli, tokotrienoli i kwasów tłuszczowych) oraz podstawowego składu chemicznego jagód rokitnika pospolitego.

Wyniki przedstawiono w **Publikacji 1**:

Tkacz K., Wojdyło A., Turkiewicz I.P., Bobak Ł., Nowicka P. 2019. Anti-oxidant and anti-enzymatic activities of sea buckthorn (*Hippophaë rhamnoides* L.) fruits modulated by chemical components. *Antioxidants*, 8, 618. doi:10.3390/antiox8120618.

Owoce rokitnika pospolitego poddano analizom podstawowego składu chemicznego, czyli zawartości cukrów, kwasów organicznych, suchej masy, ekstraktu ogólnego, popiołu, pektyn, witaminy C, pH i kwasowości ogólnej (**Publikacja 1, Tab. 1**). Niektóre z odmian *H. rhamnoides* stanowiły przedmiot zainteresowania także innych naukowców, jednak były uprawiane w odmiennych warunkach klimatycznych i glebowych, w tym na terenie Szwecji, Białorusi, Finlandii i Kanady. Co więcej, wyniki zawarte w **Publikacji 1** stanowią pierwsze doniesienie literaturowe na temat nowej odmiany 'Józef' wyselekcjonowanej w Polsce.

Zgodnie z analizą ilościową i identyfikacją cukrów w rokitniku pospolitym z użyciem metody HPLC-ELSD, w najwyższej ilości występowała glukoza (od 86,58% do 92,68% cukrów ogółem), następnie sorbitol, fruktoza i ramnoza (**Publikacja 1, Tab. 1**). Kwasy organiczne badane metodą UPLC-PDA uszeregowano pod względem ich ilości w odmianach rokitnika pospolitego w następującej kolejności: kwas jabłkowy > kwas chinowy > kwas izocytrynowy > kwas cytrynowy > kwas szczawiowy. Wyniki zaprezentowane w **Publikacji 1** wskazały na konieczność korekty smaku owoców wybranych odmian rokitnika pospolitego, w szczególności tych, w których stosunek cukrów do kwasów organicznych wyniósł poniżej 1,0, tj. 'Botaniczeskaja-Lubitelskaja', 'Luczistaja', 'Podarok Sadu' oraz nowej odmiany 'Józef'. Wyjątkowo kwaśny smak owoców potwierdziła analiza pH, które wynosiło od 2,89 do 2,95 oraz kwasowość miareczkowa równa od 2,48 g do 2,79 g kwasu jabłkowego/100 g śm (świeżej masy). Owoce rokitnika pospolitego charakteryzowała także wyższa niż w przypadku innych owoców jagodowych zawartość witaminy C (od 61,02 do 158,81mg/100 g śm), która ze względu na brak aktywnego enzymu askorbinazy w jagodach rokitnika pospolitego pozostaje stabilna po zbiorze i podczas przechowywania (Kallio i in., 2002).

Posługując się techniką UPLC-PDA oznaczono w owocach rokitnika pospolitego kwasy fenolowe i flawonole (**Publikacja 1, Tab. 1**). Całkowita zawartość związków fenolowych wyniosła od 468,60 mg do 901,11 mg/100 g sm (suchej masy). Około 98,9% związków fenolowych ogółem stanowiły flawonole, a kolejność odmian pod względem ich zawartości była następująca: 'Moskwiczka' > 'Józef' > 'Aromatnaja' > 'Podarok Sadu' > 'Botaniczeskaja-Lubitelskaja' > 'Luczistaja'. Na podstawie porównania badanych odmian z odmianami uprawianymi w Europie Wschodniej i Azji (Pop i in., 2013; Ma i in. 2016) stwierdzono

zmienność profilu flawonoli, co znajduje swoje odzwierciedlenie wynikające z oddziaływania odmiennych czynników klimatycznych, geograficznych, daty zbioru, transportu i przechowywania oraz stanowi cechę podgatunkową i odmianową (Zheng i in., 2016).

Owoce rokitnika poddano analizie karotenoidów z użyciem techniki UPLC-PDA, która pozwoliła na wyznaczenie całkowitego stężenia karotenoidów od 46,61 mg do 508,57 mg/100 g sm (**Publikacja 1, Tab. 1**). Uzyskane wartości były istotnie wyższe ($p < 0,05$) niż raportowane dla owoców rokitnika pospolitego zebranych w Szwecji (od 11,99 mg do 142,49 mg/100 g sm) (Andersson i in., 2008) i Rumunii (od 53 mg do 97 mg/100 g sm) (Pop i in., 2014). Jagody odmiany 'Józef' zawierały 3-krotnie więcej karotenoidów niż odmiany 'Luczistaja', a w owocach odmiany 'Aromatnaja' stwierdzono od 2 do 25 razy wyższą zawartość karotenów (225,42 mg/100 g sm) niż w innych odmianach. Najwyższym udziałem ksantofili w stosunku do karotenoidów ogółem, ponad 74%, charakteryzowały się owoce odmian 'Botaniczeskaja-Lubitelskaja' i 'Luczistaja'.

Przeprowadzona analiza z wykorzystaniem metody chromatograficznej (UPLC-FL) pozwoliła na stwierdzenie, że frakcja lipofilowa jagód rokitnika pospolitego zawierała obok karotenoidów także tokoferole i tokotrienole (**Publikacja 1, Tab. 1**). Sumaryczna zawartość tokoferoli i tokotrienoli wyniosła od 27,12 mg do 34,27 mg/100 g sm, odpowiednio dla owoców odmian 'Luczistaja' i 'Aromatnaja'.

Posługując się metodą chromatografii gazowej (GC-MS), zidentyfikowano sześć kwasów tłuszczowych przynależnych do omega-3, omega-6, omega-7 i omega-9, w tym dwa kwasy nasycone (SFA; palmitynowy i stearynowy), dwa kwasy jednonienasycone (MUFA, oleopalmitynowy i oleinowy) oraz dwa kwasy wielonienasycone (PUFA; linolowy i linolenowy) (**Publikacja 1, Tab. 1**). Dominującym był kwas palmitynowy (C16:0), a ogólny profil kwasów tłuszczowych owoców rokitnika pospolitego ustalono następująco: 38% kwasy nasycone, 42% kwasy jednonienasycone i 20% kwasy wielonienasycone. Wysoka zawartość kwasu palmitynowego, palmitoleinowego, oleinowego i linolowego jest charakterystyczna dla oleju z owoców rokitnika pospolitego, w przeciwieństwie do nasion bogatych w kwasy wielonienasycone (Pop i in., 2014; Vescan i in., 2010).

W **Publikacji 1**, po raz pierwszy w literaturze, zaprezentowano wyniki związane z aktywnością przeciwcukrzycową, przeciw otyłości, przeciwzapalną i przeciwutleniającą jagód odmian rokitnika pospolitego uprawianych w Polsce, w tym nowej odmiany 'Józef'. Najwyższy potencjał przeciwutleniający oznaczony metodami ABTS, FRAP i ORAC zbadano w przypadku owoców odmiany 'Aromatnaja', a najniższy dla owoców odmian 'Luczistaja' i 'Botaniczeskaja-Lubitelskaja' (**Publikacja 1, Tab. 2**). Zdolność absorpcji rodników tlenowych (metoda ORAC) wyniosła do 34,68 mmol Trolox/100 g sm, podobnie jak w przypadku jagód rokitnika pospolitego podgatunków *turkestanica* i *sinensis* zebranych w Chinach (Guo i in., 2017a). Gao i in. (2000) dowiedli, że obniżenie aktywności przeciwutleniającej ABTS podczas dojrzewania jagód korelowało z obniżeniem stężeń związków fenolowych i kwasu askorbinowego podczas gdy aktywność frakcji lipidowej wzrastała w procesie dojrzewania dzięki syntezie karotenoidów.

Najwyższą aktywność przeciwcukrzycową (IC_{50} = 26,83 mg/ml), analizowaną jako zdolność inhibicji α-amylazy, stwierdzono dla jagód odmiany 'Aromatnaja'. Z kolei, wysoką aktywność w kierunku α-glukozydazy, drugiego enzymu zaangażowanego w metabolizm cukrów, zbadano dla owoców odmiany 'Botaniczeskaja-Lubitelskaja' (IC_{50} = 41,78 mg/ml). Dla wszystkich badanych odmian rokitnika pospolitego

hamowanie α-amylazy było silniejsze niż α-glukozydazy. Efekt hipoglikemiczny potwierdziły także badania z udziałem ludzi, które dowiodły, że posiłki zawierające jagody rokitnika pospolitego mogą zmniejszać i opóźniać poposiłkową odpowiedź insulinową oraz poprawiać profil glikemiczny (Mortensen i in., 2018).

W wyniku badań zaprezentowanych **w Publikacji 1** stwierdzono wysoką aktywność jagód rokitnika pospolitego w kierunku inhibicji 15-lipooksygenazy zaangażowanej w regulację procesów zapalnych oraz lipazy trzustkowej, która determinując ilość przyswajanych tłuszczów, odgrywa kluczową rolę w mechanizmach otyłości, nadwagi i powikłań cukrzycy typu 2. Wyniki analizy korelacji Pearsona wskazały na silne korelacje między zawartością karotenoidów a zdolnością hamowania α-amylazy (r = 0,747) i 15-lipooksygenazy (r = 0,668). Nasycone kwasy tłuszczowe silniej korelowały z potencjałem przeciwzapalnym niż z aktywnością przeciwutleniającą owoców rokitnika pospolitego.

Analiza głównych składowych (PCA) pozwoliła na wyznaczenie czterech grup relacji (**Publikacja 1, Fig. 2**):

(1) jagody odmiany 'Podarok Sadu' były bogate w wielonienasycone kwasy tłuszczowe i miały silne działanie w kierunku inhibicji 15-lipooksygenazy;
(2) aktywność przeciwutleniająca i w kierunku inhibicji α-amylazy były skorelowane z zawartością karotenoidów i witaminy C, w które szczególnie zasobne były owoce odmiany 'Aromatnaja';
(3) jagody odmiany 'Moskwiczka' zawierały wysokie stężenia nasyconych kwasów tłuszczowych, związków fenolowych i glukozy, co z kolei korelowało ze zdolnością inhibicji lipazy trzustkowej i α-glukozydazy;
(4) owoce odmian 'Botaniczeskaja-Lubitelskaja', 'Luczistaja' i 'Józef' tworzył najbardziej rozległy klaster o wysokiej zawartości kwasów organicznych, jednonienasyconych kwasów tłuszczowych, tokotrienoli i tokoferoli.

Badania zaprezentowane w **Publikacji 1** dowiodły, że jagody rokitnika pospolitego mają unikatową kompozycję lipofilowych i hydrofilowych związków bioaktywnych. Uzyskane wyniki pozwoliły na wytypowanie do dalszych badań odmian o najwyższym potencjale bioaktywnym.

Dotychczas w literaturze nie przeprowadzono analizy związków fenolowych i karotenoidów w rokitniku pospolitym pod kątem ich działania przeciwstarzeniowego, istotnego w terapii choroby Alzheimera czy Parkinsona. W tym kontekście, celem kolejnej części badań była szczegółowa identyfikacja i oznaczenie ilościowe związków fenolowych i karotenoidów przy użyciu metody UPLC-PDA-ESI-Q/TOF-MS oraz ocena potencjału przeciwstarzeniowego jako inhibicji acetylocholinoesterazy (AChE) i butylocholinoesterazy (BuChE) owoców wybranych odmian rokitnika pospolitego.

Wyniki przedstawiono w **Publikacji 2**:

Tkacz K., Wojdyło A., Turkiewicz I.P., Ferreres F., Moren, D. A., Nowicka P. 2020. UPLC-PDA-Q/TOF-MS profiling of phenolic compounds and carotenoids and their influence on anticholinergic potential for AChE and BuChE inhibition and on-line antioxidant activity of selected *Hippophaë rhamnoides* L. cultivars. *Food Chemistry*, 309: 125766. doi: 10.1016/j.foodchem.2019.125766.

Wstępnie zidentyfikowano i określono zawartość 28 związków fenolowych, w tym dwóch kwasów fenolowych i reszty jako pochodnych flawonoli (**Publikacja 2, Tab. 1)**. Pasma absorpcji UV i główne jony

fragmentacyjne odpowiadały pochodnym kwasu hydroksycynamonowego, odpowiednio *O*-heksozydowi kwasu *p*-kumarowego i *O*-heksozydowi kwasu ferulowego. Dotychczasowe badania nad rokitnikiem pospolitym zebranym w indyjskim rejonie Himalajów sugerowały dominację obu oznaczonych kwasów, wraz z kwasem galusowym i *p*-hydroksybenzoesowym, jednak badane w literaturze owoce rokitnika pospolitego zawierały nawet do 107 mg kwasów fenolowych w 100 g sm (Arimboor i in., 2008; Guo i in., 2017a; Teleszko i in., 2015).

Oznaczono osiem pochodnych kwercetyny, 15 pochodnych izoramnetyny i aglikon izoramnetyny oraz dwie pochodne kemferolu (**Publikacja 2, Tab. 1)**. Głównymi strukturami glikozydów flawonolu były: -*O*-rutynozyl, -*O*-glukozyl, -*O*-soforozyl i -*O*-ramnozyl, podobnie jak wskazano to w innych badaniach nad rokitnikiem pospolitym (Zheng i in., 2016; Guo i in., 2017a). Badanie identyfikuje pochodne flawonolu podstawione w pozycji C3 (13 związków) oraz w pozycjach C3 i C7 (10 związków) jako preferencyjne pozycje glikozylacji.

Owoce rokitnika należy uznać jako bogate źródło pochodnych izoramnetyny (od 66% do 72% flawonoli ogółem), a następnie pochodnych kwercetyny (od 25% do 32% flawonoli ogółem). W przypadku wszystkich odmian najwyższą koncentrację, od 16% do 20% flawonoli ogółem, wyznaczono dla 3-*O*-rutynozydu izoramnetyny, związku ważnego w aspekcie prozdrowotnym. Badania Boubaker i in. (2011) dowiodły, że 3-*O*-rutynozydu izoramnetyny pochodzenia naturalnego sprzyjał apoptozie ludzkich komórek szpikowej erytroleukemii, z kolei badania na komórkach tłuszczowych linii 3T3-L1 wskazały, że flawonol ten może wpływać na kontrolę masy tkanki tłuszczowej, poprzez hamowanie adipogenezy (Sekii i in., 2015). Badane jagody rokitnika pospolitego charakteryzowały się także wysokim stężeniem: 3-*O*-glukozydu i 3-*O*-glukozydu-7-*O*-ramnozydu izoramnetyny oraz 3-*O*-glukozydu i 3-*O*-rutynozydu kwercetyny.

Wyniki zaprezentowane w **Publikacji 2** stanowią pierwszy w literaturze szczegółowy raport dotyczący karotenoidów badanych odmian oraz owoców rokitnika pospolitego uprawianych w Polsce. Porównanie czasów retencji, widm absorpcji UV i ich struktury oscylacyjnej, absorpcji pików *cis* oraz właściwości jonów i fragmentów molekularnych pozwoliło na wstępną identyfikację 16 karotenoidów, w tym 11 ksantofili, czterech karotenów i jednego estru. Wśród ksantofili oznaczono: all-*trans*-luteinę i jej izomery, all-*trans*-zeaksantynę i jej izomery oraz all-*trans*-β-kryptoksantynę. Z grupy karotenów zbadano all-*trans*-β-karoten i jego izomery oraz likopen, a także zidentyfikowano estryfikowany karotenoid, tj. dipalmitynian zeaksantyny (**Publikacja 2, Tab. 2)**.

Analiza UPLC-PDA pozwoliła wyznaczyć zawartość ksantofili w ilości od 16% do 81% karotenoidów ogółem (**Publikacja 2, Tab. 2)**. Stwierdzono, że owoce rokitnika pospolitego wybranych odmian uprawianych w Polsce wyróżniało kilkukrotnie wyższe stężenie all-*trans*-zeaksantyny niż odmian *H. rhamnoides* z innych regionów (Andersson i in., 2009; Pop i in., 2014). Badania wskazują, że karoteny stanowiły od 19% do 47% karotenoidów ogółem, a zawartość dominującego związku, all-*trans*-β-karotenu, wyniosła od 8,85 mg (‘Luczistaja’) do 162 mg/100 g sm (‘Aromatnaja’).

Następnie, określono aktywność przeciwstarzeniową, badaną jako zdolność do hamowania AChE i BuChE (**Publikacja 2, Tab. 3)**. Enzymy te biorą udział w rozpadzie neuroprzekaźnika acetylocholiny, którego niski poziom jest typowy dla nieuleczalnej i postępującej choroby Alzheimera, otępienia i wielu

innych zaburzeń neurodegeneracyjnych. Jagody odmian ‘Aromatnaja’, ‘Józef’ i ‘Moskwiczka’ charakteryzowała najwyższa wyznaczona aktywność. Potencjał owoców określono jako umiarkowany w kierunku AChE (IC_{50} od 20,16 do 40,60 mg/ml) i silny w kierunku BuChE (IC_{50} < 0,01 mg/ml). Aktywność BuChE wzrasta wraz z postępem upośledzenia funkcji mózgu, stąd wskazanie, iż badane odmiany rokitnika pospolitego mogą stanowić terapeutyczne uzupełnienie codziennej diety chorego.

Ustalono także aktywność przeciwutleniającą frakcji fenolowej rokitnika pospolitego za pomocą metody HPLC-PDA sprzężonej z derywatyzacją postkolumnową z użyciem odczynnika $ABTS^{\bullet+}$, dotychczas nie opisanej w literaturze dla *H. rhamnoides*. Badanie wskazało, że kwasy fenolowe charakteryzują się silniejszą zdolnością do wygaszania wolnych rodników niż flawonole, co tłumaczone jest niską aktywnością 3-*O*-glikozydów w porównaniu do aglikonów i brakiem ich zdolności do tworzenia struktur chinonowych przez utlenianie (Rösch i in., 2004).

Podsumowując, wyniki korelacji Pearsona między zdolnością inhibicji AChE i BuChE a zawartością flawonoli były wysokie (r = 0,851 i 0,614), umiarkowane dla karotenoidów (r = 0,504) i niskie w przypadku kwasów fenolowych (r = 0,388 i 0,355) (**Publikacja 2, Tab. 4**). Z kolei, szczegóły analizy głównych składowych (PCA) (**Publikacja 2, Fig. 2)** wskazały na relacje między aktywnością przeciwstarzeniową a all-*trans*-β-kryptoksantyną, 3-*O*-glukozydem kwercetyny, 3-*O*-heksozydem-7-*O*-ramnozydem kemferolu oraz 3-*O*-(2-ramnozylo)glukozydem, 3-*O*-(6-ramnozylo)heksozydem, 3-*O*-rutynozydem i 3-*O*-glukozydem izoramnetyny.

Potencjał wykorzystania całej rośliny *H. rhamnoides* postrzegany przez pryzmat gospodarki bezodpadowej skupia się na zagospodarowaniu pozostałości, takich jak pędy i liście po mechanicznym zbiorze owoców i wytłoki zawierające skórki i nasiona z endokarpem, w produkcji o wysokiej wartości dodanej (Ciesarová i in., 2020; Radenkovs i in., 2018). Postęp badań własnych nad odmianami rokitnika pospolitego i jednoczesny niedobór doniesień literaturowych na temat profilu metabolitów wtórnych i składników odżywczych części anatomicznych rokitnika pospolitego zainicjowały badania mające na celu jakościowe i ilościowe oznaczenie pentacyklicznych triterpenoidów, związków fenolowych oraz makro- i mikroelementów części anatomicznych jagód (skórki, miąższu, endokarpu, nasion), pędów i liści wybranych odmian rokitnika pospolitego.

Wyniki przedstawiono w **Publikacji 3**:

Tkacz K., Wojdyło A., Turkiewicz I.P., Nowicka P. 2021. Triterpenoids, phenolic compounds, macro- and microelements in anatomical parts of sea buckthorn (*Hippophaë rhamnoides* L.) berries, branches and leaves. *Journal of Food Composition and Analysis,* 103, 104107. doi: 10.1016/j.jfca.2021.104107.

Badania omówione w **Publikacji 3** podkreślają różnorodność anatomicznych części rokitnika pospolitego pod względem weryfikowanych składników i zapewniają kompleksowe porównanie wysokoplonujących odmian uprawianych w Europie Środkowo-Wschodniej, tj. ‘Botaniczeskaja-Lubitelskaja’, ‘Golden Rain’, ‘Luczistaja’, ‘Maryja’, ‘Podarok Sadu’, ‘Prozrocznaja’ i ‘Tatiana’.

Analiza przeprowadzona metodą UPLC-PDA-ESI-Q/TOF-MS pozwoliła na identyfikację i oznaczenie ilościowe siedmiu pochodnych kwercetyny, 12 pochodnych izoramnetyny oraz dwóch pochodnych kemferolu (**Publikacja 3, Tab. 1)**. Pochodne izoramnetyny są dobrze rozpoznane jako

dominująca frakcja fenolowa, stanowią ponad 65% flawonoli całych jagód gatunków należących do rodziny rokitnikowatych (*Elaeagnaceae*) uprawianych w różnych lokalizacjach geograficznych (Fatima i in., 2015; Ma i in., 2016; **Publikacja 1**). Jednak to badanie części anatomicznych rokitnika pospolitego wskazało na większe zróżnicowanie pochodnych izoramnetyny niż kwercetyny i kemferolu, ale jednocześnie wyższe stężenie pochodnych kwercetyny w skórce, miąższu, pędach i liściach. Najwyższe zawartości 3-*O*-glukozydu, 3-*O*-rutynozydu i 3-*O*-glukozydu-7-*O*-ramnozydu izoramnetyny oraz 3-*O*-glukozydu kwercetyny oznaczono w skórkach, przy czym w miąższu dominował 3-*O*-glukozyd kwercetyny. 3-*O*-Glukozyd-7-*O*-ramnozyd izoramnetyny był również głównym flawonolem w nasionach i endokarpie, czego wcześniej nie opisano w literaturze naukowej.

W pędach i liściach zbadano istotnie wysokie ilości 3-*O*-glukozydu-7-*O*-ramnozydu kwercetyny ($p < 0,05$), a większość liści była zasobna również w 3-*O*-glukozyd i 3-*O*-rutynozyd kwercetyny. Oprócz liści, 3-*O*-rutynozyd kwercetyny zidentyfikowano, także w skórkach, miąższu i endokarpie, w przeciwieństwie do odmian uprawianych w Kanadzie, w tym również analizowanej w tym badaniu 'Golden Rain', gdzie związek ten zidentyfikowano tylko w liściach (Fatima i in., 2015).

Obecność pochodnych kemferolu była selektywna, zależna od frakcji i odmiany; dla przykładu nasiona zawierały tylko 3-*O*-rutynozyd, natomiast pochodnych kemferolu nie zidentyfikowano w pędach. Stwierdzono, że im bardziej zewnętrzna część jagody (od nasion do skórki), tym wyższa była zawartość pochodnych kemferolu. Ponadto, analiza ilościowa przeprowadzona metodą UPLC-PDA dowiodła, że liście rokitnika pospolitego były średnio 2-krotnie zasobniejsze w kwasy fenolowe niż miąższ i skórki.

Zgodnie z oznaczeniem metodą UPLC-PDA, wśród flawan-3-oli oznaczono (+)-katechinę, (-)-epikatechinę (EC), galusan (-)-epikatechiny (ECG) i (-)-epigallokatechiny (EGC) (**Publikacja 2, Tab. 2)**. Frakcje rokitnika pospolitego pod względem zawartości flawan-3-oli uporządkowano następująco: pędy > endokarp > liście > skórki > miąższ > nasiona. Analiza UPLC-FL końcowych jednostek polimerycznych procyjanidyn (PP), powstałych w wyniku reakcji floroglucynolizy, wskazała przewagę (+)-katechiny w ilościowo zróżnicowanych strukturach polimerowych. Części wegetatywne i nasiona były lepszym źródłem PP niż miękkie tkanki jagód. Liście powszechnie uprawianych jabłoni, pigwy pospolitej, pigwowca japońskiego, żurawiny wieloowocowej i porzeczki czarnej zawierały od 239 mg do 11 215 mg PP w 100 g sm (Teleszko i Wojdyło, 2015), stąd liście rokitnika pospolitego uznano za źródło PP o umiarkowanej zawartości.

Średni stopień polimeryzacji procyjanidyn (DP) wskazujący liczbę jednostek flawan-3-olu w polimerach, oznaczono od 2,4 w pędach do 8,0 w nasionach. Obecność dimerycznych i oligomerycznych flawan-3-oli oraz słabe ujemne korelacje między DP i PP oraz DP i flawan-3-olami (odpowiednio $r = -0,30$ i $-0,39$) wskazały na niską intensywność cierpkości frakcji, ważnej z punktu widzenia potencjalnych zastosowań spożywczych.

Dotychczasowe dane literaturowe akcentowały kilkukrotnie wyższą zawartość związków fenolowych w liściach wyłącznie w porównaniu do całych jagód rokitnika pospolitego i innych owoców jagodowych i ziarnkowych, pomijając znaczenie części anatomicznych (Bittová i in., 2014; Teleszko i Wojdyło, 2015; Criste i in., 2020). Wyniki badań zaprezentowane w **Publikacji 3** wskazują na ponad 4-krotnie wyższą ilość związków fenolowych w pędach niż liściach, kolejno około 6 razy wyższą niż w nasionach i 7 razy wyższą niż w skórkach. Suma mono-, di-, oligo- i polimerycznych flawan-3-oli w endokarpie i nasionach stanowiła

ponad 80%, a w przypadku pędów ponad 99% związków fenolowych ogółem. W większości skórek i liści stosunek flawan-3-oli do flawonoli wyniósł 1:1 i proporcje te były determinowane czynnikiem odmianowym.

Posługując się metodą UPLC-PDA-ESI-Q/TOF-MS zidentyfikowano i oznaczono ilościowo 11 pentacyklicznych triterpenoidów, które podzielono na dwie grupy (**Publikacja 3, Tab. 3)**. Pierwsza z nich składała się z oznaczonych w wysokich ilościach kwasów: maslinowego, pomolowego, korozolowego, betulinowego, oleanolowego i ursolowego oraz betuliny. Drugą frakcję stanowiły kwas tormentowy, kwas α-bosweliowy, uwaol i erytrodiol (występujący tylko w pędach i liściach), których suma stanowiła około 2% triterpenoidów ogółem. Kwasy pomolowy, korozolowy, betulinowy, tormentowy i α-bosweliowy, betulinę, uwaol i erytrodiol, zidentyfikowane po raz pierwszy w częściach anatomicznych jagód, pędach i liściach różnych odmian *H. rhamnoides*.

Cechą wyróżniającą części anatomiczne jagody (skórki, miąższu, endokarpu, nasion) była dominacja kwasu pomolowego, który stanowił od 34% do 57% triterpenoidów ogółem, odpowiednio dla skórek i endokarpu. Zawartości kwasu maslinowego i kwasu pomolowego były silnie skorelowane (r = 0,98), a miąższ był ponad 20-krotnie zasobniejszy w te metabolity niż liście. Skórki charakteryzowała najwyższa ilość kwasu oleanolowego i kwasu ursolowego, powszechnie występujących w niskopolarnych i niepolarnych frakcjach wielu roślin. Pędy uznano jako najlepsze źródło kwasu korozolowego i kwasu betulinowego, podczas gdy liście zawierały kwas ursolowy w ilości 46% triterpenoidów ogółem. Ustalono, że profil triterpenoidów skórek i liści rokitnika pospolitego może mieć znaczenie w przypadku ich zastosowania w zapobieganiu stanom zapalnym, ponieważ badania *in vitro* i *in vivo*, w tym ostatnie nad pędami *H. rhamnoides*, sugerują hamowanie promocji nowotworu przez kwas ursolowy (Yasukawa i in., 2009; Marciniak i in., 2021). Zidentyfikowane triterpenoidy mają również wspólne działanie przeciwdrobnoustrojowe, hepatoprotekcyjne i przeciwutleniające (Różalska i in., 2018).

Następnie, zastosowanie metody FAAS skoncentrowano na detekcji czterech makroelementów: sodu, potasu, wapnia i magnezu oraz czterech mikroelementów: żelaza, miedzi, cynku i manganu (**Publikacja 3, Tab. 4**). Sód stanowił zaledwie od 1% do 2% badanych makroelementów. Natomiast kluczowym pierwiastkiem był potas w ilości od 74% do 93% wszystkich makroelementów odpowiednio w pędach i miąższu, z wyjątkiem liści, w których znaczny udział stanowił wapń - 55% makroelementów. Nadciśnieniu tętniczemu towarzyszy niskie stężenie potasu, wapnia i magnezu w organizmie, stąd uzasadnione wydaje się spożywanie produktów na bazie frakcji rokitnika pospolitego, zasobnych w te składniki mineralne, przez konsumentów zmagających się z tą chorobą. Co więcej, dostrzeżono potencjał w regularnym spożywaniu pokarmów zawierających jagody rokitnika pospolitego celem wspomagania utrzymania równowagi jonowej i właściwej pobudliwości tkanek wynikającej z roli potasu w organizmie człowieka (Bal i in., 2011).

Badania dowiodły, że najlepszym źródłem magnezu były nasiona (15% makroelementów), następnie pędy i liście. Wysoka zawartość magnezu oraz przewaga żelaza i cynku nad sodem były charakterystyczne również dla nasion rokitnika pospolitego badanych przez Zeb i Malook (2009). Jednak dane zaprezentowane w **Publikacji 3** dokładnie sprecyzowały 25-krotnie wyższą akumulację wapnia w endokarpie niż w nasionach, które zawierały istotnie niższe stężenie tego makroelementu ($p < 0,05$) niż pozostałe części anatomiczne rokitnika pospolitego.

Analiza mikroelementów wskazała żelazo jako dominujący mikroelement, w ilości od 36% w przypadku endokarpu do 55% mikroelementów ogółem w nasionach. Oznaczono 2,5-krotnie wyższą koncentrację żelaza w nasionach i 1,5-krotnie wyższą zawartość w skórkach i endokarpie niż w miąższu. Dlatego też, przeciery i soki mętne, czyli produkty ze zwiększoną zawartością części stałych (skórek i endokarpu), dostarczą znacznie więcej żelaza niż soki poddane klarowaniu i filtrowaniu. Dodatkowo, poprzednie badania dowiodły, że owoce rokitnika pospolitego mają wysokie stężenie witaminy C (**Publikacja 1, Tab. 1**), która zwiększa przyswajalność żelaza; stąd spożywanie produktów z tej rośliny mogłoby wspierać zarówno prawidłowe funkcjonowanie układu odpornościowego, jak i przemian metabolicznych - transportu elektronów, tlenu, aktywacji tlenu (Gutzeit i in., 2008).

Badania składników mineralnych pozwoliły na stwierdzenie, że 100 g sproszkowanych liści badanych odmian rokitnika pospolitego może pokryć zalecane dzienne spożycie (RDA) wapnia (1000 mg), żelaza (8 mg – mężczyźni, 18 mg - kobiety), miedzi (0,9 mg) i manganu (2,3 mg - mężczyźni, 1,8 mg - kobiety). Podobnie, endokarp, nasiona i pędy mogą stanowić korzystne niekonwencjonalne źródło żelaza, miedzi i manganu, bliskie zapotrzebowaniu na składniki odżywcze.

Relacje między częściami anatomicznymi rokitnika pospolitego a składem chemicznym określono poprzez analizę głównych składowych (PCA), w wyniku której wyróżniono sześć skupień (**Publikacja 3, Fig. 2**):

(1) skórki charakteryzujące się znaczną zawartością pochodnych izoramnetyny, kwercetyny i kempferolu, kwasów fenolowych, betuliny, kwasu oleanolowego i kwasu ursolowego;
(2) miąższ zasobny w kwas pomolowy, kwas maslinowy i potas;
(3) endokarp o wysokim stężeniu potasu i sodu;
(4) nasiona kumulujące polimeryczne procyjanidyny, kwas betulinowy, magnez i cynk;
(5) pędy zasobne w polimeryczne procyjanidyny, flawan-3-ole, kwas korozolowy i kwas betulinowy;
(6) liście jako najbardziej rozległa grupa reprezentująca szczególnie wysoką zawartość flawonoli, wapnia, potasu, sodu, żelaza, miedzi, manganu i kwasu ursolowego.

Podsumowując, analiza wyników zaprezentowanych w **Publikacji 3**, pozwoliła na stwierdzenie, że anatomiczne części rokitnika pospolitego (skórka, miąższ, nasiona, endokarp, pędy i liście) mają potencjał do produkcji żywności bogatej w związki fenolowe i triterpeny o wysokiej aktywności przeciwutleniającej i przeciwzapalnej, a także do uzupełniania jonów (szczególnie potasu, wapnia, żelaza, miedzi i manganu) i utrzymania ich odpowiedniej równowagi w organizmie.

Następne, działania badawcze skupiono na analizie zależności między frakcją fitozwiązków a aktywnością biologiczną soków z owoców rokitnika pospolitego (**Publikacja 4**). Produkcja soków wciąż pozostaje szybko rozwijającym się segmentem w sektorze przemysłu owocowo-warzywnego, jednocześnie stanowiąc powszechną komercyjnie formę przetworzenia owoców rokitnika pospolitego. Dotychczas, brak w literaturze doniesień na temat zróżnicowania profilu fitochemicznego i właściwości prozdrowotnych dostępnych w handlu soków z owoców tej rośliny. Dlatego też, celem badań było jakościowe i ilościowe oznaczenie tokoferoli i tokotrienoli, karotenoidów i wolnych aminokwasów oraz ocena potencjału przeciwstarzeniowego, przeciwcukrzycowego, przeciw otyłości, przeciwzapalnego i przeciwutleniającego

w dostępnych na rynku detalicznym sokach z owoców rokitnika pospolitego (J1-J5) i soku laboratoryjnym z owoców odmiany ‘Józef’ (J6). Dodatkowym novum była analiza ilościowa i jakościowa związków określanych mianem fitoprostanów (PhytoP) i fitofuranów (PhytoF).

Wyniki przedstawiono w **Publikacji 4**:

Tkacz K., Gil-Izquierdo Á., Medina S., Turkiewicz I.P., Domínguez-Perles R., Nowicka P., Wojdyło, A. 2021. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 11345. doi: 10.1002/jsfa.11345.

Po raz pierwszy zidentyfikowano fitoprostany i fitofuran w sokach z owoców rokitnika pospolitego (**Publikacja 4, Tab. 1**). Analiza wykonana metodą UHPLC-QqQ-MS/MS pozwoliła na jakościowe i ilościowe oznaczenie ośmiu fitoprostanów F_1, D_1, B_1 i L_1, ich odpowiednich enancjomerów oraz mniej powszechnej oksylipiny w matrycach roślinnych – fitofuranu. Dominującą klasą były F_1-fitoprostany (od 66% do 100% fitoprostanów ogółem), następnie pochodne D_1 (do 28% fitoprostanów ogółem). Ze względu na rosnące znaczenie fitoprostanów w ludzkim metabolizmie, biodostępności i implikacjach przeciwwirusowych, przeciwzapalnych, immunomodulujących, cytotoksycznych i cytoprotekcyjnych (Medina i in., 2018), od 20 do niemal 50-krotnie wyższą sumę fitoprostanów w sokach J1, J3 i J6 w porównaniu z pozostałymi sokami uznano za potencjalnie korzystne. Warto zauważyć, że ilości fitoprostanów w sokach z owoców rokitnika były porównywalne z tymi w roślinach i żywności uważanych za bogate źródła w oksylipiny, między innymi w marakui, migdałach i orzechach oraz różnych częściach anatomicznych roślin o potencjale leczniczym (Medina i in., 2018).

Wśród fitofuranów, określono jedynie *Ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-fitofuran w sokach J1, J4 i J6. Odmiany rokitnika pospolitego i warunki agronomiczne związane z nasileniem stresu oksydacyjnego, a następnie proces produkcji i przechowywania soków mogły istotnie modulować zawartość badanych oksylipin, ze względu na potencjalny wzrost utleniania kwasu α-linolenowego (ALA) powodowany obróbką cieplną, co poprzednio badano w olejach roślinnych (Collado-González i in., 2015).

Analiza przeprowadzona metodą UPLC-FL ujawniła w sokach z owoców rokitnika pospolitego cztery kongenery tokoferolu: α, β, γ i δ, oraz trzy kongenery tokotrienolu: α, γ i δ (**Publikacja 4, Fig. 1**). Zawartość tokoferoli była od 3,6 do 6,7 razy wyższa niż ilość tokotrienoli. Wysoka koncentracja α-tokoferolu w sokach z owoców rokitnika pospolitego (głównie J3, J4 i J6) uznano za niewątpliwie cenną, gdyż forma α jest jedyną aktywną formą spełniającą zapotrzebowanie człowieka na witaminę E. Nasiona rokitnika pospolitego zawierały wyższe ilości γ-tokoferolu (od 20% do 40%) niż miękkie części jagód, zaś δ-tokoferol dominował w skórkach (Ranjith i in., 2006; Pop i in., 2015). W związku z tym obróbka wstępna surowca mogła modulować końcową zawartość tych form w sokach, zwłaszcza w soku z owoców odmiany „Józef” o najwyższych oznaczonych stężeniach form γ i δ.

Opierając analizę wykonaną metodą UPLC-PDA-ESI-Q/TOF-MS o charakterystykę protonowanych cząsteczek i jonów fragmentacyjnych, oznaczono 18 karotenoidów, w tym 10 ksantofili (izomery all-*trans*-luteiny, all-*trans*-zeaksantyny i all-*trans*-β-kryptoksantyny), 7 karotenów (all-*trans*-β-karoten, *cis*-β-karoten, α-, γ-, δ-, ε- i ζ-karoten) oraz fitofluen (**Publikacja 4, Tab. 2**). Obecność karotenoidów determinuje pomarańczowy, niemal czerwony kolor produktów z rokitnika pospolitego, a tym samym ich atrakcyjność.

W soku J5 dominowały karoteny (82% karotenoidów ogółem), dla soku J6 stosunek karotenów do ksantofili wyniósł niemal 1:1, a w pozostałych sokach ksantofile stanowiły od 64% do 100% karotenoidów ogółem. Zatem, nie znaleziono korelacji między tymi dwiema grupami karotenoidów (r = 0,01), ale karoteny silnie korelowały z fitofluenem (r = 0,91), którego obecność mogła wynikać z zanieczyszczenia niedojrzałymi jagodami. Co istotne, all-*trans*-β-kryptoksantyna, jeden z charakterystycznych karotenoidów w rokitniku pospolitym opisany w **Publikacji 2**, nie został oznaczony w sokach J3 i J4, które nie zawierały także pochodnych karotenów (all-*trans*-β-karoten, *cis*-β-karoten, α-, γ-, δ-, ε- i ζ-karoten). Niskie ilości tych metabolitów wtórnych wynikają z eliminacji fazy tłuszczowej z soków lub technologii przetwarzania, w której surowiec podlega wstępnej obróbce wyłącznie na zimno, nie sprzyjającej wysokiej wydajności ekstrakcji karotenów (Seglina i in., 2006).

Zidentyfikowano i oznaczono ilościowo 20 wolnych aminokwasów, w tym osiem aminokwasów egzogennych (EAA) i pięć aminokwasów warunkowo niezbędnych dla organizmu człowieka (CEAA) (**Publikacja 4, Tab. 3**). Badane soki miały kompleksowy profil EAA i CEAA, ale ich zawartość była umiarkowana, od 11% do 41% aminokwasów ogółem. Zatem, tylko wyselekcjonowane produkty uznano jako atrakcyjne źródło aminokwasów.

Stosunkowo wysoką i zróżnicowaną zawartość asparaginy (Asn) w ilości od 30% do 81% aminokwasów ogółem, następnie kwasu asparaginowego (Asp) i alaniny (Ala) w sokach z owoców rokitnika pospolitego należy rozpatrywać w kontekście ryzyka wystąpienia reakcji Maillarda powodujących powstawanie potencjalnie toksycznych związków o negatywnym wpływie na zdrowie (akrylamidu - ACR, hydroksymetylofurfuralu - HMF, amin heterocyklicznych, furanu) (Collado-González i in. 2014; Constantin i in., 2019). Z drugiej strony Constantin i in. (2019) zidentyfikowali optymalną temperaturę i sekwencję czasową w celu zmniejszenia tworzenia się ACR i 5-HMF podczas obróbki termicznej purée z rokitnika, tj. 134,87 °C przez 14,82 min.

Co ważne, wśród aminokwasów nieproteogennych, GABA stanowił do 4,5%, a aminokwas siarkowy homocysteina (HCys) do 3,3% aminokwasów ogółem. Chociaż te aminokwasy nie są wbudowane w białka, ich obecność w sokach z owoców rokitnika pospolitego może być związana ze zdrowiem, ponieważ działają jako główne neuroprzekaźniki synaptyczne o działaniu hamującym i są silnymi przeciwutleniaczami (Wu, 2010; Collado-González i in. 2014). W dotychczasowych danych literaturowych nie analizowano aminokwasów w sokach z owoców rokitnika pospolitego przy użyciu metody LC-MS, a badanie opisane w **Publikacji 4** jest pierwszym, w którym zidentyfikowano i oznaczono ilościowo HCys i GABA dla produktów z *H. rhamnoides*.

Potencjał prozdrowotny soków z owoców rokitnika pospolitego selektywnie korelował z ich składem. Soki charakteryzowała niższa aktywność w kierunku inhibicji AChE i BuChE jako potencjalny sposób hamowania zmian zwyrodnieniowych poprzez zwiększenie transmisji w układzie cholinergicznym niż w przypadku jagód (**Publikacji 2, Tab. 3)**. Jednak sok uzyskany w skali laboratoryjnej miał najwyższy wśród badanych soków potencjał inhibicji AChE i BuChE. Jest to tym bardziej istotne, gdyż ostatnie doniesienia literaturowe sugerowały, że niektóre izoprostany (IsoP), izofurany (IsoF) i neurofurany (NeuroF) mogą być potencjalnymi biomarkerami stresu oksydacyjnego w zaburzeniach neurologicznych, w tym choroby Alzheimera i Parkinsona (Ahmed i in., 2020). Jednak rola oksylipin roślinnych, w tym fitoprostanów i fitofuranów, w regulacji zaburzeń neurodegeneracyjnych nie została wyjaśniona i jest to pierwsze doniesienie

na temat soków jako potencjalnych środków o aktywności hamującej AChE i BuChE w profilaktyce przyczyn otępienia.

Hipoglikemiczny wpływ soków z owoców rokitnika pospolitego analizowano pod kątem hamowania α-amylazy i α-glukozydazy (**Publikacja 4, Tab. 4**). Potencjał hamowania α-glukozydazy był silniejszy niż aktywność hamowania α-amylazy, z wyjątkiem soku J2. Tokoferole, tokotrienole i większość aminokwasów umiarkowanie lub silnie korelowały z aktywnością hamującą α-amylazę ($r \geq 0{,}50$).

Wykazano, że wszystkie analizowane soki charakteryzowały się inhibicją lipazy trzustkowej. Wiadomo, że cukrzyca typu 2 silnie koreluje z przyrostem tkanki tłuszczowej i stopniem otyłości. Zatem hamowanie lipazy trzustkowej, kluczowego enzymu w trawieniu i wchłanianiu lipidów, może mieć wpływ na leczenie zarówno cukrzycy, jak i otyłości (Justino i in., 2018). Analiza korelacji Pearsona ujawniła ksantofile (kryptoksantynę, luteinę, zeaksantynę) i niektóre fitoprostany zawarte w sokach z owoców rokitnika pospolitego jako potencjalne inhibitory lipazy trzustkowej ($r \geq 0{,}50$). Niedawne badania epidemiologiczne z udziałem ludzi dowiodły, że wyższe spożycie w diecie przekładało się na wyższe stężenie karotenoidów w surowicy krwi i istotnie wpływało na zmniejszenie stopnia otyłości (Bonet i in., 2015). Dotychczas w literaturze nie wiązano zdolności hamowania lipazy trzustkowej z obecnością fitoprostanów.

Potencjał przeciwzapalny soków z owoców rokitnika pospolitego prezentowany jako procent hamowania aktywności 15-lipoksygenazy silnie korelował z zawartością fitoprostanów i fitofuranów, z wyjątkiem *Ent*-16-epi-16-F_{1t}-fitoprostanu i *Ent*-16-F_{1t}-fitoprostanu, powszechnie występujących w badanych sokach (**Publikacja 4, Tab. 4**). Wyniki były zgodne z poprzednimi badaniami Karg i in. (2007) sugerującymi działanie przeciwzapalne fitoprostanów, ich zdolność do modulowania mechanizmów komórkowych zaangażowanych w adaptacyjną odpowiedź immunologiczną, oraz silną analogię strukturalną z endogennymi prostaglandynami w odniesieniu do pierścienia cyklopentenonu i jego elektrofilowego charakteru.

Aktywność przeciwutleniająca badana metodami ORAC, ABTS i FRAP w soku otrzymanym w skali laboratoryjnej (J6) była zbliżona do średniej aktywności soków handlowych (**Publikacja 4, Tab. 4**). Karoteny, α-tokoferol, Arg i Gln wydały się być najskuteczniejszymi przeciwutleniaczami wśród badanych związków chemicznych.

Podsumowując wyniki zawarte w **Publikacji 4**, soki z owoców rokitnika pospolitego mogą być atrakcyjną żywnością o działaniu przeciwcukrzycowym i przeciw otyłości ze względu na zawartość potencjalnych inhibitorów α-amylazy, α-glukozydazy (tokoferole, tokotrienole, wybrane aminokwasy) oraz lipazy trzustkowej (fitoprostany i ksantofile). Obecność oksylipin, tokoferoli, tokotrienoli i aminokwasów może zwiększyć funkcjonalność soków w ograniczaniu zmian neurodegeneracyjnych, co czyni je potencjalnymi środkami przeciwstarzeniowymi w profilaktyce najczęstszego typu otępienia – choroby Alzheimera.

4.2. Optymalizacja procesu fermentacji jabłkowo-mlekowej soków na bazie owoców rokitnika pospolitego

Wyniki uzyskane w pierwszym etapie badań dowiodły, że frakcje (jagody, pędy, liście) i soki z rokitnika pospolitego mają unikatową kompozycję hydrofilowych i lipofilowych związków o działaniu prozdrowotnym, stąd wpisują się w strategię poszukiwania naturalnych czynników profilaktyki przewlekłych chorób niezakaźnych. Niemniej jednak, surowiec ten stanowi dla przemysłu spożywczego istotne wyzwanie związane z opracowaniem w pełni akceptowalnego produktu pod względem sensorycznym przy zachowaniu wysokich walorów prozdrowotnych. Jagody cechuje intensywna kwasowość, cierpkość, ostry aromat i niejednorodna konsystencja wynikająca z separacji fazy tłuszczowej i osadu. Właściwości te znajdują odzwierciedlenie w niskim spożyciu i produkcji żywności z rokitnika pospolitego głównie w skali lokalnej lub stosowaniu tylko jako kilkuprocentowy dodatek do produktów owocowo-warzywnych. Dlatego też, w drugim etapie badań podjęto próbę optymalizacji procesu redukcji kwasowości soków z owoców rokitnika pospolitego na drodze fermentacji jabłkowo-mlekowej. Celem tego etapu było określenie aktywności metabolicznej szczepów *Lactobacillus plantarum*, *Lactobacillus plantarum* subsp. *argentoratensis* i *Oenococcus oeni* wraz z dynamiką zmiany zawartości kwasów organicznych, cukrów, związków fenolowych oraz aktywności przeciwutleniającej podczas 72-godzinnej fermentacji soku z owoców rokitnika pospolitego odmiany ‘Józef’ i soków mieszanych rokitnik – jabłko odmiany ‘Champion’ (1:1).

Wyniki przedstawiono w **Publikacji 5**:

Tkacz K., Chmielewska J., Turkiewicz I.P., Nowicka P., Wojdyło A. 2020. Dynamics of changes in organic acids, sugars and phenolic compounds and antioxidant activity of sea buckthorn and sea buckthorn-apple juices during malolactic fermentation. *Food Chemistry*, 332: 127382. doi: 10.1016/j.foodchem.2020.127382.

Fermentacja jabłkowo-mlekowa powszechnie stosowana jest w produkcji win czerwonych i niektórych win białych, celem modyfikacji bukietu i zwiększenia stabilności mikrobiologicznej. Wyniki opisane w **Publikacji 5** po raz pierwszy nawiązują do aktywności metabolicznej i selekcji szczepów bakterii fermentacji jabłkowo-mlekowej soków na bazie owoców rokitnika pospolitego zapewniających redukcję ilości kwasu jabłkowego przy jednoczesnym zwiększeniu potencjału prozdrowotnego poprzez wzrost ilości związków fenolowych i aktywności przeciwutleniającej.

Wcześniejsze badania nad fermentacją soków owocowych i warzywnych dowiodły, że wyższe pH sprzyja zdolności metabolicznej wielu szczepów bakterii (Filannino i in, 2014; Wei i in., 2018). Modyfikację pH (o 0,1) zbadano jedynie w sokach mieszanych zaszczepionych *L. plantarum* (**Publikacja 5, Tab. 1, 2**). Proces fermentacji jabłkowo-mlekowej przyczynił się do zmian zawartości ekstraktu ogólnego nie więcej niż o 1,2 °Bx, co silnie dodatnio korelowało z ilością kwasu jabłkowego (r = 0,92) i silnie ujemnie z ilością kwasu mlekowego (r = -0,78 i -0,85 odpowiednio dla soków jednorodnych i mieszanych). Co ważne, badane soki bioprzetwarzane spełniały obowiązujące przepisy branżowe zawarte w Codex General Standard for Fruit Juices and Nectars (Codex STAN 247–2005) określające minimalny stopień Brix dla soków z owoców rokitnika pospolitego równy 6,0, a dla soku jabłkowego - 10,0.

Wskaźnikiem procesu fermentacji była konwersja kwasu jabłkowego do łagodniejszego kwasu mlekowego. Stwierdzono umiarkowane obniżenie zawartości kwasu jabłkowego, od 3,5% do 20,9%, w sokach z owoców rokitnika pospolitego (**Publikacja 5, Tab. 1**). We wszystkich sokach jednorodnych zaobserwowano

w trakcie procesu wzrost ilości kwasu mlekowego, jednak tylko w przypadku soków fermentowanych ze szczepami DSM 20174 i DSM 10492 zmiany te były istotne statystycznie. Udowodniono, że rodzaj szczepu determinował ilość powstającego kwasu mlekowego, nie naruszając pierwotnej zawartości cukrów. Na przykład w soku jednorodnym traktowanym szczepem DSM 10492 oznaczono około 2-krotnie więcej kwasu mlekowego niż w sokach ze szczepem DSM 100813 i DSM 6872, pomimo podobnej redukcji ilości kwasu jabłkowego, średnio 13,2%. W przyszłości cenna może być próba odkwaszania soku za pomocą zrekombinowanych komórek *L. plantarum*, które zwiększają efektywność fermentacji (Schümann i in., 2012).

Redukcja zawartości kwasu jabłkowego była istotniejsza dla soków mieszanych rokitnik – jabłko (1:1) i wyniosła do 75,0% dla soku traktowanego szczepem DSM 10492 (**Publikacja 5, Tab. 2**). Najsilniejsze obniżenie ilości kwasu jabłkowego nastąpiło podczas pierwszych 24 godzin fermentacji ze szczepami *L. plantarum*. W związku z niewielkimi różnicami w stężeniu kwasu jabłkowego w kolejnych dobach procesu, optymalny czas fermentacji dla szczepów DSM 6872, DSM 100813 i DSM 20174 ustalono jako 48 godzin (**Publikacja 5, Fig. 2**). Dodatek soku jabłkowego zwiększył pH soku przeznaczonego do procesu fermentacji, zapewniając warunki procesu zbliżone do optymalnych dla bakterii, jak również dostarczył niezbędnych do ich aktywności cukrów, aminokwasów i witamin. Ponadto, wybór jabłek uznano za pożądane działanie ekonomicznie i sensorycznie.

Zastosowany szczep *O. oeni* nie wykazał aktywności w kierunku konwersji kwasu jabłkowego podczas 72-godzinnej fermentacji. Z drugiej strony, stosując ten gatunek bakterii do soku z rokitnika odmiany ‘Oranzhevaya’, osiągnięto ponad 90%-ową redukcję ilości kwasu jabłkowego (Tiitinen i in., 2006). Dane literaturowe podkreślają dominację *O. oeni* w procesie fermentacji jabłkowo-mlekowej i ich dobrą adaptację do trudnych warunków winiarskich (Cinquanta i in., 2018). Niemniej jednak, brak aktywności tłumaczone jest możliwym obniżeniem zawartości komórek bakterii poniżej minimalnej wartości niezbędnej do utrzymania fermentacji jabłkowo-mlekowej, obecnością naturalnych związków przeciwdrobnoustrojowych owoców oraz zbyt niskim pH soków i wysoką kwasowością w porównaniu do wyrobów winiarskich.

W sokach wykryto towarzyszące kwasy organiczne, tj. kwasy chinowy, cytrynowy, izocytrynowy i szczawiowy, ale proces fermentacji nie wpłynął na istotną zmianę ich zawartości ($p > 0,05$) (**Publikacja 5, Fig. 3**). Kwas chinowy ma gorzki i cierpki smak, dlatego potencjalne modyfikacje jego zawartości są ważne z punktu widzenia jakości sensorycznej soków.

Fermentacja jabłkowo-mlekowa soków jednorodnych i mieszanych nie powodowała redukcji cukrów, w tym glukozy, sacharozy, sorbitolu, fruktozy i ramnozy (**Publikacja 5, Tab. 1, 2**). Prawdopodobnie, ze względu na niskie pH soku z owoców rokitnika pospolitego, *L. plantarum* wykazał większą preferencję kwasów jako źródła węgla, a niepełna konwersja kwasu jabłkowego w kwas mlekowy nie skutkowała koniecznością bakteryjnej degradacji cukrów (Markkinen i in., 2019). Niemniej jednak, smak jagód zależy od stosunku cukrów do kwasów organicznych, który koreluje dodatnio ze słodyczą, a ujemnie z kwaśnością i cierpkością (Tiitinen i in., 2005). Fermentacja wyłącznie soków mieszanych skutkowała maksymalnie 2-krotnym spadkiem tego wskaźnika.

Wyniki uzyskane w pierwszym etapie badań wykazały, że jagody rokitnika pospolitego są bogatym źródłem związków fenolowych, w tym flawonoli, które stanowią ponad 98% całości, a pozostałe to pochodne kwasów hydroksycynamonowych (**Publikacje 1** i **2**). Dlatego też zmierzono dynamikę zmian ilości tych

metabolitów podczas 72-godzinnej fermentacji soków. Reduktazy i dekarboksylazy niektórych szczepów *L. plantarum* metabolizują kwasy zawarte w owocach rokitnika pospolitego: kwas ferulowy do *p*-winylogwajakolu, a kwasu *p*-kumarowy do kwasu floretowego i *p*-winylofenolu, czyli niepożądanych prekursorów sfermentowanego smaku (Rodríguez i in., 2008; Filannino i in., 2015). Poprzednie badania wskazały na silną redukcję ilości kwasów hydroksycynamonowych w fermentowanym soku z aronii czarnej, wzrost zawartości kwasu protokatechowego w soku z owoców rokitnika pospolitego poddanego działaniu szczepu DSM 10492 (Markkinen i in., 2019) oraz obniżenie zawartości kwasu ferulowego i kwasu *p*-kumarowego w soku z borówki bagiennej zaszczepionej *O. oeni* (Chen i in., 2019). Jednakże, zaszczepienie soków z owoców rokitnika pospolitego i soków mieszanych rokitnik – jabłko (1:1) bakteriami kwasu mlekowego nie spowodowało istotnych zmian zawartości kwasów fenolowych ($p > 0,05$).

Rodzaj szczepu bakterii kwasu mlekowego determinował różnice w stężeniu flawonoli (**Publikacja 5, Tab. 1**). Różnice w zawartości związków fenolowych ogółem w sokach z owoców rokitnika pospolitego po 72-godzinnej fermentacji wyniosły maksymalnie 10%, podobnie jak zmiany flawonoli. Fermentacja soków jednorodnych z udziałem szczepu DSM 20174 sprzyjała przyrostowi ilości flawonoli, przeciwnie do procesu z udziałem szczepów DSM 100813 i DSM 10492. Potencjalne wytrącanie, utlenianie i łączenie związków fenolowych lub ich adsorpcja w ciałach stałych i polimeryzacja mogły spowodować obniżenie ilości tych związków (Chen i in., 2018).

Fermentacja jabłkowo-mlekowa promowała wzrost zawartości związków fenolowych w sokach mieszanych rokitnik – jabłko (1:1), z wyjątkiem soku traktowanego szczepem DSM 10492 (**Publikacja 5, Tab. 2**). W sokach traktowanych szczepami DSM 16365 i DSM 100813 zmierzono wzrost flawonoli o około 27%. Inaczej niż w przypadku *L. plantarum*, w sokach poddanych działaniu *O. oeni* zaobserwowano wzrost flawonoli o 29% po 48 godzinach, następnie silną redukcję ich ilości o 20% w ostatniej dobie procesu. Zależności te wynikają z indywidualnej adaptacji szczepów bakteryjnych oraz indukcji degradacji struktury ścian komórkowych i hydrolizy glikozylowanych flawonoli do aglikonów w wyniku działania β-glikozydazy uwalnianej przez szczepy *L. plantarum* (Wei i in., 2018), oraz konwersją kompleksów fenolowych do form wolnych i depolimeryzacją podjednostek o dużej masie cząsteczkowej możliwą w reakcjach katalizowanych przez fenolooksydazę, charakterystycznych dla aktywności bakterii kwasu mlekowego (Hur i in., 2014; Kwaw i in., 2018).

Fermentacja z udziałem szczepu DSM 20174 przyczynił się do zwiększenia dostępności związków o właściwościach przeciwutleniających w sokach jednorodnych (**Publikacja 5, Tab. 1**), a podobną efektywność tego szczepu stwierdzono w badaniach, gdzie fermentacji poddano sok z owoców granatu (Mousavi i in., 2013). W przypadku soków mieszanych po profesie fermentacji, z wyjątkiem próby prowadzonej ze szczepem bakterii DSM 10492, zmierzono istotnie wyższą aktywność przeciwutleniającą mierzoną metodą ORAC ($p < 0,05$) (**Publikacja 5, Fig. 4**). Potwierdzono, że *L. plantarum* wpływa na konwersję lub ochronę związków bioaktywnych o aktywności przeciwutleniającej. Przyczyną może być zdolność bakterii do obrony przeciwutleniającej przed generowaniem rodników tlenowych poprzez udział tlenu w redukcyjnej konwersji fenoli. Związki przeciwutleniające uwalniane lub syntetyzowane podczas fermentacji mogą działać jako czynniki redukujące, wygaszacze tlenu singletowego, chelatory metali oraz donory wodoru. W rzeczywistości, na działanie przeciwutleniające wpływa wiele czynników, w tym rodzaj szczepu i jego różnorodność na poziomie fenotypowym, ekologicznym i genotypowym, stężenie komórek,

zdolność metaboliczna i zdolność rodzaju *Lactobacillus* do hamowania aktywności oksydazy polifenolowej (Hur i in., 2014; Zheng i in., 2020). W tym badaniu fermentacja jabłkowo-mlekowa promowała biotransformację składników bioaktywnych w sokach dostarczając silniejszych przeciwutleniaczy. Wzrost aktywności przeciwutleniającej był więc silnie skorelowany z zawartością flawonoli w sokach jednorodnych i sokach mieszanych, odpowiednio r = 0,92 i 0,99.

Reasumując wyniki zaprezentowane w **Publikacji 5**, fermentację jabłkowo-mlekową uznano za obiecującą metodę biologicznego odkwaszania soków z owoców rokitnika pospolitego i soków mieszanych z jego wysoką zawartością. Szczepy *Lactobacillus plantarum* DSM 10492 i DSM 20174 oraz *L. plantarum* subsp. *argentoratensis* DSM 16365 charakteryzowały najwyższa aktywność metaboliczna, sprzyjały akumulacji flawonoli i wzrostowi aktywności przeciwutleniającej w sokach z owoców rokitnika i sokach mieszanych rokitnik – jabłko (1:1).

4.3. Opracowanie recepturowe i optymalizacja technologii otrzymywania produktów funkcjonalnych z sokiem z owoców rokitnika pospolitego

W kolejnym etapie badań zaprojektowano produkty funkcjonalne zawierających wysoki udział soku z owoców rokitnika pospolitego (25% i 50%), jednocześnie o wysokim potencjale prozdrowotnym i akceptacji konsumenckiej. Celem badania była ocena potencjału przeciwstarzeniowego, przeciwcukrzycowego i przeciwutleniającego, analiza związków fenolowych, podstawowego składu chemicznego i jakości sensorycznej 18 nowatorskich smoothies na bazie owoców rokitnika pospolitego.

Wyniki przedstawiono w **Publikacji 6**:

Tkacz K., Wojdyło A., Turkiewicz I.P., Nowicka P. 2021. Anti-diabetic, anti-cholinesterase, and antioxidant potential, chemical composition and sensory evaluation of novel sea buckthorn-based smoothies. *Food Chemistry*, 338, 128105. doi: 10.1016/j.foodchem.2020.128105.

Należy podkreślić, że w dostępnych danych literaturowych dotychczas nie przeprowadzono kompleksowej analizy pomiędzy składem chemicznym, aktywnością biologiczną *in vitro* w korelacji z oceną sensoryczną produktów z rokitnika pospolitego.

Smoothies poddano analizie podstawowych właściwości chemicznych, tj. kwasowość miareczkowa, pH, zawartość suchej masy, ekstraktu ogólnego, popiołu i pektyn (**Publikacja 6, Tab. 1**). Zastosowanie dodatków owoców i warzyw spowodowało wzrost pH, obniżenie kwasowości miareczkowej i zwiększenie ekstraktu ogólnego większości smoothies. Co więcej, zastosowanie korzeni pietruszki i selera, marchwi, gruszki i moreli istotnie wzbogaciło produkty w pektyny ($p < 0,05$), co jest tym bardziej znaczące, że badane owoce rokitnika pospolitego nie są zasobne we frakcję rozpuszczalnego błonnika pokarmowego (**Publikacja 6, Tab. 1**).

Kolejnym wyróżnikiem jakości opracowanych produktów był profil cukrów i kwasów organicznych oznaczony odpowiednio metodami HPLC-ELSD i UPLC-PDA (**Publikacja 6, Tab. 2**). Pomimo znacznego wzrostu naturalnych wolnych cukrów, większość smoothies należy uznać jako niskosłodzone, gdyż 100 g produktu zawierało nie więcej niż 5 g cukrów ogółem (Rozporządzenie (WE) nr 1924/2006). Produkty wzbogacane przecierem z gruszki i jabłka ze względu na profil cukrowy (wysoka zawartość fruktozy o niskim IG w odniesieniu do IG glukozy czy sacharozy) należy uznać za produkty o niskim indeksie glikemicznym (IG). Wszystkie produkty miały także niski ładunek glikemiczny (GL), poniżej 10, co jest kluczowe z uwagi na zachowanie niskiego poziomu glukozy we krwi po spożyciu smoothies i ich potencjalnie przeciwcukrzycowego charakteru.

Całkowita zawartość kwasów organicznych w smoothies była niższa w porównaniu do soku z owoców rokitnika pospolitego i wyniosła od 1,77g do 4,53 g/100 g śm. Profil kwasów można zestawić następująco: kwas jabłkowy > kwas cytrynowy ≈ kwas chinowy > kwas szczawiowy > kwas szikimowy > kwas bursztynowy (**Publikacja 6, Tab. 2**). Większość badań przypisuje prozdrowotne właściwości warzyw i owoców, w tym rokitnika pospolitego, witaminom i związkom fenolowym, zaś mało uwagi poświęca się kwasom organicznym w kontekście właściwości terapeutycznych. Niemniej jednak, ostatnie badania Izquierdo-Vega i in. (2020) wskazały na udział kwasu cytrynowego w poprawie biodostępności składników mineralnych, regulacji równowagi kwasowo-zasadowej, działanie przeciwzapalne, przeciwutleniające

i przeciwzakrzepowe, z kolei cennymi właściwościami kwasu jabłkowego jest jego potencjał prebiotyczny, pobudzanie wydzielania soku żołądkowego oraz rozwoju niepatogennych bakterii.

Zawartość cukrów i kwasów organicznych determinuje kluczowe postrzeganie smaku i akceptację owoców i produktów z udziałem rokitnika pospolitego (Yang, 2009), a niski stosunek cukrów do kwasów organicznych równy 0,3 determinuje silnie odczuwanie kwaśnego smaku. Zastosowanie dodatku pomarańczy, winogron, jabłka, brzoskwini, korzenia pietruszki i marchwi pozwoliło na zwiększenie stosunku cukrów do kwasów organicznych powyżej 1,0 (**Publikacja 6, Tab. 2**). Chociaż wskaźnik ten silniej korelował z odczuciem kwaśnego smaku produktów owocowych niż z dodatkiem warzyw (odpowiednio r = -0,77 i -0,49), to korelacja między ogólną akceptacją a proporcją cukrów do kwasów organicznych była wyższa dla smoothies z warzywami (r = 0,66).

Zgodnie z przeprowadzoną analizą sensoryczną, produkt z owoców rokitnika pospolitego był nieakceptowalny sensorycznie (ocena 1,3), ze względu na jego intensywnie kwaśny, cierpki i gorzki smak, a następnie niesatysfakcjonującą konsystencję z tendencją do rozdzielania na fazę tłuszczową, sok właściwy i osad (**Publikacja 6, Rys. 1**). Zastosowanie dodatku przecierów owocowych i warzywnych wpłynęło na istotną poprawę ogólnej akceptacji produktów ($p < 0,05$). Ustalono silną przewagę atrakcyjnego smaku smoothies nad sokiem z owoców rokitnika pospolitego, zatem doznania smakowe determinowały ogólną ocenę produktów (r = 0,99). W tym kontekście wsad 75% owoców i 50% warzyw zapewniał najwyższe oceny. Smak kwaśny jest jednocześnie najbardziej intensywny i krótkotrwały (Obrist i in., 2014), dlatego był kluczowym wyróżnikiem w ocenie smaku nowych produktów. Stwierdzono, że im niższe odczucie kwasowości, tym wyższa była ogólna ocena smoothies (r = -0,60). Najefektywniejsze w łagodzeniu kwaśnego smaku rokitnika pospolitego okazały się warzywa – marchew, seler i pietruszka.

Sok z owoców rokitnika pospolitego zawierał jedynie 2 mg kwasów fenolowych w 100 g śm, jednak zastosowanie dodatku przecierów z gruszki, brzoskwini, jabłka, pomarańczy, marchwi i pietruszki zwiększyło udział tych związków od 2 do 7 razy (**Publikacja 6, Tab. 3**). Wzbogacenie smoothies w kwasy fenolowe jest tym bardziej korzystne, że związki te są dobrze znanymi substancjami o udowodnionym działaniu hepatoprotekcyjnym, immunomodulującym, hipotensyjnym, przeciwutleniającym, przeciwzapalnym, przeciwcukrzycowym i przeciwmiażdżycowym (Izquierdo-Vega i in., 2020). W produktach z przecierem jabłkowym w obu wariantach ilościowych, z 75%-owym udziałem pomarańczy oraz z 25%-ową ilością marchwi kwasy fenolowe stanowiły ponad 10% związków fenolowych ogółem, podczas gdy w pozostałych smoothies było to średnio 3,4%.

Flawonole były dominującymi związkami fenolowymi we wszystkich smoothies, a najwyższe ilości zbadano w produktach z pomarańczą, następnie z morelą, brzoskwinią i winogronem (średnio 60 mg/100 g śm) (**Publikacja 6, Tab. 3**). Zaprojektowane receptury zapewniają szczególnie wysokie ilości flawonoli, pochodzących głównie z rokitnika pospolitego, co odróżnia je od dotychczas badanych smoothies owocowych, w których flawonole stanowiły najmniej liczną grupę związków fenolowych (Nowicka i in., 2016; Teleszko i Wojdyło, 2014). Co więcej, doniesienia te wskazały na trudności w zaprojektowaniu produktów owocowych o wysokiej zawartości polifenoli i aktywności przeciwutleniającej w korelacji z wynikami oceny konsumenckiej. Badanie przeprowadzone w ramach tej pracy dowiodło jednak, że wybrane kompozycje owocowo-warzywne z sokiem z owoców rokitnika pospolitego zapewniają wysoki potencjał prozdrowotny przy dużej akceptacji sensorycznej.

Stwierdzono, że komponowanie soku z owoców rokitnika pospolitego z innymi owocami i warzywami istotnie zwiększyło stężenie polimerów procyjanidyn w smoothies (**Publikacja 6, Tab. 3**). W badaniu dotyczącym rokitnika pospolitego, Ma i in. (2020) zbadali szerokie zróżnicowanie ilości procyjanidyn w zależności od odmiany, jak również określili silny związek między cierpkością a proantocyjanidynami ogółem. Jednak za gorycz i cierpkość odpowiadają głównie niskocząsteczkowe procyjanidyny (Tkacz i in., 2019a). Z uwagi na fakt, iż celem tego badania były produkty o wysokiej akceptacji sensorycznej, a ilość procyjanidyn wzrosła w porównaniu do soku z owoców rokitnika pospolitego, określono stopień polimeryzacji (DP). Wskaźnik DP wzrósł średnio 1,5-krotnie (smoothies z selerem, jabłkiem, brzoskwinią) do 3-krotnie w przypadku smoothies z winogronem. Ogólna akceptacja produktów umiarkowanie korelowała z zawartością procyjanidyn ($r = 0,35$) oraz z ich DP ($r = 0,40$), z kolei silnie ujemnie ze stężeniem flawonoli ($r = -0,68$), co mogło wynikać z cierpkości pochodzącej od pochodnych izoramnetyny.

Zdolność redukcji jonów żelaza określona metodą FRAP w smoothies z gruszką, brzoskwinią i pomarańczą była istotnie wyższa ($p < 0,05$), od 3,38 mmol do 7,31 mmol Trolox/100 g śm, niż aktywność soku z owoców rokitnika pospolitego (**Publikacja 6, Tab. 4**). Z kolei, zastosowanie dodatku warzyw, jabłka, winogron i brzoskwini zapewniło wyższą zdolność smoothies do absorpcji rodników tlenowych według metody ORAC niż aktywność przeciwutleniająca FRAP.

Stwierdzono wyższą aktywność przeciwstarzeniową jako zdolność do hamowania enzymów AChE i BuChE dla produktów owocowych niż wzbogacanych warzywami (**Publikacja 6, Tab. 4**). Mieszanie soku z owoców rokitnika pospolitego z innymi półproduktami może istotnie zwiększyć aktywność hamowania AChE ($p < 0,05$) w porównaniu do samego soku (14,72%). Chociaż sok z rokitnika pospolitego silnie hamował BuChE (53,41%), to dodatek innych owoców i korzenia selera obniżył aktywność produktów o nie więcej niż 10%. Kwestia oddziaływania związków fenolowych z AChE i BuChE jest wciąż słabo zbadana i niewyjaśniona, a różnice widoczne są w stopniu powinowactwa inhibitorów do cząsteczek enzymów. Niemniej jednak, badania nad neuroprotekcją związków fenolowych wskazują na wyższy potencjał kwasów fenolowych i flawonoli (w tym kwasu ferulowego, kwasu *p*-kumarowego i kwercetyny obecnych w rokitniku pospolitym) niż flawan-3-oli (Jabir i in., 2018; Szwajgier, 2015).

Wszystkie nowo zaprojektowane smoothies miały zdolność hamowania α-amylazy i α-glukozydazy, ale wpływ na aktywność α-glukozydazy był istotnie silniejszy ($p < 0,05$). Dodatek moreli, winogron, pomarańczy i korzenia pietruszki okazał się być najbardziej korzystnym dla aktywności inhibicji α-amylazy i α-glukozydazy. Z kolei, silnymi inhibitorami lipazy trzustkowej były produkty z brzoskwinią, następnie gruszką, pomarańczą, jabłkiem i korzeniem pietruszki. Aktywność hamowania α-amylazy i α-glukozydazy korelowała za zawartością flawonoli (odpowiednio $r = 0,54$ i $0,50$), zaś zdolność inhibicji lipazy trzustkowej może wynikać z obecności polimerycznych procyjanidyn ($r = 0,50$). Wyniki te znajdują odzwierciedlenie zależności w analizie grupowania hierarchicznego (AHC) i analizie głównych składowych (PCA) (**Publikacja 6, Fig. 2**). Badania *in vitro* i z zastosowaniem modeli zwierzęcych wskazały, że perspektywa przeciwcukrzycowa związana z flawonoidami wynika z ich modulującego wpływu na metabolizm węglowodanów, poprawy funkcji komórek beta trzustki i działania insuliny, zmniejszenia insulinooporności, stanu zapalnego i stresu oksydacyjnego w mięśniach oraz zmniejszenia syntezy cholesterolu i poziomu triglicerydów (Vinayagam i Xu, 2015). Kontrolowana redukcja aktywności enzymów jest zatem obiecującym

rozwiązaniem w profilaktyce i leczeniu cukrzycy typu 2 w połączeniu ze zmniejszoną dawką leków, towarzyszącą nadwagą i wczesnym stadium otyłości oraz ich powikłaniami (Costamagna i in., 2016).

Wobec powyższego, kompozycje smoothies opracowane w ramach badań zawartych w **Publikacji 6**, można traktować jako istotną propozycję uzupełnienia diety o potencjalnych właściwościach przeciwutleniających, przeciwstarzeniowych i przeciwcukrzycowych i atrakcyjnych sensorycznie.

4.4. Analiza właściwości fizykochemicznych i potencjału prozdrowotnego mikrokapsułek otrzymanych z soku z owoców rokitnika pospolitego

Wraz z postępem prac nad produktami z owoców rokitnika pospolitego dostrzeżono potencjał soku w procesie mikroenkapsulacji, który obejmuje zamknięcie cennych, wrażliwych i docelowych składników w materiale powłokowym. Stąd, zakres badań rozszerzono w kierunku przetwarzania rokitnika pospolitego do utrwalonej formy mikrokapsułki o wydłużonym okresie przydatności do spożycia, a tym samym poprawionych właściwościach fizycznych oraz wartości prozdrowotnej. Celem tego etapu badań była ocena wpływu metod suszenia i nośników polisacharydowych na właściwości fizyczne, składniki chemiczne i aktywność przeciwutleniającą mikrokapsułek z soku z owoców rokitnika pospolitego.

Wyniki przedstawiono w **Publikacji 7**:

Tkacz K., Wojdyło A, Michalska-Ciechanowska A., Turkiewicz I.P., Lech K., Nowicka P. 2020. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules*, 25(17), 3801. doi: 10.3390/molecules25173801.

Oceniono wpływ metod suszenia rozpyłowego (180 °C), sublimacyjnego (od -30 do 30 °C) i próżniowego (w trzech wariantach temperaturowych 50, 70 i 90 °C) oraz rodzajów nośników polisacharydowych (inulina, maltodekstryna, mieszaniny inuliny i maltodekstryny w stosunku 1:2 i 2:1) na zawartość wody, aktywność wody, gęstości rzeczywistą i nasypową, porowatość, parametry barwy, wskaźnik brązowienia, zawartość hydroksymetylofurfuralu i związków fenolowych oraz aktywność przeciwutleniającą 20 wariantów mikrokapsułek z soku z owoców rokitnika pospolitego nowej odmiany 'Józef" (**Publikacja 7, Fig. 1**), przed i po sześciu miesiącach przechowywania. Nowatorski charakter wyników zaprezentowanych w **Publikacji 7** wynika z zastosowania zarówno różnych metod suszenia, jak i różnych nośników polimerowych w procesie tworzenia mikrokapsułkowanego soku z owoców rokitnika pospolitego.

Zbadano istotnie większy wpływ metody suszenia na modulację aktywności wody niż w przypadku rodzaju nośnika ($p < 0,05$) (**Publikacja 7, Tab. 1**). Inulina powodowała silniejsze zatrzymywanie wody w mikrokapsułkach niż maltodekstryna (odpowiednio 3,71 i 2,01%), co jest uzasadnione wysoką higroskopijnością inuliny wynikającą z rozgałęzionej struktury sprzyjającej tworzeniu wiązań wodorowych i absorpcji wody z otoczenia (Lacerda i in., 2016). Niemniej jednak, nowo otrzymane formuły spełniały kryterium wilgotności poniżej 5% dla bezpieczeństwa mikrobiologicznego (Aziz i in., 2018). Aktywność wody mikrokapsułek poniżej 0,1 była zasadnicza dla zminimalizowania rozwoju pleśni, drożdży i bakterii oraz zapobiegania degradacji związków biologicznie aktywnych i brunatnienia nieenzymatycznego.

Co więcej, produkty z inuliną cechowały wyższe wartości gęstości rzeczywistej niż tych z maltodekstryną, odpowiednio 1472 i 1423 kg/m^3 (**Publikacja 7, Tab. 1**). Gęstość nasypowa i porowatość znacznie różniły się ze względu na metody suszenia, a suszenie próżniowe wydaje się być użyteczną techniką otrzymywania mikrokapsułek o dużej gęstości nasypowej. Porowatość mikrokapsułek suszonych rozpyłowo i sublimacyjnie była istotnie wyższa niż formuł poddanych suszeniu próżniowemu ($p < 0,05$). Obniżona temperatura i ciśnienie w procesie suszenia sublimacyjnego zapewniają odpowiednią szybkość sublimacji, nie narażając materiału na skurcz, dlatego otrzymano produkty o dużej porowatości i zdolności rehydracyjnej (Caparino i in., 2012). Zatem porowatość i gęstość nasypowa mikrokapsułek są ważnym kryterium

aplikacyjnym, ze względu na warunki przechowywania, rodzaj i postać produktu końcowego oraz stabilność oksydacyjną i aromatów.

Następnie, określono parametry barwy w przestrzeni CIE L*a*b*, kąt barwy (h°) charakteryzujący percepcję barwy, parametr chroma (C) wskazujący na czystość i intensywność barwy oraz całkowitą różnicę barwy (dE) (**Publikacja 7, Tab. 2**). Ze względu na intensywną żółtą barwę mikrokapsułek z dodatkiem maltodekstryny (parametr barwy b* = 52,05 przy intensywności C = 55,72), stosowanie tego nośnika polimerowego było konkurencyjne w porównaniu z inuliną. Mikrokapsułki wytworzone metodą suszenia rozpyłowego i sublimacyjnego charakteryzowały wysokie wartości parametru jasności L*, dlatego uzasadnione jest wykorzystanie tych procesów suszenia w produkcji wyrobów o korzystnej, minimalnie zmienionej barwie. Uzyskane wyniki wskazały na widoczny i rozróżnialny kontrast barwy mikrokapsułek dla ludzkiego oka (dE > 5,0), ale nie sugerowały jednoznacznej oceny sensorycznej barwy (Lacerda i in., 2016).

Suszenie rozpyłowe, sublimacyjne i próżniowe w temperaturze 50 °C oraz dodatek maltodekstryny nie sprzyjały brązowieniu nieenzymatycznemu i tworzeniu hydroksymetylofurfuralu (HMF) w mikrokapsułkach. Inulina z kolei promowała wzrost indeksu brązowienia mikrokapsułek świeżych i przechowywanych (**Publikacja 7, Tab. 2**). Niskie pH i podwyższona temperatura procesów suszenia mogą sprzyjać hydrolizie inuliny w roztworach wodnych, zwiększając w ten sposób ilość cukrów redukujących uczestniczących w reakcjach Miliarda (Mensink i in., 2015). Należy to uwzględnić szczególnie przy stosowaniu soku z owoców rokitnika pospolitego o naturalnie niskim pH około 3,0 (**Publikacja 1 i 5**).

Średnia zawartość HMF w mikrokapsułkach z maltodekstryną była od 28 do 45 razy niższa niż w mikrokapsułkach z innymi nośnikami, co wskazuje na zasadność wykorzystania czystej maltodekstryny do produkcji mikrokapsułek metodą próżniową w podwyższonej temperaturze (**Publikacja 7, Tab. 3**). Należy jednak zaznaczyć, że krótkotrwałe suszenie rozpyłowe w 180 °C nie skutkowało akumulacją HMF niezależnie od rodzaju nośnika. Wzrost HMF w formułach po przechowywaniu był znacznie niższy dla mikrokapsułek suszonych rozpyłowo, co mogło wynikać z tworzenia sferycznych kapsułek podczas suszenia rozpyłowego, pozbawionych porów i tym samym pełną ochroną rdzenia (Pasrija i in., 2015).

Zgodnie z analizą związków fenolowych wykonaną metodą UPLC-PDA, suszenie rozpyłowe i suszenie próżniowe w 70 °C najbardziej sprzyjały zachowaniu odpowiednio kwasów fenolowych (2,51 mg/100 g sm) i flawonoli (271,71 mg/100 g sm) w mikrokapsułkach (**Publikacja 7, Tab. 3**). Degradacja flawonoli po procesie suszenia sublimacyjnego była najniższa (o 4,8%), zatem ich stężenie w tych mikrokapsułkach było najwyższe (około 119,76 mg/100 g sm). W mikrokapsułkach z maltodekstryną zbadano więcej związków fenolowych, jednak to inulina zapewniła minimalną redukcję ich zawartości podczas przechowywania (o 10,6%). Elastyczność szkieletu inuliny w połączeniu z wysoką temperaturą zeszklenia (Tg) czynią ten środek odpowiednim stabilizatorem składników odżywczych i bioaktywnych, na przykład białka w stanie suchym w zastosowaniach spożywczych i farmaceutycznych (Mensink i in., 2015). W związku z tym, uzyskane wyniki są uzależnione od stopnia retencji związków fenolowych i ich stabilności w mikrokapsułkach stabilizowanymi nośnikami, które wykazują różne właściwości ochronne i parametry kinetyczne. Ponadto, stężenie flawonoli korelowało z porowatością otrzymanych formuł (r = 0,913), co może tłumaczyć wydajne uwalnianie polifenoli w procesie ekstrakcji, jak również ich mniejszą retencję podczas przechowywania, i tym samym narażenie na degradację.

Mikrokapsułki otrzymane metodami suszenia rozpyłowego, sublimacyjnego i próżniowego w 50 °C miały najwyższą aktywność przeciwutleniającą określoną metodą ABTS (około 1,55 mmol Trolox/100 g sm) (**Publikacja 7, Tab. 3**). Proces przechowywania sprzyjał wzrostowi aktywności przeciwutleniającej mikrokapsułek, w tym po procesie suszenia rozpyłowego i sublimacyjnego średnio o 26%. Stwierdzono niemal 50%-owy wzrost aktywności wobec rodników kationowych formuł suszonych w warunkach próżni w 90 °C, ale ich aktywność była najniższa spośród badanych. Według Rocha-Parra i in. (2016), straty związków fenolowych w czerwonym winie kapsułkowanym metodą sublimacji również nie znalazły odzwierciedlenia w zmianie aktywności przeciwutleniającej. Reakcje między utlenionymi fenolami mogą zatem zwiększyć zdolność hamowania czynników utleniających.

Przeprowadzono profilowanie on-line przeciwutleniaczy celem weryfikacji potencjalnej aktywności HMF i furozyny, produktów powstających w wyniku obróbki termicznej i podczas przechowywania (**Publikacja 7, Fig. 2**). Brak negatywnych odpowiedzi po reakcji postkolumnowej z odczynnikiem $ABTS^{\bullet+}$ sugerował, że HMF i furozyna nie miały zdolności zmiatania rodników kationowych. Ponadto, nie stwierdzono korelacji między zawartością HMF a wzrostem aktywności przeciwutleniającej mikrokapsułek.

Wyniki uzyskane w ramach tego etapu badań, zaprezentowane w **Publikacji 7**, pozwoliły na stwierdzenie, że zastosowanie maltodekstryny było konkurencyjne w porównaniu do inuliny i mieszanek polimerowych maltodekstyny i inuliny (2:1 i 1:2), ze względu na wyższe stężenie związków fenolowych, aktywność przeciwutleniającą i niesprzyjanie brązowieniu nieenzymatycznemu i akumulacji HMF w mikrokapsułkach.

5. PODSUMOWANIE I WNIOSKI

Wyniki przedstawione w niniejszym cyklu publikacyjnym rozprawy doktorskiej pozwoliły na **potwierdzenie hipotezy badawczej**, wskazując, że owoce rokitnika pospolitego charakteryzuje unikatowy profil związków bioaktywnych o określonym potencjale prozdrowotnym oraz stanowią one wartościowy surowiec do produkcji jednocześnie funkcjonalnych i atrakcyjnych sensorycznie produktów.

Rozwiązania problemu badawczego związanego z brakiem akceptacji sensorycznej rokitnika pospolitego należy upatrywać w zastosowaniu fermentacji jabłkowo-mlekowej soków z owoców rokitnika pospolitego, komponowaniu soku z owoców rokitnika pospolitego z surowcami powszechnie stosowanym w przetwórstwie owocowo-warzywnym oraz mikroenkapsulacji soku z owoców rokitnika pospolitego.

Przeprowadzone badania pozwoliły na sformułowanie następujących **wniosków**:

1. Owoce rokitnika pospolitego odmian uprawianych w Polsce różniły się składem chemicznym, w tym kompozycji lipofilowych i hydrofilowych związków bioaktywnych. Analizowane odmiany, wraz z nową odmianą 'Józef', charakteryzował istotny potencjał przeciwcukrzycowy, przeciwstarzeniowy, przeciw otyłości, przeciwzapalny i przeciwutleniający *in vitro* w korelacji z zawartością związków fenolowych (697,56 mg/100 g sm), karotenoidów (157,93 mg/100 g sm), tokoferoli i tokotrienoli (28,23 mg/100 g sm), kwasów tłuszczowych (38% SFA, 42% MUFA, 20% PUFA) i witaminy C (64,92 mg/100 g śm). Stwarza to obiecujące perspektywy dla komercyjnej produkcji tej odmiany i jej wykorzystania w przemyśle spożywczym jako źródła substancji i właściwości prozdrowotnych. Badane odmiany jagód rokitnika pospolitego charakteryzowały się wysoką zawartością frakcji biologicznie aktywnych związków o wysokim potencjale biologicznym, co stanowi podstawę do dalszego zastosowania w projektowaniu innowacyjnych produktów funkcjonalnych, nutraceutyków i kosmetyków, oraz do wykorzystania w planowaniu i rozpowszechnianiu upraw.
2. Ponad 98% związków fenolowych owoców rokitnika pospolitego stanowiły flawonole, następnie kwasy fenolowe. Kwasy fenolowe charakteryzowały się silniejszym potencjałem przeciwutleniającym niż flawonole, które podobnie jak karotenoidy wskazały na zdolność hamowania aktywności acetylocholinoesterazy i butylocholinoesterazy. Owoce badanych odmian rokitnika pospolitego mogą stanowić składnik nowych funkcjonalnych i wartościowych produktów bogatych we flawonole i karotenoidy o działaniu przeciwstarzeniowym, stosowanych w profilaktyce zmian neurozwyrodnieniowych i przyczyn otępienia, w tym chorobie Alzheimera i Parkinsona.
3. Stwierdzono różnorodność anatomicznych części owoców (skórki, miąższu, endokarpu, nasion), pędów i liści rokitnika pospolitego w odniesieniu do triterpenoidów, związków fenolowych oraz makro- i mikroelementów. Skórka i miąższ były cennym źródłem triterpenoidów (maksymalnie 76,38 mg i 76,23 mg/100 g sm, odpowiednio), w przeciwieństwie do liści, które gromadziły znaczne stężenia składników mineralnych (głównie wapnia, żelaza, miedzi i manganu), pochodnych kwercetyny i izoramnetyny (odpowiednio 729,07 mg i 173,40 mg/100 g sm). Pędy i nasiona z endokarpem stanowiły źródło flawan-3-oli i polimerycznych procyjanidyn (maksymalnie 2 021,31 mg i 7 275,98 mg/100 g sm). Analizowane frakcje rokitnika pospolitego uznano za korzystne niekonwencjonalne źródło potasu (553,41 mg - 794,39 mg/100 g sm), a w przypadku liści również

wapnia (964,97 mg/100 g sm). Wyniki dostarczyły ważnych informacji do ukierunkowania i wdrażania formuł funkcjonalnych i nutraceutycznych, jednocześnie wskazując wykorzystanie całej rośliny w produkcji bezodpadowej zgodnie z obowiązującymi trendami.

4. Soki z owoców rokitnika pospolitego są bogatym źródłem karotenoidów (133,65 mg - 839,89 mg/100 g sm), tokoferoli (22,23 mg - 94,08 mg/100 g sm), aminokwasów (175,92 mg - 1 822,69 mg/100 g sm), fitoprostanów (32,31 ng - 1 523,51 ng/100 g sm) i fitofuranów (do 101,47 μg/100 g sm) wskazujących na potencjał zastosowania w prewencji stanów zapalnych, procesów starzenia, hiperglikemii poposiłkowej i obronie organizmu przed reakcjami wywołanymi przez wolne rodniki. Niewątpliwym osiągnięciem byłaby komercjalizacja i globalizacja produkcji owoców rokitnika pospolitego oraz szerokiej gamy produktów z nich otrzymanych, w tym produktów o charakterze płynnym i półpłynnym. Zróżnicowanie składu i bioaktywności analizowanych soków komercyjnych wskazało na istotę wyboru przez konsumenta soków z deklarowanych odmian i upraw rokitnika pospolitego.

5. Fermentację jabłkowo-mlekową uznano za obiecującą metodę redukcji kwasowości soków z owoców rokitnika pospolitego i soków mieszanych z jego wysokim udziałem, przy jednoczesnym zwiększeniu potencjału prozdrowotnego poprzez wzrost ilości związków fenolowych (maksymalnie o 23%) i aktywności przeciwutleniającej (maksymalnie o 52%). Najkorzystniejszym w tym kontekście był proces 72-godzinnej fermentacji soku mieszanego rokitnik – jabłko (1:1) z zastosowaniem szczepów *Lactobacillus planarum* DSM 10492 i DSM 20174. Uzyskane wyniki pozwolą zatem na dobór warunków i szczepów bakteryjnych do odkwaszenia na skalę przemysłową oraz przyczynią się do opracowania nowych produktów o wartości dodanej, zwiększając tym samym spożycie jagód rokitnika pospolitego, w tym w profilaktyce przewlekłych chorób niezakaźnych.

6. Komponowanie soku z owoców rokitnika pospolitego z innymi owocami i warzywami poprawiło atrakcyjność smaku, barwy i aromatu produktów typu smoothie, szczególnie przy układzie 1:3 dla owoców i 1:1 dla warzyw. Opracowane produkty rokitnikowe typu smoothies na bazie owoców i warzyw charakteryzowały się wyższą zawartością pektyn (maksymalnie 7-krotną), kwasów fenolowych (maksymalnie 7-krotną) i polimerów procyjanidyn (maksymalnie 6-krotną) w porównaniu do soku z owoców rokitnika pospolitego. Dodatek gruszki, brzoskwini, winogron, jabłka i pietruszki spowodował 3-krotny wzrost aktywności produktów w kierunku inhibicji acetylocholinoesterazy. Nowe formuły smoothies są propozycją uzupełnienia diety o ukierunkowaniu w stronę podniesienia potencjału przeciwstarzeniowego, przeciwcukrzycowego i przeciwutleniającego. Uzyskane wyniki badań stanowią podstawę do dalszego projektowania innowacyjnych produktów jednocześnie o znaczących właściwościach sensorycznych i wysokim potencjale prozdrowotnym opartych na jagodach rokitnika pospolitego – wciąż niedocenianych przez przemysł spożywczy.

7. Badania nad optymalizacją procesu mikroenkapsulacji soku z owoców rokitnika pospolitego ukierunkowują w zakresie doboru nośników i optymalizacji warunków suszenia do potencjalnego zastosowania w skali przemysłowej, stabilności wybranych związków biologicznie aktywnych oraz aktywności przeciwutleniającej soku z owoców rokitnika pospolitego po procesie suszenia i przechowywania. Zastosowanie maltodekstryny jako nośnika polimerowego sprzyjało wyższej o 20% zawartości związków fenolowych niż w mikrokapsułkach z inuliną, jednocześnie ograniczając

reakcje brązowienia nieenzymatycznego i akumulację HMF. Mikrokapsułki suszone rozpyłowo i próżniowo w 70 °C charakteryzowała najwyższa ilość odpowiednio kwasów fenolowych (2,51 mg/100 g sm) i flawonoli (271,71 mg/100 g sm), z kolei suszenie sublimacyjnego zapewniło najwyższą ochronę flawonoli podczas 6-miesięcznego przechowywania. Proces mikroenkapsulacji ograniczył niestabilność bioaktywnych związków rokitnika pospolitego podczas przetwarzania i przechowywania, co jest odpowiedzią na obecne oczekiwania producentów co do stabilności składników bioaktywnych, szczególnie ważnych w przypadku fakcji lipidowo-hydrofilowej jagód rokitnika pospolitego.

Podsumowując, ze względu na wysoki potencjał prozdrowotny owoców rokitnika pospolitego, ale niski stosunek cukrów do kwasów organicznych, należy dążyć do poprawy walorów sensorycznych jagód i produktów z nich otrzymanych. Wymiernym efektem badań nad innowacyjnymi rozwiązaniami zastosowania owoców rokitnika pospolitego był dobór warunków i szczepów bakterii do potencjalnej fermentacji jabłkowo-mlekowej soku w skali przemysłowej, wskazanie odpowiednich nośników polimerowych i metod suszarniczych w procesie mikroenkapsulacji soku, opracowanie kompozycji rokitnika pospolitego z owocami i warzywami wykorzystywanymi w przetwórstwie, co może przyczynić się do rozszerzenia upraw *H. rhamnoides* w Polsce, opracowania nowych produktów o wartości dodanej, zwiększając tym samym spożycie rokitnika pospolitego, w tym w profilaktyce przewlekłych chorób niezakaźnych.

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Anti-Oxidant and Anti-Enzymatic Activities of Sea Buckthorn (*Hippophaë rhamnoides* L.) Fruits Modulated by Chemical Components

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Abstract: The aim of this study was to analyze in vitro biological activities as anti-oxidant, anti-α-amylase, anti-α-glucosidase, anti-lipase, and anti-lipoxygenase activity, relative to bioactive components (phenolic acids, flavonols, xanthophylls, carotenes, esterified carotenoids, tocopherols, tocotrienols, and fatty acids) and the basic chemical composition (sugars, organic acid, dry matter, soluble solid, pH, titratable acidity, ash, pectins, and vitamin C) of *Hippophaë rhamnoides* berries. Six sea buckthorn cultivars commonly grown in Poland were analyzed including Aromatnaja, Botaniczeskaja-Lubitelskaja, Józef, Luczistaja, Moskwiczka, and Podarok Sadu. Berries contained 1.34–2.87 g of sugars and 0.96–4.22 g of organic acids in 100 g fresh weight, 468.60–901.11 mg of phenolic compounds, and 46.61–508.57 mg of carotenoids in 100 g dry mass. The fatty acid profile was established: palmitic > palmitoleic > oleic and linoleic > stearic and linolenic acids. The highest anti-oxidant (34.68 mmol Trolox/100 g dry mass) and anti-α-amylase potential (IC_{50} = 26.83 mg/mL) was determined in Aromatnaja, anti-α-glucosidase in Botaniczeskaja-Lubitelskaja (IC_{50} = 41.78 mg/mL), anti-lipase in Moskwiczka and Aromatnaja (average IC_{50} = 4.37 mg/mL), and anti-lipoxygenase in Aromatnaja and Podarok Sadu fruits (100% inhibition). The studied sea buckthorn berries may be a raw material for the development of functional foods and nutraceutical products rich in compounds with high biological activity.

Keywords: sea buckthorn berries; 2,2′-Azobis(2-amidinopropane)dihydrochloride (ABTS); ferric reducing ability of plasma (FRAP); oxygen radical absorbance capacity (ORAC); α-amylase; α-glucosidase; lipase; lipoxygenase; fatty acids; vitamins

1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) is a thorny, deciduous shrub belonging to the Elaeagnaceae family. Six species of *Hippophaë* and 12 subspecies are currently recognized, including ssp. *sinensis*, ssp. *mongolica*, and ssp. *rhamnoides*, which are the most economically and commercially important. There are over 150 cultivars of sea buckthorn, but new thornless and easier-to-harvest varieties are still being selected. Sea buckthorn naturally grows on sea coasts and river valleys of Central and Northern Europe, Russia, China, Mongolia, Central Asia, and slopes of the Caucasus and Himalayas. The plant is cultivated mainly in the Northern Hemisphere and its largest producer is China [1–4].

Fleshy and soft sea buckthorn fruits are yellow, orange, or red, round or oblong, and 6–15 mm in diameter [5]. Due to the similarity of berries, the plant is commonly confused with scarlet firethorn (*Pyracantha coccinea*), rock cotoneaster (*Cotoneaster horizontalis)*, or rowan (*Sorbus aucuparia*), whose raw fruits are poisonous. The sea buckthorn aroma is compared to strawberries, peach, mango, apricot, papaya, and citrus, but mostly to pineapple, which results from a similar ester profile [6]. In the food industry, sea buckthorn is used as a raw material enriching the pro-health value or increasing the acidity of fruit products. Berries are intended for the production of jams, juices, soft drinks, liqueurs, wine, or as an addition to beers, kefir, and cheeses. By contrast, oil obtained from seeds and pulp is used as a cosmetic and dietary supplement, and is used less frequently as a culinary product. Production residues can be a functional ingredient in meat or animal feed [1,2,5,7].

The therapeutic properties of bark, leaves, and fruits were already known in ancient Greece as well as in Tibetan and Mongolian medicine. In the cosmetics industry, the plant is used in dermatological diseases, hair care, revitalization of wounds and skin burns, and as a form of natural protection against UV-B radiation. The results of previous in vitro and in vivo studies [2,5,7–10] confirm the effectiveness of sea buckthorn extracts in the prevention of hyperglycemia, hyperinsulinemia, and hyperlipidemia, together with hepatoprotective, anti-carcinogenic, antibacterial, and antifungal effects, as well as positive functioning of the digestive system and eyesight. The properties of sea buckthorn are due to the high concentrations of flavonoids (mainly flavonols), carotenoids (principally β-cryptoxanthin and β-carotene), ascorbic acid, vitamin E (the most active form, α-tocopherol, dominates), and fatty acids (omega-3, omega-6, omega-7, and omega-9) present in seeds, skin, and flesh [6,11,12].

A number of studies have been carried out on sea buckthorn in different world regions, but the knowledge about biologically active compounds and pro-health potential of cultivars grown in Poland is limited. Given the above, the aim of this study was to analyze biological activities (anti-oxidant, anti-α-amylase, anti-α-glucosidase, anti-lipase, and anti-lipoxygenase effects) relative to selected bioactive components (flavonols and phenolic acids, xanthophylls, carotenes, esterified carotenoids, tocopherols and tocotrienols, fatty acids), and the basic chemical composition (sugars, organic acid, dry matter, soluble solid, pH, titratable acidity, ash, pectins, vitamin C) of berries of six commonly grown *H. rhamnoides* cultivars in Poland.

α-Amylase and α-glucosidase break down polysaccharides to glucose. Therefore, their inhibition is one of the methods of postprandial hyperglycemia reduction. This effect plays a key role in treating type 2 diabetes, which, according to WHO, affects 8.5% of the global adult population. In turn, pancreatic lipase breaks down dietary triglycerides into bioavailable forms – fatty acids and glycerol. Its inhibition can reduce energy intake at a meal, which is part of the strategy of overweight and obesity therapy. The lipoxygenase pathway, including lipoxygenase 5-LOX, 12-LOX, and 15-LOX, is associated with the production of hydroperoxy fatty acids and leukotrienes. Increased concentrations of these products correlate with the progression of, inter alia, inflammatory bowel disease, asthmatic bronchitis, rheumatoid arthritis, cancers, and cardiovascular diseases [13].

Therefore, it was assumed that the results will allow the identification of significant differences in the pro-health potential and composition of the studied sea buckthorn cultivars for further use in the design of innovative functional products, nutraceuticals, and cosmeceuticals. Additionally, this study should indicate cultivars with the highest biological potential for further use in planning and expanding cultivations. Furthermore, the results of the cultivar Józef, bred in Poland, are presented for the first time. This creates promising perspectives for commercial production of this sea buckthorn cultivar and use in the food industry as a source of health-promoting substances and antioxidant properties.

2. Materials and Methods

2.1. Chemicals

Standards of sugars, organic acids, phenolic compounds, and carotenoid compounds were purchased from Extrasynthese (Genay, France), and the rest of the reagents were bought from Merck

KgaA (Darmstadt, Germany). The samples before chromatographic analysis were filtered through a Hydrophilic PTFE 0.20 µm membrane (Millex Samplicity Filters, Merck KgaA, Darmstadt, Germany).

2.2. Plant Materials

The fruits of six sea buckthorn (*Hippophaë rhamnoides* L.) cultivars—Aromatnaja, Botaniczeskaja-Lubitelskaja, Józef, Luczistaja, Moskwiczka, and Podarok Sadu—were tested (Figure 1). Ripe berries were collected in early July and August 2018 from the Research Institute of Horticulture in Skierniewice (Poland). Fresh fruits were used to analyze the basic chemical composition. The second portion of selected berries was frozen, freeze-dried for 24 h (Christ Alpha 1–4 LSC, Martin Christ GmbH, Osterode am Harz, Germany) and crushed by a laboratory mill (A11, IKA, Darmstadt, Germany). The homogeneous materials were stored in a freezer at –80 °C until undergoing the other analysis.

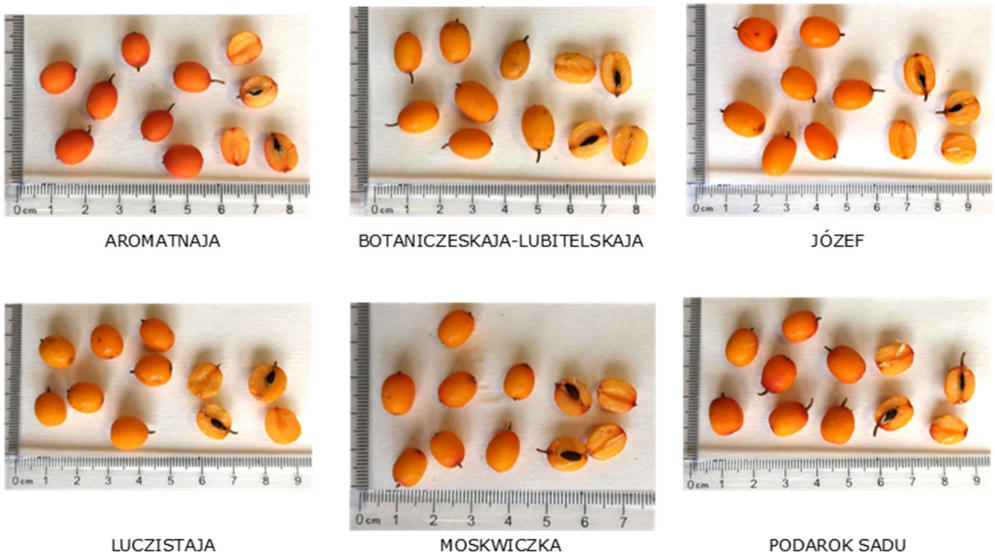

Figure 1. Berries of sea buckthorn cultivars.

2.3. Basic Chemical Composition

The soluble solids content was expressed in °Bx using a digital refractometer (Atago RX-5000, Atago Co. Ltd., Saitama, Japan). The instrument was calibrated using distilled water. Liquid and homogenized raw material was applied to the dry prism surface. The measurement was taken at 20 °C. The dry matter was determined by mixing the sample with diatomaceous earth, pre-drying, and final drying under reduced pressure. Titratable acidity (TA) was analyzed by the titration of homogenous fresh fruits with 0.1N NaOH to pH 8.1 and the result were expressed as g malic acid/100 g FW (fresh weight). TA and pH were determined using an automatic pH titrator system (TitroLine 5000, Xylem Analytics GmbH, Weilheim in Oberbayern, Germany). The soluble solids content, dry matter, and titratable acidity were taken according to European Standards, PN-EN 12143:2000, PN-EN 12145:2001, and PN-EN 12145:2000, respectively. Pectin content (g/100 g FW) was measured according to the Morris method reported by Pijanowski et al. [14]. Ash (%), L-ascorbic acid (mg/100 g FW), sugars, and organic acids contents (g/100 g FW) were determined, as reported previously by Wojdyło et al. [15]. Sugars and organic acids were analyzed using high pressure liquid chromatography including the evaporative light scattering detector (HPLC-ELSD) and ultra performance liquid chromatography-photodiode array detector (UPLC-PDA) methods. All measurements were taken three times.

2.4. Analysis of Phenolic Compounds

The extraction of the samples for phenolic compounds and their chromatographic analysis were performed exactly as described by Wojdyło et al. [15]. The samples were analyzed by an Ultra-Performance Liquid Chromatography Photodiode Array Detector (UPLC-PDA; Acquity UPLC System, Waters Corp., Milford, MA, US). The study identified phenolic acids and flavonols, and their sums were calculated as *p*-coumaric acid and isorhamnetin-3-*O*-rutinoside, respectively, which is based on dominant compounds and compared with reference standards. All results were taken in triplicate and shown as mg/100 g DM of berries (dry mass).

2.5. Analysis of Carotenoids, Tocopherols, and Tocotrienols

The extraction of the samples for carotenoid compounds was made as previously described by Wojdyło et al. [15] and Nowicka et al. [16]. The determination of carotenoids was made using the equipment as in subSection 2.4, according to the protocol given by Wojdyło et al. [15]. The powder samples of fruits (0.20 g) containing 10% $MgCO_3$ and 1% butylhydroxytoluene (BHT) to prevent oxidation were continuously shaken with 5 mL of a ternary mixture of methanol/acetone/hexane (1:1:2, *v:v:v*) at 300 rpm (DOS-10L Digital Orbital Shaker, Elmi Ltd., Riga, Latvia) for 30 min in the dark. Recovered supernatants were obtained after 4–5 times being re-extracted of solid residue. All combined fractions collected after centrifugation (4 °C, 7 min at 19,000× *g*, MPW-350, Warsaw, Poland) were evaporated to dryness. The pellet was diluted using 2 mL of 100% methanol, filtered through a hydrophilic polytetrafluoroethylene (PTFE) 0.20-μm membrane (Millex Samplicity Filter, Merck, Darmstadt, Germany) and used for analysis.

Carotenoids were carried out on an ACQUITY UPLC BEH RP C18 column being protected by the guard column of the same materials (1.7 mm, 2.1 mm 100 mm, Waters Corp., Milford, MA, USA) operated at 30 °C. The elution solvents were linear gradient of acetonitrile:methanol (70:30, *v:v*) (A) and 0.1% formic acid (B). The runs were monitored at 450 nm. The photodiode array detector PDA spectra were measured over the wavelength range of 200–700 nm in steps of 2 nm. The retention times and spectra were compared to those of the authentic standards. All incubations were done in triplicate. The results were expressed as mg per kg of dm. Samples for the analysis of tocopherols and tocotrienols were prepared as follows. The fresh sea buckthorn berries (~3g) were homogenized with two times as much of the ethanol portion mixed with 0.05% butylated hydroxytoluene (BHT). Saponification was carried out using 60% CaOH, at a temperature of 50 °C for 2 h. Then, the samples were mixed with hexane:ethyl acetate with 0.05% BHT. After that, NaOH (saturated solution) was added. The upper layer was collected, evaporated, and dissolved in methanol with 0.05% BHT. The solutions were filtered through a Hydrophilic PTFE 0.20 μm membrane and used for UPLC analysis. The analysis of tocopherols and tocotrienols was carried out by using Ultra-Performance Liquid Chromatography with a fluorescence detector (UPLC-FL). The column ACQUITY UPLC BEH RP C18 (1.7 mm, 2.1 mm × 100 mm, Waters Corp., Milford, MA, US) being protected by a guard column of the same materials was operated at 30 °C. Identification and quantification was performed based on reference standards and calibration curves. The samples (5 μL) were injected, and the elution was completed in 12 min with an isocratic method of methanol with water (88:12, *v:v*) flow rates of 0.45 mL/min. All incubations were done in triplicate. The results were expressed as mg per kg of dm.

2.6. Analysis of Fatty Acids

Fatty acids were extracted and tested with the technique of gas chromatography with mass spectrometry (GC-MS), in the same way as described by Nowacki et al. [17]. The samples were analyzed using a GC 6890 gas chromatograph coupled with a 5983 MS mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, US) equipped in a quadrupole mass detector. Measurements were taken in triplicate. The results of fatty acid studies were expressed as the percentage of total fatty acids of sea buckthorn berries.

2.7. Determintation of Biological Activity: Anti-Oxidant, Anti-α-amylase, Anti- α-glucosidase, Anti-Lipase, and Anti-Lipoxygenase

The extraction procedure was the same for all determinations and was carried out identically, as described by Nowicka et al. [16]. The ABTS, FRAP, and ORAC assays were conducted as previously reported by Re et al. [18], Benzie and Strain [19], and Ou et al. [20], respectively. The $ABTS^{\bullet+}$ (2,2′-azine-bis-(3-ethylene-benzothiazoline-6-sulfonic acid) scavenging test is based on measuring the decrease in the color intensity inversely proportional to the antioxidant content. An $ABTS^{\bullet+}$ solution was prepared with an absorbance of 0.700 ± 0.02 at a wavelength of 734 nm. Sea buckthorn extracts and the $ABTS^{\bullet+}$ solution were mixed and, after 6 min, the absorption at the wavelength above was measured. Distilled water was blank. The results were calculated based on the calibration curve ($R^2 = 0.9950$) for Trolox concentrations 0.100 to 0.900 mM.

The FRAP method involves determining the ability to reduce Fe^{3+} ions by antioxidant substances contained in sea buckthorn extracts to the blue Fe^{2+} ions complex. Sea buckthorn extracts were mixed with distilled water. The absorbance of the samples was measured 10 min after the addition of the FRAP reagent (acetate buffer, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in HCl and $FeCl_3 \times 6H_2O$ in a volume ratio of 10:1:1, *v:v:v*), at a wavelength of 593 nm. The results were calculated based on the calibration curve ($R^2 = 0.9899$) for Trolox concentrations 0.050 to 0.900 mM.

The analysis of oxygen radical absorbance capacity (ORAC) consists of a spectrofluorometric measurement of the decrease in fluorescence caused by oxidation of a fluorescent substance under the influence of free radicals, but in the presence of antioxidant substances. Samples containing sea buckthorn extract, phosphate buffer, and fluorescein were incubated at 37 °C throughout the analysis period. 2,2′-Azobis(2-amidinopropane)dihydrochloride was added and the spectrofluorometric measurement was performed every 5 min at an excitation wavelength 493 nm and an emission wavelength of 515 nm. The blank was a phosphate buffer. The antioxidant activity of the tested samples was obtained by comparing the surface under the fluorescence decrease curves over time with the surface for pure Trolox solutions (12.5, 25.0, 50.0, and 75.0 μM).

The ABTS, FRAP, and ORAC results were expressed in mmol TE (Trolox)/100 g sample.

The anti-*α*-amylase, anti-*α*-glucosidase, and anti-lipase activity were studied, according to methods reported by Nowicka et al. [16] and Podsędek et al. [21]. Briefly, analysis of the anti-*α*-amylase inhibitory activity is based on a spectrophotometric measurement of the color change as a result of a reaction of iodine in potassium iodide with the remaining starch after enzymatic hydrolysis. Basic samples contained sea buckthorn extracts, starch solution, and *α*-amylase. After incubation at 37 °C, the reaction was stopped using 0.4 M HCl. A solution of potassium iodide with iodine was added. Reference samples contained phosphate buffer instead of an enzyme. The acarbose was included as a positive control and absorbance was measured at 600 nm.

The analysis of *α*-glucosidase inhibitory activity consists of the reaction of the enzyme with a β-D-glucosidase substrate producing a yellow solution upon cleavage. Basic samples containing sea buckthorn extracts and enzymes were incubated as above. After the addition of the substrate, the mixture was incubated again and measurement was made at 405 nm. As in the above analysis, the reference samples contained buffer instead of enzymes and the acarbose was included as a positive control.

The analysis of lipase inhibitory activity is based on a spectrophotometric measurement of the amount of *p*-nitrophenol formed from *p*-nitrophenyl acetate. Basic samples contained sea buckthorn extracts, Tris-HCl buffer, and the enzyme. After 5 min of incubation at 37 °C, the substrate was added. Then incubation continued for 15 min. Reference samples contained buffer instead of the enzyme and the orlistat was used as a positive control. Absorbance was measured at 400 nm. The results of anti-*α*-amylase, anti-*α*-glucosidase, and anti-lipase activity are presented as IC_{50} in mg/mL, i.e., the amount of the sample that is able to reduce enzyme activity by 50%.

Inhibitory activity toward 15-lipoxygenase was measured in accordance with Chung et al. [13]. Basic samples containing sea buckthorn extract and enzymes were incubated at 37 °C. Then, linoleic acid was added and incubation continued for 20 min. The mixture was measured at 210 nm.

Reference samples contained Tris-HCl buffer instead of the enzyme. The results were expressed as a percentage inhibition (at the concentration of 30 mg/mL).

All tests: anti-oxidant (ABTS, ORAC, FRAP), anti-α-amylase, anti- α-glucosidase, anti-lipase, and anti-lipoxygenase were performed in triplicate using a microplate reader Synergy™ H1 (BioTek, Winooski, VT, US).

2.8. Statistical Analysis

One-way analysis of variance (ANOVA, $p < 0.05$), Tukey's test, Pearson's correlation coefficients, and Principal Component Analysis (PCA) were carried out using XLSTAT for Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, US) and Statistica 13.1 (StatSoft, Cracow, Poland). The results were presented as the mean value ($n = 3$) ± standard deviation (SD).

3. Results and Discussion

3.1. Basic Chemical Composition of Sea Buckthorn Cultivars

Table 1 presents the basic chemical composition of sea buckthorn cultivars: Aromatnaja, Botaniczeskaja-Lubitelskaja, Józef, Luczistaja, Moskwiczka, and Podarok Sadu. Analogous or closely related cultivars have been analyzed by other researchers but were cultivated in other climatic and soil conditions, including Sweden, Belarus, Finland, and Canada. However, this is the first report on the new cultivar Józef, bred in Poland.

Dry matter of *H. rhamnoides* berries was statistically different and ranged from 11.78% (Luczistaja) to 13.08% (Aromatnaja). A soluble solid was examined from 5.7 (Luczistaja) to 7.2 °Bx (Moskwiczka and Aromatnaja) and was lower than indicated in berries cultivated in Canada [22] and Finland [23] (to 12.6 °Bx). Sea buckthorn fruits had a low pH (from 2.89 to 2.95 in the case of Moskwiczka and Aromatnaja, respectively) and titratable acidity (from 2.48 to 2.79 g malic acid/100 g fresh weight (FW) for Moskwiczka and Podarok Sadu, respectively). The obtained results were in line with other reports, according to which pH was from 2.30 to 3.20 and titratable acidity from 2.00 to 4.66 g malic acid/100 g [22–24]. Yang [24] stated that pH may be dependent on the harvest period of berries because values increased from late August to mid-October, and then decreased. Additionally, low ash content was determined, from 0.31% (Moskwiczka) to 0.43% (Aromatnaja), compared to data provided by Bal et al. [1], for sea buckthorn berries cultivated in Japan (1.78% and 1.8%). Pectin content was from 0.21% (Józef) to 0.68% (Luczistaja). Aromatnaja berries were characterized by about half the amount of pectins (0.34%) and twice as high vitamin C content (158.81 mg/100 g FW) compared to other examined berries. The new cultivar (cv.) Józef contained a similar amount of vitamin C since the cultivar was the poorest in it (64.92 mg/100 g FW). In studies by Teleszko et al. [12], Aromatnaja fruits were the richest in ascorbic acid (130.97 mg/100 g FW), followed by Podarok Sadu, Moskwiczka, Botaniczeskaja-Lubitelskaja, and Luczistaja (from 82.61 to 52.86 mg/100 g FW). Kawecki et al. [25] reported two to three times more vitamin C content in Podarok Sadu and Botaniczeskaja berries than in our study. Research on sea buckthorn fruits from Germany [26], Finland [23], and Canada [22] reported a similar content of vitamin C as in the studied cultivars, and also proved that the factors determining the vitamin C content are mainly the cultivar, maturity stage, fruit size, and harvest season. Due to the lack of ascorbinase in berries and juice, vitamin C is stable for six days, whereas the annual frozen storage does not change its content [27].

The studied sea buckthorn fruits contained from 1.34 (Botaniczeskaja-Lubitelskaja) to 2.87 g of sugars/100 g FW (Moskwiczka) (Table 1). The most abundant sugar was glucose, which constituted 86.58% to 92.68% of all sugars (in the case of Podarok Sad and Moskwiczka, respectively). Significantly lower concentrations of sorbitol and fructose were determined (maximum 6.36% and 5.88% of total sugars, respectively). Only Luczistaja berries contained more fructose than sorbitol. In Luczistaja, Podarok Sadu, Józef, and Aromatnaja fruits, rhamnose below 3.5% of all sugars was quantitated, while, in other tested fruits, it was not detected. Our results corroborated those published by Kawecki et al. [25], Tiitinen et al. [23], and Zheng et al. [22]. For example, the tested cv.

Podarok Sadu contained 1.49% of sugars, compared to an analogous cultivar grown in Poland, tested by Kawacki et al. [25], and containing 1.99%.

The content of organic acids (Table 1) was from 0.96 to 4.22 g/100 g FW (for Moskwiczka and Luczistaja, respectively). In all cultivars, organic acid concentrations were studied in the following order: malic acid (from 63.11% to 85.42% of acids) > quinic acid (from 6.77% to 32.04% of acids) > isocitric acid (to 15.79% of acids) > citric acid (from 0.32% to 4.44% of acids) > oxalic acid (from 0.32% to 2.08% of acids). The exceptions were cv. Moskwiczka, in which no isocitric acid was detected, cv. Józef, which contained more citric acid than isocitric acid, and cv. Aromatnaja, in which more isocitric acid than quinic acid was identified. Furthermore, maleic and shikimic acids were identified, but were in quantities below 0.01 g/100 g FW (results not shown in the table). The newly studied cv. Józef contained the amount of sugars and acids most similar to the average amounts of sugars and organic acids in the tested sea buckthorn cultivars, 1.93 and 2.23 mg/100 g FW, respectively. Studies on sea buckthorn cultivars grown in Canada, Finland, or Poland indicated the organic acid content from 0.96% to 5.40% and the main acids were malic and then quinic [22–25].

The sugars/organic acids ratio was low and ranged from 0.40 to 2.99 (for Luczistaja and Moskwiczka, respectively). The results point to the need to correct the taste of selected cultivars, in particular those where the ratio was below 1.0, i.e., Botaniczeskaja-Lubitelskaja, Luczistaja, Podarok Sadu, and the new cv. Józef.

3.2. Analysis of Phenolic Compounds of Sea Buckthorn Cultivars

Among the phenolic compounds, phenolic acids and flavonols were quantified in tested sea buckthorn fruits and the results are summarized in Table 1. The total content of phenolic compounds in sea buckthorn berries ranged from 468.60 mg (Luczistaja) to 901.11 mg/100 g dry mass (DM) (Moskwiczka). Other reports indicated comparable phenolic compound values in *H. rhamnoides* berries from 385 to 1442 mg/100 g DM [7,28,29]. The tested Podarok Sadu fruits contained half the amount of phenolic compounds compared to the analogous cultivar collected in Belarus and studied by Zadernowski et al. [29].

The concentration of phenolic acids in studied berries was from 5.18 mg (Botaniczeskaja-Lubitelskaja) to 8.94 mg/100 g DM (Podarok Sadu). The cultivar created in Poland – Józef – contained the same amount of organic acids as Aromatnaja (6.11 mg/100 g DM), and these were richer than Botaniczeskaja-Lubitelskaja and Luczistaja berries. Other reports indicated several times higher content of phenolic acids: from 37.9 mg to 443.92 mg/100 g DM [5,7,29]. Podarok Sadu contained 43 times less phenolic acids than the analogous cultivar tested by Zadernowski et al. [29]. Research by Teleszko et al. [12] determined the phenolic acid concentration from 5.81 mg in Avgustinka to 3.11 mg/100 g FW for Luczistaja berries.

In berries of tested cultivars, approximately 98.94% of the total phenolic compounds were flavonols. Their quantity ranged from 463.14 mg to 893.92 mg/100 g DM, and the order of cultivars in terms of flavonols content was as follows: Moskwiczka > Józef > Aromatnaja > Podarok Sadu > Botaniczeskaja-Lubitelskaja > Luczistaja. Józef berries contained a statistically similar amount of flavonols as Aromatnaja and Podarok Sadu, and these values were higher than the average flavonol content in the studied cultivars (637.53 mg/100 g DM). Teleszko et al. [12] reported that, among cultivars grown in Poland, Botaniczeskaja-Lubitelskaja berries had the lowest flavonol concentration (212.89 mg/100 g FW). The obtained results were in line with those published by Pop et al. [30], where the flavonol content ranged from 563 to 1437 mg rutin equivalent/100 g DM (for Serpenta and Tiberiu, respectively). However, the analyzed berries were richer in flavonols than those examined by Ma et al. [31], who reported a concentration from 23 (Oranzhevaya collected in China) to 250 mg/100 g (wild berries of ssp. *sinensis* from China). Variation in quantitative and qualitative flavonol profile occurs within subspecies and cultivars, and is affected by the harvest date, climatic, genetic, and geographic factors, transport, and storage [32,33].

Table 1. Basic chemical composition, phenolic compounds, carotenoids, tocopherols, and tocotrienols and fatty acids contents in sea buckthorn cultivars.

Components	Aromatnaja	Botaniczeskaja--Lubitelskaja	Józef	Luczistaja	Moskwiczka	Podarok Sadu
Dry matter (%)	13.08 ± 0.23 a	11.88 ± 0.44 b	12.03 ± 0.38 b	11.78 ± 0.19 b	12.84 ± 0.34 ab	12.71 ± 0.40 ab
Soluble solid (°Bx)	7.2 ± 0.01 a	6.4 ± 0.00 a	7.1 ± 0.01 a	5.7 ± 0.01 a	7.2 ± 0.01 a	7.0 ± 0.00 a
pH	2.95 ± 0.01 a	2.90 ± 0.00 a	2.90 ± 0.01 a	2.90 ± 0.02 a	2.89 ± 0.00 a	2.93 ± 0.01 a
Titratable acidity (g malic acid/100 g) FW)	2.44 ± 0.01 c	2.62 ± 0.00 b	2.59 ± 0.08 b	2.71 ± 0.11 ab	2.48 ± 0.03 bc	2.79 ± 0.00 a
Ash (%)	0.43 ± 0.01 a	0.39 ± 0.01 a	0.40 ± 0.02 a	0.35 ± 0.04 a	0.31 ± 0.01 a	0.38 ± 0.05 a
Pectins (%)	0.34 ± 0.04 b	0.67 ± 0.16 a	0.21 ± 0.17 c	0.68 ± 0.18 a	0.64 ± 0.06 a	0.58 ± 0.18 ab
Vitamin C (mg/100 g FW)	158.81 ± 0.78 a	78.52 ± 0.64 b	64.92 ± 1.00 d	80.93 ± 2.32 b	71.32 ± 3.67 c	61.02 ± 0.21 d
Sugars (g/100 g FW)						
Rhamnose	0.05 ± 0.02 b	nd	0.04 ± 0.01 c	0.06 ± 0.00 a	nd	0.04 ± 0.02 c
Fructose	0.05 ± 0.00 d	0.04 ± 0.02 e	0.08 ± 0.02 b	0.10 ± 0.02 a	0.08 ± 0.02 b	0.07 ± 0.01 c
Sorbitol	0.14 ± 0.01 a	0.07 ± 0.00 c	0.11 ± 0.02 b	0.08 ± 0.00 c	0.13 ± 0.04 a	0.09 ± 0.02 bc
Glucose	1.96 ± 0.02 b	1.21 ± 0.02 d	1.73 ± 0.14 bc	1.49 ± 0.28 c	2.66 ± 0.65 a	1.29 ± 0.08 cd
∑ sugars	2.20 ± 0.01 b	1.34 ± 0.03 d	1.96 ± 0.19 c	1.70 ± 0.25 cd	2.87 ± 0.71 a	1.49 ± 0.13 cd
Organic acids (g/100 g FW)						
Oxalic acid	0.01 ± 0.00 b	0.01 ± 0.00 b	0.02 ± 0.00 a	0.02 ± 0.01 a	0.02 ± 0.02 a	0.02 ± 0.00 a
Citric acid	0.05 ± 0.00 b	0.01 ± 0.00 d	0.10 ± 0.05 a	0.03 ± 0.01 c	0.05 ± 0.01 b	0.02 ± 0.00 cd
Isocitric acid	0.21 ± 0.0 a	0.11 ± 0.00 a	0.02 ± 0.00 b	0.17 ± 0.01 a	nd	0.16 ± 0.00 a
Malic acid	0.96 ± 0.07 cd	1.95 ± 0.00 b	1.84 ± 0.09 b	2.87 ± 0.21 a	0.82 ± 0.03 d	1.17 ± 0.13 c
Quinic acid	0.09 ± 0.19 c	0.99 ± 0.03 a	0.27 ± 0.15 b	1.14 ± 0.22 a	0.07 ± 0.01 c	0.16 ± 0.09 c
∑ organic acids	1.33 ± 0.11 d	3.09 ± 0.27 b	2.25 ± 0.20 c	4.22 ± 0.13 a	0.96 ± 0.13 e	1.54 ± 0.14 d
Sugar: organic acid ratio	1.65 b	0.43 d	0.87 c	0.40 d	2.99 a	0.97 c
Phenolic compounds (mg/100 g DM)						
Phenolic acids	6.11 ± 1.98 c	5.18 ± 1.52 d	6.11 ± 1.88 c	5.46 ± 1.07 d	7.19 ± 2.52 b	8.94 ± 2.74 a
Flavonols	655.21 ± 46.16 b	484.22 ± 24.80 c	691.45 ± 56.36 b	463.14 ± 30.48 c	893.92 ± 54.96 a	637.22 ± 42.75 b
∑ phenolic compounds	661.32 ± 48.14 b	491.20 ± 26.71 c	697.56 ± 58.34 b	468.60 ± 31.55 c	901.11 ± 57.48 a	646.16 ± 45.52 b
Carotenoids (mg/100 g DM)						

Xanthophylls	80.73 ± 10.22 a	45.71 ± 4.62 cd	65.04 ± 5.33 b	37.76 ± 4.77 d	51.13 ± 7.18 c	60.27 ± 8.11 b
Carotenes	225.42 ± 12.27 a	16.03 ± 2.93 e	69.78 ± 4.60 c	8.85 ± 1.52 f	56.18 ± 4.27 d	115.62 ± 8.11 b
Esterified carotenoids	202.42 ± 7.02 a	nd	23.11 ± 2.04 c	nd	14.78 ± 1.35 d	70.37 ± 3.20 b
∑ carotenoids	508.57 ± 29.54 a	61.73 ± 7.55 e	157.93 ± 11.97 c	46.61 ± 6.29 e	122.09 ± 12.80 d	246.26 ± 19.42 b
∑ tocopherols and tocotrienols (mg/100 g DM)	27.12 ± 1.31 b	27.68 ± 1.42 b	28.23 ± 1.39 b	34.27 ± 2.00 a	29.29 ± 1.78 b	27.58 ± 1.55 b
			Fatty acids (%)			
Palmitic (C16:0)	34.29 ± 0.01 b	33.24 ± 0.01 b	33.45 ± 0.01 b	32.82 ± 0.01 b	38.19 ± 0.01 a	32.00 ± 0.01 b
Palmitoleic (C16:1 n-7)	25.84 ± 0.02 b	23.67 ± 0.01 b	25.87 ± 0.01 b	31.25 ± 0.01 a	26.40 ± 0.01 b	26.17 ± 0.01 b
Stearic (C18:0)	4.14 ± 0.01 a	3.71 ± 0.01 ab	3.89 ± 0.01 a	2.65 ± 0.01 b	4.49 ± 0.01 a	2.72 ± 0.01 b
Oleic (C18:1 n-9)	14.90 ± 0.01 ab	17.83 ± 0.01 a	15.03 ± 0.01 ab	12.91 ± 0.01 b	14.81 ± 0.01 b	14.49 ± 0.01 b
Linoleic (C18:2 n-6)	17.42 ± 0.03 b	17.60 ± 0.01 b	16.74 ± 0.01 b	16.93 ± 0.01 b	13.16 ± 0.01 c	20.13 ± 0.01 a
Linolenic (C18:3 n-3)	3.43 ± 0.01 b	3.94 ± 0.01 ab	3.40 ± 0.01 b	3.44 ± 0.01 b	2.95 ± 0.01 c	4.49 ± 0.01 a
∑SFAs	38.42 ± 0.04 b	36.95 ± 0.02 c	37.34 ± 0.01 bc	35.47 ± 0.02 c	42.68 ± 0.02 a	34.71 ± 0.01 c
∑MUFAs	40.73 ± 0.05 b	41.50 ± 0.05 b	40.90 ± 0.06 b	44.16 ± 0.04 a	41.21 ± 0.04 b	40.66 ± 0.01 b
∑PUFAs	20.84 ± 0.03 b	21.54 ± 0.04 b	20.14 ± 0.01 b	20.37 ± 0.02 b	16.11 ± 0.01 c	24.62 ± 0.01 a

SFAs – saturated fatty acids. MUFAs - monounsaturated fatty acids. PUFAs - polyunsaturated fatty acids. The data shown are mean values ± SD ($n = 3$). nd - not detectable. DM – dry mass. FW – fresh weight. Different letters (a–d) in the same column denote a significant difference among varieties, according to Tukey's test. $p < 0.05$.

3.3. Analysis of Carotenoids, Tocopherols, and Tocotrienols of Sea Buckthorn Cultivars

Carotenoids were classified as xanthophylls, carotenes, and esterified carotenoids. Their amounts for individual sea buckthorn cultivars are shown in Table 1. The total carotenoid concentration ranged between 46.61 mg and 508.57 mg/100 g DM, respectively, for Luczistaja and Aromatnaja. Józef berries were more than three times richer in carotenoids than Luczistaja and poorer than Aromatnaja. The values obtained were significantly higher than those observed for sea buckthorn fruits from Sweden [11] (from 11.99 mg to 142.49 mg/100 g DM) and growing in Romania [34] (from 53 mg to 97 mg/100 g DM).

Aromatnaja berries contained the most xanthophylls (80.73 mg/100 g DM). Nevertheless, Botaniczeskaja-Lubitelskaja and Luczistaja fruits were characterized by the highest percentage of these compounds with more than 74% of the total carotenoids. Moskwiczka and the new Józef berries contained similar amounts of xanthophylls and carotenes (about 41% of xanthophylls and 45% of carotenes). Fruits of cv. Aromatnaja contained 2 to 25 times more carotenes (225.42 mg/100 g DM) than other cultivars. Therefore, these berries were not yellow-orange but red (Figure 1). The remaining cultivars can be presented according to the increasing content of carotenes: Podarok Sadu > Józef > Moskwiczka > Botaniczeskaja-Lubitelskaja > Luczistaja. By comparison, in *H. rhamnoides* berries grown in Sweden, the carotene content was on average 29.66 mg/100 g DM [11]. On the other hand, research of Kruczek et al. [35] indicated that Aromatnaja and Moskwiczka berries were rich in carotenes (average 23.01 mg/100 g FW), and Botaniczeskaja-Lubitelskaja, Luczistaja, and Podarok Sadu contained less than 1 mg/100 g FW.

Esterified carotenoids were examined in four cultivars in the following order: Aromatnaja > Podarok Sadu > Józef > Moskwiczka. In the case of these berries, esterified carotenoids ranged from 12.11% to 19.80% of total carotenoids (for Moskwiczka and Aromatnaja, respectively). These contents were lower than in studies of Pop et al. [34] and Andersson et al. [11] in which esterified carotenoids accounted for an average of 71% and 55% of total carotenoids, respectively. Research on the sea buckthorn collected from Romania identified mono-esters and diesters of zeaxanthin and lutein esterified with palmitic, myristic, and stearic acid residues [34].

The lipophilic fraction of sea buckthorn berries also contains tocopherols and tocotrienols. However, the quantities determined were low and ranged between 27.12 mg of tocopherols and tocotrienols for Aromatnaja and 34.27 mg/100 g DM for Luczistaja (on average 29.03 mg/100 g DM). Cultivars grown in Sweden contained from 40.6 mg to 80.1 mg/100 g DM [6], and those from Finland contained tocopherols and tocotrienols from similar concentrations to almost four times higher [27].

3.4. Analysis of Fatty Acids of Sea Buckthorn Cultivars

In this research, omega-3, omega-6, omega-7, and omega-9 fatty acids were identified and divided into saturated fatty acids (SFAs) without C=C double bonds, monounsaturated fatty acids (MUFAs) with one such bond, and polyunsaturated fatty acids (PUFAs) with two or more double bonds between two connected carbon atoms. The fatty acid content in berries of sea buckthorn is summarized in Table 1.

Six fatty acids were identified in berries of the studied sea buckthorn cultivars, including two unsaturated acids (palmitic and stearic), two monounsaturated acids (palmitoleic and oleic), and two polyunsaturated acids (linoleic and linolenic). The dominant fatty acid was palmitic acid (C16:0), which ranged from 32.00% (Podarok Sadu) to 38.19% (Moskwiczka) of total fatty acid content. The berries were also abundant in palmitoleic acid (C16:1 n-7). Similar contents of oleic (C18:1 n-9) and linoleic acids (C18:2 n-6) as well as stearic (C18:0) and linolenic acids (C18:3 n-3) were determined. MUFAs dominated in the sea buckthorn berries (from 40.66% to 44.16%), except for cv. Moskwiczka in which saturated acids predominated (SFAs). PUFAs ranged from 16.11% (Moskwiczka) to 24.62% (Podarok Sadu). Generally, the fatty acid profile of the studied cultivars, including the newly bred cv. Józef, can be presented as mean 37.60% SFAs, 41.53% MUFAs, and 20.60% PUFAs.

The results obtained were in line with those given for the analogous Aromatnaja, Botaniczeskaja-Lubitelskaja, Luczistaja, Moskwiczka, and Podarok Sadu, rich in palmitic, palmitoleic, and linoleic

acids (up to 38.25%, 38.51%, and 14.11%, respectively) [12]. Yang and Kallio [36] observed high contents of palmitic, palmitoleic, oleic, and linoleic acids (up to 29.2%, 31.0%, 24.8%, and 33.9%, respectively) in oil from whole berries grown in Finland. In sea buckthorn berries grown in Romania, the main acids were oleic (up to 45.9%) and palmitic acids (up to 40.2%), in contrast with seeds rich in polyunsaturated acids [9,34,37].

Other studies also examined vaccenic acid (C18:1 n-7) in the sea buckthorn fruits in an amount of 4.5% to 9.8%, as well as myristic (C14:0), pentadecanoic (C15:0), hexadecanoic (C16:1 n-9), margaric (C17:0), arachidic (C20:0), and eicosenoic acids (C20:1 n-9) below 1% of the total amount of fatty acids [12,27,37].

3.5. Analysis of Biological Activity of Sea Buckthorn Cultivars: Anti-Oxidant, Anti-α-amylase, Anti-α-glucosidase, Anti-Lipase, and Anti-Lipoxygenase Effects

Table 2 presents anti-oxidant capacity measured by ABTS, FRAP, and ORAC assays in analyzed sea buckthorn fruits. According to all three methods, the highest anti-oxidant potential was observed for Aromatnaja berries, and the lowest for Luczistaja and Botaniczeskaja-Lubitelskaja. In the oxygen radical absorbance capacity (ORAC) test, the results ranged from 15.47 mmol to 34.68 mmol TE/100 g DM. The mean ABTS and FRAP activity values were 1.86 mmol and 2.59 mmol TE/100 g, respectively. Similarly, sea buckthorn berries harvested in China had ORAC activity from 26.6 to 36.9 mmol TE/100 g DM, in Turkestanica and Sinensis subspecies, respectively [7]. In line with Sharma et al. [38], the diversity of activity may result from the method (higher results in DPPH than ABTS test) and extraction because microwave application caused the highest activity of sea buckthorn berries in comparison to maceration, ultrasound, and Soxhlet. In addition, Gao et al. [39] reported that the reduction of ABTS activity during maturation correlated with decreasing concentrations of phenolic compounds and ascorbic acid. The lipid fraction activity increased due to the carotenoid synthesis, but this fraction did not significantly affect the anti-oxidant activity of berries. For example, fractions from ripe Aromatnaja berries had activities equal to 1.30, 0.45, and 0.56 mmol TE/100 g, for the phenolic, ascorbic, and lipophilic fractions, respectively.

Table 2 also shows anti-α-amylase, anti-α-glucosidase, and anti-lipase activity, as IC_{50} (mg/mL). The inhibitory activity against α-amylase ranged from 26.83 mg to 35.12 mg/mL (for Aromatnaja and Józef berries, respectively), while α-glucosidase inhibition was between 41.79 mg and 60.32 mg/mL (Botaniczeskaja-Lubitelskaja and Luczistaja, respectively). In all studied cultivars, α-amylase inhibition was stronger than that of α-glucosidase. In human trials, meals containing sea buckthorn berries reduced and delayed the postprandial insulin response and improved the glycemic profile [40,41]. Moreover, studies of Sharma et al. [38] and Xue et al. [42], carried out on rats and mice with type 2 diabetes, also confirmed the hypoglycemic effect of *H. rhamnoides* fruits. The inhibition toward pancreatic lipase in Aromatnaja and Moskwiczka berries was below 5.00 mg/mL. In the remaining cultivars, activity from 6.07 mg (Józef) to 14.02 mg/mL (Podarok Sadu) was recorded. The positive influence of sea buckthorn on lipid metabolism is confirmed by the examination of Linderborg et al. [43], according to which the addition of berries or their extract residues to meals delayed postprandial lipemia in humans.

The potential effect of sea buckthorn berries in relation to 15-lipoxygenase activity was analyzed, and the results were presented as the percentage of inhibition (at the concentration of 30 mg/mL) (Table 2). High anti-lipoxygenase activity of all cultivars ranged from 92.01% (Moskwiczka) to 100.00% (Aromatnaja and Podarok Sadu). Józef berries inhibited the enzyme activity equal to the others at 94.10%. The studied sea buckthorn berries can constitute a remedy in the concept of prevention and treatment of inflammatory diseases. Therefore, a good proposition is to use these fruits as a major component of functional foods. Zadernowski et al. [44] reported that lipophilic and ethanolic hydrophilic extracts from sea buckthorn decreased lipase activity, but the inhibition of lipoxygenase was higher than that of lipase.

Table 2. Anti-oxidant (mmol TE/100g DM), anti-α-amylase, anti- α-glucosidase, anti-lipase (IC_{50}), and anti-lipoxygenase (percentage of inhibition) activities of sea buckthorn cultivars.

Properties	Aromatnaja	Botaniczeskaja--Lubitelskaja	Józef	Luczistaja	Moskwiczka	Podarok Sadu
			Anti-oxidant activity			
ABTS	3.58 ± 0.36 a	1.27 ± 0.10 d	1.12 ± 0.40 d	1.28 ± 0.01 d	2.22 ± 0.04 b	1.69 ± 0.12 c
FRAP	4.70 ± 0.14 a	1.84 ± 0.17 c	1.98 ± 0.12 c	1.85 ± 0.29 c	2.89 ± 0.09 b	2.29 ± 0.04 bc
ORAC	34.68 ± 2.14 a	18.41 ± 0.79 d	20.04 ± 0.62 c	15.47 ± 2.38 e	28.71 ± 0.41 b	27.30 ± 1.15 b
Enzyme inhibitory activity						
anti-α-amylase	26.83 ± 0.22 a	32.84 ± 0.09 c	35.12 ± 0.11 d	32.93 ± 0.48 c	29.62 ± 0.41 bc	28.49 ± 0.34 b
anti-α-glucosidase	44.45 ± 0.35 ab	41.79 ± 0.42 a	54.76 ± 0.72 c	60.32 ± 0.87 d	46.26 ± 0.31 b	58.89 ± 0.11 cd
anti-lipase	4.55 ± 0.16 a	9.20 ± 0.20 c	6.07 ± 0.19 b	10.07 ± 0.11 d	4.19 ± 0.17 a	14.02 ± 0.10 e
anti-lipoxygenase	100.00 a	92.22 d	94.10 c	97.43 b	92.01 d	100.00 a

The data shown are mean values ± SD (n = 3). α-amylase, α-glucosidase, and lipase inhibition are presented as IC_{50} in mg/mL, and anti-lipoxygenase effect as the percentage of inhibition (at the concentration of 30 mg/mL). DM – dry mass. Different letters (a–d) in the same column denote a significant difference among varieties, according to Tukey's test. $p < 0.05$.

3.6. Pearson's Correlation and Principal Component Analysis (PCA)

Pearson's correlation coefficients (r) between chemical composition and biological activity were determined. According to Rösh et al. [26], the dominant anti-oxidant compound in sea buckthorn is ascorbic acid, which is followed by flavan-3-ols and phenolic acids with catechol structures, in which the tested cultivars were not abundant. High correlations of vitamin C content with ABTS and FRAP anti-oxidant effects (r = 0.864 and 0.886, respectively) and between flavonols and the oxygen radical absorbance capacity (ORAC) (r = 0.617) were found. Carotenoids strongly influenced α-amylase inhibition (r = 0.747), lipoxygenase (r = 0.668), and antioxidant potential measured by ABTS, FRAP, and ORAC methods (mean r = 0.875). Carotenes and esterified carotenoids had a stronger effect on these activities than xanthophylls. SFAs correlated more strongly with the anti-lipase and anti-lipoxygenase potential (r = 0.601 and 0.710, respectively) than with antioxidant activity. Nevertheless, correlations of stearic acid were stronger with anti-oxidant, anti-α-glucosidase, and anti-lipase activity than for palmitic acid. However, oleic acid positively correlated with anti-α-glucosidase potential (r = 0.759). The correlation of linoleic and linolenic acids with the anti-lipoxygenase effect was positively moderate (r = 0.633). This could be due to the LOX function consisting in the catalysis of PUFAs.

Nutritionally, pectin is one of the soluble fiber fractions and may delay gastric emptying, which, in turn, is associated with reducing glycemic response [45]. There was, however, no correlation between pectins and biological activity, including α-amylase inhibition. Weickert and Pfeiffer [46] stated that a diet rich in insoluble dietary fiber in the form of cereals and whole grains may significantly reduce diabetes risk. However, there is no indisputable evidence that soluble dietary fibers from fruits and vegetables play a key role in this process, which may explain our results.

It should not be ruled out that the correlations between biological effects and lipophilic compounds may be apparent or false. The biological activities of sea buckthorn berries were tested on methanol-water solutions. Therefore, correlations for in vitro tests should be performed, with regard to lipophilic and hydrophilic compounds in *H. rhamnoides*. Other research on fruits also proved to be a selective correlation between chemical composition and biological activities. For example, Wang et al. [47] reported that flavonoids isolated from goji berries showed the most pronounced effect in scavenging free radicals (DPPH and ABTS) and chelating metal ions, while the zeaxanthin fraction was a strong scavenger of free hydroxyl radicals. In the studies of Wojdyło et al. [48], figs with a high content of sugars did not correlate with high anti-diabetic activity, but glycosylated derivatives of kaempferol, cyanidin, apigenin, polymeric procyanidins, and (−)-epicatechin correlated with the ability to inhibit α-amylase and α-glucosidase, and with the anti-oxidant effect (for the ORAC test). Ado et al. [49] found that a potent lipase inhibitor was kaempferol-3-*O*-rhamnoside isolated from *C. cauliflora* leaves. According to Stahl and Sies [50], carotenoids are effective anti-oxidants scavenging singlet molecular oxygen and peroxyl radicals. Principal component analysis (PCA) was conducted on the average contents of each chemical component (basic chemical composition, phenolic and carotenoid compounds, fatty acids), biological effects (anti-oxidant as ABTS, FRAP, and ORAC tests, enzyme inhibitory potential for α-amylase, α-glucosidase, lipase, and lipoxygenase), and berries of the tested *H. rhamnoides* cultivars. The outcomes are shown on the PCA biplot (Figure 2). The first two principal components (PC1 and PC2) explained 68.10% of the total variance (41.48% and 26.61%, respectively). The correlation biplot indicated the following: (1) Podarok Sadu berries were rich in PUFAs and had a strong anti-lipoxygenase effect, (2) anti-oxidant and anti-α-amylase activities were strongly correlated with the content of carotenoids and vitamin C, in which Aromatnaja fruits were particularly rich, (3) cv. Moskwiczka contained high concentrations of SFAs, phenolic compounds, and glucose, which, in turn, correlated with anti-lipase and anti-glucosidase activity, (4) Botaniczeskaja-Lubitelskaja, Luczistaja, and the new cv. Józef formed the most extensive berry cluster with high content of organic acids, MUFAs, tocotrienols, and tocopherols.

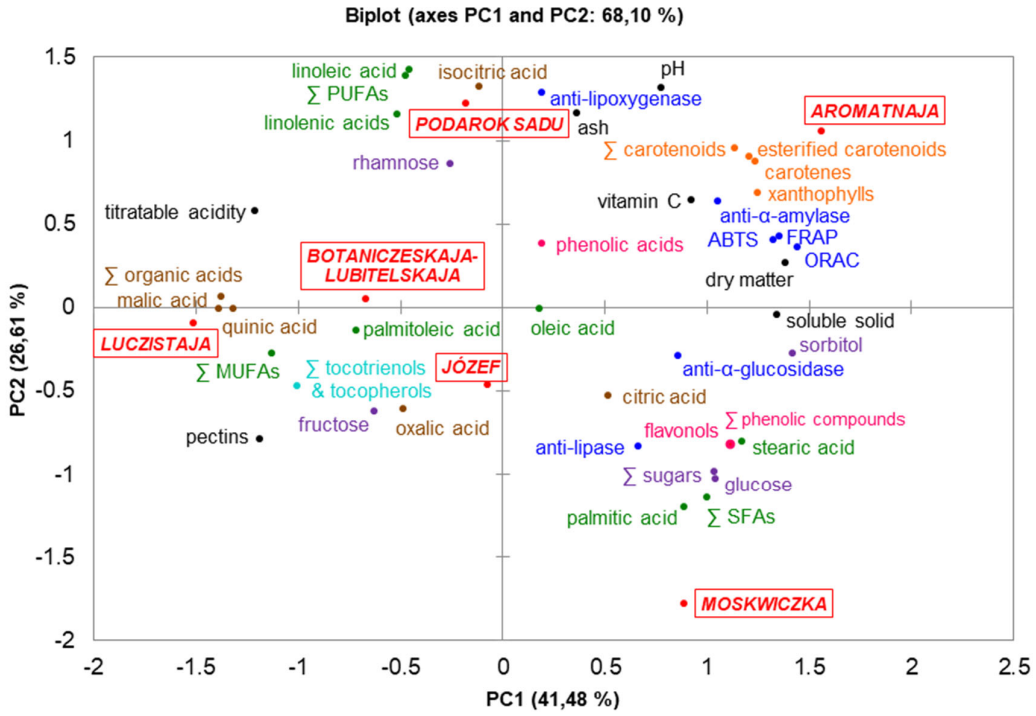

Figure 2. Principal component analysis biplot of chemical composition and biological effects of sea buckthorn cultivars. SFAs – saturated fatty acids. MUFAs - monounsaturated fatty acids. PUFAs - polyunsaturated fatty acids.

4. Conclusions

The study confirmed biochemical and functional differences among six cultivars of sea buckthorn berries cultivated in Poland. In conclusion, the analyzed sea buckthorn cultivars, including cv. Józef (reach in tocopherols and tocotrienols, MUFAs, palmitoleic acid (C16:1 n-7), fructose, quinic and oxcalic acids, and pectin) with biological potency against anti-α-glucosidase and anti-lipase activity, studied for the first time, can be a raw material in the development of innovative functional foods, nutraceuticals, and cosmeceuticals rich in chemical compounds with high biological activity. Research provides valuable information for selecting high-activity cultivars for further cultivation, and may also direct further *in vivo* testing for non-communicable diseases.

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Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, przygotowaniu materiału badawczego, oznaczeniu podstawowego składu chemicznego i związków fenolowych, tokoferoli, tokotrienoli i karotenoidów metodami LC-MS, oznaczeniu potencjału przeciwcukrzycowego, przeciwutleniającego i przeciwzapalnego *in vitro* odmian rokitnika pospolitego. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

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mój udział polegał na współtworzeniu koncepcji i planu badań, pozyskaniu materiału badawczego, uczestnictwie w analizach związków bioaktywnych metodami LC-MS, oznaczeniu potencjału prozdrowotnego *in vitro* odmian rokitnika pospolitego. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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mój udział polegał na uczestnictwie w etapie przygotowania materiału badawczego, oznaczeniach podstawowego składu chemicznego i potencjału prozdrowotnego *in vitro* odmian rokitnika pospolitego.

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mój udział polegał na przeprowadzeniu analizy kwasów tłuszczowych metodą GC-MS, przygotowaniu wyników z tego zakresu oraz merytorycznym współredagowaniu publikacji.

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w analizach potencjału przeciwcukrzycowego, przeciwutleniającego i przeciwzapalnego *in vitro* odmian rokitnika pospolitego oraz współredagowaniu manuskryptu pod względem merytorycznym.

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UPLC-PDA-Q/TOF-MS profiling of phenolic and carotenoid compounds and their influence on anticholinergic potential for AChE and BuChE inhibition and on-line antioxidant activity of selected *Hippophaë rhamnoides* L. cultivars

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ABSTRACT

This study aimed to identify by UPLC-PDA-Q/TOF-MS and quantify by UPLC-PDA phenolic compounds (26 flavonols and 2 phenolic acids) and carotenoids (16) from berries of different cultivars of *Hippophaë rhamnoides* and determine correlations between these variables and *in vitro* anticholinergic and on-line antioxidant potential. Isorhamnetin derivatives presented over 65% of total flavonols, but quercetin and kaempferol derivatives were also determined. Carotenes accounted for 19 to 47%, xanthophylls 16 to 81% of total carotenoids. Pearson's correlations between AChE and BuChE inhibition and phenolic acid content were low (r = 0.388 and 0.355), moderate for carotenoids (0.504 and 0.437) and high for flavonols (0.851 and 0.614). The PCA biplot showed the highest correlation between anticholinergic activity and all-*trans*-ß-cryptoxanthin, quercetin-3-*O*-glucoside, isorhamnetin-3-*O*-(2-rhamnosyl)glucoside, kaempferol-3-*O*-hexoside-7-*O*-rhamnoside, isorhamnetin-3-*O*-(6-rhamnosyl)hexoside, isorhamnetin-3-*O*-rutinoside, and isorhamnetin-3-*O*-glucoside concentrations. The results obtained can be used to identify sea buckthorn cultivars, develop crops and production, and design functional products rich in flavonols and carotenoids with anticholinergic properties.

1. Introduction

Rich sources of flavonols include onions, broccoli, kale, red grapes, apples, tea, and berries (Panche, Diwan, & Chandra, 2016; Testa, Bonfigli, Genovese, De Nigris, & Cariello, 2016). But previous studies also confirmed the high concentrations of these compounds in sea buckthorn fruits (Guo, Guo, Li, Fu, & Liu, 2017; Ma et al., 2016; Pop et al., 2013; Teleszko, Wojdyło, Rudzińska, Oszmiański, & Golis, 2015; Zheng, Kallio, & Yang, 2016). Sea buckthorn (*Hippophaë rhamnoides* L.) belongs to the *Elaeagnaceae* olive family and is a spiny fruiting and deciduous shrub. In some countries, inter alia, Germany, Finland, Czech Republic, Ukraine, Estonia, Belarus, Latvia, Russia, China, Japan, Slovakia, Chile, and Canada, sea buckthorn is used on an industrial scale (Rafalska, Abramowicz, & Krauze, 2017; Ruan, Rumpunen, & Nybom, 2013). The global area of wild and grown sea buckthorn is estimated at about 3.0 mln ha, of which about 85% is in China, followed by Mongolia, India and Pakistan. In China, this area is successively expanded by about 10,000 ha per year. Global yields of sea buckthorn fruits are not established due to the use of shrubs not only for the production of berries, but also for firewood, planting for soil remediation and harvesting methods. In Poland and other European countries, the average yield from industrial crops is about 4 t/ha/year, using harvest with cutting whole shoots with berries. For example, the crop obtained from one shrub of cv. Podarok Sadu is within a range of 12.5–20.5 kg of fruits (Piłat, Bieniek, & Zadernowski, 2015; Ruan et al., 2013). According to the International Sea Buckthorn Association (ISA), juices, beverages, tea, oils, jams, snacks, liquor, food supplements, cosmetics and feeding stuff are produced from sea buckthorn fruits.

Sea buckthorn berries are spherical or oval, shiny, the color of the skin and flesh varying from yellow to red. This characteristic coloration is caused by the high content of carotenoid compounds with at least 7 conjugated double bonds (Andersson, Olsson, Johansson, & Rumpunen, 2009; Pop et al., 2014). Nevertheless, the primary sources of carotenoids in the human diet are yellow and orange vegetables and fruits.

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Carrots, sweet potatoes, pumpkins, peppers, and apricots contain high concentrations of β-carotene; papayas and citruses are rich in α-cryptoxanthin and zeinoxanthin; tomatoes contain lycopene; and lutein, violaxanthin and neoxanthin are present in green vegetables – kale, spinach and broccoli (Saini, Nile, & Park, 2015). Sea buckthorn berries may contain high concentrations of most carotenoids mentioned above, which makes them unique (Andersson et al., 2009; Pop et al., 2014).

Application of sea buckthorn for therapeutic purposes is derived from Tibetan and Mongolian medicine and ancient Greece, but healthful properties of compounds from this plant are currently being researched (Piłat et al., 2015; Rafalska et al., 2017). Studies conducted on cardiac patients proved that flavonoids from sea buckthorn contributed to lowering total cholesterol, triacylglycerides and the low-density lipoprotein (LDL) fraction and increasing the high-density lipoprotein (HDL) fraction (Rafalska et al., 2017). Guo, Yang, Cai, & Li (2017) reported that the cardioprotective effect of sea buckthorn intake can be attributed to its content of flavonoids and *ß*-sitosterol. Flavonol extracts from sea buckthorn berries reduced levels of serum glucose, serum triglyceride and serum cholesterol in mice, and effects on glycometabolism may be associated with glycogenesis (Cao et al., 2003), whereas Yohendra Kumar, Tirpude, Maheshwari, Bansal, and Misra (2013) investigated the high inhibitory activity of the phenolic fraction of *H. rhamnoides* towards *E. coli*, *S. typhi*, *S. dysenteriae*, *S. pneumoniae* and *S. aureus*. Furthermore, sea buckthorn oil with a high concentration of carotenoids may be beneficial in the treatment and prevention of atherosclerotic artery diseases, because it effectively inhibits platelet aggregation (Xu, Kaur, Dhillon, Tappia, & Dhalla, 2011). Chew et al. (2014) reported that increased oral intake of lutein and zeaxanthin may be more potent than *ß*-carotene in reducing the risk of age-related macular degeneration.

Currently Poland is becoming one of the major countries in Europe interested in growing sea buckthorn on an industrial scale. So far, analysis of phenolic and carotenoid compounds in reference to anticholinergic activity in sea buckthorn has not been carried out. In this context, the first aim of this paper was detailed identification and quantification of phenolic and carotenoid compounds from berries of selected *H. rhamnoides* cultivars grown in Poland. The second objective was to assess the anticholinergic activity of berries and to determine the correlations between the composition and pro-health potential, as well as to establish the activity of the phenolic fraction in relation to the $ABTS^{\cdot+}$ reagent by on-line HPLC-PDA analysis.

2. Materials and methods

2.1. Chemicals

The chemicals required to carry out anticholinergic activity analysis, antioxidant on-line profiling with post-column derivatization with $ABTS^{\cdot+}$, and solvents for LC/MS grade were acquired from Sigma-Aldrich (Steinheim, Germany). Acetonitrile for ultraperformance liquid chromatography (UPLC; gradient grade) and ascorbic acid were bought from Merck (Darmstadt, Germany). The standards of phenolic and carotenoid compounds were bought from Extrasynthese (Genay, France).

2.2. Plant materials

Six sea buckthorn (*Hippophae rhamnoides* L.) cultivars: Botaniczeskaja-Lubitelskaja, Luczistaja, Moskwiczka, Podarok Sadu, Józef, and Aromatnaja were analyzed. Mature raw fruits were collected between July and August 2018 from orchard located in Dąbrowice (51°56′N 20°06′E) of Research Institute of Horticulture (Skierniewice, Poland). General samples were washed with cold water, frozen at 80 °C, freeze-dried for 24 h (Alpha 1–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH; Osterode am Harz, Germany) and milled (IKA, A11 basic analytical mill; Darmstadt, Germany). The homogeneous laboratory samples were subjected to extraction.

2.3. Identification and quantification of phenolic compounds

For identification and quantification of phenolic compounds by UPLC-PDA-Q/TOF-MS and UPLC-PDA methods, the extraction protocols were the same as that applied by Tkacz, Wojdyło, Nowicka, Turkiewicz, and Golis (2019). Briefly, the sea buckthorn samples (~0.50 g) were mixed with 5 mL methanol:water (3:7, v/v) with 2% ascorbic acid and 1% acetic acid, and sonicated (Sonic-6D, Polsonic; Warsaw, Poland). The extraction process was repeated after storage for 24 h at 4 °C, and then, the samples were centrifuged (19,000*g*, 10 min, 4 °C; MPW-350; Warsaw, Poland). Supernatants were filtered through a hydrophilic membrane (PTFE, 0.20 μm; Millex Samplicity Filter, Merck; Germany) and used for analysis.

The analysis of phenolic compounds was carried out using an ACQUITY Ultra Performance Liquid Chromatography system (Waters Corporation; Milford, MA, USA) with a binary solvent manager and PDA detector coupled to G2 Q/TOF micro-mass spectrometer (Waters; Manchester, UK) fitted with an electrospray ionization ESI source acting on negative and positive modes. The parameters of the UPLC-PDA-Q/TOF-MS analysis were analogous to that explained previously by Wojdyło, Nowicka, and Bąbelewski (2018). The UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters Corporation; Milford, MA, USA) was kept at 30 °C. The injection volume was 5 μL, and the elution was completed in 30 min with a flow rate of 0.420 mL/min. Solvent A (2.0% formic acid) and solvent B (100% acetonitryle) were used in the following gradients: elution start with 98.0% A; next solvent A reduction to 65% (to 32.00 min), and to 0% (to 33.00 min); 98% A from 33.50 to 35.00 min to re-equilibrate the column. The PDA spectra for phenolic acids and flavonols were measured at 320 and 360 nm, respectively. The optimized MS parameters were as follows: source temperature of 100 °C, desolvation temperature of 300 °C, cone gas flow 40 L/h, desolvation gas flow 300 L/h, capillary voltage of 2500 V, and cone voltage of 30 V. The MS analysis was performed using a mass scanning from *m/z* 100 to 1200. Empower 3 software and MassLynx 4.0 ChromaLynx Application Manager software were used to develop quantitative and qualitative data. Quantification was made by injection of solutions of known concentrations ranging between 0.05 and 5 mg/mL ($R2 \leq 0.9998$) of coumaric and ferulic acids, isorhamnetin, quercetin, and kaempferol-3-*O*-rutinoside, -glucoside, and -galactoside, as standards. The remaining flavonol derivatives were expressed as the corresponding 3-*O*-glucoside derivatives. The results of ULPC-PDA analyses were reported as the average of three replicates and expressed as mg per 100 g of dry matter (dm).

2.4. Identification and quantification of carotenoids

For identification and quantification of carotenoids by UPLC-PDA-Q/TOF-MS and UPLC-PDA assays, the extraction and all parameters were the same as that given by Wojdyło et al. (2018). The sea buckthorn samples (~0.20 g) enriched with 10% $MgCO_3$ were shaken in the dark, with 5 mL hexane:acetone:methanol (2:1:1, v/v/v) with 1% BHT (300 rpm, 30 min; DOS-10L Digital Orbital Shaker, ELMI; Riga, Latvia). Then, the samples were centrifuged (as in subsection 2.3), supernatants were collected, and the extraction procedure was repeated two more times. The combined supernatants were evaporated to dryness. The residues were dissolved in 2 mL of 100% methanol, filtered through a hydrophilic 0.20 μm membrane (as before) and used for analysis.

The ACQUITY UPLC system and software were used as previously (subsection 2.3). A ACQUITY UPLC BEH RP C18 column (2.1mmx 100 mm, 1.7 μm; Waters Corporation; Milford, MA, USA) with a C18 guard column were maintained at 32 °C. The injection volume of sea buckthorn extract was 10 μL. The mobile phase consisted and 0.1% formic acid (solvent A) and acetonitrile:methanol (7:3, v/v) (solvent B). A gradient with a flow rate of 0.500 mL/min was used: 25% A

0–0.60 min; 4.9% A to 6.50 min; 0% A to 13.60 min; 25% A to 16.60 min. The detection wavelength for carotenoid compounds was 450 nm. The MS parameters were as in subsection 2.3, except for desolvation gas flow equal 350 L/h. The MS analysis was performed using a mass scanning from *m/z* 200 to 1100. The retention times and UV spectra were compared to authentic standards of lutein, zeaxanthin, ß-cryptoxanthin, lycopene, zeaxanthin dipalmitate, and ß-carotene. Quantification was performed on the basis of standard curves constructed similarly as above. The results of ULPC-PDA studies were shown as the average of three replicates and as mg per 100 g dm.

2.5. Determination of anticholinergic activity as AChE and BuChE inhibition

The extraction procedure for this analysis was the same as that reported by Turkiewicz, Wojdyło, Tkacz, Nowicka, and Hernández (2019). The anticholinergic activity was examined as acetylcholinesterase (AChE) and butylcholinesterase (BuChE) inhibition methods reported by Wojdyło et al. (2018). The results were expressed as IC_{50} (mg of dried sample/ml) and % of inhibition. Tests were performed using multi-mode microplate reader Synergy™ H1 (BioTek; Winooski, Vermont, U.S.).

2.6. Antioxidant on-line profiling by HPLC-PDA coupled with post-column derivatization with $ABTS^{\cdot+}$ reagent

The antioxidant activity of individual phenolic peaks was studied using an on-line HPLC antioxidant detector system. The procedure and conditions of this assay were the same as reported by Tkacz et al. (2019). Briefly, a Cadenza CD-C18 column (75 mm × 4.6 mm, 3 µm; Tokyo, Japan) being protected by C18 guard column was operated at 30 °C. The injection volume of sea buckthorn extract was 10 µL. The solvents and gradient profile were analogous to those in Section 2.3. A flow rate was 0.600 mL/min. The detection wavelength for phenolic compounds was set at 360 nm. The mobile phase after passing through the PDA detector was mixed with the $ABTS^{\cdot+}$ solution (a flow rate was 0.2 mL/min). The mixture flowed through reaction coil (PTFE; 40°C; 25 m long, 0.25 mm I.D.) to the UV detector. Decolorisation of the HPLC eluate and $ABTS^{\cdot+}$ solution was monitored as a negative peaks at 734 nm. The results are presented in the form of chromatograms.

2.7. Statistical analysis

One-way analysis of variance (ANOVA; $p < 0.05$) and Tukey's HSD test were carried out using Statistica 13.1 (StatSoft; Cracow, Poland). The statistical software XLSTAT for Microsoft Excel 2010 was used to perform the Principal Component Analysis (PCA) and determine Pearson's correlation coefficients.

3. Results and discussion

3.1. Identification of phenolic compounds of H. rhamnoides

Liquid chromatography–mass spectrometry–photodiode array–quadrupole time-of-flight (UPLC-PDA-Q/TOF-MS) analysis was conducted for extracts of six selected sea buckthorn cultivars and 28 phenolic compounds were tentatively identified, including two phenolic acids, and the rest were flavonol derivatives. The compounds were tested at negative ionization and at 320 and 360 nm, respectively for phenolic acids and flavonols. The identification was made on the basis of reference standards, as well as MS fragmentation, the UV spectrum, and literature data (tentative identification) (Ferreres et al., 2017; Ma et al., 2016; Pop et al., 2013; Rösch, Krumbein, Mügge, & Kroh, 2004; Zheng et al., 2016). The results are presented in Table 1 and Fig. 1 in the example of Podarok Sadu berries.

In all cultivars, two hydroxycinnamic acid derivatives were tentatively identified: *p*-coumaric acid-*O*-hexoside (compound **1**) and ferulic acid-*O*-hexoside (compound **2**). UV spectrum analysis of both compounds presented the absorption typical of these phenolic acids: peak **1** (rt = 2.871 min) showed absorption bands at 312 nm and 284 nm, and peak **2** (rt = 3.753 min) at 323 nm and 251 nm. The major fragment ion at *m/z* 163.04 corresponded to *p*-coumaric acid and *m/z* 193.02 was specific to ferulic acid.

The study tentatively identified 26 flavonol derivatives, including eight derivatives of quercetin (compounds **3**, **6**, **8–10**, **13**, **15**, **23**), 15 isorhamnetin derivatives (compounds **4**, **5**, **7**, **11**, **12**, **14**, **16**, **17**, **19–22**, **25–27**), and aglycon (compound **28**) and two derivatives of kaempferol (compound **18** and **24**). For all flavonols, the characteristic wavelengths at band I (348–370 nm) and band II (248–266 nm) were observed (Chen, Zhang, Xiao, Yong, & Bai, 2007). The flavonols were detected at mass-to-charge ratio *m/z* equal to 301.03 for quercetin, 315.05 for isorhamnetin, and 285.00 in the case of kaempferol. The study tentatively identified flavonol derivatives substituted at position 3 (13 compounds) and position 7 as preferential glycosylation position (10 compounds). The main structures of flavonol glycosides were *O*-rutinosyl, -*O*-glucosyl, -*O*-sophorosyl, and -*O*-rhamnosyl, as was indicated in other research on sea buckthorn (Guo, Guo et al., 2017; Rösch et al., 2004; Zheng et al., 2016).

Compound **3** (rt = 2.407 min and $[M-H]^-$ at *m/z* 771.20) was tentatively identified as quercetin-3-*O*-sophoroside-7-*O*-rhamnoside due to the loss of 324 Da indicating sophorose. But according to the specificity of the disaccharides linked at position 3, this fragment can also be considered a combination of two hexosyls: hexosyl(1 → 2) hexoside. Hence, this compound would be quercetin-3-*O*-(2-hexosyl) hexoside-7-*O*-rhamnoside. An analogous situation can be concluded for compound **7** isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside (rt = 5.164 min and $[M-H]^-$ at *m/z* 785.20), which is the isomer of compound **3** differing in the basic flavonol ring. Similarly, for peak **5** (rt = 4.213 min and $[M-H]^-$ at *m/z* 785.20), with the loss of 308 Da (rutinoside), two compounds can be labeled as isorhamnetin-3-*O*-rutinoside-7-*O*-glucoside and isorhamnetin-3-*O*-(6-rhamnosyl)glucoside-7-*O*-glucoside. The variants of creating flavonol patterns were described in detail by Ferreres et al. (2017) in a high-performance liquid chromatography–diode array detector–electrospray ionization–mass spectrometry (HPLC-DAD-ESI/MS^n) study on *Lathyrus cicera* seeds. In reports specifically on sea buckthorn berries, a series of flavonol combinations with sophorosides and rutinosides were given (Ma et al., 2016; Pop et al., 2013; Rösch et al., 2004; Teleszko et al., 2015; Zheng et al., 2016).

The loss of 162 Da points to hexose, and according to previous research on sea buckthorn extracts, glucose was the main sugar on the molecules of flavonol glycosides (Ma et al., 2016; Pop et al., 2013; Rösch et al., 2004). Thus, compound **4** isorhamnetin-3,7-*O*-dihexoside (rt = 3.188 min and $[M-H]^-$ at *m/z* 639.15) can be tentatively recognized as isorhamnetin-3,7-*O*-diglucoside and equally for compounds **8**, **11**, **16**, **18**, **19**.

Compound **6** (rt = 4.502 min and $[M-H]^-$ at *m/z* 771.20) lost −162/−180 Da, which is due to the loss of 18 Da (H_2O) and indicates a direct link of sugar residues. This compound should therefore be considered as quercetin-3-*O*-(2-rutinosyl)glucoside and such a structure has not yet been identified in *H. rhamnoides*.

Analyzing compound **8** (rt = 6.678 min), MS/MS fragmentation *m/z* 755.20 → 609.14 resulted in loss of 146 Da (rhamnosyl) and then led to *m/z* 301.03 by loss of 308 Da (rhamnosyl + hydroxyl). Additionally, the link in position 6 is more stable than in position 2 and, therefore, during fragmentation there was an internal rupture of hexose linked with rhamnose at position 6 and the loss of 120 Da. The confirmation is the loss of 266 Da (*m/z* 755.20 → 489.11) (Ferreres et al., 2017). Consequently, this peak was tentatively identified as quercetin-3-*O*-(2,6-dirhamnosyl)hexoside. Compounds **8** and **12** (rt = 9.374 min and $[M-H]^-$ at *m/z* 769.21) belong to dirhamnosylglucosides (triglycosides) and are isomers with two different flavonols – quercetin and

Table 1
UPLC-PDA-Q/TOF-MS data of phenolic acids and flavonols identification and their quantification in *H. rhamnoides* cultivars.

Peak no.	Rt (min)	λ_{max} (nm)	MS $[M-H]^-$ (*m/z*)	MS/MS $[M-H]^-$ (*m/z*)	Phenolic content (mg/100 g dm)					
					Botaniczeskaja-Lubitelskaja	Luczistaja	Moskwiczka	Podarok Sadu	Józef	Aromatnaja
Phenollic acids										
1	2.871	284/312	325.09	163.04/119.05	2.84 ± 0.64	2.88 ± 0.32	4.19 ± 1.43	5.50 ± 1.24	3.12 ± 0.95	4.26 ± 1.06
2	3.753	251/323	401.14	193.02	2.34 ± 0.88	2.58 ± 0.75	3.00 ± 1.09	3.44 ± 1.50	2.99 ± 1.03	2.72 ± 0.85
Flavonols										
3	2.407	258/356	771.20	625.20/301.03	7.28 ± 0.21	2.83 ± 0.22	8.16 ± 1.00	4.92 ± 0.51	5.22 ± 0.71	2.76 ± 0.28
4	3.188	254/356	639.15	477.10/315.05	5.35 ± 0.18	9.59 ± 1.11	12.1 ± 2.1	13.1 ± 1.4	10.5 ± 2.5	11.1 ± 1.8
5	4.213	256/352	785.20	623.16/315.03	7.03 ± 0.14	4.77 ± 0.14	16.8 ± 2.4	8.53 ± 1.27	5.06 ± 0.84	5.59 ± 0.73
6	4.502	258/352	771.20	609.14/591.20/301.03	6.30 ± 0.38	4.56 ± 0.27	11.4 ± 1.8	7.05 ± 1.08	8.13 ± 1.79	6.72 ± 1.00
7	5.164	256/355	785.20	639.15/315.05	21.4 ± 1.1	23.7 ± 2.1	41.4 ± 3.6	44.5 ± 3.5	40.8 ± 3.0	38.1 ± 2.7
8	6.678	254/352	755.20	625.20/609.14/489.11/301.03	26.1 ± 0.9	16.2 ± 2.0	29.7 ± 2.3	22.1 ± 1.9	24.4 ± 1.5	22.2 ± 1.4
9	7.716	258/354	609.14	447.04/301.03	1.02 ± 0.02	1.14 ± 0.11	0.992 ± 0.314	1.20 ± 0.23	1.10 ± 0.43	1.07 ± 0.55
10	7.908	256/356	609.14	447.04/301.03	6.32 ± 0.17	1.62 ± 0.05	5.07 ± 0.89	3.64 ± 2.02	3.19 ± 1.53	5.29 ± 0.91
11	8.961	248/336	977.31	831.18/771.20/639.03/625.20/445.12/315.05	1.34 ± 0.38	1.22 ± 0.43	1.93 ± 0.57	1.57 ± 0.53	1.04 ± 0.81	1.83 ± 0.40
12	9.374	254/356	769.21	623.16/605.09/503.22/315.05	2.31 ± 0.65	3.89 ± 0.76	3.53 ± 0.43	2.95 ± 1.34	4.09 ± 1.17	4.69 ± 1.74
13	9.758	260/354	609.15	301.03	43.0 ± 1.0	38.5 ± 2.1	67.9 ± 4.1	40.4 ± 2.0	54.4 ± 4.3	51.9 ± 3.0
14	10.200	254/352	623.16	477.10/315.05	40.4 ± 1.0	37.4 ± 4.0	83.6 ± 5.0	52.8 ± 3.4	86.2 ± 4.6	45.7 ± 2.8
15	10.776	256/354	463.08	301.03	62.7 ± 3.0	68.7 ± 3.0	98.3 ± 5.3	81.2 ± 4.7	79.1 ± 4.2	88.2 ± 4.5
16	11.668	255/353	623.16	461.01/443.04/315.05	17.2 ± 1.9	12.4 ± 1.1	24.7 ± 2.6	16.5 ± 1.1	22.7 ± 3.0	32.7 ± 3.6
17	12.297	254/360	593.04	447.04/315.05	2.01 ± 0.61	2.13 ± 0.54	1.98 ± 0.61	2.18 ± 0.26	2.03 ± 0.45	2.42 ± 0.11
18	14.057	265/348	593.04	447.04/285.00	6.25 ± 0.47	9.01 ± 0.76	19.6 ± 1.0	13.3 ± 1.6	14.1 ± 3.3	14.4 ± 2.4
19	14.555	257/348	623.16	461.01/315.05	3.88 ± 0.57	4.09 ± 1.62	5.43 ± 0.67	4.51 ± 1.33	4.79 ± 1.28	4.82 ± 1.27
20	15.036	255/354	623.16	315.05	96.4 ± 3.4	102 ± 4	228 ± 9	129 ± 4	172 ± 6	160 ± 6
21	15.575	255/353	477.10	315.05	79.4 ± 3.1	64.1 ± 2.1	208 ± 9	119 ± 3	105 ± 4	122 ± 6
22	19.960	254/350	447.04	315.05	2.66 ± 0.96	2.00 ± 0.05	nd	2.82 ± 0.84	2.75 ± 0.93	2.60 ± 0.94
23	22.256	256/366	447.04	301.03	3.69 ± 1.84	2.06 ± 0.14	4.95 ± 0.78	6.00 ± 0.05	3.72 ± 1.14	4.52 ± 1.37
24	24.768	265/352	593.04	285.00	3.72 ± 0.07	4.49 ± 1.53	1.02 ± 0.05	1.05 ± 0.35	3.01 ± 1.10	2.55 ± 0.16
25	25.567	256/368	461.01	315.05	2.02 ± 0.85	1.99 ± 0.77	nd	6.91 ± 1.63	2.09 ± 0.78	1.31 ± 0.05
26	27.007	260/354	707.21	545.16/477.10/315.05	5.95 ± 0.10	9.87 ± 1.04	2.71 ± 0.17	1.20 ± 0.84	2.74 ± 1.65	0.712 ± 0.113
27	27.217	256/358	707.21	545.16/477.10/315.05	25.8 ± 1.2	29.3 ± 2.4	15.6 ± 1.3	44.5 ± 2.2	30.3 ± 3.9	21.0 ± 2.0
28	28.158	256/370	315.05	301.01	4.69 ± 0.55	4.83 ± 0.84	0.743 ± 0.041	5.46 ± 1.60	3.28 ± 1.51	1.45 ± 0.23

Identification of peak numbers: (**1**) *p*-Coumaric acid-*O*-hexoside; (**2**) Ferulic acid-*O*-hexoside; (**3**) Quercetin-3-*O*-sophoroside-7-*O*-rhamnoside; (**4**) Isorhamnetin-3,7-*O*-dihexoside; (**5**) Isorhamnetin-3-*O*-rutinoside-7-*O*-glucoside; (**6**) Quercetin-3-*O*-(2-rutinosyl)glucoside; (**7**) Isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside; (**8**) Quercetin-3-*O*-(2,6-dirhamnosyl)hexoside; (**9**) Quercetin-3-*O*-galactoside-7-*O*-rhamnoside; (**10**) Quercetin-3-*O*-glucoside-7-*O*-rhamnoside; (**11**) Isorhamnetin-3-*O*-hydroxyferuloyl-glucosyl-glucoside-7-*O*-rhamnoside; (**12**) Isorhamnetin-3-*O*-(2,6-dirhamnosyl)glucoside; (**13**) Quercetin-3-*O*-rutinoside; (**14**) Isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside; (**15**) Quercetin-3-*O*-glucoside; (**16**) Isorhamnetin-3-*O*-(2-rhamnosyl)hexoside; (**17**) Isorhamnetin-3-*O*-pentoside-7-*O*-rhamnoside; (**18**) Kaempferol-3-*O*-hexoside-7-*O*-rhamnoside; (**19**) Isorhamnetin-3-*O*-(6-rhamnosyl)hexoside; (**20**) Isorhamnetin-3-*O*-rutinoside; (**21**) Isorhamnetin-3-*O*-glucoside; (**22**) Isorhamnetin-3-*O*-pentoside; (**23**) Quercetin-3-*O*-rhamnoside; (**24**) Kaempferol-3-*O*-rutinoside; (**25**) Isorhamnetin-3-*O*-rhamnoside; (**26**) Derivative of isorhamnetin I; (**27**) Derivative of isorhamnetin II; (**28**) Isorhamnetin.
The data shown are mean values ± SD (n = 3); nd - not detectable.

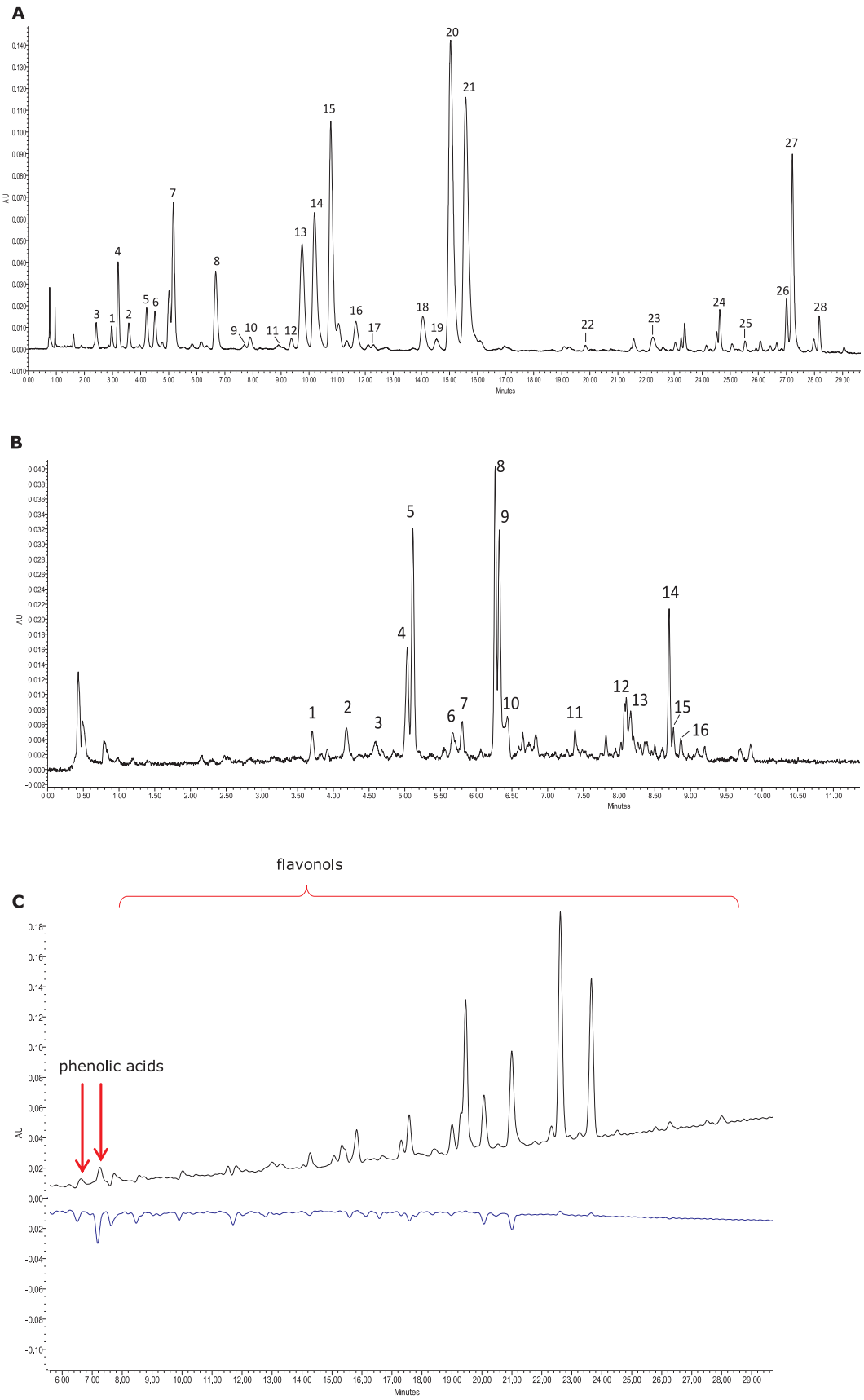

Fig. 1. UPLC-PDA chromatogram of phenolic compounds at 360 nm (**A**) and carotenoids at 450 nm of Podarok Sadu (**B**) and chromatographic profile HPLC-PDA obtained before and after the derivatization using the $ABTS^{\cdot+}$ reagent for Botaniczeskaja-Lubitelskaja (**C**). The identification of peak numbers is given in Table 1 for phenolic compounds (A) and in Table 2 for carotenoids (B).

isorhamnetin, respectively. Also, despite the most common glucose substitution at positions 2 and 6, rhamnosyl substituents may also be linked at positions 3/4 and 6. Hence these compounds may be called quercetin-3-*O*-(3/4,6-dirhamnosyl)hexoside and isorhamnetin-3-*O*-(3/4,6-dirhamnosyl)hexoside. Rösh et al. (2004) reported the presence of quercetin dirhamnosylglucoside; however, this combination of sugars with isorhamnetin was first tentatively identified.

Compounds **9** and **10** (rt = 7.716 and 7.908 min, respectively) had the same MS/MS fragmentation $[M-H-162-146]^-$ yielding an ion at *m/z* 301.03. Because of the elution order of sugar residues, peaks were recognized as quercetin-3-*O*-galactoside-7-*O*-rhamnoside and quercetin-3-*O*-glucoside-7-*O*-rhamnoside (Wojdyło & Nowicka, 2019). Flavonol **11** (rt = 8.961 min and $[M-H]^-$ at *m/z* 977.31) was acylated with hydroxycinnamic acid derivative which results from shifted bands of spectra at 248 and 336 nm (Ma et al., 2016) and MS/MS fragmentation which corresponded with the loss of hydroxyferulic acid (192 Da), 2 molecules of glucose (162 Da) and rhamnose (146 Da). As in the study of Rösh et al. (2004), this compound was considered to be isorhamnetin-3-*O*-hydroxyferuloyl-glucosyl-glucoside-7-*O*-rhamnoside. This research group also tentatively identified derivatives with coumaric, caffeic and hydroxyvanillic acids but our research, like the studies of Ma et al. (2016), Pop et al. (2013), and Zheng et al. (2016), did not provide such results.

Compound **13** (rt = 9.758 min and $[M-H]^-$ at *m/z* 609.15) had identical mass as flavonols **9** and **10**. However, its fragmentation caused a direct loss of 308 Da (rutinoside) yielding an ion at *m/z* 301.03, whereas the fragmentation of compounds **9** and **10** was gradual and led to ions at *m/z* 447.04 and then *m/z* 301.03. Quercetin-3-*O*-glucoside-7-*O*-rhamnoside (**10**) and quercetin-3-*O*-rutinoside (**13**) were commonly present in sea buckthorn extracts examined by Ma et al. (2016), Pop et al. (2013), Rösch et al. (2004), and Zheng et al. (2016). In all LC analyses using the silica-based stationary phases, as well as in our work, quercetin-3-*O*-glucoside-7-*O*-rhamnoside eluted before quercetin-3-*O*-rutinoside. Compound **14** (rt = 10.200 min and $[M-H]^-$ at *m/z* 623.16) lost rhamnose at position 7 in the first step of fragmentation, yielding ions at *m/z* 477.10, and then glucose at position 3 $[M-H-146-162]^-$. Thus, this compound was tentatively identified as isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside and is an isomer of compound **10**.

In further retention times from 9.758 to 26.067 min, 8 flavonol monoglycosides were tentatively identified. MS/MS fragmentation demonstrated that compounds **13**, **15** and **23** were the precursors of quercetin ($[M-H]^-$ at *m/z* 301.03), peaks **20–22** and **25** corresponded to isorhamnetin ($[M-H]^-$ at *m/z* 315.05), whilst compound **24** was a derivative of kaempferol ($[M-H]^-$ at *m/z* 285.00). Compounds **15** and **21** were identified based on reference standards, as quercetin-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside, respectively. MS/MS fragmentation *m/z* 463.08 → 301.01 and *m/z* 477.10 → 315.05 (peaks **15** and **21**, respectively) caused the loss of hexose units (162 Da). Furthermore, the LC-MS analysis proved the formation of a dimer with a proton of isorhamnetin-3-*O*-glucoside (compound **21**), yielding an ion at *m/z* 955.

Peaks **16**, **19**, and **20** (rt = 11.668, 14.555 and 15.036 min, respectively) had pseudomolecular ions at *m/z* 623.26 and were tentatively identified as isorhamnetin derivatives considering the loss of 315.05 Da. MS/MS fragmentation of compound **20** explicitly indicated a loss of rutinoside at position 3 $[M-H-308]^-$; hence the peak was identified as isorhamnetin-3-*O*-rutinoside, also based on the reference standard. Referring to compounds **16** and **19**, subsequent fragmentation steps resulted in the generation of ions at *m/z* 461.01, but in the case of compound **16**, there appeared an additional signal at *m/z* 443.04. Compound **19** was more stable during ion separation than flavonol **16**, so it can be assumed that the rhamnosyl residue was linked by a bond difficult to splitting at position 6 (Ferreres et al., 2017). Accordingly, compounds **16** and **19** were tentatively identified as isorhamnetin-3-*O*-(2-rhamnosyl)hexoside and isorhamnetin-3-*O*-6-rhamnosyl)hexoside, respectively.

Compounds **17** (rt = 12.297 min and $[M-H]^-$ at *m/z* 583.04) and **22** (rt = 19.960 min and $[M-H]^-$ at *m/z* 447.04) contained pentose linked at position 3, but xylose may be assumed. Thus, these flavonols can be tentatively identified as isorhamnetin-3-*O*-xyloside-7-*O*-rhamnoside and isorhamnetin-3-*O*-xyloside (peaks **17** and **22**, respectively). Kaempferol derivatives were found based on an ion at *m/z* 285.00 and the UV spectrum which is specific at 265 nm for this flavonol compared to others (Tallini et al., 2015). The MS/MS fragmentation $[M-H-146-162]^-$ of compound **18** (rt = 14.057 min) indicated the presence of rhamnose and hexose molecules, whereas for flavonol **24** (rt = 25.068 min) the loss of rutinose (308 Da) was examined. The elution order was analogous to the compounds **10** and **13** described above, and peaks **18** and **24** were denoted as kaempferol-3-*O*-hexoside-7-*O*-rhamnoside and kaempferol-3-*O*-rutinoside, respectively. Quercetin-3-*O*-rhamnoside and isorhamnetin-3-*O*-rhamnoside (compounds **23**, rt = 22.256 min and **25**, rt = 26.067 min, respectively) were considered as isomers that showed the main fragment ions at *m/z* 301.03 and 315.05, which arose after the loss of 146 Da (rhamnose).

At retention time 27.007 and 27.217 min, there were tentatively identified isorhamnetin derivatives (compounds **26** and **27**) with the loss of two hexose units, probably a combination of glucose and galactose, along with an unidentified acyl with *m/z* 68. HPLC-DAD-ESI-MS studies on sea buckthorn fruits also reported isorhamnetin glycosides with an undefined structure at high retention times (Ma et al., 2016; Rösch et al., 2004; Zheng et al., 2016). Compound **28** giving a single base peak at *m/z* 315.05 and with absorption maxima at band I (370 nm) and II (256 nm), corresponded to an aglycon – isorhamnetin, similarly as in a report by Pop et al. (2013).

3.2. Quantification of phenolic compounds of *H. rhamnoides* cultivars

The content of phenolic compounds listed in Table 1 was made on the basis of the UPLC-PDA method and calculations using data from the calibration curves.

The *p*-coumaric acid derivative dominated in all tested cultivars and Podarok Sadu berries were the richest in this acid (5.50 mg/100 g dm). The content of the ferulic acid derivative ranged from 2.34 to 3.44 mg/100 g dm (in Botaniczeskaja-Lubitelskaja and Podarok Sadu, respectively). In sea buckthorn berries collected from the region of the Himalayas, both these acids together with gallic and *p*-hydroxybenzoic acids were dominant (Arimboor, Kumar, & Arumughan, 2008). However, in sea buckthorn grown in Sweden, no hydroxycinnamic acid was detected, while in blueberries and black chokeberries it was particularly abundant (Olsson, Gustavsson, Andersson, Nilsson, & Duan, 2004). The previous studies suggest that sea buckthorn may contain protocatechuic, vanillic, salicylic, cinnamic, and caffeic acids, and total phenolic acids content may amount to 107 mg/100 g dm (Arimboor et al., 2008; Guo, Guo et al., 2017; Teleszko et al., 2015).

In the case of all samples, isorhamnetin-3-*O*-rutinoside (compound **20**) had the highest concentration and a similar result was obtained by Olas (2018) in the study of *E. rhamnoides* A. Nelson. It is worth mentioning that isorhamnetin-3-*O*-rutinoside of natural origin promotes apoptosis of human myelogenous erythroleukaemia cells (Boubaker et al., 2011). Other studies have shown that isorhamnetin-3-*O*-rutinoside may affect the control of adipose tissue mass because it inhibits adipogenesis in 3 T3-L1 adipocytes (Sekii et al., 2015). The content of compound **20** ranged from 96.4 (Botaniczekaja-Lubitelskaja) to 228 mg/100 g dm (Moskwiczka) and accounted for 20 and 16% of flavonols content. Our results are in accordance with those obtained by Zheng et al. (2016) for two sea buckthorn cultivars, Terhi and Tytti (29% and 17% of total flavonols, respectively). In berries grown in Finland and Canada, isorhamnetin-3-*O*-rutinoside was also the main flavonol (from 11% to 43% of total flavonol glycosides) (Ma et al., 2016).

Our study also proves high concentration of: isorhamnetin-3-*O*-

glucoside, quercetin-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside and quercetin-3-*O*-rutinoside (**21**, **15**, **14** and **13**, respectively). Moskwiczka presented the highest contents of compounds **21**, **15** and **13**: 208, 98.3 and 67.9 mg/100 g dm, respectively. The highest concentration of compound **14** was determined in cv. Józef (86.2 mg/100 g dm), which has not been analyzed in the literature before. Chen et al. (2007) reported that isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside was the most abundant flavonol glycoside (2.17 mg/g) among several sea buckthorn cultivars grown in China. Additionally, isorhamnetin-3-*O*-pentoside and isorhamnetin-3-*O*-rhamnoside (compound **22** and **25**, respectively) were not identified in Moskwiczka.

Referring to Moskwiczka, Podarok Sadu, Józef and Aromatnaja, the contents of isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside (peak **7**) were two-fold higher than in the other cultivars. The amounts of compounds **9**, **11**, **17**, **22** and **25** were relatively low (below 2.82 mg/100 g dm), except isorhamnetin-3-*O*-rhamnoside in Podarok Sadu berries (6.91 mg/100 g dm).

In all studied cultivars, flavonols were present in the following order of concentration: monoglycosides (from 61% for Botaniczeskaja-Lubitelskaja to 68% of total flavonols for Moskwiczka) > diglycosides (from 24% for Luczistaja to 30% for Józef) > triglycosides (from 3.9% for Moskwiczka to 6.1% for Botaniczeskaja-Lubitelskaja). Isorhamnetin derivatives ranged from 66% (Botaniczeskaja-Lubitelskaja) to 72% of total flavonols (Moskwiczka). By comparison, Zheng et al. (2016) found that cultivars grown in Canada had over 85% of isorhamnetin glycosides and from 10 to 15% of quercetin glycosides. Research by Ma et al. (2016) found from 45 to 78% of isorhamnetin derivatives and from 22 to 50% of quercetin derivatives among all flavonols. In our study, the percentage of quercetin derivatives ranged from 25% (Moskwiczka) to 32% (Botaniczeskaja-Lubitelskaja). The compounds containing kaempferol had a low impact on the total flavonol content and constituted ca. 2.4% of their amount. Nevertheless, in the sea buckthorn berries collected in Finland and Canada (Ma et al., 2016; Zheng et al., 2016), kaempferol derivatives were not identified, in contrast to fruits grown Romania and Germany (Pop et al., 2013; Rösch et al., 2004).

In our research no flavan-3-ols were found, although Guo et al. (2017) reported wide variation in the levels of these compounds depending on the sea buckthorn subspecies. According to their studies, the main forms were (+)-catechin and (−)-epicatechin, which maximum content were 11.9 and 4.51 g/100 g dm for Yunnanensis. It should be noted that Luczistaja fruits studied by Teleszko et al. (2015) were the richest in polymeric proanthocyanidins (5.76 mg/100 g fresh weight).

3.3. Identification of carotenoid compounds of H. rhamnoides

The carotenoid compounds were studied using UPLC-PDA-Q/TOF-MS at positive ionization mode and at 425 and 450 nm. The identification was done on the basis of reference standards, and also retention time, UV–Vis spectra, MS fragmentation and literature data (tentative identification) (Da Silva, Rodrigues, Mercadante, & de Rosso, 2014; De Rosso & Mercadante, 2007; Petry & Mercadante, 2016; Pop et al., 2014). The results are shown in Table 2 and Fig. 1. This is the first detailed report on carotenoids from the studied cultivars as well as sea buckthorn berries grown in Poland.

The analysis indicates that 16 compounds were tentatively identified, including 11 xanthophylls, four carotenes and one carotenoid ester. Among the xanthophylls all-*trans*-lutein and its isomers ($[M+H]^+$ at *m/z* 569.40), all-*trans*-zeaxanthin and its isomers ($[M+H]^+$ at *m/z* 569.40) and all-*trans*-ß-cryptoxanthin ($[M-H]^+$ at *m/z* 553.32) were examined. Additionally, all-*trans*-ß-carotene and its isomers ($[M+H]^+$ at *m/z* 537.38) and lycopene ($[M+H]^+$ at *m/z* 537.37) from carotenes were tested, as well as an esterified carotenoid, i.e. zeaxanthin dipalmitate ($[M+H]^+$ at *m/z* 1045.10). MS/MS fragmentation resulted in the loss of toluene ($[M + H\text{-}92]^+$) deriving from intra-chain fragmentation in most carotenoids, except for all-*trans*-ß-cryptoxanthin and lycopene (compounds **11** and **12**, respectively).

Table 2
UPLC-PDA-Q/TOF-MS data of carotenoids identification and their quantification in *H. rhamnoides* cultivars.

Peak no.	Compounds	Rt (min)	λ_{max} (nm)	MS $[M+H]^+$ (*m/z*)	MS/MS $[M+H]^+$ (*m/z*)	Carotenoid content (mg/100 g dm)					
						Botaniczeskaja-Lubitelskaja	Luczistaja	Moskwiczka	Podarok Sadu	Józef	Aromatnaja
1	Lutein isomer I	3.702	336/447/475	569.40	476.03	nd	nd	nd	0.53 ± 0.07	nd	nd
2	Lutein isomer II	4.188	336/447/475	569.40	551.11/476.03	nd	nd	0.613 ± 0.037	0.59 ± 0.10	0.638 ± 0.021	0.758 ± 0.109
3	Lutein isomer III	4.605	336/447/475	569.40	551.11/476.03	0.344 ± 0.019	nd	nd	0.29 ± 0.04	nd	0.973 ± 0.281
4	All-*trans*-lutein	5.039	336/447/475	569.40	551.11/476.03	1.84 ± 0.31	1.39 ± 0.44	nd	2.12 ± 0.48	nd	2.22 ± 0.52
5	All-*trans*-zeaxanthin	5.115	453/481	569.40	551.11/476.03	37.3 ± 2.7	32.1 ± 3.6	26.6 ± 2.7	22.3 ± 2.8	33.2 ± 2.0	26.7 ± 1.5
6	Zeaxanthin isomer I	5.673	453/481	569.40	551.11/476.03	1.25 ± 0.47	nd	2.88 ± 0.59	3.49 ± 0.72	3.43 ± 0.27	5.34 ± 0.99
7	Zeaxanthin isomer II	5.805	453/481	569.40	551.11/476.03	2.31 ± 0.52	2.76 ± 0.46	3.37 ± 0.71	3.30 ± 0.91	4.05 ± 0.53	5.70 ± 1.57
8	Zeaxanthin isomer III	6.266	453/481	569.40	551.11/476.03	1.32 ± 0.23	0.742 ± 0.114	12.8 ± 1.8	22.0 ± 1.8	17.8 ± 1.9	31.9 ± 3.3
9	Lutein isomer IV	6.324	336/447/475	569.40	551.11/476.03	0.161 ± 0.034	0.104 ± 0.041	2.24 ± 0.45	3.66 ± 0.82	3.10 ± 0.34	4.01 ± 0.61
10	Lutein isomer V	6.437	336/447/475	569.40	551.11/476.03	nd	nd	0.331 ± 0.088	1.04 ± 0.34	0.564 ± 0.089	1.63 ± 0.69
11	All-*trans*-ß-cryptoxanthin	7.384	455/478	553.32	535.20/497.10	1.24 ± 0.35	0.674 ± 0.090	2.21 ± 0.73	0.990 ± 0.047	2.35 ± 0.12	1.54 ± 0.73
12	Lycopene	8.101	364/446/472/502	537.37		nd	nd	2.06 ± 0.52	3.81 ± 0.99	2.80 ± 0.53	9.93 ± 1.35
13	Zeaxanthin dipalmitate	8.165	453/481	1045.10	789.20/533.06/441.07	nd	nd	14.8 ± 1.4	70.4 ± 3.2	23.1 ± 2.0	202 ± 7
14	All-*trans*-ß-carotene	8.701	425/454/478	537.38	444.01	16.0 ± 2.9	8.85 ± 1.52	47.4 ± 2.6	85.1 ± 3.8	57.8 ± 3.4	162 ± 7
15	*Cis*-ß-carotene I	8.760	363/450/482	537.38	444.01	nd	nd	6.67 ± 1.11	13.9 ± 1.7	6.77 ± 0.54	25.1 ± 2.0
16	*Cis*-ß-carotene II	8.866	342/450/480	537.38	444.01	nd	nd	nd	12.8 ± 1.6	2.40 ± 0.18	28.5 ± 2.5

The data shown are mean values ± SD (n = 3); nd – not detectable.

Signals derived from all-*trans*-lutein (compound **4**) and all-*trans*-zeaxanthin (compound **5**) were identified at rt = 5.039 and 5.115 min, respectively. As expected, MS/MS fragmentations (*m/z* 569.40 → 551.11 → 476.03) were the same for both compounds so did not allow their unambiguous identification. But, the lower intensity of the protonated molecule peak ($[M+H]^+$ at *m/z* 569.40) than the fragment created after losing 18 Da ($[M+H]^+$ at *m/z* 551.11) indicated lutein. This diversity was caused by two ß-rings in zeaxanthin, while lutein possesses one β-ring and one ε-ring (De Rosso et al., 2007). Nevertheless, the identity of compounds **4** and **5** was confirmed by commercial standards, and their derivatives were tentatively tested according to the characteristic λ_{max} values – 336/447/475 nm and 453/481 nm for lutein and zeaxanthin, respectively. Peak **11** (rt = 7.384 min) had the MS/MS fragmentation ($[M+H-18-92]^+$) yielding an ion at *m/z* 197.10 and was identified as all-*trans*-ß-cryptoxanthin, which was confirmed by comparison with the reference standard. Lycopene (compound **12**; rt = 8.101 min) was confirmed via the authentic standard as well as broad spectra (364/446/472/502 nm) and protonated molecule at *m/z* 537.37. Compound **14** (rt = 8.701 min) was identified based on reference standard, as all-*trans*-ß-carotene with maximum absorbance at 425/454/478 nm. The protonated molecule was detected at *m/z* 537.38 and a fragment at *m/z* 444.01 was generated from the loss of toluene. The *cis*-isomers of ß-carotene were reported by comparison to literature data and tentatively they may be 15- or 15′-*cis*-, 13- or 13′-*cis*- and/or 9- or 9′-*cis*-ß-carotene (Da Silva et al., 2014; De Rosso & Mercadante, 2007). Other research on sea buckthorn examined γ-, σ- and α-carotene, 15,15-*cis*-ß-carotene and *cis*-ß-carotene (Andersson et al., 2009; Pop et al., 2014).

Compound **13** (rt = 8.165 min) was eluted before ß-carotene and its MS/MS fragmentation *m/z* 1045.10 → 789.20 → 533.06 resulted in the loss of two palmitic acid molecules ($[M+H-256-256]^+$), then led to *m/z* 441.07 $[M+H-256-256\text{-}92]^+$ (Petry & Mercadante, 2016). Based on the above and comparing it with reference standard, this compound was considered as physalien i.e. zeaxanthin diplamitate ($C_{16:0}$, $C_{16:0}$). Our analysis did not identify chlorophylls and their derivatives, which indicates the harvesting of ripe sea buckthorn berries.

3.4. Quantification of carotenoid compounds of H. rhamnoides cultivars

The quantification of carotenoids was determined based on the UPLC-PDA analysis, standard curves and peak areas, and the results are displayed in Table 2.

Our research indicated that xanthophylls concentration ranged from 16% (for Moskwiczka) to 81% of the total carotenoids (for Luczistaja). The dominant compound in this group was *all-trans*-zeaxanthin (compound **5**) – from 22.3 for Podarok Sadu to 37.3 mg/100 g dm in Botaniczeskaja-Lubitelskaja berries. The all-*trans*-zeaxanthin concentration was determined several times higher than in the *H. rhamnoides* cultivars studied by other authors: max of 2.5 mg/100 g dm in the case of cv. Şerbăneşti and 9.50 mg/100 g dm for cv. BHI 72,587 (Pop et al., 2014; Andersson et al., 2009, respectively). Zeaxanthin dominated over lutein and ß-cryptoxanthin, similarly as in the study of Andersson et al. (2009). *All-trans*-lutein (compound **4**) was not identified in Moskwiczka and Józef berries, and all lutein isomers appeared only in Podarok Sadu fruits (8.23 mg/100 g dm). The tested lutein content was comparable to the amount of lutein (1.56 mg/100 g dm) determined in sea buckthorn harvested in Sweden and tested by Olsson et al. (2004). But cryptoxanthin was identified, and the contents of lycopene, ß-carotene and carotoid ester were higher, in contrast to the results of this research team. The concentration of all-*trans*-lutein (compound **4**; to 2.22 mg/100 g dm for Aromatnaja), all-*trans*-ß-cryptoxanthin (compound **11**; from 0.67 for Luczistaja to 2.35 mg/100 g dm for Józef), and lycopene (compound **12**; to 9.93 mg/100 g dm for Aromatnaja) were tested, and our results corroborated those published by Andersson et al. (2009) and Pop et al. (2014).

Comparatively, in sea buckthorn berries grown in Sweden xanthophylls accounted for 9.1%, carotenes 36% and esterified carotenoids 55% of all carotenoids (Andersson et al., 2009). In studies on analogous cultivars collected in Poland to those tested, carotenes predominated in Aromatnaja and Moskwiczka (above 80% of the carotenoids sum), whereas in Botaniczeskaja-Lubitelskaja, Luczistaja and Podarok Sadu, xanthophylls were the main carotenoids (from 61 to 77% of the carotenoids sum) (Kruczek, Świderski, Mech-Nowak, & Król, 2012).

Our study showed that carotenes accounted for total carotenoids from 19% (Luczistaja) to 47% (Podarok Sadu). In this group, all-*trans*-ß-carotene (compound **14**) was the main compound and amounted from 8.85 mg (Luczistaja) to 162 mg/100 g dm (Aromatnaja). The lycopene concentration (compound **12**) was between 2.06 mg for Moskwiczka and 9.93 mg/100 g dm for Aromatnaja (not detected in Botaniczeskaja-Lubitelskaja and Luczistaja berries) and it was in line with lycopene content in berries from Romania – from 1.4 mg to 2.3 mg/100 g dm – and not detected in cv. Tiberiu (Pop et al., 2014).

Zeaxanthin esterified with two molecules of palmitic acid (compound **13**) was not detected in Botaniczeskaja-Lubitelskaja and Luczistaja fruits, while in other cultivars it represented from 12% (Moskwiczka) to 40% of all carotenoids (Aromatnaja). Weller and Breithaupt (2003) found that sea buckthorn fruits are a rich source of zeaxanthin esters, alongside red pepper, wolfberry, zucchini blossom, and Chinese lantern (fruit and husk), and the dominant ester was also zeaxanthin dipalmitate, similar to the research of Pop et al. (2014).

The general carotenoids content in sea buckthorn berries increases during maturation (Gao, Ohlander, Jeppsson, Björk, & Trajkovski, 2000). However, according to Andersson et al. (2009) the concentration of esterified carotenoids and cryptoxanthin increased, while lutein content decreased in sea buckthorn berries during ripening, but cultivar has a greater impact on the carotenoid content than year and harvest period.

3.5. Anticholinergic activity of H. rhamnoides cultivars

Anticholinergic activity of selected cultivars of *H. rhamnoides* was examined as the ability to inhibit acetylcholinesterase (AChE) and butylcholinesterase (BuChE). The results are shown as IC_{50} (mg of dried sample/ml) and percentage inhibition at the concentration of 35 mg dm/ml enzyme (Table 3). These enzymes are involved in the breakdown of the neurotransmitter acetylcholine, a low level of which is typical for incurable and progressive Alzheimer's disease, dementia and many other neurodegenerative disorders.

The anti-AChE activity, as IC_{50}, fluctuated from 20.16 (Aromatnaja) to 40.60 (Luczistaja). Statistically similar activity as in the case of Aromatnaja was observed for Moskwiczka and Józef (IC_{50} = 20.96 and 21.01, respectively). Wszelaki, Kuciun, and Kiss (2010) found that among the plants used in traditional European medicine to treat central

Table 3
Anticholinergic activity of *H. rhamnoides* cultivars.

Cultivars	Anticholinergic activity			
	IC_{50}		% of inhibition	
	AChE	BuChE	AChE	BuChE
Botaniczeskaja-Lubitelskaja	35.34 ± 0.71b	< 0.01a	41.00 ± 0.24b	98.86 ± 0.14a
Luczistaja	40.60 ± 0.11a	< 0.01a	30.25 ± 0.13c	98.48 ± 0.20a
Moskwiczka	20.96 ± 0.50c	< 0.01a	65.18 ± 0.59a	98.92 ± 0.61a
Podarok Sadu	32.89 ± 0.43b	< 0.01a	43.66 ± 0.74b	98.80 ± 0.25a
Józef	21.01 ± 0.51c	< 0.01a	63.72 ± 0.43a	99.01 ± 0.64a
Aromatnaja	20.16 ± 0.07c	< 0.01a	63.37 ± 0.68a	98.95 ± 0.57a

The data shown are mean values ± SD (n = 3). Different letters in the same column denote a significant difference among varieties according to Tukey's test, $p < 0.05$.

nervous system diseases and improve memory, *Arnicae flos*, *Hyperici herba,* and *Rutae herba* extracts (IC_{50} < 0.20 in the case of AChE and < 0.08 for BuChE) were the most active. In our study, BuChE inhibition of all cultivars was stronger than AChE, at the level below 0.01. Percent inhibition of AChE was from 30.25% for Luczistaja to 65.18% for Moskwiczka, respectively. Referring to BuChE inhibition, the results did not differ significantly and were ca. 99.00%. BuChE activity increases with the progression of brain function impairment; hence tested sea buckthorn berries should be a therapeutic supplement to the patient's diet.

3.6. Antioxidant on-line profiling by HPLC-PDA coupled with post-column derivatization with $ABTS^{\cdot+}$ reagent

Fig. 1 shows the chromatographic profile of sea buckthorn extract obtained before and after the derivatization process with $ABTS^{\cdot+}$ reagent serving as a negative control. Chromatograms are presented in the example of Botaniczeskaja-Lubitelskaja berries but the results for all tested cultivars were very similar. The upper chromatogram refers to the absorbance at 360 nm (due to the high content of flavonols), and the lower chromatogram is the response after the reaction with the radical cation solution at 734 nm. So far, on-line profiling with $ABTS^{\cdot+}$ reagent on *H. rhamnoides* berries has not been reported.

Negative responses after the post-column reaction suggest that phenolic acids had a higher radical scavenging capacity than flavonols. However, phenolic acid content was relatively low, so their activity does not affect the antioxidant effect of the phenolic fraction of sea buckthorn. Ferulic acid, which eluted as the second acid, contains one methoxy group and therefore is more active than *p*-coumaric acid with one hydroxyl group in its molecule. The activity of phenolic acids may be modulated by the alkyl, methoxy groups and electron donors substituted at the *ortho* position (Tkacz et al., 2019).

Flavonols did not show or had very low activity against the $ABTS^{\cdot+}$ reagent. Generally, quercetin is a stronger antioxidant than isorhamnetin, whilst -3-*O*-glycosides have lower antioxidant activity than their aglycones. Our results are in line with the research on sea buckthorn juices, in which isorhamnetin-3-*O*-glycosides were tested using electron spin resonance spectroscopy. These flavonols were unable to form quinonic structures by oxidation; therefore, they were weak radical scavengers (Rösch et al., 2004; Rösch, Bergmann, Knorr, & Kroh, 2003). On the other hand, Chen et al. (2013) examined acylated flavonol glycosides from sea buckthorn berries with high scavenging activities towards DPPH and ABTS radicals. In the case of these studies, the appearance of a sinapoyl moiety could significantly increase the antioxidant potential of flavonol glycosides. The very low antioxidant activity of the flavonol fraction from sea buckthorn should nevertheless be confirmed by other methods including *in vitro* studies.

3.7. Pearson's correlation and principal component analysis (PCA)

Table 4 contains Pearson's correlation coefficients (r) of phenolic and carotenoid concentrations with anticholinergic activity (% inhibition was used). The correlations between the amount of phenolic acids and the inhibitory activity against AChE and BuChE were low (r = 0.268 and 0.226, respectively).

The high anticholinergic potential of tested sea buckthorn cultivars may be explained by the strong correlation with the total flavonol content and activity against AChE and BuChE (0.834 and 0.616, respectively). Regarding AChE inhibition, the highest correlation was computed with the content of quercetin-3-*O*-rutinoside (r = 0.868), isorhamnetin-3-*O*-(2-rhamnosyl)hexoside (r = 0.872), isorhamnetin-3-*O*-(6-rhamnosyl)hexoside (r = 0.854), and isorhamnetin-3-*O*-rutinoside (r = 0.867) (compounds **13, 16**, **19**, and **20**, respectively). In the case of BuChE inhibition, the strongest correlation was found for quercetin-3-*O*-(2,6-di-rhamnosyl)hexoside (r = 0.754), isorhamnetin-3-*O*-(2-rhamnosyl)hexoside (r = 0.740), and isorhamnetin-3-*O*-pentoside (r = 0.852) (compounds **8**, **16**, and **22**, respectively). The coefficients of correlations above r = 0.600 were calculated for the inhibition of both enzymes and the content of quercetin-3-*O*-(2-rutinosyl)glucoside, quercetin-3-*O*-(2,6-dirhamnosyl)hexoside, quercetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside-7-*O*-rhamnoside, isorhamnetin-3-*O*-(2-rhamnosyl)hexoside, and isorhamnetin-3-*O*-pentoside (compounds **6**, **8**, **13**, **14**, **16**, and **22**, respectively). In the case of quercetin-3-*O*-galactoside-7-*O*-rhamnoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rhamnoside, two isorhamnetin derivatives, and isorhamnetin (compound **9**, **24–28**), negative correlations were obtained. This result may stem from the high variability of concentration of these compounds in cultivars.

Pearson's correlation coefficients between the total carotenoids and anticholinergic activity were higher than for phenolic acids and lower than in the case of flavonols, and consequently amounted to 0.504 for AChE and 0.437 for BuChE inhibition. The highest and at the same time very strong correlations were determined between all-*trans*-ß-cryptoxanthin concentration (compound **11**) and AChE inhibition (r = 0.903)

Table 4
Pearson's correlation values of phenolic compound and carotenoids content and anticholinergic activity of *H. rhamnoides* cultivars.

Peak no.	Compounds	AChE	BuChE
1[a]	*p*-Coumaric acid-*O*-hexoside	0.242	0.212
2	Ferulic acid-*O*-hexosice	0.304	0.233
	Total phenolic acids	**0.268**	**0.226**
3	Quercetin-3-*O*-sophoroside-7-*O*-rhamnoside	0.258	0.417
4	Isorhamnetin-3,7-*O*-dihexoside	0.404	0.119
5	Isorhamnetin-3-*O*-rutinoside-7-*O*-glucoside	0.387	0.246
6	Quercetin-3-*O*-(2-rutinosyl)glucoside	0.765	0.639
7	Isorhamnetin-3-*O*-sophoroside-7-*O*-rhamnoside	0.689	0.564
8	Quercetin-3-*O*-(2,6-dirhamnosyl)hexoside	0.649	0.754
9	Quercetin-3-*O*-galactoside-7-*O*-rhamnoside	−0.467	−0.442
10	Quercetin-3-*O*-glucoside-7-*O*-rhamnoside	0.392	0.629
11	Isorhamnetin-3-*O*-hydroxyferuloyl-glucosyl-glucoside-7-*O*-rhamnoside	0.433	0.263
12	Isorhamnetin-3-*O*-(2,6-dirhamnosyl)glucoside	0.483	0.116
13	Quercetin-3-*O*-rutinoside	0.868	0.646
14	Isorhamnetin-3-*O*-glucoside-7-O-rhamnoside	0.752	0.621
15	Quercetin-3-*O*-glucoside	0.783	0.483
16	Isorhamnetin-3-*O*-(2-rhamnosyl)hexoside	0.872	0.740
17	Isorhamnetin-3-*O*-pentoside-7-*O*-rhamnoside	0.085	−0.010
18	Kaempferol-3-*O*-hexoside-7-*O*-rhamnoside	0.793	0.489
19	Isorhamnetin-3-*O*-(6-rhamnosyl)hexoside	0.854	0.559
20	Isorhamnetin-3-*O*-rutinoside	0.867	0.594
21	Isorhamnetin-3-*O*-glucoside	0.710	0.502
22	Isorhamnetin-3-*O*-pentoside	0.607	0.852
23	Quercetin-3-*O*-rhamnoside	0.451	0.553
24	Kaempferol-3-*O*-rutinoside	−0.546	−0.494
25	Isorhamnetin-3-*O*-rhamnoside	−0.240	−0.088
26	Derivative of isorhamnetin I	−0.761	−0.807
27	Derivative of isorhamnetin II	−0.491	−0.274
28	Isorhamnetin	−0.841	−0.555
	Total flavonols	**0.834**	**0.616**
1[b]	Lutein isomer I	−0.249	−0.095
2	Lutein isomer II	0.844	0.702
3	Lutein isomer III	0.221	0.300
4	All-*trans*-lutein	−0.489	−0.292
5	All-*trans*-zeaxanthin	−0.266	−0.078
6	Zeaxanthin isomer I	0.786	0.757
7	Zeaxanthin isomer II	0.690	0.512
8	Zeaxanthin isomer III	0.662	0.586
9	Lutein isomer IV	0.687	0.623
10	Lutein isomer V	0.500	0.449
11	All-*trans*-ß-cryptoxanthin	0.903	0.819
12	Lycopene	0.573	0.473
13	Zeaxanthin dipalmitate	0.419	0.346
14	All-*trans*-ß-carotene	0.570	0.499
15	*Cis*-ß-carotene I	0.536	0.451
16	*Cis*-ß-carotene II	0.328	0.291
	Total carotenoids	**0.504**	**0.437**

[a] Peak numbers according to Table 1.
[b] Peak numbers according to Table 2.

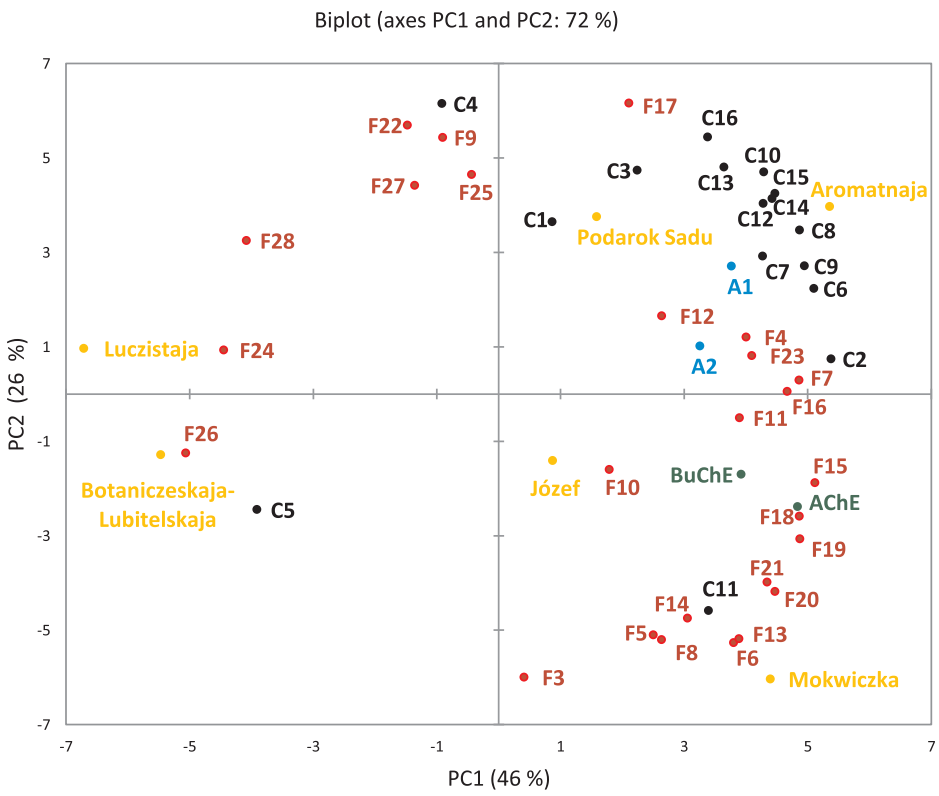

Fig. 2. Principal component analysis biplot of phenolic and carotenoid compounds and anticholinergic activity for *H. rhamnoides* cultivars. (PC1 – principal component 1; PC2 – principal component 2; **A1**-**A2** – phenolic acids and **F3**-**F28** – flavonols with the numbers according to Table 1; **C1**-**C16** – carotenoids with the numbers according to Table 2).

and BuChE inhibition (r = 0.819). Negative correlations were calculated for the inhibition of both enzymes and lutein isomer I, all-*trans*-lutein, and all-*trans*-zeaxanthin (compound **1**, **4**, and **5**). But positive high correlations were found for most isomers of these compounds. Anticholinergic activity correlated more strongly with the total amount of xanthophylls (r = 0.706 for AChE and r = 0.696 for BuChE inhibition) than with the content of all carotenes (r = 0.535 for AChE and r = 0.465 for BuChE inhibition).

Principal component analysis (PCA) was performed on the average contents of each phenolic and carotenoid compounds, anti-cholinergic activity and fruits of six sea buckthorn cultivars. The results are presented on the PCA biplot (Fig. 2), from which on the basis of links and rays, the connections between variables were proposed. The first two principal components (PC1 and PC2) explained 72% of total variance (46% and 26%, respectively). The close position of the points suggests the highest correlation between the ability to inhibit AChE and BuChE and the concentration of flavonols **15**, **18**, **19**, **20** and **21** and carotenoid **11**. The most abundant in flavonols were Moskwiczka and Józef berries. Flavonols had a stronger effect on anticholinergic activity, but carotenoids formed a smaller coherent cluster with Aromatnaja and Podarok Sadu. The content of flavonols **9**, **25** and **26** and carotenoids **1**, **4** and **5** weakly correlated with the anticholinergic activity as indicated by nearly perpendicular links between the vertices of these elements. Considering the longest links, the highest variability (no fixed proportion) was between anticholinergic activity and the amounts of isorhamnetin (flavonol **28**) and all-*trans*-lutein (carotenoid **4**).

4. Conclusions

Analyses of phenolic and carotenoid compounds by UPLC-PDA-Q/TOF-MS, and anticholinergic potential using the *in vitro* method of Botaniczeskaja-Lubitelskaja, Luczistaja, Moskwiczka, Podarok Sadu, Józef, and Aromatnaja berries were carried out for the first time. Twenty-eight phenolic compounds were tentatively identified and the content of each was determined; however, over 98% of phenolic compounds were flavonols. Sea buckthorn berries were a rich source of isorhamnetin derivatives (from 66% to 72% of total flavonols), followed by quercetin (from 25% to 32% of total flavonols). The substitution of the flavonol structure of sea buckthorn compounds is predominant at the 3-position and 3,7–position. There were tentatively identified 16 carotenoids, whereas xanthophylls predominated in Botaniczeskaja-Lubitelskaja and Luczistaja berries (74 and 81% of total carotenoids) and carotenes were the main compounds in the remaining cultivars (from 44 to 45% of total carotenoids). The anticholinergic potential was tested as the ability to inhibit AChE and BuChE and respectively moderate and strong activity was found. The highest anticholinergic potential was tested for Aromatnaja, Józef, and Moskwiczka berries. The post-column derivatization with $ABTS^{\cdot+}$ reagent proved that phenolic acids were stronger free radical scavengers than flavonols contained in tested *H. rhamnoides* fruits. According to Pearson's correlation coefficients and PCA analysis, this potential was dependent on cultivar, phenolic and carotenoid compounds. The results obtained provide information to determine identity and purity, check the origin and perform quality control of sea buckthorn as well as to target the selection of cultivars for industrial crops. Furthermore, the fruits of tested sea buckthorn cultivars may be a component of new functional and added-value products rich in flavonols and carotenoids.

Ethics statement

Research did not include any human subjects and animal experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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OŚWIADCZENIE

Oświadczam, że jestem współautorem publikacji pt.:

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Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, przygotowaniu materiału badawczego, oznaczeniu ilościowym i identyfikacji związków fenolowych i karotenoidów metodą LC-MS, analizie potencjału przeciwstarzeniowego *in vitro* i profilowaniu przeciwutleniaczy on-line na drodze derywatyzacji postkolumnowej w ekstraktach owoców wybranych odmian rokitnika pospolitego. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, a następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

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mój udział polegał na współtworzeniu koncepcji i planu badań, pozyskaniu materiału badawczego, uczestnictwie w oznaczeniu ilościowym i identyfikacji związków fenolowych i karotenoidów metodą LC-MS, analizie potencjału przeciwstarzeniowego *in vitro* i profilowaniu przeciwutleniaczy on-line na drodze derywatyzacji postkolumnowej w ekstraktach owoców wybranych odmian rokitnika pospolitego. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

Awojdyło

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mój udział polegał na uczestnictwie w etapie przygotowania materiału badawczego i analizach potencjału przeciwstarzeniowego *in vitro* w ekstraktach owoców wybranych odmian rokitnika pospolitego.

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DECLARATION

I declare that in the publication entitled:

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my participation was to conduct the analysis of the identification of phenolic compounds by the LC-MS method and substantive co-editing. The research is the result of collaboration with the PhD student during her research internship at Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) under the "PROM Program" - International scholarship exchange of PhD candidates and academic staff (NAWA).

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Original Research Article

Triterpenoids, phenolic compounds, macro- and microelements in anatomical parts of sea buckthorn (*Hippophaë rhamnoides* L.) berries, branches and leaves

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ABSTRACT

This study aimed to identify and quantify triterpenoids, phenolic compounds (by UPLC/ESI-Q/TOF-MS) and minerals (by FAAS) of the anatomical berry parts (skin, flesh, endocarp, seed), branches and leaves of seven sea buckthorn cultivars. The flavonols exceeded 0.7 g/100 g dry weight of leaves and skins. The ratio of quercetin to isorhamnetin derivatives was higher than 1.0 for skins, flesh, branches and leaves. The most flavan-3-ols and polymeric procyanidins were found in branches, and phenolic acids in leaves. 11 triterpenoids were identified, including some new ones such as pomolic acid dominating in berry parts. Triterpenoids were five times more abundant in the flesh than the leaves, where ursolic acid constituted 46 % of these triterpenes. High contents of corosolic acid and betulinic acid were specific for branches, and betulin and oleanolic and ursolic acids for skins. The best sources of sodium were endocarp and leaves, potassium – flesh and endocarp, calcium – leaves, and magnesium – seeds. Leaves and branches were rich in iron and copper, while seeds were rich in zinc. A characteristic feature of leaves was an iron to manganese ratio of 1:1. Diverse fractions of sea buckthorn give the potential of non-waste food production with the desired profile of pro-health components.

1. Introduction

Common sea buckthorn (*Hippophaë rhamnoides* L., Elaeagnaceae), also known as seaberry, Siberian pineapple, sandthorn and sallow thorn, is widespread from dry, sandy, through mountainous areas, to the sea coasts and river valleys of Europe, Canada and Asia, where China has the highest concentration of crops (Ciesarová et al., 2020; Tkacz et al., 2020). Globally, the most important and best-known part of the plant is the berries, which are several times richer in vitamin C than popular fruits such as strawberries, lemons or blackberries and are distinguished by high content of oil (average 10 % of the fresh fruit weight) rich in n-3, n-6, n-7 and n-9 fatty acids (Teleszko et al., 2015; Tkacz et al., 2019).

However, sea buckthorn is a multi-purpose plant, the production of which includes food industry products (juice, drink, smoothie, jam, sauce, oil) and alcohols (wine, liqueur, beer additive) from berries, herbal leaf teas providing high access to flavonoids and detoxifying properties, production of fodder supplements of sea buckthorn by-products, cosmetics, pharmaceuticals, and fuel as firewood. The second aspect of sea buckthorn use concerns good soil erosion protection, reclamation of polluted areas, afforestation of marginal areas and planting as an ornamental shrub (Bal et al., 2011; Ciesarová et al., 2020; Madawala et al., 2018; Michel et al., 2012).

Sea buckthorn berries and preserves attract attention due to their comprehensive nutritional and health-promoting properties known from traditional medicine (reduction of fever and inflammation, antitoxic effect, a positive effect on the regeneration and condition of the skin and hair) and then well established scientifically (Ciesarová et al., 2020; Maheshwari et al., 2011; Tkacz et al., 2019). These properties of sea buckthorn are associated with the wide range of active biological substances found in berries and leaves, rich in vitamins, carotenoids, flavonoids, sterols, and tocopherols. A few reports have indicated antioxidant, cardioprotective, hypoglycemic, hypolipidemic, and antibacterial properties of phenolic seed extracts (Arimboor and Arumughan, 2012; Wang et al., 2011). In turn, the importance of leaves can be attributed to anti-influenza activities, cytotoxic effects (Enkhtaivan et al., 2017) and protection against CCl-4-induced liver oxidative

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damage in the case of phenolic rich fraction of sea buckthorn leaves containing mainly gallic acid, isorhamnetin, quercetin, kaempferol, and myricetin (Maheshwari et al., 2011). Enkhtaivan et al. (2017) showed that the anti-influenza activity strongly correlated with flavonol aglycones and monoglycosides, while di- and triglycosides showed a strong correlation with cytotoxic activity towards normal and cancer cells.

Nowadays, traditionally grown plants are the center of attention and the demand for their production is increasing, due to the strategy of inhibiting or delaying diseases using a natural diet (Ciesarová et al., 2020). Young branches with leaves are a waste product after mechanical harvesting of sea buckthorn berries, and the pomace containing skins and seeds with endocarp accounts for 20 % of the weight of sea buckthorn berries after juice pressing. Therefore, the potential for using the whole plant is seen in non-waste technology and focuses on the management of residues for production with high added value (Ciesarová et al., 2020; Radenkovs et al., 2018). According to these reports, the residues after pressing juices and oils (pulp, press cake) can be used to extract pigments, and then used as a natural food colouring, for the production of fodder, tea-type infusion, powders, nutraceuticals, antioxidant additives used to stabilize and fortify food, such as bread and other baked goods, as well as an unconventional source of bio-oil with a potential use in the food, cosmetic, and pharmaceutical industries. However, literature reports on the profile of many bioactive components of the anatomical parts of *H. rhamnoides* are still very limited. The necessity to characterize secondary metabolites and nutrients also results from previous reports on sea buckthorn (Fatima et al., 2015; Kaur et al., 2017; Michel et al., 2012; Tkacz et al., 2020).

Hence, this study aimed at qualitative and quantitative determination of pentacyclic triterpenoids, phenolic compounds (flavonols, phenolic acids, flavan-3-ols, polymeric procyanidins), macro- and microelements of the anatomical parts of berries (skin, flesh, endocarp, seed), young branches and leaves of selected sea buckthorn cultivars. It was assumed that this would allow differentiation and identification of plant fractions in terms of the profile of health-promoting components for unconventional and innovative applications in food and nutraceutical production. Previous studies have reported flavonols in fruits (Yang et al., 2009), leaves (Fatima et al., 2015), sea buckthorn seeds (Arimboor and Arumughan, 2012), phenolic acids in seeds and leaves (Arimboor et al., 2008), flavan-3-ols in shoots and fruits (Bittová et al., 2014), some triterpenes in sea buckthorn fractions (Marciniak et al., 2021), some minerals in berries (Sabir et al., 2005) and seeds (Zeb and Malook, 2009). Nevertheless, this work is the first to discuss all sea buckthorn fractions such as skin, flesh, endocarp, seeds, branches, and leaves, for the identification and quantification of triterpenoids, phenolic compounds and minerals, providing a comprehensive and valuable comparison of high-yielding varieties in Central and Eastern Europe.

2. Materials and methods

2.1. Reagents and standards

The reference standards of flavonols (isorhamnetin, quercetin, and kaempferol-3-*O*-rutinoside, -rhamnoside, -glucoside, and –galactoside), phenolic acids (*p*-coumaric, ferulic, gallic, and chlorogenic acids), flavan-3-ols [(+)-catechin, (-)-epicatechin, (-)-epicatechin-gallate, (-)-epigallo-catechin], and triterpenoids (maslinic acid, pomolic acid, corosolic acid, betulinic acid, oleanolic acid, ursolic acid, betulin, tormentic acid, erythrodiol, α-boswellic acid, and uvaol) were purchased from Extrasynthese (Genay Cedex, Lyon Nord, France) and Sigma-Aldrich (Steinheim, Germany). The reference standards for macro- and microelements (sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese) were provided by AccuStandard (New Haven, CT, US). Reagents for extraction, chromatographic analysis and atomic absorption spectroscopy analysis were purchased from Merck KGaA (Darmstadt, Germany).

2.2. Plant materials and sample preparation

Fresh sea buckthorn berries, young branches and leaves were collected from seven cultivars: 'Botaniczeskaja-Ljubitelskaja' (also as 'Botaniczeskaja-Lubitelskaja'), 'Golden Rain' (also as 'Goldrain'), 'Luczistaja', 'Maryja' (also as 'Mary'), 'Podarok Sadu', 'Prozrocznaja' (also as 'Prozrachnaya'), and 'Tatiana' (also as 'Tatjana'). The material was obtained in mid-September 2020 from a sea buckthorn plantation with an area of 33 ha from the horticultural farm in Podlaskie Voivodeship (Sokółka, Poland). Fruits, branches and leaves at the optimal harvest date were collected manually from the same bushes for each cultivar and sent to the laboratory in refrigerated packages as general samples. The leaves were manually separated from the young branches from two batches in the amount of 0.25 kg. In turn, the two batches of berries (each 0.25 kg) were manually fractionated into anatomical parts such as skins, flesh, endocarp (the thin innermost layer of the pericarp directly surrounding the seed) and seeds, and treated immediately with liquid nitrogen. The individual fractions pooled from both batches (0.5 kg) constituted laboratory samples in the following amounts, taking into account material loss: leaves ~110 g; branches ~310 g; skins ~150 g; flesh ~250 g; endocarp ~4 g; and seeds ~15 g. All samples were freeze-dried (Christ Alpha 1–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), then crushed by a basic analytical mill (IKA A11, Darmstadt, Germany), and stored vacuum-sealed under freezing conditions until analysis.

2.3. Identification (UPLC-PDA/ESI-Q/TOF-MS) and quantification (UPLC-PDA) of flavonols, phenolic acids and flavan-3-ols

The profile and content of phenolic compounds were determined by ultra performance liquid chromatography (Acquity UPLC System) with a binary solvent manager and photodiode array detector PDA (Waters Corp., Milford, MA, US) coupled to G2 Q/TOF micro-mass spectrometer fitted with an electrospray ionization ESI source acting on negative modes (Waters Corp., Manchester, UK), as previously described by Tkacz et al. (2020).

The extraction of these compounds was as follows. Sample (~0.5 *g*) was mixed with 1.8 ml of solvent methanol-water-acetic acid-ascorbic acid (30:68:1:1; *v/v/v/m*) and sonicated twice (Sonic 6D, Polsonic, Warsaw, Poland) for 20 min with an interval of 24 h at 4 °C, thus ensuring the highest extraction efficiency of the desired compounds. The amount of endocarp was reduced in proportion to the volume of the extraction reagent due to the low yield of this fraction from the sea buckthorn berries. The sample solution was centrifuged at 19,000 ×*g* for 10 min using MPW-150R (MPW Med. Instruments, Warsaw, Poland), and before injection the supernatant was filtered through a 0.20 µm pore size hydrophilic PTFE membrane (Millex Samplicity™ Filter, Merck KGaA, Darmstadt, Germany).

The separation of flavonols, phenolic acids and flavan-3-ols was performed on an UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm, Waters Corp., Milford, MA, US). The optimized UPLC and MS parameters were as previously reported (Tkacz et al., 2020). Briefly, the elution lasted 30 min with a flow rate of 0.420 ml/min and the injection volume equal 5 µL. 2.0 % formic acid (solvent A) and 100 % acetonitrile (solvent B) were used in the following gradients: (1) elution start with 98.0 %solvent A; (2) solvent A reduction to 65 % (to 32.00 min), and to 0% (to 33.00 min); (3) 98 % solvent A from 33.50 to 35.00 min to re-equilibrate the column. The MS parameters were as follows: cone gas flow: 40 L/h; desolvation gas flow: 300 L/h; capillary voltage: 2500 V; cone voltage: 30 V; source temperature: 100 °C; desolvation temperature: 300 °C. The runs were monitored at 280 nm (flavan-3-ols), 320 nm (phenolic acids), and 360 nm (flavonols). Empower 3 Chromatography Data Software and MassLynx™ 4.0 Software were used to develop records. Quantitative determination was based on injections of the phenolic calibration standards at concentrations ranging between 0.05 and 5 mg/ml and under the same conditions as above mentioned for the samples ($R^2 \geq$

0.9995). The sums of phenolic acids, flavan-3-ols, and derivatives of quercetin, isorhamnetin and kaempferol were calculated as the sums of ferulic acid, (+)-catechin, quercetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, and kaempferol-3-*O*-rutinoside, respectively. The results of the ULPC-PDA quantification were expressed as mg per 100 g of dry matter (dm).

2.4. Analysis of polymeric procyanidins by UPLC-FL

Analysis of polymeric procyanidins was performed by direct phloroglucinolysis method, exactly as in the protocol previously described by Teleszko and Wojdyło (2015). The UPLC-FL Acquity System and the UPLC BEH Shield RP18 column (2.1 × 50 mm, 1.7 µm, Waters Corp., Milford, MA, US) were used. The flow rate was 0.500 mL/min, sample injection volume - 5 µL, elution time – 8.80 min. Solvent A (2.5 % acetic acid in water) and solvent B (100 % acetonitrile) were used in the following linear gradients: 0 − 0.6 min, 2% B; 0.6 − 2.17 min, 2–3% B; 2.17 − 3.22 min, 3–10% B; 3.22 − 5.00 min, 10–15% B; 5.00 − 6.00 min, 100 % B; and then, re-equilibrate until the end of the process time. The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves were established using (+)-catechin, (-)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (-)-epicatechin--phloroglucinol adduct standards. Empower 3 Chromatography Data Software (Waters Corp., Milford, MA, US) was used to develop records. The results were expressed as mg polymeric procyanidins per 100 g of dm and average procyanidins polymerization degree (DP).

2.5. Analysis of pentacyclic triterpenoids by UPLC-PDA

Analysis of pentacyclic triterpenoids was performed by chromatographic technique using PAH (polycyclic aromatic hydrocarbons) polymeric C18 bonded phase based on the method previously described by Zhang et al. (2013) with significant modifications.

The two-step extraction of these compounds was as follows. Sample (~0.1 *g*) was mixed with 4 ml of solvent hexane-ethyl acetate (1:1; v/v) and sonicated for 30 min at 40 °C (Sonic 6D, as in Section 2.3). After 24 h at 4 °C, the sample solution was centrifuged at 19,000 ×*g* for 10 min using the MPW-150R (as in Section 2.3), and supernatant was preserved. The residue was mixed with 4 ml of solvent chloroform-chloromethane (1:1; v/v) and sonicated and centrifuged again as before. Both supernatants were combined, evaporated to dryness with nitrogen (XCV-5400 XcelVap, Horizon Technology, Salem, NH, US), then residues were dissolved in 100 % UPLC-grade methanol and filtered through a 0.20 µm hydrophilic PTFE membrane (as in Section 2.3) prior to injection.

The UPLC-PDA Acquity System (as in Section 2.3) and the ZORBAX Elipse PAH column (2.1 × 150 mm, 3.5 µm) with guard column (2.1 × 12.5 mm, 5 µm) (Agilent Technologies, Santa Clara, CA, US) were used. The flow rate was 0.250 mL/min, sample injection volume - 3 µL, column temperature – 30 °C. Solvent A (100 % UPLC-grade water) and solvent B (100 % acetonitrile). The runs were monitored at 210 nm for oleanolic acid and ursolic acid, and 200 nm for the other triterpenes. Empower 3 Chromatography Data Software (as in Section 2.4) was used to develop records. Quantitative determination was based on injections of the triterpenoid calibration standards at known concentrations and under the same conditions ($R^2 \geq 0.9990$). The identification of the triterpenes was further confirmed by mass spectrometry and the $[M-H]^-$ at *m/z* were as follows: 487.30 for tormentic acid, 471.30 for maslinic, pomolic and corosolic acids, 455.30 for betulinic, oleanolic, ursolic and α-boswellic acids, and 441.31 for betulin, erythrodiol and uvaol. The parameters of LC-ESI-Q/TOF-MS analysis were as given in Section 2.3. The results of the ULPC-PDA quantification were expressed as mg per 100 g of dm.

2.6. Analysis of macro- and microelements by FAAS

Analysis of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), cupper (Cu), zinc (Zn) and manganese (Mn) was performed by flame atomic absorption spectrophotometry (FAAS) technique. Wet digestion was as follows. To the sample (0.5 g) in a PTFE-TFM vessel was added 7 mL of nitric acid-hydrochloric acid (6:1, *v/v*) and then 0.5 mL of 30 % hydrogen peroxide. The amount of endocarp was reduced in proportion to the volume of the extraction reagents, as in Section 2.3. The mixture was subjected to microwave digestion at 180 °C for 20 min with temperature control *via* IR sensors. After cooling, the sample was placed in a 10 mL volumetric flask and made up to the mark with distilled water.

The concentrations of each macro- and micronutrients were measured using an AA-7000 series Atomic Absorption Spectrophotometer (Shimadzu Corp., Kyoto, Japan) equipped with ASC-7000 Autosampler and hollow cathode lamps (Hamamatsu Photonics K.K., Japan), and using an air-acetylene flame. The samples were determined for Na at 589 nm, K at 766.5 nm, Ca at 422.7 nm, Mg at 285.2 nm, Fe at 248.3 nm, Cu at 324.8 nm, Zn at 213.9 nm, and Mn at 279.5 nm. Quantitative determination was based on the calibration curve of the reference standards ($R2 \geq 0.9990$). WizzAArd software was used to develop records and the results were expressed as mg per 100 g of dry matter (dm).

2.7. Statistical analysis

All measurements were conducted in triplicate and the results were presented as mean value ± standard deviation (SD). Statistical analysis data were obtained from Tukey's HSD test preceded by the one-way analysis of variance (ANOVA), as well Pearson's correlation coefficients and principal component analysis (PCA). Parameters were measured in different units and ranges; therefore autoscaling was used to give each variable a unit variance and therefore the same weight for PCA analysis. The main factors were obtained based on the correlation matrix. Statistical differences at level $p < 0.05$ were marked with consecutive lowercase letters in Tables. XLSTAT Statistical Software version 2016.4 (Addinsoft Inc, New York, NY, US) integrated with Microsoft Excel 2017 (Microsoft Corp., Redmond, WA, US) were used.

3. Results and discussion

3.1. Phenolic compounds in anatomical parts of sea buckthorn berries, branches and leaves

3.1.1. Flavonols

Ultra high performance liquid chromatography coupled to quadrupole time-of-flight tandem mass spectrometer (UPLC-PDA/ESI-Q/TOF-MS) analysis allowed the identification and quantification of seven quercetin (Q), 12 isorhamnetin (I), and two kaempferol (K) derivatives ($[M-H]^-$ at *m/z* 301.01, 315.03, 285.00, respectively) (Tables 1 and 2). The identification was performed on the basis of reference standards (compounds 9, 11, 15–19), as well as the UV spectrum, MS fragmentation, and literature data - tentative identification (Ma et al., 2016; Pop et al., 2013; Rösch et al., 2004; Tkacz et al., 2020). Flavonols were identified with preferential glycosylation positions 3 and 7, in structures with -*O*-glucoside, -*O*-rhamnoside, -*O*-rutinoside, -*O*-sophoroside, and with combinations of two hexosyls: hexosyl(1→2)hexoside.

Isorhamnetin derivatives are well recognized as the dominant polyphenol fraction (over 65 % of total flavonols) of whole berries of species belonging to the *Elongaceae* family from different geographical locations (Fatima et al., 2015; Ma et al., 2016; Tkacz et al., 2019). However, this analysis of the plant anatomical parts indicated a greater variation in I derivatives than Q and K derivatives but higher concentrations of Q derivatives in most fractions (skins, flesh, branches, leaves). Thus, the highest concentrations of I-3-*O*-glucoside (peak 16, $[M-H]^-$ at *m/z* 477.11), I-3-*O*-rutinoside (peak 15, $[M-H]^-$ at *m/z* 623.10),

Table 1
Identification of flavonols in anatomical parts of sea buckthorn berries, branches and leaves by UPLC-PDA-Q/TOF-MS.

Peak no.	Compound	R_t (min)	λ_{max} (nm)	Molecular formula	MS $[M-H]^-$ (*m/z*)		Mass error (ppm)	MS/MS $[M-H]^-$ (*m/z*)	Number of cultivars					
					Exact mass	Accurate mass			Skins	Flesh	Endocarp	Seeds	Branches	Leaves
Quercetin derivatives														
1	Q-3-*O*-sophoroside-7-*O*-rhamnoside	1.71	258, 357	$C_{33}H_{40}O_{21}$	771.2062	771.1847	−27.9	625.18/ 301.01	1–7	1–7	nd	1–7	1	nd
4	Q-3-*O*-(2-rutinosyl) glucoside	3.57	258, 353	$C_{33}H_{40}O_{21}$	771.2062	771.1845	−28.2	609.14/ 591.20/ 301.01	1–7	1–7	1–7	nd	nd	1–7
6	Q-3-*O*-(2,6-dirhamnosyl) glucoside	5.45	255, 353	$C_{33}H_{40}O_{20}$	755.2113	755.1838	−36.4	625.18/ 609.13/ 489.09/ 301.01	1–7	1–7	1–7	1–7	nd	1–7
7	Q-3-*O*-glucoside-7-*O*-rhamnoside	6.51	255, 355	$C_{27}H_{30}O_{16}$	609.1534	609.1345	−31.0	447.03/ 301.01	1–7	1–7	1–7	nd	1–7	1–7
9	Q-3-*O*-rutinoside	8.29	258, 355	$C_{27}H_{30}O_{16}$	609.1534	609.1349	−30.4	301.01	1–7	1–7	1–6	nd	nd	1–5, 7
11	Q-3-*O*-glucoside	9.27	254, 355	$C_{21}H_{20}O_{12}$	463.0877	463.0991	+24.6	301.01	1–7	1–7	1–7	1–7	nd	1–7
17	Q-3-*O*-rhamnoside	20.34	255/ 365	$C_{21}H_{20}O_{11}$	447.1006	447.0341	−148.7	301.01	1–7	1–7	nd	nd	nd	nd
Isorhamnetin derivatives														
2	I-3,7-*O*-diglucoside	2.41	255, 354	$C_{28}H_{32}O_{17}$	639.1639	639.1545	−14.7	477.11/ 315.03	1–7	1–7	1–7	1–7	1	1–7
3	I-3-*O*-rutinoside-7-*O*-glucoside	3.26	254, 350	$C_{34}H_{42}O_{21}$	785.2219	785.1839	−48.4	623.10/ 315.03	1–7	1–7	1–7	1–7	nd	1–7
5	I-3-*O*-sophoroside-7-*O*-rhamnoside	4.18	255, 354	$C_{34}H_{42}O_{21}$	785.2219	785.1842	−48.0	639.15/ 315.03	1–7	1–7	1–7	1–7	nd	nd
8	I-3-*O*-(2,6-dirhamnosyl) glucoside	8.08	254, 355	$C_{34}H_{41}O_{20}$	769.2197	769.2037	−20.8	623.10/ 605.06/ 503.20/ 315.03	1–7	1–7	1–7	1–7	nd	1–7
10	I-3-*O*-glucoside-7-*O*-rhamnoside	8.58	355, 352	$C_{28}H_{32}O_{16}$	623.1690	623.1030	−105.9	477.11/ 315.03	1–7	1–7	1–7	1–7	nd	1–7
12	I-3-*O*-(2-rhamnosyl) glucoside	9.89	255, 353	$C_{28}H_{32}O_{16}$	623.1690	623.0997	−111.2	461.00/ 443.02/ 315.03	1–7	1–7	nd	1–7	nd	nd
14	I-3-*O*-(6-rhamnosyl) glucoside	12.61	257, 349	$C_{28}H_{32}O_{16}$	623.1690	623.1015	−108.3	461.00/ 315.03	1–7	1–7	nd	nd	nd	1–5, 7
15	I-3-*O*-rutinoside	13.32	255, 354	$C_{28}H_{32}O_{16}$	623.1690	623.0996	−111.4	315.03	1–7	1–7	1–7	nd	nd	1–3, 5–7
16	I-3-*O*-glucoside	13.62	256, 352	$C_{22}H_{22}O_{12}$	477.1111	477.1078	−7.4	315.03	1–7	2–7	nd	nd	1–7	nd
19	Isorhamnetin	25.69	255, 368	$C_{16}H_{12}O_7$	315.0583	315.0341	−79.8	301.01	1–7	1–7	1–7	1–7	nd	1–7
20	I derivative I	25.86	256, 355	–	–	707.09	–	545.15/ 477.11/ 315.03	1–7	1–7	2, 4–7	1–7	nd	1–7
21	I derivative II	26.04	256, 356	–	–	707.09	–	545.15/ 477.11/ 315.03	1–7	1–7	nd	1–7	nd	2–7
Kaempferol derivatives														
13	K-3-*O*-glucoside-7-*O*-rhamnoside	12.29	266, 349	$C_{27}H_{30}O_{15}$	593.1585	593.0546	−175.2	447.03/ 285.00	1–6	1–7	3–7	nd	nd	1–5, 7
18	K-3-*O*-rutinoside	23.79	266, 350	$C_{27}H_{30}O_{15}$	593.1585	593.0539	−176.3	285.00	1–7	1–7	1–7	1–7	nd	1–7

Q – quercetin; I – isorhamnetin; K – kaempferol; numbers of cultivars: 1 – Botaniczeskaja-Ljubitelskaja; 2 – Golden Rain; 3 – Luczistaja; 4 – Maryja; 5 – Podarok Sadu; 6 – Prozrocznaja; 7 – Tatiana; R_t – retention time; λ_{max} - UV absorption maxima. Exact massses and molecular formulas based on https://pubchem.ncbi.nlm.nih.gov/.

Q-3-*O*-glucoside (peak 11, $[M-H]^-$ at *m/z* 463.10) and I-3-*O*-glucoside-7-*O*-rhamnoside (peak 10, $[M-H]^-$ at *m/z* 623.10) were determined in skins, but in flesh the dominant one was Q-3-*O*-glucoside, followed by flavonols specific to skins, depending on the berry cultivars. The exception was flesh of 'Botaniczeskaja-Ljubitelskaja' in which I-3-*O*-glucoside was not identified, as in endocarp, seeds and leaves of the analyzed cultivars. According to previous reports, these derivatives were most prevalent in homogenized berries of Russian, Finnish and Chinese cultivars (Yang et al., 2009) but contrary to this research, I-3-*O*-glucoside was one of the dominant compounds in leaves (Pop et al., 2013).

I-3-*O*-glucoside-7-*O*-rhamnoside was also the dominant flavonol in

Table 2
Quantification of phenolic compounds (mg/100 g dm) in anatomical parts of sea buckthorn berries, branches and leaves by UPLC-PDA-FL.

No.	Fractions and cultivars	Phenolic compounds (mg/100 g dm)							
		Flavonols			Phenolic acids	Flavan-3-ols	Polymeric procyanidins	DP	Total phenolic compounds
		Quercetin derivatives	Isorhamnetin derivatives	Kaempferol derivatives					
Skins									
1	Bot.-Ljubitel.	630.02 ± 5.12 e	466.88 ± 3.49 b	11.76 ± 0.66 i	2.57 ± 0.20 i-l	46.86 ± 1.32 r-v	425.76 ± 4.01 qr	3.4	1583.85 ± 14.8 jk
2	Golden Rain	260.58 ± 4.08 k	124.29 ± 2.14 m	7.97 ± 0.54 j	2.85 ± 0.18 h-k	69.00 ± 1.34 o	497.49 ± 5.23 pq	4.7	962.17 ± 5.39 mn
3	Luczistaja	376.41 ± 4.55 i	414.31 ± 3.52 c	14.30 ± 0.60 h	3.67 ± 0.32 e-g	59.80 ± 1.25 o-q	514.34 ± 6.43 p	4.4	1382.82 ± 11.4 l
4	Maryja	244.91 ± 3.63 l	138.51 ± 1.95 L	25.90 ± 0.81 bc	2.95 ± 0.21 h-j	62.32 ± 1.29 o-q	396.11 ± 4.75 r	5.1	870.69 ± 8.25 no
5	Podarok Sadu	481.14 ± 4.89 g	401.42 ± 2.26 d	13.55 ± 0.48 h	5.00 ± 0.38 cd	85.23 ± 1.43 n	619.55 ± 5.39 o	5.4	1605.89 ± 10.4 jk
6	Prozrocznaja	731.54 ± 5.23 d	580.73 ± 3.58 a	18.12 ± 0.34 f	3.44 ± 0.35 e-g	90.60 ± 1.53 n	471.03 ± 4.42 q	3.6	1895.46 ± 16.2 i
7	Tatiana	55.03 ± 1.52 u	29.64 ± 1.03 w	21.07 ± 0.63 d	0.88 ± 0.13 m	45.93 ± 1.27 r-v	599.67 ± 5.45 op	5.1	752.22 ± 14.6 op
Flesh									
1	Bot.-Ljubitel.	216.22 ± 5.04 m	94.43 ± 2.12 q	4.01 ± 0.53 lm	1.72 ± 0.19 lm	50.42 ± 1.41 q-u	115.55 ± 2.59 t	3.8	482.35 ± 5.13 rs
2	Golden Rain	150.60 ± 4.23 p	63.05 ± 1.74 tu	4.69 ± 0.40 l	2.11 ± 0.15 j-l	40.61 ± 1.30 t-x	90.90 ± 2.43 u	4.0	351.94 ± 4.30 s
3	Luczistaja	196.95 ± 4.68 n	168.89 ± 2.30 hi	7.00 ± 0.62 jk	2.36 ± 0.15 i-l	53.57 ± 1.26 p-s	175.43 ± 2.35 st	4.3	604.20 ± 6.28 p-r
4	Maryja	185.35 ± 4.92 n	97.30 ± 2.53 pq	16.94 ± 0.89 g	3.16 ± 0.20 g-i	58.17 ± 1.64 o-r	182.88 ± 2.94 s	4.2	543.78 ± 3.57 q-s
5	Podarok Sadu	164.13 ± 4.53 o	125.11 ± 2.05 m	7.29 ± 0.57 j	3.68 ± 0.26 e-g	63.19 ± 1.27 op	136.51 ± 3.02 t	6.6	499.92 ± 5.26 rs
6	Prozrocznaja	303.36 ± 3.99 j	217.24 ± 2.86 f	5.96 ± 0.56 k	1.93 ± 0.22 k-l	61.96 ± 1.80 oq	117.60 ± 2.85 t	3.1	708.03 ± 6.85 o-q
7	Tatiana	89.44 ± 1.05 q	32.56 ± 1.14 w	21.20 ± 0.91 d	1.70 ± 0.16 lm	45.09 ± 1.54 s-w	169.92 ± 3.11 st	5.1	359.91 ± 3.70 s
Endocarp									
1	Bot.-Ljubitel.	49.84 ± 1.64 v	73.70 ± 1.55 rs	2.09 ± 0.13 n	nd	120.23 ± 2.45 lm	636.21 ± 8.20 o	3.2	882.05 ± 10.3 no
2	Golden Rain	47.50 ± 1.50 vw	57.30 ± 1.12 uv	4.80 ± 0.33 l	nd	89.49 ± 1.95 n	380.62 ± 3.49 r	3.5	579.71 ± 6.25 p-r
3	Luczistaja	64.03 ± 1.62 s-u	109.34 ± 2.85 n	4.82 ± 0.40 l	nd	192.18 ± 3.92 h	290.09 ± 3.92 s	1.9	660.46 ± 5.17 p-r
4	Maryja	60.64 ± 1.62 tu	66.05 ± 1.45 st	13.62 ± 0.57 h	nd	168.45 ± 2.53 jk	359.68 ± 3.74 r	3.1	668.44 ± 6.30 p-r
5	Podarok Sadu	61.97 ± 1.38 tu	75.27 ± 1.94 r	3.77 ± 0.21 lm	nd	83.12 ± 1.45 n	474.91 ± 3.80 q	4.3	699.05 ± 4.90 o-q
6	Prozrocznaja	75.29 ± 2.04 rs	75.91 ± 1.85 r	3.18 ± 0.25 lm	nd	127.24 ± 2.54 l	456.84 ± 4.12 qr	3.0	738.45 ± 5.12 o-q
7	Tatiana	47.74 ± 1.09 v	51.26 ± 1.24 v	17.36 ± 0.74 fg	nd	178.60 ± 2.83 ij	375.81 ± 3.05 r	3.8	670.77 ± 3.06 p-r
Seeds									
1	Bot.-Ljubitel.	59.48 ± 1.64 tu	109.01 ± 2.43 no	1.32 ± 0.10 n	nd	51.58 ± 1.33 p-t	1282.71 ± 24.8 k	10.2	1504.10 ± 13.5 j-l
2	Golden Rain	54.80 ± 1.56 u	101.35 ± 2.23 o-q	1.05 ± 0.08 o	nd	36.29 ± 1.20 v-x	1144.60 ± 30.2 l	5.3	1338.09 ± 12.6 l
3	Luczistaja	50.79 ± 1.35 v	78.24 ± 2.06 r	1.31 ± 0.13 n	nd	30.08 ± 1.54 x	967.49 ± 19.4 mn	7.8	1127.91 ± 16.9 m
4	Maryja	60.16 ± 1.20 tu	103.33 ± 2.64 n-p	1.03 ± 0.13 o	nd	33.18 ± 1.09 wx	1297.65 ± 48.3 k	5.5	1495.36 ± 14.0 j-l
5	Podarok Sadu	82.17 ± 1.65 qr	150.24 ± 2.39 k	1.22 ± 0.05 n	nd	47.47 ± 2.07 rv	1623.86 ± 50.3 h	7.6	1904.96 ± 22.4 i
6	Prozrocznaja	68.13 ± 1.93 st	109.79 ± 1.95 n	1.49 ± 0.21 n	nd	53.25 ± 1.49 p-s	1190.61 ± 47.0 l	10.5	1423.26 ± 22.7 kl
7	Tatiana	84.55 ± 2.05 qr	137.90 ± 2.83 l	1.16 ± 0.09 n	nd	39.14 ± 1.28 u-x	1360.63 ± 28.3 j	9.1	1623.38 ± 13.8 j
Branches									
1	Bot.-Ljubitel.	36.66 ± 1.67 w	5.40 ± 2.57 xy	nd	nd	1823.77 ± 8.02 d	5630.83 ± 53.4 f	2.5	7496.66 ± 23.5 e
2	Golden Rain	19.60 ± 0.95 y	1.51 ± 1.30 xy	nd	nd	2215.51 ± 9.23 c	7028.88 ± 60.8 d	2.4	9265.49 ± 30.2 c
3	Luczistaja	24.61 ± 1.36 wx	1.69 ± 1.82 xy	nd	nd	2690.62 ± 8.45 b	9481.02 ± 73.5 a	2.5	12197.94 ± 26.1 a
4	Maryja	145.95 ± 3.93 p	9.47 ± 2.40 x	nd	nd	24325.10 ± 8.83 a	9083.70 ± 66.1 b	2.4	11531.76 ± 22.3 b
5	Podarok Sadu	7.12 ± 1.85 z	1.91 ± 0.73 xy	nd	nd	1773.83 ± 7.34 e	6341.54 ± 48.3 e	2.4	8124.40 ± 30.4 d
6	Prozrocznaja	25.16 ± 2.04 wx	2.22 ± 0.93 xy	nd	nd	1719.38 ± 6.20 f	5587.42 ± 32.2 f	2.6	7334.18 ± 31.5 e

(*continued on next page*)

Table 2 (*continued*)

No.	Fractions and cultivars	Phenolic compounds (mg/100 g dm)							
		Flavonols			Phenolic acids	Flavan-3-ols	Polymeric procyanidins	DP	Total phenolic compounds
		Quercetin derivatives	Isorhamnetin derivatives	Kaempferol derivatives					
7	Tatiana	7.41 ± 1.42 z	0.71 ± 0.21 xy	nd	nd	1493.52 ± 6.08 g	7778.49 ± 63.5 c	2.3	9280.13 ± 38.2 c
Leaves									
1	Bot.-Ljubitel.	574.90 ± 5.03 f	118.86 ± 3.51 m	19.41 ± 1.64 e	5.69 ± 0.78 b	85.00 ± 3.47 n	324.60 ± 5.43 rs	2.9	1128.46 ± 6.63 m
2	Golden Rain	632.45 ± 6.22 e	157.83 ± 3.69 jk	26.56 ± 1.83 b	4.44 ± 0.45 c-e	116.63 ± 3.66 lm	921.46 ± 8.05 n	2.5	1859.37 ± 8.21 i
3	Luczistaja	786.94 ± 6.40 c	193.99 ± 3.53 g	25.01 ± 1.95 c	5.35 ± 1.12 bc	186.97 ± 3.94 hi	1040.88 ± 14.0 m	2.7	2239.15 ± 11.5 h
4	Maryja	1035.21 ± 7.60 a	238.28 ± 4.20 e	31.81 ± 1.49 a	4.16 ± 0.83 d-f	119.66 ± 2.50 lm	1721.07 ± 12.5 g	2.2	3150.19 ± 13.5 f
5	Podarok Sadu	805.22 ± 6.34 b	167.70 ± 3.47 hi	14.50 ± 0.48 h	8.12 ± 0.94 a	91.54 ± 2.07 n	927.20 ± 8.93 n	2.6	2014.28 ± 9.61 i
6	Prozrocznaja	463.30 ± 5.09 h	161.88 ± 3.74 ij	5.98 ± 0.35 k	5.93 ± 0.56 b	112.39 ± 2.54 m	345.97 ± 5.35 r	3.3	1095.45 ± 6.56 m
7	Tatiana	805.47 ± 6.37 b	175.24 ± 4.02 h	11.64 ± 0.82 i	4.12 ± 0.54 d-f	160.87 ± 2.87 k	1540.35 ± 12.6 i	2.3	2697.69 ± 11.7 g
		Tukey's multiple comparison							
Skins		397.09 B	307.97 A	16.09 B	3.05 B	65.68 D	503.42 D		1293.30 D
Flesh		186.58 C	114.08 C	9.58 C	2.38 B	53.29 E	141.25 F		507.16 F
Endocarp		58.15 D	72.69 D	7.09 D	nd	137.04 B	424.88 E		669.85 E
Seeds		65.73 D	112.84 C	1.23 E	nd	41.57 F	1266.79 B		1488.15 C
Branches		38.07 E	3.27 E	nd	nd	2021.31 A	7275.98 A		9318.65 A
Leaves		729.07 A	173.40 B	19.27 A	5.40 A	124.72 C	974.50 C		2026.37 B

Bot.-Ljubitel. – Botaniczeskaja-Ljubitelskaja; dm – dry matter; DP - average procyanidins polymerization degree by thiolysis; nd – not detectable. Data are shown as mean (n = 3) ± standard deviation (SD); for each parameter tested values with different letters differ significantly (Tukey's HSD test, $p < 0.05$).

seeds and endocarp, which was not reported in the literature before. Arimboor and Arumughan (2012) identified Q-3-*O*-rutinoside and I-3-*O*-rutinoside as the main flavonoid glycosides of sea buckthorn seeds from the Indian Himalayan Region; however, this study did not identify these monoglucosides either in seeds or in branches.

The branches and leaves had a significantly high content of Q-3-*O*-glucoside-7-*O*-rhamnoside (peak 7, $[M-H]^-$ at *m/z* 609.13) ($p < 0.05$), and most of the leaves were also abundant in Q-3-*O*-glucoside and Q-3-*O*-rutinoside (peak 9, $[M-H]^-$ at *m/z* 609.13) (except for 'Prozroczznaja'). Rutin was identified in addition to leaves, also in skins, flesh and endocarp (except for 'Tatiana'), contrary to cultivars grown in Canada, including 'Golden Rain', also analyzed in this study, where rutin was identified only in leaves (Fatima et al., 2015). It was one of the main flavonols in sea buckthorn berries grown in Romania (Criste et al., 2020) and sea buckthorn berries and leaves from the Czech Republic (Bittová et al., 2014). Similar to Romanian sea buckthorn leaves (Pop et al., 2013), no Q-3-*O*-rhamnoside (peak 17, $[M-H]^-$ at *m/z* 6447.03) was detected in leaves and branches, although it was the most abundant flavonol in leaves and shoots studied by Criste et al. (2020) and Bittová et al. (2014).

The above-mentioned reports analyzed cultivars grown in the same USDA hardiness zone for Europe (6) and using liquid chromatography with DAD/PDA detection, as in this study. Hence, the differences in the qualitative and quantitative profile of the fraction can be explained by the accumulation of flavonols depending on the plant age, development phase of the vegetative and generative parts, harvesting date, subspecies, cultivars and genetic, agronomic, annual or seasonal variations, origins and growth place, post factors and plant defense (Fatima et al., 2015; Morgenstern et al., 2014; Šne et al., 2013; Yang et al., 2009). Moreover, according to Sharma et al. (2008), the flavonol content in seeds, leaves, pulp and whole sea buckthorn fruits was strongly dependent on extraction, and Soxhlet and microwave-assisted extraction seem to be more efficient than maceration and ultrasound.

Fatima et al. (2015) reported that the putative flavonoid biosynthetic genes identified in the *H. rhamnoides* seed transcriptome were expressed in both leaves and pulp with seed according to RT-PCR analysis, although the relative expression patterns of some genes may be different in these fractions. Thus, the flavonoid biosynthesis genes are expressed in a tissue-specific and developmentally regulated manner, which explains the differences and disproportionality in the phenolic profile of the sea buckthorn fraction. For example, Bittová et al. (2014) investigated the increase in Q-3-*O*-rhamnoside content along with the elongation of berries and leaves (even 10 times), while the concentration in shoots did not change depending on harvesting time.

Other Q derivatives identified in this study: Q-3-*O*-sophoroside-7-*O*-rhamnoside (peak 1, $[M-H]^-$ at *m/z* 771.18), Q-3-*O*-(2-rutinosyl) glucoside (peak 4, $[M-H]^-$ at *m/z* 771.18), Q-3-*O*-(2,6-dirhamnosyl) glucoside (peak 6, $[M-H]^-$ at *m/z* 755.18), and Q-3-*O*-rhamnoside (peak 17, $[M-H]^-$ at *m/z* 447.03) were among the most abundant in skins and flesh, while the profile of other berry parts was similar to that for branches and leaves (Table 1). In the anatomical berry parts and leaves of all cultivars, I derivatives were identified: I-3,7-*O*-diglucoside (peak 2, $[M-H]^-$ at *m/z* 639.15), I-3-*O*-rutinoside-7-*O*-glucoside (peak 3, $[M-H]^-$ at *m/z* 785.18), I-3-*O*-sophoroside-7-*O*-rhamnoside (peak 5, $[M-H]^-$ at *m/z* 785.18; except for leaves), I-3-*O*-(2,6-dirhamnosyl) glucoside (peak 8, $[M-H]^-$ at *m/z* 769.20) and I aglycone (peak 19).

Flavonols tentatively identified as I-3-*O*-glucoside with rhamnosyl residues in position 2 and 6 (peaks 12 and 14, $[M-H]^-$ at *m/z* 623.10) and unknown isorhamnetin derivatives (peaks 20 and 21, $[M-H]^-$ at *m/z* 707.09) were present only in skins and flesh, and in endocarp, seeds and leaves depending on cultivars (Table 1). Generally, skins and flesh had a more complex flavonol profile (21 compounds each) than branches, with only one Q and I derivative identified, (except for 'Botaniczeskaja-Ljubitelskaja', in which flavonols 1 and 2 were also detected).

The content of Q and I derivatives within the individual fractions of sea buckthorn was strongly correlated and the Pearson correlation coefficient (r) was between 0.82 and 0.95, except for endocarp ($r = 0.55$). The ratio of the sum of Q derivatives to I derivatives was higher than 1.0 for skins, flesh, branches and leaves (1.3, 1.6, 1.6 and 4.2, respectively). The cultivar 'Prozrocznaja' contained about 15 times in the case of skins and about 4 times in the case of flesh more Q and I derivatives than those

parts of 'Tatiana' – the poorest in flavonols (Table 2). Conversely, seeds of 'Tatiana' and 'Podarok Sadu' contained the most Q and I derivatives (82.17 mg of Q derivatives to 150.24 mg of I derivatives per 100 g dm). Branches and leaves of 'Maryja' contained significantly higher concentrations of flavonols than other cultivars (9.47 mg of I derivatives in branches to 1035.21 mg of Q derivatives per 100 g dm in leaves), which was up to 20 times more flavonols than in other cultivars.

The LC–MS fingerprint also showed the presence of two K derivatives ($[M-H]^-$ at *m/z* 593.05): K-3-*O*-glucoside-7-*O*-rhamnoside and K-3-*O*-rutinoside (peaks 13 and 18) (Table 1). Contrary to the cultivars analyzed in this study, compound 13 was one of the dominant flavonols in young shoots and leaves of 'Botaniczeskaja-Ljubitelskaja' and 'Prozrocznaja' collected in Latvia (Radenkovs et al., 2018). In turn, K-3-*O*-rutinoside was the main derivative, alongside K-3-*O*-neohesperidoside (not identified here) in leaves of well-established cultivars in Romania (Pop et al., 2013). The presence of K derivatives was selective and dependent on the fraction and cultivar (for example, seeds contained only rutinoside derivative), while none of the K derivatives were found in branches. The skins, flesh and endocarp of 'Maryja' and 'Tatiana' contained the highest concentration of kaempferols. The deeper the berry part (from skin to seed), the lower the content of K derivatives. Moreover, leaves were the richest in K derivatives, and 'Golden Rain', 'Luczistaja' and 'Maryja' contained on average 20 times more of these compounds than seeds.

In the research by Fatima et al. (2015), the cultivar 'Golden Rain' had the lowest flavonoid concentration among sea buckthorn berries of Canadian grown cultivars, and kaempferol in leaves differed almost 3 times between consecutive harvest years. But the sums of flavonols of berry fractions and branches of 'Golden Rain' harvested in Poland were below the average for all cultivars.

Other studies also report the presence of vitexin and luteolin in leaves and berries, myricetin in leaves (Criste et al., 2020; Yogendra Kumar et al., 2013) or myricetin only in whole berries but not leaves (Fatima et al., 2015), which were not detected in sea buckthorn harvested in Poland.

In terms of the total flavonol levels, the sea buckthorn parts can be ranked as follows: branches < endocarp < seeds < flesh < skins < leaves (mean total levels between 41.34 mg and 921.74 mg/100 g dm) (Table 2). Leaves and skins were the most abundant in flavonols, with leaves being the main source of Q derivatives and skins of I derivatives. Branches contained on average from 17 to 22 times lower flavonol concentration in relation to leaves and skins, respectively.

3.1.2. Phenolic acids

Quantification using the UPLC-PDA method indicated that sea buckthorn leaves were on average 2 times more abundant in phenolic acids than the flesh and skin (Table 2). Phenolic acids were not detected in endocarp, seeds and branches. Strong correlations were found between the content of phenolic acids in skins and leaves ($r = 0.80$) and skins and flesh ($r = 0.72$). The sea buckthorn cultivar 'Podarok Sadu' contained the most phenolic acids and were 2 times (in the case of leaves) to almost 6 times (in the case of skins) more abundant in these metabolites than 'Tatiana', in which the lowest concentrations were determined.

Previous studies showed about 5 times higher content of hydroxybenzoic and hydroxycinnamic acid derivatives in seeds and leaves than in pulp of *H. rhamnoides* berry, with dominant gallic acid (Arimboor et al., 2008). This acid was found in a high concentration in leaves of 'Golden Rain', also analyzed in this study (Fatima et al., 2015). Other reports also indicated high levels of *p*-coumaric acid hexoside in berries and shoot with leaves of 'Botaniczeskaja-Ljubitelskaja', 'Prozrocznaja', 'Luczistaja', 'Podarok Sadu' (Radenkovs et al., 2018; Tkacz et al., 2020), and salicylic acid in whole berries of 'Podarok Sadu' (Zadernowski et al., 2005).

In addition to free phenolic acids, sea buckthorn contains phenolic acids liberated from glycosidic bonds predominant in pulp, and phenolic acids bound as esters, which are the main fraction in leaves, seed kernel and coat. Therefore, their detection depends on solvent polarity, extraction and chromatographic conditions (Arimboor et al., 2008). Moreover, autumn leaves, annual green shoots and sea buckthorn fruits contained lower concentrations of phenolic acids than the spring samples (Bittová et al., 2014).

3.1.3. Flavan-3-ols and polymeric procyanidins

The content of flavan-3-ols, as (+)-catechin, (-)-epicatechin, (-)-epicatechin-gallate, (-)-epigallo-catechin, determined by the UPLC-PDA method was on average from 41.57–2021.31 mg/100 dm and the sea buckthorn fractions in this respect can be arranged as follows: seeds < flesh < skins < leaves < endocarp < branches (Table 2). Branches contained about 15 times more flavan-3-ols than endocarp and leaves, and 30–48 times more than other berry parts. Endocarp, branches and leaves of 'Luczistaja', and skins and flesh of 'Prozrocznaja' and 'Podarok Sadu' had the highest flavan-3-ol concentrations within the fractions. Flavan-3-ols of skins and flesh were moderately correlated ($r = 0.64$), in contrast to the negative correlations of seeds with branches, leaves and endocarp ($r = -0.75$, -0.72, and -0.56, respectively).

The UPLC-FL analysis of terminal units of polymeric procyanidins (PP) after phloroglucinol cleavage revealed the dominance of (+)-catechin in quantitatively differentiated polymer structures. Vegetative parts and seeds were better sources of PP than the soft tissues of berry, and the fractions can be ranked by PP content as follows: flesh < endocarp < skins < leaves < seeds < branches (mean from 141.25 mg to 7275.98 mg/100 g dm).

Research on sea buckthorn seeds by Fan et al. (2007) detected, in addition to catechin and epicatechin, also epigallocatechin and gallocatechin, especially abundant in terminal units in highly heterogeneous polymers, similar to sea buckthorn pomace studied by Rösch et al. (2004). This is all the more important as epigallocatechin contained in *H. rhamnoides* branches shows significantly stronger inhibition of tumor promoter-induced inflammation than catechin and epicatechin (Yasukawa et al., 2009).

Bittová et al. (2014) identified catechin as the dominant unit in shoots and fruits, as in this research, but leaves were more abundant in epicatechin and levels of these compounds were up to 35 times lower than in the fractions tested. In 100 g dm of apple, quince, Japanese quince, cranberry and blackcurrant leaves there was from 239 to 11.22 mg of PP (Teleszko and Wojdyło, 2015); hence sea buckthorn leaves could be considered a source of PP with an average content. It is known that as fruit and leaves grow and mature, the PP amount decreases, and conversely, catechins can increase in branches (Bittová et al., 2014). In addition to the specificity of the method and type of plant and its fraction, the harvest period may therefore explain the differences in the proportions between polymeric procyanidin units.

The highest concentrations of PP within the fraction were determined in skins and seeds of 'Podarok Sadu' and 'Tatiana' and in flesh and branches of 'Luczistaja' and 'Maryja'. Leaves of these cultivars were almost 3–5 times more abundant in PP than leaves of 'Botaniczeskaja-Ljubitelskaja', whose endocarp had exceptionally two times more PP than leaves. The low-polymerized flavan-3-ols and procyanidin polymers strongly correlated ($r = 0.97$), and the ratio of PP to flavan-3-ols ranged from approx. 2.7 in flesh, followed by 3.6 in endocarp and shoots, 8.0 in peel and leaves, to 31.3 in seeds.

The average degree of procyanidin polymerization (DP) (number of flavan-3-ol units in polymers) ranged from 2.4 (branches), followed by 2.6 (leaves), to an average of 8.0 (seeds). The presence of dimeric and oligomeric flavan3-ols and the weak negative correlations of DP and PP, and DP and flavan-3-ols ($r = -0.30$ and -0.39) indicate a potentially low intensity of astringency in food application.

3.1.4. Total phenolic compounds

The total phenolic compounds (TPC) accounted for on average from 0.51 % to 9.32 % of dry weight of flesh and branches, respectively. The

sea buckthorn parts can be ranked according to the increasing level of TPC: flesh < endocarp < skins < seeds < leaves < branches (507.16 mg–9318.65 mg/100 g dm). Previous studies only reported that TPC in leaves is several times higher than that of whole sea buckthorn berries and other types of berries and pome fruits, leaving aside their anatomical parts (Bittová et al., 2014; Criste et al., 2020; Teleszko and Wojdyło, 2015). This research however showed that branches are 4.6 times more abundant in phenolic compounds than leaves, about 6 times more than seeds, and about 7 times more than skins. Flesh and endocarp were found to contain from 3 to several times less of these compounds than the fractions. The sum of mono-, di-, oligo- and polymeric flavan-3-ols in endocarp and seeds accounted for over 80 %, and in the case of shoots more than 99 % of total phenolic compounds. The flesh was the source of flavonols with about 60 % of TPC. In most skins and leaves the ratio of flavan-3-ols to flavonols was 1:1, but the TPC proportions were determined by cultivars.

Contrary to results obtained previously, seeds and roots were significantly more abundant in TPC than leaves, stems or bark (Michel et al., 2012; Saikia and Handique, 2013). Nevertheless, the difference can be attributed to the spectrophotometric method using Folin--Ciocâlteu reagent used in the earlier works, which, due to interferences with other bioactive components of sample, is burdened with greater error than the UPLC technique.

3.2. Triterpenoids in anatomical parts of sea buckthorn berries, branches and leaves

All 11 triterpenoids were identified on the basis of reference standards using ultra high performance liquid chromatography analysis UPLC-PDA and additionally confirmed by mass spectrometry LC-ESI-Q/TOF-MS; the retention times, UV spectrum, exact mass, accurate mass, mass errors, and molecular formulas are presented in Table 3. The pentacyclic triterpenoids determined in sea buckthorn fractions can be divided into two groups. The first one was composed of maslinic acid, pomolic acid, corosolic acid, betulinic acid, oleanolic acid, ursolic acid, and betulin determined in significant quantities (Table 3). In contrast, tormentic acid, α-boswellic acid, uvaol, and erythrodiol (only in branches and leaves) were found in amounts below 1 mg/100 g dm, and their sum represented about 2% of all triterpenoids in each parts.

Oleanolic and ursolic acids are commonly reported triterpenoids in the low-polar and non-polar fractions of many plants (Różalska et al., 2018). So far, the presence of these two acids, along with maslinic acid and their derivatives, has been investigated in whole berries, seeds, leaves, roots, twigs and extract of branch bark of sea buckthorn (Grey et al., 2010; Marciniak et al., 2021; Michel et al., 2012; Olas et al., 2018; Yang et al., 2007). Previous research also indicated that leaves contain high levels of triterpenoid saponins, unlike twigs and fruits, which in turn are abundant in free triterpenoids and triterpenoids acylated with phenolic acids, mainly coumaric and ferulic acids (Różalska et al., 2018; Yang et al., 2007).

This study was the first to identify 11 triterpenoids for individual berry fractions, and also indicated new triterpenes, pomolic acid, corosolic acid, betulinic, betulin, tormentic acid, α-boswellic acid, uvaol, and erythrodiol, for berry parts, branches and leaves of various *H. rhamnoides* cultivars. The distinctive feature of berry fractions (skins, flesh, endocarp, seed) was the dominance of pomolic acid, which constituted from 34 % to 57 % of the total triterpenoids, for skins and endocarp, respectively. Maslinic acid and pomolic acid were strongly correlated ($r = 0.98$), and flesh was over 20 times richer than leaves. In turn, the best source of corosolic acid and betulinic acid were branches (on average 10.94 mg and 1.93 mg/100 g dm, respectively). Skins were characterized by the highest average amounts of oleanolic acid, ursolic acid and betulin. However, in the case of acids, the content differed even 10 times between cultivars. As with maslinic acid and pomolic acid, leaves were the least abundant fraction in oleanolic acid.

However, leaves contained significantly high concentrations of ursolic acid, from 6.10 to 9.14 mg/100 g dm (except for 'Botaniczeskaja-Ljubitelskaja'), *i.e.* on average 46 % of the total triterpenoids. This may be important for the use of sea buckthorn skins and leaves in the prevention of inflammation, as *in vitro* and *in vivo* studies suggest inhibition of tumor promotion by ursolic acid, including recent research on branches of *H. rhamnoides* (Marciniak et al., 2021; Yasukawa et al., 2009). All triterpenoids identified also share antimicrobial, hepatoprotective, and antioxidant activities (Różalska et al., 2018). In the future use of plant parts, the extraction efficiency and extract polarity should be taken into account, as these determine the concentration of triterpenoids in sea buckthorn and their biological potential. For example, in a study by Grey et al. (2010), the ethyl acetate fraction of 'Podaruk Sadu' berries ('Podarok Sadu' in this paper) was exclusively found to contain high contents of ursolic acid (190 mg/100 g dw), together with low levels of phenolic compounds.

And finally, the amounts of betulin in skins were significantly higher ($p < 0.05$) (21 % of the total triterpenoids), compared to leaves – over 11 times, and to seeds and endocarp – over 40 times. The total content of triterpenoids determined the following relationships between sea buckthorn fractions: leaves < seeds ≈ endocarp ≈ branches < skins < flesh, with a clear ratio of these compounds 1: 2: 2: 2: 4: 5, respectively. The fraction determined the triterpenoid content more strongly than the cultivars. Nevertheless, skins, flesh, endocarp and seeds of 'Prozroczna-ja' were the best sources of triterpenoids within each fraction. 'Botaniczeskaja-Ljubitelskaja' had significantly high amounts of triterpenoids in skins and seeds, in contrast to endocarp, branches and leaves in which the lowest concentrations of these analytes were determined. The common feature of 'Golden Rain' and 'Tatiana' were the highest triterpenoid levels in branches and the lowest in seeds. Hence, there were strong positive correlations between skins and seeds ($r = 0.72$), leaves and endocarp ($r = 0.77$) and leaves and branches ($r = 0.75$), compared to negative correlations of seeds with branches and leaves ($r = -0.61$ and -0.66, respectively).

3.3. Minerals in anatomical parts of sea buckthorn berries, branches and leaves

The application of flame atomic absorption spectroscopy (FAAS) was focused on the detection of four macroelements: sodium, potassium, calcium and magnesium, and four microelements: iron, copper, zinc and manganese, a complete overview of which in sea buckthorn fractions is presented in Table 4. All eight minerals were identified based on reference standards.

3.3.1. Macroelements

The macroelement contents were more heterogeneous within cultivars and fractions than microelements, especially in the case of leaves. The best sources of sodium were endocarp and leaves, potassium – flesh and endocarp, and calcium – leaves. Seeds provided the least sodium, potassium and calcium, but were distinguished by the highest concentration of magnesium (Table 4). Sodium constituted just 1% to 2 % of macroelements. All fractions of 'Golden Rain' had significantly higher sodium concentrations, as did endocarp and branches of 'Botaniczeskaja-Ljubitelskaja'. Therefore, the sea buckthorn consumption does not threaten to reach the tolerable upper intake level for sodium (2,300 mg per day), the higher dose of which adversely affects blood pressure (Gutzeit et al., 2008). Hypertension is accompanied by a low concentration of potassium, calcium and magnesium in body; hence it seems justified for consumers with this disease to use products based on sea buckthorn parts much richer in these macroelements.

Potassium was the dominant element (from 74 % in branches to 93 % of macroelements in flesh), except for leaves in which calcium constituted a significant portion (55 % of macroelements). In terms of potassium, the fractions can be divided into 2 groups: flesh, endocarp and leaves (706.16–794.39 mg/100 g dm) and skins, seeds and branches (553.41–578.62 mg/100 g dm). Regular consumption of food with sea

Table 3
Quantification of triterpenoids (mg/100 g dm) in anatomical parts of sea buckthorn berries, branches and leaves by UPLC-PDA.

		Triterpenoids (mg/100 g dm)							
		Maslinic acid	Pomolic acid	Corosolic acid	Betulinic acid	Oleanolic acid	Ursolic acid	Betulin	
	R_t (min)	5.946	6.087	6.359	8.781	11.248	13.591	14.185	
	λ_{max} (nm)	200	194	200	205	212	214	204	
MS [M-H]$^-$ (m/z)	Exact mass	471.3553	471.3553	471.3553	455.3603	455.3603	455.3603	441.3811	
	Accurate mass	471.3001	471.3012	471.3005	455.2998	455.2988	455.2988	441.3098	Total
Mass error (ppm)		−117.1	−114.8	−116.3	−132.9	−135.1	−135.1	−161.5	
No.	Fractions and cultivars	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_3$	$C_{30}H_{48}O_3$	$C_{30}H_{48}O_3$	$C_{30}H_{50}O_2$	
Skins									
1	Bot.-Ljubitel.	7.88 ± 0.54 e	34.17 ± 1.64 d	10.52 ± 0.75 cd	0.37 ± 0.05 p-r	1.64 ± 0.12 o-q	4.70 ± 0.44 g	12.38 ± 1.55 bc	73.02 ± 3.26 ab
2	Golden Rain	3.34 ± 0.52 k-n	16.46 ± 1.48 f-h	10.63 ± 0.71 bc	0.42 ± 0.03 o-q	3.97 ± 0.39 e-h	7.06 ± 0.45 d	17.08 ± 1.70 a	60.49 ± 3.05 de
3	Luczistaja	6.41 ± 0.55 fg	31.40 ± 1.53 d	11.00 ± 0.73 ab	0.42 ± 0.03o-q	2.79 ± 0.20 mn	5.62 ± 0.40 ef	11.31 ± 1.63 c	70.41 ± 3.82 bc
4	Maryja	4.98 ± 0.51 g-i	21.79 ± 1.47 e	6.61 ± 0.56 jk	0.85 ± 0.04 ij	7.21 ± 0.31 c	6.89 ± 0.47d	12.75 ± 1.40 bc	62.75 ± 3.01 cd
5	Podarok Sadu	2.20 ± 0.59 no	2.70 ± 1.18 n-p	7.91 ± 0.61 hi	0.60 ± 0.03 mn	3.05 ± 0.27 lm	8.45 ± 0.49 bc	13.30 ± 1.58 b	39.74 ± 2.45 f
6	Prozrocznaja	7.12 ± 0.50 ef	31.37 ± 1.52 d	8.39 ± 0.75 gh	0.41 ± 0.02 o-q	2.63 ± 0.18 n	7.91 ± 0.52 c	16.99 ± 1.82 a	76.38 ± 3.23 a
7	Tatiana	4.14 ± 0.58 i-k	12.23 ± 1.47 i-k	7.89 ± 0.62 hi	1.55 ± 0.08 f	11.81 ± 0.14 a	4.54 ± 0.45 g	9.11 ± 1.43 d	53.03 ± 2.87 e
Flesh									
1	Bot.-Ljubitel.	9.80 ± 0.93 cd	42.60 ± 1.71 bc	9.31 ± 0.64 fg	0.53 ± 0.05 no	0.52 ± 0.14 y	0.37 ± 0.14 l	0.72 ± 0.19 f	65.05 ± 3.57 cd
2	Golden Rain	10.06 ± 0.88 cd	40.58 ± 1.78 c	8.56 ± 0.68 gh	0.70 ± 0.04 k-m	1.85 ± 0.26 o	0.51 ± 0.09 kl	0.63 ± 0.10 f	63.75 ± 3.45 cd
3	Luczistaja	10.93 ± 0.87 c	44.62 ± 1.60 b	10.00 ± 0.61 ef	0.82 ± 0.07 i-k	1.58 ± 0.22 o-r	0.38 ± 0.08 l	0.78 ± 0.14 f	70.05 ± 3.81 bc
4	Maryja	14.61 ± 0.93 a	41.50 ± 1.67 bc	11.89 ± 0.65 a	1.13 ± 0.14 g	4.61 ± 0.31 d	0.50 ± 0.05 kl	0.84 ± 0.16 ef	76.23 ± 3.86 a
5	Podarok Sadu	10.08 ± 0.99 cd	42.95 ± 1.73 bc	9.94 ± 0.62 ef	0.61 ± 0.09 mn	0.76 ± 0.15 w-y	0.46 ± 0.05 kl	0.72 ± 0.10 f	66.54 ± 3.07 cd
6	Prozrocznaja	12.59 ± 0.87 b	49.11 ± 1.74 a	10.22 ± 0.70 de	0.91 ± 0.06 hi	0.72 ± 0.15 xy	0.41 ± 0.04 l	1.11 ± 0.23 f	75.90 ± 3.65 a
7	Tatiana	9.35 ± 0.86 d	40.17 ± 1.51 c	7.96 ± 0.67 hi	1.41 ± 0.08 f	8.50 ± 0.38 b	0.61 ± 0.09 kl	0.38 ± 0.05 f	69.76 ± 3.48 bc
Endocarp									
1	Bot.-Ljubitel.	2.56 ± 0.22 L-o	10.20 ± 1.26 kl	3.98 ± 0.37 no	0.15 ± 0.05 t	0.58 ± 0.10 xy	4.31 ± 0.36 gh	0.17 ± 0.12 f	22.46 ± 2.04 mn
2	Golden Rain	5.19 ± 0.35 g-i	20.63 ± 2.27 e	4.99 ± 0.23 lm	0.29 ± 0.08 r-t	1.12 ± 0.21 s-u	0.83 ± 0.13 j-l	0.43 ± 0.25 f	33.97 ± 2.43 f
3	Luczistaja	5.52 ± 0.36 gh	19.92 ± 1.47 ef	6.60 ± 0.38 jk	0.29 ± 0.05 r-t	1.04 ± 0.17 u-w	0.71 ± 0.15 j-l	0.39 ± 0.23 f	34.92 ± 2.56 f
4	Maryja	5.03 ± 0.30 g-i	19.31 ± 1.30 e-g	5.36 ± 0.36 lm	0.36 ± 0.04 p-r	1.73 ± 0.25 op	0.79 ± 0.12 j-l	0.36 ± 0.20 f	33.43 ± 2.12 f
5	Podarok Sadu	3.89 ± 0.36 i-l	16.29 ± 1.38 gh	5.53 ± 0.29 lm	0.18 ± 0.02 st	0.86 ± 0.23 v-x	0.86 ± 0.12 j-l	0.22 ± 0.19 f	28.21 ± 2.62 ij
6	Prozrocznaja	4.96 ± 0.28 g-i	19.84 ± 1.41 ef	4.87 ± 0.27 lm	0.51 ± 0.08 n-p	3.47 ± 0.48 i-k	0.42 ± 0.12 l	0.33 ± 0.22 f	34.73 ± 2.03 f
7	Tatiana	4.37 ± 0.41 h-j	18.48 ± 1.37 e-g	5.16 ± 0.39 lm	0.23 ± 0.04 r-t	1.06 ± 0.17 t-v	1.25 ± 0.27 j	0.30 ± 0.26 f	31.41 ± 2.25 f-h
Seeds									
1	Bot.-Ljubitel.	3.49 ± 0.26 k-m	16.83 ± 0.63 f-h	6.28 ± 0.51 kl	4.26 ± 0.35 a	4.29 ± 0.36 de	0.44 ± 0.14 kl	0.48 ± 0.10 f	37.08 ± 2.71 f
2	Golden Rain	3.29 ± 0.25 k-n	13.67 ± 0.50 i-k	6.72 ± 0.52 jk	1.24 ± 0.05 g	1.03 ± 0.14 uw	0.38 ± 0.17 l	0.20 ± 0.14 f	27.28 ± 1.96 ik
3	Luczistaja	2.78 ± 0.25 L-o	13.41 ± 0.51 i-k	6.22 ± 0.45 kl	2.36 ± 0.12 b	2.65 ± 0.25 n	0.44 ± 0.07 kl	0.28 ± 0.11 f	29.08 ± 2.50 ij
4	Maryja	3.04 ± 0.31 L-o	14.43 ± 0.43 hi	6.97 ± 0.42 ij	1.12 ± 0.23 g	1.26 ± 0.13 r-t	0.38 ± 0.09 l	0.25 ± 0.12 f	28.28 ± 2.87 ij
5	Podarok Sadu	2.85 ± 0.20 L-o	13.62 ± 0.47 i-k	7.28 ± 0.54 ij	1.07 ± 0.20 gh	1.45 ± 0.13 p-r	0.32 ± 0.12 l	0.20 ± 0.12 f	27.55 ± 2.49 ij
6	Prozrocznaja	4.13 ± 0.33 i-k	20.89 ± 1.58 e	7.01 ± 0.47 ij	0.76 ± 0.17 j-m	0.87 ± 0.08 v-x	0.45 ± 0.16 kl	0.34 ± 0.15 f	35.22 ± 2.90 f
7	Tatiana	2.99 ± 0.43 L-o	13.50 ± 1.04 i-k	6.25 ± 0.44 kl	1.56 ± 0.32 f	1.38 ± 0.16 q-s	0.31 ± 0.10 l	0.21 ± 0.08 f	26.97 ± 2.44 kl
Branches									
1	Bot.-Ljubitel.	1.64 ± 0.11 o-r	4.83 ± 0.44 m-o	8.88 ± 0.54 gh	2.00 ± 0.05 d	3.66 ± 0.14 h-j	4.22 ± 0.30 gh	0.70 ± 0.23 f	26.61 ± 2.35 lm
2	Golden Rain	3.29 ± 0.40 k-n	13.88 ± 1.52 h-j	10.77 ± 1.43 bc	1.46 ± 0.04 f	3.38 ± 0.15 jk	3.90 ± 0.28 h	0.64 ± 0.14 f	38.24 ± 2.45 f

(*continued on next page*)

Table 3 (*continued*)

		Triterpenoids (mg/100 g dm)							
	R_t (min)	Maslinic acid 5.946	Pomolic acid 6.087	Corosolic acid 6.359	Betulinic acid 8.781	Oleanolic acid 11.248	Ursolic acid 13.591	Betulin 14.185	
	λ_{max} (nm)	200	194	200	205	212	214	204	
MS [M-H]$^-$ (m/z)	Exact mass	471.3553	471.3553	471.3553	455.3603	455.3603	455.3603	441.3811	
	Accurate mass	471.3001	471.3012	471.3005	455.2998	455.2988	455.2988	441.3098	Total
Mass error (ppm)		−117.1	−114.8	−116.3	−132.9	−135.1	−135.1	−161.5	
No.	Fractions and cultivars	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_3$	$C_{30}H_{48}O_3$	$C_{30}H_{48}O_3$	$C_{30}H_{50}O_2$	
3	Luczistaja	1.97 ± 0.15 n-q	6.66 ± 0.94 mn	10.90 ± 1.48 bc	1.77 ± 0.04 e	3.80 ± 0.15 g-i	3.83 ± 0.28 h	0.98 ± 0.25 ef	30.70 ± 2.90 hi
4	Maryja	2.12 ± 0.16 n-p	7.57 ± 0.82 lm	11.35 ± 1.03 ab	1.92 ± 0.04 de	3.76 ± 0.25 g-i	4.39 ± 0.33 gh	0.42 ± 0.14 f	32.42 ± 2.24 fg
5	Podarok Sadu	2.85 ± 0.17 L-o	10.41 ± 1.20 j-l	11.40 ± 1.00 ab	1.89 ± 0.03 de	3.19 ± 0.14 kl	2.79 ± 0.25 i	0.31 ± 0.18 f	33.88 ± 2.91 f
6	Prozrocznaja	2.33 ± 0.20 no	7.77 ± 0.95 lm	11.47 ± 0.95 ab	2.30 ± 0.09 bc	4.10 ± 0.30 ef	6.04 ± 0.40 ef	0.57 ± 0.16 f	35.33 ± 2.05 f
7	Tatiana	2.82 ± 0.23 L-o	11.56 ± 0.99 i-k	11.77 ± 1.27 a	2.19 ± 0.10 c	4.04 ± 0.41 e-g	4.81 ± 0.31 g	0.54 ± 0.23 f	39.09 ± 2.16 f
Leaves									
1	Bot.-Ljubitel.	0.59 ± 0.18 qr	2.34 ± 0.23 op	3.31 ± 0.38 o	0.67 ± 0.12 l-n	0.66 ± 0.14 xy	1.05 ± 0.11 jk	1.48 ± 0.14 e	10.40 ± 1.59 o
2	Golden Rain	0.55 ± 0.21 g	2.17 ± 0.28 op	2.99 ± 0.24 o	0.37 ± 0.05 p-r	0.61 ± 0.12 xy	9.14 ± 0.63 a	1.26 ± 0.17 e	17.32 ± 1.88 no
3	Luczistaja	0.69 ± 0.10 p-r	2.60 ± 0.30 op	4.02 ± 0.30 mn	0.53 ± 0.03 no	0.71 ± 0.13 xy	6.77 ± 0.59 d	1.17 ± 0.08 e	16.71 ± 1.53 no
4	Maryja	0.35 ± 0.14 r	1.19 ± 0.19 p	3.61 ± 0.28 o	0.33 ± 0.05 q-s	0.55 ± 0.20 xy	5.45 ± 0.52 f	1.41 ± 0.14 e	13.07 ± 1.47 o
5	Podarok Sadu	0.48 ± 0.19 r	1.91 ± 0.27op	3.29 ± 0.31 o	0.77 ± 0.06 i-l	0.64 ± 0.18 xy	6.10 ± 0.58 e	0.83 ± 0.11 ef	14.32 ± 1.50 o
6	Prozrocznaja	0.53 ± 0.23 qr	2.05 ± 0.29 op	3.43 ± 0.26 o	0.88 ± 0.06 i	0.51 ± 0.18 y	6.15 ± 0.51 e	1.10 ± 0.15 e	14.91 ± 1.48 no
7	Tatiana	0.37 ± 0.10 r	1.62 ± 0.18 op	3.09 ± 0.21 o	0.39 ± 0.05 p-r	0.72 ± 0.10 xy	9.00 ± 0.55 ab	0.92 ± 0.16 ef	16.28 ± 1.79 no
	Tukey's multiple comparison								
Skins	5.15 BC	21.45 B	8.99 C	0.66 C	4.73 A	6.45 A	13.27 A	62.26 B	
Flesh	11.06 A	43.08 A	9.70 B	0.87 C	2.65 C	0.46 D	0.74 BC	69.61 A	
Endocarp	4.50 CD	17.81 C	5.21 E	0.29 D	1.41 D	1.31 C	0.31 D	31.30 C	
Seeds	3.22 DE	15.19 D	6.67 D	1.77 B	1.85 D	0.39 D	0.28 D	30.21 C	
Branches	2.43 E	8.95 E	10.94 A	1.93 A	3.71 B	4.28 B	0.60 CD	33.75 C	
Leaves	0.51 F	1.98 F	3.39 F	0.56 CD	0.63 E	6.24 A	1.17 B	14.72 D	

Bot.-Ljubitel. – Botaniczeskaja-Ljubitelskaja; dm – dry matter; nd – not detectable; R_t – retention time; λ_{max} - UV absorption maxima.
The table contains selected triterpenoids at the highest concentrations. The sum of triterpenoids includes maslinic acid, pomolic acid, corosolic acid, betulinic acid, oleanolic acid, ursolic acid, betulin, as well as tormentic acid, erythrodiol, α-boswellic acid, and uvaol which were below 1 mg/100 g dm. Data are shown as mean (n = 3) ± standard deviation (SD); for each parameter tested values with different letters differ significantly (Tukey's HSD test, $p < 0.05$).

buckthorn parts can therefore help maintain the ionic balance and proper tissue excitability, resulting from the role of potassium in the human body (Bal et al., 2011).

The calcium amounts were not significantly different ($p > 0.05$) among the cultivars; hence they can be sequentially ranked: seeds < flesh < skins < endocarp. Leaves were on average eight times more abundant in calcium than branches.

The observation of potassium dominance was consistent with other studies of sea buckthorn berries and juice (Sabir et al., 2005; Stobdan et al., 2010). This research showed the advantage of magnesium over calcium in skins and flesh (up to two times), as well as in berries of different populations of sea buckthorn investigated by Arif et al. (2010); Gutzeit et al. (2008), and Sabir et al. (2005); however, Stobdan et al. (2010) found in juice almost eight times more calcium than magnesium. Ercisli et al. (2007) also reported nitrogen, followed by potassium, phosphorus, calcium and magnesium as the most common elements in sea buckthorn berries.

The lowest and the highest concentrations of magnesium were determined in leaves, respectively 10.52 mg for 'Prozrocznaja' and 148.05 mg/100 g dm for 'Golden Rain'. However, seeds were the best source of magnesium (15 % of macroelements), followed by branches and leaves, and finally flesh, skins and endocarp. High magnesium content and the advantage of iron and zinc over sodium were characteristic of sea buckthorn seeds also in the study by Zeb and Malook (2009). This research team reported the predominant amount of calcium in seeds; however, this study more accurately concluded that the accumulation of calcium is 25 times higher in endocarp than seeds, which contained significantly lower concentrations of this macroelement than other parts.

Strong correlations were found for calcium with manganese ($r = 0.97$), with iron ($r = 0.70$), and with sodium ($r = 0.51$), and for iron with manganese ($r = 0.73$) and with copper ($r = 0.52$).

3.3.2. Microelements

Leaves and branches were the richest in iron and copper, unlike the flesh, and a particularly valuable source was the 'Podarok Sadu' cultivar (Table 4). Skins, flesh, endocarp and branches of 'Tatiana' were characterized by the highest amounts of iron within each fraction. In turn, skins and flesh of 'Golden Rain', as well as endocarp and seeds of 'Botaniczeskaja-Ljubitelskaja' were considered the best sources of copper.

Iron dominated as a microelement, constituting from 36 % (for endocarp) to 55 % of total microelements (for seeds). In anatomical parts of berries, the lowest percentage of microelements was copper, while in branches and leaves it was zinc.

Gutzeit et al. (2008) observed a reduction in the amount of iron during sea buckthorn juice processing by up to 45 %. This study clearly

Table 4
Quantification of minerals (mg/100 g dm) in anatomical parts of sea buckthorn berries, branches and leaves by FAAS.

No.	Fractions and cultivars	Minerals (mg/100 g dm)							
		Macroelements				Microelements			
		Na	K	Ca	Mg	Fe	Cu	Zn	Mn
Skins									
1	Bot.-Ljubitel.	13.64 ± 0.15 ef	779.79 ± 46.4 bc	28.23 ± 1.27 j-n	38.00 ± 0.90 jk	2.83 ± 0.02 L	0.66 ± 0.01 o	1.20 ± 0.01 l	1.25 ± 0.03 rs
2	Golden Rain	15.87 ± 0.48 cd	542.66 ± 50.2 jk	19.69 ± 0.03 l-n	22.26 ± 0.62 q-s	1.91 ± 0.04 m-o	1.45 ± 0.02 j	1.25 ± 0.01 l	1.01 ± 0.02 uv
3	Luczistaja	4.85 ± 0.13 m-p	375.50 ± 33.8 qr	23.89 ± 1.00 k-n	26.70 ± 0.74 m-q	1.92 ± 0.02 m-o	0.29 ± 0.01 uv	1.27 ± 0.01 l	1.01 ± 0.01 uv
4	Maryja	4.46 ± 0.07 n-p	573.68 ± 63.7 ij	18.91 ± 0.52 l-n	25.08 ± 0.84 o-r	2.42 ± 0.05 lm	0.24 ± 0.01 vw	0.98 ± 0.01 o	1.22 ± 0.01 st
5	Podarok Sadu	5.69 ± 0.81 l-n	374.84 ± 63.8 qr	25.01 ± 2.87 k-n	28.02 ± 1.16 m-q	2.53 ± 0.04 lm	1.18 ± 0.01 k	1.41 ± 0.02 i	1.28 ± 0.03 qr
6	Prozrocznaja	5.62 ± 0.57 l-n	871.92 ± 57.0 bc	25.86 ± 1.93 k-n	29.48 ± 0.51 m-p	2.22 ± 0.02 l-n	0.72 ± 0.01 n	1.37 ± 0.01 j	1.18 ± 0.03 st
7	Tatiana	6.08 ± 0.01 k-n	443.22 ± 63.6 lm	23.54 ± 0.13 k-n	32.70 ± 0.50 k-m	4.52 ± 0.03 j	0.43 ± 0.01 r	1.21 ± 0.01 l	1.34 ± 0.02 pq
Flesh									
1	Bot.-Ljubitel.	7.86 ± 0.17 i-k	624.93 ± 73.6 gh	11.25 ± 0.55 m	30.83 ± 0.83 l-p	1.27 ± 0.03 o	0.45 ± 0.01 r	1.27 ± 0.01 l	1.06 ± 0.01 u
2	Golden Rain	19.92 ± 0.06 b	653.64 ± 86.6 fg	10.10 ± 0.19 m	18.64 ± 0.94 s	1.38 ± 0.03 o	0.80 ± 0.01 m	1.24 ± 0.01 l	0.83 ± 0.04 w
3	Luczistaja	5.74 ± 0.01 L-n	436.81 ± 77.9 ln	16.06 ± 0.12 m	22.64 ± 1.07 q-s	1.33 ± 0.03 o	0.21 ± 0.01 wx	1.15 ± 0.01 m	0.88 ± 0.01 vw
4	Maryja	5.73 ± 0.41 L-n	747.53 ± 65.1 cd	14.71 ± 0.79 m	35.90 ± 1.75 j-l	1.60 ± 0.04 no	0.17 ± 0.01 x	0.66 ± 0.01 s	1.22 ± 0.04 st
5	Podarok Sadu	7.06 ± 0.13 j-l	873.60 ± 92.3 bc	12.00 ± 0.57 m	36.69 ± 0.99 j-l	1.48 ± 0.02 no	0.58 ± 0.01 pq	0.92 ± 0.01 p	1.20 ± 0.02 st
6	Prozrocznaja	7.96 ± 0.01 ij	913.39 ± 90.1 bc	14.68 ± 0.12 m	32.13 ± 0.13 k-n	1.54 ± 0.01 no	0.55 ± 0.02 pq	1.29 ± 0.01 kl	1.11 ± 0.02 tu
7	Tatiana	10.99 ± 0.84 gh	1310.80 ± 63.5 a	14.21 ± 1.17 m	57.51 ± 0.17 i	3.71 ± 0.10 k	0.36 ± 0.01 t	1.14 ± 0.01 m	1.58 ± 0.04 no
Endocarp									
1	Bot.-Ljubitel.	17.10 ± 0.12 c	684.25 ± 57.3 ef	57.14 ± 0.84 h-k	31.04 ± 1.75 l-o	2.74 ± 0.01 L	1.67 ± 0.02 g	1.59 ± 0.01 g	1.75 ± 0.03 lm
2	Golden Rain	16.97 ± 0.14 c	542.52 ± 43.1 jk	52.41 ± 1.40 h-l	26.30 ± 0.58 n-q	2.45 ± 0.01 lm	1.60 ± 0.02 h	1.54 ± 0.01 h	1.42 ± 0.01 op
3	Luczistaja	15.02 ± 0.10 de	638.16 ± 60.1 fg	56.04 ± 1.72 h-k	19.01 ± 1.23 rs	2.63 ± 0.01 lm	0.93 ± 0.01 L	1.52 ± 0.01 h	1.39 ± 0.02 p
4	Maryja	15.54 ± 0.23 cd	894.14 ± 57.3 bc	42.63 ± 1.03 i-m	28.22 ± 1.43 m-q	2.57 ± 0.01 lm	1.59 ± 0.02 h	1.38 ± 0.01 j	1.69 ± 0.01 mn
5	Podarok Sadu	15.74 ± 0.04 cd	791.10 ± 34.5 bc	58.99 ± 1.70 h-k	26.84 ± 1.03 m-q	2.56 ± 0.01 lm	1.50 ± 0.02 i	1.50 ± 0.01 h	1.68 ± 0.02 mn
6	Prozrocznaja	16.23 ± 0.24 cd	803.65 ± 69.4 bc	58.15 ± 1.75 h-k	24.65 ± 1.54 p-s	2.42 ± 0.01 lm	1.58 ± 0.02 hi	1.67 ± 0.02 f	1.70 ± 0.02 mn
7	Tatiana	15.80 ± 0.15 cd	863.02 ± 74.5 bc	55.73 ± 1.45 h-k	29.14 ± 0.93 m-p	2.71 ± 0.01 l	0.96 ± 0.01 l	1.60 ± 0.01 g	1.80 ± 0.02 kl
Seeds									
1	Bot.-Ljubitel.	3.63 ± 0.09 o-r	488.20 ± 64.0 kl	1.51 ± 0.18 n	108.81 ± 1.19 c	5.63 ± 0.01 e-g	0.84 ± 0.01 m	2.68 ± 0.01 a	1.27 ± 0.04 qr
2	Golden Rain	10.75 ± 0.86 gh	358.85 ± 31.7 qr	0.86 ± 0.05 n	97.25 ± 1.67 d	4.78 ± 0.01 h-j	0.80 ± 0.01 m	2.09 ± 0.01 c	0.86 ± 0.01 vw
3	Luczistaja	1.89 ± 0.47 r	407.19 ± 48.8 op	1.24 ± 0.24 n	81.93 ± 2.12 ef	4.37 ± 0.01 jk	0.45 ± 0.01 r	2.43 ± 0.01 b	0.74 ± 0.02 w
4	Maryja	7.11 ± 0.12 j-l	772.59 ± 60.5 bc	0.95 ± 0.01 n	100.86 ± 1.13 d	4.69 ± 0.03 ij	0.38 ± 0.01 st	2.53 ± 0.01 b	0.81 ± 0.02 w
5	Podarok Sadu	2.58 ± 0.17 qr	575.72 ± 68.8 ij	2.07 ± 0.28 n	98.93 ± 0.99 d	4.78 ± 0.01 h-j	0.60 ± 0.01 op	2.52 ± 0.01 b	1.17 ± 0.01 tu
6	Prozrocznaja	3.36 ± 0.21 o-r	610.70 ± 69.2 g-i	5.45 ± 0.37 n	88.20 ± 1.98 e	4.90 ± 0.03 g-j	0.72 ± 0.01 n	2.38 ± 0.01 bc	1.08 ± 0.01 u
7	Tatiana	3.07 ± 0.04 p-r	660.59 ± 80.3 fg	3.00 ± 0.20 n	101.39 ± 0.65 d	4.31 ± 0.12 jk	0.37 ± 0.01 st	2.30 ± 0.01 bc	0.88 ± 0.05 vw
Branches									
1	Bot.-Ljubitel.	12.02 ± 0.46 fg	518.07 ± 56.5 jk	220.06 ± 9.88 g	100.49 ± 0.84 d	5.30 ± 0.06 f-i	1.68 ± 0.03 g	1.34 ± 0.01 k	3.57 ± 0.08 g
2	Golden Rain	10.66 ± 1.07 gh	476.33 ± 20.5 kl	61.97 ± 6.26 h-j	78.12 ± 3.06 fg	5.99 ± 0.08 ef	4.46 ± 0.02 c	1.39 ± 0.01 j	1.99 ± 0.01 j
3	Luczistaja	4.78 ± 0.03 m-p	769.35 ± 65.2 cd	69.23 ± 1.16 hi	85.73 ± 0.53 e	4.69 ± 0.03 ij	0.52 ± 0.01 q	1.76 ± 0.01 e	1.92 ± 0.01 jk
4	Maryja	4.41 ± 0.31 n-q	625.46 ± 21.3 gh	66.59 ± 3.90 hi	65.90 ± 4.41 h	5.51 ± 0.02 f-h	0.20 ± 0.01 wx	1.90 ± 0.01 d	1.89 ± 0.01 jk
5	Podarok Sadu	6.53 ± 0.10 j-m	415.84 ± 29.2 lo	84.60 ± 4.66 h	72.56 ± 1.71 g	6.32 ± 0.04 e	4.73 ± 0.01 b	1.42 ± 0.04 i	2.62 ± 0.03 i

(*continued on next page*)

Table 4 (*continued*)

No.	Fractions and cultivars	Minerals (mg/100 g dm)							
		Macroelements				Microelements			
		Na	K	Ca	Mg	Fe	Cu	Zn	Mn
6	Prozrocznaja	10.45 ± 0.07 gh	543.26 ± 27.5 jk	233.67 ± 12.41 g	73.00 ± 1.00 g	5.01 ± 0.04 g-j	2.50 ± 0.01 f	1.37 ± 0.04 j	2.97 ± 0.05 h
7	Tatiana	5.05 ± 0.33 m-o	702.05 ± 14.9 de	67.86 ± 6.93 hi	65.30 ± 0.53 h	11.70 ± 0.10 c	0.33 ± 0.01 tu	1.13 ± 0.01 m	1.78 ± 0.01 lm
Leaves									
1	Bot.-Ljubitel.	16.12 ± 0.59 cd	832.19 ± 32.9 bc	1141.93 ± 14.9 b	127.26 ± 2.24 b	15.56 ± 0.19 b	2.66 ± 0.01 e	1.23 ± 0.01 l	12.00 ± 0.04 a
2	Golden Rain	36.62 ± 1.06 a	296.55 ± 15.5 r	1100.98 ± 2.08 c	148.05 ± 0.19 a	8.43 ± 0.80 d	4.11 ± 0.05 d	0.83 ± 0.01 r	10.25 ± 0.09 e
3	Luczistaja	7.79 ± 0.40 i-k	631.64 ± 40.4 gh	652.43 ± 16.9 f	40.72 ± 1.21 j	4.98 ± 0.58 g-j	0.35 ± 0.01 t	1.04 ± 0.01 n	9.80 ± 0.13 f
4	Maryja	8.26 ± 0.43 ij	702.52 ± 37.2 de	841.45 ± 23.3 d	60.53 ± 1.43 hi	5.99 ± 0.47 ef	0.16 ± 0.01 x	1.16 ± 0.01 m	11.72 ± 0.09 b
5	Podarok Sadu	9.57 ± 0.87 hi	892.77 ± 41.7 bc	742.23 ± 56.4 e	29.32 ± 3.80 m-p	19.78 ± 0.63 a	5.19 ± 0.06 a	1.33 ± 0.01 k	11.53 ± 0.16 c
6	Prozrocznaja	12.89 ± 0.04 f	968.39 ± 89.9 b	1074.79 ± 13.8 c	10.52 ± 1.37 t	8.47 ± 0.65 d	2.50 ± 0.02 f	1.21 ± 0.01 l	12.11 ± 0.15 a
7	Tatiana	9.30 ± 0.02 hi	619.04 ± 27.4 gh	1200.96 ± 5.58 a	85.90 ± 4.07 e	11.50 ± 0.33 c	0.21 ± 0.01 wx	0.85 ± 0.01 r	10.61 ± 0.10 d
	Tukey's multiple comparison								
Skins		8.03 CD	565.94 C	23.59 D	28.89 D	2.62 D	0.71 C	1.24 BC	1.18 D
Flesh		9.32 C	794.39 A	13.29 E	33.48 CD	1.76 E	0.45 F	1.10 C	1.12 D
Endocarp		16.06 A	745.26 AB	54.44 C	26.46 D	2.58 D	1.40 D	1.54 B	1.63 C
Seeds		4.63 E	553.41 C	2.16 F	96.77 A	4.78 C	0.59 E	2.42 A	0.97 D
Branches		7.70 D	578.62 C	114.85 B	77.30 B	6.36 B	2.06 B	1.47 B	2.39 B
Leaves		14.36 B	706.16 B	964.97 A	71.76 B	10.67 A	2.17 A	1.09 C	11.14 A

Bot.-Ljubitel. – Botaniczeskaja-Ljubitelskaja; dm – dry matter; nd – not detectable. Data are shown as mean (n = 3) ± standard deviation (SD); for each parameter tested values with different letters differ significantly (Tukey's HSD test, $p < 0.05$).

showed 2.5 times higher iron concentration in seeds and 1.5 times higher amounts in skins and endocarp than in flesh. Therefore, purees and smoothies without clarification and with an increased proportion of skins and endocarp will provide much more iron than juices. Sea buckthorn has a high vitamin C concentration, which increases the absorption of this element; hence the consumption of products from this plant can support both the proper functioning of the immune system and metabolism energy transformations (electron or oxygen transports, oxygen activation) (Gutzeit et al., 2008; Tkacz et al., 2019).

Characteristic of leaves was the average iron to manganese ratio of 1 to 1, indicating an exceptionally high manganese content, four to 11 times higher than in branches and seeds, respectively. Compared to all other cultivars, 'Tatiana' contained the most manganese in skins, flesh and endocarp, while 'Botaniczeskaja-Ljubitelskaja' and 'Prozrocznaja' contained the most in branches and leaves.

Seeds, despite the lowest amount of manganese, contained 1.5 to two times more zinc than other sea buckthorn fractions. No significant differences in the zinc content were found for other berry parts, branches and leaves. Consequently, leaves and seeds may have nutritional value due to the manganese and zinc concentrations, which affect the metabolism of carbohydrates, amino acids and cholesterol, strengthen the osteoarticular system and sexual performance (manganese), and the proper functioning of the immune system and effective action of insulin (zinc), and both protect against oxidative stress (Gutzeit et al., 2008; Stobdan et al., 2010).

Previous studies have found a higher content of calcium, iron, sodium, zinc and manganese in sea buckthorn than in apricot, peach, banana, mango, and orange (Stobdan et al., 2010). 100 g of the leaf powder of the studied sea buckthorn cultivars covers the recommended daily allowances (RDAs) of calcium (1000 mg), iron (8 mg men and 18 mg women), copper (0.9 mg) and manganese (2.3 mg men, 1.8 mg women). Likewise, endocarp, seeds, and branches provide a favorable unconventional source of iron, copper, and manganese, close to meeting nutrient requirements.

3.3.3. Total mineral content

The total content of macro- and microelements in the sea buckthorn fractions was from 430.94 mg to 2117.51 mg/100 g dm and from 3.57 mg to 37.83 mg/100 g dm, respectively (Fig. 1). Leaves of all cultivars and flesh of 'Tatiana' contained over 1 g of macroelements per 100 g dm, while flesh, branches, skins and seeds of some cultivars did not differ significantly ($p > 0.05$). Moreover, leaves were 2–10 times more abundant in microelements than flesh (average 4.43 mg/100 g dm), and the other fractions can be ranked as follow: shoots > seeds > endocarp > skins (average 12.29, 8.76, 7.17 and 5.76 mg/100 g dm). Skins and flesh of 'Tatiana', endocarp and seeds of 'Botaniczeskaja-Ljubitelskaja' as well as leaves and branches of 'Podarok Sadu' contained the highest amounts of microelements within individual fractions.

For skins, flesh and seeds, the ratio of macro- to microelements above 100 was tested, and for other fractions it ranged from 63 (branches) to 75 (seeds). Macro- to microelements strongly correlated ($r = 0.79$), but only a few relationships were found within the fractions. The macroelement contents strongly correlated between skins and leaves, flesh and seeds, flesh and endocarp, and also seeds and endocarp ($r > 0.60$). In the case of microelements, positive correlations of leaves with all plant fractions, except flesh, were significant.

Differences in the elemental composition of sea buckthorn lie in the selective and sensitive FAAS technique used compared to older measurement methods hampered by the interference of other ions in analytes. On the other hand, the plant material was collected from cultivars coming from the same area; hence the soil type and growth environment, reported as the most common factor differentiating minerals in plants, can be omitted from this inference (Ercisli et al., 2007; Zeb and Malook, 2009). For example, the potassium content in sea buckthorn leaves may be higher with the use of natural mulch and higher soil moisture (Boivin et al., 2007), there is a strong correlation between the calcium and magnesium content in soil and leaves (Nowakowska et al., 2017), and their concentrations are higher in berries at full maturity (Arif et al., 2010). The present study revealed, however, an important and variable varietal effect and the fraction type not analyzed so far,

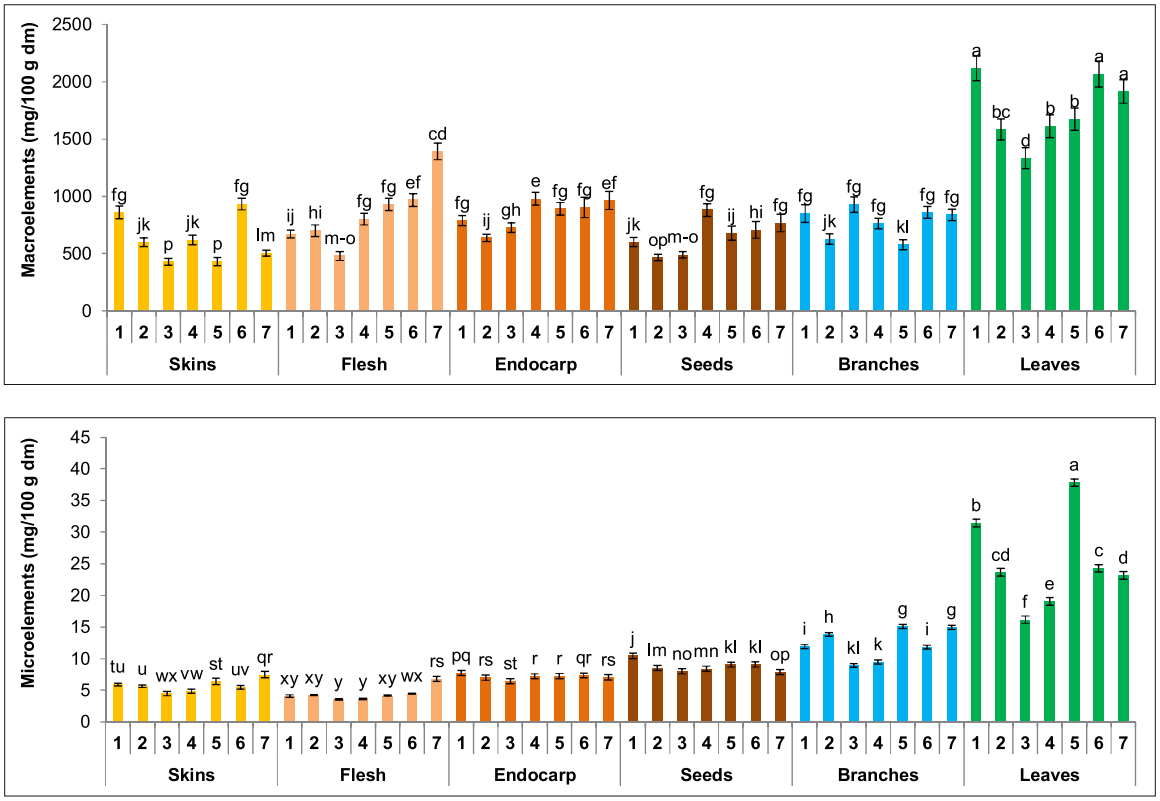

Fig. 1. Total contents of macroelements (sodium, potassium, calcium and magnesium) and microelements (iron, copper, zinc and manganese) (mg/100 g dm) in anatomical parts of sea buckthorn berries, branches and leaves. Numbers of cultivars: 1 – Botaniczeskaja-Ljubitelskaja; 2 – Golden Rain; 3 – Luczistaja; 4 – Maryja; 5 – Podarok Sadu; 6 – Prozrocznaja; 7 – Tatiana.

taking into account berry parts, leaves and branches, as factors strongly differentiating the qualitative and quantitative mineral profile.

3.4. Classification of sea buckthorn fractions with principal component analysis (PCA)

To better understand the relationships between sea buckthorn fractions and chemical components, principal component analysis (PCA) was applied (Fig. 2). The close location of the variable points distinguished six clusters:

- group 1 – skins characterized by significant amounts of isorhamnetin, quercetin and kaempferol derivatives, phenolic acids, betulin, oleanolic and ursolic acids;
- group 2 – flesh rich in pomolic and maslinic acids, and potassium;
- group 3 – endocarp with high concentrations of potassium and sodium;
- group 4 – seeds that accumulated polymeric procyanidins, betulinic acid, magnesium, and zinc;
- group 5 – branches rich in polymeric procyanidins, flavan-3-ols, corosolic and betulinic acids;
- group 6 – leaves as the most extensive cluster representing particularly high abundance in quercetin, kaempferol and isorhamnetin derivatives, calcium, potassium, sodium, iron, copper, manganese, and ursolic acid as the only triterpenoid.

Based on the vector lengths, the influence of phenolic compounds, pomolic, malinic, betulinic and corosolic acids, calcium, iron and manganese on the principal components was the strongest. Small angles between vectors of flavan-3-ols, betulin acid and zinc, between quercetin derivatives, ursolic acid, manganese and calcium, as well as between isorhamnetin derivatives and betulin, indicated strong positive correlations of these variables.

4. Conclusions

This study highlights the diversity of the anatomical berry parts (skins, flesh, endocarp, seed), branches and leaves of sea buckthorn with regard to triterpenoids, phenolic compounds, and macro- and microelements. Skins and flesh are a valuable source of triterpenoids, unlike leaves, which in turn accumulate significant concentrations of minerals, and quercetin and isorhamnetin derivatives. Branches and seeds with endocarp, despite the low amounts of flavonols and the lack of phenolic acids, can provide extreme doses of flavan-3-ols and polymeric procyanidins. Moreover, sea buckthorn fractions are favorable unconventional sources of potassium, and in the case of the leaves also calcium. Vegetative parts and seeds with endocarp are close to meeting the iron, copper and manganese needs.

Morphological parts of sea buckthorn have the potential to produce food rich in high-antioxidant and anti-inflammatory components, such as phenolic compounds and triterpenes, and for ion replenishment and proper balance. The results provide important information to guide current technological processes to achieve the desired phytochemical profiles and to further design and implement nutraceutical-rich applications and food with high pharmacological potential, while supporting the use of the entire plant in non-waste production. In the future, it will be beneficial to analyze lipophilic components and formulations based on the sea buckthorn fraction in *in vivo* studies.

Ethic statement

Research did not include any human subjects and animal experiments.

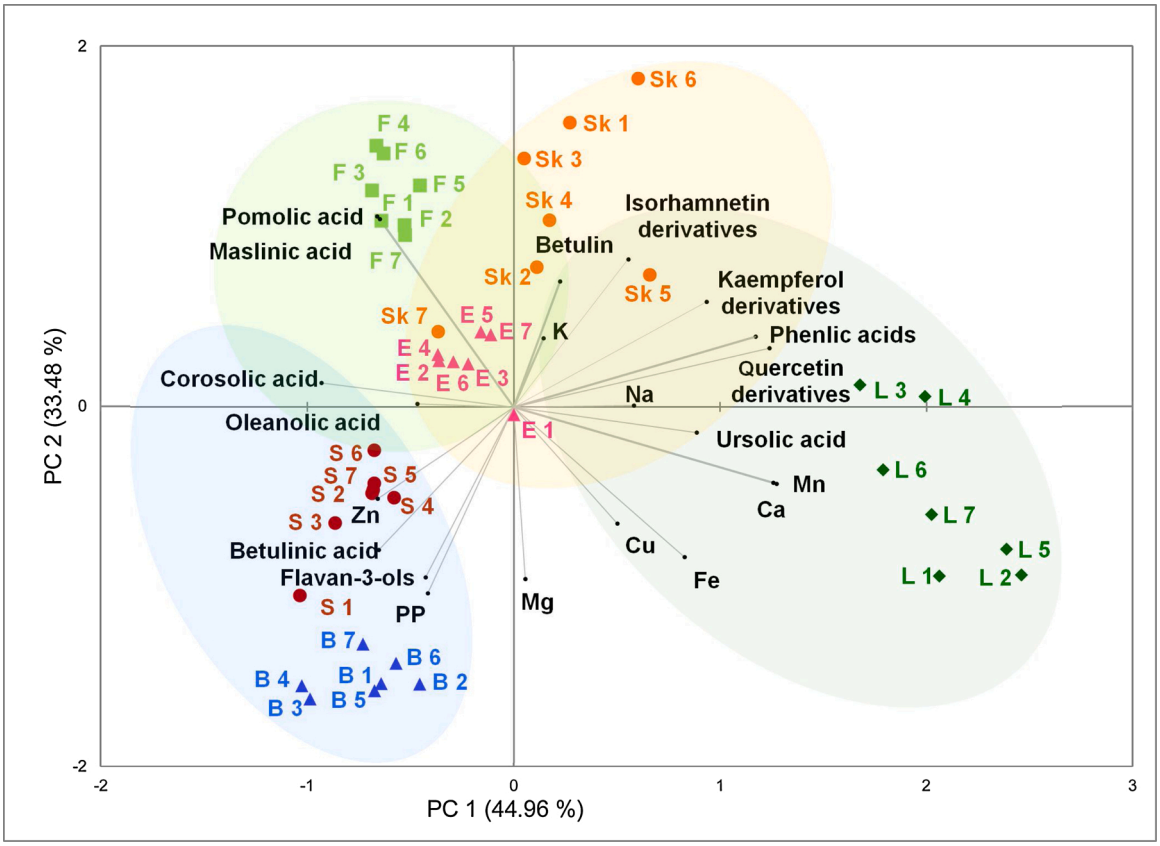

Fig. 2. Principal component analysis (PCA) biplot of anatomical parts of sea buckthorn berries, branches and leaves and phenolic compounds, triterpenoids and minerals.
The biplot shows the PC scores of chemical components (black vectors) and anatomical fractions of each of the seven sea buckthorn cultivars (colored marks) clustered (colored circles) based on the two main principal components (PC 1 and PC 2) explained 78.44 % of total variance.
Sk1-Sk7 – skins; F1-F7 – flesh; E1-E7 – endocarp; S1-S7 – seeds; B1-B7 – branches; L1-L7 – leaves; PP – polymeric procyanidins; numbers of cultivars: 1 – Botaniczeskaja-Ljubitelskaja; 2 – Golden Rain; 3 – Luczistaja; 4 – Maryja; 5 – Podarok Sadu; 6 – Prozrocznaja; 7 – Tatiana.

CRediT authorship contribution statement

Karolina Tkacz: Conceptualization, Writing - review & editing, Formal analysis, Methodology, Visualization. **Aneta Wojdyło:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Igor Piotr Turkiewicz:** Formal analysis, Methodology. **Paulina Nowicka:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, przygotowaniu materiału badawczego, oznaczeniu ilościowym i identyfikacji triterpenów i związków fenolowych metodą LC-MS oraz składników mineralnych metodą FAAS w częściach anatomicznych jagód, młodych gałązkach i liściach rokitnika pospolitego. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, a następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

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mój udział polegał na współtworzeniu koncepcji i planu badań, pozyskaniu materiału badawczego, uczestnictwie w oznaczeniu ilościowym i identyfikacji triterpenów i związków fenolowych metodą LC-MS w częściach anatomicznych jagód, młodych gałązkach i liściach rokitnika pospolitego. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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mój udział polegał na uczestnictwie w etapie przygotowania materiału badawczego i oznaczeniu składników mineralnych metodą FAAS w częściach anatomicznych jagód, młodych gałązkach i liściach rokitnika pospolitego.

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w analizie jakościowej triterpenów, związków fenolowych metodą LC-MS i składników mineralnych metodą FAAS w częściach anatomicznych jagód, młodych gałązkach i liściach rokitnika pospolitego oraz współredagowaniu manuskryptu pod względem merytorycznym.

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Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices

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Abstract

BACKGROUND: Juices are currently a fast-growing segment in the fruit and vegetable industry sector. However, there are still no reports on the diversity of the phytochemical profile and health-promoting properties of commercial sea buckthorn (*Hippophaë rhamnoides*) juices. This study aimed to identify and quantify phytoprostanes, phytofurans by ultra high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS), tocopherols, tocotrienols by ultra-performance liquid chromatography coupled with a fluorescence detector (UPLC-FL), carotenoids, and free amino acids by ultra-performance liquid chromatography coupled with a photodiode detector-quadrupole and tandem time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS), and assess their anti-cholinergic, anti-diabetic, anti-obesity, anti-inflammatory, and antioxidant potential by *in vitro* assays of commercial sea buckthorn juices.

RESULTS: Phytoprostanes (PhytoPs) and phytofurans (PhytoFs) in sea buckthorn juices were identified for the first time. Juices contained eight F_1-, D_1-, B_1- and L_1-phytoprostanes and one phytofuran (32.31–1523.51 ng and up to 101.47 μg/100 g dry weight (DW)), four tocopherol congeners (22.23–94.08 mg 100 g^{-1} DW) and three tocotrienols (5.93–25.34 mg 100 g^{-1} DW). Eighteen carotenoids were identified, including ten xanthophylls, seven carotenes and phytofluene, at a concentration of 133.65 to 839.89 mg 100 g^{-1} DW. Among the 20 amino acids (175.92–1822.60 mg 100 g^{-1} DW), asparagine was dominant, and essential and conditionally essential amino acids constituted 11 to 41% of the total. The anti-enzyme and antioxidant potential of juices correlated selectively with the composition.

CONCLUSION: Sea buckthorn juice can be a valuable dietary source of vitamins E and A, oxylipins and amino acids, used in the prevention of metabolic syndrome, inflammation, and neurodegenerative processes. The differentiation of the composition and the bioactive potential of commercial juices indicate that, for the consumer, it should be important to choose juices from the declared berry cultivars and crops.

Supporting information may be found in the online version of this article.

Keywords: *Hippophaë rhamnoides*; UHPLC–QqQ-MS/MS; UPLC-PDA-Q/TOF-MS; BuChE; α-Glucosidase; 15-lipoxygenase

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides*) is a thorny shrub of economic and nutritional importance, native to Europe and Asia but currently cultivated or growing wild in many regions of the Northern Hemisphere. Its berries are aptly defined as superfruits due to the abundance of lipophilic and hydrophilic bioactive phytochemicals, such as (on a fresh weight basis) vitamins C (0.3–5 g kg^{-1}), K (1.1–2.3 g kg^{-1}) and E (0.03–0.21 g kg^{-1}), carotenoids (0.01–0.40 g kg^{-1}), phytosterols (13–20 g kg^{-1}), flavonoids (1.7–10 g kg^{-1}), organic acids (10–54 g kg^{-1}), fatty acids, coumarins, and triterpenes.[1-3] The interest in its composition is constantly growing, with an expanding pool of proven antioxidant, anti-

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cancer, anti-stress, anti-diabetic, anti-atherosclerotic, antimicrobial, hepatoprotective, cardioprotective, neuroprotective, cytoprotective, radioprotective, and immunomodulating properties.[1,3] The wide occurrence of sea buckthorn, undemanding growing conditions, rich composition, and health-promoting effects are not reflected in the global consumption of sea buckthorn and food production from it due to the difficult harvest, technological processing, and sensory values.[2,4] The plant is a valuable raw material in the cosmetics and pharmaceutical industries but has strong untapped potential as a food ingredient.

The most common form of commercial and processed sea buckthorn is juices, followed by beverages, preserves, jams, jellies, powders, syrups, teas, beers, wines, and essential oils, but such products are more often produced and available on local markets than globally.[5,6] Previous research on *H. rhamnoides* focused mainly on the assessment of the chemical composition of its berries, seeds, leaves, roots, and oils, and the study of the biosynthetic pathways of their metabolites, the impact of composition variability depending on cultivars, growing conditions, harvest dates, and maturation over the years for different geographic areas.[7,8] In the literature there are several reports of sea buckthorn juices. Möller *et al.*[9] (2011) found that sea buckthorn juice is significantly richer in carotenoids and vitamin E than carrot, tomato and orange juices. Several reports indicate a high concentration of vitamin C, selected mineral elements, amino acids, and organic acids in juices from cultivars of various origins.[10,11] Rösh *et al.* (2003)[12] identified flavonols as the main phenolic compounds of sea buckthorn juices, and phenolic acids and catechins represented minor components. Ascorbic acid contributes to approximately 75% of the total antioxidant activity of juices. Research on production methods of sea buckthorn juices has proven that the vitamin C contained in them is very stable, and that briefly heating fruits before pressing gives a higher recovery of carotenoids.[13] In turn, Eccleston *et al.*[14] characterized the antioxidant profile of sea buckthorn juice and assessed its positive effect on the regulation of risk factors for coronary heart disease in humans.

Juices are currently a fastest growing segment in the fruit and vegetable industry sector. By 2023, EU fruit juice and nectar consumption is forecast to stand at around 8.2 billion liters.[15] The main factors are the growing appreciation of Eastern European consumers (in countries such as Poland, Hungary, and the Czech Republic) for the wellness and health benefits of fruit juice and an increase in their living standards. The growing interest in the health risks associated with inadequate nutrition increases pressure to seek authenticity, transparency, and 'all-natural' claims for foods, shifting the focus to innovation and product development with natural ingredients.[16] However, there are still no reports on the diversity of the phytochemical profile and health-promoting properties of commercial sea buckthorn juices.

The role of vitamin E as the main antioxidant in human plasma against oxidative stress is well established and the current recommended dietary allowance (RDA) by Food and Nutrition Board of the Institute of Medicine (in the US) for adults is 15 mg. Similarly, carotenoids, including β-carotene – the major precursor of vitamin A and a significant dietary source of it – are potent antioxidants with proven effects on redox modulation, inflammation, and prevention of cardiometabolic diseases. The results of epidemiological studies have shown that the preventive health potential of carotenoids can be achieved with a daily intake of 2 to 4 mg of β-carotene.[17] Nevertheless, the role of amino acids in nutrition has been ignored until recently, but nowadays the suggested daily intake of essential amino acids is estimated at 184 mg kg^{-1} body weight. Amino acids are essential precursors for the synthesis of a wide range of nitrogenous substances of biological importance, including neurotransmitters and hormones, and are also key regulators of metabolic pathways that are significant in growth, development, immunity, and health.[18,19] Moreover, sea buckthorn berries are among the few fruits containing saturated, monounsaturated, and polyunsaturated fatty acids, in an approximate ratio of 2:2:1, with an average α-linolenic acid (C18:3 n-3) content of 3.60%.[3] Phytoprostanes (PhytoPs) and phytofurans (PhytoFs), which are products of nonenzymatic oxidation of alanine (ALA) triggered by reactive oxygen species (ROS), therefore seem to be an important fraction of sea buckthorn berry juices. Data from the literature do not provide unequivocal evidence of a correlation between PhytoPs and ALA[20] but indicate moderate linear correlations with fatty acids.[21] Currently, the importance of PhytoPs and PhytoFs is based not only on their use as biomarkers of oxidative degradation of plant-derived foods but also on proven biological effects despite the reduction of ALA. Previous studies suggest the regulation of immune functions, anti-inflammatory, and apoptosis-inducing activity, and a potential for action in the pathophysiology of metabolic, respiratory, and cardiovascular diseases.[22] To the best of our knowledge, there are no previous investigations of PhytoPs and PhytoFs in species belonging to the genus *Hippophaë* and the family Elaeagnaceae.

Hence, the aim of this study was to perform the qualitative and quantitative determination of phytoprostanes and phytofurans by ultra high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC-QqQ-MS/MS), tocopherols and tocotrienols by ultra-performance liquid chromatography coupled with a fluorescence detector (UPLC-FL), carotenoids, and free amino acids by ultra-performance liquid chromatography coupled with a photodiode detector-quadrupole and tandem time-of-flight mass spectrometry (UPLC-PDA-Q/TOF-MS) and assessment of the anti-cholinergic, anti-diabetic, anti-obesity, anti-inflammatory, and antioxidant potential by *in vitro* assays in commercially available sea buckthorn juices. The report is a valuable comparison of commercial juices with juice from berries of the Polish cv. 'Józef'. For the first time, plant oxylipins, and inhibitory potential against key enzymes linked to metabolic syndrome, inflammation, and neurodegenerative processes in sea buckthorn juices were determined.

MATERIALS AND METHODS

Materials

The samples were sea buckthorn (*Hippophaë rhamnoides*) juices generally available on the Polish retail market. All five commercial juices were pasteurized, unfiltered, and not from concentrate. Laboratory samples constituted three equal parts of fresh juices of the same type (primary samples) and were labeled from J1 to J5. The average energy value was 154 kJ 37 kcal per 100 mL^{-1} of juice, with the following composition: fat – 1.1 g, including saturated fatty acids – 0.4 g, carbohydrates – 5.6 g, including sugars – 1 g, dietary fiber – 0.9 g, protein – 0.6 g, salt – 0 g. The dry weight of the juices was on average 7.30 g 100 g^{-1} and the soluble solids content was 5.5 °Brix (determined according to European Standards PN-EN). J6 was the juice from berries of the 'Józef' cultivar collected in August 2018 from the Experimental Orchard in Dąbrowice of the Research Institute of Horticulture in Skierniewice (Poland). Berries (0.60 kg) were harvested from three bushes,

0.20 kg each batch. Sea buckthorn juice cv. 'Józef' was squeezed from selected berries using a laboratory hydraulic press (SRSE, Warsaw, Poland), and then pure juice was heated to 100 °C for 4 min, hot-filled into a 900 mL glass bottle, kept for 10 min at 95 °C, and finally cooled to 4 °C. The pasteurization parameters used for the juice, with a low pH of 2.9, ensured the destruction of pathogenic microorganisms and the extension of the shelf life due to the almost complete neutralization of vegetative forms, which was similar to the commercial juices. The products were stored in refrigerated conditions without access to light, and after opening they were sent for analysis.

Analysis of phytoprostanes and phytofurans by UHPLC-ESI-QqQ-MS/MS

The extraction combined with a dispersive liquid–liquid extraction and solid-phase extraction (SPE), and liquid chromatography coupled to mass spectrometry (LC–MS) conditions were described by Collado-González *et al.*,[23] with modifications. The lyophilized juice sample was sonicated with methanol with 0.1% butylhydroxyanisole (BHA), centrifuged, and the supernatant mixed with hexane:methanol:Bis-Tris (5:1:1). Solid-phase extraction was performed using Strata™ x-Aw columns (weak anion mixed mode phase). The process included conditioning with hexane, methanol, and water (1:1:1), loading the sample, removing undesirable compounds using hexane, water, 50% methanol, and acetonitrile (1:1:1:1), and finally eluting PhytoPs and PhytoFs with methanol. Compounds were dried using a SpeedVac™ vacuum concentrator (Savant™ SPD121P, Thermo Scientific, Waltham, MA, USA), dissolved in 50% methanol and filtered using MF-Millipore membrane filters (0.45 µm, Merck, Darmstadt, Germany). The identification and quantification of PhytoFs and PhytoPs were performed using ultra-high performance liquid chromatography (UHPLC) coupled to a 6460 triple quadrupole-MS/MS (Agilent Technologies, Waldbronn, Germany), with a Ethylene Bridged Hybrid (BEH) C18 column (1.7 µm, 2.1 × 50 mm) (Waters Corp., Milford, MA, USA), and gradient flow of water and methanol (each with 0.01% acetic acid). Analysis was carried out by multiple reaction monitoring (MRM) in the negative mode. Results were expressed as ng PhytoP or µg PhytoF per 100 g of DW (dry weight).

Analysis of tocotrienols and tocopherols by UPLC-FL

The extraction procedure and conditions for the ultra-performance liquid chromatography (UPLC) analysis of tocotrienols and tocopherols were previously described by Tkacz *et al.*[3] Briefly, the juice sample was homogenized with ethanol with 0.05% BHT, saponified with 60% KOH at 50 °C for 2 h, then 1% NaCl was added. The ice-cooled mixture was stirred with hexane: ethyl acetate (9:1) with 0.05% BHT, then saturated NaCl solution was added and the top layer of juice extracted was collected for evaporation. The evaporation residue was dissolved in methanol with 0.05% BHT and filtered before injection using polytetrafluoroethylene (PTFE) Millex Samplicity™ Filters (25 mm, 0.20 µm, Merck, Darmstadt, Germany). Analysis of tocotrienols and tocopherols was performed using UPLC (Acquity UPLC System) with a binary solvent manager and a fluorescence (FL) detector (Waters Corp., Milford, MA, USA). The separation was performed on an Acquity UPLC BEH RP C18 column (1.7 µm, 2.1 mm × 100 mm, Waters Corp., Milford, MA, USA) using isocratic flow of methanol: water (88:12). The compounds were identified and quantified with reference standards of α-, β-, γ-, Δ-tocotrienols and -tocopherols (Extrasynthese, Lyon, France; Sigma-Aldrich, Steinheim, Germany) and their calibration curves ($R^2 \geq 0.998$). Results were expressed as mg per 100 g of DW.

Analysis of carotenoids by UPLC-PDA-Q/TOF-MS

The extraction procedure and conditions for the LC–MS analysis of carotenoids were described previously by Tkacz *et al.*[3] Briefly, the lyophilized juice sample was shaken five times for 30 min with methanol:acetone:hexane (1:1:2), 1% BHT and $4MgCO_3 \times Mg(OH)_2 \times 5H_2O$. The decanted portions were centrifuged, the combined supernatants evaporated and the residue was dissolved in methanol. Samples were filtered before injection using hydrophilic polypropylene membrane (GHP) Minispike syringe filters (13 mm, 0.45 µm, Acrodisc®, Waters Corp., Milford, MA, USA), and all analysis was carried out in the absence of light. Analysis of carotenoids was performed using ultra performance liquid chromatography (Acquity UPLC System) with a binary solvent manager and a photodiode array (PDA) detector (Waters Corp., Milford, MA, USA). The separation was performed on an Acquity UPLC BEH reversed-phase (RP) C18 column using a gradient flow of acetonitrile:methanol (70:30) and 0.1% formic acid. The qualitative determination of carotenoids was carried out using a mass detector-quadrupole and tandem time-of-flight (Q/TOF) micro-mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionization (ESI) source operating in positive modes, as given by Tkacz *et al.*[24] Compounds were quantified according with calibration curves ($R^2 \geq 0.998$) of reference standards of lutein, zeaxanthin, β-cryptoxanthin, α-, β-, γ-, Δ-, ε-, ζ-carotene, and phytofluene (Extrasynthese, Lyon, France; Carote*Nature* GmbH, Münsingen, Switzerland). Results were expressed as mg per 100 g of DW.

Analysis of amino acids by UPLC-PDA-Q/TOF-MS

Analysis of free amino acids was determined as described previously by Collado-González *et al.*[25] with modifications. Double extraction of the lyophilized sample (~20 mg) was carried out using 50% methanol. Extracts centrifuged at 19000×g for 7 min at 4 °C (MPW M-Universal, MPW MED. Instruments; Warsaw, Poland) were directly intended for derivatization. Derivatization consisted of mixing of derivatization buffer (0.2 mol L^{-1} boric acid with 5 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) calcium disodium salt; pH 8.8 established with NaOH), supernatant and AQC solution (10 mmol L^{-1} 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate in acetonitrile), in a proportion of 7:1:2 (v/v/v), respectively. This solution was vortexed for 1 min and then placed in a heating block (Eppendorf ThermoMixer® C, Eppendorf AG, Hamburg, Germany) for 10 min at 55 °C. The samples prepared were immediately used for directed to UPLC-PDA-Q/TOF-MS analysis.

Qualitative (LC/MS Q-TOF) and quantitative (UPLC-PDA) estimation of free amino acids was carried out using an Acquity UPLC system equipped with a PDA detector with a binary solvent manager (Waters Corp., Milford, MA, USA) series with a mass detector G2 Q/TOF micro-mass spectrometer (Waters, Manchester, UK) equipped with an ESI source operating in positive modes. The separation of individual amino acids was carried out using a AccQ Tag Ultra BEH column (2.1 × 100 mm, 1.7 µm) (Waters Corp., Milford, MA, USA). The column was kept at 50 °C, the samples at 20 °C, the injection volume was 3 µL, with a flow rate of 0.50 mL min^{-1}. The mobile phase consisted of solvent A (50 mL of solution: acetonitrile, formic acid, and 5 mmol L^{-1} ammonium acetate in water (10:6:84, v/v/v) and 950 mL water) and solvent B (acetonitrile and formic acid; 99.9:0.1, v/v). The optimized MS

parameters were as follows: source temperature of 100 °C, desolvation temperature of 350 °C, cone gas flow 40 L h^{-1}, desolvation gas flow 535 L h^{-1}, capillary voltage of 2500 V, and cone voltage of 30 V. Quantification was based on reference standards, retention times, and spectra. Results were expressed as mg per 100 g of DW.

Determination of enzyme inhibitory and antioxidant potential by *in vitro* tests

Bioassays for the inhibition of enzymes and oxidants were performed using spectrophotometric and spectrofluorometric methods. Anti-cholinesterase activity was tested for acetylcholinesterase (AChE) and butylcholinesterase (BuChE) inhibition as reported previously by Tkacz *et al.*[24] The anti-diabetic potential was examined as the ability to inhibit α-amylase, α-glucosidase, the anti-obesity effect for pancreatic lipase inhibition, and the anti-inflammatory effect for 15-lipoxygenase (15-LOX) inhibition. The anti-cholinesterase and anti-diabetic effects were expressed as IC_{50} (the half maximal inhibitory concentration; mg of sample/mL). The anti-15-LOX activity was given as percentage inhibition (%) at the concentration of 30 mg mL^{-1}. The antioxidant capacity was tested as oxygen radical absorbance capacity (ORAC), ferric reducing ability (FRAP), and free radical-scavenging activity (ABTS), and the results were expressed as mmol TE (Trolox) 100 g FW^{-1}. These activities were determined based on the protocol previously described by Tkacz *et al.*[3] All tests were performed using a hybrid multi-mode microplate reader Synergy™ H1 (BioTek, Winooski, VT, USA).

Statistical analysis

Data were analyzed using the Kruskal–Wallis test, and then the *post hoc* Dunn's multiple comparison test. Statistical differences at $P < 0.05$ were marked with consecutive lowercase letters. All results were means of three measurements ± standard deviation (SD). Pearson's correlations coefficients (r) was determined between chemical composition and biological potential of samples. XLSTAT Statistical Software version 2016.4 (Addinsoft Inc, New York, NY, USA) integrated with Microsoft Excel 2017 (Microsoft Corp., Redmond, WA, USA) was used.

RESULTS AND DISCUSSION

Phytoprostanes and phytofurans of sea buckthorn juices

Ultra-high performance liquid chromatography coupled to a triple quadrupole (UHPLC–QqQ-MS/MS) analysis allowed the identification and quantification of eight F_1-, D_1-, B_1- and L_1-PhytoPs and their respective enantiomers and one phytofuran (Table 1). The most intense MRM transitions were characteristic of pairs of PhytoP diastereoisomers. The F_{1t}-PhytoP isomers corresponded to two typical transition 327.2 → 251.2 (*Ent*-16-*epi*-16-F_{1t}-PhytoP and *Ent*-16-F_{1t}-PhytoP, rt 1.81 min) and 327.2 → 171.1 for 9-F_{1t}-PhytoP and 9-*epi*-9-F_{1t}-PhytoP (rt 1.91 min and 2.13 min). In turn, a pair of D_{1t}-PhytoP isomers, that is 9-*epi*-9-D_{1t}-PhytoP and 9-D_{1t}-PhytoP (rt 2.14 min and 2.49 min), were identified for the transition 325.2 → 307.2. One B_1- and L_1-isomer each was detected for the transitions 307.2 → 235.2 (16-B_1-PhytoP, rt 3.23 min) and 307.2 → 185.2 (9-L_1-PhytoP, 3.51 min), respectively, as described by Collado-González *et al.*[23]

The total content of PhytoPs ranged from 32.31 (J1) to 1523.51 ng 100 g^{-1} DW in the juice of berries of cv. 'Józef' (J6), in which all eight PhytoPs were determined (Table 1). The dominant PhytoP class was F_1-PhytoP (from 66 to 100% of the sum of PhytoPs), followed by D_1-PhytoP (up to 28% of the sum of PhytoPs). The F_1-isomers were the only PhytoPs determined in J2, J4 and J5 juices. In this case, the sums of *Ent*-16-*epi*-16-F_{1t}-PhytoP and *Ent*-16-F_{1t}-PhytoP were given together as they were indistinguishable by retention times. In turn, for J1, J3, and J6 juices, 9-*epi*-9-F_{1t}-PhytoP represented 50% and more of the sum of these oxylipins. Due to the growing importance of PhytoPs in human metabolism, bioavailability, and biological implications (antiviral, anti-inflammatory, immunomodulatory, cytotoxic and cytoprotective),[22] a 20- to almost 50-fold higher sum of PhytoPs in J1, J3 and J6 compared with the rest of the juices can be considered potentially beneficial.

As a result of this first analysis, statistically significantly different amounts of these compounds were obtained in sea buckthorn juices, and the concentration of PhytoPs correlated moderately with the PhytoFs (r = 0.56). It is notable that the PhytoP concentrations in the juices were comparable with those in plants and foods considered rich in these compounds, for example passion fruit (130 ng to 2166.0 µg 100 g^{-1} DW), almonds, and nuts (500 ng to 23.8 µg 100 g^{-1} DW), and various anatomical parts of plants with medicinal potential (450 ng to 3244 µg 100 g^{-1} DW).[22]

Lastly, among the PhytoFs, only *Ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF (rt 1.65 min) with MRM transition 344.0 → 300.0 was determined. Three juices contained this compound (J1, J4 and J6) at a concentration of 34.19 (J1) to 101.47 µg 100 g^{-1} DW (J6). PhytoFs are less common oxylipins in plant matrices but can be synthesized preferentially at higher partial pressure of oxygen, that is above 21%.[26] Previous liquid chromatography–tandem mass spectrometry reports provided data for the presence of PhytoFs in rice, nuts and seeds, vegetable oils, and brown and red macroalgae.[22] *Ent*-9-(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF determined in the juices was a characteristic PhytoF for pea[26] and some cacao beans,[21] up to 50 µg and 0.30 µg 100 g^{-1} DW, respectively.

The berry cultivars and agronomical conditions related to the rate of oxidative stress, and then the production process and storage of juices could significantly modulate the content of PhytoPs and PhytoFs, due to the potential increase in ALA oxidation by the heat treatment, which was previously investigated in vegetable oils.[23] León-Perez *et al.*[21] additionally reported the influence of chemical composition on the PhytoP profile in relation to oleic, linoleic, and α-linolenic acids, other lipids, and tocopherols. This research, however, showed a negligible Pearson's correlation between PhytoP and PhytoF levels and tocopherol and tocotrienol content (r close to zero). On the other hand, data in the literature provide information on the profile of PhytoPs and PhytoFs based on such analytical methods as nuclear magnetic resonance (NMR) spectroscopy, enzyme-linked immunosorbent assays (ELISA), gas chromatography coupled to mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), and LC–MS. The latter method, used in this study, allows the separation and identification of isomers and is the most specific and sensitive,[22] which may also explain the quantitative variation between juices as well as the results of other research teams.

Tocotrienols and tocopherols of sea buckthorn juices

A UPLC-FL analysis of the vitamin E-related compounds revealed four tocopherol congeners: alpha (α-T), beta (β-T), gamma (γ-T) and delta (Δ-T), and three tocotrienol congeners: alpha (α-T3), gamma (γ-T3) and delta (Δ-T3) in sea buckthorn juices (Fig. 1). The concentrations of tocopherols and tocotrienols strongly correlated (r = 0.82), but the sum of tocopherols was 3.6 to 6.7 times higher than that of tocotrienols. The content of tocopherols

Table 1. UHPLC-QqQ-MS/MS identification and quantification of phytoprostanes (PhytoPs) and phytofurans (PhytoFs) in sea buckthorn juices

Peak no.	Compounds	Rt (min)	MS $[M-H]^-$ (*m/z*)	Product ions (*m/z*)	Sea buckthorn juices					
					J1	J2	J3	J4	J5	'Józef' juice
							Phytoprostanes (ng/100 g DW)			
1	*Ent*-16-*epi*-16-F_{1t}-PhytoP + *Ent*-16-F_{1t}-PhytoP	1.81	327.2	251.2	87.38 ± 1.20 a	32.31 ± 0.75 c	23.61 ± 0.30 e	40.80 ± 0.81 b	34.50 ± 0.34 c	26.31 ± 0.92 d
2	9-F_{1t}-PhytoP	1.91	327.2	171.1	72.78 ± 1.23 c	Traces	116.91 ± 1.45 b	Traces	Traces	223.75 ± 2.58 a
3	9-*epi*-9-F_{1t}-PhytoP	2.13	327.2	171.1	313.76 ± 2.38 c	Traces	715.97 ± 3.04 b	Traces	Traces	753.17 ± 3.73 a
4	9-*epi*-9-D_{1t}-PhytoP	2.14	325.2	307.2	78.87 ± 1.67 c	Traces	199.95 ± 1.98 a	Traces	Traces	113.19 ± 1.21 b
5	9-D_{1t}-PhytoP	2.49	325.2	307.2	72.33 ± 1.80 b	Traces	159.80 ± 1.57 a	Traces	Traces	72.89 ± 1.86 b
6	16-B_1-PhytoP	3.23	307.2	235.2	Traces	Traces	57.02 ± 0.54 b	Traces	Traces	154.87 ± 1.24 a
7	9-L_1-PhytoP	3.51	307.2	185.2	Traces	Traces	Traces	Traces	Traces	179.32 ± 1.46
				Total PhytoPs	625.12 ± 2.43 c	32.31 ± 0.75 e	1273.26 ± 3.12 b	40.80 ± 0.81 d	34.50 ± 0.34 e	1523.51 ± 3.84 a
							Phytofurans (µg/100 g DW)			
8	*Ent*-9-(*RS*)-12-*epi*-ST-$\Delta^{10,13}$-PhytoF	1.65	344.0	300.0	34.19 ± 0.83 c	Traces	Traces	38.63 ± 0.79 b	Traces	101.47 ± 1.19 a

The data shown are mean values ± SD (n = 3); values followed by the same letter, within the row, were significantly different ($P < 0.05$); PhytoP, phytoprostane; PhyoF, phytofuran; J1-J5, Juices 1–5; DW, dry weight.

ranged from 22.23 (J1) to 94.08 mg 100 g^{-1} DW (J4), and tocotrienols from 5.93 (J1) to 25.34 mg 100 g^{-1} DW (J4). Thus, the total amount of all vitamers in the juices was between 28.17 and 119.42 mg 100 g^{-1} DW, and tocopherols averaged 82% of the total.

The dominant tocotrienol was γ-T3 (42 to 49% of the total), then α- and Δ-T3 were roughly equal to the rest of tocotrienols. These trends were in line with research on juices rich in lipophilic fractions, including sea buckthorn, carrot, tomato and orange juices.[9] In the case of tocopherols, α-T accounted for between 71 and 79%, while the γ- and Δ-T, being the precursors of the α- and β-forms, represented 1.3 and 0.5% of the total tocopherols, respectively. Previous studies also did not identify β-T3 in sea buckthorn berries; however, contrary to the results of Andersson *et al.*,[7] significant amounts of β-T (23% of the total tocopherols) were found in the juices. The dominance of α-T (on average 40 to 85% of tocopherols and tocotrienols) was also found in whole sea buckthorn berries,[7,27] pulp oil and seed oils,[5,28] seeds and leaves, except for peels.[29] The high concentration of α-T in sea buckthorn juices (mainly J3, J4 and J6) is especially valuable as it is the only vitamer recognized to meet human vitamin E requirements.

The content of tocopherols and tocotrienols in sea buckthorn may be differentiated by cultivars, climate and growth conditions, yearly variations, and the degree of berry ripeness;[7] these factors should therefore be taken into account in the production of juices. The most extreme differences in the amount of tocopherols between juices were found for α- and Δ-T, which could result from the strong influence of berry varieties on the profile of these forms. The low content of Δ-T (0.14 to 0.53 mg 100 g^{-1} DW) in all juices could indicate the use of fruits in the early stage of maturity, because this homologue increases with the ripening period. In turn, average fourfold differences in the content of γ-T, α-T3 and Δ-T3 can be explained by the correlation with maturation conditions (temperature and irradiation), as proved by research investigating the composition of sea buckthorn cultivars over the years.[7] Sea buckthorn seeds also have a higher proportion of γ-T (20–40%) than the soft parts of berry,[27] and Δ-T is dominant in peels.[29] Therefore, the pre-treatment of the raw material could modulate the final content of these forms in juices, especially in the 'Józef' juice with the highest concentrations of γ- and Δ-T.

The content of tocopherols and tocotrienols in the juice obtained in the laboratory were similar to the average concentrations for commercial juices (J1–J5). However, different results were obtained by Górnaś *et al.*[30] in their research on juices and nectars available in Baltic countries. They explained the lower concentrations of these compounds in commercial sea buckthorn products by the potential addition of cheaper substitutes or removing the lipid fraction, different berry varieties and species, and storage time and conditions.

Carotenoids of sea buckthorn juices

Eighteen carotenoids were identified in sea buckthorn juices using UPLC-PDA-Q/TOF-MS analysis, including 10 xanthophylls, 7 carotenes, and phytofluene (Table 2). Among the xanthophylls, all-*trans*-lutein (rt 5.04 min) and its three isomers, all-*trans*-zeaxanthin (rt 5.11 min) and its four isomers, and all-*trans*-β-cryptoxanthin were determined with protonated molecules at *m/z* 553.32 and MS/MS fragment ions at *m/z* 535.20 and 497.10. Lutein and zeaxanthin, characterized by the same fragmentations, were identified based on the lower signal intensity

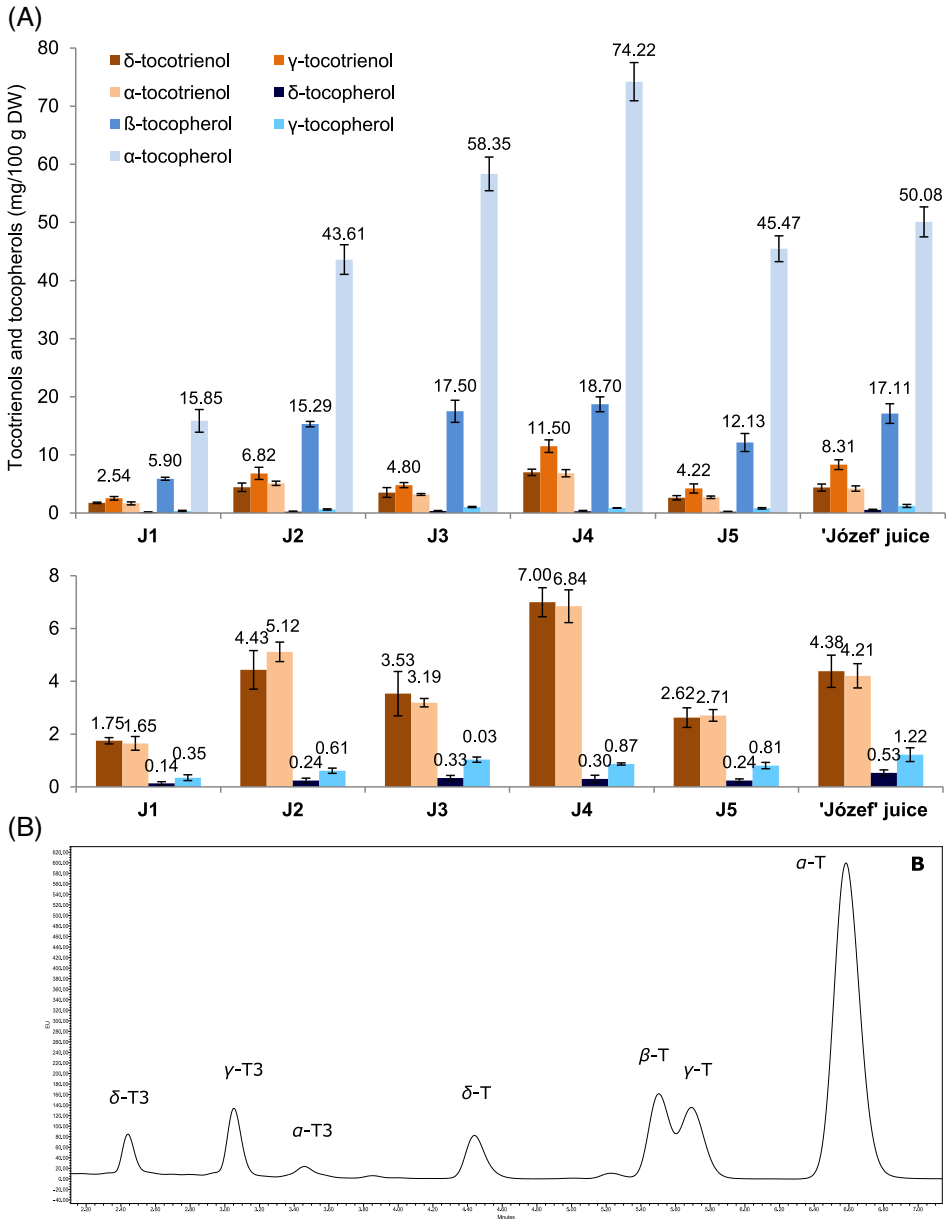

Figure 1. Tocotrienol and tocopherol content (A) and UPLC-FL chromatograph of α-, β-, γ-, and Δ-isomers in sea buckthorn juices (B). J1-J5, Juices 1–5; Peaks: Δ-T3 (Δ-tocotrienol); γ-T3 (γ-tocotrienol); α-T3 (α-tocotrienol); Δ-T (Δ-tocopherol); γ-T (γ-tocopherol); β-T (β-tocopherol); α-T (α-tocopherol).

of parental ions (*m/z* 569.40) than fragment ions (*m/z* 551.11 and 476.03) characteristic for lutein, and also different absorption bands, as given in Table 2. Of the carotenes, Δ-, γ-, α-, and ε-carotene, all-*trans*-ß-carotene, and *cis*-ß-carotene (rt 7.53 to 8.77 min) showed MS/MS fragmentation with toluene loss $[M + H\text{-}92]^+$ yielding ions at *m/z* 444.01. Protonated ions at *m/z* 541.41 and fragment ions at *m/z* 404.00 corresponded to ζ-carotene (rt 8.39 min), while phytofluene (rt 9.84 min) was identified at *m/z* 549.39 → 449.02, for the specific absorption band 323, 349, and 367 nm, according to Pop *et al.*[31] and Tkacz *et al.*[24]

The sum of xanthophylls ranged from 133.65 (J3) to 552.37 mg 100 g^{-1} DW (J1), and the dominant one was all-*trans*-zeaxanthin, constituting 45 to 83% of total xanthophylls for J2 and J5 juices, respectively. Interestingly, lutein and its isomers were not determined in J3 and J5 juices, but this compound is characteristic only for some sea buckthorn cultivars.[24,31] The content of these compounds decreases with the maturation process and may depend on interaction of harvest date, year and cultivar.[32] In turn, all-*trans*-β-cryptoxanthin, one of the dominant compounds for sea buckthorn, was not detected in J3 and J4 juices, which also did not contain carotenes. The low amounts of these secondary metabolites can be explained by the elimination of the fat phase from the juices or by the processing technology with only cold pre-treatment of the raw material. Seglina *et al.*[13] found that heating sea buckthorn berries (5 min, 98 °C) immediately before juice pressing significantly increased the carotenoid amounts (up to 56%) due to their release from a particularly abundant skin. In turn, Andersson *et al.*[32] explained the low amounts of zeaxanthin and β-cryptoxanthin by their presence in esterified carotenoids. However, this study did not investigate esters, even though zeaxanthin dipalmitate was previously investigated

Table 2. LC/MS-QTOF data of carotenoid identification and their UPLC-PDA quantification in sea buckthorn juices

Peak no.	Compounds	Rt (min)	λ_{max} (nm)	MS $[M + H]^+$ (*m/z*)	MS/MS $[M + H]^+$ (*m/z*)	Carotenoid content (mg/100 g DW)					
						J1	J2	J3	J4	J5	'Józef' juice
1	Lutein isomer I	4.60	447/476	569.40	476.03	7.18 ± 0.82 a	5.14 ± 0.50 b	nd	nd	nd	nd
2	All-*trans*-lutein	5.04	447/476	569.40	551.11/476.03	7.09 ± 0.39 a	4.85 ± 0.65 b	nd	3.05 ± 0.16 c	nd	nd
3	All-*trans*-zeaxanthin	5.11	453/481	569.40	551.11/476.03	367.36 ± 5.24 a	180.87 ± 3.11 b	106.59 ± 3.47 e	113.82 ± 4.04 d	126.17 ± 3.78 c	128.66 ± 4.03 c
4	Zeaxanthin isomer I	5.21	453/481	569.40	551.11/476.03	17.09 ± 1.54 a	12.52 ± 1.18 b	nd	nd	nd	nd
5	Zeaxanthin isomer II	5.55	453/481	569.40	551.11/476.03	28.22 ± 1.17 a	12.43 ± 1.44 b	nd	nd	3.83 ± 1.94 d	10.24 ± 1.73 c
6	Zeaxanthin isomer III	5.80	453/481	569.40	551.11/476.03	38.05 ± 2.35 b	41.35 ± 1.87 a	27.06 ± 1.30 c	35.99 ± 1.07 b	14.56 ± 1.37 d	26.65 ± 1.38 c
7	Zeaxanthin isomer IV	6.27	453/481	569.40	551.11/476.03	37.73 ± 2.48 b	68.10 ± 2.74 a	nd	nd	nd	27.07 ± 2.46 c
8	Lutein isomer II	6.32	447/476	569.40	476.03	30.65 ± 1.05 b	49.85 ± 1.90 a	nd	nd	nd	15.25 ± 1.70 c
9	Lutein isomer III	6.43	447/476	569.40	476.03	8.04 ± 1.44 b	15.87 ± 0.72 a	nd	nd	nd	2.68 ± 0.15 c
10	All-*trans*-ß-cryptoxanthin	7.38	453/478	553.32	535.20/497.10	10.96 ± 1.20 a	11.04 ± 1.37 a	nd	nd	6.66 ± 0.88 b	7.17 ± 0.37 b
11	Δ-Carotene	7.53	432/457/489	537.38	444.01	Nd	nd	nd	nd	0.66 ± 0.13	nd
12	γ-Carotene	8.10	436/461/492	537.38	444.01	nd	nd	nd	nd	8.20 ± 0.96	nd
13	α-Carotene	8.13	422/445/472	537.38	444.01	nd	nd	nd	nd	15.82 ± 1.84 a	1.96 ± 0.10 b
14	ζ-Carotene	8.39	380/401/426	541.41	404.00	nd	nd	nd	nd	5.04 ± 0.42	nd
15	ε-Carotene	8.49	417/441/470	537.38	444.01	nd	nd	nd	nd	9.34 ± 0.66	nd
16	All-*trans*-ß-carotene	8.70	425/453/480	537.38	444.01	183.78 ± 3.37 b	179.19 ± 3.95 b	nd	nd	409.90 ± 6.15 a	149.99 ± 4.64 c
17	*Cis*-ß-carotene	8.77	363/450/482	537.38	444.01	44.79 ± 1.63 c	43.64 ± 2.35 c	nd	nd	238.55 ± 3.53 a	86.72 ± 1.36 b
18	Phytofluene	9.84	323/349/367	543.39	449.02	0.35 ± 0.31 b	nd	nd	nd	1.16 ± 0.29 a	nd
	Total carotenoids					781.27 ± 5.30 b	624.85 ± 4.02 c	133.65 ± 3.51 f	152.86 ± 4.77 e	839.89 ± 6.23 a	456.39 ± 4.72 d

The data shown are mean values ± SD (n = 3); values followed by the same letter, within the row, were significantly different ($P < 0.05$); J1-J5, Juices 1–5; DW, dry weight; nd, not detectable.

Table 3. LC/MS-QTOF data of free amino acid identification and their UPLC-PDA quantification in sea buckthorn juices

Peak no.	Amino acid	Rt (min)	λ_{max} (nm)	Parent ion $[M + H]^+$ (*m/z*)	Daughter ion $[M + H]^+$ (*m/z*)	Free amino acid content (mg/100 g DW) J1	J2	J3	J4	J5	'Józef' juice
Essential amino acids											
1	**His**	3.32	259	326.21	171.11	9.72 ± 0.32 a	9.55 ± 0.54 a	8.15 ± 0.31 c	5.59 ± 0.40 e	8.68 ± 0.19 b	7.03 ± 0.27 d
2	**Thr**	6.15	259	290.20	171.11	6.35 ± 0.44 b	4.32 ± 0.40 c	2.15 ± 0.26 d	6.92 ± 0.31 ab	7.14 ± 0.63 ab	7.44 ± 0.82 a
3	**Lys**	7.27	259	487.35/244.18	171.11	55.59 ± 2.40 a	10.46 ± 1.62 e	8.53 ± 1.53 f	34.26 ± 1.15 c	27.54 ± 1.85 d	41.91 ± 2.00 b
4	**Val**	8.48	260	288.22	171.11	7.99 ± 1.55 b	6.83 ± 1.45 c	3.45 ± 1.54 d	8.87 ± 1.99 ab	9.11 ± 1.40 a	8.37 ± 1.06 b
5	**Ile**	9.78	260	302.24	171.11	2.38 ± 0.38 a	1.48 ± 0.88 b	0.94 ± 0.25 c	2.06 ± 0.38 ab	1.92 ± 0.62 b	2.89 ± 0.29 a
6	**Leu**	9.84	260	302.24	171.11	0.78 ± 0.03 d	1.47 ± 0.13 c	1.10 ± 0.40 c	2.55 ± 0.45 b	2.21 ± 0.56 b	3.88 ± 0.96 a
7	**Phe**	9.38	260	336.24	171.11	34.81 ± 1.59 c	20.18 ± 1.36 d	16.70 ± 1.11 e	40.75 ± 2.06 b	22.47 ± 1.26 d	48.16 ± 2.13 a
8	**Trp**	10.04	260	375.25	171.11	8.60 ± 0.93 c	10.18 ± 0.99 b	16.44 ± 1.42 a	12.67 ± 1.64 b	15.14 ± 1.00 a	10.54 ± 0.88 b
Conditionally essential amino acids											
9	**Arg**	4.12	259	345.24	171.11	6.71 ± 0.66 b	1.72 ± 0.28 e	2.85 ± 0.50 d	6.49 ± 0.33 b	7.69 ± 0.28 a	6.07 ± 0.91 c
10	**Gln**	4.50	259	317.21	171.11	23.91 ± 1.16 b	6.33 ± 1.25 d	6.95 ± 1.08 d	41.05 ± 2.10 a	40.48 ± 1.57 a	17.81 ± 1.12 c
11	**Gly**	4.83	259	246.16	171.11	4.83 ± 0.18 c	4.33 ± 0.76 c	2.29 ± 0.23 d	6.82 ± 0.38 b	4.74 ± 0.64 c	7.84 ± 0.53 a
12	**Pro**	7.44	259	286.20	171.11	4.73 ± 0.62 d	10.18 ± 0.81 b	1.39 ± 0.27 e	21.89 ± 1.11 a	8.42 ± 0.71 bc	7.38 ± 0.80 c
13	**Tyr**	8.12	260	352.23	171.11	3.71 ± 0.48 a	0.60 ± 0.10 c	1.85 ± 0.21 b	3.66 ± 0.31 a	3.93 ± 0.25 a	3.53 ± 0.28 a
Non-essential amino acids											
14	**Asn**	3.74	259	303.02	171.11	818.70 ± 5.05 b	185.85 ± 4.10 e	53.04 ± 2.20 f	1467.97 ± 7.63 a	709.04 ± 5.93 c	563.96 ± 4.49 d
15	**Ser**	4.48	259	276.18	171.11	18.99 ± 0.72 c	24.41 ± 0.67 b	8.74 ± 0.32 d	19.61 ± 0.85 c	8.81 ± 0.94 d	34.21 ± 1.41 a
16	**Asp**	5.39	259	304.17	171.11	20.27 ± 0.60 a	7.08 ± 0.74 c	3.82 ± 0.47 d	23.96 ± 1.09 a	19.16 ± 0.88 a	14.95 ± 0.55 b
17	**Glu**	5.79	259	318.19	171.11	33.05 ± 1.72 a	28.26 ± 0.83 b	8.82 ± 0.73 c	26.18 ± 0.55 b	31.44 ± 1.01 a	31.19 ± 1.26 a
18	**Ala**	6.70	259	260.16	171.11	35.22 ± 1.37 d	31.10 ± 1.00 e	16.34 ± 1.04 f	52.95 ± 1.86 a	48.43 ± 1.80 b	39.34 ± 1.25 c
19	**GABA**	6.83	259	274.22	171.11	22.83 ± 1.02 b	16.23 ± 1.25 c	6.60 ± 0.46 d	30.92 ± 1.54 a	31.69 ± 1.59 a	30.25 ± 1.77 a
20	**Hcys**	7.76	259	306.01	171.11	5.47 ± 0.60 c	7.20 ± 0.52 b	5.77 ± 0.66 c	7.52 ± 0.74 ab	8.17 ± 0.46 a	5.20 ± 0.94 c
Total essential amino acids						126.22 ± 2.42 a	64.47 ± 1.65 d	57.46 ± 1.58 d	113.68 ± 2.09 b	94.21 ± 1.88 c	130.23 ± 2.16 a
Total conditionally essential amino acids						43.90 ± 1.20 c	23.17 ± 1.27 d	15.33 ± 1.11 e	79.91 ± 2.14 a	65.26 ± 1.60 b	42.63 ± 1.16 c
Total non-essential amino acids						954.53 ± 5.09 b	300.14 ± 4.14 e	103.13 ± 2.23 f	1629.10 ± 7.68 a	856.75 ± 5.95 c	719.11 ± 4.53 d
Total amino acids						1124.66 ± 5.11 b	387.77 ± 4.15 e	175.92 ± 2.21 f	1822.69 ± 7.66 a	1016.22 ± 5.96 c	891.98 ± 451 d

His, histidine; **Thr**, threonine; **Lys**, lysine; **Val**, valine; **Ile**, isoleucine; **Leu**, leucine; **Phe**, phenylalanine; **Trp**, tryptophan; **Arg**, arginine; **Gln**, glutamine; **Gly**, glycine; **Pro**, proline; **Tyr**, tyrosine; **Asn**, asparagine; **Ser**, serine; **Asp**, aspartic acid; **Glu**, glutamic acid; **Ala**, alanine; **GABA**, γ-amino-n-butyric acid; **HCys**, homocysteine.
The data shown are mean values ± SD (n = 3); values followed by the same letter, within the row, were significantly different ($P < 0.05$); J1-J5, Juices 1–5; DW, dry weight.

using the LC–MS method in berries of some sea buckthorn cultivars grown in Poland.[24]

The concentration of carotenes in juices J1 and J2 and the juice obtained on a laboratory scale were statistically similar (mean 230.02 mg 100 g^{-1} DW), whereas J5 juice contained all of the carotenes identified in the total amount of 687.51 mg 100 g^{-1} DW. All-*trans*-ß-carotene in these juices constituted from 60 to 80% of the total carotenes. This was in line with previous studies that also found zeaxanthin and ß-carotene to be the predominant carotenoids in *H. rhamnoides* fruits.[9,31,32] Lycopene was not detected in the juices, despite being present in a considerable amount in previously tested berries of sea buckthorn varieties (up to 8%)[31] and commercially available sea buckthorn juice (13%).[9]

It is known that the influence of cultivar on the carotenoid content is the strongest but in the case of carotenes, the year could have had a greater influence than harvest date, and conversely for xanthophylls. High variability could result from subspecies, sea buckthorn origin, and growth conditions (temperature, insolation, rainfall) during the vegetation period.[31,32] Nevertheless, the presence of carotenoids determines the yellow to almost red color of sea buckthorn products and thus their attractiveness. Carotenes dominated in J5 juice (82 and 52% of total carotenoids), for J6 juice the carotene: xanthophyll ratio was almost 1:1, and for the rest of the juices xanthophylls were dominant (64 to 100% of total carotenoids). Thus, no correlation was found between these two carotenoid groups (r = 0.01), but carotenes strongly correlated with phytofluene (r = 0.91). Its presence in J1 and J5 juices corresponded to no more than 0.2% of the total carotenoids. Phytofluene is a colorless acyclic intermediate in carotenoid biosynthesis, so it could be concluded that its presence in juices is caused by impurity of unripe berries. However, no chlorophylls were detected in the juices, which indicates fully maturated berries.[32] On the other hand, Trebolazabala *et al.*[33] observed an increase in phytofluene in the middle stage of tomato ripening, which could be due to the activation of the carotenoid biosynthesis pathway before reaching full maturity. The presence of phytofluene in sea buckthorn berries or juices has not been reported so far, but significant amounts have been found in carrots, tomatoes, peppers, watermelon, red grapefruits, and apricot. Its final content in food has not been studied fully and probably does not correlate with cultivar and growing conditions.[34]

Free amino acids of sea buckthorn juices

The amino acids analyzed by the UPLC-PDA-Q/TOF-MS method, along with their corresponding retention times, maximum absorption wavelengths, and specific parent and daughter ions ($[M + H]^+$ *m/z*), are presented in Table 3. In the sea buckthorn juices, 20 free amino acids were identified and quantified, including eight essential (EAAs) and five conditionally essential amino acids (CEAAs) for the human body.

The total sum of amino acids ranged from 175.92 (J3) to 1822.60 mg 100 g^{-1} DW (J4), including EAAs and CEAAs between 72.79 (J3) and 193.59 mg 100 g^{-1} DW (J4). The correlation between these two groups of amino acids was moderate (r = 0.60). The results obtained were in line with the data included in the reviews by Bekker and Glushenkova[35] and Zeb[11] concerning the composition of *H. rhamnoides*. These authors presented 18 free amino acids in sea buckthorn juices with a total content of 94.5–188.3 mg 100 mL^{-1} and 51.57 mg 100 g^{-1} of juice, respectively, which in terms of the average dry weight of sea buckthorn juices is about 706 to 2580 mg 100 g^{-1} DW. The juices tested showed a rich profile of EAAs and CEAAs but their content was moderate, from 11 (J4) to 41% of the total amino acid pool (J3). Only selected juices can therefore be an interesting source of amino acids and protein with nutritional importance. The research of Zenkova and Pinchykova[36] demonstrated that EAAs in sea buckthorn berries were differentiated by cultivar (38 to 59% of total amino acids). Juice J3, with the lowest amino acid content, was characterized by the highest percentage of EAAs and CEAAs, 33 and 9% of total amino acids, respectively, similar to the previously studied sea buckthorn juices (23 to 50% of EAAs).[35] The ratio of EAAs to CEAAs ranged from 1.4 (J4 and J5) to 3.7 (J3). In the juice obtained on a laboratory scale, the contents of EAAs and CEAAs were similar to those in J1 juice, followed by J4 and J5.

The contents of individual amino acids in the juices varied significantly, but the dominant one – asparagine (Asn) – represented from 30 (J3) to 81% (J4) of the total amino acids. In all juices, glutamine (Gln), glutamic acid (Glu), alanine (Ala), γ-amino-n-butyric

Table 4. Inhibitory activity against acetyl- and butylcholinesterase, α-amylase, α-glucosidase, pancreatic lipase and 15-lipoxygenase and antioxidant capacity of sea buckthorn juices

	Sea buckthorn juices					
Biological potential	J1	J2	J3	J4	J5	'Józef' juice
Enzyme inhibitory activity						
AChE (IC_{50})	110.43 ± 2.42 b	239.95 ± 4.56 f	128.99 ± 2.60 c	137.54 ± 2.05 d	186.5 ± 2.77 e	87.22 ± 1.44 a
BuChE (IC_{50})	174.10 ± 4.56 f	130.19 ± 3.53 d	140.56 ± 3.56 e	80.93 ± 2.04 b	101.28 ± 2.92 c	56.15 ± 1.13 a
α-Amylase (IC_{50})	80.25 ± 3.40 c	75.55 ± 2.43 b	81.17 ± 2.62 c	68.94 ± 1.98 a	74.52 ± 2.43 b	72.41 ± 2.30 b
α-Glucosidase (IC_{50})	37.94 ± 1.24 d	89.17 ± 3.54 f	33.35 ± 1.45 c	29.45 ± 1.28 b	44.10 ± 1.59 e	23.97 ± 1.14 a
Pancreatic lipase (IC_{50})	0.54 ± 0.14 b	0.05 ± 0.01 a	0.05 ± 0.03 a	3.39 ± 0.53 e	1.35 ± 0.31 d	1.12 ± 0.52 c
15-Lipoxygenase (% of inhibition)	15.21 ± 1.07 e	46.71 ± 2.26 c	56.29 ± 2.65 b	41.02 ± 2.17 d	47.01 ± 2.95 c	61.01 ± 3.61 a
Antioxidant capacity (mmol Trolox/100 g FW)						
ORAC	9.27 ± 0.34 c	9.79 ± 0.54 c	9.47 ± 0.62 c	13.55 ± 0.24 a	13.45 ± 0.81 a	12.78 ± 0.35 b
FRAP	0.24 ± 0.05 c	0.25 ± 0.04 c	0.45 ± 0.07 b	0.47 ± 0.08 b	0.68 ± 0.10 a	0.45 ± 0.06 b
ABTS	0.16 ± 0.02 e	0.18 ± 0.03 e	0.36 ± 0.04 c	0.62 ± 0.04 a	0.29 ± 0.02 d	0.55 ± 0.04 b

The data shown are mean values ± SD (n = 3); values followed by the same letter, within the row, were significantly different ($P < 0.05$); AChE, acetylcholinesterase; BuChE, butylcholinesterase; IC_{50}, the half maximal inhibitory concentration (mg of sample/mL); J1-J5, Juices 1–5; FW, fresh weight.

acid (GABA), lysine (Lys), and phenylalanine (Phe) were also detected in significant amounts, and histidine (His), serine (Ser), and tryptophan (Trp) were detected in J2 and J4 juices. According to Bekker and Glushenkova (2001)[35] and Zeb (2004),[11] in juices and berries, proline (Pro), aspartic acid (Asp) and threonine (Thr) were also dominant in addition to Asn, Ser, Lys, and Phe, highlighted in this study. However, the amino acid profile of the juices studied was more similar to the recent report by Constantin *et al.*,[6] according to which the predominant amino acids in sea buckthorn berries were Asn, Gln, Ala, and Asp (Table 3).

The relatively high and diverse content of Asn, Asp, and Ala in the juices tested should be considered in the context of the risk of Maillard reactions causing formation of potentially toxic compounds with negative health effects (acrylamide, hydroxymethylfurfural, heterocyclic amines, furans).[6,25] On the other hand, Constantin *et al.*[6] identified the optimal temperature and time sequence to reduce ACR and 5-HMF formation during thermal treatment of sea buckthorn purée, that is 134.87 °C for 14.82 min.

Sea buckthorn seeds are particularly rich in Asp, Glu, and Arg;[37] hence the high content of these amino acids in juices could be determined by the pressing process and potential crumbling of seed coats. In turn, Prandi *et al.*[38] found that, of the fruit waste, sea buckthorn spent pulp contained significant amounts of EAAs (35%), including high levels of Lys and Leu. Therefore, when assessing the concentrations of these two amino acids in sea buckthorn juices (especially the high content of Lys in J1, J4, J5 and J6), the pressing efficiency and composition of pomace should be taken into account. In addition to the aforementioned factors, amino acids could be determined by growing conditions (soil, habitat and climatic conditions, irrigation) and extraction conditions.[6,25]

In all juices tested, 18 proteinogenic amino acids were identified, without cysteine and methionine, contrary to the previous reports on *H. rhamnoides*.[6,11,36] However, according to the investigation by Zenkova and Pinchykova[36] cysteine was characteristic only for some sea buckthorn cultivars. Importantly, among the non-proteinogenic amino acids, GABA constituted from 1.7 (J4) to 4.5% (J2), and the sulfur-containing amino acid homocysteine (HCys) from 0.4 (J4) to 3.3% of total amino acids (J3) were determined. Although these amino acids are not incorporated into proteins, their presence in sea buckthorn juices may be health related as they act as principal neurotransmitters at inhibitory synapses and a potent antioxidant.[18,25] Bekker and Glushenkova[35] reported the presence of only the S-methylated derivative of cysteine in sea buckthorn berries, determined by an automated amino acid analyzer. However, to the best of our knowledge, there is no amino acid analysis using LC–MS for sea buckthorn juices, and this study is the first to identify and quantify HCys and GABA for *H. rhamnoides*.

Enzyme inhibitory activity of sea buckthorn juices

In vitro *anti-cholinesterase effect*

In the therapy of neurodegenerative diseases, an important role is played by cholinesterase inhibitors that inhibit the development of lesions with relatively low side effects, although they do not eliminate their causes. The activity of sea buckthorn juices against acetyl- and butylcholinesterase as a potential way to inhibit degenerative changes by increasing transmission in the cholinergic system was investigated (Table 4). IC_{50} values ranged from 87.22 (J6) to 239.95 mg mL^{-1} (J2) for AChE and between 56.15 (J6) and 174.10 mg mL^{-1} (J1) for inhibition of BuChE. In the case of juices J2, J4, J5 and J6, the activity towards BuChE was stronger than for AChE, which was in line with previous results for sea buckthorn cultivars.[24] The juices tested were significantly less active than berries, but juice obtained on a laboratory scale had the highest anti-aging potential.

PhytoPs, PhytoF and selected amino acids (Lys, Tyr, Ile, Phe) correlated strongly with anti-AChE activity ($r \geq 0.52$), and the correlation with HCys was strongly negative ($r = -0.73$) (supplementary material Table S1). In the case of anti-BuChE activity, a strong correlation with tocopherols and tocotrienols ($r \geq 0.63$), selected PhytoPs, PhytoF and amino acids (His, Gly, Thr, Ala, GABA, Pro, Leu, Phe), and strong negative correlations with lutein, zeaxanthin, and some of their isomers were found. Previous reports indicate the potential of vitamin E in slowing the progression of dementia, modulating signaling and gene regulation. α-Tocopherol was able to modulate pathways that are altered in Alzheimer's disease (AD). Vitamin E supplementation normalized behavioral and cognitive functions, was able to counteract oxidative stress and β-amyloid toxicity, and exerted beneficial effects in AD animal models.[39] Furthermore, recent reports suggest that some isoprostanes (IsoPs), isofurans (IsoFs), and neurofurans (NeuroFs), for example F_2-IsoPs, 15-F_{2t}-IsoP and F_4-NeuroPs, may be potential oxidative stress biomarkers of neuronal diseases, including Alzheimer's and Parkinson's.[40] However, the role of plant oxylipins including PhytoPs and PhytoFs in the regulation of neurodegenerative disorders has not been elucidated, and this is the first report of their anticholinergic potential. Abnormal amino acid levels may indicate a pathogenesis associated with mild cognitive impairment and preclinical dementia. For instance, a prospective cohort study indicated that the essential branched-chain amino acids (Val, Ile, and Leu) are associated with lower dementia risk and Alzheimer's disease.[41] In this research, these amino acids correlated more strongly with the anti-BuChE effect ($r = 0.45$ for Val, Ile; $r = 0.97$ for Leu). The high content of PhytoPs, PhytoFs, tocopherols, tocotrienols, and amino acids, in selective correlation with anti-AChE and/or anti-BuChE activity, underscores the importance of sea buckthorn juices in the diet used in the treatment of neurodegenerative diseases.

In vitro *anti-diabetic and anti-obesity effects*

The hypoglycemic effect of sea buckthorn juices was analyzed for inhibition of α-amylase and α-glucosidase (IC_{50}). The inhibitory potential against α-glucosidase (68.92 to 81.17 mg mL^{-1}) was stronger than for α-amylase (23.97 to 89.17 mg mL^{-1}), except for J2 juice. The juice from the berries of cv. 'Józef' was similarly active to the J2 and J5 juices, and only the J4 juice was more active than them. In the case of anti-glucosidase activity, laboratory juice was the strongest, and J2 juice showed 2 to 3.7 times weaker potential than the others.

Surprisingly, the anti-amylase and anti-glucosidase activities did not correlate ($r = 0.13$), which could indicate a different effect of bioactive compounds contained in *H. rhamnoides* on these activities. Correlations between anti-amylase and the sum of tocotrienols, tocopherols, and most amino acids (Asn, Gln, Gly, Asp, Thr, Ala, GABA, Pro, Val, Leu, Phe) were strong ($r \geq 0.50$). However, in contrast to vitamin E, amino acids were less closely correlated with anti-glucosidase activity. Only Leu was able to positively inhibit glucosidase ($r = 0.65$).

The analysis of the correlation for carotenoids and anti-hyperglycemic activity of juices showed differences with regard to the well-documented activities of some carotenoids, including β-carotene and β-cryptoxanthin, against type 2 diabetes.[42]

However, in assessing the potential of sea buckthorn juices, the potential isomerization of naturally occurring (*all*-E)-carotenoid configuration to (Z)-isomers as a result of heat treatment and exposure to light and acids, resulting in loss of provitamin A activity and changes in bioavailability, should be considered.[9] On the other hand, biological activity may result from synergistic or antagonistic effects of compounds in juices, including those not determined in this study, such as polyphenols, triterpenes, fruit acids, and sugars contained in *H. rhamnoides*.

All juices showed high pancreatic lipase inhibitory activity, with the highest levels of J2 and J3 juices (0.05 mg mL^{-1}). Type 2 diabetes is known to correlate strongly with adipose tissue gain and the degree of obesity. Hence inhibition of pancreatic lipase (a key enzyme in lipid digestion and absorption) may have implications for the treatment of both diabetes and obesity.[43] Studies on the risk factors of coronary heart disease in humans indicated that supplementation with sea buckthorn juice caused a moderate decrease in the susceptibility of low-density lipoproteins (LDL) to oxidation and an increase in the concentration of high density lipoprotein cholesterol (HDL—C) and triacylglycerol (TAG) in plasma.[14] The Pearson correlation study revealed xanthophylls (cryptoxanthin, lutein, zeaxanthin, and their isomers) and some PhytoPs contained in juices as potent pancreatic lipase inhibitors ($r \geq 0.50$). Recent cell and animal tests have associated carotenoids and their derivatives with reducing obesity and influencing key aspects of adipose tissue, while human epidemiological studies have shown that higher dietary intake of carotenoids and their serum levels are associated with decreased adiposity.[44] However, to the best of our knowledge, this research is the first to show PhytoPs' ability to inhibit pancreatic lipase.

In vitro *anti-inflammatory effect*

The anti-inflammatory potential of sea buckthorn juices as a percentage of inhibition of 15-lipoxygenase activity at a concentration of 30 mg mL^{-1} was analyzed (Table 4). The inhibition results were between 15.21 and 61.01%, and the juices in terms of activity can be ranked as follows: J6 > J3 > J2 ≈ J5 > J4 > J1. Phytoprostanes and PhytoFs ($r = 0.95$ and 0.50) correlated most strongly with anti-15-LOX activity, except for *Ent*-16-*epi*-16-F_{1t}-PhytoP and *Ent*-16-F_{1t}-PhytoP ($r = -0.19$), which were most common in sea buckthorn juices. The F_{1t}-PhytoP isomers seemed to have the strongest effect against 15-LOX ($r = 0.94$). The results are in line with previous studies suggesting the anti-inflammatory activity of PhytoPs and their ability to modulate the cellular mechanisms involved in the adaptive immune response. The biological effects are explained by the strong structural analogy between PhytoPs and endogenous prostaglandins, with regard to the cyclopentenone ring and electrophilic character. Karg *et al.*[20] reported that the cyclopentenone A_1-PhytoPs and deoxy-J_1-PhytoPs exhibit potent anti-inflammatory and apoptosis-inducing effects similar to A_1- and deoxy-J_2-prostaglandins. Immunomodulatory activity is also associated with the analogy between E_2-prostaglandin and 16-E_1-PhytoP, while the ability to hinder signaling mediated by nuclear factor kappa B (NF-κB) suggests multidirectional potential in immune and inflammatory processes, oncogenesis, and tumor progression.[22,45]

For tocopherols and tocotrienols and carotenoids, the correlation with anti-15-LOX activity was negligible ($r = -0.22$ and -0.07), but the effect of amino acids was varied. Most of them, but not Asn, Gln, Ala, Pro and HCys, correlated weakly with activity against 15-LOX (r between −0.46 and 0.31). Previous studies have identified amino acids as anti-inflammatory, immunomodulatory, and cytoprotective agents, as their role is related to the synthesis of cytokines and antibodies, as well as the regulation of metabolic pathways of the immune response to infectious pathogens. For instance, Trp and Tyr are associated with inhibition of the production of inflammatory cytokines and superoxide, GABA administration may inhibit development of a pro-inflammatory T-cell response in mice, and Gly, in turn, reduced inflammatory responses and improved the survival rate in pathogen-infected animals.[46]

Antioxidant capacity of sea buckthorn juices

The antioxidant capacity of sea buckthorn juices was assessed as oxygen radical absorbance capacity (ORAC), ferric reducing ability (FRAP), and free radical-scavenging activity (ABTS) (Table 4). Activity in the ORAC test was the highest, between 9.27 (J1) and 13.55 mmol TE/100 g FW (J4), while the activities in the other two tests were similar and ranged from 0.16 (J1) to 0.68 mmol TE/100 g FW (J5). The activity of sea buckthorn juices varied depending on the type of assay, similar to the studies by Möller *et al.*,[9] who observed the highest activity for sea buckthorn juice (0.74 mmol α-TE/100 g) in the luminol-chemiluminescence based peroxyl radical scavenging capacity (LPSC) test based on peroxyl radical scavenging capacity. Despite the differentiation, the results of antioxidant activity strongly correlated ($r = 0.71$ for ORAC-FRAP; $r = 0.83$ for ORAC-ABTS; $r = 0.88$ for ABTS-FRAP). Thus, the J4 and J5 juices were 1.5 to 3.8 times more active towards oxidants than the J1 and J2 juices. The activity of the juice obtained on a laboratory scale was also close to the average antioxidant activity of commercial juices.

No strong correlations were found between the antioxidant activity of the juices and PhytoPs and PhytoF (supplementary material Table S1). Currently, the influence of PhytoPs on human physiological mechanisms is associated with metabolic processes. These oxylipins can activate the transcription factor Nrf2, which regulates the resistance of cells to oxidants, and controls and induces the expression of the antioxidant response. For sea buckthorn juices, the interactions of PhytoPs, carotenoids, polyphenols, diterpenes, and triterpenes present in *H. rhamnoides* berries may be particularly important in the context of these biological activities.[21,47]

Tocopherols and tocotrienols are known to have antioxidant properties, it has been suggested that they prevent lipid peroxidation in plant membranes, and the ability of their antioxidant system depends on the stress level.[7] The total content of tocopherols and tocotrienols in sea buckthorn juices moderately correlated with their antioxidant activity ($r = 0.38$ to 0.54), and tocopherols were more active than tocotrienols according to ABTS and FRAP tests. α-Tocopherol was the most effective antioxidant of the tocochromanols tested ($r = 0.48$ to 0.55). This is agreement with antioxidant activity of β, γ and Δ forms from which represented 10 to 50% of α-T activity.[2] In research on antioxidant activity assays,[9] the lipophilic extract of sea buckthorn juice was found to be more active towards oxidants than tomato and carrot juice extracts, rich in carotenoids and tocopherols, and showed 20 times higher activity than orange juice. In this study, the antioxidant activity of sea buckthorn juices was more strongly related to the carotene content ($r = 0.24$ to 0.81) than to xanthophylls, which negatively correlated with the ORAC, FRAP, and ABTS effects ($r = -0.33$ to -0.91).

The results should be considered with regard to mechanisms of antioxidant action and test conditions. Previous analyses of the α- and β-carotene standards indicated their higher antioxidant activity than lutein and zeaxanthin in the α-tocopherol equivalent

antioxidant capacity (α-TEAC test), in contrast with the FRAP test, and significantly similar activities in the LPSC assay.[9] However, in sea buckthorn juices, a diverse effect of carotene forms on the activity was found. So Δ-, γ-, α-, ζ- and ε-carotene correlated more strongly with antioxidant activity than ß-carotene. The antioxidant activity of carotenoids depends on their structural features, including the number of double bonds, functional groups on rings and type of ring, while the degree and pattern of methylation affect the potential of tocopherols and derivatives.[27] The accumulation of these metabolites during the ripening of sea buckthorn berries corresponds to the increase in antioxidant capacity of lipophilic extracts.[27] However, the bioassays in this study were carried out on water–methanol extracts, which may explain the differences.

Antioxidant activity correlated positively with amino acids, and especially strongly for the ORAC effect (r = 0.65). According to the three measurements, Arg and Gln followed by Thr, Ala, GABA, Tyr, Leu, and Trp had the strongest antioxidant effect (r above 0.50 for at least two tests). Improving the oxidative defense of Arg, Gln, Tyr, and Trp (along with Ser, Gly, Glu, Pro) has also been emphasized in previous reports.[18,46] For example, supplementation with Gln increased intestinal expression of genes necessary for cell growth and removal of oxidants, and decreased expression of genes promoting oxidative stress and immune activation.[18] Literature data provide several mechanisms explaining the antioxidant properties of amino acids, including quenching singlet oxygen, chelating prooxidative metals, synergistic reactions with tocopherols and other primary antioxidants, and the activity caused by secondary compounds of the reactions between amino acids and oxidized lipids. Moreover, the antioxidant activity of Arg, Lys, HCys, and Trp in sea buckthorn juices could be due to the presence of thiol or an additional amino group in these amino acids.[48]

CONCLUSIONS

Phytoprostanes and PhytoFs in sea buckthorn juices were identified for the first time and their concentrations strongly correlated with the inflammation alleviation potential investigated as 15-LOX inhibition. Sea buckthorn juices can be an interesting anti-diabetic and anti-obesity food due to the content of potential inhibitors of α-amylase, α-glucosidase (tocopherols, tocotrienols, selected amino acids) and pancreatic lipase (PhytoPs, xanthophylls). The presence of PhytoPs, PhytoF, tocopherols, tocotrienols, and amino acids increases the functionality of juices in reducing neurodegenerative changes, which makes them potential anti-aging agents in the prevention of the most common dementia type – Alzheimer's disease. Sea buckthorn juices may play an important role in the body's defense mechanism against pathologies related to free radical attack.

The commercialization and globalization of sea buckthorn berries and a wide range of products from them, including juices, would undoubtedly be an achievement. The differentiation of the composition and the bioactive potential of commercial juices indicates that it is important for the consumer to choose juices from the declared berry cultivars and crops. The results of the study may direct *in vivo* analyses of the pro-health potential of juices, and in the future it will be valuable to supplement the research with the profiles of other biologically active compounds.

ETHICS STATEMENT

This research did not include any human subjects or animal experiments.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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OŚWIADCZENIE

Oświadczam, że jestem współautorem publikacji pt.:

Tkacz K., Gil-Izquierdo Á., Medina S., Turkiewicz I.P., Domínguez-Perles R., Nowicka P., Wojdyło A. 2022. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 102(1), 185-197. doi: 10.1002/jsfa.11345.

Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, pozyskaniu i przygotowaniu materiału badawczego, oznaczeniu ilościowym i identyfikacji fitoprostanów i fitofuranów metodą UHPLC-ESI-QqQ-MS/MS, tokoferoli i tokotrienoli metodą UPLC-FL, karotenoidów i wolnych aminokwasów metodą UPLC-PDA-Q/TOF-MS, oznaczeniu potencjału przeciwstarzeniowego, przeciw-cukrzycowego, przeciwutleniającego i przeciwzapalnego *in vitro* soków z owoców rokitnika pospolitego. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

Kierowałam projektem naukowym Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy. Analizy oksylipin są efektem współpracy z Naukowcami z Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) w ramach odbytego stażu naukowego „Program PROM" – Międzynarodowa wymiana stypendialna doktorantów i kadry akademickiej (NAWA).

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Oświadczam, że w pracy pt.:

Tkacz K., **Gil-Izquierdo Á.**, Medina S., Turkiewicz I.P., Domínguez-Perles R., Nowicka P., Wojdyło A. 2022. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 102(1), 185-197. doi: 10.1002/jsfa.11345

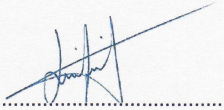
mój udział polegał na koordynowaniu analizą ilościową i identyfikacją fitoprostanów i fitofuranów metodą UHPLC-ESI-QqQ-MS/MS, przygotowaniu wyników z tego zakresu oraz merytorycznym współredagowaniu publikacji. Badania są efektem współpracy z Doktorantką podczas odbytego przez nią stażu naukowego w Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) w ramach „Programu PROM" – Międzynarodowa wymiana stypendialna doktorantów i kadry akademickiej (NAWA). Pełniłem funkcję opiekuna naukowego Doktorantki.

DECLARATION

I declare that in the publication entitled:

Tkacz K., **Gil-Izquierdo Á.**, Medina S., Turkiewicz I.P., Domínguez-Perles R., Nowicka P., Wojdyło A. 2022. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 102(1), 185-197. doi: 10.1002/jsfa.11345

my participation was to coordinate the identification and quantitative analysis of phytoprostanes and phytofurans using the UHPLC-ESI-QqQ-MS/MS method, preparation of results, and substantive co-editing. The research is the result of collaboration with the PhD student during her research internship at Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) under the "PROM Program" - International scholarship exchange of PhD candidates and academic staff (NAWA). I was a research supervisor for the PhD student.



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Oświadczam, że w pracy pt.:

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mój udział polegał na przeprowadzeniu analizy ilościowej i identyfikacji fitoprostanów i fitofuranów metodą UHPLC-ESI-QqQ-MS/MS, uczestnictwie w opracowaniu podrozdziału dotyczącego metodologii w tym zakresie oraz merytorycznym współredagowaniu publikacji. Badania są efektem współpracy z Doktorantką podczas odbytego przez nią stażu naukowego w Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) w ramach „Programu PROM" – Międzynarodowa wymiana stypendialna doktorantów i kadry akademickiej (NAWA).

DECLARATION

I declare that in the publication entitled:

Tkacz K., Gil-Izquierdo Á., **Medina S.**, Turkiewicz I.P., Domínguez-Perles R., Nowicka P., Wojdyło A. 2022. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 102(1), 185-197. doi: 10.1002/jsfa.11345

my participation was to conduct the identification and quantitative analysis of phytoprostanes and phytofurans using the UHPLC-ESI-QqQ-MS/MS method, participation in the development of the subchapter on the methodology in this area, and substantive co-editing. The research is the result of collaboration with the PhD student during her research internship at Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) under the "PROM Program" - International scholarship exchange of PhD candidates and academic staff (NAWA).

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mój udział polegał na uczestnictwie w etapie przygotowania materiału badawczego, analizie tokoferoli i tokotrienoli metodą UPLC-FL i wolnych aminokwasów metodą UPLC-PDA-Q/TOF-MS w sokach z owoców rokitnika pospolitego.

Turkiewicz Igor

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Oświadczam, że w pracy pt.:

Tkacz K., Gil-Izquierdo Á., Medina S., Turkiewicz I.P., **Domínguez-Perles R.**, Nowicka P., Wojdyło A. 2022. Phytoprostanes, phytofurans, tocopherols, tocotrienols, carotenoids and free amino acids and biological potential of sea buckthorn juices. *Journal of the Science of Food and Agriculture*, 102(1), 185-197. doi: 10.1002/jsfa.11345

mój udział polegał na przeprowadzeniu analizy ilościowej i identyfikacji fitoprostanów i fitofuranów metodą UHPLC-ESI-QqQ-MS/MS oraz przygotowaniu wyników z tego zakresu. Badania są efektem współpracy z Doktorantką podczas odbytego przez nią stażu naukowego w Consejo Superior de Investigaciones Científicas - Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) w ramach „Programu PROM" – Międzynarodowa wymiana stypendialna doktorantów i kadry akademickiej (NAWA).

DECLARATION

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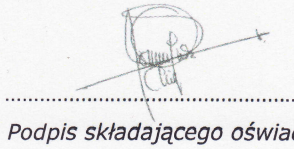

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w analizie tokoferoli i tokotrienoli metodą UPLC-FL, karotenoidów i wolnych aminokwasów metodą UPLC-PDA-Q/TOF-MS, oznaczeniu potencjału prozdrowotnego *in vitro* soków z owoców rokitnika pospolitego oraz współredagowaniu manuskryptu pod względem merytorycznym.

Paulina Nowicka

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w oznaczeniu ilościowym i identyfikacji tokoferoli i tokotrienoli metodą UPLC-FL, karotenoidów i wolnych aminokwasów metodą UPLC-PDA-Q/TOF-MS, oznaczeniu potencjału prozdrowotnego *in vitro* soków z owoców rokitnika pospolitego. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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Podpis składającego oświadczenie

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Dynamics of changes in organic acids, sugars and phenolic compounds and antioxidant activity of sea buckthorn and sea buckthorn-apple juices during malolactic fermentation

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ORAC

ABSTRACT

Sea buckthorn (*Hippophaë rhamnoides* L.) berries have high biological value as a rich source of phenolic compounds, fatty acids and vitamins A, C, E. Due to the high organic acid content and sour taste, the fruits are rarely used in juice production. Therefore, the study aimed to determine the metabolic activity of *Lactobacillus plantarum*, *Lactobacillus plantarum* subsp. *argentoratensis* and *Oenococcus oeni* strains along with the dynamics of changes in organic acids, sugars, phenolic compounds, and antioxidant activity during 72-h fermentation of 100% sea buckthorn and mixed with apple (1:1) juices. The strongest malolactic conversion was in mixed juices (to 75.0%). The most efficient strains were *L. plantarum* DSM 10492, 20174 and 6872. *L. plantarum* strains caused an increase in flavonols and antioxidant activity of sea buckthorn-apple juices. The results can be used to select conditions and strains in industrial-scale fermentation, to produce novel sea buckthorn products and increase their consumption.

1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) is a shrub whose fruit can be used as a remedy supporting treatment and prevention of gastrointestinal disorders, hyperlipidemia, hyperglycemia, hyperinsulinemia, and nervous system and cardiovascular diseases (Bal, Meda, Naik, & Satya, 2011). The positive effects of the plant on the functioning of eyesight, hair and skin have been proven, and antiproliferative effects have been investigated for colon, liver and breast cancer cells and leukemia cells (Grey, Widén, Adlercreutz, Rumpunen, & Duan, 2010; Bal et al., 2011; Guo, Guo, Li, Fu, & Liu, 2017). Previous studies have shown that sea buckthorn berries are notably rich in flavonols, xanthophylls, carotenes, tocopherols and tocotrienols, vitamin C, and n-3, n-6, n-7, and n-9 fatty acids (Tkacz, Wojdyło, Turkiewicz, Bobak, & Nowicka, 2019). Hence, the use of the berries is mainly based on the production of dietary supplements and cosmetics, while in food industry, fruit is only a component in products with added value and high pro-health properties. The berries can also be used for production of jams and jellies, oils, soft drinks, alcoholic beverages and dairy products (Rafalska, Abramowicz, & Krauze, 2017).

Nevertheless, the taste of sea buckthorn fruit is unattractive and described as sour, astringent and with low sweetness. This correlates with the high amount of organic acids (up to 5.4%), including dominant malic acid, low pH 2.9 and sugar:organic acid ratio of 1.2 on average (Tiitinen, Hakala, & Kallio, 2005; Tkacz et al., 2019). In order to increase the consumption and application in the food industry, the solution may be to correct the sour taste of sea buckthorn berries through malolactic fermentation (MLF). This process involves decarboxylation of malic acid to lactic acid and carbon dioxide. MLF is widely used to reduce acidity, increase microbiological stability and modify the aroma, flavor and texture of red wines and some white wines. Among lactic acid bacteria, genus *Oenococcus*, followed by *Lactobacillus* and *Pediococcus*, shows high efficiency and adaptation to unfavorable conditions (du Toit, Engelbrecht, Lerm, & Krieger-Weber, 2011; Bartowsky, Costello, & Chambers, 2015; Wojdyło, Samoticha, & Chmielewska, 2020).

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The potential use of MLF has also been studied in fruit and vegetable juices and pulps. The results suggested that fermentation with *L. plantarum* may have a beneficial effect on the physico-chemical properties, content and profile of bioactive compounds, antioxidant potential and/or sensory evaluation of, among others, olives (Kachouri et al., 2015), kiwifruit pulp (Zhou et al., 2020), pomegranate (Mousavi et al., 2013), mulberry (Kwaw et al., 2018), and *Momordica charantia* juices (Gao et al., 2019). Fermentation with *L. plantarum* and *L. brevis* enhanced the antioxidant and immune modulation features of cactus cladode pulp (Filannino et al., 2016). In addition, *L. plantarum* is commercially relevant as a starter culture for food fermentation, and as a probiotic culture (Zheng et al., 2020). Hence, there is a growing interest in innovative fermented products based on vegetables and fruits as a good source of probiotic bacteria (Di Cagno, Coda, De Angelis, & Gobbetti, 2013).

Reduction of acidity and astringency and the increase of fruity and fermented taste were studied in fermented sea buckthorn juices by Tiitinen, Vahvaselkä, Laakso, and Kallio (2007). However, the process was carried out with unadapted *O. oeni*, the fermentation reaction differed between berry cultivars, and the reaction size was not proportional to the initial malic acid amount and pH in juices. On the other hand, Markkinen, Laaksonen, Nahku, Kuldjärv, and Yang (2019) showed that certain *L. plantarum* strains can perform MLF in sea buckthorn juices without additional adaptation and nutrients.

There is still a lack of detailed and complete literature data on the impact of fermentation using different strains on the chemical composition and antioxidant activity of sea buckthorn juice, including a new cultivar grown in Poland. Therefore, this study aimed to determine the metabolic activity of *L. plantarum*, *L. plantarum* subsp. *argentoratensis* and *O. oeni* strains along with the dynamics of changes in the content of organic acids, sugars, phenolic compounds, and antioxidant activity during 72-h MLF of sea buckthorn and sea buckthorn-apple juices. It is known that MLF in wine depends on pH and lasts longer than in fruit juices. Cinquata, De Stefano, Formato, Niro and Panfili (2018) reported that *O. oeni* and *L. plantarum* metabolized malic acid in white wines with pH 3.2 for 35 and 56 d, respectively, but no conversion took place in wines with a lower pH 3.2. The addition of apple juice will not only increase the pH of sea buckthorn juice, but will also be a good medium for *O. oeni* (Viljakainen & Laakso, 2002). Apples, in addition to lower acidity, will provide sugars, amino acids and vitamins necessary for bacterial activity, and their selection is economically and sensorially attractive. This paper presents for the first time the appropriate conditions and strains of malolactic fermentation in sea buckthorn and sea buckthorn-apple juices to achieve maximum reduction of malic acid while increasing the health-promoting potential by the rise in phenolic compounds and antioxidant activity.

2. Materials and methods

2.1. Plant materials

Sea buckthorn berries of the cultivar 'Józef' were collected from the Research Institute of Horticulture in Skierniewice (Poland). Apples of the cultivar 'Champion' were purchased on the retail market. The choice of apple variety was dictated by the lack of tendency to enzymatic darkening. Sea buckthorn and apple juices were squeezed using a hydraulic press (SRSE, Warsaw, Poland) and slow juicer (Hurom HG 2G, Puregreen S.C., Sławno, Poland), respectively. The juices were pasteurized and immediately cooled. 100% sea buckthorn juice and sea buckthorn juice mixed with apple juice (in the ratio 1:1) were intended for inoculation.

2.2. Malolactic fermentation

The process of biological deacidification was carried out using malolactic fermentation. Freeze-dried cultures of strains of *Lactobacillus plantarum* (DSM 100813, DSM 13273, DSM 20174, DSM 10492, and DSM 6872), *Lactobacillus plantarum* subsp. *argentoratensis* (DSM 16365) and *Oenococcus oeni* (DSM 20255; Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) were used for this purpose.

The rehydration of *L. plantarum* and *L. plantarum* subsp. *argentoratensis* was performed in MRS broth (casein peptone, tryptic digest, meat extract, yeast extract, glucose, Tween 80, K_2HPO_4, Na-acetate, $(NH_4)_3$ citrate, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$ in distilled water; pH 6.2–6.5), and *O. oeni* in medium from casein peptone, tryptic digest, yeast extract, glucose, fructose, Tween 80, $(NH_4)_3$ citrate, $MgSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}H_2O$, tomato juice, cysteine-HCl·H_2O in distilled water (pH 4.8) for up to 30 min. Then bacterial cells were cultured as recommended for 24 h in 10 mL of sterile (autoclaved at 121 °C for 20 min) liquid media (as above), transferred into 100 mL of dedicated media and incubated at 30 °C (*L. plantarum* DMS 6872, DMS 10492 and DMS 20174, *L. plantarum* subsp. *argentoratensis* DMS 16365 and *O. oeni* DMS 20255) or 37 °C (*L. plantarum* DMS 100813 and DMS 13273) for 48 h. All biomass was centrifuged at 5500 rpm for 10 min using an MPW-351R centrifuge (MPW Med. Instruments, Warsaw, Poland). The supernatant was decanted and the remaining biomass was suspended in 5 mL of sterile 0.9% NaCl solution. The sea buckthorn and sea buckthorn-apple juices were inoculated with a 1% (vol.) cell suspension. Fermentation was carried out in sealed glass flasks for 72 h at 30 °C in the absence of lights, in a laboratory incubator (ST2, POL-EKO-APARATURA, Wodzisław Śląski, Poland). Samples were mixed regularly.

The above described steps were performed under sterile conditions. Fermentation was carried out in duplicate. Three samples were taken from the fermentation vessels. An additional vessel was a sample, from which no juices were taken during the process, only at the beginning and after 72 h. During the process, access to oxygen was inhibited, and during sampling minimized. Samples of juices were taken for analyses every 12 h and immediately placed at −80 °C before the next step of analysis.

2.3. Microscopy

Microscopic images were obtained using a Zeiss Axio Imager M2 microscope (Carl Zeiss Meditec France S.A.S., Le Pecq, France) with a 100 × objective lens. Immersion was used. AxioVision 4.8 Software (Carl Zeiss Meditec France S.A.S., Le Pecq, France) was used for image acquisition. Pictures were taken just before inoculation of juices and are shown in Fig. 1.

2.4. Optical density

Cell growth was examined by optical density (OD_{560}) of bacterial suspensions measured spectrophotometrically at 560 nm using a UV-2401 PC spectrophotometer (Shimadzu Corp., Kyoto, Japan). The measurement was made for bacterial suspensions just before inoculation of the juices. The blank were pure bacterial media diluted as suspensions of bacteria. All measurements were taken three times and the results were expressed as mean with standard deviation.

2.5. Measurement of pH and soluble solids

pH of juices was determined using an automatic pH titrator system TitroLine 5000 (Xylem Analytics GmbH, Weilheim in Oberbayern, Germany). The soluble solids content (°Bx) was measured with a digital refractometer (Atago RX-5000, Atago Co. Ltd., Saitama, Japan). All measurements were taken three times and the results were expressed as mean with standard deviation.

2.6. Determination of organic acids and sugars

Organic acids were studied using ultra performance liquid

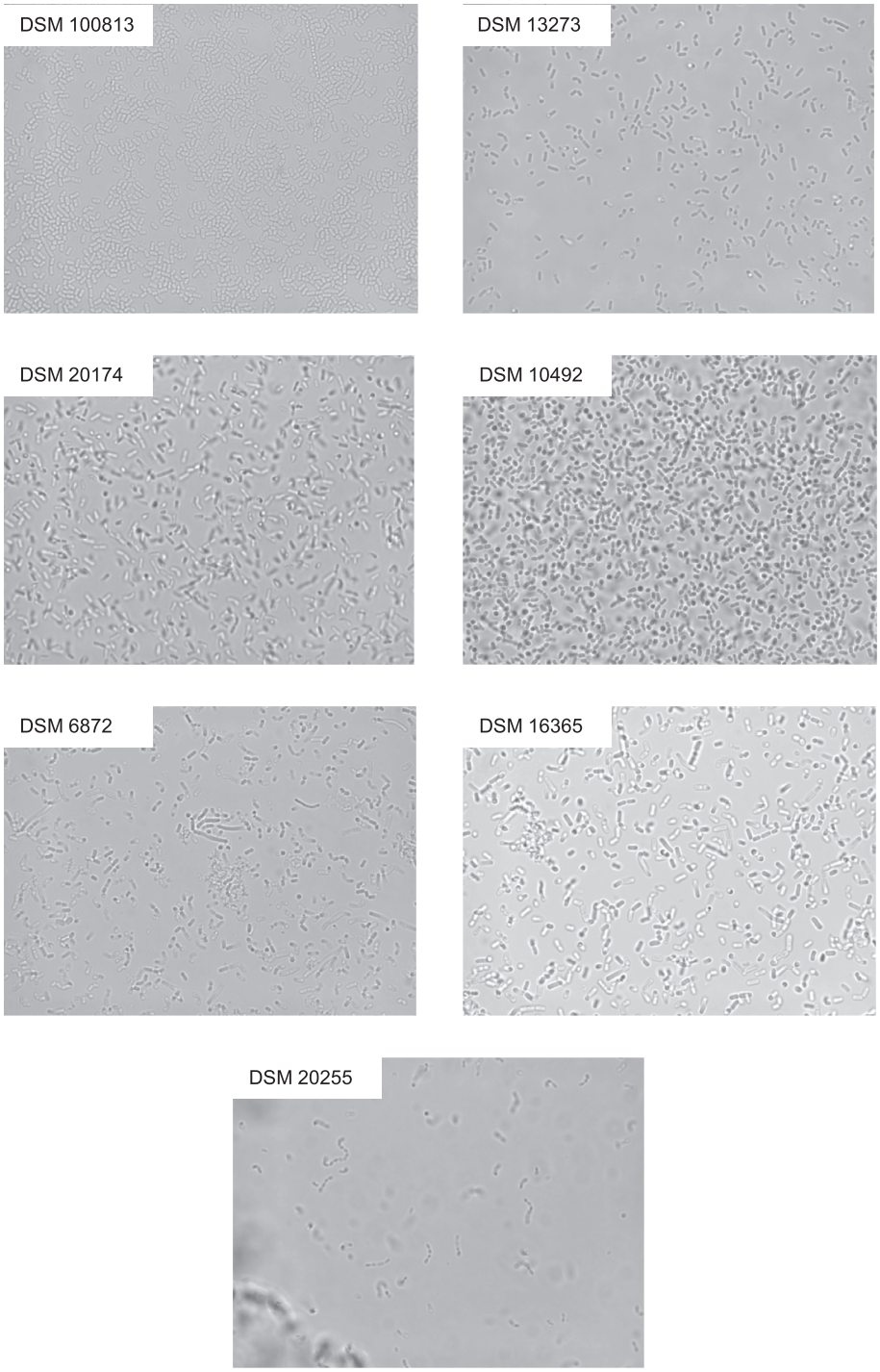

Fig. 1. Microscopic images of *Lactobacillus plantarum* (DSM 100813, DSM 13273, DSM 20174, DSM 10492, DSM 6872), *Lactobacillus plantarum* subsp. *argentoratensis* (DMS 16365) and *Oenococcus oeni* (DSM 20255) under 100x magnification.

chromatography with photodiode array detector (UPLC-PDA, Acquity UPLC System, Waters Corp., Milford, MA, US). Sugars were analyzed by high pressure liquid chromatography (HPLC-ELSD, Merck-Hitachi L-7455, Merck KGaA, Darmstadt, Germany) equipped with an evaporative light scattering detector (ELSD, PL-ELS 1000, Polymers Labs Inc., Amherst, MA, US). The analyzes were carried out exactly as reported previously by Wojdyło, Nowicka, and Bąbelewski (2018). Identification of organic acids (malic acid, lactic acid, oxalic acid, citric acid, isocitric acid, quinic acid) and sugars (rhamnose, fructose, glucose, sucrose) was based on reference standards (Merck KGaA, Darmstadt, Germany). All measurements were taken three times and the results were expressed in g/100 mL as mean with standard deviation.

2.7. Determination of phenolic compounds

Phenolic compounds were analyzed using an ultra performance liquid chromatography with photodiode array detector (UPLC-PDA, Acquity UPLC System; Waters, MA, USA) and the UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) (Waters Corp., Milford, MA, USA). Before injection, the juice samples were centrifuged at 15,000 rpm for 7 min at 4 °C using MPW-150R centrifuge (MPW Med. Instruments, Warsaw, Poland). The supernatants were then filtered through 0.20 μm

Table 1
pH, soluble solids, content of organic acids, sugars and phenolic compounds, sugar:organic acid ratio and antioxidant activity of **sea buckthorn juices** fermented with different lactic acid bacteria strains.

Properties	Uninoculated juice				*L. plantarum*								
					DSM 100813			DSM 13273			DSM 20174		
	0 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
pH	3.012 ± 0.01b	3.012 ± 0.0b	3.013 ± 0.01b	3.012 ± 0.01b	–	–	3.019 ± 0.01b	–	–	3.021 ± 0.01b	–	–	3.108 ± 0.01a
Soluble solids (°Bx)	7.1 ± 0.1a	7.3 ± 0.1a	7.0 ± 0.0a	7.1 ± 0.1a	7.4 ± 0.0a	6.9 ± 0.1b	6.3 ± 0.1c	7.0 ± 0.1a	6.7 ± 0.1b	6.5 ± 0.1bc	7.4 ± 0.1a	6.8 ± 0.1b	6.0 ± 0.1c
Organic acids (g/100 mL)													
Malic acid	6.06 ± 0.11a	6.06 ± 0.15a	6.09 ± 0.14a	6.08 ± 0.15a	5.83 ± 0.11a	5.74 ± 0.10ab	5.32 ± 0.11c	5.98 ± 0.12b	5.94 ± 0.10a	5.85 ± 0.10a	5.60 ± 0.10b	5.13 ± 0.10d	4.80 ± 0.10e
Lactic acid	nd	nd	nd	nd	0.48 ± 0.04d	0.49 ± 0.06d	0.57 ± 0.02d	0.42 ± 0.03d	0.43 ± 0.05d	0.51 ± 0.05d	0.89 ± 0.08c	1.45 ± 0.10b	1.82 ± 0.13a
Oxalic acid	0.10 ± 0.01a	0.10 ± 0.04a	0.09 ± 0.02a	0.10 ± 0.02a	0.10 ± 0.03a	0.11 ± 0.03a	0.11 ± 0.03a	0.10 ± 0.02a	0.10 ± 0.02a	0.09 ± 0.01a	0.08 ± 0.02a	0.10 ± 0.03a	0.08 ± 0.02a
Citric acid	0.26 ± 0.01a	0.26 ± 0.05a	0.26 ± 0.05a	0.25 ± 0.07a	0.25 ± 0.06a	0.23 ± 0.04a	0.23 ± 0.03a	0.24 ± 0.05a	0.21 ± 0.03a	0.21 ± 0.05a	0.24 ± 0.05a	0.25 ± 0.04a	0.25 ± 0.03a
Isocitric acid	0.18 ± 0.04a	0.19 ± 0.05a	0.20 ± 0.04a	0.20 ± 0.04a	0.20 ± 0.03a	0.20 ± 0.05a	0.22 ± 0.05a	0.17 ± 0.03a	0.20 ± 0.05a	0.18 ± 0.06a	0.19 ± 0.05a	0.20 ± 0.05a	0.23 ± 0.01a
Quinic acid	0.64 ± 0.06a	0.63 ± 0.10a	0.63 ± 0.09a	0.63 ± 0.09a	0.62 ± 0.08a	0.62 ± 0.10a	0.61 ± 0.07a	0.59 ± 0.05a	0.61 ± 0.06a	0.61 ± 0.09a	0.62 ± 0.09a	0.59 ± 0.05a	0.61 ± 0.04a
Total organic acids	7.24 ± 0.10b	7.24 ± 0.09b	7.27 ± 0.08b	7.26 ± 0.10b	7.50 ± 0.07a	7.39 ± 0.09b	7.06 ± 0.09c	7.50 ± 0.10a	7.49 ± 0.08a	7.45 ± 0.08a	7.62 ± 0.09a	7.15 ± 0.08c	7.79 ± 0.09a
Sugars (g/100 mL)													
Rhamnose	0.14 ± 0.01a	0.14 ± 0.01a	0.14 ± 0.02a	0.15 ± 0.01a	0.16 ± 0.02a	0.14 ± 0.01a	0.15 ± 0.01a	0.17 ± 0.02a	0.13 ± 0.02a	0.15 ± 0.01a	0.14 ± 0.01a	0.15 ± 0.03a	0.16 ± 0.01a
Fructose	0.10 ± 0.02a	0.10 ± 0.01a	0.11 ± 0.01a	0.12 ± 0.02a	0.11 ± 0.01a	0.11 ± 0.02a	0.10 ± 0.02a	0.09 ± 0.01a	0.11 ± 0.02a	0.10 ± 0.01a	0.09 ± 0.01a	0.10 ± 0.01a	0.12 ± 0.03a
Sorbitol	0.23 ± 0.04a	0.22 ± 0.03a	0.22 ± 0.04a	0.22 ± 0.04a	0.22 ± 0.03a	0.22 ± 0.03a	0.21 ± 0.04a	0.23 ± 0.02a	0.23 ± 0.02a	0.22 ± 0.02a	0.20 ± 0.03a	0.22 ± 0.03a	0.21 ± 0.03a
Glucose	3.15 ± 0.12a	3.12 ± 0.17a	3.25 ± 0.10a	3.28 ± 0.21a	3.11 ± 0.11a	3.18 ± 0.22a	3.20 ± 0.25a	3.20 ± 0.13a	3.20 ± 0.20a	3.25 ± 0.09a	3.18 ± 0.17a	3.24 ± 0.24a	3.29 ± 0.30a
Total sugars	3.62 ± 0.11a	3.58 ± 0.18a	3.72 ± 0.11a	3.77 ± 0.21a	3.60 ± 0.12a	3.65 ± 0.23a	3.66 ± 0.26a	3.69 ± 0.14a	3.67 ± 0.21a	3.72 ± 0.09a	3.61 ± 0.18a	3.71 ± 0.25a	3.78 ± 0.30a
Sugar:organic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phenolic compounds (mg/100 mL)													
Phenolic acids	0.63 ± 0.02a	0.63 ± 0.01a	0.62 ± 0.02a	0.60 ± 0.01a	0.59 ± 0.04a	0.62 ± 0.01a	0.64 ± 0.02a	0.62 ± 0.01a	0.63 ± 0.01a	0.62 ± 0.01a	0.63 ± 0.02a	0.63 ± 0.02a	0.63 ± 0.04a
Flavonols	45.40 ± 1.00b	41.52 ± 0.65c	44.38 ± 0.94b	44.59 ± 0.99b	41.50 ± 1.31c	41.52 ± 0.80c	40.88 ± 1.03c	41.54 ± 0.53c	44.25 ± 0.43b	44.36 ± 0.88b	45.62 ± 1.17b	46.13 ± 0.70b	49.26 ± 0.57a
Total phenolic compounds	46.03 ± 0.98b	42.15 ± 0.66c	45.00 ± 0.86b	45.19 ± 0.94b	42.09 ± 1.27c	42.14 ± 0.79c	41.52 ± 0.10c	42.16 ± 0.52c	44.88 ± 0.43b	44.98 ± 0.87b	46.25 ± 1.15b	46.76 ± 0.68b	49.89 ± 0.53a
Antioxidant activity (mmol TE/100 mL)	7.42 ± 1.00b	7.98 ± 0.94b	7.03 ± 0.82b	7.05 ± 0.78b	6.77 ± 1.21bc	6.90 ± 1.63c	5.32 ± 0.58c	7.11 ± 1.01b	7.24 ± 1.25b	7.94 ± 0.99b	7.32 ± 0.69b	8.60 ± 1.48a	9.40 ± 1.22a

Properties	*L. plantarum*						*L. plantarum* subsp. *argentoratensis*			*O. oeni*		
	DSM 10492			DSM 6872			DSM 16365			DSM 20255		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
pH	–	–	3.029 ± 0.01b	–	–	3.020 ± 0.01b	–	–	3.015 ± 0.01b	–	–	3.014 ± 0.01b
	7.1 ± 0.1a	6.6 ± 0.1b	6.3 ± 0.1c	6.7 ± 0.1b	6.6 ± 0.1b	6.2 ± 0.1c	7.0 ± 0.1a	6.8 ± 0.1b	6.6 ± 0.1b	7.0 ± 0.1a	6.7 ± 0.1b	6.5 ± 0.1bc

(*continued on next page*)

Table 1 (*continued*)

Properties	*L. plantarum*						*L. plantarum* subsp. *argentoratensis*			*O. oeni*		
	DSM 10492			DSM 6872			DSM 16365			DSM 20255		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Soluble solids (°Bx)												
Organic acids (g/100 mL)												
Malic acid	5.74 ± 0.11ab	5.45 ± 0.10bc	5.28 ± 0.10c	5.96 ± 0.10a	5.94 ± 0.10a	5.21 ± 0.10d	5.89 ± 0.10a	5.67 ± 0.10b	5.55 ± 0.10b	6.01 ± 0.10a	5.65 ± 0.10b	5.43 ± 0.10bc
Lactic acid	0.69 ± 0.03 d	0.92 ± 0.05c	1.02 ± 0.10c	0.38 ± 0.08d	0.43 ± 0.09d	0.47 ± 0.04d	0.48 ± 0.06d	0.56 ± 0.09d	0.64 ± 0.05d	0.38 ± 0.9d	0.39 ± 0.05d	0.39 ± 0.04d
Oxalic acid	0.10 ± 0.02a	0.09 ± 0.03a	0.12 ± 0.03a	0.11 ± 0.01a	0.11 ± 0.02a	0.08 ± 0.01a	0.11 ± 0.01a	0.10 ± 0.02a	0.10 ± 0.03a	0.08 ± 0.05a	0.10 ± 0.03a	0.11 ± 0.03a
Citric acid	0.24 ± 0.03a	0.25 ± 0.04a	0.22 ± 0.02a	0.25 ± 0.06a	0.25 ± 0.05a	0.23 ± 0.01a	0.24 ± 0.04a	0.21 ± 0.04a	0.23 ± 0.05a	0.27 ± 0.03a	0.24 ± 0.03a	0.23 ± 0.04a
Isocitric acid	0.21 ± 0.01a	0.21 ± 0.03a	0.23 ± 0.04a	0.19 ± 0.03a	0.20 ± 0.01a	0.22 ± 0.05a	0.20 ± 0.05a	0.20 ± 0.05a	0.21 ± 0.04a	0.19 ± 0.05a	0.21 ± 0.03a	0.23 ± 0.05a
Quinic acid	0.62 ± 0.07a	0.62 ± 0.09a	0.62 ± 0.08a	0.62 ± 0.10a	0.63 ± 0.11a	0.62 ± 0.08a	0.59 ± 0.08a	0.60 ± 0.11a	0.60 ± 0.05a	0.59 ± 0.07a	0.61 ± 0.05a	0.61 ± 0.07a
Total organic acids	7.60 ± 0.09a	7.54 ± 0.08a	7.49 ± 0.10a	7.51 ± 0.09a	7.56 ± 0.10a	6.83 ± 0.09d	7.51 ± 0.09a	7.34 ± 0.10b	7.33 ± 0.07b	7.52 ± 0.08a	7.20 ± 0.06c	7.00 ± 0.09c
Sugars (g/100 mL)												
Rhamnose	0.13 ± 0.02a	0.13 ± 0.01a	0.15 ± 0.02a	0.15 ± 0.01a	0.17 ± 0.02a	0.16 ± 0.01a	0.17 ± 0.01a	0.15 ± 0.02a	0.14 ± 0.03a	0.15 ± 0.01a	0.15 ± 0.02a	0.15 ± 0.01a
Fructose	0.08 ± 0.02a	0.09 ± 0.02a	0.08 ± 0.02a	0.11 ± 0.02a	0.11 ± 0.01a	0.10 ± 0.01a	0.12 ± 0.01a	0.12 ± 0.01a	0.13 ± 0.02a	0.09 ± 0.01a	0.09 ± 0.02a	0.10 ± 0.01a
Sorbitol	0.20 ± 0.04a	0.19 ± 0.03a	0.21 ± 0.03a	0.19 ± 0.01a	0.21 ± 0.01a	0.20 ± 0.02a	0.19 ± 0.06a	0.21 ± 0.02a	0.22 ± 0.07a	0.21 ± 0.05a	0.22 ± 0.05a	0.23 ± 0.07a
Glucose	3.22 ± 0.16a	3.25 ± 0.15a	3.29 ± 0.19a	3.21 ± 0.11a	3.24 ± 0.20a	3.24 ± 0.18a	3.21 ± 0.21a	3.28 ± 0.14a	3.28 ± 0.17a	3.21 ± 0.28a	3.21 ± 0.16a	3.30 ± 0.15a
Total sugars	3.63 ± 0.17a	3.66 ± 0.16a	3.73 ± 0.20a	3.66 ± 0.12a	3.73 ± 0.21a	3.70 ± 0.19a	3.69 ± 0.21a	3.76 ± 0.15a	3.77 ± 0.18a	3.66 ± 0.29a	3.67 ± 0.17a	3.78 ± 0.16a
Sugar:organic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Phenolic compounds (mg/100 mL)												
Phenolic acids	0.61 ± 0.01a	0.62 ± 0.01a	0.64 ± 0.02a	0.60 ± 0.03a	0.59 ± 0.03a	0.59 ± 0.02a	0.63 ± 0.02a	0.64 ± 0.03a	0.63 ± 0.01a	0.60 ± 0.01a	0.63 ± 0.02a	0.61 ± 0.02a
Flavonols	41.75 ± 1.38c	46.23 ± 0.35b	40.01 ± 1.22c	45.72 ± 1.02b	45.12 ± 0.82b	44.33 ± 1.04b	46.06 ± 0.92b	46.10 ± 0.83b	44.20 ± 0.53b	40.04 ± 0.29c	44.87 ± 1.11b	45.94 ± 0.10b
Total phenolic compounds	42.36 ± 1.37c	46.85 ± 0.34b	40.65 ± 1.18c	46.32 ± 0.98b	45.71 ± 0.66b	44.92 ± 1.06b	46.69 ± 1.02b	46.74 ± 0.86b	44.83 ± 0.52b	40.64 ± 0.28c	45.50 ± 1.09b	46.55 ± 0.09b
Antioxidant activity (mmol TE/ 100 mL)	6.64 ± 1.05bc	5.88 ± 1.31c	5.02 ± 1.14c	7.14 ± 1.22b	7.24 ± 1.09b	7.99 ± 1.32b	7.54 ± 1.54b	7.84 ± 1.24b	7.43 ± 1.46b	7.27 ± 0.94b	7.00 ± 0.73b	7.07 ± 1.41b

Data are shown as mean (n = 3) ± standard deviation; for each parameter tested, values with different letters, within the row, differ significantly (Tukey's test, $p < 0.05$); h-hours.

PTFE hydrophilic membranes. Chromatographic analysis conditions were the same as described by Tkacz et al. (2020), including injection volume − 5 μL, flow rate − 0.420 mL/min, run time − 30 min, and column temperature 30 °C. Solvent A (2.0% formic acid) and solvent B (100% acetonitrile) were used in the following gradients: elution start with 98.0% A; next solvent A reduction to 65% (to 32 min), and to 0% (to 33 min); 98% A from 33.5 to 35 min to re-equilibrate the column. The PDA spectra were measured over the wavelength range 200–800 nm, every 2 nm. The runs for phenolic acids and flavonols were monitored at 320 and 360 nm, respectively. Quantitative determination was based on injections of the phenolic calibration standards at concentrations ranging 0.05 to 5 mg/mL ($R^2 \geq 0.9998$). The sums of phenolic acids and flavonols were calculated as *p*-coumaric acid and isorhamnetin-3-*O*-rutinoside, respectively. These compounds were the dominant phenolic compounds, as confirmed by reference standards (Extrasynthèse S.A., Genay, France; Merck KGaA, Darmstadt, Germany). Empower 3 software (Waters Corp., Milford, MA, US) was used to develop data. All measurements were taken three times and the results were expressed in mg/100 mL as mean with standard deviation.

2.8. Determination of antioxidant activity

Antioxidant activity was tested as oxygen radical absorbance capacity (ORAC) and the analysis was conducted as previously described by Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002). Spectrofluorometric measurement of fluorescence decrease caused by fluorescent substance oxidation by free radicals with the antioxidants presence was measured using a microplate reader Synergy™ H1 (BioTek, Winooski, VT, US). Before analysis, the juice samples were centrifuged the same as in subsection 2.7. Samples containing juice supernatant, phosphate buffer, and fluorescein were incubated at 37 °C until the end of the analysis. 2,2′-Azobis(2-amidinopropane)dihydrochloride was inserted and the spectrofluorometric measurement was performed every 5 min at excitation and emission wavelengths of 493 and 515 nm, respectively. The phosphate buffer was blank. The antioxidant activity was calculated by comparing the surface under the fluorescence decrease curves over time with the surface for pure Trolox solutions at 12.5, 25.0, 50.0, and 75.0 μM. All measurements were taken three times and the results were expressed in mmol TE (Trolox)/100 mL as mean with standard deviation.

2.9. Statistical analysis

Analysis of variance (ANOVA, $p < 0.05$), Tukey's HSD test and Pearson's correlation coefficient (r) were done using a Statistica 13.1 software (StatSoft, Cracow, Poland) and XLSTAT for Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, US). Data are shown as mean (n = 3) ± standard deviation (SD). For each parameter tested, values with different letters differ statistically significantly.

3. Results and discussion

3.1. Optical density

Optical density (OD_{560}) was measured to compare bacterial growth in suspension intended for inoculated juices. OD_{560} values of the bacterial suspension were 2.1 ± 0.1 to 2.3 ± 0.1 for *L. plantarum* strains, 2.0 ± 0.1 for the *L. plantarum* subsp. *argentoratensis* strain, and 1.9 ± 0.2 for the *O. oeni* strain. No significant differences ($p > 0.05$) were found between these results. Measurement of optical density takes into account inactive cells with preserved integrity of cytoplasmic membranes; therefore the OD_{560} value does not fully reflect the physiological state of the culture and its potential. No correlation was found between the optical density and the malic acid conversion and the phenolic content for both types of juice tested.

3.2. pH and soluble solids

Previous studies on the fermentation of fruit and vegetable juices indicated that a higher pH promotes the metabolic capacity of bacterial strains (Gao, Vasantha Rupasinghe, & Pitts, 2013; Filannino et al., 2014; Wei et al., 2018). The pH values of uninoculated sea buckthorn and sea buckthorn-apple juices were constant during 72-h incubation and were 3.012 and 3.238. The changes in pH (Table 1) of sea buckthorn juices inoculated with bacteria were not significant ($p > 0.05$), except for *L. plantarum* DSM 20174 strain with increase by 0.1 units. Mixed juices inoculated with *L. plantarum* had the highest final pH, from 3.299 to 3.324, respectively, for DSM 13273 and DSM 20174 strains. Inoculation of mixed juice with *O. oeni* did not change the pH significantly compared ($p > 0.05$) to uninoculated juice.

In applying analogous *L. plantarum* strains, a rise in pH to 0.1 and 0.26 units was achieved for chokeberry and sea buckthorn juices, respectively (Markkinen et al., 2019). Tiitinen et al. (2007) studied the pH change in sea buckthorn juices treated with *O. oeni*, and the maximum increase was 0.2 units for Oranzhevaya and Avgustinka berry juices. A similar change in pH, by 0.1–0.2 units, was found after malolactic fermentation of white and black currant, apple, and bilberry juices using *O. oeni* (Viljakainen & Laakso, 2002). In contrast, pH changes during MLF were not significant ($p > 0.05$) for apple juices (Gao, Vasantha Rupasinghe, & Pitts, 2013), lingonberry material (Viljanen, Heiniö, Juvonen, Kössö, & Puupponen-Pimiä, 2014), and bog bilberry juice (Wei et al., 2018).

The soluble solids content, one of the quality indicator, was 7.1 and 10.6 °Bx for uninoculated sea buckthorn and sea buckthorn-apple juices, respectively (Tables 1–2). The fermentation process resulted in a decrease in value by a maximum of 1.0 in the case of sea buckthorn juice with DSM 20174 strain and by 1.2 for sea buckthorn-apple juice with DSM 16365 strain. Changes in the soluble solids content were strongly correlated with malic acid (r = 0.92) and lactic acid (r = -0.78 and −0.85 for sea buckthorn and sea buckthorn-apple juices, respectively) (Table 3). In turn, the correlation with sugars, the major soluble solids in juices, was low due to their constant concentration during MLF. Codex General Standard for Fruit Juices and Nectars (Codex STAN 247–2005) defines the minimum °Bx level for sea buckthorn juices equal 6.0, and for apple juice 10.0, which may naturally differ for countries. Hence, both types of deacidified juices contain soluble solids in accordance with applicable industry regulation.

3.3. Organic acids

3.3.1. Fermentation of sea buckthorn juices

An indicator of the fermentation process was the reduction of malic acid, which is converted to lactic acid. For *L. plantarum* strains, the largest reduction in malic acid content was observed between 48 and 72 h of incubation. The reduction of malic acid involving *L. plantarum* subsp. *argentoratensis* (DSM 16365) and *O. oeni* (DSM 20255) was stronger between 24 and 48 h, but the final acid reduction was relatively small (below 10.5%) (Table 1). For the *O. oeni* strain, lactic acid concentration was constant despite a slight reduction of malic acid. This may indicate the course of other metabolic pathways using malic acid as a carbon source through *O. oeni* or potentially yeast with the affinity of malic enzyme to its substrate, through malate dehydrogenase activity or during the tricarboxylic acid cycle (Subden, Krizus, Osothsilp, Viljoen, & Van Vuuren, 1998; Volschenk, Van Vuuren, & Viljoen-Bloom, 2006).

Incubation had no effect on the content of organic acids, and the malic acid amount in uninoculated sea buckthorn juice was on average 6.07 g/100 mL. Moderate malic acid conversion was found in sea buckthorn juices and ranged from 3.5% to 20.9% (for inoculated juices with DSM 13273 and DSM 20174 strains, respectively). The DSM 20174 and DMS 10492 strains showed the highest metabolic activity in black chokeberry juice and sea buckthorn juice, respectively, with complete

reduction of malic acid (Markkinen et al., 2019). In the future, an attempt to deacidify the juice using recombinant *L. plantarum* cells that increase fermentation efficiency may be valuable (Schümann et al., 2012).

In all inoculated sea buckthorn juices, during the process there were observed increasing amounts of lactic acid, ranging from 0.38 to 0.89 g/100 mL after 24 h, from 0.39 to 1.45 g/100 mL after 48 h, and from 0.39 to 1.82 g/100 mL after 72 h of incubation. However, only in the case of juices with DSM 20174 and DSM 10492 strains were these changes significant, and the highest content of lactic acid, in proportion to the strongest reduction of malic acid, was found in sea buckthorn juice inoculated with the first strain. In the research of Markkinen et al. (2019) analogous strains of *L. plantarum* – DSM 100813, DSM 20174, DSM 10492, and DSM 16365 – were able to metabolize malic acid in black chokeberry and sea buckthorn (except DSM 16365) juices, but not in lingonberry juice rich in citric acid.

It was found that the type of strain determined the amount of lactic acid formed. For example, approximately twice as much lactic acid as in juices with DSM 100813 and DSM 6872 strains was determined in juice with the DSM 10492 strain, despite similar malic acid reduction (13.2% on average).

The sum of organic acids of sea buckthorn juices inoculated with DSM 13273, DSM 20174 and DSM10492 strains increased compared to the initial value (average 7.27 g/100 mL) due to the accumulation of lactic acid. Additional organic acids in decreasing quantities were also detected in sea buckthorn-apple juices – quinic > citric > isocitric > oxalic acids (Table 2) – but their content did not change significantly ($p > 0.05$) during juice fermentation. Quinic acid has a bitter and astringent taste, which is relevant in terms of sensory quality of juices. Fermentation of papaya juice by *L. acidophilus* and *L. plantarum* also did not change the oxalic acid concentration (Chen, Chen, Chen, Zhang, & Chen, 2018). Similarly, the metabolic activity of *O. oeni* did not affect the quinic acid content of sea buckthorn juice in research of Tiitinen et al. (2007). The reduction of quinic acid along with the shikimic acid formation by *L. plantarum* was found in bog bilberry juice with pH 3.50 (Wei et al., 2018), and sea buckthorn juices inoculated with DSM 10492 and DSM 100813 strains after combination with enzymatic treatment (Markkinen et al., 2019).

3.3.2. Fermentation of sea buckthorn-apple juices (1:1)

The kinetics of changes (every 12 h) in malic and lactic acid content are shown in Fig. 2 comparing the dynamics of change in analyzed juices during incubation with *L. plantarum* DSM 10492 strain (**A**), *L. plantarum* subsp. *argentoratensis* DSM13635 (**B**), *O. oeni* DSM 20255 (**C**). The reduction in malic acid was stronger for mixed juices (1:1) and was up to 75.0% for juice inoculated with the DSM 10492 strain, following by 63.8% for juice with the DSM 20174 strain (Table 2). Identification and changes in organic acids after 12 and 72 h of MLF with DMS 10492 strain for sea buckhorn-apple juice are also shown in the UPLC-PDA chromatograms (Fig. 3). Reduction of malic acid in juices with other bacterial strains ranged from 55.6 to 60.9%, with a final acid content on average 1.44 g/100 mL. The exception was juice inoculated with *O. oeni,* in which there was no significant change ($p > 0.05$) in malic acid content after 72-h incubation compared to uninoculated juice (3.40 g/100 mL).

The largest reduction in malic acid occurred during the first 24 h of incubation with *L. plantarum* strains, except for juice treated with *L. plantarum* subsp. *argentoratensis*. Therefore, based on the slight differences in malic acid concentration between 48 and 72 h, the optimal fermentation time for DSM 6872, DSM 100813, and DSM 20174 strains can be determined as 48 h. In the research of Mousavi et al. (2013) the same strain DSM 20174 was used for fermentation of pomegranate juice at pH 3.1 at 30 °C. Weak metabolism of organic acids for the first 24 h due to the lag phase and a significant increase in lactic acid in the logarithmic growth stage between 24 and 48 h were found. In comparison, Markkinen et al. (2019) investigated the termination of fermentation with the DSM 20174 strain after 30 h in black chokeberry juice, but for sea buckthorn juices the optimal malolactic conversion time was 72 h. The consumption of half malic acid content in apple juice treated with *O. oeni* occurred within 2 d of the fermentation, then during the next 14 d the conversion was slow and incomplete (Viljakainen & Laakso, 2002).

Lactic acid concentration increased with fermentation time to 9.21 g/100 mL for sea buckthorn-apple juice inoculated with DSM 13273 and DSM 100813 strains. However, the application of DSM 20174, DSM 6872, and DSM 16365 strains was not conducive to such a strong lactic acid increase (up to 5.68 g/100 mL).

In the case of *O. oeni*, lactic acid formation was weaker in sea buckthorn-apple juice than in 100% sea buckthorn juice. A small increase in lactic acid, with no significant changes in the reduction of sugars and acids, was also found in black chokeberry juice treated with *O. oeni* (Markkinen et al., 2019). Conversely, an over 90% reduction in malic acid was achieved using this species for sea buckthorn juice of the Oranzhevaya cultivar (Tiitinen et al., 2007). *O. oeni* completely degraded malic acid in white and black currant juices, incompletely in bilberry juice, and was not able to ferment lingonberry juices (Viljakainen & Laakso, 2002). Low *O. oeni* activity may result from lowering of its cell content below the minimum value necessary to maintain malolactic fermentation (10^6 CFU mL^{-1}; Maicas, Natividad, Ferrer, & Pardo, 2000; Viljakainen & Laakso, 2002), especially since a decrease in *O. oeni* growth in the first stage of bog berry juice fermentation has been proven (Chen et al., 2019). The causes may also be associated with too low pH of juice and the presence of natural antimicrobial compounds of fruits, including benzoic acid (Viljakainen & Laakso, 2002; Gao et al., 2013), although UPLC-PDA analysis did not show even trace amounts of this acid in the juices studied. Literature data emphasize the dominance of *O. oeni* in MLF and their good adaptation to difficult wine-making conditions. The optimum pH for growth is from 4.3 to 4.8, but bacteria can also grow at pH 3.2 (Cinquanta, De Stefano, Formato, Niro, & Panfili, 2018). However, sea buckthorn juice had higher acidity, lower pH and different chemical compositions than wine. It should be noted that only one strain of *O. oeni* was tested, therefore further research using many strains available for industrial deacidification and modified will be desirable.

Sea buckthorn-apple juice also contained other organic acids in decreasing contents: quinic > citric and isocitric > oxalic acids (Table 2). Concentrations of these organic acids did not change significantly ($p > 0.05$), similarly as during sea buckthorn juices' fermentation. Only in sea buckthorn-apple juices fermented with the most efficient DSM 10492 and DSM 20174 strains, were trace amounts of acetic acid, below 0.04 g/100 mL, detected (results not shown). *L. plantarum* is one of the facultatively heterofermentative bacteria (heterolactic metabolism), which means that they are capable of forming lactic acid and other compounds, including acetic acid, depending on the conditions (Gerardi et al., 2019). Stronger acetic acid production was observed in *Prunus mahaleb* fruits fermented by a mixed starter of *L. plantarum* and *S. cerevisiae* (Gerardi et al., 2019), stored pomegranate juice (Mantzourani et al., 2019), papaya juices (Chen et al., 2018), and tomato, pineapple, cherry, and carrot juices as a result of activation of the acetate kinase route of the phosphogluconate pathway by *L. plantarum* strains (Filannino et al., 2014). In turn, the DSM 20174 strain produced a considerable quantity of propionic acid rather than acetic acid (Mousavi et al., 2013).

3.4. Sugars and sugar:organic acid ratio

Non-fermented sea buckthorn and sea buckthorn-apple juices contained 3.62 and 8.11 g sugars/100 mL, respectively, and glucose was dominant (Tables 1–2). Other sugars were identified in decreasing concentrations in sea buckthorn juice (sorbitol > rhamnose > fructose), and in sea buckthorn-apple juice (sucrose > fructose > rhamnose). No differences in sugar concentration were found between

Table 2
pH, soluble solids, content of organic acids, sugars and phenolic compounds and sugar:organic acid ratio of **sea buckthorn-apple juices** fermented with different lactic acid bacteria strains.

Properties	Uninoculated juice				*L. plantarum*								
					DSM 100813			DSM 13273			DSM 20174		
	0 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
pH	3.238 ± 0.01c	3.239 ± 0.01c	3.238 ± 0.01c	3.238 ± 0.01c	–	–	3.316 ± 0.01a	–	–	3.299 ± 0.01b	–	–	3.324 ± 0.0-1a
Soluble solids (°Bx)	10.6 ± 0.1a	10.6 ± 0.1a	10.6 ± 0.1a	10.7 ± 0.1a	10.3 ± 0.1b	9.7 ± 0.1c	9.5 ± 0.1c	10.4 ± 0.1c	9.7 ± 0.0c	9.6 ± 0.1c	10.3 ± 0.0b	9.8 ± 0.1c	9.9 ± 0.0bc
Organic acids (g/100 mL)													
Malic acid	3.40 ± 0.20a	3.39 ± 0.21a	3.41 ± 0.24a	3.40 ± 0.22a	2.29 ± 0.18b	1.51 ± 0.12d	1.50 ± 0.11d	2.48 ± 0.19b	1.55 ± 0.10d	1.41 ± 0.11d	1.71 ± 0.12d	1.28 ± 0.13d	1.23 ± 0.12d
Lactic acid	nd	nd	nd	nd	2.47 ± 0.11 g	4.84 ± 0.14d	7.36 ± 0.18b	1.98 ± 0.09 g	6.14 ± 0.19c	9.21 ± 0.33a	2.85 ± 0.18 g	4.29 ± 0.25d	5.68 ± 0.24c
Oxalic acid	0.07 ± 0.01a	0.06 ± 0.02a	0.07 ± 0.01a	0.07 ± 0.02a	0.05 ± 0.01a	0.05 ± 0.01a	0.05 ± 0.01a	0.07 ± 0.01a	0.06 ± 0.02a	0.07 ± 0.01a	0.05 ± 0.02a	0.07 ± 0.01a	0.07 ± 0.01a
Citric acid	0.16 ± 0.02a	0.16 ± 0.04a	0.15 ± 0.05a	0.16 ± 0.08a	0.13 ± 0.03a	0.11 ± 0.05a	0.11 ± 0.03a	0.14 ± 0.04a	0.12 ± 0.07a	0.13 ± 0.02a	0.15 ± 0.04a	0.14 ± 0.06a	0.13 ± 0.07a
Isocitric acid	0.16 ± 0.05a	0.16 ± 0.06a	0.17 ± 0.04a	0.16 ± 0.07a	0.17 ± 0.08a	0.17 ± 0.03a	0.18 ± 0.08a	0.17 ± 0.09a	0.17 ± 0.05a	0.16 ± 0.06a	0.17 ± 0.04a	0.17 ± 0.04a	0.18 ± 0.03a
Quinic acid	0.45 ± 0.09a	0.45 ± 0.10a	0.45 ± 0.11a	0.43 ± 0.15a	0.44 ± 0.18a	0.44 ± 0.12a	0.45 ± 0.10a	0.42 ± 0.11a	0.44 ± 0.10a	0.44 ± 0.16a	0.41 ± 0.15a	0.43 ± 0.14a	0.41 ± 0.11a
Total organic acids	4.24 ± 0.20f	4.22 ± 0.19f	4.25 ± 0.22f	4.22 ± 0.19f	5.55 ± 0.23e	7.12 ± 0.34c	9.65 ± 0.51b	5.26 ± 0.40e	8.48 ± 0.55b	11.42 ± 0.29a	5.34 ± 0.24e	6.35 ± 0.30d	7.70 ± 0.41c
Sugars (g/100 mL)													
Rhamnose	0.07 ± 0.02a	0.08 ± 0.01a	0.06 ± 0.02a	0.06 ± 0.02a	0.08 ± 0.03a	0.08 ± 0.02a	0.07 ± 0.03a	0.09 ± 0.02a	0.07 ± 0.03a	0.06 ± 0.02a	0.08 ± 0.02a	0.08 ± 0.02a	0.07 ± 0.04a
Fructose	2.22 ± 0.11a	2.17 ± 0.13a	2.17 ± 0.20a	2.20 ± 0.19a	2.20 ± 0.18a	2.22 ± 0.17a	2.22 ± 0.25a	2.18 ± 0.18a	2.19 ± 0.15a	2.19 ± 0.16a	2.26 ± 0.17a	2.25 ± 0.13a	2.23 ± 0.20a
Glucose	3.18 ± 0.27a	3.17 ± 0.21a	3.20 ± 0.25a	3.22 ± 0.30a	3.17 ± 0.23a	3.17 ± 0.28a	3.16 ± 0.33a	3.22 ± 0.20a	3.30 ± 0.41a	3.16 ± 0.34a	3.21 ± 0.21a	3.21 ± 0.10a	3.18 ± 0.34a
Sucrose	2.64 ± 0.19a	2.62 ± 0.12a	2.65 ± 0.22a	2.65 ± 0.32a	2.57 ± 0.32a	2.60 ± 0.23a	2.66 ± 0.41a	2.64 ± 0.12a	2.57 ± 0.32a	2.70 ± 0.11a	2.67 ± 0.10a	2.51 ± 0.23a	2.64 ± 0.30a
Total sugars	8.11 ± 0.28a	8.04 ± 0.22a	8.08 ± 0.26a	8.13 ± 0.31a	8.02 ± 0.33a	8.07 ± 0.29a	8.11 ± 0.34a	8.13 ± 0.21a	8.13 ± 0.40a	8.11 ± 0.35a	8.22 ± 0.22a	8.05 ± 0.24a	8.12 ± 0.35a
Sugar:organic acid	1.9	1.9	1.9	1.9	1.4	1.1	0.8	1.5	1.0	0.7	1.5	1.3	1.1
Phenolic compounds (mg/100 mL)													
Phenolic acids	1.30 ± 0.01a	1.22 ± 0.01b	1.21 ± 0.02b	1.07 ± 0.03b	1.24 ± 0.01b	1.22 ± 0.04b	1.16 ± 0.06b	1.24 ± 0.03b	1.22 ± 0.01b	1.21 ± 0.01b	1.19 ± 0.02b	1.20 ± 0.04b	1.19 ± 0.02b
Flavonols	17.57 ± 1.35c	17.24 ± 1.22c	17.21 ± 1.09c	17.09 ± 1.42c	20.52 ± 1.54b	23.19 ± 1.06a	23.71 ± 1.32a	18.57 ± 1.00c	18.78 ± 1.03c	20.20 ± 1.21b	18.38 ± 1.00c	20.62 ± 0.99b	22.48 ± 1.1-7ab
Total phenolic compounds	18.87 ± 1.34c	18.46 ± 1.21c	18.42 ± 1.07c	18.16 ± 1.43c	21.76 ± 1.53b	24.41 ± 1.09a	24.87 ± 1.35a	19.81 ± 1.03c	20.00 ± 1.03c	21.41 ± 1.20b	19.57 ± 1.01c	21.82 ± 0.96b	23.67 ± 1.1-8ab

Properties	*L. plantarum*						*L. plantarum* subsp. *argentoratensis*			*O. oeni*		
	DSM 10492			DSM 6872			DSM 16365			DSM 20255		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
pH	–	–	3.320 ± 0.01a	–	–	3.305 ± 0.01b	–	–	3.307 ± 0.01b	–	–	3.241 ± 0.01c
Soluble solids (°Bx)	10.3 ± 0.1a	9.4 ± 0.1c	9.5 ± 0.1c	10.2 ± 0.1a	9.5 ± 0.1c	9.5 ± 0.1c	10.6 ± 0.1a	9.4 ± 0.1c	9.4 ± 0.1c	10.6 ± 0.1a	10.5 ± 0.1a	10.5 ± 0.1a
Organic acids (g/100 mL)												
Malic acid	1.47 ± 0.10d	1.19 ± 0.10de	0.85 ± 0.10e	2.02 ± 0.10c	1.37 ± 0.10d	1.33 ± 0.10d	3.35 ± 0.10 a	1.55 ± 0.10d	1.51 ± 0.10d	3.35 ± 0.26a	3.17 ± 0.25a	3.47 ± 0.20a
Lactic acid	3.79 ± 0.21f	6.26 ± 0.40c	7.44 ± 0.39b	2.57 ± 0.13g	3.94 ± 0.16ef	4.42 ± 0.34e	2.24 ± 0.20g	4.06 ± 0.19e	5.33 ± 0.48d	0.24 ± 0.10*h*	0.27 ± 0.11*h*	0.29 ± 0.09h
Oxalic acid	0.06 ± 0.01a	0.05 ± 0.01a	0.05 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.02a	0.07 ± 0.01a	0.06 ± 0.01a	0.06 ± 0.01a	0.07 ± 0.01a	0.05 ± 0.02a	0.06 ± 0.01a	0.06 ± 0.01a
Citric acid	0.14 ± 0.06a	0.14 ± 0.05a	0.14 ± 0.05a	0.15 ± 0.07a	0.14 ± 0.04a	0.14 ± 0.07a	0.16 ± 0.05a	0.14 ± 0.02a	0.14 ± 0.01a	0.17 ± 0.04a	0.16 ± 0.04a	0.15 ± 0.05a
Isocitric acid	0.17 ± 0.09a	0.18 ± 0.07a	0.16 ± 0.07a	0.15 ± 0.06a	0.16 ± 0.03a	0.17 ± 0.04a	0.16 ± 0.07a	0.16 ± 0.05a	0.16 ± 0.04a	0.15 ± 0.05a	0.17 ± 0.06a	0.18 ± 0.02a
Quinic acid	0.44 ± 0.12a	0.43 ± 0.09a	0.44 ± 0.15a	0.45 ± 0.08a	0.44 ± 0.09a	0.44 ± 0.11a	0.45 ± 0.10a	0.45 ± 0.07a	0.46 ± 0.14a	0.43 ± 0.12a	0.45 ± 0.13a	0.42 ± 0.10a
Total organic acids	6.07 ± 0.42de	8.25 ± 0.52b	9.08 ± 0.57b	5.40 ± 0.47e	6.11 ± 0.38d	6.57 ± 0.39d	6.42 ± 0.32d	6.42 ± 0.43d	7.67 ± 0.80c	4.39 ± 0.36f	4.28 ± 0.45f	4.57 ± 0.56f
Sugars (g/100 mL)												

(*continued on next page*)

Table 2 (*continued*)

Properties	*L. plantarum*						*L. plantarum* subsp. *argentoratensis*			*O. oeni*		
	DSM 10492			DSM 6872			DSM 16365			DSM 20255		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
Rhamnose	0.09 ± 0.03a	0.11 ± 0.05a	0.09 ± 0.02a	0.08 ± 0.02a	0.10 ± 0.02a	0.11 ± 0.03a	0.09 ± 0.04a	0.10 ± 0.02a	0.08 ± 0.02a	0.09 ± 0.05a	0.08 ± 0.03a	0.07 ± 0.02a
Fructose	2.20 ± 0.34a	2.23 ± 0.17a	2.21 ± 0.33a	2.23 ± 0.11a	2.18 ± 0.32a	2.25 ± 0.28a	2.17 ± 0.26a	2.22 ± 0.20a	2.20 ± 0.28a	2.17 ± 0.23a	2.19 ± 0.27a	2.22 ± 0.27a
Glucose	3.16 ± 0.19a	3.20 ± 0.26a	3.28 ± 0.29a	3.26 ± 0.14a	3.30 ± 0.28a	3.28 ± 0.19a	3.28 ± 0.22a	3.20 ± 0.27a	3.23 ± 0.31a	3.30 ± 0.25a	3.27 ± 0.24a	3.34 ± 0.19a
Sucrose	2.60 ± 0.30a	2.65 ± 0.29a	2.71 ± 0.28a	2.54 ± 0.22a	2.61 ± 0.30a	2.64 ± 0.42a	2.64 ± 0.18a	2.64 ± 0.31a	2.70 ± 0.20a	2.54 ± 0.20a	2.55 ± 0.21a	2.61 ± 0.17a
Total sugars	8.05 ± 0.35a	8.19 ± 0.30a	8.29 ± 0.34a	8.11 ± 0.23a	8.19 ± 0.33a	8.28 ± 0.29a	8.18 ± 0.27a	8.16 ± 0.32a	8.21 ± 0.32a	8.10 ± 0.26a	8.09 ± 0.28a	8.24 ± 0.28a
Sugar:organic acid	1.3	1.0	0.9	1.5	1.3	1.0	1.3	1.3	1.1	1.8	1.9	1.8
Phenolic compounds (mg/100 mL)												
Phenolic acids	1.22 ± 0.04b	1.19 ± 0.05b	1.20 ± 0.03b	1.25 ± 0.07b	1.25 ± 0.02b	1.19 ± 0.06b	1.25 ± 0.03b	1.24 ± 0.05b	1.24 ± 0.02b	1.22 ± 0.02b	1.21 ± 0.04b	1.21 ± 0.05b
Flavonols	17.34 ± 1.28c	17.36 ± 1.52c	18.24 ± 1.35c	19.03 ± 1.11bc	20.43 ± 1.24b	20.56 ± 1.63b	20.53 ± 1.22b	21.03 ± 1.60b	23.33 ± 1.61a	18.20 ± 1.13c	24.02 ± 1.21a	19.00 ± 1.06bc
Total phenolic compounds	18.56 ± 1.29c	18.55 ± 1.55c	19.44 ± 1.37c	20.28 ± 1.13c	21.68 ± 1.23c	21.75 ± 1.67b	21.78 ± 1.23b	22.27 ± 1.63b	24.57 ± 1.60a	19.42 ± 1.13c	25.23 ± 1.22a	20.21 ± 1.08bc

Data are shown as mean (n = 3) ± standard deviation; for each parameter tested, values with different letters, within the row, differ significantly (Tukey's test, $p < 0.05$); h-hours.

juices treated with *L. plantarum* strains and those with *O. oeni*. The sugar content in both juices inoculated with bacterial cells did not change significantly ($p > 0.05$) after 72-h incubation. Deacidification of berry juices from various sea buckthorn cultivars with *O. oeni* likewise did not result in a reduction of sugars, including glucose and fructose, as demonstrated by Tiitinen et al. (2007). In contrast, the DSM 20174 strain was able to reduce reducing sugars in pomegranate juice for 72 h after inoculation (Mousavi et al., 2013).

Wei et al. (2018) found that the decrease in reducing sugars during the fermentation by *L. plantarum* strains was stronger in bog bilberry juice with a lower pH (2.65) than in juice with a pH of 3.50. In contrast, Markkinen et al. (2019) reported that probably due to the low pH of sea buckthorn juice, *L. plantarum* revealed a higher preference for acids than sugars as a carbon source. However, the higher pH of sea buckthorn-apple juice (1:1) in our study was not conducive to the use of sugars by the bacteria used. Malic acid was not completely converted to lactic acid; hence bacterial degradation of sugars was not necessary, contrary to the study of Viljakainen and Laakso (2002) on acidity reduction in black currant, white currant, bilberry, and lingonberry juices.

The taste of the berries depends on the sugar:organic acid ratio, which correlates positively with sweetness and negatively with sourness and astringency (Tiitinen et al., 2005). This ratio for sea buckthorn juice did not change during fermentation with the tested strains and was 0.5 (Table 1). In the case of non-fermented sea buckthorn-apple juice, the ratio was inverse and was 1.9 regardless of the incubation time (Table 2). Fermentation of these juices resulted in a decrease in sugar:organic acid ratio over time, except for the process using *O. oeni* (DSM 20255 strain). The strongest change in the ratio was noted for sea buckthorn-apple juices fermented with the DSM 13273 strain (1.5→0.7), followed by the DSM 100813 strain (1.4→0.8).

3.5. Phenolic compounds

3.5.1. Fermentation of sea buckthorn juices

Previous studies have proven that sea buckthorn berries are a rich source of phenolic compounds, including flavonols, which account for over 98% of the total, and the others are hydroxycinnamic acids (Tkacz et al., 2020). Therefore, in this study, the dynamics of concentration changes of these compounds during juice fermentation was measured. Phenolic acids and flavonols in fresh sea buckthorn juice were constant at 0.63 and 45.40 mg/100 mL, respectively, and their concentrations did not change after a 72-h incubation period (Table 1).

Inoculation of sea buckthorn juices with lactic acid bacteria did not cause significant changes ($p > 0.05$) in phenolic acids content (Table 1). However, Markkinen et al. (2019) observed a strong reduction of hydroxycinnamic acids in black chokeberry juice and an increase in protocatechuic acid content in sea buckthorn juice using the same DSM 10492 strain. The content of *p*-coumaric and ferulic acids, which sea buckthorn berries contain (Tkacz et al., 2020), increased in mulberry juice treated with *L. plantarum* (Kwaw et al., 2018), but decreased in bog bilberry juice inoculated with *O. oeni* (Chen et al., 2019). The results can be explained by metabolism of ferulic acid to 4-vinyl guaiacol and *p*-coumaric acid to phloretic acid and *p*-vinylphenol (fermented flavor precursors) by reductases and decarboxylases of certain *L. plantarum* strains (Rodríguez, Landete, de las Rivas & Muñoz, 2008; Filannino, Bai, Di Cagno, Gobbetti, & Gänzle, 2015; Kachouri et al., 2015).

The type of lactic acid bacteria strain determined the differences in flavonol concentrations. A decrease in flavonol content was observed after fermentation in juices inoculated with DSM 100813 and DSM 10492 strains, by 8.3 and 10.3%, respectively. In turn, the juice inoculated with the DSM 20174 strain had a higher final flavonol concentration by 9.5% compared to uninoculated juice. *L. plantarum* subsp. *argentoratensis* and *O. oeni* did not significantly affect ($p > 0.05$) the content of flavonols in sea buckthorn juices. Similar trends in reducing

Table 3
Pearson's correlation matrix of selected chemical composition and antioxidant activity for juices after malolactic fermentation.

	pH	Soluble solids	Malic acid	Lactic acid	Total organic acids	Total sugars	Phenolic acids	Flavonols	Total phenolic compounds
Sea buckthorn juices									
pH	–								
Soluble solids	−0,62	–							
Malic acid	**−0,71**	**0,92**	–						
Lactic acid	**0,91**	**−0,78**	**−0,82**	–					
Total organic acids	**0,71**	−0,18	−0,19	**0,72**	–				
Total sugars	0,30	0,30	0,07	0,12	0,42	–			
Phenolic acids	0,29	−0,35	−0,33	0,55	0,55	−0,16	–		
Flavonols	0,62	−0,13	−0,24	0,36	0,31	0,66	−0,35	–	
Total phenolic compounds	0,62	−0,13	−0,24	0,36	0,31	0,66	−0,34	**1,00**	–
Antioxidant activity	0,56	−0,19	−0,20	0,33	0,29	0,48	−0,41	**0,92**	**0,92**
Sea buckthorn-apple juices									
pH	–								
Soluble solids	**−0,89**	–							
Malic acid	**−0,98**	**0,92**	–						
Lactic acid	**0,85**	**−0,85**	**−0,88**	–					
Total organic acids	**0,75**	**−0,77**	**−0,78**	**0,98**	–				
Total sugars	0,02	−0,18	−0,11	−0,17	−0,28	–			
Phenolic acids	**0,75**	−0,61	−0,50	0,47	0,43	0,39	–		
Flavonols	0,65	−0,62	−0,51	0,48	0,44	−0,31	0,46	–	
Total phenolic compounds	0,65	−0,68	−0,51	0,49	0,45	−0,30	0,48	**1,00**	–
Antioxidant activity	0,58	−0,52	−0,42	0,38	0,33	−0,37	0,33	**0,99**	**0,98**

the total flavonol glycoside content were reported for chokeberry juices inoculated with DSM 10492 and 100813 strains, but not for sea buckthorn (Markkinen et al., 2019). Fermentation of bog bilberry juice with *O. oeni* promoted phenolic acid reduction (Chen et al., 2019), whilst treatment of mulberry juice with *L. plantarum* resulted in an almost two-fold increase in total flavonols (Kwaw et al., 2018).

The differences in total phenolic compound content in sea buckthorn juices after 72-h fermentation amounted to a maximum of 10%, as did flavonol changes. Potential precipitation, oxidation and combination of phenolic compounds or their adsorption to solids and polymerization could cause a decrease in concentrations of these compounds (Chen et al., 2018).

3.5.2. Fermentation of sea buckthorn-apple juices (1:1)

The phenolic acid content in sea buckthorn-apple juice was 1.30 mg/100 mL and decreased during 72-h incubation by 17.7% (Table 2). Like in sea buckthorn juices, bacterial strains did not metabolize phenolic acids, whose final content in juices had not changed significantly ($p > 0.05$, Table 2).

Incubation of sea buckthorn-apple juice did not change the flavonol content, equal to 17.57 mg/100 mL in fresh juice. Bacterial inoculation promoted an increase in flavonol content in all fermented juices, except for the juice treated with the DSM 10492 strain. Therefore, a strong correlation was found between the malic acid conversion and the final flavonol content in juices (r = 0.78). The largest increase after 72 h was noted in juices inoculated with DSM 16365 and DSM 100813 strains (by 27.9 and 26.7%, respectively). Unlike in the case of *L. plantarum*, in juices treated with *O. oeni* (DSM 20255) the largest amount of flavonols was observed after 48 h of MLF (a rise of 28.9%); and in the next 24 h a reduction of 20% relative to the maximum was found. The increase in content of flavonols, isorhamnetin and kaempferol was studied in fermented cactus cladode pulp by *L. plantarum* in the research of Filannino et al. (2016). Glycosylated flavonols may have been hydrolyzed to the corresponding aglycons due to the action of β-glycosidase released by *L. plantarum* strains (Wei et al., 2018). Differences in concentrations of phenolic compounds between the strains tested may result from their individual adaptation. Fermentation induces the structural degradation of plant cell walls and thus the release of compounds from the plant matrix. *L. plantarum* have a special ability to efficiently produce hydrolytic enzymes and to deglycosylate phenolic compounds during fermentation, thereby breaking down them into simpler forms. Additionally, phenolic complex conversion into free forms and depolymerization of high molecular weight subunits is possible in phenoloxidase-catalyzed reactions (laccase and tyrosinase) present in lactic acid bacteria (Hur, Lee, Kim, Choi, & Kim, 2014; Kwaw et al., 2018).

The sum of phenolic acids and flavonols of fermented sea buckthorn-apple juices was higher (maximal to 24.87 mg/100 mL) in relation to fresh juice.

3.6. Antioxidant activity

Antioxidant activity of juices was analyzed as the oxygen radical absorbance capacity (ORAC test). Seventy-two-hour incubation did not affect the antioxidant activity of sea buckthorn juice, which was 7.35 mmol TE/100 mL on average (Table 1). DSM 100813 and DSM 10492 strains reduced antioxidant activity during the entire fermentation period, finally resulting in a drop by 24.5 and 28.8%, respectively (Table 2). In turn, a rise in final antioxidant activity (after 72 h) by 25.0% was noted in sea buckthorn juice treated with the DSM 20174 strain. Fermentation with this bacterial strain also increased the antioxidant activity of pomegranate juice analyzed by the DPPH test (Mousavi et al., 2013). Finally, it was established that *L. plantarum* strains contributed to increasing the availability of compounds with antioxidant capacity more strongly than juices treated with *O. oeni*.

The antioxidant activity of fresh sea buckthorn-apple juice (1:1) was 5.54 mmol TE/100 mL and did not change during 72-h incubation. The dynamics of changes in antioxidant activity during fermentation with lactic acid bacterial strains are shown in Fig. 4. It was found that the variations during MLF were dependent on bacteria, and all strains, except for DSM 10492, significantly increased the antioxidant activity ($p < 0.05$) of juices. There was also no significant ($p > 0.05$) change in the amount of phenolic compounds in fermented juice with this strain. Recent studies on the antioxidant capacity of lactobacilli have shown that some strains can both reduce the risk of accumulation of reactive oxygen species (ROS) and degrade hydrogen peroxide and superoxide anion (Liu & Pan, 2010). However, strains differ in their enzymatic and non-enzymatic antioxidant mechanisms and their ability to minimize the ROS production. It is confirmed that *L. plantarum* influences the conversion or protection of bioactive compounds that positively participate in the increase of antioxidant activity. The cause may be by the antioxidant defense ability of bacteria to oxygen radical generation by oxygen involvement in reductive conversion of phenols.

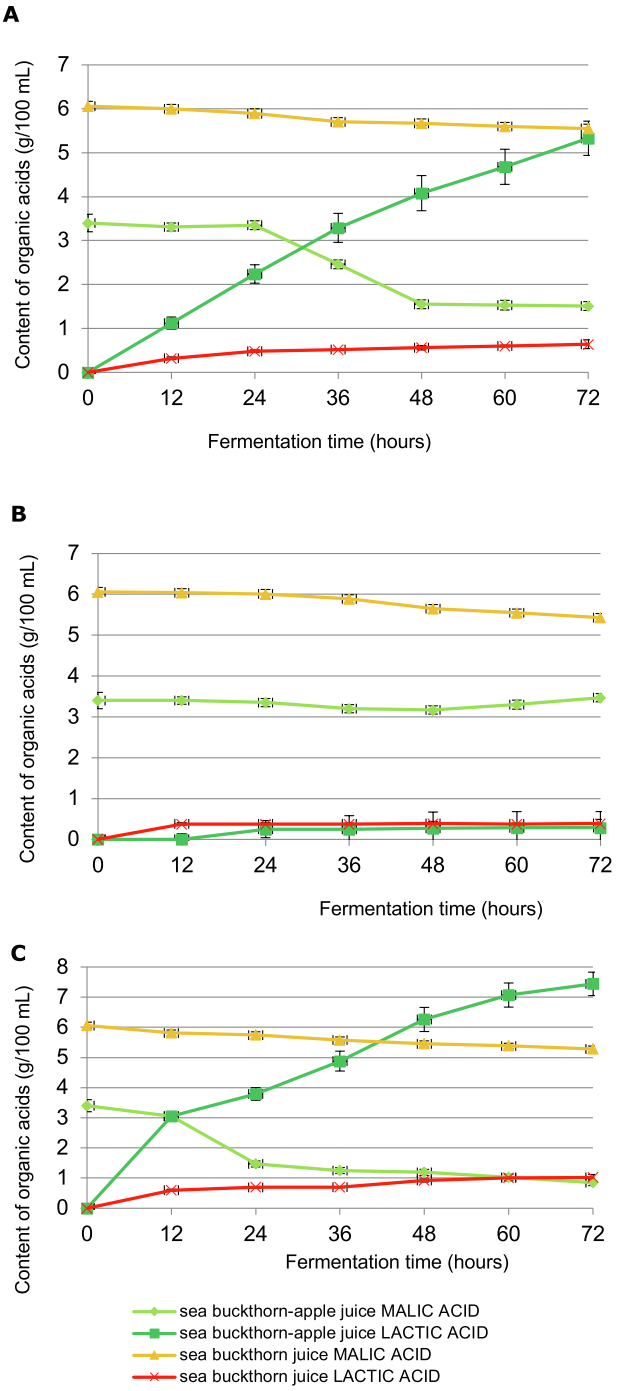

Fig. 2. Dynamics of changes in the content of malic and lactic acids in juices fermented with *L. plantarum* subsp. *argentoratensis* DSM13635 (**A**), *O. oeni* DSM 20255 (**B**), *L. plantarum* DSM 10492 strain (**C**).

Antioxidant compounds released or synthesized during fermentation can act as singlet oxygen quenchers, metal chelators, hydrogen donors to radicals and free radical terminators. In fact, many factors affect the antioxidant effect, including the type of strain and their diversity at the phenotypic, ecological and genotypic levels, cell concentration, metabolic capacity and ability of genus *Lactobacillus* to inhibit polyphenol oxidase activity (Hur et al., 2014; Kachouri et al., 2015; Zheng et al., 2020). In this research, fermentation promoted the biotransformation of bioactive components in juices, mainly flavonols, providing stronger antioxidants. Therefore, the rise in antioxidant activity was strongly correlated with the flavonol content in sea buckthorn juices (r = 0.92) and sea buckthorn-apple juices (r = 0.99) (Table 3).

During the first 24 h, a significant increase ($p < 0.05$) in activity was only observed in sea buckthorn-apple juices treated with DSM 16365 and DSM 100813 strains. Between 24 and 36 h of incubation, a strong rise in activity was also noted in juices treated with DSM 20174 and DSM 6872 strains. It was found that *O. oeni* (DSM 20255 strain) did not modulate activity as strongly as most *L. plantarum* strains. Zhou et al. (2020) reported that *L. plantarum* was able to increase the antioxidant activity of kiwifruit pulp just during 28-h fermentation at 37 °C. In this study, incubation for the last 12 h (after 60 h of the process) did not significantly change the activity ($p > 0.05$) in juices fermented with DSM 6872, DSM 13273, DSM 20255, and DSM 10492 strains. Finally, a considerable increase in antioxidant activity was recorded in juices after fermentation with three strains (DSM 100813, DSM 16363 and DSM 20174) and reached a maximum increase of 46.6 to 51.6% compared to fresh juice.

In vitro measurement of antioxidant activity provides a complete picture and preliminary indications in one measure, and can be a quick reference point for *in vivo* studies. However, it should be emphasized the possible interference of measurement by factors unrelated to the antioxidant concentration, it is highly dependent on the extraction procedure, and different measurement methods provide dissimilar results (Pellegrini, Vitaglione, Granato, & Fogliano, 2018). Zhao, Zhang, Zhang, Liu, and Meng (2019) and Di Cagno, Minervini, Rizzello, De Angelis, and Gobbetti (2011) found significant decreases ($p < 0.05$) in antioxidant activity of fermented jujube juice, and red and green smoothies, respectively. Chen et al. (2018) reported a slight increase in activity for fermented papaya juice. However, most previous studies, as well as this, confirmed the positive effect of certain *L. plantarum* strains on antioxidant activity, including those on cactus cladodes (Filannino et al., 2016), mulberry (Kwaw et al., 2018), *Momordica charantia* (Gao et al., 2019), kiwifruit (Zhou et al., 2020), and pomegranate juices (Mantzourani et al., 2019).

4. Conclusions

In this study, biological deacidification of sea buckthorn and sea buckthorn-apple juices was performed using malolactic fermentation. The results provided information on the kinetics of changes in the content of organic acids, sugars, phenolic compounds and antioxidant activity during 72-h fermentation. The metabolic activity of lactic acid bacteria can be ranked in the following descending order: *L. plantarum* > *L. plantarum* subsp. *argentoratensis* > *O. oeni*. Low pH inhibited the fermentation capacity of bacterial strains; therefore stronger malolactic conversion was observed in sea buckthorn-apple juices (up to 75%) than sea buckthorn juices (up to 21%). Fermentation of sea buckthorn-apple juice with DSM 10492 and DSM 20174 strains proved to be the most favorable. In addition, *L. plantarum* increased the final flavonol content and antioxidant activity in sea buckthorn-apple juices more effectively than *O. oeni*. During malolactic fermentation, no significant changes in the content of phenolic acids, sugars (glucose, sorbitol, fructose, rhamnose), or organic acids except for malic and lactic acids (quinic, citric, isocitric, and oxalic acids) were observed. In summary, malolactic fermentation can be considered as a promising method of reducing malic acid in sea buckthorn juices or mixed juices with high content of sea buckthorn addition. The results obtained will allow the selection of appropriate conditions and bacterial strains for industrial-scale deacidification. Reduction of excessive amounts of malic acid may improve the sensory attractiveness and contribute to the development of novel value-added fermented sea buckthorn products, thus increasing its consumption. In the future, it will be valuable to supplement these studies with the identification of other biologically active compounds and their metabolic pathways, sensory analysis, including volatile compounds, shelf life tests and broad assessment of the health-promoting effects of fermented sea buckthorn juices.

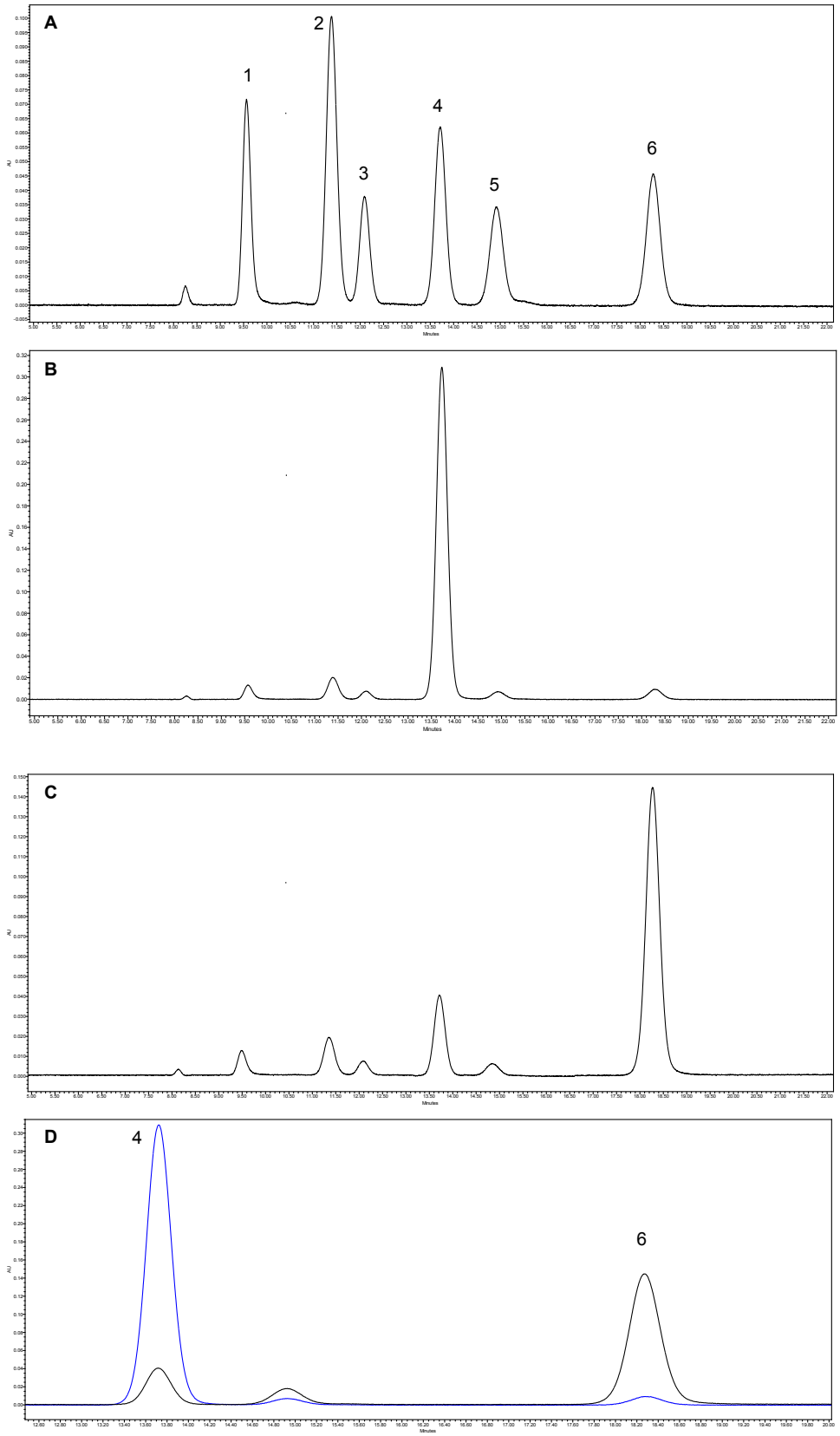

Fig. 3. UPLC–PDA chromatograms at 210 nm of organic acid standards (A), sea buckthorn-apple juice after 12 h (B) and after 72 h malolactic fermentation (C). Changes in malic acid and lactic acid during malolactic fermentation (D). Peak number: 1 – oxalic acid, 2 – citric acid, 3 – isocitric acid, 4 – malic acid, 5 – quinic acid, 6 – lactic acid.

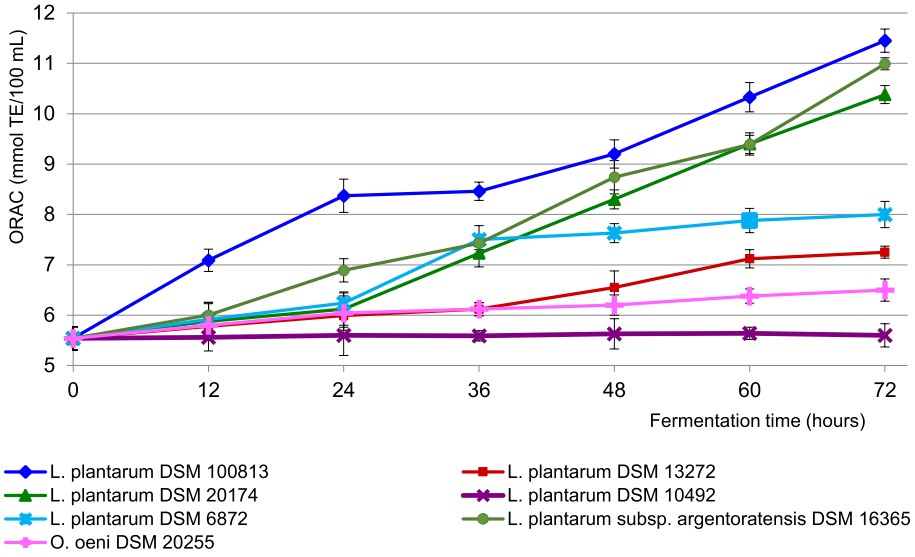

Fig. 4. Dynamics of changes in oxygen radical absorbance capacity of sea buckthorn-apple juices during fermentation with various bacterial strains. Data are shown as mean (n = 3) ± standard deviation.

Ethical statement

Research did not include any human subjects and animal experiments.

CRediT authorship contribution statement

Karolina Tkacz: Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Joanna Chmielewska:** Formal analysis, Writing - review & editing. **Igor Piotr Turkiewicz:** Formal analysis. **Paulina Nowicka:** Formal analysis. **Aneta Wojdyło:** Supervision, Conceptualization, Writing - original draft, Writing - review & editing, Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Oświadczam, że jestem współautorem publikacji pt.:

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Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, pozyskaniu i przygotowaniu materiału badawczego, uczestnictwie w badaniu mikroskopowym inokulowanych soków, optymalizacji warunków i monitorowaniu procesu fermentacji malolaktycznej, wyznaczeniu dynamiki zmian składu chemicznego, w tym cukrów metodą HPLC-ELDS, kwasów organicznych i związków fenolowych metodą UPLC-PDA, i aktywności przeciwutleniającej *in vitro* bioprzetwarzanych soków z owoców rokitnika pospolitego. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

Kierowałam projektem naukowym Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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mój udział polegał na współopracowaniu i koordynowaniu technologią bioprzetwarzania soków z owoców rokitnika pospolitego, przygotowaniu kultur bakterii, uczestnictwie w badaniu mikroskopowym inokulowanych soków i optymalizacji warunków procesu fermentacji malolaktycznej. Brałam udział w opracowaniu podrozdziału manuskryptu dotyczącego metodologii fermentacji malolaktycznej i merytorycznym współredagowaniu publikacji.

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mój udział polegał na uczestnictwie w etapie przygotowania materiału badawczego oraz podczas monitorowania procesu fermentacji malolaktycznej soków z owoców rokitnika pospolitego.

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w analizie aktywności przeciwutleniającej *in vitro* bioprzetwarzanych soków z owoców rokitnika pospolitego oraz współredagowaniu manuskryptu pod względem merytorycznym.

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w wyznaczeniu dynamiki zmian zawartości kwasów organicznych i związków fenolowych metodą UPLC-PDA i aktywności przeciwutleniającej *in vitro* bioprzetwarzanych soków z owoców rokitnika pospolitego. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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Anti-diabetic, anti-cholinesterase, and antioxidant potential, chemical composition and sensory evaluation of novel sea buckthorn-based smoothies

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ABSTRACT

Sea buckthorn berries fit into the strategy of seeking natural factors in the non-communicable diseases prevention, but their sensory qualities are a challenge for consumers and food industry. The study aimed to evaluate anti-cholinesterase (anti-acetylcholinesterase and -butylcholinesterase), anti-diabetic (anti-α-amylase, -α-glucosidase, -pancreatic lipase) and antioxidant potential (FRAP, ORAC), phenolic compounds (UPLC-PDA-FL), basic chemical composition, and sensory quality of sea buckthorn-based smoothies. Eighteen novel products containing sea buckthorn (25–50%) with other fruits and vegetables were analyzed. Sea buckthorn enriched the smoothies in flavonols (25.46–95.13 mg/100 g), and fruits and vegetables provided phenolic acids and procyanidins. The anti-BuChE effect was higher than anti-AChE, while products with apricot, orange, grape and parsley root were strong inhibitors of carbohydrates digesting enzymes. Lipase inhibition by all smoothies was over 50%. Products with 75% fruits or 50% vegetables were the most sensory attractive. The results will be valuable in designing innovative food with rarely used berries.

1. Introduction

Regular consumption of fruits and vegetables has been found to be related to a reduced risk of diet-related diseases such as heart disease, stroke, obesity, diabetes, some types of cancer, and others (Kuntz et al., 2015). The World Health Organization (WHO) recommends a daily intake of at least 400 g of fruits and vegetables, while the U.S. Department of Agriculture (USDA) suggests five to nine portions per day. Due to current lifestyle and eating habits, fruit and vegetable intake on a global scale is below recommended levels (Castillejo, Martínez-Hernández, Gómez, Artés, & Artés-Hernández, 2016; González-Tejedor et al., 2017). Consequently, consumer trends are oriented on ready-to-eat and ready-to-drink products, and the food industry develops innovative and alternative products with functional additives, interesting flavor combinations, forgotten plants, and superfruits and –vegetables (Baiano, Mastromatteo, & Del Nobile, 2012; Di Cagno, Minervini, Rizzello, De Angelis & Gobbetti, 2011).

On the other hand, bioactive compounds in food have been gaining much attention due to their possible beneficial implications for human health (Agbenorhevi & Marshall, 2012). Many studies are underway to determine antioxidant capacity and the ability to inhibit various enzymes as potential pathways in minimizing many non-communicable diseases. The prevention and treatment of neurodegenerative disorders, including Alzheimer's disease and dementia, are currently focused on increasing levels of the neurotransmitter acetylcholine and preventing its rapid degradation. Inhibition of acetylcholinesterase (AChE) and butylcholinesterase (BuChE) activities can therefore be one of the main strategies (Nanasombat, Thonglong, & Jitlakha, 2015; Szwajgier & Borowiec, 2012). In turn, α-amylase and α-glucosidase break down polysaccharides into glucose, and pancreatic lipase breaks down triglycerides into bioavailable fatty acids and monoglyceride or glycerol molecules, so their inhibition can reduce postprandial hyperglycemia and energy intake, respectively. The controlled reduction of enzyme activities is therefore a promising solution in the prevention and treatment of type 2 diabetes mellitus in combination with reduced dose of drugs, overweight and the early stage of obesity and their complications (Costamagna et al., 2016).

Natural inhibitors are constantly being sought, and a plant which is an ideal complex of phenolic compounds, vitamins A, E, C, microelements, fatty acids and phytosterols is sea buckthorn (*Hippophaë rhamnoides* L.). A number of reports indicate the potential of its berries associated with reducing the risk of cardiovascular disease, the prevention of hyperglycemia, hyperinsulinemia, hyperlipidemia and atherosclerosis, as well as hepatoprotective, anti-cancer,

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neuroprotective effects, and many others (Bal, Meda, Naik, & Satya, 2011; Ma et al., 2020; Tkacz, Wojdyło, Turkiewicz, Bobak, & Nowicka, 2019b). However, sea buckthorn is a challenge for both the food industry and consumers because of the perceived sensory quality. The berry flavor is dominated by the intense acidity resulting from the very low ratio of sugars to organic acids, as well as astringency correlated with some flavonols glycosides, proanthocyanidins, ethyl-β-D-glucopyranoside and malic acid, and bitterness related to the ratio of acids and phenolic compounds. The aroma is described as intensely pungent with fermented notes. In turn, the consistency of pure juice is heterogeneous due to the susceptibility of separation of the fat phase and sediment (Laaksonen, Knaapila, Niva, Deegan, & Sandell, 2016; Ma et al., 2020; Tang, Kälviäinen, & Tuorila, 2001; Tiitinen, Vahvaselkä, Hakala, Laakso, & Kallio, 2006).

Several percent quantities of sea buckthorn are added to food products to improve acidity and health benefits. Voicu et al. (2009) applied only 2–3% of fruit juice to apple-quince nectar. Selvamuthukumaran, Khanum, and Bawa (2007) developed jellies based on sea buckthorn berries and grapes (1:1), which exhibited good organoleptic characteristics with a high sensory score, compared to sea buckthorn jelly and sea buckthorn-papaya, and –watermelon jellies. According to Teleszko and Wojdyło (2014), even a small proportion of sea buckthorn (just 6.7%) reduced the taste of fruit smoothies with apple, quince, chokeberry, and blackcurrant, and was definitely unacceptable. Previously in a study conducted by Hartvig, Hausner, Wendin, and Bredie (2014) only children among consumers from Denmark accepted sweet and sour sea buckthorn juice, which is, however, a common component of the Nordic diet. There are still no reports on products with a high proportion of these berries that would be attractive to a potential consumer in terms of sensory but also pro-health properties.

Therefore, the aim of the study was to evaluate anti-cholinesterase (anti-AChE and -BuChE), anti-diabetic (anti-α-amylase, -α-glucosidase and -pancreatic lipase) and antioxidant potential (ferric reducing ability – FRAP and oxygen radical absorbance capacity – ORAC), phenolic compounds, basic chemical composition, and sensory quality of novel sea buckthorn-based smoothies. The paper will provide comprehensive knowledge about novel fruit and vegetable compositions with high proportions (25% and 50%) of sea buckthorn juice. It should be emphasized that so far there are no data on *in vitro* biological activity and composition analyzed by ultra performance liquid chromatography and high pressure liquid chromatography (UPLC-PDA-FL, HPLC-ELSD) methods in correlation with the consumer sensory assessment of novel sea buckthorn products.

2. Materials and methods

2.1. Plant materials and production of smoothies

Sea buckthorn (*Hippophaë rhamnoides*) berries of the cultivar 'Józef' were collected in August from the Experimental Orchard in Dąbrowice (51°56′N 20°06′E) of the Research Institute of Horticulture in Skierniewice (Poland). Some relevant agronomic conditions were as follows: soil category – medium; growing season average temperature (GST) − 16.6 to 20.7 °C; precipitation sum − 29.4 to 52.5 mm; irrigation and fertilization were used. Other raw materials such as pear (*Pyrus communis*), apricot (*Prunus armeniaca*), peach (*Prunus persica*), orange (*Citrus sinensis)*, green grape (*Vitis vinifera*), apple (*Malus domestica*), celery root (*Apium graveolens*), carrot (*Daucus carota*), and parsley roots (*Petroselinum crispum*) were purchased in the first half of September on the retail market in Poland. The origin of the raw materials was as follows: Poland – pear, apple, carrot, celery and parsley root; Spain – apricot, peach, orange; Italia – green grape. All of them were commercially mature, with no signs of post-harvest physiological disorders, stored under refrigerated conditions at a relative humidity (RH) of 90–95%, in gunny bags or corrugated fiber board boxes. Before further processing, the raw materials were washed in running water at 50 °C, the roots were scraped but not peeled, in accordance with the industrial practice.

The production of smoothies consisted of four main stages: (1) making of sea buckthorn juice, (2) processing of other fruit and vegetable pulps, (3) creating smoothies by mixing the juice with fruit/vegetable pulps, (4) thermal treatment of products, as described below.

(1) Sea buckthorn juice was squeezed from selected berries using a laboratory hydraulic press (SRSE, Warsaw, Poland).
(2) Other fruits and vegetables were ground using a Thermomix device (Vorwerk & Co. KG, Wuppertal, Germany). Apricot and peach were previously pitted. To prevent enzymatic browning, ascorbic acid (10 mL 10% solution per 1 kg of raw material) was added to pears, peaches, apples and parsley roots. Fineness of raw materials was to form a pulp. Shredded grapes were passed through a sieve to remove seeds.
(3) The pulps formed were combined with sea buckthorn juice in ratios of 50:50 and 75:25 on a weight/weight basis (*w/w*).
(4) These smoothies were heated to 100 °C, then pasteurized in 80 g glass jars for 10 min at 90 °C, and finally cooled to 20 °C. The heat treatment conditions were established based on the pH values of the products ranged of 2.86 to 3.95, ensuring microbiological stability. The products were stored under refrigerated conditions and intended for analysis 24 h after manufacture.

In summary, eighteen smoothies containing sea buckthorn juice (SB) and one fruit/vegetable pulp in two weight proportions were created and labeled as: SB-PEAR, SB-APRICOT, SB-PEACH, SB-ORANGE, SB-GRAPE, SB-APPLE, SB-CELERY ROOT, SB-CARROT, and SB-PARSLEY ROOT. Each smoothie variant was prepared in triplicate, one of which was intended for sensory analysis. 100% sea buckthorn juice (SB 100%), after pasteurization as in stage 4, was analyzed as the 19th variant.

2.2. Basic chemical properties: Dry matter, soluble solids, titratable acidity, pH, ash, pectin content

Basic chemical properties were determined according to European Standards (PN-EN) and as described previously by Tkacz et al. (2019b). Briefly, dry matter (%) was studied by mixing the smoothie sample with diatomaceous earth, pre-drying, and final drying by the vacuum-oven method at 70 °C and pressure of 100 Pa for 24 h, using a Vacucell ECO line (MMM Medcenter Einrichtungen GmbH, Planegg/München, Germany). The soluble solids content at °Brix (°Bx) was measured with a digital refractometer (Atago RX-5000, Atago Co. Ltd., Saitama, Japan). Titratable acidity (g malic acid/100 g) and pH were determined using a dedicated automatic pH titrator system (TitroLine 5000, Xylem Analytics GmbH, Weilheim in Oberbayern, Germany). The ash content (%) was determined by dry mineralization. About 1.0 g of a homogeneous smoothie sample was weighed into a tared porcelain crucible. The sample was charred on an electric stove under a fume hood, and then placed in a muffle furnace at 550 °C to complete ashing. The sample cooled in the desiccator was weighed and the ash content calculated based on the weight difference with fresh sample. Pectin content (g/100 g) was determined by a modified Morris method. Pectins were precipitated from a smoothie sample using acetone, filtered through filters, and then dried at 75 °C to constant weight. The pectins amount was calculated based on the weight of precipitate on the filter after drying. All data were mean of three measurements ± SD.

2.3. Analysis of sugars by HPLC-ELSD and organic acids by UPLC-PDA methods

Smoothie sample (~6 g) was diluted with 50 mL redistilled water and incubated at 90 °C for 30 min with constant shaking. The smoothie solution was centrifuged at 19,000 g for 10 min using MPW-350 (MPW

Med. Instruments, Warsaw, Poland); the supernatant was filtered through Sep-Pak C18 cartridges (Waters Corp., Milford, MA, US), and then - before injection - through a 0.20 μm pore size hydrophilic PTFE membrane (Millex Samplicity™ Filter, Merck KGaA, Darmstadt, Germany). Smoothie extracts thus prepared were directed for analysis of sugars and acids.

The profile and content of sugars were determined by high pressure liquid chromatography (HPLC) method, as described previously by Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). A Merck-Hitachi L-7455 liquid chromatograph (Merck KGaA, Darmstadt, Germany) with an evaporative light scattering detector ELSD (PL-ELS 1000, Polymer Laboratories Inc., Amherst, MA, US) were used. The separation of sugars was performed on an Alltech® Prevail™ Carbohydrate ES HPLC Column-W (5 μm particle size, 250 × 4.6 mm, Columbia, MD, USA). 87% acetonitrile was used as the mobile phase for isocratic elution. The flow rate was 0.7 mL/min, sample injection volume − 4 μL, elution time − 17 min. Quantification of sugars was performed based on calibration curves (R^2 = 0.9998) of reference standards of rhamnose, fructose, sorbitol, glucose, and sucrose injected at known concentrations and under the same conditions. Merck-Hitachi D-7000 HPLC System Manager was used to develop records.

The profile and content of organic acids were determined by ultra performance liquid chromatography method, as described by Nowicka, Wojdyło, and Laskowski (2019). An Acquity UPLC System with photodiode array detector PDA (Waters Corp., Milford, MA, US) were used. The separation of organic acids was performed on an Aminex® HPX-87H column (9 μm particle size, 300 × 7.8 mm, Bio-Rad, Hercules, CA, US). 1 mM phosphoric acid solution was used as the mobile phase for isocratic elution. The flow rate was 0.5 mL/min, sample injection volume − 10 μL, elution time − 19 min. Quantification of organic acids was performed based on calibration curves (R^2 = 0.9998) of reference standards of oxalic, citric, malic, quinic, shikimic, and succinic acids injected at known concentrations and under the same conditions. Empower 3 Chromatography Data Software (Waters Corp., Milford, MA, US) was used to develop records. All data were mean of three measurements ± SD, and results were expressed as g/100 g of sample.

2.4. Sensory analysis

The sensory analysis was carried out in accordance with ISO 13299:2016 Sensory analysis — Methodology — General guidance for establishing a sensory profile. 23 trained panelists, including 13 women and 10 men, aged 19–60, evaluated the products in the laboratory. Products were given to panelists in random order, in coded plastic disposable 60 mL cups, at room temperature. The following attributes were assessed: color, aroma, consistency, taste, acidity flavor, foreign flavor, and overall acceptance on a 5-point scale with increments of 0.5. The color, aroma, consistency, taste, and overall acceptance were rated from very unattractive (1) to very attractive (5). In turn, sensations of acidity and foreign flavors were assessed between undetectable (1) to very intense (5). The results are presented in a graphic form.

2.5. Analysis of phenolic compounds (phenolic acids, flavonols and polymeric procyanidins) by UPLC-PDA-FL method

The profile and content of phenolic compounds were determined by ultra performance liquid chromatography (Acquity UPLC System) with photodiode array detector PDA for analysis of phenolic acids and flavonols, and with fluorescence detector FL for analysis of polymeric procyanidins (Waters Corp., Milford, MA, US). Extraction and UPLC analysis of phenolic acids and flavonols were performed as previously described by Tkacz et al. (2020). The sums of phenolic acids and flavonols were calculated as *p*-coumaric acid and isorhamnetin-3-*O*-rutinoside, respectively. Analysis of polymeric procyanidins was performed by direct phloroglucinolysis method, exactly as in the protocol previously described by Wojdyło, Teleszko, and Oszmiański (2014). The calibration curves were established using (+)-catechin, (−)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (−)-epicatechin-phloroglucinol adduct standards. Empower 3 Chromatography Data Software (Waters Corp., Milford, MA, US) was used to develop records. All data were mean of three measurements ± SD, and results were expressed as mg/100 g of sample.

2.6. Determination of biological activities: antioxidant, anti-cholinesterase, anti-α-amylase, anti-α-glucosidase and anti-lipase

Smoothie sample (~3 g) was mixed with 6 mL of solvent methanol–water (80:20; *v/v*) with 1% hydrochloric acid and sonicated twice for 20 min with an interval of 24 h (Sonic 6D, Polsonic, Warsaw, Poland). The smoothie solution was centrifuged at 19,000 g for 10 min using MPW-150R (MPW Med. Instruments, Warsaw, Poland), and before injection the supernatant was filtered through a 0.20 μm pore size hydrophilic PTFE membrane (Millex Samplicity™ Filter, Merck KGaA, Darmstadt, Germany).

The antioxidant capacity was examined by spectrophotometric method as ferric reducing ability and spectrofluorometric method as oxygen radical absorbance capacity. The FRAP and ORAC results were mean of three measurements ± SD and expressed as mmol Trolox (TE)/100 g of sample. The anti-diabetic potential was tested as the ability to inhibit *α*-amylase, *α*-glucosidase, and pancreatic lipase. The antioxidant and anti-diabetic activities were investigated as given previously by Tkacz et al. (2019b). Anti-cholinesterase activity was tested for acetylcholinesterase and butylcholinesterase inhibition as reported previously by Tkacz et al. (2020). The results were mean of three measurements ± SD and expressed as percentages inhibition (at concentrations of 0.25 g/mL for anti-AChE, anti-BuChE, anti-*α*-amylase and anti-*α*-glucosidase effects, and 0.30 mg/mL for anti-lipase effect). All measurements were made using a multi-mode microplate reader Synergy™ H1 (BioTek, Winooski, Vermont, US).

2.7. Statistical analysis

Each variant of the product was prepared in duplicate (primary samples), and the laboratory sample was a combination of equal parts. Each analysis was performed for three analytical samples. All data were subjected to one-way analysis of variance (ANOVA) and then Tukey's multiple-range test to compare the means. Values with different letters in tables differed significantly at $p < 0.05$. Additionally, Pearson's correlations coefficients (r), Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) were determined. AHC was obtained by Euclidian distance dissimilarity using the aggregation criterion - Ward's method. Statistical analyzes were done using XLSTAT Statistical Software for Microsoft Excel 2017 (Microsoft Corp., Redmond, WA, US).

3. Results and discussion

3.1. Basic chemical properties (dry matter, soluble solids, titratable acidity, pH, ash, pectin content) of sea buckthorn-based smoothies

Basic chemical properties such as dry matter, pH, soluble solid, titratable acidity, ash and pectin contents in sea buckthorn-based smoothies are presented in Table 1. The dry matter content depended on the type of semi-products and their proportions in smoothies. Products with a higher percentage of sea buckthorn juice had a lower dry matter. Thus, the dry matter ranged from 10.25% (SB-celery root 50:50) to 20.23% (SB-parsley root 25:75).

Sea buckthorn juice had the lowest pH, 2.86, which was consistent with previous studies on *H. rhamnoides* cultivars (Tkacz et al., 2019b). The addition of fruits and vegetables caused an increase in pH of smoothies, but at the same time keeping the pH below 4, ensuring the potential stability of final products. The largest change in pH – by more

Table 1
Dry matter, pH, soluble solids, titratable acidity and ash and pectin contents of sea buckthorn-based smoothies.

Smoothie	Ratio (*w/w*)	Dry matter (%)	pH	Soluble solids (°Bx)	Titratable acidity (g malic acid/100 g)	Ash (%)	Pectin (%)
SB-PEAR	25:75	17.83 ± 0.11b	3.26 ± 0.01d	14.7 ± 0.1b	0.85 ± 0.02 h	0.26 ± 0.02f	2.09 ± 0.14c
	50:50	16.03 ± 0.09bc	3.03 ± 0.01e	13.7 ± 0.0c	1.40 ± 0.02e	0.37 ± 0.05de	1.66 ± 0.11de
SB-APRICOT	25:75	13.55 ± 0.14d	3.08 ± 0.02e	12.1 ± 0.1de	2.23 ± 0.01c	0.54 ± 0.01c	1.13 ± 0.07f
	50:50	12.88 ± 0.10d	2.96 ± 0.02e	11.8 ± 0.1e	2.41 ± 0.04b	0.50 ± 0.01 cd	1.36 ± 0.14e
SB-PEACH	25:75	13.17 ± 0.08d	3.10 ± 0.02e	12.3 ± 0.0d	1.58 ± 0.02e	0.41 ± 0.02de	0.57 ± 0.08 g
	50:50	12.45 ± 0.12e	3.10 ± 0.02e	11.7 ± 0.0e	1.96 ± 0.02d	0.36 ± 0.02de	0.98 ± 0.09f
SB-ORANGE	25:75	13.55 ± 0.10d	3.18 ± 0.06de	12.5 ± 0.1d	1.28 ± 0.02f	0.33 ± 0.02e	0.65 ± 0.11 g
	50:50	12.93 ± 0.05e	3.05 ± 0.01e	11.7 ± 0.1e	1.71 ± 0.02de	0.41 ± 0.02de	0.82 ± 0.18f
SB-GRAPE	25:75	12.33 ± 0.05e	3.23 ± 0.03d	11.3 ± 0.1f	0.92 ± 0.02 g	0.46 ± 0.02d	0.29 ± 0.05 h
	50:50	11.80 ± 0.07e	3.08 ± 0.01e	10.6 ± 0.1 g	1.59 ± 0.01e	0.47 ± 0.01d	0.21 ± 0.09 h
SB-APPLE	25:75	15.25 ± 0.10c	3.02 ± 0.04e	13.4 ± 0.1c	1.30 ± 0.02f	0.37 ± 0.03de	1.03 ± 0.15f
	50:50	14.50 ± 0.09c	2.90 ± 0.05ef	12.0 ± 0.0e	1.80 ± 0.01d	0.39 ± 0.01de	1.13 ± 0.04f
SB-CELERY ROOT	25:75	10.48 ± 0.12f	3.68 ± 0.01b	8.2 ± 0.1i	0.92 ± 0.01 g	0.82 ± 0.01b	1.96 ± 0.09d
	50:50	10.25 ± 0.03f	3.20 ± 0.01de	8.1 ± 0.1i	1.46 ± 0.01e	0.67 ± 0.03bc	1.89 ± 0.09d
SB-CARROT	25:75	12.11 ± 0.04e	3.55 ± 0.03b	9.8 ± 0.1 h	0.78 ± 0.02 h	0.58 ± 0.01c	1.65 ± 0.13de
	50:50	11.45 ± 0.06e	3.18 ± 0.03de	9.5 ± 0.0 h	1.41 ± 0.02e	0.55 ± 0.01c	1.53 ± 0.07e
SB-PARSLEY ROOT	25:75	20.23 ± 0.11a	3.95 ± 0.01a	17.0 ± 0.0a	0.85 ± 0.04 h	1.08 ± 0.02a	4.21 ± 0.14a
	50:50	16.92 ± 0.08b	3.37 ± 0.01c	14.5 ± 0.0b	1.47 ± 0.01e	0.87 ± 0.01b	3.73 ± 0.10b
SB 100%	–	12.00 ± 0.09e	2.86 ± 0.07f	7.6 ± 0.0j	2.54 ± 0.01a	0.43 ± 0.03de	0.61 ± 0.15 g

All data are mean of three measurements ± standard deviation. Values followed by the same letter, within the column, were significantly different ($p < 0.05$; Tukey's test); SB – sea buckthorn juice.

than one unit – was observed in SB-parsley root 25:75. Smoothies with carrot, celery root, pear, and grape had a much higher pH than juice ($p < 0.05$).

Wide variation in soluble solid contents from 7.6 °Bx (SB 100%) to 17.0 °Bx (SB-parsley root 25:75) was found. The use of other fruits (pear, apricot, peach, orange, grape, apple) and parsley root doubled the soluble solids, an important quality differentiator in the food industry. Soluble solids of the products were similar to the contents in other smoothies studied by Di Cagno et al. (2011), Castillejo et al. (2016), and Rodríguez-Verástegui et al. (2016), i.e. around 13 °Bx for fruit smoothies and 8 °Bx for smoothies with vegetables.

The titratable acidity of all smoothies was significantly lower than SB 100% (2.54 g malic acid/100 g), especially in mixed products with vegetables (0.78 g malic acid/100 g for SB-carrot 25:75).

In terms of reduction of the titratable acidity, fruits can be ordered as follows: apricot < peach < apple < orange < grape < celery and parsley roots ≈ carrot ≈ pear. Thus, the titratable acidity of smoothies with 50% of sea buckthorn was between 1.40 g (SB-pear) and 2.41 g malic acid/100 g (SB-apricot), whereas with 25% of sea buckthorn it ranged from 0.78 g (SB-carrot) to 2.23 g malic acid/100 g (SB-apricot).

The ash content was found to be in the range from 0.26% (SB-pear 25:75) to 10.8% (SB-parsley root 25:75). The type of semi-product had a stronger effect on its content than the proportions in smoothies, which with vegetables had significantly higher ($p < 0.05$) ash content. The ash content in 100% SB was close to the average amount in smoothies containing only fruits (0.41%).

The content of pectin, which is one of the fractions of soluble dietary fiber, was also examined. As previous research on sea buckthorn cultivars proved, its fruits are poor in pectin (Tkacz et al., 2019b). SB 100% contained 0.61% pectin. However, it was found that the use of other vegetables and fruits, especially parsley and celery roots, carrot, pear, and apricot, can significantly enrich ($p < 0.05$) sea buckthorn-based products with pectin. In the case of SB-parsley, this increase was seven times (4.21% and 3.73% for 25:75 and 50:50 variants, respectively). Other smoothies in terms of pectin concentration can be ordered as follows: SB-pear > SB-celery root > SB-carrot > SB-apricot and SB-apple > SB-peach and SB-orange > SB-grape (2.09% to 0.21% pectin). Similarly, Nowicka, Wojdyło, and Samoticha (2016a) indicated peach fruit as a rich source of pectin in fruit smoothies. Moreover, products providing a minimum of 20% of the daily dietary fiber requirement (25 g per day for adults) are recognized as an excellent source of fiber, and red vegetable smoothies can cover up to 50% of this requirement (Castillejo et al., 2016). According to the U.S. Food and Drug Administration (FDA) recommendations, one portion (250 g) of SB-parsley root, SB-pear and SB-celery root can certainly be considered a good source of fiber.

3.2. Sugars, organic acid and their ratio in sea buckthorn-based smoothies

The next quality attributes were sugars and organic acids, the quantitative and qualitative determination of which are presented in Table 2. The total sugar content of smoothies ranged from 1.44 (SB-celery root 25:75) to 7.45 g/100 g (SB-grape 25:75). Only in the case of SB-celery root did the total sugar content not differ significantly ($p > 0.05$) with low sugar SB 100% (1.55 g/100 g). Other semi-products increased the sugar concentration in smoothies, which in this regard can be ranked as follow: SB-grape > SB-apple ≈ SB-pear > SB-orange > SB-parsley root > SB-peach > SB-carrot ≈ SB-apricot. Sea buckthorn berries are known for their low sugar content compared to other fruits (Kallio et al., 2009; Ma et al., 2020; Tiitinen et al., 2006; Tkacz et al., 2019b; Yang, 2009). Despite the significant increase in natural free sugars, most smoothies can be considered low-sugar because they contain a maximum of 5 g of total sugars per 100 g (Regulation (EC) no 1924/2006). The exceptions were products enriched with 75% addition of pear, grape and apple with average sugar level near the lower limit. This is relevant with regard to smoothies as potentially anti-diabetic pro-health products. The sugar profile can affect both sweetness and blood glucose level after smoothie consumption. Consequently, the glycemic index (GI), classifying products in terms of their effect on blood glucose increases, and the glycemic load (GL), determining how smoothies will affect postprandial glycemia, were calculated. Thus, SB-pear and SB-apple were considered products with low GI (27.2 to 46.6), which resulted from higher fructose concentrations with a lower GI than glucose or sucrose. However, all smoothies had a low GL, below 10 (between 1.3 and 5.0 for SB-celery root and SB-grape 25:75, respectively). Given the effect of fiber and organic acids on the reduction of postprandial glycemia, obtained smoothies can be considered important for people with type 2 diabetes and cardiovascular diseases (Izquierdo-Vega et al., 2020), especially since recent studies support dietary recommendations regarding the consumption of 100% fruit products, which is not associated with the risk of developing type 2 diabetes (Murphy, Barrett, Bresnahan, & Barraj, 2017).

In the smoothies with vegetables (carrot, celery and parsley roots) sugars were identified in decreasing concentration in the order glucose > fructose > sucrose > rhamnose, while those with fruits

Table 2
Contents of sugars (HPLC-ELSD) and organic acids (UPL-PDA) and sugar:organic acid ratios in sea buckthorn-based smoothies.

Smoothie	Ratio (*w/w*)	Sugars (g/100 g)				
		rhamnose	fructose	glucose	sucrose	total
SB-PEAR	25:75	0.01 ± 0.11b	3.60 ± 0.15b	0.80 ± 0.05 h	nd	6.17 ± 0.26b
	50:50	0.03 ± 0.01b	2.20 ± 0.12d	0.93 ± 0.07 g	nd	4.21 ± 0.14d
SB-APRICOT	25:75	0.01 ± 0.00b	0.89 ± 0.10 fg	1.55 ± 0.12e	0.24 ± 0.02c	2.79 ± 0.17f
	50:50	0.02 ± 0.01b	0.62 ± 0.11 g	1.45 ± 0.11ef	0.05 ± 0.02d	2.18 ± 0.10 g
SB-PEACH	25:75	0.01 ± 0.01b	1.06 ± 0.10f	1.49 ± 0.03e	0.44 ± 0.05b	3.01 ± 0.18f
	50:50	0.03 ± 0.00b	0.83 ± 0.05 fg	1.60 ± 0.02e	0.11 ± 0.02d	2.58 ± 0.15f
SB-ORANGE	25:75	0.02 ± 0.01b	1.70 ± 0.19e	2.30 ± 0.13c	0.36 ± 0.14bc	4.37 ± 0.21d
	50:50	0.04 ± 0.01b	1.15 ± 0.07f	2.06 ± 0.10d	0.09 ± 0.01d	3.34 ± 0.13e
SB-GRAPE	25:75	0.01 ± 0.00b	3.07 ± 0.18c	4.37 ± 0.21a	nd	7.45 ± 0.28a
	50:50	0.05 ± 0.01b	1.86 ± 0.09e	3.45 ± 0.16b	nd	5.35 ± 0.20c
SB-APPLE	25:75	0.01 ± 0.00b	4.76 ± 0.04a	1.58 ± 0.05e	0.24 ± 0.10c	6.62 ± 0.24b
	50:50	0.04 ± 0.01b	3.35 ± 0.20b	1.68 ± 0.05de	0.05 ± 0.02d	5.12 ± 0.20c
SB-CELERY ROOT	25:75	0.03 ± 0.01b	0.13 ± 0.09 h	1.23 ± 0.01f	0.04 ± 0.02d	1.44 ± 0.09 h
	50:50	0.02 ± 0.01b	0.19 ± 0.04 h	1.38 ± 0.08ef	nd	1.58 ± 0.13 h
SB-CARROT	25:75	0.01 ± 0.00b	0.80 ± 0.09 fg	1.64 ± 0.11de	0.49 ± 0.08b	2.95 ± 0.17f
	50:50	0.02 ± 0.00b	0.57 ± 0.05 g	1.55 ± 0.14e	0.09 ± 0.01d	2.24 ± 0.09 g
SB-PARSLEY ROOT	25:75	0.02 ± 0.01b	0.62 ± 0.11 g	1.85 ± 0.03d	1.07 ± 0.14a	3.56 ± 0.23e
	50:50	0.04 ± 0.01b	0.56 ± 0.13 g	1.88 ± 0.07d	0.23 ± 0.06c	2.71 ± 0.10f
SB 100%	–	0.08 ± 0.02a	0.03 ± 0.01i	1.40 ± 0.16ef	nd	1.55 ± 0.19 h

Smoothie	Organic acids (g/100 g)						Sugar:organic acid
	oxalic acid	citric acid	malic acid	quinic acid	shikimic acid	total	
SB-PEAR	0.03 ± 0.01gh	0.12 ± 0.02e	1.43 ± 0.07d	0.18 ± 0.02d	0.02 ± 0.01ab	1.77 ± 0.01f	3.5
	0.05 ± 0.01 g	0.18 ± 0.03e	3.78 ± 0.24a	0.49 ± 0.02b	0.03 ± 0.01a	4.53 ± 0.27a	0.9
SB-APRICOT	0.02 ± 0.01 h	1.48 ± 0.06a	1.30 ± 0.10d	0.20 ± 0.02d	nd	3.00 ± 0.12c	0.9
	0.02 ± 0.01 h	0.98 ± 0.03b	1.96 ± 0.09c	0.39 ± 0.02c	0.01 ± 0.01b	3.36 ± 0.21bc	0.6
SB-PEACH	0.02 ± 0.01 h	0.29 ± 0.02d	1.65 ± 0.12 cd	0.19 ± 0.02d	0.01 ± 0.01b	2.15 ± 0.13e	1.4
	0.01 ± 0.01 h	0.23 ± 0.02de	2.28 ± 0.22bc	0.44 ± 0.02bc	0.01 ± 0.01b	2.97 ± 0.16c	0.9
SB-ORANGE	0.02 ± 0.00 h	0.76 ± 0.08c	1.13 ± 0.09d	0.10 ± 0.02e	0.01 ± 0.01b	2.01 ± 0.10e	2.2
	0.02 ± 0.00 h	0.56 ± 0.07c	1.71 ± 0.15c	0.41 ± 0.02c	0.01 ± 0.01b	2.71 ± 0.10 cd	1.2
SB-GRAPE	0.05 ± 0.01 g	0.60 ± 0.09c	1.56 ± 0.16d	0.18 ± 0.02d	0.01 ± 0.01b	2.44 ± 0.18d	3.1
	0.06 ± 0.01 g	0.61 ± 0.05c	2.57 ± 0.08b	0.36 ± 0.02c	0.01 ± 0.01b	3.61 ± 0.12b	1.5
SB-APPLE	0.01 ± 0.01 h	0.03 ± 0.01f	1.86 ± 0.03c	0.22 ± 0.02d	0.01 ± 0.01b	2.13 ± 0.13e	3.1
	0.02 ± 0.00 h	0.12 ± 0.02e	2.59 ± 0.16b	0.51 ± 0.02b	0.01 ± 0.01b	3.25 ± 0.17bc	1.6
SB-CELERY ROOT	0.29 ± 0.08d	0.12 ± 0.02e	2.25 ± 0.25bc	0.24 ± 0.02d	nd	2.99 ± 0.11c	0.5
	0.42 ± 0.11c	0.13 ± 0.01e	1.10 ± 0.11d	0.34 ± 0.02c	nd	1.99 ± 0.15e	0.8
SB-CARROT	0.16 ± 0.01e	0.25 ± 0.05d	1.92 ± 0.15c	0.17 ± 0.02d	nd	2.64 ± 0.14d	1.1
	0.10 ± 0.01f	0.13 ± 0.01e	1.97 ± 0.08c	0.31 ± 0.02c	0.01 ± 0.01b	2.58 ± 0.09d	0.9
SB-PARSLEY ROOT	0.84 ± 0.13a	0.28 ± 0.02d	1.23 ± 0.11d	0.19 ± 0.02d	nd	2.54 ± 0.22d	1.4
	0.52 ± 0.01b	0.20 ± 0.03de	1.96 ± 0.10c	0.41 ± 0.02c	nd	3.08 ± 0.11c	0.9
SB 100%	0.03 ± 0.01gh	0.36 ± 0.07d	3.58 ± 0.20a	0.89 ± 0.02a	0.01 ± 0.01b	4.87 ± 0.31a	0.3

All data are mean of three measurements ± standard deviation. Values followed by the same letter, within the column, were significantly different ($p < 0.05$; Tukey's test); SB – sea buckthorn juice. The Table contains the main sugars and organic acids in smoothies. The sums of sugars consist of rhamnose, fructose, sorbitol, glucose, and sucrose. The sums of organic acids contain oxalic, citric, malic, quinic, shikimic, succinic, and maleic acids.

contained dominant glucose and fructose, as well as sucrose, sorbitol and/or rhamnose in quantities depending on semi-products. Glucose dominated in all smoothies with vegetables (up to 70% total sugars in SB-carrot and SB-parsley root 50:50) and with apricot, peach, orange and grapes (up to 65% total sugars for SB-grape 50:50). However, there were no significant differences ($p > 0.05$) in the glucose content between 100% SB and smoothies with apricot, peach, apple, celery root and carrot. In sea buckthorn juice, glucose accounted for 90% of sugars, which was in line with previous studies on berries (Ma et al., 2020; Tkacz et al., 2019b). Recent reports have shown the active role of the biochemical pathway converting glucose into its derivatives in some subspecies and cultivars of sea buckthorn. In the sugar fraction of sea buckthorn, ethyl β-D-glucopyranoside, correlated with bitterness, was identified as major sugar metabolite in *H. rhamnoides* ssp. *rhamnoides* (Yang, 2009). Berries also contain cyclitol derivatives from sugars, methyl inositol and l-quebrachitol, with confirmed biological and physiological effects, in the field of regulation of sugar metabolism, protection of cells against oxidative, mutagenic and cytotoxic damage, and signal transduction of cells (Kallio et al., 2009).

The concentration of rhamnose in smoothies was due to its presence in sea buckthorn berry juice (0.08 g/100 g) and averaged 0.02 g/100 g products. The use of pulp, especially fruity, enriched smoothies with fructose, and the most abundant in it were products with apples, followed by pear and grape (1.86 to 4.76 g/100 g). Fructose accounted for < 40% and 27% of the total sugars in other smoothies with fruits and vegetables, respectively. Research of Kolniak-Ostek (2016) proved that pear pulp contains up to 60% fructose compared to total sugars. The same studies reported a high concentration of sorbitol in pear, 13% of total sugars. Therefore, high sorbitol concentration was only found in SB-pear smoothies (28.5% and 24.8% of total sugars for 75:25 and 50:50 variants, respectively). Sorbitol in SB-apricot and SB-apple accounted for < 3.7% of total sugars (below 0.10 g/100 g), whereas in other smoothies this sugar alcohol was not detected.

Furthermore, the addition of semi-products (except pear and grape) enriched smoothies with sucrose, whose concentration was between 0.04 (SB-celery root 75:25) and 1.07 g/100 g (SB-parsley). In products with vegetables, sucrose constituted up to 30.1% (SB-parsley 25:75), and in fruit smoothies up to 14.8% (SB-peach 25:75). Sucrose accounts for about 60% of total sugars in peaches (Nowicka et al., 2019), and the effect of peach, as well as apricot purees, on the enrichment of fruit smoothies with sucrose was previously found in studies by Nowicka et al. (2016a).

The total organic acid content in smoothies was reduced compared to SB 100% (4.87 g/100 g) and ranged from 1.77 to 4.53 g/100 g (SB-pear 25:75 and 50:50, respectively) (Table 2). Smoothies with 50% fruits and vegetables contained more organic acids than in ratios 25:75, except SB-celery root and SB-carrot. The following organic acids were identified in decreasing concentrations: malic > citric ≈ quinic > oxalic > shikimic > succinic acid.

Smoothies with vegetables contained a higher concentration of oxalic acid (up to 33.1% total organic acids). The most abundant in citric acid were smoothies with apricot, followed by smoothies with orange and grape (16.9% to 49.3% total organic acids). At the same time, these products contained more citric acid than SB 100%. Most studies attribute the health promoting properties of vegetables and fruits, including sea buckthorn, to vitamins and phenolic compounds, and little attention is paid to organic acids in the context of therapeutic properties. Citric acid improves the bioavailability of minerals, including calcium, non-heme iron and zinc, prevents the formation of kidney stones, regulates the acid-base balance, and has anti-inflammatory, antioxidant and anticoagulant effects (Izquierdo-Vega et al., 2020).

Malic acid was dominant in smoothies and accounted for up to 87.3% of total organic acids (SB-apple 25:75). However, the addition of semi-products reduced its content in smoothies compared to SB 100% (3.58 g/100 g). No significant differences were found between malic acid content in smoothies with vegetables and fruit smoothies ($p > 0.05$). The extremely valuable properties of malic acid are its prebiotic properties, increasing peristalsis, stimulating the secretion of gastric juices and the development of non-pathogenic bacteria, while a higher concentration has a preservative effect and exhibits bacteriostatic and bactericidal properties (Hassaan, Soltan, Jarmołowicz, & Abdo, 2018; Izquierdo-Vega et al., 2020). This acid has been found to stimulate salivation and is clinically effective in the treatment of xerostomia, and has a significant cytotoxic and apoptotic effect in human keratinocyte cell lines (HaCaT) (Izquierdo-Vega et al., 2020).

Finally, in SB 100%, quinic acid constituted 18.3% of total organic acids, while in smoothies its content decreased significantly ($p < 0.05$) and ranged from 4.95% (SB-orange 25:75) to 16.4% of total organic acids (SB-celery root 50:50). Shikimic acid constituted mean 0.45% of total organic acids, and was identified only in SB 100%, smoothies with pear, peach, orange, grape, apple (both variants), and in SB-apricot and SB-carrot 50:50. In most smoothies and 100% SB, no succinic acid was identified. Only the addition of carrot, celery root and grape enriched products in it (max 0.15 g/100 g, which was 5.7% of total organic acids for SB-carrot 25:75). In SB 100% and smoothies with pear, orange and apple, trace amounts of maleic acid were identified (below 0.01 g/100 g).

Both the absolute content and the relative abundance of sugars and organic acids determine the key perception of taste and consumer acceptance of sea buckthorn products (Tiitinen et al., 2006; Yang, 2009). The low ratio of sugars to organic acids in SB 100% (0.3) causes a strong sensation of sour taste. Similarly, research on sea buckthorn cultivars showed a strong relationship between the sugar:organic acid ratio and acceptance assessment (Tang et al., 2001; Tiitinen et al., 2006). The addition of fruits and vegetables increased this ratio in all smoothies, mainly in 25:75 variants. Exceptions were smoothies with celery root due to having the lowest sugar content. The use of 75% pear, grape and apple resulted in the highest increase in sugar:organic acid ratio (to over 3.0). Interestingly, the same ratios were determined in SB-peach and SB-parsley root smoothies. A sugar:organic acid ratio above 1.0 was achieved in SB-orange, SB-grape, and SB-apple, as well in 25:75 variants of SB-peach, SB-carrot and SB-parsley root.

Moreover, the ratio moderately correlated with the perception of sour taste and the general acceptance of smoothies ($r = -0.48$ and 0.47, respectively). Although the sensation of sour taste correlated more strongly with sugar:organic acid ratio for fruit smoothies than those with vegetables ($r = -0.77$ and -0.49, respectively), the correlation between overall acceptance and the ratio was higher for smoothies with vegetables ($r = 0.66$). However, the study should take into account the marked variability in sugar accumulation pattern and reduction of organic acids during ripening and harvesting, trends which depend on the genetic background of sea buckthorn and different local climates (Yang, 2009).

3.3. Sensory analysis of sea buckthorn-based smoothies

Fig. 1 shows sensory analysis profiles of sea buckthorn-based smoothies including color, aroma, consistency, taste, acidity and foreign flavors, and overall acceptance on a 5-point scale. SB 100% was not accepted (score 1.3), due to its intensely acidic, astringent and bitter taste, followed by consistency. The obtained results were in line with the sensory assessment of *H. rhamnoides* berry juices of various cultivars (Ma et al., 2020; Tang et al., 2001; Tiitinen et al., 2006). However, the use of fruit and vegetable supplements significantly improved ($p < 0.05$) the overall acceptance of smoothies.

The analysis of smoothies clearly showed the attractiveness of their color depending on the orange tone. The color rating was more strongly determined by the type of semi-products than by their proportions. An increase in the color attractiveness compared to the SB 100% color was noted for smoothies with apricot and carrot (average 4.7 and 4.8 points, respectively). SB–celery root (both variants) and SB-parsley root 25:75

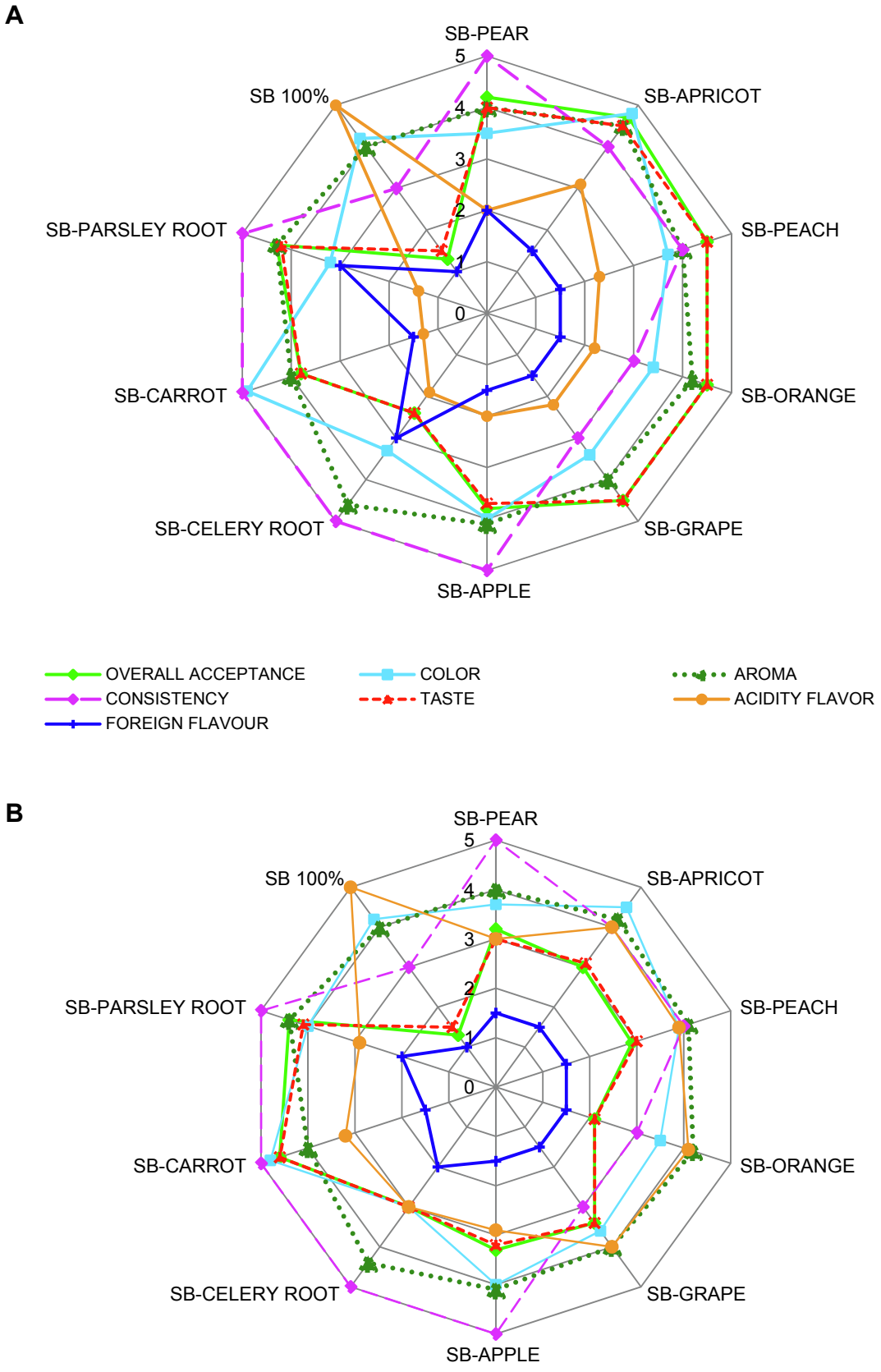


Fig. 1. Sensory analysis profiles of sea buckthorn-based smoothies with fruits and vegetables in ratios 25:75 (*w/w*) (A) and 50:50 (*w/w*) (B). SB – sea buckthorn juice; color, aroma, consistency, taste and overall acceptance ranged from very unattractive (1) to very attractive (5); sensations of acidity and foreign flavors were between undetectable (1) to very intense (5).

Table 3
Contents of phenolic compounds (UPLC-PDA-FL) in sea buckthorn-based smoothies.

Smoothie	Ratio (*w/w*)	Phenolic acids	Flavonols	Procyanidin polymers	DP
		mg/100 g			
SB-PEAR	25:75	7.40 ± 1.01d	31.89 ± 3.74 g	7.94 ± 1.86e	3.7
	50:50	6.98 ± 0.88d	58.77 ± 3.24c	4.01 ± 1.38hi	3.4
SB-APRICOT	25:75	2.17 ± 0.34f	38.44 ± 2.55f	4.44 ± 1.28 h	1.9
	50:50	2.05 ± 0.42f	62.08 ± 4.00bc	3.00 ± 1.96j	2.3
SB-PEACH	25:75	4.30 ± 0.52e	34.02 ± 3.72 g	18.31 ± 2.80a	3.2
	50:50	2.09 ± 0.32f	59.99 ± 3.48bc	8.29 ± 1.83e	2.9
SB-ORANGE	25:75	13.32 ± 0.61a	48.77 ± 3.10d	5.50 ± 1.17 g	4.4
	50:50	9.71 ± 0.94c	65.26 ± 2.88b	3.84 ± 1.55i	3.0
SB-GRAPE	25:75	1.98 ± 0.31f	32.64 ± 3.70 g	13.15 ± 2.51c	5.8
	50:50	2.21 ± 0.46f	59.33 ± 4.04bc	9.50 ± 1.36d	4.8
SB-APPLE	25:75	11.71 ± 1.14b	25.46 ± 2.53 h	16.07 ± 3.03b	3.1
	50:50	9.41 ± 1.64c	46.43 ± 3.63e	9.55 ± 2.11d	2.2
SB-CELERY ROOT	25:75	2.03 ± 0.30f	28.34 ± 3.22 h	1.33 ± 0.28 l	2.8
	50:50	1.12 ± 0.13 g	49.79 ± 3.33d	1.72 ± 0.68 k	2.4
SB-CARROT	25:75	9.68 ± 1.66c	30.77 ± 3.14 g	0.81 ± 0.70 *m*	1.7
	50:50	0.75 ± 0.09 h	51.28 ± 3.08d	1.61 ± 0.73 k	2.0
SB-PARSLEY ROOT	25:75	13.87 ± 1.82a	26.23 ± 2.04 h	6.27 ± 1.09f	4.7
	50:50	9.53 ± 0.10c	38.92 ± 3.12f	4.66 ± 1.93 h	4.9
SB 100%	–	2.01 ± 0.13f	95.13 ± 4.50a	2.92 ± 1.50j	1.9

All data are mean of three measurements ± standard deviation. Values followed by the same letter, within the column, were significantly different ($p < 0.05$; Tukey's test); SB – sea buckthorn juice; DP- mean degree of polymerization.

were rated the lowest in terms of color (average score 3.2).

The aroma of all smoothies was considered as attractive, and the highest scores were given to products with a 25% share of celery root and apricot (4.6 and 4.5, respectively). The use of pear, orange, grape and apple did not significantly change ($p > 0.05$) the aroma rate in relation to SB 100% (score 4.0). The results obtained were in line with research of Nowicka, Wojdyło, Teleszko, and Samoticha (2016b), in which aroma of apple and pear juices was highly rated, and their use as ingredients for smoothies with cherry puree positively affected the aroma.

The consistency of the smoothies depended on the semi-products, but not their proportions. The most attractive consistency was obtained for products from pear, apple, carrot, celery and parsley roots (mean 5.0 points). Sea buckthorn juice tends to separate into a fat phase, proper juice and sediment (Beveridge, Harrison, & Drover, 2002); hence the consistency was not rated as attractive (score 3.0). An improvement in consistency relative to SB 100% was not found for SB-orange and SB-grape. Changes in color, aroma and consistency had the least impact on the overall acceptance of smoothies (negligible correlations). Color and intensity of aroma – contrary to sweetness – also slightly affected pleasantness ratings of juices from berries of six sea buckthorn genotypes (Tang et al., 2001). However, the overall assessment should take into account the impact of complex product matrices, and interaction of the attributes analyzed, including taste–aroma interaction (Ma et al., 2020).

Finally, a strong advantage of attractive taste of the smoothies over sea buckthorn juice was established. The taste experience determined the overall assessment of the products ($r = 0.99$). Smoothies with 75% fruits were rated the highest (3.7 for SB-apple to 4.6 for SB-apricot and SB-peach). In turn, compositions of sea buckthorn juice and vegetable pulp in a 50:50 ratio were more favorable in taste than proportions of 25:75. Although previous research has shown that apple juice perfectly blends with other fruits, and the taste of smoothies with it was highly rated (Nowicka et al., 2016b; Teleszko & Wojdyło, 2014), SB-apple smoothies were the least attractive in taste (3.0 points) compared to products with other fruits.

Acid taste is the most intense and short-lived (Obrist et al., 2014), and is therefore a key differentiator in taste evaluation during sensory analysis of new products. As expected, the perception of acidity was less intense in smoothies with 25% sea buckthorn content (1.3 for SB-carrot to 3.1 for SB-apricot). The lower the feeling of acidity, the higher the overall rating of smoothies ($r = -0.60$). The vegetables used – carrot, celery and parsley roots – turned out to be the most beneficial to alleviate the sourness of sea buckthorn (≤ 1.9 and ≤ 3.2 points for 25:75 and 50:50 variants, respectively). Apricot, orange and grapes in amounts of 50% reduced the sour taste intensity by only 1 unit. In turn, in studies on sea buckthorn-based jellies, 50% grape significantly improved the taste and overall acceptance of jelly (Selvamuthukumaran et al., 2007).

Foreign flavors in the smoothies were unambiguously very weak (mainly 1.5 points), except for products with 75% celery and parsley roots (average score 3.0).

3.4. Phenolic compounds of sea buckthorn-based smoothies

3.4.1. Phenolic acids

One of the analyzed groups of phenolic compounds was phenolic acids, whose contents in smoothies are listed in Table 3. Phenolic acids were measured in a wide range from 0.75 (SB-carrot 25:75) to 13.87 mg/100 g (SB-parsley root 25:75). Sea buckthorn juice contained only 2.01 mg of phenolic acids, and therefore the addition of pear, peach, apple, orange, carrot and parsley root significantly ($p < 0.05$) enriched smoothies with phenolic acids (two to seven times). This was in line with previous studies that identified the same fruits and vegetables, as well as apricot, as dietary sources of hydroxycinnamic acids (Naczk & Shahidi, 2006). The enrichment of sea buckthorn-based smoothies with phenolic acids is all the more beneficial because these compounds are well-known agents with proven hepatoprotective, immunomodulatory, antioxidant, anti-inflammatory, anti-diabetic, anticancer, anti-atherosclerotic, antihypertensive, anti-mutagenic, antiparasitic and antimicrobial activity (Izquierdo-Vega et al., 2020).

Smoothies with 75% addition of these semi-products can be ordered by decreasing phenolic acid content: SB-parsley root ≈ SB-orange > SB-apple > SB-carrot > SB-pear > SB-peach. Additionally, 50:50 variants of SB-orange, SB-apple and SB-parsley root did not differ significantly ($p > 0.05$) in the content of phenolic acids, on average 9.55 mg/100 g. In turn, products with apricot, grape and celery root did not differ significantly ($p > 0.05$) compared to SB 100%. In SB-apple (both variants), SB-orange (75:25) and SB-carrot (50:50), phenolic acids constituted over 10% of the total phenolic

compounds, while in other smoothies they constituted on average 3.4%.

3.4.2. Flavonols

The type of semi-products strongly modulated the flavonol content of smoothies (Table 3). Sea buckthorn berries are a good source of flavonols, as previously studied in various cultivars (Tkacz et al., 2020). Accordingly, SB 100% contained the largest amount of flavonols from the samples tested, 95.13 mg/100 g. As expected, smoothies with a smaller addition of other fruits and vegetables (50:50 variant) had a higher concentration of flavonols, between 38.92 and 65.26 mg/100 g (SB-parsley root and SB-orange, respectively).

The most abundant in flavonols were smoothies with orange, followed by apricot, peach, and grape (on average 60 mg/100 g). By contrast, the use of vegetables and apples was the least favorable with regard to flavonol concentration. The results obtained were in accordance with the research of Tsanova-Savova and Ribarova (2013), which proved that carrot, celery and parsley roots did not contain flavonols, but are a rich source of flavones in the diet.

An average three-fold lower concentration of flavonols compared to 100% SB was measured in products with 25% sea buckthorn juice, except SB-orange. Among them, SB-pear, SB-peach, SB-grape, and SB-carrot did not differ significantly in flavonol contents (mean 32 mg/100 g), nor did SB-apple, SB-celery root and SB-parsley root, which were the least abundant in flavonols (mean 27 mg/100 g).

Furthermore, flavonols were the dominant phenolic compounds in all smoothies. In previously studied fruit smoothies (Nowicka et al., 2016a; Teleszko & Wojdyło, 2014), flavonols were the smallest group of phenolic compounds. But *Prunus* fruits, among them peach and apricot, were found not rich in these compounds, which was in line with the results obtained.

3.4.3. Procyanidin polymers

The contents of procyanidin polymers in smoothies were determined by direct phloroglucinolysis methods (Table 3). Sea buckthorn juice contained a relatively low concentration of procyanidins, 2.92 mg/100 g. However, the use of fruits and vegetables increased their concentration in all smoothies, except for SB-celery root and SB-carrot. The other products in the 25:75 variant contained 4.44 to 18.31 mg of procyanidin polymers/100 g and can be ranked according to decreasing concentration: SB-peach > SB-apple > SB-grape > SB-pear > SB-parsley root > SB-orange > SB-apricot. The results obtained were in line with the naturally high content of these polymeric flavonoids in apple, grape, peach, and pear (Naczk & Shahidi, 2006). For instance, procyanidins accounted for about 50% in apple and pear juices (Nowicka et al., 2016b), 60% in pear pulp (Kolniak-Ostek, 2016), on average 85% in peach (Nowicka, Wojdyło, & Laskowski, 2018), and as much as 99% of total phenolic compounds in flesh of white grapes (Tkacz, Wojdyło, Nowicka, Turkiewicz, & Golis, 2019a).

In a study on sea buckthorn, Ma et al. (2020) found large variation in procyanidin concentrations, from 74.3 to 221 mg/100 g of fresh berries, depending on the cultivars. The same research team also found a strong relationship between mouth-drying astringency and total proanthocyanidins. However, mainly low-molecular procyanidins are responsible for bitterness and astringency (Tkacz et al., 2019a). As this study targeted smoothies with good sensory acceptance, and the amount of procyanidins increased, the degree of polymerization (DP) was determined. Interestingly, the DP for SB 100% was the lowest, like for SB-apricot and SB-carrot. For other products, DP increased from an average of 1.5 times (SB-celery root, SB-apple, SB-peach) to three times in the case of SB-grape. The overall acceptance of smoothies moderately correlated with the content of procyanidins ($r = 0.35$), and with their DP ($r = 0.40$). Moreover, flavonols strongly correlated with the general acceptance of products ($r = -0.68$), which was in line with the studies of Ma et al. (2020) indicating some isorhamnetin derivatives as factors for the undesirable astringency of sea buckthorn fruits.

As in the previous studies on fruit and vegetable-based smoothies (Agbenorhevi & Marshall, 2012; Baiano et al., 2012; Kuntz et al., 2015; Teleszko & Wojdyło, 2014), the content of phenolic compounds was attributed to their ingredients, and smoothies based on purees and pulps, especially with berries, were richer in these compounds than juices. It is important to consider the large variety of other phenolic compounds in semi-products. Sea buckthorn-based smoothies with high concentrations of flavonols, mainly isorhamnetin and quercetin glycosides (Tkacz et al., 2020), can also be enriched with flavones such as apigenin, luteolin and diosmetin glycosides (orange, parsley and celery), flavanones such as hesperetin and naringenin derivatives

Table 4
Anti-oxidant capacity, anti-cholinesterase (anti-AChE and anti-BChE), anti-diabetic (anti-α-amylase, anti-α-glucosidase and anti-lipase) activities of sea buckthorn-based smoothies.

Smoothie	Ratio (*w/w*)	Antioxidant capacity (mmol TE/100 g)		Enzyme inhibitory activities (% inhibition)				
		FRAP	ORAC	anti-AChE	anti-BuChE	anti-α-amylase	anti-α-glucosidase	anti-lipase
SB-PEAR	25:75	7.31 ± 0.18a	3.27 ± 0.19hi	47.57 ± 1.33ab	26.21 ± 0.95f	13.67 ± 0.57 g	63.77 ± 1.89f	89.71 ± 0.33b
	50:50	3.38 ± 0.05d	3.60 ± 0.12 h	41.06 ± 1.11b	46.31 ± 0.31b	25.17 ± 1.34e	60.48 g ± 0.61	82.84 ± 1.58c
SB-APRICOT	25:75	2.25 ± 0.02e	1.51 ± 0.10j	27.80 ± 1.86d	49.73 ± 1.54ab	41.61 ± 1.24c	90.59 ± 1.01b	50.80 ± 1.80 g
	50:50	2.40 ± 0.05e	3.68 ± 0.18 h	28.92 ± 1.34d	51.41 ± 1.58ab	46.71 ± 1.07b	90.92 ± 0.72b	58.20 ± 1.46f
SB-PEACH	25:75	3.74 ± 0.04d	7.66 ± 0.29e	49.12 ± 2.01a	37.85 ± 1.36de	20.66 ± 1.40f	60.29 ± 0.96 g	96.31 ± 1.57a
	50:50	2.86 ± 0.12e	2.66 ± 0.31i	35.92 ± 1.30c	51.38 ± 1.88ab	20.78 ± 0.93f	82.21 ± 1.42c	81.21 ± 0.96c
SB-ORANGE	25:75	4.48 ± 0.57c	2.28 ± 0.69i	24.39 ± 1.83de	40.35 ± 0.90 cd	20.53 ± 1.52f	63.45 ± 1.55f	79.44 ± 1.73c
	50:50	6.53 ± 0.23b	3.90 ± 0.29 h	44.73 ± 0.97b	47.95 ± 1.47b	32.47 ± 1.81d	86.68 ± 1.09b	87.15 ± 1.01b
SB-GRAPE	25:75	2.09 ± 0.03f	4.52 ± 0.74 g	46.27 ± 2.01ab	43.51 ± 1.64c	24.25 ± 1.27e	83.27 ± 0.68c	74.59 ± 1.50d
	50:50	2.34 ± 0.01ef	5.34 ± 0.22f	42.33 ± 1.75b	47.64 ± 0.49b	32.89 ± 1.30d	90.86 ± 1.27b	63.63 ± 0.68e
SB-APPLE	25:75	2.78 ± 0.05e	9.97 ± 0.47c	34.99 ± 1.88c	34.84 ± 1.20e	26.16 ± 1.94e	56.83 ± 1.00 h	82.60 ± 1.94c
	50:50	2.61 ± 0.03e	15.09 ± 0.48ab	47.98 ± 1.30ab	44.00 ± 2.44c	48.96 ± 0.53ab	79.79 ± 0.99d	82.19 ± 1.40c
SB-CELERY ROOT	25:75	2.22 ± 0.06e	4.36 ± 0.42gh	9.26 ± 1.31 g	39.37 ± 2.35d	21.56 ± 1.24f	26.21 ± 1.20j	57.86 ± 1.15f
	50:50	2.48 ± 0.03e	7.59 ± 0.11e	34.64 ± 2.07c	48.86 ± 1.57b	21.09 ± 1.40f	6.12 ± 0.33 l	58.57 ± 2.05f
SB-CARROT	25:75	3.06 ± 0.05de	8.61 ± 0.18d	16.45 ± 1.44f	18.65 ± 1.99 g	20.03 ± 2.18f	17.01 ± 0.81 k	56.18 ± 1.66f
	50:50	2.53 ± 0.04e	14.04 ± 0.83b	21.46 ± 2.05e	39.43 ± 0.84d	26.46 ± 1.70e	74.53 ± 1.62e	57.66 ± 1.23f
SB-PARSLEY	25:75	2.14 ± 0.10f	15.70 ± 1.50a	40.62 ± 1.73b	15.39 ± 1.35 h	26.99 ± 1.65e	47.69 ± 1.73i	85.83 ± 0.72bc
ROOT	50:50	2.03 ± 0.15f	15.93 ± 0.94a	13.73 ± 1.86f	39.88 ± 1.81d	33.18 ± 1.22d	87.79 ± 1.40b	83.94 ± 2.75bc
SB 100%	–	2.89 ± 0.03e	14.69 ± 1.19b	14.72 ± 1.20f	53.41 ± 1.10a	49.82 ± 0.29a	98.61 ± 1.39a	96.14 ± 2.00a

All data are mean of three measurements ± standard deviation. Values followed by the same letter, within the column, were significantly different ($p < 0.05$; Tukey's test); SB – sea buckthorn juice; TE – Trolox; AChE – acetylcholinesterase; BuChE – butylcholinesterase; % inhibition – the percentage of inhibition at the concentration 0.25 g/mL for anti-AChE, anti-BuChE, anti-α-amylase and anti-α-glucosidase activities, and 30 mg/mL for anti-lipase activity.

(orange), stilbenes (grape), as well as chalcones (apple, orange) (Bresciani et al., 2015; Naczk & Shahidi, 2006).

The study showed that one portion of smoothies (100 g) can provide 31.70 to 62.56 mg of total phenolic compounds for smoothies with vegetables, and 45.04 to 78.82 mg/100 g for fruit smoothies. According to previous reports, 100 g of green smoothies contained 15.1 mg of phenolic compounds (Castillejo et al., 2017), fruit and vegetable purple smoothies contained 26.7 mg (González-Tejedor et al., 2017), whilst red vegetable smoothies contained up to 46.2 mg (Rodríguez-Verástegui et al., 2016). Greater diversity was found for fruit smoothies, including apple, quince, pear, chokeberry, blackcurrant, cherry, flowering quince, and berries and tropical fruits. The phenolic compounds in these smoothies ranged between 0.2 and 729 mg/100 g or 100 mL (Agbenorhevi & Marshall, 2012; Nowicka et al., 2016b; Teleszko & Wojdyło, 2014).

3.5. Biological activities of sea buckthorn-based smoothies

3.5.1. Antioxidant capacity

Antioxidant potential of smoothies was assessed as ferric reducing ability and oxygen radical absorbance capacity, and the results are summarized in Table 4. The ferric reducing ability for SB 100% was 2.89 mmol TE/100 g. However, compositions with pear, peach and orange favorably increased antioxidant activity from 3.38 mmol to 7.31 mmol TE/100 g. The use of other fruits (apricot, grape, apple) and vegetables (carrot, celery and parsley roots) did not significantly affect ($p > 0.05$) ferric reducing ability of smoothies compared to SB 100%. Oxygen radical absorbance capacity was higher than FRAP for smoothies with vegetables, apple, grape and peach. Only SB-apple and SB-parsley root were more active than SB 100% (14.69 mmol TE/100 g). For other products, the antioxidant activity ranged from 1.51 mmol to 14.04 mmol TE/100 g in the following order: SB-apricot < SB-orange < SB-pear < SB-celery < SB-grape < SB-peach < SB-carrot. Antioxidant capacity of sea buckthorn-based smoothies was similar to the average activity of previously tested smoothies (1.3 mg to 59.1 mg TE/100 g) composed of different combinations of vegetables (cucumber, broccoli, spinach, beet, tomato, red pepper, carrot) and fruits (grape, sour cherry, apricot, peach, plum) (Castillejo et al., 2017; González-Tejedor et al., 2017; Nowicka et al., 2016a; Rodríguez-Verástegui et al., 2016).

Antioxidant capacity tested by the ORAC method of SB 100% was five times higher than FRAP, as was the case with berries of different cultivars (7 to 12 times higher ORAC) (Tkacz et al., 2019b). Additionally, the study found low correlations between antioxidant activity and each group of phenolic compounds. Opposite results were obtained by González-Tejedor et al. (2017), who found that the FRAP method best reflected the concentration of antioxidant compounds in purple smoothie made from grape, cucumber, beet, and broccoli. On the other hand, DPPH activity showed a low correlation with bioactive compounds. Additionally, studies on fruit and vegetable smoothies (Keenan et al., 2010; Rodríguez-Verástegui et al., 2016) proved three times higher FRAP than DPPH. In turn, the correlation of ORAC and phenolic compounds, including their groups, was higher than for ABTS in *Prunus*-fruit smoothies (Nowicka et al., 2016a). Teleszko and Wojdyło (2014) did not find correlations for ABTS with phenolic acids and flavonols in smoothies based on pome and berry fruits. The antioxidant activity of phenolic compounds may be based on their reducing properties related to the direct inactivation of reactive radicals. For example, the reaction with peroxyl radical requires a concerted transfer of hydrogen cation from phenol to $ROO^{\bullet}$, and thus the formation of a transition state of an H−O bond with electron. In addition, polyphenols are able to indirectly reduce the effect of oxidants by chelating transition metals, regenerating other antioxidants (e.g. carotenoids, tocopherols), and inhibiting the activity or expression of free radical-producing enzymes or by enhancing antioxidant enzymes. However, *in vitro* antioxidant compounds do not necessarily retain their *iv vivo* radical reduction properties due to the fact that these are second order reactions, free radicals spread easily, and some have short life spans (Santos-Sánchez, Salas-Coronado, Villanueva-Cañongo, & Hernández-Carlos, 2019). Bearing in mind the heterogeneity found, the method of antioxidant activity measurement should be adapted to each of the smoothies, especially since various types were analyzed. For example, flavonols of smoothies in the 50:50 variant strongly correlated with FRAP ($r = 0.66$), while for 25:75 variants the correlation was negligible. It is known that synergistic and antagonistic interactions occur between phenolic compounds that can explain the results of antioxidant activity for smoothies tested (Hidalgo, Sánchez-Moreno, & de Pascual-Teresa, 2010).

3.5.2. Anti-cholinesterase activity

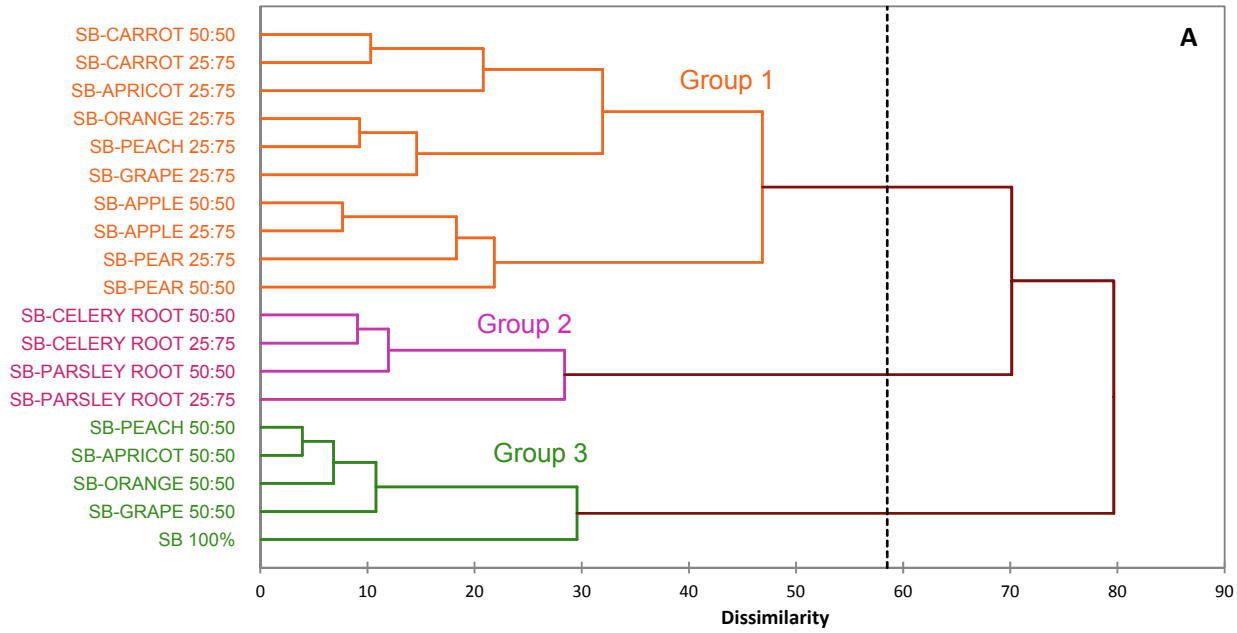
Anti-aging activity was analyzed as the ability to inhibit acetylcholinesterase and butylcholinesterase, and the results as % inhibition are summarized in Table 4. Higher activity towards both enzymes was determined for fruit smoothies than those with vegetables. It was found that the mixing of sea buckthorn juice with other semi-products can significantly increase ($p < 0.05$) anti-AChE activity compared to SB 100% (14.72%). The inhibition efficiency depended on the type of blend, with the highest potential for 25:75 variants of SB-peach, SB-pear and SB-grape (46.26% to 49.12%). In turn, anti-AChE activity was twice as low in the case of SB-orange and SB-carrot.

BuChE inhibition activity was higher for smoothies in 50:50 variants, conversely to AChE. Although SB 100% strongly inhibited BuChE (53.41%), addition of other fruits and celery root reduced the activity of smoothies by no more than 10%. This may indicate the synergistic effect of bioactive compound complexes of semi-products, not just phenolic compounds, as was found in research on functional beverages Nanasombat et al. (2015). The lowest anti-BuChE activity was determined in smoothies from carrot and parsley root (15.39% to 39.88%). The results obtained were in line with research of Szwajgier and Borowiec (2012), which indicated peach and apple juices and celery root extract as a source of anti-cholinesterase inhibitors with the potential to restore cognitive function and improve memory.

The diverse anti-cholinesterase potential of smoothies can be explained by the Pearson's correlation found between its phenolic compounds and the effects: anti-AChE with polymeric procyanidins ($r = 0.68$), anti-BuChE with flavonols and total phenolic compounds ($r = 0.70$ and 0.62, respectively). Despite the fact that AChE and BuChE have many structural similarities, the different inhibitory effects of phenolic compounds are associated with slight differences in the structure of the enzymes, e.g. active site, oxyanion hol. Phenolic compounds interact with the amino acid residues defining the active site through hydrogen bond, $\pi - \pi$ and hydrophobic interactions. It is known that the methoxy and hydroxyl groups in bioactive compounds can enhance the enzyme inhibitory effect due to stronger binding capacity. However, the issue of docking phenolic compounds with AChE and BuChE is still poorly researched and explained, and the differences are seen in the degree of affinity of the inhibitors to enzyme molecules. Nevertheless, studies on the neuroprotection of phenolic compounds indicate a higher potential of phenolic acids and flavonols (including ferulic acid, *p*-coumaric acid and quercetin present in sea buckthorn) than flavan-3-ols (Jabir, Khan, & Tabrez, 2018; Szwajgier, 2015).

3.5.3. Anti-α-amylase, anti-α-glucosidase and anti-lipase activities

α-Amylase, α-glucosidase and pancreatic lipase inhibition effects for smoothies are shown as % inhibition in Table 4. All smoothies were able to inhibit α-amylase and α-glucosidase, but the effect towards the latter was significantly stronger ($p < 0.05$). The correlation coefficient ($r = 0.61$) showed a strong relationship between these activities. SB 100% had the highest anti-α-amylase and anti-α-glucosidase activity (49.82% and 98.61%, respectively); therefore smoothies in the 50:50 variant were more active. SB-apple, followed by SB-apricot, SB-grape, SB-orange and SB-parsley root, inhibited α-amylase most strongly (25%



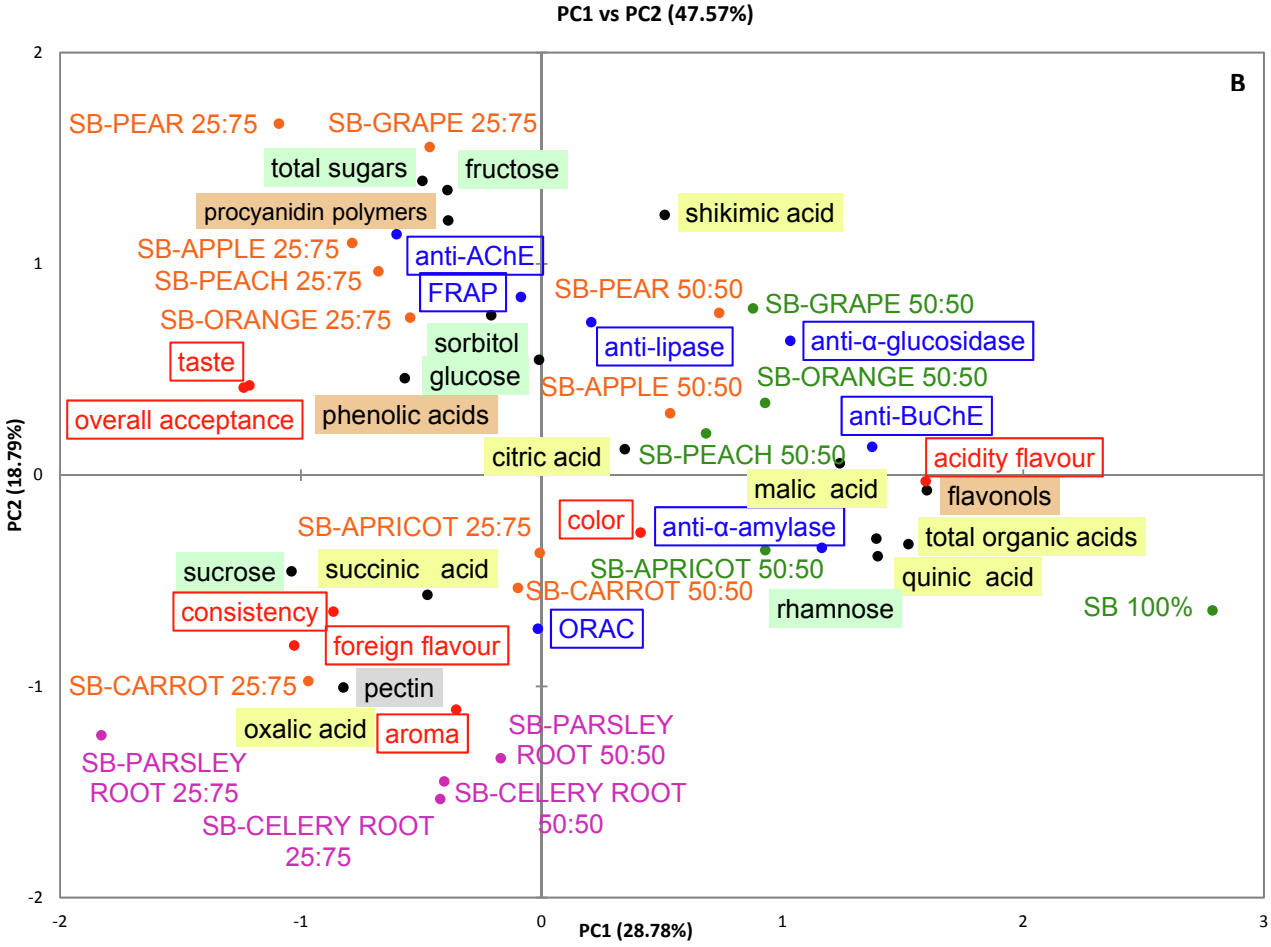


Fig. 2. Dendrogram of Agglomerative Hierarchical Clustering (AHC) (A) and biplot of Principal Component Analysis (PCA) (B) representing dissimilarity relationships of sensory attributes, chemical compounds and biological activities among sea buckthorn-based smoothies.

to 48%). Smoothies with celery root, carrot, pear, and peach were on average twice as weak α-amylase inhibitors. Strong α-glucosidase inhibitory activity was found for blends of sea buckthorn juice with apricot, orange, grape, and parsley root (86.68% to 90.92%). In contrast, SB-pear, SB-celery root and SB-carrot showed significantly lower inhibition ($p < 0.05$) of α-glucosidase.

SB 100% also indicated the strongest inhibition towards pancreatic lipase. Previous studies confirmed the potential of sea buckthorn as an anti-diabetic agent (Tkacz et al., 2019b; Xue et al., 2015). Lipase inhibition activity depended more on the type of semi-products than their percentage share in smoothies. The strongest inhibitors were products with peach, followed by pear, orange, parsley root, and apple. Similar to the anti-hyperglycemic potential, smoothies with celery root and carrot showed the weakest lipase inhibition (mean 57.15%).

Correlations between inhibition of α-amylase and α-glucosidase, and flavonol content were found ($r = 0.54$ and 0.50, respectively). In turn, the anti-lipase effect correlated more strongly with polymeric procyanidins ($r = 0.50$). No relationship was revealed for phenolic acids with anti-α-amylase and anti-α-glucosidase effects; and for anti-lipase activity the correlation was low ($r = 0.39$). Studies on *in vitro* and animal models indicated that the anti-diabetic perspective associated with flavonoids results from their modulating effects on carbohydrate metabolism, improvement of pancreatic β-cell function and insulin action, reduction of insulin resistance, inflammation and oxidative stress in muscles, and reduction of cholesterol synthesis and triglyceride levels (Vinayagam & Xu, 2015). Antidiabetic activity is related to the molecular mechanisms of phenolic compounds in pathways of AMP-activated protein kinase, beta cell apoptosis, glucose transporter, hepatic enzymes, NF kappa B, peroxisome proliferator-activated receptor, tyrosine kinase inhibitor. Additionally, their activity is associated with hydroxyl groups and α, β ketones. Despite a number of positive studies of the antidiabetic effects of polyphenols, the mechanisms of action and side effects have not been thoroughly investigated (AL-Ishaq, Abotaleb, Kubatka, Kajo, & Büsselberg, 2019). Thus, developed sea buckthorn smoothies can have a high potential to protect against diabetes, obesity and their complications.

3.6. Agglomerative hierarchical clustering (AHC) and principal components analysis (PCA)

Agglomerative hierarchical clustering and principal component analysis were conducted to summarize the dissimilarity relationships of sensory properties, chemical compounds and biological activities among sea buckthorn-based smoothies. The dendrogram of AHC and the biplot of PCA are shown in Fig. 2. The binary clustering tree (Fig. 2A) explicitly shows the dissimilarity between smoothies with vegetables and smoothies with 50% and 75% of fruits. The largest and the most diverse group, Group 1, contains SB-carrot, SB-apple and SB-pear in both variants, and SB-apricot, SB-orange, SB-peach, SB-grape only in the ratio 25:75. Group 2 was the most homogeneous and included smoothies with celery and parsley roots, while Group 3 concentrated SB 100% and other fruit smoothies in 50:50 variants. According to the biplot (Fig. 2B), Group 1 was characterized by the highest overall acceptance and taste ratings, which was closely related to the sugar content, except for rhamnose. Strong anti-lipase, anti-AChE and antioxidant capacity of these smoothies correlated with phenolic acids and procyanidin polymers. Group 2 was more strongly associated with foreign flavors, aroma, and consistency scores in correlation with pectin, succinic acid, oxalic acid, and sucrose. Smoothies in Group 3 with high anti-hyperglycemic and anti-BuChE potential were abundant in flavonols and organic acids. However, the strongly acidic flavor caused their low consumer acceptance. These products were most similar to SB 100%. Previous studies on fruit smoothies revealed that antioxidant activity and polyphenol content were not correlated with consumer evaluation scores (Nowicka et al., 2016b; Teleszko & Wojdyło, 2014). However, this study proved that selected fruit and vegetable compositions with sea buckthorn juices provide high health potential with high consumer acceptance.

4. Conclusions

The study provides comprehensive insight into the subject of chemical composition (sugar, organic acid, phenolic compound contents and basic chemical properties), biological potential (antioxidant, anti-neurodegenerative, anti-diabetic effects), as well as consumer sensory evaluation for eighteen novel sea buckthorn-based smoothies. The results obtained lead to the following key conclusions:

(1) Composing sea buckthorn juice with fruits and vegetables can significantly influence the sensory qualities, including reducing the acidity by increasing sugar:organic acid ratio, as well as improve the attractiveness of taste, color and aroma. The most favorable were blends of sea buckthorn juice with 75% fruits and 50% vegetables, especially SB-apricot and SB-carrot. Additionally, analysis of volatile aroma compounds may be interesting for future research into sea buckthorn-based products.
(2) The use of sea buckthorn berries enriches the products in flavonols, while other fruits and vegetables provide significant amounts of phenolic acids (pear, peach, orange, apple, carrot, parsley root) and procyanidin polymers (mainly peach, grape, apple).
(3) FRAP activity increased significantly for smoothies with pear, orange and peach, while ORAC increased significantly for SB-apple, SB-carrot and SB-parsley. The anti-BuChE effect of smoothies was higher than towards AChE, but the addition of all fruits and vegetables improved the ability to inhibit this second enzyme. Products from apricot, orange, grape and parsley root were stronger inhibitors of both α-amylase and α-glucosidase. Over 50% lipase inhibition by all smoothies was determined. Thus, the developed smoothies can be considered as supplements to the diet, with potential anti-aging, anti-diabetic and antioxidant properties.
(4) Sea buckthorn-based smoothies with apricot, peach, orange and grape were the most attractive in terms of sensory properties and the content of biologically active compounds, and thus antioxidant and anti-enzymatic effects.
(5) The results will be useful in the future design of innovative products with good sensory values and at the same time health-promoting potential using sea buckthorn berries – still underrated by the food industry. They can also direct further *in vivo* studies for non-communicable diseases.

5. Ethic statement

Research did not include any human subjects and animal experiments.

CRediT authorship contribution statement

Karolina Tkacz: Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Aneta Wojdyło:** Supervision, Conceptualization, Writing - original draft, Writing - review & editing, Resources, Funding acquisition. **Igor Piotr Turkiewicz:** Formal analysis. **Paulina Nowicka:** Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Oświadczam, że jestem współautorem publikacji pt.:

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Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, pozyskaniu materiału badawczego i wykonaniu produktów na bazie owoców rokitnika pospolitego zgodnie z założoną technologią, zbadaniu podstawowych właściwości chemicznych, analizie związków fenolowych metodą UPLC-PDA, przeprowadzeniu panelu sensorycznego oraz określeniu aktywności biologicznych *in vitro*. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w opracowaniu technologii produkcji produktów na bazie owoców rokitnika pospolitego, analizie związków fenolowych metodą UPLC-PDA, panelu sensorycznym oraz określeniu aktywności biologicznych *in vitro*. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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Paulina Nowicka

Podpis składającego oświadczenie

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Influence Carrier Agents, Drying Methods, Storage Time on Physico-Chemical Properties and Bioactive Potential of Encapsulated Sea Buckthorn Juice Powders

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Abstract: Sea buckthorn (*Hippophaë rhamnoides* L.) juice with inulin, maltodextrin, and inulin:maltodextrin (1:2 and 2:1) were spray-, freeze- and vacuum-dried at 50, 70 and 90 °C. The study aimed to assess the impact of drying methods and carrier agents on physical properties (moisture content, water activity, true and bulk density, porosity, color parameters, browning index), chemical components (hydroxymethylfurfural and phenolic compounds) and antioxidant capacity of sea buckthorn juice powders. Storage of powders was carried out for six months. Inulin caused stronger water retention in powders than maltodextrin. Vacuum drying provided powders with the highest bulk density. Maltodextrin did not promote browning and HMF formation as strongly as inulin. More phenolic compounds were found in powders with maltodextrin. Storage increased the antioxidant capacity of powders. The results obtained will be useful in optimizing the powders production on an industrial scale, designing attractive food ingredients.

Keywords: *Hippophaë rhamnoides* L.; inulin; maltodextrin; spray drying; freeze drying; vacuum drying; flavonols; HMF

1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) belongs to the Elaeagnaceae family and occurs mainly in the northern hemisphere. The high content of flavonols, L-ascorbic acid and lipophilic compounds including carotenoids, tocopherols, fatty acids and phytosterols provides unique health-promoting properties and thus enables a wide range of applications of this plant [1,2]. Juices, beverages, jams, oils, teas, pharmaceuticals, cosmetics, dairy and spirits as well as feedstuff are produced from sea buckthorn fruits, leaves, bark and seeds. To date, anti-radical activity, protection against UV radiation, efficacy in dermatological diseases, cardioprotective, hepatoprotective, anti-inflammatory, anti-hyperlipidemic, anti-cholinergic, anti-hypertensive, anti-hyperinsulinemia and antimicrobial properties have been studied [2,3]. Due to the high fat content, liquid and semi-liquid products from sea buckthorn separate into two phases and thus are not attractive to consumers. An alternative can therefore be the process of juice encapsulation using drying methods leading to the formation of powders. However, drying pure fruit juices is hindered due to agglomeration of material particles and adhesion to the surface of dryer

installations. Fruit juices, including sea buckthorn, contain organic acids and sugars with a low glass transition temperature (Tg). Therefore, the high-molecular weight carrier agents are mixed with the juices before drying to increase the Tg of the product and, as a consequence, avoid a viscoplastic state and caking [4,5]. The carrier agents used in the production of powders may be maltodextrin, inulin, gum arabic, carrageenan gum, carboxymethyl cellulose (CMC), starch, pectin, whey protein, gelatin, casein and others; however, each of them affects the physical and chemical properties of products [6–8]. The most commonly used techniques are spray drying and freeze drying. However, the potential for vacuum drying, drum drying, reactance window drying, microwave-vacuum and other combined drying is increasing [4,6,9].

Powders from whole fruits, juice, extract and pomace are produced, depending on the form of the fruit. For instance, powders from mango [10], apple juice [11], Roselle extract [8], orange juice with incorporated lactic acid bacteria [12], grape skin phenolic extract [13], purple sweet potato [6], grape wastes [14], pomegranate peel phenolics [15], blackberry phenolics [16], herb extract [7] and with probiotics in raspberry juice [9] have been produced and studied thus far.

Encapsulation involves entrapment of valuable, sensitive or target components or fractions within the coating material. Processing fruit juice into powder can extend its shelf life and thus improve its physical properties and nutritional and pro-healthy value, as in the research by Bąkowska-Barczak and Kołodziejczyk [17], Aziz et al. [4] and Çam et al. [15]. The development of sea buckthorn powders may facilitate the potential use of health benefits of sea buckthorn with their prolonged shelf life and lower transport and storage costs. The encapsulated juice form offers flexibility for innovative formulas and uses as a replacement for juices and concentrates and in new markets, including bakery products, confectionery, sauces, ice cream, dairy and nutritional and functional snacks. Juice powders can fit well with the trend of using natural thickeners and agents that change or enhance the taste, color and health value of products. Additionally, reducing the instability of sea buckthorn bioactive compounds during processing and storage, as well as digestion in the digestive system, may meet the expectations of the cosmetics and pharmaceutical industries [6,8,10]. Thus, the formation of sea buckthorn juice powders can be equally beneficial.

This study aimed to assess the impact of drying methods (spray drying, freeze drying and vacuum drying at 50, 70 and 90 °C) and types of carrier agents (inulin, maltodextrin and mixtures inulin:maltodextrin in the ratio of 1:2 and 2:1) on physical properties (moisture content, water activity, true and bulk density, porosity, color parameters, browning index), chemical components (hydroxymethylfurfural [HMF] and phenolic compounds) and antioxidant capacity of sea buckthorn juice powders before and after six-month storage. To the best of our knowledge, this is the first detailed report on powders from *H. rhamnoides* juice. It will provide valuable information on the selection of carrier agents and optimal drying conditions, stability of chemical compounds and antioxidant activity of sea buckthorn juice after drying processes and then after storage.

2. Results and Discussion

2.1. Physical Properties of Sea Buckthorn Juice Powders

Physical properties, such as moisture content, water activity, true and bulk density and porosity of sea buckthorn juice powders are summarized in Table 1. Color parameters, chroma parameter, the total color change, hue angle, and browning index are presented in Table 2. Color of sea buckthorn juice powders are presented on Figure 1. Tested properties can be used in processing control, quality of the final product and its storage stability, but also for estimation of the texture of powders [4,18].

Table 1. Moisture content, water activity, true density, bulk density and porosity of sea buckthorn juice powders.

Drying Method	Carrier Agent	Moisture Content (%)	Water Activity (a_w)	True Density (kg m^{-3})	Bulk Density (kg m^{-3})	Porosity (%)
SD	INU	2.62 ± 0.11 d	0.086 ± 0.001 d	1408 ± 15 c	488.2 ± 13 d	65.34 ± 0.6 d
	MALTO	2.01 ± 0.16 de	0.090 ± 0.002 cd	1240 ± 9 e	389.5 ± 5 f	68.52 ± 0.1 c
	I:M 2:1	1.64 ± 0.10 e	0.080 ± 0.001 d	1375 ± 13 d	459.6 ± 23 de	66.59 ± 1.3 d
	I:M 1:2	2.19 ± 0.12 de	0.089 ± 0.000 cd	1471 ± 12 b	466.5 ± 10 de	68.29 ± 0.4 c
FD	INU	4.75 ± 0.18 b	0.101 ± 0.001 c	1543 ± 10 a	448.1 ± 4 e	70.95 ± 0.1 b
	MALTO	2.55 ± 0.12 d	0.096 ± 0.000 c	1529 ± 13 ab	548.9 ± 18 bc	64.10 ± 0.9 d
	I:M 2:1	3.28 ± 0.14 c	0.097 ± 0.001 c	1485 ± 15 b	474.9 ± 11 d	68.03 ± 0.4 c
	I:M 1:2	3.45 ± 0.16 c	0.098 ± 0.001 c	1519 ± 8 ab	373.1 ± 3 f	75.43 ± 0.1 a
VD 50 °C	INU	4.96 ± 0.20 ab	0.099 ± 0.001 c	1508 ± 11 ab	541.9 ± 12 bc	64.08 ± 0.5 d
	MALTO	2.42 ± 0.10 d	0.096 ± 0.001 c	1485 ± 13 b	550.5 ± 18 bc	62.94 ± 0.9 de
	I:M 2:1	3.68 ± 0.13 c	0.097 ± 0.000 c	1480 ± 15 b	541.0 ± 19 bc	63.45 ± 0.9 d
	I:M 1:2	4.04 ± 0.11 bc	0.100 ± 0.001 c	1462 ± 12 b	519.1 ± 11 c	64.49 ± 0.5 d
VD 70 °C	INU	4.31 ± 0.13 b	0.098 ± 0.000 c	1479 ± 10 b	542.7 ± 12 bc	63.30 ± 0.6 d
	MALTO	1.69 ± 0.15 e	0.088 ± 0.002 d	1467 ± 9 b	549.4 ± 9 bc	62.57 ± 0.4 de
	I:M 2:1	1.99 ± 0.18 de	0.088 ± 0.001 d	1473 ± 12 b	539.9 ± 21 bc	63.35 ± 1.1 d
	I:M 1:2	3.98 ± 0.22 bc	0.096 ± 0.001 c	1475 ± 13 b	515.9 ± 6 c	65.03 ± 0.1 d
VD 90 °C	INU	1.89 ± 0.11 e	0.076 ± 0.000 e	1421 ± 11 c	551.7 ± 3 bc	61.19 ± 0.1 e
	MALTO	1.36 ± 0.17 ef	0.074 ± 0.000 e	1393 ± 14 cd	524.5 ± 5 c	62.34 ± 0.1 de
	I:M 2:1	1.62 ± 0.12 e	0.076± 0.002 e	1412 ± 11 c	597.1 ± 8 b	57.71 ± 0.3 f
	I:M 1:2	1.29 ± 0.19 f	0.075 ± 0.002 e	1406 ± 12 c	573.1 ± 15 b	59.23 ± 0.7 e
Pure carrier agents	INU	2.08 ± 0.01 de	0.129 ± 0.001 b	1387 ± 11 d	644.3 ± 9 a	53.53 ± 0.3 h
	MALTO	5.70 ± 0.25 a	0.397 ± 0.001 a	1228 ± 12 ef	472.3 ± 2 d	61.65 ± 0.2 e
	I:M 2:1	5.07 ± 0.00 ab	0.383 ± 0.001 a	1273 ± 13 e	547.5 ± 5 bc	57.00 ± 0.1 f
	I:M 1:2	4.65 ± 0.08 b	0.368 ± 0.001 a	1308 ± 9 f	575.4 ± 6 b	56.01 ± 0.2 g
Duncan's Multiple Range Test						
Drying method	SD	2.12 C	0.086 B	1374 D	451.2 D	67.19 A
	FD	3.51 A	0.098 A	1519 A	461.3 C	69.63 A
	VD 50 °C	3.78 A	0.098 A	1484 B	538.1 B	63.74 B
	VD 70 °C	2.99 B	0.093 AB	1474 B	537.0 B	63.56 B
	VD 90 °C	1.54 D	0.075 C	1408 C	561.6 A	60.12 C
Carrier agent	INU	3.71 A	0.092 A	1472 A	514.5 B	64.97 AB
	MALTO	2.01 D	0.089 B	1423 D	512.7 B	64.09 B
	I:M 2:1	2.44 C	0.088 B	1445 C	522.5 A	63.83 B
	I:M 1:2	2.99 B	0.092 A	1467 B	489.6 C	66.49 A

Data are shown as mean (n = 3) ± standard deviation; for each parameter tested, values with different letters differ significantly (Duncan's test, $p < 0.05$); SD–spray drying; FD, freeze drying; VD, vacuum drying; INU, inulin; MALTO, maltodextrin; I:M, inulin:maltodextrin.

2.1.1. Moisture Content

The moisture content of sea buckthorn juice powders ranged from 1.29% (vacuum-dried powder at 90 °C with inulin:maltodextrin 1:2) to 4.96% (vacuum-dried powder at 50 °C with inulin) (Table 1). All powders met the moisture criterion below 5% for microbiological safety [4]. Nevertheless, the kind of carrier agents, the drying methods and their parameters modulated the moisture content. The moisture-differentiating factors could be temperature during freeze drying (too low cause sublimation barrier) [13], inlet and outlet temperatures during spray drying and percentage of carrier agents [19]. Selvamuthukumaran and Khanum [20] found that the inlet air temperature, followed by maltodextrin concentration, had the maximum effect on the moisture content of the spray-dried sea buckthorn juice. In the same research, they also found that the optimal values of these parameters were, respectively, 162.5 °C and 1:4 for maltodextrin:fruit slurry. Thus, the determination of water sorption isotherms for powders can be extremely helpful in modeling drying, conditioning and storage processes. The sorption of water by sugar is weaker at higher temperatures, therefore research could be directed towards the effect of the degree of maltodextrin dextrinization causing an increase in water absorption and sugar content in plant material and powders [21].

Table 2. Color parameters and browning index of sea buckthorn juice powders.

Drying Method	Carrier Agent	Color Parameters						Browning Index (AU)	
		L*	a*	b*	Chroma (C)	dE	Hue Angle (h°)	0 Months	6 Months
SD	INU	86.57 ± 0.14 bc	1.56 ± 0.03 g	40.81 ± 0.08 d	40.84 ± 0.38 d	36.67 ± 0.13 c	87.81 ± 0.04 d	0.22 ± 0.00 de	0.74 ± 0.02 e
	MALTO	89.26 ± 0.17 b	−1.21 ± 0.05 i	36.77 ± 0.05 e	36.79 ± 0.41 e	41.64 ± 0.16 b	91.88 ± 0.06 c	0.24 ± 0.01 d	0.50 ± 0.01 g
	I:M 2:1	88.53 ± 0.09 b	−0.63 ± 0.11 i	38.76 ± 0.02 de	38.77 ± 0.67 de	40.08 ± 0.08 b	90.93 ± 0.13 c	0.43 ± 0.02 b	0.58 ± 0.01 f
	I:M 1:2	87.12 ± 0.16 bc	0.97 ± 0.05 h	40.70 ± 0.08 d	40.71 ± 0.65 d	37.47 ± 0.15 c	88.64 ± 0.06 d	0.28 ± 0.01 cd	0.59 ± 0.01 f
FD	INU	87.58 ± 0.17 bc	−0.36 ± 0.23 hi	46.67 ± 0.05 c	46.67 ± 0.44 c	37.38 ± 0.15 c	90.44 ± 0.24 c	0.30 ± 0.02 c	0.72 ± 0.01 e
	MALTO	81.99 ± 0.04 c	6.32 ± 0.06 d	50.16 ± 0.06 bc	50.56 ± 0.37 b	28.46 ± 0.03 e	82.82 ± 0.07 g	0.16 ± 0.00 e	0.52 ± 0.01 fg
	I:M 2:1	78.87 ± 0.06 d	9.66 ± 0.04 c	57.43 ± 0.02 ab	58.24 ± 0.20 a	24.55 ± 0.05 f	80.45 ± 0.05 g	0.14 ± 0.01 e	0.59 ± 0.01 f
	I:M 1:2	83.79 ± 0.19 c	3.55 ± 0.03 ef	55.24 ± 0.07 b	55.35 ± 0.85 ab	31.79 ± 0.16 d	86.32 ± 0.04 de	0.11 ± 0.01 ef	0.47 ± 0.01 g
VD 50 °C	INU	84.59 ± 0.20 bc	1.64 ± 0.10 g	47.89 ± 0.06 c	47.92 ± 0.34 c	33.71 ± 0.18 d	88.04 ± 0.12 d	0.19 ± 0.01 e	0.61 ± 0.01 f
	MALTO	77.00 ± 0.09 d	11.98 ± 0.02 bc	55.53 ± 0.03 b	56.81 ± 0.40 ab	21.31 ± 0.07 fg	77.83 ± 0.04 h	0.15 ± 0.02 e	0.64 ± 0.01 f
	I:M 2:1	80.99 ± 0.03 c	6.05 ± 0.05 d	53.57 ± 0.02 b	53.91 ± 0.12 b	27.91 ± 0.01 e	83.57 ± 0.06 f	0.11 ± 0.01 ef	0.66 ± 0.01 f
	I:M 1:2	82.88 ± 0.28 c	2.96 ± 0.04 f	53.70 ± 0.04 b	53.78 ± 0.54 b	31.40 ± 0.24 d	86.85 ± 0.05 de	0.18 ± 0.01 e	0.55 ± 0.01 fg
VD 70 °C	INU	77.48 ± 0.03 d	4.62 ± 0.04 e	50.32 ± 0.02 bc	50.53 ± 0.79 b	26.48 ± 0.01 ef	84.75 ± 0.05 f	0.17 ± 0.01 e	1.16 ± 0.01 d
	MALTO	77.59 ± 0.17 d	9.12 ± 0.06 c	59.19 ± 0.03 a	59.89 ± 0.98 a	24.43 ± 0.15 f	81.24 ± 0.07 g	0.09 ± 0.00 f	0.70 ± 0.01 ef
	I:M 2:1	77.64 ± 0.04 d	9.19 ± 0.06 c	58.86 ± 0.10 a	59.57 ± 0.11 a	24.32 ± 0.03 f	81.13 ± 0.07 g	0.32 ± 0.02 c	0.74 ± 0.01 e
	I:M 1:2	77.62 ± 0.14 d	4.56 ± 0.05 e	52.87 ± 0.05 b	53.07 ± 0.31 b	26.61 ± 0.13 ef	85.57 ± 0.06 e	0.25 ± 0.01 d	1.00 ± 0.01 d
VD 90 °C	INU	54.85 ± 0.09 f	12.03 ± 0.07 b	33.06 ± 0.05 f	35.80 ± 0.69 e	23.34 ± 0.08 f	70.00 ± 0.08 i	0.71 ± 0.02 a	2.98 ± 0.03 a
	MALTO	71.52 ± 0.03 e	10.57 ± 0.08 bc	58.60 ± 0.01 a	59.55 ± 0.87 a	19.33 ± 0.03 g	79.78 ± 0.08 g	0.28 ± 0.01 cd	1.57 ± 0.01 c
	I:M 2:1	54.55 ± 0.04 f	35.78 ± 0.04 a	33.70 ± 0.09 f	49.15 ± 0.83 b	21.91 ± 0.03 fg	43.29 ± 0.09 k	0.30 ± 0.01 c	2.84 ± 0.03 ab
	I:M 1:2	56.21 ± 0.12 f	13.26 ± 0.07 b	39.62 ± 0.07 d	41.78 ± 0.69 d	17.30 ± 0.10 h	71.50 ± 0.08 i	0.42 ± 0.01 b	2.72 ± 0.01 b
Pure carrier agents	INU	97.58 ± 0.01 a	−1.51 ± 0.01 i	4.53 ± 0.01 g	4.78 ± 0.01 f	65.60 ± 0.01 a	108.44 ± 0.01 b	-	-
	MALTO	98.03 ± 0.00 a	−1.24 ± 0.01 i	2.74 ± 0.01 h	3.01 ± 0.01 f	67.05 ± 0.01 a	114.35 ± 0.01 a	-	-
	I:M 2:1	97.90 ± 0.01 a	1.47 ± 0.01 i	3.75 ± 0.01 gh	4.03 ± 0.01 f	65.22 ± 0.01 a	68.60 ± 0.02 ij	-	-
	I:M 1:2	98.02 ± 0.00 a	1.37 ± 0.01 i	3.33 ± 0.01 gh	3.60 ± 0.01 f	65.63 ± 0.01 a	67.64 ± 0.01 j	-	-
Duncan's Multiple Range Test									
Drying method	SD	87.87 A	0.17 C	39.26 D	39.28 C	38.96 A	89.82 A	0.29 B	0.60 C
	FD	83.06 AB	4.79 B	52.38 B	52.70 A	30.55 B	85.01 B	0.18 CD	0.58 C
	VD 50 °C	81.37 B	5.66 B	52.67 B	53.10 A	28.58 B	84.07 BC	0.16 D	0.62 C
	VD 70 °C	77.58 C	6.87 B	55.31 A	55.76 A	25.46 C	83.17 C	0.21 C	0.90 B
	VD 90 °C	59.28 D	17.91 A	41.25 C	46.41 B	20.47 D	66.14 D	0.43 A	2.53 A
Carrier agents	INU	78.21 AB	3.90 D	43.75 C	44.23 D	31.52 A	70.17 A	0.32 A	1.24 A
	MALTO	79.47 A	7.36 B	52.05 A	55.72 A	27.03 C	68.93 A	0.18 C	0.79 C
	I:M 2:1	76.12 C	12.01 A	48.46 B	51.93 B	27.75 BC	62.23 B	0.26 B	1.08 B
	I:M 1:2	77.52 BC	5.06 C	48.43 B	48.94 C	28.91 B	69.81 A	0.25 B	1.07 B

Data are shown as mean (n = 3) ± standard deviation; for each parameter tested, values with different letters differ significantly (Duncan's test, $p < 0.05$); SD–spray drying; FD, freeze drying; VD, vacuum drying; INU, inulin; MALTO, maltodextrin; I:M, inulin:maltodextrin; dE, total color change; AU, arbitrary units.

Figure 1. Color of sea buckthorn juice powders modulated by drying methods and carrier agents. SD, spray drying; FD, freeze drying; VD, vacuum drying; INU, inulin; MALTO, maltodextrin; I:M, inulin:maltodextrin.

In this study, the water content for sea buckthorn juice powders after freeze drying and vacuum drying at 50 °C was comparable moisture (approximately 3.51% and 3.78%, respectively). Higher temperatures (spray drying at 180 °C similar to vacuum drying at 90 °C) significantly reduced the moisture content (approximately 2.12% and 1.54%, respectively). Similar observations were obtained by Bąkowska-Barczak and Kołodziejczyk [17] in the research on encapsulated blackcurrant polyphenols; however, our study also revealed differences between powders with maltodextrin and inulin. The addition of inulin caused stronger water retention than in the case of maltodextrin powders (approximately 3.71% and 2.01%, respectively), which can be explained by the high hygroscopicity of inulin resulting from the branched structure that promotes hydrogen bonding and moisture absorption from ambient air [22]. The influence of maltodextrin on moisture content is ambiguous, as dextrinization causes an increase in the number of ramifications with hydrophilic groups, but lower DE was associated with better binder properties [23]. Nevertheless, other studies also confirm lower moisture content in powders containing maltodextrin, compared to other carrier agents, e.g., gum arabic, corn starch, pectin, carboxymethylcellulose and yucca starch [8,24].

The results obtained for sea buckthorn juice powders are typical of the average moisture content of previously created powders, including blackcurrant polyphenol extracts (1.8–3.9%) [17], chokeberry

powders (1.13–4.05%) [25], spray-dried cinnamon infusions with maltodextrin (1.34–1.99%) [21] and spray-dried grape skin phenolic extract (2.41–2.57%) [13].

2.1.2. Water Activity

The water activity of sea buckthorn juice powders ranged from 0.074 (vacuum-dried powder containing maltodextrin) to 0.101 (freeze-dried powder containing inulin) (Table 1). Maintaining the water activity of powders at the level obtained will prevent or minimize the growth of mold, yeast and bacteria, degradation of biologically active compounds and non-enzymatic browning.

The type of drying method caused greater variation in the water activity than the type of carrier agents. The water activity of powders increases with equilibrium moisture content at a constant temperature [26], therefore the lowest water activity was characterized by powders vacuum-dried at 90 °C (approximately 0.075). The values for powders after freeze drying and vacuum drying at 50 °C was the same (approximately 0.098), and similar with that for powders vacuum-dried at 70 °C (approximately 0.093). Freeze-dried powders may have higher water activity than those after other methods due to their higher porosity which facilitates water penetration in the pores [11]. Additionally, the nozzle diameter and feed flow rate in spray drying [23,24], and structure and content of carrier agents (higher concentration causes a decrease in the a_w) [19] could modulate the a_w in powders. Statistically similar water activity was observed for powders with inulin and with inulin:maltodextrin (1:2) (approximately 0.092), as well as those with maltodextrin and with inulin:maltodextrin (2:1) (0.089 and 0.088, respectively).

The water activity results obtained were similar or lower than those for probiotic orange powders (0.34–0.42) [12], spray-dried watermelon powders (0.20–0.29) [19], microencapsulated Andes berry extracts (0.199–0.422) [24], spray-died microencapsulated betalains cactus (0.176–0.205) [27], microencapsulated Bordo grape skin extract (0.160–0.360) [13] and cantaloupe juice powders (0.15–0.19) [28].

2.1.3. True and Bulk Density

The values of true density for sea buckthorn juice powders ranged between 1240 (spray-dried powder with maltodextrin) and 1543 kg m^{-3} (freeze-dried powder with inulin) (Table 1). Generally, spray dried particles have lower true density values than freeze dried and vacuum dried products [11]. The same relationship was found for sea buckthorn powders, which can be ranked according to decreasing true density values as follows: freeze-dried > vacuum-dried at 50 and 70 °C > vacuum-dried at 90 °C > spray-dried powders (approximately 1519, 1479, 1408 and 1374 kg m^{-3}, respectively). The largest diversity in true density values was recorded for spray-dried powders (1240–1471 kg m^{-3}), whereas powders containing inulin had significantly higher true density values than those with maltodextrin (approximately 1472 and 1423 kg m^{-3}, respectively). As expected, true density of all powders was higher than true density of corresponding pure carrier agents. Furthermore, the largest increase in true density was determined for powder with maltodextrin after freeze drying (difference of approximately 300 kg m^{-3}).

Bulk density values did not correlate with true density (r = 0.187). The lowest bulk density values were determined for freeze-dried powder with inulin:maltodextrin (1:2) and spray-dried powder with maltodextrin (373.1 and 389.5 kg m^{-3}, respectively). For this first powder, the largest difference between its bulk density and bulk density of the pure carrier agent (575.4 kg m^{-3}) was also calculated. Sea buckthorn juice powder with inulin:maltodextrin (2:1), vacuum-dried at 90 °C, had the highest bulk density (597.1 kg m^{-3}) and this value did not differ significantly from the bulk density of the analogous pure carrier agent (547.5 kg m^{-3}). Moreover, extremely bulk density values were observed for powders with blend of inulin and maltodextrin 1:2 and 2:1, respectively, compared to powders with simple carrier agents. This differentiation was explained by polymer interactions between carbohydrates and juice powder in the study by Ferrari et al. [26]. Powders encapsulated with maltodextrin had a minor bulk density than that produced with a mix of gum arabic and maltodextrin.

Bulk density and porosity depend on particle size, inter-particle voids of powders, properties of juice and carrier agents, drying methods and temperatures [10]. Particles with higher moisture amount tend to have a higher bulking weight due to the presence of water which is denser than dried solids [26]. However, in this study, no correlation was found between moisture content and bulk density (r = −0.183) and weak correlation between moisture content and porosity (r = 0.406). In addition, sea buckthorn fruits contain low sugar levels (average 2%), so the powder obtained by spray drying was not sticky. Drying methods had a larger impact on bulk density than carrier agents. Thus, powders in terms of decreasing bulk density can be ordered as follows: vacuum-dried at 90 °C > vacuum-dried at 50 and 70 °C > freeze-dried > spray-dried powder (approximately 561.6, 538.1, 461.3 and 451.2 kg m^{-3}, respectively). This behavior can be explained by the crystal structure of the powders obtained in a vacuum and by the reduction of interstitial air content between the particles and the reduction of the volume occupied [11,26].

High bulk density is economically positive, since products require smaller packaging, and transport and storage costs are lower. Lower bulk density is associated with entrapment of air in voids, thereby facilitating oxidation and less stability [4]. Pure inulin had a higher value than maltodextrin (644.3 and 472.3 kg m^{-3}, respectively) due to its molecular weight and thus heavier material and limited spaces between particles. Nevertheless, the bulk density of sea buckthorn juice powders with inulin (approximately 514.5 kg m^{-3}) decreased relative to the pure carrier agent, but with maltodextrin favorably increased (mean 512.7 kg m^{-3}). The studied powders had similar bulk density values to those determined for spray-dried cinnamon infusions with maltodextrin (536–554 kg m^{-3}) [21] and spray-dried Roselle extract with maltodextrin, pectin, carboxymethylcellulose, gum arabic and whey powder (427–588 kg m^{-3}) [8]. However, this second research team investigated higher differentiation, with double the bulk density for the powder with carrageenan gum (849 kg m^{-3}) than for gelatin (392 kg m^{-3}).

2.1.4. Porosity

The drying methods had a greater impact on the powder porosity than the type of carrier agents. Measured porosity values were between 57.71% (powder with inulin:maltodextrin in the ratio of 2:1 after vacuum drying at 90 °C) and 75.43% (freeze-dried powder with inulin:maltodextrin 1:2). The porosity of sea buckthorn juice powders obtained by vacuum drying at 50 and 70 °C was similar and averaged 62.47%. Consequently, in terms of porosity, powders can be ranked as follows: freeze-dried (approximately 69.63%) ≈ spray-dried (approximately 67.19%) > vacuum-dried (between approximately 60.12% and 63.74%). Based on scanning electron micrographs, Azizpour et al. [29] stated that the high temperature promotes the increase in porosity of dried material. However, other studies suggested that surface roughness is typical for spray dried powders at low temperatures. Such a structure promotes higher humidity and suppleness, and, consequently, it causes a reduced volume of particles during cooling [22,23].

The porosity of the pure carrier agents differed significantly, ranging between 53.53% for inulin and 61.65% for maltodextrin. Despite this, the porosity of the sea buckthorn juice powders with inulin was higher by less than 1% compared to those with maltodextrin (64.97% and 64.09%, respectively). Increased porosity in powders with maltodextrin and thus a significant reduction in bulk density was investigated for spray-dried mango puree [10]. In turn, the smoother surface of the insulin particles can be ascribed to the relatively low polydispersity and higher molecular flexibility enabling many conformations. An increase in temperature and a low concentration of this oligosaccharide in final powders (20%) may have contributed to a decrease in its crystallinity and viscosity [30].

The largest variation in the porosity was found between powders produced by freeze drying. Reduced temperature and pressure in this process ensure an appropriate sublimation rate; the material is not exposed to shrinkage, and, as a result, products with high porosity and rehydration capacity are created [10]. Thus, the porosity and bulk density of sea buckthorn juice powders may be an important

criterion of application, due to the storage conditions, the type and form of the final product and oxidative and aromatic stability.

2.1.5. Color Parameters

The color parameters of sea buckthorn juice powders are given in Table 2, while images, showing clear differences in color, are presented in Figure 1.The color of the powders was determined with reference to the CIE L*a*b* color space, in which parameter L* indicates brightness from blackness (0) to whiteness (100), parameter a* determines the color from green (−) to red (+) and parameter b* is from blue (−) to yellow (+). In the sea buckthorn juice powders, coordinate L* ranged from 54.55 (vacuum-dried powder with inulin:maltodextrin in the ratio of 2:1) to 89.26 (spray-dried powder with maltodextrin). Powders produced by freeze drying and spray drying had higher parameter L* values than those after vacuum drying (especially in higher temperature variants). In previous studies, similar trends were obtained, which indicates the beneficial use of these drying processes in the production of powders with favorable, minimally changed color. Prolonged exposure to 90 °C under vacuum could have promoted browning reactions such as caramelization and Maillard reactions [12,25].

Moreover, the powders after vacuum drying at 90 °C were the darkest (L* = approximately 59.28) but the powder with maltodextrin had a significantly higher parameter L* equal to 71.52. Such a large difference between the brightness of powders with maltodextrin and the remaining powders was found only in this drying method. Other researchers have also described higher parameter L* after the addition of maltodextrin in raspberry powders, orange juice powders and mango powders [9,10,12]. The superiority of maltodextrin over insulin in the context of final brightness may find corroboration in various interactions between juice matrix and carrier agents and, consequently, the release of compounds that undergo further reactions.

The hue angle (h°), which characterizes the perception of color, showed values from 43.29° (vacuum dried powder at 90 °C with inulin:maltodextrin 2:1) to 91.88° (sprayed powder with maltodextrin), thus indicating values between red (0°) and yellow (90°). For spray-dried powders, followed by freeze-dried and vacuum-dried at 50 and 70 °C, the highest h° values were recorded, i.e. the closest to the angle for yellow. The type of carrier agent did not significantly affect h° but the parameters a* and b* were significantly different for powders with inulin, maltodextrin and their mixtures. The lowest h° values were recorded for powders dried in vacuum at 90 °C, which could still be caused by browning reactions. Coordinate b* indicates a more yellow color of the powders with maltodextrin than those with inulin (b* = approximately 52.05 and 43.75, respectively), however the h° values indicate such results only for drying techniques at higher temperatures (spray drying and vacuum drying at 90 °C).

The chroma parameter (C), indicating the purity and intensity of color, of the sea buckthorn juice powders ranged from 35.80 (powder with inulin after vacuum drying at 90 °C) to 59.89 (powder with maltodextrin after vacuum drying at 70 °C). The highest intensity of color was characterized by powders with maltodextrin, and the lowest with inulin (C = approximately 55.72 and 44.25, respectively). The average values of the parameter C for powders after freeze drying and vacuum drying at 50 and 70 °C did not differ significantly. The color intensity of sea buckthorn powders obtained by spray drying was the lowest, as was the case with orange, mango and apple powders [10–12]. Nevertheless, opposite results were obtained by Kuck and Noreña [13], and thus higher parameter C values were observed for spray-dried grape skin extracts than after freeze drying. The reason may be the use of gum arabic, polydextrose and guar gum as encapsulating agents. The values of the chroma parameter strongly correlated with the value of parameter b* (r = 0.922) and poorly with the parameter a* (r = 0.218), indicating a strong yellow color of powders.

The total color change (dE) was calculated using sea buckthorn juice as a reference (L* = 60.44; a* = 24.84; b* = 51.75). It was found that the color change resulted from the parameter L* increase and the parameter a* decrease in the powders obtained. Thus, the dE ranged between 17.30 (powder with inulin:maltodextrin 1:2, after vacuum drying at 90 °C) to 41.64 (spray-dried powder with maltodextrin).

All results indicate visible and distinguishable differences in the color of powders for the human eye (dE > 5.0) [22] but do not suggest explicit sensory assessments of color.

Drying methods had a stronger impact on the color change than the type of carrier agents. The highest average dE was observed for spray-dried powders, and the lowest after vacuum drying 90 °C (approximately 38.96 and 20.47, respectively). Powders with maltodextrin were characterized by the smallest color change (dE = approximately 27.03), again suggesting the appropriateness of this carrier agent to produce sea buckthorn powders.

2.1.6. Browning Index

The browning index was presented as an indicator of non-enzymatic browning of powders. In powders before storage, the browning index ranged from 0.09 AU (powder with maltodextrin after vacuum drying at 70 °C) to 0.71 AU (powder with inulin after vacuum drying at 90 °C). Freeze drying and vacuum drying at 50 °C resulted in the lowest browning of powders, in contrast to vacuum drying at 90 °C (approximately 0.16, 0.18 and 0.43 AU, respectively). Inulin promoted browning reactions, so powders can be ranked according to the increasing browning index: powders with maltodextrin < powders with inulin:maltodextrin 1:2 and 2:1 < powders with inulin.

Research on model systems has proved that browning reactions at temperatures above 60 °C occur significantly more quickly in a model containing glucose than in one containing galactose. Glucose is the dominant sugar in sea buckthorn; hence, the long-term vacuum drying at 90 °C resulted in the highest browning index. In addition, the browning index could be modulated by carrier agents, drying temperature, storage process, content of sugars and compounds with a free amino group in powders [31]. Generally, inulin is non-reducing and does not contain or form reactive ketone and aldehyde groups. However, it is a polydisperse mixture and may contain more reactive mono- and disaccharides and as a consequence may participate in reactions with groups of other compounds, including in Maillard reactions. Importantly, low pH and elevated temperature may promote its hydrolysis in aqueous solutions, thereby increasing the amount of reducing sugars. This should be particularly taken into account when using sea buckthorn juice with a naturally low pH about 3.0 [30].

After six months of storage, the browning index varied between 0.50 AU (spray-dried powder with maltodextrin) and 2.98 AU (vacuum-dried powder at 90 °C with inulin). Powders vacuum-dried at 90 °C had the highest browning index (approximately 2.53 AU), and simultaneously almost six times higher compared to powders before storage. As with fresh powders, stored powders with inulin had the highest average index, and the lowest with maltodextrin (approximately 1.24 and 0.79 AU).

2.2. *Hydroxymethylfurfural in Sea Buckthorn Juice Powders*

The hydroxymethylfurfural (HMF) content was tested in powders before and after six months of storage (Table 3). In fresh powders, the HMF content ranged from 0.05 (powder with inulin:maltodextrin 2:1 after vacuum drying at 70 °C) to 75.21 mg/100 g DM (powder with inulin after vacuum drying at 90 °C). The average HMF content in powders spray-dried, freeze-dried and vacuum-dried at 50 °C did not differ significantly and ranged between 0.15 and 0.94 mg/100 g DM. Moßhammer et al. [32] also reported that cactus pear powders after freeze drying and spray drying had low and similar HMF concentration.

Table 3. Content of hydroxymethylfurfural (HMF) and phenolic compounds (mg/100 g DM) and antioxidant capacity (mmol Trolox/100 g DM) of sea buckthorn juice powders.

Drying Method	Carrier Agent	HMF		Phenolic Acids		Flavonols		Antioxidant Capacity	
		0 Months	6 Months	0 Months	6 Months	0 Months	6 Months	0 Months	6 Months
SD	INU	1.50 ± 0.13 e	1.79 ± 0.19 f	2.86 ± 0.11 ab	2.71 ± 0.15 a	210.83 ± 4.05 f	203.92 ± 2.23 e	1.62 ± 0.08 ab	2.25 ± 0.23 c
	MALTO	0.39 ± 0.09 g	0.72 ± 0.11 g	3.00 ± 0.29 a	2.96 ± 0.19 a	222.21 ± 2.45 e	209.51 ± 2.46 e	1.56 ± 0.08 b	1.43 ± 0.19 ef
	I:M 2:1	0.82 ± 0.10 f	1.89 ± 0.20 f	2.49 ± 0.09 b	2.26 ± 0.13 b	242.05 ± 5.17 d	174.96 ± 2.01 f	1.60 ± 0.02 ab	2.20 ± 0.03 c
	I:M 1:2	1.05 ± 0.02 ef	1.88 ± 0.18 f	1.70 ± 0.14 c	1.26 ± 0.10 de	266.43 ± 3.55 b	144.05 ± 2.12 g	1.64 ± 0.10 ab	2.27 ± 0.22 c
FD	INU	0.04 ± 0.01 i	0.67 ± 0.14 gh	1.71 ± 0.10 c	1.67 ± 0.21 d	213.28 ± 4.44 ef	200.40 ± 2.32 ef	1.73 ± 0.19 a	2.10 ± 0.34 cd
	MALTO	0.41 ± 0.10 g	0.57 ± 0.11 h	1.85 ± 0.24 bc	1.80 ± 0.14 cd	251.48 ± 4.64 c	249.02 ± 3.54 ab	1.45 ± 0.20 c	2.09 ± 0.12 cd
	I:M 2:1	0.07 ± 0.01 i	0.15 ± 0.08 i	1.44 ± 0.18 d	1.37 ± 0.24 d	255.78 ± 2.47 c	233.09 ± 3.53 c	1.56 ± 0.07 b	1.85 ± 0.22 d
	I:M 1:2	0.09 ± 0.01 i	0.17 ± 0.10 i	2.12 ± 0.22 b	2.07 ± 0.07 c	245.40 ± 3.89 d	236.53 ± 2.79 c	1.40 ± 0.07 c	1.72 ± 0.29de
VD 50 °C	INU	0.45 ± 0.06 g	0.51 ± 0.10 h	1.39 ± 0.16 d	1.00 ± 0.25 e	217.69 ± 2.56 e	150.42 ± 3.72 g	1.56 ± 0.08 b	1.80 ± 0.34 d
	MALTO	0.44 ± 0.03 g	0.50 ± 0.07 h	1.57 ± 0.20 cd	1.49 ± 0.24 d	274.25 ± 3.52 b	256.46 ± 2.64 a	1.46 ± 0.11 c	1.96 ± 0.17 d
	I:M 2:1	0.13 ± 0.01 i	0.89 ± 0.22 g	1.60 ± 0.15 cd	1.23 ± 0.17 de	248.55 ± 3.12 cd	222.51 ± 2.70 d	1.55 ± 0.14 b	2.61 ± 0.17 b
	I:M 1:2	0.32 ± 0.09 h	0.90 ± 0.06 g	1.02 ± 0.09 e	0.92 ± 0.15 e	273.93 ± 2.25 b	223.91 ± 3.75 d	1.46 ± 0.05 c	2.35 ± 0.08 bc
VD 70 °C	INU	14.09 ± 1.53 c	21.12 ± 2.14 c	1.21 ± 0.13 de	0.94 ± 0.13 e	243.86 ± 2.51 d	212.50 ± 2.34 e	1.40 ± 0.18 c	1.65 ± 0.20 e
	MALTO	0.46 ± 0.08 g	2.15 ± 0.67 ef	1.59 ± 0.08 cd	1.12 ± 0.13 e	290.34 ± 4.02 a	244.73 ± 2.27 b	1.29 ± 0.05 d	2.92 ± 0.11 a
	I:M 2:1	0.05 ± 0.01 i	2.94 ± 0.23 e	0.24 ± 0.01 g	0.18 ± 0.09 g	267.62 ± 1.87 b	234.59 ± 2.75 c	1.64 ± 0.33 ab	1.99 ± 0.18 d
	I:M 1:2	9.22 ± 0.45 d	15.35 ± 2.53 d	1.06 ± 0.11 e	0.79 ± 0.17 f	285.03 ± 2.16 a	232.87 ± 3.64 c	1.32 ± 0.11 cd	1.42 ± 0.17 ef
VD 90 °C	INU	75.21 ± 1.74 a	94.20 ± 4.35 a	0.71 ± 0.12 ef	0.14 ± 0.03 g	191.24 ± 1.54 g	195.87 ± 1.58 ef	1.12 ± 0.06 d	1.82 ± 0.25 d
	MALTO	0.39 ± 0.02 gh	11.53 ± 1.04 c	1.16 ± 0.17 de	0.89 ± 0.12 ef	298.68 ± 5.34 a	200.13 ± 2.28 ef	1.29 ± 0.03 c	1.35 ± 0.19 f
	I:M 2:1	75.07 ± 1.19 a	101.40 ± 4.93 a	0.71 ± 0.09 ef	0.25 ± 0.08 g	219.68 ± 3.74 e	188.92 ± 2.22 f	1.42 ± 0.05 c	1.76 ± 0.22 de
	I:M 1:2	47.12 ± 0.80 b	70.53 ± 3.66 b	0.24 ± 0.03 g	0.10 ± 0.02 g	181.80 ± 2.81 h	175.41 ± 3.64 f	0.85 ± 0.07 e	2.19 ± 0.11 c
Duncan's Multiple Range Test									
Drying method	SD	0.94 C	1.57 C ↑67.0%	2.51 A	2.30 A ↓8.4%	235.38 BC	183.11 C ↓22.2%	1.61 A	2.04 A ↑26.7%
	FD	0.15 C	0.33 C ↑120.0%	1.78 B	1.73 B ↓2.8%	241.49 BC	229.76 A ↓4.8%	1.54 A	1.94 AB ↑26.0%
	VD 50 °C	0.33 C	0.90 C ↑173%	1.39 B	1.16 B ↓16.5%	253.61 B	213.33 B ↓15.9%	1.51 A	2.18 A ↑44.4%
	VD 70 °C	5.95 B	10.39 B ↑74,6%	1.03 BC	0.76 C ↓26.2%	271.71 A	231.17 A ↓14.9%	1.41 AB	2.00 A ↑41.8%
	VD 90 °C	49.45 A	69.42 A ↑40.4%	0.71 C	0.35 D ↓50.7%	222.85 C	190.08 C ↓14.7%	1.17 B	1.75 B ↑49.6%
Carrier agents	INU	18.26 A	29.45 A ↑61.3%	1.58 B	1.29 B ↓18.4%	215.38 C	192.62 C ↓10.6%	1.49 A	1.92 A ↑28.9%
	MALTO	0.41 D	4.80 C ↑1070.7%	1.83 A	1.65 A ↓9.8%	267.39 A	231.97 A ↓13.2%	1.41 AB	1.93 A ↑36.9%
	I:M 2:1	15.23 B	26.59 A ↑74.6%	1.30 C	1.06 C ↓18.5%	246.74 B	210.81 B ↓14.6%	1.55 A	2.08 A ↑34.2%
	I:M 1:2	11.56 C	17.77 B ↑53.7%	1.23 C	1.03 C ↓16.3%	250.52 B	202.55 BC ↓19.1%	1.33 B	1.99 A ↑49.6%

Data are shown as mean (n = 3) ± standard deviation; for each parameter tested, values with different letters differ significantly (Duncan's test, $p < 0.05$); SD–spray drying; FD, freeze drying; VD, vacuum drying; INU, inulin; MALTO, maltodextrin; I:M, inulin:maltodextrin.

The temperature increase during vacuum drying significantly increased the amount of HMF formed, and thus powders vacuum-dried at 90 °C had 47.12–75.21 mg/100 g DM, except for that with maltodextrin (0.39 mg/100 g DM). Similarly, Michalska et al. [33] reported that the HMF formation was slow at temperatures above 60 °C, and rapid above 80 °C, during convection drying of blackcurrant pomace powders.

The average HMF content in powders with maltodextrin was 28–45 times lower than in powders with other carrier agents (approximately between 11.56 and 18.16 mg/100 g DM). This indicates the appropriateness of using pure maltodextrin for the production of powders by the vacuum method at higher temperatures. The results obtained were in line with the research on plum powders, in which maltodextrin also impeded HMF creation [34]. It should be noted, however, that short-term spray drying at 180 °C did not result in high HMF concentration regardless of carrier agents.

HMF is one of the products of the advanced Maillard reaction, occurring in products with hexose, as a result of thermal treatment and storage, or a degradation product of ascorbic acid. Therefore, in stored powders, the HMF amount increased and equaled 0.15–101.40 mg/100 g DM (powder with inulin:maltodextrin 2:1 after freeze drying and after vacuum drying at 90 °C, respectively). Powders spray-, freeze- and vacuum-dried at 50 °C had significantly similar average HMF content, similar as in fresh powders (approximately between 0.33 and 1.57 mg/100 g DM).

However, the increase in HMF after storage was significantly lower for spray-dried powders. This could be due to the formation of spherical capsules in spray drying, without pores and thus with full core protection [35]. The increase in HMF after storage was the highest in the case of powders with maltodextrin (almost 12 times), whereas the content of this compound was the lowest in comparison to powders with other carrier agents (4.80 vs. 17.77–29.45 mg/100 g DM). In addition, the correlation between HMF content and browning index was higher for powders after storage than before (r = 0.970 and 0.669).

2.3. Phenolic Compounds in Sea Buckthorn Juice Powders

The content of phenolic compounds before and after six months of storage is presented in Table 3. Sea buckthorn berries are a rich source of flavonols, which account for over 98% of the total phenolic compounds, as proven in previous studies [2]. Similarly, in the case of powders, flavonols were also the dominant phenolic compounds. The greatest variation in their content was tested within powders vacuum-dried at 90 °C (between 181.80 for powders with inulin:maltodextrin 1:2 and 298.68 mg/100 g DM for powders with maltodextrin). Vacuum drying at 70 °C had the most beneficial effect on retaining sea buckthorn flavonols (approximately 271.71 mg/100 g DM). In terms of the type of carrier agents, powders can be ranked according to decreasing flavonol concentration: powders with: maltodextrin > with inulin:maltodextrin 1:2 and 2:1 > with inulin (from 267.35 to 215.38 mg/100 g DM). Flavonols in stored powders were found in concentrations from 144.05 (spray-dried powder with inulin:maltodextrin 1:2) to 256.46 mg/100 g DM (vacuum-dried powder at 70 °C with maltodextrin). Flavonol degradation after freeze drying was the lowest (by 4.8%) and thus their concentration in these powders was the highest (approximately 119.76 mg/100 g DM). The lowest amount of flavonols was determined in inulin powders, and their reduction after six months was the smallest (by 10.6%). The flexibility of the inulin skeleton combined with high glass transition temperature (Tg) make this agent a proper stabilizer of nutritional and bioactive components. For example, in food and pharmaceutical applications it is a suitable stabilizer of proteins in the dry state [30]. Therefore, the results could be strongly dependent on the retention degree of polyphenols and their stability in the powders encapsulated with carrier agents which display different protection characteristics and kinetic parameters.

Phenolic acid content was from 0.24 (powder vacuum-dried at 70 °C with inulin:maltodextrin 2:1 and vacuum-dried powder at 90 °C with inulin:maltodextrin 1:2) to 3.00 mg/100 g DM (spray-dried powder with maltodextrin). Spray-dried powders contained on average 3.5 times more phenolic acids than powders vacuum-dried at 90 °C (2.51 and 0.71 mg/100 g DM, respectively). The results obtained were in line with those obtained by Horszwald et al. [25], who reported that spray drying caused the

least degradation of flavonoids in chokeberry powders compared to freeze drying and vacuum drying. The highest average phenolic acid content (1.83 mg/100 g DM) was found in powders with maltodextrin. Stored powders contained from 0.10 (vacuum-dried powder at 90 °C with inulin:maltodextrin 1:2) to 2.96 mg phenolic acids/100 g DM (spray-dried powder with maltodextrin). Six-month storage resulted in stronger degradation of phenolic acids in powders after vacuum drying at 90 °C (by 50.7%) than after freeze drying (by 2.8%). The highest concentration of phenolic acids (approximately 1.65 mg/100 g DM) was measured in powders with maltodextrin, and the degradation of these compounds (by 9.8%) was the lowest compared to powders with other carrier agents (up to 18.5%).

The contents of flavonols and phenolic acids in pure sea buckthorn juice were 578.30 and 5.25 mg/100 g DM, respectively. Thus, the powders contained on average 2–3 times less phenolic compounds immediately after drying and 2–4 times less after the storage process compared to pure juice. The loss of phenolic compounds after drying may result from the use of high temperatures, exposure to oxygen, formation of fissures, concavities, microspheres and pores, which cause release and degradation of the encapsulated component [13]. The sea buckthorn powders were stored in the presence of oxygen and moisture, which favor the phenol compound degradation reactions and changes in the structure of the carrier agents [36]. Moreover, flavonol concentration correlated with porosity of sea buckthorn powders with different agents ($r = 0.913$), which may explain the easier release of polyphenols in extraction process, as well as their lower retention during storage and thus exposure to degradation. On the other hand, previous research on stored blackcurrant microcapsules obtained by spray drying showed that inulin created a more stable product with polyphenols than maltodextrins, whereas the stability of polyphenols in powders with maltodextrin was dependent on dextrose equivalent (DE) and those with DE 11 provided greater protection for polyphenols during storage than with DE 18 and DE 21 [17]. Higher degree of carrier polymerization results in lower retention of compounds present in the encapsulated material due to the sensitivity of shorter carrier carbohydrate units to temperature and thus their deformation [36]. In research on spray-dried Roselle extract, Díaz-Bandera et al. [8] observed that maltodextrin, similar to carrageenan gum, carboxymethyl cellulose, gelatin and gum arabic, showed low polyphenol release values at the steady state. Mensink et al. [30] emphasized, however, that both the mean DP and actual size distribution of carrier agents determining rheological and thermal properties should be taken into account.

2.4. Antioxidant Capacity of Sea Buckthorn Juice Powders

Antioxidant capacity of sea buckthorn juice powders estimated by the $ABTS^{\cdot+}$ method ranged from 0.85 (powders vacuum-dried at 90 °C with inulin:maltodextrin 1:2) to 1.73 mmol Trolox/100 g DM (powders freeze-dried with inulin). Powders obtained by spray, freeze and vacuum drying at 50 °C had the highest antioxidant capacity (approximately 1.55 mmol Trolox/100 g DM). The average antioxidant effects of powders, except for powders with inulin and maltodextrin (1:2), were similar and ranged from 1.41 to 1.55 mmol Trolox/100 g DM.

Higher antioxidant capacity was measured for powders stored for six months than for fresh powders. An almost 50% increase in antioxidant capacity was found for powders dried at 90 °C under vacuum, but they showed the lowest activity (1.75 mmol Trolox/100 g DM). The increase in antioxidant capacity of spray- and freeze-dried powders averaged 26.4%. Similar to research on blackcurrant polyphenol microcapsules [17], the antioxidant capacity of powders with inulin was the most stable after storage, but powders with maltodextrin showed stronger activity towards the cation radicals.

Spray drying had the most favorable effect on preserving the antioxidant capacity of powders. There was only 4.6 times less antioxidant activity than for pure juice (7.53 mmol Trolox/100 g DM), compared to almost nine times difference for powders obtained by vacuum drying at 90 °C. However, as noted by Santiago-Adame et al. [21], the activity of products dried by this method is conditioned by parameters, and 180 °C and feed rate at 10 mL/min are the most desired. Browning compounds did not affect the increase in antioxidant capacity, and there was also no correlation between changes in antioxidant activity and content of flavonols and phenolic acids.

On-line profiling was conducted to verify the potential antioxidant capacity of HMF and furosine. Furosine is an early product of the Maillard reaction and, similar to HMF, is formed during high temperature processing and storage. Figure 2 shows the chromatographic HMF and furosine profile obtained before and after the derivatization process with $ABTS^{\cdot+}$ reagent serving as a negative control. The upper chromatogram refers to the absorbance at 280 nm, and the lower one is the response after the reaction with $ABTS^{\cdot+}$ reagent at 734 nm. The absence of negative responses after the post-column reaction suggests that HMF and furosine had no radical scavenging capacity. Moreover, there was no correlation between the HMF content and the increase in antioxidant effect. In research on blackcurrant pomace powders, Michalska et al. [33] also concluded that compounds with antioxidant capacity are not formed during the Maillard reaction.

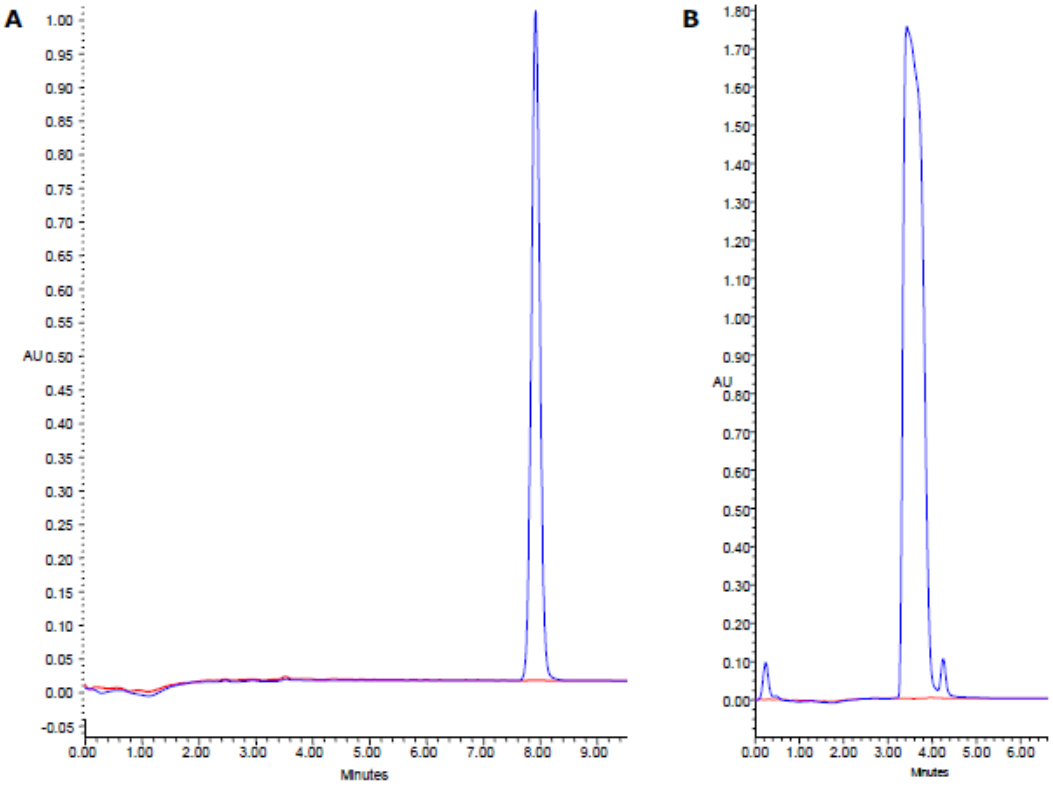

Figure 2. Chromatographic profile HPLC-PDA obtained before and after the derivatization using the $ABTS^{\cdot+}$ reagent for: hydroxymethylfurfural (**A**); and furosine (**B**). The measurement was performed three times for independent samples and there were no significant differences between the results. ($p > 0.05$).

To explain the increase in antioxidant capacity, further analysis of powder structure and other compounds found in sea buckthorn powders and the kinetics of their degradation or formation should be performed. Analysis of phenolic and other compounds by LC-MS in powders before and after storage can be valuable. According to Rocha-Parra et al. [37], losses of phenolic compounds in freeze-dried encapsulated red wine were also not reflected in antioxidant capacity changes. Reactions between oxidized phenolics can therefore increase the antioxidant capacity.

3. Materials and Methods

3.1. Chemicals

All standards used for Ultra-Performance Liquid Chromatography Photodiode Array Detector (UPLC-PDA) assays were bought from Extrasynthese (Lyon, France). Ascorbic acid and acetonitrile for

ultraperformance liquid chromatography UPLC (gradient grade), carrier agents and the rest of the reagents were procured from Merck (Darmstadt, Germany).

3.2. Material and Sample Preparation

Sea buckthorn berries of cultivar "Józef" were collected from the Experimental Orchard in Dąbrowice of the Research Institute of Horticulture in Skierniewice (Poland). Sea buckthorn juice was squeezed from selected fruits using a laboratory hydraulic press (SRSE, Warsaw, Poland), centrifuged at 5000× *g* for 10 min (Sigma 6 K15, Shrewsbury, UK) and portioned into four parts. Each portion was mixed with 20% (*w*/*w*) commercial inulin (INU), maltodextrin (MALTO) and inulin with maltodextrin in 2:1 (I:M) and 1:2 proportions (I:M), separately. The 20% addition of carrier agents was determined experimentally on the basis of solubility in juice, drying tests and the properties of finished products.

3.3. Drying Methods

Each variant of the juice with carrier agent was divided into five parts (ca. 100 mL each) to undergo various drying methods: spray drying (SD), freeze drying (FD) and vacuum drying (VD) at three different temperatures. The spray drying process of the sea buckthorn juices with different carrier agents was performed using a Büchi Mini Spray-Dryer B-290 (Büchi AG, Flawil, Switzerland). The initial temperature of the juices was 21 °C. The spray dryer operated at an inlet temperature of 180 °C and the feeding rate was 40 mL min^{-1}. The freeze-drying process was performed at temperatures from −30 to +30 °C, a pressure of 0.22 mbar and for 24 h using a Christ Alpha 1–4 LSC (Martin Christ GmbH; Osterode am Harz, Germany). The choice of carrier agents and drying parameters was determined experimentally. The drying time was determined on the basis of previous drying tests for juices in the temperature range of 50–90 °C using the determination of water. The water content was determined on the basis of the mass losses of samples during drying in a previous drying test. The process was stopped when the moisture content of samples reached below 5%. The vacuum drying processes at temperatures of 50, 70, and 90 °C were done using a Vacucell ECO line (MMM Medcenter Einrichtungen GmbH, Planegg/München, Germany), at a pressure below 0.1 mbar for 24, 20 and 16 h, respectively. The conditions used in the three drying methods were adequate to ensure complete drying of the samples with a final moisture content below 5%. All drying processes were performed in triplicate. The sea buckthorn juice powders obtained (Figure 1) were vacuum-sealed in transparent polyamide/polyethylene (PA/PE) moisture-resistant bags and stored at −18 °C for further analyses. Physical analyses and sample extractions for chemical analyses and evaluation of antioxidant activity were performed within 5 days from the production of the powders.

3.4. Storage

To determine the potential progress of the browning reaction, HMF and phenolic compounds contents, and the antioxidant activity of powders, a storage test was carried out. The second batch of sea buckthorn juice powders was stored in transparent polyamide/polyethylene (PA/PE) bags, for six months, at 20 °C and relative humidity 40%, with access to oxygen, in darkness. A laboratory incubator (ST2, POL-EKO-APARATURA, Wodzisław Śl.; Poland) was used to maintain stable conditions. After this period, the powders were again subjected to selected analyses.

3.5. Physical Properties

Moisture content (%) of the sea buckthorn juice powders was determined by the vacuum-oven method at 70 °C and pressure of 100 Pa for 24 h, using the vacuum dryer from Section 3.3. Water activity (a_w) was studied at 20 °C using a dedicated device a Novasina (LabMaster-aw, Lachen, Switzerland). True and bulk density (kg m^{-3}) and porosity (%) were studied and calculated as previously described by Turkiewicz et al. [38]. Color parameters were measured using a spectrophotometer Minolta Chrome Meter CM-700d (Konica Minolta, Inc.; Osaka, Japan) and expressed in scale of CIE L*a*b* space (10°, D65). Chroma parameter (C), hue angle (h°) and the total color change (dE) were calculated according

to Kuck and Noreña [13] and Šumić et al. [39]. Browning index was determined in powder extracts (1 g of powder in 100 mL of distilled water). The results were measured at 420 nm using a multi-mode microplate reader Synergy^{TM} H1 (BioTek, Winooski, VT, USA) and shown in arbitrary units (AU).

3.6. Determination of Phenolic Compounds and Hydroxymethylfurfural (HMF)

Analysis of phenolic compounds and hydroxymethylfurfural (HMF) were performed using an Ultra-Performance Liquid Chromatography with Photodiode Array Detector (UPLC-PDA, Acquity UPLC System, Waters Corp.; Milford, WA, USA). The extraction procedure and analysis conditions of phenolic compounds and HMF were analogous to those given previously by Tkacz et al. [2] and Turkiewicz et al. [40], respectively. Quantification was made on the basis of standard curves, using HMF; *p*-coumaric and ferulic acids; 3-*O*-glucosides, 3-*O*-rutinosides and 3-*O*-rhamnosides of isorhamnetin; quercetin; and kaempferol as standards. The other flavonol derivatives were calculated as the corresponding 3-*O*-glucoside derivatives. Phenolic acids, flavonols and HMF were detected at wavelengths 320, 360 and 284 nm, respectively. The results were expressed as mg per 100 g of dry matter (DM).

3.7. Determination of Antioxidant Capacity and Antioxidant On-Line Profiling by HPLC-PDA Coupled with Post-Column Derivatization with ABTS·$^{+}$ Reagent

The antioxidant capacity was tested as free radical-scavenging activity (ABTS·$^{+}$). The extraction and assay were conducted as previously described by Tkacz et al. [41]. The multi-mode microplate reader discussed in Section 3.5 was used. The results of antioxidant effects were calculated as mmol Trolox/100 g DM.

An on-line HPLC system was applied to verify the possible antioxidant capacity of HMF and furosine. The same ABTS·$^{+}$ reagent was used as in antioxidant capacity assay. Conditions and procedure of the assay were analogous as reported by Tkacz et al. [42]. The detection wavelengths for HMF and furosine were set at 280 nm, and discoloration of mobile phase after reaction with radical cation was detected as negative peaks at 734 nm. The chromatograms are shown as results.

3.8. Statistical Analysis

One-way analysis of variance (ANOVA) with a significance below 0.05, Duncan's multiple range test and Pearson's correlation coefficients (r) were determined to compare the samples. XLSTAT Statistical Software (Addinsoft Inc, New York, NY, USA) integrated with Microsoft Excel 2017 (Microsoft Corp.; Redmond, WA, USA) were used. Drying tests were performed three times and replicates were samples from each trial. Each of the analyses was performed three times and the results were summarized in the form of the mean with standard deviation (SD).

4. Conclusions

For the first time, research was conducted on the optimization of microencapsulation of sea buckthorn juice using both different drying methods and different carrier agents. The main results of this paper can be summarized as follows:

(1) Inulin caused stronger water retention of powders than maltodextrin. The drying method modulated the water activity more strongly than the type of carrier agents.

(2) Powders with inulin had higher true density values than those with maltodextrin. Bulk density and porosity were significantly differentiated by drying methods, and vacuum drying seems to be a useful technique to obtain powders with high bulk density. The porosity of the spray-dried and freeze-dried powders was higher than after vacuum drying.

(3) In view of the yellow color and its intensity, the use of maltodextrin was competitive compared to inulin. Moreover, spray-, freeze- and vacuum-drying at 50 °C and the addition of maltodextrin were not conducive to browning and HMF formation.

(4) Powders spray- and vacuum-dried at 70 °C had the highest concentrations of phenolic acids and flavonols, respectively. However, in stored freeze-dried powders, phenolic compound losses were the lowest. More phenolic compounds were determined in powders with maltodextrin.
(5) Storage for six months increased antioxidant capacity, but browning compounds, HMF and furosine did not affect this effect.

In conclusion, the results obtained will be useful in the selection of carrier agents and optimization of drying conditions on an industrial scale. Encapsulation technique can be valuable for extending the stability of sea buckthorn juice and for designing innovative and high-quality products, such as attractive functional foods or food ingredients, improving physical and health-promoting properties. The choice of carrier agent and its interaction with the juice should be further investigated to ensure minimal degradation of biologically active compounds and beneficial properties of finished powders. In the future, it will also be valuable to study the stability, bioavailability and kinetics of biologically active compounds released from powders or real food systems by in vitro and in vivo methods.

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Sample Availability: Samples are available from the authors.

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OŚWIADCZENIE

Oświadczam, że jestem współautorem publikacji pt.:

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Mój udział w przygotowaniu tej publikacji polegał na zaproponowaniu i tworzeniu koncepcji i planu badań, przygotowaniu materiału badawczego, uczestnictwie w procesie suszenia soku z owoców rokitnika pospolitego z nośnikami polisacharydowymi, przeprowadzeniu kontroli prób przechowalniczych i analizie właściwości fizycznych, analizie związków fenolowych i HMF metodą UPLC-PDA, profilowaniu przeciwutleniaczy on-line na drodze derywatyzacji postkolumnowej oraz aktywności przeciwutleniających *in vitro* zaprojektowanych proszków. Otrzymane wyniki opracowałam pod względem statystycznym i merytorycznym, przygotowując manuskrypt, następnie uczestniczyłam we współredagowaniu tekstu w procesie recenzji.

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mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w opracowaniu technologii produkcji proszków z soku z owoców rokitnika pospolitego, analizie związków fenolowych i HMF metodą UPLC-PDA, profilowaniu przeciwutleniaczy on-line na drodze derywatyzacji postkolumnowej oraz aktywności przeciwutleniających *in vitro* zaprojektowanych proszków. Współredagowałam manuskrypt pod względem merytorycznym, koordynowałam pracą Doktorantki, pełniłam rolę autora korespondującego w procesie publikacji oraz opiekuna naukowego w projekcie Diamentowy Grant VII (nr DI2017007047) obejmującym badania zaprezentowane w tej pracy.

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Podpis składającego oświadczenie

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OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Tkacz K., Wojdyło A., **Michalska-Ciechanowska A.**, Turkiewicz I.P., Lech K., Nowicka P. 2020. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules*, 25(17), 3801. doi: 10.3390/molecules25173801

mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w opracowaniu i koordynowaniu technologii produkcji proszków z soku z owoców rokitnika pospolitego, analizach właściwości fizycznych i aktywności przeciwutleniających *in vitro* zaprojektowanych proszków oraz merytorycznym współredagowaniu manuskryptu. Badania są efektem współpracy z Doktorantką w ramach udziału w programie szkoleniowo-mentoringowym TopMinds 2019 z inicjatywy Stowarzyszenia Top500 Innovators i Polsko-Amerykańskiej Komisji Fulbrighta.

Anna Michalska-Ciechanowska

Podpis składającego oświadczenie

mgr inż. Igor Piotr Turkiewicz

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Wrocław, 07.02.2022 r.

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Tkacz K., Wojdyło A., Michalska-Ciechanowska A., **Turkiewicz I.P.**, Lech K., Nowicka P. 2020. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules*, 25(17), 3801. doi: 10.3390/molecules25173801

mój udział polegał na uczestnictwie w etapie przygotowania materiału badawczego, procesie suszenia soku z owoców rokitnika pospolitego z nośnikami polisacharydowymi i analizie właściwości fizycznych otrzymanych proszków.

TURKIEWICZ IGOR

.....

Podpis składającego oświadczenie

dr hab. inż. Krzysztof Lech, prof. uczelni

Instytut Inżynierii Rolniczej
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ul. Chełmońskiego 37/41
51-630 Wrocław

Wrocław, 07.02.2022 r.

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Tkacz K., Wojdyło A., Michalska-Ciechanowska A., Turkiewicz I.P., **Lech K.**, Nowicka P. 2020. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules*, 25(17), 3801. doi: 10.3390/molecules25173801

mój udział polegał na koordynowaniu technologii produkcji proszków z soku z owoców rokitnika pospolitego, uczestnictwie w analizach właściwości fizycznych i przygotowaniu wyników z tego zakresu.

.....

Podpis składającego oświadczenie

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Katedra Technologii Owoców, Warzyw
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ul. Chełmońskiego 37
51-630 Wrocław

Wrocław, 07.02.2022 r.

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Tkacz K., Wojdyło A., Michalska-Ciechanowska A., Turkiewicz I.P., Lech K., **Nowicka P.** 2020. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules*, 25(17), 3801. doi: 10.3390/molecules25173801

mój udział polegał na współtworzeniu koncepcji i planu badań, uczestnictwie w analizie aktywności przeciwutleniających *in vitro* proszków z soku z owoców rokitnika pospolitego oraz współredagowaniu manuskryptu pod względem merytorycznym.

Paulina Nowicka

Podpis składającego oświadczenie

DOROBEK NAUKOWY

WYKSZTAŁCENIE:

10.2018 – nadal	**Uniwersytet Przyrodniczy we Wrocławiu, Wydział Biotechnologii i Nauk o Żywności** kierunek: Technologia Żywności i Żywienie Człowieka (studia III stopnia)
02.2017 – 06.2018	**Uniwersytet Przyrodniczy we Wrocławiu, Wydział Biotechnologii i Nauk o Żywności** kierunek: Technologia Żywności i Żywienie Człowieka specjalność: technologia żywności (dyplom z wyróżnieniem) tytuł: magister inżynier
10.2013 – 02.2017	**Uniwersytet Przyrodniczy we Wrocławiu, Wydział Biotechnologii i Nauk o Żywności** kierunek: Technologia Żywności i Żywienie Człowieka tytuł: inżynier

STAŻE ZAGRANICZNE:

01.03 – 01.07.2022	**University of Lisbon, School of Agriculture,** Lizbona, Portugalia Program Bekker (NAWA), realizacja badań pt. Functionality of hydrogels based on fruit pomace and psyllium husk: texture, rheology and bioactivity
03.02 – 03.03.2020	**University of Minnesota, Department of Food Science and Nutrition, Csallany Laboratory**, St. Paul, Minneapolis, USA Program PROM (NAWA), realizacja badań pt. Evaluation lipid peroxidation in response to a diet enriched with fatty acids
05.04 – 04.05.2019	**CEBAS-CSIC, Department of Food Science and Technology, Research Group on Quality, Safety & Bioactivity of Plant Foods**, Murcia, Hiszpania Program PROM (NAWA), realizacja badań pt. Analysis of phytoprostanes and phenolic compounds in the biologically active fraction of sea buckthorn berries by LC-MS
01.03 – 31.05.2018	**Universidad Miguel Hernández de Elche**, Departamento Tecnología Agroalimentaria, Grupo Calidad y Seguridad Alimentaria, Orihuela, Hiszpania Narodowa Agencja Erasmus+, realizacja badań pt. How Millennial generation perceives the commercial novel smoothies?

DOŚWIADCZENIE ZAWODOWE:

16.07 – 14.08.2018	**Maspex – Tymbark MWS Sp. z o.o. sp. k.**, Olsztynek, Polska (staż naukowy)
14.08 – 08.09.2017	**Maspex – Tymbark MWS Sp. z o.o. sp. k.,** Tymbark, Polska (staż zawodowy)

03.07 – 28.07.2017	**Wrocławskie Zakłady Zielarskie "Herbapol" Wrocław S.A.**, Legnica, Polska (staż zawodowy – **beneficjent konkursu stażowego**)
01.08 – 26.08.2016	**Podhalański Zakład Produkcyjny Mlekovita**, Zakopane, Polska (staż zawodowy)

PROJEKTY BADAWCZE:

01.09.2022 – 01.09.2025	**Kierownik - Preludium 20**, **Narodowe Centrum Nauki**, projekt nr 2021/41/Z/NZ9/02790, pt. Amplifikacja formuł synbiotycznych związkami fenolowymi jako czynnik modulujący biodostępność w kontekście hiperglikemii poposiłkowej
08.09.2018 – 08.09.2021	**Kierownik - Diamentowy Grant VII**, **Ministerstwo Edukacji i Nauki**, projekt nr DI2017007047, pt. Opracowanie atrakcyjnego sensorycznie produktu funkcjonalnego na bazie owoców rokitnika pospolitego z wyznaczeniem właściwości biologicznych metodami *in vitro*
26.03.2020 – 31.12.2021	**Kierownik - Innowacyjny Doktorat IV**, **Uniwersytet Przyrodniczy we Wrocławiu**, nr N070/0016/20, pt. Naturalne mikrosfery polimerowe na bazie soku z rokitnika zwyczajnego o potencjale przeciwcukrzycowym

WSPÓŁPRACA W PROJEKTACH BADAWCZYCH:

01.06.2020 – 31.05.2023	**Wykonawca - Sonata 15**, **Narodowe Centrum Nauki**, projekt nr UMO-2019/35/D/NZ9/02951, pt. Nanoemulsje jako sposób modulowania właściwości prozdrowotnych i biodostępności związków bioaktywnych izolowanych z różnych matryc roślinnych. Kierownik projektu: dr hab. inż. Paulina Nowicka, prof. uczelni
01.06.2020 – 31.05.2023	**Wykonawca - Projekt Programu Operacyjnego Inteligentny Rozwój POIR 2014-2020**, 1.1.1 Badania przemysłowe i prace rozwojowe realizowane przez przedsiębiorstwa, nr POIR.01.01.01-00-1170/19-00. pt. Innowacyjne rozwiązania technologiczne w procesie opracowywania produktów o wyższym poziomie związków bioaktywnych. Projekt współfinansowany ze środków Europejskiego Funduszu Rozwoju Regionalnego. Kierownik projektu: prof. dr hab. inż. Aneta Wojdyło
19.01.2018 – 02.02.2021	**Wykonawca - Projekt Program Operacyjny Innowacyjna Gospodarka POIG 2014-2020**, 1.1.1 Badania przemysłowe i prace rozwojowe realizowane przez przedsiębiorstwa, pt. Opracowanie nowych przetworów warzywno-owocowych o ukierunkowanych właściwościach prozdrowotnych. Kooperacja z firmą TYMBARK MWS Sp. z o.o. Sp. k., Olsztynek. Kierownik projektu po stronie UPWr: prof. dr hab. inż. Aneta Wojdyło
1.04 – 05.12.2019	**Wykonawca - Projekt finansowany przez Ministerstwo Rolnictwa i Rozwoju Wsi 04-12.2019**, nr PJ.re.027.3.2019. Innowacyjne rozwiązania w zastosowaniu warzyw i owoców. Kierownik projektu: prof. dr hab. inż. Aneta Wojdyło

01.04 – 05.12.2018	**Wykonawca - Projekt finansowany przez Ministerstwo Rolnictwa i Rozwoju Wsi 04-12.2018**, nr HOR.re.027.9.2018, pt. Badania nad innowacyjnymi rozwiązaniami w celu poprawy cech i parametrów sensorycznych produktów przetwórstwa owoców ekologicznych z uwzględnieniem zachowania składników odżywczych otrzymywanych produktów. Kierownik projektu: prof. dr hab. inż. Aneta Wojdyło
21.11.2016 – 30.06.2017	**Wykonawca - Projekt Krajowy Naukowy Ośrodek Wiodący KNOW** w ramach wsparcia naukowego doktorantów, studentów i Studenckich Kół Naukowych, pt. Oznaczanie zawartości związków bioaktywnych i aktywności przeciwutleniającej w owocach rokitnika pospolitego (*Hippophaë rhamnoides*). Uniwersytet Przyrodniczy we Wrocławiu. Opiekun projektu: dr hab. inż. Paulina Nowicka, prof. uczelni

SZKOLENIA NAUKOWE:

15.12 – 17.12.2021	**Szkolenie z obsługi wysokosprawnego cieczowego Acquity UPLC sprzężonego z wysokorozdzielczym spektrometrem mas Xevo G2 - QTof**, Waters Corp., Wrocław, Polska
19.09 – 22.09.2021	**V Akademia Chemii Analitycznej, „Spektrometria mas w chromatografii cieczowej – praktyczne zastosowania”**, Jachranka, Polska
01 – 06.2019	**Top Minds 2019 Program szkoleniowo-mentoringowy,** Stowarzyszenie Top 500 Innovators i Polsko-Amerykańska Komisja Fulbright, Warszawa, Polska
06.2019	**Chromatography and Tandem Mass Spectrometry Techniques (LC-MS/MS) in Quantitative Determinations** MS Ekspert Sp. z o. o., Warszawa, Polska
03.2018	**III Jornadas Prácticas Agroecológicas** Universitas Miguel Hernández, Escuela Politécnica Superior de Orihuela, Hiszpania
02 – 07.2016	**Ekspert Systemów Zarządzania Jakością** (IFS, BRC, Auditor HACCP i ISO 9001, Pełnomocnik ISO 22000), Swisscert Sp. z o. o., Kraków, Polska
06 – 09.2015	**Ekspert Dietetyki**, Akademia Dietetyki, Łódź, Polska

NAJWAŻNIEJSZE NAGRODY I WYRÓŻNIENIA:

12.2021	**Beneficjent Programu Bekker**, Narodowa Agencja Wymiany Akademickiej
01.09.2021– 31.08.2024	**Beneficjent Stypendium Ministra Nauki i Szkolnictwa Wyższego dla wybitnych młodych naukowców w 2021 roku**, Warszawa

01.06 – 30.06.2019	**Beneficjent Programu Szkoleniowo – Mentoringowego Top Minds 2019**, Inicjatywa Stowarzyszenia Top 500 Innovators i Polsko-Amerykańskiej Komisji Fulbrighta, Dialog, MEiN, Warszawa
04.2019 i 02.2020	**Beneficjent Programu PROM** – Międzynarodowa wymiana stypendialna doktorantów i kadry akademickiej, Narodowa Agencja Wymiany Akademickiej
15.11.2019	**Nagroda dla najlepszych prac naukowych**, praca pt. Czerwone owoce jako źródło związków biologicznie aktywnych, I Ogólnopolska Konferencja Naukowa „Dolny Śląsk jako lider w sektorze nutraceutyków, żywności prozdrowotnej i suplementów diety", Wrocław
25.04.2019	**Nagroda od Edytora *Polish Journal of Food and Nutrition Sciences* za najlepszą pracę naukową**, praca pt. Potencjał prozdrowotny owoców rokitnika pospolitego, XXIV Sesja Naukowa Sekcji Młodej Kadry Naukowej PTTŻ i VII International Session of Young Scientific Staff „Żywność - wczoraj, dziś i na zdrowej jutro", Olsztyn
03.10.2018	**Nagroda „Najlepszy Absolwent Technologii Żywności i Żywienia Człowieka"**, Władze Wydziału Biotechnologii i Nauk o Żywności, Uniwersytet Przyrodniczy we Wrocławiu, Wrocław
03.10.2018	**Nagroda „Najlepszy Dyplom Roku 2018"**, Marszałek Województwa Dolnośląskiego, Wrocław
01.10.2018 – 31.09.2022	**Beneficjent Stypendium Rektora dla najlepszych doktorantów** i **Stypendium Doktoranckiego z dotacji na zadania projakościowe**, Uniwersytet Przyrodniczy we Wrocławiu, Wrocław
04.11.2017	**Nagroda „Najlepsza Prezentacja w panelu Nauki Medyczne, Farmaceutyczne i o Zdrowiu"**, pt. Korekta smaku przecieru z rokitnika pospolitego. Ogólnopolska Konferencja Naukowa „Nauka, Badania, Rozwój", Warszawa
25.04.2017	**Nagroda za zajęcie II miejsca** za referat ustny pt. Właściwości prozdrowotne sorbetów owocowych, możliwość ich wzbogacania i akceptowalność konsumencka. XLVI Międzynarodowe Seminarium Kół Naukowych „Koła naukowe – szkołą twórczego działania", Olsztyn
06.12.2017	**Wyróżnienie dla najlepszych i najbardziej zaangażowanych studentów Kół Naukowych**, Rektor Uniwersytetu Przyrodniczego we Wrocławiu, Wrocław
03.07.2017	**Beneficjent konkursu stażowego organizowanego przez Wrocławskie Zakłady Zielarskie "Herbapol" Wrocław S.A.**, Legnica, Polska
08.12.2016	**Wyróżnienie za działalność informacyjno-edukacyjną na rzecz Uczelni**, Rektor Uniwersytetu Przyrodniczego we Wrocławiu, Wrocław

PUBLIKACJE NAUKOWE NIEWCHODZĄCE W SKŁAD ROZPRAWY DOKTORSKIEJ:

1. **Tkacz K.**, Wojdyło A., Nowicka P., Turkiewicz I.P., Golis T. 2019. Characterization *in vitro* potency of biological active fractions of seeds, skins and flesh from selected *Vitis vinifera* L. cultivars and interspecific hybrids. *Journal of Functional Foods*, *56*, 353-363. doi: 10.1016/j.jff.2019.03.029.
IF 4,451; 100 punktów MEiN; cyt. 16

2. **Tkacz K.**, Turkiewicz I., Nowicka P. 2018. Wzbogacanie przecieru z owoców rokitnika pospolitego (*Hippophaë rhamnoides* L.) niekonwencjonalnymi substancjami słodzącymi. Nauka, Badania i Doniesienia Naukowe 2018. Nauki przyrodnicze i medyczne. Idea Knowledge Future, Świebodzice, 293 – 302. ISBN 978-83-945311-7-1.

3. Wojdyło A., Nowicka P., **Tkacz K**., Turkiewicz I. P. 2021. Fruit tree leaves as unconventional and valuable source of chlorophyll and carotenoid compounds determined by liquid chromatography-photodiode-quadrupole/time of flight-electrospray ionization-mass spectrometry (LC-PDA-qTof-ESI-MS). *Food Chemistry*, *349*, 129156. doi: 10.1016/j.foodchem.2021.129156.
IF 7,514; 200 punktów MEiN; cyt. 4

4. Wojdyło A., Nowicka P., Turkiewicz I.P., **Tkacz K.** 2021. Profiling of polyphenols by LC-QTOF/ESI-MS, characteristics of nutritional compounds and in vitro effect on pancreatic lipase, α-glucosidase, α-amylase, cholinesterase and cyclooxygenase activities of sweet (*Prunus avium*) and sour (*P. cerasus*) cherries leaves and fruits. *Industrial Crops and Products*, *174*, 114214.
doi: https://doi.org/10.1016/j.indcrop.2021.114214.
IF 5,645; 200 punktów MEiN

5. Wojdyło A., Turkiewicz I. P., **Tkacz K**., Hernández F. 2021. Fruit tree leaves as new valuable source of tocopherol and tocotrienol compounds. *Journal of the Science of Food and Agriculture*, *102*(4), 11481. doi: 10.1002/jsfa.11481.
IF 3,638; 100 punktów MEiN

6. Wojdyło, A., Nowicka, P., Turkiewicz, I.P., **Tkacz, K.**, Hernández, F. 2021. Comparison of bioactive compounds and health promoting properties of fruits and leaves of apple, pear and quince. *Scientific Reports*, *11*(1), 1-17. doi: 10.1038/s41598-021-99293-x.
IF 3,998; 140 punktów MEiN; cyt. 1

7. Turkiewicz I.P., Wojdyło A., **Tkacz K.,** Nowicka P. 2021. UPLC/ESI-Q-TOF-MS analysis of (poly)phenols, tocols and amino acids in *Chaenomeles* leaves versus in vitro anti-enzyme activities. *Industrial Crops and Products*, *181*, 114829. doi: 10.1016/j.indcrop.2022.114829
IF 5,645; 200 punktów MEiN

8. Turkiewicz I.P., Wojdyło A., **Tkacz K.**, Nowicka P. 2021. Comprehensive characterization of Chaenomeles seeds as a potential source of nutritional and biologically active compounds. *Journal of Food Composition and Analysis,* 102, 104065. doi: 10.1016/j.jfca.2021.104065.
IF 4,556; 100 punktów MEiN; cyt. 1

9. Turkiewicz I.P., Wojdyło A., **Tkacz K.**, Nowicka P., Lech K., Michalska-Ciechanowska A. 2021. Physicochemical characterization and biological potential of Japanese quince polyphenol extract treated by different drying techniques. *LWT-Food Science and Technology,* 152, 112247. doi: 10.1016/j.lwt.2021.112247.
IF 4,952; 100 punktów MEiN; cyt. 1

10. Vieira M. V., Turkiewicz I.P., **Tkacz K.**, Fuentes-Grünewald C., Pastrana L. M., Fuciños P., Wojdyło A., Nowicka P. 2021. Microalgae as a potential functional ingredient: evaluation of the phytochemical profile, antioxidant activity and in-vitro enzymatic inhibitory effect of different species. *Molecules*, 26(24), 7593. doi: https://doi.org/10.3390/molecules26247593.
IF 4,412; 140 punktów MEiN

11. Cano-Lamadrid M., **Tkacz K.**, Turkiewicz I. P., Nowicka P., Hernández F., Lech, K., Carbonell-Barrachina A.A., Wojdyło A. 2021. Inhibition of enzymes associated with metabolic and neurological disorder by dried pomegranate sheets as a function of pomegranate cultivar and fruit puree. *Journal of the Science of Food and Agriculture*, *101*(6), 2294-2303. doi: 10.1002/jsfa.10850.
IF 3,638; 100 punktów MEiN; cyt. 1

12. Yusuf E., **Tkacz K**., Turkiewicz I. P., Wojdyło A., Nowicka P. 2021. Analysis of chemical compounds' content in different varieties of carrots, including qualification and quantification of sugars, organic acids, minerals, and bioactive compounds by UPLC. *European Food Research and Technology*, doi: 10.1007/s00217-021-03857-0.
IF 2,998; 70 punktów MEiN; cyt. 2

13. Haładyn K., **Tkacz K**., Wojdyło A., Nowicka P. 2021. The types of polysaccharide coatings and their mixtures as a factor affecting the stability of bioactive compounds and health-promoting properties expressed as the ability to inhibit the α-amylase and α-glucosidase of chokeberry extracts in the microencapsulation process. *Foods*, 10(9), 1994, 128105. doi: 10.3390/foods10091994.
IF 4,350; 70 punktów MEiN; cyt. 1

14. Wojdyło A., Nowicka P., **Tkacz K.**, Turkiewicz I. P. 2020. Sprouts vs. microgreens as novel functional foods: variation of nutritional and phytochemical profiles and their *in vitro* bioactive properties. *Molecules*, *25*(20), 4648. doi: 10.3390/molecules25204648.
IF 4,412; 100 punktów MEiN; cyt. 13

15. Turkiewicz I.P., Wojdyło A, **Tkacz K.**, Lech K., Michalska-Ciechanowska A., Nowicka P. 2020. The influence of different carrier agents and drying techniques on physical and chemical characterization of Japanese quince (*Chaenomeles japonica*) microencapsulation powder. *Food Chemistry*, 323: 126830. doi: 10.1016/j.foodchem.2020.126830.
IF 7,514; 140 punktów MEiN; cyt. 6

16. Turkiewicz I.P., Wojdyło A, **Tkacz K.**, Nowicka P., Golis T., Bąbelewski P. 2020. ABTS on-line antioxidant, α-amylase, α-glucosidase, pancreatic lipase, acetyl-and butyrylcholinesterase inhibition activity of *Chaenomeles* fruits determined by polyphenols and other chemical compounds. *Antioxidants*, 9, 60. doi: 10.3390/antiox9010060.
IF 6,313; 100 punktów MEiN; cyt. 5

17. Turkiewicz I.P., Wojdyło A., Lech K., **Tkacz K.**, Nowicka P. 2020. Osmotic dehydration as a pretreatment modulating the physicochemical and biological properties of Japanese quince fruit dried by the combined method. *Food and Bioprocess Technology*: 13(10). doi:10.1007/s11947-020-02522-w.
IF 4,465; 100 punktów MEiN; cyt. 2

18. Turkiewicz I.P., Wojdyło A., **Tkacz K.**, Nowicka P. 2020. Carotenoids, chlorophylls, vitamin E and amino acids profile in fruits of nineteen *Chaenomeles* cultivars. *Journal of Food Composition and Analysis:* 103608. doi: 10.1016/j.jfca.2020.103608.
IF 4,556; 100 punktów MEiN; cyt. 3

19. Cano-Lamadrid M., **Tkacz K**., Turkiewicz I. P., Clemente-Villalba J., Sánchez-Rodríguez L., Lipan L., García-García E., Carbonell-Barrachina Á.A., Wojdyło A. 2020. How a Spanish group of millennial generation perceives the commercial novel smoothies? *Foods*, *9*(9), 1213. doi: 10.3390/foods9091213.
IF 4,350; 70 punktów MEiN; cyt. 5

20. Cano-Lamadrid M., Turkiewicz I.P., **Tkacz K**., Sánchez-Rodríguez L., López-Lluch D., Wojdyło A., Sendra E., Carbonell-Barrachina Á.A. 2019. A critical overview of labeling information of pomegranate juice-based drinks: phytochemicals content and health claims. *Journal of Food Science*, 84(4), 886-894. doi: 10.1111/1750-3841.14497.
IF 3,167; 70 punktów MEiN; cyt. 5

21. Turkiewicz I.P., Wojdyło A., Lech K., **Tkacz K.**, Nowicka P. 2019. Influence of different drying methods on the quality of Japanese quince fruit. *LWT-Food Science and Technology*, 114, 108416. doi: 10.1016/j.lwt.2019.108416.
IF 4,952; 100 punktów MEiN; cyt. 8

22. Turkiewicz I.P., Wojdyło A., **Tkacz K**., Nowicka P., Hernández F. 2019. Antidiabetic, anticholinesterase and antioxidant activity vs. terpenoids and phenolic compounds in selected new cultivars and hybrids of artichoke *Cynara scolymus* L. *Molecules*, 28, 24(7). doi:10.3390/molecules24071222.
IF 4,412; 100 punktów MEiN; cyt. 17

23. Nowicka P., Turkiewicz I., Wojdyło A., **Tkacz K.** 2018. Kwiaty jadalne – właściwości prozdrowotne oraz potencjalne kierunki ich wykorzystania w przemyśle spożywczym. *Przemysł Fermentacyjny i Owocowo-Warzywny*, 01, 8-10. doi: 10.15199/64.2018.1.1.
20 punktów MEiN

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1. **Tkacz K.**, Wojdyło A., Turkiewicz I.P., Nowicka P. Natural polymeric microspheres based on sea buckthorn fruit juice with antidiabetic potential (plakat naukowy). *II Congreso Anual Internacional de Estudiantes de Doctorado*, Spain, Orihuela, 02-03.02.2022.

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INNE FORMY AKTYWNOŚCI:

09.2021	**Udział w realizacji filmów naukowo-dydaktycznych** w ramach projektu pt. „B-Innova - Master in Food Technology - wspólne studia magisterskie Uniwersytetu Przyrodniczego we Wrocławiu i University Miguel Hernandez de Elche w dyscyplinie technologia żywności i żywienie człowieka”. Koordynator: dr hab. Anna Michalska-Ciechanowska, prof. uczelni
20-21.05.2021	**Współorganizator** XXV Jubileuszowej Sesji Naukowej Sekcji Młodej Kadry Naukowej PTTŻ „Przyszłość w żywności – żywność w przyszłości”, Wrocław
30.11.2019	**Współorganizator** Konferencji pt. „Zielona Dolina”, Naczelna Organizacja Techniczna, Wrocław
26-27.03.2019	**Współorganizator** IV Konferencji Naukowej „Rolnictwo XXI wieku – problemy i wyzwania”, Krzyżowa
16.11.2018	**Współorganizator** Konferencji pt. „Odpady w gospodarce obiegu zamkniętego”, XLIV Wrocławskie Dni Nauki i Techniki, Wrocław
1.10.2018 - nadal	**Członek** Polskiego Towarzystwa Technologów Żywności (PTTŻ), Oddział we Wrocławiu
1.10.2018 - nadal	**Opiekun pomocniczy** Studenckiego Koła Naukowego FRUCTUS działającego w Katedrze Technologii Owoców, Warzyw i Nutraceutyków Roślinnych na Uniwersytecie Przyrodniczym we Wrocławiu
2016 - nadal	**Działalność wolontariacka** w ramach współpracy z Biurem Rekrutacji Uniwersytetu Przyrodniczego we Wrocławiu, Naczelną Organizacją Techniczną we Wrocławiu (NOT) i Ogólnopolskim Miesięcznikiem Ekologicznym Ekonatura

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