

Uniwersytet Przyrodniczy we Wrocławiu
Wydział Biotechnologii i Nauk o Żywności
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mgr inż. Igor Piotr Turkiewicz

**Potencjalne wykorzystanie owoców pigwowca
(*Chaenomeles* ssp.) w otrzymaniu innowacyjnych
produktów o zaprogramowanych właściwościach
prozdrowotnych**

Potential use of *Chaenomeles* ssp. fruits in obtaining innovative
products with programmed, health-promoting properties

Rozprawa doktorska

Promotor:
prof. dr hab. inż. Aneta Wojdyło

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„Jeśli potrafisz o czymś marzyć, to
potrafisz także tego dokonać”

Walt Disney

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Streszczenie

Na całym świecie obserwuje się dynamiczny wzrost liczby zachorowań na przewlekłe choroby niezakaźne, wśród których cukrzyca typu 2 i otyłość osiągnęły status epidemii XXI wieku. Jedną z głównych przyczyn rozwoju tych stanów patologicznych w organizmie człowieka jest niewłaściwy sposób odżywiania. Dieta charakteryzująca się nadmierną podażą węglowodanów i tłuszczów nasyconych, przy jednoczesnym niskim spożyciu warzyw i owoców prowadzi do zachwiania homeostazy. Kluczową rolę w prewencji i leczeniu chorób metabolicznych ogrywa zbilansowana dieta, bogata w owoce i warzywa, będące źródłem związków bioaktywnych. Jednocześnie wśród konsumentów zauważalny jest trend dbania o ciało i zdrowie poprzez stosowanie różnego typu diet oraz aktywność fizyczną. Powoduje to konieczność oferowania przez producentów żywności coraz to nowych produktów, odznaczających się nie tylko zasobnością związków bioaktywnych, ale i atrakcyjną formą spożycia. Owoce *Chaenomeles* to przykład surowca o dużym potencjale przetwórczym, ale jednocześnie wysoka zawartość kwasów organicznych dyskwalifikuje go do bezpośredniego spożycia. Na rynku wciąż brak atrakcyjnego asortymentu z owoców pigwowca, a wśród dostępnych przetworów dominują produkty wysokosłodzone i/lub zawierające niewielki dodatek tych owoców.

W związku z powyższym, celem pracy było określenie i wykorzystanie potencjału bioaktywnego owoców pigwowca (*Chaenomeles* ssp.) w otrzymaniu innowacyjnych produktów funkcjonalnych o zaprogramowanych właściwościach prozdrowotnych ukierunkowanych na prewencję i terapię wybranych stanów patologicznych.

Cel ten realizowano poprzez określenie potencjału biologicznego związków zawartych w owocach pigwowca wybranych gatunków (*C. japonica*, *C. speciosa*, *C. × superba*) i odmian (i) a następnie opracowanie i optymalizację technologii suszenia wybranymi metodami (także skojarzonymi z procesem odwadniania osmotycznego) (ii). Dodatkowo opracowano technologię mikroenkapsulacji soku z pigwowca oraz preparatu polifenolowego przy użyciu suszenia sublimacyjnego, rozpyłowego i próżniowego (iii).

W wyniku przeprowadzonych badań dowiedziono, że owoce pigwowca to atrakcyjny surowiec charakteryzujący się bogactwem składu chemicznego oraz posiadający potencjalne właściwości prozdrowotne. Zidentyfikowano 15 związków polifenolowych, 5 karotenoidów, 8 chlorofili, wszystkie izomery tokoferoli i tokotrienoli. Ponadto owoce *Chaenomeles* charakteryzowały się obecnością wszystkich aminokwasów niezbędnych oraz wykazywały zdolność do inhibicji enzymów kluczowych w prewencji cukrzycy typu 2 (α -amylaza i α -glukozydaza), otyłości (lipaza trzustkowa), chorób neurodegeneracyjnych (acetylocholinoesteraza i butyrylocholinoesteraza) i stanów zapalnych (15-lipooxygenaza). Dowiedziono, że proces suszenia kombinowanego (podsuszanie konwekcyjne i dosuszanie mikrofalowo-próżniowe) pozwala na otrzymanie suszu o jakości i zawartości związków biologicznie aktywnych na poziomie zbliżonym (51,04 g związków polifenolowych/kg suchej masy) do metody sublimacyjnej. Ponadto proces odwadniania osmotycznego w koncentratkach soków owocowych prowadzi do skrócenia czasu suszenia, obniżenia zawartości kwasów

organicznych w owocach (od 47 do 77%) oraz wzrostu potencjału antycholinergicznego. Mikroenkapsulacja soku z użyciem maltodekstryny jako nośnika oraz suszenia sublimacyjnego i rozpyłowego zapewniała otrzymanie proszków o największym stężeniu związków polifenolowych, niskiej zawartości 5-hydroksymetylofurfuralu oraz wysokim potencjale przeciwutleniającym. Natomiast najwyższą retencję związków fenolowych, w tym z grupy flawan-3-oli, w preparacie polifenolowym uzyskano po suszeniu rozpyłowym, podczas gdy suszenie próżniowe zapewniało korzystniejsze parametry fizyczne otrzymanych proszków.

Na podstawie przeprowadzonych badań chemicznych i technologicznych dowiedziono, że owoce *Chaenomeles* są dobrym surowcem do produkcji atrakcyjnych produktów suszonych a także, że technika mikroenkapsulacji to innowacyjny sposób na wykorzystanie tych owoców w przemyśle spożywczym, zapewniając wysokie stężenie związków bioaktywnych o właściwościach przeciwutleniających, przeciwcukrzycowych, przeciw otyłości i antycholinergicznym.

Słowa kluczowe: suszenie sublimacyjne, suszenie próżniowe, suszenie rozpyłowe, suszenie konwekcyjne, mikroenkapsulacja, odwadnianie osmotyczne, związki bioaktywne, właściwości przeciwutleniające, cukrzyca typu 2, choroby neurodegeneracyjne

Abstract

The incidence of chronic, non-communicable diseases is increasing rapidly worldwide, and among them type 2 diabetes and obesity have reached the status of 21st century epidemics. One of the main reasons for the development of these pathological conditions is improper nutrition. A diet containing an excessive supply of carbohydrates and saturated fats, with a low intake of fruits and vegetables, leads to an imbalance in homeostasis. Balanced diet rich in fruits and vegetables, which are a source of bioactive compounds, plays a key role in the prevention and treatment of metabolic diseases. There is also a noticeable trend among consumers to take care of their bodies and health through various types of diets and physical activity. Consequently, food manufacturers are forced to offer new products characterized not only by the abundance of bioactive compounds but also by an attractive form of consumption. *Chaenomeles* fruits are an example of a raw material with high processing potential, but high content of organic acids makes them unfit for direct consumption. Still, the market lacks an attractive *Chaenomeles* fruit assortment, and the available preserves are dominated by high-sugar products and/or those containing a small addition of *Chaenomeles* fruit.

Therefore, the purpose of this study was to determine and exploit the bioactive potential of *Chaenomeles* ssp. fruits in obtaining innovative, functional products with programmed health-promoting properties aimed at prevention and therapy of selected pathological conditions.

This objective was realized by determining the biological potential of compounds contained in *Chaenomeles* fruits of selected species (*C. japonica*, *C. speciosa*, *C. × superba*) and cultivars (*i*), and then developing and optimizing a drying technology based on selected methods (also combined with osmotic dehydration) (*ii*). Additionally, the technology of microencapsulation of *Chaenomeles* juice and a polyphenol extract by means of sublimation, spray, and vacuum drying was developed (*iii*).

The study proved that *Chaenomeles* fruits are an attractive raw material characterized by a rich chemical composition and potential health-promoting properties. Fifteen polyphenolic compounds, 5 carotenoids, 8 chlorophylls, all tocopherol and tocotrienol isomers were identified. In addition, *Chaenomeles* fruits contained all essential amino acids and were capable of inhibiting enzymes crucial in the prevention of type 2 diabetes (α -amylase and α -glucosidase), obesity (pancreatic lipase), neurodegenerative diseases (acetylcholinesterase and butyrylcholinesterase), and inflammation (15-lipoxygenase).

The combined drying (convective pre-drying followed by microwave-vacuum drying) yielded a dried product of the quality and content of biologically active compounds similar to the sublimation method (51.04 g of polyphenolic compounds/kg of dry matter). In addition, the osmotic dehydration of fruit juice concentrates reduced the drying time, lowered (from 47 to 77%) the organic acid content, and increased anticholinergic potential. Microencapsulation of the juice with maltodextrin as a carrier and sublimation and spray drying provided powders with the highest concentration of polyphenolic compounds, low content of 5-hydroxymethylfurfural, and high antioxidant potential. The highest retention of phenolic

compounds, including flavan-3-ols, in polyphenolic extract, was measured after spray drying, while vacuum drying provided more favorable physical parameters of the resulting powders.

The chemical and technological experiments performed during the study demonstrated that *Chaenomeles* fruits are a good raw material for the production of attractive dried products. They also confirmed that the microencapsulation is an innovative way to use the fruits in the food industry due to their high concentration of bioactive compounds with antioxidant, antidiabetic, anti-obesity, and anticholinergic properties.

Keywords: sublimation drying, vacuum drying, spray drying, convection drying, microencapsulation, osmotic dehydration, bioactive compounds, antioxidant properties, type 2 diabetes, neurodegenerative diseases

1. Wprowadzenie

Pigwowiec (*Chaenomeles*) należy do rodziny różowatych (Rosaceae) a jego nazwa łacińska stworzona jest z połączenia dwóch greckich słów *chainein* (dzielić, otwierać się) oraz *melon* (jabłko). Rośliny te spokrewnione są z pigwą pospolitą (*Cydonia oblonga*) i pigwą chińską (*Pseudocydonia sinensis*), lecz różnią się nie tylko wyglądem owoców, morfologią liści i kwiatów, ale przede wszystkim składem chemicznym. Wyróżnia się pięć gatunków w obrębie rodzaju *Chaenomeles*: *cathayensis*, *japonica*, *sinensis*, *speciosa* i *thibetica* oraz cztery mieszańce stworzone w wyniku krzyżowania roślin w obrębie gatunku: *C. × superba* (hybryda *C. speciosa × C. japonica*), *C. × vilmoriniana* (hybryda *C. speciosa × C. cathayensis*), i *C. × clarkiana* (hybryda *C. japonica × C. cathayensis*). Z kolei *C. × californica* jest hybrydą trójgatunkową (*C. × superba × C. cathayensis*) (Antoniewska i wsp. 2017; Nahorska i wsp. 2014; Zhang i wsp. 2019). W Polsce spotykane są głównie trzy gatunki, tj. *C. japonica* - pigwowiec japoński, *C. speciosa* - pigwowiec okazały i *C. × superba* - pigwowiec pośredni, przy czym najczęściej uprawiany jest pigwowiec japoński, ponieważ posiada najmniejsze wymagania oraz wykazuje największe przystosowanie do warunków klimatycznych. Doskonalenie metod uprawy krzewów pigwowca, celem uzyskania najwyższej jakości owoców, było prowadzone w latach 90. XX wieku w ramach specjalnego programu realizowanego w Polsce, Finlandii, Szwecji, na Litwie, Łotwie i Ukrainie (Baranowska-Bosiacka i wsp. 2017; Bieniasz i wsp. 2017; Rusnak 2012).

W stanie naturalnym pigwowiec występuje w Azji Wschodniej (Chiny i Japonia), natomiast w Europie największy areał upraw odnotowuje się w krajach nadbałtyckich. Gatunki *Chaenomeles* stały się popularnymi krzewami ozdobnymi w niektórych częściach Europy i Ameryki Północnej, uprawianymi w ogrodach zarówno ze względu na barwne kwiaty, jak i kolczaste pędy, wykorzystywane jako żywopłoty (Antoniewska i wsp. 2017; Byczkiewicz i wsp. 2019). Warto zauważyć, że pomimo niewątpliwych walorów dekoracyjnych, krzewy pigwowca uprawiane były i są dla owoców, które w tradycyjnej chińskiej medycynie wykorzystuje się od tysięcy lat. Owoce różnych gatunków *Chaenomeles* znalazły zastosowanie w leczeniu czerwonki, prozopalgii, reumatoidalnego zapalenia stawów, cholery, beri-beri, zespołu niedoboru witaminy C, zapalenia jelit i zapalenia wątroby ze względu na obecność w ich składzie wielu składników bioaktywnych, w tym głównie związków fenolowych i triterpenów (Huang i wsp. 2018; Zhang i wsp. 2014).

Dotychczas w badaniach *in vitro* i *in vivo* dokonano pozytywnej weryfikacji następujących właściwości farmakologicznych owoców pigwowca: przeciwutleniających, przeciwzapalnych, immunomodulacyjnych, przeciwdrobnoustrojowych i przeciwnowotworowych (Huang i wsp. 2018). Sproszkowane owoce *C. speciosa* wykorzystano z powodzeniem w leczeniu miażdżycy,

której patogenezą wywodzi się od utleniania lipoprotein o małej gęstości tworzących blaszkę miażdżycową. Owoce pigwowca dzięki wysokiej zawartości przeciwutleniaczy powodowały obniżenie poziomu cholesterolu we krwi (Tang i wsp. 2010). Polisacharyd (złożony z glukozy, ramnozy, galaktozy i arabinozy) wyekstrahowany z owoców *C. speciosa* hamuje wzrost guza u myszy wraz z poprawą opóźnionej nadwrażliwości i wyższym wydzielaniem interleukiny-(IL-)2, TNF- α i IFN- γ w surowicy krwi (Xie i wsp. 2015). Ponadto kwercetyna, kwas 3,4-dihydroksybenzoesowy i estry metylo-3-hydroksybutanodiowe, które zostały wyekstrahowane z *C. speciosa*, wykazują efekt synergiczny podczas leczenia ptasiej grypy i dlatego okazały się potencjalnymi i silnymi związkami przeciwwirusowymi (Zhang i wsp. 2010).

Skład chemiczny owoców *Chaenomeles* jest złożony i różni się w zależności od gatunku, wieku krzewów, środowiska, warunków klimatycznych jak i metod ekstrakcji. Badania fitochemiczne *Chaenomeles* pozwoliły na wyizolowanie około 150 substancji chemicznych, wśród których najczęściej opisywano triterpenoidy, seskwiterpenoidy, kwasy organiczne, związki fenolowe, olejki lotne i kwasy tłuszczowe (Zhang i wsp. 2019). Triterpenoidy są jednymi z bardziej reprezentatywnych składników bioaktywnych występujących w owocach pigwowca. Dotychczas wśród pentacyklicznych triterpenoidów zidentyfikowano związki z grupy ursulanów, lupulanów i oleanolanów. Na szczególną uwagę zasługuje kwas ursolowy i oleanolowy będące wskaźnikami wykorzystywanymi do oceny i klasyfikacji jakości owoców *Chaenomeles* (Zhang i wsp. 2019). Stwierdzono również, iż związki fenolowe są istotnymi substancjami bioaktywnymi obecnymi we wszystkich częściach anatomicznych *Chaenomeles* (owocach, nasionach, kwiatach, liściach, pędach i korzeniach). Do tej pory z owoców i płatków pigwowca wyizolowano i zidentyfikowano około 37 flawonoidów które można przyporządkować do 6 następujących grup: flawony, flawanony, flawano-3-ole, flawanole, izoflawony oraz antocyjany (Watychowicz i wsp. 2017; Zhang i wsp. 2019). Wśród kwasów fenolowych obecnych w owocach *Chaenomeles* w największej ilości występuje kwas chlorogenowy (Turkiewicz i wsp. 2020d; P1).

W przemyśle spożywczym wzrasta zainteresowanie wykorzystaniem zapomnianych lub mało znanych gatunków roślin. Owoce pigwowca są przykładem surowca o dużym potencjale przetwórczym o czym świadczy jego bogaty skład chemiczny. Czynnikiem limitującym wykorzystanie owoców *Chaenomeles* w produkcji żywności jest ich wysoka kwasowość, wpływająca na ich nieakceptowalny profil sensoryczny. W Polsce badania nad składem oraz zastosowaniem owoców wybranych gatunków z rodzaju *Chaenomeles* w latach 70., 80. i 90. XX wieku prowadziła Elżbieta Lesińska (Akademia Rolnicza im. H. Kołłątaja w Krakowie), w których wskazany został potencjał do zastosowania tego surowca jako składnika nadzień cukierniczych, karmelków i produktów scukrzonych. Asortyment produktowy z owoców

pigwowca dostępny na rynku jest ubogi, a najczęściej pojawiają się produkty wysokosłodzone dżemy, konfitury, marmolady i przeciery. W roku 2002 opatentowano na Litwie technologię przetwarzania pigwowca japońskiego celem poszerzenia asortymentu produktowego wytwarzanego z tego surowca, otrzymując syrop i owoce kandyzowane (LV 12779 B, 2001). Podejmowane były także próby zastosowania przetworzonych owoców pigwowca jako dodatku do wyrobów cukierniczych i nabiałowych, lecz wymagały one aplikacji substancji słodzących, celem złagodzenia odczucia smaku kwaśnego lub połączenia z innymi owocami (np. jabłkami) lecz wówczas udział procentowy pigwowca był na niskim poziomie (Antoniewska i wsp. 2017).

Dlatego też w niniejszej pracy podjęto się badań nad opracowaniem nowych, innowacyjnych kierunków wykorzystania owoców pigwowca, przy zachowaniu ich pełnego potencjału bioaktywnego.

2. Cel i hipoteza

Celem pracy było określenie i wykorzystanie potencjału bioaktywnego owoców pigwowca (*Chaenomeles* ssp.) w otrzymaniu innowacyjnych produktów funkcjonalnych o zaprogramowanych właściwościach prozdrowotnych ukierunkowanych na prewencję i terapię wybranych stanów patologicznych związanych z chorobami cywilizacyjnymi.

Postawiono następującą hipotezę badawczą: owoce pigwowca to wartościowy surowiec, którego wykorzystanie w przemyśle spożywczym można upatrywać poprzez technologię suszenia (także w połączeniu z procesem odwadniania osmotycznego) oraz mikroenkapsulację, co prowadzi do otrzymania atrakcyjnego produktu i/lub półproduktu o wysokim potencjale bioaktywnym.

Tak postawioną hipotezę zweryfikowano w obrębie celów szczegółowych prezentowanej pracy:

- a) określenie potencjału biologicznego związków zawartych w owocach pigwowca wybranych gatunków i odmian oraz otrzymanych z nich produktach;
- b) określenie przydatności owoców pigwowca w technologii suszenia poprzez wybór metody i parametrów procesu;
- c) weryfikację zastosowania odwadniania osmotycznego owoców przed suszeniem celem zwiększenia wartości biologicznej;
- d) weryfikację zastosowania procesu mikroenkapsulacji soku i preparatu polifenolowego z owoców pigwowca z użyciem różnych metod suszenia i typów nośników.

3. Organizacja badań

3.1. Materiał badawczy

Materiał do badań (**P1** i **P2**) stanowiły owoce 19 odmian *Chaenomeles* należących do trzech gatunków:

- owoce *Chaenomeles* × *superba* ‘Crimson and Gold’ i ‘Colour Trail’, *Chaenomeles japonica* ‘Cameo’ i ‘Red Joy’ oraz *Chaenomeles speciosa* ‘Nivalis’ i ‘Rubra’ pozyskano z ogrodu doświadczalnego Uniwersytetu Przyrodniczego we Wrocławiu,

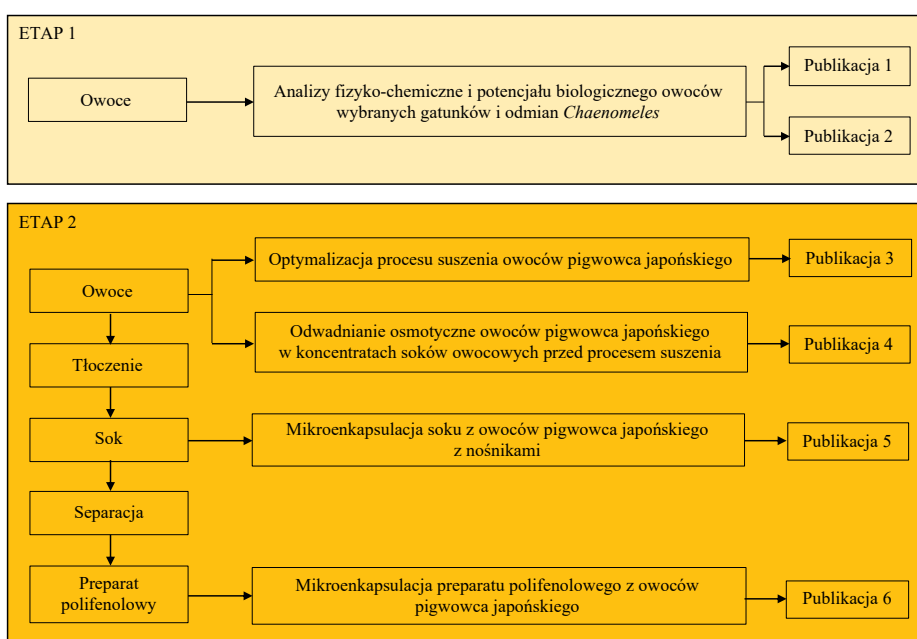
- owoce *Chaenomeles* × *superba* ‘Texas Scarlet’, ‘Nicoline’, ‘Andenken an Karl Ramcke’, ‘Pink Lady’, ‘Flavon Rose’, ‘Hollandia’, ‘Jet Trail’; *Chaenomeles japonica* ‘Cido’, n1; *Chaenomeles speciosa* ‘Simonii’ pozyskano z Instytutu Ogrodnictwa w Skierniewicach,

- owoce *Chaenomeles* × *superba* wild i *Chaenomeles japonica* wild #1 i #2 zostały zebrane z krzewów dziko rosnących we Wrocławiu.

W pozostałych badaniach (**P3**, **P4**, **P5** i **P6**) wykorzystano owoce *Chaenomeles japonica* zakupione z ekologicznej plantacji (firma Pigwowiec.eu) k. Puław.

Koncentraty soków owocowych (jabłkowy, gruszkowy, ananasowy, wiśniowy, aroniowy i z owoców czarnej porzeczki) wykorzystane w procesie odwadniania osmotycznego (**P4**) jak i nośniki (**P5**) użyte w procesie mikroenkapsulacji (inulina i maltodekstryna DE 20-30) zostały zakupione w handlu detalicznym, odpowiednio firma Grupa Maspex sp. z o.o. oraz Brenntag Polska sp. z o.o.

3.2. Schemat organizacyjny



Schemat 1. Ogólny schemat organizacyjny doświadczeń

3.3. Metodyka

3.3.1. Podstawowe analizy fizyko-chemiczne

- a) oznaczenie zawartości popiołu ogólnego (**P1**) wg PN-90/A-75101/08
- b) oznaczenie zawartości suchej masy metodą wagową (**P1, P3, P4, P5, P6**) wg PN-90/A-75101/03
- c) oznaczenie zawartości ekstraktu ogólnego metodą refraktometryczną (**P1, P4**) wg PN-90/A-75101/02
- d) oznaczenie kwasowości ogólnej metodą miareczkową (**P1**) wg PN-90/A-75101/04
- e) oznaczenie pH metodą potencjometryczną (**P1**) wg PN-90/A-75101/06
- f) oznaczenie aktywności wody (**P3, P4, P5, P6**) wg instrukcji obsługi urządzenia LabMaster-aw (Novasina, Lachen, Niemcy)
- g) oznaczenie barwy w modelu CIELab (**P3, P4, P5, P6**) wg instrukcji obsługi spektrofotometru CM-700d (Konica Minolta Sensing Inc., Osaka, Japonia)
- h) oznaczenie lepkości (**P4**) wg instrukcji obsługi wiskozymetru wibracyjnego SV-10 (A&D Company Ltd., Tokio, Japonia)
- i) oznaczenie gęstości rzeczywistej, usypowej i porowatości (**P5, P6**) wg Michalska i wsp. (2016) oraz Bhusari i wsp. (2014)
- j) oznaczenie zawartości pektyn metodą Morrisa (**P1**) wg Pijanowski i wsp. (1973) z modyfikacjami
- k) proces suszenia metodą sublimacyjną (**P1, P2, P3, P4, P5, P6**), konwekcyjną (**P3, P4**) i mikrofalowo-próżniową (**P3, P4**) przeprowadzono zgodnie z procedurą opisaną przez Turkiewicz i wsp. (2019a; **P3**) oraz Turkiewicz i wsp. (2020b; **P4**)
- l) charakterystykę kinetyki suszenia (**P3, P4**) wyznaczono w oparciu o model podany przez Lech i wsp. (2018a) oraz Lech i wsp. (2018b) z modyfikacjami
- m) proces odwadniania osmotycznego (**P4**) przeprowadzono zgodnie z procedurą opisaną przez Turkiewicz i wsp. (2020b; **P4**)
- n) proces mikroenkapsulacji metodą suszenia sublimacyjnego, próżniowego i rozpyłowego (**P5, P6**) przeprowadzono zgodnie z procedurą opisaną przez Turkiewicz i wsp. (2020c; **P5**)
- o) proces izolacji związków fenolowych na żywicy polimerowej (**P6**) przeprowadzono zgodnie z procedurą opisaną przez Michalska i wsp. (2017) i Turkiewicz i wsp. (2021; **P6**).

3.3.2. Analizy chromatograficzne

- a) oznaczenie zawartości kwasu L-askorbinowego HPLC-DAD (**P1, P3, P4**) wg Wojdyło i wsp. (2013)
- b) oznaczenie ilościowe i jakościowe cukrów HPLC-ELSD (**P1, P4, P6**) wg Wojdyło i wsp. (2017) oraz Turkiewicz i wsp. (2020d; **P1**)
- c) oznaczenie ilościowe i jakościowe kwasów organicznych UPLC-PDA (**P1, P4, P6**) wg Wojdyło i wsp. (2017) oraz Turkiewicz i wsp. (2020b; **P4**)
- d) identyfikacja LC-MS/QTOF i oznaczenie ilościowe UPLC-PDA związków fenolowych (**P1, P3, P4, P5, P6**) wg Wojdyło i wsp. (2016)
- e) oznaczenie polimerów procyjanidyny UPLC-FL wraz ze stopniem polimeryzacji metodą floroglucynolizy (**P1, P3, P4, P5, P6**) wg Wojdyło i wsp. (2013)
- f) profilowanie przeciwutleniaczy UPLC-PDA techniką derywatywacji postkolumnowej z użyciem kationorodnika ABTS^{•+} (**P1**) wg Turkiewicz i wsp. (2020d; **P1**)
- g) identyfikacja LC-MS/QTOF i oznaczenie ilościowe UPLC-PDA karotenoidów i chlorofili (**P2**) wg Wojdyło i wsp. (2018)
- h) oznaczenie ilościowe i jakościowe UPLC-FL tokoferoli i tokotrienoli (**P2**) wg Tkacz i wsp. (2019b)
- i) identyfikacja LC-MS/QTOF i oznaczenie ilościowe UPLC-PDA aminokwasów techniką derywatywacji z karbaminianem 6-aminochinolinylu-N-hydroksysukcynimidylu (AQC) (**P2**) wg Turkiewicz i wsp. (2020a; **P2**)
- j) oznaczenie ilościowe i jakościowe 5-hydroksymetylofurfuralu UPLC-PDA (**P3, P5, P6**) wg Gökmen i Şenyuva (2016) z modyfikacjami.

3.3.3. Analizy spektro- i fluorometryczne

- a) oznaczenie aktywności przeciwutleniającej metodą z kationorodnikiem ABTS^{•+} (**P1, P3, P5, P6**) wg Re i wsp. (1999) z modyfikacjami
- b) oznaczenie aktywności przeciwutleniającej metodą redukcji jonów żelaza Fe²⁺ FRAP (**P1, P3, P5, P6**) wg Benzie i Strain (1996) z modyfikacjami
- c) oznaczenie aktywności przeciwutleniającej metodą absorpcji rodników tlenowych ORAC (**P1, P3, P4, P5, P6**) wg Ou i wsp. (2002) z modyfikacjami
- d) oznaczenie aktywności przeciw cukrzycowej jako zdolności do inhibicji α -amylazy (**P1, P4, P6**) wg Podsędek i wsp. (2014) z modyfikacjami
- e) oznaczenie aktywności przeciw cukrzycowej jako zdolności do inhibicji α -glukozydazy (**P1, P4, P5, P6**) wg Podsędek i wsp. (2014) z modyfikacjami

- f) oznaczenie zdolności do inhibicji lipazy trzustkowej (**P1, P4, P5, P6**) wg Podsędek i wsp. (2014) z modyfikacjami
- g) oznaczenie aktywności antycholinergiczej jako zdolności do inhibicji acetylocholinoesterazy (AChE) (**P1, P4, P5, P6**) wg Gironés-Vilaplana i wsp. (2015) z modyfikacjami
- h) oznaczenie aktywności antycholinergiczej jako zdolności do inhibicji butyrylocholinoesterazy (BuChE) (**P1, P4, P6**) wg Gironés-Vilaplana i wsp. (2015) z modyfikacjami
- i) oznaczenie aktywności przeciwzapalnej jako zdolności do inhibicji 15-lipooxygenazy (15-LOX) (**P1, P5, P6**) wg Chung i wsp. (2009) z modyfikacjami.

3.3.4. Analiza statystyczna

Otrzymane wyniki poddano analizie statystycznej z wykorzystaniem programu Addinsoft XLSTAT 2017 - statistical and data analysis solution (New York, USA). Przeprowadzono jednokierunkową analizę wariancji (ANOVA) przy poziomie istotności $\alpha=0,05$. Grupy jednorodne określano za pomocą testu Duncana i Tukeya. Wykorzystano także test Kruskal-Wallis i procedurę Dunna do analizy nieparametrycznej. Ponadto obliczono współczynniki korelacji Pearsona (R^2) pomiędzy badanymi cechami, przeprowadzono analizę składowych głównych (PCA) oraz analizę hierarchicznego grupowania metodą aglomeracyjną (AHC).

4. Wyniki i dyskusja

4.1. Określenie składu chemicznego i potencjału biologicznego owoców *Chaenomeles* wybranych gatunków i odmian

Badania w niniejszej pracy rozpoczęto od pełnej charakterystyki składu chemicznego i właściwości biologicznych wybranych gatunków i odmian owoców pigwowca (3 gatunki, 19 odmian). Etap ten miał na celu określenie właściwości fizyko-chemicznych surowca, ze szczególnym uwzględnieniem (ilościowym i jakościowym): związków fenolowych, karotenoidów, chlorofili, tokoferoli, tokotrienoli i aminokwasów. Ponadto przeprowadzono szeroki zakres analiz potencjału biologicznego, w tym aktywności przeciwutleniającej (ABTS, FRAP, ORAC), właściwości hamowania α -amylazy, α -glukozydazy, lipazy trzustkowej, acetylocholinoesterazy (AChE), butyrylocholinoesterazy (BuChE) i 15-lipooxygenazy (15-LOX). Uzyskane wyniki zostały opublikowane w pracy **P1** (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*, 2020) oraz w pracy **P2** (Carotenoids, chlorophylls, vitamin E and amino acid profile in fruits of nineteen *Chaenomeles* cultivars, *Journal of Food Composition and Analysis*, 2020). Ten etap badań miał na celu wyselekcjonowanie odmian o najwyższym potencjale bioaktywnym oraz dostarczył informacji dla właściwego ukierunkowania dalszych badań.

W pracy **P1** (**Tab. 1**) przedstawiono podstawowy skład chemiczny i właściwości fizyczne analizowanych owoców *Chaenomeles*. Zawartość suchej masy (sm) owoców zawierała się w zakresie od 10,09% (*C. japonica* n1) do 20,40% (*C. × superba* wild) a popiołu od 0,32% (*C. × superba* 'Crimson and Gold') do 0,64% (*C. × superba* 'Pink Lady'). Wyniki te odnajdują odzwierciedlenie w danych literaturowych (Lesińska 1986, Rubinskień i wsp. 2014). Słodki smak owoców jest zależny od zawartości ekstraktu, który odgrywa ważną rolę zarówno w owocach przeznaczonych do przetwórstwa, jak i do bezpośredniego spożycia. Średnia zawartość ekstraktu analizowanych owoców pigwowca wynosiła 9°Brix. Podobne wyniki (od 5,2 do 8,8°Brix) dla 21 różnych genotypów *Chaenomeles* uzyskali Ros i i wsp. (2004). Owoce należące do rodzaju *Chaenomeles* uważane są za bogate w związki pektynowe, które znajdują się głównie w miąższu. Przeciętna zawartość pektyn w tych owocach (1,4% w świeżych owocach) jest porównywalna do wartości oznaczonych w jabłkach (Nahorska i wsp. 2014, Thomas i wsp. 2003). Analizowane owoce wykazywały duże zróżnicowanie zawartości pektyn ($p \leq 0,05$) od 0,65% (*C. japonica* n1) do 1,72% (*C. × superba* wild). Niewątpliwie cechą charakterystyczną owoców pigwowca jest wysoka kwasowość miareczkowa, która dla analizowanych odmian wynosiła średnio 4,64 g kwasu jabłkowego/100

g świeżej masy (śm). Wysokiej kwasowości towarzyszyły niskie wartości pH (2,71-2,99). Uzyskane wyniki są zgodne z danymi prezentowanymi przez Ros i wsp. (2004).

Owoce *Chaenomeles* charakteryzują się znacząco niską zawartością cukrów w porównaniu z wieloma innymi owocami. *C. × superba* ‘Texas Scarlet’ była odmianą o najwyższej zawartości cukru 3,98 g/100 g śm, natomiast *C. × superba* ‘Jet Trail’ zawierała ich ponad 9-krotnie mniej (0,44 g/100 g śm). Głównym zidentyfikowanym sacharydem była fruktoza, a następnie sorbitol i glukoza, stanowiące średnio 40,10%, 32,89% i 26,70% wszystkich zidentyfikowanych cukrów. Ksylozę w śladowych stężeniach oznaczono tylko w dwóch odmianach *C. × superba* ‘Crimson and Gold’ oraz ‘Andenken an Karl Ramcke’. Dla porównania Hellín i wsp. (2003) przeanalizowali zawartość cukru w dziesięciu genotypach *Chaenomeles* uprawianych w Szwecji i na Litwie i stwierdzili, że głównymi cukrami są fruktoza, glukoza, sorbitol i sacharoza. Zawartość cukrów redukujących była zbliżona do wartości uzyskanych we wcześniejszych badaniach, lecz zgłaszano także obecność maltozy, mannitolu, stachiozy, rafinozy, ramnozy i inozytolu (Hellín i wsp. 2003, Lesińska 1987, Lesińska i wsp. 1988, Tarko i wsp. 2014).

W przeciwieństwie do zawartości cukrów, owoce *Chaenomeles* zawierają bardzo duże ilości kwasów organicznych (**P1, Tab. 1**). W analizowanych próbkach oznaczono: kwas szczawiowy, maleinowy, cytrynowy, jabłkowy, chinowy i szikimowy. Całkowita zawartość kwasów wahała się od 41,64 (*C. japonica* ‘Cido’) do 110,31 g/kg śm (*C. × superba* wild), wykazując tym samym duże zróżnicowanie w obrębie analizowanych genotypów ($p \leq 0,05$). Głównym kwasem był kwas jabłkowy (81,94% wszystkich kwasów), obecny w próbkach w stężeniach od 32,08 do 88,75 g/kg śm, co jest zgodne z wynikami Hellín i wsp. (2003). Kwas chinowy stanowił średnio 15,63% wszystkich kwasów, a pozostałe, tj. cytrynowy, szikimowy, szczawiowy i maleinowy stanowiły mniejszość i występowały w owocach pigwowca poniżej 1,5% całkowitej ilości kwasów organicznych.

Kolejnym istotnym parametrem jakości owoców jest stosunek cukrów do kwasów. W przypadku owoców przeznaczonych do bezpośredniego spożycia przyjmuje się, aby zawartość cukrów przekraczała dziesięciokrotnie zawartość kwasów. W owocach rokitnika i cytrynach stosunek ten wynosi 1:1, a w analizowanych owocach *Chaenomeles* 0,3:1. Ze względu na powyższe technologicznie wyprodukowane 100% soki owocowe nie znajdują zainteresowania wśród konsumentów, a tym samym wśród producentów. Jako alternatywę dla dosładzania soków zaproponowano metodę biologicznego odkwaszania, polegającą na zaszczerpieniu soku bakteriami fermentacji mlekowej, które przekształcają kwas jabłkowy w kwas mlekowy charakteryzujący się łagodniejszymi właściwościami sensorycznymi. Wysokie stężenie kwasu jabłkowego powodujące bardzo niskie pH (wartość poniżej 3) nie

pozwoili na efektywny przebieg tego procesu (badania własne, nie publikowane). Stąd był to jeden z powodów odrzucenia koncepcji przygotowania produktów płynnych jako potencjalnego sposobu wykorzystania owoców pigwowca. Również niezadowolające były próby zastosowania soku pigwowcowego w mieszaninie z innymi surowcami (gruszki, jabłka) gdyż dodatek był ograniczony do poziomu 10-20% co stanowiło niezadowolający udział tego surowca w produkcie finalnym, nie wykorzystując tym samym pełnego potencjału biologicznego owoców pigwowca.

Przeprowadzona analiza związków fenolowych techniką LC-MS/QTOF i UPLC-PDA pozwoliła zidentyfikować w sumie 15 związków polifenolowych (**P1, Tab. 2**) w 19 odmianach trzech gatunków *Chaenomeles*. Wśród tych związków 13 należało do grupy flawan-3-oli (monomery, dimery, trimery i tetramery procyanidyn) a dwa pozostałe były pochodnymi kwasu kawoilochinowego.

Owoce *C. × superba* 'Nicoline' zawierały najwyższe stężenie związków fenolowych (170,38 g/kg sm), a owoce *C. speciosa* 'Rubra' najniższe (57,84 g/kg sm) ($p \leq 0,05$). Procyjanidyna B2 była związkiem obecnym w największej ilości, w zakresie od 3,39 do 18,16 g/kg sm. Zawartość polimerycznych procyanidyn we wszystkich badanych genotypach wahała się od 34,60 (*C. × superba* 'Colour Trail') do 109,67 g/kg sm (*C. × superba* 'Nicoline'), przy średniej zawartości 63,27 g/kg sm. Stopień polimeryzacji (DP) wahał się od 2,43 do 4,25, co wskazuje, że analizowane flawan-3-ole były oligomerami ($2 < DP < 10$) o niskim stopniu polimeryzacji. Niskie wartości DP w owocach pigwowca sprawiają, że w smaku nie są one bardzo cierpkie i gorzkie, tak jak np. owoce aronii, które również zawierają znaczne ilości związków procyanidynowych, ale o znacznie wyższym stopniu polimeryzacji (Wojdyło i wsp. 2014). Oznaczona całkowita zawartość związków fenolowych, tak jak ilość polimerów procyanidyn, była istotnie wyższa w analizowanych owocach niż oznaczone przez Du i wsp. (2013) oraz Teleszko i Wojdyło (2015).

Po raz pierwszy przeprowadzono szczegółową charakterystykę chlorofili i karotenoidów w owocach pigwowca przy użyciu techniki LC-MS/QTOF i UPLC-PDA. Zidentyfikowano pięć związków z grupy karotenoidów (pochodne karotenu i luteiny) oraz osiem pochodnych chlorofilu (w tym chlorofilidy, feoforbidy i feofityny) (**P2, Tab. 1**). Owoce *C. × superba* 'Nicoline' zawierały najwyższe (314,94 i 227,19 mg/kg sm), a *C. × superba* 'Pink Lady' najniższe (32,44 i 19,27 mg/kg sm) stężenie karotenoidów i chlorofili ($p \leq 0,05$). Wśród karotenoidów głównym zidentyfikowanym związkiem był all-*trans*- β -karoten (średnio 33,17 mg/kg sm), a wśród chlorofili feofityna *a* (średnio 20,60 mg/kg sm). Ponadto wykazano, że zawartość karotenoidów była istotnie wyższa niż chlorofili (dla *C. speciosa* 'Simonii' nawet 3 razy). Podobną zależność wykazali Ponder i Hallmann (2017). Porównując zawartość

naturalnych barwników w owocach *Chaenomeles* z innymi owocami o jasnym miąższu stwierdzono, że koncentracja karotenoidów i chlorofili w badanych owocach jest od 2 do 8 razy wyższa niż w miąższu jabłek i jest zbliżona do ich stężenia w skórce (58,72-1510,77 mg/kg sm) (Delgado-Pelayo i wsp. 2014).

W analizowanych owocach pigwowca zidentyfikowano cztery izomery tokoferoli (T) i (po raz pierwszy) cztery izomery tokotrienoli (T3) (**P2, Tab. 3**). Owoce *C. speciosa* 'Rubra' były najzasobniejsze w tokoferole (37,58 mg/kg sm), a owoce *C. × superba* 'Crimson and Gold' w tokotrienole (67,35 mg/kg sm). W największej ilości został zidentyfikowany α -tokoferol (α -T), który stanowił średnio 50% sumy tych związków. Spośród wszystkich izomerów witaminy E, α -T odgrywa zasadniczą rolę w organizmie człowieka, wykazując najwyższą aktywność biologiczną (Górnaś 2015). W grupie tokoferoli poszczególne izomery uszeregowano według malejącej ilości: α -T > β -T > γ -T > δ -T, natomiast w grupie tokotrienoli: γ -T3 > β -T3 > α -T3 > δ -T3. Analizując stosunek T do T3, stwierdzono, że tokoferoli było statystycznie dwukrotnie więcej niż tokotrienoli (prawie 5-krotnie więcej w owocach *C. japonica* 'Cido'), z wyjątkiem *C. × superba* 'Crimson i Gold', 'Colour Trail' i *C. japonica* wild #1, gdzie dominowały tokotrienole ($p \leq 0,05$). Dla porównania analizowane owoce pigwowca zawierały więcej tokoferoli niż popularne owoce o jasnym miąższu, np. jabłka, gruszki lub brzoskwinie (odpowiednio 4,2; 3,0 i 7,4 mg/kg sm), ale mniej niż maliny (34,6 mg/kg sm (Chun i wsp. 2006).

Po raz pierwszy dokonano analizy pełnego profilu aminokwasowego w owocach *Chaenomeles* techniką LC-MS/QTOF i UPLC-PDA z wykorzystaniem derywatywacji z karbaminianem 6-aminochinolinylu-N-hydroksysukcynimidylu (AQC). Zidentyfikowano 10 aminokwasów, w tym trzy należące do grupy aminokwasów niezbędnych (**P2, Tab. 1 i 4**). Owoce odmiany *C. × superba* 'Jet Trail' charakteryzowała najwyższa (2326,33 mg/100 g sm) a *C. japonica* 'Cameo' najniższa (15,87 mg/100 g sm) zawartość aminokwasów ($p \leq 0,05$). W największej ilości oznaczono asparaginę i kwas glutaminowy, które stanowiły (w zależności od badanego gatunku owoców) średnio 55 i 23% (*C. × superba*), 44 i 31% (*C. japonica*) oraz 67 i 17% sumy aminokwasów (*C. speciosa*). Pozostałe aminokwasy były w stężeniach zawierających się poniżej 8% całkowitej sumy tych związków. W owocach *Chaenomeles* zidentyfikowano trzy aminokwasy egzogenne, tj. treoninę, walinę i izoleucynę. Są to aminokwasy, których organizm ludzki nie jest w stanie syntetyzować i muszą być dostarczane z pożywieniem. Treonina uczestniczy w syntezie kolagenu i elastyny, walina reguluje metabolizm mięśni i odbudowuje tkanki, a do najważniejszych funkcji izoleucyny należą regulacja poziomu cukru oraz udział w procesach energetycznych i krwiotwórczych (Wu 2013). Najwyższe stężenie treoniny stwierdzono w owocach *C. × superba* 'Jet Trail', a waliny

i izoleucyny odpowiednio w *C. speciosa* 'Simonii' i 'Rubra'. Dla porównania Zhang i wsp. (2011) w badanych owocach *C. speciosa* określili całkowitą zawartość aminokwasów na poziomie 260-500 mg/100 g sm. Podobnie Chung i wsp. (1988) w owocach *C. sinensis* (Thouin) Koehne oznaczyli zawartość wolnych aminokwasów równą 383,3 mg/100 g sm.

Zainteresowanie związkami o właściwościach przeciwutleniających wzrosło na przestrzeni ostatnich dziesięcioleci, do czego przyczyniło się odkrycie roli aktywnych form tlenu w patogenezie przewlekłych chorób niezakaźnych, do których zaliczane są m.in. choroby układu krążenia, choroby neurodegeneracyjne i cukrzyca typu 2. Obecnie istnieje wiele metod określania zdolności przeciwutleniającej, dostosowanych do specyfiki badanego materiału i uwzględniających potencjalne reakcje uboczne. W omawianej pracy wykorzystano trzy metody do oznaczenia właściwości przeciwutleniających owoców pigwowca: oparte na zdolności wychwytywania syntetycznych rodników (ABTS^{•+}), redukcji jonów metali, np. żelaza (FRAP) i pomiarze wpływu przeciwutleniaczy na szybkość procesów utleniania zachodzących w próbkach (ORAC).

Najwyższą zdolność przeciwutleniającą, zarówno ABTS, jak i FRAP, posiadały owoce *C. × superba* 'Nicoline' (20,61 i 21,32 mmol Trolox/100 g sm) (**P1, Tab. 4**). Średnia aktywność przeciwutleniająca mierzona metodami ABTS i FRAP dla analizowanych gatunków wyniosła odpowiednio dla *C. × superba* (17,39 i 17,18 mmol Trolox/100 g sm), dla *C. japonica* (14,98 i 13,90 mmol Trolox/100 g sm) oraz dla *C. speciosa* (15,27 i 14,55 mmol Trolox/100 g sm) ($p \leq 0,05$). Dla porównania Teleszko i Wojdyło (2015) dla czterech odmian pigwowca japońskiego uzyskały wyższe wartości aktywności mierzonej metodami ABTS i FRAP, odpowiednio od 44,98 do 68,37 i od 30,73 do 46,57 mmol Trolox/100 g sm. Natomiast Du i wsp. (2013) dla *C. japonica* i *C. speciosa* określili podobne wartości, odpowiednio dla ABTS (36,54 i 14,61 mmol Trolox/100 g sm) i FRAP (11,39 i 2,80 mmol Trolox/100 g sm). Najsilniejszy potencjał przeciwutleniający zmierzony w teście ORAC wykazały owoce *C. × superba* 'Colour Trail' (66,59 mmol Trolox/100 g sm). Średnia aktywność ORAC dla 19 analizowanych odmian owoców *Chaenomeles* wyniosła 48,35 mmol Trolox/100 g sm i była wyższa niż dla karczochów (27,86 mmol Trolox/100 g sm) (Turkiewicz i wsp. 2019b) i pestek winogron (36,46 mmol Trolox/100 g sm) (Tkacz i wsp. 2019a).

Ponadto przeprowadzono profilowanie przeciwutleniaczy za pomocą UPLC-PDA techniką derywatywacji postkolumnowej z użyciem kationorodnika ABTS^{•+} (**P1, Tab. 4**), które nie było jak dotąd stosowane w analizie owoców *Chaenomeles*. Miało ono na celu weryfikację potencjału przeciwutleniającego poszczególnych zidentyfikowanych związków fenolowych. Uzyskane wyniki potwierdziły, że związki fenolowe z grupy flawan-3-oli wyróżniają się silnymi właściwościami przeciwutleniającymi oraz że (-)-epikatechina i jej polimery

charakteryzują się silniejszymi właściwościami przeciwutleniającymi niż (+)-katechina i jej pochodne. Co więcej, w wyniku przeprowadzonej analizy stwierdzono istotne właściwości przeciwutleniające polimerów procyjanidyn, zgodnie z Raudone i wsp. (2016), którzy również dowiedli większą aktywność oligomerów i polimerów procyjanidyn niż form monomerycznych. Ponadto, zgodnie z Zhang i wsp. (2018) potwierdzono, że procyjanidyny B3, B2, C1 i (-)-epikatechina są w głównej odpowiedzialne za zdolność przeciwutleniającą owoców pigwowca.

Kluczową kwestią w przeciwdziałaniu rozwojowi cukrzycy typu 2 jest znalezienie skutecznych inhibitorów α -amylazy trzustkowej i α -glukozydazy jelitowej, odpowiedzialnych za zmniejszenie glikemii poposiłkowej. Ponadto jako doustne środki hipoglikemiczne stosuje się związki o działaniu hamującym α -glukozydazę (Tkacz i wsp. 2019b). Wartości IC_{50} (mg owoców/mL) analizowanych owoców pigwowca w przypadku inhibicji α -amylazy wahały się od 13,88 (*C. × superba* ‘Nicoline’) do 18,48 (*C. speciosa* ‘Nivalis’) i α -glukozydazy od 5,08 (*C. × superba* ‘Texas Scarlet’) do 15,19 (*C. japonica* ‘Red Joy’) (P1, Tab. 4). Miao i wsp. (2018b) przeanalizowali zdolność inhibicji α -glukozydazy przez ekstrakty otrzymane ze skórek 13 genotypów owoców *Chaenomeles* w zakresie 0,05-0,35 mg/mL i miąższu 0,04-0,43 mg/mL. Dla porównania owoce *Actinidia* wybranych odmian wykazywały również wyższą zdolność hamowania α -amylazy (4,13-6,40 mg/mL) i α -glukozydazy (0,18-10,00 mg/mL) niż badane owoce pigwowca (Wojdyło i wsp. 2017).

Hamowanie aktywności lipazy trzustkowej wykorzystywane jest w profilaktyce otyłości, ponieważ odpowiada ona za hydrolizę ponad połowy spożywanych trójglicerydów do związków niskocząsteczkowych i wolnych kwasów tłuszczowych (Nowicka i wsp. 2018). Prowadzi to w konsekwencji do zmniejszenia ilości tłuszczu wchłanianego do krwiobiegu a inhibitory tego enzymu mogą być stosowane w kontroli masy ciała. Działanie hamujące wobec lipazy trzustkowej (P1, Tab. 4) analizowanych owoców pigwowca wyrażone jako IC_{50} wahało się od 0,04 (*C. × superba* ‘Andenken an Karl Ramcke’ i *C. speciosa* ‘Rubra’) do 0,35 mg/mL (*C. japonica* wild #1) ($p \leq 0,05$). Warto zauważyć, że dla pięciu analizowanych odmian, tj. *C. × superba* ‘Colour Trail’, ‘Flavon Rose’, ‘Hollandia’, wild oraz *C. japonica* wild #2, wartości inhibicji lipazy trzustkowej oznaczono jako $<0,01$. Oznacza to, że bardzo niskie stężenie próbki miało istotny i silny potencjał hamujący. Wyniki były podobne do uzyskanych przez Nowicka i wsp. (2018) gdzie w analizie 20 odmian brzoskwini uzyskano wartości w zakresie od 0,07 do 2,06 mg/mL. Należy podkreślić, że dotychczas w literaturze brak było danych na temat aktywności owoców *Chaenomeles* w kontekście ich potencjału do hamowania lipazy trzustkowej.

Choroba Alzheimera jest uważana za jedno z najczęściej występujących zaburzeń neurodegeneracyjnych i odpowiada za ponad 80% demencji wśród osób starszych na całym świecie. Szacuje się, że do 2050 roku co minutę mogą pojawić się trzy nowe przypadki zachorowań (Honarvar i wsp. 2017). Acetylocholinesteraza (AChE) i butyrylocholinesteraza (BuChE) są kluczowymi enzymami w rozkładzie neuroprzekaźnika - acetylocholiny, a jego inhibitory są wykorzystywane w leczeniu zaburzeń neurodegeneracyjnych (Honarvar i wsp. 2017, Tkacz i wsp. 2019a). Potencjał do inhibicji AChE i BuChE przez wybrane gatunki i odmiany *Chaenomeles* wynosił $IC_{50}=6,65-20,42$ i $6,06-31,59$ mg owoców/mL enzymu (**P1, Tab. 4**). Odmianami cechującymi najwyższą zdolność hamowania AChE i BuChE były *C. speciosa* 'Rubra' i *C. japonica* 'Red Joy', podczas gdy najmniej skuteczne okazały się *C. speciosa* 'Simonii' i *C. × superba* wild #1. W literaturze niniejszego tematu informacje na temat właściwości przeciwneurodegeneracyjnych dotychczas były ograniczone, dlatego podjęto się analizy na tak szerokim spektrum genotypów. Sancheti i wsp. (2013) podczas badań *in vivo* na szczurach z indukowaną cukrzycą zaobserwowali spadek aktywności AChE po podaniu ekstraktu z owoców *Chaenomeles sinensis*.

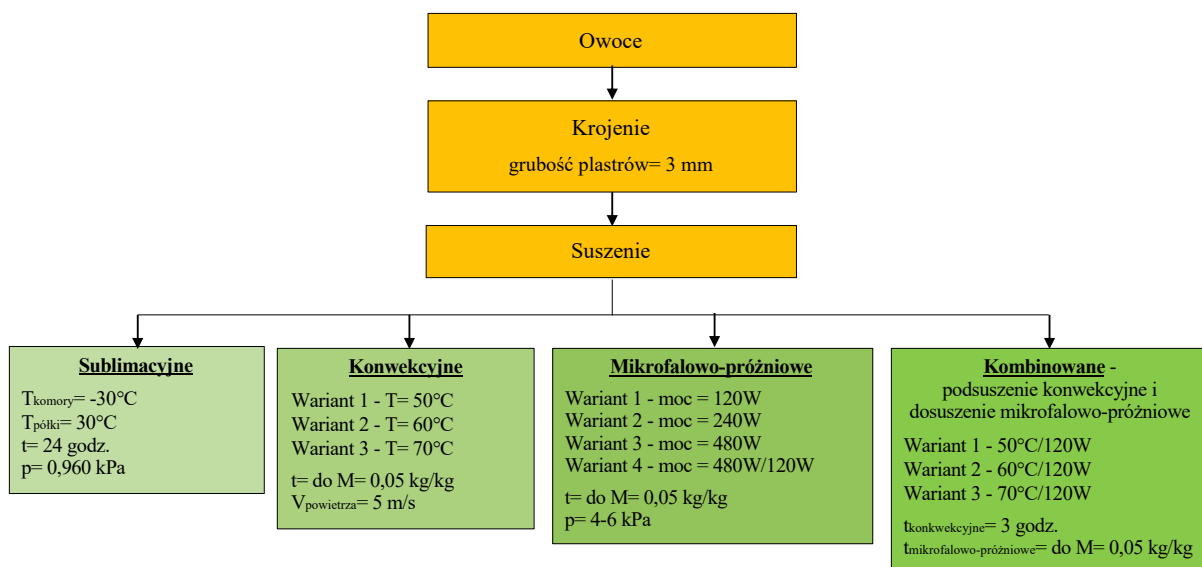
Lipoksygenazy są ważnymi enzymami w metabolizmie lipidów, które przekształcają wielonienasycone kwasy tłuszczowe, kwas arachidonowy i kwas linolowy w odpowiadające im metabolity. Inhibitory 15-lipooxygenazy (15-LOX) były głównie przedmiotem zainteresowania w leczeniu stanów zapalnych, ale także chorób nowotworowych (Orafaie i wsp. 2018). Przeprowadzone analizy i uzyskane wyniki inhibicji 15-LOX wyraźnie pokazały istotne zróżnicowanie właściwości przeciwzapalnych (wyrażonych jako % hamowania przy stężeniu próbki 5,77 mg/mL) pomiędzy badanymi gatunkami i odmianami owoców pigwowca ($p \leq 0,05$). Największym potencjałem charakteryzowały się owoce *C. × superba* 'Crimson and Gold' (99,81%), a najniższym *C. speciosa* 'Rubra' (14,29%). *C. × superba* 'Pink Lady', 'Hollandia', 'Jet Trail' i wild uzyskały wartości poza zakresem (1-100%), co oznacza, że zastosowane ekstrakty miały bardzo silne właściwości hamujące 15-LOX (**P1, Tab. 4**). Dotychczas nie prowadzono badań w tym zakresie i było to pierwsze doniesienie na temat właściwości przeciwzapalnych owoców *Chaenomeles*.

Tak dokładna analiza właściwości fizyko-chemicznych analizowanych gatunków i odmian surowca pozwoliła na szczegółową charakterystykę owoców pigwowca a także wskazanie genotypów najzasobniejszych w związki bioaktywne i wykazujących największy potencjał prozdrowotny. W kolejnych etapach badań wykorzystano owoce *Chaenomeles* gatunku *japonica* ze względu na optymalną zawartość związków fenolowych (55,67-101,96 g/kg sm), karotenoidowych (15,74-140,44 mg/kg sm) i tokoferoli (10,86-30,05 mg/kg sm) oraz

ponadprzeciętny potencjał przeciwutleniający ORAC (33,99-53,45 mmol Trolox/100 g sm). Ponadto, spośród wszystkich analizowanych gatunków, krzewy pigwowca japońskiego należą do najczęściej uprawianych na nielicznych plantacjach w Polsce, co tym samym przekłada się na ich dostępność handlową.

4.2. Wpływ wybranych metod i parametrów procesu suszenia owoców pigwowca dla zachowania najwyższej jakości suszu

W drugim etapie badań owoce pigwowca zostały poddane procesowi suszenia. Zastosowano różne metody (suszenie sublimacyjne, konwekcyjne, mikrofalowo-próżniowe i kombinowane) oraz parametry tych procesów (**Schemat 2**) w kontekście otrzymania suszu charakteryzującego się najwyższą zawartością związków biologicznie czynnych oraz aktywnością prozdrowotną określaną metodami *in vitro*. Doświadczenie to pozwoliło na finalne wytypowanie metody i parametrów procesu jako najkorzystniejszych do utrwalania owoców *Chaenomeles* otrzymując tym samym atrakcyjny produkt suszony. Uzyskane wyniki zostały opublikowane w pracy **P3** (*Influence of different drying methods on the quality of Japanese quince fruit*, *LWT*, 2019). Podjęta w tej części pracy tematyka wydaje się być kluczowa w odniesieniu do optymalizacji otrzymywania suszu z owoców pigwowca. Właściwy dobór parametrów procesu, tj. czasu, temperatury, a także mocy magnetronu w przypadku suszenia mikrofalowo-próżniowego odgrywa kluczową rolę w kształtowaniu cech fizyko-chemicznych produktu.



Schemat 2. Proces technologiczny suszenia owoców pigwowca japońskiego wybranymi metodami

T-temperatura [$^{\circ}\text{C}$], t-czas [min], p-ciśnienie [kPa], M-współczynnik wilgotności [kg/kg], V-prędkość powietrza [m/s]

Czas suszenia konwekcyjnego owoców pigwowca do tej samej wartości względnej zawartości wody wynosił od 360 do 480 min. Najdłuższy czas zmierzono przy zastosowaniu najniższej temperatury suszenia (50°C). Podniesienie temperatury powietrza suszącego o 20°C

spowodowało skrócenie czasu suszenia o 25%, co jest zgodne z wcześniejszymi wynikami Szychowski i wsp. (2018). Czas suszenia innych owoców o jasnym miąższu był znacząco dłuższy niż wymagany do wysuszenia owoców pigwowca do podobnej końcowej wilgotności - np. dla owoców jujube podwyższenie temperatury powietrza z 50 do 70°C skróciło czas suszenia o ponad 60% (Wojdyło i wsp. 2016). W przypadku suszenia mikrofalowo-próżniowego czas suszenia wahał się od 28 min dla mocy magnetronu 480 W do 96 min przy zastosowanej mocy równej 120 W. Czterokrotne zwiększenie mocy (ze 120 do 480 W) spowodowało skrócenie czasu suszenia o prawie 70%. Z kolei wzrost mocy mikrofal ze 120 do 240 W spowodował dwukrotne skrócenie czasu suszenia. W badaniach wykorzystujących metodę mikrofalowo-próżniową Wojdyło i wsp. (2016, 2019) wskazali na zależność pomiędzy mocą magnetronu lub temperatury od czasu trwania procesu.

W pracy **P3 (Rys. 1B)** przedstawiono zmiany temperatury owoców pigwowca podczas procesu suszenia mikrofalowo-próżniowego. Na początku procesu, od 2 do 8 min suszenia, w zależności od zastosowanej mocy magnetronu, zaobserwowano szybki wzrost temperatury materiału. Przy najniższej mocy magnetronu materiał suszony przez 72 min charakteryzował się maksymalną temperaturą 84°C. Podwojenie mocy mikrofal spowodowało, że temperatura wzrosła aż do 98°C w czasie poniżej 30 min. Przy zastosowaniu mocy 480 W ta sama maksymalna temperatura materiału została osiągnięta już po 26 min. Natomiast w suszeniu kombinowanym (konwekcyjno-mikrofalowo-próżniowym) dla zastosowanych temperatur powietrza suszącego maksymalne temperatury osiągane przez suszone owoce były statystycznie równoważne i osiągnęły średnio 75°C ($p \leq 0,05$). Końcowy etap procesu suszenia charakteryzował się stabilizacją temperatury, co jest związane z wyczerpaniem pokładów wolnej wody w owocach.

W prowadzonym doświadczeniu monitorowano aktywność wody (a_w) otrzymanych suszy jako wskaźnik kontroli jakości produktów suszonych, zapewniający trwałość przechowalniczą. Uzyskane wartości a_w mieszczą się w akceptowalnym, niskim zakresie gwarantującym stabilność mikrobiologiczną suszy pigwowcowych. Najniższe wartości a_w uzyskano implementując suszenie sublimacyjne, a najwyższe dla suszenia konwekcyjnego w najniższej temperaturze (**P3, Tab. 2**). W procesie suszenia konwekcyjnego wzrost temperatury o 20°C powodował spadek a_w o ponad 35%. Jednak przy suszeniu kombinowanym wpływ temperatury nie był statystycznie istotny dla uzyskiwanych wartości aktywności wody ($p \leq 0,05$). Dla porównania, Samoticha i wsp. (2016) badając wpływ metody suszenia na jakość i właściwości suszu aroniowego również uzyskali najniższe wartości a_w po suszeniu sublimacyjnym.

Owoce *Chaenomeles* należą do grupy owoców bogatych w kwas L-askorbinowy, którego stabilność jest w dużym zakresie limitowana takimi czynnikami jak: podwyższona temperatura

(>40°C), pH środowiska (obojętne i zasadowe), obecność tlenu, jonów miedzi, żelaza i srebra (Bieniasz i wsp. 2017). Dlatego też ze względu na prozdrowotne działanie kwasu L-askorbinowego ważne jest dobranie takich parametrów procesu suszenia, które zapewnią jego maksymalną retencję w produkcie końcowym. Najwyższe stężenie kwasu L-askorbinowego zmierzono po suszeniu owoców metodą mikrofalowo-próżniową przy 120 W (7669 mg/kg sm), a najniższe po suszeniu kombinowanym w 50°C (5738 mg/kg sm). Zawartość kwasu L-askorbinowego w owocach suszonych metodą mikrofalowo-próżniową była istotnie wyższa niż po procesie sublimacyjnym ($p \leq 0,05$). Wzrost temperatury w suszeniu konwekcyjnym i mocy magnetronu w suszeniu mikrofalowo-próżniowym przyczynił się do obniżenia zawartości witaminy C w podobnym stopniu. Tożsame wyniki do uzyskanych otrzymali Zaki i wsp. (2007), którzy analizowali zawartość witaminy C w owocach papai w zależności od temperatury suszenia. Doszli do wniosku, że niższa moc magnetronu korelowała z wyższym stężeniem kwasu L-askorbinowego w produkcie. Co więcej, owoce wysuszone metodą mikrofalowo-próżniową zachowały kwas L-askorbinowy na równie wysokim poziomie jak po suszeniu sublimacyjnym (7306 mg/kg sm).

W analizowanych suszach pigwowcowych oznaczono zawartość 5-hydroksymetylofurfuralu (5-HMF) (**P3, Tab. 2**). Jego obecność jest powszechnie uważana jako wskaźnik obróbki cieplnej produktów. Synteza 5-HMF odbywa się w wyniku reakcji Maillarda, które inicjowane są poprzez niskie pH środowiska, obecność cukrów i aminokwasów, oraz wysoką temperaturę (Moßhammer i wsp. 2006). Zawartość 5-HMF w owocach poddanych suszeniu różnymi metodami wahała się od 1,16 do 1,47 mg/kg sm ($p \leq 0,05$). Największe stężenie 5-HMF zmierzono w próbkach poddanych procesowi suszenia mikrofalowo-próżniowego przy 480 i 240 W, natomiast susze otrzymane metodą konwekcyjną w 60 i 70°C, mikrofalowo-próżniową z redukcją mocy magnetronu (z 480 do 120 W) oraz sublimacyjną charakteryzowała najmniejsza formacja tego związku (<1,24 mg/kg sm). Zawartość 5-HMF w próbkach suszonych mikrofalowo-próżniowo wzrastała wraz z mocą magnetronu, natomiast w suszeniu konwekcyjnym temperatura powietrza suszącego była odwrotnie proporcjonalna do stężenia 5-HMF w suszu. Dlatego należy pamiętać, że temperatura procesu nie jest jedynym czynnikiem wpływającym na stężenie tego związku w finalnym produkcie. Równie ważny jest czas trwania procesu, obecność lub brak tlenu i działanie fal magnetycznych, na co wskazują uzyskane wyniki (**P3**). Ponadto związki polifenolowe takie jak kwas chlorogenowy, który występuje w owocach pigwowca były dodatnio skorelowane z formacją 5-HMF, na co zwróciła uwagę również Michalska i wsp. (2016).

W otrzymanych suszach pigwowcowych oznaczono parametry barwy ($L^*a^*b^*$), gdyż kolor i wygląd produktu to kluczowe parametry w ocenie jakości produktu i akceptowalności konsumenckiej. Analizując zmiany parametru L^* określającego jasność barwy, we wszystkich wariantach odnotowano pociemnienie. Najniższą wartość uzyskano w próbkach suszonych mikrofalowo-próżniowych przy 480 W, a najbardziej zbliżoną wartość w porównaniu do świeżego materiału uzyskano po suszeniu sublimacyjnym. Wojdyło i wsp. (2016) i Wojdyło i wsp. (2019) podają, że suszone owoce jujuby uzyskane metodą sublimacyjną miały najjaśniejszy kolor, lecz z uwagi na kosztocłonność procesu metoda ta jest rzadziej stosowana na skalę przemysłową. Jednak suszenie mikrofalowo-próżniowe (wariant z redukcją mocy 480/120 W i 120 W) pozwala na uzyskanie produktu o jakości zbliżonej do materiału suszonego metodą sublimacyjną.

Wykorzystując metodę UPLC-PDA w suszonych owocach oznaczono związki fenolowe z grupy kwasów fenolowych, flawonoli, i flawan-3-oli, a polimery procyjanidyn dodatkowo oznaczano metodą floroglucynolizy (**P3, Tab. 3**). Zastosowane metody i parametry suszenia miały istotny wpływ na zawartość związków fenolowych ($p \leq 0,05$). Spośród wszystkich badanych grup związków przeważającą większość stanowiły polimeryczne procyjanidyny, następnie monomeryczne flawan-3-ole, kwasy fenolowe i flawonole. Najwyższe stężenie sumy związków fenolowych uzyskano po zastosowaniu procesu sublimacyjnego (57,06 g/kg sm) a największą degradację tych związków zaobserwowano po zastosowaniu najwyższej mocy magnetronu w suszeniu mikrofalowo-próżniowym (38,06 g/kg sm). Analizując całkowitą zawartość związków fenolowych stwierdzono, że w suszeniu konwekcyjnym czas suszenia był czynnikiem ograniczającym zawartość tych związków, dlatego korzystne jest podniesienie temperatury powietrza suszącego na rzecz skrócenia czasu trwania procesu. W suszeniu kombinowanym (konwekcyjno-mikrofalowo-próżniowym) zastosowanie temperatury 70°C przyczyniło się do uzyskania suszu o największej zawartości związków polifenolowych, z kolei dla suszenia mikrofalowo-próżniowego najbardziej korzystny był wariant z redukcją mocy mikrofal 480/120 W (zwiększona retencja polifenoli o ponad 20% w porównaniu do wariantu bez redukcji). Dla porównania Szychowski i wsp. (2018) badali wpływ metody suszenia na zawartość związków bioaktywnych i aktywność przeciwutleniającą owoców pigwy. Dowiedli, że oprócz techniki sublimacyjnej, susze otrzymane metodą konwekcyjną i kombinowaną charakteryzują się istotnie większą retencją związków fenolowych. Uzyskane wyniki są zgodne z wnioskami badań prowadzonych przez Miao i wsp. (2017) i Samoticha i wsp. (2016), którzy wskazali, że kluczowe znaczenie dla jakości produktu suszonego ma nie tylko dobór metody suszenia, ale również optymalizacja jej parametrów, tj. temperatury i/lub mocy magnetronu,

które mogą w istotny sposób wpływać na zawartość flawan-3-oli, flawonoli i kwasów fenolowych.

W otrzymanych próbkach suszy pigwowcowych oznaczono aktywność przeciwutleniającą za pomocą metody ABTS, FRAP i ORAC (**P3, Tab. 4**). Najwyższe wartości aktywności przeciwutleniającej jako zdolność do redukcji kationorodnika ABTS^{•+} (150,19 mmol Trolox/kg sm) jak i jonów Fe²⁺ (146,42 mmol Trolox/kg sm) zmierzono w próbkach suszonych mikrofalowo-próżniowo przy 480/120 W. Wykazano istotną zależność pomiędzy aktywnością przeciwutleniającą, która ulegała zmniejszeniu w suszeniu mikrofalowo-próżniowym wraz ze spadkiem mocy magnetronu, a w suszeniu konwekcyjnym wraz z dłuższą ekspozycją na gorące powietrze, uzyskując najmniejsze wartości dla suszenia kombinowanego. Również w metodzie ORAC owoce pigwowca poddane suszeniu mikrofalowo-próżniowemu uzyskały najwyższe wartości (805,31 mmol Trolox/kg sm po zastosowaniu mocy 480 W), podczas gdy suszenie konwekcyjne i konwekcyjno-mikrofalowo-próżniowe w 50°C i przy 120 W spowodowało 2-krotne obniżenie aktywności przeciwutleniającej. Uzyskane wyniki są zgodne z wynikami prezentowanymi w literaturze (Michalska i wsp. 2016), gdzie suszenie mikrofalowo-próżniowe przyczyniło się do wzrostu aktywności przeciwutleniającej wraz ze wzrostem mocy magnetronu.

Obliczone współczynniki korelacji Pearsona pomiędzy aktywnością przeciwutleniającą mierzoną metodami ABTS, FRAP i ORAC a całkowitą zawartością związków fenolowych wynosiły odpowiednio $R^2=0,456$; $0,253$ i $0,028$. Stwierdzono, większy udział kwasu L-askorbinowego ($R^2=0,657$) w formowaniu aktywności przeciwutleniającej ORAC niż kwasów fenolowych ($R^2=0,153$) czy 5-HMF ($R^2=0,417$).

Przeprowadzone w tym doświadczeniu badania (**P3**) dowiodły, że suszenie sublimacyjne zapewnia otrzymanie produktu charakteryzującego się najkorzystniejszymi parametrami fizyko-chemicznymi oraz najwyższą retencją związków bioaktywnych. Niemniej jednak, metoda kombinowana, łącząca konwekcyjne suszenie wstępne w temperaturze 70°C i dosuszanie mikrofalowo-próżniowe przy mocy 120 W, może być konkurencyjna szczególnie w aspekcie ekonomicznym poprzez istotne skrócenie czasu suszenia. Co więcej susze otrzymane tą metodą charakteryzowały się najwyższą retencją związków fenolowych (51,04 g/kg sm).

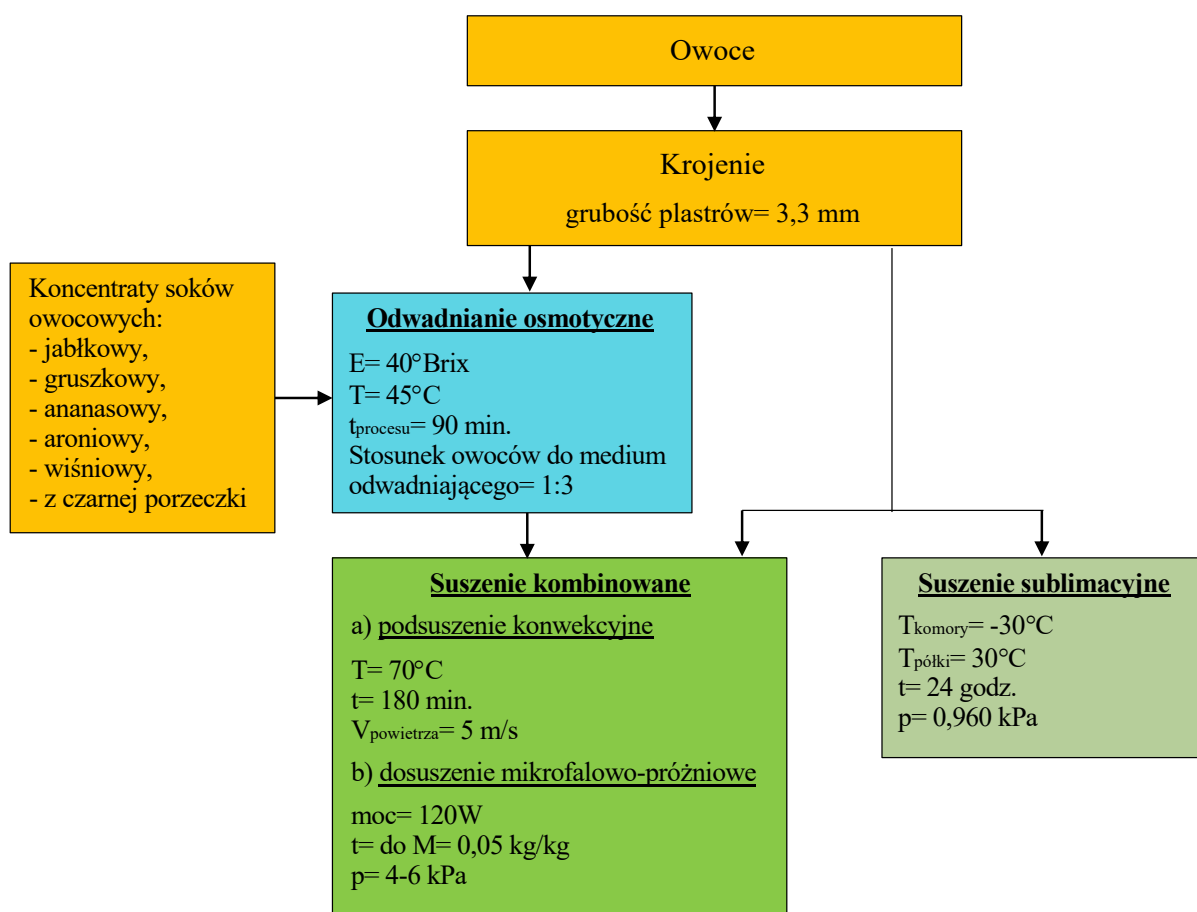
Uzyskane wyniki pozwalają stwierdzić, że suszone owoce *Chaenomeles* są ciekawą, zdrową alternatywą dla tradycyjnych, często wysokosłodzonych lub solonych przekąsek, szczególnie mając na uwadze wysoką zawartość kwasu L-askorbinowego i bogaty profil polifenolowy.

4.3. Opracowanie i optymalizacja otrzymywania suszu pigwowcowego z zastosowaniem odwadniania osmotycznego jako modulatora właściwości funkcjonalnych

Kolejnym etapem badań stanowiących część składową niniejszej rozprawy było zastosowanie zabiegu wstępnego, przed procesem suszenia, mającego na celu nie tylko skrócenie czasu suszenia, ale przede wszystkim modyfikację składu chemicznego i właściwości biologicznych suszu pigwowcowego. Realizacja tego celu opierała się na opracowaniu i optymalizacji procesu odwadniania osmotycznego w wybranych koncentratkach soków owocowych (**Schemat 3**) oraz zastosowaniu optymalnej metody suszenia na podstawie danych uzyskanych w pracy **P3**. Uzyskane wyniki zostały opublikowane w pracy **P4** (*Osmotic dehydration as a pretreatment modulating the physicochemical and biological properties of the Japanese quince fruit dried by the convective and vacuum-microwave method, Food and Bioprocess Technology, 2020*). Proces suszenia niejednokrotnie jest czasochłonny i wymaga poniesienia znaczących nakładów finansowych ze względu na jego energochłonny charakter. Zaproponowany w tym etapie badań proces odwadniania osmotycznego prowadzi do redukcji wyjściowej zawartości wody w surowcu przeznaczonym do suszenia, tym samym skracając czas tego procesu. Kolejnym aspektem jest modulacja składu fizyko-chemicznego owoców, dzięki zastosowaniu koncentratów soków owocowych, zamiast np. roztworu sacharozy. W przypadku owoców pigwowca niewątpliwą zaletą zastosowania odwadniania poprzez osmozę jest redukcja zawartości kwasów organicznych oraz nadanie odmiennych cech sensorycznych np. barwy.

W niniejszej pracy wykorzystano zmodyfikowany model Page do opisanie kinetyki procesu suszenia. Uzyskano bardzo wysoki współczynnik dopasowania ($r^2 > 0,9599$) oraz niski podstawowy błąd średniokwadratowy ($RMSE < 0,0162$) dla zastosowanej kombinowanej metody suszenia, łączącej podsuszanie konwekcyjne i dosuszanie mikrofalowo-próżniowe (**P4, Tab. 1**). Analizując zmiany współczynnika wilgotności (MR) w funkcji czasu stwierdzono, że zastosowanie procesu odwadniania osmotycznego pozwoliło uzyskać 4-krotną redukcję wartości MR w porównaniu z owocami nie odwadnianymi. W czasie 0 min podczas suszenia konwekcyjnego rodzaj użytego koncentratu nie miał istotnego wpływu ($p \leq 0,05$) na wartość MR, za wyjątkiem owoców poddanych obróbce w koncentracie ananasowym (**P4, Rys. 3a**). Podobną zależność zaobserwowano podczas odwadniania owoców wiśni (Nowicka i wsp. 2015). Uzyskanie MR na poziomie 0,25 (wartość wyjściowa dla większości próbek, z wyjątkiem wariantu odwadnianego w koncentracie ananasowym) przez owoce nie

odwadnianie zajęło ponad 70 min. Intensywna utrata wilgoci przez konwekcyjnie suszone owoce miała miejsce w pierwszych 90 minutach procesu. Następnie krzywa ulegała wyłuszczeniu, wartości MR nie zmieniały się istotnie ($p > 0,05$), przez co dalszy proces był nieefektywny i czasochłonny. Uzyskane wyniki są zgodne z badaniami prowadzonymi przez Bchir i wsp. (2012) nad odwadnianymi owocami granatu poddawanych suszeniu owiewowemu, gdzie kinetykę suszenia można również podzielić na dwie fazy. Co więcej, stabilizacja wartości MR (na poziomie 0,03-0,04) dla wszystkich odwadnianych próbek nastąpiła w 75 min procesu, podczas gdy dla owoców nie odwadnianych był to czas dwukrotnie dłuższy. Wyniki te są zbliżone do tych obserwowanych przez Bchir i wsp. (2020), w których wspomagane ultradźwiękami odwadnianie osmotyczne skróciło czas suszenia nasion granatu o ponad 40%. Po etapie podsuszania konwekcyjnego w temperaturze 70°C zawartość wilgoci we wszystkich uprzednio odwodnionych próbkach wynosiła średnio 0,086 kg/kg sm.



Schemat 3. Proces technologiczny odwadniania osmotycznego i suszenia owoców pigwowca japońskiego wybranymi metodami

T-temperatura [°C], t-czas [min], p-ciśnienie [kPa], M-współczynnik wilgotności [kg/kg], V-prędkość powietrza [m/s], E-ekstrakt [°Brix]

Ostatnim etapem suszenia było dosuszanie metodą mikrofalowo-próżniową przy mocy magnetronu 120 W. Podczas tego procesu spadek wilgotności nie był już tak znaczący jak w przypadku procesu konwekcyjnego ($p \leq 0,05$), gdzie wartości MR zmniejszyły się 25-krotnie dla wszystkich odwadnianych próbek. Na tym etapie wartości MR uległy redukcji o 15% w porównaniu z próbką nie odwadnianą. Ze wszystkich użytych koncentratów koncentrat z czarnej porzeczki obniżył wartość MR o 50% w porównaniu z owocami pigwowca bez odwadniania. Zastosowanie koncentratu ananasowego i wiśniowego skutkowało uzyskaniem wyższej wartości MR niż w przypadku pozostałych wariantów.

Sucha masa suszy pigwocowych była w zakresie od 91,89% (próbka suszona sublimacyjnie) do 95,10% (próba kontrolna, nie odwadniana). Zastosowanie procesu odwadniania osmotycznego spowodowało zmniejszenie zawartości suchej masy w porównaniu do próby kontrolnej ($p \leq 0,05$). Dla wszystkich odwodnionych wariantów, z wyjątkiem próbek procesowanych w koncentracie ananasowym, aktywność wody była poniżej 0,300, a zastosowanie procesu odwadniania osmotycznego skutkowało niższą a_w (od 8 do 17%) w porównaniu z próbą kontrolną.

Zawartość kwasu L-askorbinowego zmieniała się istotnie ($p \leq 0,05$) w zależności od użytego koncentratu owocowego (**P4, Tab. 2**). Najwyższe stężenie kwasu L-askorbinowego występowało w świeżych owocach pigwowca (770,14 mg/100 g sm), a najniższe w suszu odwadnianym w koncentracie gruszkowym (ponad 12-krotna redukcja w porównaniu ze świeżymi owocami). W odniesieniu do próby kontrolnej (nie odwadnianej), w której stężenie kwasu L-askorbinowego wynosiło 424,75 mg/100 g sm, zastosowanie tego procesu doprowadziło do obniżenia jego zawartości we wszystkich wariantach (od 69 do 85%), z wyjątkiem owoców odwadnianych w koncentracie z owoców czarnej porzeczki.

Kolejnym oznaczeniem w omawianym artykule, stanowiącym integralną część rozprawy doktorskiej, była analiza cukrów i kwasów organicznych. W analizowanych próbach suszy poddanych procesowi odwadniania osmotycznego stwierdzono znaczne zróżnicowanie w profilu cukrów ($p \leq 0,05$). Całkowita zawartość cukru wahała się od 11,56 do 43,45 g/100 g sm (odpowiednio dla owoców odwadnianych w koncentracie porzeczkowym i jabłkowym). Zastosowanie odwadniania spowodowało wzrost stężenia cukru we wszystkich próbkach od 5 do 20 razy w porównaniu z nieodwadnianymi owocami pigwowca (2,15 g/100 g sm). Biorąc pod uwagę zawartość poszczególnych cukrów, susze pigwocowe odwadniane w koncentraty z jabłka, gruszki, ananasa i czarnej porzeczki nie zawierały sorbitolu, a po zastosowaniu koncentratów z wiśni, czarnej porzeczki i aronii w próbkach nie zidentyfikowano sacharozy. Na tle wszystkich próbek wyróżniał się wariant odwadniany w koncentracie

aroniowym, w którym ponad 70% całkowitej zawartości cukrów stanowił sorbitol, co wynika z przewagi tego cukru w owocach aronii (Djuric i wsp. 2015).

Zawartość kwasów organicznych w odwadnianych suszach pigwowcowych różniła się istotnie między próbkami i wynosiła od 14,11 (wariant odwadniany w koncentracie gruszkowym) do 32,10 g/100 g sm (wariant odwadniany w koncentracie porzeczkowym) (**P4, Tab. 2**). Proces odwadniania osmotycznego, niezależnie od rodzaju użytego koncentratu, obniżył istotnie ($p \leq 0,05$) zawartość kwasów organicznych od 47 do 77% w porównaniu do próby kontrolnej (61,64 mg/100 g sm). Dla porównania Nowicka i wsp. (2015) przeanalizowali skład chemiczny suszonych owoców wiśni wstępnie odwodnionych w koncentratkach owocowych i uzyskali mniejszą redukcję kwasowości (w przeliczeniu na kwas jabłkowy) o 10-18% w porównaniu z owocami nieodwodnionymi. Dominującym kwasem we wszystkich próbkach (z wyjątkiem wariantu odwadnianego w koncentracie porzeczkowym, gdzie dominował kwas malonowy) był kwas jabłkowy i stanowił od 60 do 97% całkowitej zawartości kwasów organicznych.

Uzyskane w tej części badań wyniki są szczególnie istotne ze względu na nieakceptowalny smak świeżych owoców pigwowca, powodowany ich wysoką kwasowością. Odwadnianie osmotyczne w koncentratkach soków owocowych powodowało zrównoważenie smaku słodko-kwaśnego, co jest podstawowym aspektem dla akceptowalności konsumenckiej produktów z owoców *Chaenomeles*. Dla próbki kontrolnej (suszonej, nie odwadnianej) stosunek cukrów do kwasów był na poziomie 1:29, a zastosowany proces odwadniania osmotycznego zmodyfikował tę zależność do wartości od 1:0,4 (dla koncentratu jabłkowego) do 1:2,8 (dla koncentratu z czarnej porzeczki). Była to pierwsza próba wykorzystania procesu odwadniania osmotycznego w związku z postawionym celem utrwalenia technologicznego owoców pigwowca.

Wartości jasności barwy (L^*), zaczerwienienia (a^*), żółtości (b^*) i całkowitej zmiany barwy ($\Delta E_{a^*b^*}$) dla suszy z owoców pigwowca przedstawiono w pracy **P4 (Tab. 2)**. Parametr L^* miał wartości od 22,29 (próbka odwadniana w koncentracie aroniowym) do 84,20 (próbka suszona sublimacyjnie). Wariant odwodniony w koncentracie ananasowym miał taką samą wartość L^* jak świeże owoce pigwowca. Generalnie proces odwadniania osmotycznego spowodował znaczne przyciemnienie barwy, a tym samym obniżenie wartości parametru L^* w zakresie od 13 aż do 70% (dla koncentratu aroniowego) ($p \leq 0,05$). Odwodnienie osmotyczne w koncentracie z wiśni, czarnej porzeczki i aronii spowodowało istotny wzrost udziału koloru czerwonego w produkcie finalnym. Są to owoce bogate w antocyjany, które w warunkach kwaśnych (pH owoców pigwowca: 2,71-2,99; Turkiewicz i wsp. 2020d; **P1**) są najbardziej stabilne, co doprowadziło do zmiany barwy w kierunku odcienia czerwonego (Torskangerpoll

i Andersen 2005). Znaczne nasycenie odcieniem zielonym nastąpiło po użyciu koncentratów z wiśni, czarnej porzeczki i aronii, dla których zaobserwowano ponad 160-krotny spadek wartości koordynanty b^* . Parametr $\Delta E_{a^*b^*}$ wskazywał na wielkość różnicy koloru w porównaniu z kolorem świeżych owoców pigwowca ($L^*=64,60$, $a^*=-1,67$ i $b^*=17,78$). Przyjmuje się, że zmiana barwy dwóch próbek jest zauważalna przez obserwatora, gdy wartość $\Delta E_{a^*b^*}$ jest większa niż 5. W analizowanych próbkach wartości te wahały się od 14,70 (próbki odwadniane w koncentracie jabłkowym) do 49,64 (próbki odwadniane w koncentracie aroniowym). Ponadto zastosowanie koncentratu jabłkowego skutkowało mniejszą zmianą barwy suszu niż w przypadku próbki kontrolnej ($\Delta E_{a^*b^*}=15,91$).

Dla odwadnianych osmotycznie prób przeprowadzono identyfikację i analizę ilościową związków fenolowych (**P4, Tab. 3**). Proces odwadniania miał istotny wpływ na zawartość związków fenolowych ($p \leq 0,05$). W analizowanych suszach polimery procyjanidyny wraz z ich formami monomerycznymi (flawan-3-olami) stanowiły istotną większość w całkowitej zawartości związków fenolowych (odpowiednio 57,31 i 35,64%). Odwadnianie w koncentracie aroniowym skutkowało najmniejszą redukcją zawartości związków fenolowych (12,48 g/kg sm), natomiast największą odnotowano po zastosowaniu koncentratu gruszkowego (7,32 g/kg sm). Podsumowując, proces odwadniania osmotycznego, niezależnie od zastosowanego koncentratu, doprowadził do zmniejszenia stężenia związków fenolowych od 70 do 82% w porównaniu z próbą kontrolną (41,49 g/kg sm), co jest zgodne z wynikami uzyskanymi wcześniej przez Kucner i wsp. (2013). Podobny efekt odwadniania osmotycznego zaobserwowali Bchir i wsp. (2012) podczas badań nad nasionami granatu, gdzie po suszeniu konwekcyjnym w 60°C całkowita zawartość związków fenolowych uległa zmniejszeniu o 60% (w porównaniu z nasionami świeżymi). Obserwując zmiany w poszczególnych grupach związków fenolowych stwierdzono 4-krotną redukcję zawartości flawan-3-oli oraz 7-krotną polimerów procyjanidyn. Regres w stężeniu polifenoli po procesie odwadniania osmotycznego spowodowany był ich migracją do roztworu osmotycznego, wynikającą z różnicy potencjału osmotycznego. Wyjątkiem było wyższe stężenie kwasów fenolowych w suszach pigwowcowych odwadnianych w koncentraty gruszkowym, wiśniowym i aroniowym, oraz flawonoli po zastosowaniu koncentratu aroniowego. Dodatkowo, poprzez zastosowanie koncentratów z owoców czerwonych, wybrane susze zostały wzbogacone w antocyjany (natywnie nieobecne w owocach *Chaenomeles*) w ilości od 0,75 do 1,52 g/kg sm. Badania epidemiologiczne wskazują na odwrotną korelację między wysokim spożyciem polifenoli a występowaniem niektórych przewlekłych chorób. Antocyjany są grupą związków polifenolowych, charakterystyczną dla czerwonych owoców i warzyw, posiadającą silne działanie przeciwutleniające. Liczne badania *in vitro* i *in vivo* wskazują na ich działanie

przeciwcukrzycowe i przeciw otyłości. Związki te mogą być również przydatne jako środki o działaniu ochronnym na układ nerwowy i sercowo-naczyniowy, a także w zapobieganiu i hamowaniu rozwoju nowotworów (Smeriglio i wsp. 2016).

W omawianym doświadczeniu aktywność przeciwutleniającą suszy pigwowcowych zbadano za pomocą testu ORAC, a wyniki przedstawiono w pracy **P4 (Tab. 3)**. Najwyższą zdolnością przeciwutleniającą charakteryzował się świeży pigwowiec (128,51 mmol Trolox/100 g sm), podczas gdy najniższą zmierzono dla suszu odwadnianego w koncentracji ananasowym (32,35 mmol Trolox/100 g sm). Zastosowanie koncentratów z owoców czerwonych w procesie odwadniania osmotycznego spowodowało wzrost wartości aktywności mierzonej testem ORAC (średnio o 22%) w porównaniu z koncentratami z owoców o jasnym miąższu. Wynika to z wyższego stężenia związków fenolowych, co potwierdza wyliczony współczynnik korelacji Pearsona ($R^2=0,760$).

Aktywność hamująca wobec α -amylazy (IC_{50}) zawierała się w zakresie od 23,58 do 118,27 mg/mL (odpowiednio dla próbki suszonej sublimacyjnie i próbki kontrolnej), podczas gdy hamowanie α -glukozydazy było na poziomie od 1,01 do 20,01 mg/mL (odpowiednio dla próbki kontrolnej i suszu odwadnianego w koncentracji gruszkowym) (**P4, Tab. 3**). Dla wszystkich odwadnianych próbek zdolność hamowania α -glukozydazy była średnio 8-krotnie wyższa niż α -amylazy ($p \leq 0,05$), co według Unuofin i wsp. (2018), korzystnie wpływa na prawidłowe funkcjonowanie układu pokarmowego i potencjalne właściwości przeciwcukrzycowe. Ponadto wykazano, że próby odwadniane w koncentraty z wiśni, czarnej porzeczki i aronii wykazywały wyższy potencjał hamowania α -amylazy niż po zastosowaniu koncentratów z jabłek, gruszek i ananasów co związane było z zawartością związków fenolowych, w tym obecnością antocyjanów (**P4, Tab. 3**). W otrzymanych suszach z owoców pigwowca oznaczono także potencjał do inhibicji lipazy trzustkowej, który dla wszystkich próbek wynosił poniżej $IC_{50}=0,59$ mg/mL, podczas gdy najsilniejszy potencjał hamujący odnotowano dla świeżych owoców pigwowca ($IC_{50}=0,13$ mg/mL). Ponadto obliczony współczynnik korelacji Pearsona potwierdził silną dodatnią korelację całkowitej zawartości związków fenolowych ze zdolnością do hamowania α -glukozydazy ($R^2=0,652$) oraz między zawartością polimerycznych procyjanidyn a zdolnością do hamowania lipazy trzustkowej ($R^2=0,888$).

Wyniki z analizy aktywności antycholinergiczej przedstawiono jako % hamowania przy stężeniu 50 mg próbki na mL enzymu (**P4, Tab. 3**). Zdolność do inhibicji AChE zawierała się w przedziale się od 32,54 (wariant odwadniany w koncentracji wiśniowym) do 87,69% (świeże owoce pigwowca). Dodatkowo zaobserwowano bardzo niską aktywność inhibicji wobec AChE przez próbki suszone sublimacyjnie i próbkę kontrolną (wartości $<0,01$). Podobnie jak w przypadku zdolności do hamowania AChE, świeże owoce pigwowca odznaczały się

najwyższym potencjałem inhibicji BuChE (99,98%), a najniższym próbka kontrolna (40,46%). Warto zauważyć, że proces odwadniania osmotycznego zwiększył istotnie aktywność antycholinergiczną zarówno dla AChE (ponad 98%), jak i BuChE (ponad 30%) w porównaniu z nieodwadnianymi owocami *Chaenomeles*. Były to pierwsze badania weryfikujące wpływ procesu odwadniania osmotycznego w kontekście modyfikacji właściwości antycholinergicznym otrzymanego produktu suszonego z owoców pigwowca.

Analizie poddano także płyny osmotyczne przed i po procesie odwadniania (**P4, Tab. 2, 3, 5**). Stwierdzono zmianę parametrów fizycznych (sm, a_w , ekstrakt i lepkość) spowodowaną rozcieńczeniem roztworu wodą migrującą z owoców pigwowca, zmniejszeniem zawartości cukru i wzrostem stężenia kwasów organicznych. Ponadto roztwory odwadniające, w wyniku przeprowadzonego procesu, zostały wzbogacone w związki fenolowe i charakteryzowały się zwiększonym potencjałem przeciwutleniającym i przeciwcukrzycowym (inhibicja α -amylazy i α -glukozydazy).

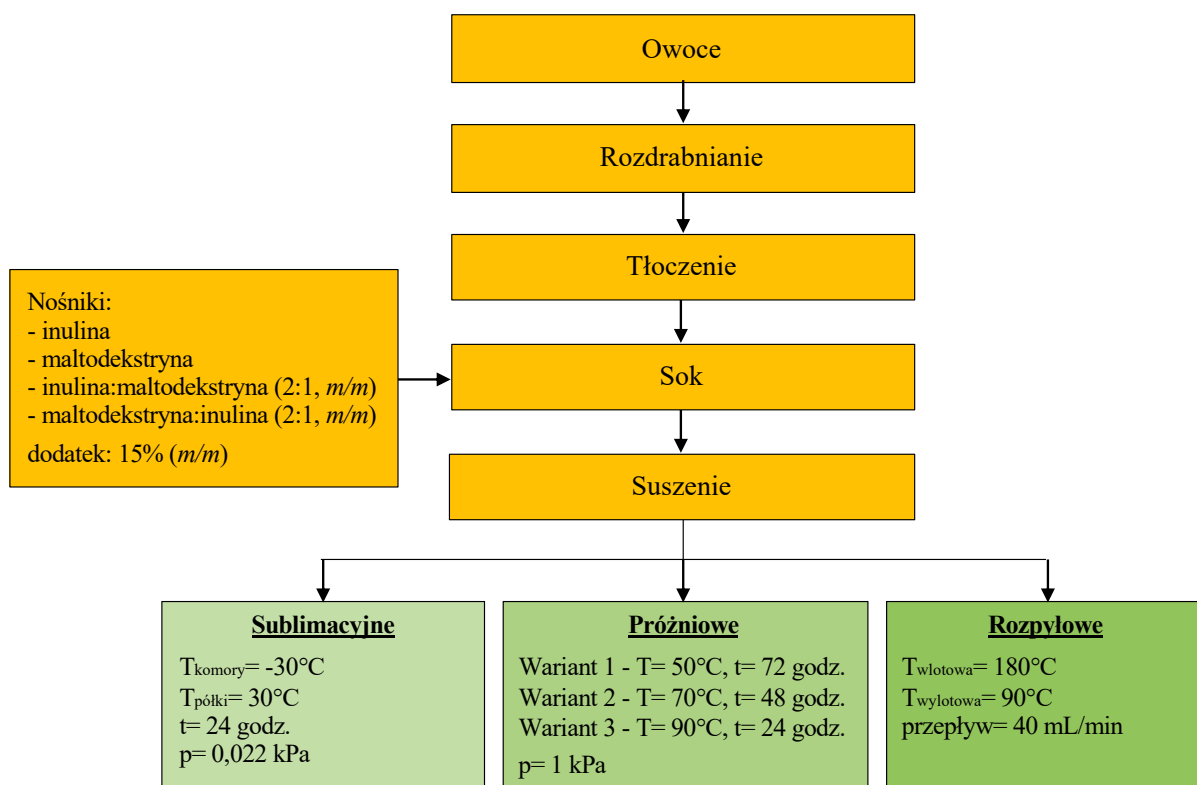
Podsumowując, proces odwadniania osmotycznego istotnie kształtował czas procesu, finalnie powodując przyspieszenie suszenia kombinowanego, co uzyskano dzięki redukcji wyjściowego współczynnika wilgotności. Zmniejszenie czasu ekspozycji produktu suszonego na działanie wysokiej temperatury oraz tlenu powoduje większe zachowanie cennych związków bioaktywnych zwiększając wartość biologiczną produktu suszonego. Co więcej uzyskano zmniejszenie charakterystycznej kwasowości owoców pigwowca, co jest jedną z głównych przyczyn ograniczających spożycie tych owoców. Była to pierwsza próba zastosowania procesu odwadniania osmotycznego m.in. celem redukcji zawartości kwasów organicznych w owocach *Chaenomeles*, otwierająca nowe możliwości aplikacji tego surowca w przemyśle spożywczym.

Dodatkowo wzrost potencjału antycholinergicznego (inhibicja AChE i BuChE) w wyniku tego procesu niewątpliwie wskazuje na potencjał wykorzystania obróbki osmotycznej jako zabiegu wstępnego przed procesem suszenia, skutkującego wielopłaszczyznowymi korzyściami.

4.4. Mikroenkapsulacja jako innowacyjny sposób wykorzystania owoców pigwowca i stabilizacji związków bioaktywnych

Mikroenkapsulacja została zaproponowana jako kolejna innowacyjna metoda wykorzystania owoców pigwowca. Produkcja proszków owocowych to interesujący i przyszłościowy kierunek dla przemysłu spożywczego, szczególnie biorąc pod uwagę zainteresowanie konsumentów zwiększeniem podaży składników bioaktywnych w diecie, celem ochrony przed rozwojem licznych chorób dietozależnych, tj. cukrzyca typu 2. Proszki owocowe otrzymywane na drodze mikroenkapsulacji mogą być aplikowane do szerokiej gamy produktów, podnosząc ich finalną wartość odżywczą. Założono, że na tym etapie badań zostanie dokonana optymalizacja procesu mikroenkapsulacji soku z owoców pigwowca z użyciem różnych metod suszenia (sublimacyjnego, próżniowego i rozpyłowego) oraz ich warunków. W przypadku soków i koncentratów owocowych nie wystarczy jedynie dobór najkorzystniejszej techniki suszenia ze względu na specyfikę tych produktów. Obecność cukrów i/lub kwasów organicznych, przy jednoczesnej wysokiej lepkości, zapobiega bezpośredniemu sproszkowaniu. Dlatego do procesu mikroenkapsulacji zastosowano wybrane nośniki (inulina i maltodekstryna) zmieniające właściwości fizyko-chemiczne proszków i ułatwiające ten proces. Na **schemacie 4** przedstawiono proces technologiczny mikroenkapsulacji z nośnikami soku z owoców pigwowca japońskiego. Uzyskane wyniki zostały opublikowane w pracy **P5** ([The influence of different carrier agents and drying techniques on physical and chemical characterization of Japanese quince \(*Chaenomeles japonica*\) microencapsulation powder, *Food Chemistry*, 2020](#)).

W omawianej pracy dokonano oznaczenia wyróżników fizycznych (sucha masa, aktywność wody) w otrzymanych proszkach a wyniki zestawiono w pracy **P5** (**Tab. 1**). Zarówno zastosowany nośnik, jak i sposób suszenia, miały istotny wpływ na zawartość sm ($p \leq 0,05$). Proszki z maltodekstryną charakteryzowały się najwyższą zawartością sm, a dodatek inuliny powodował wzrost wilgotności. Analizując wpływ metody suszenia na zawartość sm stwierdzono, że proszki z mikroenkapsulowanego soku z owoców pigwowca japońskiego otrzymane metodą suszenia próżniowego w 50°C charakteryzowały się najniższą wartością sm, a wraz ze wzrostem temperatury procesu, wzrastała zawartość sm. Spośród wszystkich metod suszenia najwyższą temperaturę (180°C) zastosowano w suszeniu rozpyłowym, niemniej jednak wartości sm dla proszków uzyskanych tą metodą były niższe niż po suszeniu próżniowym w 90°C.



Schemat 4. Proces technologiczny mikroenkapsulacji z nośnikami soku z owoców pigwowca japońskiego

T-temperatura [$^{\circ}\text{C}$], t-czas [min], p-ciśnienie [kPa]

W przeciwieństwie do wyników z analizy zawartości suchej masy, istotnie statystycznie różnice w aktywności wody były spowodowane zastosowaną metodą suszenia, a nie rodzajem użytego nośnika w procesie mikroenkapsulacji ($p \leq 0,05$). Najniższą a_w oznaczono dla wariantu z inuliną po suszeniu sublimacyjnym (0,072), a najwyższą dla wariantu z inuliną:maltodekstryną 1:2 po suszeniu próżniowym w 50°C (0,154). Obserwując wpływ metody mikroenkapsulacji odnotowano najniższe wartości a_w po suszeniu sublimacyjnym, a najwyższe dla proszków po suszeniu próżniowym przy najniższej temperaturze (50°C). Wartości a_w dla mikroenkapsulowanych proszków jabłkowych w badaniach Michalska i Lech (2018) były ok. 2 razy wyższe niż uzyskane w niniejszych badaniach, a suszenie rozpyłowe umożliwiło otrzymanie proszków o niższych wartościach a_w niż po suszeniu sublimacyjnym. Różnice te mogą wynikać m.in. z różnicy w składzie chemicznym soków owocowych (większa zawartość cukrów w soku jabłkowym).

Gęstość substancji sypkich jest ważną właściwością wpływającą na zastosowanie i funkcje wielu materiałów, a wyniki z obliczonej gęstości rzeczywistej i usypowej zestawiono w pracy **P5 (Tab. 1)**. Gęstość rzeczywista (ρ_t) nie różniła się istotnie między mikroenkapsulowanymi proszkami z soku z owoców pigwowca japońskiego w kontekście zastosowanego biopolimeru

($p > 0,05$). Dla wariantów z dodatkiem inuliny wartości ρ_t były na poziomie $1,5180 \text{ g/cm}^3$, a dla wariantów z maltodekstryną ok. $1,5410 \text{ g/cm}^3$. Istotny wpływ na uzyskane wartości ρ_t miała użyta metoda suszenia, a co za tym idzie temperatura i czas trwania procesu. Suszenie sublimacyjne przyczyniło się do uzyskania proszków o najniższej ρ_t , natomiast suszenie próżniowe w 90°C skutkowało uzyskaniem najwyższej wartości. Wyniki wskazują na związek z zawartością suchej masy - im wyższa wartość sm, tym wyższa gęstość rzeczywista (Koc i wsp. 2008). W przeciwieństwie do gęstości rzeczywistej, nośniki miały istotne znaczenie dla uzyskiwanych wartości gęstości usypowej (ρ_b). Proszki zawierające inulinę charakteryzowały się najniższą gęstością usypową, a najwyższą proszki z mieszanymi biopolimerami, zawierającymi w przewodzie maltodekstrynę. Korzystne, wysokie wartości ρ_b uzyskano podczas suszenia próżniowego w temperaturze 90°C . Ponadto po suszeniu próżniowym (niezależnie od temperatury suszenia) proszki charakteryzowały się wyższymi wartościami ρ_b w porównaniu z wariantami suszonymi sublimacyjnie i rozpyłowo (Caparino i wsp. 2012, Michalska i Lech 2018, Michalska i wsp. 2016). Zależność ta wynika z faktu, że po suszeniu w próżni uzyskuje się proszek o strukturze bardziej krystalicznej, czyli o mniejszej objętości, gdzie finalnie proszki charakteryzują się porowatą i płaską powierzchnią. Koszty pakowania i transportu rosną wraz ze spadkiem gęstości nasypowej (Michalska i wsp. 2017), dlatego też suszenie sublimacyjne okazało się najmniej korzystne pod tym względem.

Kolejnym parametrem opisującym właściwości fizyczne materiałów sypkich jest porowatość (ϵ) (**P5, Tab. 1**). Wśród zastosowanych nośników występowało zróżnicowanie w porowatości otrzymanych proszków ($p \leq 0,05$). Najwyższą porowatością charakteryzowały się proszki z inuliną a dodatek maltodekstryny powodowały zmniejszenie porowatości. Porównując wybrane metody suszenia, dla proszków suszonych sublimacyjnie i rozpyłowo uzyskano wyższe wartości ϵ (do 20%) w porównaniu do wariantów uzyskanych w wyniku suszenia próżniowego. Większa porowatość będzie wiązała się m.in. z większą zdolnością retencji wody, a proszki suszone w próżni będą charakteryzowały się zwartą, twardą strukturą i mniejszą rozpuszczalnością (Michalska i wsp. 2016).

Kolejnym oznaczeniem w niniejszej pracy była analiza obecności i zawartości 5-HMF w mikroenkapsułkowanych proszkach na bazie soku z owoców pigwowca. Zawartość 5-HMF (**P5, Tab. 3 i 4**) zawierała się w przedziale od 0,1 (proszki z maltodekstryną i mieszanką inuliny:maltodekstryny 2:1 suszone sublimacyjnie) do $315,6 \text{ mg/100 g sm}$ (wariant z inuliną po suszeniu próżniowym w 90°C). 5-HMF nie został zidentyfikowany w soku świeżym, co świadczy o tym, że występuje on tylko w produktach przetworzonych. Porównując zastosowane metody suszenia, proszki otrzymane w wyniku suszenia sublimacyjnego,

rozpyłowego i próżniowego w 50 i 70°C nie różniły się pod względem zawartości 5-HMF ($p>0,05$). Zastosowanie temperatury 90°C w suszeniu próżniowym spowodowało 223-krotny wzrost stężenia 5-HMF w porównaniu z proszkami po suszeniu sublimacyjnym. Podwyższenie temperatury suszenia próżniowego z 50 do 70°C zwiększyło zawartość 5-HMF 20-krotnie, a wzrost temperatury o kolejne 20°C przyczynił się do uzyskania stężenia 5-HMF prawie 260 razy wyższego. Dla porównania, dwukrotny wzrost temperatury z 40 do 80°C w procesie suszenia próżniowego soku śliwkowego spowodował 14-krotny wzrost zawartości 5-HMF (Michalska i wsp. 2017). Ponadto w badaniu Michalska i wsp. (2016) nad proszkami śliwkowymi, 5-HMF nie został wykryty w próbkach po suszeniu sublimacyjnym. Użyte nośniki miały istotny wpływ na zawartość 5-HMF w mikroenkapsulowanych proszkach z soku z owoców pigwowca ($p\leq 0,05$). Zastosowanie jako nośnika inuliny spowodowało 3-krotny wzrost zawartości 5-HMF w porównaniu z maltodekstryną. Należy stwierdzić, że wyższy udział maltodekstryny w procesie mikroenkapsulacji powoduje mniejszą formację 5-HMF.

Parametry chromatyczne L^* , a^* , b^* dla mikroenkapsulowanych proszków z soku z owoców pigwowca japońskiego przedstawiono w pracy **P5 (Tab.1)**. Wartości parametru jasności L^* wynosiły od 60,37 (wariant z mieszanką inuliny i maltodekstryny 2:1 po suszeniu próżniowym w 90°C) do 98,03 (czysta maltodekstryna). Analizując wpływ użytych nośników na wartość parametru L^* , wyższe wartości odnotowano dla proszków z maltodekstryną, a wraz ze wzrostem udziału inuliny w mieszance kolor ciemniał. Jasność proszku różniła się istotnie ($p\leq 0,05$) dla wybranych metod suszenia. Najjaśniejsze proszki uzyskano po suszeniu sublimacyjnym, a zastosowanie suszenia próżniowego w 90°C spowodowało obniżenie wartości L^* o 30%. Suszenie sublimacyjne i rozpyłowe gwarantowało otrzymanie produktu o jaśniejszej barwie niż po suszeniu w próżni. Podobną obserwację poczyniono w przypadku proszków jabłkowych (Michalska i Lech 2018). Spośród wszystkich proszków najniższe wartości koordynanty a^* odnotowano przy zastosowaniu inuliny i suszenia sublimacyjnego. Wzrost temperatury w suszeniu próżniowym o 40°C spowodował, że parametr a^* wzrósł o 5 jednostek i tym samym kolor wysycił się odcieniem czerwonym. Zastosowanie maltodekstryny przyczyniło się do uzyskania proszków o większym udziale koloru niebieskiego, natomiast inulina powodowała żółknięcie otrzymywanych proszków. Ze względu na charakterystyczną żółtą barwę owoców pigwowca i soku wyższe wartości parametru b^* będą korzystniejsze; zatem właściwe w tym kontekście wydaje się zastosowanie inuliny i suszenia sublimacyjnego w procesie mikroenkapsulacji.

Po raz pierwszy przeprowadzono szczegółową analizę profilu związków fenolowych w mikroenkapsulowanych proszkach na bazie soku z owoców pigwowca japońskiego.

Zidentyfikowano 14 związków z wykorzystaniem metody LC-MS/QTOF i UPLC-PDA głównie należących do grupy flawan-3-oli i kwasów fenolowych (**P5, Tab. 2** oraz **Rys. 1**). Całkowita zawartość związków fenolowych w proszkach otrzymanych przy użyciu różnych nośników i metod suszenia wynosiła od 133,2 do 1359,9 mg/100 g sm, odpowiednio dla wariantu z mieszanką inuliny i maltodekstryny 2:1 suszonego próżniowo w 90°C oraz próbki z maltodekstryną suszoną sublimacyjnie (**P5, Tab. 3** i **4**). Główną grupę (ponad 80% wszystkich polifenoli) stanowiły flawan-3-ole na które składało się 11 pochodnych katechiny. Trimery procyjanidyn, procyjanidyna B2 i C1 (odpowiednio 17, 14 i 11% całkowitej zawartości związków fenolowych) były dominującymi związkami w grupie flawan-3-oli, co jest zgodne z wcześniejszymi doniesieniami (Nahorska i wsp. 2014). Polimery procyjanidyn oznaczone z wykorzystaniem metody floroglucynolizy stanowiły 17%, a kwasy fenolowe 12% całkowitej zawartości polifenoli.

Wybrane metody suszenia miały istotny wpływ na zawartość związków fenolowych w otrzymanych proszkach ($p \leq 0,05$). Stężenie związków fenolowych ulegało redukcji zgodnie z uszeregowaniem metod suszenia: sublimacyjne > rozpyłowe > próżniowe w 50°C > próżniowe w 70°C > próżniowe w 90°C. Suszenie próżniowe w 90°C powodowało obniżenie stężenia polifenoli aż o 87%, w porównaniu do suszenia sublimacyjnego. W przypadku związków z grupy flawan-3-oli nie zaobserwowano statystycznie istotnych różnic w zawartości tych związków po suszeniu sublimacyjnym i rozpyłowym, natomiast wzrost temperatury z 50 do 70°C podczas suszenia próżniowego powodował spadek stężenia flawan-3-oli o 50%. Zawartość kwasów fenolowych w proszkach poddanych suszeniu rozpyłowemu była dwukrotnie niższa niż po suszeniu sublimacyjnym i próżniowym w 50°C, w przeciwieństwie do wyników uzyskanych przez Michalska i wsp. (2016), gdzie nie stwierdzono istotnych różnic pomiędzy zawartością kwasów fenolowych w proszkach śliwkowych otrzymywanych metodą suszenia sublimacyjnego i próżniowego w 60°C ($p > 0,05$). Zawartość polimerów procyjanidyn zmieniała się analogicznie jak w przypadku kwasów fenolowych. Stopień polimeryzacji dla proszków otrzymanych w procesie suszenia sublimacyjnego wyniósł ok. 1,5, podczas gdy suszenie próżniowe w 90°C spowodowało jego prawie 2,5-krotny wzrost. Suszenie sublimacyjne zapewniało największą retencję związków fenolowych, natomiast proces próżniowy, niezależnie od zastosowanej temperatury, powodował ich największą degradację.

W odniesieniu do wpływu nośnika na zawartość związków fenolowych stwierdzono istotną zmienność między analizowanymi próbkami ($p \leq 0,05$). Wyższą zawartość związków fenolowych uzyskano w proszkach z dodatkiem maltodekstryny niż inuliny, a ich stężenie zmniejszało się zgodnie z uszeregowaniem nośników: maltodekstryna >

inulina:maltodekstryna 2:1 > inulina > inulina:maltodekstryna 1:2. Zawartość flawan-3-oli, kwasów fenolowych i polimerów procyjanidyn, analogicznie do całkowitej zawartości polifenoli, była niższa dla proszków z większym udziałem inuliny (redukcja o ok. 20% w stosunku do wariantów z maltodekstryną). Podobnie do uzyskanych wyników, Michalska i wsp. (2017) w badaniu nad proszkami otrzymanymi z preparatu soku śliwkowego stwierdzili, że dodatek maltodekstryny w wysokim stężeniu działał ochronnie na wybrane związki (np. kwas chlorogenowy) podczas procesu suszenia. Rodzaj użytego nośnika nie wpłynął istotnie na zawartość polimerycznych procyjanidyn ($p>0,05$). Stopień polimeryzacji był wyższy dla proszków z inuliną (DP=2,3) niż dla proszków z maltodekstryną (DP=2,0). Można zatem wnioskować, że inulina promuje łączenie jednostek flawan-3-oli w dłuższe łańcuchy oligomeryczne.

W niniejszej pracy dokonano weryfikacji właściwości przeciwutleniających otrzymanych mikrokapsulek metodami ABTS, FRAP i ORAC (**P5, Tab. 5**). Najwyższą zdolność przeciwutleniającą, zarówno w teście ABTS, jak i FRAP, wykazał proszek z maltodekstryną po suszeniu sublimacyjnym (odpowiednio 8,0 i 6,7 mmol Trolox/100 g sm). Przeprowadzona analiza statystyczna dla wyników aktywności przeciwutleniającej w obrębie zastosowanych metod wykazała, że nie miały one istotnego wpływu na uzyskiwane wartości ($p>0,05$). Z kolei najwyższą zdolnością absorpcji rodników tlenowych (ORAC) charakteryzował się proszek z mieszkanką inuliny i maltodekstryny 1:2 po suszeniu w próżni w 50°C (26,5 mmol Trolox/100 g sm). Ponadto, w przeciwieństwie do testu z kationorodnikiem ABTS^{•+}, jak i jonami Fe²⁺, metoda suszenia miała istotny wpływ na aktywność przeciwutleniającą ORAC ($p\leq 0,05$), gdzie proces suszenia rozpyłowego i próżniowego w 70°C gwarantował otrzymanie mikroenkapsułkowanego proszku z soku z owoców pigwowca japońskiego posiadającego wyższą aktywność przeciwutleniającą w stosunku do suszenia sublimacyjnego. Co więcej, zastosowane nośniki nie miały istotnego wpływu na aktywność przeciwutleniającą ORAC ($p\leq 0,05$), w przeciwieństwie do testu ABTS i FRAP, gdzie proszki z maltodekstryną wykazywały wyższe wartości w porównaniu z innymi. Dla porównania, Michalska i wsp. (2017) stwierdzili, że im większy był dodatek maltodekstryny w proszkach z soku śliwkowego, tym niższa aktywność przeciwutleniająca. Spośród wszystkich grup związków fenolowych kwasy fenolowe i flawan-3-ole wykazały najwyższą dodatnią korelację z aktywnością przeciwutleniającą ORAC ($R^2=0,405$ i $0,309$). W niniejszym badaniu zaobserwowano dodatnią korelację między zawartością 5-HMF a aktywnością przeciwutleniającą mierzoną testami ABTS, FRAP i ORAC (odpowiednio $R^2=0,210$; $0,175$ i $0,162$), co sugeruje możliwy udział 5-HMF w formowaniu aktywności przeciwutleniającej otrzymanych proszków.

W omawianej publikacji, stanowiącej składową część rozprawy doktorskiej, oprócz aktywności przeciwutleniających po raz pierwszy dokonano weryfikacji potencjału *in vitro* do hamowania α -glukozydazy, lipazy trzustkowej, AChE i 15-LOX w mikroenkapsułkowanych proszkach na bazie soku z owoców pigwowca japońskiego (P5, Tab. 5).

IC₅₀ (mg owoców/mL) dla potencjału inhibicji α -glukozydazy wynosiło od 13,8 (wariant z maltodekstryną po suszeniu sublimacyjnym) do 21,8 mg/mL (wariant z maltodekstryną po suszeniu próżniowym w 90°C). Nie stwierdzono wpływu zastosowanych nośników na zdolność hamowania α -glukozydazy ($p>0,05$), jednak istotne różnice były widoczne po zastosowaniu wybranych metod suszenia ($p\leq 0,05$). Suszenie sublimacyjne, rozpyłowe i próżniowe w 50°C zapewniały uzyskanie najniższych wartości IC₅₀, natomiast wzrost temperatury w metodzie próżniowej skutkował spadkiem aktywności przeciwcukrzycowej (zmniejszenie aktywności o 20% po suszeniu w próżni w 90°C w porównaniu do suszenia sublimacyjnego). Miao i wsp. (2018b) przeanalizowali zdolność hamowania α -glukozydazy przez owoce *Chaenomeles* uzyskując wartości w zakresie 0,04-0,43 mg/mL. Zdolność do hamowania α -glukozydazy wykazała silną pozytywną korelację z całkowitą zawartością związków fenolowych ($R^2=0,666$) i aktywnością przeciwutleniającą mierzoną metodą ABTS ($R^2=0,439$).

Analizowane mikroenkapsułkowane proszki z soku z owoców pigwowca charakteryzowała duża zmienność w odniesieniu do działania hamującego wobec lipazy trzustkowej ($p\leq 0,05$). Należy podkreślić, że dla 11 analizowanych próbek (głównie po suszeniu sublimacyjnym i rozpyłowym) wartości IC₅₀ hamowania lipazy trzustkowej oznaczono jako $<0,01$. Dla pozostałych wariantów wartości te były poniżej 0,05 mg/mL, co świadczy o bardzo wysokiej aktywności analizowanych proszków. Biorąc pod uwagę wpływ metody suszenia na potencjał inhibicji lipazy trzustkowej w analizowanych proszkach stwierdzono, że największą aktywnością cechowały się próbki po suszeniu rozpyłowym i próżniowym w 50°C. Analizując wpływ nośnika na analizowany parametr, proszki z inuliną wyróżniały się niższymi wartościami IC₅₀, a więc większym potencjałem hamowania lipazy trzustkowej. Uzyskane wyniki wskazują, że proszki owocowe mają duży potencjał do stosowania u osób borykających się z problemem nadwagi, ponieważ ograniczając aktywność lipazy trzustkowej zmniejsza się ilość tłuszczu wchłanianego do krwiobiegu, a tym samym wspomaga to utrzymanie prawidłowej masy ciała.

Ze wszystkich zastosowanych metod suszenia technika rozpyłowa znacznie zmniejszyła potencjał hamowania AChE w porównaniu z innymi metodami - prawie dwukrotnie niższa aktywność w porównaniu do proszków poddanych suszeniu sublimacyjnemu. Analizując wpływ nośników na inhibicję AChE nie stwierdzono istotnych ($p>0,05$) różnic pomiędzy

proszkami z inuliną i maltodekstryną. Zaobserwowano ujemną korelację między całkowitą zawartością związków fenolowych a zdolnością hamowania AChE ($R^2=-0,145$). Jest to zgodne z wynikami uzyskanymi przez Wojdyło i wsp. (2018) dla owoców goji, gdzie stwierdzono, że zdolność hamowania AChE jest modulowana zawartością karotenoidów, a nie polifenoli.

Aktywność hamowania 15-lipoksygenazy wyrażono jako procent hamowania przy stężeniu próbki 2,5 mg/mL. Warto podkreślić, że była to pierwsza próba określenia potencjału przeciwzapalnego jako zdolności inhibicji 15-LOX mikroenkapsulowanych proszków na bazie soku z owoców pigwowca japońskiego. Największy potencjał charakteryzował proszki z inuliną po suszeniu rozpyłowym (90,4%), a najmniejszy wariant z maltodekstryną po suszeniu próżniowym w 90°C (29,4%) ($p\leq 0,05$). Porównując wpływ metody suszenia na inhibicję 15-LOX można je uszeregować według malejącej aktywności: suszenie sublimacyjne > suszenie rozpyłowe > suszenie próżniowe w 70°C > suszenie próżniowe w 50°C > suszenie próżniowe w 90°C. Zastosowanie metody próżniowej w 90°C przyczyniło się do redukcji potencjału hamowania 15-LOX o 38% w porównaniu z metodą sublimacyjną. Stwierdzono, że zdolność inhibicji 15-LOX jest modulowana zawartością związków fenolowych ($R^2=0,465$), i że istnieje dodatnia korelacja między zdolnością do hamowania α -glukozydazy i 15-LOX ($R^2=0,480$).

W niniejszym opracowaniu zaproponowano nową formę wykorzystania potencjału bioaktywnego owoców pigwowca poprzez mikroenkapsulację soku z wybranymi biopolimerami przy zastosowaniu wybranych metod suszenia. Na profil związków fenolowych w otrzymanych proszkach miał wpływ zarówno sposób suszenia, jak i zastosowany nośnik. Metoda sublimacyjna spowodowała największą retencję polifenoli, natomiast spośród użytych nośników maltodekstryna okazała się najlepszym biopolimerem do uzyskania wysokiej jakości proszku owocowego. Również skojarzenie tych dwóch czynników zapewniło otrzymanie proszków o najniższej zawartości niepożądanego 5-HMF. Wybrane metody suszenia nie miały istotnego wpływu na aktywność przeciwutleniającą (ABTS, FRAP i ORAC), ale aplikacja maltodekstryny przyczyniła się do otrzymania proszków o większej aktywności (od 8 do 15%). Proszki (niezależnie od zastosowanego nośnika) otrzymane poprzez suszenie sublimacyjne charakteryzowały się wyższą aktywnością hamowania α -glukozydazy (16,2 mg/mL), AChE (24,7 mg/mL) i 15-LOX (72,8%) w porównaniu z innymi, natomiast proszki suszone rozpyłowo i próżniowo w 50°C charakteryzowały się większym potencjałem do inhibicji lipazy trzustkowej (24,7 mg/mL). Podsumowując proszki owocowe na bazie soku z owoców pigwowca japońskiego otrzymane metodą mikroenkapsulacji mogą stać się alternatywnym sposobem aplikacji tego surowca w szerokiej gamie produktów spożywczych, zwiększając ich

właściwości prozdrowotne (działanie przeciwutleniające, przeciwcukrzycowe, antycholinergiczne i przeciwzapalne) m.in. dzięki wysokiej zawartości związków polifenolowych (1359,9 mg/100 g sm). Kluczowy jest dobór odpowiedniej metody i warunków procesu a najbardziej korzystna pod względem analizowanych wyróżników do otrzymania mikrokapsułek jest metoda sublimacyjna i rozpyłowa.

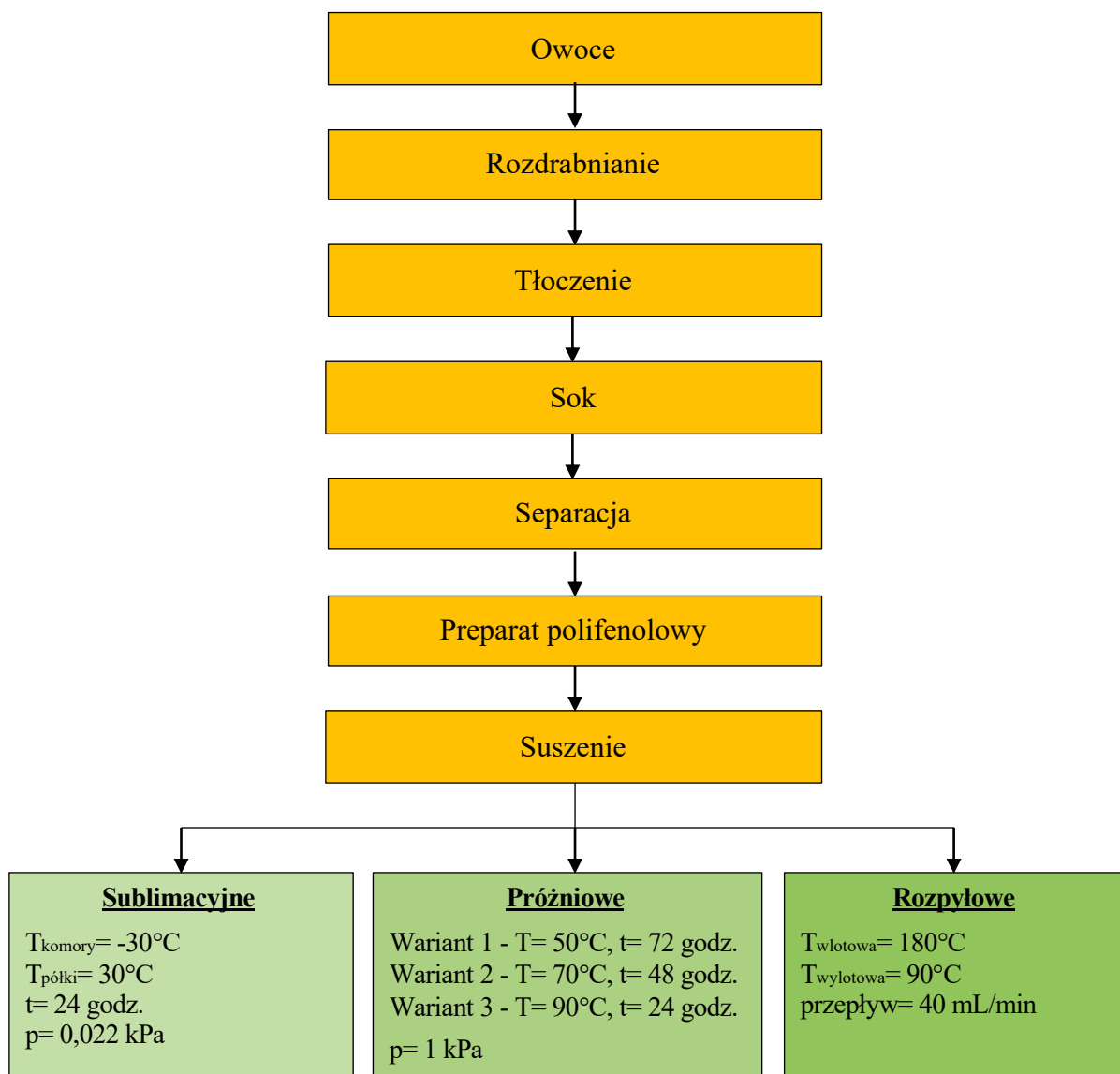
4.5. Optymalizacja technologii otrzymywania preparatu polifenolowego z owoców pigwowca metodami suszarniczymi

Do kolejnego etapu badań zaproponowano wykorzystanie preparatu polifenolowego otrzymanego z soku z owoców pigwowca japońskiego, pozbawionego substancji balastowych, takich jak pektyny, cukry i kwasy organiczne i inne związki organiczne. Zastosowanie separacji frakcji biologicznie aktywnej na żywicy jonowymiennej pozwoliło na bezpośredni proces mikroenkapsulacji otrzymanego preparatu polifenolowego, z pominięciem substancji nośnikowych. Założono, że takie podejście pozwoli na uzyskanie produktu charakteryzującego się wyższym stężeniem związków fenolowych, w porównaniu do produktu otrzymanego w pracy **P5**. Przeprowadzono walidacje różnych metod i warunków suszenia (sublimacyjne, rozpyłowe i próżniowe) celem wyboru techniki i parametrów zapewniających otrzymanie mikroenkapsulowanego preparatu polifenolowego zawierającego najwyższe stężenie substancji aktywnych (**Schemat 5**). W otrzymanych proszkach dokonano oceny potencjału przeciwutleniającego oraz właściwości przeciwcukrzycowych, antycholinergicznym i przeciwzapalnych. Uzyskane wyniki zostały opublikowane w pracy **P6** (*Physicochemical characterization and biological potential of Japanese quince polyphenol extract treated by different drying techniques, LWT, 2021*). Była to pierwsza próba otrzymania preparatu polifenolowego z owoców pigwowca japońskiego w procesie mikroenkapsulacji z wykorzystaniem wybranych metod suszenia.

Parametry fizyczne, tj. sucha masa, aktywność wody, gęstość rzeczywista i usypowa oraz porowatość dla mikroenkapsulowanego ekstraktu polifenolowego z owoców pigwowca japońskiego zostały przedstawione w pracy **P6** (**Tab. 1**).

Zawartość suchej masy w proszkach wynosiła od 92,95% (po suszeniu sublimacyjnym) do 98,82% (po suszeniu próżniowym w 70°C). Parametr ten ma bezpośredni wpływ na sypkość i lepkość proszków i jest traktowany jako miara efektywności suszenia (Aziz i wsp. 2018). Obliczony współczynnik korelacji Pearsona potwierdził wyniki Young i wsp. (2007) gdzie zawartość suchej masy była dodatnio skorelowana z gęstością usypową ($R^2=0,308$) i ujemnie skorelowana z gęstością rzeczywistą ($R^2=-0,350$). Wartości a_w w mikroenkapsulowanym preparacie polifenolowym różniły się między próbkami ($p \leq 0,05$) i zawierały się w przedziale od 0,090 (wariant suszony rozpyłowo i próżniowo w 90°C) do 0,105 (wariant suszony próżniowo w 70°C). Podobnie niskie wartości a_w w zakresie od 0,074 do 0,101 uzyskali Tkacz i wsp. (2020) dla sproszkowanego soku z rokitnika pospolitego. Utrzymywanie jak najniższej

a_w w produktach sypkich zapobiega ich aglomeracji, zbrylaniu i degradacji związków bioaktywnych (Ramakrishnan i wsp. 2018).



Schemat 5. Proces technologiczny mikroenkapsulacji preparatu polifenolowego z owoców pigwowca japońskiego

T-temperatura [$^{\circ}\text{C}$], t-czas [min], p-ciśnienie [kPa]

Gęstość rzeczywista otrzymanych proszków wynosiła od 1,036 (po suszeniu rozpyłowym) do 1,527 g/cm^3 (po suszeniu sublimacyjnym) i występowały istotne w wartościach ρ_t w zależności od zastosowanej metody suszenia ($p \leq 0,05$). Wartość ρ_t wzrastały wraz ze wzrostem temperatury w suszeniu próżniowym, ale przeprowadzona analiza statystyczna nie zaklasyfikowała tych zależności jako istotnych statystycznie ($p > 0,05$). Proces suszenia miał również istotny wpływ na wartości ρ_b , które wahały się od 0,265 (proszek suszony rozpyłowo)

do $0,626 \text{ g/cm}^3$ (proszek suszony próżniowo w 70°C) ($p \leq 0,05$). Im niższe wartości ρ_b , tym więcej powietrza jest uwięzione w pustych przestrzeniach, a tym samym większa możliwość utleniania związków bioaktywnych (Aziz i wsp. 2018). Dodatkowo stwierdzono, że stosowanie metody próżniowej (niezależnie od zastosowanej temperatury) ma korzystne następstwa poprzez zmniejszenie opakowań i obniżenie kosztów transportu produkowanych proszków, w porównaniu z metodą sublimacyjną i rozpyłową. Porównując otrzymane wyniki z wcześniejszymi ustaleniami dla mikroenkapsułkowanego soku (**P5, Tab. 1**) zauważono, że dodatek polisacharydów do proszków miał większy wpływ na wartość ρ_b niż temperatura w suszeniu próżniowym.

Najwyższą porowatością charakteryzował się mikroenkapsułkowany preparat polifenolowy po suszeniu rozpyłowym (74,42%), a najniższą po suszeniu próżniowym w 50°C (58,32%). Porowatość wykazuje umiarkowaną ujemną korelację z gęstością rzeczywistą ($R^2 = -0,500$) i silną ujemną korelację z gęstością usypową ($R^2 = -0,953$). Według Saifullah i wsp. (2016) porowatość jest bardzo ważna z punktu widzenia rozpuszczalności dowolnego materiału sypkiego - im wyższe wartości ε , tym większa rozpuszczalność.

Zawartość 5-HMF (**P6, Tab. 2**) w mikroenkapsułkowanym preparacie polifenolowym były na poziomie od 4,9 (po suszeniu sublimacyjnym) do 17,4 mg/kg sm (po suszeniu próżniowym w 90°C). Porównując metody suszenia otrzymane proszki różniły się istotnie pod względem zawartości 5-HMF ($p \leq 0,05$). Zastosowanie metody próżniowej w 90°C spowodowało ponad 3,5-krotny wzrost stężenia 5-HMF w porównaniu z techniką sublimacyjną, a podwyższenie temperatury w suszeniu próżniowym z 70 do 90°C zwiększyło zawartość tego związku ponad 2-krotnie. Ponadto proszki otrzymane w wyniku suszenia rozpyłowego i próżniowego w 50°C nie różniły się istotnie pod względem zawartości 5-HMF ($p > 0,05$). Dla porównania stężenie 5-HMF w otrzymanych proszkach było niższe niż w suszonych jabłkach, gruszkach i brzoskwiniach (40,0-100,0 mg/kg) (Choudhary i wsp. 2020).

Proszki odznaczające się najjaśniejszą barwą otrzymano w wyniku suszenia rozpyłowego ($L^* = 83,77$), a najciemniejsze po suszeniu próżniowym w 90°C ($L^* = 74,20$) (**P6, Tab. 1**). Natomiast w odniesieniu do badań prezentowanych w pracy **P5** najwyższe wartości parametru L^* uzyskano dla proszków z soku z owoców pigwowca japońskiego po suszeniu sublimacyjnym ($L^* = 91,27$). Z drugiej strony 5-HMF powstający podczas długotrwałego ogrzewania może być związany z ciemniejszym kolorem próbek po suszeniu próżniowym w 90°C ($R^2 = 0,511$). Największą zmianę parametru a^* pomiędzy zastosowanymi metodami suszenia zaobserwowano, gdy temperaturę w procesie próżniowym zwiększono z 70 do 90°C - wówczas nastąpił prawie 12-krotny wzrost wartości koordynanty a^* , co w konsekwencji

doprowadziło do wysycenia proszków odcieniem czerwonym. Wartości parametru b^* wahały się od 16,51 (wariant po suszeniu próżniowym w 50°C) do 20,93 (wariant po suszeniu rozpyłowym). Wraz ze wzrostem temperatury w suszeniu próżniowym, kolor ulegał wysyceniu kolorem żółtym. Te same zależności zmian współrzędnych a^* i b^* uzyskali Tkacz i wsp. (2020) dla proszków z soku rokitnika pospolitego.

Dla uzyskania pełnego obrazu zmian przeprowadzono identyfikację i oznaczenie ilościowe związków fenolowych techniką LC-MS/QTOF i UPLC-PDA (**P6, Tab. 2**). Zidentyfikowano 15 związków fenolowych należących do dwóch różnych grup (flawan-3-oli i kwasów fenolowych). Flawan-3-ole stanowiły ok. 50% całkowitej zawartości związków fenolowych, polimery procyjanidyn 29,2%, a kwasy fenolowe 15,3%. Ekstrakt polifenolowy (nie poddany obróbce cieplnej) charakteryzował się stężeniem polifenoli na poziomie 886,2 g/kg sm, a suszenie sublimacyjne i próżniowe (niezależnie od zastosowanej temperatury) prowadziły do redukcji ich zawartości. Rodzaj zastosowanej metody i jej parametry miały istotny wpływ na zawartość związków fenolowych ($p \leq 0,05$). Najwyższe stężenia poszczególnych grup związków fenolowych uzyskano po suszeniu rozpyłowym, z wyjątkiem flawan-3-oli, gdzie technika próżniowa prowadzona w 70°C powodowała najmniejsze straty tych związków. Największą degradację flawan-3-oli (obniżenie zawartości o 20%) stwierdzono w próbkach po zastosowaniu najwyższej temperatury podczas suszenia w próżni. Z kolei zawartość kwasów fenolowych w mikroenkapsulowanym preparacie polifenolowym zmniejszała się zgodnie z zastosowaną metodą suszenia i parametrami: rozpyłowe > sublimacyjne > próżniowe w 70°C > próżniowe w 50°C > próżniowe w 90°C. Analizując wpływ metody suszenia zaobserwowano, że największą retencję polimerów procyjanidyn, oprócz suszenia rozpyłowego, uzyskano dla metody próżniowej prowadzonej w 50°C oraz sublimacyjnej - odpowiednio 231,1 i 230,8 g/kg sm. W odniesieniu do całkowitej zawartości związków fenolowych wzrost temperatury suszenia próżniowego z 70 do 90°C doprowadził do redukcji ich zawartości o ponad 20%. Natomiast Miao i wsp. (2017) w badaniach nad wpływem obróbki cieplnej i metod suszenia na jakość owoców *Chaenomeles* zaobserwowali wzrost całkowitej zawartości polifenoli o 12% po podwyższeniu temperatury w suszeniu próżniowym z 60 do 80°C. Z kolei Michalska i wsp. (2017) w badaniach nad mikroenkapsulacją ekstraktu z soku śliwkowego uzyskali redukcję zawartości kwasu chlorogenowego o ponad 60% po podwyższeniu temperatury suszenia próżniowego z 60 do 80°C.

Zdolność antyoksydacyjną otrzymanych proszków zmierzono trzema metodami (ABTS, FRAP i ORAC), a wyniki przedstawiono w pracy **P6 (Tab. 3)**. Wystąpiły istotne różnice w aktywnościach analizowanych próbek w zależności od użytej metody suszenia ($p \leq 0,05$).

Najwyższą aktywnością przeciwutleniającą (zarówno w teście ABTS jak i FRAP) charakteryzował się preparat otrzymany metodą próżniową w 50°C (430,3 i 294,6 mmol Trolox/100 g sm), natomiast najniższą po suszeniu sublimacyjnym (319,2 i 208,8 mmol Trolox/100 g sm). W przypadku metody ORAC nie stwierdzono różnicy w uzyskanych wartościach pomiędzy zastosowaniem temperatury 50 i 90°C w metodzie próżniowej ($p>0,05$), natomiast po zastosowaniu 70°C udało się uzyskać 65% wyższą aktywność niż po procesie sublimacyjnym (886,7 mmol Trolox/100 g sm). Porównując aktywność przeciwutleniającą mikroenkapsułkowanego preparatu polifenolowego do aktywności wybranych owoców *Chaenomeles* stwierdzono, że aktywność uzyskanych proszków była 18-krotnie większa (dla metody z kationorodnikiem ABTS⁺), oraz 44-krotnie większa (dla metody redukcji jonów Fe²⁺ FRAP) (Du i wsp. 2013). Natomiast wartości aktywności przeciwutleniającej ORAC dla suszonego preparatu polifenolowego były niemal 20-krotnie wyższe niż dla suszonych owoców *Chaenomeles japonica* (Turkiewicz i wsp. 2019b; **P3**). Obliczony współczynnik korelacji Pearsona potwierdził dodatnią korelację między zawartością 5-HMF a aktywnością przeciwutleniającą ABTS i ORAC (odpowiednio $R^2=0,651$ i $0,605$), co jest zgodne z wynikami Michalska i wsp. (2017). Ponadto we wcześniejszych badaniach nad mikroenkapsulacją soku z owoców pigwowca japońskiego potwierdzono istnienie korelacji pomiędzy stężeniem 5-HMF a aktywnością przeciwutleniającą (Turkiewicz i wsp. 2020c; **P5**).

W pracy **P6** dokonano po raz pierwszy oceny potencjału przeciwcukrzycowego i przeciw otyłości jako zdolności do hamowania α -amylazy, α -glukozydazy i lipazy trzustkowej (IC₅₀; mg/mL) mikroenkapsułkowanego preparatu polifenolowego z soku z owoców pigwowca japońskiego. Analizowane próbki wykazały duże zróżnicowanie ($p\leq 0,05$) a wpływ na to miały zastosowane metody suszenia. Aktywność hamująca wobec α -amylazy wahała się od 7,1 do 21,8 mg/mL (odpowiednio dla próbek suszonych próżniowo w 50°C i sublimacyjnie), podczas gdy potencjał inhibicji α -glukozydazy zawierał się w przedziale od 1,6 do 3,1 mg/mL (odpowiednio dla wariantu po suszeniu próżniowym w 90 i 70°C). Zastosowanie procesu suszenia, niezależnie od metody, przyczyniło się do zwiększenia działania przeciwcukrzycowego i przeciw otyłości ekstraktu polifenolowego. W porównaniu z proszkiem otrzymanym techniką sublimacyjną, ekstrakt bez obróbki termicznej charakteryzował się ponad 5-krotnie niższym potencjałem hamowania α -amylazy, prawie 2-krotnie niższym α -glukozydazy i aż 9-krotnie mniejszą aktywnością hamowania lipazy trzustkowej. Co więcej, metody suszenia sublimacyjnego i rozpyłowego okazały się najkorzystniejsze pod względem potencjalnych właściwości przeciw otyłości analizowanych mikroenkapsułkowanych proszków (0,4 mg/mL). Uzyskane wyniki potwierdzają wcześniejsze doniesienia (Miao i wsp. 2018a; Zakł \acute{o} s-Szydła i wsp. 2015), że owoce *Chaenomeles* mogą być

obiecującym naturalnym źródłem związków aktywnych o właściwościach przeciwuczkrywcowych. Ponadto współczynnik korelacji Pearsona obliczony dla zdolności do hamowania α -amylazy i potencjałem przeciwutleniającym (ABTS, FRAP i ORAC) potwierdza istnienie silnej dodatniej korelacji między nimi (odpowiednio $R^2=0,867$; $0,874$ i $0,909$). Dodatkowo stwierdzono silną korelację między aktywnością hamowania α -glukozydazy a zawartością 5-HMF ($R^2=0,803$); do tej pory w literaturze nie wykazano związku pomiędzy tymi dwoma składowymi. Zwrócono również uwagę, że spośród analizowanych grup związków fenolowych największy wpływ na modulowanie aktywności hamowania lipazy trzustkowej miały polimery procyjanidyn ($R^2=0,806$), co również było artykułowane w badaniach Wojdyło i wsp. (2020) nad składem i aktywnością biologiczną wybranych kiełków i mikroliści.

Właściwości antycholinergiczne analizowanych proszków wyrażono jako zdolność do hamowania AChE i BuChE (**P6, Tab. 3**). Potencjał inhibicji AChE i BuChE wyrażony jako IC_{50} wynosił odpowiednio od 11,3 do 39,8 i od 10,1 do 22,1 mg próbki/mL wykazując istotne różnice między zastosowanymi metodami suszenia ($p \leq 0,05$). Aktywność inhibicji AChE wzrastała wraz ze wzrostem temperatury podczas suszenia próżniowego, podczas gdy nie odnotowano różnicy między suszeniem sublimacyjnym i rozpyłowym ($p > 0,05$). Z kolei biorąc pod uwagę zdolność do hamowania BuChE, analizowane próbki można uporządkować w zależności od rosnącej aktywności zgodnie z szeregiem zastosowanych metod: suszenie sublimacyjne = suszenie próżniowe $70^\circ C <$ suszenie próżniowe $50^\circ C <$ suszenie rozpyłowe $<$ suszenie próżniowe $90^\circ C$. Warto zauważyć, że po raz pierwszy antycholinergiczne działanie owoców *Chaenomeles* zostało opisane w pracy **P1** gdzie wartości były na poziomie 11,8 i 16,5 mg/mL, odpowiednio dla AChE i BuChE (Turkiewicz i wsp. 2020d; **P1**). Stwierdzono, że spośród zidentyfikowanych związków fenolowych największy udział w tworzeniu właściwości antycholinergicznym mikroenkapsułkowanego preparatu polifenolowego mają polimery procyjanidyn ($R^2=0,285$ i $0,536$, odpowiednio dla AChE i BuChE). Ponadto, podobnie jak w przypadku właściwości przeciwutleniającej, współczynnik korelacji Pearsona wskazywał na dodatnią korelację między zawartością 5-HMF a zdolnością do hamowania AChE i BuChE - odpowiednio $R^2=0,689$ i $0,700$. Co więcej, aktywność inhibicji AChE mikroenkapsułkowanego ekstraktu polifenolowego była średnio dwukrotnie wyższa niż aktywność mikroenkapsułkowanego soku z owoców pigwowca japońskiego (**P5, Tab. 5**).

W niniejszej pracy podjęto również próbę oceny zdolności hamowania 15-LOX jako regulatora peroksydacji lipidów komórkowych, a wyniki wyrażono jako procent inhibicji przy stężeniu próbki 2,5 mg/mL. Najwyższy potencjał wykazał wariant suszony próżniowo w $70^\circ C$ (81,6%), natomiast najniższy po suszeniu tą samą metodą, ale w $50^\circ C$ (40,7%) ($p \leq 0,05$).

Podobnie jak w przypadku zdolności do hamowania α -glukozydazy i AChE, w odniesieniu do 15-LOX potwierdzono, że przy suszeniu próżniowym korzystniejsze jest skrócenie czasu suszenia niż stosowanie niższej temperatury.

Podsumowując, mikroenkapsułkowany preparat polifenolowy z soku z owoców pigwowca japońskiego jest bogatym źródłem związków fenolowych, w szczególności z grupy flawan-3-oli (średnio 53,4% całkowitej ich zawartości). Suszenie rozpyłowe wydaje się być najbardziej korzystną metodą z punktu widzenia retencji związków bioaktywnych, przy jednoczesnym zapewnieniu niskiej zawartości 5-HMF (porównywalnej z suszeniem sublimacyjnym). Z drugiej strony, biorąc pod uwagę parametry fizyczne, tj. gęstość rzeczywistą i usypową oraz porowatość, technika próżniowa pozwoliła uzyskać najkorzystniejsze parametry proszków, zwłaszcza w kontekście potencjalnej redukcji kosztów transportu i pakowania oraz małej podatności na procesy utleniania. Biorąc pod uwagę wpływ metody suszenia i jej parametrów na właściwości biologiczne *in vitro* stwierdzono, że mikroenkapsułkowany preparat polifenolowy charakteryzował się wysokim potencjałem przeciwutleniającym, zwłaszcza po suszeniu próżniowym w 50 i 70°C. Ponadto właściwości przeciwcukrzycowe otrzymanych proszków były istotniejsze po suszeniu próżniowym niż sublimacyjnym i rozpyłowym, w przeciwieństwie do właściwości antycholinergicznyc.

5. Podsumowanie i wnioski

W niniejszej pracy podjęto się określenia i wykorzystania potencjału bioaktywnego owoców pigwowca (*Chaenomeles* ssp.) w otrzymaniu innowacyjnych produktów funkcjonalnych o zaprogramowanych właściwościach prozdrowotnych. Osiągnięto powyższy cel oraz potwierdzono założoną hipotezę badawczą. Dowiedziono, że owoce pigwowca to wartościowy surowiec, który można z powodzeniem wykorzystać do stworzenia atrakcyjnych produktów i/lub półproduktów, jako suszone przekąski lub mikroenkapsułkowane proszki, z potencjałem do aplikacji m.in. w funkcjonalnych napojach owocowo-warzywnych.

Realizacja szczegółowych celów pracy pozwoliła na sformułowanie następujących wniosków:

1. Owoce pigwowca były zasobne w kwas L-askorbinowy (30,26-195,05 mg/100 g owoców) oraz kwasy organiczne (41,64-110,31 g/100 g owoców), wśród których w największej ilości oznaczono kwas jabłkowy, stanowiący ponad 80% wszystkich kwasów. Owoce *Chaenomeles* zawierały niewielkie ilości cukrów (0,60-3,98 g/100 g owoców), z dominującą zawartością fruktozy, sorbitolu i glukozy.
2. Najzasobniejsze w karotenoidy, chlorofile i związki fenolowe były owoce *C. × superba* ‘Nicoline’, natomiast w aminokwasy - *C. × superba* ‘Jet Trail’. Owoce *C. speciosa* ‘Rubra’ charakteryzowały się największym stężeniem tokoferoli i tokotrienoli. Wśród polifenoli, dominującą frakcją owoców *Chaenomeles* były polimery procyanidyn (65% sumy tych związków).
Potencjał przeciwutleniający owoców *Chaenomeles* był formowany głównie przez polimery procyanidyn i pozostałe flawan-3-ole, co wykazało profilowanie on-line z kationorodnikiem ABTS^{•+}. Największą aktywność przeciwutleniającą w teście ORAC posiadały owoce *C. × superba* ‘Colour Trail’ (66,59 mmol Trolox/100 g sm).
3. Badane owoce pigwowca charakteryzowały się aktywnością przeciwcukrzycową, wykazując wysoki potencjał hamowania α -amylazy (IC₅₀=13,88 mg/mL dla *C. × superba* ‘Nicoline’) i α -glukozydazy (IC₅₀=5,08 mg/mL dla *C. × superba* ‘Texas Scarlet’). Potwierdzono także ich potencjał antycholinergiczny, uzyskując największy stopień inhibicji acetylocholinoesterazy dla *C. speciosa* ‘Rubra’ i butyrylocholinoesterazy dla *C. japonica* ‘Red Joy’. Owoce *Chaenomeles* charakteryzowały się silną inhibicją lipazy trzustkowej (IC₅₀=0,04-0,35 mg/mL) oraz zróżnicowaną aktywnością hamowania 15-lipooxygenazy.

4. Metoda kombinowana (podsuszanie konwekcyjne w temperaturze 70°C i dosuszanie mikrofalowo-próżniowe przy 120 W) pozwoliło na zachowanie koloru produktów i cennych związków bioaktywnych niż zastosowanie tych technik oddzielnie. Ponadto czas suszenia był 7-krotnie krótszy, zawartość związków fenolowych dla tej metody była na zbliżonym poziomie do suszenia sublimacyjnego.
5. Odwadnianie osmotyczne w koncentratkach owocowych skutkowało obniżeniem wilgotności owoców średnio o 70% oraz skróceniem czasu suszenia konwekcyjnego o 60 min. Susze po procesie odwadniania osmotycznego charakteryzowały się zwiększoną zawartością cukrów (zwłaszcza po zastosowaniu koncentratu jabłkowego) oraz znacznym zmniejszeniem zawartości kwasów organicznych (ponad 4-krotna redukcja przy użyciu koncentratu gruszkowego). Zawartość związków fenolowych oraz aktywność przeciwutleniająca zmniejszyły się istotnie (odpowiednio 70-82% i 38-57%) w wyniku odwadniania przy jednoczesnym wzroście potencjału antycholinergicznego otrzymanych suszy (ponad 30% dla inhibicji butyrylocholinoesterazy). Ponadto wykorzystane do tego celu koncentraty z owoców czerwonych wzbogacały produkt finalny w cenne związki fenolowe z grupy antocyjanów.
6. Na profil polifenolowy otrzymanych mikroenkapsułkowanych proszków na bazie soku z owoców pigwowca japońskiego wpływ miała zarówno metoda suszenia, jak i zastosowany nośnik. Suszenie sublimacyjne gwarantowało największą retencję polifenoli, natomiast wśród nośników najlepszym biopolimerem do uzyskania wysokiej jakości proszku owocowego była maltodekstryna. Skojarzenie tych dwóch czynników doprowadziło do otrzymania proszku o najniższej zawartości niepożądanego 5-HMF. Zastosowanie inuliny przyczyniło się do zmniejszenia aktywności wody, większej porowatości oraz pojaśnieniu barwy. Proszki o największej aktywności przeciwutleniającej ABTS i FRAP (odpowiednio 8,0 i 6,7 mmol Trolox/100 g sm) uzyskano w wyniku suszenia sublimacyjnego z użyciem maltodekstryny. Mikroenkapsułkowane proszki wykazywały aktywność hamowania α -glukozydazy, acetylocholinoesterazy i 15-LOX.
7. Preparat polifenolowy otrzymany z owoców pigwowca japońskiego jest bogatym źródłem związków fenolowych, w szczególności z grupy flawan-3-oli (>53% całkowitej zawartości). Suszenie sublimacyjne było najkorzystniejszą metodą pod względem retencji związków bioaktywnych, przy jednoczesnym zapewnieniu niskiej zawartości 5-HMF. Najkorzystniejsze parametry fizyczne, tj. gęstość rzeczywistą, usypową i porowatość uzyskano stosując próżniową metodę suszenia. Największy potencjał przeciwutleniający miały proszki suszone próżniowo w 50 i 70°C. Potencjał

przeciwcukrzycowy mikroenkapsułkowanego ekstraktu polifenolowego był wyższy po suszeniu próżniowym ($IC_{50}=7,1-15,8$ i $1,6-31$ mg/mL odpowiednio dla α -amylazy i α -glukozydazy), w przeciwieństwie do właściwości antycholinergiczych, gdzie korzystniejsze wartości uzyskano dla preparatu polifenolowego otrzymanego metodą suszenia próżniowego ($IC_{50}=16,6$ i $11,9$ mg/mL odpowiednio dla acetylo- i butyrylocholinoesterazy).

8. Wymiernym efektem przeprowadzonych badań było ustalenie metody i parametrów procesu suszenia dla nowego kierunku przetwarzania owoców pigwowca. Opracowano metodę odwadniania osmotycznego w wybranych koncentratkach soków owocowych, które może być stosowane przed suszeniem, modyfikując właściwości fizykochemiczne otrzymywanego suszu i zwiększając jego właściwości antycholinergiczne. Dokonano optymalizacji procesu mikroenkapsulacji soku i preparatu polifenolowego z owoców pigwowca, stwarzając potencjalny, nowy kierunek wykorzystania potencjału bioaktywnego tych owoców.

Owoce *Chaenomeles* stanowią wartościowy surowiec z potencjałem do produkcji żywności o charakterze funkcjonalnym. Proces suszenia (opcjonalnie w połączeniu z odwadnianiem osmotycznym) oraz mikroenkapsulacja gwarantują otrzymanie produktu cechującego się wysokim potencjałem przeciwutleniającym. Przeprowadzone analizy inhibicji enzymów *in vitro* potwierdziły, że zarówno susz, jak i mikroenkapsułkowane proszki na bazie owoców pigwowca wykazują potencjał do zastosowania w prewencji i leczeniu otyłości oraz cukrzycy typu 2, lecz konieczne są dalsze prace pozwalające na przełożenie uzyskanych wyników na model *in vivo*.

6. Literatura

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Article

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

Igor Piotr Turkiewicz ¹, Aneta Wojdyło ^{1,*}, Karolina Tkacz ¹, Paulina Nowicka ¹, Tomasz Golis ² and Przemysław Bąbalewski ³

¹ Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chełmońskiego Str., 51-630 Wrocław, Poland; igor.turkiewicz@upwr.edu.pl (I.P.T.); karolina.tkacz@upwr.edu.pl (K.T.); paulina.nowicka@upwr.edu.pl (P.N.)

² Research Institute of Horticulture, Department of Pomology, Gene Resources and Nurseries, 1/3 Konstytucji 3 Maja Str., 96-100 Skierniewice, Poland; tomasz.golis@inhort.pl

³ Department of Horticulture, Wrocław University of Environmental and Life Sciences, 24a Grunwaldzki Str., 53-363 Wrocław, Poland; przemyslaw.babalewski@upwr.edu.pl

* Correspondence: aneta.wojdylo@upwr.edu.pl; Tel.: +48-71320-7706

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Abstract: This study aimed to identify and quantify the chemical composition and polyphenolic profile of 19 cultivars of *Chaenomeles* × *superba*, *Chaenomeles japonica*, and *Chaenomeles speciosa* by liquid chromatography coupled with photodiode array detector and quadrupole time-of-flight electrospray ionization mass spectrometry (LC-PDA-QTOF-ESI-MS). Antioxidant (ABTS on-line, ABTS, FRAP, and ORAC), as well as in vitro biological activities, i.e., the ability to inhibit α -amylase, α -glucosidase, pancreatic lipase, acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and 15-lipoxygenase (15-LOX) were determined. Most of the *Chaenomeles* species and cultivars analyzed in this study have not been examined in this respect until now. Fruits contained 30.26 to 195.05 mg of vitamin C, 0.65 to 1.69 g of pectin, 0.32 to 0.64 g of ash, 0.60 to 3.98 g of sugars, and 41.64 to 110.31 g of organic acids in 100 g fresh weight. The lowest content of total polyphenols showed *C. speciosa* ‘Rubra’ (57.84 g/kg dry weight, dw) while *C. × superba* ‘Nicoline’ (170.38 g/kg dw) exhibited the highest concentration of those compounds. In the phenolic compounds, polymeric procyanidin fraction predominated (65%) with procyanidin B2, C1, and (–)-epicatechin the most abundant. The antioxidant capacity measured by ABTS assay was mainly formed by polymeric procyanidins and flavan-3-ols, which was confirmed by ABTS on-line profiling. *Chaenomeles* fruits showed high potential for inhibition of α -glucosidase and pancreatic lipase. The analyzed cultivars displayed greater potential for acetylcholinesterase (AChE) inhibition than for butyrylcholinesterase (BuChE). The data indicate that *Chaenomeles* fruits could be regarded as a promising source of bioactive functional food.

Keywords: Japanese quince; flowering quince; LC-PDA-QTOF-ESI-MS; α -amylase; α -glucosidase; pancreatic lipase; AChE; BuChE; 15-LOX; antioxidant capacity; ABTS on-line; AHC analysis

1. Introduction

Chaenomeles species belong to the Rosaceae family (Maloideae subfamily) and have been known widely in China for thousands of years. In Europe, interest in these fruits has been systematically growing over the last twenty years. The generic name is associated with the anatomy of the fruit,

it comes from the Greek words “chainein” (divide, open) and “melon” (apple). The systematic affiliation and naming of Maule’s quince were ordered only a dozen years ago and currently four basic species belong to the genus *Chaenomeles*, while in Poland the following three species are mainly grown: *C. speciosa* (Sweet) Nakai (flowering or Chinese quince), *C. japonica* (Thunb.) Lindl. (Japanese quince), and *C. × superba* (Frahm) Rehd. (intermediate quince) which is made from the last two because of the easy crossing within the species [1].

Flowering quince grows up to 2 to 3 meters in height, has spiny shoots, broad and green leaves on the edges, and dark red flowers. The fruits of this species are spherical, slightly elongated, yellow, and aromatic. The Japanese quince is a much lower shrub (1 to 1.5 m tall) and more broadly spread. It has smaller, almost round leaves, and the flowers usually have an orange-red color. Yellow fruit with a round shape due to the intense aroma are most often recommended for preserves. Intermediate quince, formed from the aforementioned species, is most commonly found. It is very changeable in appearance, because it has many cultivars differing in height, shape, size of fruit, or the color of flowers (e.g., ‘Crimson and Gold’ blooms in red, and ‘Jet Trail’ in white).

Chaenomeles fruit has been widely used in traditional medicine of the Far East, which confirms their presence in the Pharmacopeia of the People’s Republic of China (2010). It describes *Fructus Chaenomeles speciosa*, as a source of medicinal raw materials, but the fruit of other species also have medicinal properties [2]. In vitro and in vivo studies have confirmed the anti-inflammatory, analgesic, antispasmodic, antioxidant, immunoregulatory, and antibacterial effects of this species. The potential to use Japanese quince fruit in the treatment of Parkinson’s disease has also been found [3]. Consumption of the fruit of the *Chaenomeles* genus has been recommended for the following: rheumatism, beri-beri disease, cholera, dysentery, and enterocolitis. In particular, Japanese quince products have been suggested in the therapy for stomach diseases, alleviation of diarrheal symptoms and vomiting, and also protective in liver diseases [1]. Gorlach et al. [4] proved that procyanidin extracted from Japanese quince have proapoptotic activity of HT-29 colon and Caco-2 large intestine carcinoma cells, where fractions containing higher proanthocyanidin oligomers are more active than that of the lower ones.

The aim of this study was to compare the following: (i) basic chemical composition (content of dry matter, soluble solid, ash, pectins, L-ascorbic acid, sugars, and organic acids, as well as titratable acidity and pH); (ii) the content of bioactive compounds such as polyphenolics including polymeric procyanidins (identification by liquid chromatography coupled with photodiode array detector and quadrupole time-of-flight electrospray ionization mass spectrometry (LC-PDA-QTOF-ESI-MS) and quantification by ultra performance liquid chromatography-photodiode array detector-fluorescence detector (UPLC-PDA-FL)); and (iii) in vitro biological activities (antioxidant, α -amylase, α -glucosidase, AChE, BuChE, and 15-LOX inhibition activity) in the nineteen cultivars of *Chaenomeles* fruits. Our secondary aim was to determine the relationships between the basic chemical composition, polyphenolic, and specific biological activities of selected species and cultivars of *Chaenomeles* fruits. It should be emphasized that this is the first such comprehensive work characterizing the chemical composition and biological activities including as many as 19 cultivars of three different species of *Chaenomeles*. The research literature contains no reports on the effects of *Chaenomeles* extracts on pancreatic lipase, AChE, BuChE, and 15-lipoxygenase inhibition activity. In addition, research on α -amylase and α -glucosidase activity of specific cultivars, described in this paper, have not been reported by the other authors.

2. Materials and Methods

2.1. Plant Material and Sample Preparation

Three different species and nineteen cultivars of *Chaenomeles* fruits were used for research. Fruit samples (*C. × superba* ‘Crimson and Gold’, ‘Colour Trail’, and ‘Cameo’; *C. japonica* ‘Red Joy’, and *C. speciosa* ‘Nivalis’, and ‘Rubra’) were collected manually from bushes grown in a field trial

established in 2016 at the experimental orchard at Wrocław (51°07' 02.0''N, 17°04'25.0'' E). *C. × superba* 'Texas Scarlet', 'Nicoline', 'Andenken an Karl Ramcke', 'Pink Lady', 'Flocon Rose', 'Hollandia', and 'Jet Trail'; *C. japonica* 'Cido'; *C. speciosa* 'Simonii'; and new genotype (n1) were collected from an experimental field from the Research Institute of Horticulture in Skierniewice (51° 55' 41.688" N, 20° 9' 9.896" E). *C. × superba* wild and *C. japonica* wild #1 and #2 were collected from wild bushes located near the Centennial Hall in Wrocław (51° 6' 26.548'' N, 17° 4' 56.782'' E) in September 2018. Approximately 0.5 kg fruits (of each cultivar) were collected and then were washed with distilled water. The first part of the study included measurements on fresh fruits of dry matter, ash, soluble solids, pH, titratable acidity, pectin, L-ascorbic acid, sugars, and organic acids content. The second part included freeze dried using a freeze drier (Christ Alpha 2–4; Braun Biotech Int., Melsungen, Germany) for 24 h at the pressure of 0.220 mbar. The samples were subsequently ground using a pestle and mortar to a fine powder and stored vacuum packed in a freezer at -80°C until the analysis but no longer than 5 weeks.

2.2. Extraction Procedure

Methanol extracts for determination of polyphenolic compounds were prepared as follows: The freeze-dried powder of fruits (~1 g) was vortexed for 1 min with 6 mL methanol/water/acetic acid/ascorbic acid (30:68:1:1, *v/v/v/m*), sonicated for 20 min (Sonic 6D; Polsonic, Warsaw, Poland) and left for 24 hours at 4°C . Then, the extract was sonicated again for 20 min, and centrifuged at $19,000 \times g$ for 10 min at 4°C . Finally, the extract was filtered by $0.20\ \mu\text{m}$ hydrophilic PTFE membrane (Millex Simplicity Filter; Merck, Germany) and used for phenolic compounds identification by LC-PDA-QTOF-ESI-MS and quantification by UPLC-PDA. For the determination of antioxidant and in vitro biological activities, the same protocol as that described above was used, but a methanol/water (80:20, *v/v*) with 1% hydrochloric acid mixture was used for extraction.

2.3. Physicochemical Analyses

The dry matter was measured using a vacuum dryer (SPT-200; ZEAMiL Horyzont, Kraków, Poland) according to Turkiewicz et al. [5], while the soluble solids content was determined in fresh juices with a refractometer (Atago Rx 5000; Atago Co.Ltd., Kyoto, Japan) and expressed as °Brix. Total ash content was performed as reported previously by Wojdyło et al. [6]. Pectin content was analyzed according to the Morris method described by Pijanowski et al. [7] and expressed as g per 100 g of fresh weight (fw). Titratable acidity was determined by titration aliquots of homogenate of fresh fruits by 0.1 N NaOH to an end point of pH 8.1 using an automatic pH titration system (pH-meter type IQ 150; Warsaw, Poland) and expressed as g of malic acid per 100 g fw. The pH was measured with the same equipment.

L-ascorbic acid was analyzed according to the HPLC method described previously by Wojdyło et al. [8], and expressed as milligrams per 100 g fw. Sugars were determined by HPLC-ELSD while organic acids by UPLC-PDA method as described previously by Wojdyło et al. [6]. All samples were assayed in triplicate and the results were expressed as g of total sugar content or g of organic acid per kg of fw, respectively.

2.4. Identification and Quantification of Phenolic Compounds by the LC-PDA-QTOF-ESI-MS and UPLC-PDA Methods

Identification and quantification of polyphenols from *Chaenomeles* fruits was carried out using an Acquity UPLC system (Waters Corp., Milford, MA, USA) equipped with a photodiode detector (PDA) with 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 electrospray ionization (ESI) source operating in negative modes. An Acquity UPLC BEH C18 column ($2.1 \times 100\ \text{mm}$, $1.7\ \mu\text{m}$; Waters Corporation, Milford, USA) at 30°C was used to perform the chromatographic separation of $5\ \mu\text{L}$ of each sample. Elution at a flow rate of $0.42\ \text{mL/min}$ was completed within 15 min using a sequence of elution modes, linear gradients and isocratic. The mobile phase was composed of

solvent A (4.5% formic acid) and solvent B (acetonitrile). Samples were eluted according to a linear gradient: 0 to 12 min, 1% to 25% B; 12 to 12.5 min, 100% B; 12.5 to 13.5 min, 1% B; and, then, held constant to re-equilibrate the column. Analysis was carried out using full scan, data-dependent MS scanning from m/z 100 to 1700. Leucine enkephalin was used as the mass reference compound, to ensure that mass was measured accurately, at a concentration of 500 pg/ μ L. The mass spectrometer was operated in a negative ion mode and set to the base peak intensity (BPI) chromatograms and scaled to 12,400 counts per second (cps) (=100%). The optimized MS conditions were as follows: capillary voltage of 2000 V, cone voltage of 35 V, source and desolvation temperature were of 100 and 250 °C, respectively, and desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as the collision gas, with voltage ramping cycles from 0.3 to 2 V. The characterization of the single components was carried out via the retention time and the accurate molecular masses. Phenolic acids, flavan-3-ols and flavonols compound were optimized to their estimated molecular masses $[M-H]^-$ in the negative mode before and after fragmentation. The data were collected by MassLynx™ 4.1 ChromaLynx Application Manager (Waters Corp., Milford, MA, USA) software.

For quantification, elution was the same gradient as LC-PDA-QTOF-ESI-MS analysis. The PDA spectra were measured over the wavelength range of 200 to 600 nm in steps of 2 nm. The runs were monitored at the following wavelengths: flavan-3-ols at 280 nm, phenolic acids at 320 nm, and flavonols at 360 nm. Retention times (R_t) and spectra were compared with those of pure standards. Quantification was achieved by injection of solutions of known concentrations ranging from 0.05 to 5 mg/mL ($R^2 \leq 0.9998$) made from (–)-epicatechin, (+)-catechin, procyanidin B1, B2, B3, and C1, chlorogenic acid, neochlorogenic acid, 3,5-di-caffeoylquinic acid, quercetin and kaempferol-3-*O*-glucoside, and -3-*O*-rutinoside. 4-*p*-Coumaroylquinic acid was expressed as caffeic acid. Acylated quercetin and kaempferol were expressed as quercetin and kaempferol-3-*O*-glucoside, respectively. All samples were assayed in triplicate and the results were expressed as g per kg of dry weight (dw).

2.5. Quantification of Polymeric Procyanidins by the UPLC-PDA-FLMethod

Analysis of polymeric procyanidins was performed by the phloroglucinolysis method as described previously by Wojdyło et al. [8]. The analysis was carried out on a UPLC system Acquity (Waters Corp., Milford, MA, USA) consisting of a binary solvent manager and fluorescence detector (FL). The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (–)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (–)-epicatechin-phloroglucinol adduct standards. All incubations were done in triplicate. Results were expressed as g per kg of dw.

2.6. Determination of Antioxidant and In Vitro Biological Activities

Antioxidant activities were determined using the ABTS method described by Re et al. [9] and the FRAP method described by Benzie and Strain [10]. The ORAC assay was determined following the method previously described by Ou et al. [11]. All samples were assayed in triplicate and the results were expressed as mM of Trolox per 100 g of dw.

The inhibition of α -amylase, α -glucosidase, pancreatic lipase acetylcholinesterase, and butyrylcholinesterase were measured as reported previously by Wojdyło et al. [12] and 15-lipoxygenase inhibition activity was measured using ferric oxidation of xylenol orange (FOX) assay previously described by Chung et al. [13].

All samples were assayed in triplicate and the results were expressed as IC_{50} (mg of dried sample per mL of enzyme) or % of inhibition. All spectrophotometric measurements were performed using a plate reader Synergy H1 (BioTek Instruments, Inc., Winooski, VT, USA).

2.7. Antioxidant On-Line Profiling by HPLC-PDA Coupled with Post-Column Derivatization with ABTS

The antioxidant activity of individual HPLC peaks was measured as reported previously by Turkiewicz et al. [14] using an on-line HPLC antioxidant detector system. The detection wavelength was set at 280 and 734 nm, while the injection volume of sample was 10 μ L. The separation was achieved using CADENZA C18 column (75mm \times 4.6mm i.d., 3 μ m; Tokyo, Japan) with a C18 guard column at 30 °C. The gradient elution solvent was formic acid solution (solvent A, 2%) and acetonitrile (solvent B, 100%) at a flow rate of 0.6 mL/min, 0 to 30 min, 2% to 40% B, and up to 45 min column was recognition. ABTS radical cations were produced in accordance with the method described by Re et al. [9]. The second pump delivered the ABTS solution (at a flow rate of 0.2 mL/min) which was mixed with the mobile phase after the first PDA detector. The mixture was guided through PTFE reaction coil (25 m long, 0.25 mm internal diameter, at 40 °C) to a second UV detector, where decolorization of the mixture was detected as a negative peak at 734 nm.

2.8. Statistical Analysis

Statistical analysis was conducted using XLSTAT2017, Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France). Significant differences ($p \leq 0.05$) between means were evaluated by one-way ANOVA and Tukey's test. Agglomerative hierarchical clustering (AHC) analysis was performed to highlight correlations.

3. Results and Discussion

3.1. Physicochemical Analysis

Table 1 shows basic chemical composition and physical properties of the analyzed *Chaenomeles* fruits. Dry matter content of the fruits varied significantly ($p \leq 0.05$) from 10.09% (*C. japonica* n1) to 20.40% (*C. \times superba* wild). Thomas et al. [15] reported dry matter content of Japanese quince genotypes grown in Sweden and Lithuania as ranging from 10.60% to 11.70%, while Tarko et al. [16] determined dry matter content at the level of 12.90%. Lesińska [17] investigated dry matter in Japanese quince fruit grown in Poland and obtained results from 13% to 18% (average 15.5%) depending on the harvest year. In addition, the key parameter affecting the dry matter content is sun exposure, the shortage of which results in lower dry weight, but also maturity stage, cultivar, climatic conditions, and agrotechnical techniques. Ash content of the analyzed *Chaenomeles* fruits was from 0.32% (*C. \times superba* 'Crimson and Gold') to 0.64% (*C. \times superba* 'Pink Lady') ($p \leq 0.05$). The results are consistent with those obtained by Rubinskienė et al. [18], which indicates that the range of ash content in Japanese quince fruit is 0.38% to 0.46%. The content of soluble solids (mainly sugars) is an important indicator of the quality of fruit. The sweet taste of fruits depends on the amount of soluble solid content (SSC), which plays an important role for fruit intended for processing, as well as those for direct consumption. The SSC in *Chaenomeles* fruits of selected species and cultivars was from 5.8 (*C. speciosa* 'Simonii') to 12.1 °Brix (*C. \times superba* wild) ($p \leq 0.05$). Higher values of the SSC were recorded in the fruit of Japanese quince by Rubinskienė et al. [18], i.e., from 14 to 17 °Brix, while results similar to ours (9.4 °Brix) were obtained by Tarko et al. [16] and Ros et al. [19], for 21 different genotypes of *Chaenomeles* fruits SSC ranged from 5.2 to 8.8 °Brix (average 7.1 °Brix). Fruits belonging to the genus *Chaenomeles* are considered rich in pectin compounds, which are located mainly in the fruit pulp. The source of pectin is mainly immature fruit (0.85% to 1.28%), because, during the ripening of fruit, pectin is partially transformed to monosaccharides. The average pectin content in fruits (1.4% of fresh fruit) is equal to or sometimes higher than the values determined in apples [1,15]. The analyzed fruits showed a large variation in pectin content ($p \leq 0.05$) and the results ranged from 0.65% (*C. japonica* n1) to 1.72% (*C. \times superba* wild). Undoubtedly, the characteristic feature of *Chaenomeles* fruits is high titratable acidity (TA). For the fruit of *C. japonica*, *C. speciosa*, and *C. \times superba* values of TA ranged from 3.11 (*C. japonica* 'Cido') to 6.16 g malic acid/100 g fresh weight (fw) (*C. \times superba* wild). Other authors reported acidity values for Japanese quince fruit in the range from 2.6 to 5.6 g of malic acid/100 g fw [19] and 4.10 g of malic

acid/100g fw [16]. These values outweigh the acidity of apple juice (0.2 to 0.7 g malic acid/100 g fw) and are comparable with lemon (5.0 to 9.0 g malic acid/100 g fw) [19]. Therefore, the fruits are classified as extremely acidic, unsuitable for direct consumption [19]. The high acidity of the *Chaenomeles* juice was accompanied by a low pH from 2.71 (*C. × superba* wild) to 2.99 (*C. speciosa* ‘Simonii’). To confirm the results, Ros et al. [19] determined the pH of *Chaenomeles* fruits genotypes in the range 2.40–2.99 (average 2.60). For comparison, the pH of apple juice is 3.50 to 3.80 and lemon 2.00 to 2.30 [19].

Large variation ($p \leq 0.05$) was found in the content of L-ascorbic acid. Among the taxa studied, the sample of *C. × superba* ‘Hollandia’ had the highest amount of L-ascorbic acid (195.05 mg/100 g fw) while *C. speciosa* ‘Rubra’ had the lowest amount (30.26 mg/100 g fw). For comparison, Ros et al. [19] determined the average content of L-ascorbic acid in *Chaenomeles* fruit at 128.26 mg/100 g. Bieniasz et al. [20] studied the influence of storage and harvesting year on the content of vitamin C in Japanese quince fruit. They obtained results of L-ascorbic acid content in nine *Chaenomeles* genotypes in the range of 90.0–243.0 and 73.1–172.6 mg/100 g of fresh fruit in two successive years of harvest, respectively. The obtained results are higher than for lemon (50.4 mg/100 g fw), strawberry (60.0 mg/100 g fw), and blackcurrant (86.0 mg/100 g fw) [21].

The *Chaenomeles* fruits are characterized by an extremely low content of sugars in comparison with many other fruits. *C. × superba* ‘Texas Scarlet’ was the cultivar with the highest sugar content, 3.98 g/100 g fw ($p \leq 0.05$), while ‘Jet Trail’ ranked at the other end of the scale (0.44 g/100 g fw). The main identified saccharide was fructose, followed by sorbitol and glucose. Each one accounted for approximately 40.10%, 32.89%, and 26.70% of the total identified sugars, respectively. Xylose was only found in two cultivars of *C. × superba*, ‘Crimson and Gold’ and ‘Andenken an Karl Ramcke’, in trace concentrations. For comparison, Hellín et al. [22] analyzed the sugar content in ten *Chaenomeles* genotypes grown in Sweden and Lithuania and found that the main sugars are fructose, glucose, sorbitol, and sucrose. The content of reducing sugars was similar to the values obtained in earlier studies but also the presence of sucrose, maltose, mannitol, stachyose, raffinose, rhamnose, and inositol was reported before [16,22–24]. Differences in the quantitative and qualitative composition may result from, among other reasons, the fact that during the analysis of the whole fruit, apart from sugars, the carbohydrate constituents of the structural polysaccharides of the *Chaenomeles* fruit cell wall are also determined, which does not occur during juice analysis. One of the indicators characterizing fruit quality is the ratio of fructose to glucose (fructose is more resistant to heating than glucose, which slows the browning process, e.g., during the manufacture of preserves and marmalades). In the analyzed fruits it was 1.5, while Lesińska [23] found this index for Japanese quince fruit equal to 1.8. In contrast, Tarko et al. [16] reported this ratio equal to 0.3. For comparison, in apples it is 1.8 and in pears 2.0 [23].

Table 1. Basic chemical composition of selected species and cvs. of *Chaenomeles* fruits.

Species	<i>Chaenomeles</i> × <i>Superba</i>										
Cultivar	Crimson and Gold	Texas Scarlet	Nicoline	Andenken an Karl Ramcke	Pink Lady	Colour Trail	Flocon Rose	Hollandia	Jet Trail	Wild	Cameo
dry matter (%)	13.46 ± 0.20h	16.21 ± 0.21d	17.51 ± 0.30bc	17.13 ± 0.13c	14.76 ± 0.20f	13.82 ± 0.20gh	11.95 ± 0.25ij	15.58 ± 0.18e	13.92 ± 0.22gh	20.40 ± 0.20a	12.31 ± 0.21i
ash content (%)	0.32 ± 0.12j	0.43 ± 0.33fghi	0.48 ± 0.15defg	0.53 ± 0.13bcdef	0.64 ± 0.24a	0.41 ± 0.11ghij	0.46 ± 0.15fg	0.41 ± 0.10ghij	0.51 ± 0.11cdefg	0.57 ± 0.14abcde	0.34 ± 0.44ij
SSC (°Brix)	6.8 ± 0.1h	11.6 ± 0.0b	10.4 ± 0.1c	7.9 ± 0.1f	9.4 ± 0.0d	9.5 ± 0.1d	7.3 ± 0.0g	10.3 ± 0.1c	5.9 ± 0.0j	12.1 ± 0.1a	7.2 ± 0.1g
pectin (%)	1.23 ± 0.10def	1.57 ± 0.10abc	1.62 ± 0.12ab	1.69 ± 0.09ab	1.10 ± 0.10efg	0.99 ± 0.11efgh	0.68 ± 0.18ij	0.98 ± 0.08fghi	1.41 ± 0.10bcd	1.72 ± 0.20a	0.71 ± 0.10hij
TA (g of malic acid/100 g of fw)	4.27 ± 0.13ef	4.60 ± 0.10cde	4.66 ± 0.49cde	5.30 ± 0.15b	4.64 ± 0.49cde	5.20 ± 0.12bc	4.20 ± 0.10ef	4.25 ± 0.15ef	3.45 ± 0.10gh	6.16 ± 0.16a	4.66 ± 0.10cde
pH	2.927 ± 0.01d	2.801 ± 0.00h	2.782 ± 0.01hi	2.738 ± 0.01j	2.772 ± 0.00i	2.897 ± 0.01e	2.855 ± 0.02g	2.842 ± 0.00g	2.975 ± 0.01ab	2.713 ± 0.01j	2.892 ± 0.01ef
L-ascorbic acid (mg/100 g of fw)	40.83 ± 0.55jk	175.32 ± 0.68ab	134.38 ± 0.23cd	144.17 ± 0.50c	111.81 ± 0.88def	47.86 ± 0.74ijk	110.99 ± 0.29ef	195.05 ± 0.30a	70.96 ± 0.55gh	143.09 ± 1.00c	70.29 ± 0.67ghi
Sugars (g/100g fw)											
xylose	0.03 ± 0.00b	nd	nd	0.05 ± 0.00a	nd	nd	nd	nd	nd	nd	nd
fructose	0.58 ± 0.02g	1.80 ± 0.35a	0.60 ± 0.02g	0.51 ± 0.03gh	1.34 ± 0.13bcd	1.10 ± 0.07def	0.94 ± 0.02f	1.62 ± 0.03ab	0.12 ± 0.02i	1.31 ± 0.07cde	1.18 ± 0.04def
sorbitol	0.30 ± 0.00jk	0.95 ± 0.16b	0.56 ± 0.00fgh	0.75 ± 0.03cde	0.72 ± 0.06def	0.72 ± 0.04def	0.47 ± 0.01hij	0.79 ± 0.01bcde	0.22 ± 0.01k	1.40 ± 0.05a	0.37 ± 0.00ijk
glucose	0.29 ± 0.00ghi	1.23 ± 0.26a	0.44 ± 0.02efg	0.29 ± 0.01ghi	0.91 ± 0.07bc	0.88 ± 0.06bc	0.51 ± 0.01def	0.91 ± 0.02bc	0.10 ± 0.01i	0.85 ± 0.04c	0.93 ± 0.03bc
total	1.20 ± 0.02ij	3.98 ± 0.37a	1.60 ± 0.04hi	1.60 ± 0.27hi	2.97 ± 0.26cde	2.70 ± 0.17def	1.92 ± 0.04gh	3.32 ± 0.06bcd	0.44 ± 0.04k	3.56 ± 0.17abc	2.48 ± 0.08fg
fructose:glucose ratio	2.0	1.5	1.4	1.8	1.5	1.2	1.8	1.8	1.2	1.5	1.3
Organic Acids (g/kg fw)											
oxalic	0.06 ± 0.00e	0.70 ± 0.05b	0.24 ± 0.06bcde	0.27 ± 0.00bcde	0.18 ± 0.02bcde	0.23 ± 0.02bcde	0.20 ± 0.03bcde	0.59 ± 0.09a	0.23 ± 0.03bcde	0.28 ± 0.04bcd	0.17 ± 0.02bcde
maleic	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	nd	0.01 ± 0.00a	0.01 ± 0.00a
citric	0.78 ± 0.07de	0.90 ± 0.12de	0.41 ± 0.02fg	0.75 ± 0.05de	0.66 ± 0.09ef	1.20 ± 0.02ab	1.29 ± 0.10ab	1.35 ± 0.09ab	0.31 ± 0.02g	1.42 ± 0.07a	0.91 ± 0.02cde
malic	62.52 ± 1.33efg	56.12 ± 1.00gh	48.61 ± 1.97hi	64.34 ± 3.54ef	64.87 ± 3.43ef	81.44 ± 2.12abc	62.14 ± 1.70efg	57.64 ± 0.39fg	38.83 ± 1.47j	88.75 ± 1.32a	65.94 ± 2.52e
quinic	10.51 ± 0.16fg	7.52 ± 0.12ij	14.50 ± 0.32bc	12.37 ± 0.15de	9.91 ± 0.04fgh	10.70 ± 0.48efg	14.52 ± 0.18bc	17.28 ± 1.39a	11.50 ± 0.79def	17.15 ± 0.29a	8.70 ± 0.04hi
shikimic	0.11 ± 0.01fg	0.14 ± 0.01fg	0.73 ± 0.05de	0.91 ± 0.07cd	0.12 ± 0.01fg	0.07 ± 0.00g	0.22 ± 0.01efg	0.17 ± 0.00efg	0.65 ± 0.00def	2.70 ± 0.06a	0.10 ± 0.00fg
total	73.99 ± 1.57de	65.07 ± 1.29ef	64.50 ± 2.37ef	78.65 ± 3.82d	75.75 ± 3.55d	93.65 ± 2.64c	78.39 ± 1.96d	77.04 ± 1.96d	51.52 ± 2.26g	110.31 ± 1.12a	75.82 ± 2.60d
sugars:acids ratio	0.2	0.6	0.2	0.2	0.4	0.3	0.2	0.4	0.1	0.3	0.3
Species	<i>Chaenomeles Japonica</i>					<i>Chaenomeles Speciosa</i>					
Cultivar	Cido	Red Joy	Wild #1	Wild #2	n1 (New)	Nivalis			Rubra	Simonii	
dry matter (%)	13.45 ± 0.15h	10.95 ± 0.15k	17.76 ± 0.14b	14.35 ± 0.15fg	10.09 ± 0.11l	17.04 ± 0.24c			11.95 ± 0.15j	11.71 ± 0.20j	
ash content (%)	0.47 ± 0.23efg	0.35 ± 0.15hij	0.61 ± 0.31abc	0.58 ± 0.22abcd	0.41 ± 0.21ghij	0.43 ± 0.33fghi			0.45 ± 0.15fgh	0.63 ± 0.23ab	
SSC (°Brix)	8.4 ± 0.0e	6.3 ± 0.1i	10.3 ± 0.0c	8.6 ± 0.0e	6.3 ± 0.1i	10.5 ± 0.0c			6.6 ± 0.0h	5.8 ± 0.1j	
pectin (%)	0.76 ± 0.16hij	0.95 ± 0.15fghij	1.08 ± 0.18efg	0.90 ± 0.10ghij	0.65 ± 0.15j	1.29 ± 0.09cde			0.85 ± 0.15ghij	0.88 ± 0.18ghij	
TA (g of malic acid/100 g of fw)	3.11 ± 0.11h	4.90 ± 0.10bcd	5.50 ± 0.15b	5.32 ± 0.12b	3.97 ± 0.10fg	5.44 ± 0.14b			4.58 ± 0.12de	3.45 ± 0.10gh	

Table 1. Cont.

Species	<i>Chaenomeles × Superba</i>										
Cultivar	Crimson and Gold	Texas Scarlet	Nicoline	Andenken an Karl Ramcke	Pink Lady	Colour Trail	Flocon Rose	Hollandia	Jet Trail	Wild	Cameo
pH	2.867 ± 0.01fg	2.941 ± 0.01cd	2.965 ± 0.01bc	2.966 ± 0.01bc	2.843 ± 0.00g		2.862 ± 0.00g		2.992 ± 0.01ab		2.994 ± 0.00a
L-ascorbic acid (mg/100 g of fw)	132.33 ± 0.35cde	57.82 ± 0.23hij	101.72 ± 0.21f	114.13 ± 0.21def	91.19 ± 0.57fg		154.97 ± 0.33bc		30.26 ± 0.20k		53.6 ± 0.09hij
Sugars (g/100 g fw)											
xylose	nd	nd	nd	nd	nd		nd		nd		nd
fructose	1.57 ± 0.01abc	0.18 ± 0.02i	1.11 ± 0.04def	1.02 ± 0.01ef	0.62 ± 0.03g		1.70 ± 0.02a		0.24 ± 0.01hi		0.25 ± 0.02hi
sorbitol	0.66 ± 0.01efg	0.25 ± 0.02k	1.38 ± 0.13a	0.92 ± 0.12bc	0.33 ± 0.02jk		0.88 ± 0.00bcd		0.53 ± 0.03ghi		0.25 ± 0.02k
glucose	1.07 ± 0.03ab	0.17 ± 0.01i	0.65 ± 0.02d	0.62 ± 0.02de	0.42 ± 0.01fgh		1.21 ± 0.01a		0.22 ± 0.00hi		0.15 ± 0.01i
total	3.30 ± 0.05bcd	0.60 ± 0.04jk	3.14 ± 0.20cde	2.56 ± 0.10ef	1.37 ± 0.07hi		3.79 ± 0.03ab		0.99 ± 0.04ijk		0.65 ± 0.04jk
fructose:glucose ratio	1.5	1.1	1.7	1.6	1.5		1.4		1.1		1.6
Organic Acids (g/kg fw)											
oxalic	0.13 ± 0.02cde	0.20 ± 0.00bcde	0.17 ± 0.05bcde	0.25 ± 0.01bcde	0.65 ± 0.25a		0.26 ± 0.00bcde		0.09 ± 0.01de		0.32 ± 0.08bc
maleic	nd	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a	0.01 ± 0.00a		nd		0.01 ± 0.00a		0.01 ± 0.00a
citric	0.30 ± 0.06g	1.23 ± 0.10ab	1.35 ± 0.04ab	1.33 ± 0.05ab	0.94 ± 0.17cd		1.16 ± 0.09bc		0.81 ± 0.07de		0.75 ± 0.11de
malic	32.08 ± 6.19j	79.51 ± 3.82bc	86.14 ± 1.30ab	86.04 ± 0.78ab	56.20 ± 1.79gh		74.30 ± 3.99cd		67.02 ± 1.20de		47.93 ± 1.74i
quinic	9.05 ± 0.76ghi	9.15 ± 0.36ghi	14.22 ± 0.80bc	15.10 ± 0.56b	13.09 ± 0.20cd		16.89 ± 1.06a		6.21 ± 0.02j		12.33 ± 0.43de
shikimic	0.08 ± 0.01g	1.12 ± 0.08bcd	1.34 ± 0.56bc	0.72 ± 0.53de	0.12 ± 0.01fg		1.48 ± 0.12b		1.17 ± 0.05bcd		0.10 ± 0.01fg
total	41.64 ± 7.04h	91.21 ± 4.36c	103.23 ± 2.58ab	103.47 ± 1.81ab	71.01 ± 1.52de		94.09 ± 5.26bc		75.30 ± 1.30d		61.45 ± 2.37f
sugars:acids ratio	0.8	0.1	0.3	0.2	0.2		0.4		0.1		0.1

nd, not detected; SSC, soluble solid content; TA, tritable acidity; fw, fresh weight. ± Standard deviation, value in the same columns followed by different letters are significantly different at $p < 0.05$ according to Tukey's test.

Contrary to sugars, *Chaenomeles* fruits contain very large amounts of organic acids. In the analyzed samples the following six organic acids were detected: oxalic, maleic, citric, malic, quinic, and shikimic (Table 1). Other typical organic acids normally found in fruits, such as succinic, fumaric, and tartaric acid, were not found in detectable amounts. The content of organic acids in *Chaenomeles* fruits differed between samples ($p \leq 0.05$). Total content of organic acid ranged from 41.64 (*C. japonica* 'Cido') to 110.31 g/kg fw (*C. × superba* wild). Malic acid, the main organic acid (81.94% of total acids), was present in the samples at concentrations from 32.08 to 88.75 g/kg of fw, which is consistent with Hellín et al. [22]. The highest amount was found in a sample of *C. × superba* wild. The range of quinic acid (15.63% of total acids) was 6.21–16.89 g/kg fw. The remaining acids, i.e., citric, shikimic, oxalic, and maleic were in the minority and accounted for 1.20, 0.89, 0.34, and 0.01%, respectively, of the total amount of organic acids. Another parameter of fruit quality is the ratio of sugars to acids. For fruit intended for direct consumption, it is required that sugars must exceed acids ten-fold. In the case of the analyzed *Chaenomeles* fruits this ratio is only 0.3:1, and thus is even lower than among the fruits of sea buckthorn, wild growing trees, and shrubs or lemons, where this ratio is 1:1. Due to the high acidity, *Chaenomeles* fruits or juices are unsuitable for drinking without the addition of sweeteners. Additionally, for juices a better alternative seems to be the inoculation of the malolactic microorganism *Oenococcus oeni*, which transforms malic acid into lactic acid, characterized by lower acidity. In addition, malic acid is used in the food industry as an acidifying additive, and therefore *Chaenomeles* juice, like lemon juice, can be used as a natural acidifying agent in a wide range of food products [19,22].

3.2. Polyphenol Compounds

A total of 15 polyphenol compounds were found in the nineteen cultivars of the three *Chaenomeles* species by using LC-PDA-QTOF-ESI-MS (Table 2). Structural formulas of selected phenolic compounds identified in *Chaenomeles* fruits are shown on Figure S1 (Supplementary Material). In the chromatogram profiles (Figure 1) obtained at 280 nm, the labeled peaks 1 to 15 followed an elution order. Among these compounds, 13 were flavan-3-ols (monomers and procyanidins dimers, trimers, and tetramers) and two were caffeoylquinic acid derivatives.

Table 2. Identification of phenolic compounds in *Chaenomeles* fruits on example *Chaenomeles × superba* 'Texas Scarlet' by using liquid chromatography coupled with photodiode array detector and quadrupole time-of-flight electrospray ionization mass spectrometry (LC-PDA-QTOF-ESI-MS).

Peak	Compound	R _t (min)	λ _{max} (nm)	Molecular Formula	MS [M-H] ⁻ (m/z)	MS/MS (m/z)
1	Procyanidin B3	11.09	280	C ₃₀ H ₂₆ O ₁₂	577.13	425.08/451.00/407.05/289.05
2	(+)-Catechin	12.53	240/280	C ₁₅ H ₁₃ O ₆	289.06	245.06/205.03/125.01
3	Procyanidin trimer	12.79	280	C ₄₅ H ₃₇ O ₁₈	865.21	577.13/425.08/289.06
4	5-O-Caffeoylquinic acid (chlorogenic)	13.20	246/326	C ₂₂ H ₂₇ O ₁₄	353.08	191.04
5	4-O-Caffeoylquinic acid (cryptochlorogenic)	13.33	246/326	C ₂₂ H ₂₇ O ₁₄	353.08	191.04
6	Procyanidin trimer	13.84	280	C ₄₅ H ₃₇ O ₁₈	865.21	577.13/425.08/289.06
7	Procyanidin B2	14.19	280	C ₃₀ H ₂₆ O ₁₂	577.13	425.08/451.00/407.05/289.05
8	Procyanidin dimer	15.21	280	C ₃₀ H ₂₆ O ₁₂	577.13	425.08/289.05
9	(-)-Epicatechin	15.58	240/280	C ₁₅ H ₁₃ O ₆	289.06	245.06/205.03/125.01
10	Procyanidin dimer	15.80	280	C ₃₀ H ₂₆ O ₁₂	577.13	425.08/289.05
11	Procyanidin C1	16.18	280	C ₄₅ H ₃₇ O ₁₈	865.21	577.13/289.06/245.06/125.01
12	Procyanidin tetramer	16.79	280	C ₆₀ H ₄₉ O ₂₄	1153.3	865.21/576.12/289.05
13	Procyanidin tetramer	17.00	280	C ₆₀ H ₄₉ O ₂₄	1153.3	865.21/576.12/289.05
14	Procyanidin dimer	17.20	280	C ₃₀ H ₂₆ O ₁₂	577.13	425.08/289.05
15	Procyanidin dimer	17.92	280	C ₃₀ H ₂₆ O ₁₂	577.13	425.08/289.05

The m/z values of flavan-3-ol ions were as follows: [M-H]⁻ 289 for monomer of (+)-catechin or (-)-epicatechin, and for B-type procyanidin dimer as [M-H]⁻ 577, trimer as [M-H]⁻ 865, and for tetramer as [M-H]⁻ 1153 [2,25]. Six procyanidin dimers (peaks 1, 7, 8, 9, 14, and 15) were detected at different retention times (R_t) in the electrospray-ionization quadrupole time-of-flight (ESI-QTOF) mass spectrometry in negative ion mode. All compounds gave the same [M-H]⁻ parental ion at m/z 577.13 in

accordance with the molecular formula $C_{30}H_{26}O_{12}$ [26]. Their molecular ions showed a fragment ion (MS/MS) at m/z 425.08. In addition, each of these compounds had a fragmentation ion at m/z 289.05, which confirms that the procyanidins in *Chaenomeles* fruit are made of (+)-catechin and (–)-epicatechin units. Through analyzing the samples with standards and based on a comparison of R_t , it was found that peaks 1 and 7 are procyanidin B3 and B2, respectively, which is in accordance with Du et al. [2] and Teleszko and Wojdyło [27]. Moreover, the negative ESI-QTOF spectra of procyanidin B1 and procyanidin B2 gave $[M-H-170]^-$ fragment ions at m/z 407 from the retro Diels–Alder F reaction of the heterocyclic ring and loss of H_2O at m/z 451 ($[M-H-126]^-$) from cleavages between C_4 – C_5 [28]. Therefore, peaks 8, 10, 14 and 15 have been suggested to be procyanidin dimers [25]. Peak 2 (m/z 289.06) yielded fragment ions at m/z 245.06, 205.03, and 125.01, while peak 9 gave the same fragment ions. Due to the fact that stereoisomers could not be distinguished by mass spectrometry, the retention times have been compared with the standards, and those compounds have been assigned to (+)-catechin and (–)-epicatechin, respectively.

Moreover, according to Bravo et al. [29], the $[M-H-44]^-$ fragment at m/z 245.06 could result from the loss of a CO_2 group or the loss of a $-CH_2-CHOH-$ group and the ion at m/z 205.03 is probably due to the loss of a flavonoid A-ring. The presence of an ion at m/z 125.01 is considered to be diagnostic for the presence of two (–OH) groups on the A-ring of flavan-3-ols [30]. The elution order of procyanidin monomers, dimers, and trimers was procyanidin B3 < (+)-catechin < procyanidin B2 < (–)-epicatechin < procyanidin C1. Whereas, peaks 3 and 6 with a precursor ion at m/z 865.21 and identical molecular formula $C_{45}H_{37}O_{18}$ were designated as procyanidin trimers. Peak 11 ($R_t = 16.18$ min) exhibited a deprotonated molecule at m/z 865.21 and a MS/MS fragment at m/z 577.13, 289.06, 245.06, and 125.01. The comparative analysis with standards confirmed that this signal came from procyanidin C1. Additionally, this compound was reported in Japanese quince before by Teleszko and Wojdyło [27] and Lewandowska et al. [31]. Finally, peaks 12 and 13 at $R_t = 16.79$ and 17.00 min, respectively, with the molecular ion at m/z 1153.3 and molecular formula $C_{60}H_{49}O_{24}$, have been proposed to be procyanidin tetramers.

With regards to caffeoylquinic acid derivatives, two compounds were detected at $R_t = 13.20$ min and $R_t = 13.33$ min. Peaks 4 and 5, with the identical molecular formula $C_{22}H_{27}O_{14}$, demonstrated the same UV absorption bands and the same $[M-H]^-$ at m/z 353.08. Moreover, a product ion $[M-H-162]^-$ at m/z 191.04, which was ascribed to quinic acid, was also the same for both compounds. On the basis of the correct MS and MS/MS data but also the literature [5,26,31], these compounds were designated as 5-O-caffeoylquinic acid (chlorogenic) and 4-O-caffeoylquinic acid (cryptochlorogenic), respectively.

The content of each polyphenol compound was calculated using UPLC-PDA analysis. The flavan-3-ol content including (+)-catechin, (–)-epicatechin, and procyanidin oligomers accounts for 96.02% to 99.85% of all phenolic compounds. This indicates clearly that procyanidins were the main polyphenol compounds in *Chaenomeles* fruits. Generally, there were three main compounds (procyanidin B2, (–)-epicatechin, and procyanidin C1) in the analyzed species and cultivars of *Chaenomeles* (Table 3).

Total phenolic content, calculated as the sum of individual phenolic compounds, varied significantly between genotypes ($p \leq 0.05$), with *C. × superba* ‘Nicoline’ displaying the highest (170.38 g/kg dw), and *C. speciosa* ‘Rubra’ the lowest content (57.84 g/kg dw). Procyanidin B2 was the compound present in the largest amount, in the range from 3.39 (*C. japonica* ‘Red Joy’) to 18.16 g/kg dw (*C. × superba* ‘Nicoline’). Analyzing the content of (+)-catechin and (–)-epicatechin in particular species, *C. × superba* contained 0.27 to 1.07 and 2.35 to 7.68 g/kg dw, *C. japonica* 0.22 to 0.86 and 1.77 to 4.93 g/kg dw while *C. speciosa* contained 0.69 to 0.99 and 1.97 to 4.99 g/kg dw, respectively. The content of polymeric procyanidins in all the tested genotypes ranged from 34.60 (*C. × superba* ‘Color Trail’) to 109.67 g/kg dw (*C. × superba* ‘Colour Trail’), with an average of 63.27 g/kg dw. The degree of polymerization (DP) ranged from 2.43 (*C. × superba* ‘Colour Trail’) to 4.25 (*C. × superba* ‘Pink Lady’), indicating that the analyzed flavan-3-ols were oligomers ($2 < DP < 10$) with a low degree of polymerization. Moreover, the low DP in the *Chaenomeles* fruit causes them to not be astringent

and bitter, such as chokeberry, which also contains significant amounts of procyanidin compounds but with a much higher degree of polymerization [32]. Total phenolic content, as well as polymeric proanthocyanidin concentrations, in this study, were higher than reported by Du et al. [2] and Teleszko and Wojdyło [27]. *C. × superba* ‘Cameo’ accumulated the greatest amounts of phenolic acids (3.30 g/kg dw), and in ‘Cido’ their content was the lowest (0.15 g/kg dw). Additionally, 4-*O*-caffeoylquinic acid (cryptochlorogenic) was absent in some samples, i.e., *C. × superba* ‘Andenken an Karl Ramcke’, ‘Pink Lady’, wild, *C. japonica* wild #2, and *C. speciosa* ‘Rubra’.

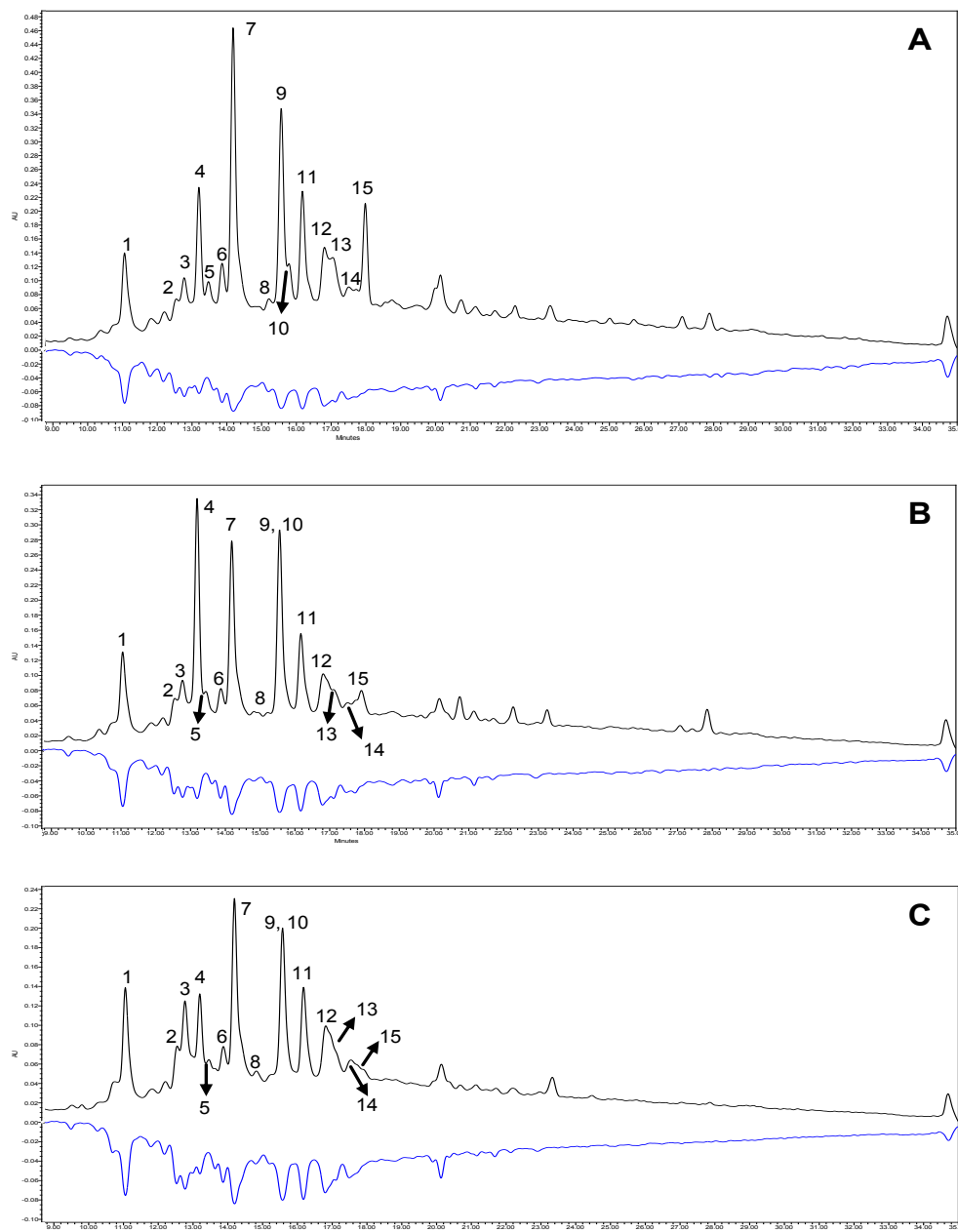


Figure 1. Segment (9.0 to 35.0 min) of chromatographic profiles obtained before and after the derivatization process using the ABTS reagent in samples of *Chaenomeles × superba* ‘Texas Scarlet’ (A), *Chaenomeles × superba* ‘Cameo’ (B), and *Chaenomeles speciosa* ‘Nivalis’ (C). Peak number identities are displayed in Table 2.

Table 3. Content of phenolic compounds (g/kg dw) in various species and cvs. of *Chaenomeles* fruits.

<i>Chaenomeles × Superba</i>											
Peak no	Crimson and Gold	Texas Scarlet	Nicoline	Andenken an Karl Ramcke	Pink Lady	Colour Trail	Flocon Rose	Hollandia	Jet Trail	Wild	Cameo
Phenolic acids											
4	0.43 ± 0.15ef	1.68 ± 0.20bc	1.34 ± 0.21cd	1.35 ± 0.10cd	0.79 ± 0.13de	0.23 ± 0.08ef	1.65 ± 0.27bc	1.08 ± 0.11cd	1.12 ± 0.19cd	3.04 ± 0.21a	3.05 ± 0.15a
5	0.46 ± 0.12bc	0.35 ± 0.10bc	0.04 ± 0.00c	nd	nd	0.36 ± 0.08bc	0.41 ± 0.22bc	0.45 ± 0.11bc	0.06 ± 0.00c	nd	0.25 ± 0.14bc
Sum	0.89 ± 0.15ef	2.03 ± 0.20bc	1.38 ± 0.00de	1.35 ± 0.31de	0.79 ± 0.20ef	0.59 ± 0.10fg	2.06 ± 0.33bc	1.53 ± 0.18cd	1.18 ± 0.27def	3.04 ± 0.11a	3.30 ± 0.19a
Flavan-3-ols											
1	0.28 ± 0.08i	2.86 ± 0.22a	1.85 ± 0.21cde	1.47 ± 0.18efg	0.65 ± 0.09hi	1.00 ± 0.12gh	2.02 ± 0.44bcde	1.66 ± 0.36def	1.76 ± 0.15cde	1.75 ± 0.27cde	2.75 ± 0.22a
2	0.27 ± 0.10de	0.29 ± 0.11cde	0.42 ± 0.08bcde	0.37 ± 0.10cde	0.42 ± 0.15bcde	0.75 ± 0.15abcde	0.62 ± 0.22abcde	0.69 ± 0.19abcde	1.07 ± 0.08a	0.86 ± 0.31abcd	0.86 ± 0.28abcd
3	nd	2.86 ± 0.44a	2.64 ± 0.39a	2.40 ± 0.30ab	nd	0.40 ± 0.18f	1.07 ± 0.19de	0.88 ± 0.24def	0.94 ± 0.09def	1.16 ± 0.12de	1.97 ± 0.33bc
6	1.59 ± 0.18c	2.33 ± 0.28b	3.00 ± 0.30a	2.28 ± .21b	1.03 ± 0.18cde	0.87 ± 0.22de	0.91 ± 0.10de	1.48 ± 0.19cd	2.73 ± 0.28ab	1.46 ± 0.17cd	1.39 ± 0.19cde
7	10.92 ± 1.21d	13.40 ± 1.30c	18.16 ± 1.02a	14.19 ± 1.00b	5.53 ± 0.50i	7.90 ± 0.67f	5.74 ± 0.78hi	9.29 ± 0.62e	14.72 ± 1.05b	9.60 ± 0.77e	8.24 ± 0.46f
8	1.27 ± 0.62cde	2.59 ± 0.80a	2.55 ± 0.55a	1.84 ± 0.42bc	1.34 ± 0.42cde	1.28 ± 0.33cde	1.20 ± 0.27de	1.34 ± 0.19cde	2.16 ± 0.55ab	1.17 ± 0.31def	1.42 ± 0.41cd
9	6.8 ± 0.99bc	5.03 ± 0.55d	6.99 ± 0.89b	7.68 ± 0.77a	2.35 ± 0.60gh	5.02 ± 0.63d	2.79 ± 0.49g	4.35 ± 0.55f	6.30 ± 0.68c	4.59 ± 0.70def	4.93 ± 0.66def
10	nd	0.91 ± 0.10a	0.48 ± 0.25abc	nd	0.23 ± 0.11c	nd	0.51 ± 0.21abc	0.65 ± 0.10abc	0.65 ± 0.10abc	nd	0.79 ± 0.13abc
11	3.90 ± 0.54de	5.88 ± 0.52c	7.58 ± 1.12a	6.71 ± 0.66b	1.87 ± 0.28ij	3.05 ± 0.65fgh	3.16 ± 0.33fg	4.12 ± 0.33de	5.84 ± 0.74c	4.31 ± 0.56gh	4.20 ± 0.60de
12	2.39 ± 0.10fgh	3.78 ± 0.25bc	4.36 ± 0.85ab	3.61 ± 0.33cd	1.29 ± 0.00jk	1.81 ± 0.31hij	2.26 ± 0.55gh	2.41 ± 0.33fgh	3.07 ± 0.59de	2.05 ± 0.27hi	2.05 ± 0.55ghi
13	4.28 ± 0.87d	6.03 ± 0.54bc	7.12 ± 0.42a	5.81 ± 0.77c	2.68 ± 0.28gh	3.70 ± 0.44de	2.44 ± 0.36ghi	3.49 ± 0.50e	6.60 ± 0.45ab	3.38 ± 0.22ef	2.87 ± 0.85fg
14	0.61 ± 0.55fgh	1.58 ± 0.30c	2.33 ± 0.54b	1.40 ± 0.26cde	0.62 ± 0.40fgh	nd	0.93 ± 0.58defgh	0.90 ± 0.63efgh	1.14 ± 0.28cdef	1.12 ± 0.24cdefg	0.67 ± 0.00fgh
15	2.80 ± 0.60c	4.67 ± 0.47a	1.85 ± 0.32efg	1.49 ± 0.40fgh	0.93 ± 0.35hi	0.48 ± 0.09ij	2.20 ± 0.45cde	3.55 ± 0.27b	2.08 ± 0.65def	1.17 ± 0.33h	1.39 ± 0.14gh
Sum	35.11 ± 1.18d	52.21 ± 1.33b	59.33 ± 2.15a	49.25 ± 1.22c	18.94 ± 1.66j	26.26 ± 0.99hi	25.85 ± 1.15hi	34.81 ± 1.66de	49.04 ± 1.44c	32.62 ± 2.03efg	33.53 ± 1.88def
Polymeric procyanidins	51.73 ± 0.99g	100.47 ± 1.33b	109.67 ± 1.11a	88.37 ± 1.15c	74.33 ± 2.33d	34.60 ± 1.21k	48.46 ± 1.65hi	63.69 ± 1.35f	73.53 ± 0.87d	68.15 ± 0.98e	54.04 ± 2.22g
DP	2.91	3.77	3.98	3.44	4.25	2.43	3.41	3.45	3.24	3.80	3.35
Total	87.73 ± 2.22j	154.71 ± 3.00b	170.38 ± 1.25a	138.97 ± 1.98c	94.06 ± 1.98h	61.45 ± 1.24m	76.37 ± 1.56k	100.03 ± 1.54f	123.75 ± 1.88d	103.81 ± 2.22e	90.87 ± 1.78i

Table 3. Cont.

Peak no	<i>Chaenomeles Japonica</i>				<i>Chaenomeles Speciosa</i>			
	Cido	Red Joy	Wild #1	Wild #2	n1 (New)	Nivalis	Rubra	Simonii
Phenolic acids								
4	0.09 ± 0.02f	1.07 ± 0.17cd	0.31 ± 0.15ef	1.15 ± 0.10cd	2.12 ± 0.20b	2.05 ± 0.26b	1.29 ± 0.15cd	0.25 ± 0.05ef
5	0.06 ± 0.01c	0.04 ± 0.01c	0.70 ± 0.22b	nd	0.28 ± 0.16bc	0.19 ± 0.10c	nd	2.80 ± 0.33a
Sum	0.15 ± 0.05g	1.11 ± 0.21def	1.01 ± 0.06def	1.15 ± 0.15def	2.40 ± 0.17b	2.24 ± 0.25b	1.29 ± 0.10de	3.05 ± 0.44a
Flavan-3-ols								
1	0.38 ± 0.10i	1.88 ± 0.19cde	0.48 ± 0.11hi	1.09 ± 0.17fgh	2.29 ± 0.38abc	2.08 ± 0.30bcd	2.51 ± 0.50ab	0.37 ± 0.14i
2	0.39 ± 0.13cde	0.71 ± 0.19abcde	0.22 ± 0.13e	0.56 ± 0.14abcde	0.86 ± 0.21abc	0.69 ± 0.38abcde	0.99 ± 0.39ab	0.81 ± 0.25abcd
3	nd	1.04 ± 0.24de	nd	0.75 ± 0.28ef	1.20 ± 0.19de	1.37 ± 0.26d	1.40 ± 0.30cd	nd
6	2.23 ± 0.15b	0.84 ± 0.20e	1.17 ± 0.26cde	0.91 ± 0.15de	1.34 ± 0.08cde	1.09 ± 0.33cde	1.13 ± 0.16cde	2.32 ± 0.47b
7	11.40 ± 0.99d	3.39 ± 0.96j	6.19 ± 0.54h	5.36 ± 0.66i	8.40 ± 0.72f	7.15 ± 0.60g	3.80 ± 0.58j	8.49 ± 0.50f
8	1.57 ± 0.50bcd	0.48 ± 0.33g	1.09 ± 0.44defg	0.77 ± 0.20efg	1.57 ± 0.00bcd	1.11 ± 0.24def	0.56 ± 0.10fg	nd
9	4.37 ± 0.22f	1.77 ± 0.11h	4.40 ± 0.43ef	2.31 ± 0.39gh	2.92 ± 0.18g	2.88 ± 0.52g	1.97 ± 0.12h	4.99 ± 0.12de
10	0.36 ± 0.09abc	0.41 ± 0.20abc	0.32 ± 0.15bc	nd	0.53 ± 0.22abc	0.42 ± 0.29abc	0.43 ± 0.08abc	0.83 ± 0.00ab
11	4.05 ± 0.61de	1.54 ± 0.88j	2.61 ± 0.71gh	2.43 ± 0.70hi	3.94 ± 0.59de	3.63 ± 0.20ef	1.75 ± 0.55j	2.79 ± 0.80gh
12	0.49 ± 0.14l	0.39 ± 0.00l	3.21 ± 0.27cde	1.17 ± 0.40k	2.67 ± 0.00efg	4.68 ± 0.33a	1.58 ± 0.22ijk	2.96 ± 0.47ef
13	2.41 ± 0.35ghi	0.89 ± 0.61j	2.16 ± 0.33hi	1.91 ± 0.54i	3.45 ± 0.96ef	2.86 ± 0.46fg	0.82 ± 0.28j	2.55 ± 0.27gh
14	4.74 ± 0.19a	0.87 ± 0.18efgh	0.56 ± 0.11fghi	0.54 ± 0.27ghi	0.72 ± 0.33fgh	1.50 ± 0.21cd	0.38 ± 0.20hi	0.39 ± 0.15hi
15	1.94 ± 0.30efg	0.27 ± 0.08j	2.64 ± 0.33cd	0.45 ± 0.24ij	1.15 ± 0.51h	2.42 ± 0.36cde	0.31 ± 0.36j	1.41 ± 0.22gh
Sum	34.33 ± 1.54de	14.48 ± 1.11k	25.05 ± 2.29i	18.25 ± 1.55j	31.04 ± 1.43g	31.88 ± 2.00fg	17.63 ± 1.55j	27.10 ± 1.48h
Polymeric procyanidins	67.48 ± 1.99e	40.08 ± 1.64j	37.37 ± 1.00jk	51.35 ± 1.70gh	62.31 ± 1.46f	90.83 ± 2.15c	38.92 ± 1.02j	45.83 ± 0.99i
DP	4.18	2.72	3.16	3.87	3.53	3.95	2.70	2.74
Total	101.96 ± 1.14ef	55.67 ± 2.15n	63.43 ± 1.11m	70.75 ± 1.77l	95.75 ± 1.65g	124.95 ± 1.14d	57.84 ± 2.05n	76.79 ± 1.19k

nd, not detected; ± standard deviation; DP, degree of polymerization; value in the same columns followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

3.3. Antioxidant and In Vitro Biological Activities

The interest in compounds with antioxidant properties has been increasing over the last decades, mainly due to the discovery of the role of active oxygen species in chronic non-infectious diseases, such as cardiovascular diseases and cancer. Currently, there are many methods for determining the antioxidant capacity, tailored to the specifics of the test material and taking into account the potential side reactions. Chemical methods for determining the antioxidant capacity are based on the ability to capture synthetic radicals (ABTS), the reduction of metal ions, for example, iron (FRAP), and the measurement of the antioxidant effect on the rate of oxidation processes occurring in the sample (ORAC).

In this study, these three methods were used after measuring the antioxidant capacity of the test samples (Table 4). The analyzed fruits of selected *Chaenomeles* species and cultivars showed large variation ($p \leq 0.05$) among the samples. The highest antioxidant capacity, both ABTS and FRAP, was shown by *C. × superba* ‘Nicoline’ (20.61 and 21.32 mmol Trolox/100 g dw) while the lowest was shown by *C. speciosa* ‘Rubra’ (10.91 and 10.24 mmol Trolox/100 g dw). The average antioxidant activity measured by ABTS and FRAP methods from the analyzed species was respectively, for *C. × superba* (17.39 and 17.18 mmol Trolox/100 g dw), for *C. japonica* (14.98 and 13.90 mmol Trolox/100 g dw), and for *C. speciosa* (15.27 and 14.55 mmol Trolox/100 g dw). For comparison, Teleszko and Wojdyło [27] for four Japanese quince cultivars obtained higher values of the activity measured by ABTS and FRAP assays from 44.98 to 68.37 and from 30.73 to 46.57 mmol Trolox/100 g dw. while Du et al. [2] for *C. japonica* and *C. speciosa*, determined similar values for ABTS (36.54 and 14.61 mmol Trolox/100 g dw) and for FRAP (11.39 and 2.80 mmol Trolox/100 g dm), respectively. The strongest antioxidant potential measured by the ORAC test was shown by *C. × superba* ‘Colour Trail’ (66.59 mmol Trolox/100 g dw) and the lowest by *C. speciosa* ‘Rubra’ and *C. japonica* wild #2 (33.82 and 33.99 mmol Trolox/100 g dw, respectively). The average ORAC activity for the 19 analyzed varieties of *Chaenomeles* fruits was 48.35 mmol Trolox/100 g dw and was higher than for the average ORAC value for the artichoke (27.86 mmol Trolox/100 g dw) [5] or grape seeds (36.46 mmol Trolox/100 g dw) [33]. The Global Report on Diabetes WHO [34] states that diabetes had become a serious chronic disease worldwide, and by 2030 could become the seventh greatest killer in the world. The key issue in the fight against type 2 diabetes is finding effective inhibitors of pancreatic α -amylase and intestinal α -glucosidase, responsible for reducing the postprandial glycemia. In addition, agents with α -glucosidase inhibitory are used as oral hypoglycemic agents. Nevertheless, previous studies [35,36] indicate that *Chaenomeles* fruits may be a potential inhibitor of α -glucosidase. IC₅₀ (mg of dried fruit/mL) for α -amylase and α -glucosidase ranged from 13.88 (*C. × superba* ‘Nicoline’) to 18.48 (*C. speciosa* ‘Nivalis’), and from 5.08 (*C. × superba* ‘Texas Scarlet’) to 15.19 (*C. japonica* ‘Red Joy’), respectively ($p \leq 0.05$). Miao et al. [35] analyzed the α -glucosidase inhibition ability of skin from 13 *Chaenomeles* fruit genotypes in the range 0.05–0.35 mg/mL and flesh 0.04–0.43 mg/mL. To compare, the *Actinidia* fruits of selected cultivars also showed a higher capacity to inhibit α -amylase (4.13 to 6.40 mg/mL) and α -glucosidase (0.18 to 10.00 mg/mL) [6].

Table 4. Antioxidant (mmol Trolox/100 g dw), α -amylase, α -glucosidase, pancreatic lipase, acetylcholinesterase, butyrylcholinesterase (IC₅₀, mg/mL), and 15-lipoxygenase inhibition activity (% of inhibition) of various species and cvs. of *Chaenomeles* fruits.

Species	Cultivar	Antioxidant Capacity			In Vitro Inhibition Activities					
		ABTS	FRAP	ORAC	α -amylase	α -glucosidase	Pancreatic Lipase	AChE	BuChE	15-LOX
<i>Chaenomeles</i> × <i>superba</i>	Crimson and Gold	16.03 ± 1.04cdef	16.00 ± 0.99de	54.93 ± 1.11bc	17.49 ± 0.88a	7.03 ± 0.20def	0.29 ± 0.01ab	11.84 ± 0.24efg	10.13 ± 0.97efgh	99.81 ± 0.15a
	Texas Scarlet	19.63 ± 0.99ab	17.90 ± 1.22bcde	53.89 ± 1.37cd	14.34 ± 0.48b	5.08 ± 0.22g	0.09 ± 0.00def	17.43 ± 0.56ab	8.90 ± 0.70fghi	43.23 ± 0.73f
	Nicoline	20.61 ± 1.13a	21.32 ± 0.85a	51.86 ± 0.90cde	13.88 ± 0.98b	2.67 ± 0.17h	0.07 ± 0.02ef	16.02 ± 0.25bcd	16.14 ± 0.79d	71.24 ± 0.18d
	Andenken an Karl Ramcke	18.80 ± 0.88abc	15.51 ± 1.14def	40.38 ± 0.83h	16.28 ± 0.77ab	6.71 ± 0.72defg	0.04 ± 0.02f	13.03 ± 0.81defg	9.96 ± 0.17efgh	74.81 ± 0.18c
	Pink Lady	17.65 ± 1.50abcde	17.29 ± 0.63bcde	57.86 ± 1.00b	18.01 ± 0.89a	5.90 ± 0.85fg	0.20 ± 0.10bcd	15.94 ± 0.13bcd	12.14 ± 0.87e	>100.00
	Colour Trail	11.02 ± 0.72h	10.56 ± 0.55h	66.59 ± 0.55a	17.56 ± 0.99a	7.95 ± 0.16cde	<0.01	17.53 ± 0.41ab	7.85 ± 0.77hi	75.94 ± 0.29c
	Flocon Rose	15.38 ± 1.80defg	15.32 ± 0.88efg	45.25 ± 0.99g	15.59 ± 0.66ab	7.18 ± 0.11def	<0.01	11.73 ± 0.47efg	22.70 ± 0.63bc	98.15 ± 1.00a
	Hollandia	18.36 ± 0.63abcd	19.44 ± 1.11abc	40.72 ± 0.78h	16.49 ± 0.32ab	6.10 ± 1.23fg	<0.01	10.73 ± 0.74gh	15.89 ± 0.44d	>100.00
	Jet Trail	18.91 ± 0.91abc	20.04 ± 1.37ab	54.80 ± 0.46bc	17.97 ± 1.00a	6.97 ± 0.77def	0.29 ± 0.01ab	12.22 ± 0.65efg	8.09 ± 0.99ghi	>100.00
<i>wild</i>		17.46 ± 0.81bcde	18.45 ± 0.45abcd	50.18 ± 0.89ef	18.25 ± 0.39a	7.17 ± 0.57def	<0.01	11.05 ± 0.57g	31.59 ± 0.95a	>100.00
	Cameo	15.24 ± 0.24efg	11.93 ± 0.33h	51.63 ± 1.62de	16.75 ± 0.57ab	8.54 ± 0.34cd	0.12 ± 0.02def	11.56 ± 0.84fg	12.37 ± 1.22e	36.84 ± 0.44g
<i>Chaenomeles japonica</i>	Cido	18.06 ± 1.52abcde	18.00 ± 0.65bcde	48.48 ± 1.55f	16.47 ± 0.56ab	6.49 ± 0.49efg	0.17 ± 0.00cde	14.75 ± 0.75bcde	16.42 ± 0.31d	42.11 ± 0.56f
	Red Joy	13.50 ± 0.50fgh	12.76 ± 1.12fgh	53.43 ± 0.87cd	17.45 ± 0.54a	15.19 ± 0.14a	0.06 ± 0.01ef	7.74 ± 0.34hi	6.06 ± 0.41i	73.31 ± 0.74cd
	wild #1	12.41 ± 0.41gh	11.53 ± 0.55h	40.28 ± 0.66h	16.66 ± 0.87ab	6.11 ± 0.19efg	0.35 ± 0.05a	12.14 ± 0.20efg	32.11 ± 1.13a	90.60 ± 0.69b
	wild #2	13.72 ± 0.72fgh	12.30 ± 0.62gh	33.99 ± 1.74i	16.11 ± 1.13ab	9.57 ± 0.55c	<0.01	10.13 ± 0.30gh	20.68 ± 0.56c	70.37 ± 0.55d
	n1 (new)	16.96 ± 0.96bcde	16.90 ± 0.22cde	51.07 ± 0.77def	16.89 ± 0.98ab	6.09 ± 1.22fg	0.25 ± 0.05abc	14.18 ± 0.49cdef	11.17 ± 0.66efg	66.05 ± 0.99e
<i>Chaenomeles speciosa</i>	Nivalis	17.54 ± 0.54abcde	16.39 ± 0.47cde	44.30 ± 1.50g	18.48 ± 0.43a	6.56 ± 0.46efg	0.20 ± 0.02bcd	16.52 ± 35bc	11.97 ± 0.20ef	74.81 ± 0.45c
	Rubra	10.91 ± 0.91h	10.24 ± 1.20h	33.82 ± 0.49i	18.38 ± 0.77a	5.74 ± 0.84fg	0.04 ± 0.00f	6.65 ± 0.73i	11.07 ± 0.81efg	14.29 ± 0.99i
	Simonii	17.37 ± 1.37bcde	17.02 ± 0.98bcde	45.18 ± 1.15g	16.88 ± 1.00ab	12.48 ± 0.68b	0.20 ± 0.05bcd	20.42 ± 0.99a	25.79 ± 0.11b	27.37 ± 0.30h

± standard deviation; value in the same columns followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

The inhibitory activity of pancreatic lipase is used in the prevention of obesity, because it is responsible for hydrolyzing more than half of the consumed triglycerides, to low-molecular compounds and free fatty acids [37]. Therefore, it reduces the amount of fat absorbed into the blood stream and can be used for weight loss control. Among the *Chaenomeles* species and cultivars with reference to the inhibitory activity toward pancreatic lipase, significant differences ($p \leq 0.05$) were observed. The inhibitory effect (IC_{50}) of the analyzed fruits ranged from 0.04 (*C. × superba* ‘Andenken an Karl Ramcke’ and *C. speciosa* ‘Rubra’) to 0.35 mg/mL (*C. japonica* wild #1). It should be noted that for five analyzed cultivars, i.e., *C. × superba* ‘Color Trail’, ‘Flavon Rose’, ‘Hollandia’, wild, and *C. japonica* wild #2, the values of pancreatic lipase inhibition were designated as <0.01 . This means that a lower concentration of *Chaenomeles* had the greatest inhibitory potential. The results were similar to those obtained by Nowicka et al. [37] in 20 peach cultivars in the range from 0.07 to 2.06 mg/mL. It should be emphasized that so far there are no data on the activity of *Chaenomeles* fruit in the literature as regards the inhibition of pancreatic lipase.

Alzheimer’s disease is considered as one of the most prevalent neurodegenerative disorders and accounts for more than 80% of dementia worldwide in the aged population. It is estimated that by 2050, three new case may develop every minute [38]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE, pseudocholinesterase) are key enzymes in the breakdown of an important neurotransmitter, acetylcholine (ACh). Several clinical trials have confirmed that ACh inhibitors could be used to treat this pathology [33,38]. IC_{50} inhibition of AChE and BuChE ranged from 6.65 to 20.42 and from 6.06 to 31.59 mg of dried fruit/mL with significant differences between samples ($p \leq 0.05$). The cultivars showing the highest ability to inhibit AChE and BuChE were found to be *C. speciosa* ‘Rubra’ and *C. japonica* ‘Red Joy’ while the least effective were *C. speciosa* ‘Simonii’ and *C. × superba* wild #1, respectively. The analyzed *Chaenomeles* genotypes showed similar mean AChE and BuChE inhibition values (IC_{50}), 13.24 and 15.32 mg/mL, respectively. It is worth noting that Sancheti et al. [39] during in vivo studies in rats observed a positive effect of the ethyl acetate fraction from *Chaenomeles sinensis*, which caused a strong decrease in AChE activity in diabetic rats.

Lipoxygenases (LOXs) are important enzymes in lipid metabolism that convert the polyunsaturated fatty acids, arachidonic acid (AA), and linoleic acid (LA), to their corresponding metabolites. The inhibitors of 15-LOX have mainly been of interest in the treatment of inflammatory conditions. Recently, multiple studies have provided evidence to elucidate the relationship of 15-LOX-1 and cancer cell growth and development [40]. The results of 15-LOX inhibition clearly showed the great variation of obtained values between tested genotypes ($p \leq 0.05$). The 15-lipoxygenase inhibition activity was expressed as % inhibition at a sample concentration of 5.77 mg/mL. The highest potential was exhibited by *C. × superba* ‘Crimson and Gold’ and ‘Flacon Rose’ (99.81% and 98.15%), while the lowest was shown by *C. speciosa* ‘Rubra’ (14.29%). *C. × superba* ‘Pink Lady’, ‘Hollandia’, ‘Jet Trail’ and wild obtained values out of the range (>100.00), which means that the used *Chaenomeles* extracts had very strong inhibitory properties against LOX. The obtained results may be a clue to continue research on cell lines and using a simulated digestive system to verify their biological potential. Moreover, it is advisable to carry out in vivo studies, as there is not enough information in this area.

3.4. Antioxidant On-Line Profiling by HPLC-PDA Coupled with Post-Column Derivatization with ABTS

Nowadays, sensitive on-line HPLC-ABTS methods for analyzing free radical scavenging activity have been developed. They combine the liquid chromatography system with additional pumps and detectors allowing the individual active components to be characterized with high sensitivity and evaluation of the antioxidant potential of individual compounds from complex mixtures [41]. Figure 1A–C shows the analysis of the three cultivars of *Chaenomeles* (*C. × superba* ‘Texas Scarlet’, *C. × superba* ‘Cameo’, and *C. speciosa* ‘Nivalis’). The upper chromatogram (positive, black) shows the response after passing through the first detector at a wavelength of 280 nm, and the lower one (negative, blue) is characterized by the response of the eluted compounds after reaction with the radical

cation ABTS after passing through the second detector ($\lambda = 734$ nm). The high area of negative peaks on the lower chromatogram is proportional to the activity of individual compounds.

The characteristic elevation of the baseline in the middle part of the upper chromatogram (Figure 1A–C) is caused by the presence of polymeric procyanidins. A mirror reflection of this elevation in the lower chromatogram indicates that these compounds exhibit significant antioxidant activity. Comparing the intensity of the negative response for (–)-epicatechin (peak 9), which is the second highest peak in the order, it can be clearly seen that its antioxidant activity is disproportionate, because this response is negligible. Comparing the activity of (–)-epicatechin and procyanidin C1 (peak 11), whose signal in the upper chromatogram is almost 50% lower, they have a very similar response in the lower chromatogram, and hence similar activity. Numerous *in vitro* and *in vivo* studies [1] confirm that polyphenol compounds belonging to the flavan-3-ol group have antioxidant and anti-inflammatory properties. Furthermore, the chemical structure of (–)-epicatechin and its polymers makes them better antioxidants than (+)-catechin and its derivatives, but also the type B procyanidins are better antioxidants than the A type procyanidins, and the degree of polymerization (its increase causes an increase in activity) of the compound is important for their pro-health activity [42]. These results are confirmed by Raudone et al. [43], who also observed greater activity of procyanidin oligomers and polymers than (+)-catechin and (–)-epicatechin. The activity of phenolic acids (peaks 4 and 5) is lower than catechins [44], which can be clearly seen in the blue chromatogram. The signal from chlorogenic acid (peak 4) at 280 nm is significant, whereas its response after reaction with a radical cation is very small. It is caused, among others, by the fact that antioxidant activity increase with the number and position of the –OH groups on the molecule. To summarize, among all identified polyphenolic compounds, procyanidin B3, B2, C1, and (–)-epicatechin were found to be predominant in building antioxidant capacity of *Chaenomeles* fruit, in accordance with Zhang et al. [26] and Raudone et al. [43].

3.5. Agglomerative Hierarchical Clustering (AHC)

Dendrograms of agglomerative hierarchical clustering (AHC) analysis (Figure 2A,B) showed dissimilarity of biological activities and chemical compounds (A) and between studied cultivars (B) of *Chaenomeles* fruits obtained by Euclidian distance dissimilarity (within the interval 0 to 65 and 0 to 18, respectively) using the aggregation criterion, Ward's method.

The horizontal axis of the dendrogram represents the dissimilarity between clusters, while the vertical axis represents the objects. Each leaf corresponds to one object and objects that are similar to each other are combined into branches. The greater the height of the horizontal line, the less similar the objects are. By analyzing Figure 2A, it is visible that two groups are approximately the same size, and the third one has only two states. The first group (displayed in orange color) includes objects showing similarity to the second group (displayed in green color). This confirms the calculated Pearson correlation coefficient (r), which for phenolic acids and inhibition of 15-LOX is equal to 0.23, and 0.36 for organic acids and inhibition of AChE. In the second group, the branch created between flavan-3-ols and polymeric procyanidins and the activity of ABTS and FRAP is flatter than the others in this cluster. They are more homogeneous with each other (ABTS:flavan-3-ols, $r=0.86$ and ABTS:polymeric procyanidins, $r=0.76$). This is further confirmation (apart from on-line ABTS antioxidant profiling) that flavan-3-ols and their polymers are responsible for the antioxidant capacity of *Chaenomeles* fruits. The third group (displayed in purple) formed between the BuChE and ORAC inhibition activity ($r=0.41$) is more homogeneous with the remaining two clusters (it is flatter on the dendrogram). This is confirmed by comparing the within-class variable, which is almost 70% lower. From the analysis of the dendrogram it can be concluded that inhibition of AChE, BuChE and 15-LOX are influenced by the content of phenolic acids and organic acids, while the polyphenol compounds from the flavan-3-ols group, L-ascorbic acid, and sugars formed the activity of *Chaenomeles* fruit against α -amylase and α -glucosidase. Considering the relationship between the *Chaenomeles* genotypes, in the lower dendrogram (Figure 2B), the three clusters can be seen as three branches that occur at about the same horizontal distance. The two outliers, between 'Jet Trail' and 'Crimson and Gold', and 'Red Joy'

and 'Colour Trail', fused rather arbitrarily at much greater distances. Moreover, two wild genotypes of *C. japonica* are similar in the context of analyzed parameters and form a cluster with *C. × superba* wild with dissimilarity less than one. Not with standing, it can be concluded that there is significant variation within the analyzed species, as well as internal cultivars.

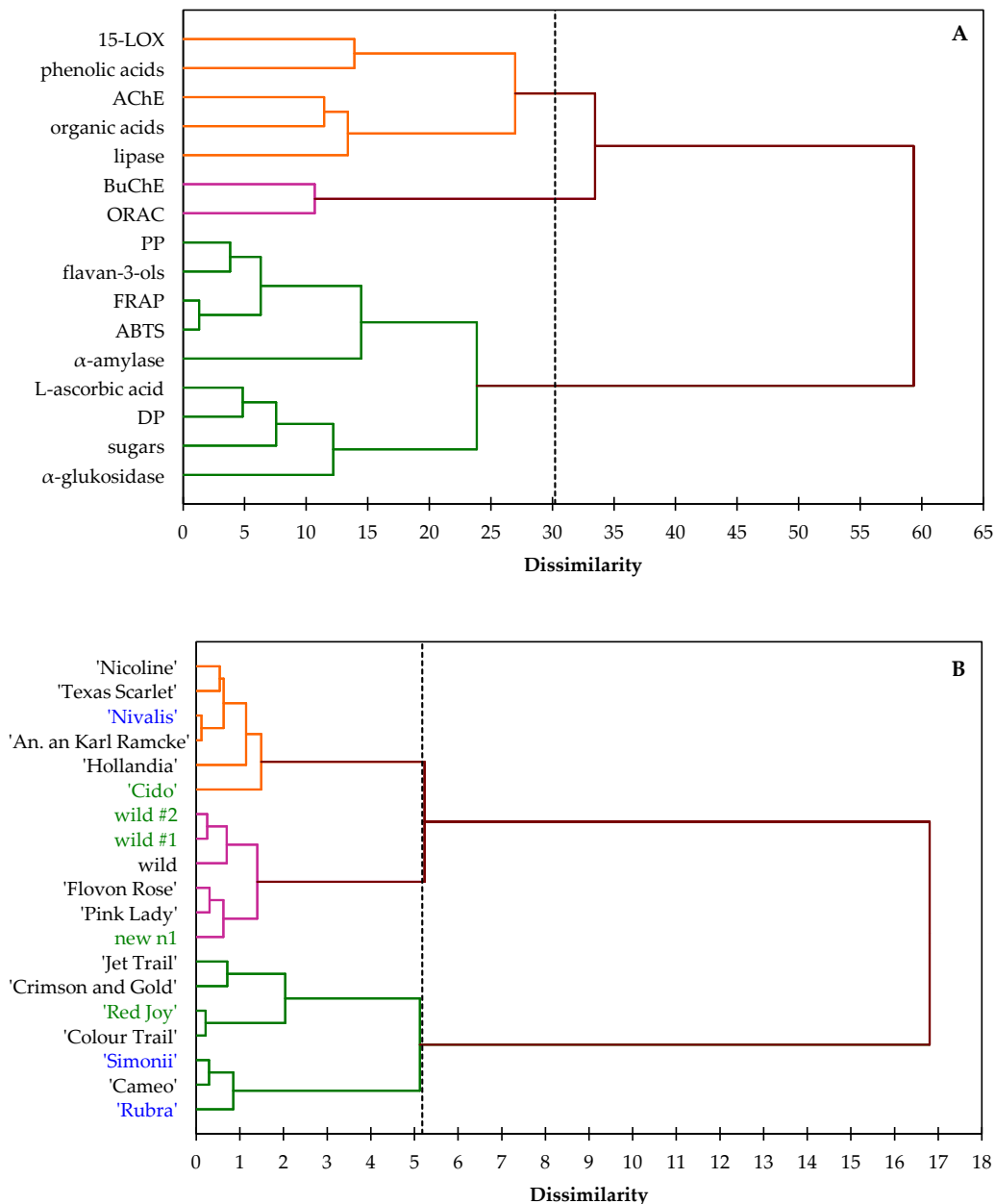


Figure 2. Dendrograms of Agglomerative Hierarchical Clustering (AHC) analysis representing dissimilarity relationship of biological activities and chemical compounds (A) and between studied cultivars (B) of *Chaenomeles × superba* (black), *C. japonica* (green) and *C. speciosa* (blue). PP – polymeric procyanidins; DP – degree of polymerization.

4. Conclusions

Physiochemical composition and biological activities of the nineteen *Chaenomeles* species and cultivars evaluated in this study revealed a diverse range of polyphenolic compounds and in vitro biological properties (antioxidant, α -amylase, α -glukosidase, AChE, BuChE, and 15-LOX inhibition activity). The analyzed fruits are rich in polymeric procyanidins and contain a high level of organic

acids. *Chaenomeles × superba* ‘Nicoline’ displayed the highest total phenol content (170.38 g/kg dw) while *Chaenomeles × superba* ‘ColourTrail’ was characterized by the highest concentration of polymeric procyanidins (109.67 g/kg dw). *Chaenomeles* fruits are a good source of malic acid (41.64 to 110.31 g/100 g fw), L-ascorbic acid (30.26 to 1195.05 mg/100 g fw), and pectins (0.65% to 1.72%). In addition, *Chaenomeles × superba* ‘Nicoline’ showed high potential for inhibition α -amylase and α -glucosidase (as compared with all analyzed species and cultivars), while *Chaenomeles japonica* ‘Red Joy’ proved to be an effective inhibitor for AChE and BuChE. The study established *Chaenomeles* fruits as a source of functional ingredients with possible pharmacological use. However, in order to verify the thesis that *Chaenomeles* fruits are a source of bioactive compounds showing pro-health properties, it is necessary to use in vivo models in further studies. Gastrointestinal systems should be used to determine the bioavailability and digestibility of the *Chaenomeles* bioactive compounds. Therefore, the fruits could be important dietary sources of natural antioxidants.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-3921/9/1/60/s1>, Figure S1: Structural formulas of selected phenolic compounds identified in *Chaenomeles* fruits.

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mgr inż. Igor Piotr Turkiewicz

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Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
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51-630 Wrocław

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Oświadczam, że jestem współautorem publikacji pt.:

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Mój udział w przygotowaniu tej publikacji polegał na kierowaniu projektem naukowym obejmującym badania opisane w tej pracy (Diamentowy Grant VII, nr DI2017 006347), współtworzeniu koncepcji prowadzonych badań, wykonaniu analiz fizykochemicznych, chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca. Przygotowałem tekst publikacji, opracowałem merytorycznie otrzymane wyniki, przeprowadziłem dyskusję oraz współredagowałem odpowiedzi na recenzje.

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prof. dr hab. inż. Aneta Wojdyło

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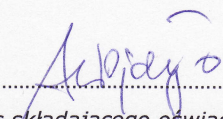
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Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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mgr inż. Karolina Tkacz

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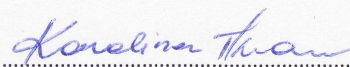
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Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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dr hab. inż. Paulina Nowicka, prof. uczelni

Wrocław, 07.02.2022 r.

Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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mój udział polegał na analizie formalnej publikacji oraz opracowaniu metodyki dotyczącej
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Paulina Nowicka

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mgr inż. Tomasz Golis

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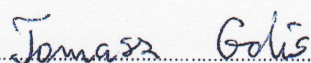
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Instytut Ogrodnictwa w Skierniewicach
ul. Konstytucji 3 Maja 1/3
96-100 Skierniewice

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mój udział polegał na dostarczeniu materiału badawczego do analiz fizykochemicznych różnych gatunków i odmian owoców pigwowca. Ponadto współredagowałem informacje o pochodzeniu odmianowym badanych owoców.


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dr hab. inż. Przemysław Bąbalewski, prof. uczelni

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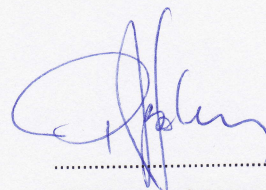
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Wydział Przyrodniczo-Technologiczny
Uniwersytet Przyrodniczy we Wrocławiu
pl. Grunwaldzki 24A
50-363 Wrocław

OŚWIADCZENIE

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.....
Podpis składającego oświadczenie

Publikacja 2

Turkiewicz, I. P., Wojdyło, A., Tkacz, K., Nowicka, P. (2020). Carotenoids, chlorophylls, vitamin E and amino acid profile in fruits of nineteen *Chaenomeles* cultivars. *Journal of Food Composition and Analysis*, 93, 103608.



Original Research Article

Carotenoids, chlorophylls, vitamin E and amino acid profile in fruits of nineteen *Chaenomeles* cultivars

Igor Piotr Turkiewicz, Aneta Wojdyło*, Karolina Tkacz, Paulina Nowicka

Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chelmońskiego Str., 51-630 Wrocław, Poland

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ABSTRACT

This study aimed to identify and quantify carotenoids, chlorophylls, tocopherols, tocotrienols and the free amino acid profile of 19 fruit cultivars of *Chaenomeles × superba*, *Chaenomeles japonica*, and *Chaenomeles speciosa*. For this purpose ultra-performance liquid chromatography (UPLC) with a photodiode array (PDA) and fluorescence (FL) detector coupled with quadrupole time-of-flight electrospray ionization mass spectrometry (QToF-ESI-MS) was used. Most of the *Chaenomeles* species and cultivars analyzed in this study have not been examined in this respect until now. Fruits contained 32.44–314.94 mg of carotenoids, 19.27–227.19 mg of chlorophylls, 5.51–37.58 mg of tocopherols, and 2.06–42.30 mg of tocotrienols per kg of dry weight (dw). Analysis of the amino acid profile revealed the presence of 10 amino acids, three of them essential (L-threonine, L-valine and L-isoleucine). The highest content of total amino acids was shown by *C. × superba* 'Jet Trail' (2326.33 mg/100 g dw). Among tested species, *C. speciosa* was characterized by the highest content of all analyzed compounds, except for chlorophylls, present in the highest concentration in *C. × superba* fruits. These results indicate that *Chaenomeles* fruits could be regarded as a promising source of bioactive functional food containing vitamins A and E and indispensable amino acids.

1. Introduction

Chaenomeles is a genus of four species of deciduous shrubs, usually 1–3 m high, with thick and thorny shoots, from the *Rosaceae* family. They are popular and widely cultivated in Japan, Korea, China, Bhutan and Burma (Ros et al., 2004). These plants are related to quince (*Cydonia oblonga* Mill.) and Chinese quince (*Pseudocydonia sinensis*, *Chaenomeles sinensis* (Thouin) Koehne) but differ in many respects. The fruits – apple (pome) shaped, yellow or green and fragrant – are edible.

Chaenomeles japonica naturally occurs in central and southern Japan, while in Europe it was introduced in 1869 (Baranowska-Bosiacka et al., 2017). *Chaenomeles speciosa*, which is the source of (Sweet) Nakai fruits, is widely cultivated in Korea, Japan and especially in China (Sichuan, Yunnan, Chongqing and Zhejiang provinces). Mugua fruit, as is well known in China, is cultivated as an important crop with high commercial potential to produce tea, wine, vinegar, medicinal liquor or even seasoning (Miao et al., 2017). Research carried out in Poland, Ukraine, Lithuania, Finland, and Latvia confirmed that in comparison with other species, *C. japonica* is best adapted to the climatic conditions of northern Europe (Baranowska-Bosiacka et al., 2017). *Chaenomeles × superba*,

which was formed through the intersection of *C. japonica* and *C. speciosa*, is very diverse and mostly cultivated in Poland.

Consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic non-infectious diseases such as cancer, diabetes, and cardiovascular disease. Researchers estimated that changing the lifestyle and diet would reduce the cancer-induced morbidity in the United States by almost a third (Liu, 2013). Therefore, over the past twenty years, the attention of scientists from around the world has focused on the analysis of the chemical composition of plant raw materials and their biological potential in the context of human health protection. The health-promoting effect of fruits and vegetables is related to a wide range of nutrients and biologically active substances, including vitamins, minerals, and phenolic compounds.

The fruits of various species of *Chaenomeles* have been used for thousands of years in traditional Asian medicine. They contain many bioactive components, including organic acids, pectins, micro- and macroelements, vitamin C and polyphenols. The most important properties of these fruits include a protective effect against diabetes, anti-tumoral, anti-inflammatory activities (Zhang et al., 2014). Nevertheless, from a nutritional point of view, carotenoids and

* Corresponding author.

E-mail address: aneta.wojdylo@upwr.edu.pl (A. Wojdyło).<https://doi.org/10.1016/j.jfca.2020.103608>

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chlorophylls, apart from being responsible for the green, yellow, orange and red colors of fruits and vegetables, have many important functions in the human body. β -Carotene is an important dietary source of vitamin A while chlorophylls affect cell renewal and play an important role in preventing skin diseases (Lanfer-Marquez et al., 2005; Rao and Rao, 2007). Vitamin E is a powerful polyunsaturated fatty acid and phospholipid, forming part of cell membranes. In children, especially newborns, its deficiency is extremely dangerous because it can cause the development of anemia and retinopathy, disturb the development of the respiratory system and even result in death (Eitenmiller et al., 2016). In the case of vitamin E deficiency in the body, the risk of cancer, infectious, cardiovascular, inflammatory processes and neurological disorders resulting from neuronal degeneration increases (Zielińska and Nowak, 2014). The presence of tocopherols and tocotrienols protects the body against free radicals. The enormous physiological importance of amino acids in human body is well known. They are building blocks for tissue proteins and essential substrates for the synthesis of many low molecular-weight substances. Furthermore, the recent years have witnessed growing interest in amino acids in health prevention. It is predicted that they will have a great promise in treatment of metabolic diseases, infertility, and intestinal and neurological dysfunction (Wu, 2013).

The literature on the composition of *Chaenomeles* \times *superba* is scarce. Only one scientific article has reported some data on its amino acid composition (Hellín et al., 2003), while carotenoids, chlorophylls and vitamin E have never been investigated before for this species, or for specific cultivars described in this paper. Therefore the aim of this study was to investigate (i) the content of bioactive compounds such as carotenoids and chlorophylls (identification by LC-PDA-QTOF-MS, and quantification by UPLC-PDA); (ii) the content of tocopherols and tocotrienols by UPLC-FL and (iii) the profile of free amino acids with the AQC derivatization method (identification by LC-PDA-QTOF-MS, and quantification by UPLC-PDA) of 19 cultivars of *Chaenomeles* \times *superba*, *C. japonica* and *C. speciosa* fruits.

2. Material and methods

2.1. Reagents and standards

All standards of carotenoids, chlorophylls and tocotrienols were purchased from Extrasynthese (Lyon, France). All amino acids and tocopherols standards were purchased from Sigma-Aldrich (Steinheim, Germany). The aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) reagent was from Synchem (Felsberg-Altenburg, Germany). UPLC grade water, prepared by using an HPL SMART 1000s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22 μ m membrane filter immediately before use. Acetonitrile, formic acid, and methanol for ultraperformance liquid chromatography (UPLC; Gradient grade) were from Merck (Darmstadt, Germany). Hexane, ethyl acetate, acetone, sodium borate, calcium disodium EDTA, ammonium acetate, and BHT were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Plant material and sample preparation

Fruit samples (*C.* \times *superba*: 'Crimson and Gold', 'Colour Trail'; *C. japonica*: 'Cameo', 'Red Joy'; *C. speciosa*: 'Nivalis', and 'Rubra') were collected manually from bushes grown in a field trial established in 2018 at the Experimental Orchard at Wrocław (51°07' 02.0" N, 17°04' 25.0" E), (*C.* \times *superba*: 'Texas Scarlet', 'Nicoline', 'Andenken an Karl Ramcke', 'Pink Lady', 'Flavon Rose', 'Hollandia', 'Jet Trail'; *C. japonica*: 'Cido'; *C. speciosa* 'Simonii' and new genotype (n1)) from an experimental field from the Research Institute of Horticulture in Skierniewice (51° 55' 41.688" N, 20° 9' 9.896" E) and (*C.* \times *superba* wild and *C. japonica* wild #1 and #2) from wild bushes located near the Centennial Hall in Wrocław (51° 6' 26.548" N, 17° 4' 56.782" E) in September 2018. Information about agronomic management and postharvest treatment are

included in the Supplementary materials. In total, the fruits of 19 cultivars of three different species of *Chaenomeles* were analyzed.

Approximately 0.5 kg fruits of each cultivar was collected, washed with distiller water, cut into smaller pieces, and frozen at -80 °C. Then fruits were freeze dried (24 h; Christ Alpha 1-4 LSC, Melsungen, Germany) and crushed using a closed laboratory mill (IKA A 11, Staufen, Germany) to obtain the homogeneous dry material for further analysis.

2.3. Identification and quantification of carotenoids and chlorophylls

Extracts for determination of carotenoids and chlorophylls were prepared as follow: the freeze-dried powder of fruits (\sim 0.3 g) was vortexed for 30 min in the dark at 300 rpm (DOS-10 L Digital Orbital Shaker, Elmi Ltd.; Riga, Latvia) with 5 mL methanol:acetone:hexane (1:1:2, v/v/v). To prevent oxidation, also 10 % MgCO_3 and 1% butylated hydroxytoluene (BHT) were added. The extraction process of solid residue was repeated 4 times, each time centrifuging (4 °C, 7 min at 19 000 g; MPW-350; Warsaw, Poland) and collecting the supernatants, which were finally evaporated to dryness (XCV-5400 XcelVap® Evaporation System, Horizon Technology, Inc.; Salem, USA). The pellet was diluted using 2 mL of 100 % methanol, and filtered through Millex Smplicity™ Filters (Hydrophilic PTFE, 0.20 μ m, Millipore, Merck; Darmstadt, Germany) before analysis.

Qualitative (LC-PDA-QTOF-MS) and quantitative (UPLC-PDA) analysis of carotenoids (at 425 nm) and chlorophylls (at 650 nm) were performed as described previously by Wojdyło et al. (2018). Compounds were separated with an ACQUITY UPLC BEH C18 column (2.1 \times 100 mm, 1.7 μ m, Waters Corp.; Milford, USA) at 32 °C. The elution solvents were ACN:MeOH (7:3, v/v) (A) and 0.1 % formic acid (B). Samples (10 μ L) were eluted at linear gradient at flow rate of 0.42 mL/min. Identification of carotenoids was carried out using QTOF mass spectrometer (Waters Corp.; Milford, USA) equipped with an ESI source, operating in positive mode, on the basis of fragmentation patterns and PDA profiles. The retention times and spectra were compared to those of the authentic standards. PDA spectra were measured over the wavelength range of 200–700 nm in steps of 2 nm. Calibration curves were made from all-*trans*- β -carotene, α -carotene, all-*trans*-lutein, all-*trans*- β -cryptoxanthin, chlorophyll *a*, and pheophorbide *a*. Chlorophyll *b*, chlorophyllide and pheophytin derivatives were expressed as chlorophyll *a*. Calibration curves, range of concentration (linearity), limit of quantification (LOQ) and limit of detection (LOD) of studied compounds are included in the Supplementary materials. All incubations were done in triplicate. The results were expressed as mg per kg of dry weight (dw).

2.4. Identification and quantification of tocopherols and tocotrienols by the UPLC-FL

Samples for the analysis of tocopherols and tocotrienols were prepared as follows. The fresh fruits (\sim 3 g) were homogenized with 5 mL of ethanol with 0.05 % BHT. Saponification was carried out using 1 mL of 60 % KOH, at a temperature of 70 °C and for 90 min. Then, the samples were mixed with 10 mL hexane:ethyl acetate (9:1, v/v) with 0.05 % BHT. After 30 min, NaOH (saturated solution) was added. The upper layer was collected, evaporated and dissolved in 1 mL methanol with 0.05 % BHT. The solutions were filtered through a Minispike Syringe Filter (GHP, 0.20 μ m, Acrodisc®, Waters Corp.; Milford, USA) and used for UPLC analysis.

The analysis of tocopherols and tocotrienols were performed as described previously by Tkacz et al. (2019) using Ultra-Performance Liquid Chromatography with fluorescence detector (UPLC-FL). The column ACQUITY UPLC BEH RP C18 (1.7 mm, 2.1 mm \times 100 mm, Waters Corp.; Milford, USA) being protected by guard column of the same materials was operated at 30 °C. The wavelengths of excitation/emission were 290/330 nm. Identification and quantification was performed based on reference standards and calibration curves. Calibration curves were made from α -, β -, γ -, δ -tocopherol and tocotrienol.

Calibration curves, range of concentration (linearity), limit of quantification (LOQ) and limit of detection (LOD) of studied compounds are included in the Supplementary materials. The samples (5 μ L) were injected, and the elution was completed in 12 min with an isocratic of methanol with water (88:12) flow rates of 0.45 mL/min. All incubations were done in triplicate. The results were expressed as mg per kg of dw.

2.5. Identification and quantification of free amino acids by the LC-PDA-MS/QToF

Free amino acids were determined by following a method described previously Collado-González et al. (2014). Briefly, the freeze-dried powder of fruits (~0.2 g) with 0.5 mL methanol:water (1:1, v/v) was sonicated for 15 min (Sonic 6D; Polsonic, Warsaw, Poland) and centrifugated at 19 000 g for 10 min at 4 °C (MPW-350; Warsaw, Poland). The extraction procedure was repeated twice. Supernatants were combined and immediately derivatized. The derivatization of amino acids and amino thiols were accomplished as follow: 350 μ L of borate derivatization buffer (0.2 M sodium borate, pH 8.8, with 5 mM calcium disodium EDTA), 50 μ L of amino acid standard or sample extract, and 100 μ L of 10 mM AQC in ACN were mixed into propylene vial and vortexed for several seconds. After 1 min at room temperature, the vials were placed into ThermoMixer® C (Eppendorf AG, Hamburg, Germany) and heated at 55 °C for 10 min. After that, the samples were analysed immediately.

The identification and quantification of free amino acids were performed using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corp.; Milford, USA) series with a mass detector G2 Q/TOF micro-mass spectrometer (Waters; Manchester, UK) equipped with an electrospray ionization (ESI) source operating in positive modes. Separations of individual amino acids were carried out using a AccQ Tag Ultra BEH column (2.1 \times 100 mm, 1.7 μ m) (Waters Corp.; Milford, USA). The column was kept at 50 °C, the samples at 20 °C. The injection volume was 3 μ L and the elution was performed at a flow rate of 0.50 mL/min. The mobile phase consisted of solvent A (50 mL of solution: acetonitrile, formic acid, and 5 mM ammonium acetate (10:6:84, v/v/v) in 950 mL water) and solvent B (acetonitrile and formic acid; 99.9:0.1, v/v). The gradient profile was: 99.0 % A at 0–0.30 min, 97.0 % A at 3.20 min, 88.0 % at 6.80 min, 82.0 % A at 8.95 min, 74.0 % A at 9.50 min, 67 % A at 9.80 min, 40.0 % A at 10.65 min, and 99.0 % A at 14.50–15.00 min. The PDA spectra for amino acids were measured at wavelength 260 nm. Retention times and spectra were compared to those of the authentic standards of amino acids. Quantification was achieved by injection of solutions of known concentrations ranging from 20.00–100.00 mg/L ($R^2 \geq 0.9900$) of amino acid standards. Calibration curves, limit of quantification (LOQ) and limit of detection (LOD) of studied compounds are included in the Supplementary materials. All incubations were done in triplicate. The results were expressed as mg per 100 g of dw.

2.6. Statistical analysis

Statistical analysis was made using XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft; Paris, France). Significant differences ($p \leq 0.05$) between means were evaluated by one-way ANOVA and Tukey's test while to highlight correlations Principal Components Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) have been performed.

3. Results and discussion

3.1. Identification of carotenoids and chlorophylls

A total of 13 compounds were found in the 19 cultivars of the three *Chaenomeles* species by using LC-PDA-MS/QToF (Table 1). In the chromatogram profiles (Fig. 1A) obtained at 425 nm, the labeled peaks

1–13 followed an elution order. Among these compounds, five belong to the group of carotenoids (lutein, β -cryptoxanthin, α - and β -carotene) and eight were chlorophyll derivatives. It is worth noting that detection of all-*trans*- β -cryptoxanthin, α -carotene and chlorophyll derivatives (including pheophorbide *a*, chlorophyllide *b* and pheophytin epimers) in *Chaenomeles* fruits is reported for the first time. All the detected compounds were characterized along with their retention times (R_t), UV spectrum, and MS data together with the interpretation of the observed MS/MS spectra and comparison with standards.

3.1.1. Carotenoids

Carotenoids are biosynthesized by bacteria, algae, fungi, and plants, but not by animals, which must obtain them from their food. They can be found in colored fruits and vegetables. For example, carrots, apricots, pumpkin and sweet potato are sources of α -carotene and β -carotene, while tomatoes and watermelon are sources of lycopene, ζ -carotene, β -carotene, phytofluene and phytoene. Mango, papaya, spinach, peaches and prunes are sources of lutein, zeaxanthin, α -, β - and ζ -carotene and β -cryptoxanthin (Jaswir et al., 2011). More than 600 natural carotenoids have been identified while only about 50 have provitamin A activity. Among them, three are the most important precursors of vitamin A (which are converted into vitamin A or retinol) in humans: α -carotene, β -carotene and β -cryptoxanthin (Jaswir et al., 2011). In the human organism, carotenoids are part of the antioxidant defense system. Carotenoids, as well as tocopherols, are known to be efficient antioxidants and capable of scavenging reactive oxygen species generated during photo-oxidative stress (Stahl et al., 2000). Moreover, the beneficial effects on human health, such as lowering the risk of cancer and enhancement of immune system function, have been confirmed (Das et al., 2007; Rock, 2009). In addition, research Maeda et al. (2008) confirmed the positive effect of daily consumption of carotenoids on the regulation of metabolism, decreased body fat deposition and consequently weight reduction.

Peak 3 gave an $[M+H]^+$ ion at m/z 569.40 with the molecular formula $C_{40}H_{56}O_2$. The MS/MS spectra yielded fragment ions at m/z 551.11 $[M+H-18]^+$ and at m/z 553.11 $[M+H-18-18]^+$, indicating the loss of molecules of water. Those fragment ions were observed for lutein by Delgado-Pelayo et al. (2014) and Kolniak-Ostek (2016). Comparison with the standard allowed this peak to be assigned to all-*trans*-lutein.

Peak 4 ($R_t = 7.39$), which displayed a molecular ion at m/z 553.32 with the molecular formula $C_{40}H_{56}O$, was characterized as all-*trans*- β -cryptoxanthin. The characterization of this compound was based on the reference standard.

Peaks 7, 8 and 11, with the identical molecular formula $C_{40}H_{56}$ and a precursor ion at m/z 537.38, showed a product ion at m/z 444.01 $[M+H-92]^+$. The first compound ($R_t = 8.71$ min) displayed additionally a fragment ion at m/z 137.01 $[M+H-399]^+$ which has been recognized by Vallverdú-Queralt et al. (2012) as characteristic of α -carotene. Two other peaks were therefore tentatively identified as β -carotene isomers. The presence of all-*trans*- β -carotene (peak 8) was confirmed by co-elution with an authentic standard. Based on the elution order and in accordance with Kolniak-Ostek (2016) and Wojdyło et al. (2018), peak 11 ($R_t = 9.46$ min) was suggested to be 9 or 9'-*cis*- β -carotene.

3.1.2. Chlorophylls

Chlorophylls are pigments that are responsible for the green color of plants. This natural colorant, whose structure is similar to hemoglobin in blood, is essential along with sunlight for photosynthesis. Leafy greens tend to be very high in chlorophyll, and the two foods with the highest chlorophyll content are spinach and parsley. Chlorophyll and its derivatives have a long history in traditional medicine, and various pro-health properties, including as a wound healing and anti-inflammatory agent, internal deodorant, potent anti-mutagen and anti-carcinogen, have been described (Mishra et al., 2011). Most chlorophyll-based supplements on the nutraceutical market contain a water-soluble derivative of natural chlorophyll – chlorophyllin – which is potentially

Table 1Identification of carotenoids, chlorophylls and free amino acids in *Chaenomeles* fruits using LC–PDA–QTOF–MS method.

Peak no	Compound	R _t (min)	λ _{max} (nm)	Proposed molecular formula	LC–PDA–QTOF–MS fragmentation pattern m/z	
					[M+H] ⁺	MS/MS
Carotenoids and chlorophylls						
1	Pheophorbide a	3.99	411/653	C ₃₅ H ₃₆ N ₄ O ₅	593.37	533.37/505.37
2	Chlorophyllide b	4.55	460/648	C ₃₅ H ₃₂ MgN ₄ O ₆	629.37	597.40/569.40
3	all- <i>trans</i> -lutein	5.05	336/447/475	C ₄₀ H ₅₆ O ₂	569.40	551.11/533.11
4	all- <i>trans</i> -β-cryptoxanthin	7.39	455/478	C ₄₀ H ₅₆ O	553.32	535.20/497.10
5	Chlorophyll a	8.53	430/615/665	C ₅₅ H ₇₂ MgN ₄ O ₅	893.30	615.40/555.30
6	Chlorophyll a'	8.64	430/615/663	C ₅₅ H ₇₂ MgN ₄ O ₅	893.30	615.40/555.30
7	α-carotene	8.71	445/478	C ₄₀ H ₅₆	537.38	137.01/444.01
8	all- <i>trans</i> -β-carotene	8.81	425/454/478	C ₄₀ H ₅₆	537.38	444.01
9	Chlorophyll b or b'	9.00	453/590/641	C ₅₅ H ₇₀ MgN ₄ O ₆	907.30	629.30
10	Pheophytin b or b'	9.27	433/599/653	C ₅₅ H ₇₂ N ₄ O ₆	885.72	553.70
11	9 or 9'- <i>cis</i> -β-carotene	9.46	425/454	C ₄₀ H ₅₆	537.38	444.01
12	Pheophytin a	9.58	408/503/666	C ₅₅ H ₇₄ N ₄ O ₅	871.38	593.01
13	Pheophytin a'	9.90	408/503/666	C ₅₅ H ₇₄ N ₄ O ₅	871.38	623.01/593.01
Amino acids						
1	6-aminoquinoline	2.01	–	C ₇ H ₁₀ N ₂ O ₄	145.11	–
2	Nonspecific side compound	2.32	–	–	171.09 (188.12)	145.11
3	L-asparagine	3.15	260.1	C ₄ H ₈ N ₂ O ₃	303.18	171.11
4	L-serine	3.94	260.1	C ₃ H ₇ NO ₃	276.18	171.11
5	Glycine	4.30	260.1	C ₂ H ₅ NO ₂	246.16	171.11
6	L-aspartic acid	5.04	260.1	C ₄ H ₇ NO ₄	304.17	171.11
7	L-glutamic acid	5.58	260.1	C ₅ H ₉ NO ₄	318.19	171.11
8	L-threonine*	5.89	260.1	C ₄ H ₉ NO ₃	290.20	171.11
9	Derivatization peak	6.30	254.1	–	203.12	171.11
10	L-alanine	6.51	260.1	C ₃ H ₇ NO ₂	260.16	171.11
11	γ-amino-n-butyric acid	6.67	260.1	C ₄ H ₉ NO ₂	274.22	171.11
12	L-valine*	8.45	260.1	C ₅ H ₁₁ NO ₂	288.22	171.11
13	L-isoleucine*	9.64	260.1	C ₆ H ₁₃ NO ₂	302.24	171.11

* -essential amino acid.

better absorbed by the human body than other isomers.

Peak 1 (R_t = 3.99 min) exhibited a deprotonated molecule at m/z 593.37 and a MS/MS fragment at m/z 533.37 and 505.37. The loss of m/z 60 is most likely due to loss of the HCOO + CH₃ group, which is consistent with Delpino-Rius et al. (2018). Thus, after comparison with an authentic standard, this compound has been identified as pheophorbide a. Peak 2 at the precursor ion m/z 629.37 displayed a molecular formula C₃₅H₃₂MgN₄O₆ given by the formula editor. In the MS/MS spectra, ions at m/z 597.40 01 [M+H-32]⁺ (loss of –CH₃OH) and 569.40 [M+H-60]⁺ (loss of –COOCH₃) were observed. Thus, in accordance with Kolniak-Ostek (2016) and Milenković et al. (2012) it was tentatively characterized as chlorophyllide b.

The two peaks (5 and 6) detected at R_t = 8.53 and 8.64 min displayed the same molecular ion at m/z 893.30 and product ions at m/z 615.40 and 555.30. These fragment ions resulted from the successive loss of C₂₀H₃₈ [M+H-278]⁺ and CH₃COOC₂₀H₃₉ [M+H-338]⁺, respectively (Turkiewicz et al., 2019). Therefore, those compounds were characterized as isomers of chlorophyll a. On the other hand, peak 9 detected at R_t = 9.00 min (m/z 907.30), with the molecular formula C₅₅H₇₀MgN₄O₆, showed a neutral loss of a phytol chain [M+H-278]⁺. Previous data (Delpino-Rius et al., 2018; Turkiewicz et al., 2019) indicate that this peak came from a chlorophyll b isomer.

The molecular ion at m/z 885.72 (peak 10) showed the molecular formula C₅₅H₇₂N₄O₆. This compound was suggested as an isomer of pheophytin b, based on data from UV absorption and the MS/MS fragment ion at m/z 553.70 demonstrated by the neutral loss [M+H-332]⁺, which can be interpreted as the loss of a phytol and –CH₃OH group.

Finally, peaks 12 and 13 detected at R_t = 9.58 and 9.90 min have been proposed to be pheophytin a and a', respectively. Those two corresponding epimers of the chlorophyll a derivatives with the molecular formula C₅₅H₇₄N₄O₅ showed an identical molecular ion at m/z 871.38. Both compounds released an MS/MS fragment ion at m/z 593.01, while pheophytin a' showed additionally [M+H-248]⁺ loss.

3.2. Quantification of carotenoids and chlorophylls

Carotenoid and chlorophyll content, calculated as the sum of individual compounds (Table 2), varied significantly between genotypes (p ≤ 0.05), with *C. × superba* 'Nicoline' displaying the highest (314.94 and 227.19 mg/kg dw), and *C. × superba* 'Pink Lady' the lowest content (32.44 and 19.27 mg/kg dw). The carotenoids account for on average 59 % of all analyzed compounds. Generally, all-*trans*-β-carotene was the main compound among carotenoids (average 33.17 mg/kg dw) and among chlorophylls it was pheophytin a (average 20.60 mg/kg dw) in the analyzed species and cultivars of *Chaenomeles*. Moreover, *C. × superba* 'Nicoline' contained almost 17 times more of the mentioned compounds than *C. × superba* 'Pink Lady' (regarding carotenoids as well as chlorophylls). In addition, carotenoids were on average 1.5 times more abundant than chlorophylls (for *C. speciosa* 'Simonii' even 3 times more). A similar ratio of these two dyes was calculated in the study by Ponder and Hallmann (2017), where it was 1.24 for the fruit of *C. × superba* and two *C. japonica* cultivars.

Ponder and Hallmann (2017) in four fruits of *Chaenomeles* ssp. identified and quantified lutein (38.00–101.73 mg/kg dw), zeaxanthin (42.76–177.06 mg/kg dw) and β-carotene (67.30–72.35 mg/kg dw). Differences in carotenoid content may result, among other factors, from sun exposure, cultivation method, level of nitrogen in the soil (increase along with nitrogen fertilization) or cultivar. Moreover, average total chlorophyll content was higher (87.24 mg/kg dw) (Ponder and Hallmann, 2017) than in our study (73.72 mg/kg dw). Comparing the content of natural pigments in *Chaenomeles* fruit to other fruit with light flesh (apples), it was found that the concentration of carotenoids and chlorophylls in the studied fruits is 2–8 times higher than in apple flesh and is very close to their concentration in the peel (58.72–1510.77 mg/kg dw) (Delgado-Pelayo et al., 2014).

The primary pigment of photosynthesis is chlorophyll a, while chlorophyll b is not necessary for photosynthesis to occur, and that is why not all cells that perform photosynthesis have chlorophyll b. An

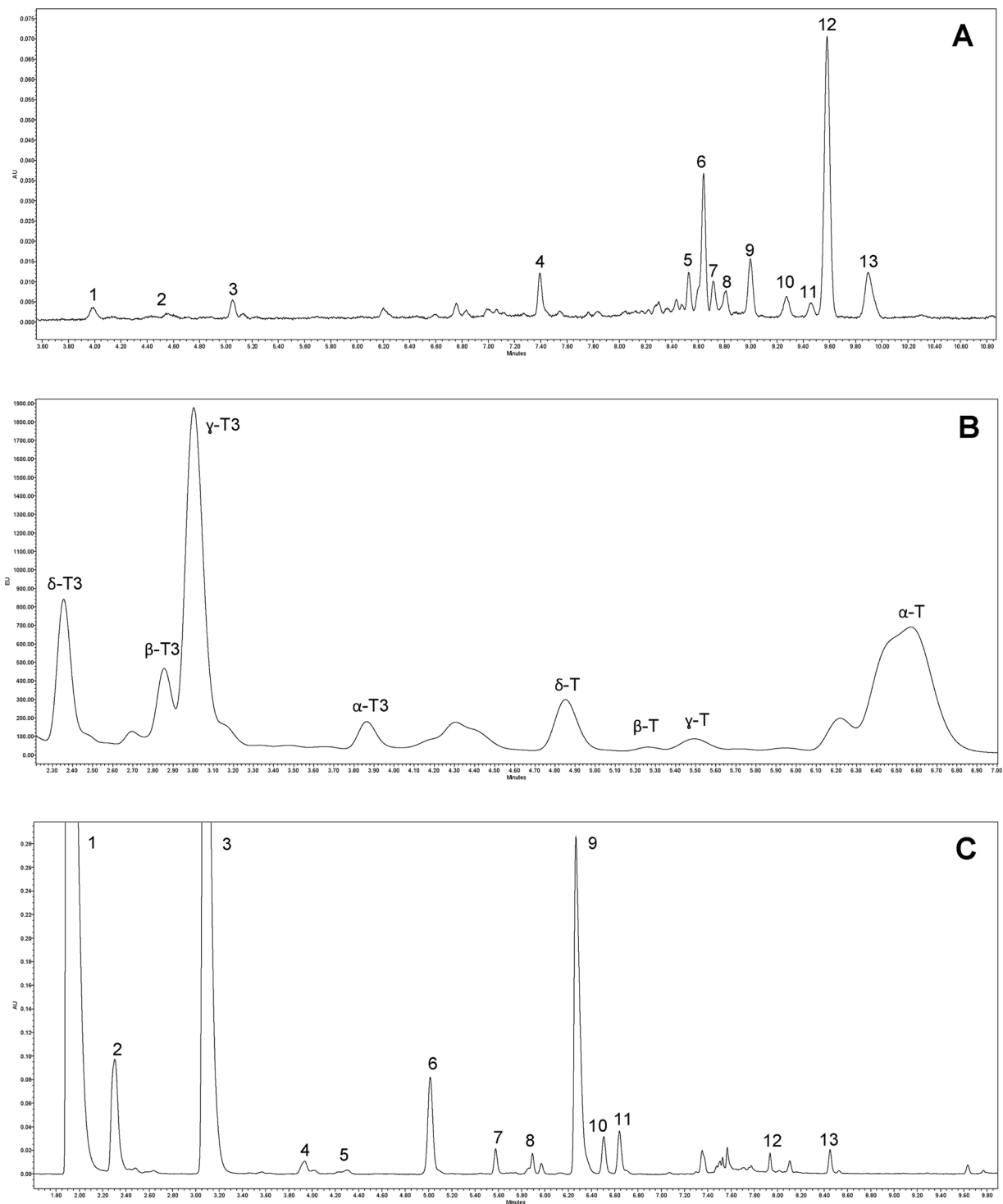


Fig. 1. Chromatograms from the analysis of *Chaenomeles* fruit: carotenoids and chlorophylls by UPLC-PDA at 425 nm in *C. x superba* 'Nicoline' (A), tocopherols and tocotrienols by UPLC-FL at 290/330 nm in *C. speciosa* 'Rubra' (B) and amino acids by UPLC-PDA at 260 nm in *C. x superba* 'Crimson and Gold' (C). Peak number identities are displayed in Table 1.

increase in chlorophyll *b* is an adaption to the shade, as it allows the plant to absorb a broader range of wavelengths of light. Therefore, when analyzing the ratio of chlorophyll *a* to *b*, it can be concluded, inter alia, under which conditions of sun exposure the fruits grew, or one can assume their color, because chlorophyll *b* is responsible more for the yellow color (Khan Academy, 2019). In the analyzed fruits of selected species and cultivars of *Chaenomeles* this ratio was at a similar level – chlorophyll *a* was up to 5 times more abundant than chlorophyll *b*. An

exception was *C. japonica* wild #1, having 17 times less chlorophyll *b*. In addition, *C. x superba* 'Flavon Rose' contained no chlorophyll, while in *C. x superba* 'Hollandia', *C. japonica* wild #1, 'Cido' and n1 no chlorophyll *b* was detected.

Bearing in mind the latest regulations established by the U.S. Food and Drug Administration (FDA) since January 2020 vitamin A has been listed on the new Nutrition Facts and Supplement Facts labels as retinol activity equivalents (RAE) (FDA, 2016). Therefore, knowing the content

Table 2
Content of carotenoids and chlorophylls (mg/kg dm) in various species and cvs. of *Chaenomeles* fruits.

Group of compounds	Peak no	<i>Chaenomeles × superba</i>										
		'Crimson and Gold'	'Texas Scarlet'	'Nicoline'	'Andenken an Karl Ramcke'	'Pink Lady'	'Colour Trail'	'Flovon Rose'	'Hollandia'	'Jet Trail'	wild	
Carotenoids	3	nd	0.74 ± 0.08bc	2.81 ± 0.20a	0.57 ± 0.24cd	nd	0.82 ± 0.29b	nd	0.73 ± 0.09bc	0.23 ± 0.00ef	0.37 ± 0.16def	
	4	6.49 ± 0.85de	8.94 ± 0.54bc	14.20 ± 0.22a	1.86 ± 0.56gh	0.74 ± 0.00h	2.07 ± 0.58gh	7.35 ± 0.20bcd	7.43 ± 0.16bcd	4.70 ± 0.79ef	3.17 ± 0.93fg	
	7	20.21 ± 1.11d	21.59 ± 1.44d	34.02 ± 1.72a	12.68 ± 0.25fg	12.42 ± 0.65fgh	10.30 ± 1.08ghij	17.28 ± 0.72e	21.91 ± 0.93cd	9.59 ± 0.87ij	5.28 ± 0.73k	
	8	17.02 ± 0.95gh	16.98 ± 0.87gh	159.07 ± 2.85a	62.69 ± 0.81c	nd	32.56 ± 0.99ef	nd	28.87 ± 0.67ef	nd	nd	
	11	nd	nd	104.84 ± 1.46a	nd	nd	37.46 ± 1.42d	nd	33.59 ± 1.61d	nd	20.84 ± 0.75e	
Sum		43.72 ± 2.56ghi	48.24 ± 1.74gh	314.94 ± 3.88a	77.80 ± 1.92de	13.17 ± 0.88k	83.22 ± 1.47de	24.62 ± 1.01jk	92.53 ± 2.00d	14.52 ± 1.64k	29.65 ± 1.71ijk	
Chlorophylls	1	nd	nd	5.76 ± 0.72a	1.46 ± 0.17cd	1.62 ± 0.19c	1.12 ± 0.19de	nd	nd	0.65 ± 0.05fg	nd	
	2	15.65 ± 0.76c	7.41 ± 0.88e	2.37 ± 0.17fg	0.69 ± 0.10i	1.08 ± 0.34ghi	1.05 ± 0.10hi	34.68 ± 1.11a	22.27 ± 0.93b	1.57 ± 0.41ghi	1.26 ± 0.22ghi	
	5	1.50 ± 0.23e	1.05 ± 0.11e	10.79 ± 0.85a	1.08 ± 0.34e	1.13 ± 0.26e	1.13 ± 0.38e	1.43 ± 0.45e	3.49 ± 0.21d	1.77 ± 0.17e	3.21 ± 0.76d	
	6	1.05 ± 0.18f	2.09 ± 0.35f	44.78 ± 0.99a	7.25 ± 0.77d	2.03 ± 0.30f	5.35 ± 0.29d	nd	nd	2.64 ± 0.70ef	0.64 ± 0.04f	
	9	1.27 ± 0.33ef	1.06 ± 0.44f	18.67 ± 0.81a	7.41 ± 0.48c	1.33 ± 0.21ef	2.32 ± 0.46ef	nd	nd	1.21 ± 0.95ef	1.40 ± 0.28ef	
	10	nd	1.68 ± 0.41d	8.01 ± 0.37a	1.69 ± 0.33d	nd	1.55 ± 0.54d	nd	1.18 ± 0.55d	nd	1.36 ± 0.32d	
	12	14.12 ± 1.00defg	15.96 ± 1.14def	111.65 ± 2.18a	27.91 ± 1.12c	7.70 ± 0.72fgh	22.54 ± 1.06cd	8.76 ± 0.72fgh	10.45 ± 0.49fgh	6.92 ± 0.31gh	6.49 ± 0.53gh	
	13	11.94 ± 0.78d	6.45 ± 0.22gh	25.17 ± 1.63a	18.17 ± 1.00b	4.39 ± 0.37i	7.66 ± 0.14fg	4.70 ± 0.85hi	6.93 ± 0.19g	6.65 ± 0.94g	3.56 ± 0.67ij	
	Sum		45.53 ± 2.21ef	35.70 ± 1.74gh	227.19 ± 2.18a	65.66 ± 1.59d	19.27 ± 1.14i	42.71 ± 1.64fg	49.57 ± 0.99ef	44.32 ± 0.66f	21.40 ± 1.54i	17.91 ± 0.98i
	Total		89.26 ± 2.87ef	83.94 ± 2.00fg	542.13 ± 2.22a	143.47 ± 2.05d	32.44 ± 1.03i	125.93 ± 1.70fgh	74.19 ± 2.01fgh	136.84 ± 1.55d	35.92 ± 1.22hi	47.56 ± 1.99ghi
Ch a : Ch b ratio		2.00	2.95	2.98	1.12	2.38	2.80	–	–	3.65	2.75	
C : Ch ratio		0.96	1.35	1.39	1.18	0.68	1.95	0.50	2.09	0.68	1.66	

Group of compounds	Peak no	<i>Chaenomeles japonica</i>						<i>Chaenomeles speciosa</i>			
		'Cido'	'Cameo'	'Red Joy'	wild #1	wild #2	n1 (new)	'Nivalis'	'Rubra'	'Simonii'	
Carotenoids	3	0.73 ± 0.28bc	0.74 ± 0.38bc	0.44 ± 0.16de	nd	0.29 ± 0.06ef	0.19 ± 0.15f	nd	0.67 ± 0.13bc	0.88 ± 0.21b	
	4	nd	3.65 ± 0.58fg	6.90 ± 0.90cd	9.14 ± 0.20b	4.63 ± 0.44ef	4.90 ± 0.90ef	4.88 ± 0.21ef	2.43 ± 0.98gh	15.30 ± 0.74a	
	7	8.700.60ij	7.95 ± 0.95j	24.46 ± 0.88b	14.51 ± 0.57f	10.00 ± 1.00hij	10.65 ± 0.74ghi	11.00 ± 0.93ghi	22.31 ± 1.15bcd	24.22 ± 1.62bc	
	8	25.02 ± 1.02fg	24.57 ± 1.00fg	59.84 ± 1.11c	9.53 ± 0.63h	41.69 ± 1.88d	nd	34.71 ± 1.44de	86.83 ± 2.25b	30.87 ± 2.04ef	
	11	nd	nd	48.80 ± 1.16c	nd	34.04 ± 1.54d	nd	9.33 ± 0.93f	63.52 ± 1.81b	nd	
Sum		34.45 ± 1.34hij	36.91 ± 0.97hij	140.44 ± 2.69c	33.17 ± 1.33hij	90.65 ± 1.29d	15.74 ± 0.30k	59.93 ± 0.44fg	175.75 ± 1.77b	71.26 ± 1.82ef	
Chlorophylls	1	1.16 ± 0.00de	2.34 ± 0.50b	2.34 ± 0.50b	0.53 ± 0.13g	0.98 ± 0.10ef	nd	0.87 ± 0.16efg	5.80 ± 0.39a	nd	
	2	2.29 ± 0.39fgh	8.41 ± 0.86e	3.16 ± 0.39f	9.95 ± 0.96d	3.25 ± 0.69f	10.59 ± 0.36d	2.00 ± 0.41fghi	1.04 ± 0.63hi	10.46 ± 1.30d	
	5	0.98 ± 0.20e	1.17 ± 0.09e	3.91 ± 0.30d	8.37 ± 0.25b	5.36 ± 0.66c	1.13 ± 0.40e	1.54 ± 0.31e	5.87 ± 0.88c	5.13 ± 0.54c	
	6	2.06 ± 0.66f	2.55 ± 0.70f	10.28 ± 1.14f	11.67 ± 0.98bc	5.21 ± 0.60de	0.72 ± 0.28f	2.19 ± 0.60f	13.69 ± 1.66b	nd	
	9	nd	0.95 ± 0.24f	4.97 ± 0.58d	1.15 ± 0.55ef	3.00 ± 0.55e	nd	1.13 ± 0.42ef	9.41 ± 0.57b	1.03 ± 0.23f	
	10	2.04 ± 0.18d	nd	3.40 ± 0.61c	nd	2.07 ± 0.85d	nd	1.09 ± 0.18d	5.41 ± 0.42b	nd	
	12	7.59 ± 0.96fgh	7.74 ± 0.52fgh	43.39 ± 1.07b	13.28 ± 1.11efg	20.14 ± 1.32cde	6.05 ± 0.66gh	8.95 ± 0.30fgh	49.79 ± 1.34b	2.04 ± 0.68h	
	13	6.39 ± 0.38gh	10.67 ± 1.21de	11.95 ± 1.76d	7.63 ± 0.87fg	9.33 ± 0.49ef	2.49 ± 0.52j	3.00 ± 0.54ij	15.48 ± 1.00c	2.40 ± 0.34j	
	Sum		22.49 ± 1.67i	31.50 ± 1.44h	83.41 ± 2.12c	52.58 ± 1.72e	49.35 ± 1.32ef	20.99 ± 0.99i	20.76 ± 1.64i	106.48 ± 2.56b	21.07 ± 1.11i
	Total		56.95 ± 1.52fghi	68.41 ± 1.71fghi	223.85 ± 2.95c	85.76 ± 1.14efg	140.00 ± 2.17d	36.73 ± 1.46hi	80.69 ± 1.07fg	282.23 ± 2.00b	92.33 ± 1.75ef
Ch a : Ch b ratio		–	3.93	2.86	17.41	3.52	–	3.30	2.08	4.98	
C : Ch ratio		1.53	1.17	1.68	0.63	1.84	0.75	2.89	1.65	3.38	

nd–not detected; ± standard deviation; Ch–chlorophylls; C–carotenoids; value in the same columns followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

Table 3
Content of tocopherols and tocotrienols (mg/kg dm) in various species and cvs. of *Chaenomeles* fruits.

Species	Cultivars	Tocopherols (T)					Tocotrienols (T3)					Total	T : T3 ratio
		alfa	beta	gamma	delta	sum	alfa	beta	gamma	delta	sum		
<i>Chaenomeles × superba</i>	'Crimson and Gold'	23.22 ± 1.22 cd	0.33 ± 0.03efgh	0.29 ± 0.10def	1.21 ± 0.10b	25.05 ± 3.05c	5.23 ± 0.23b	5.18 ± 0.18b	23.61 ± 1.00a	8.28 ± 0.20a	42.30 ± 1.00a	67.35 ± 2.35a	0.59
	'Texas Scarlet'	5.45 ± 0.45 j	0.18 ± 0.02efgh	0.02 ± 0.00 g	0.16 ± 0.01ij	5.81 ± 0.19i	2.31 ± 0.31ef	0.24 ± 0.04f	0.91 ± 0.09ij	0.22 ± 0.10k	3.69 ± 0.19 j	9.50 ± 0.32 j	
	'Nicoline'	23.08 ± 3.08 cd	0.09 ± 0.00gh	0.13 ± 0.03efg	0.86 ± 0.04d	24.16 ± 1.16c	2.69 ± 0.30de	1.25 ± 0.25cde	5.22 ± 0.22e	1.86 ± 0.10 h	11.03 ± 0.50 g	35.18 ± 0.18f	2.19
	'Andenken an Karl Ramcke'	8.25 ± 0.25hij	0.19 ± 0.06efgh	0.05 ± 0.00efg	0.34 ± 0.04gh	8.84 ± 0.16hi	0.80 ± 0.20ij	0.29 ± 0.09f	1.15 ± 0.15hij	0.98 ± 0.02ij	3.22 ± 0.11jk	12.06 ± 1.00 j	2.74
	'Pink Lady'	6.86 ± 0.16ij	0.16 ± 0.04fgh	0.04 ± 0.00fg	0.21 ± 0.01ji	7.27 ± 0.27i	1.39 ± 0.09gh	0.38 ± 0.08ef	1.77 ± 0.10ghi	0.62 ± 0.02jk	4.17 ± 0.07ij	11.44 ± 0.40 j	1.74
	'Colour Trail'	21.95 ± 1.00de	0.55 ± 0.05de	0.66 ± 0.06c	0.96 ± 0.24 cd	24.11 ± 2.11c	6.23 ± 0.13a	2.11 ± 0.11c	13.88 ± 0.88c	4.16 ± 0.16de	26.37 ± 1.37c	50.48 ± 1.32c	0.91
	'Flovon Rose'	10.69 ± 0.69gh	0.07 ± 0.00gh	0.09 ± 0.09efg	0.27 ± 0.09hi	11.13 ± 0.10gh	1.86 ± 0.06fg	0.22 ± 0.11f	1.43 ± 0.03hij	0.50 ± 0.00jk	4.01 ± 0.01ij	15.14 ± 0.18i	2.77
	'Hollandia'	14.80 ± 0.80f	0.49 ± 0.01def	0.05 ± 0.01efg	0.41 ± 0.07fg	15.75 ± 0.25ef	2.87 ± 0.07d	0.95 ± 0.05def	2.22 ± 0.22gh	0.56 ± 0.06jk	6.60 ± 0.15 h	22.35 ± 0.33 h	2.39
	'Jet Trail'	26.99 ± 1.00ab	0.38 ± 0.02defgh	0.44 ± 0.04 cd	1.07 ± 0.17bc	28.89 ± 0.20b	2.82 ± 0.22d	1.90 ± 0.00c	16.11 ± 1.00b	6.36 ± 0.30b	27.19 ± 0.19c	56.07 ± 2.08b	1.06
	wild	14.61 ± 0.61f	1.41 ± 0.42c	0.12 ± 0.02efg	0.94 ± 0.06 cd	17.07 ± 0.07de	0.80 ± 0.00ij	1.39 ± 0.09 cd	2.87 ± 0.30fg	0.27 ± 0.03k	5.32 ± 0.32hi	22.39 ± 0.37 h	3.21
	'Cido'	11.88 ± 0.20fg	0.75 ± 0.05 cd	0.28 ± 0.08defg	0.30 ± 0.00hi	13.21 ± 0.21fg	0.36 ± 0.01 j	0.46 ± 0.06ef	1.79 ± 0.21ghi	0.25 ± 0.05k	2.86 ± 0.06jk	16.07 ± 0.99i	4.61
	'Cameo'	14.44 ± 0.40f	0.40 ± 0.10defgh	0.02 ± 0.00 g	0.86 ± 0.04d	15.71 ± 0.00ef	3.88 ± 0.12c	1.48 ± 0.40 cd	6.11 ± 0.11e	3.16 ± 0.16f	14.63 ± 0.13d	30.33 ± 0.32 g	1.07
	'Red Joy'	18.46 ± 0.41e	3.61 ± 0.20b	1.65 ± 0.25b	1.85 ± 0.05a	25.57 ± 0.50c	4.18 ± 0.18c	5.49 ± 0.50b	0.46 ± 0.04 j	3.77 ± 0.07e	13.91 ± 0.09def	39.48 ± 0.41e	1.84
	<i>Chaenomeles japonica</i>	wild #1	9.56 ± 0.00ghi	0.49 ± 0.01def	0.30 ± 0.10def	0.51 ± 0.01f	10.86 ± 0.06gh	1.16 ± 0.16hi	1.56 ± 0.06 cd	5.86 ± 0.06e	4.36 ± 0.36d	12.94 ± 0.14ef	23.80 ± 0.28 h
wild #2		18.75 ± 0.25e	0.44 ± 0.04defg	0.13 ± 0.03efg	0.97 ± 0.03 cd	20.29 ± 0.29d	3.11 ± 0.11d	1.68 ± 0.02 cd	5.19 ± 0.19e	2.56 ± 0.50 g	12.54 ± 0.04fg	32.82 ± 0.85fg	1.62
new n1		28.92 ± 0.08a	0.17 ± 0.03efgh	0.31 ± 0.01de	0.65 ± 0.05e	30.05 ± 1.95b	5.23 ± 0.23b	1.52 ± 0.10 cd	6.16 ± 0.16e	1.24 ± 0.04i	14.15 ± 0.85def	44.19 ± 1.20d	2.12
'Nivalis'		7.30 ± 0.15hij	0.02 ± 0.00 h	0.08 ± 0.00efg	0.06 ± 0.00 j	7.46 ± 0.40i	0.70 ± 0.05ij	0.22 ± 0.13f	0.90 ± 0.10ij	0.25 ± 0.05k	2.06 ± 0.06k	9.52 ± 0.01 j	3.62
<i>Chaenomeles speciosa</i>	'Rubra'	26.12 ± 1.12abc	6.21 ± 0.21a	4.20 ± 0.20a	1.05 ± 0.13c	37.58 ± 1.00a	5.21 ± 0.11b	14.65 ± 1.00a	3.78 ± 0.10f	5.45 ± 0.05c	29.09 ± 1.00b	66.67 ± 0.67a	1.29
	'Simonii'	23.91 ± 3.00bcd	0.15 ± 0.15fgh	0.18 ± 0.02defg	0.53 ± 0.03ef	24.77 ± 0.77c	2.74 ± 0.04de	2.05 ± 0.05c	7.62 ± 0.30d	1.95 ± 0.05 h	14.36 ± 0.36de	39.14 ± 1.14e	1.72
Tukey's Multiple Range Test for mean values													
<i>Chaenomeles × superba</i>		15.59 ± 1.02c	0.39 ± 0.09c	0.19 ± 0.00b	0.64 ± 0.20b	16.81 ± 1.44c	2.70 ± 0.41b	1.39 ± 0.18c	6.92 ± 0.60a	2.38 ± 0.10a	13.39 ± 0.63b	30.20 ± 2.01b	1.26
	<i>Chaenomeles japonica</i>	17.00 ± 1.38b	0.98 ± 0.10b	0.45 ± 0.02b	0.86 ± 0.11a	19.28 ± 1.25b	2.99 ± 0.39a	2.03 ± 0.22b	4.26 ± 0.26b	2.56 ± 0.19a	11.84 ± 0.55c	31.12 ± 1.99b	1.63
<i>Chaenomeles speciosa</i>		19.11 ± 1.22a	2.13 ± 0.98a	1.49 ± 0.11a	0.55 ± 0.05b	23.27 ± 1.00a	2.88 ± 0.47ab	5.64 ± 0.44a	4.10 ± 0.17b	2.55 ± 0.15a	15.17 ± 0.41a	38.44 ± 1.80a	1.53

± standard deviation; value in the same columns followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

of α -, β -, γ -carotene and β -cryptoxanthin, one can calculate the amount of RAE. *Chaenomeles* fruit, depending on the studied cultivar, contains from 6.53–420.15 μg RAE in 100 g of fresh fruit (*C. japonica* n1 and *C. × superba* 'Nicoline', respectively). Compared to other fruits, *Chaenomeles* fruit on average contains more vitamin A than nectarines, apples or oranges (17, 12 and 11 μg RAE) but less than cantaloupe melon, apricots and loquats (169, 96 and 76 μg RAE) (MYFOODDATA, 2020). Referring the RAE content to the recommended daily intake (RDI), it was found

that 100 g of fresh fruit covers 9% of the requirement for vitamin A in men and 11 % in women.

3.3. Quantification of tocopherols and tocotrienols

One of the most effective antioxidants that inhibit cell aging is vitamin E, which protects the epidermis against the harmful effects of the external environment (creating a barrier to UV rays) (Zielińska and

Nowak, 2014). Vitamin E is a group of fat-soluble compounds that include tocopherols (T) and tocotrienols (T3). This group includes 8 naturally occurring homologues – four of tocopherol and four of tocotrienol, respectively α -, β -, γ - and δ - (Chun et al., 2006). Tocopherols are present in all plant tissues (e.g. roots, seeds, petals, leaves and fruits) and predominate in the form of α -T, except for seeds (α - or γ -isomers). Tocotrienols on the other hand are not usually found in the green parts

of higher plants, but are found in high amounts in seeds. Total tocopherol content usually ranged from <1 mg/kg dw (potato tubers) to very high levels >1000 mg/kg dw in leaves and seeds (Munné-Bosch and Alegre, 2002). Antioxidant properties of tocopherols isomers in living organisms increase as follows: α -T > β -T > γ -T > δ -T with their *in vitro* activity being exactly the opposite (Nogala-Kalucka and Siger, 2011; Seppanen et al., 2010).

Table 4
Content of free amino acids (mg/100 g dm) in various species and cvs. of *Chaenomeles* fruits.

Species	Cultivars	L-asparagine	L-serine	L-glycine	L-aspartic acid	L-glutamic acid	L-threonine*	L-alanine	γ -amino-n-butyric acid	L-valine*	L-isoleucine*	Total	
<i>Chaenomeles × superba</i>	'Crimson and Gold'	133.38 ± 2.13f	19.76 ± 1.12bc	4.66 ± 0.45e	77.77 ± 1.12ef	31.06 ± 0.89 g	3.20 ± 0.21e	21.66 ± 1.25efg	32.01 ± 1.32d	10.67 ± 1.17e	3.42 ± 0.20c	337.58 ± 20.17 g	
	'Texas Scarlet'	234.15 ± 5.82e	13.41 ± 0.67e	2.51 ± 0.21 h	72.02 ± 1.28efg	72.50 ± 1.54b	3.17 ± 0.33e	38.63 ± 1.57bc	15.98 ± 1.18efgh	8.01 ± 0.56f	4.80 ± 0.18b	465.17 ± 21.22f	
	'Nicoline'	75.40 ± 2.11fgh	16.62 ± 0.88d	3.59 ± 0.32fg	41.69 ± 2.01ghij	89.37 ± 1.67a	3.33 ± 0.27e	30.95 ± 1.82cde	20.55 ± 1.23efg	15.62 ± 0.98b	2.55 ± 0.14d	299.67 ± 18.14 g	
	'Andenken an Karl Ramcke'	6.86 ± 0.68gh	5.61 ± 0.42 h	1.05 ± 0.11i	8.82 ± 1.00kl	16.37 ± 1.05k	1.50 ± 0.16ghij	14.96 ± 1.03ghi	12.72 ± 1.14gh	12.97 ± 1.13d	0.48 ± 0.08ij	81.34 ± 3.42jk	
	'Pink Lady'	84.17 ± 2.20fg	3.21 ± 0.32hi	2.10 ± 0.12 h	101.43 ± 3.87e	28.30 ± 1.63gh	1.24 ± 0.18hij	19.90 ± 1.22fgh	12.19 ± 1.11 h	2.04 ± 0.08 j	0.61 ± 0.08hij	255.21 ± 14.32gh	
	'Colour Trail'	1296.71 ± 63.36b	10.03 ± 1.01 g	17.76 ± 0.54a	575.11 ± 10.41a	49.56 ± 1.88e	8.38 ± 0.67b	44.15 ± 1.63b	72.08 ± 2.19b	5.74 ± 0.25 h	1.17 ± 0.23efg	2080.69 ± 28.12b	
	'Flovon Rose'	65.85 ± 3.54fgh	13.03 ± 1.21ef	6.96 ± 0.75c	47.75 ± 1.44fghi	20.43 ± 1.21 j	1.48 ± 0.20hij	14.82 ± 1.00ghi	22.96 ± 1.03e	3.81 ± 0.33i	1.69 ± 0.28e	198.78 ± 3.99hi	
	'Hollandia'	372.15 ± 7.96d	17.25 ± 1.33 cd	7.08 ± 0.63c	182.28 ± 2.87d	24.96 ± 1.29hi	2.62 ± 0.13efg	27.75 ± 1.41def	20.48 ± 2.09efg	8.13 ± 0.42f	3.10 ± 0.30c	665.81 ± 14.63e	
	'Jet Trail'	1509.07 ± 22.87a	53.79 ± 2.08a	13.86 ± 0.67b	458.17 ± 5.73b	61.10 ± 1.49c	10.84 ± 0.60a	76.56 ± 2.03a	121.53 ± 3.13a	15.21 ± 0.76bc	6.19 ± 0.19a	2326.33 ± 32.17a	
	wild	24.55 ± 1.63gh	10.24 ± 1.10fg	2.59 ± 0.32 h	33.86 ± 1.03hijkl	12.93 ± 1.07kl	1.84 ± 0.07fghi	12.20 ± 1.18ghi	12.85 ± 1.17gh	7.39 ± 0.45fg	0.39 ± 0.07 j	118.84 ± 7.22ij	
<i>Chaenomeles japonica</i>	'Cido'	288.28 ± 5.57e	21.39 ± 1.47b	3.59 ± 0.33fg	371.00 ± 6.00c	70.71 ± 1.99b	5.72 ± 0.19d	33.65 ± 1.13 cd	21.03 ± 1.32ef	10.62 ± 0.93e	1.40 ± 0.00ef	827.39 ± 19.37d	
	'Cameo'	3.49 ± 0.41 h	0.96 ± 0.09i	0.23 ± 0.07 j	4.00 ± 0.99 L	2.37 ± 0.54n	0.55 ± 0.05 j	1.47 ± 0.19 j	2.14 ± 0.14i	0.24 ± 0.00k	0.40 ± 0.04 j	15.87 ± 1.11k	
	'Red Joy'	108.71 ± 8.22f	8.45 ± 0.84 g	2.09 ± 0.24 h	64.41 ± 1.01fgh	23.41 ± 1.62ij	2.27 ± 0.08efgh	18.55 ± 1.33fgh	31.95 ± 1.39d	2.48 ± 0.10 j	1.14 ± 0.06fgh	263.47 ± 17.73gh	
	wild #1	542.53 ± 7.41c	19.68 ± 1.29bc	4.14 ± 0.35ef	203.17 ± 3.09d	36.09 ± 1.50f	2.89 ± 0.10ef	36.83 ± 1.46cde	69.36 ± 1.83b	6.81 ± 0.32gh	1.50 ± 0.10ef	923.00 ± 27.28c	
	wild #2	17.73 ± 0.97gh	4.62 ± 0.65 h	1.44 ± 0.20i	18.23 ± 1.00ijkl	14.28 ± 1.37k	1.18 ± 0.06hij	8.31 ± 0.87ij	13.65 ± 0.88fgh	3.66 ± 0.27i	1.00 ± 0.18fghi	84.10 ± 7.49jk	
	new n1	8.32 ± 0.54gh	4.94 ± 0.55 h	5.39 ± 0.39d	19.99 ± 0.47ijkl	7.44 ± 0.42 m	0.76 ± 0.00ij	11.64 ± 1.03hi	20.08 ± 0.97efgh	2.09 ± 0.11 j	0.52 ± 0.09j	81.17 ± 6.09jk	
	'Nivalis'	61.52 ± 2.82fgh	8.58 ± 0.64 g	3.56 ± 0.27 g	37.43 ± 1.43hijk	10.12 ± 0.66 lm	1.32 ± 0.06hij	8.38 ± 0.99ij	19.13 ± 1.09efgh	4.54 ± 0.19i	0.86 ± 0.07hij	155.44 ± 2.33ij	
	<i>Chaenomeles speciosa</i>	'Rubra'	1490.69 ± 28.30a	19.66 ± 1.23bc	5.71 ± 0.38d	343.85 ± 6.85c	29.33 ± 0.98 g	7.23 ± 0.17c	34.12 ± 1.63 cd	39.90 ± 1.37 cd	14.25 ± 0.58c	6.41 ± 0.41a	1991.14 ± 37.16b
		'Simonii'	9.64 ± 0.89gh	17.33 ± 1.08 cd	6.75 ± 0.86c	12.04 ± 1.07jkl	54.88 ± 1.61d	1.50 ± 0.10ghij	27.27 ± 1.11def	43.27 ± 1.58c	16.72 ± 0.60a	4.73 ± 0.26b	194.13 ± 2.45hi
	Tukey's Multiple Range Test for mean values												
<i>Chaenomeles × superba</i>		380.23 ± 5.88b	16.30 ± 1.21a	6.22 ± 0.22a	159.89 ± 2.88a	40.66 ± 1.01a	3.76 ± 0.13a	30.16 ± 0.39a	34.33 ± 0.50a	8.96 ± 0.54b	2.44 ± 0.10b	682.94 ± 3.88b	
<i>Chaenomeles japonica</i>		161.51 ± 3.82c	10.01 ± 0.59b	2.81 ± 0.30c	113.47 ± 1.67b	25.72 ± 0.99c	2.23 ± 0.34b	18.41 ± 0.11c	26.37 ± 0.99b	4.32 ± 0.20c	0.99 ± 0.09c	365.83 ± 2.67c	
<i>Chaenomeles speciosa</i>		520.62 ± 7.78a	15.19 ± 0.97a	5.34 ± 0.45b	131.11 ± 1.93b	31.44 ± 0.71b	3.35 ± 0.28a	23.25 ± 0.49b	34.10 ± 0.67a	11.84 ± 0.22a	4.00 ± 0.14a	780.24 ± 3.99a	

GABA- γ -amino-N-butyric acid; *-essential amino acid; \pm standard deviation; value in the same columns followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

In the studied *Chaenomeles* fruits, regardless of the cultivar, four isomers of tocopherols and four isomers of tocotrienols were identified (Table 3, Fig. 1B). All the detected compounds were characterized along with their retention times (R_t), observed absorption bands (λ_{max}) and comparison with authentic standards. Total tocopherol and tocotrienol content, calculated as the sum of individual compounds (Table 3), varied significantly between genotypes ($p \leq 0.05$), with *C. × superba* 'Crimson and Gold' displaying the highest (67.35 mg/kg dw), and *C. speciosa* 'Nivalis' the lowest content (9.50 mg/kg dw). In the largest quantity we detected α -T, which represented on average 50 % of the sum of T and T3, ranging from 5.45 to 28.92 mg/kg dw depending on cultivar. Among all isomers of vitamin E, α -T plays an essential role in the human body, showing the highest biological activity (Górnaś, 2015). In the tocopherol group, individual isomers can be ranked according to their decreasing amount: α -T > β -T > γ -T > δ -T, whereas in the tocotrienol group: γ -T3 > β -T3 > α -T3 > δ -T3. Analyzing the ratio of T to T3, it was found that tocopherols were on average twice as abundant as tocotrienols (almost 5-fold more in *C. japonica* 'Cido'), except for *C. × superba* 'Crimson and Gold', 'Color Trail' and *C. japonica* wild # 1, where tocotrienols

dominated. Attempts have been made in the literature to determine tocopherols in *C. japonica* seeds. Górnaś et al. (2019) confirmed α -, β - and γ -T in different Japanese quince seeds from 1117 to 1266 mg/kg oil. It should be noted that the tocopherol profile did not contain the δ -T isomer, whereas only β -T3 was determined from tocotrienols (Górnaś et al., 2015). As was mentioned before, this is the first report on the composition of T and T3 in *Chaenomeles* fruit. For comparison, analyzed fruits contain more tocopherols than popular fruit with a light flesh, e.g. apples, pears or peaches (4.2; 3.0 and 7.4 mg/kg fresh weight (fw), respectively), but less than raspberries (34.6 mg/kg fw) (Chun et al., 2006).

The new FDA guidelines also included vitamin E, whose content in food products is expressed in mg of α -tocopherol. Depending on the cultivar, 100 g of fresh *Chaenomeles* fruits contained on average 0.34 mg of α -tocopherol. Assuming that the RDI for an adult is 10 mg (for men) and 8 mg (for women), a portion of fruit covers 3 and 4%, respectively, of the need for vitamin E. This is more than for typical citrus fruits (limes, lemons, and oranges) but less than for kiwi fruits (up to 1.5 mg of α -tocopherol in 100 g) (MYFOODDATA, 2020).

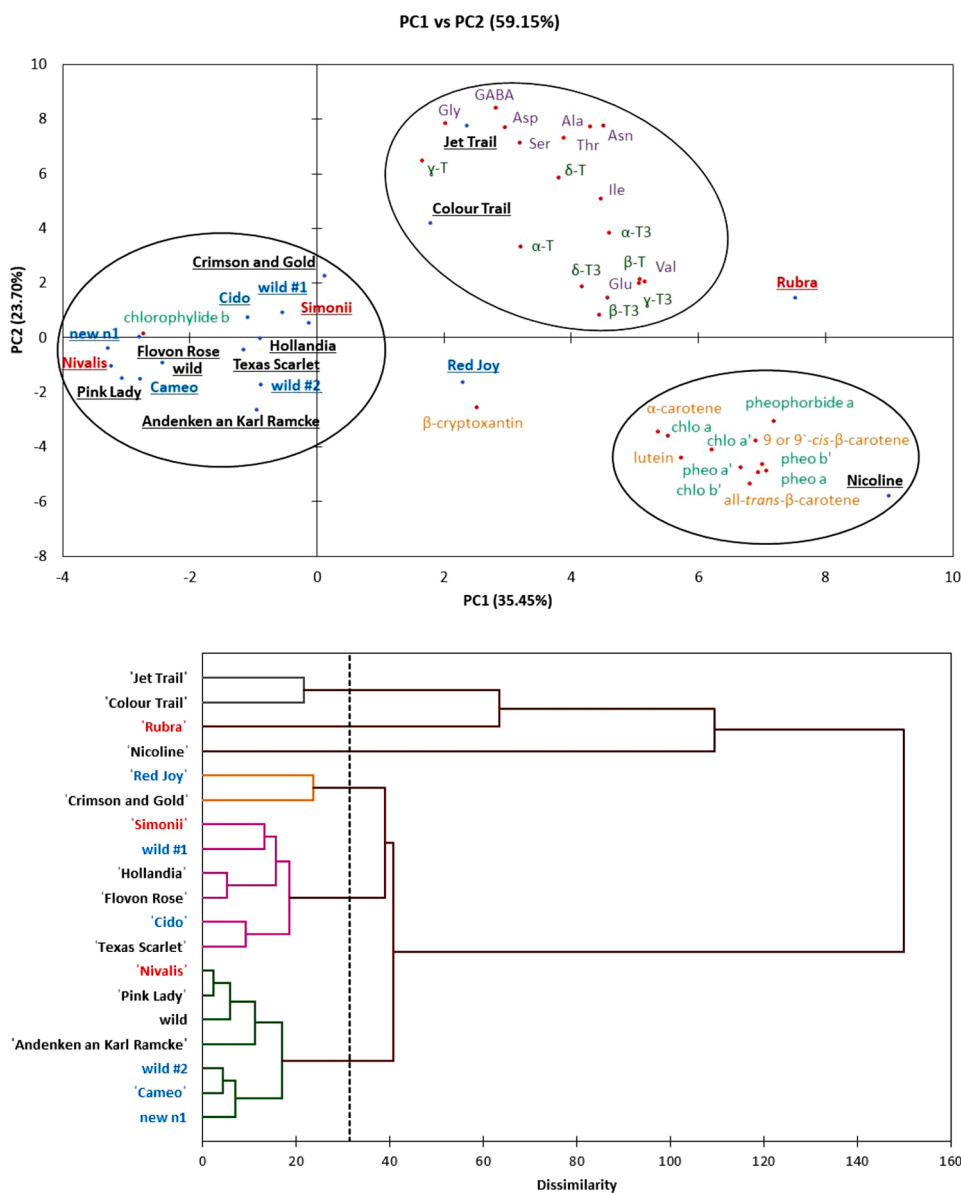


Fig. 2. PCA biplot showing the relationship among samples and chemical constituents (A); AHC dendrogram based on dissimilarities with respect to *Chaenomeles* fruit cultivars (B). *C. japonica* (blue), *C. speciosa* (red) and *C. × superba* (black). chlo-chlorophyll; pheo-pheophytin; T-tocopherol; T3-tocotrienol.

3.4. Identification and quantification of amino acids

Animal as well as plant proteins are made up of about 20 common amino acids (among more than 300 occurring in nature). The proportion of these amino acids varies as a characteristic of a specific protein, but all food proteins contain some of each. Amino nitrogen accounts for approximately 16 % of the weight of proteins. Amino acids are required for the synthesis of body protein and other important nitrogen-containing compounds, such as creatine, peptide hormones, and some neurotransmitters. However, non-protein amino acids such as ornithine, citrulline, and homocysteine also play important roles in cell metabolism (Wu, 2009, 2013). Although the most common recommended intakes are established for protein, WHO (2007) also provides estimated requirements for essential amino acids. Thus, it is assumed that the total adult suggested intake for indispensable amino acids is 184 mg per kg body weight per day.

In the present work, a qualitative and quantitative analysis of the free amino acids in *Chaenomeles* fruits was performed using a derivatization with AQC and UPLC-PDA-MS/QToF operated in positive ionization mode. Thus 10 amino acids were characterized, of which three were essential. For the cultivars analyzed in this study, this is the first attempt of determination of the free amino acid profile. All the detected compounds (Table 1, Fig. 1C) were characterized by means of their detectable UV spectrum and MS/MS data, together with comparison with authentic standards.

Peak 1 ($R_t = 2.01$ min) gave an $[M+H]^+$ ion at m/z 145.11, which is characteristic for 6-aminoquinoline (AMQ) which is the breakdown product of AQC reagent. This compound eluted for all samples as the major peak before polar amino acids in early retention time, which is consistent with Fiechter and Mayer (2011). Peak 2 detected at 2.32 min and with an $[M+H]^+$ ion at m/z 171.09 gave a product ion at m/z 145.11 ($[M-H-26]^+$). Moreover, this peak, like the previous one, did not have characteristic absorption bands. According to Spáčil et al. (2010) these ions are nonspecific and were formed as a result of breaking the cleavage of the bond between the amide nitrogen and the carbonyl carbon. According to other authors (Jama, 2013; Nagumo et al., 2009) this peak was assigned as derived from the NH_4^+ ion. Peak 9 with a parent ion at m/z 203.12 and maximum absorption band (λ_{max}) at 254.1 nm displayed the $[M-H]^+$ daughter ion at m/z 171.11, which indicates the loss of CH_3OH (32 Da). Thus the compound was tentatively assigned to be derivatization peak which is consistent with the findings of Jama (2013) based on a comparison of retention time. According to literature data (Salazar et al., 2012) the AQC reagent gives a common fragment ion at m/z 171.11, generated by a loss of the AMQ moiety. Therefore, in all remaining peaks (3, 4, 5, 6, 7, 8, 10, 11, 12 and 13) the combination of the parent ion (from specific amino acids or amino thiols) and this common fragment (daughter) ion ($[M + AQC+H]^+$) was selected for identification (Table 1).

Total amino acid content, calculated as the sum of individual compounds (Table 4), varied significantly between genotypes ($p \leq 0.05$), with *C. × superba* 'Jet Trail' displaying the highest (2326.33 mg/100 g dw), and *C. japonica* 'Cameo' the lowest content (15.87 mg/100 g dw). L-asparagine and L-glutamic acid were determined in the largest amount, representing on average 55 and 23 % of the total amino acid content in *C. × superba*, 44 and 31 % in *C. japonica* and 67 and 17 % in *C. speciosa*. The remaining amino acid content was below 8% of the total amino acid content. As mentioned before, in the fruits of *Chaenomeles* three essential (exogenous) amino acids, i.e. L-threonine, L-valine and L-isoleucine, were identified. Threonine participates in the synthesis of collagen and elastin, valine regulates muscle metabolism and rebuilds tissues, while the most important functions of isoleucine include the regulation of sugar levels, and participation in energy and hematopoietic processes (Wu, 2013). These are amino acids that the human body is unable to synthesize, and must be supplied with food. The highest concentration of L-threonine was observed in the fruit of *C. × superba* 'Jet Trail', and L-valine and L-isoleucine in *C. speciosa* 'Simonii' and 'Rubra', respectively.

For comparison, Zhang et al. (2011) in a study of *C. speciosa* fruit determined the total amino acid content at the level of 260–500 mg/100 g dw. Similarly, Chung et al. (1988) in the fruits of *C. sinensis* (Thouin) Koehne confirmed a free amino acids content of 383.3 mg/100 g dw. Moreover, additionally to the amino acids determined in this study, they confirmed the presence of proline, cysteine, phenylalanine and histidine. In contrast, Hellfn et al. (2003) analyzed the composition of *Chaenomeles* fruit juice of selected species and cultivars and obtained higher values for *C. japonica* (352.17–5771.96 mg/100 g dw), *C. speciosa* (444.83 mg/100 g dw) and *C. cathayensis* (1062.79 mg/100 g dw), with glutamic acid as the dominant amino acid. Differences in amino acid concentrations according to various authors may result, among other reasons, from the method of determination, the derivatizing agent used, the sample pre-treatment and the detector used in HPLC analysis (Mandrioli et al., 2013).

3.5. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC)

PCA biplots represent distances between the observations and also the inner products between observations and variables. Therefore a 2-dimensional biplot (Fig. 2A) represents the information contained in two of the principal components. The first two principal components explained 59.15 % ($PC1 = 35.45$ % and $PC2 = 23.70$ %, respectively) of the total variation of the experimental data. There can be noted three large, separate groups on the chart formed from the examined fruits and the chemical components analyzed in them. The first cluster from the left contains the fruits of almost all analyzed cultivars, except for *C. × superba* 'Jet Trail', 'Color Trail' and 'Nicoline', *C. japonica* 'Red Joy' and *C. speciosa* 'Rubra'. Interestingly, only chlorophyllide *b* was found in this group of all the compounds analyzed. The second cluster (visible in the middle of the biplot) was formed from amino acids, tocopherols and tocotrienols identified in *Chaenomeles* fruits. The Pearson correlation coefficient between amino acids – tocopherols and tocotrienols – was $r^2 = 0.529$ and 0.460 , respectively. In addition, it contained *C. × superba* 'Jet Trail' and 'Color Trail' fruits, so it can be said that these cultivars were particularly rich in the abovementioned compounds. The last group was represented by carotenoids (except β -cryptoxanthin) and chlorophylls as well as *C. × superba* 'Nicoline' fruits, which were characterized (in comparison with the others) by a particularly high concentration of these compounds. The fruits of *C. japonica* 'Red Joy' and *C. speciosa* 'Rubra' were not found in any of the 3 discussed groups, which indicates that they were different from the others in their chemical composition. In addition, the calculated Pearson correlation coefficient showed a higher correlation of carotenoids with tocotrienols than with tocopherols ($r^2 = 0.380$ and 0.100 , respectively). The same relationship also occurred in the case of chlorophylls, where for tocotrienols $r^2 = 0.300$ and for tocopherols $r^2 = 0.100$.

Fig. 2B presents the AHC dendrogram obtained from fruit cultivars based on Euclidean distance dissimilarity (within the interval 0–160) using Ward's agglomeration method. The dotted line on the chart represents the automatic truncation, leading to formation of six homogeneous groups. The two last groups (displayed in purple and green color, respectively) are approximately the same size. Despite this, they are made of cultivars belonging to different species, which show a large diversity in relation to the analyzed compounds. The third has only two states (*C. speciosa* 'Red Joy' and *C. × superba* 'Crimson and Gold'; yellow) and is less homogeneous than the previous two. Moreover, 'Nicoline' is a single state on the dendrogram (dissimilarity over 110), which reflected the obtained results of the content of carotenoids and chlorophylls (their concentration in fruit of this cultivar is 4-fold higher than their average content for the *C. × superba* species). The conclusions resulting from the analyzed chart are consistent with the results of Turkiewicz et al. (2020), where the authors for the same cultivars analyzed the basic chemical composition, phenolic content and *in vitro* biological activities. Selected *Chaenomeles* fruits are characterized by great diversity within species.

4. Conclusions

This study undertook the first such comprehensive analysis of carotenoids, chlorophylls, tocopherols, tocotrienols and the amino acid profile in the fruit of 19 cultivars of *C. japonica*, *C. speciosa* and *C. × superba*. The study confirmed the large qualitative and quantitative diversity of the analyzed compounds. Five compounds from the carotenoid group and eight chlorophyll derivatives have been identified. Fruits of *C. × superba* 'Nicoline' had the highest concentration of carotenoids and chlorophylls, 314.94 and 227.19 mg/kg dw, with a predominant content of β -carotene and pheophytin *a*, respectively. Thus, the fruit of this cultivar contained 420.15 μ g RAE in 100 g of fresh fruit, covering 9% of the daily requirement of vitamin A in men and 11 % in women. *Chaenomeles* fruits, regardless of the cultivar, contained all isomers (α , β , γ , δ) of tocopherols and tocotrienols, with the most active form – α -tocopherol – being determined in the largest amount (representing on average half of the sum of T and T3). Thus, fruit of *C. × superba* 'Nicoline' containing the highest amount of this isomer is the richest source of vitamin E (covering 6 and 8% of the RDI for men and women, respectively). Of the 10 amino acids identified, three of them belong to the group of essential ones. The highest content of the sum of amino acids was found in the fruit of *C. × superba* 'Jet Trail' (2326.33 mg/100 g dw) with L-asparagine constituting over 50 % of the total amino acids. In addition, the calculated Pearson correlation coefficient proved a strong positive correlation between the content of amino acids and the concentrations of tocopherols and tocotrienols ($r^2 = 0.529$ and 0.460 , respectively).

To conclude, this study confirmed that *Chaenomeles* fruits may be part of a balanced diet, providing vitamins A and E, and essential amino acids necessary for the proper functioning of the human body.

CRedit authorship contribution statement

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

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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mgr inż. Igor Piotr Turkiewicz

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Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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Mój udział w przygotowaniu tej publikacji polegał na kierowaniu projektem naukowym obejmującym badania opisane w tej pracy (Diamentowy Grant VII, nr DI2017 006347), współtworzeniu koncepcji prowadzonych badań, wykonaniu analiz fizykochemicznych, chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca. Przygotowałem tekst publikacji, opracowałem merytorycznie otrzymane wyniki, przeprowadziłem dyskusję oraz współredagowałem odpowiedzi na recenzje.

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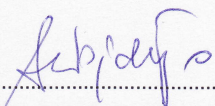
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ul. Chełmońskiego 37
51-630 Wrocław

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mój udział polegał na tworzeniu i nadzorze koncepcji projektu (Diamentowy Grant VII, nr DI2017 006347), w ramach którego realizowana była praca doktorska, uczestnictwie w analizach chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca i ich produktów, koordynowaniu prac Doktoranta, współredagowaniu publikacji i merytorycznej ocenie wyników.


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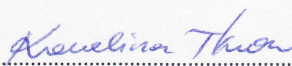
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mój udział polegał na współredagowaniu publikacji oraz uczestniczeniu w opracowaniu metodyki dotyczącej analizy karotenoidów i chlorofili.



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Podpis składającego oświadczenie

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51-630 Wrocław

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mój udział polegał na analizie formalnej i statystycznej wyników.


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Podpis składającego oświadczenie

Publikacja 3

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*, *114*, 108416.



Influence of different drying methods on the quality of Japanese quince fruit

Igor Piotr Turkiewicz^a, Aneta Wojdyło^{a,*}, Krzysztof Lech^b, Karolina Tkacz^a, Paulina Nowicka^a

^a Department of Fruit, Vegetable and Plant Nutraceutical Technology, 37 Chełmońskiego Street, Wrocław University of Environmental and Life Sciences, 51-630, Wrocław, Poland

^b Institute of Agricultural Engineering, 37/41 Chełmońskiego Street, Wrocław University of Environmental and Life Sciences, 51-630, Wrocław, Poland

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ABSTRACT

The aim of this study was to determine the effect of different drying methods – convective drying (at 50, 60, 70 °C), vacuum-microwave drying (at 120, 240, 480 W and 480 W with reduction to 120 W), a combination (of convective pre-drying (at 50, 60, 70 °C) and by vacuum-microwave finish drying (at 120 W)) and freeze-drying – on the quality factors of Japanese quince fruit, including phenolic compounds, antioxidant activity, and color. Drying kinetics were determined. The highest content of bioactive compounds was in samples after freeze-drying (total phenolic content – 57 g/kg dw; polymeric proanthocyanidins – 41 g/kg dw). The antioxidant activity measured by oxygen radical absorbance capacity (ORAC) assay was the highest for samples after vacuum-microwave drying at 480 W (0.8 mol Trolox/kg dw). Unfavorable changes in color, formation of hydroxymethylfurfural (HMF) and degradation of L-ascorbic acid as well as phenolic compounds were noted along with the increasing drying temperature and increasing magnetron power. The method that will ensure the proper appearance of dried fruits, storage stability and at the same time guarantee the retention of bioactive compounds seems to be freeze-drying.

1. Introduction

Chaenomeles belongs to the Rosaceae family. Currently four species belong to the *Chaenomeles* genus, and in Poland the most common is *C. japonica* (Thunb.) Lindl. (Japanese quince), originating in Japan. They are widely cultivated in Japan, Korea, China and the countries of the Baltic Sea basin (Antoniewska, Rutkowska, & Adamska, 2017).

In Poland, these shrubs are widely known for their decorative qualities, while their use in the food industry is negligible. Although information on the chemical composition and health properties of Japanese quince fruit (JQF) is limited, they nevertheless point to their exceptional nutritional value (Antoniewska et al., 2017). The content of L-ascorbic acid ranges from 0.4 to 1.1 g/kg of fruits and is comparable to the content in citrus fruits (Seglina, Krasnova, Heidemane, & Ruisa, 2009). Japanese quince fruit is also rich in pectin, dietary fiber and the minerals Fe, Mg, P, Zn, Mo and Cu (Nahorska, Dzwoniarska, & Thiem, 2014). Particularly valuable ingredients found in JQF are polyphenols, with more than 95% of their total content (6 g/kg fresh weight) being proanthocyanidins, which are condensed flavan-3-ols (Du et al., 2013). Detailed analysis revealed the presence of catechin, epicatechin, procyanidin B1, B2, oligomers and polymers of flavan-3-ols. Among phenolic acids, chlorogenic acid was detected in significant quantities, and quercetin derivatives among flavonols (Du et al., 2013; Nahorska et al.,

2014).

Japanese quince fruit is not consumed in the form of raw fruits, due to its sour taste (acidity between 35 and 45 g/kg). It is mainly processed into juices, which may be a potential acidifier in the food industry. Moreover, it can enrich the final products with Vitamin C, whose retention is quite large in comparison to raw fruits (Seglina et al., 2009). Due to the high content of pectin, storage stability and sensory profile, JQF may be processed into jams, marmalades, purees and candied fruit. They can also be added to ice cream, yogurt or jelly. Fruits can form the basis for teas or filling for confectionery products (Antoniewska et al., 2017). Apart from the traditional food uses of JQF, some novel food applications have also been proposed. For example, dried snacks produced from JQF offer a new perspective for the food industry and consumers focused on high bioactive snacks. However, as yet, there is no literature on the influence of drying conditions on physicochemical and phytochemical characteristics of JQF. Considering consumer preferences and characteristics of the raw material, the method of drying should be selected to ensure retention of the maximum quantity of bioactive compounds in the product and only slightly changed appearance (including color, taste and aroma) compared to fresh fruit.

Therefore, the aim of this study was to investigate the influence of different methods of drying (convection, vacuum-microwave, convection-vacuum-microwave and freeze drying) and their parameters on the

* Corresponding author.

E-mail address: aneta.wojdylo@upwr.edu.pl (A. Wojdyło).

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physicochemical parameters (water activity, color, HMF content), content of bioactive compounds (ι -ascorbic acid, polyphenolic compounds) and antioxidant activity (ORAC, ABTS, FRAP). The use of various drying methods was aimed at choosing the parameters which ensure the best preservation of these characteristics.

2. Material and methods

2.1. Chemicals

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). All standards of polyphenolic compounds were purchased from Extrasynthese (Lyon, France). Acetonitrile for ultra-performance liquid chromatography (UPLC; gradient grade) and ascorbic acid were from Merck (Darmstadt, Germany).

2.2. Plant material

Japanese quince fruits (*Chaenomeles japonica*; 3 kg) were obtained from cultivation in Lublin Province (Poland) at processing maturity in September 2018 and were immediately subjected to further processing at the University. Fresh fruits were characterized by water activity ($a_w = 0.987$) and values of color determinants equal to $L^* = 78$, $a^* = -1$ and $b^* = 25$. The content of ι -ascorbic acid in fresh fruits was 8.0 g/kg dry weight (dw).

2.3. Drying experiments

Just before drying, JQF were cut in slices of approximately 3 mm wide and pitted. The moisture content of fresh fruits was $15.35 \pm 0.3 \text{ kg kg}^{-1}$ dw.

Japanese quince fruits were dried with 4 methods: (i) convective drying - CD (convective drier designed and made at the Agricultural Engineering Institute of Wrocław University of Environmental and Life Sciences), (ii) vacuum-microwave drying - VMD (VM-200; Plazmatronika S.A., Wrocław, Poland), (iii) combined method with a pre-treatment by convective drying and finished by vacuum-microwave drying - CVMD and (iv) freeze drying - FD (24 h; Alpha 1-4 LSC; Martin Christ GmbH, Osterode am Harz, Germany). These 4 drying technologies led to the final application of 11 drying treatments: CD (50, 60 and 70 °C), VMD (120, 240, 480 W and 480/120 W), combined methods (CVMD), and FD as control.

The process of dehydration using all the above-mentioned methods was continued until moisture content in the dried samples was 0.05 kg kg^{-1} dw. In the case of FD, the samples were kept in the drying chamber for 24 h (Alpha 1-4 LSC; Martin Christ GmbH, Osterode am Harz, Germany). Hot air temperatures during convective drying were 50, 60 and 70 °C (± 1 °C); air velocity was $0.5 \pm 0.1 \text{ ms}^{-1}$. During the vacuum-microwave drying, the initial microwave power was set to 120, 240, 480 W and 480 W reduced to 120 W. The pressure in the vacuum-microwave drying chamber varied between 4 and 6 kPa.

In convective-vacuum-microwave drying, the fresh material was pre-dried first at a temperature of 50, 60 and 70 °C for 3 h to achieve the same dry weight before further final drying and finished by vacuum-microwave drying with 120W.

The temperatures of the vacuum-microwave treated JQF samples were measured just after taking the samples out of the dryer using an infrared camera Flir i50 (Flir Systems Inc., Stockholm, Sweden).

2.4. Modeling of drying kinetics

Based on the weight losses of JQF samples, drying kinetics were determined. The moisture ratio (MR) was calculated as described previously by Lech, Figiel, Michalska, Wojdyło, and Nowicka (2018a,b).

2.5. Determination of water activity (a_w), moisture and color measurement

The water activity determination was performed on the Novasina (LabMas-terav., Lachen, Switzerland) at 20 °C. The moisture content of the dried samples, as well as fresh fruits, was determined by drying ground samples in a vacuum dryer (SPT-200; ZEAMiL Horyzont, Krakow, Poland) for 24 h at 70 °C until reaching a constant weight. The color of dried JQF powders was determined using an A5 Chroma-Meter (Minolta CR300; Osaka, Japan), referring to color space CIE $L^*a^*b^*$. Data were mean of three measurements.

2.6. Determination of L-ascorbic acid

ι -ascorbic acid was analyzed according to the HPLC method described previously by Wojdyło, Oszmiański, and Bielicki (2013). Fresh fruits (2–3 g) or dried fruits (~1 g) were mixed with 50 mL of 0.1 mol/L phosphoric acid and centrifuged at $20,000 \times g$ for 10 min. The estimation of ι -ascorbic acid was carried out using a Waters liquid chromatograph with a tunable absorbance detector (Waters 486), and a quaternary pump with Waters 600 Controller apparatus (Waters Associates, Milford, USA). A sample of 20 μL was injected into a Chromolith Performance RP-18e column ($100 \times 4.6 \text{ mm}$) (Merck, Darmstadt, Germany). The elution was carried out using 0.1 mol/L phosphoric acid at the flow rate of 1.0 mL/min. The absorbance was monitored at 254 nm. ι -ascorbic acid was identified by comparison with the standard. The calibration curve was prepared by plotting different concentrations of the standard versus the area measurements in HPLC. All determinations were done in triplicate and were expressed as g/kg dw.

2.6.1. Quantification of polyphenols (flavanols, phenolic acids, flavan-3-ols), polymeric procyanidins and hydroxymethylfurfural

The extract of dried JQF for polyphenols analysis was prepared as described previously by Wojdyło, Carbonell-Barrachina, Legua, and Hernández (2016). Dried fruits (~1 g) were mixed for 1 min with 5 mL methanol/water/acetic acid/ascorbic acid (300:680:10:10, v/v/v/m) and sonicated for 20 min (Sonic 6D; Polsonic, Warsaw, Poland). The extraction was repeated twice, all supernatants were pooled after centrifugation at $19,000 \times g$ for 10 min at 4 °C. Finally, the extract was filtered by 0.20 μm hydrophilic PTFE membrane (Millex Simplicity Filter; Merck, Germany) and used for analysis.

Quantitative (UPLC-PDA) analysis of polyphenols (flavan-3-ols, flavanols, phenolic acids) and HMF (at 284 nm) were performed as described previously by Wojdyło et al. (2013) and Gökmen and Şenyuva (2006), respectively. Separations of individual analyzed compounds were carried out using a ACQUITY UPLC BEH C18 column ($1.7 \mu\text{m}$, $2.1 \times 100 \text{ mm}$; Waters Corporation, Milford, USA) at 30 °C. The samples (5 μL) were injected and the program began with gradient elution at flow rate of 0.42 mL/min with 99–65% solvent A (0–12 min), and then lowering solvent A to 0% for condition column (12.5–13.5 min), the gradient returned to the initial composition (99% A) until 15 min for held constant to re-equilibrate the column. The mobile phase consisted of solvent A (20 mL/L formic acid in water) and solvent B (acetonitrile). The runs were monitored at the following wavelengths: flavan-3-ols at 280 nm, phenolic acids at 320 nm, and flavonol glycosides at 360 nm. Retention times (R_t) and spectra were compared with those of pure standards. Calibration curves at concentrations ranging from 0.05 to 5 mg/mL ($R^2 = 0.9998$) were made from (–)-epicatechin, (+)-catechin, procyanidin B1, B2 and C1, chlorogenic acid, cryptochlorogenic acid, 3,5-di-caffeoylquinic acid, quercetin and kaempferol -3-O-glucoside and -3-O-rutinoside. Phenolic acids were expressed as chlorogenic and cryptochlorogenic acids, quercetin and kaempferol derivatives were expressed as quercetin- and kaempferol-3-O-glucoside, respectively.

Analysis of polymeric procyanidins was performed by phloroglucinolysis as described previously by Wojdyło et al. (2013). The analysis was carried out on a ACQUITY UPLC system (Waters Corp.,

Milford, MA, USA) consisting of a binary solvent manager, and fluorescence detector (FL). The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (-)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (-)-epicatechin-phloroglucinol adduct standards. All measurements were repeated three times and were expressed as g/kg dw.

2.7. Determination of antioxidant activity

The solvent for analysis of antioxidant capacity was prepared as described previously by Wojdyło, Carbonell-Barrachina, et al. (2016) and Wojdyło, Figiel, et al. (2016). Briefly, the dried fruits (~1 g) were extracted with 10 mL of 800:190:10 (v/v/v) methanol:water:acetic acid; the extraction protocol was the same as described above.

Free radical scavenging capacities were determined using the ABTS (radical cation decolorization assay) method described by Re et al. (1999), and FRAP (ferric reducing antioxidant power) method described by Benzie and Strain (1996). Spectrophotometric measurements were performed using a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). The ORAC assay was determined on Shimadzu RF-5301 PC spectrofluorometer (Shimadzu, Kyoto, Japan) follow the method previously described by Ou, Hampsch-Woodill, and Prior (2001). All antioxidant capacity analyses were run in triplicate, and the results were expressed as mmol Trolox/kg dw.

2.8. Statistical analysis

Statistical analysis was conducted using Statistica version 13.3 (StatSoft, Kraków, Poland). Relative importance rankings on the attributes and significant differences were evaluated by non-parametric tests – Kruskal-Wallis test and Dunn's procedure. All analyses were done in triplicate.

3. Results and discussion

3.1. Physical properties

3.1.1. Drying kinetics

Table 1 presents the parameters used to describe the drying kinetics model:

Table 1
Parameters of model describing the drying kinetics, drying time and final moisture content of Japanese quince fruit as affected by the drying method.

Drying method	Drying conditions	Parameters model*			Statistics		Drying time (min)	MC _{wb} (g/kg)
		A	k	n	RMSE	R ²		
CD	50 °C	1	0.0029	1.34	0.0126	0.9988	480 ± 10	33.6 ± 0.3a
	60 °C	1	0.0033	1.36	0.0122	0.9989	420 ± 10	27.3 ± 0.1bc
	70 °C	1	0.0028	1.46	0.0127	0.9988	360 ± 10	21.9 ± 0.3f
VMD	120W	1	0.0017	1.83	0.0088	0.9994	96 ± 4	25.3 ± 1.0e
	240W	1	0.0080	1.76	0.0130	0.9986	48 ± 3	26.8 ± 1.1bc
	480W	1	0.0550	1.43	0.0290	0.9962	28 ± 2	27.6 ± 0.2b
	480W/120W	1	0.0610	1.38	0.0204	0.9961	62 ± 4	26.6 ± 0.3cd
CVMD	50 °C/120W	0.0300	0.3650	0.45	0.0004	0.9969	180/40 ± 4	21.4 ± 0.2fg
	60 °C/120W	0.0155	0.1110	0.68	0.0007	0.9412	180/32 ± 4	20.7 ± 0.1g
	70 °C/120W	0.0095	0.0252	0.95	0.0003	0.9764	180/24 ± 4	25.8 ± 0.1de

CD-convective drying; VMD-vacuum-microwave drying; CVMD-convective-vacuum-microwave drying; *Modified Page model; A, k and n are constants of the modified Page model; RMSE-mean square errors; R²-determination coefficient; MC_{wb}-moisture content wet basis; ± standard deviation; in each column different letters mean significant differences between samples.

$$MR = A \cdot e^{-k \cdot t^n} \quad (1)$$

While Fig. 1 illustrates changes in MR of JQF dehydrated by convective drying at temperatures of 50, 60 and 70 °C (A), vacuum-microwave drying at microwave power 120, 240, 480 W and reduced 480/120 W (B), and convective-vacuum-microwave drying at temperatures 50, 60 and 70 °C and microwave power 120 W (C).

According to Szychowski et al. (2018) the modified Page model is the best model taking into account the highest value of the determination coefficient (R²) and the lowest value of the root-mean-square errors (RMSE) as well as easy comparison. In this study, in the modified Page model for all the drying methods used, the R² ranged from 0.9412 to 0.9994 and the RMSE from 0.0003 to 0.0290.

The convective drying time of JQF for the same relative water content ranged from 360 to 480 min. The longest time was measured when using the lowest temperature. Raising the temperature of the drying air by 20° resulted in the reduction of the drying time by 25%, which is consistent with Szychowski et al. (2018). The drying time for other fruits was significantly higher than that required to dry JQF to a similar final moisture content; for example in jujubes increasing the temperature of air from 50 to 70 °C decreased the drying time by over 60% (Wojdyło et al., 2016). In the case of vacuum-microwave drying, the drying time ranged from 28 min for 480 W to 96 min at 120 W. The increase of power from 120 to 480 W resulted in shortening the drying time by almost 70%. Increasing the microwave power from 120 to 240 W resulted in a two-fold reduction in the drying time. Szychowski et al. (2018), Wojdyło, Carbonell-Barrachina, et al. (2016), Wojdyło, Figiel, et al. (2016); and Wojdyło, Lech et al. (2019)) in their studies obtained the same relationships for vacuum-microwave drying.

Fig. 1B shows the changes of temperature of the material during the vacuum-microwave drying process. In the first 2–8 min of drying, depending on the applied magnetron power, a rapid increase in the temperature of the material was observed. At the lowest power of 120 W, the material after 72 min warmed up to a maximum temperature of 84 °C. Doubling power caused the material temperature to increase to 98 °C in almost half the time. When 480 W was used, the same maximum temperature of material was reached after 26 min. On the other hand, in convective-vacuum-microwave drying at different convective drying temperatures, the maximum temperatures reached were statistically equivalent, and reached a mean of 75 °C. The final stage of the drying process is characterized by temperature stabilization, which is related to the depletion of free water in the material.

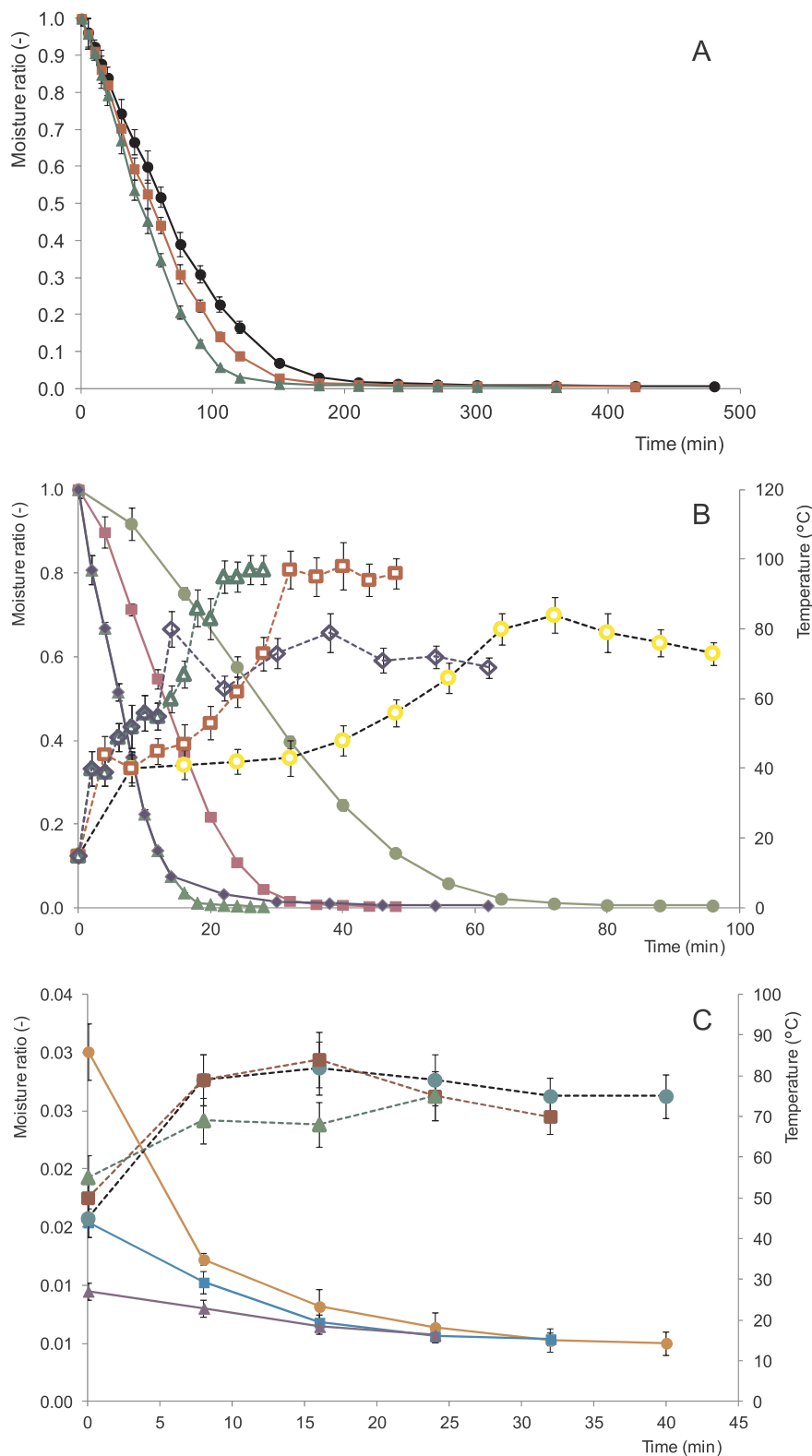


Fig. 1. Drying kinetics of Japanese quince fruit samples treated by different drying techniques. (A) convective drying at temperature 50 °C, 60 °C and 70 °C, (B) vacuum-microwave drying at microwave power 120 W (and MR) and 240 W (and MR) and 480 W (and MR) and reduced 480–120 W (and MR) and (C) and combined convective-vacuum-microwave drying at temperature 50 °C (and MR) and 60 °C (and MR) and 70 °C and microwave power 120 W (and MR). MR- moisture ratio.

3.1.2. Water activity

The water activities (a_w) are useful to control the quality of dried products. The lower the a_w , the greater the assurance that any microbial growth and other chemical reactions in food products will be inhibited (Ng et al., 2018; Samoticha, Wojdyło, & Lech, 2016). The obtained values are at an acceptable, low range guaranteeing their microbiological stability because bacteria, yeast, and molds cannot grow in such a low a_w . The results of a_w are the lowest for freeze-drying and the

highest for convective drying at 50 °C (Table 2). In the convective process, a temperature increase of 20° caused a decrease in a_w by over 35%. However, in convective-vacuum-microwave drying, the influence of temperature is not as significant and can be observed in the values of water activity. After vacuum-microwave drying at 120 W the a_w was 0.140 and a fourfold increase in power caused a 30% decrease in a_w , the variant with power reduction from 480 to 120 W, water activity was similar to the value obtained in CD at 70 °C. Samoticha et al. (2016)

Table 2

Effects of drying method on water activity, color parameters and hydroxymethylfurfural content (mg/kg dw) in Japanese quince fruit.

Drying method	Drying conditions	Water activity	Color			HMF
			L*	a*	b*	
CD	50 °C	0.180	70.30 ± 0.20b	4.06 ± 0.17g	31.21 ± 0.01b	1.29 ± 0.10c
	60 °C	0.158	69.48 ± 0.04cd	4.17 ± 0.03g	30.50 ± 0.05d	1.18 ± 0.09e
	70 °C	0.116	67.20 ± 0.27g	5.69 ± 0.08c	29.27 ± 0.08h	1.24 ± 0.04d
CVMD	50 °C/120W	0.134	68.28 ± 0.09e	5.00 ± 0.03e	29.77 ± 0.05f	1.26 ± 0.03cd
	60 °C/120W	0.150	67.65 ± 0.24fg	5.15 ± 0.07d	30.10 ± 0.06e	1.29 ± 0.05c
	70 °C/120W	0.155	69.08 ± 0.02cd	4.54 ± 0.03f	30.73 ± 0.08c	1.40 ± 0.03b
VMD	120W	0.148	68.01 ± 0.22ef	4.99 ± 0.05e	31.78 ± 0.14a	1.39 ± 0.04b
	240W	0.109	65.73 ± 0.40h	6.31 ± 0.09b	29.51 ± 0.15g	1.42 ± 0.00b
	480W	0.105	63.30 ± 0.32i	7.76 ± 0.08a	28.72 ± 0.17i	1.47 ± 0.10a
FD	480W/120W	0.117	69.61 ± 0.05cd	4.61 ± 0.05f	31.11 ± 0.02b	1.19 ± 0.30e
	–	0.089	74.42 ± 0.59a	–0.67 ± 0.05h	26.74 ± 0.19j	1.16 ± 0.20e

CD-convective drying; VMD-vacuum-microwave drying; CVMD-convective-vacuum-microwave drying; HMF-hydroxymethylfurfural; ± standard deviation; in each column different letters mean significant differences between samples.

investigating the effect of the drying method on the quality and properties of chokeberry also stated that the lowest values of a_w are obtained after FD.

3.1.3. Color parameters

The color and appearance of the product are key parameters in assessing quality, so on their basis we can determine the state of food spoilage. These changes are caused by the degradation of vitamin, colorants and polyphenolic compounds as well as enzyme activity and the chemical reactions occurring between the product compounds (Mac Dougall, 2010, pp. 312–342). Analyzing the changes in the L* parameter defining the brightness of the color, darkening was noted in all variants. The lowest value was obtained for the vacuum-microwave drying at 480 W and the most similar value compared to the fresh material was obtained in FD. Seglina et al. (2009) noted that candied JQF obtained by vacuum-microwave drying has a brighter color than the dried fruits obtained by convective drying. Moreover, Wojdyło, Carbonell-Barrachina, et al. (2016), Wojdyło, Lech, et al. (2019) and Wojdyło, Figiel, et al. (2016) report that dried jujube fruits obtained by freeze-drying had the lightest color, but the method is very expensive and not often applied at industry level. However, vacuum-microwave drying (480/120 W and 120 W) gives similar results and the obtained products have near-natural color. The value of the green-red coordinate a* changed in the same way in all drying methods and parameters – increasing towards the red color. Its highest, six-fold increase was recorded in vacuum-microwave drying at 480 W. As in the case of the parameter L*, the freeze-drying provided the smallest changes of coordinate a*. In contrast, Samoticha et al. (2016) found that different methods of chokeberry drying had various effects on the value of the a* parameter. Convective drying and vacuum-microwave drying caused a decrease in the proportion of green color. The value of the blue-yellow coordinate b* increased in all obtained dried Japanese quince fruits, as for the previous parameter. The increase in temperature in convective drying and power in vacuum-microwave drying resulted in a decrease in coordinate b*. Freeze-drying caused the smallest color changes.

3.2. Chemical properties

3.2.1. L-ascorbic acid

Japanese quince fruits belongs to the group of fruits rich in L-ascorbic acid. However, Vitamin C has limited stability due to its sensitivity to such factors as heating, especially in a water, neutral and alkaline environment, presence of oxygen, copper, iron and silver ions (Bieniasz, Dziedzic, & Kaczmarczyk, 2017). Therefore, due to the health-promoting effect of L-ascorbic acid, it is important to choose parameters of the drying process that provide its maximum retention in the final product. The content of L-ascorbic acid in JQF depending on

the drying method is shown in Table 3. The highest concentration of L-ascorbic acid was in vacuum-microwave drying fruits at 120 W and the lowest convective-vacuum-microwave drying at 50 °C. The content of L-ascorbic acid in fruit dried by vacuum-microwave method was higher than in those after freeze-drying. Degradation of ascorbic acid depends on several factors, which include besides oxygen and temperature also moisture content (Cui, Li, Song, & Song, 2008). Under the influence of microwaves, non-specific new compounds could also be formed, which caused an increase in the content of L-ascorbic acid. However, there are no reports in the literature on this topic, so it is advisable to continue research in this area. The increase of temperature in convective drying and power in vacuum-microwave drying contributed to a decrease in L-ascorbic acid content to a similar extent. Within the vacuum-microwave drying, the use of 480 W with reduction to 120 W resulted in the largest loss of ascorbic acid content. Consistent results were obtained by Zaki, Muhamad, and Salleh (2007), who studied the content of Vitamin C in the dried fruits of papaya depending on the drying temperature. They concluded that lower microwave power helped to maintain a higher content of L-ascorbic acid in the product. Freeze-drying contributed to the preservation of L-ascorbic acid at an equally high level (7306 mg/kg dw) as vacuum-microwave drying. In previous studies it was emphasized that vacuum microwave drying preserved the vitamin C content better than by convective drying (Kamiloglu et al., 2016; Lin, Durance, & Scaman, 1998; Wojdyło et al., 2016). Moreover, Asami, Hong, Barrett, and Mitchell (2003) investigated the effect of the drying method on the content of biologically active compounds in strawberries, and demonstrated that convective drying causes three times greater losses of Vitamin C than freeze-drying.

3.2.2. Hydroxymethylfurfural content

Low pH, the presence of sugars and amino acids, as well as a high temperature drying process, are factors contributing to the Maillard reaction and formation of HMF. Its presence is commonly used as an indicator of heat treatment of products (Moffhammer, Stintzing, & Carle, 2006). The content of HMF in fruits subjected to drying by different methods ranged from 1.16 to 1.47 mg/kg dw. The highest HMF value was recorded for vacuum-microwave drying at 480 W and 240 W, while convective drying at 60 and 70 °C and vacuum-microwave drying at 480/120 W and freeze-drying resulted in the lowest amount of this compound (< 1.24 mg/kg dw). The HMF content increased along with the magnetron power in vacuum-microwave drying, while in convective drying the drying air temperature was inversely proportional to the HMF concentration in the dried fruits. Therefore, it should be remembered that the temperature of the drying process is not the only factor affecting the concentration of HMF in the product. The duration of the process and the presence or absence of oxygen and magnetic waves are equally important. In addition, compounds such as

chlorogenic acid, which occurs in JQF in large quantities, have been shown to contribute to the formation of HMF (Michalska, Wojdyło, Lech, Lysiak, & Figiel, 2016). Although the presence of HMF was in the analyzed dried JQF, its concentration was very low compared to the mean range in other dried fruits, which was from 1 to 780 mg/kg dw (Murkovic & Pichler, 2006).

3.2.3. Polyphenolic compounds

Flavonols, phenolic acids, and flavan-3-ols were evaluated in JQF by UPLC-PDA, and polymeric proanthocyanidins were additionally determined by UPLC-FL (Table 3). The methods and parameters of drying had a significant influence on the content of polyphenolic compounds (total phenols). Of all investigated groups of compounds, polymeric proanthocyanidins formed a large majority, followed by monomeric flavan-3-ols, phenolic acids and flavonols.

The highest concentration of polymeric proanthocyanidins was in fruits after freeze-drying, which was adopted as a reference method. In turn, JQF dried by vacuum-microwave drying at 480 W was characterized by their lowest concentration. The greatest retention of polymeric proanthocyanidins, besides freeze-drying, was obtained by convective-vacuum-microwave drying at 70 °C. In vacuum-microwave drying, reduction of magnetron power from 480 to 120 W allowed obtain of fruit with higher polymeric proanthocyanidins content. Freeze

processing time. Among the convective-vacuum-microwave drying treatments, the use of a temperature of 70 °C contributed to obtaining a dried sample with the highest total phenols content and in vacuum-microwave drying at 480/120 W. Teleszko and Wojdyło (2015) analyzed the content of total phenols in the fruits of four Japanese quince cultivars and the total polyphenol content ranged from 54 to 92 g/kg dw. The content of individual groups decreased in the order polymeric proanthocyanidins > flavan-3-ols > flavonols > phenolic acids. Szychowski et al. (2018) investigated the effect of the drying method on the content of bioactive compounds and the biological activity of quince fruit. They reported that, apart from freeze-drying, convective drying and convective-vacuum-microwave drying provide the greatest retention of total phenols. In accordance with the conclusion of Miao et al. (2017), Samoticha et al. (2016) and Wojdyło, Carbonell-Barrachina, et al. (2016) and Wojdyło, Figiel, et al. (2016), not only drying method but also drying conditions, i.e. temperature, magnetron power and presence or absence of oxygen, can affect polyphenolic composition, including flavan-3-ols, flavonols and phenolic acids. Summarizing all drying methods, freeze-drying still ensures the highest content of total phenols, but it should be borne in mind that this is one of the most expensive methods, the use of which in the food industry is marginal precisely because of its costs.

Table 3

Effects of drying method on L-ascorbic acid (mg/kg dw) and phenolic compounds (g/kg dw) in Japanese quince fruit.

Drying method	Drying conditions	L-ascorbic acid	Phenolic compounds				
			PP	FL	PA	F	ΣTP
CD	50 °C	6723 ± 28d	32.22 ± 1.00d	8.75 ± 0.30g	1.59 ± 0.09c	0.15 ± 0.06d	42.72 ± 0.20h
	60 °C	6445 ± 40e	34.95 ± 1.05c	7.80 ± 0.03i	1.48 ± 0.10c	0.12 ± 0.06f	44.35 ± 0.30f
	70 °C	6007 ± 38g	34.47 ± 0.03c	8.82 ± 0.22g	1.60 ± 0.05c	0.11 ± 0.03f	45.00 ± 0.12d
CVMD	50 °C/120W	5738 ± 32h	34.91 ± 0.09c	7.97 ± 0.06h	1.50 ± 0.10c	0.10 ± 0.02g	44.48 ± 0.35e
	60 °C/120W	6501 ± 48e	29.79 ± 0.20f	9.39 ± 0.40e	1.52 ± 0.02c	0.14 ± 0.02de	40.84 ± 0.19i
	70 °C/120W	6236 ± 61f	35.82 ± 0.10b	13.47 ± 0.11b	1.59 ± 0.01c	0.16 ± 0.04c	51.04 ± 0.28b
VMD	120W	7669 ± 36a	29.10 ± 0.10f	9.89 ± 0.06d	0.90 ± 0.05d	0.16 ± 0.04b	40.05 ± 0.30j
	240W	7012 ± 21c	31.33 ± 0.33e	9.80 ± 1.00d	1.55 ± 0.05c	0.13 ± 0.10e	42.82 ± 0.23g
	480W	6948 ± 10c	27.35 ± 0.05g	8.86 ± 0.22f	1.71 ± 0.01b	0.14 ± 0.02de	38.06 ± 0.16k
	480W/120W	6778 ± 19d	34.52 ± 0.12c	10.38 ± 0.07c	2.14 ± 0.04a	0.15 ± 0.00cd	47.18 ± 0.28c
FD	–	7306 ± 54b	40.68 ± 0.08a	14.41 ± 0.09a	1.77 ± 0.07b	0.20 ± 0.02a	57.06 ± 0.20a

CD-convective drying; VMD-vacuum-microwave drying; CVMD-convective-vacuum-microwave drying; PP-polymeric proanthocyanidins; FL-mono-, di- and oligomeric flavan-3-ols; PA-phenolic acids; F-flavonols; ΣTP-total polyphenols; ± standard deviation; in each column different letters mean significant differences between samples.

drying also resulted in the least degradation of flavan-3-ols. By contrast, convective drying at 60 °C led to the lowest flavan-3-ols content. The negative effects of microwave power were evident, and significantly reduced the content of flavan-3-ols by about 30% compared to freeze-drying. Japanese quince fruit dried using convective-vacuum-microwave drying at 70 °C was characterized by the flavan-3-ols content closest to that of freeze-drying, as was the case with polymeric proanthocyanidins.

The content of phenolic acids, in comparison to the previous two groups of compounds, varied in a different way. Vacuum-microwave drying with power reduction contributed to obtaining the highest content of these compounds, while the use of power 120 W led to the highest degradation of phenolic acids. Within the convective drying and convective-vacuum-microwave drying, there were no statistically significant differences in phenolic acids content depending on the used temperature. Flavonols had the lowest initial concentrations. Japanese quince fruit subjected to freeze-drying was characterized by the highest content of flavonols (0.20 g/kg dw), and convective-vacuum-microwave drying at 50 °C led to the reduction of their content by 50%. Analyzing the total phenols, it was stated that in convective drying the drying time is a factor limiting the content of polyphenols, so it is preferable to raise the temperature of the drying air and reduce the

3.2.4. Antioxidant activity

The antioxidant activity cannot be evaluated using only one method, due to the complexity of the composition of plant raw materials and possible reactions between them (Valadez-Carmona, Cortez-García, Plazola-Jacinto, Necochea-Mondragón, & Ortiz-Moreno, 2016). In this study, the antioxidant activity of dried JQF was investigated by ABTS, FRAP and ORAC assays (Table 4). The highest values of antioxidant activity were observed for samples dried by vacuum-microwave drying, obtaining for the ABTS and FRAP methods (both for vacuum-microwave drying 480 W). Subsequently, the antioxidant activity values decreased in the drying of vacuum-microwave drying together with the decreasing magnetron power, and in convective drying along with the longer exposure to hot air, obtaining the smallest values for convective-vacuum-microwave drying.

Also in the ORAC assay JQF dried by vacuum-microwave drying obtained the highest value, while convective drying and convective-vacuum-microwave drying at 50 °C led to the lowest values. Correlation coefficients between antioxidant activity measured by ABTS, FRAP and ORAC methods and total phenols content were: $R^2 = 0.456$; 0.253 and 0.028 , respectively. The contribution of L-ascorbic acid and HMF content to antioxidant activity measured by ORAC assay was found to be more important than that for total phenols ($R^2 = 0.657$ and 0.417 ,

respectively). Vacuum-microwave drying contributed to the increase of antioxidant activity (ABTS, FRAP and ORAC) of the dried JQF (increased with the increase of the magnetron power), which is in accordance with Michalska et al. (2016).

and after applying vacuum-microwave drying it increased from 3 to 18% for the ORAC assay. Most likely under the influence of the high power of microwaves, new chemical compounds were created that increased the antioxidant capacity of dried fruit.

Table 4
Effects of drying method on antioxidant activity (mmol Trolox/kg dw) in Japanese quince fruit.

Drying method	Drying conditions	Antioxidant activity		
		ABTS	FRAP	ORAC
CD	50 °C	123.85 ± 5.8e	114.33 ± 0.9de	368.90 ± 5.0g
	60 °C	140.05 ± 12.6abc	138.38 ± 7.8abc	459.41 ± 11.2f
	70 °C	131.11 ± 1.0cde	116.08 ± 6.8de	378.48 ± 26.4g
CVMD	50 °C/120W	120.93 ± 3.4e	103.42 ± 9.8e	370.04 ± 7.3g
	60 °C/120W	124.08 ± 5.2e	112.08 ± 7.3de	328.43 ± 22.9h
	70 °C/120W	147.24 ± 3.8 ab	140.32 ± 10.3 ab	661.18 ± 15.7e
VMD	120W	127.07 ± 3.9de	121.48 ± 3.1cd	718.01 ± 12.6c
	240W	128.97 ± 4.1cde	124.57 ± 0.50bcd	704.84 ± 2.8cd
	480W	141.17 ± 7.8abc	147.40 ± 19.1a	805.31 ± 1.9a
	480W/120W	150.19 ± 6.2a	146.42 ± 8.1a	744.22 ± 16.8b
FD	–	144.82 ± 12.2 ab	140.78 ± 6.7 ab	681.89 ± 2.6de

CD-convective drying; VMD-vacuum-microwave drying; CVMD-convective-vacuum-microwave drying; ± standard deviation; in each column different letters mean significant differences between samples.

3.3. Principal components analysis (PCA)

For visualization of the analyzed variables (phenolic compounds, L-ascorbic acid and HMF content but also antioxidant activity) of the JQF after different drying methods, PCA was performed (Fig. 2). The first two PC explained 69.54% of the total variation of the experimental data. One group of elements showing the largest positive correlation between variables and factors was created on the chart. Three protocols measuring antioxidant activity (ABTS, FRAP and ORAC) were positively correlated with each other, and the greatest part in the formation of this activity have oligomeric flavan-3-ols and flavonols. As a result of the principal component analysis, it can be also stated that the content of HMF was mostly related to the content of L-ascorbic acid.

4. Conclusions

Drying resulted in a decrease in the content of total phenols by 10–33%, regardless of the method used. Antioxidant activity was not directly proportional to the content of identified bioactive compounds

Among applied techniques, the greatest qualitative changes occurred during convective drying especially and the smallest after freeze-drying. In the method using convection, at a temperature of 70 °C, dried fruits had a higher total phenolic content than those dried at 50 °C, due to the 2 h longer drying process at a lower temperature. Convective-vacuum-microwave drying allowed better preservation of the color of products and valuable bioactive compounds than convective drying and vacuum-microwave drying separately.

To conclude, freeze-drying ensures that the highest quality product is obtained. Nevertheless, combined method of convective pre-drying at 70 °C and vacuum-microwave drying at 120 W can be a competitive way of drying taking into account the economic aspect and reduction of the drying time (204 ± 4 min) almost seven-fold while ensuring similar quality of the freeze-drying product. Considering the low use of JQF in the food industry despite the high pro-health value, resulting mainly from the content of polyphenolic compounds and L-ascorbic acid, it seems reasonable to extend the range of products based on these fruits on the functional food market. Dried JQF can be an interesting, healthy alternative to traditional, often high-sugar or high-salted

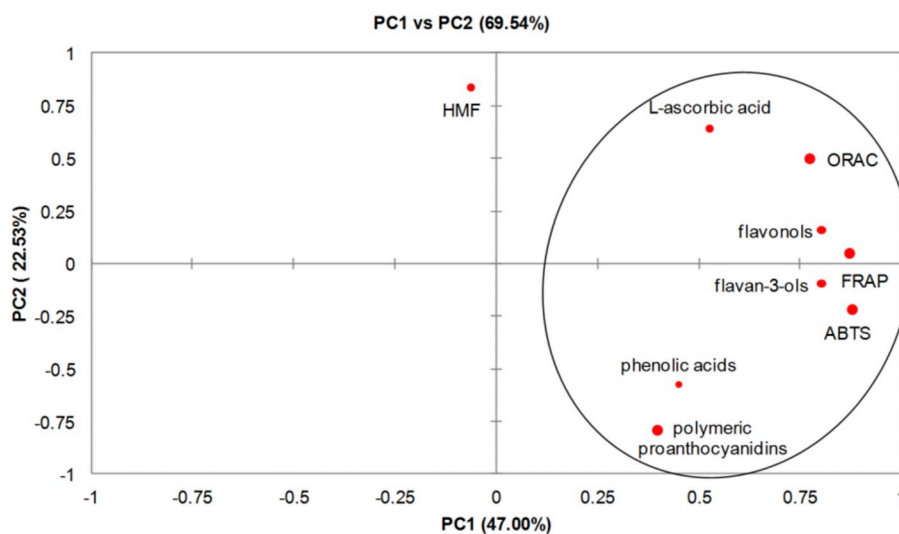


Fig. 2. PCA scores plot showing the relationship among chemical parameters and biological activity of Japanese quince fruit dried by different methods.

snacks. Low sugar content, high acidity and a rich polyphenolic profile perfectly fit into the functional food segment, especially for people who care about well-being and a healthy body.

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Conflicts of interest

The authors declare no conflict of interest.

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mgr inż. Igor Piotr Turkiewicz

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Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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Mój udział w przygotowaniu tej publikacji polegał na kierowaniu projektem naukowym obejmującym badania opisane w tej pracy (Diamentowy Grant VII, nr DI2017 006347), współtworzeniu koncepcji prowadzonych badań, wykonaniu analiz fizykochemicznych, chromatograficznych i potencjału biologicznego *in vitro* produktów z owoców pigwowca. Opracowałem technologię otrzymywania suszy pigwowcowych, przygotowałem tekst publikacji, opracowałem merytorycznie otrzymane wyniki, przeprowadziłem dyskusję oraz współredagowałem odpowiedzi na recenzje.

Turkiewicz Igor

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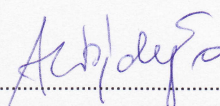
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51-630 Wrocław

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mój udział polegał na tworzeniu i nadzorze koncepcji projektu (Diamentowy Grant VII, nr DI2017 006347), w ramach którego realizowana była praca doktorska, uczestnictwie w analizach chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca i ich produktów, koordynowaniu prac Doktoranta, współredagowaniu publikacji i merytorycznej ocenie wyników.



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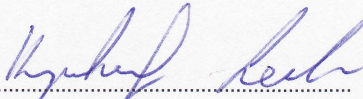
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Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37/41
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

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mój udział polegał na przygotowaniu wraz z mgr. inż. Igorem Turkiewiczem suszy pigwowcowych według wcześniej przyjętej koncepcji technologii oraz pomoc w opracowaniu wyników dotyczących kinetyki suszenia.



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Podpis składającego oświadczenie

mgr inż. Karolina Tkacz

Wrocław, 07.02.2022 r.

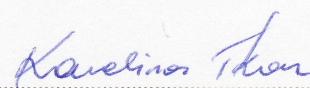
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Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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mój udział polegał na współredagowaniu publikacji oraz uczestniczeniu w analizie statystycznej wyników.



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dr hab. inż. Paulina Nowicka, prof. uczelni

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Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
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51-630 Wrocław

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mój udział polegał na analizie formalnej publikacji oraz wizualizacji wyników w postaci wykresów.

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Publikacja 4

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Osmotic Dehydration as a Pretreatment Modulating the Physicochemical and Biological Properties of the Japanese Quince Fruit Dried by the Convective and Vacuum-Microwave Method

Igor Piotr Turkiewicz¹ · Aneta Wojdyło¹ · Karolina Tkacz¹ · Krzysztof Lech² · Paulina Nowicka¹

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Abstract

The aim of this study was to determine the effect of osmotic dehydration (OD) using fruit concentrates (apple, pear, pineapple, sour cherry, blackcurrant, and chokeberry), before combined drying involving convective drying and vacuum-microwave finish drying, on the drying kinetics and physicochemical parameters (dry weight, water activity, content of L-ascorbic acid, sugars, organic acids, and phenolic compounds). Moreover, biological activities, including antioxidant, antidiabetic, and anticholinergic activities, of the dried Japanese quince fruit and osmotic fluids before and after osmotic dehydration have been assessed. The chokeberry concentrate reduced the final moisture ratio by half compared with the non-OD Japanese quince fruit, and the pineapple and sour cherry concentrates hindered the dehydration process during vacuum-microwave drying. OD significantly shortened the combined drying time compared with non-OD samples. The OD Japanese quince fruit was characterized by an increased content of sugars (up to 20 times more) and a significant reduction in the content of organic acids (even 77% reduction compared with non-OD fruit). Total phenolic content and antioxidant capacity of OD fruits decreased, but increased inhibition potential of α -amylase, acetylcholinesterase, and butyrylcholinesterase was observed. Osmotic fluids were also analyzed before and after the OD, and the following changes were found: reduction of sugars and increase of organic acid content, increase in phenolic content, antioxidant and antidiabetic potential, regardless of the concentrate used. To sum up, the osmotic dehydration process has the potential to modulate the chemical composition and biological properties of the Japanese quince fruit.

Keywords *Chaenomeles* · Osmotic dehydration · Combined drying · UPLC-PDA-FL · α -Amylase · α -Glucosidase · Antioxidant capacity

Introduction

In recent years, one of the most important subjects of interest in the food industry has been the production of high-quality food, having a sensory profile and chemical characteristics as close as possible to fresh raw material (Kowalska et al. 2016).

Therefore, a category of minimally processed food has been created, which is subjected to mild methods of fixation, which, in addition to extension of shelf-life, will guarantee high nutritional value and storage stability (Yadav and Singh 2014). This type of food includes, among others, intermediate moisture food (Ahmed et al. 2016). Another challenge faced by food technologists is to develop processing techniques for raw plant materials that enrich their composition with bioactive compounds (mainly vitamins and phenolic compounds) often lost during thermal treatment and/or compounds that are not found in raw material but are new, additional ingredients which increase their nutritional value (Ciużyńska et al. 2016). A potential way to achieve the above goals is by using osmotic dehydration (OD).

For the osmotic dehydration process, hypertonics with consumer-acceptable sensory characteristics are used, and

✉ Aneta Wojdyło
aneta.wojdylo@upwr.edu.pl

¹ Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chelmońskiego Street, 51-630 Wrocław, Poland

² Institute of Agricultural Engineering, Wrocław University of Environmental and Life Sciences, 41 Chelmońskiego Street, 51-630 Wrocław, Poland

they are most often sugar solutions (glucose, fructose, sucrose), sorbitol, sodium chloride, starch and corn syrups, maltodextrins with different dextrose contents, glycerol, or fructooligosaccharides (Ahmed et al. 2016; Piasecka et al. 2009). However, due to the adverse effects of sugars and salts on the human body, attempts have been made to use fruit juice concentrates, e.g., chokeberry (Jiménez et al. 2020; Lech et al. 2015), cherry (Nowicka et al. 2015b), or blackcurrant (Nowicka et al. 2015a), which, in addition to ensuring adequate osmotic pressure, can enrich the product with biological active compounds.

According to Chandra and Kumari (2015) and Yadav and Singh (2014), the main advantages of the osmotic dehydration process include the improvement of the quality of the final product in terms of color, smell, and texture. The OD process leads to weight reduction, so it saves energy in subsequent processes (e.g., drying) and reduces packaging and distribution costs. The raw material during the process is constantly immersed in the liquid and the anaerobic conditions protect against oxidative and enzymatic browning. The disadvantages include the increase in sweetness (excessive salinity when using NaCl) and the reduction of the perception of the sour taste, which can distort the natural characteristics of the product. Nonetheless, in the case of the Japanese quince fruit (JQF), lowering the acidity is undoubtedly an advantage and can increase the consumer acceptability of final products.

Osmotic dehydration can be an independent or pretreatment process improving functional and nutritional properties (Masztalerz et al. 2020; Yadav and Singh 2014). OD can precede processes such as freezing, deep-frying, pasteurization, air-drying, vacuum drying, microwave drying, or freeze-drying (Piasecka et al. 2009; Ahmed et al. 2016; Chandra and Kumari 2015). Combined methods are used to reduce drying time and, as a result, spare energy consumption and increase the quality of the dried product (Wojdyło et al. 2016). One such method involves combining convective drying with vacuum-microwave drying (VMD). The dried material is subjected to convective predrying to reduce humidity and mass and is finally dried by the vacuum-microwave method—convective-vacuum-microwave drying (CVMD). In current literature on osmotic dehydration, the authors used this process before drying by air, vacuum, microwave, or unconventional processing techniques such as ohmic heating or pulsed electromagnetic field (Czurzyńska et al. 2016).

It is worth mentioning that the authors in earlier studies (Turkiewicz et al. 2019a) attempted to dry the Japanese quince fruit to investigate the impact of various drying techniques and parameters on the final quality of dried fruit. In these studies, the combined method (CVMD) allowed to obtain a product of the highest quality comparable to the effects of freeze-drying (FD).

However, there is limited information on the use of OD as a pretreatment before the combined CVMD method. Moreover,

most publications on the application of OD process of fruit focus only on modeling kinetics and the impact of OD on physical parameters (Allali, Marchal, & Vorobiev 2010; Bchir et al. 2012a, b; Ferrari et al. 2013; Uribe et al. 2011), while there are no reports on the possible impact of OD on biological activity of both dehydrated material and osmotic fluids. So far, no comprehensive analysis of osmotic fluids for phenolic compounds, organic acids, and sugars using UPLC methods has been performed. It is worth noting that this is the first attempt to dehydrate the Japanese quince fruit, which seems to be a great raw material for this process, taking into account its chemical composition (low sugar content and high acidity).

In this study, the authors focused on qualitative and quantitative changes in the composition of dehydrated JQF and liquids before and after the OD process. The aim of this study was to investigate (i) basic physicochemical properties of the dehydrated and dried Japanese quince fruit (water activity, color, content of dry matter, L-ascorbic acid, sugars, and organic acids) and fluids (fruit concentrates) before and after the dehydration process (additionally soluble solid content (SSC) and viscosity); (ii) identification and quantification of phenolic compounds, including polymeric procyanidins, by UPLC-PDA-FL; and (iii) in vitro biological properties (antioxidant, antidiabetic, and anticholinergic activities).

Material and Methods

Chemicals

UPLC-grade water, prepared by using an HPLC SMART 1000 s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22- μ m membrane filter immediately before use. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), phloroglucinol, hydrochloric acid, acetic acid, formic acid, sulfuric acid, ascorbic acid, acetonitrile, methanol for UPLC (gradient grade), sodium acetate, and sodium hydroxide were purchased from Sigma-Aldrich (Steinheim, Germany). All standards for the quantification of phenolic compounds were from Extrasynthese (Genay, France).

Plant Material

Japanese quince fruits (*Chaenomeles japonica*; 5 kg) were obtained from cultivation in Lublin Province (Poland) at processing maturity in October 2019 and were immediately subjected to further processing. Fruits were washed and cut into the cylinders of 5.0 ± 0.1 mm in diameter and 3.3 ± 0.2 mm thickness. Concentrated fruit juices (from apple, pear, pineapple, sour cherry, blackcurrant, and chokeberry) were purchased in a retail store. The parameters of Japanese quince

fruits and concentrated fruit juices are presented in Tables 2 and 3, and Table 4, respectively.

Osmotic Dehydration

The commercial concentrated fruit juices were used as an osmotic solution. Each juice was adjusted to 40 °Bx by diluting the concentrate with 5-mL portions of water until the appropriate degrees Brix was measured by a Pocket PAL-3 refractometer. The osmotic dehydration process of Japanese quince fruits (JQF) was performed in water baths at 45 °C for 1.5 h. The ratio of fruit slices to osmotic solution was maintained at 1:3 (100 g:300 mL), and the mixture was manually agitated every 5 min. The mass transfer during osmotic dehydration (OD) was calculated according to the method previously described by Lech et al. (2017).

Drying Process

Directly after OD, JQF were dried using the combined method (CVMD) and were first predried using the convective method (CD) at a temperature of 70 °C for 180 min before further finally drying using the vacuum-microwave method (VMD) with a magnetron power of 120 W. In Figs. 1 and 2, convective and vacuum-microwave dryers are presented. The control was Japanese quince fruit not subjected to osmotic dehydration (non-OD JQF). For comparison, freeze-drying (FD) was also used for non-OD JQF. The equipment for drying was characterized previously by Lech et al. (2018b). Air velocity in CD was 0.5 ± 0.1 m/s and the pressure in the VMD varied between 4000 and 6000 Pa. Parameters of VMD are as follows: frequency: 2450 MHz, power density: 120 W/100 g fresh sample = 1.2 W per g of fresh sample. The samples in FD were kept in the drying chamber for 24 h (Alpha 1-4 LSC;

Martin Christ GmbH, Osterode am Harz, Germany). The temperature of the dried samples was measured using an infrared camera (FLIR i50, FLIR Systems Inc., Stockholm, Sweden). The sample weight was 100 g and the tests were carried out in two technological replications.

Based on the weight losses of Japanese quince fruit samples, drying kinetics were determined. The moisture ratio (MR) was calculated according to Eq. 1:

$$MR = \frac{M(t)}{M_0} \quad (1)$$

where $M(t)$ and M_0 denote moisture content achieved after the drying time t and initial moisture content, respectively. Preliminary tests conducted that the best fitting was obtained for the modified Page model (Eq. 2), where A , n , and k are constants and t is the drying time.

$$MR = A \cdot e^{(-k \cdot t^n)} \quad (2)$$

Physical Analysis (Water Activity (a_w), Dry Weight, Color, Soluble Solid Content (SSC), and Juice Viscosity)

The a_w determination was performed using LabMaster-aw (Novasina AG, Lachen, Switzerland) at 25 °C. The dry weight was determined by drying samples in a vacuum dryer (VACUCELL 111 ECO LINE, Medcenter Einrichtungen GmbH, Planegg, Germany) for 24 h at 70 °C and pressure of 100 kPa and expressed as percentage. The color of the samples was measured using a spectrophotometer (CM-700d, Konica Minolta Sensing, Inc., Osaka, Japan), referring to the color space CIE $L^*a^*b^*$. The concentration of fruit juices was measured using a Digital Refractometer Pocket PAL-3 (Atago Co. Ltd., Tokyo, Japan) and expressed as

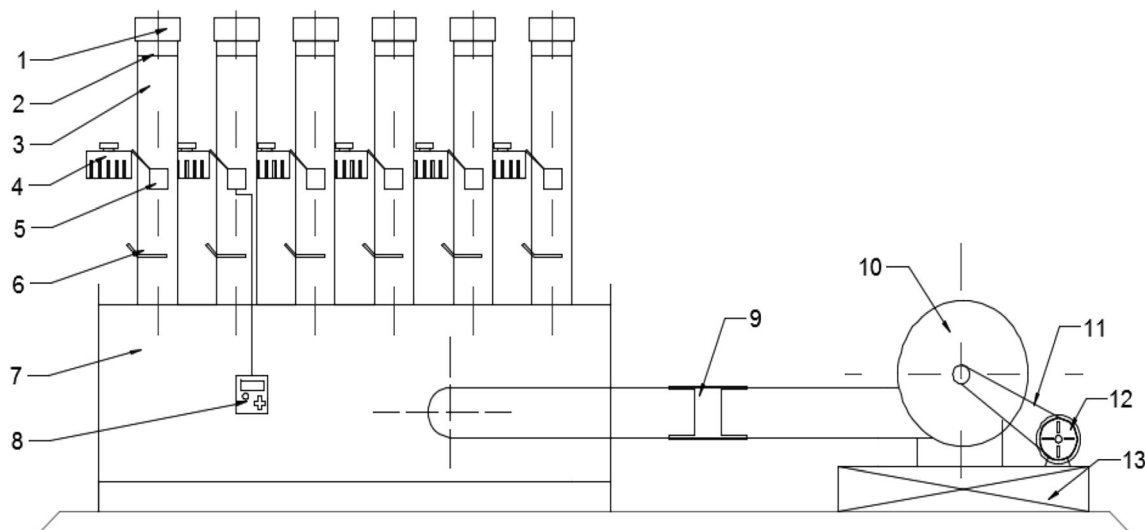


Fig. 1 Convective dryer. 1 basket for dried material, 2 support element, 3 air supply duct, 4 autotransformer, 5 heaters, 6 regulating slide, 7 expansion tank, 8 thermocouple, 9 flexible connector, 10 fan, 11 transmission belt, 12 motor, and 13 base

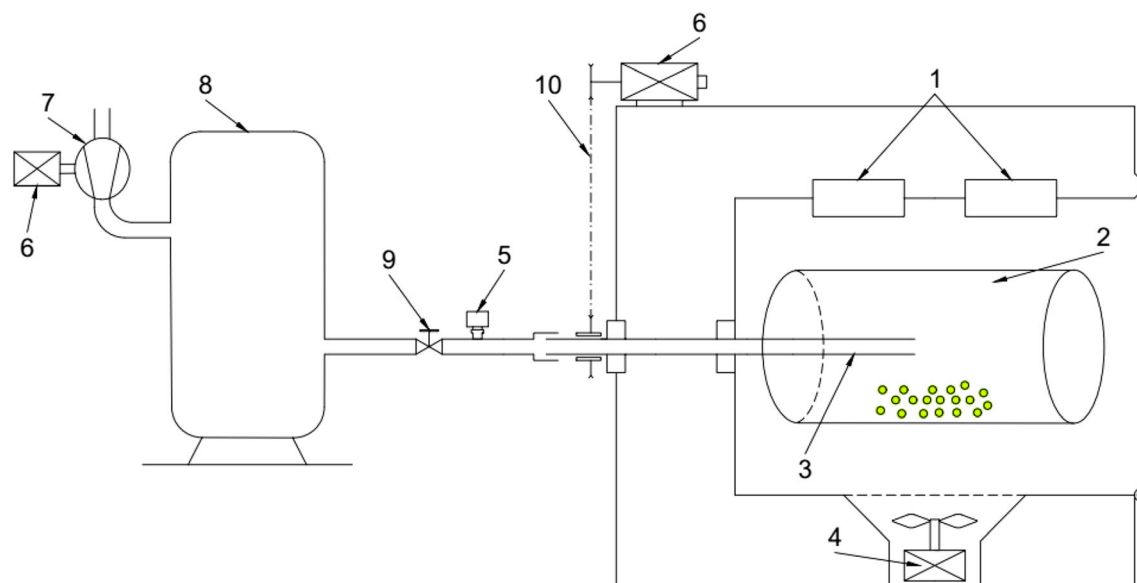


Fig. 2 Vacuum-microwave dryer. 1 magnetrons, 2 drying chamber, 3 wire, 4 fan, 5 pressure regulator, 6 motor, 7 vacuum pump, 8 expansion tank, 9 closing valve, and 10 transmission chain

degrees Brix. Viscosity was measured using a Vibro Viscometer SV-10 (A&D Company, Limited, Tokyo, Japan) for osmotic solutions (at 45 °C) before and after OD and expressed as millipascal-second. All measurements were performed in triplicate.

Chemical Analysis

Determination of L-Ascorbic Acid, Sugars, and Organic Acids

L-Ascorbic acid was analyzed as described previously by Turkiewicz et al. (2019a). Briefly, approx. 1 g of the dried sample was mixed with 20 mL of 0.1 M phosphoric acid and centrifuged at 19,000g for 10 min using MPW-350 (MPW Med. Instruments, Warsaw, Poland), and supernatants were filtered through Millex Smplicity™ Filters (Hydrophilic PTFE, 0.20 μm, Millipore, Merck; Darmstadt, Germany) before analysis. Determination of vitamin C was achieved using ultra-performance liquid chromatography (UPLC) with a photodiode array (PDA) detector. A sample of 20 μL was injected into a Chromolith® Performance RP-18 endcapped column (100 × 4.6 mm; 2 μm, Merck, Darmstadt, Germany), and the elution was carried out using 0.1 M phosphoric acid at the flow rate of 1.0 mL/min. The absorbance was monitored at 254 nm. The measurements were performed in triplicate and the results were expressed as milligrams per 100 g dry weight (dw).

The extract of the dried Japanese quince fruit for sugar and organic acid analysis was prepared as follows: dried sample (~0.5 g) was placed into a 20-mL volumetric flask, diluted with redistilled water, and incubated at 98 °C for 30 min with constant shaking using water bath (GFL 1083, Gesellschaft

für Labortechnik, Burgwedel, Germany). Then, the part of the sample was placed into a 10-mL plastic tube and centrifuged at 19,000g for 10 min using MPW-350 (MPW Med. Instruments, Warsaw, Poland); the supernatant was filtered through ISOLUTE® C18 columns (Biotage®, Uppsala, Sweden) using PRESSURE+ 48 positive pressure manifold (Biotage®, Uppsala, Sweden), and then—before injection—through Millex Smplicity™ Filters (Hydrophilic PTFE, 0.20 μm, Millipore, Merck; Darmstadt, Germany).

The profile and content of sugars were determined the by high-performance liquid chromatography (HPLC) method using the 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, USA). The separation of sugars was performed on an Unison UK-Amino column (250 × 3 mm; 3 μm, Imtakt, Portland, OR, USA). Eighty-seven percent of acetonitrile (v/v) was used as the mobile phase for isocratic elution. The flow rate was 0.7 mL/min, sample injection volume 4 μL, and elution time 17 min.

The analysis of organic acids was performed using ultra-performance liquid chromatography (UPLC) with photodiode array (PDA) detector. The column Polymex IEX H (250 × 8 mm; 8 μm, Watrex, Praha, Czech Republic) being protected by the guard column of the same materials was operated at 25 °C. The samples (10 μL) were injected, and the isocratic elution was completed in 27 min with 9 mM sulfuric acid (v/v) at a flow rate of 0.8 mL/min. The results were monitored at 210 nm.

The measurements were performed in triplicate and the results were expressed as grams of sugar or grams of organic acid per 100 g of dw.

Identification and Quantification of Polyphenols (Flavonols, Anthocyanidins, Phenolic Acids, Flavan-3-ols) and Polymeric Procyanidins

The extract of the dried Japanese quince fruit for polyphenol analysis was prepared as described previously by Wojdyło et al. (2013).

UPLC-PDA analyses of polyphenols (flavan-3-ols at 280 nm, flavonols at 360 nm, phenolic acids at 320 nm, and anthocyanidins at 520 nm) were performed as described previously by Turkiewicz et al. (2020b). Separations of individual polyphenols were carried out using the ACQUITY UPLC BEH C18 column (100 × 2.1 mm; 1.7 μm, Waters Corporation, Milford, USA) at 30 °C. The samples (5 μL) were injected and the program began with gradient elution with 99–65% solvent A (0–12 min), and then lowering solvent A to 0% for condition column (12.5–13.5 min), the gradient returned to the initial composition (99% A) until 15 min for held constant to re-equilibrate the column. The mobile phase consisted of solvent A (2% formic acid, v/v) and solvent B (acetonitrile). Retention times (R_t) and spectra were compared with those of analytical standards. Calibration curves at concentrations ranging from 0.05 to 5 mg/mL ($R^2 = 0.9998$) were made from (–)-epicatechin; (+)-catechin; procyanidin B1, B2, and C1; chlorogenic acid; cryptochlorogenic acid; 3,5-dicaffeoylquinic acid; quercetin- and kaempferol-3-*O*-glucoside and quercetin- and kaempferol-3-*O*-rutinoside; and cyanidin-3-*O*-glucoside. Flavan-3-ols were expressed as (–)-epicatechin, flavonol derivatives as quercetin-3-*O*-glucoside, and phenolic acids as chlorogenic acid while anthocyanidins as cyanidin-3-*O*-glucoside.

Analysis of polymeric procyanidins was performed by the phloroglucinolysis method as described previously by Turkiewicz et al. (2020a). The analysis was carried out on a UPLC system Acquity (Waters Corp., Milford, MA, USA) consisting of a binary solvent manager, and fluorescence (FL) detector. The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (–)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (–)-epicatechin-phloroglucinol adduct standards. All measurements were repeated three times. The results were expressed as milligrams per 100 g dw.

Determination of In Vitro Biological Activities

The extract for following analysis was prepared as described previously by Turkiewicz et al. (2019b).

The antioxidant activity was determined using the oxygen radical absorbance capacity (ORAC) assay as previously described by Wojdyło et al. (2018). The results were expressed as millimoles of Trolox per 100 g of dw. The antidiabetic

activity was measured as inhibition of α-amylase, α-glucosidase, and pancreatic lipase, while the anticholinergic activity was measured as inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) as reported previously by Tkacz et al. (2019b). All samples were assayed in triplicate and the result was expressed as IC_{50} (mg of dried sample per mL of enzyme) or percentage of inhibition (for concentration 50 mg dried sample per mL of enzyme).

All measurements were performed using a plate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA).

Statistical Analysis

Statistical analysis was conducted using *XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel* (Addinsoft, Paris, France). Significant differences ($p < 0.05$) between means were evaluated by the non-parametric Kruskal-Wallis test and Dunn's procedure. All analyses were done in triplicate. To highlight correlations, principal component analysis (PCA) has been done.

Results and Discussion

Drying Kinetics

Table 1 presents the parameters used to describe the drying kinetics by a modified Page model, which is the best model taking into account the highest value of the determination

Table 1 Parameters of model describing the drying kinetics of the Japanese quince fruit dehydrated in various fruit juices

Drying method	Sample	Parameter model*			Statistics		
		A	k	n	RMSE	R ²	
CD	Control	1.000	0.003	1.430	0.0163	0.9979	
	Apple	0.265	0.019	1.060	0.0062	0.9948	
	Pear	0.276	0.022	1.020	0.0045	0.9974	
	Pineapple	0.262	0.018	1.060	0.0045	0.9972	
	Sour cherry	0.272	0.017	1.110	0.0068	0.9941	
	Blackcurrant	0.282	0.013	1.150	0.0049	0.9973	
	Chokeberry	0.299	0.007	1.300	0.0068	0.9957	
	VMD	Control	0.011	0.100	0.399	0.0001	0.9884
		Apple	0.014	0.760	0.611	0.0003	0.9599
Pear		0.013	0.062	0.387	0.0001	0.9692	
Pineapple		0.013	0.139	0.221	0.0001	0.9944	
Sour cherry		0.013	0.015	0.658	0.0001	0.9686	
Blackcurrant		0.011	0.041	0.482	0.0001	0.9712	
Chokeberry		0.012	0.049	0.354	0.0001	0.9931	

*Modified Page model; A, k, and n are constants of the modified Page model; RMSE, root mean square errors; R², determination coefficient

coefficient (R^2) and the lowest value of the root mean square errors (RMSE) (Petković et al. 2020). This model has been used to predict the drying behavior of many fruit materials, such as quinces (Szychowski et al. 2018) and apples (Masztalerz et al. 2020).

Figure 3a, b illustrates changes in moisture content (Mc) of OD JQF dehydrated by CD at a temperature of 70 °C while Fig. 3c, d shows the changes observed for dehydration by VMD at microwave power 120 W. In this study, in the modified Page model for convective and vacuum-microwave drying, the R^2 was above 0.9599 and the RMSE was below 0.0163. High R^2 and low RMSE proved good agreement between the thin-layer modeling equation and the experimental data, in agreement with studies of Cano-Lamadrid et al. (2017). The analysis of Fig. 3a, b (the change in moisture ratio (MR) as a function of time during the CD process) shows that MR of non-OD JQF was more than 4 times higher than after using OD. The MR at 0 min of CD was not influenced by the concentrate used for the osmotic dehydration, except for OD JQF in pineapple (Fig. 3a). A similar relationship was observed during sour cherry dehydration described by Nowicka et al. (2015a). Obtaining MR = 0.25 (baseline for OD samples) by non-OD JQF took over 70 min. Moisture loss was relatively quick only in the first 90 min of the CD process; later on, the curve flattened, MR no longer changed significantly, and the process was ineffective and time-consuming.

The obtained results are consistent with the research by Bchir et al. (2012c) over pomegranate seeds after OD and CD, where the drying kinetics could also be divided into two phases. In addition, MR value stabilization (at 0.03–0.04) for all OD samples occurred after 75 min of the process, while for non-OD JQF, this time was twice as long. These results are close to those observed by Bchir et al. (2020) where ultrasound-assisted osmotic dehydration reduced the drying time of pomegranate seeds by over 40%. This is due to the fact that they contained much more moisture to remove than samples that lost some of the water in the OD process. After CD, the moisture content in all sour cherries previously osmo-dehydrated in fruit concentrates was on average 0.086 kg/kg dw. The last stage of drying was VMD. During this process, the decrease in moisture content was no longer as significant as in the case of CD, where the MR was reduced 25 times for all OD JQF samples. In the case of VMD, MR for OD JQF decreased on average by 15% compared with that for non-OD JQF. In contrast to CD, OD had a significant effect on MR changes during VMD (Fig. 3c, d). Of all the concentrates used, the blackcurrant concentrate reduced MR by 50% compared with non-OD JQF. In this way, the composition of the osmotic solution affected the final MR of samples, and the application of pineapple and sour cherry concentrates resulted in obtaining a higher MR value than the other variants. For comparison, Cano-Lamadrid et al. (2017) in the research on

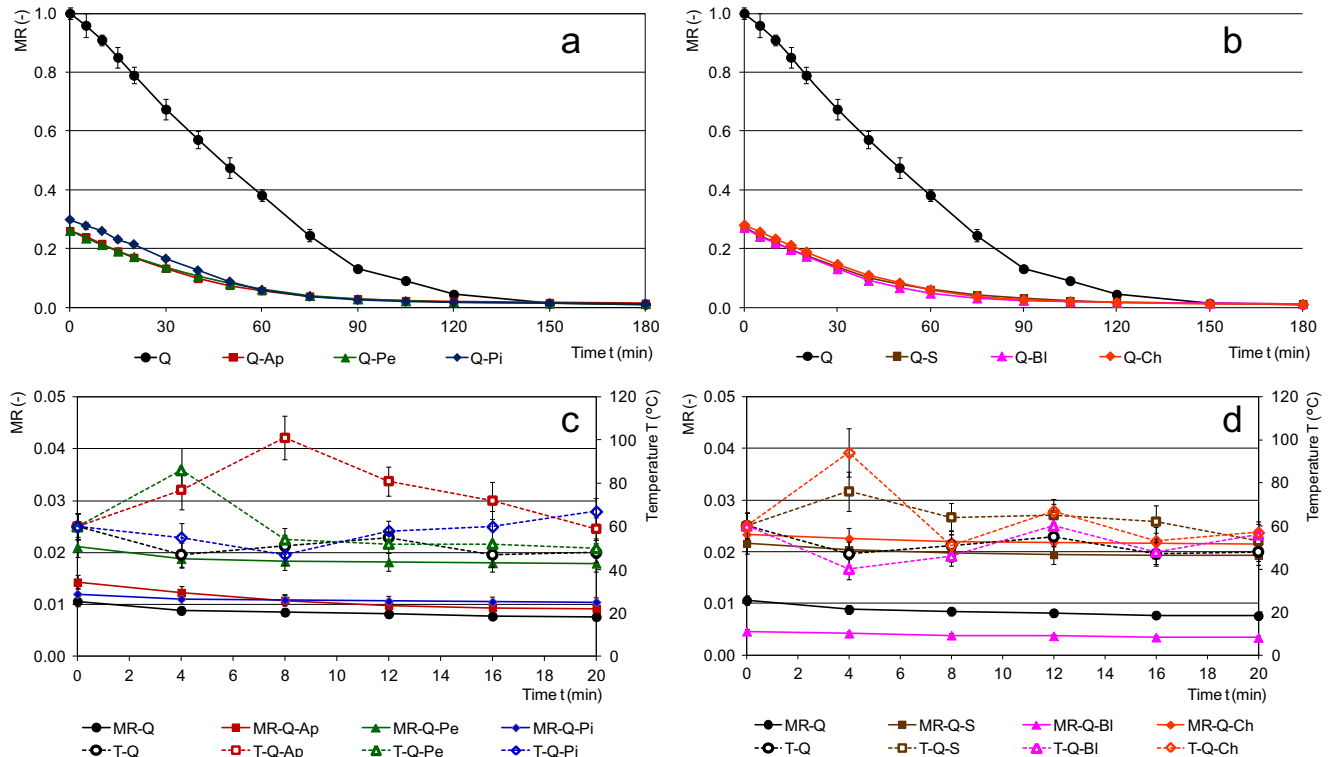


Fig. 3 Drying kinetics of the Japanese quince fruit (JQF) after osmotic dehydration (OD) process in: Ap apple, Pe pear, Pi pineapple, S sour cherry, BI blackcurrant, and Ch chokeberry concentrates treated by

convective drying at 70 °C (a, b), and by vacuum-microwave drying at microwave power 120 W (c, d); Q, non-OD JQF; MR, t , and T stand for moisture ratio, time, and temperature, respectively

pomegranate arils found that the use of pomegranate concentrate shortens the process of CVMD, while apple concentrate hinders the drying process.

Moreover, the values of constant A (Table 1) indicated the MR of JQF samples dehydrated by combination of OD-CVMD at the initial time of the VMD. The largest MR changes and thus the largest water losses during VMD were noted during the first 12 min of the process. The highest temperature of dried OD JQF samples was in the initial phase of VMD. The sample for which apple concentrate was used for OD obtained a temperature over 100 °C. A significant increase in temperature was associated with achieving critical moisture content (0.0730 kg/kg dw). JQF was impregnated with a concentrate which blocked the capillaries and thus the possibility of free water evaporation. According to Nowicka et al. (2015a), the increase in intracellular pressure and the accumulation of energy from microwaves led to temporary overheating of the material. Szychowski et al. (2018), who analyzed the drying kinetics of quince fruit, reported that the maximum temperature of the samples in microwave-vacuum drying depended on the balance of energy generated by water contained in fruit under the influence of the magnetron and energy necessary for its evaporation. In turn, samples dehydrated in apple, pineapple, and blackcurrant concentrates were characterized by similar temperature variability during the VMD process as non-OD JQF. In addition, these samples obtained the lowest final MR values.

Physiochemical Changes in OD JQF

Table 2 presents the physical and chemical properties of fresh, dehydrated, and dried Japanese quince fruits (JQF). Dry matter content in the JQF ranged from 91.89% (JQF after FD) to 95.10% (non-OD JQF). The use of the osmotic dehydration (OD) process resulted in a decrease in the dry matter content (in the case of all fruit concentrates) compared with non-dehydrated fruit ($p < 0.05$). In all dehydrated variants, except for the samples dehydrated in pineapple concentrate, water activity (a_w) was below 0.300. Moreover, the use of the OD process resulted in lower water activity (from 8 to 17%) compared with the control sample, except for the JQF OD in pineapple and blackcurrant concentrates. Similar results were obtained by Nowicka et al. (2015a) for dehydrated cherry fruits.

Compared with the variety of other parameters which require an analytical approach in a food laboratory, the fresh, appealing, and appetizing color of food products represents the only and immediately perceptible quality aspect. Color influences not only our tastes but even more so our purchasing decisions (*to buy or not to buy*) as consumers of foods. The color lightness (L^*), redness (a^*), yellowness (b^*), and total change in color (ΔEa^*b^*) values of JQF after OD and non-OD JQF are shown in Table 2. The value of the L^* parameter

ranged from 22.29 (JQF OD in chokeberry) to 84.20 (JQF after FD). JQF dehydrated in the pineapple concentrate had the same final level of L^* as the fresh JQF. As in the studies by Silva et al. (2014), where apple slices were dehydrated in sucrose solution, FD provided the product with the lightest color. In general, the OD-CVMD process caused significant darkening of the color and thus a decrease in the L^* parameter value, from 13 up to 70% for chokeberry concentrate, compared with non-OD JQF ($p < 0.05$). Similar results for OD pumpkin in chokeberry concentrate were obtained by Lech et al. (2018b). Osmotic dehydration in the sour cherry, blackcurrant, and chokeberry concentrates caused a significant increase in the share of red color (a^*) in the final product, amounting to 21.87, 19.32, and 17.39, respectively. These are fruits rich in anthocyanins, which in acidic conditions (pH of the JQF 2.71–2.99; Turkiewicz et al. (2020a)) are the most stable and therefore significantly caused color saturation with a red shade (Torskangerpoll and Andersen 2005). In addition, the OD process in pear, apple, and pineapple concentrates caused an increase in parameter a^* (from 1- to 7-fold) compared with non-OD JQF (1.36), while causing a small change ($p < 0.05$) in the value of the b^* parameter compared with the non-OD JQF (29.36)—an increase from 8 to 16%. Significant color saturation with green followed after the use of other concentrates, i.e., sour cherry, blackcurrant, and chokeberry, for which a more than 160-fold decrease in the value of the b^* parameter was observed. The same tendency to lower the value of parameter b^* for different pumpkin cultivars subjected to the OD process, among others in chokeberry concentrate, was observed by Lech et al. (2018a). Another parameter is ΔEa^*b^* , which indicates the size of the color difference in comparison with the color of fresh JQF ($L^* = 64.60$, $a^* = -1.67$, and $b^* = 17.78$). It is assumed that the color change of two samples is noticeable by the observer when the ΔEa^*b^* value is greater than 5. In the analyzed samples, those values ranged from 14.70 (JQF OD in apple) to 49.64 (JQF OD in chokeberry). The use of apple concentrate resulted in a smaller color change of JQF than for the non-OD sample (15.91).

L-Ascorbic acid is one of the important water-soluble vitamins. For humans, it is an essential nutrient and it can be taken as an index of quality of processes (Santos and Silva 2008). The content of L-Ascorbic acid in OD JQF depending on the used fruit concentrate is shown in Table 2. The highest concentration of L-Ascorbic acid was in fresh JQF and the lowest in JQF dehydrated in pear. Compared with the control sample (non-OD), where the concentration of L-Ascorbic acid was 424.75 mg/100 g dw, the use of OD led to a reduction in its content in all variants (from 69 to 85%), except for JQF OD in blackcurrants.

Japanese quince fruits are known for their very low sugar concentration, which makes them more similar to vegetables than fruits. The main identified saccharide in JQF was fructose, followed by glucose and sorbitol. Sucrose was not

Table 2 Effects of osmotic dehydration on dry weight, water activity (a_w), color parameters, L-Ascorbic acid (mg/100 g dw), sugars, and organic acid content in the Japanese quince fruit

Parameters	Fresh	FD	Control	Apple	Pear	Pineapple	Sour cherry	Blackcurrant	Chokeberry
Dry weight (%)	12.76 ± 0.22g	91.89 ± 0.90f	95.10 ± 0.18a	94.14 ± 0.15b	93.73 ± 0.10c	93.40 ± 0.30d	93.25 ± 0.28d	92.72 ± 0.11e	94.11 ± 0.32b
a_w	0.967 ± 0.010a	0.362 ± 0.000b	0.259 ± 0.000e	0.216 ± 0.010g	0.238 ± 0.010f	0.304 ± 0.010c	0.214 ± 0.000g	0.281 ± 0.010d	0.217 ± 0.010g
Color									
L	64.60 ± 2.01c	84.20 ± 1.41a	75.08 ± 1.41b	54.82 ± 1.22d	54.82 ± 1.43d	64.80 ± 1.72c	25.53 ± 1.99e	25.05 ± 1.00e	22.29 ± 2.31f
a^*	-1.67 ± 0.01e	1.20 ± 0.00d	1.36 ± 0.01d	2.81 ± 0.00d	9.59 ± 0.01c	1.71 ± 0.01d	21.87 ± 1.64a	19.32 ± 1.23b	17.39 ± 1.50b
b^*	17.78 ± 0.00c	18.97 ± 0.91c	29.36 ± 0.50b	31.72 ± 0.81ab	34.10 ± 0.50a	32.79 ± 0.90a	3.37 ± 0.20d	1.85 ± 0.12de	0.18 ± 0.09e
ΔE_{ab}^*	-	19.84 ± 0.81c	15.91 ± 0.71d	14.70 ± 0.11d	22.11 ± 0.31b	15.39 ± 0.41d	47.84 ± 1.41a	47.53 ± 2.09a	49.64 ± 1.99a
L-Ascorbic acid	770.14 ± 21.30a	441.35 ± 15.11b	424.75 ± 10.17b	81.14 ± 2.18d	62.40 ± 2.11ef	129.58 ± 8.36c	75.21 ± 1.14de	434.54 ± 15.22b	50.25 ± 2.00e
Sugars (g/100 g dw)									
Fructose	2.72 ± 0.23d	1.26 ± 0.18e	1.49 ± 0.17e	31.31 ± 1.01a	22.58 ± 1.46b	7.07 ± 0.19b	7.01 ± 0.54b	7.41 ± 0.38b	3.16 ± 0.11d
Sorbitol	0.54 ± 0.09c	0.13 ± 0.00c	0.15 ± 0.02c	nd	nd	nd	1.55 ± 0.30b	nd	11.08 ± 0.57a
Glucose	1.29 ± 0.14f	0.34 ± 0.08 g	0.52 ± 0.01 g	10.50 ± 0.73a	3.41 ± 0.54e	8.25 ± 0.33b	7.44 ± 0.45c	4.15 ± 0.08d	1.44 ± 0.02f
Sucrose	nd	nd	nd	1.64 ± 0.46b	0.48 ± 0.03c	2.92 ± 0.36a	nd	nd	nd
Total	4.54 ± 0.44f	1.73 ± 0.39g	2.15 ± 0.18g	43.45 ± 1.41a	26.47 ± 1.07b	18.24 ± 0.99c	16.00 ± 0.82d	11.56 ± 0.71e	15.68 ± 0.85d
Organic acids (g/100 g dw)									
Oxalic	0.13 ± 0.02de	0.19 ± 0.04c	0.20 ± 0.02c	0.11 ± 0.00de	0.16 ± 0.01cd	0.34 ± 0.07b	0.08 ± 0.00e	0.45 ± 0.04a	0.17 ± 0.01cd
Citric	0.68 ± 0.01c	1.16 ± 0.20a	0.84 ± 0.01bc	0.75 ± 0.07bc	0.34 ± 0.04e	nd	1.13 ± 0.22a	0.89 ± 0.05b	0.40 ± 0.02d
Isocitric	nd	nd	nd	0.35 ± 0.03d	0.28 ± 0.09d	7.92 ± 0.72a	0.58 ± 0.21d	2.02 ± 0.44c	3.17 ± 0.00b
Malonic	0.78 ± 0.05c	1.93 ± 0.23b	1.61 ± 0.11b	0.19 ± 0.01c	nd	0.17 ± 0.01c	nd	16.57 ± 0.87a	nd
Malic	62.26 ± 1.12a	53.41 ± 0.87c	58.87 ± 1.00b	13.91 ± 0.56ef	13.10 ± 0.41ef	13.02 ± 0.63ef	17.49 ± 0.69d	11.72 ± 0.66f	15.29 ± 0.40de
Succinic	nd	nd	nd	0.18 ± 0.01b	0.17 ± 0.04bc	0.35 ± 0.05a	0.32 ± 0.02a	0.13 ± 0.01c	0.08 ± 0.00d
Shikimic	0.12 ± 0.00c	0.09 ± 0.00 cd	0.10 ± 0.02c	0.03 ± 0.00d	0.06 ± 0.00 cd	0.03 ± 0.00d	0.08 ± 0.01 cd	0.31 ± 0.02a	0.20 ± 0.01b
Total	63.97 ± 1.45a	56.79 ± 1.23c	61.64 ± 1.03b	15.52 ± 0.87g	14.11 ± 0.89g	21.84 ± 0.99e	19.68 ± 1.00f	32.10 ± 1.04d	19.30 ± 0.87f

± Standard deviation; in each column, different letters mean significant differences between samples ($p < 0.05$); nd, not detected

detected. These results are in line with previous research on JQF by Turkiewicz et al. (2020a). In the analyzed samples of OD JQF, there was considerable variation ($p < 0.05$) in the content of sugars and their type. For the JQF after osmotic dehydration, values of total sugar content ranged from 11.56 (blackcurrant) to 43.45 g/100 g dw (apple). The use of the OD process resulted in an increase in sugar concentration in all samples by 5 to 20 times compared with non-OD JQF (2.15 g/100 g dw). Considering the content of individual sugars, JQF OD in apple, pear, pineapple, and blackcurrant did not contain sorbitol, and sucrose was not present in the samples after the application of sour cherry, blackcurrant, and chokeberry concentrates. Among all samples, JQF OD in chokeberry stands out, with sorbitol accounting for over 70% of the total sugar content, which is due to the predominance of this sugar in chokeberry fruit (Djuric et al. 2015).

JQF contains large amounts of organic acids, which makes them unsuitable for direct consumption. In fresh, FD, and non-OD JQF, the following five organic acids were detected: oxalic, citric, maleic, malic, and shikimic (Table 2). The content of organic acids in OD JQF samples differed between samples ($p < 0.05$) and ranged from 14.11 (JQF OD in pear) to 63.97 g/100 g dw (fresh JQF). The osmotic dehydration process, irrespective of the type of concentrate used, reduced the content of organic acids by 47 to 77% compared with non-OD JQF. For comparison, Nowicka et al. (2015a) analyzed the chemical composition of dried sour cherry fruits predehydrated in fruit concentrates and obtained a smaller reduction of acidity (calculated as malic acid) by 10–18% compared with non-dehydrated fruit. The JQF samples after the OD process can be ordered by increasing acidity: pear < apple < chokeberry = sour cherry < pineapple < blackcurrant. Compared with fresh JQF, the OD samples in apple and blackcurrant concentrates contained isocitric and succinic acids, and malonic acid was absent in the OD samples in sour cherry and chokeberry. Citric acid was not detected only in JQF OD in pineapple. The dominant organic acid in all samples (except JQF OD in blackcurrant, where malonic acid predominated) was malic acid and constituted from 60 (pineapple) to 97% (fresh JQF) of total organic acid content.

Flavonols, phenolic acids, and flavan-3-ols were evaluated in fresh, FD, non-OD, and OD JQF by UPLC-PDA, while polymeric procyanidins were determined by UPLC-FL. In addition, the degree of polymerization (DP) was calculated and the results are shown in Table 3. The osmotic dehydration had a significant influence ($p < 0.05$) on the content of individual phenolic compounds as well as total phenolic content (TPC). In JQF, polymeric procyanidins with monomeric flavan-3-ols formed a large majority (57.31 and 35.64% of TPC), followed by phenolic acids. Flavonols were measured in very small quantities and anthocyanins were not detected. The highest concentration of TPC was in fresh and non-OD JQF—42.56 and 41.49 g/kg dw, respectively. These results

are consistent with those obtained by Turkiewicz et al. (2019a) for JQF dried by different methods. OD in chokeberry resulted in the least degradation of TPC, while OD in pear led to the lowest total phenolic content. In general, the osmotic dehydration process in all concentrates led to a reduction of the TPC by 70 to 82% compared with non-OD JQF, which is consistent with the results obtained by Kucner et al. (2013). A similar effect of the OD process was observed by Bchir et al. (2012c) in a study of pomegranate seeds, where after the OD and CD at 60 °C, TPC was reduced by almost 60% compared with fresh seeds. A similar relationship occurred when observing individual groups of compounds—the concentration of flavan-3-ols decreased on average by 4 times, and procyanidins by over 7 times. The regress in TPC after OD process was due to the migration of phenolic compounds to osmotic solution caused by the difference in osmotic potential, which is in line with that of Bchir et al. (2012c). The exception is higher concentration of phenolic acids in OD JQF in pear, sour cherry, and chokeberry concentrates, flavonols in OD JQF in chokeberry, and anthocyanins, which appeared when red concentrates were used for dehydration, i.e., sour cherry, blackcurrant, and chokeberry. Similar results were obtained by Nowicka et al. (2015b) dehydrating sour cherry fruits in apple concentrate, obtaining a reduction TPC from 23 to 41% depending on the variety of fruit. In contrast, Lech et al. (2018a) obtained a significant, almost 9-fold increase in the TPC in pumpkin dehydrated in chokeberry concentrate. These differences may result from the fact that the osmotic dehydration process is influenced by many factors, including duration of the process, temperature, type, and concentration of osmotic fluid and intensity of agitation (Ahmed et al. 2016). In addition, the method and parameters of the drying process could significantly affect the TPC in the final product. The degree of polymerization (DP) increased compared with non-OD JQF, the highest value being obtained for OD JQF in chokeberry (2.72) and the lowest value for OD JQF in pineapple (1.10).

In this study, the antioxidant activity was tested using the ORAC assay and the results for fresh and dehydrated JQF are shown in Table 3. The highest antioxidant capacity was shown by fresh JQF (128.51 mmol Trolox/100 g dw), while the lowest was measured for JQF OD in pineapple concentrate (32.35 mmol Trolox/100 g dw). Non-OD JQF had almost twice as high antioxidant activity as JQF after FD ($p < 0.05$). Similar relationships have been reported earlier, where the use of microwaves was followed by an increase in antioxidant capacity (Michalska et al. 2017; Turkiewicz et al. 2019a). Similar results have been reported by Bchir et al. (2012c) for the dried pomegranate seeds after OD. They concluded that a reduction in antioxidant activity (over 60% compared with fresh seed) could be explained due to loss of biologically active compounds as phenolics and vitamins, as a result of the OD and high temperature during drying. The

Table 3 Effects of osmotic dehydration on phenolic compounds and antioxidant, antidiabetic, and anticholinergic activities in the dried Japanese quince fruit

Parameters	Fresh	FD	Control	Apple	Pear	Pineapple	Sour cherry	Blackcurrant	Chokeberry
Phenolic compounds (g/kg dw)									
Phenolic acids	0.38 ± 0.02d	0.39 ± 0.03 cd	0.42 ± 0.04cd	0.18 ± 0.02f	0.47 ± 0.07c	0.16 ± 0.00f	1.62 ± 0.31b	0.30 ± 0.02e	4.12 ± 0.05a
Flavonols	2.63 ± 0.35a	1.36 ± 0.11c	2.05 ± 0.12b	0.42 ± 0.02d	0.59 ± 0.05d	0.55 ± 0.07d	1.21 ± 0.03c	2.02 ± 0.20b	2.21 ± 0.14b
Anthocyanins	nd	nd	nd	nd	nd	nd	0.75 ± 0.02b	1.52 ± 0.09a	1.45 ± 0.08a
Flavan-3-ols	15.17 ± 0.29c	18.07 ± 0.78b	20.35 ± 0.86a	5.54 ± 0.12e	5.22 ± 0.28e	6.17 ± 0.14d	6.24 ± 0.39d	3.00 ± 0.00f	3.06 ± 0.18f
Polymeric procyanidins	24.39 ± 0.54a	16.98 ± 0.61c	18.68 ± 0.57b	1.84 ± 0.05g	1.04 ± 0.02h	5.59 ± 0.02d	2.32 ± 0.03f	2.93 ± 0.02e	1.63 ± 0.09g
TPC	42.56 ± 1.11a	36.80 ± 1.21b	41.49 ± 1.54a	7.98 ± 0.89e	7.32 ± 0.55e	12.47 ± 0.36c	12.13 ± 0.65c	9.77 ± 0.42d	12.48 ± 0.33c
DP	1.01	1.04	1.05	1.19	1.26	1.10	1.78	1.54	2.72
Antioxidant activity (mmol Trolox/100 g dw)									
ORAC	128.51 ± 2.82a	45.86 ± 1.45d	85.80 ± 1.98b	39.94 ± 1.32e	35.69 ± 1.11f	32.35 ± 1.54g	49.02 ± 1.87c	43.46 ± 1.66d	46.53 ± 1.39d
Antidiabetic activity (IC ₅₀ : mg/mL)									
α-Amylase	< 0.01	23.58 ± 1.02f	118.27 ± 2.46a	57.67 ± 1.57c	122.48 ± 3.55a	79.11 ± 1.99b	63.59 ± 1.55c	36.98 ± 1.32e	46.53 ± 0.92d
α-Glucosidase	3.66 ± 0.52f	2.74 ± 0.33g	1.01 ± 0.11h	7.37 ± 0.98c	20.01 ± 0.86a	8.55 ± 0.48b	6.08 ± 0.88d	7.00 ± 0.00c	4.71 ± 0.17c
Pancreatic lipase	0.13 ± 0.02g	0.25 ± 0.03f	0.22 ± 0.00f	0.45 ± 0.02c	0.59 ± 0.05a	0.50 ± 0.05b	0.39 ± 0.09d	0.33 ± 0.01e	0.51 ± 0.07b
Anticholinergic activity (% inhibition; 50 mg/mL of enzyme)									
AChE	87.69 ± 1.64a	< 0.01	< 0.01	44.92 ± 0.68d	60.20 ± 0.80b	57.24 ± 0.87bc	32.54 ± 0.66e	47.60 ± 0.75d	55.10 ± 1.00c
BuChE	99.98 ± 1.94a	45.43 ± 0.83d	40.26 ± 0.57e	56.24 ± 0.51bc	60.08 ± 0.77b	59.79 ± 0.92b	54.92 ± 0.69c	60.70 ± 0.53b	60.36 ± 0.87b

± Standard deviation ; *nd*, not detected; *TPC*, total phenolic content; *DP*, degree of polymerization; in each column, different letters mean significant differences between samples ($p < 0.05$)

use of concentrates from red fruits in the OD process resulted in increased values of ORAC activity (on average by 22%) compared with concentrates from fruit with light flesh. This is probably due to the higher concentration of phenolic compounds, which is confirmed by the calculated Pearson correlation coefficient ($R^2 = 0.76$).

Table 3 also shows α -amylase, α -glucosidase, and pancreatic lipase inhibition activity, as IC₅₀ (mg/mL). The inhibitory activity against α -amylase ranged from 23.58 to 118.27 mg/mL (for FD and non-OD JQF, respectively), while α -glucosidase inhibition was between 1.01 and 20.01 mg/mL (non-OD JQF and JQF OD in pear concentrate, respectively). Moreover, for fresh JQF, the value of α -amylase inhibition was designated as < 0.01 . This indicates the highest inhibitory potential for this enzyme and the possibility of using a lower concentration. For all OD JQF samples, the ability to inhibit α -glucosidase was on average 8 times higher than that of α -amylase, which, according to Unuofin et al. (2018), is beneficial for the proper functioning of the digestive system and potential antidiabetic properties. In contrast, Tkacz et al. (2019b), analyzing various cultivars of sea buckthorn fruit, found almost 2-fold higher inhibition of α -amylase compared with α -glucosidase. In addition, it was noted that JQF OD in concentrates of sour cherry, blackcurrant, and chokeberry showed a higher inhibition potential for α -amylase than after using apple, pear, and pineapple concentrates. The inhibition toward pancreatic lipase for all samples was below 0.59 mg/mL, while the strongest inhibitory potential was recorded for fresh JQF (0.13 mg/mL). The study of Maqsood et al. (2017) suggests that the ability to inhibit pancreatic lipase activity, which in turn reduces the entry of lipids into the blood stream, can be used to control obesity. The calculated Pearson correlation coefficient confirmed the strong positive correlation of TPC and the ability to inhibit α -glucosidase ($R^2 = 0.65$) and between the content of polymeric procyanidins and the ability to inhibit pancreatic lipase ($R^2 = 0.88$).

The human brain contains two major forms of cholinesterases: acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The two forms differ in structure and function. In brains of Alzheimer disease patients, AChE activity decreases progressively, while BuChE activity shows some increase (Giacobini 2004). Therefore, finding effective inhibitors of these enzymes may be important for the prevention and treatment of neurodegenerative diseases. The results of JQF anticholinergic activity are shown as percentage of inhibition at the concentration of 50 mg of dried sample per milliliter of enzyme (Table 3). The anti-AChE activity ranged from 32.54 (JQF OD in sour cherry) to 87.69% (fresh JQF). Additionally, very low AChE inhibition by FD and non-OD JQF (< 0.01) was observed. As in the case of the ability to inhibit AChE, fresh JQF was the strongest inhibitor (99.98%), while the weakest was non-OD JQF (40.46%). It is worth noting that the osmotic dehydration process increased anticholinergic

activity in both AChE (over 98%) and BuChE (over 30%) compared with non-OD JQF. The calculated Pearson correlation coefficient confirmed the strong negative correlation of the ability to inhibit AChE and BuChE with monomeric flavan-3-ols ($R^2 = -0.93$ and -0.97 , respectively) as well as with polymerized forms ($R^2 = -0.91$ and -0.93 , respectively). This is consistent with the research of Tkacz et al. (2019a) on *Vitis vinifera*, where it was found that among all groups of phenolic compounds, flavan-3-ols are the weakest cholinesterase inhibitors.

Physiochemical Changes in OD Fluids

Table 4 presents the physical parameters, including color, of fruit juice concentrates before and after osmotic dehydration. The dry weight and soluble solid content in selected fruit concentrates before the OD process were on average 40% and 40 °Bx, respectively, and decreased as a result of the process. Apple and pineapple concentrates had the highest final dw and SSC, while chokeberry had the lowest. On average, dw and SSC decreased by over 16%. It is the result of water migration from Japanese quince fruit tissues during the OD process and thus dilution of solutions. Water activity in fruit juice concentrates before and after the osmotic dehydration process showed significant differentiation ($p < 0.05$). Before OD, a_w values were above 0.938 and increased for pear, sour cherry, and blackcurrant concentrates. For others (apple, pineapple, and chokeberry), a_w values were lower than before the OD, but remained above 0.900. The viscosity of the solution is one of the factors influencing the process of osmotic dehydration, and when the solution is concentrated, its penetration deeper into the dehydrated tissue is directly limited by viscosity (Phisut 2012). The viscosity of the concentrates before the OD was on average 3.84 mPa, except for the pineapple concentrate, whose viscosity was almost 4 times higher compared with the others. The OD process reduced the viscosity by an average of one third, which is consistent with the results of Lech et al. (2017).

The brightness of concentrates expressed as the L^* value (Table 4) was the lowest for blackcurrant and chokeberry concentrates, and for pineapple, it was more than twice as high. As a result of OD, the color of pineapple, sour cherries, and blackcurrant concentrates brightened, while for the others, L^* values decreased. The values of parameter a^* , after OD, for pear, sour cherry, and chokeberry concentrates increased, which was associated with a shift in color saturation toward red. In turn, observing changes in the proportion of blue and yellow, expressed by the value of b^* , increase of the share of yellow in pear and chokeberry concentrates was noted.

In order to examine the chemical composition of fruit concentrates used as osmotic agents, the sugar and organic acid content was evaluated, and the results are provided in Table 4. For the fruit juice concentrates before osmotic dehydration,

values of total sugar content ranged from 39.41 (blackcurrant) to 67.28 g/100 g dw (pineapple). The OD process resulted in a decrease in sugar concentration in all samples by up to 20% compared with samples after OD. Sugars were absorbed by the Japanese quince fruit tissue, which can be observed by analyzing the sugar content in dried JQF after the OD in Table 2. The opposite situation occurred in the case of organic acid content. The osmotic dehydration process increased the content of organic acids in concentrates after the OD by up to 50% (apple concentrate) compared with the initial values. The exception was the blackcurrant and chokeberry concentrates, where the acid content increased after the OD. The reason may be that these two concentrates had the highest initial content of organic acids (109.48 and 89.47 g/100 g, respectively). Thus, the concentration difference between the acid content in JQF was not sufficient for the exchange to take place, as was the case for apple concentrate, whose initial content was the lowest of all, and the increase in concentration after the OD was the largest.

Total phenolic content, calculated as the sum of individual phenolic compounds, varied significantly between used fruit juice concentrates ($p < 0.05$), with chokeberry displaying the highest (41.71 g/kg dw) and apple the lowest content (2.26 g/kg dw; Table 5). The main detected phenolic group was flavan-3-ols, followed by polymeric procyanidins, phenolic acids, anthocyanidins, and flavonols. For concentrates before OD, they accounted for 65, 16, 12, 9, and 5% of total phenolic compounds, respectively. The exception was the chokeberry concentrate, for which phenolic acids were the majority. Anthocyanins were absent in apple, pear, and pineapple concentrates, while flavonols were not detected in pear and pineapple concentrates. The osmotic dehydration process led to an increase in the total phenolic content by up to 75% (apple concentrate), except for the blackcurrant concentrate, for which a slight decrease in TPC was noted (36.98 g/kg dw). Analyzing the changes of individual groups of phenolic compounds, there were mainly increases in the concentrations of polymeric procyanidins (on average 27%) and flavan-3-ols (on average 21%), because these two groups form the majority in the content of TPC in JQF (Turkiewicz et al. 2020a). Anthocyanins stand out from all the groups, because their concentration has decreased, while pear and pineapple concentrates have been enriched with flavonols, which were not detected before the OD, as was mentioned before. The degree of polymerization has decreased as a result of the OD process, which indicates a decrease in the number of catechin units in the procyanidin chains.

What is more, the content of phenolic compounds is correlated with the antioxidant capacity (Pearson's correlation coefficient $R^2 = 0.87$). It can be observed that the antioxidant capacity values measured in ORAC assay increase significantly ($p < 0.05$) for the fruit juice concentrates after OD (Table 5), except for chokeberry concentrate. The largest, almost 4-fold

Table 4 Effects of osmotic dehydration on dry weight, water activity (a_w), soluble solid content (SSC), viscosity, color parameters, sugars, and organic acid content in osmotic fluids before and after dehydration

Parameters	After OD											
	Before OD					After OD						
	Apple	Pear	Pineapple	Sour cherry	Blackcurrant	Chokeberry	Apple	Pear	Pineapple	Sour cherry	Blackcurrant	Chokeberry
Dry weight (%)	40.58 ± 0.19c	41.10 ± 0.12a	40.91 ± 0.20b	40.41 ± 0.22d	39.19 ± 0.17e	40.91 ± 0.13f	34.91 ± 0.20g	34.27 ± 0.22h	34.80 ± 0.38g	33.05 ± 0.25i	32.78 ± 0.22j	32.61 ± 0.14k
a_w	0.950 ± 0.000ab	0.938 ± 0.000b	0.968 ± 0.010a	0.939 ± 0.000b	0.941 ± 0.000ab	0.968 ± 0.000ab	0.901 ± 0.010c	0.954 ± 0.000ab	0.955 ± 0.010ab	0.952 ± 0.010ab	0.954 ± 0.000ab	0.955 ± 0.010ab
SSC (°Bx)	39.00 ± 0.00d	40.00 ± 0.00b	39.50 ± 0.05c	40.40 ± 0.10a	39.60 ± 0.15c	39.50 ± 0.15c	33.60 ± 0.00e	33.20 ± 0.00f	33.60 ± 0.15c	32.80 ± 0.10g	32.60 ± 0.00g	32.80 ± 0.10g
Viscosity (mPa s)	3.98 ± 0.05d	3.67 ± 0.06e	15.60 ± 0.22a	3.55 ± 0.01e	3.63 ± 0.06e	4.40 ± 0.07c	2.57 ± 0.01g	2.53 ± 0.01g	9.29 ± 0.05b	2.59 ± 0.00g	2.70 ± 0.01fg	2.86 ± 0.01f
Color												
L^*	20.89 ± 0.87c	18.41 ± 0.90e	36.24 ± 1.01b	17.88 ± 0.93i	17.85 ± 0.61i	18.20 ± 0.71fg	19.77 ± 1.21d	18.34 ± 1.77ef	38.05 ± 1.95a	18.52 ± 0.88e	17.98 ± 0.72hi	18.09 ± 1.20gh
a^*	1.86 ± 0.01a	0.17 ± 0.01d	-1.16 ± 0.00h	-0.28 ± 0.05g	-0.26 ± 0.01fg	-0.20 ± 0.02ef	1.28 ± 0.30b	0.31 ± 0.09c	-1.73 ± 0.10i	-0.15 ± 0.07e	-0.24 ± 0.04fg	-0.13 ± 0.03e
b^*	4.74 ± 0.01c	1.01 ± 0.01f	18.18 ± 1.20a	0.81 ± 0.11hi	0.91 ± 0.20g	0.84 ± 0.63ghi	3.21 ± 0.66d	1.39 ± 0.10e	17.73 ± 0.99b	0.76 ± 0.01i	0.86 ± 0.01gh	0.91 ± 0.10g
Sugars (g/100 g dw)												
Fructose	47.59 ± 2.25b	50.61 ± 2.45a	11.09 ± 0.87g	17.84 ± 1.01ef	23.27 ± 0.99d	8.67 ± 0.52gh	42.13 ± 1.49c	46.30 ± 1.37b	9.57 ± 0.28gh	16.50 ± 0.74f	20.07 ± 0.88e	7.26 ± 0.49h
Sorbitol	nd	nd	nd	3.50 ± 0.33c	nd	34.16 ± 1.76a	nd	nd	nd	3.75 ± 0.06c	nd	28.83 ± 1.89b
Glucose	5.26 ± 0.19fg	6.27 ± 0.25ef	16.43 ± 0.75c	22.86 ± 1.03a	16.14 ± 0.88c	3.24 ± 0.12h	4.82 ± 0.23g	6.59 ± 0.34e	13.12 ± 0.66d	19.49 ± 0.55b	12.67 ± 0.72d	3.04 ± 0.11h
Sucrose	6.82 ± 0.18c	4.35 ± 0.21d	39.77 ± 1.44a	nd	nd	nd	6.23 ± 0.25c	4.15 ± 0.33d	30.75 ± 1.02b	nd	nd	nd
Total	59.67 ± 1.85bc	61.23 ± 1.12b	67.28 ± 1.03a	44.21 ± 1.22e	39.41 ± 1.35f	46.07 ± 1.99e	53.18 ± 1.45d	57.04 ± 1.63c	53.44 ± 1.60d	39.74 ± 1.22f	32.74 ± 1.07g	39.13 ± 0.99f
Organic acids (g/100 g dw)												
Oxalic	0.39 ± 0.02d	0.87 ± 0.02d	0.61 ± 0.06d	4.19 ± 0.12b	16.52 ± 1.01a	3.09 ± 0.37c	0.35 ± 0.05d	0.74 ± 0.11d	0.56 ± 0.02d	4.27 ± 0.10b	16.64 ± 1.12a	2.84 ± 0.09c
Citric	1.12 ± 0.03e	2.69 ± 0.06e	0.31 ± 0.05e	34.02 ± 1.48c	49.21 ± 2.03ab	10.38 ± 0.47d	1.48 ± 0.06e	3.46 ± 0.12de	1.17 ± 0.12e	36.00 ± 2.00bc	45.53 ± 2.78a	10.38 ± 0.49d
Isocitric	1.88 ± 0.10e	3.52 ± 0.42e	14.75 ± 1.01d	nd	25.97 ± 1.98c	39.60 ± 1.88a	4.10 ± 0.18e	4.12 ± 0.03e	17.16 ± 0.65d	nd	23.33 ± 2.07c	30.08 ± 1.06b
Malonic	2.00 ± 0.22b	1.26 ± 0.19d	0.12 ± 0.01e	nd	nd	nd	3.15 ± 0.00a	1.67 ± 0.45c	0.76 ± 0.63f	nd	nd	nd
Malic	10.28 ± 0.32gh	7.43 ± 0.34i	3.52 ± 0.28i	36.87 ± 1.55a	11.22 ± 0.88fg	29.23 ± 1.02b	14.62 ± 0.88e	12.47 ± 0.66f	8.95 ± 0.88hi	37.85 ± 1.22a	17.36 ± 0.99d	26.26 ± 0.95c
Succinic	0.45 ± 0.14ij	0.68 ± 0.09g	0.33 ± 0.04j	7.96 ± 0.89a	6.08 ± 0.71d	6.61 ± 0.69c	0.49 ± 0.08i	0.66 ± 0.04gh	0.52 ± 0.12hi	7.80 ± 0.72b	5.15 ± 0.58e	3.16 ± 0.32f
Shikimic	0.03 ± 0.00e	0.03 ± 0.00e	0.02 ± 0.00e	0.21 ± 0.09d	0.48 ± 0.39b	0.56 ± 0.69a	0.03 ± 0.00e	0.02 ± 0.00e	0.02 ± 0.00e	0.16 ± 0.01d	0.42 ± 0.10c	0.39 ± 0.11c
Total	16.14 ± 0.89f	16.49 ± 0.99f	19.65 ± 1.11ef	83.24 ± 1.47b	109.48 ± 2.05a	89.47 ± 1.32b	24.20 ± 1.00de	23.13 ± 0.98de	29.14 ± 1.34d	86.09 ± 1.12b	108.43 ± 2.20a	73.11 ± 0.13c

± Standard deviation; nd, not detected; in each column, different letters mean significant differences between samples ($p < 0.05$)

Table 5 Effects of osmotic dehydration on phenolic compounds and antioxidant, antidiabetic, and anticholinergic activities in osmotic fluids before and after dehydration

	Before					After						
	Apple	Pear	Pineapple	Sour cherry	Blackcurrant	Chokeberry	Apple	Pear	Pineapple	Sour cherry	Blackcurrant	Chokeberry
Phenolic compounds (g/kg dw)												
Phenolic acids	0.39 ± 0.02de	0.77 ± 0.10cd	0.20 ± 0.01e	4.31 ± 0.30b	0.55 ± 0.03cde	11.55 ± 0.14a	0.36 ± 0.09e	0.82 ± 0.10c	0.21 ± 0.00e	4.22 ± 0.26b	0.52 ± 0.04cde	11.28 ± 0.10a
Flavonols	0.03 ± 0.00f	nd	nd	2.32 ± 0.21e	3.35 ± 0.54c	4.94 ± 0.28a	0.05 ± 0.00f	0.10 ± 0.01f	0.06 ± 0.00f	3.17 ± 0.14cd	3.04 ± 0.32d	4.37 ± 0.09b
Anthocyanins	nd	nd	nd	4.16 ± 0.08e	9.45 ± 0.33a	7.03 ± 0.23c	nd	nd	nd	4.14 ± 0.17e	8.91 ± 0.44b	6.74 ± 0.00d
Flavan-3-ols	1.40 ± 0.10 k	6.04 ± 0.21 g	4.37 ± 0.11i	18.72 ± 0.17c	19.43 ± 0.98bc	8.65 ± 0.27e	2.80 ± 0.08j	7.54 ± 0.17f	5.24 ± 0.45 h	23.79 ± 1.14a	19.50 ± 0.83b	10.33 ± 0.66d
Polymeric procyanidins	0.44 ± 0.05e	0.41 ± 0.08e	1.39 ± 0.24de	4.85 ± 0.10c	4.40 ± 0.02c	9.54 ± 0.63b	0.73 ± 0.11de	1.04 ± 0.01de	1.99 ± 0.22d	5.07 ± 0.57c	5.01 ± 0.04c	10.98 ± 0.50a
TPC	2.26 ± 0.10i	7.22 ± 0.22fg	5.96 ± 0.13g	34.37 ± 1.24d	37.18 ± 1.50c	41.71 ± 1.87b	3.94 ± 0.54h	9.50 ± 0.60e	7.50 ± 0.52f	40.39 ± 1.87b	36.98 ± 1.99c	43.70 ± 1.67a
DP	2.16	1.56	1.51	1.74	2.19	1.30	1.80	1.27	1.37	1.55	1.96	1.27
Antioxidant activity (mmol Trolox/100 g dw)												
ORAC	1.44 ± 0.21f	2.07 ± 0.37f	1.30 ± 0.22f	16.32 ± 0.98d	21.04 ± 0.78c	54.89 ± 1.12a	1.80 ± 0.01f	2.86 ± 0.00ef	4.81 ± 0.54e	23.07 ± 0.46c	23.16 ± 0.62c	43.54 ± 0.99b
Antidiabetic activity (IC ₅₀ ; mg/mL)												
α-Amylase	453.07 ± 10.15b	368.58 ± 14.18c	518.87 ± 22.11a	103.89 ± 7.25f	< 0.01	< 0.01	141.43 ± 5.56e	187.24 ± 8.22d	124.27 ± 6.31ef	74.95 ± 1.11g	< 0.01	< 0.01
α-Glucosidase	35.84 ± 0.55b	40.60 ± 0.68a	28.62 ± 0.48c	< 0.01	< 0.01	< 0.01	27.41 ± 0.64c	23.97 ± 0.59d	22.73 ± 0.49d	< 0.01	< 0.01	< 0.01
Pancreatic lipase	1.25 ± 0.34b	2.82 ± 0.48a	0.34 ± 0.01f	1.01 ± 0.00c	< 0.01	1.17 ± 0.22b	0.71 ± 0.08e	1.03 ± 0.03c	< 0.01	0.39 ± 0.05f	< 0.01	0.86 ± 0.06d
Anticholinergic activity (% inhibition; 50 mg/mL of enzyme)												
ACHE	10.88 ± 0.35d	17.18 ± 0.41a	12.53 ± 0.55c	0.81 ± 0.04h	7.55 ± 0.21e	6.00 ± 0.11ig	10.87 ± 0.50d	5.91 ± 0.69ig	13.77 ± 0.34b	5.72 ± 0.16g	6.50 ± 0.23fg	6.89 ± 0.21ef
BuChE	12.17 ± 0.31e	12.13 ± 0.52e	11.56 ± 0.47f	10.58 ± 0.65g	10.81 ± 0.44g	12.81 ± 0.68cd	13.11 ± 0.66c	12.48 ± 0.47de	14.36 ± 0.64b	13.18 ± 0.59c	14.68 ± 0.33ab	14.88 ± 0.74a

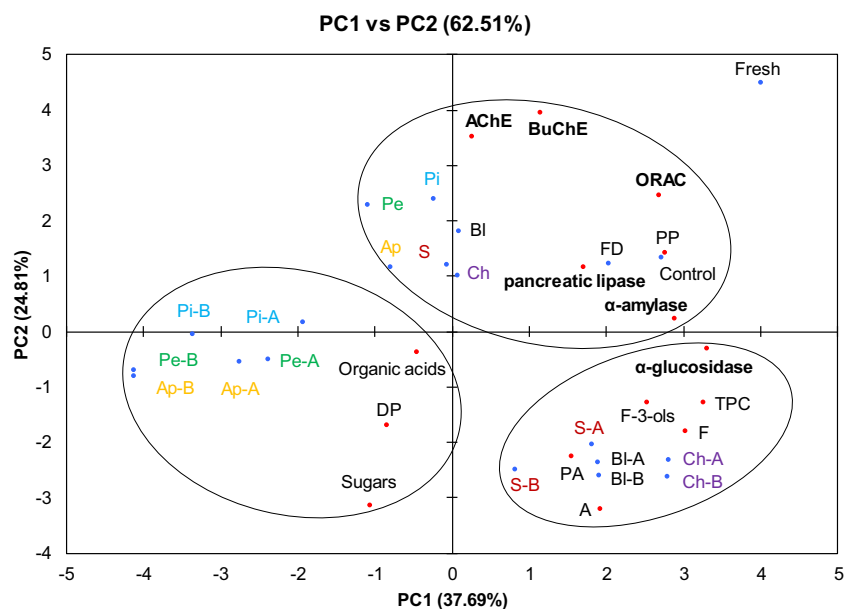
± Standard deviation; nd, not detected; TPC, total phenolic content; DP, degree of polymerization; in each column, different letters mean significant differences between samples ($p < 0.05$)

increase in antioxidant activity was noted for pineapple concentrate (4.81 mmol Trolox/100 g dw). In contrast, Lech et al. (2017) dehydrated zucchini and carrots in chokeberry concentrate and obtained an increase in the antioxidant activity of the osmotic fluid. The results indicate that the fruit concentrates after the process of osmotic dehydration can be a valuable product with increased antioxidant potential for reuse. As in the case of OD JQF, antidiabetic and anticholinergic properties were also evaluated in the analyzed concentrates before and after the OD process (Table 5). Blackcurrant and chokeberry concentrates, both before and after OD, were characterized by very high α-amylase inhibition activity (designated IC₅₀ < 0.01 mg/mL) and α-glucosidase (here also sour cherry concentrate). Other concentrates, i.e., apple, pear, and pineapple (for α-amylase also cherry), showed considerable variation in antidiabetic activity ($p < 0.05$). In general, the osmotic dehydration process increased α-amylase inhibition activity by 28 to 76% and α-glucosidase by 21 to 41% compared with concentrates before the OD. Regarding pancreatic lipase inhibiting activity, IC₅₀ values for all concentrates before the OD were below 3 mg/mL, and as a result of OD, they decreased (almost 3 times for the pear concentrate). Anticholinergic activity expressed as a percentage of AChE inhibition decreased after the OD process (almost a 3-fold decrease in activity for pear concentrate), except for pineapple and chokeberry concentrates, where a slight increase was observed. In contrast, the OD process had a positive effect on BuChE inhibition activity, where a slight increase was noted for all concentrates. So far no reports in the literature can be found on the impact of osmotic dehydration on antidiabetic and anticholinergic activities in osmotic fluids.

Principal Component Analysis

For an easy visualization of all the studied variables (physical and chemical), a PCA was run for all samples (Fig. 4). The first two principal components explained 62.51% (PC1 = 37.69% and PC2 = 24.81%, respectively) of the total variation of the experimental data. Three distinct clusters formed on the plot, with fresh Japanese quince fruit not included in any of them. This means that in terms of physicochemical properties, it was radically different from the other samples. Starting from the left, the first group was created from concentrate samples of fruit with light flesh (apple, pear, and pineapple) both before and after the osmotic dehydration process. This group also contained organic acids, sugars, and DP. Another cluster was formed by selected biological activities and dried JQF after OD. AChE and BuChE inhibition activity levels were strongly positively correlated (Pearson’s correlation coefficient $R^2 = 0.96$), as were ORAC antioxidant capacity and content of polymeric procyanidins ($R^2 = 0.70$), which was in agreement with the results of Wojdyło et al. (2018) on goji fruits. In addition, OD JQF correlated more strongly with

Fig. 4 Principal component analysis score plot showing the relationship among Japanese quince fruit samples and osmotic fluids vs. chemical parameters and biological activities. Ap apple, Pe pear, Pi pineapple, S sour cherry, Bl blackcurrant, and Ch chokeberry concentrates; B and A after dash stand for before and after; TPC, total phenolic content; PP, polymeric procyanidins; F-3-ols, flavan-3-ols; PA, phenolic acids; F, flavonols; A, anthocyanins; DP, degree of polymerization; FD, freeze-drying; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase



anticholinergic activity than non-OD and FD JQF, which in turn were closer to α -amylase and pancreatic lipase inhibiting activity. The third group was created from concentrate samples of fruits with dark flesh (cherry, blackcurrant, and chokeberry), TPC and α -glucosidase inhibitory activity. The content of flavan-3-ols correlated more strongly with the inhibition of α -glucosidase ($R^2 = 0.53$) than the content of phenolic acids ($R^2 = 0.13$), oppositely to the study of Wojdyło et al. (2017) on *Actinidia* fruits. In addition, anthocyanins and flavonols were found close to concentrate samples, the content of which was much higher than for other samples.

Conclusions

The study revealed that the osmotic dehydration of Japanese quince fruit in fruit concentrates resulted in a reduction of the moisture ratio by an average of 70% compared with the non-OD JQF. In addition, the use of the OD process resulted in a reduction of convection drying time by 60 min. The chokeberry concentrate reduced the final MR by 50% compared with the non-OD JQF, and the pineapple and sour cherry concentrates hindered the dehydration process during VMD. The use of OD in fruit concentrates generally resulted in darkening and warming of the color of the final product. OD JQF was characterized by an increased content of sugars (especially after using apple concentrate) and a significant reduction in the content of organic acids (more than a 4-fold reduction with the use of pear concentrate). TPC and ORAC antioxidant capacity decreased as a result of osmotic dehydration, but increased inhibition potential of α -amylase (blackcurrant concentrate) was obtained. It should also be noted that a significant increase in anticholinergic potential was found after

application of pear concentrate (AChE inhibition) and blackcurrant (BuChE) after the OD process. Osmotic fluids were also analyzed before and after the OD. A change in physical parameters (d_w , a_w , SSC, and viscosity) was found due to dilution during the OD, reduction of sugar content, and increase in organic acid concentration. Importantly, there was an increase in TPC and antioxidant and antidiabetic potential, regardless of the concentrate used. To sum up, the osmotic dehydration process significantly influenced the acceleration of the CVMD drying process, the decrease of the characteristic acidity of JQF, and the increase of anticholinergic potential, which was not observed for non-OD fruits. Osmotic fluids after dehydration were richer in phenolic compounds and were characterized by increased antioxidant and antidiabetic potential.

Code Availability Not applicable.

Authors' Contributions Igor Piotr Turkiewicz: formal analysis, data curation, writing—original draft, writing—review and editing, visualization; Aneta Wojdyło: supervision, conceptualization, writing—original draft, writing—review and editing, resources, funding acquisition; Karolina Tkacz: formal analysis; Krzysztof Lech: methodology, conceptualization; Paulina Nowicka: formal analysis

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Data Availability All data and materials are available from the authors

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Abbreviations *OD*, osmotic dehydration; *JQF*, Japanese quince fruit; *CD*, convective drying; *VMD*, vacuum-microwave drying; *CVMD*, convective-vacuum-microwave drying; *FD*, freeze-drying; *MR*, moisture ratio; *ORAC*, oxygen radical absorbance capacity; *TPC*, total phenolic content

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mgr inż. Igor Piotr Turkiewicz

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Mój udział w przygotowaniu tej publikacji polegał na kierowaniu projektem naukowym obejmującym badania opisane w tej pracy (Diamentowy Grant VII, nr DI2017 006347), współtworzeniu koncepcji prowadzonych badań, wykonaniu analiz fizykochemicznych, chromatograficznych i potencjału biologicznego *in vitro* produktów z owoców pigwowca. Opracowałem technologię otrzymywania suszy pigwowcowych, przygotowałem tekst publikacji, opracowałem merytorycznie otrzymane wyniki, przeprowadziłem dyskusję oraz współredagowałem odpowiedzi na recenzje.

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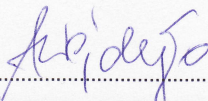
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51-630 Wrocław

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mój udział polegał na tworzeniu i nadzorze koncepcji projektu (Diamantowy Grant VII, nr DI2017 006347), w ramach którego realizowana była praca doktorska, uczestnictwie w analizach chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca i ich produktów, koordynowaniu prac Doktoranta, współredagowaniu publikacji i merytorycznej ocenie wyników.


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Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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mój udział polegał na współredagowaniu publikacji oraz wykonaniu części analiz potencjału biologicznego *in vitro*.



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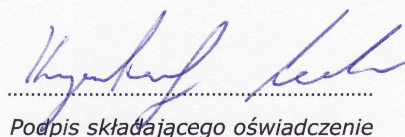
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mój udział polegał na przeprowadzeniu procesu odwadniania osmotycznego i przygotowaniu wraz z mgr. inż. Igozem Turkiewiczem suszy pigwowcowych według wcześniej przyjętej koncepcji technologii oraz pomoc w opracowaniu i wizualizacji wyników dotyczących kinetyki suszenia.



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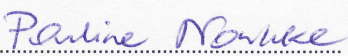
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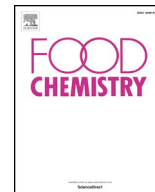
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mój udział polegał na analizie formalnej publikacji oraz identyfikacji związków polifenolowych metodą LC/MS.


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Publikacja 5

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The influence of different carrier agents and drying techniques on physical and chemical characterization of Japanese quince (*Chaenomeles japonica*) microencapsulation powder

Igor Piotr Turkiewicz^a, Aneta Wojdyło^{a,*}, Karolina Tkacz^a, Krzysztof Lech^b, Anna Michalska-Ciechanowska^a, Paulina Nowicka^a

^a Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chelmońskiego Street, 51-630 Wrocław, Poland

^b Institute of Agricultural Engineering, Wrocław University of Environmental and Life Sciences, 37-41 Chelmońskiego Street, 51-630 Wrocław, Poland

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ABSTRACT

Fruit powders can become a new and innovative direction of using the potential of Japanese quince (JQ) fruit in an affordable form. Therefore, physical (dry matter, true and bulk density, porosity and color) and chemical parameters of JQ juice powders obtained by using different carrier agents and drying techniques were evaluated. The juice was mixed with maltodextrin, inulin and a mixture of both in different proportions and dried using freeze, spray, and vacuum (50, 70, and 90 °C) drying techniques. The identification and quantification of phenolic compounds in JQ juice powders were performed by LC-PDA-QTOF-MS and UPLC-PDA, respectively, while antioxidant capacity was measured using ABTS, FRAP and ORAC assays. In addition, enzymatic *in vitro* inhibition tests of α -glucosidase, pancreatic lipase, acetylcholinesterase and 15-lipoxygenase were performed. Among the drying techniques applied, freeze-drying resulted in the highest retention of polyphenols, while among the carrier agents maltodextrin was found to be the best biopolymer for obtaining high-quality fruit powder and also ensured powders with the lowest content of undesirable hydroxymethylfurfural.

1. Introduction

The production of food powders is growing day by day. The global fruit powder market in 2017 was valued at \$13.52 billion and a compound annual growth rate (CAGR) of 7.4% is expected to be reached by 2025. Northern Europe and Asia are the two most important markets for fruit powders, while the growing interest in functional beverages has strengthened the position of the European market (Hexa Research, 2018).

The increase in consumer interest in forgotten or unpopular fruits, observed over the past two decades, has made *Chaenomeles* the object of interest for the food industry (Antoniewska, Rutkowska, & Adamska, 2017). Japanese quince (JQ) fruit is an example of a raw material with high processing potential, but at the same time the high content of acids disqualifies it from direct consumption (Nahorska, Dzwoniarska, & Thiem, 2014). The natural growth area of JQ fruits is the central and southern part of Japan, although it is cultivated in many other countries of the world (Lithuania, Latvia, Russia, Belarus, Ukraine, Sweden, Finland and Poland) (Jakobija & Bankina, 2018; Mihova, Kondakova, &

Mondeshka, 2012). For example, the total area of *Chaenomeles* plantations in Latvia was 200 ha in 2016 (Mihova et al., 2012). JQ fruit can be used as a rich source of polyphenols, the most largest fraction being proanthocyanidins (57.06 g/kg dry weight [dw], of which 70% is polymeric procyanidins), which is reflected in the significant antioxidant activity measured by the ORAC test (68.19 mmol Trolox/100 g dw) (Turkiewicz, Wojdyło, Lech, Tkacz, & Nowicka, 2019), and it exhibits a number of health-promoting activities (Strek et al., 2007; Zhang, Han, Zhang, & Xin, 2014).

Therefore, these fruits are a valuable raw material for which powder production can be an innovative method of application for food, increasing their final nutritional value and pro-health qualities. This is an interesting and future-oriented direction considering consumers who are becoming more health conscious and are focusing on increasing their nutritional intake to prevent chronic disorders resulting from nutrient deficiencies. Fruit powders can be used in the bakery and confectionery industry, dairy products, beverages, snacks, and others. Above all, beverages dominate the market owing to the high application of fruit powders in smoothies, fruit drinks, energy drinks, and

* Corresponding author.

E-mail address: aneta.wojdylo@upwr.edu.pl (A. Wojdyło).

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carbonated drinks. Enhanced water and ready-to-drink beverages are seeing an increased consumer demand (Hexa Research, 2018).

For many years, the most common drying technique has involved spraying liquid in a hot air stream, known as spray drying (SD). However, despite some of the mentioned advantages having been confirmed in research (Minh, 2019; Shishir et al., 2018; Tchabo et al., 2019), SD of juices containing significant amounts of low molecular weight sugars requires the use of carriers that lower the viscosity of the powder, thus increasing the efficiency of the process (Fazaeli, Emam-Djomeh, Ashtari, & Omid, 2012; Tonon, Freitas, & Hubinger, 2011). Nevertheless, attempts were also made to obtain fruit powders using other drying methods, such as freeze-drying (FD) (Michalska & Lech, 2018; Michalska, Wojdyło, Łysiak, & Figiel, 2017; Shishir et al., 2018), which is currently considered the best because of the largest retention of labile bioactive compounds (phenolic compounds, vitamin C) and color parameters (Turkiewicz et al., 2019). Nevertheless, the disadvantage of FD is the high cost of the process, slowness and the difficulty of using it on an industrial scale (Szychowski et al., 2018; Wojdyło, Nowicka, & Bąbelewski, 2018). Vacuum drying (VD) is a new method in the production of fruit powders that can become an alternative to SD and FD. This method allows one to reduce energy consumption by lowering the pressure and thus the temperature needed to evaporate the water. In addition, the use of VD can help in obtaining powders with better sensory values (than after SD) and with a higher nutritional value (Michalska et al., 2017).

In the case of fruit juices and concentrates, it is not enough to choose the most favorable drying method due to the specificity of these products. The high content of sugars and organic acids at the same time as high viscosity prevents direct pulverization. Therefore, various types of biopolymers are used as carrier agents altering the physicochemical properties of fruit powders. Such substances include maltodextrins, natural gums, proteins, waxes and fructooligosaccharides such as inulin, which will affect the physicochemical properties of fruit powders (Michalska et al., 2017). Maltodextrin (MA) is the most commonly used carrier due to its transparency, neutral smell and taste, good solubility and low price (Chong & Wong, 2017). Another biopolymer obtained mainly from chicory root (*Cichorium intybus*) is inulin (IN). Previous studies have reported that the most important properties of inulin are its high thermal stability, resistance to sticking, agglomeration, and crystallization (Leyva-Porras, López-Pablos, Alvarez-Salas, Pérez-Urizar, & Saavedra-Leos, 2015). The use of these carrier agents or their mixtures in different proportions can result in powders with different physical and chemical properties.

Therefore, the aim of this study was to investigate the influence of different carrier agents (inulin, maltodextrin and a mixture of them) and drying methods (spray, freeze and vacuum (SD, FD and VD) drying) on the physical parameters (dry matter, water activity, true and bulk density, porosity and color parameters), content of phenolic compounds, antioxidant capacity (ABTS, FRAP, ORAC) and enzymatic *in vitro* inhibition tests of α -glucosidase, pancreatic lipase, acetylcholinesterase and 15-lipoxygenase. The use of various biopolymers and drying techniques was aimed at choosing the best to ensure the highest quality of the final product.

2. Material and methods

2.1. Chemicals

All standards of polyphenolic compounds were purchased from Extrasynthese (Lyon, France). Water for chromatography analysis prepared by using an HPL SMART 1000 s system (HydroLab, Gdańsk, Poland), was additionally filtered through a 0.22 μ m membrane filter immediately before use. Acetonitrile and methanol for ultra-performance liquid chromatography (UPLC; Gradient grade) were from Merck (Darmstadt, Germany). Maltodextrin (dextrose equivalent: 20–30) and Amberlite® XAD-16 resin were supplied by Brenntag

(Kędzierzyn-Koźle, Poland) while inulin by Beneo-Orafti (Tienen, Belgium). The rest reagents and solvents for biological activities analysis were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Plant material and sample preparation

JQ fruits (*Chaenomeles japonica* ssp.) were obtained from cultivation in Lublin Province (Poland) at processing maturity in September 2018. The fruits (5 kg) were washed, pitted, and mixed using a Thermomix (Wuppertal, Vorkwek, Germany). The obtained mash was pressed by a laboratory hydraulic press (SRSE, Warsaw, Poland) and centrifuged for 10 min at room temperature at 5,000 g (Sigma 6 K15, Shrewsbury, UK). FQ juice was mixed with 15% (w/w) commercial inulin, maltodextrin and mix of those two (inulin: maltodextrin 2:1 and 1:2 w/w, respectively). All samples were subjected to different drying techniques and further analysis.

2.3. Drying methods

Freeze drying (FD) was carried out in freeze dryer (Christ Alpha 2–4; Braun Biotech Int., Melsungen, Germany) for 24 h at the pressure of 0.022 kPa. The temperature within the drying chamber was -30 °C, while the heating plate reached 30 °C. The FD sample was considered as the control sample. Spray drying (SD) was performed by Mini Buchi Spray-dryer B190 (Buchi, Flawil, Switzerland). The spray dryer was operated at an inlet temperature of 180 °C while the rate of feeding was 40 mL/min. Vacuum drying (VD) at 50, 70 and 90 °C was performed in a vacuum dryer VACUCELL 111 ECO LINE (MMM Medcenter Einrichtungen GmbH; Planegg, Germany) at a pressure of 1 kPa for, respectively, 72 h, 48 h and 24 h. Finally, 5 variants of drying were obtained. All process was made in technological duplicate.

2.4. Physical analysis

2.4.1. Dry matter content and water activity

The dry matter content (dm) of powders were performed in a vacuum dryer (SPT-200, ZEAMiL Horyzont, Kraków, Poland) at 80 °C for 72 h at the pressure of 1 kPa. The determination of water activity (a_w) was performed on the Novasina (LabMas-terav., Lachen, Switzerland) at 20 °C. The measurements were done in triplicate.

2.4.2. Color

The color of the samples was determined with reference to the International Commission on Illumination color space ($L^*a^*b^*$) using a spectrophotometer (CM-700d; Konica Minolta Sensing, Inc., Osaka, Japan). The measurements were done in triplicate and data were presented as an average.

2.4.3. True density, bulk density and porosity

True density (ρ_t), bulk density (ρ_b) and porosity (ϵ) were measured as reported previously by Michalska and Lech (2018). True density (g/cm^3) was calculated as a ratio of the sample mass (m) to its total volume (V_s), excluding the air pores according to Eq. (1):

$$\rho_t = \frac{m}{V_s} \quad (1)$$

Bulk density (g/cm^3) was calculated as a ratio of the sample mass (m) to its bulk volume (V_b), according to Eq. (2):

$$\rho_b = \frac{m}{V_b} \quad (2)$$

Porosity (%) of the powders was calculated using the relationship between the bulk (ρ_b) and the true density (ρ_t) as in Eq. (3):

$$\epsilon = \left(1 - \frac{\rho_b}{\rho_t}\right) \times 100 \quad (3)$$

The powders were weighed with an analytical balance with an accuracy of 0.0001 g (XA 60/220/X Radwag, Radom, Poland), the total volume was measured with an HumiPyc™/model 2 Gas Pycnometer (InstruQuest Inc., Coconut Creek, FL, USA) while bulk volume was measured with a graduated cylinder. The container was filled with the samples (10 ± 0.5 mL) and then gently shaken to obtain the smallest volume of the samples. All measurements were done in triplicate.

2.5. Chemical analysis

2.5.1. Identification of polyphenols by the LC-PDA-QTOF-MS method

The samples for polyphenol analysis were prepared as previously described by Turkiewicz et al. (2019). The presence of polyphenols in JQ juice powders was identified using Acquity UPLC system (Waters Corporation, Milford, USA) with a QToF mass spectrometer (Waters, Manchester, UK). An Acquity UPLC BEH C18 column (2.1×100 mm, $1.7 \mu\text{m}$; Waters Corp.) was used to perform the chromatographic separation previously described by Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). Samples ($5 \mu\text{L}$) were injected into a gradient system at a flow rate of 0.42 mL/min. The mobile phase consisted of 4.5% formic acid in deionized water (A) and acetonitrile (B). Samples were eluted according to a linear gradient: 0–12 min, 1–25% B; 12–12.5 min, 100% B; 12.5–13.5 min, 1% B. The analysis was prepared by ionization mode at negative ($\text{M}-\text{H}$)⁻ before and after fragmentation within mass scanning from m/z 100 to 1700. The data were collected by Mass-Lynx TM v 4.1 software.

2.5.2. Quantification of polyphenols, polymeric procyanidins and hydroxymethylfurfural (HMF) using the UPLC-PDA system

The analysis of polyphenolic compounds was carried out using the Acquity UPLC system (Waters Corp., Milford, USA). Polyphenolic compounds were monitored at the following wavelengths at 280 nm (flavan-3-ols), 320 nm (phenolic acids) and 360 nm (flavonols and flavanons). Quantification was achieved by injection of solutions of known concentrations ranging from 0.05 to 0.5 mg/mL ($R^2 \leq 0.9998$) of (-)-epicatechin, (+)-catechin, chlorogenic acid, quercetin, and kaempferol-3-O-glucoside, -galactoside, and -rutinoside, as standards.

Analysis of polymeric procyanidins was performed by phloroglucinolysis method as described previously by Wojdyło, Oszmiański, and Bielicki (2013). The analysis was carried out on a UPLC system Acquity (Waters Corp., Milford, MA, USA) consisting of a binary solvent manager, and fluorescence detector (FL). The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (-)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (-)-epicatechin-phloroglucinol adduct standards. To calculate the degree of polymerisation (DP) of polymeric procyanidins, the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts), corrected by subtracting the content of flavan-3-ol monomers separately (assayed by UPLC-FL), was divided by the sum of all flavan-3-ol monomers, again corrected by subtracting the content of flavan-3-ol monomers assayed separately. All incubations were done in triplicate. Results were expressed as mg per kg of dw.

The analysis of HMF was performed using the Acquity UPLC system (Waters Corp., Milford, USA) according to Turkiewicz et al. (2019). HMF was detected at 284 nm and quantification was achieved by injection of HMF standard solutions of known concentrations ranging from 0.05 to 2 $\mu\text{g}/\text{mL}$ ($R^2 \leq 0.9998$). All incubations were done in triplicate. Results were expressed as mg per kg of dw.

2.5.3. Analysis of antioxidant activities and enzymatic in vitro inhibition tests

The extracts for following analysis was prepared as described as follows: the sample (~ 0.5 g) was vortexed for 1 min with 7 mL methanol/water (80:20, v/v) with 1% hydrochloric acid mixture,

sonicated for 20 min (Sonic 6D; Polsonic, Warsaw, Poland) and left for 24 h at 4 °C. Then, the extract was sonicated again for 20 min, and centrifuged at $19.000 \times g$ for 10 min at 4 °C. Finally, the extract was filtered by $0.20 \mu\text{m}$ hydrophilic PTFE membrane (Millex Simplicity Filter; Merck, Germany) and used.

Antioxidant activities were determined using the ABTS method, FRAP and ORAC as previously described by Turkiewicz et al. (2019) protocols. All samples were assayed in triplicate and the results were expressed as mmol of Trolox per 100 g of dw.

The inhibition of α -glucosidase, pancreatic lipase and acetylcholinesterase were measured as reported previously by Turkiewicz, Wojdyło, Tkacz, Nowicka, and Hernández (2019) while 15-lipoxygenase inhibiting activity was investigated according to Chung et al. (2009). All samples were assayed in triplicate and the result was expressed as IC_{50} (mg of dried sample per mL of enzyme) while for 15-lipoxygenase inhibition activity in % of inhibition.

All spectrophotometric and spectrofluorometric measurements were performed using a plate reader Synergy H1 (BioTek Instruments, Inc., Winooski, VT, USA).

2.6. Statistical analysis

Statistical analysis was conducted using XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France). Significant differences ($p \leq 0.05$) between means were evaluated by one-way ANOVA and Duncan's multiple-range test. Principal components analysis (PCA) also has been done.

3. Results and discussion

3.1. Physical analysis

The dry matter content (dm) in JQ juice powders present in Table 1. In general, both the used biopolymer and the drying method had a significant impact on the content of dm in JQ juice powders ($p \leq 0.05$). In general, maltodextrin powders had an average highest dry matter content and the addition of inulin caused an increase in humidity. Differences in dm content in the analyzed powders may result, among other factors, from different sorption capacities (Michalska & Lech, 2018). Analyzing the impact of the drying method on the dry matter content, it was found that the powders obtained as a result of VD at 50 °C were characterized by the lowest dm value, and an increase in temperature to 90 °C allowed the driest powders to be obtained. Of all drying methods, the highest temperature (180 °C) was used in SD, but nevertheless the average dm values were lower than for VD 90 °C. In addition, the drying time for FD and VD 90 °C was the same, and the dm values after FD were much lower.

Bacteria, yeast and molds cannot grow in products with low water activity (a_w), which is why this parameter is an important issue affecting the microbiological stability of powders and other food products (Turkiewicz et al., 2019). Water activity results (Table 1) are the lowest for inulin powder after FD and the highest for IN:MA 1:2 after VD at 50 °C. Analyzing the effect of the carrier agent (inulin, maltodextrin and mixtures thereof), no significant differences ($p > 0.05$) were found in the mean a_w values for the fruit powders. In the case of drying methods, the lowest average values of a_w were observed after FD, while the highest average values of a_w were observed for powders after VD at 50 °C. The a_w values of apple powders in the research of Michalska and Lech (2018) were on average 2 times higher than those obtained in this study. Moreover, SD made it possible to obtain powders with lower values of a_w than after FD. These differences may result, among other factors, from the difference in the chemical composition of the fruit juices (higher sugar content in apple juice), which also translated into differences in porosity. Compared to fresh juice with $a_w = 0.878$, the water activity of JQ juice powders was reduced on average by 90%.

The density of bulk materials, particles and powders is an important

Table 1
Physical parameters of Japanese quince fruit juice powders.

Sample	Drying method	Dry matter (%)	a _w	True density ρ _t (g/cm ³)	Bulk density ρ _b (g/cm ³)	Porosity ε (%)	Color parameters			
							L*	a*	b*	
Juice fresh	–	14.19 ± 0.10	0.878 ± 0.00	–	–	–	62.72 ± 0.10	0.80 ± 0.01	27.69 ± 0.01	
	IN (100%)	97.92 ± 0.01 ^{de}	0.133 ± 0.000 ^{cd}	1.387 ± 0.010 ^{def}	0.644 ± 0.000 ^e	53.53 ± 1.10 ^f	97.58 ± 0.01 ^c	–1.51 ± 0.01 ^m	4.53 ± 0.01 ^t	
IN	FD	96.40 ± 0.13 ⁱ	0.072 ± 0.000 ⁱ	1.540 ± 0.010 ^{ab}	0.388 ± 0.010 ^m	74.78 ± 2.08 ^s	93.49 ± 0.01 ^d	–5.92 ± 0.01 ^v	11.95 ± 0.05 ^s	
	SD	96.46 ± 0.12 ⁱ	0.107 ± 0.000 ^{fg}	1.347 ± 0.000 ^{ef}	0.480 ± 0.000 ^k	64.40 ± 1.05 ^d	73.79 ± 0.01 ^q	0.71 ± 0.00 ^f	12.05 ± 0.01 ^r	
	VD 50 °C	94.93 ± 0.18 ^m	0.104 ± 0.000 ^{fg}	1.545 ± 0.010 ^{ab}	0.643 ± 0.010 ^e	58.38 ± 1.08 ^l	83.56 ± 0.01 ^m	–1.58 ± 0.01 ⁿ	23.99 ± 0.01 ^d	
	VD 70 °C	95.44 ± 0.04 ^{kl}	0.108 ± 0.010 ^{fg}	1.557 ± 0.000 ^a	0.645 ± 0.000 ^e	58.57 ± 1.00 ^k	70.79 ± 0.01 ^s	0.31 ± 0.01 ^s	19.60 ± 0.01 ⁱ	
	VD 90 °C	98.31 ± 0.04 ^c	0.094 ± 0.000 ^{ghi}	1.601 ± 0.000 ^a	0.679 ± 0.000 ^{cd}	57.58 ± 1.00 ⁿ	60.45 ± 0.01 ^v	3.61 ± 0.01 ^b	15.40 ± 0.01 ^o	
	MA (100%)	95.35 ± 0.06 ^l	0.397 ± 0.010 ^a	1.230 ± 0.010 ^h	0.472 ± 0.000 ^{cd}	61.61 ± 1.01 ^g	98.03 ± 0.00 ^a	–1.24 ± 0.01 ^j	2.74 ± 0.01 ^w	
	FD	96.50 ± 0.06 ^l	0.101 ± 0.000 ^{efgh}	1.566 ± 0.100 ^a	0.564 ± 0.010 ^{hi}	63.96 ± 2.10 ^e	90.42 ± 0.02 ^f	–5.37 ± 0.01 ^t	15.30 ± 0.01 ^p	
	SD	97.57 ± 0.14 ^g	0.094 ± 0.000 ^{ghi}	1.397 ± 0.000 ^{de}	0.520 ± 0.010 ⁱ	62.76 ± 1.90 ^f	83.63 ± 0.01 ^h	0.09 ± 0.01 ^h	20.02 ± 0.01 ^h	
	VD 50 °C	96.39 ± 0.06 ^l	0.114 ± 0.000 ^{def}	1.555 ± 0.010 ^a	0.696 ± 0.000 ^e	55.24 ± 1.04 ^f	85.04 ± 0.00 ⁱ	–1.91 ± 0.01 ^p	22.38 ± 0.01 ^f	
	VD 70 °C	98.00 ± 0.09 ^d	0.107 ± 0.010 ^{fg}	1.589 ± 0.010 ^a	0.622 ± 0.010 ^f	60.89 ± 1.00 ⁱ	82.91 ± 0.01 ⁿ	–1.78 ± 0.01 ^o	26.63 ± 0.01 ^a	
VD 90 °C	99.01 ± 0.14 ^a	0.092 ± 0.000 ^{ghi}	1.596 ± 0.100 ^a	0.670 ± 0.000 ^d	58.05 ± 1.05 ^m	64.28 ± 0.00 ^u	2.25 ± 0.01 ^c	16.22 ± 0.01 ^m		
IN : MA (1:2)	IN : MA (1:2)	94.93 ± 0.00 ^m	0.387 ± 0.050 ^{ab}	1.273 ± 0.000 ^{gh}	0.548 ± 0.000 ^f	57.00 ± 1.20 ^o	98.02 ± 0.01 ^k	–1.37 ± 0.01 ^k	3.34 ± 0.01 ^v	
	FD	95.61 ± 0.13 ^k	0.109 ± 0.060 ^{hi}	1.534 ± 0.020 ^{ab}	0.591 ± 0.010 ^g	61.47 ± 1.07 ^h	89.17 ± 0.01 ^s	–4.21 ± 0.01 ^s	16.81 ± 0.01 ⁱ	
IN : MA (2:1)	SD	97.24 ± 0.28 ^h	0.098 ± 0.000 ^{efgh}	1.427 ± 0.000 ^{cd}	0.515 ± 0.000 ^j	63.89 ± 1.60 ^e	86.49 ± 0.01 ^h	0.09 ± 0.01 ^h	18.05 ± 0.00 ^k	
	VD 50 °C	94.48 ± 0.13 ⁿ	0.154 ± 0.010 ^e	1.576 ± 0.010 ^a	0.696 ± 0.020 ^e	55.84 ± 1.04 ^q	82.86 ± 0.01 ^o	–2.10 ± 0.01 ^r	23.85 ± 0.01 ^e	
	VD 70 °C	97.77 ± 0.03 ^{ef}	0.096 ± 0.000 ^{efghi}	1.578 ± 0.100 ^a	0.741 ± 0.030 ^{ab}	53.01 ± 1.31 ^u	80.34 ± 0.01 ^p	–0.75 ± 0.01 ⁱ	25.11 ± 0.01 ^b	
	VD 90 °C	98.73 ± 0.19 ^b	0.111 ± 0.010 ^{def}	1.570 ± 0.000 ^a	0.755 ± 0.010 ^f	51.94 ± 0.00 ^v	65.01 ± 0.01 ^t	1.81 ± 0.01 ^d	14.99 ± 0.01 ^q	
	IN : MA (2:1)	94.31 ± 0.18 ⁿ	0.368 ± 0.010 ^b	1.321 ± 0.100 ^{fg}	0.575 ± 0.000 ^{gh}	56.44 ± 1.24 ^p	97.90 ± 0.01 ^b	–1.47 ± 0.01 ^u	3.75 ± 0.01 ^u	
	FD	96.14 ± 0.18 ^j	0.084 ± 0.000 ^{ghi}	1.572 ± 0.000 ^a	0.558 ± 0.000 ^{hi}	64.48 ± 1.70 ^e	91.99 ± 0.01 ^e	–5.41 ± 0.01 ^u	15.57 ± 0.01 ⁿ	
IN : MA (2:1)	SD	97.41 ± 0.19 ^{gh}	0.099 ± 0.000 ^{efghi}	1.386 ± 0.010 ^{def}	0.430 ± 0.010 ^j	68.94 ± 1.04 ^b	85.02 ± 0.01 ^j	0.83 ± 0.00 ^e	19.01 ± 0.01 ^j	
	VD 50 °C	92.34 ± 0.10 ^o	0.120 ± 0.010 ^{de}	1.472 ± 0.000 ^{bc}	0.592 ± 0.000 ^g	59.96 ± 1.50 ^j	84.22 ± 0.01 ^k	–2.00 ± 0.01 ^q	24.85 ± 0.01 ^c	
	VD 70 °C	94.36 ± 0.00 ⁿ	0.112 ± 0.010 ^{def}	1.552 ± 0.000 ^a	0.566 ± 0.000 ^d	57.04 ± 1.04 ^o	73.47 ± 0.01 ^r	0.09 ± 0.01 ^h	20.57 ± 0.00 ^g	
	VD 90 °C	98.44 ± 0.14 ^c	0.104 ± 0.000 ^{efg}	1.576 ± 0.010 ^a	0.731 ± 0.000 ^b	53.65 ± 1.05 ^s	60.37 ± 0.01 ^w	3.92 ± 0.01 ^a	15.41 ± 0.01 ^o	
	Duncan's Multiple Range Test for mean values									
	FD	96.16 ± 0.10 ^c	0.083 ± 0.020 ^e	1.553 ± 0.100 ^{ab}	0.525 ± 0.200 ^f	66.17 ± 1.65 ^a	91.27 ± 0.01 ^a	–5.23 ± 0.01 ^e	14.91 ± 0.01 ^c	
SD	97.17 ± 0.11 ^b	0.100 ± 0.110 ^b	1.389 ± 0.110 ^c	0.486 ± 0.320 ^d	65.00 ± 1.07 ^a	82.23 ± 0.01 ^b	0.43 ± 0.01 ^b	17.28 ± 0.01 ^b		
VD 50 °C	94.53 ± 0.19 ^d	0.123 ± 0.010 ^b	1.537 ± 0.100 ^b	0.657 ± 0.190 ^b	57.31 ± 1.09 ^b	83.92 ± 0.01 ^b	–1.90 ± 0.01 ^d	23.77 ± 0.01 ^a		
VD 70 °C	96.39 ± 0.22 ^c	0.106 ± 0.100 ^b	1.569 ± 0.120 ^{ab}	0.669 ± 0.100 ^b	57.38 ± 1.11 ^b	76.88 ± 0.01 ^c	–0.53 ± 0.01 ^c	22.98 ± 0.01 ^a		
VD 90 °C	98.62 ± 0.29 ^a	0.100 ± 0.050 ^b	1.586 ± 0.130 ^a	0.709 ± 0.180 ^a	55.31 ± 1.49 ^b	62.53 ± 0.01 ^d	2.90 ± 0.01 ^a	15.51 ± 0.01 ^c		
IN	96.31 ± 0.09 ^{bc}	0.097 ± 0.050 ^a	1.518 ± 0.100 ^a	0.567 ± 0.120 ^c	62.74 ± 1.09 ^a	76.42 ± 0.01 ^b	–0.57 ± 0.01 ^a	16.60 ± 0.00 ^b		
MA	97.49 ± 0.16 ^a	0.102 ± 0.000 ^a	1.541 ± 0.110 ^a	0.614 ± 0.120 ^b	60.18 ± 1.19 ^b	81.26 ± 0.02 ^a	–1.35 ± 0.01 ^a	20.11 ± 0.01 ^a		
IN :MA (1:2)	96.76 ± 0.13 ^b	0.107 ± 0.010 ^a	1.537 ± 0.100 ^a	0.560 ± 0.110 ^a	57.23 ± 1.29 ^c	80.77 ± 0.01 ^a	–1.03 ± 0.01 ^b	19.76 ± 0.01 ^a		
IN :MA (2:1)	95.74 ± 0.04 ^c	0.104 ± 0.000 ^a	1.511 ± 0.200 ^a	0.596 ± 0.090 ^{bc}	60.77 ± 1.14 ^b	79.01 ± 0.01 ^a	–0.51 ± 0.05 ^a	19.08 ± 0.01 ^a		

IN–inulin; MA–maltodextrin; FD–freeze drying; SD–spray drying; VD–vacuum drying at 50, 70 and 90 °C; ± –standard deviation; a–w—different lowercase letters within the column in the group of powders indicate significant differences between samples (p ≤ 0.05) according to Duncan's test.

Table 2
Identification of phenolic compounds in Japanese quince fruit juice using LC–PDA–QTOF–MS.

Peak	Compound	R _t (min)	λ _{max} (nm)	MS [M – H] [–] (m/z)	MS/MS (m/z)
1	5-O-Caffeoylquinic acid (chlorogenic acid)	3.86	246/326	353.09	191.06
2	p-Coumaroyl hexoside	4.01	316/326	325.09	163.04/145.03
3	Procyanidin B1	4.07	280	577.13	425.08/289.05
4	Procyanidin trimer	4.13	280	865.20	577.13
5	Procyanidin B2	4.26	280	577.13	456.15/425.09
6	Procyanidin dimer	4.41	280	577.13	425.08/289.05
7	(-)-Epicatechin	4.74	240/280	289.06	245.08/187.05
8	Procyanidin C1	5.06	280	865.21	565.21/403.16/289.07
9	Procyanidin trimer	5.21	280	865.20	695.16/525.10/289.07
10	Procyanidin tetramer	5.39	280	1153.26	865.20/720.16/289.07
11	Procyanidin trimer	5.54	280	865.20	695.16/525.10/289.07
12	Syringic acid hexoside derivative	5.85	353	403.16	241.10/197.12
13	Procyanidin dimer	6.39	280	577.13	456.15/425.09/289.09
14	Procyanidin dimer	6.48	280	577.13	456.15/425.09/289.09

property affecting the use and function of many materials. The true density (ρ_t) did not differ significantly between JQ juice powders in the context of the used biopolymer (Table 1). The average ρ_t value for variants with inulin was 1.5180 g/cm³, and for those with maltodextrin 1.5410 g/cm³. The effect of drying, and thus the temperature and duration of the process, had a significant impact on the true density ($p \leq 0.05$). SD contributed to obtaining powders with the lowest average ρ_t value, while VD at 90 °C resulted in obtaining the highest value. The results show a relationship with the dry matter content. The higher the dm value, the higher the true density (Koc, Eren, & Ertekin, 2008). Bulk density (ρ_b) is a characteristic of a volume of divided material such as powders, grains, and granules. In contrast to the true density, the carrier agents had significant importance for ρ_b values. Inulin powders had an average lowest bulk density and the highest with mixed biopolymers with a predominance of maltodextrin. Favorable, high average values of bulk density were obtained during VD at 90 °C. Moreover, the highest bulk density values were recorded after VD (regardless of the drying temperature) compared to FD and SD (Caparino et al., 2012; Michalska & Lech, 2018; Michalska, Wojdyło, Lech, Łysiak, & Figiel, 2016). This dependence is due to the fact that after VD a powder with a more crystalline structure is obtained, i.e. with a smaller volume than after FD and SD, where finally the powders are characterized by a porous flat surface with occluded air content. The costs of packaging and transport increase with decreasing values of bulk density (Michalska et al., 2017). Therefore, SD turned out to be the least financially advantageous, obtaining the lowest average ρ_b values (0.4860 g/cm³). Another parameter describing the physical properties of loose materials is porosity (ϵ). Among the carrier agents there was diversity in porosity ($p \leq 0.05$). The highest porosity characterized powders with inulin and the addition of maltodextrin caused a decrease in the porosity of the analyzed powders. Comparing selected drying methods, generally FD and SD resulted in higher porosity of powders (up to 20%) compared to VD (regardless of drying temperature). Higher porosity will be associated with, among others, greater water absorption of the material, while the VD process will result in a product with a compact, hard structure and lower solubility (Michalska et al., 2016).

The chromatic parameters L*, a*, b* of fresh JQ juice were 62.72, 0.80 and 27.69, respectively and for other samples present in Table 1. The L* parameter values ranged from 60.37 (IN:MA 2:1 after VD 90 °C) to 98.03 (MA), reaching a very high value almost equal to the brightness of pure white. Analyzing the effect of the carrier agents ($p \leq 0.05$) on the L* parameter value, the highest average L* values were observed for powders with maltodextrin, and with increasing inulin in the blend, the color darkened to the value of 76.42 for pure biopolymer. Powder brightness differed significantly ($p \leq 0.05$) for selected drying methods. The highest average L* value was obtained for FD and the use of 90 °C in VD caused a decrease of almost one-third, contrary to Michalska et al. (2017). Powders obtained as a result of SD and those

after VD 50 °C did not differ in terms of the discussed equalizer, despite the difference in the drying temperature used in these two methods. Obtaining the darkest powders by VD 90 °C is associated with partial charring of the material and a significant reduction in the L* value. In general, FD and SD resulted in products with a lighter color than those obtained as a result of VD. A similar observation was made in the case of apple powders (Michalska & Lech, 2018). Shortening the drying time and eliminating the influence of oxygen affects the brightening of the color. Values of parameter a* ranged from –5.92 (IN after FD) to 3.92 (IN:MA 2:1 after VD 90 °C). Of all powders, the lowest values of the attribute a* were noted when inulin and the FD process were used, which indicates a tendency to a greenish color. An increase in VD temperature by 40 degrees caused the a* parameter to increase by almost 5 units and thus the color shifted towards a red tone. The use of maltodextrin contributed to obtaining powders with greater proportion of blue, while inulin caused yellowing of the color. In turn, the value of blue-yellow coordinate b*, FD and VD at 90 °C allowed more yellow powders to be obtained compared to VD at 50 °C, which caused color cooling towards a blue color. Contrary to the results obtained by Michalska and Lech (2018) for apple powders, the increase in temperature in VD caused a decrease in the value of parameter b*. Due to the characteristic yellow color of JQ fruit and juice, higher values of the parameter b* will be more favorable; thus in this context it seems appropriate to choose freeze-drying and inulin as the carrier agent.

3.2. Chemical analysis

3.2.1. Identification and quantification of phenolic compounds

About 14 compounds were identified by using LC–PDA–MS/QTOF, mainly flavan-3-ols and phenolic acid derivatives as a minor components in analyzed JQ juice powders. Compounds (peaks 1–14) were numbered by their order of elution and are displayed in Table 2 and Fig. 1. The identifications were made according to PDA spectra, MS and MS/MS data, and molecular ions [M – H][–]; data available in the scientific literature and authentic standards were also used. Additionally, some derivatives were putatively assigned, on the basis of analogous fragmentations.

Peak 1 exhibited [M – H][–] ions at m/z 353.09 and an MS/MS fragment at m/z 191.06 was characterized as 5-O-caffeoylquinic acid by comparison with analytical standards. The presence of chlorogenic acid in JQ fruit is also confirmed by previous studies (Du et al., 2013; Teleszko & Wojdyło, 2015; Zhang et al., 2018). Peak 2 showed [M – H][–] ions at m/z 325.09 and MS/MS fragments at m/z 145.03 (base peak; [coumaric acid-H₂O][–]) and 163.04 (-162 Da; hexosyl moiety) and thus could be identified as p-coumaroyl hexoside

Five procyanidins dimers (peaks 3, 5, 6, 13 and 14) were detected at different retention times in the ESI-QTOF in negative ion mode. All compounds gave the same [M – H][–] parental ion at m/z 577.13. Peak 3)

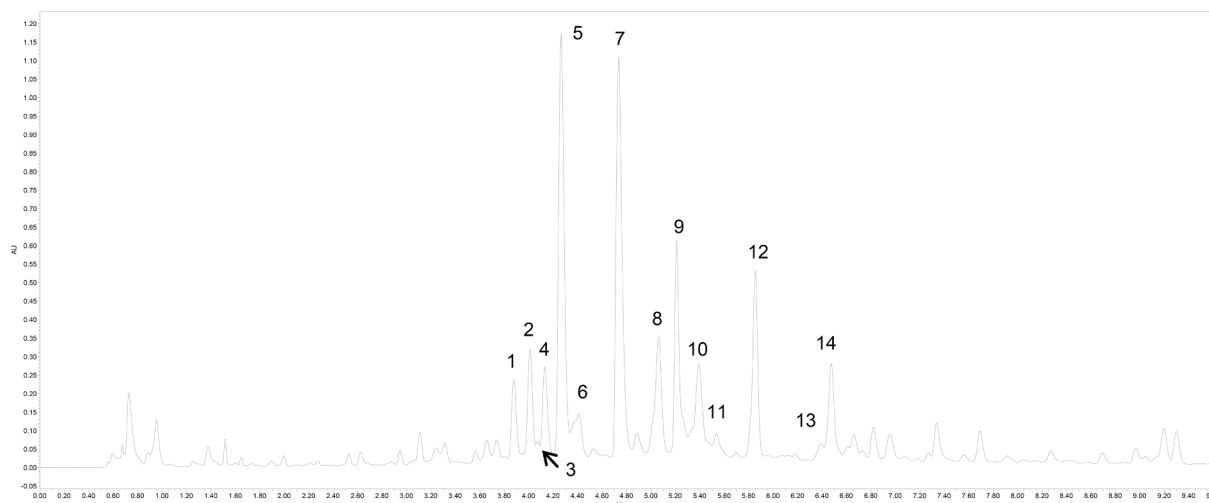


Fig. 1. UPLC-PDA chromatogram at 280 nm of Japanese quince juice. Peak number identities are displayed in Table 2.

showed fragment ions at m/z 425.08 ($[M-H-152]^-$; loss of $C_8H_8O_3$) and 289.05 ($[M-H-288]^-$; loss of $C_{15}H_{12}O_6$), which indicate the loss of a catechin unit. Comparing the retention time and MS data with the standard, this compound was assigned as procyanidin B1. The MS/MS spectrum of peak 5 yielded ions at m/z 456.15 and 425.09. Based on literature data (Teleszko & Wojdyło, 2015; Zhang et al., 2018) and after comparison with an authentic standard, this compound was identified as procyanidin B2. Peak 6 with product ions at m/z 425.08 and 289.05 has been proposed to be a procyanidin dimer (Owczarek et al., 2017). Two peaks (13 and 14) displayed the same molecular ion at m/z 577.13 and product ions at m/z 456.15, 425.08 and 289.05. The resulting fragment ions indicate the successive reduction of the flavan-3-ol skeleton. Therefore, those compounds were characterized as procyanidin dimers. Peak 4 exhibited a deprotonated molecule at m/z 865.20 and an MS/MS fragment at m/z 577.13, indicating the presence of three catechin units in the chemical structure. Thus, this compound was tentatively suggested to be a procyanidin trimer (Du et al., 2013; Owczarek et al., 2017). Additionally, Peaks 9 and 11 also showed a molecular ion at m/z 865.20. Each of these compounds had fragmentation ions at m/z 695.16, 525.10 and 289.07. Thus those compounds were identified as procyanidin trimers by comparison of their fragmentation behavior with previous research (Spínola & Castilho, 2017). Peak 7 displayed the $[M-H]^-$ parental ion at m/z 289.06 along with characteristic fragment ions at m/z 245.08 and 187.05. The retention time has been compared with the standards, and this compound has been assigned to (-)-epicatechin. Additionally, this compound was reported in JQ before by Owczarek et al. (2017), Du et al. (2013), Teleszko and Wojdyło (2015) and Lewandowska et al. (2013). Peak 8 exhibited a deprotonated molecule at m/z 865.21 and MS/MS fragments at m/z 565.21, 403.16 and 289.07. The comparison analysis with standards confirmed that this signal came from procyanidin C1, which additionally express in other fruits (Wojdyło & Nowicka, 2019). Peak 10 displayed an $[M-H]^-$ ion at m/z 1153.26 and its MS/MS spectrum yielded fragment ions at m/z 865.20 ($[M-H-288]^-$; loss of $C_{15}H_{12}O_6$), 720.16 and 289.07 ($[M-H-864]^-$), indicating loss of a procyanidin trimer. Therefore, this compound based on literature data and the MS fragmentation pattern was tentatively identified as a procyanidin tetramer (Owczarek et al., 2017). Finally, Peak 12 with a precursor ion at m/z 403.16 and observation of MS/MS fragmentation ions at m/z 197.12 ([syringic acid-H] $^-$) and at m/z 241 (from the loss of a hexose) pointed to syringic acid hexoside derivative (Barros, Dueñas, Pinela, Carvalho, Buelga, & Ferreira, 2012; Spínola & Castilho, 2017). It bears mentioning that this compound has not previously been reported in plants of *Chaenomeles*.

The content of each polyphenol compound was calculated using UPLC-PDA analysis. The total phenolic content (TPC) in JQ juice

powders obtained using different carrier agents and drying methods ranged from 1359.9 to 133.2 mg/100 g dw for, respectively, MA after FD and IN:MA 2:1 after VD at 90 °C (Tables 3 and 4). The main group (over 80% of all phenolics) comprised flavan-3-ols consisting of 11 catechin derivatives identified by LC-PDA-MS/QTOF. Procyanidin trimers, procyanidin B2 and C1 (17, 14 and 11% of TPC, respectively) were predominant compounds in the flavan-3-ol group, which is in line with previous reports (Nahorska et al., 2014). Polymers of procyanidins determined in the phloroglucinolysis assay account for 17% and phenolic acids account for 12% of the total polyphenol content. Among phenolic acids, the largest amount of syringic acid hexoside derivative (11%) was identified, while chlorogenic acid and *p*-coumarylhexose were found in similar concentrations, reaching respectively 11 and 7% of the sum of phenolic acids. The total content of phenolic compounds in the juice was 3056.7 mg/100 g dw, of which flavan-3-ols accounted for 58% (1792.2 mg/100 g dw). The selected drying methods had a significant impact on the phenolic content ($p \leq 0.05$). Comparing the influence of the drying method on the average content of phenolic compounds in JQ juice powders, they can be ranked by decreasing content: FD > SD > VD 50 °C > VD 70 °C > VD 90 °C. The highest TPC content was found in powders after FD, and VD at 90 °C in relation to FD caused their reduction by as much as 87%. For comparison, Horszwald, Julien, and Andlauer (2013) for chokeberry powders obtained by three methods (FD, SD and VD at 40, 60 and 80 °C) did not find significant differences ($p > 0.05$) between the values of total phenolic content. In the case of compounds from the flavan-3-ol group, no statistically significant differences in the concentrations of these compounds were observed using FD and SD, while an increase in temperature in VD from 50 to 70 °C caused a decrease in the content of flavan-3-ols by 50%. The content of phenolic acids in powders subjected to SD was two times lower than after FD and VD at 50 °C, contrary to results obtained by Michalska et al. (2016), where there were no differences ($p > 0.05$) between phenolic acid content in plum powders after FD and VD at 60 °C. The content of polymeric procyanidins changed analogically as in the case of phenolic acids, reaching the average highest value for FD and the lowest after VD at 90 °C. The average degree of polymerization (DP) for powders obtained in the freeze-drying process was 1.5, while VD at 90 °C resulted in its almost 2.5-fold increase. The degree of polymerization of procyanidins is responsible for their bitter taste, and the taste may deteriorate with its increase. In general, freeze-drying provided the largest retention of phenolic compounds, while VD, regardless of the temperature used, caused their greatest degradation. Turkiewicz et al. (2019), who investigated the influence of different drying methods on the quality of JQ fruit, also found that freeze-drying allows one to obtain the highest

Table 3
Content of phenolic compounds and hydroxymethylfurfural (HMF) (mg/100 g dm) in Japanese quince fruit juice and powders.

Phenolic compounds	Peak	IN				MA								
		FD	SD	VD	90 °C	70 °C	90 °C	FD	SD	VD 50 °C	VD 70 °C	VD 90 °C		
Flavan-3-ols	3	10.0 ± 0.2	0.4 ± 0.1 ⁿ	4.6 ± 0.2 ^f	0.1 ± 0.0 ^k	3.4 ± 0.1 ^h	0.1 ± 0.0 ^o	4.9 ± 0.2 ^g	3.1 ± 0.0 ⁱ	4.1 ± 0.0 ^l	4.8 ± 0.2 ^g	0.4 ± 0.0 ⁿ		
	4	93.6 ± 7.1	54.2 ± 5.5 ^d	34.8 ± 2.9 ^g	23.0 ± 1.1 ^j	14.8 ± 1.3 ^k	23.0 ± 1.1 ^j	51.9 ± 2.0 ^d	30.7 ± 1.6 ^h	35.7 ± 1.3 ^f	28.6 ± 2.5 ^{hi}	31.9 ± 3.3 ^{gh}		
	5	538.1 ± 12.2	272.3 ± 11.6 ^a	110.7 ± 6.1 ^h	2.5 ± 0.0 ^p	10.0 ± 0.4 ^{no}	2.5 ± 0.0 ^p	256.2 ± 5.0 ^b	101.9 ± 1.8 ^g	101.9 ± 1.8 ^g	119.5 ± 1.8 ^g	7.8 ± 0.0 ^o		
	6	81.1 ± 1.0	39.8 ± 0.6 ^b	156.6 ± 7.4 ^a	7.9 ± 0.1 ^{ij}	1.2 ± 0.1 ⁱ	7.9 ± 0.1 ^{ij}	70.8 ± 1.7 ^f	117.0 ± 10.0 ^c	117.0 ± 10.0 ^c	141.0 ± 1.7 ^b	5.1 ± 0.0 ^j		
	7	308.2 ± 12.5	157.8 ± 10.0 ^b	34.0 ± 2.7 ^k	5.3 ± 0.1 ^p	5.3 ± 0.1 ^p	2.2 ± 0.0 ^r	161.3 ± 16.9 ⁿ	64.4 ± 1.4 ⁱ	89.8 ± 1.8 ^e	83.6 ± 0.7 ^f	1.2 ± 0.1 ^{qr}		
	8	178.2 ± 11.6	88.5 ± 2.0 ^f	153.7 ± 9.8 ^a	28.4 ± 0.2 ⁱ	10.9 ± 0.9 ^{kl}	28.4 ± 0.2 ⁱ	87.0 ± 2.8 ^e	128.5 ± 1.9 ^d	138.0 ± 2.8 ^c	95.5 ± 1.6 ^c	40.9 ± 1.8 ⁱ		
	9	236.4 ± 9.9	132.1 ± 9.9 ^e	366.6 ± 12.9 ^a	118.3 ± 2.6 ^k	58.9 ± 2.6 ^k	18.3 ± 3.5 ⁿ	131.1 ± 1.7 ^{gh}	261.2 ± 8.8 ^d	257.8 ± 2.9 ^e	128.7 ± 1.9 ^h	35.5 ± 1.1 ⁱ		
	10	170.2 ± 8.4	102.7 ± 7.8 ^e	36.7 ± 4.4 ^b	258.6 ± 13.4 ^a	43.9 ± 1.5 ^f	0.6 ± 0.0 ^o	155.8 ± 9.9 ^d	27.5 ± 0.5 ^j	24.4 ± 0.5 ^k	11.2 ± 0.1 ^l	5.1 ± 0.2 ^{mn}		
	11	34.9 ± 1.8	10.5 ± 0.6 ^f	70.2 ± 2.6 ^a	33.4 ± 0.6 ^c	4.0 ± 0.1 ^{hi}	0.5 ± 0.0 ^{op}	21.9 ± 0.3 ^h	38.4 ± 0.3 ^d	25.1 ± 0.6 ^k	1.6 ± 0.0 ^{mo}	2.4 ± 0.0 ^{mn}		
	13	20.9 ± 1.6	7.2 ± 0.4 ^f	11.7 ± 0.5 ^{def}	11.4 ± 0.1 ^{sig}	11.8 ± 0.1 ^{sig}	4.1 ± 0.4 ^m	10.6 ± 0.1 ^{gh}	11.0 ± 0.0 ^{gh}	13.2 ± 0.1 ^{bc}	13.9 ± 0.1 ^b	14.7 ± 0.3 ^a		
	14	120.6 ± 5.0	50.6 ± 4.6 ^e	45.1 ± 3.7 ^{gh}	46.0 ± 7.3 ^f	41.7 ± 1.3 ^k	32.9 ± 1.4 ⁱ	63.2 ± 0.4 ^h	43.0 ± 0.4 ^{ij}	55.3 ± 0.9 ^d	56.2 ± 0.5 ^d	49.9 ± 0.0 ^{ef}		
	Sum	1792.2 ± 22.8	916.1 ± 16.0 ^e	993.6 ± 19.4 ^b	634.8 ± 17.0 ^f	205.8 ± 6.8 ^o	118.5 ± 8.8 ^f	1014.9 ± 11.9 ⁿ	826.7 ± 14.2 ^h	904.1 ± 18.8 ^f	582.2 ± 9.9 ^k	195.0 ± 8.7 ^g		
	Phenolic acids	1	25.0 ± 0.6	12.5 ± 0.9 ^b	13.3 ± 0.6 ^a	10.1 ± 0.1 ^f	10.1 ± 0.1 ^f	13.3 ± 0.1 ^a	12.2 ± 0.2 ^{bc}	12.1 ± 0.5 ^{bc}	11.5 ± 0.2 ^f	10.7 ± 0.0 ^h		
		2	18.0 ± 0.5	8.9 ± 0.2 ^b	8.6 ± 0.2 ^{bc}	8.6 ± 0.2 ^{bed}	6.7 ± 0.0 ^h	3.6 ± 0.1 ^k	9.7 ± 0.1 ^a	7.8 ± 0.2 ^{ef}	8.0 ± 0.2 ^{def}	5.5 ± 0.9 ^k		
Polymeric procyanidins	12	383.7 ± 17.4	193.1 ± 7.9 ^c	105.3 ± 7.9 ^g	52.5 ± 0.5 ^k	5.0 ± 0.0 ^r	232.6 ± 11.9 ⁿ	78.9 ± 4.4 ⁱ	139.7 ± 5.7 ^e	63.4 ± 2.3 ^j	3.2 ± 0.1 ⁿ			
	Sum	426.6 ± 11.8	214.4 ± 4.0 ^c	127.2 ± 2.9 ^f	74.2 ± 0.8 ^j	21.8 ± 0.2 ^m	12.9 ± 0.2 ^m	255.6 ± 6.8 ^a	98.9 ± 1.3 ^h	159.8 ± 1.3 ^e	83.6 ± 0.7 ^f			
Total	Sum	3056.7 ± 28.3	1201.5 ± 20.0 ^{abc}	1180.0 ± 15.0 ^{bc}	772.0 ± 16.5 ^c	255.0 ± 7.0 ^h	1359.9 ± 35.4 ^a	957.6 ± 18.3 ^d	1119.6 ± 15.5 ^c	704.4 ± 10.1 ^{ef}	220.7 ± 8.5 ^h			
	DP	2.1	1.4	1.9	2.1	2.2	4	1.9	1.5	1.9	1.8			
HMF	nd	0.2 ± 0.0 ^m	2.6 ± 0.2 ^k	1.2 ± 0.1 ^l	27.1 ± 0.9 ^e	315.6 ± 11.0 ⁿ	0.1 ± 0.0 ^m	8.3 ± 0.2 ^h	0.7 ± 0.2 ^{lm}	6.9 ± 1.3 ⁱ	99.8 ± 8.5 ^d			
Phenolic compounds	Peak	IN : MA (1:2)												
		IN : MA (2:1)												
		Flavan-3-ols	3	5.8 ± 0.2e	9.5 ± 0.5 ^b	3.8 ± 0.3 ⁱ	0.3 ± 0.0 ⁿ	7.7 ± 0.2 ^c	0.3 ± 0.0 ⁿ	4.9 ± 0.4 ^g	10.4 ± 0.7 ⁿ	6.1 ± 0.3 ^d	3.9 ± 0.4 ^d	2.1 ± 0.0 ^m
			4	44.2 ± 1.4 ^e	138.3 ± 1.8 ^b	30.0 ± 2.4 ^h	8.4 ± 0.1 ⁱ	46.3 ± 3.3 ^c	8.4 ± 0.1 ⁱ	45.4 ± 2.1 ^e	144.2 ± 8.8 ⁿ	69.7 ± 1.6 ^c	17.5 ± 0.8 ^k	5.0 ± 0.0 ^l
			5	229.2 ± 12.9 ^d	132.9 ± 2.7 ^e	93.4 ± 1.6 ^k	8.8 ± 0.1 ^{no}	100.9 ± 5.8 ^j	8.8 ± 0.1 ^{no}	243.9 ± 8.8 ^e	128.2 ± 7.8 ^f	108.6 ± 9.9 ^h	18.6 ± 0.6 ^m	11.4 ± 0.2 ⁿ
			6	83.3 ± 5.8 ^e	15.0 ± 0.9 ^f	106.5 ± 9.7 ^d	2.9 ± 0.1 ^j	14.2 ± 0.1 ⁱ	2.9 ± 0.1 ^j	83.9 ± 1.2 ^e	15.3 ± 0.5 ^f	15.2 ± 0.1 ⁱ	2.4 ± 0.0 ^l	3.4 ± 0.3 ^j
			7	131.4 ± 8.9 ^d	7.6 ± 0.1 ^o	76.4 ± 2.5 ^g	5.9 ± 0.0 ^p	49.7 ± 1.6 ^f	5.9 ± 0.0 ^p	139.2 ± 2.9 ^e	9.7 ± 0.7 ⁿ	60.9 ± 0.6 ⁱ	19.7 ± 0.7 ⁱ	16.8 ± 0.4 ^{am}
			8	71.9 ± 1.8 ^e	137.3 ± 7.2 ^c	125.0 ± 1.7 ^d	40.0 ± 1.3 ⁱ	90.7 ± 2.8 ^f	40.0 ± 1.3 ⁱ	75.5 ± 0.6 ^g	145.6 ± 8.9 ^b	150.4 ± 6.7 ⁿ	65.5 ± 1.7 ^h	16.4 ± 0.2 ^k
			9	124.9 ± 2.8 ^f	297.2 ± 9.9 ^c	227.4 ± 7.9 ^f	26.0 ± 0.9 ^m	4.2 ± 0.0 ^o	26.0 ± 0.9 ^m	123.6 ± 2.1 ⁱ	334.4 ± 11.4 ^b	2.9 ± 0.0 ^o	37.9 ± 1.2 ⁱ	1.7 ± 0.0 ^o
			10	183.2 ± 3.0 ^b	31.5 ± 1.2 ⁱ	21.8 ± 0.8 ^k	4.4 ± 0.0 ^{mn}	0.1 ± 0.0 ^o	4.4 ± 0.0 ^{mn}	171.0 ± 1.8 ^c	40.1 ± 1.6 ^g	6.7 ± 0.0 ^m	3.7 ± 0.0 ⁿ	2.2 ± 0.0 ^{no}
			11	19.7 ± 0.4 ^f	49.0 ± 1.5 ^h	19.7 ± 0.3 ⁱ	2.4 ± 0.0 ^{mn}	1.4 ± 0.0 ^{no}	2.4 ± 0.0 ^{mn}	26.7 ± 0.6 ^f	58.6 ± 1.8 ^h	0.0 ± 0.0 ^o	4.9 ± 0.0 ^h	2.9 ± 0.0 ^{lm}
			13	10.6 ± 0.6 ^{ghi}	10.4 ± 0.2 ^{hi}	12.2 ± 0.1 ^{de}	9.6 ± 0.1 ^j	15.6 ± 0.8 ^g	9.6 ± 0.1 ^j	10.3 ± 0.3 ^{hij}	9.7 ± 0.2 ^{ij}	12.5 ± 0.6 ^{cd}	13.7 ± 0.4 ^b	8.7 ± 0.1 ^k
			14	58.1 ± 2.7 ^e	43.8 ± 2.4 ^{hi}	51.4 ± 1.5 ^e	43.6 ± 1.7 ^{hi}	49.7 ± 1.5 ^{ef}	43.6 ± 1.7 ^{hi}	61.1 ± 0.1 ^b	45.8 ± 1.5 ^g	48.6 ± 0.9 ^f	46.5 ± 1.6 ^g	40.8 ± 1.5 ^k
			Sum	962.3 ± 11.3 ^c	872.4 ± 11.2 ^g	767.6 ± 11.8 ^l	152.3 ± 9.7 ⁱ	380.5 ± 14.7 ^m	152.3 ± 9.7 ⁱ	986.2 ± 22.0 ^b	941.6 ± 21.7 ^d	481.9 ± 18.1 ⁱ	234.3 ± 10.9 ⁿ	111.3 ± 8.8 ^r
Phenolic acids	1		11.7 ± 0.2 ^{bed}	12.3 ± 0.2 ^{bc}	11.1 ± 0.2 ^{de}	9.3 ± 0.3 ^h	10.7 ± 0.1 ^{ef}	9.3 ± 0.3 ^h	12.2 ± 0.6 ^{bc}	12.0 ± 0.2 ^{bc}	10.7 ± 0.1 ^{ef}	11.2 ± 0.5 ^{de}	6.8 ± 0.1 ^h	
	2		8.1 ± 0.1 ^{cde}	8.3 ± 0.1 ^{bede}	7.5 ± 0.1 ^{fg}	5.8 ± 0.1 ^g	7.0 ± 0.0 ^{gh}	5.8 ± 0.1 ^g	8.5 ± 0.2 ^{bed}	7.1 ± 0.1 ^{gh}	7.1 ± 0.1 ^{gh}	6.7 ± 0.1 ^h	4.4 ± 0.2 ⁱ	
Polymeric procyanidins	12	187.8 ± 4.4 ^d	94.3 ± 0.5 ^h	110.7 ± 3.8 ^f	3.7 ± 0.5 ⁿ	39.7 ± 1.4 ⁱ	3.7 ± 0.5 ⁿ	198.2 ± 1.9 ^h	95.3 ± 1.8 ^h	65.0 ± 0.4 ^j	17.8 ± 0.7 ^m	0.5 ± 0.1 ^o		
	Sum	207.6 ± 7.9 ^d	115.0 ± 7.8 ^e	129.3 ± 6.0 ^f	18.8 ± 0.4 ^m	57.4 ± 5.4 ^k	18.8 ± 0.4 ^m	218.9 ± 7.0 ^h	115.4 ± 10.9 ^g	82.8 ± 5.7 ⁱ	35.7 ± 2.3 ^j	11.7 ± 0.2 ⁿ		
Total	Sum	129.5 ± 10.4 ⁱ	232.6 ± 12.2 ^c	162.2 ± 9.5 ^h	22.9 ± 5.0 ^{op}	47.9 ± 2.8 ^m	290.6 ± 5.6 ^f	193.6 ± 7.6 ^e	112.3 ± 6.3 ^j	31.7 ± 1.5 ⁿ	30.1 ± 2.3 ⁿ			
	DP	1.3	1.9	1.8	1.8	1.6	1.5	1.5	1.9	1.5	1.8			
HMF	3.4 ± 0.2 ^j	3.5 ± 0.2 ^j	0.6 ± 0.1 ^{lm}	144.0 ± 9.8 ^c	10.5 ± 0.3 ^g	144.0 ± 9.8 ^c	0.1 ± 0.0 ^m	3.3 ± 0.2 ^{jk}	0.7 ± 0.0 ^{lm}	21.2 ± 0.1 ⁶	289.3 ± 11.1 ^b			

peak number identities are displayed in Table 2; IN—inulin; MA—maltodextrin; ± –standard deviation; nd—not detected; FD—freeze drying; SD—spray drying; VD—vacuum drying at 50, 70 and 90 °C; DP—degree of polymerization; a–r—different lowercase letters within the column in the group of powders indicate significant differences between samples (p ≤ 0.05) according to Duncan's test. peak number identities are displayed in Table 2; IN—inulin; MA—maltodextrin; ± –standard deviation; FD—freeze drying; SD—spray drying; VD—vacuum drying at 50, 70 and 90 °C; DP—degree of polymerization; a–r—different lowercase letters within the column in the group of powders indicate significant differences between samples (p ≤ 0.05) according to Duncan's test.

Table 4
Duncan's Multiple Range Test for mean values of phenolic compounds (mg/100 g dm) in Japanese quince fruit juice powders.

Phenolic compounds	Peak	Method of drying				Carrier agent					
		FD	SD	VD 50 °C	VD 70 °C	VD 90 °C	IN	MA	IN:MA (1:2)	IN:MA (2:1)	
Flavan-3-ols	3	4.0 ± 0.5 ^b	7.1 ± 0.4 ^a	4.6 ± 0.0 ^b	5.0 ± 0.4 ^b	0.7 ± 0.0 ^c	2.8 ± 0.4 ^b	3.5 ± 0.8 ^b	5.4 ± 0.5 ^a	5.5 ± 0.7 ^a	
	4	48.9 ± 1.2 ^b	87.0 ± 2.0 ^b	40.3 ± 1.4 ^b	26.8 ± 1.1 ^{bc}	17.1 ± 1.0 ^c	30.5 ± 2.8 ^c	35.8 ± 3.1 ^{bc}	53.4 ± 5.0 ^{ab}	56.4 ± 3.2 ^a	
	5	250.4 ± 9.9 ^a	118.4 ± 4.7 ^b	101.8 ± 5.0 ^b	58.7 ± 1.7 ^c	7.7 ± 0.4 ^d	96.2 ± 3.4 ^b	118.1 ± 4.5 ^a	113.0 ± 2.0 ^{ab}	102.2 ± 3.4 ^{ab}	
	6	69.5 ± 1.3 ^a	76.0 ± 1.5 ^a	66.8 ± 1.0 ^a	20.2 ± 0.7 ^b	4.8 ± 0.1 ^b	42.0 ± 0.9 ^b	79.4 ± 1.1 ^a	44.4 ± 0.9 ^b	24.1 ± 0.5 ^b	
	7	147.4 ± 5.2 ^a	20.9 ± 1.3 ^d	65.3 ± 0.5 ^b	39.6 ± 2.9 ^c	6.0 ± 0.0 ^e	39.9 ± 1.8 ^c	80.1 ± 4.6 ^a	54.2 ± 2.7 ^b	49.3 ± 3.0 ^{bc}	
	8	80.7 ± 2.5 ^c	141.3 ± 8.4 ^a	106.6 ± 4.0 ^b	65.6 ± 2.3 ^c	31.4 ± 0.9 ^d	58.9 ± 2.6 ^b	98.0 ± 2.0 ^a	93.0 ± 2.4 ^a	90.7 ± 1.1 ^a	
	9	127.9 ± 2.0 ^b	314.8 ± 5.3 ^a	151.6 ± 2.5 ^b	57.4 ± 1.0 ^c	20.4 ± 0.9 ^c	138.9 ± 4.4 ^{ab}	162.9 ± 5.9 ^a	135.9 ± 2.6 ^{ab}	100.1 ± 1.7 ^b	
	10	153.2 ± 1.6 ^a	34.0 ± 0.9 ^c	77.9 ± 1.3 ^b	14.7 ± 0.4 ^e	3.1 ± 0.1 ^c	88.5 ± 0.9 ^a	44.8 ± 0.4 ^b	48.2 ± 0.6 ^b	44.7 ± 0.4 ^b	
	11	19.7 ± 0.4 ^b	54.1 ± 0.9 ^b	19.6 ± 0.4 ^b	3.0 ± 0.0 ^c	2.0 ± 0.1 ^c	23.7 ± 0.7 ^a	17.9 ± 0.7 ^a	18.5 ± 0.7 ^a	18.6 ± 1.0 ^a	
	13	9.7 ± 0.4 ^c	10.7 ± 0.3 ^c	12.3 ± 0.3 ^b	13.7 ± 0.0 ^a	9.3 ± 0.1 ^c	9.3 ± 0.1 ^c	12.7 ± 0.4 ^a	11.6 ± 0.4 ^{ab}	11.0 ± 0.5 ^b	
	14	58.3 ± 0.9 ^a	44.4 ± 1.0 ^c	50.3 ± 1.3 ^b	48.5 ± 0.7 ^b	41.8 ± 0.7 ^d	43.2 ± 0.8 ^c	53.5 ± 0.5 ^a	49.3 ± 0.9 ^b	48.6 ± 0.7 ^b	
	Sum	969.7 ± 18.5 ^a	908.7 ± 17.5 ^a	697.1 ± 11.4 ^b	353.2 ± 10.6 ^c	144.3 ± 10.5 ^d	573.9 ± 9.8 ^{bc}	706.7 ± 12.3 ^a	551.2 ± 9.9 ^c	626.9 ± 10.0 ^b	
	Phenolic acids	1	12.4 ± 0.7 ^a	12.4 ± 0.6 ^a	11.5 ± 1.0 ^b	10.9 ± 0.6 ^b	8.4 ± 0.5 ^c	10.9 ± 0.7 ^b	12.0 ± 0.7 ^a	10.6 ± 0.6 ^b	10.0 ± 0.4 ^b
		2	8.8 ± 0.5 ^a	8.2 ± 0.0 ^b	7.8 ± 0.4 ^{bc}	7.3 ± 0.3 ^c	4.8 ± 0.5 ^d	7.3 ± 0.4 ^b	7.9 ± 0.6 ^a	7.0 ± 0.7 ^b	7.3 ± 0.7 ^b
12		202.9 ± 4.9 ^a	93.5 ± 2.1 ^b	92.0 ± 2.0 ^b	31.5 ± 1.0 ^c	2.5 ± 0.2 ^d	71.7 ± 3.8 ^c	103.6 ± 3.0 ^b	75.4 ± 2.4 ^{bc}	87.2 ± 2.1 ^b	
Sum	224.1 ± 7.5 ^a	114.1 ± 6.1 ^b	111.3 ± 6.3 ^b	49.7 ± 2.7 ^c	15.7 ± 0.9 ^d	89.9 ± 2.3 ^c	123.5 ± 5.4 ^a	93.0 ± 2.9 ^{bc}	104.5 ± 4.7 ^b		
Polymeric procyanidins	223.0 ± 6.3 ^a	173.6 ± 6.4 ^b	155.9 ± 5.9 ^b	68.5 ± 1.1 ^c	24.5 ± 1.9 ^d	135.0 ± 4.8 ^a	130.6 ± 3.4 ^a	131.7 ± 4.5 ^a	119.0 ± 3.9 ^a		
	1416.8 ± 49.0 ^a	1196.4 ± 28.9 ^b	964.3 ± 15.4 ^c	471.4 ± 10.8 ^d	184.5 ± 6.8 ^e	798.8 ± 12.4 ^b	960.8 ± 11.8 ^a	775.9 ± 9.5 ^b	850.4 ± 10.0 ^{ab}		
Total	1.5	1.8	1.8	1.9	3.7	2.3	2.0	1.9	2.4		
DP	0.95 ± 0.1 ^a	4.43 ± 0.0 ^b	0.82 ± 0.2 ^b	16.44 ± 1.1 ^b	212.16 ± 2.8 ^a	69.34 ± 0.9 ^a	23.17 ± 0.5 ^b	32.40 ± 0.7 ^b	62.92 ± 1.3 ^a		
HMF											

peak number identities are displayed in Table 2; IN–inulin; MA–maltodextrin; ± –standard deviation; FD–freeze drying; SD–spray drying; VD–vacuum drying at 50, 70 and 90 °C; DP–degree of polymerization; a–e–different lowercase letters within the column indicate significant differences between samples ($p \leq 0.05$) according to Duncan's test.

content of polyphenols, and contrary to our results, TPC increased with increasing temperature in VD.

Regarding the effect of the carrier agent on the content of biologically active compounds, significant variance was found between samples ($p \leq 0.05$). The highest average total phenolic content value was obtained for maltodextrin and the lowest for powders with a mix of inulin and maltodextrin in a ratio of 1:2. JQ juice powders can be ranked by decreasing TPC: MA > IN:MA 2:1 > IN > IN:MA 1:2. As well as TPC, flavan-3-ol, phenolic acid and polymeric procyanidin contents were the highest for powders with inulin. The use of inulin, compare to maltodextrin, reduced the value of total polyphenols by 20%. Michalska et al. (2017) in a study of powders obtained from different plum juice formulations stated that addition of maltodextrin at high concentration had a protective effect on the selected compounds (e.g. chlorogenic acid) during the drying process. Analyzing the average contents of individual identified compounds, the concentrations of all were higher for powders with maltodextrin than with inulin except for the trimer and tetramer procyanidin (peaks 10 and 11). The carrier agent used did not affect the content of polymeric procyanidins ($p > 0.05$). The mean degree of polymerization was higher for powders with inulin (DP = 2.3) than for those with maltodextrin (DP = 2.0). It can therefore be concluded that inulin promotes the combination of flavan-3-ol units into longer oligomer chains.

3.2.2. Hydroxymethylfurfural analysis

Hydroxymethylfurfural (HMF) is a widespread food contaminant formed as a result of the heating process and can be used as a quality marker for many processed foods, including fruit, coffee, honey and milk (Murkovic & Pichler, 2006). Previously, HMF was identified in dried JQ fruit (Turkiewicz et al., 2019). The HMF content (Tables 3 and 4) in JQ juice powders ranged from 0.1 (MA and IN:MA 2:1 after FD) to 315.6 mg/100 g dw (IN after VD at 90 °C). HMF was not detected in fresh juice, which is confirmed by the fact that it is only present in processed products. Comparing the drying methods, the powders obtained as a result of freeze-drying, SD and VD at 50 and 70 °C did not differ in terms of HMF content ($p > 0.05$). The use of VD at 90 °C resulted in a 223-fold increase in HMF concentration compared to FD. Increasing the temperature in VD from 50 to 70 °C increased the HMF content in powders 20-fold, and the increase in temperature by another 20° contributed to achieving a HMF value almost 260 times higher. For comparison, a two-fold increase in vacuum drying plum juice powders from 40 to 80 °C caused a 14-fold increase in HMF content (Michalska et al., 2017). Moreover in the study of Michalska et al. (2016) on whole plum powders, HMF was not detected in samples after FD. The carrier agents had a significant impact on the HMF content in JQ juice powders ($p \leq 0.05$). The use of inulin resulted in a 3-fold increase in HMF content as compared to maltodextrin. It can be stated that a higher proportion of maltodextrin in the fruit powder causes less formation of HMF, which is in line with Michalska et al. (2017).

3.2.3. Antioxidant capacity and enzymatic in vitro inhibition tests

In the current study, the antioxidant capacity of the obtained JQ juice powders was measured by the ABTS, FRAP and ORAC methods (Table 5). The highest antioxidant capacity, both ABTS and FRAP, was shown by maltodextrin powder after FD. The results of average antioxidant capacity measured by those methods showed no differences, suggesting that drying methods had no influence on ABTS and FRAP values ($p > 0.05$). In turn, the highest oxygen radical absorption capacity (ORAC) was shown by inulin and maltodextrin powder (1:2) after VD at 50 °C, while the lowest was observed for inulin after VD at 50 °C. It was found that the drying method had a significant effect on the value of ORAC antioxidant capacity ($p \leq 0.05$). Powders obtained by SD and VD at 70 °C had the lowest antioxidant capacity, while after FD and VD at 50 and 90 °C they had the highest values of antioxidant capacity. Moreover, the carrier agents had no effect on the ORAC antioxidant capacity, unlike ABTS and FRAP, where powders with

maltodextrin showed higher antioxidant capacity compared to others. In contrast, Michalska et al. (2017) stated that the greater the addition of maltodextrin in plum juice powders was, the lower were the antioxidant capacity values. Of all groups of phenolic compounds, phenolic acids and flavan-3-ols showed the highest positive correlation with antioxidant activities measured by ORAC assay ($r^2 = 0.40$ and 0.30). In the current study, a positive correlation between HMF and antioxidant activity measured by ABTS, FRAP and ORAC assay was observed ($r^2 = 0.21$, 0.17 and 0.16 , respectively), suggesting possible participation of HMF in the formation of antioxidant capacity in the studied powders.

The α -glucosidase inhibitory activities of JQ juice powders are presented in Table 5, and α -glucosidase inhibitory activities of powders vary significantly ($p \leq 0.05$). In fact, oral agents that inhibit α -glucosidase are used as oral hypoglycemic agents, which is why finding natural foods with such properties may be useful in diabetes prevention. IC_{50} (mg of dried fruit/mL) for α -glucosidase ranged from 13.8 (MA after FD) to 21.8 mg/mL (MA after VD at 90 °C). There was no effect of the carrier agents on the ability to inhibit α -glucosidase. However, differences in IC_{50} values were visible after the application of selected drying methods. FD, SD and VD 50 °C, provided the highest activity in inhibition of α -glucosidase, while the increase in temperature in VD caused a decrease in activity (reduction of activity by > 20% after using VD 90 °C compared to FD). For comparison, Miao et al. (2018) analyzed the α -glucosidase inhibition ability of *Chaenomeles* in the range 0.04–0.43 mg/mL (for fruit flesh). The ability to inhibit α -glucosidase showed a strong positive correlation with the total phenolic content ($r^2 = 0.66$) and with the antioxidant capacity of ABTS ($r^2 = 0.43$). For comparison, Wojdyło, Nowicka, Oszmiański, and Golis (2017) showed that inhibition of α -glucosidase was highly associated with the content of polymeric procyanidins and phenolic acids.

Among the JQ juice powders with reference to the inhibitory activity toward pancreatic lipase, significant differences ($p \leq 0.05$) were observed. It should be emphasized that for 11 analyzed samples (mainly after FD and SD), the values of pancreatic lipase inhibition (IC_{50}) were designated as < 0.01. For other powders, these values were below 0.05 mg/mL. Considering the effect of the drying method on pancreatic lipase inhibition activity in the analyzed powders, it was found that powders after SD and VD 50 °C showed the greatest inhibitory ability. In turn, when considering the effect of the biopolymer, powders with inulin stood out from the others with lower IC_{50} values, and therefore greater potential for pancreatic lipase inhibition. The obtained results indicate that the fruit powders have a high potential for application in people struggling with the problem of overweight, because limiting the activity of pancreatic lipase reduces the amount of fat absorbed into the bloodstream and thus helps in maintaining normal body weight.

Of all drying methods used, SD significantly reduced the ability to inhibit acetylcholinesterase (AChE) compared to other methods – almost two times lower average activity compared to powders subjected to FD. Analyzing the effect of the carrier agents on acetylcholinesterase inhibition, there were no significant ($p > 0.05$) differences between powders with inulin and maltodextrin. Nevertheless, the use of a mixture containing these two biopolymers resulted in a decrease in AChE inhibition from 28 to 54%, for a mixture with a predominance of inulin and maltodextrin, respectively. A negative correlation between total phenolic content and inhibition of AChE was observed ($r^2 = -0.14$). This is in agreement with the results obtained by Wojdyło et al. (2018) for goji fruit, where it was found that the ability to inhibit AChE depends on the presence of carotenoids, not on TPC.

The 15-lipoxygenase inhibition activity was expressed as percentage inhibition at a sample concentration of 2.5 mg/mL. The highest potential was exhibited by JQ juice powder with inulin after SD (90.4%), while the lowest was shown by maltodextrin powder after VD at 90 °C (29.4%). Comparing the influence of the drying method on the average 15-LOX inhibition by JQ juice powders, they can be ranked by decreasing activity: FD > SD > VD 70 °C > VD 50 °C > VD 90 °C. The

Table 5

Antioxidant capacity (mmol Trolox/100 g dw), antidiabetic, antiobesity, anticholinesterase (IC₅₀; mg/mL) and 15-lipoxygenase (LOX) inhibition activity (%) of Japanese quince fruit juice and powders.

Sample	Drying method	Antioxidant capacity			α -Glucosidase	Pancreatic lipase	AChE	15-LOX
		ABTS	FRAP	ORAC				
Juice	fresh	15.3 ± 1.2	13.5 ± 0.0	83.7 ± 5.8	14.2 ± 1.4	< 0.1	15.6 ± 1.7	> 100.0
IN	FD	4.0 ± 0.1 ^g	3.3 ± 0.0 ^f	25.4 ± 2.7 ^{abc}	19.4 ± 0.2 ^{de}	0.4 ± 0.0 ^b	16.6 ± 0.3 ^b	68.0 ± 0.6 ^g
	SD	5.6 ± 0.1 ^{de}	4.9 ± 0.4 ^{cde}	21.6 ± 1.2 ^f	16.7 ± 0.4 ^c	< 0.1	14.7 ± 0.1 ^a	90.4 ± 0.6 ^a
	VD 50 °C	5.6 ± 0.3 ^{de}	5.0 ± 0.1 ^{cde}	16.6 ± 1.3 ^h	18.5 ± 0.6 ^d	< 0.1	24.0 ± 0.8 ^{ef}	43.9 ± 0.6 ^o
	VD 70 °C	5.4 ± 0.5 ^{def}	4.3 ± 0.9 ^e	17.1 ± 1.2 ^{gh}	17.2 ± 0.8 ^c	< 0.1	29.8 ± 0.5 ^h	72.5 ± 0.8 ^e
	VD 90 °C	7.2 ± 0.5 ^b	6.3 ± 1.2 ^{ab}	26.2 ± 1.2 ^{ab}	20.4 ± 0.9 ^{ef}	0.4 ± 0.1 ^b	19.8 ± 0.8 ^c	63.8 ± 0.9 ⁱ
MA	FD	8.0 ± 0.2 ^a	6.7 ± 0.1 ^a	23.5 ± 1.0 ^{cdef}	13.8 ± 1.0 ^a	< 0.1	23.7 ± 0.6 ^{ef}	70.4 ± 0.2 ^f
	SD	5.5 ± 0.0 ^{de}	4.6 ± 0.5 ^{de}	21.7 ± 1.4 ^f	16.7 ± 0.4 ^c	< 0.1	23.9 ± 0.3 ^{ef}	43.2 ± 0.3 ^o
	VD 50 °C	6.6 ± 0.1 ^{bc}	5.6 ± 0.1 ^{bcd}	25.0 ± 1.2 ^{abcd}	17.3 ± 0.7 ^c	< 0.1	27.4 ± 0.3 ^g	58.9 ± 0.6 ^k
	VD 70 °C	6.9 ± 0.0 ^{bc}	5.7 ± 0.2 ^{abc}	23.3 ± 1.0 ^{cdef}	19.1 ± 0.8 ^d	0.4 ± 0.0 ^b	33.6 ± 0.8 ^j	46.6 ± 0.9 ⁿ
	VD 90 °C	5.1 ± 0.2 ^{def}	4.8 ± 0.0 ^{cde}	23.1 ± 0.9 ^{def}	21.8 ± 0.5 ^{ab}	0.5 ± 0.0 ^b	36.4 ± 0.5 ^k	29.4 ± 0.6 ^q
IN : MA (1:2)	FD	6.4 ± 0.2 ^c	5.6 ± 0.0 ^{bcd}	21.8 ± 0.3 ^{ef}	14.7 ± 0.5 ^{ab}	< 0.1	36.0 ± 0.02 ^k	75.2 ± 0.7 ^d
	SD	5.6 ± 0.0 ^d	4.7 ± 0.2 ^{cde}	24.2 ± 1.4 ^{bcd}	15.2 ± 0.4 ^b	< 0.1	97.5 ± 1.3 ^m	65.8 ± 0.4 ^h
	VD 50 °C	5.6 ± 0.1 ^{de}	5.0 ± 0.2 ^{cde}	26.5 ± 1.4 ^a	16.8 ± 0.2 ^c	0.1 ± 0.0 ^a	30.9 ± 0.2 ^j	50.1 ± 0.4 ^m
	VD 70 °C	5.6 ± 0.1 ^d	4.9 ± 0.1 ^{cde}	18.6 ± 0.2 ^{gh}	21.2 ± 0.9 ^{fg}	0.5 ± 0.0 ^b	41.8 ± 0.4 ^l	60.1 ± 0.9 ^j
	VD 90 °C	4.7 ± 0.1 ^f	4.5 ± 0.1 ^e	17.9 ± 0.5 ^{gh}	22.9 ± 0.5 ^h	0.5 ± 0.0 ^b	24.3 ± 0.5 ^f	32.0 ± 0.8 ^p
IN : MA (2:1)	FD	6.4 ± 0.2 ^c	5.2 ± 0.2 ^{cde}	24.0 ± 0.3 ^{cde}	16.9 ± 0.8 ^c	0.4 ± 0.0 ^b	22.6 ± 0.5 ^d	77.5 ± 0.5 ^b
	SD	5.0 ± 0.2 ^{ef}	4.5 ± 0.4 ^e	19.1 ± 0.8 ^g	19.5 ± 0.7 ^{de}	< 0.1	27.1 ± 0.7 ^g	75.7 ± 0.6 ^{cd}
	VD 50 °C	5.4 ± 0.3 ^{def}	5.1 ± 0.0 ^{cde}	22.8 ± 0.9 ^{def}	16.3 ± 0.3 ^c	< 0.1	23.2 ± 0.3 ^{de}	50.9 ± 0.4 ^m
	VD 70 °C	5.2 ± 0.7 ^{def}	5.3 ± 0.6 ^{bcd}	19.0 ± 0.9 ^g	18.8 ± 0.7 ^d	0.1 ± 0.0 ^a	20.3 ± 0.9 ^c	76.6 ± 0.7 ^{bc}
	VD 90 °C	6.3 ± 0.4 ^c	4.9 ± 1.4 ^{cde}	22.9 ± 1.0 ^{def}	18.7 ± 0.7 ^d	0.4 ± 0.0 ^b	16.2 ± 0.2 ^b	55.3 ± 0.5 ^l
Duncan's Multiple Range Test for mean values								
	FD	6.2 ± 0.2 ^a	5.2 ± 0.4 ^a	23.7 ± 1.4 ^a	16.2 ± 1.2 ^a	0.2 ± 0.0 ^a	24.7 ± 0.5 ^a	72.8 ± 1.7 ^a
	SD	5.4 ± 0.4 ^a	4.7 ± 0.4 ^a	21.7 ± 1.0 ^{ab}	17.0 ± 1.4 ^a	< 0.1	40.8 ± 0.7 ^b	68.8 ± 0.9 ^{ab}
	VD 50 °C	5.8 ± 0.2 ^a	5.2 ± 0.7 ^a	22.7 ± 1.1 ^a	17.2 ± 1.9 ^a	< 0.1	26.4 ± 0.9 ^a	51.0 ± 1.2 ^c
	VD 70 °C	5.8 ± 0.5 ^a	5.1 ± 0.4 ^a	19.5 ± 1.4 ^b	19.7 ± 0.9 ^b	0.3 ± 0.0 ^b	28.7 ± 1.0 ^b	55.1 ± 1.4 ^b
	VD 90 °C	5.9 ± 0.2 ^a	5.1 ± 0.8 ^a	22.5 ± 1.6 ^a	20.9 ± 1.1 ^c	0.4 ± 0.0 ^c	24.2 ± 0.6 ^a	45.1 ± 0.8 ^c
	IN	5.5 ± 0.5 ^b	4.8 ± 0.7 ^b	21.4 ± 1.0 ^a	18.4 ± 1.3 ^a	0.1 ± 0.0 ^a	21.0 ± 0.2 ^a	67.7 ± 0.7 ^a
	MA	6.4 ± 0.6 ^a	5.5 ± 0.6 ^a	23.3 ± 1.2 ^a	18.0 ± 1.2 ^a	0.2 ± 0.0 ^b	21.9 ± 0.2 ^a	67.2 ± 0.7 ^a
	IM:MA (1:2)	5.6 ± 0.1 ^b	5.0 ± 0.9 ^{ab}	21.8 ± 1.4 ^a	18.2 ± 1.2 ^a	0.2 ± 0.0 ^b	46.1 ± 0.7 ^b	56.6 ± 0.7 ^b
	IM:MA (2:1)	5.7 ± 0.2 ^b	5.0 ± 0.5 ^{ab}	21.5 ± 1.2 ^a	17.7 ± 1.6 ^a	0.2 ± 0.0 ^b	29.0 ± 0.9 ^a	49.7 ± 0.7 ^b

IN–inulin; MA–maltodextrin; ± –standard deviation; FD–freeze drying; SD–spray drying; VD–vacuum drying at 50, 70 and 90 °C; a–q—different lowercase letters within the column in the group of powders indicate significant differences between samples ($p \leq 0.05$) according to Duncan's test.

use of VD at 90 °C reduced the ability of inhibition of 15-lipoxygenase by 38% compared to FD. The mean activity values for inulin and maltodextrin were at a similar level, while the use of a mixture of these two carrier agents resulted in a reduction of percentage inhibition. It was found that the ability to inhibit 15-lipoxygenase is moderated by the total phenolic content ($r^2 = 0.46$) and that there is a positive correlation between the ability to inhibit α -glucosidase and 15-LOX ($r^2 = 0.48$). However, no relationship was found with the HMF content ($r^2 = -0.24$).

3.3. Principal component analysis (PCA)

To better understand the trends and relationships among the studied variables and factors, principal component analysis (PCA) was applied (Fig. 2). PCA examined the relationships between chemical composition (polyphenolic profile and HMF content) and biological activities (antioxidant, α -glucosidase, pancreatic lipase, AChE and 15-LOX inhibition) of JQ juice powders. The two main principal components identified (PC1 and PC2) explained 79.51% of the total data variance. The other principal components with a minor effect on the model were discarded. In Fig. 2 two clusters created between variables and factors can be seen. The first cluster consisted of HMF which affected the degree of polymerization of procyanidins and correlated with 15-lipoxygenase inhibition activity. Moreover, this cluster also includes samples with inulin and a mixture of inulin and maltodextrin in two variants. This indicates similar characteristics of the powders obtained using these biopolymers. This group also includes powders dried under vacuum at 70 and 90 °C, which indicates the lack of significant impact of those temperatures in this drying method on the physicochemical properties of the final product. In the second, a clear correlation

between the inhibition of acetylcholinesterase and antioxidant capacity measured by ABTS, FRAP and ORAC assays was observed. The same relationship between antioxidant capacity and the ability to inhibit AChE was previously reported by Turkiewicz et al. (2019) and Turkiewicz et al. (2019). Moreover, phenolic compounds (TPC, phenolic acid, flavan-3-ols and polymeric procyanidins) were positively correlated with the activity for inhibiting α -glucosidase and pancreatic lipase (Wojdyło et al., 2017). The highest positive correlation was found between maltodextrin powders after FD (located closest to each other in the second, positive quadrant of the biplot).

4. Conclusions

In this study, the influence of the drying method and the carrier agents on the chemical composition (content of polyphenolic compounds and HMF) and physical properties of JQ juice powders of polyphenols was evaluated. The major group of phenolic compounds present in the powders comprised flavan-3-ols consisting of 11 derivatives, among which procyanidin B2, C1 and (-)-epicatechin were predominant. Moreover, three phenolic acid derivatives were detected and polymeric procyanidins made up approximately 17% of the mean total phenolic content. As well as the drying method, also the biopolymer used affected the phenolic profile in the obtained powders. Among the drying techniques applied, FD resulted in the highest retention of polyphenols, while among the carrier agents maltodextrin was found to be the best biopolymer for obtaining high-quality fruit powder. Also the use of FD and maltodextrin ensured that powders with the lowest content of undesirable HMF were obtained. The used drying methods and carrier agents had a significant impact on the physical parameters of JQ juice powders. FD for most of the analyzed parameters (dry

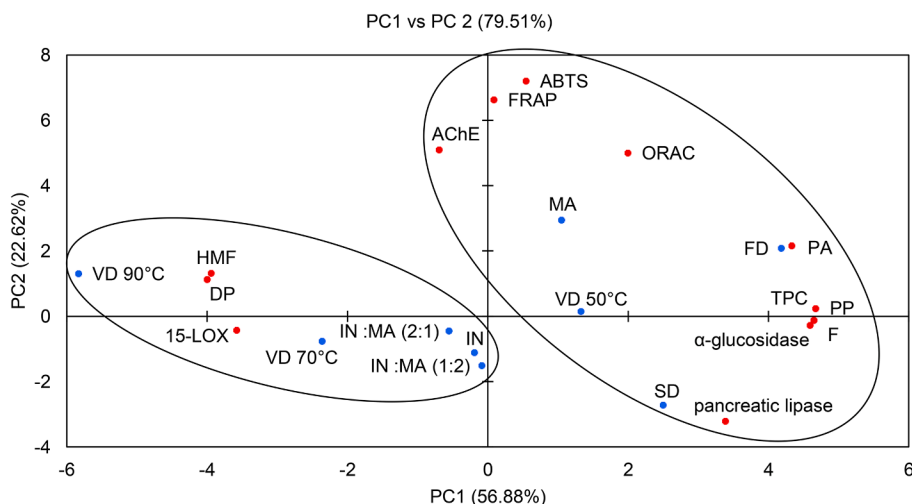


Fig. 2. Principal component analysis (PCA) scores plot showing correlations between variables and factors of Japanese quince fruit juice powders. IN–inulin; MA–maltodextrin; SD–spray drying; FD–freeze drying; VD–vacuum drying at 50, 70 and 90 °C; HMF–hydroxymethylfurfural; DP–degree of polymerization; PP–phenolic acids; F–flava-3-ols; TPC–total phenolic content.

matter, water activity, porosity and color) had a positive effect on the powders quality. The use of inulin has contributed to the reduction of water activity, higher porosity and more favorable color parameters. The selected drying methods did not have a significant impact on antioxidant capacity (ABTS, FRAP and ORAC), but the use of maltodextrin allowed powders with higher activity to be obtained. Powders (regardless of the carrier agent used) obtained as a result of FD were characterized by higher activity for inhibition of α -glucosidase, AChE and 15-LOX in comparison to others, whereas SD and VD at 50 °C were more advantageous in terms of pancreatic lipase inhibition. To sum up, VD to obtain JQ juice powders caused deterioration of all discussed parameters compared to FD and SD.

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CRediT authorship contribution statement

Igor Piotr Turkiewicz: Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Aneta Wojdyło:** Supervision, Conceptualization, Writing - original draft, Writing - review & editing, Funding acquisition. **Karolina Tkacz:** Formal analysis. **Krzysztof Lech:** Methodology. **Anna Michalska-Ciechanowska:** Conceptualization, 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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mgr inż. Igor Piotr Turkiewicz

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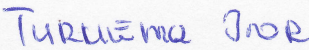
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i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chelmońskiego 37
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że jestem współautorem publikacji pt.:

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.

Mój udział w przygotowaniu tej publikacji polegał na kierowaniu projektem naukowym obejmującym badania opisane w tej pracy (Diamentowy Grant VII, nr DI2017 006347), współtworzeniu koncepcji prowadzonych badań, wykonaniu analiz fizykochemicznych, chromatograficznych i potencjału biologicznego *in vitro* produktów z owoców pigwowca. Opracowałem technologię otrzymywania proszków pigwowcowych, przygotowałem tekst publikacji, opracowałem merytorycznie otrzymane wyniki, przeprowadziłem dyskusję oraz współredagowałem odpowiedzi na recenzje.


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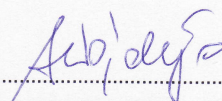
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mój udział polegał na tworzeniu i nadzorze koncepcji projektu (Diamentowy Grant VII, nr DI2017 006347), w ramach którego realizowana była praca doktorska, uczestnictwie w analizach chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca i ich produktów, koordynowaniu prac Doktoranta, współredagowaniu publikacji i merytorycznej ocenie wyników.


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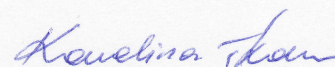
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mój udział polegał na współredagowaniu publikacji oraz uczestniczeniu w tworzeniu technologii otrzymywania proszków pigwocowych metodami suszarniczymi.



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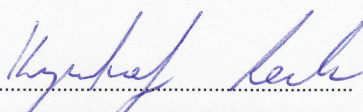
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Wydział Przyrodniczo-Technologiczny
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chelmońskiego 37/41
51-630 Wrocław

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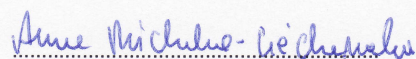
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Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chelmońskiego 37
51-630 Wrocław

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i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

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mój udział polegał na analizie formalnej publikacji oraz opracowaniu metodyki dotyczącej analizy potencjału biologicznego *in vitro*.


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Publikacja 6

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Physicochemical characterization and biological potential of Japanese quince polyphenol extract treated by different drying techniques

Igor Piotr Turkiewicz^a, Karolina Tkacz^a, Paulina Nowicka^a, Anna Michalska-Ciechanowska^a, Krzysztof Lech^b, Aneta Wojdyło^{a,*}

^a Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences, 37 Chelmońskiego Street, 51-630, Wrocław, Poland

^b Institute of Agricultural Engineering, Wrocław University of Environmental and Life Sciences, 41 Chelmońskiego Street, 51-630, Wrocław, Poland

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ABSTRACT

The aim of this study was to determine the effect of different drying methods – freeze drying, spray drying and vacuum drying (at 50, 70, 90 °C) – on the quality factors of Japanese quince polyphenol extract, including physical parameters, phenolic compounds, and *in vitro* biological activities (antioxidant, anti-diabetic, anti-obesity, and anticholinesterase). The highest content of bioactive compounds was observed in samples after freeze drying (total phenolic content – 912.7 g/kg dry weight [dw]; flavan-3-ols – 467.3 g/kg dw). The antioxidant activity measured by Oxygen Radical Absorbance Capacity (ORAC) assay was the highest for samples vacuum dried at 70 °C (1455.5 mol Trolox/kg dw). Moreover, strong anti-diabetic properties were obtained after vacuum drying, and the samples subjected to freeze drying and spray drying showed the most favorable anticholinergic potential. Unfavorable changes in color, formation of 5-hydroxymethylfurfural (5-HMF) and degradation of phenolics were noted along with the increasing drying temperature in vacuum drying. For vacuum drying, the most optimal temperature is 70 °C, as the final product obtained in this way is characterized by favorable physical properties, a beneficial content of biological compounds, a low concentration of undesirable 5-hydroxymethylfurfural and satisfactory biological properties.

1. Introduction

Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) is an endemic species, whose homeland is in central and southern Japan. It occurs mainly on mountain slopes (even at altitudes of 2,000 m above sea level) and on the banks of rivers and lakes. In Europe it quickly spread as an ornamental shrub and is now planted in parks and gardens. Its popularity is evidenced by the fact that currently over half a thousand varieties of these plants are cultivated. The decorative and nutritional advantages of the shrubs and their aromatic fruits have been appreciated in many countries and now they are grown almost in the entire temperate climate zone.

Numerous studies (Du et al., 2013; Turkiewicz, Wojdyło, Tkacz, Nowicka, et al., 2020; Urbanavičiūtė et al., 2020) on Japanese quince fruits prove that, apart from the high content of organic acids, they are a rich source of phenolic compounds, with the dominant group of polymeric proanthocyanidins (PACs), which can constitute up to 79% of the total phenolics. PACs (also known as condensed tannins) have been

quantified in a large amount in grape seeds, chokeberry, nuts, cocoa and black beans (Rauf et al., 2019). The building blocks of PACs include (+)-catechin and (–)-epicatechin linked by C–C and occasionally C–O–C bonds. Based on their B-rings, the four most common B-type PAC dimers are B1, B2, B3 and B4 (Ky et al., 2016). The main pharmacological activities ascribed to PACs are: antioxidant and radical scavenging, anti-cancer, antimicrobial, cardioprotective, anti-diabetic and anti-obesity (Smeriglio, Barreca, Bellocco, & Trombetta, 2017).

Fruits and vegetables are good sources of pectin, dietary fiber, oligosaccharides, organic colors, vitamins and, more importantly, polyphenols. These bioactive compounds can be isolated and/or purified in order to obtain them in a concentrated form (extract). They can then be used in the production of fortified functional foods or dietary supplements (Aziz et al., 2018). The worldwide polyphenols market size was valued at \$1.28 billion in 2018 and is expected to reach an estimated CAGR of 7.2% from 2019 to 2025. The main factors contributing to such significant growth in this market over the last 20 years is the developing functional food and beverage industry, as well as technological advances

* Corresponding author.

E-mail address: aneta.wojdylo@upwr.edu.pl (A. Wojdyło).

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in the polyphenol extraction process (GVR, 2019).

Drying is one of the indispensable techniques for large-scale food preservation, offering numerous benefits including storage stability, lowering packaging requirements, and reducing the mass for transportation (Hnin, Zhang, Mujumdar, & Zhu, 2019). However, the techniques and processing conditions used to produce fruit extract powders depend on the characteristics of the phytochemicals present in the extract. Among the commonly known drying techniques, spray drying (SD) is usually applied to produce the fruit juice powder. However, the high process temperature and the high exposure of labile bioactive compounds to oxygen are the two major disadvantages of this process. In addition, in the case of sticky food, e.g., rich in sugars or oligosaccharides, agglomerates clog the dryer, which in turn reduces the efficiency of the process (Muzaffar, Nayik, & Kumar, 2018). Freeze drying (FD) in many industries is used for the reliable preservation of a wide spectrum of heat-sensitive products. Unfortunately, its disadvantages include still unsatisfactory energy- and cost-effectiveness. Furthermore, the high porosity of the dried materials has a negative effect on their storage stability (Ciużyńska & Lenart, 2011). Therefore, vacuum drying (VD) seems to be an effective compromise between SD and FD, while providing an anaerobic environment for the protection of labile chemicals, shorter process times, and the possibility of using it on a larger scale due to the lower cost.

In the production of powders based on fruit juices, it is necessary to use carrier agents (such as inulin or maltodextrin) to eliminate unfavorable physicochemical changes occurring during the drying process (sticking to the dryer wall, clumping) (Aziz et al., 2018). However, in the production of juice extract powders, free from ballast substances (pectic compounds, sugars, and organic acids), it is possible to carry out the drying process without the use of coating compounds.

Therefore, this study aimed to investigate the influence of different drying methods (vacuum, spray and freeze drying) on the physical parameters (water activity, true and bulk density, porosity, and color parameters), content of phenolic compounds, antioxidants (ABTS, FRAP, ORAC) and biological (anti-diabetic, anti-obesity, anticholinesterase, and anti-15-LOX) activities on Japanese quince polyphenol extract (JQPE). So far, no attempt has been made to determine the effect of the drying method and parameters on the quality of the JQPE, including the content of undesirable 5-hydroxymethylfurfural (5-HMF).

2. Material and methods

2.1. Chemicals

Chromatographic solvents were from Merck (Darmstadt, Germany). Chemicals for antioxidant and biological activities were purchased from Sigma-Aldrich (Steinheim, Germany). All standards of phenolic compounds were purchased from Extrasynthese (Lyon, France). UPLC grade water, prepared by using an HPL SMART 1000 s system (Hydrolab, Gdańsk, Poland), was additionally filtered through a 0.22 µm membrane filter immediately before use.

2.2. Plant material and preparation of Japanese quince polyphenol extract (JQPE)

Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) fruits were harvested from one bush from cultivation in Lublin Province (Poland) at processing maturity in September 2018. The fruits (30 kg) were washed, pitted, and mixed using a Thermomix (Vorkwek; Wuppertal, Germany). The obtained mash was pressed by a laboratory hydraulic press (SRSE, Warsaw, Poland) and centrifuged (10 min, 4 °C, 8000 g) using a refrigerated laboratory centrifuge (MPW-380R, MPW Med. Instruments; Warsaw, Poland). To obtain a polyphenol extract, saccharides and organic acids were removed from juice using a glass column filled with Amberlite™ polymeric resin XAD-16 and 80% (v/v) ethanol as a solvent to the elution process. Next, solvent was removed by

a scale rotary evaporator (Hei-VAP Expert, Heidolph; Schwabach, Germany) at 40 °C, thus producing a Japanese quince polyphenol extract (JQPE) which was subjected to different drying techniques and further analysis. In order to verify the removal of sugars and organic acids from JQPE, a chromatographic analysis (HPLC-ELSD and UPLC-PDA, respectively) was performed according to the protocol previously described by Turkiewicz, Wojdyło, Tkacz, Lech, and Nowicka (2020). From 30 kg of fruit, 15 L of Japanese quince juice were produced, from which 1500 mL of JQPE was obtained (after removing ballast substances and ethanol).

2.3. Drying methods – preparation of JQPE powders

Freeze drying (FD) was carried out in freeze dryer (Christ Alpha 2–4, Braun Biotech Int.; Melsungen, Germany) for 24 h at the pressure of 0.220 mbar. The temperature within the drying chamber was –30 °C, while the heating plate reached 30 °C. Spray drying (SD) was performed by a Mini Buchi Spray-dryer (Buchi; Flawil, Switzerland). The spray dryer was operated at an inlet temperature of 180 °C while the rate of feeding was 40 mL/min. Vacuum drying (VD) at 50, 70 and 90 °C was performed in a VACUCELL 111 ECO LINE (Medcenter Einrichtungen GmbH; Planegg, Germany) at a pressure of 1 kPa for, respectively, 72 h, 48 h and 24 h. Finally, five variants of drying were obtained and 90 mL of JQPE was used for each. All processes were performed in duplicate.

2.4. Physical analysis of JQPE and JQPE powders

2.4.1. Dry matter, water activity and color parameters

The dry matter content (dm) of powders was determined in a vacuum dryer (SPT-200, ZEAMiL Horyzont; Kraków, Poland) at 80 °C for 72 h at the pressure of 100 Pa. The determination of water activity (a_w) was performed using a LabMaster water activity analysis instrument (Novasina AG; Lachen, Switzerland) at 20 °C. The measurements were made in triplicate. The color of the samples was determined with reference to the CIE L*a*b* color space using a portable sphere type spectrophotometer with vertical alignment (CM-700d, Konica Minolta Sensing, Inc.; Osaka, Japan). The data were presented as an average of three replications.

2.4.2. True density, bulk density and porosity

True density (ρ_t ; g/cm³), bulk density (ρ_b ; g/cm³) and porosity (ϵ ; %) were measured as reported previously (Michalska, Wojdyło, Lech, Lysiak, & Figiel, 2016).

The powders were weighed with an analytical balance with an accuracy of 0.0001 g (XA 60/220/X Radwag; Radom, Poland), the total volume was measured with an HumiPyc/model 2 Gas Pycnometer (InstruQuest Inc.; Coconut Creek, USA) while bulk volume was measured with a graduated cylinder. The container was filled with the samples (10 ± 0.50 mL) and then gently shaken to obtain the smallest volume of the samples. All measurements were made in triplicate.

2.5. Chemical analysis of JQPE and JQPE powders

2.5.1. Identification of polyphenols by the UPLC-ESI-Q-TOF-MS method

The polyphenol extracts were prepared as previously described Wojdyło, Nowicka, Carbonell-Barrachina, and Hernández (2016). The presence of polyphenols in Japanese quince juice extract was identified using Acquity UPLC system (Waters Corporation, Milford, USA) with a QToF mass spectrometer (Waters, Manchester, UK). An Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm; Waters Corp.) was used to perform the chromatographic separation previously described by Wojdyło, Figiel, et al., (2016). Samples (5 µL) were injected into a gradient system at a flow rate of 0.42 mL/min. The mobile phase consisted of 2% formic acid in deionized water (A) and acetonitrile (B). Samples were eluted according to a linear gradient: 0–12 min, 1–25% B; 12–12.5 min, 100% B; 12.5–13.5 min, 1% B. The analysis was prepared by ionization

mode at negative (M–H)[–] before and after fragmentation within mass scanning from *m/z* 100 to 1700. The data were collected by Mass-Lynx TM v 4.1 software.

2.5.2. Quantification of polyphenols, polymeric proanthocyanidins (PACs) and 5-hydroxymethylfurfural (5-HMF) using the UPLC-PDA-FL system

The analysis of polyphenolic compounds was carried out using the Acquity UPLC system (Waters Corp., Milford, USA). Polyphenolic compounds were monitored at the following wavelengths at 280 nm (flavan-3-ols), 320 nm (phenolic acids) and 360 nm (flavonols and flavanols). Quantification was achieved by injection of solutions of known concentrations ranging from 0.05 to 5 mg/mL ($R^2 \leq 0.9997$) of (–)-epicatechin, (+)-catechin, chlorogenic acid, quercetin, and kaempferol 3-O-glucoside, -galactoside, and -rutinoside, as standards.

Analysis of polymeric proanthocyanidins (PACs) was performed by phloroglucinolysis method as described previously by Wojdyło, Oszmiański, and Bielicki (2013). The analysis was carried out on a UPLC system Acquity (Waters Corp., Milford, MA, USA) consisting of a binary solvent manager, and fluorescence detector (FL). The fluorescence detection was recorded at an excitation wavelength of 278 nm and an emission wavelength of 360 nm. The calibration curves, which were based on peak area, were established using (+)-catechin, (–)-epicatechin, and procyanidin B1 after phloroglucinol reaction as (+)-catechin- and (–)-epicatechin-phloroglucinol adduct standards. All incubations were done in triplicate. Results were expressed as g per kg of dw.

The analysis of 5-HMF was performed using the Acquity UPLC system (Waters Corp., Milford, USA) according to Turkiewicz, Wojdyło, Lech, Tkacz, and Nowicka (2019). Detection of 5-HMF was at 284 nm and quantification was achieved by injection of solutions of known concentrations ranging from 5.00 to 50.00 mg/L ($R^2 \leq 0.9998$) of 5-HMF standard. All incubations were done in triplicate. Results were expressed as mg per kg of dw.

2.5.3. Analysis of *in vitro* antioxidant and biological activities

The extracts for following analysis was prepared as described previously (Wojdyło, Nowicka, Laskowski, & Oszmiański, 2014).

Antioxidant activities were determined using the ABTS method, FRAP and ORAC as previously described by Wojdyło, Nowicka, Tkacz, and Turkiewicz (2020). All samples were assayed in triplicate and the results were expressed as mmol of Trolox per 100 g of dw. The anti-diabetic and anti-obesity activities were measured as inhibition of α -amylase, α -glucosidase and pancreatic lipase (Nowicka, Wojdyło, & Laskowski, 2018) while anticholinesterase properties were measured as inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE). Activity against 15-lipoxygenase (15-LOX) were investigated according to Turkiewicz, Wojdyło, Tkacz, Nowicka, et al. (2020). All samples were assayed in triplicate and the result was expressed as IC₅₀ (mg of sample per mL of enzyme) while for 15-LOX inhibition activity as % inhibition (for 2.5 mg of sample per mL of enzyme). All spectrophotometric and spectrofluorometric measurements were performed using a plate reader Synergy H1 (BioTek Instruments, Inc.; Winooski, USA).

2.6. Statistical analysis

Statistical analysis was conducted using XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft; Paris, France). Significant differences ($p \leq 0.05$) between means were evaluated by non-parametric Kruskal-Wallis test and Dunn's procedure. Principal components analysis (PCA) also has been done.

3. Results and discussion

3.1. Physical analysis

The dry matter (dm) content of Japanese quince polyphenol extract

(JQPE) was 9.28%, while for powders ranged from 92.95% (freeze-dried) to 98.82% (vacuum-dried at 70 °C) (Table 1). This parameter has a direct impact on the flowability and stickiness of powders and is taken as a measure of drying efficiency (Aziz et al., 2018). Michalska, Wojdyło, Lysiak, and Figiel (2017) in the study of plum juice extract also obtained powders with the lowest dm after freeze drying (FD). In addition, ÇalıřkanKoç (2020) proved that the moisture content of red pepper powder can be lowered by applying a microwave finish drying after FD. The calculated Pearson correlation coefficient (r^2) confirmed the results of Young et al. (2007), where dm is weakly positively correlated with bulk density (ρ_b) – $r^2 = 0.30$, and weakly negatively correlated with true density (ρ_t) – $r^2 = -0.35$.

The water activity (a_w) is used by food designers to create shelf-stable food products – the lower the a_w value, the longer the shelf life. Furthermore, keeping the a_w as low as possible prevents agglomeration, caking and degradation of bioactive compounds (Ramakrishnan, Adzahan, Yusof, & Muhammad, 2018). The a_w in dried JQPE samples differed between samples ($p \leq 0.05$) and ranged from 0.090 (SD and VD 90 °C) to 0.105 (VD 70 °C). Similarly low a_w values in the range from 0.074 to 0.101 were obtained by Tkacz, Wojdyło, Michalska-Ciechanowska et al. (2020) for powders obtained from different sea buckthorn juice formulations.

The density of powder plays an important role in determining the mixing and packing properties as well as forming the powder into tablet form. True density (ρ_t) corresponds to the real solid density and does not consider the spaces between particles. Table 1 shows that ρ_t ranges from 1.036 (for SD) 1.527 g/cm³ (for FD) and there are significant differences in ρ_t depending on the drying method used. In the studies by Turkiewicz, Wojdyło, Tkacz, Lech, and Nowicka (2020) on fruit powders from *Chaenomeles* juice, it was proved that the drying method influenced ρ_t , in contrast to the carriers used, where no significant differences were noted. Moreover, a tendency can be noticed, according to which the value of ρ_t increases with the temperature increase in VD, but the performed statistical analysis did not classify these relations as significant ($p \geq 0.05$). Bulk density (ρ_b) is related to the porosity of the powder and is one of the properties used to specify loose products. The drying process had a significant influence ($p \leq 0.05$) on the ρ_b values, which ranged, as in case of dm, from 0.265 (for SD) to 0.626 g/cm³ (for VD 70 °C). The lower the ρ_b values, the more air is entrapped in the voids, and therefore the greater the possibility of oxidation (Aziz et al., 2018). In addition, it can be stated that the use of VD (regardless of the applied temperature) may have practical consequences for reducing packaging and transport costs, compared to the FD and SD method. In contrast, ÇalıřkanKoç (2020) obtained almost two times higher ρ_b for red pepper powders after FD than after VD at 80 °C. The reasons for such differences probably can be found in the properties of ballast substances removed in JQPE. Moreover, comparing the obtained results to the earlier findings Turkiewicz, Wojdyło, Tkacz, Lech, and Nowicka (2020) it can be notice that the addition of polysaccharides to powders has a greater impact on the ρ_b value than the drying temperature in VD.

Porosity (ϵ) is defined as the void fraction in the sample. The highest ϵ value was observed for the JQPE after SD (74.42%) and the lowest after VD at 50 °C (58.32%). Porosity shows a moderate negative correlation with true density ($r^2 = -0.50$) and strong negative correlation with bulk density ($r^2 = -0.95$). According to Saifullah, Yusof, Chin, and Aziz (2016) the porosity is very important from the point of view of solubility of any powdery material – the higher the ϵ values, the greater the solubility.

The color parameters of JQPE and JQPE powders are given in Table 1. The chromatic parameters were measured in reference to the CIE L*a*b* color space. The lightest powders were obtained after the SD process (83.77), while the darkest were obtained after VD at 90 °C (74.20). In contrast, Turkiewicz, Wojdyło, Tkacz, Lech, and Nowicka (2020) obtained the highest values of the L* parameter for Japanese quince juice powders after FD (average 91.27). On the other hand, the hydroxymethylfurfural (HMF) formed during prolonged heating could

Table 1
Physical parameters of Japanese quince juice extract powders.

drying method	dry matter (%)	a_w	true density ρ_t (g/cm ³)	bulk density ρ_b (g/cm ³)	porosity ϵ (%)	color parameters		
						L*	a*	b*
JQPE*	9.28 ± 0.00	0.848 ± 0.00	–	–	–	81.23 ± 0.01	−0.80 ± 0.01	20.00 ± 0.01
FD	92.95 ± 0.05 ^E	0.100 ± 0.010 ^{AB}	1.527 ± 0.010 ^A	0.435 ± 0.005 ^D	71.50 ± 1.10 ^B	80.91 ± 0.01 ^B	−0.86 ± 0.01 ^D	20.52 ± 0.01 ^C
SD	97.97 ± 0.04 ^D	0.090 ± 0.010 ^B	1.036 ± 0.005 ^C	0.265 ± 0.005 ^E	74.42 ± 1.00 ^A	83.77 ± 0.01 ^A	−1.21 ± 0.01 ^E	20.93 ± 0.02 ^B
VD 50 °C	98.58 ± 0.06 ^B	0.099 ± 0.005 ^{AB}	1.428 ± 0.100 ^B	0.595 ± 0.010 ^B	58.32 ± 1.12 ^D	76.52 ± 0.01 ^C	−0.28 ± 0.00 ^C	16.51 ± 0.01 ^E
VD 70 °C	98.82 ± 0.02 ^A	0.105 ± 0.010 ^A	1.430 ± 0.010 ^B	0.626 ± 0.005 ^A	56.25 ± 1.05 ^E	72.67 ± 0.01 ^E	−0.26 ± 0.01 ^B	17.61 ± 0.01 ^D
VD 90 °C	98.16 ± 0.04 ^C	0.090 ± 0.010 ^B	1.458 ± 0.005 ^{AB}	0.518 ± 0.005 ^C	64.50 ± 1.00 ^C	74.20 ± 0.01 ^D	2.63 ± 0.01 ^A	21.41 ± 0.01 ^A

JQPE—without applying any heat treatment (before drying) and therefore not included in the statistical analysis; ±—standard deviation; FD—freeze drying; SD—spray drying; VD—vacuum drying at 50, 70 and 90 °C; a_w —water activity; A–E—different capital letters within the column in the group of extract indicate significant differences between samples ($p \leq 0.05$).

be related to the darker color of samples after VD at 90 °C (Pearson correlation coefficient $r^2 = 0.51$). The largest change of the a^* parameter between the drying methods used was observed when the temperature in the VD process increased from 70 to 90 °C – then there was an almost 12-fold increase in the a^* coordinate value, which thus led to a redder color of the powders. Values of parameter b^* ranged from 16.51 (powder after VD 50 °C) to 20.93 (powder after SD). Along with the increase in temperature in drying VD, the color was warming towards yellow. The same relationships in changes in a^* and b^* coordinates were

obtained by Tkacz, Wojdyło, Michalska-Ciechanowska et al. (2020) for sea buckthorn powders.

3.2. Chemical analysis

3.2.1. Identification and quantification of phenolic compounds

An overview of all the main identified compounds in the JQPE by UPLC-ESI-Q-TOF-MS using the negative mode is given in Table 2 and Fig. 1. The compounds are summarized along with their retention time

Table 2
Identification and quantification of phenolic compounds (g/kg dm) and 5-hydroxymethylfurfural (5-HMF; mg/kg dm) in Japanese quince polyphenol extract powders using LC–PDA–QTOF–MS.

Compound	R_t (min)	λ_{max} (nm)	MS [M-H] [−] (m/z)	MS/MS (m/z)	JQPE*	FD	SD	VD 50 °C	VD 70 °C	VD 90 °C
Chlorogenic acid (5-O-caffeoylquinic)	3.86	246/326	353.09	191.06	19.3 ± 0.6	17.6 ± 0.8 ^B	18.8 ± 0.3 ^{AB}	18.2 ± 0.9 ^{AB}	19.8 ± 0.2 ^A	15.1 ± 0.3 ^C
<i>p</i> -Coumaroyl hexose	4.01	316/326	325.09	163.04/145.03	5.2 ± 0.2	4.7 ± 0.1 ^{ABC}	5.0 ± 0.0 ^{AB}	4.9 ± 0.0 ^{ABC}	5.3 ± 0.4 ^A	4.2 ± 0.1 ^C
Syringic acid hexoside	5.85	353	403.16	241.10/197.12	150.8 ± 9.8	142.0 ± 6.8 ^A	143.5 ± 9.1 ^A	106.5 ± 1.8 ^C	118.7 ± 2.7 ^B	12.6 ± 0.2 ^D
Sum of phenolic acids					175.3 ± 6.9	164.3 ± 7.2 ^A	167.4 ± 7.1 ^A	129.7 ± 5.6 ^C	175.3 ± 6.9	31.9 ± 1.4 ^D
Procyanidin B1	4.07	280	577.13	425.08/289.05	3.5 ± 0.1	3.5 ± 0.1 ^B	3.0 ± 0.2 ^C	4.3 ± 0.2 ^A	3.8 ± 0.2 ^B	4.5 ± 0.3 ^A
Procyanidin trimer	4.13	280	865.20	577.13/289.07	27.9 ± 1.3	26.9 ± 1.3 ^C	26.4 ± 0.9 ^C	27.1 ± 0.8 ^B	29.4 ± 1.3 ^A	22.8 ± 0.3 ^D
Procyanidin B2	4.26	280	577.13	456.15/425.09	152.1 ± 8.9	149.4 ± 8.8 ^B	154.8 ± 4.9 ^A	142.8 ± 2.6 ^C	153.9 ± 3.0 ^A	97.3 ± 1.8 ^D
Procyanidin dimer	4.41	280	577.13	456.15/425.08/289.05	8.6 ± 0.6	10.6 ± 0.5 ^A	8.2 ± 0.3 ^C	9.3 ± 0.7 ^B	8.9 ± 0.4 ^{BC}	5.4 ± 0.5 ^D
(−)-Epicatechin	4.74	240/280	289.06	245.08/187.05	98.0 ± 2.8	92.2 ± 2.6 ^{BC}	97.9 ± 2.8 ^B	93.0 ± 1.7 ^{BC}	100.5 ± 2.9 ^A	80.1 ± 1.1 ^C
Procyanidin C1	5.06	280	865.21	565.21/403.16/289.07	48.6 ± 1.5	46.2 ± 1.5 ^B	49.7 ± 1.4 ^A	45.8 ± 1.5 ^B	50.1 ± 1.7 ^A	36.4 ± 0.6 ^C
Procyanidin trimer	5.21	280	865.20	695.16/577.13/525.10/289.07	58.7 ± 1.8	55.8 ± 1.4 ^B	58.6 ± 1.5 ^A	57.0 ± 1.6 ^{AB}	55.5 ± 1.6 ^B	52.1 ± 1.5 ^C
Procyanidin tetramer	5.39	280	1153.26	865.20/720.16/289.07	19.1 ± 0.9	19.7 ± 0.9 ^A	18.8 ± 0.2 ^B	17.8 ± 0.7 ^C	16.7 ± 0.5 ^C	20.3 ± 0.9 ^A
Procyanidin trimer	5.54	280	865.20	695.16/577.13/525.10/289.07	8.1 ± 0.2	8.1 ± 0.2 ^{AB}	8.4 ± 0.0 ^A	7.7 ± 0.1 ^{BC}	7.4 ± 0.3 ^{BC}	6.3 ± 0.1 ^C
Procyanidin dimer	6.39	280	577.13	456.15/425.09/289.09	8.1 ± 0.1	7.4 ± 0.0 ^{AB}	7.5 ± 0.1 ^{AB}	7.6 ± 0.1 ^{AB}	8.1 ± 0.1 ^A	7.9 ± 0.0 ^A
Procyanidin dimer	6.48	280	577.13	456.15/425.09/289.09	35.4 ± 1.6	32.7 ± 1.7 ^C	34.0 ± 0.9 ^{BC}	35.5 ± 1.4 ^{BC}	37.4 ± 0.9 ^B	41.6 ± 0.8 ^A
Sum of flavan-3-ols					468.0 ± 11.4	452.6 ± 10.9 ^B	467.3 ± 9.4 ^{AB}	447.9 ± 11.1 ^B	3.5 ± 0.1	374.7 ± 11.7 ^C
Polymeric proanthocyanidins (PACs)					242.9 ± 12.0	230.8 ± 10.1 ^B	278.0 ± 8.9 ^A	231.1 ± 6.5 ^B	200.8 ± 7.7 ^C	223.8 ± 10.0 ^{BC}
Total Phenolic Content (TPC)					886.2 ± 21.5	847.7 ± 22.8 ^B	912.7 ± 18.9 ^A	808.6 ± 16.5 ^C	816.7 ± 17.5 ^C	630.4 ± 14.4 ^D
DP					1.6	1.5	1.6	1.6	1.4	1.7
5-hydroxymethylfurfural (5-HMF)					0.1 ± 0.0	0.5 ± 0.0 ^D	0.6 ± 0.0 ^C	0.6 ± 0.1 ^C	0.7 ± 0.0 ^D	1.7 ± 0.5 ^A

JQPE—without applying any heat treatment (before drying) and therefore not included in the statistical analysis; ±—standard deviation; FD—freeze drying; SD—spray drying; VD—vacuum drying at 50, 70 and 90 °C; DP—degree of polymerization; A–D—different lowercase letters within the column indicate significant differences between samples ($p \leq 0.05$).

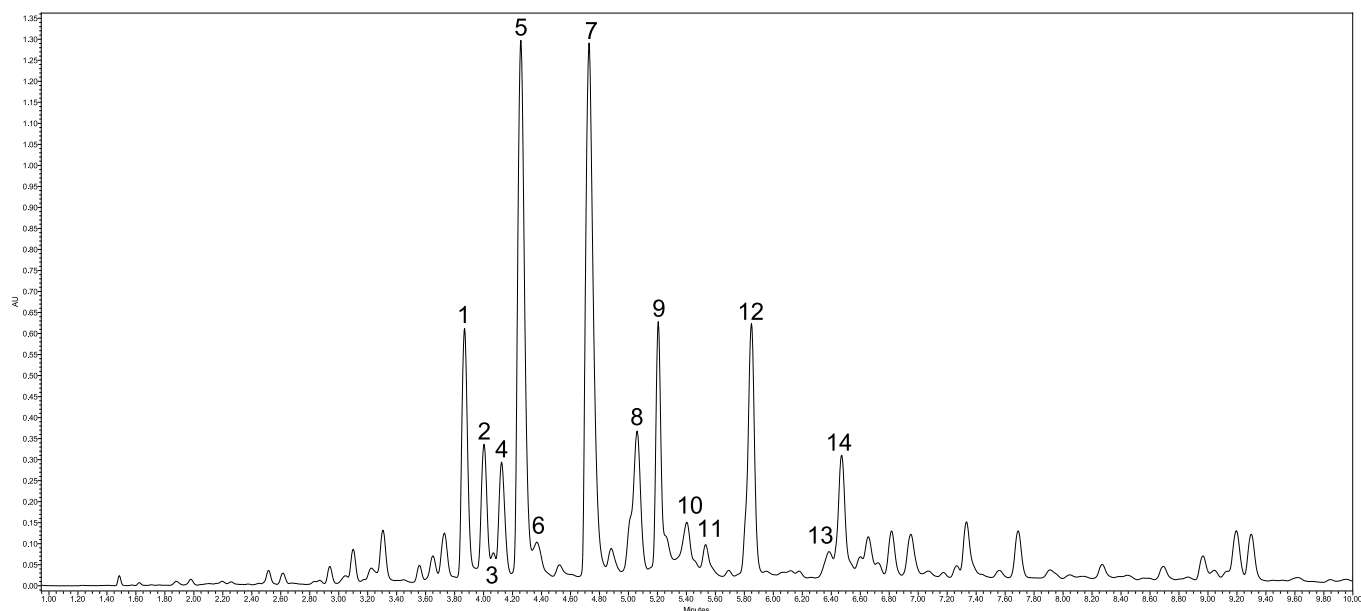


Fig. 1. UPLC–PDA chromatogram segment (1.00–10.00 min) at 280 nm of freeze-dried Japanese quince polyphenol extract. 1-Chlorogenic acid (5-*O*-caffeoylquinic), 2-*p*-coumaroyl hexose, 3-Procyanidin B1, 4-Procyanidin trimer, 5-Procyanidin B2, 6-Procyanidin dimer, 7-(–)-epicatechin, 8-Procyanidin C1, 9-Procyanidin trimer, 10-Procyanidin tetramer, 11-Procyanidin trimer, 12-Syrngic acid hexoside, 13-Procyanidin dimer, 14-Procyanidin dimer.

(R_f), UV–vis maxima (λ_{max}), m/z for the deprotonated molecule and MS/MS fragments. In the present study 15 phenolic compounds belonging to two different groups were tentatively identified. A detailed description of fragmentation is described in the publication of Turkiewicz, Wojdylo, Tkacz, Lech, and Nowicka (2020).

The content of each phenolic compound was quantified using UPLC–PDA, while polymeric proanthocyanidins (PACs) were additionally determined by UPLC–FL (Table 2). The flavan-3-ols including (–)-epicatechin and procyanidins oligomers account for 51.2–59.4% of the total phenolic content (TPC), indicating that this group was in the majority in JQPE. Polymeric PACs constituted on average 29.2% of the TPC, and phenolic acids constituted 15.3%. Overall, there were two representative compounds (procyanidin B2, and syringic acid hexoside). JQPE without heat treatment was characterized by a TPC of 886.2 g/kg dw, and FD and VD (regardless of the temperature used) led to a reduction in the content of polyphenols. SD was an exception, where TPC increased by 2.9%. The methods and parameters of drying had a significant influence on the content of phenolic compounds ($p \leq 0.05$) in JQPE powders. The highest concentrations of individual groups of phenolic compounds were obtained after SD, except for flavan-3-ols, where the VD at 70 °C resulted in the lowest losses of these compounds. The highest degradation of flavan-3-ols (a decrease of one fifth compared to its contents after VD at 70 °C) was found in samples after application of the highest temperature in VD. In turn, the content of phenolic acids in JQPE decreased in the following order: SD > VD 70 °C > VD 50 °C > VD 90 °C, leading to more than 80% degradation of these compounds after the use of VD 90 °C. For example, Michalska et al. (2017) in research on plum juice extract noted a reduction in the content of chlorogenic acid by more than 6% after increasing the temperature in VD from 60 to 80 °C. Analyzing the effect of the drying method, it was observed that the greatest retention of polymeric PACs, besides SD, was obtained by VD at 50 °C and FD – 231.1 and 230.8 g/kg dw, respectively. Referring to TPC, an increase in temperature in VD from 70 to 90 °C caused a decrease in TPC content by over 20%. In contrast, Miao et al. (2017), in research on the effect of heat treatment and drying methods on the quality of *Chaenomeles* fruits, observed an increase in TPC by 12% after increasing the temperature in VD from 60 to 80 °C.

3.2.2. 5-HMF analysis

During thermal processes (such as drying) in food processing, non-enzymatic browning reactions take place, including the Maillard reaction and caramelization, imparting desirable organoleptic characteristics to food products (Lee et al., 2019). However, compounds such as 5-hydroxymethylfurfural (5-HMF) formed during non-enzymatic browning can have negative effects on the human body. Ingestion of 5-HMF above the recommended dietary limit may cause carcinogenicity, genotoxicity and organotoxicity (Choudhary et al., 2020). At the same time, the limits of its dietary intake have so far been established only for a few products (including honey), which is why it is so important to control its concentration in food. The 5-HMF content (Table 2) in JQPE powders ranged from 4.9 (after FD) to 17.4 mg/kg dw (after VD at 90 °C). Comparing the drying methods, the obtained powders differed significantly in terms of 5-HMF content ($p \leq 0.05$). The use of VD at 90 °C resulted in an over 3.5-fold increase in 5-HMF concentration compared to FD. Increasing the temperature in vacuum drying from 70 to 90 °C increased the 5-HMF content in powders over 2-fold. Moreover, SD and VD at 50 °C did not differ significantly in terms of 5-HMF content. For comparison, the content of 5-HMF in the obtained powders was similar to that in fruit juices (0.4–21.9 mg/kg) but lower than in dried fruit (1.0–2200.0 mg/kg) (Choudhary et al., 2020). Michalska et al. (2017) obtained values for plum juice extract powders below 0.1 mg/kg dw with no clear differences between the drying methods used. This value is close to the 5-HMF content in JQPE without heat treatment (0.01 mg/kg dw).

3.2.3. Antioxidant activities and enzymatic *in vitro* inhibition tests

The antioxidant capacity of the dried JQPE was measured by three methods (ABTS, FRAP and ORAC), as shown in Table 3. There were significant differences in activities between samples dried using different methods ($p \leq 0.05$). The highest antioxidant capacity for powders was demonstrated by VD samples at 50 °C (430.3 and 294.6 mmol Trolox/100 g dw), while the lowest was observed for FD (319.2 and 208.8 mmol Trolox/100 g dw) – both in the ABTS and FRAP methods, respectively. In the case of the assessment of oxygen radical absorbance capacity (ORAC), no difference was found between the application of temperature 50 and 90 °C in the vacuum method, while by application of 70 °C in VD it was possible to obtain 65% higher

Table 3

Antioxidant capacity (mmol Trolox/100 g dw), anti-diabetic, anti-obesity, anticholinesterase (IC₅₀; mg/mL) and 15-lipoxygenase (15-LOX) inhibition activity (%) of Japanese quince polyphenol extract powders.

Drying method	Antioxidant capacity			α -Amylase	α -Glucosidase	Pancreatic lipase	AChE	BuChE	15-LOX
	ABTS	FRAP	ORAC						
JQPE*	259.8 ± 9.2	142.6 ± 4.6	460.3 ± 4.5	120.8 ± 1.2	5.6 ± 0.0	3.6 ± 0.0	51.0 ± 0.9	49.8 ± 1.2	99.8 ± 0.8
FD	319.2 ± 2.8 ^E	208.8 ± 5.1 ^D	886.7 ± 0.5 ^C	21.8 ± 0.8 ^D	2.9 ± 0.2 ^{BC}	0.4 ± 0.0 ^A	16.0 ± 0.7 ^B	22.1 ± 0.4 ^D	42.9 ± 0.4 ^D
SD	411.2 ± 5.9 ^D	257.3 ± 5.1 ^B	1178.0 ± 2.5 ^{BC}	15.8 ± 0.4 ^C	2.6 ± 0.6 ^B	0.4 ± 0.5 ^A	16.6 ± 0.8 ^B	11.9 ± 0.2 ^B	48.6 ± 0.0 ^C
VD 50 °C	430.3 ± 2.0 ^A	294.6 ± 4.6 ^A	1249.5 ± 4.5 ^B	7.1 ± 0.2 ^A	2.5 ± 0.3 ^B	0.6 ± 0.0 ^B	39.8 ± 0.6 ^D	14.4 ± 0.2 ^C	40.7 ± 0.8 ^E
VD 70 °C	390.9 ± 1.1 ^C	239.6 ± 3.7 ^C	1455.5 ± 2.5 ^A	10.8 ± 0.5 ^B	3.1 ± 0.0 ^C	1.0 ± 0.2 ^C	28.2 ± 0.6 ^C	22.0 ± 0.6 ^D	81.6 ± 0.8 ^A
VD 90 °C	417.8 ± 5.6 ^B	229.7 ± 4.1 ^C	1255.7 ± 1.4 ^B	15.9 ± 0.9 ^C	1.6 ± 0.1 ^A	0.7 ± 0.2 ^B	11.3 ± 0.5 ^A	10.1 ± 0.4 ^A	69.8 ± 0.6 ^B

JQPE—without applying any heat treatment (before drying) and therefore not included in the statistical analysis; ±—standard deviation; FD—freeze drying; SD—spray drying; VD—vacuum drying at 50, 70 and 90 °C; A–E—different capital letters within the column in the group extract indicate significant differences between samples ($p \leq 0.05$).

activity than after the FD process (886.7 mmol Trolox/100 g dw). It was noted that for JQPE without heat treatment, the activity measured by ABTS, FRAP and ORAC assays were respectively 18.8; 31.7 and 48.1% lower compared to JQPE powder after FD. The presence of ethanol in JQPE and the potential side compounds that may be formed during the drying process and absent in JQPE, most likely may have contributed to the reduction of antioxidant activity, but requires further research to determine the cause of this trend. Comparing the antioxidant capacity of dried JQPE to the activity of selected *Chaenomeles* species fruits, it was found that on average for the ABTS method, the JQPE activity is 18 times higher, and for the FRAP method more than 44 times (Du et al., 2013). On the other hand, for the ORAC method, the values obtained for JQPE powder are on average over 20 times higher than for the *Chaenomeles japonica* fruits dried by different methods (Turkiewicz et al., 2019). The calculated Pearson correlation coefficient confirmed the positive correlation between the 5-HMF content and the antioxidant capacity of ABTS and ORAC ($r^2 = 0.65$ and 0.60 , respectively), which is consistent with the results of Michalska et al. (2017). Moreover, in the study of Turkiewicz, Wojdyło, Tkacz, Lech, and Nowicka (2020) on Japanese quince juice powders the dependence in the presence of 5-HMF on antioxidant capacity was proven.

Table 3 also shows the anti-diabetic and anti-obesity potential of dried JQPE expressed as ability to inhibit α -amylase, α -glucosidase, and pancreatic lipase (IC₅₀; mg/mL). The analyzed samples showed large variation ($p \leq 0.05$) among the used drying methods. The inhibitory activity against α -amylase ranged from 7.1 to 21.8 mg/mL (for VD 50 °C and FD, respectively), while α -glucosidase inhibition was between 1.6 and 3.1 mg/mL (VD 90 and 70 °C, respectively). The use of the drying process, regardless of the method, contributed to the increase in anti-diabetic and anti-obesity activity of Japanese quince polyphenol extract. Compared to the powder obtained after FD, JQPE without thermal treatment was characterized by over 5 times lower potential for inhibiting α -amylase, almost 2 times lower for α -glucosidase and as much as 9 times lower activity for inhibiting pancreatic lipase. The use of vacuum drying at 90 °C resulted in obtaining a value similar to the inhibition of α -amylase as after the SD. Moreover, FD and SD turned out to be the most advantageous in terms of potential anti-obesity properties of JQPE powders (0.4 mg/mL). The obtained results confirm previous reports (Miao et al., 2018; Zaklos-Szyda, Majewska, Redzynia, & Koziolkiewicz, 2015) that *Chaenomeles* fruits may be promising natural sources for active compounds with antidiabetic properties. The Pearson correlation coefficient calculated for ability to inhibit α -amylase and antioxidant capacity (ABTS, FRAP and ORAC) confirms a strong positive correlation between them ($r^2 = 0.86$, 0.87 and 0.90 , respectively). Moreover, a strong correlation between the α -glucosidase inhibition activity and the content of 5-HMF ($r^2 = 0.80$) was found; until now there has been no information on the correlation of these two factors in the literature. It was also noted that among the analyzed groups of phenolic compounds, the polymeric PACs had the greatest influence on modulating pancreatic lipase inhibition activity ($r^2 = 0.80$), which was also confirmed in the recent study by Wojdyło et al. (2020) on the biological

activity of sprouts and microgreens.

The anticholinergic properties in the analyzed dried JQPE were expressed as the ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Attempts were also made to assess the ability to inhibit 15-lipoxygenase (15-LOX) as a regulator of cellular lipid peroxidation. IC₅₀ inhibition of AChE and BuChE ranged from 11.3 to 39.8 and from 10.1 to 22.1 mg of sample/mL (Table 3) with significant differences between samples dried by different methods ($p \leq 0.05$). Activity against AChE increased with increasing temperature in VD, while no difference was noted between FD and SD. In turn, considering the ability to inhibit BuChE, JQPE samples can be ordered according to increasing activity as follows: FD = VD 70 °C < VD 50 °C < SD < VD 90 °C. It is worth noting that for the first time the anticholinergic activities of *Chaenomeles* fruits were investigated by Turkiewicz, Wojdyło, Tkacz, Nowicka, et al. (2020) and for the Japanese quince were on average 11.8 and 16.5 mg/mL for AChE and BuChE, respectively. The polymeric PACs were found to have the greatest contribution to the formation of anticholinergic properties of the dried JQPE among the phenolic compounds identified ($r^2 = 0.28$ and 0.53 for AChE and BuChE, respectively). Moreover, as in the case of antioxidant capacity, the Pearson correlation coefficient indicates a positive correlation between the 5-HMF content and the ability to inhibit AChE and BuChE – 0.68 and 0.70 , respectively. As with the antioxidant, anti-diabetic and anti-obesity activity, the AChE and BuChE inhibition activity of the JQPE without thermal treatment was respectively two and three times lower, than after FD. On the other hand, the AChE inhibition activity of powders obtained from JQPE is on average twice as high as the activity of powders from *Chaenomeles* juice (Turkiewicz, Wojdyło, Tkacz, Lech, & Nowicka, 2020).

The 15-LOX inhibition activity was expressed as % inhibition at a sample concentration of 2.5 mg/mL. The highest potential was exhibited by VD JQPE at 70 °C (81.6%), while the lowest was shown by VD JQPE at 50 °C (40.7%) ($p \leq 0.05$). As in the case of the ability to inhibit α -glucosidase and AChE, with regard to 15-LOX it is confirmed that it is more advantageous in vacuum drying to shorten the drying time instead of decreasing the temperature. Moreover, of all the methods used to measure the antioxidant capacity, the anti-15-LOX activity is most strongly correlated with the ORAC assay ($r^2 = 0.73$) and there is no correlation with the content of phenolic acids ($r^2 = 0.00$).

3.3. Principal component analysis (PCA)

A PCA biplot (Fig. 2) shows both PC scores of samples and loadings of variables and allowed better understanding of the relationship between bioactive compounds and *in vitro* biological activities of JQPE dried by different methods. The first two modes of the PCA explained more than 80% of the observed variance, and therefore it can be assumed that the demonstrated dependencies are reliable. The upper left quadrant contains the variables that show the greatest correlation with each other. Thus, it can be seen that the three methods used to assess the antioxidant capacity (ABTS, FRAP and ORAC) are correlated. Moreover, the activity

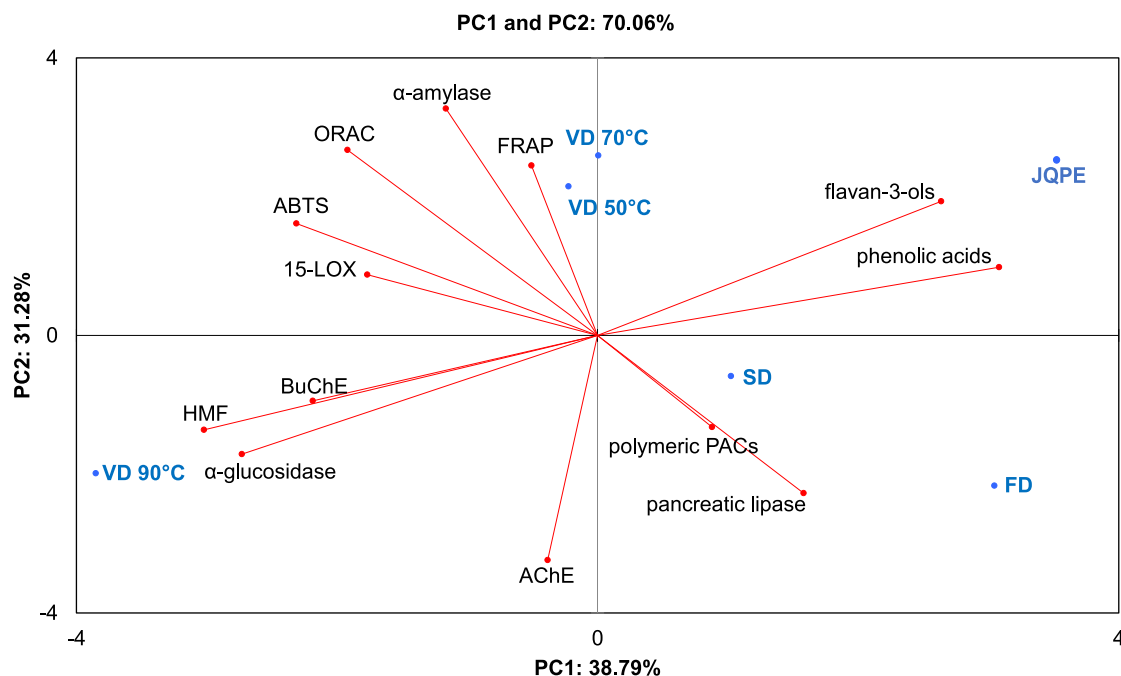


Fig. 2. PCA biplot showing scores of samples (blue dots) and loadings of variables (red vectors). JQPE—without applying any heat treatment (before drying); FD—freeze drying; SD—spray drying; VD—vacuum drying at 50, 70 and 90 °C; PACs—proanthocyanidins; AChE—acetylcholinesterase; BuChE—butyrylcholinesterase; 15-LOX—15-lipoxygenase. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to inhibit α -amylase and the ability to inhibit 15-LOX have a strong positive correlation with antioxidant capacity. These results are confirmed by the observations of [Tkacz, Wojdyło, Turkiewicz, and Nowicka \(2020\)](#) on sea buckthorn berries. This group included samples dried at the lowest temperature (VD at 50 °C). In the lower left quadrant there are, among others, anticholinergic activities (against AChE and BuChE) affected by the presence of 5-HMF. Therefore, the VD at 90 °C was in the same group, in which the largest amounts of this compound were formed. In addition, as in the [Cano-Lamadrid et al. \(2020\)](#) study on pomegranate fruit sheets, a dependence of anticholinergic activity on the ability to inhibit α -glucosidase was found. The upper right corner was occupied by flavan-3-ols and phenolic acids, the content of which in the analyzed samples of dried JQPE was dependent on each other. The last group was composed of polymeric PACs modulating anti-obesity activity and the FD and SD method, which ensured the greatest retention of polymeric PACs.

4. Conclusions

The polyphenol extract from Japanese quince fruit was found to be a rich source of phenolic compounds, in particular from the flavan-3-ol group (on average 53.4% of the total phenolic content). SD seems to be the most advantageous method in terms of the retention of bioactive compounds, while ensuring a low content of 5-HMF (comparable to FD). On the other hand, taking into account the physical parameters, i.e. true and bulk density and porosity, VD provided the most favorable parameters, especially in the context of the potential reduction of transport and packaging costs and the low susceptibility to oxidation processes. Conducting the VD process at 90 °C resulted in negative chemical changes (3.5-fold increase in 5-HMF concentration compared to FD) as well as deterioration of color parameters (significantly darkening). Considering the influence of the drying method and its parameters on biological properties, it was found that the dried JQPE was characterized by a high antioxidant potential, especially after the application of VD at 50 and 70 °C. In addition, the antidiabetic properties of JQPE powders were greater after VD than FD and SD, in contrast to anticholinergic properties. In summary, VD may be an alternative to the previously widely

used SD in the industry, but it is necessary to conduct further research, e.g. using polysaccharide carrier agents, to improve the physical properties and potentially protect labile phenolic compounds.

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CRediT authorship contribution statement

Igor Piotr Turkiewicz: Writing – review & editing, Formal analysis, Methodology, Visualization. **Karolina Tkacz:** Formal analysis, Methodology. **Paulina Nowicka:** Formal analysis. **Anna Michalska-Ciechanowska:** Conceptualization, Formal analysis, Methodology. **Krzysztof Lech:** Formal analysis, Methodology. **Aneta Wojdyło:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Conflict of 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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mgr inż. Igor Piotr Turkiewicz

Wrocław, 07.02.2022 r.

Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że jestem współautorem publikacji pt.:

Turkiewicz, I.P., Tkacz, K., Nowicka, P., Michalska-Ciechanowska, A., Lech, K.,
Wojdyło, 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.

Mój udział w przygotowaniu tej publikacji polegał na kierowaniu projektem
naukowym obejmującym badania opisane w tej pracy (Diamentowy Grant VII,
nr DI2017 006347), współtworzeniu koncepcji prowadzonych badań, wykonaniu analiz
fizykochemicznych, chromatograficznych i potencjału biologicznego *in vitro* produktów
z owoców pigwowca. Opracowałem technologię otrzymywania proszków pigwocowych,
przygotowałem tekst publikacji, opracowałem merytorycznie otrzymane wyniki,
przeprowadziłem dyskusję oraz współredagowałem odpowiedzi na recenzje.

TURKIEWICZ IGOR

.....
Podpis składającego oświadczenie

mgr inż. Karolina Tkacz

Wrocław, 07.02.2022 r.

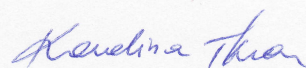
Katedra Technologii Owoców, Warzyw
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Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Turkiewicz, I.P., **Tkacz, K.**, Nowicka, P., Michalska-Ciechanowska, A., Lech, K., Wojdyło, 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

mój udział polegał na współredagowaniu publikacji oraz identyfikacji związków polifenolowych metodą LC/MS.



.....
Podpis składającego oświadczenie

dr hab. inż. Paulina Nowicka, prof. uczelni

Wrocław, 07.02.2022 r.

Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Turkiewicz, I.P., Tkacz, K., **Nowicka, P.**, Michalska-Ciechanowska, A., Lech, K., Wojdyło, 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

mój udział polegał na analizie formalnej publikacji oraz wykonaniu części analiz potencjału biologicznego *in vitro*.

Paulina Nowicka

Podpis składającego oświadczenie

dr hab. Anna Michalska-Ciechanowska, prof. uczelni

Wrocław, 07.02.2022 r.

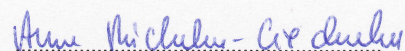
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Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chelmońskiego 37
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Turkiewicz, I.P., Tkacz, K., Nowicka, P., **Michalska-Ciechanowska, A.**, Lech, K., Wojdyło, 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

mój udział polegał na kreowaniu koncepcji technologii otrzymywania proszków pigwowcowych metodami suszarniczymi oraz pomocy przy merytorycznym współredagowaniu publikacji.



Podpis składającego oświadczenie

dr hab. inż. Krzysztof Lech, prof. uczelni

Wrocław, 07.02.2022 r.

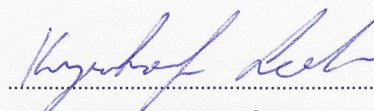
Instytut Inżynierii Rolniczej
Wydział Przyrodniczo-Technologiczny
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37/41
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Turkiewicz, I.P., Tkacz, K., Nowicka, P., Michalska-Ciechanowska, A., **Lech, K.**, Wojdyło, 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

mój udział polegał na uczestnictwie w wykonaniu oznaczeń właściwości fizycznych proszków pigwcowych oraz merytorycznym współredagowaniu publikacji.



.....
Podpis składającego oświadczenie

prof. dr hab. inż. Aneta Wojdyło

Wrocław, 07.02.2022 r.

Katedra Technologii Owoców, Warzyw
i Nutraceutyków Roślinnych
Wydział Biotechnologii i Nauk o Żywności
Uniwersytet Przyrodniczy we Wrocławiu
ul. Chełmońskiego 37
51-630 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy pt.:

Turkiewicz, I.P., Tkacz, K., Nowicka, P., Michalska-Ciechanowska, A., Lech, K., **Wojdyło, 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

mój udział polegał na tworzeniu i nadzorze koncepcji projektu (Diamentowy Grant VII, nr DI2017 006347), w ramach którego realizowana była praca doktorska, uczestnictwie w analizach chromatograficznych i potencjału biologicznego *in vitro* owoców pigwowca i ich produktów, koordynowaniu prac Doktoranta, współredagowaniu publikacji i merytorycznej ocenie wyników.

.....
Podpis składającego oświadczenie

8. Dorobek naukowy

Wykształcenie:

- 2018 - 2022 Uniwersytet Przyrodniczy we Wrocławiu, Technologia żywności i żywienia, Studia doktoranckie III°
- 2017 - 2018 Uniwersytet Przyrodniczy we Wrocławiu, Technologia żywności i żywienia człowieka, Studia magisterskie II° (egzamin 20.06.2018), Ocena: bardzo dobra
- 2013 - 2017 Uniwersytet Przyrodniczy we Wrocławiu, Technologia żywności i żywienia człowieka, Studia inżynierskie I° (egzamin 07.02.2017), Ocena: bardzo dobra

Staże naukowe:

- 01.03. - 01.07.2022 University of Lisbon, Lisbon, Portugalia (Bekker, NAWA)
- 01.02. - 01.03.2020 University of Minnesota, Saint Paul, USA (PROM-NAWA)
- 05.04. - 04.05.2019 CEBAS-CSIC, Murcia, Hiszpania (PROM-NAWA)
- 15.07. - 14.08.2018 Tymbark MWS Sp. z o.o. Sp. k., Olsztynek
- 01.03. - 31.05.2018 Universidad Miguel Hernández, Orihuela, Hiszpania (ERASMUS+)

Szkolenia:

- 15-17.12.2021 Szkolenie z obsługi wysokosprawnego chromatografu cieczowego Acquity UPLC sprzężonego z wysokorozdzielczym spektrometrem mas Xevo G2-QToF, Waters, Wrocław.
- 19-22.09.2021 V edycja Akademii Chemii Analitycznej "Spektrometria mas w chromatografii cieczowej - praktyczne zastosowania", SHIM-POL A.M. Borzymowski, Warszawa.
- 15-17.05.2019 Podstawy technik chromatografii i tandemowej spektrometrii mas (LC-MS/MS) w oznaczeniach ilościowych, MS Ekspert Sp. z o.o., Warszawa.
- 01.01. - 30.06.2019 Program Szkoleniowo - Mentoringowy TopMinds 2019. Inicjatywa Stowarzyszenia Top 500 Innovators i Polsko-Amerykańskiej Komisji Fulbrighta, Warszawa.
- 04-05.06.2016 Wymagania Standarów sieciowych IFS i BRC, Swisscert Sp. z o.o., Kraków.
- 16-17.04.2016 Auditor wewnętrzny HACCP, Swisscert Sp. z o.o., Kraków.
- 19-20.03.2016 Auditor wewnętrzny systemu zarządzania jakością ISO 9001, Swisscert Sp. z o.o., Kraków.
- 20-21.02.2016 Pełnomocnik systemu zarządzania bezpieczeństwem żywności ISO 22000, Swisscert Sp. z o.o., Kraków.

Projekty badawcze:

a) kierownik

Diamentowy Grant VII. Ministerstwo Nauki i Szkolnictwa Wyższego. DI2017 006347. 07.09.2018-06.09.2021: „Potencjalne wykorzystanie owoców pigwowca (*Chaenomeles* spp.) w otrzymaniu innowacyjnych produktów o zaprogramowanych właściwościach prozdrowotnych”.

Innowacyjny Doktorat. Uniwersytet Przyrodniczy we Wrocławiu. N070/0015/20. 01.01.2020-31.12.2020: „Modulowanie wartości odżywczej i właściwości funkcjonalnych owoców *Chaenomeles japonica* procesem odwadniania osmotycznego”.

b) wykonawca

POIR.01.01.01-00-1170/19-00. POIR 2014-2020. 01.06.2020-31.05.2023: „Innowacyjne rozwiązania technologiczne w procesie opracowywania produktów o wyższym poziomie związków bioaktywnych”.

Preludium 18. Narodowe Centrum Nauki. 09.06.2020-08.06.2023; „Wpływ dodatków pochodzenia naturalnego na formowanie się produktów reakcji Maillarda i karmelizacji oraz na właściwości biologiczne proszków owocowych”.

POIR.01.01.01-00-0261/17. POIR 2014-2020. 19.01.2018-02.02.2021. „Opracowanie nowych przetworów warzywno-owocowych o ukierunkowanych właściwościach prozdrowotnych”.

PJ.re.027.3.2019. Ministerstwo Rolnictwa i Rozwoju Wsi. 01.04.2019-31.12.2019: „Innowacyjne rozwiązania w zakresie wykorzystania warzyw i owoców”.

HOR.re.027.9.2018. Ministerstwo Rolnictwa i Rozwoju Wsi. 01.04.2018-31.12.2018: „Badania nad innowacyjnymi rozwiązaniami w celu poprawy cech i parametrów sensorycznych produktów przetwórstwa owoców ekologicznych z uwzględnieniem zachowania składników odżywczych otrzymywanych produktów”.

Publikacje nie wchodzące w skład rozprawy doktorskiej:

Turkiewicz, I. P., Wojdyło, A., Tkacz, K., Nowicka, P. (2021). UPLC/ESI-Q-TOF-MS analysis of (poly)phenols, tocopherols 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 MEiN: 200 pkt

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: 10.1016/j.indcrop.2021.114214 IF: 5,645 MEiN: 200 pkt

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: 10.3390/molecules26247593 IF: 4,412 MEiN: 140 pkt

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: 4,380 MEiN: 140 pkt

Yusuf, E., Tkacz, K., **Turkiewicz, I. P.**, Wojdyło, A., Nowicka, P. (2021). Analysis of physiochemical 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*, *247*(12), 3053-3062. doi: 10.1007/s00217-021-03857-0 IF: 2,998 MEiN: 70 pkt

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), 1466-1474. doi: 10.1002/jsfa.11481 IF: 3,639 MEiN: 100 pkt

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 MEiN: 100 pkt

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 MEiN: 100 pkt

Tkacz, K., Gil-Izquierdo, Á., Medina, S., **Turkiewicz, I. P.**, Domínguez-Perles, R., Nowicka, P., Wojdyło, A. (2021). Phytoprostanes, phytofuranos, 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 IF: 3,639 MEiN: 100 pkt

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 MEiN: 200 pkt

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 MEiN: 140 pkt

Cano-Lamadrid, M., Tkacz, K., **Turkiewicz, I. P.**, Nowicka, P., Hernández, F., Lech, K., Carbonell-Barrachina, Á. 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: 3,639 MEiN: 100 pkt

Tkacz, K., Wojdyło, A., **Turkiewicz, I. P.**, Nowicka, P. (2020). 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 MEiN: 200 pkt

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 MEiN: 100 pkt

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 IF: 4,412 MEiN: 140 pkt

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 MEiN: 200 pkt

Ali, A., Choo, C. O., Chua, B. L., Figiel, A., Chong, C. H., Wojdyło, A., **Turkiewicz, I. P.**, Szumny, A., Łyczko, J. (2020). Volatile and polyphenol composition, anti-oxidant, anti-diabetic and anti-aging properties, and drying kinetics as affected by convective and hybrid vacuum microwave drying of *Rosmarinus officinalis* L. *Industrial Crops and Products*, 151, 112463. doi: 10.1016/j.indcrop.2020.112463 IF: 5,645 MEiN: 200 pkt

Tkacz, K., Bobak, Ł., **Turkiewicz, I. P.**, Nowicka, P., Wojdyło, A. (2019). Anti-oxidant and anti-enzymatic activities of sea buckthorn (*Hippophaë rhamnoides* L.) fruits modulated by chemical components. *Antioxidants*, 8(12), 618. doi:10.3390/antiox8120618 IF: 6,313 MEiN: 100 pkt

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ORCID: 0000-0002-3837-6308

WoS ResearcherID: U-2678-2018

Scopus Author ID: 57207861322