Wrocław University of Environmental and Life Sciences
Faculty of Biotechnology and Food Science
Scientific discipline: Nutrition and Food Technology

M.Sc. Jessica Brzezowska

Creation of high-quality plant-based powders with targeted health-oriented properties

Kształtowanie wysokojakościowych proszków roślinnych o ukierunkowanych właściwościach prozdrowotnych

Doctoral dissertation

Supervisor:
Dr hab. Anna Michalska-Ciechanowska

Second Supervisor:
Prof. Maria Dolores del Castillo

Wrocław 2023

"Inspiration exists, but it has to find you working"

Pablo Picasso

Table of contents

PUBLICATIONS AND SUPPLEMENTARY STUDIES INCLUDED IN THE HYBRID DOCTORAL DISSERTATION

Publication 1: Michalska-Ciechanowska, A.; Hendrysiak, A.; **Brzezowska, J.**; Wojdyło, A.; Gajewicz-Skretna, A. (2021). How do the different types of carrier and drying techniques affect the changes in physico-chemical properties of powders from chokeberry pomace extracts? *Foods*, 10, 1864. https://doi.org/10.3390/foods10081864

IF 4.927; 140 MSHE points, number of citations: 5

***Publication 2*: Brzezowska, J.**, Skrzypczak, K., Radzki, W., Turkiewicz, I.P., Ziaja-Sołtys, M., Bogucka-Kocka, A., Wojdyło, A., Michalska-Ciechanowska, A. (2023). Comparative study of antioxidant, antiglycation and chemoprotective potential of beetroot juice powder formulations with functional carriers. *Food Bioscience*, 55, 103049. doi: https://doi.org/10.1016/j.fbio.2023.103049

IF 5.200; 70 MSHE points, number of citations: 0

Total:
Impact Factor: **10.127**
MSHE score: **210**
Citations: **5** (*WoS*), **5** (*Scopus*), **7** (*Google Scholar*) as of 16.09.2023

Supplementary study 1: Extraction-depended and thermally-modulated physical and chemical properties of powders produced from cranberry pomace extracts

Supplementary study 2: How do the (poly)phenolics and amino acids affect the antioxidant, antidiabetic and antiglycation properties of selected fruit powders?

Supplementary study 3: The influence of different drying techniques and type of carrier on the formation of Maillard reaction products - the case of fruit juice resembling model powders

Supplementary study 4: Matrix changes driven by cultivar diversity, inulin addition and drying techniques - shaping the antioxidant, antimicrobial and anti-inflammatory properties of blueberry powders

Doctoral dissertation carried out under the:

POWER 3.5. programme *"UPWR 2.0: international and interdisciplinary programme of development of Wrocław University of Environmental and Life Sciences"*, co-financed by the European Social Fund under the Operational Program Knowledge Education Development, under contract No. POWR.03.05.00-00-Z062 / 18 of June 4, 2019.

PRELUDIUM-18 project (2019/35/N/NZ9/03208) financed by the National Science Centre (Poland) entitled *"The effect of the natural food additives on the formation of Maillard reaction/caramelisation products and on the biological activity of fruit powders"*

SONATA project (2016/23/D/NZ9/02671) financed by the National Science Centre (Poland) entitled *"The analysis of physical and chemical properties of powders obtained from the juices and pomace of selected fruits using different drying methods and determination of their influence on the chosen indicators of the immune system in in vivo model studies"*

ABSTRACT

Considering the global practices promoting sustainable agriculture, as well as in line with the latest trends in the food industry responding to consumer needs, the production of plant-based powders offers a strong potential in this context. The quality of such products is a resultant of initial plant matrix composition subjected to multi-step processing, which in turn can trigger formation of hazardous process contaminants. Thus, moderation of their quality in terms of the presence of native bioactives and minimized process contaminants formation is of great importance.

The study aimed at the benefit-risk evaluation of the processing conditions for plant-based powders obtainment depending on the matrix composition using holistic approach including physico-chemical and biological characterization, and to provide the recommendations for their production. The research was structured in three main stages designed to investigate: (**I**) processing-induced changes towards improved properties of plant powder products; (**II**) plant-based matrix complexity vs. bioactive response and process contaminants drivers; and (**III**) cross-factors influencing the biological properties of powdered plant products.

In the first phase, a multidirectional approach was adopted to recover bioactive components from botanical by-products into ready-to-use powders. For powdered preparations from chokeberry pomace, the inclusion of selected carrier(s) during particular drying techniques provided the highest retention of (poly)phenolics and the lowest hydroxymethyl-*L*-furfural (HMF) content. The formation of the latest was attributed to the inulin application for powder production. Cranberry preparations showed substantial differences in (poly)phenolics and HMF presence and content depending on solvent used for pomace extraction, with the indication of acidified 50% ethanol as the preferred extraction medium, while using acetone favored the formation of this undesirable compound in powders. Suitably adjusted processing was proved to be an effective strategy in minimizing the content of process contaminants in the final product. Overall, the findings confirmed the feasibility of converting chokeberry and cranberry pomace into high quality powders as well as their extraction-dependent and thermo-modulated quality modification.

The second stage was designed to recognize the effectiveness of selected components of different fruit matrices in shaping the bioactive potential of the powders obtained from them. The heterogeneity of the matrix in which the bioactives were present conditioned their anti-diabetic ability, while flavonols affected antiglycation potential of freeze-dried fruit products according to the reaction stage. In order to explore the matrix-originated drivers that influence the formation of process contaminants, model systems resembling simplified composition of selected fruit juices were adopted by including major organic acid, sugar, and ascorbic acid. Furfural (FF) was formed when drying at 90 °C, while HMF was formed even at 60 °C and above and in considerably lower quantities under spray drying conditions. No process contaminants were detected in lyophilized powders, proving that their formation in real matrix depends on other components. Freeze- and spray drying ensured comparable retention of vitamin C, and no linkage was found between this compound and process contaminants presence. Finally, based on compositional differences between models applied and resulting FF

and HMF contents it was possible to surmised, that the organic acids may also take part in the process contaminants formation under specific processing conditions in plant-based matrix. Consistently, matrix complexity was presented as a critical differentiating factor determining the properties of plant powders, which should be carefully considered when designing their powdering technology.

The final stage included examination of biological potential of powders obtained from plant-based matrices that were diversified in terms of cultivar (blueberry matrix) as well as its pretreatment (beetroot matrix) and subjected to different processing conditions. The cultivar differentiation resulted in substantial antioxidant capacity differences pointing at blueberry powders from Bluecrop and Bluejay displaying the most desired potential. The higher antibacterial activity against *Helicobacter pylori* when compared to *Campylobacter jejuni* was presented for all blueberry products, however carrier application significantly weakened this effect. Treating at relatively high temperatures during vacuum drying tended to improve the anti-inflammatory properties of such products, depending on the cultivar used. In case of beetroot powders, juice pretreatment reduced betalains content in final products, while inulin was the most effective in protection of syringic acid derivatives. Products with oligofructose and inulin exhibited stronger antioxidant capacity than those with Nutriose® and maltodextrin. Nutriose®-added products showed selective antiproliferative activity toward leukemia cell lines, while oligofructose-based stimulated the *in vitro* proliferation of cancer cells. Moreover, oligofructose induced HMF formation, regardless processing applied. Finally, it was proven that the specific biological response of plant-based powders was dependent on joint matrix-processing interrelation.

Conclusively, in the study the comprehensive overview about relationship between plant-based matrix, applied processing and resulting properties was presented. The potential of processing to produce powders with the best possible properties has been demonstrated, depending on the botanical material used. The complexity of the constantly changing matrix has been identified as a major challenge in the design of high-quality powder products, as even small variations can alter their properties. Therefore, the development of plant powders with targeted health-related properties should be preceded by preliminary studies dedicated to the specific material to be processed.

Keywords: plant-based matrix, probiotic fermentation, functional carriers, processing, freeze-drying, vacuum drying, spray drying, powdering technology, powders, (poly)phenolics, process contaminants, furfural, hydroxymethyl-*L*-furfural, antioxidant properties, anti-glycation properties, anti-diabetic properties, anti-bacterial properties, anti-inflammatory properties, chemoprotective properties

STRESZCZENIE

Proszkowanie surowców roślinnych o wysokim potencjale prozdrowotnym jest odpowiedzią na aktualne wymagania konsumentów, które znajdują odzwierciedlenie w najnowszych trendach przemysłu spożywczego i jednocześnie wpisują się w światową politykę zrównoważonego rolnictwa. Jakość sproszkowanych produktów na bazie roślin jest ściśle związana ze składem wyjściowym matrycy poddawanej wieloetapowemu procesowi przetwarzania, co z kolei może prowadzić do tworzenia się szkodliwych zanieczyszczeń procesowych, czyli substancji powstających podczas przetwarzania żywności. W związku z tym, moderowanie jakości proszków pod względem obecności natywnych składników bioaktywnych i minimalizacji powstawania zanieczyszczeń procesowych ma ogromne znaczenie dla konsumentów.

Badania miały na celu ocenę pozytywnych i negatywnych aspektów związanych z warunkami przetwarzania produktów roślinnych w zależności od składu matrycy przy użyciu wielokierunkowego podejścia obejmującego charakterystykę fizykochemiczną i biologiczną proszków oraz przedstawienie zaleceń dotyczących ich produkcji. Badania zostały podzielone na trzy główne etapy dotyczące: (**I**) zmian wywołanych przetwarzaniem w kierunku poprawy właściwości sproszkowanych produktów roślinnych; (**II**) złożoności matrycy roślinnej a odpowiedzi bioaktywnej i czynników powodujących powstawanie zanieczyszczeń procesowych; oraz (**III**) czynników wpływających na właściwości biologiczne sproszkowanych produktów roślinnych.

W pierwszym etapie przyjęto wielokierunkowe podejście do odzyskiwania bioaktywnych składników z roślinnych produktów ubocznych w postaci gotowych do użycia proszków. W przypadku sproszkowanych preparatów z wytłoków aroniowych, zastosowanie wybranych nośników podczas suszenia zapewniło najwyższą retencję (poli)fenoli i najniższą zawartość hydroksymetylo-*L*-furfuralu (HMF), którego powstawanie powiązano z zastosowaniem inuliny. Z kolei, preparaty z żurawiny wykazały znaczne różnice w zawartości i obecności (poli)fenoli i HMF w zależności od rozpuszczalnika użytego do ekstrakcji wytłoków. Wskazano zastosowanie zakwaszonego 50% etanolu jako preferowanego medium ekstrakcyjnego, podczas gdy użycie acetonu sprzyjało tworzeniu się HMF w proszkach. Odpowiednio dostosowane warunki produkcji okazały się skuteczną strategią w minimalizowaniu zawartości zanieczyszczeń procesowych. Wyniki badań potwierdziły możliwość przetwarzania wytłoków z aronii i żurawiny w wysokiej jakości proszki, a także zależną od ekstrakcji i modulowaną termicznie modyfikację ich jakości.

Drugi etap miał na celu rozpoznanie skuteczności wybranych składników różnych matryc owocowych w kształtowaniu potencjału bioaktywnego uzyskanych z nich proszków. Heterogeniczność matrycy, w której obecne były substancje bioaktywne, warunkowała ich zdolność przeciwcukrzycową. Flawonole wpływały na potencjał antyglikacyjny liofilizowanych produktów owocowych w zależności od etapu reakcji. W celu zbadania czynników wywodzących się z matrycy początkowej, które wpływają na powstawanie zanieczyszczeń procesowych, wykorzystano układy modelowe imitujące uproszczony skład wybranych soków owocowych. Udowodniono, że furfural (FF) powstawał podczas suszenia w temperaturze 90 °C, podczas gdy formowanie HMF następowało już w temperaturze 60 °C i wyższej oraz w znacznie mniejszych ilościach podczas suszenia rozpyłowego.

W liofilizowanych produktach nie wykryto zanieczyszczeń procesowych, co dowodzi, że ich powstawanie w rzeczywistej matrycy zależy od innych jej komponentów. Liofilizacja i suszenie rozpyłowe zapewniły porównywalną retencję witaminy C w proszkach. W badaniu tym nie powiązano jej obecności z powstawaniem zanieczyszczeń procesowych. W oparciu o różnice w składzie między zastosowanymi modelami a zawartością FF i HMF, można przypuszczać, że kwasy organiczne również mogą brać udział w powstawaniu zanieczyszczeń procesowych pod wpływem określonych warunków suszenia. Złożoność matrycy została przedstawiona jako kluczowy czynnik różnicujący właściwości proszków roślinnych, który należy dokładnie rozważyć przy dobieraniu technologii ich otrzymywania.

Ostatnim etapem było zbadanie potencjału biologicznego proszków uzyskanych z matryc roślinnych zróżnicowanych pod względem odmiany (matryca borówkowa), a także jej wstępnej obróbki przez fermentację (matryca buraczana) i poddanych przetwarzaniu w różnych warunkach. Zróżnicowanie odmianowe surowca generowało odmienną zdolność przeciwutleniającą, ze wskazaniem na proszki z borówki amerykańskiej odmiany Bluecrop i Bluejay jako tych o najbardziej pożądanym potencjale. Proszki wykazywały wyższą zdolność przeciwbakteryjną wobec *Helicobacter pylori* w porównaniu do *Campylobacter jejuni*, a zastosowanie nośnika znacznie osłabiało ten efekt. Wysokotemperaturowa obróbka sprzyjała poprawie właściwości przeciwzapalnych wybranych produktów, w zależności od zastosowanej odmiany tych owoców. W przypadku proszków z buraka, obróbka wstępna soku wpłynęła na mniejszą zawartość betalain w produktach końcowych, podczas gdy inulina była najbardziej skuteczna w ochronie pochodnych kwasu syringowego. Produkty z oligofruktozą i inuliną wykazywały większą zdolność przeciwutleniającą niż te z nutriozą (Nutriose®) i maltodekstryną. Proszki z dodatkiem nutriozy (Nutriose®) charakteryzowała selektywna aktywność antyproliferacyjną wobec linii komórkowych białaczki, podczas gdy oligofruktoza stymulowała proliferację komórek nowotworowych *in vitro*. Nośnik ten indukował również powstawanie HMF, niezależnie od zastosowanego procesu przetwarzania. Udowodniono, że specyficzna odpowiedź biologiczna proszków roślinnych była zależna od wzajemnych powiązań między matrycą a procesem przetwarzania.

Podsumowując, w pracy przedstawiono kompleksowy przegląd zależności między matrycą roślinną, zastosowanym sposobem przetwarzania a właściwościami proszków. Przedstawiono potencjał jaki oferuje odpowiednio dobrany proces przetwarzania w moderowaniu jakości sproszkowanych produktów roślinnych. Złożoność matrycy została zidentyfikowana jako główne wyzwanie w ich otrzymywaniu, ponieważ nawet niewielkie różnice w jej składzie mogą spowodować zmianę właściwości produktów końcowych. Z tego względu, opracowanie proszków roślinnych o ukierunkowanych właściwościach biologicznych powinno być poprzedzone badaniami wstępnymi poświęconymi konkretnej matrycy, która ma zostać poddana przetwarzaniu.

Słowa kluczowe: matryca roślinna, fermentacja probiotyczna, nośniki funkcjonalne, przetwarzanie, liofilizacja, suszenie próżniowe, suszenie rozpyłowe, technologia proszkowania, (poli)fenole, zanieczyszczenia procesowe, furfural, hydroksymetylofurfural, właściwości przeciwutleniające, właściwości przeciwglikacyjne, właściwości przeciwcukrzycowe, właściwości przeciwbakteryjne, właściwości przeciwzapalne, właściwości chemoprotekcyjne

1. INTRODUCTION

Numerous aspects are in favor of the production of plant-based powders, consider as a modern food product type, including their availability throughout the year and longer shelf-life compared to fresh raw material. Another pros are easy-to-handle form facilitating transport, storage and versatile application (Bhandari, 2013). Manufacturing of such kind of plant-originated additives is in line with recent European Union priorities which address among others promotion of sustainable agriculture and circular economy (European Council, 2019). It also follows '*The 2030 Agenda for Sustainable Development*', namely, the good health and well-being (goal 3) and responsible consumption and production (goal 12) (United Nations, 2015). Moreover, observing the latest trends in food science and industry, the production of plant powders seems to meet the vast majority of them, such as the transition from animal to plant-based foods or the management of botanical by-products by converting them into high-quality products with improved characteristics (Raak et al., 2017; Hassoun et al., 2022).

Although plant-based powders are rich source of numerous health-promoting constituents, they were perceived as an ultra-processed foodstuffs with a questionable connotation (Hess et al., 2023). According to the latest deliberations, one of the main challenges facing the processing of plant raw materials is to transform them in such a way that the final product not only retains the best possible nutritional value and bioactive properties, but is additionally enriched with functional constituents and thus increases its health-promoting potential. Several factors may contribute to the improvement of the product's bioactive properties, such as the appropriate choice of process parameters, which, if properly adjusted to a specific plant matrix, can result even in an enhancement of the product's quality (Michalska-Ciechanowska, Brzezowska, et al., 2021). Another approach is introducing various ingredients into the primary plant-based matrix that would upgrade the desired characteristics already possessed by the formulation or even impart entirely new ones.

On the other hand, although botanicals are an abundant source of beneficial bioactive compounds their inappropriate processing may results not only in their degradation, but also evoke undesirable alterations. Recently, trends in food science and technology are also linked with the risk assessment of nutritional foods and analyzing the fate of certain components including formation of process contaminants, which is of high importance from the consumer point of view (Lin et al., 2023). So far, the effects of selected processing factors on physicochemical properties (including the formation of unwanted substances) and, rarely, biological potential have been extensively studied in the literature (Santhalakshmy et al., 2015;

Michalska et al., 2016; Tontul & Topuz, 2017; Silvan, Michalska-Ciechanowska, et al., 2020). Nevertheless, in the literature of the subject there are remain issues that have not been sufficiently covered and the clarification of which could significantly contribute to the development of the field. One of the examples are interactions of bioactives caused by thermal processing and its parameters, addition of carriers, etc., seems to be difficult and sometimes impossible to established due to the complexity of the plant-based matrix composition. Moreover, it is not fully known how exactly the formation of Maillard reaction and caramelisation products (MRCPs) may be controlled by appropriate selection of powdering technology (liquid feed composition, drying parameters, handling). Finally, there is a significant deficiency on the possibility of shaping the biological potential of such products, resulting from the physico-chemical changes induced during the powdering process.

In line with the above, it should be cautioned that the inherent bioactive response exhibited by the final product is directly dependent on the broadly understood food matrix, which is subjected to specific processing conditions, and can be ambiguous with even the slightest change in one of the variables (Capuano et al., 2018). Up to date, the focus has been on a specific raw material subjected to a tailor-made powdering process (selective parameters). Therefore, research findings refer only to the particular raw material used in the study. Consequently, when any changes occur in the matrix subjected to the same processing, the observed relationships may not be applicable anymore. For this reason, the properties should be studied in the context of the broadly understood matrix, rather than the raw material with differences based solely on its origin.

In light of the above, since processing may lead to both, positive (boosting the bioactive response) and negative (degradation of selected bioactives and/or formation of process contaminants) alterations which determine the final quality of plant-based powders, their production should be considered in a more holistic view taking into account two key factors:

(1) **plant-based matrix** (including its botanical origin, cultivar, maturation stage, consistency, microstructure, specific chemical composition and pretreatment),
(2) **processing** (including addition of substances facilitating drying i.a. carrier agents and their concentration, drying technique and its parameters, further packaging and storage, etc.) (**Figure 1**).

Therefore, the new concept that has emerged over the past few years is actually the opposite of what has been followed previously (the impact of processing on the quality of the final product). Recently, a new approach that is becoming adopted in the body of literature is to define the

effect of the food matrix which, in response to specific processing, allows to create particular product's traits, although not the only possible ones (Capuano et al., 2018; Aguilera, 2019; Shahidi & Pan, 2022; Wang et al., 2023).

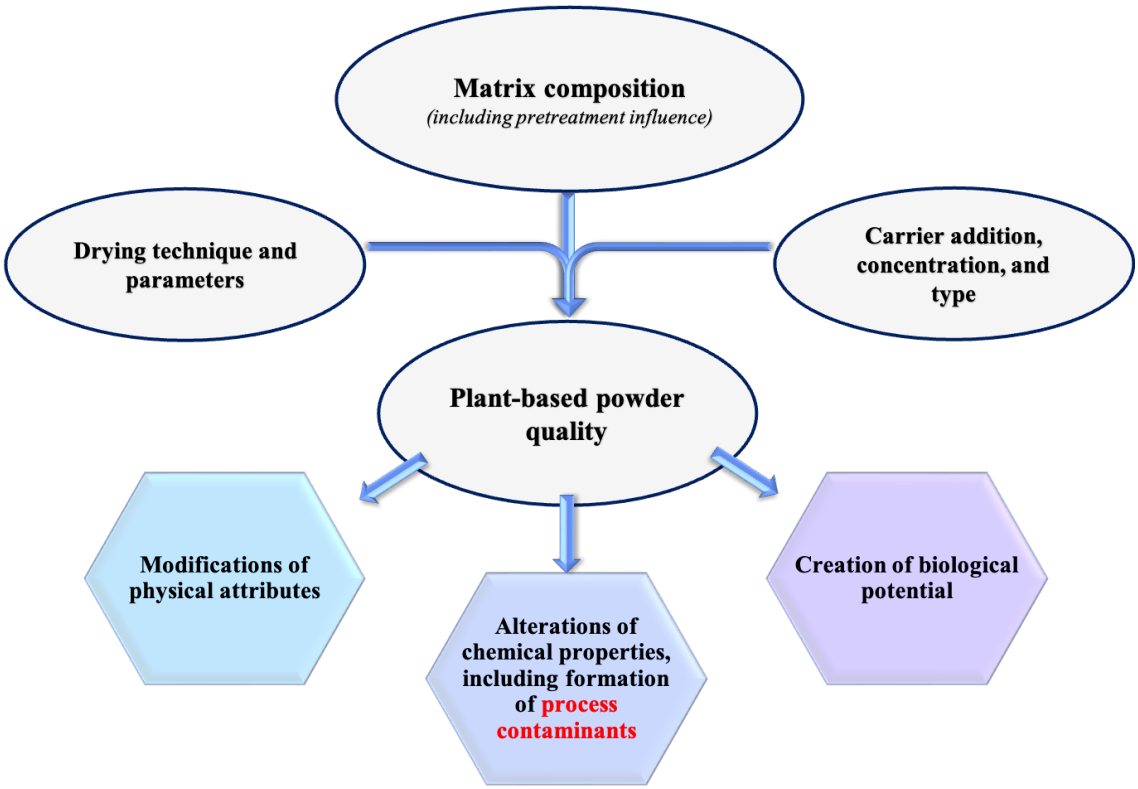


Figure 1. Interrelation of contributing factors and induced changes in the plant-based powders quality.

The powdering technology itself is a complex, often multi-stage process (including initial matrix manipulation, formula design and preparation, the drying technique, and sometimes post-processing, such as grinding, packaging, storage, etc.) that must be very carefully tailored to suit the type of matrix. Finding the balance between the quality and characteristics of the raw material, the conditions of production technology used, and the quality of the powders that combines physical attributes, chemical properties, biological potential, without or with possibly minimized content of process contaminants, is a challenge that has been undertaken in this doctoral thesis.

The novelty of the research is based on tracking processing-induced alterations of native and newly formed components of different botanical matrices, including monitoring progress of Maillard reaction and caramelization, as well as assessment of powdering technology effect on plant-based powders' biological potential.

2. HYPOTHESIS AND RESEARCH OBJECTIVE

The following **research hypothesis** was stated: by adapting the tailor-made processing for specific botanical matrices, it is possible to shape broadly defined quality of powders towards their functional properties and minimized process contaminants presence.

The general research **objective** was to make a benefit-risk evaluation of the processing conditions for plant-based powders obtainment depending on the matrix composition using holistic approach including physico-chemical and biological characterization, and to provide the recommendations for their production. The specific objectives of the present investigation were as follows:

1. Conversion of pomace-originated (poly)phenolic preparations into soluble type products. Recognition of processing parameters influence on the physical attributes, (poly)phenolics profile, antioxidant capacity and hydroxymethyl-*L*-furfural (HMF) presence in plant-based powders (**Publication 1, Supplementary study 1**).
2. Evaluation of matrix effect on (poly)phenolics and amino acid profile during freeze-drying as well as scrutinizing the responsiveness of fruit powders potential in terms of antidiabetic, antiglycation and antioxidant properties (**Supplementary study 2**).
3. Recognition of the matrix-originated drivers triggering the formation of process contaminants under particular processing conditions by employing model systems resembling simplified composition of selected fruit juices (**Supplementary study 3**).
4. Assessment the influence of matrix-driven differences depending on the cultivar, drying techniques and parameters as well as carrier addition on the physico-chemical characteristics, antimicrobial potential and anti-inflammatory properties of plant-based powders (**Supplementary study 4**).
5. Determination the impact of matrix fermentation, drying technique and carrier agent type on the (poly)phenolics alterations, antioxidant capacity, antiglycation potential and antiproliferative activities toward human leukemia cell lines of plant-based powders (**Publication 2**).

3. EXPERIMENTAL DESIGN

In order to recognize the relationship in the most comprehensive context, the plant-based matrices subjected to the powdering process differed depending on the origin, cultivar, consistency and microstructure (fraction), and chemical composition (**Figure 2)**.

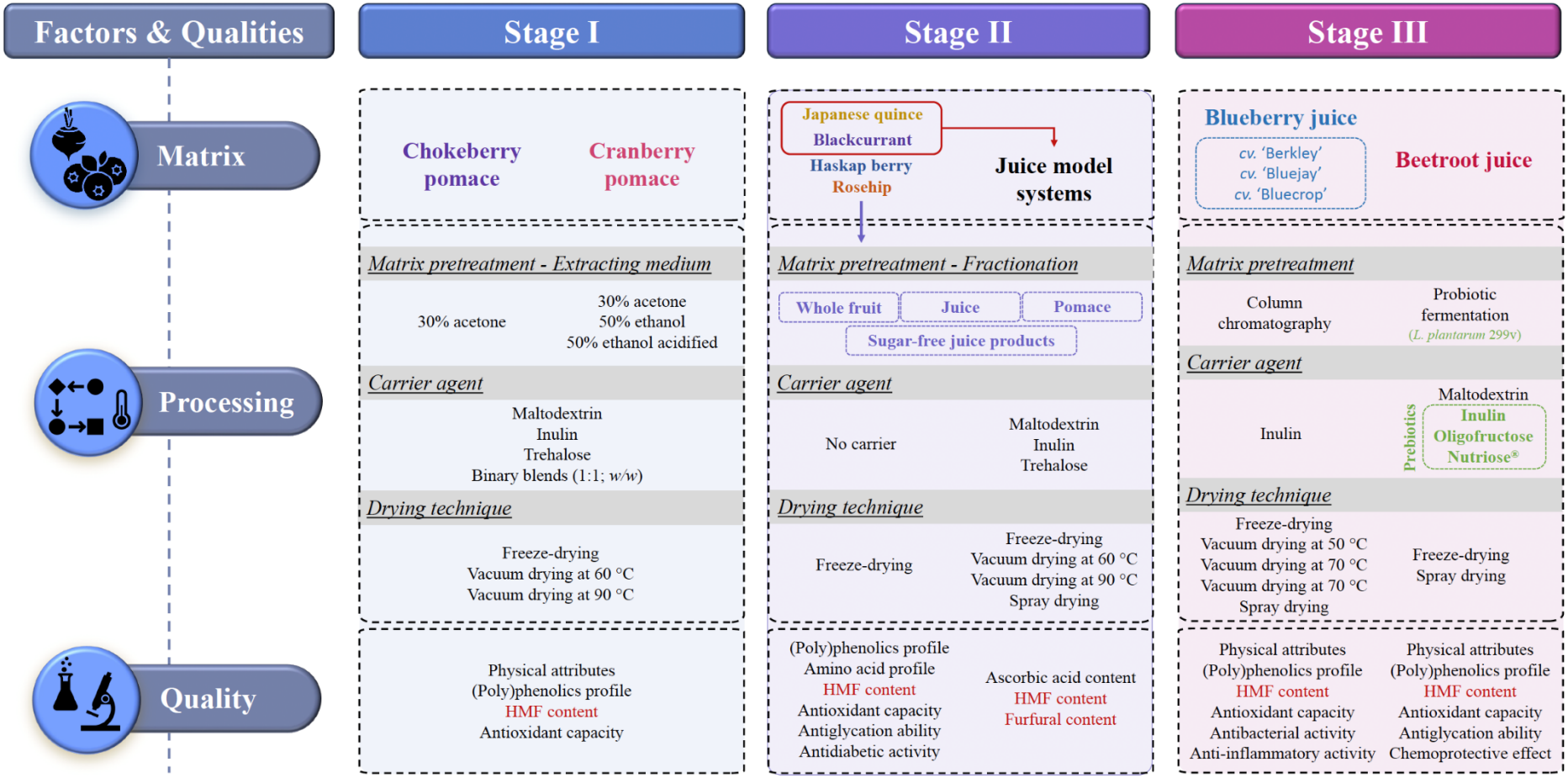


Figure 2. Scheme of core investigations on the quality of plant powders in the context of their physico-chemical properties and biological potential.

4. MATERIAL AND METHODS

4.1. Research material

Since the main focus of the study was to identify the effect of processing on the changes occurring in the matrix during the powdering of plant-based products, the material for the study was fruit and vegetable that were heterogeneous in terms of chemical composition, especially (poly)phenolics profile (**Figure 3**). Such a research plan made it possible to reveal the factors that, regardless of the matrix interaction, cause changes of the same direction, as well as those that, driven by the matrix interaction - cause ambiguous alterations.

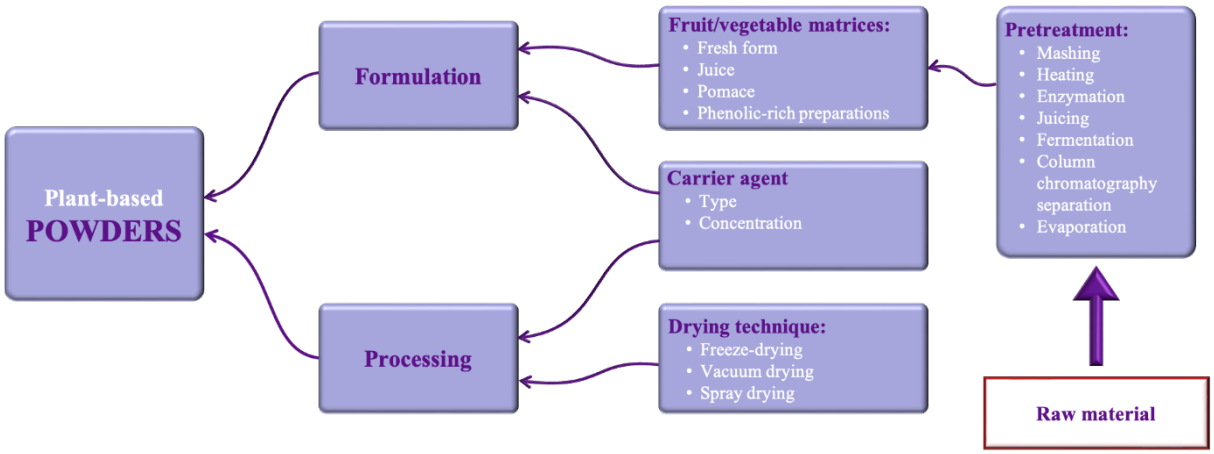


Figure 3. Schematic representation of research material preparation.

Stage I: The chokeberry (*Aronia melanocarpa* L.) and cranberry (*Vaccinium macrocarpon* L.) fruit were bought from the experimental station 'Przybroda' (Rokietnica near Poznań, Poland). The pomace obtained from the fruit squeezing were extracted with 30% acetone (chokeberry pomace) as well as 30% acetone (*w/w*), 50% ethanol (*w/w*), and acidified (pH = 2) 50% ethanol (*w/w*) in the 1:4 ratio (w/v; experimentally established) (cranberry pomace). The resulting extracts after solvent evaporation were subjected to column chromatography, and the condensed (poly)phenolic-rich preparations were mixed with 10% (*w/w*) of maltodextrin (DE 9.3; PEPEES S.A, Poland), inulin GR (Beneo-Orafti, Belgium), trehalose (Hayashibara, Co., Okayama, Japan), and their diblends (1:1; *w/w*). The powders without carrier agent were considered as controls. Prepared formulations were subjected to freeze- and vacuum drying (60 and 90 °C) (**Manuscript 1**, **Supplementary study 1**).

Stage II: Blackcurrant (*Ribes nigrum* L.), rosehip (*Rosa canina* L.) (Wrzesina, Poland), haskap berry (*Lonicera caerulea* L.) (Białężyn, Poland) and Japanese quince (*Chaenomeles japonica* L.) (Leokadiów, Poland) were used to prepare powders from their various fractions. Whole fruits, juice, pomace and sugar-free juice preparations were lyophilized without the addition of a carrier (**Supplementary study 2**).

Simplified fruit matrix in the form of sugar-organic acid models were prepared on the basis of the content of dominant sugar and organic acid present in Japanese quince and blackcurrant fruit juices that were determined according to Wojdyło et al. (2017). Moreover, two variants of model system were prepared (with and without vitamin C) according to recipe given in a **Table 1**. The ascorbic acid content was adjusted based on the measurement in fruit juices and was performed according to PN-90/A-75101/11. To each model solution 30% of carrier (*w/w*; maltodextrin, Pepees S.A., Poland; trehalose, Hayashibara Co., Japan; and inulin GR, Beneo-Orafti, Belgium; established experimentally) was added. The compositions were submitted to freeze-drying, vacuum drying (60 and 90 °C), and spray drying (inlet and outlet temperatures: 180 °C and 70 °C; volume flow: 38 m^3/h; feed flow: 5mL/min) in order to obtain model powders (**Supplementary study 3**).

Table 1. The composition of model system resembling Japanese quince and blackcurrant juices without (Variant I) and with (Variant II) ascorbic acid addition.

Component	***Model system***	
	Variant I	***Variant II***
Japanese quince juice model		
Fructose	43.4 g/L	43.4 g/L
Malic acid	33.7 g/L	33.7 g/L
Ascorbic acid	-	0.65 g/L
Blackcurrant juice model		
Fructose	67.9 g/L	67.9 g/L
Citric acid	22.4 g/L	22.4 g/L
Ascorbic acid	-	1.84 g/L

Stage III: The blueberry (*Vaccinium corymbosum* L.) fruits from three different cultivars ('Berkey', 'Blucrop', and 'Bluejay') were used for juice squeezing that was subsequently subjected for column chromatography. Resulting sugar-free juice preparations were mixed without (controls) or with 5% (*w/w*) of inulin addition and dried using freeze-, spray- (inlet and outlet temperatures: 180 °C and 70 °C; volume flow: 35 m^3/h; feed flow: 9 mL/min) and vacuum drying (50, 70, and 90 °C) (**Supplementary study 4**). The beetroot (*Beta vulgaris* L. *cv.* Opolski) was bought in a local store (Lublin, Poland). After squeezing, the juice was pasteurized and divided into two parts; the first one was fermented with probiotic strain *Lactobacillus plantarum* 299v (Sanprobi IBS, Sanum Poland) and pasteurized again, while the pasteurized non-fermented one served as a control. Such prepared juice variants were mixed with 20% (*w/w*) of maltodextrin (DE = 9.3; PEPEES S.A., Poland) and prebiotic carriers: Nutriose® (Roquette, Lestrem, France), inulin GR and oligofructose (Beneo-Orafti, Belgium). Prepared formulations were subjected to freeze- and spray drying (**Publication 2**).

4.2. Research methodology

4.2.1. Processes applied for powders preparation

Juice and pomace obtainment:

The fruit juice preparation was performed using Thermomix (Wuppertal, Vorkwek, Germany) for the grinding of raw material, and subsequent pulp squeezing with hydraulic press (SRSE, Warsaw, Poland). Resulting juice was filtered and centrifuged (MPW-380R, MPW - Med. Instruments, Warsaw, Poland) before usage (**Supplementary study 2, 4**), while pomace was packed in polyethylene bags, and kept at −20 °C until analyses (**Supplementary study 1, 2, 3**). For selected raw materials, pressing was preceded by an appropriately tailored enzymatic treatment with the application of Pectinex® Ultra SP-L and Berry XXL (Novozyme A/S, Bagswaerd, Denmark). In turn, for beetroot juice obtainment a screw press 20K-GS (Angel, Korea) was used, and pasteurization was performed (DEST-25 696, Destiller, Poland) (**Publication 2**).

Sugar-free juice/pomace extracts obtainment:

The obtainment of (poly)phenolic-dense preparations from juice (**Supplementary study 2, 4**) or pomace extracts (**Publication 1; Supplementary study 1**) included application of column chromatography filled with XAD-16 amberlite polymer resin (Brenntag, Poland). Resulting eluate was subjected to evaporation using an Unipan 350P vacuum rotary evaporator (Warsaw, Poland).

Fermentation

The fermentation of beetroot juice was carried out in a designed fermentation vessel (Gestar, Poland), and an automatic pH control system was used to monitor the process (**Publication 2**).

Drying:

Freeze-drying was performed using FreeZone freeze dryer (Labconco Corp., MO, USA) (**Publication 1, 2; Supplementary study 1, 2, 3, 4**) at 65 Pa for 24 h (drying chamber: −60 °C; heating plates: +25 °C). Vacuum drying was carried out in the Vacucell 111 Eco Line vacuum dryer (MMM Medcenter Einrichtungen GmbH, Germany) at 0.1 mbar (temperature range: 50 – 90 °C; time range: 16 – 24 h) (**Publication 1; Supplementary study 1, 3, 4**). Spray drying was conducted using B290 spray dryer (**Publication 2; Supplementary study 3, 4**) (Buchi, Flawil, Switzerland) at an inlet temperature of 150 - 190 °C and feed flow rate in the range of 4 – 9 mL/min in dependence of liquid feed composition and properties.

4.2.2. Characterization of powders properties

The powders quality was assessed in terms of their physical attributes, chemical properties as well as biological potential *in vitro*. Such characterization allowed for identification main drivers responsible for majority of occurring alterations caused by processing applied for their production.

Physical attributes

- Dry matter (dm) content was done according to PN-90/A-75101/03
- Water activity (a_w) measurement was performed using Dew Point Water Activity Meter 4TE (AQUA LAB, Pullman, WA, USA) following the operating guidelines included with the device
- The bulk density (ρ_b), true density (ρ_t) and porosity (ε) were performed and calculated as described by Michalska & Lech (2018)
- Color measurement was done by determining the L^*, a^* and b^* coordinates with a Minolta Chroma Meter CR-410 (Minolta Co. Ltd., Osaka, Japan) according to Michalska & Lech (2018)

Chemical properties

- Total extract content by refractometric method was performed according to PN-90/A-75101/02
- pH measurement by potentiometric method was made according to PN-90/A-75101/06
- Vitamin C content determination by titration method was performed according to PN-90/A-75101/11
- Total phenolics content (TPC) assay was carried out according to Gao et al. (2000)
- Betalains content was measured according to Gościnna et al. (2012)
- Quantitative and qualitative determination of sugars (HPLC-DAD) and organic acids (UPLC-PDA) was made according to Wojdyło et al. (2017)
- Quantitative and qualitative determination of (poly)phenolic compounds by liquid chromatography with a photodiode array coupled with a tandem quadrupole time-of-flight mass spectrometer (UPLC-PDA-ESI-Q/TOF-MS) was analysed according to Wojdyło et al. (2018)
- Quantification of procyanidin polymers by direct phloroglucinolysis using ultraperformance liquid chromatography (UPLC) coupled to fluorescence detection (FL) was made according to Teleszko & Wojdyło (2015)

- Quantitative and qualitative determination of free amino acids by UPLC-PDA-Q/TOF-MS method was done according to Turkiewicz et al. (2020)
- Qualitative and quantitative determination of hydroxymethyl-*L*-furfural (HMF) by UPLC-PDA method was made according to Tkacz et al. (2020)
- Qualitative determination of ascorbic acid (AA), furfural (FF) and HMF by HPLC method (Supplemenatary study 3 only) was performed accordingly: extracts of each sample were prepared in duplicate (n = 2) by dissolving 50 mg of powder in diluent for 3 min (LLG-uni TEXER 1 homogeniser; Germany), filtered through nylon syringe filters (pore size: 0.2 µm), and stored at 5 °C before analysis (not longer than 48 h). A Shimadzu LC-2050C system (Japan) equipped with a PDA detector and a HyperClone™ 5 µm BDS C8 130 Å chromatography column (250 x 4.6 mm; USA) was used for the quantification of AA, FF and HMF. Sequential separation was performed over 15 minutes *via* isocratic elution using a mobile phase containing methanol and sodium acetate buffer with tetrabutylammonium bromide (TBABr) (5:95; *v/v*). The flow rate of an injected sample (20 µl) was 1 mL/min at a temperature of 25 °C, while the autosampler temperature was 5 °C. The stock standard solutions were dissolved using sodium acetate buffer prepared by mixing 1 L of distilled water with 1.8 g of acetic acid, 3 g of TBABr, AA preservative: 2 g dithiothreitol, and 10% of sodium hydroxide solution pH 4.5 (diluent)/methanol; 95:5, *v/v*). No methanol was used to dissolve the standards. The AA, FF and HMF were identified at the following wavelengths: λ = 264 nm, λ = 276 nm and λ = 284 nm, respectively. Results were calculated using LABSolutions LC software and expressed as µg/mg dm.

Biological activity in vitro

- Antioxidant capacity was measured by:

  TEAC ABTS assay according to Re et al. (1999)

  FRAP assay according to Benzie & Strain (1996)
- Anti-glycation activity was carried out by applying systems imitating three different stages of glycation according to Wang et al. (2011)

  Bovine Serum Albumin (BSA) – glucose/fructose model

  BSA – Methylglyoxal (MGO) model

  MGO – *L*-arginine model

- Antidiabetic activity as the ability to inhibit digestive enzymes was performed according to Nowicka et al. (2016) by applying:
    α-Amylase inhibiting activity assay
    α-Glucosidase inhibiting activity assay
- Antibacterial activity against *C. jejuni, H. pylori*, *E. coli, S. enterica, S. aureus, and L. monocytogenes* was done according to Silvan, Gutiérrez-Docio, et al. (2020). Respective bacterial cultures were prepared following corresponding protocols:
    The *C. jejuni* culture was prepared according to Silvan et al. (2023)
    The *E. coli, S. enterica*, *S. aureus,* and *L. monocytogenes* bacteria cultures were prepared according to Silvan, Michalska-Ciechanowska, et al., (2020)
    The culture of *H. pylori* culture was prepared according to Villalva et al. (2022)
- Anti-inflammatory activity on AGS cells (a human gastric adenocarcinoma cell-line) infected with *H. pylori* performed according to Silvan et al. (2021)
- Cancer cell lines antiproliferative activity was tested on the HL-60/MX2 (ATCC® CRL2257 ™) and J-45.01 (ATCC® CRL1990 ™) human leukemia cell lines according to the protocol described by **Brzezowska** et al. (2023).

4.3. Statistical analysis and data visualization

The results of the determination of physical parameters, chemical properties and biological activity of the powders were subjected to statistical analysis using Statistica 10 and Statistica 13 software (Statsoft, Tulsa, OK, USA). In order to estimate the significance ($p < 0.05$) of differences between the averages, the results were subjected to one-way analysis of variance (ANOVA) and the least significance test HSD Tukey was performed (**Publication 1, 2; Supplementary study 1, 2, 3, 4**). The Shapiro-Wilk test was applied to examine the normality of the distribution of the variables (**Publication 2**). Pearson's correlation coefficient (r) was calculated to determine the relationships between selected variables (**Publication 1, 2; Supplementary study 1, 2, 3, 4**). The R statistical computing environment (R Core Team, 2016) was adopted for selected data visualization: the 'corrplot' package was used for correlation matrix preparation (**Publication 2; Supplementary study 2, 3**) (Wei & Simko, 2021), while 'ggplot2' package was for polar plots creation (**Publication 1, 2; Supplementary study 2, 4**) (Wickham, 2016). The Principal Component Analysis (PCA) was done using XLSTAT Software (**Publication 1; Supplementary study 1, 2, 4**) (Addinsoft, 2022) integrated with Microsoft Excel software (Microsoft Corp., Redmond, WA, USA).

5. RESULTS

The research was structured into three main stages, aimed at identifying:

(I) processing-induced changes toward improved characteristics of fruit powdered products
(II) plant-based matrix complexity *vs*. bioactive response and process contaminants drivers
(III) cross-factors affecting the biological properties of powdered plant products

The experiment designed in this manner permitted the recognition of the relationships that occur during the conversion of the fresh form of plants into their powdered counterparts, and thus the identification of common and divergent points depending on the type of matrix being processed.

5.1. Stage I: *Processing-induced changes toward improved characteristics of fruit powdered products*

The **first stage** covered issues related to physico-chemical changes (including formation of process contaminants) induced by processing in plant matrices that vary in terms of origin, fraction, and thus their chemical composition (**Figure 4**). The research, being the initial stage of the PhD fieldwork, was at the same time a continuation of the experiments carried out in the framework of the master's thesis. The goal then was to discern the effect of the same processing conditions on two different matrices (chokeberry and blackcurrant juice), the results of which have been published previously (Michalska, Wojdyło, **Brzezowska** et al., 2019; Michalska-Ciechanowska, **Brzezowska**, Wojdyło et al., 2021). For this reason, the initial stage of PhD research also focused on the recognition of uniform processing conditions, but for an even more pre-transformed matrix, which was (poly)phenolic-dense pomace preparations that included chokeberry (**Publication 1**) and cranberry (**Supplementary study 1**) pomace extracts.

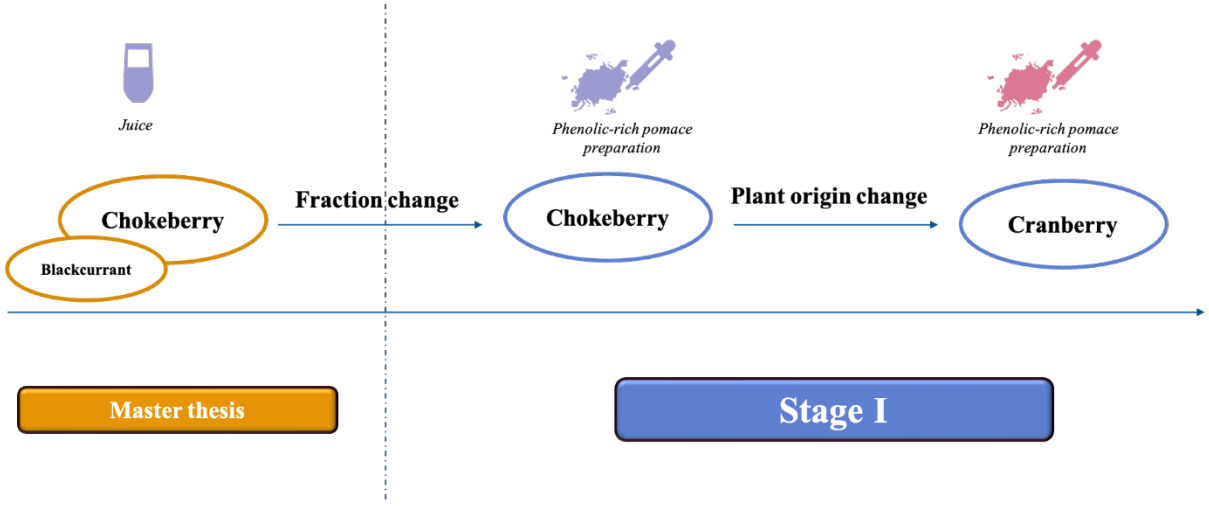


Figure 4. Schematic representation of the differentiating criteria of the matrix used for the study - stage I.

Fruit pomace and juice are a highly complex matrix, abundant in numerous bioactive constituents such as organic acids and sugars, the presence of which hinders the recognition of changes occurring under the powdering process. Consistently, their (nearly all) removal from the matrix was crucial in order to ascertain the fate of the compounds mainly responsible for the powders' health-promoting potential - the (poly)phenolics. Such a procedure makes it possible to obtain a powder from a liquid form without the need to add high-molecular-weight carrier substances, as the low-molecular-weight sugars and organic acids with a low glass transition temperature are eliminated. Furthermore, one of the challenges undertaken in this research (**Publication 1** and **Supplementary study 1**) was to valorize fruit pomace, considered a residual product that poses a problem in terms of industrial by-products management, into a high-quality soluble type powder with a wide potential for use in the food industry.

Therefore, both studies aimed to assess the influence of freeze and vacuum drying at 60 and 90 °C as well as different carrier types (maltodextrin, inulin, trehalose and their binary blends) presence/absence (controls – samples without encapsulant) on the changes in (poly)phenolic profile (qualitative and quantitative), antioxidant capacity and hydroxymethyl-*L*-furfural formation in chokeberry and cranberry pomace extract powders. Additionally, in the case of cranberry pomace matrix, the different extractions were applied to evaluate the influence of the pre-treatment on the above-mentioned properties of the powders.

5.1.1. Publication 1: *Chokeberry pomace preparation powders case study*

Despite the fact that attempts have already been made to valorize chokeberry pomace into an edible food ingredient in the form of a liquid preparation rich in anthocyanins (Oszmiański & Krzywicki, 1993; Roda-Serrat et al. 2022), the impact of subsequent processing on the quality of the final product was usually overlooked, especially when the conversion included drying. For this reason, the study presented in the **Publication 1** address this issue in the context of chokeberry pomace extracts processed to powdered form (**Figure 4**).

For chokeberry pomace preparations powders made with an application of acetone for the extraction, when considering the physical properties tested, PCA analysis showed that freeze-dried samples indicated more variation, particularly in moisture content, water activity, color and browning index, than those obtained by vacuum drying at 60 and 90 °C, for which the bulk density was higher. Accordingly, as the high bulk density is desirable (savings in packaging and shipping costs), the vacuum-dried powders seem to be the most attractive products from economical and practical point of view (Barbosa-Cánovas et al., 2005). When considering the influence of the carrier on the bulk density, an interesting observation was made in the case of trehalose, which showed an inverse trend compared to the other carriers (and their blends added), depending on the drying techniques applied. In addition, among the encapsulants used, trehalose resulted in products with the highest moisture content values in the case of freeze-dried powders and the lowest in the case of vacuum-dried ones at 90 °C. Application of inulin resulted in freeze-dried products with the lowest water activity among all the carriers used, whereas the highest when the vacuum drying at 60 °C was applied. It is a clear illustration of the fact that the same carrier can have different effects on physical properties of powders. When the color of the powders was analysed, the fluctuations in L^*, a^*, and b^* coordinates values were due to the interplay between temperature and carrier addition; however, the differences could be barely noticeable to a potential observer. The browning index (BI) resulting from the measurement of color in the CIE $L^*a^*b^*$ system which was used to generally describe the color changes during the browning process (Pathare et al., 2013), showed that freeze-dried powders had the highest BI values, regardless of the type of added carrier. The brown pigments present in the powders analysed were thought to be masked by the predominant reddish color in vacuum-dried samples, or it can be linked to the formation of (poly)phenolics complexes under freeze-drying (Liu et al., 2003; Michalska et al., 2016). This was in contradiction to the results obtained by Tkacz et al. (2020), who confirmed that drying

techniques applied to different plant matrices can cause their ambiguous responses in terms of physical properties.

When the (poly)phenolics composition was considered, their content ranged from 3.3 – 22.7 g/100 g dm, and the following groups were determined with the corresponding number of compounds identified within each of them; phenolic acids (3), anthocyanins (4), and flavonols (8). In general, the (poly)phenolics of the powdered products obtained with the addition of selected carriers were significantly influenced by the drying techniques. As expected, freeze-drying resulted in the highest retention of thermolabile anthocyanins along with phenolic acids, but the highest content of flavonols was found when vacuum drying at 90 °C was applied. This tendency has been attributed to the probable release of this group from more polymerized structures, as previously indicated by Hamrouni-Sellami et al. (2013). Furthermore, Sharma et al. (2015) demonstrated that heating from 80 to 120 °C increased quercetin and its glucoside content. Therefore, it is important to note that the presence of individual groups of (poly)phenolics can be shaped to some extent by the use of a suitably adapted drying technique. Going into the details, despite the fact that the sum of flavonols showed relatively high stability compared to anthocyanins and phenolic acids, the detailed analysis revealed that the individual compounds identified within this group responded very differently to the processing parameters applied. Therefore, no specific processing conditions for powder production have been identified that would simultaneously provide the highest content of each of the individual flavonols. For anthocyanins and phenolic acids, the use of maltodextrin and trehalose during lyophilization and vacuum drying at 60 °C allowed their highest retention in the powders, but not in the case of vacuum-dried samples at 90 °C, for which trehalose resulted in the lowest anthocyanin content. Nevertheless, when the sum of (poly)phenolics were referred to, the addition of maltodextrin and its mixture with trehalose during freeze-drying and vacuum drying at 90 °C gave the most favorable results, while inulin and its binary blends during vacuum drying at 60 °C.

Another aspect that is currently of great importance is the tracking of the possible formation of process contaminants, which are the result of a series of reactions that take place during the multi-stage processing of a given raw material. One of the most common indicators used to determine the processing level of a product is hydroxymethyl-*L*-furfural. Surprisingly, in chokeberry pomace extract powders its lowest content was noted for vacuum-dried samples at 60 °C (not freeze-dried ones), while the highest - as expected – in those obtained by vacuum drying at 90 °C. These results were supported by the literature, stating that even mild heat treatment can cause the formation of this compound in food products (Fitzpatrick et al., 2013),

as well as the fact that its increased formation occurs at temperatures above 80 °C in plant-based matrix (Michalska, Wojdyło, Łysiak, & Figiel, 2017). In addition, as previously found by Michalska-Ciechanowska, **Brzezowska**, et al. (2021), powders with inulin (or its binary blends) were characterized by higher HMF content compared to other carriers used. Therefore, its application to the fruit-based matrix combined with high-temperature treatment should be reconsidered, as it may evoke uncontrolled formation of unwanted process contaminants such as hydroxymethyl-*L*-furfural.

In conclusion, the physical properties, the (poly)phenolics content and the formation of HMF were influenced both by the initial composition of the powdered matrix and the processing applied. The drying technique and its parameters affected qualities of the chokeberry powders to a greater extent than the carrier agent, however the interplay between these two factors was noticeable. Finally, the findings presented in this publication may serve as a starting point for further research on the development of functional foods based on fruit powders, as well as provide crucial hints for food industry if the intention was to manage the chokeberry pomace and to valorize it into a high-quality soluble type product with broad application potential.

Recommendation: The incorporation of a maltodextrin-trehalose blend for lyophilization and vacuum drying at 90 °C resulted in the highest retention of (poly)phenols along with the lowest content of hydroxymethyl-*L*-furfural in chokeberry pomace preparation powders. However, achieving a high-quality, soluble type powdered product form fruit pomace requires a tailor-made and comprehensive approach adjusted to particular plant-based matrix intended to be processed.

5.1.2. Supplementary study 1: *Cranberry pomace preparation powders case study*

A trial of recycling of bioactives from cranberry by-products into valuable products in order to stabilize (poly)phenolics was previously made (Roopchand et al., 2013); however, the evaluation of drying techniques and parameters influence on the (poly)phenolics' alterations during powdering process remained unknown. Therefore, the second study (**Supplementary study 1**) focused on identifying the effects of the same processing on the same fraction (pomace extract), but from a different raw material (cranberries) (**Figure 4**).

Previously, for the extraction of (poly)phenolics from berry pomace, the 30% acetone was recommended (Oszmiański & Krzywicki, 1993). Taking into account the GRAS status of this solvent, green solution was proposed for production of powders from cranberry pomace preparations. To this end, the 50% ethanol (*w/w*) and 50% acidified ethanol (*w/w*) (pH = 2) was applied for the extraction of (poly)phenolics from cranberry pomace (matrix pre-treatment) in order to produce (poly)phenolic concentrated powders and to follow the possible changes caused either by freeze-drying and vacuum drying at 60 and 90 °C, as well as by different carrier types used for their production. Moreover, bearing in mind the probable formation of process contaminants during processing, the presence of hydroxymethyl-*L*-furfural was monitored.

Physical properties

The moisture content (Mc) of powders ranged from 0.56% to 11.81% and was a resultant of drying technique, carrier addition and type as well as the solvent used to obtain cranberry pomace extracts (**Table 2**). Among samples analyzed, freeze-drying led to products with the highest moisture content regardless of solvent used for pomace extraction, while vacuum drying at 60 and 90 °C resulted in comparable values (Michalska-Ciechanowska, Hendrysiak, et al., 2021). The exceptions were control samples after 50% ethanol extraction, for which freeze-drying and vacuum drying at 90 °C yielded in products with relatively similar values of this parameter. This was contrary to expectations, as generally higher temperatures during drying reduce moisture content of powders, however in this case the interplay between matrix composition and applied processing generated ambiguous observations. One possible explanation could be the faster crust formation in matrix during drying, which might hinder evaporation of water and leave it trapped in the product as observed for red pepper powder (Çalışkan Koç, 2020). In the case of freeze-dried powders, those obtained by application of acetone showed the highest moisture content values among the solvents used, both for the control (10.17%) and the products with carriers (8.55%, averaged value). The inclusion of

inulin yielded powders with the lowest average *Mc* values for (poly)phenolic-rich samples extracted with acetone and 50% ethanol, while the inulin-trehalose blend gave the highest, regardless of drying technique. A different pattern was observed for products obtained from acidified 50% ethanol extracts, for which the maltodextrin-inulin mixture resulted in the highest and maltodextrin-trehalose in the lowest mean values of this parameter. These differences might be connected to various carriers' stability in a given matrix composition and under specific processing conditions. For instance, unambiguous inulin response was observed when subjected to changing thermal and pH conditions (Ozyurt & Ötles, 2016; Li et al., 2019) which, as a result, can lead to divergent properties of final product, including moisture content.

Water activity (a_w) ranged between 0.075 and 0.481 (**Table 2**). Consistent with Michalska-Ciechanowska, Hendrysiak, et al. (2021), freeze-drying resulted in the highest a_w, while vacuum drying at 90 °C in the lowest, for both control powders and those with carriers (averaged values). When solvent type was considered, acetone yielded powders with the highest water activity, while 50% ethanol and its acidified counterpart gave comparable results. This may be related to the extraction of different compounds from cranberry pomace, including various chemical structures or in diverse quantities and therefore with different water-binding abilities (Jin et al., 2019). Taking into account carrier type, inulin was recommended for ethanol-extracted samples toward low a_w, while maltodextrin for those obtained with acetone application. The inulin-trehalose mixture caused the highest a_w values exerted by powders obtained after acetone and acidified 50% ethanol extracted samples, while trehalose (followed by inulin-trehalose blend) in case of those produced with 50% ethanol application, regardless of the drying technique. Overall, obtained powders may be considered as microbiologically stable, as the a_w did not exceed 0.6 value (Fontana, 2020).

The bulk density (ρb) of cranberry pomace extract powders ranged from 0.06 to 0.85 g/cm^3 (**Table 2**). Drying techniques the most differentiated samples in terms of ρb since the lowest bulk density was reported for freeze-dried powders, while vacuum drying at 60 and 90 °C resulted in products with comparable values, for control and carrier-added samples (**Table 2**) (Michalska & Lech, 2018). The differences may be due to structural particularities caused by different drying parameters. Moreover, strong negative correlations were found between bulk density and moisture content ($r = –0.72$) as well as water activity ($r = –0.71$). When carriers were considered, for freeze-dried samples trehalose and inulin-trehalose blend gave powders with higher bulk density. On the other hand, for vacuum-dried samples at 90 °C, the same single carrier resulted in powders with the lowest values.

Table 2. Moisture content (%), water activity (a_w), bulk density (g/cm^3), and color (CIE $L^*a^*b^*$) of cranberry pomace preparations powders.

Solvent type	Drying technique	Carrier type	Physical parameters					
			Moisture content	Water activity	Bulk density	Color		
						*L**	*a**	*b**
Acetone	Freeze-drying	Controls (no carrier)	10.17 ± 0.95 ^{c}	0.397 ± 0.039 ^{f}	0.06 ± 0.01 ^{a}	33.54 ± 0.40 ^{f}	31.27 ± 0.24 ^{f}	3.23 ± 0.09 ^{e}
	Vacuum drying at 60 °C		1.80 ± 0.85 ^{a}	0.223 ± 0.004 $^{b-d}$	0.58 ± 0.10 ^{b}	20.84 ± 0.77 ^{bc}	8.62 ± 0.12 ^{a}	0.71 ± 0.03 ^{a}
	Vacuum drying at 90 °C		2.17 ± 0.02 ^{ab}	0.172 ± 0.010 ^{ab}	0.72 ± 0.01 ^{bc}	19.80 ± 0.57 ^{ab}	12.14 ± 0.12 ^{b}	1.53 ± 0.05 ^{c}
50% Ethanol	Freeze-drying		3.44 ± 0.73 ^{ab}	0.323 ± 0.017 ^{e}	0.15 ± 0.02 ^{a}	26.43 ± 0.74 ^{d}	21.99 ± 0.51 ^{d}	3.49 ± 0.11 ^{f}
	Vacuum drying at 60 °C		1.76 ± 0.22 ^{a}	0.181 ± 0.007 $^{a-c}$	0.68 ± 0.04 ^{bc}	18.78 ± 0.45 ^{a}	8.86 ± 0.56 ^{a}	1.25 ± 0.06 ^{b}
	Vacuum drying at 90 °C		3.09 ± 0.54 ^{ab}	0.136 ± 0.019 ^{a}	0.77 ± 0.00 ^{c}	19.90 ± 0.13 ^{ab}	9.16 ± 0.21 ^{a}	0.81 ± 0.02 ^{a}
50% Ethanol (pH = 2)	Freeze-drying		4.99 ± 1.48 ^{b}	0.288 ± 0.016 ^{de}	0.07 ± 0.00 ^{a}	31.14 ± 0.91 ^{e}	26.78 ± 0.05 ^{e}	3.16 ± 0.10 ^{e}
	Vacuum drying at 60 °C		2.76 ± 0.64 ^{ab}	0.243 ± 0.001 ^{cd}	0.74 ± 0.01 ^{bc}	20.28 ± 0.08 ^{ab}	14.20 ± 0.03 ^{c}	2.08 ± 0.03 ^{d}
	Vacuum drying at 90 °C		2.43 ± 0.39 ^{ab}	0.153 ± 0.015 ^{a}	0.59 ± 0.10 ^{bc}	21.57 ± 0.10 ^{c}	9.18 ± 0.24 ^{a}	0.74 ± 0.02 ^{a}
Acetone	Freeze-drying	Maltodextrin	8.97 ± 0.55 ^{de}	0.353 ± 0.028 $^{i-k}$	0.15 ± 0.00 ^{a}	25.20 ± 0.10 ^{d}	19.77 ± 0.09 ^{h}	1.88 ± 0.01 ^{gh}
		Inulin	5.76 ± 0.29 ^{c}	0.338 ± 0.004 $^{h-j}$	0.15 ± 0.00 ^{a}	32.94 ± 0.43 ^{f}	35.14 ± 0.05 ^{m}	2.59 ± 0.10 ^{j}
		Trehalose	6.98 ± 0.73 ^{cd}	0.365 ± 0.014 ^{jk}	0.43 ± 0.01 ^{b}	21.13 ± 0.52 ^{b}	17.50 ± 0.26 ^{f}	2.71 ± 0.02 ^{j}
		Maltodextrin-Inulin	9.44 ± 1.50 ^{e}	0.393 ± 0.032 ^{kl}	0.15 ± 0.01 ^{a}	36.96 ± 0.34 ^{h}	32.03 ± 0.05 ^{k}	1.45 ± 0.06 ^{f}
		Maltodextrin-Trehalose	9.68 ± 0.45 ^{e}	0.427 ± 0.024 ^{l}	0.13 ± 0.01 ^{a}	34.17 ± 0.07 ^{g}	30.67 ± 0.20 ^{j}	2.09 ± 0.02 ^{i}
		Inulin-Trehalose	10.47 ± 1.30 ^{e}	0.481 ± 0.007 ^{m}	0.18 ± 0.00 ^{a}	33.27 ± 0.42 ^{f}	32.73 ± 0.36 ^{l}	2.99 ± 0.13
	Vacuum drying at 60 °C	Maltodextrin	3.28 ± 0.11 ^{b}	0.206 ± 0.003 $^{c-f}$	0.71 ± 0.03 $^{c-e}$	19.47 ± 0.07 ^{a}	5.87 ± 0.04 ^{a}	-0.41 ± 0.03 ^{b}
		Inulin	3.26 ± 0.14 ^{b}	0.296 ± 0.001 ^{gh}	0.63 ± 0.02 ^{cd}	25.29 ± 0.19 ^{d}	19.80 ± 0.21 ^{h}	1.04 ± 0.04 ^{de}
		Trehalose	3.03 ± 0.29 ^{ab}	0.307 ± 0.007 $^{g-i}$	0.70 ± 0.02 $^{c-e}$	27.18 ± 0.29 ^{e}	21.14 ± 0.20 ^{i}	1.92 ± 0.02 ^{hi}
		Maltodextrin-Inulin	3.12 ± 0.05 ^{ab}	0.226 ± 0.004 ^{ef}	0.74 ± 0.00 ^{de}	23.30 ± 0.14 ^{c}	10.62 ± 0.10 ^{b}	-0.10 ± 0.00 ^{c}
		Maltodextrin-Trehalose	2.89 ± 0.44 ^{ab}	0.257 ± 0.002 ^{fg}	0.68 ± 0.00 $^{c-e}$	20.99 ± 0.10 ^{b}	10.45 ± 0.13 ^{b}	1.11 ± 0.01 ^{e}
		Inulin-Trehalose	2.84 ± 0.08 ^{ab}	0.235 ± 0.005 ^{ef}	0.74 ± 0.04 ^{de}	25.41 ± 0.32 ^{d}	18.57 ± 0.12 ^{g}	1.74 ± 0.01 ^{g}
	Vacuum drying at 90 °C	Maltodextrin	1.37 ± 0.29 ^{ab}	0.097 ± 0.011 ^{a}	0.64 ± 0.01 ^{cd}	21.16 ± 0.04 ^{b}	13.75 ± 0.04 ^{d}	1.14 ± 0.03 ^{e}
		Inulin	1.03 ± 0.12 ^{ab}	0.107 ± 0.014 ^{ab}	0.84 ± 0.11 ^{e}	23.54 ± 0.08 ^{c}	12.30 ± 0.09 ^{c}	-0.66 ± 0.02 ^{a}
		Trehalose	1.09 ± 0.19 ^{ab}	0.152 ± 0.008 ^{bc}	0.45 ± 0.11 ^{b}	27.53 ± 0.56 ^{e}	18.10 ± 0.16 ^{fg}	1.02 ± 0.09 ^{de}
		Maltodextrin-Inulin	1.33 ± 0.26 ^{ab}	0.192 ± 0.007 $^{c-e}$	0.72 ± 0.01 ^{de}	23.73 ± 0.13 ^{c}	16.79 ± 0.56 ^{e}	0.93 ± 0.10 ^{d}
		Maltodextrin-Trehalose	0.99 ± 0.18 ^{a}	0.172 ± 0.009 ^{cd}	0.80 ± 0.01 ^{de}	26.95 ± 0.32 ^{e}	18.45 ± 0.36 ^{g}	1.09 ± 0.03 ^{de}
		Inulin-Trehalose	2.83 ± 0.24 ^{ab}	0.215 ± 0.001 $^{d-f}$	0.56 ± 0.00 ^{bc}	23.50 ± 0.11 ^{c}	10.96 ± 0.21 ^{b}	-0.15 ± 0.06 ^{c}
50% Ethanol	Freeze-drying	Maltodextrin	6.98 ± 1.39 $^{d-f}$	0.284 ± 0.005 ^{f}	0.12 ± 0.00 ^{a}	23.67 ± 0.08 ^{gh}	21.65 ± 0.23 ^{g}	1.84 ± 0.02 ^{i}
		Inulin	2.73 ± 0.38 $^{a-c}$	0.282 ± 0.014 ^{f}	0.14 ± 0.00 ^{ab}	36.08 ± 0.07 ^{k}	35.28 ± 0.02 ^{j}	-0.43 ± 0.02 $^{b-d}$
		Trehalose	4.99 ± 1.39 $^{c-e}$	0.402 ± 0.014 ^{h}	0.18 ± 0.00 ^{ab}	27.06 ± 0.73 ^{ij}	28.76 ± 0.25 ^{i}	3.22 ± 0.22 ^{l}
		Maltodextrin-Inulin	9.75 ± 0.22 ^{f}	0.346 ± 0.017 ^{g}	0.15 ± 0.00 ^{ab}	28.33 ± 0.74 ^{j}	28.38 ± 0.05 ^{i}	2.35 ± 0.17 ^{j}
		Maltodextrin-Trehalose	8.07 ± 1.31 ^{ef}	0.221 ± 0.014 ^{de}	0.15 ± 0.00 ^{ab}	26.26 ± 0.21 ^{ij}	23.24 ± 0.04 ^{h}	1.44 ± 0.02 ^{h}
		Inulin-Trehalose	6.70 ± 0.47 $^{d-f}$	0.290 ± 0.015 ^{f}	0.18 ± 0.01 ^{ab}	27.93 ± 0.25 ^{j}	28.03 ± 0.11 ^{i}	2.76 ± 0.09 ^{k}

Table 2. (*continued*).

50% Ethanol	Vacuum drying at 60 °C	Maltodextrin	1.01 ± 0.13 [a]	0.099 ± 0.003 [a]	0.78 ± 0.05 [de]	17.56 ± 0.54 [bc]	9.21 ± 1.29 [a]	0.50 ± 0.19 [f]
		Inulin	0.92 ± 0.08 [a]	0.090 ± 0.005 [a]	0.73 ± 0.03 [de]	18.80 ± 0.52 [cd]	11.23 ± 0.25 [b]	-0.56 ± 0.09 [ab]
		Trehalose	3.29 ± 1.01 [a-c]	0.229 ± 0.000 [de]	0.56 ± 0.03 [cd]	22.44 ± 0.57 [fg]	20.30 ± 0.06 [f]	1.27 ± 0.06 [gh]
		Maltodextrin-Inulin	1.88 ± 0.40 [a-c]	0.118 ± 0.004 [ab]	0.70 ± 0.02 [de]	16.11 ± 1.67 [ab]	9.56 ± 0.37 [a]	-0.14 ± 0.06 [de]
		Maltodextrin-Trehalose	1.36 ± 0.32 [ab]	0.155 ± 0.002 [bc]	0.55 ± 0.10 [cd]	14.22 ± 0.88 [a]	12.93 ± 0.18 [c]	1.00 ± 0.05 [g]
		Inulin-Trehalose	3.70 ± 0.32 [a-d]	0.263 ± 0.000 [ef]	0.85 ± 0.17 [de]	17.87 ± 1.08 [bc]	19.79 ± 0.23 [ef]	1.48 ± 0.02 [h]
	Vacuum drying at 90 °C	Maltodextrin	1.48 ± 0.10 [ab]	0.075 ± 0.009 [a]	0.57 ± 0.05 [cd]	19.73 ± 0.02 [c-e]	8.42 ± 0.08 [a]	-0.44 ± 0.08 [bc]
		Inulin	1.77 ± 0.47 [a-c]	0.075 ± 0.011 [a]	0.79 ± 0.00 [de]	25.32 ± 0.12 [hi]	18.93 ± 1.04 [de]	-0.23 ± 0.06 [c-e]
		Trehalose	1.83 ± 0.96 [a-c]	0.161 ± 0.018 [bc]	0.39 ± 0.14 [bc]	27.26 ± 1.49 [ij]	20.05 ± 0.24 [ef]	-0.10 ± 0.12 [e]
		Maltodextrin-Inulin	0.56 ± 0.52 [a]	0.093 ± 0.012 [a]	0.69 ± 0.07 [de]	20.99 ± 0.03 [d-f]	9.17 ± 0.21 [a]	-0.81 ± 0.04 [a]
		Maltodextrin-Trehalose	1.65 ± 0.71 [ab]	0.105 ± 0.012 [a]	0.60 ± 0.01 [c-e]	21.53 ± 0.36 [e-g]	8.88 ± 0.20 [a]	-0.61 ± 0.05 [ab]
		Inulin-Trehalose	4.47 ± 1.73 [b-d]	0.187 ± 0.016 [cd]	0.79 ± 0.03 [de]	27.20 ± 0.20 [ij]	18.29 ± 0.04 [d]	0.37 ± 0.04 [f]
50% Ethanol (pH = 2)	Freeze-drying	Maltodextrin	6.24 ± 1.72 [de]	0.346 ± 0.031 [f]	0.11 ± 0.01 [a]	29.71 ± 0.13 [e]	28.26 ± 0.38 [f]	2.20 ± 0.10 [f]
		Inulin	5.96 ± 1.32 [c-e]	0.276 ± 0.007 [e]	0.17 ± 0.00 [ab]	33.77 ± 0.16 [g]	36.77 ± 0.03 [i]	1.99 ± 0.08 [f]
		Trehalose	7.05 ± 0.74 [e]	0.375 ± 0.010 [f]	0.29 ± 0.01 [b]	26.51 ± 0.60 [d]	30.27 ± 0.26 [g]	4.18 ± 0.20 [i]
		Maltodextrin-Inulin	11.81 ± 1.07 [f]	0.394 ± 0.007 [f]	0.18 ± 0.00 [ab]	31.40 ± 1.15 [f]	34.24 ± 0.14 [h]	2.71 ± 0.29 [g]
		Maltodextrin-Trehalose	3.29 ± 0.40 [a-d]	0.364 ± 0.015 [f]	0.14 ± 0.00 [ab]	33.02 ± 0.28 [g]	31.48 ± 0.04 [g]	2.16 ± 0.06 [f]
		Inulin-Trehalose	4.97 ± 1.29 [b-e]	0.372 ± 0.012 [f]	0.24 ± 0.00 [ab]	31.27 ± 0.36 [f]	34.52 ± 0.41 [h]	3.56 ± 0.04 [h]
	Vacuum drying at 60 °C	Maltodextrin	2.54 ± 0.17 [ab]	0.187 ± 0.006 [c]	0.75 ± 0.06 [ef]	18.08 ± 0.29 [a]	10.56 ± 0.36 [b]	0.55 ± 0.03 [c]
		Inulin	1.54 ± 0.00 [a]	0.216 ± 0.003 [cd]	0.74 ± 0.01 [d-f]	21.53 ± 0.21 [b]	16.14 ± 0.43 [d]	0.12 ± 0.03 [b]
		Trehalose	2.10 ± 0.46 [ab]	0.253 ± 0.001 [de]	0.63 ± 0.01 [c-f]	21.71 ± 0.21 [b]	15.12 ± 0.14 [d]	1.01 ± 0.05 [d]
		Maltodextrin-Inulin	2.86 ± 0.43 [a-d]	0.238 ± 0.002 [c-d]	0.62 ± 0.12 [c-e]	18.72 ± 0.77 [a]	13.34 ± 1.74 [c]	0.53 ± 0.14 [c]
		Maltodextrin-Trehalose	3.11 ± 0.37 [a-d]	0.285 ± 0.002 [e]	0.69 ± 0.06 [d-f]	19.43 ± 0.81 [a]	15.89 ± 0.45 [d]	1.28 ± 0.14 [de]
		Inulin-Trehalose	2.30 ± 0.00 [ab]	0.282 ± 0.000 [e]	0.66 ± 0.00 [d-f]	21.04 ± 0.77 [b]	18.93 ± 0.58 [e]	1.34 ± 0.05 [e]
	Vacuum drying at 90 °C	Maltodextrin	4.15 ± 1.89 [a-e]	0.124 ± 0.016 [ab]	0.63 ± 0.01 [d-f]	21.81 ± 0.16 [b]	7.55 ± 0.09 [a]	-0.52 ± 0.03 [a]
		Inulin	2.58 ± 0.51 [a-c]	0.124 ± 0.017 [ab]	0.76 ± 0.02 [ef]	23.46 ± 0.04 [c]	10.08 ± 0.08 [b]	-0.83 ± 0.03 [a]
		Trehalose	3.00 ± 0.40 [a-d]	0.180 ± 0.026 [bc]	0.46 ± 0.07 [c]	24.77 ± 0.07 [c]	13.39 ± 0.06 [c]	0.17 ± 0.01 [b]
		Maltodextrin-Inulin	2.23 ± 0.28 [ab]	0.079 ± 0.011 [a]	0.79 ± 0.02 [f]	21.70 ± 0.05 [b]	7.71 ± 0.11 [a]	-0.78 ± 0.01 [a]
		Maltodextrin-Trehalose	1.33 ± 0.29 [a]	0.188 ± 0.021 [c]	0.57 ± 0.05 [cd]	21.90 ± 0.06 [b]	7.80 ± 0.14 [a]	-0.54 ± 0.02 [a]
		Inulin-Trehalose	3.31 ± 0.11 [a-d]	0.234 ± 0.022 [c-e]	0.75 ± 0.05 [ef]	26.37 ± 0.10 [d]	16.36 ± 0.05 [d]	0.61 ± 0.01 [c]

a, b, c, … – different letters within groups: controls, carrier-added samples (acetone), carrier-added samples (50% ethanol), carrier-added samples (50% acidified ethanol) standing for different cranberry pomace extract powders indicate significant differences (ANOVA, HSD Tukey, $p < 0.05$).

In almost every case, freeze-drying yielded powders with higher values of *L**, *a** and *b** coordinates, compared to controls and carrier-added samples after vacuum drying at 60 and 90 °C (**Table 2**). Acetone and acidified 50% ethanol resulted in products lighter and more yellow than those extracted with 50% ethanol, with exception of controls in case of *b** parameter (comparable results). Regardless solvent type and drying technique, inulin led to powders obtainment with the highest *L** and *a** coordinates, while maltodextrin the lowest. Moreover, trehalose and its blends gave products with the highest *b** parameter values. Nevertheless, the discrepancies between values were within a very narrow range, and thus the differences were visually imperceptible.

Chemical properties

Previously, attempts were made to extract (poly)phenolics from fruit by-products and various solvents as well as additives were tested (Oszmiański & Krzywicki, 1993; White et al., 2010b; Michalska-Ciechanowska, Hendrysiak, et al., 2021) with the emphasis on the search for an effective food-compatible solvent. Water with different pH range (2-5) and 50% ethanol solutions were used for extraction of (poly)phenolics from fruit pomace with water being significantly less effective (Roopchand et al., 2013). Thus, in the study the 30% acetone (commonly used solvent), 50% ethanol and acidified 50% ethanol (as more food-compatible solvents) were compared for extraction of these constituents.

In order to track changes caused by drying, cranberry pomace preparations were produced by removing ballast substances to examine the alterations of major (poly)phenolics group without influence of other cranberry pomace components. An absorber technology was used due to high selectivity, absorption capacity, economic feasibility and low toxicity when compared to other separation techniques (Soto et al., 2011), excluding toxic solvents that could cause degradation of selected bioactives. Additionally, previous study on cranberry products elucidated that matrix composition (juice, sugar-free juice preparations) plays an important role and significantly influences presence and content of particular (poly)phenolics in resulting powders, and therefore their quality (Michalska et al., 2018), which should be assessed in light of these differences.

(Poly)phenolics determination

Controls (no carrier addition)

Consistent with White et al. (2010a) and Roopchand et al. (2013), 4 major groups of (poly)phenolics were quantified in powders produced without carriers' addition, i.e., flavonols (64% of all quantified (poly)phenolics), phenolic acids (13.4%), flavan-3-ols (11.6%) and anthocyanins (11%) (**Table 3**), regardless of the solvent applied and drying technique used. The highest retention of (poly)phenolics in controls was noted when acidified 50% ethanol was used, followed by 50% ethanol and 30% acetone, an application of which resulted in, on average, 15% lower content of those constituents in powders (**Figure 5**). It was in agreement with Klavins et al. (2022) who recommended acidified ethanol for efficient extraction of (poly)phenolics from cranberry press residue.

Drying techniques affected (poly)phenolics content in the following order: freeze-drying < vacuum drying at 60 °C < vacuum drying at 90 °C. Freeze-drying and vacuum drying at 60 °C resulted in products with comparable quantities of all identified (poly)phenolics when acidified 50% ethanol was used, while vacuum drying at 90 °C turned out to be only approx. 8% less effective in terms of (poly)phenolics retention. This was in contrast to vacuum drying of cranberry juice extracts that resulted in approx. 30% and 90% lower content of (poly)phenolics when temperature of 60 and 90 °C was used for powders preparation in comparison to freeze-drying (Michalska et al., 2018). This indicates that types of fruit matrix (i.e., juice, pomace) have a significant effect on alterations of (poly)phenolics during thermal treatment. Considering their relatively low degradation and a high operating costs of freeze-drying being approx. 50% higher than vacuum drying (Peighambardoust et al., 2011) the latter can be recommended for production of cranberry pomace extracts powders. The application of acidified 50% ethanol (pH = 2) for extracts' preparation and vacuum drying at 90 °C led to powders obtained with a higher content of (poly)phenolics than in case of products gained by using 30% acetone, even when freeze-drying was used. In conclusion, selection of appropriate solvent plays a pivotal role in designing of the final quality of cranberry pomace extract powders, and at the same time may enable to reduce costs associated with long-time drying, especially lyophilization (Ratti, 2001).

Among four identified groups of (poly)phenolics in powders, the highest content of flavonols (approx. 64% of all identified (poly)phenolics) was indicated. Extraction solvent influenced their retention that was also moderated by drying technique and parameters. Among solvent applied, 30% acetone resulted in the lowest content of flavonols in powders, regardless

of the drying technique used (**Figure 5**). Application of 50% ethanol yielded the highest content of these constituents in freeze-dried products followed by samples produced by vacuum drying at 60 °C. In the case of acidified 50% ethanol, no significant differences in powders produced by freeze-drying and vacuum drying at 60 °C were noted, which might indicate flavonols stability during heating (Michalska et al., 2018). Regardless of extraction solvent, difference between an average content of flavonols after freeze-drying and vacuum drying at 60 as well as 90 °C was, respectively, 8% and 14%.

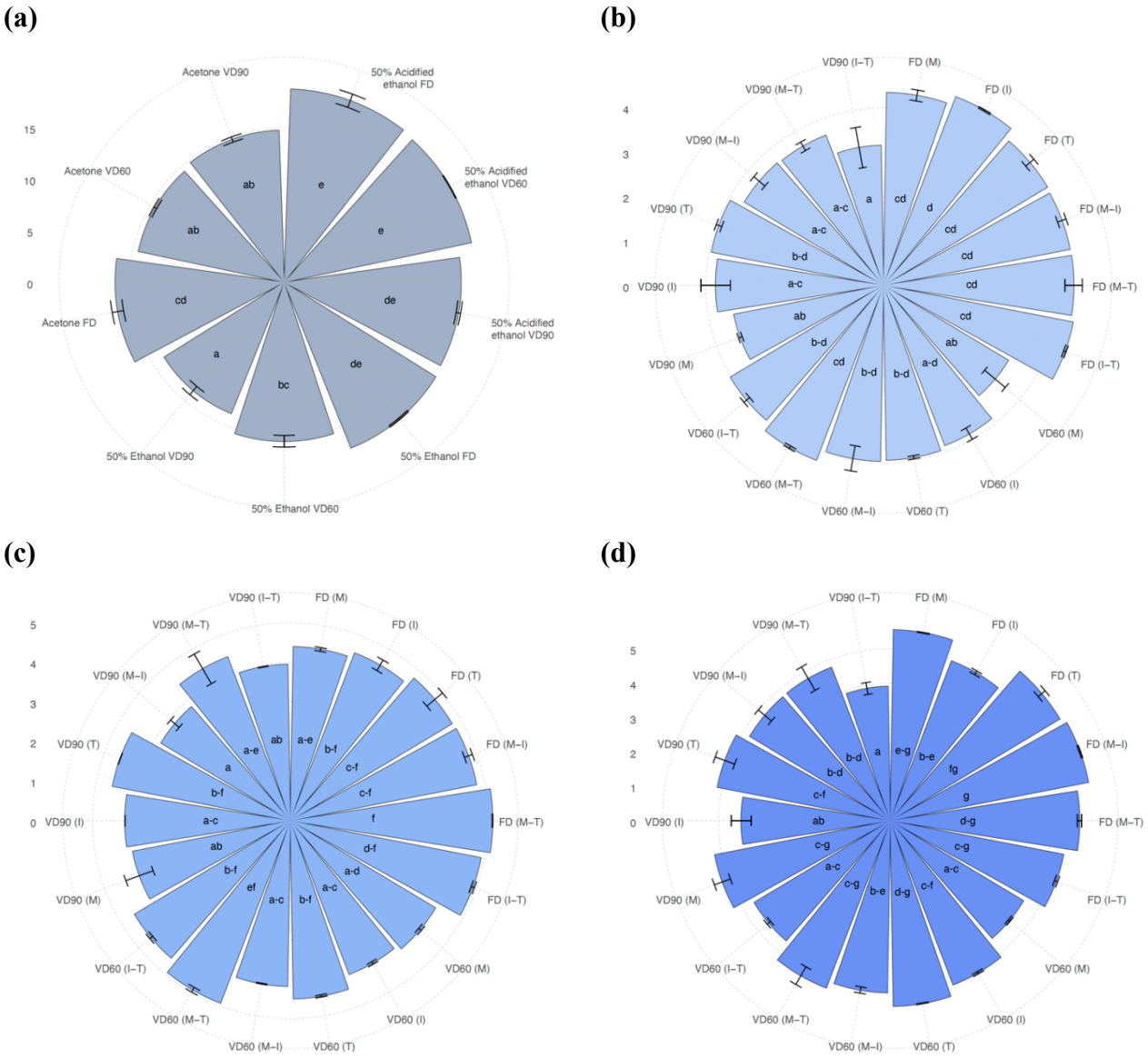


Figure 5. The sum of (poly)phenolics [g/100 g dm] in cranberry pomace extract powders obtained without carrier addition (**a**), as well as, with different carrier agents obtained by extraction with: (**b**) 30% acetone, (**c**) 50% ethanol and (**d**) acidified 50% ethanol. FD – freeze-drying; VD60 – vacuum drying at 60 °C; VD90 – vacuum drying at 90 °C; M – maltodextrin; I – inulin; T – trehalose; M-I – blend composed of maltodextrin and inulin; M-T – blend composed of maltodextrin and trehalose; I-T – blend composed of inulin and trehalose; a, b, c, … – different letters within groups: controls, carrier-added samples (acetone), carrier-added samples (50% ethanol), carrier-added samples (50% acidified ethanol) standing for different cranberry pomace extract powders indicate significant differences (ANOVA, HSD Tukey, $p < 0.05$).

Table 3. (Poly)phenolics content [g/100 g dm], antioxidant capacity [mmol Trolox/100 g dm] and hydroxymethyl-*L*-furfural (HMF) content [μg/100 g dm] in cranberry pomace extract control powders.

Solvent type	Drying technique	(Poly)phenolics				Antioxidant capacity		Hydroxymethyl-*L*-furfural
		Flavonols	Flavan-3-ols	Phenolic acids	Anthocyanins	TEAC ABTS	FRAP	
Acetone	Freeze-drying	9.62 ± 0.32 $^{a-c}$	3.01 ± 0.16 ^{b}	2.09 ± 0.06 ^{ab}	1.66 ± 0.02 ^{bc}	398.86 ± 1.35 ^{ab}	264.38 ± 2.41 $^{a-c}$	38.63 ± 1.31 ^{b}
	Vacuum drying at 60 °C	8.99 ± 0.15 ^{a}	2.05 ± 0.00 ^{a}	1.94 ± 0.02 ^{a}	1.47 ± 0.01 ^{ab}	403.28 ± 18.40 ^{b}	271.07 ± 12.39 $^{a-c}$	48.18 ± 3.55 ^{c}
	Vacuum drying at 90 °C	9.06 ± 0.11 ^{ab}	2.34 ± 0.07 ^{a}	1.95 ± 0.02 ^{a}	1.38 ± 0.05 ^{a}	379.98 ± 1.75 ^{ab}	259.68 ± 6.44 $^{a-c}$	47.13 ± 1.56 ^{c}
50% EtOH	Freeze-drying	12.61 ± 0.06 ^{e}	tr	2.43 ± 0.01 ^{d}	2.28 ± 0.01 ^{e}	395.10 ± 18.86 ^{ab}	279.84 ± 19.36 ^{c}	tr
	Vacuum drying at 60 °C	11.04 ± 0.39 $_{d}$	tr	2.37 ± 0.09 ^{cd}	2.01 ± 0.09 ^{d}	375.54 ± 3.37 ^{ab}	262.60 ± 9.14 $^{a-c}$	tr
	Vacuum drying at 90 °C	10.18 ± 0.44 ^{cd}	tr	2.03 ± 0.00 ^{a}	1.50 ± 0.08 ^{ab}	371.77 ± 18.52 ^{ab}	273.18 ± 0.41 ^{bc}	tr
50% EtOH (pH = 2)	Freeze-drying	10.87 ± 0.41 ^{d}	3.43 ± 0.14 ^{c}	2.32 ± 0.06 ^{cd}	2.11 ± 0.07 ^{de}	363.82 ± 4.70 ^{ab}	243.68 ± 0.51 ^{ab}	6.80 ± 0.35 ^{a}
	Vacuum drying at 60 °C	10.84 ± 0.04 ^{d}	3.45 ± 0.06 ^{c}	2.23 ± 0.00 ^{bc}	2.02 ± 0.02 ^{d}	358.30 ± 6.17 ^{a}	238.69 ± 1.54 ^{a}	6.64 ± 0.02 ^{a}
	Vacuum drying at 90 °C	10.11 ± 0.05 $^{b-d}$	3.20 ± 0.11 ^{bc}	2.09 ± 0.03 ^{ab}	1.75 ± 0.03 ^{c}	397.69 ± 3.07 ^{ab}	276.12 ± 3.18 ^{bc}	6.55 ± 0.33 ^{a}

tr – trace amounts; a, b, c, … – different letters within groups standing for different cranberry pomace extract powders indicate significant differences (ANOVA, HSD Tukey, $p < 0.05$).

The third group of (poly)phenolics determined in cranberry extracts powders were phenolic acids. In controls, the highest content of these constituents was noted in products gained when 50% ethanol was used in the case of freeze-drying and vacuum drying at 60 °C (**Table 3**). Application of 30% acetone caused the lowest content of phenolic acids in all powders. Freeze-drying led to obtainment of powders with the highest content of phenolic acids, whereas the influence of VD differed due to temperature applied for powders production. The temperature of 90 °C caused a lower content of these components in powders for production of which the 50% ethanol and acidified 50% ethanol was used. Interestingly, application of 30% acetone led to powders obtained by vacuum drying with similar content of phenolic acids, regardless of the temperature used for powders production.

Previously, cranberry procyanidins belonging to the flavan-3-ols family, were mainly extracted by acetone at different concentrations with acidifying agents used for lowering the pH of an extraction medium (Wallace & Giusti, 2010). It was also proved that absorber technology, including type of resin and solvents used for desorption of components, had an impact on the presence and content of procyanidins in products obtained (Gao et al., 2018), and thus in their powdered form. In the study, for controls, the usage of 50% ethanol resulted in only trace amount of flavan-3-ols in these products, whereas their content in powders produced with application of 30% acetone and acidified 50% ethanol ranged from 2.05 up to 3.25 mg/100 g dm (**Table 3**). This confirmed that acidification of the extraction medium significantly improved extractability of cranberry flavan-3-ols. Klavins et al. (2022) pointed that acidified acetone at various concentrations was the most effective solvent for procyanidins extraction of dried cranberry press residue when compared to ethanolic solution, however, effect of drying and its parameters was neglected. Moreover, the measurement of procyanidins was made by a spectrophotometric method which may not be precise for accurate determination of these constituents. In the study, it was supposed that absorber technology modified composition of initial matrix of cranberry pomace extracts (Gao et al., 2018) which may influence presence of flavan-3-ols in powders gained. Thus, 50% ethanol at pH = 2 can be recommended for production of powders with improved content of flavan-3-ols. It can be concluded that extraction solvent should be chosen on the basis of composition of the material submitted to extraction. In the case of acidified 50% ethanol extracts, drying techniques and parameters used led to obtainment of products with similar content of flavan-3-ols. This indicated a thermal stability of these components extracted with 50% ethanol at pH = 2. One possible explanation might be that different structures of compounds belonging to this group were extracted from cranberry pomace by using specific type of solvent, and therefore with

different thermal stability depending on the degrees of polymerization (higher polymerization resulted in a lower heat resistance) (Qi et al., 2022).

Among (poly)phenolics groups quantified in powders, anthocyanins were present in the smallest quantities (**Table 3**). Regardless of drying technique, the highest content of these components was noted in products extracted with 50% ethanol and its acidified counterpart (Klavins et al., 2022), that was on average 23% higher when compared to 30% acetone. Drying techniques affected anthocyanins' content as their presence in powders were in the following order: freeze-drying > vacuum drying at 60 °C > vacuum drying at 90 °C. Irrespective of solvent type used, vacuum drying at 60 and 90 °C diminished their content, by 9 and 24%, accordingly, compared to freeze-drying. Therefore, the anthocyanins' presence, as particularly temperature-sensitive compounds, was noticeably moderated by parameters used during selected drying techniques, which was especially linked to interplay between temperature and time applied (Reque et al., 2016; Zhu et al., 2017).

Carrier-added powders

In the next part of the study, powders were produced with addition of carriers in order to verify how the carrier type (maltodextrin, inulin and trehalose) and their binary blends (mixes) affect (poly)phenolics stability during drying (**Table 4**). The addition of carriers at the level of 10% (*w/w*) resulted in, on average, 3.6-times lower content of (poly)phenolics in return for higher process efficiency (data not shown) (Michalska et al., 2018). Similar to controls, a strong influence of solvent type was indicated as acidified 50% ethanol (pH = 2) led to obtainment of powders with a higher content of (poly)phenolics that was 28% and 13% higher when compared to those produced using 30% acetone and 50% ethanol, respectively (**Table 4**).

In general, content of all identified (poly)phenolics was more affected by drying technique than the carrier type used (one-dimensional significance test; data not shown). Freeze-drying led to powders with the highest content of (poly)phenolics, followed by vacuum drying at 60 and 90 °C (decrease of, respectively, 10% and 17%). That was more linked to the content of flavonols and flavan-3-ols, while considerably greater differences were noted for anthocyanins - in this case the degradation was at the level of 15% (vacuum drying at 60 °C) and 51% (vacuum drying at 90 °C) compared to freeze-dried powders. Among single carriers applied, trehalose resulted in the highest retention of sum of identified (poly)phenolics in powders gained, regardless of solvent used and drying technique applied. Among carrier blends used for powders production, the inulin-trehalose binary blend led to lower content of (poly)phenolics than maltodextrin-trehalose, regardless of extraction solvent and drying

technique. This composition of blend (maltodextrin-trehalose) also resulted in the highest content of total (poly)phenolics in chokeberry pomace extracts powders (Michalska-Ciechanowska, Hendrysiak, et al., 2021).

Going into the details, addition of carriers and their blends influenced the content of compounds that belong to different (poly)phenolic' groups. In the case of flavonols, such an approach led to their lower content, similarly to total (poly)phenolics content, approx. 3.6-times the content of flavonols in powders. In general, the sum of flavonols was the highest in powders produced using pomace extracted with 50% ethanol, that was on average 9.4% and 36% higher than in powders produced with acidified 50% ethanol and 30% acetone, respectively, regardless carrier type. Among single carriers, trehalose preserved the flavonols to the highest extent when compared to maltodextrin and inulin. Similar to the sum of (poly)phenolics, addition of a maltodextrin-trehalose blend resulted in a higher content of flavonols in powders when compared to inulin-trehalose. When drying technique was concerned, the highest content of flavonols was noted after freeze-drying, regardless of the carrier type used, that was higher of approx. 9% and 13% when compared to vacuum drying at 60 and 90 °C, respectively. Difference between flavonols content during vacuum drying at 60 and 90 °C was less than 5%, which was similar to the difference between powders produced without a carrier. This indicated that the carriers did not play a decisive role in flavonols protection during thermal processes.

For flavan-3-ols, when the carrier-added powders were concerned, the solvent type played the most influential role as powders prepared from acidified 50% ethanol-derived extracts had approx. 30% more flavan-3-ols than those obtained with 30% acetone application. As with the control, again extraction with only 50% ethanol yielded residual amounts of flavan-3-ols identified in carrier-added powders, compared to 30% acetone and 50% acidified ethanol. Freeze-drying gave products with the greatest amount of these constituents, while vacuum drying at 60 and 90 °C resulted in their comparable content, which were about 12% lower than lyophilized samples. Amongst applied carriers and its mixes, i.a., trehalose, maltodextrin and their blend seemed to ensure the highest protection during drying, regardless of technique or solvent type. Previously, trehalose was indicated to stabilize selected (poly)phenolics, e.g., anthocyanins by ensuring thermal protection during elevated-temperature treatment by forming hydrogen bonds with them and therefore hindering the structure breakdown (Castagnini et al., 2021). Moreover, this substance was indicated as distinctive amongst others due to its superior ability to replace water molecules during dehydration process via hydrogen-bonding to biomolecules (Lerbret et al., 2005) known as a ‘water replacement’ hypothesis proposed by (Crowe et al., 1994).

Table 4. (Poly)phenolics content [g/100 g dm], antioxidant capacity [mmol Trolox/100 g dm] and hydroxymethyl-*L*-furfural content [µg/100 g dm] in cranberry pomace extract powders with carriers.

Solvent type	Drying technique	Carrier type	(Poly)phenolics				Antioxidant capacity		Hydroxymethyl-*L*-furfural
			Flavonols	Flavan-3-ols	Phenolic acids	Anthocyanins	TEAC ABTS	FRAP	
Acetone	Freeze-drying	Maltodextrin	2.58 ± 0.07 $^{d-f}$	0.75 ± 0.03 ^{cd}	0.56 ± 0.01 ^{b}	0.45 ± 0.01 ^{ef}	112.48 ± 1.34 ^{cd}	78.74 ± 1.10 ^{cd}	8.84 ± 0.75 ^{a}
		Inulin	2.67 ± 0.01 ^{f}	0.82 ± 0.03 ^{d}	0.58 ± 0.00 ^{b}	0.48 ± 0.00 ^{f}	110.65 ± 8.74 $^{b-d}$	77.92 ± 2.71 ^{cd}	10.31 ± 1.02 $^{a-c}$
		Trehalose	2.61 ± 0.09 $^{d-f}$	0.65 ± 0.00 $^{a-d}$	0.58 ± 0.02 ^{b}	0.47 ± 0.01 ^{f}	99.23 ± 6.28 $^{a-c}$	68.88 ± 4.58 $^{a-c}$	10.12 ± 1.22 $^{a-c}$
		Maltodextrin-Inulin	2.57 ± 0.07 $^{d-f}$	0.67 ± 0.01 $^{a-d}$	0.58 ± 0.02 ^{b}	0.45 ± 0.01 ^{ef}	87.96 ± 8.10 ^{a}	60.86 ± 6.34 ^{a}	16.48 ± 1.04 ^{e}
		Maltodextrin-Trehalose	2.58 ± 0.12 $^{d-f}$	0.67 ± 0.04 $^{a-d}$	0.57 ± 0.02 ^{b}	0.46 ± 0.01 ^{f}	105.09 ± 0.58 $^{b-d}$	72.78 ± 0.91 $^{b-d}$	15.37 ± 0.26 ^{de}
		Inulin-Trehalose	2.64 ± 0.00 ^{ef}	0.68 ± 0.01 $^{b-d}$	0.58 ± 0.01 ^{b}	0.44 ± 0.02 ^{ef}	106.16 ± 6.08 $^{b-d}$	76.09 ± 1.02 ^{cd}	16.35 ± 0.01 ^{e}
	Vacuum drying at 60 °C	Maltodextrin	1.98 ± 0.20 ^{a}	0.51 ± 0.02 ^{a}	0.47 ± 0.04 ^{ab}	0.32 ± 0.04 $^{b-d}$	108.08 ± 2.29 $^{b-d}$	72.49 ± 1.39 $^{b-d}$	10.37 ± 1.70 $^{a-c}$
		Inulin	2.39 ± 0.09 $^{a-f}$	0.55 ± 0.02 ^{ab}	0.53 ± 0.02 ^{ab}	0.39 ± 0.01 ^{de}	108.77 ± 0.21 $^{b-d}$	73.31 ± 1.15 $^{b-d}$	12.17 ± 0.98 $^{a-d}$
		Trehalose	2.43 ± 0.02 $^{b-f}$	0.57 ± 0.00 ^{ab}	0.55 ± 0.01 ^{ab}	0.39 ± 0.01 ^{de}	104.08 ± 2.69 $^{b-d}$	72.87 ± 6.58 $^{b-d}$	13.19 ± 0.89 $^{c-e}$
		Maltodextrin-Inulin	2.39 ± 0.18 $^{a-f}$	0.63 ± 0.03 $^{a-c}$	0.55 ± 0.04 ^{ab}	0.38 ± 0.03 $^{c-e}$	94.54 ± 1.22 ^{ab}	64.09 ± 4.63 ^{ab}	11.78 ± 0.64 $^{a-d}$
		Maltodextrin-Trehalose	2.57 ± 0.03 $^{d-f}$	0.68 ± 0.02 $^{a-d}$	0.55 ± 0.03 ^{ab}	0.41 ± 0.01 ^{ef}	110.97 ± 2.15 ^{cd}	72.96 ± 0.13 $^{b-d}$	12.92 ± 0.47 $^{b-e}$
		Inulin-Trehalose	2.43 ± 0.06 $^{b-f}$	0.64 ± 0.00 $^{a-c}$	0.56 ± 0.01 ^{b}	0.39 ± 0.01 ^{de}	106.34 ± 0.02 $^{b-d}$	75.63 ± 1.24 ^{cd}	9.14 ± 0.27 $^{a-c}$
	Vacuum drying at 90 °C	Maltodextrin	2.09 ± 0.03 $^{a-c}$	0.59 ± 0.05 $^{a-c}$	0.48 ± 0.02 ^{ab}	0.27 ± 0.00 ^{b}	106.26 ± 0.92 $^{b-d}$	73.72 ± 0.11 $^{b-d}$	9.53 ± 1.09 $^{a-c}$
		Inulin	2.30 ± 0.14 $^{a-f}$	0.70 ± 0.07 $^{b-d}$	0.51 ± 0.08 ^{ab}	0.27 ± 0.04 ^{b}	109.41 ± 0.62 $^{b-d}$	78.34 ± 0.18 ^{cd}	9.36 ± 1.94 $^{a-c}$
		Trehalose	2.45 ± 0.05 $^{c-f}$	0.68 ± 0.00 $^{a-d}$	0.55 ± 0.01 ^{ab}	0.27 ± 0.01 ^{b}	106.78 ± 5.55 $^{b-d}$	75.14 ± 1.76 $^{b-d}$	9.30 ± 0.11 $^{a-c}$
		Maltodextrin-Inulin	2.21 ± 0.08 $^{a-e}$	0.62 ± 0.05 $^{a-c}$	0.51 ± 0.01 ^{ab}	0.31 ± 0.02 ^{bc}	109.88 ± 1.07 $^{b-d}$	80.05 ± 1.09 ^{cd}	10.50 ± 0.43 $^{a-c}$
		Maltodextrin-Trehalose	2.17 ± 0.08 $^{a-d}$	0.61 ± 0.01 $^{a-c}$	0.51 ± 0.02 ^{ab}	0.33 ± 0.00 $^{b-d}$	118.15 ± 3.69 ^{d}	82.78 ± 0.02 ^{d}	9.05 ± 1.04 ^{ab}
		Inulin-Trehalose	1.99 ± 0.26 ^{ab}	0.56 ± 0.12 ^{ab}	0.43 ± 0.06 ^{a}	0.18 ± 0.02 ^{a}	108.66 ± 1.72 $^{b-d}$	73.92 ± 1.15 $^{b-d}$	12.62 ± 1.60 $^{a-e}$
50% Ethanol	Freeze-drying	Maltodextrin	3.18 ± 0.04 $^{a-e}$	tr	0.64 ± 0.00 $^{a-e}$	0.57 ± 0.00 $^{e-i}$	107.62 ± 1.42 ^{ab}	76.79 ± 0.42 $^{a-c}$	tr
		Inulin	3.25 ± 0.11 $^{a-f}$	tr	0.69 ± 0.03 $^{a-f}$	0.59 ± 0.02 $^{f-i}$	94.13 ± 5.50 ^{a}	68.99 ± 0.72 ^{a}	tr
		Trehalose	3.45 ± 0.20 $^{c-f}$	tr	0.71 ± 0.03 $^{c-f}$	0.60 ± 0.03 $^{g-i}$	112.07 ± 2.39 ^{b}	79.72 ± 0.65 $^{a-c}$	tr
		Maltodextrin-Inulin	3.45 ± 0.07 $^{c-f}$	tr	0.72 ± 0.02 $^{c-f}$	0.62 ± 0.02 ^{hi}	108.30 ± 2.00 ^{ab}	72.40 ± 1.61 $^{a-c}$	tr
		Maltodextrin-Trehalose	3.68 ± 0.01 ^{f}	tr	0.77 ± 0.01 ^{f}	0.66 ± 0.01 ^{i}	110.32 ± 2.34 ^{b}	79.99 ± 0.77 ^{bc}	tr
		Inulin-Trehalose	3.54 ± 0.03 $^{d-f}$	tr	0.73 ± 0.00 $^{d-f}$	0.64 ± 0.01 ^{hi}	108.98 ± 0.14 ^{ab}	77.46 ± 2.64 $^{a-c}$	tr
	Vacuum drying at 60 °C	Maltodextrin	3.09 ± 0.03 $^{a-d}$	tr	0.67 ± 0.00 $^{a-e}$	0.51 ± 0.02 $^{d-f}$	99.70 ± 12.27 ^{ab}	71.48 ± 7.18 ^{ab}	tr
		Inulin	2.99 ± 0.03 $^{a-c}$	tr	0.64 ± 0.00 $^{a-d}$	0.52 ± 0.01 $^{d-g}$	104.43 ± 4.26 ^{ab}	73.97 ± 3.93 $^{a-c}$	tr
		Trehalose	3.24 ± 0.02 $^{a-f}$	tr	0.69 ± 0.00 $^{b-f}$	0.56 ± 0.00 $^{e-h}$	109.70 ± 2.49 ^{b}	78.73 ± 0.42 $^{a-c}$	tr
		Maltodextrin-Inulin	3.02 ± 0.01 $^{a-c}$	tr	0.64 ± 0.00 $^{a-c}$	0.53 ± 0.00 $^{d-g}$	107.02 ± 1.03 ^{ab}	77.18 ± 5.12 $^{a-c}$	tr
		Maltodextrin-Trehalose	3.58 ± 0.04 ^{ef}	tr	0.74 ± 0.01 ^{ef}	0.61 ± 0.02 ^{hi}	107.76 ± 1.48 ^{ab}	77.38 ± 1.25 $^{a-c}$	tr
		Inulin-Trehalose	3.34 ± 0.04 $^{b-f}$	tr	0.70 ± 0.00 $^{b-f}$	0.55 ± 0.01 $^{e-h}$	108.06 ± 0.15 ^{ab}	78.17 ± 1.62 $^{a-c}$	tr

Table 4. (*continued*).

	Vacuum drying at 90 °C	Maltodextrin	2.93 ± 0.24 ^{ab}	tr	0.65 ± 0.07 $^{a-e}$	0.48 ± 0.06 $^{c-e}$	103.86 ± 1.63 ^{ab}	73.41 ± 1.95 $^{a-c}$	tr
		Inulin	3.07 ± 0.01 $^{a-d}$	tr	0.65 ± 0.00 $^{a-e}$	0.46 ± 0.01 $^{b-d}$	108.56 ± 0.94 ^{ab}	78.06 ± 1.79 $^{a-c}$	tr
		Trehalose	3.44 ± 0.00 $^{c-f}$	tr	0.71 ± 0.00 $^{c-f}$	0.43 ± 0.01 ^{bc}	112.27 ± 0.39 ^{b}	78.39 ± 0.90 $^{a-c}$	tr
		Maltodextrin-Inulin	2.82 ± 0.12 ^{a}	tr	0.60 ± 0.01 ^{ab}	0.39 ± 0.01 ^{b}	107.90 ± 4.13 ^{ab}	75.08 ± 3.71 $^{a-c}$	tr
		Maltodextrin-Trehalose	3.24 ± 0.33 $^{a-f}$	tr	0.68 ± 0.06 $^{a-f}$	0.51 ± 0.04 $^{c-f}$	114.10 ± 2.00 ^{b}	82.86 ± 1.63 ^{c}	tr
		Inulin-Trehalose	3.11 ± 0.01 $^{a-e}$	tr	0.60 ± 0.00 ^{a}	0.24 ± 0.00 ^{a}	112.53 ± 0.32 ^{b}	78.37 ± 0.65 $^{a-c}$	tr
50% Ethanol (pH = 2)	Freeze-drying	Maltodextrin	3.23 ± 0.00 $^{e-g}$	1.04 ± 0.00 $^{e-g}$	0.67 ± 0.00 $^{f-h}$	0.61 ± 0.01 $^{g-i}$	104.32 ± 2.36 $^{a-e}$	71.83 ± 3.38 $^{a-d}$	3.96 ± 0.16 $^{a-c}$
		Inulin	2.89 ± 0.05 $^{a-e}$	0.93 ± 0.00 $^{b-f}$	0.59 ± 0.01 $^{b-e}$	0.55 ± 0.01 $^{e-g}$	97.18 ± 0.37 ^{ab}	68.60 ± 0.37 ^{ab}	3.68 ± 0.03 $^{a-c}$
		Trehalose	3.37 ± 0.07 ^{fg}	1.06 ± 0.04 ^{fg}	0.68 ± 0.01 ^{gh}	0.64 ± 0.01 ^{hi}	112.75 ± 0.91 $^{c-f}$	79.30 ± 0.98 ^{cd}	4.09 ± 0.48 ^{c}
		Maltodextrin-Inulin	3.44 ± 0.02 ^{g}	1.07 ± 0.00 ^{g}	0.71 ± 0.00 ^{h}	0.66 ± 0.00 ^{i}	111.58 ± 1.55 $^{c-f}$	78.68 ± 1.42 $^{b-d}$	4.29 ± 0.48 ^{c}
		Maltodextrin-Trehalose	3.23 ± 0.04 $^{e-g}$	1.01 ± 0.02 $^{d-g}$	0.66 ± 0.01 $^{e-h}$	0.61 ± 0.01 $^{f-i}$	104.24 ± 2.03 $^{a-e}$	68.84 ± 2.44 ^{ab}	4.04 ± 0.08 ^{bc}
		Inulin-Trehalose	2.99 ± 0.02 $^{b-g}$	0.95 ± 0.02 $^{c-g}$	0.62 ± 0.00 $^{b-g}$	0.57 ± 0.01 $^{e-h}$	105.62 ± 0.57 $^{a-e}$	70.79 ± 0.62 $^{a-c}$	3.58 ± 0.21 $^{a-c}$
	Vacuum drying at 60 °C	Maltodextrin	2.64 ± 0.02 $^{a-c}$	0.85 ± 0.00 ^{bc}	0.57 ± 0.00 $^{a-c}$	0.45 ± 0.01 ^{bc}	105.63 ± 1.25 $^{a-e}$	76.50 ± 2.34 $^{a-d}$	3.89 ± 0.17 $^{a-c}$
		Inulin	2.95 ± 0.02 $^{a-f}$	0.98 ± 0.02 $^{c-g}$	0.63 ± 0.00 $^{b-g}$	0.54 ± 0.00 $^{d-f}$	103.68 ± 1.36 $^{a-d}$	67.51 ± 5.52 ^{a}	3.67 ± 0.02 $^{a-c}$
		Trehalose	3.14 ± 0.02 $^{d-g}$	1.00 ± 0.01 $^{d-g}$	0.67 ± 0.01 $^{e-h}$	0.58 ± 0.01 $^{e-h}$	111.30 ± 1.07 $^{c-f}$	78.36 ± 0.11 $^{b-d}$	4.21 ± 0.10 ^{c}
		Maltodextrin-Inulin	2.90 ± 0.06 $^{a-e}$	0.94 ± 0.01 $^{b-g}$	0.62 ± 0.01 $^{b-g}$	0.54 ± 0.01 $^{d-f}$	108.29 ± 6.52 $^{b-f}$	75.69 ± 4.03 $^{a-d}$	3.82 ± 0.02 $^{a-c}$
		Maltodextrin-Trehalose	3.05 ± 0.19 $^{b-g}$	0.96 ± 0.04 $^{c-g}$	0.65 ± 0.03 $^{c-h}$	0.54 ± 0.03 $^{d-g}$	111.24 ± 0.66 $^{c-f}$	73.60 ± 5.27 $^{a-d}$	3.72 ± 0.30 $^{a-c}$
		Inulin-Trehalose	2.69 ± 0.05 $^{a-d}$	0.87 ± 0.00 ^{bc}	0.58 ± 0.00 $^{b-d}$	0.48 ± 0.01 ^{cd}	101.75 ± 1.89 $^{a-c}$	68.53 ± 1.09 ^{ab}	3.77 ± 0.30 $^{a-c}$
	Vacuum drying at 90 °C	Maltodextrin	3.08 ± 0.15 $^{c-g}$	0.95 ± 0.05 $^{c-g}$	0.65 ± 0.02 $^{d-h}$	0.51 ± 0.03 $^{c-e}$	118.65 ± 3.36 ^{f}	81.94 ± 0.66 ^{d}	3.87 ± 0.05 $^{a-c}$
		Inulin	2.59 ± 0.18 ^{ab}	0.81 ± 0.05 ^{ab}	0.55 ± 0.04 ^{ab}	0.39 ± 0.03 ^{b}	96.84 ± 6.17 ^{a}	70.79 ± 2.63 $^{a-c}$	3.75 ± 0.63 $^{a-c}$
		Trehalose	3.10 ± 0.20 $^{c-g}$	0.91 ± 0.05 $^{b-e}$	0.65 ± 0.03 $^{d-h}$	0.45 ± 0.02 ^{bc}	113.62 ± 5.10 $^{d-f}$	79.54 ± 1.04 ^{cd}	2.93 ± 0.07 ^{a}
		Maltodextrin-Inulin	2.77 ± 0.16 $^{a-e}$	0.91 ± 0.04 $^{b-e}$	0.60 ± 0.03 $^{b-f}$	0.48 ± 0.02 ^{cd}	115.03 ± 0.99 ^{ef}	78.79 ± 1.66 $^{b-d}$	3.38 ± 0.04 $^{a-c}$
		Maltodextrin-Trehalose	2.81 ± 0.24 $^{a-e}$	0.88 ± 0.08 $^{b-d}$	0.60 ± 0.03 $^{b-f}$	0.48 ± 0.03 ^{cd}	109.66 ± 0.21 $^{c-f}$	77.99 ± 1.44 $^{b-d}$	2.98 ± 0.03 ^{ab}
		Inulin-Trehalose	2.51 ± 0.11 ^{a}	0.70 ± 0.03 ^{a}	0.50 ± 0.02 ^{a}	0.20 ± 0.02 ^{a}	109.16 ± 0.32 $^{c-f}$	77.69 ± 2.12 $^{a-d}$	3.92 ± 0.33 $^{a-c}$

tr – trace amounts; a, b, c, … – different letters within groups: carrier-added samples (acetone), carrier-added samples (50% ethanol), carrier-added samples (50% acidified ethanol) standing for different cranberry pomace extract powders indicate significant differences (ANOVA, HSD Tukey, $p < 0.05$).

Similar to controls, solvent type had the strongest influence on extraction of phenolic acids. Application of 30% acetone resulted in the lowest content of these compounds, regardless of the type of carrier used for drying, while 50% ethanol and its acidified counterpart gave products with, on average, 27% and 16% higher content, respectively. The drying technique had a slight effect on phenolic acid sum, since compared to freeze-drying, vacuum drying at 60 and 90 °C only resulted in a 6 and 11% reduction in their content, respectively. Previously, Szwajgier et al. (2014) pointed out that depending on fruit type and its processing, the content of phenolic acids could decrease, as well as remain unchanged or even increase. In addition, composition of matrix in which the individual phenolic acids occur was singled out as one of the key factors. Recognizing the impact of carrier type, the most preferable levels of phenolic acids were found when trehalose and maltodextrin-trehalose mixture were used for powders production. However, taking into account powders obtained by a given drying technique, freeze-dried products were characterized by a comparable content of phenolic acids (7% difference between the lowest and highest content depending on the carrier used), while in the case of vacuum drying at 60 and 90 °C, the difference was 14 and 25%, respectively.

In comparison to controls, the average sum of anthocyanins in powders produced with 10% carrier addition was 3.8-times lower. Compared to lyophilizates, products after vacuum drying at 60 and 90 °C retained 16 and 34% less of these constituents, respectively, despite the inclusion of protective substances. Intriguingly, despite the contrasting levels of these compounds in control powders and those with a carrier, the percentage difference in the decrease in anthocyanin content caused by high drying temperature, is markedly smaller in products where no protective substance - a carrier - was used (9 and 24% lower content, respectively, for vacuum-dried samples at 60 and 90 °C compared to freeze-dried ones). Such phenomenon may be linked with other protective mechanisms which could take place in such a (poly)phenolic-dense matrix under thermal processing, as copigmentation of anthocyanins by other compounds (Gençdağ et al., 2022). In general, among single carriers, maltodextrin addition resulted in lowest content of anthocyanins when compared to trehalose which led to powders with the highest amount of these constituents (Castagnini et al., 2021). Among diblends the average content of anthocyanins was the lowest when inulin-trehalose mix was applied, while maltodextrin-trehalose blend turned out to ensure the greatest protection for this group.

Antioxidant capacity

The antioxidant capacity of extract powders ranged between 88 to 403 and between 61 to 280 mmol Trolox/100 g dm for TEAC ABTS and FRAP measurements, respectively (**Table 3** and **Table 4**). Despite the discrepancies in results mainly reflected in lower antioxidant capacity values measured by FRAP method compared to TEAC ABTS assay, which can be attributed to the different reaction mechanisms underlying the principle of the employed methods (Pérez-Burillo et al., 2019), a very similar pattern was observed among the samples.

Controls

For control powders, acidified 50% ethanol extraction resulted in the lowest average values of antioxidant capacity measured by both assays, while the highest were noted for those obtained with acetone pomace extraction in case of TEAC ABTS measurement and by 50% ethanol for FRAP method, regardless of drying technique. Independently of solvent type, the vacuum drying at 60 °C led to powders with the lowest antioxidant properties, while interestingly, lyophilization and vacuum drying at 90 °C yielded products with relatively higher average values. However, it needs to be noted that differences between discussed results are not substantive, and all control samples exerted antioxidant potential on a comparable level. In general, control powders exerted the highest values, while carrier addition caused approximately 70% lower antioxidant capacity. One reason for this is lower ratio of preparation in the drying solution (preparation to carrier agent proportion was 90% : 10%) compared to the control samples where the preparation was 100%. Another possible explanation is the high ability of encapsulating materials to protect bioactives (strong (poly)phenolics-wall material interaction) resulting in a lower response in the *in vitro* tests, compared to unencapsulated samples (Radünz et al., 2019).

Carrier-added powders

For carrier-added products, solvent type did not affect the antioxidative effect exhibited by these powders to the same extent as in case of (poly)phenolics content or HMF formation (described below), and therefore differences between recorded average values were barely noticeable. Taking into account drying technique, the strongest antioxidant capacity was observed for vacuum drying at 90 °C, while freeze-drying and vacuum drying at 60 °C gave comparable quality powders, regardless of solvent type. This in turn may be connected to formation of other Maillard reaction and/or caramelization products such as melanoidins which

exhibit varying antioxidant potential (Feumba Dibanda et al., 2020). Despite emerging statistically significant differences between the powders with carrier addition, there are no clear distinctions or recurring patterns depending on the specific type of carrier/binary mixture. However, taking into account TEAC ABTS results, it is worth noting that all samples exhibited considerably higher antioxidant potential compared to cranberry juice powders with 15% maltodextrin addition (1.21 - 5.15 mmol Trolox/100 g dm) or sugar-free juice extract powders without carrier agent (71.86 - 188.83 mmol Trolox/100 g dm) obtained by analogous drying methods (Michalska et al., 2018). Therefore, it can be concluded that sustainable pomace extract powders obtained both, with and without addition of an encapsulating substance, constitute a high-value additive with potentially functional properties, which may be used for improving the quality of the foodstuff.

HMF determination

The presence of hydroxymethyl-*L*-furfural was confirmed in powders gained after extraction of cranberry pomace in 30% acetone and acidified 50% ethanol, regardless of drying technique. In carrier-free powders, the HMF content was 6.7-times higher in 30% acetone extracts' products when compared to those produced after usage of acidified 50% ethanol (**Table 3**). This was in line with the finding of Bicker et al. (2003) who indicated that water and acetone mixture promote the rearrangement of carbohydrates to furanoid form influencing formation of HMF. Only a trace amount of HMF was detected in 50% ethanolic extract powders. Thus, the solvent applied for cranberry pomace extraction played a key role in moderation of chemical composition of powders produced. Drying had an influence on HMF content in powders. Similar to Michalska-Ciechanowska, **Brzezowska**, et al. (2021) and Michalska-Ciechanowska, Hendrysiak, et al. (2021) the formation of HMF was confirmed in freeze-dried fruit-based products. Herein, the usage of 30% acetone for powders production by freeze-drying resulted in products with 23.4% lower content of this process contaminant than vacuum drying, regardless of temperature applied. The difference between vacuum drying at 60 and 90 °C was not statistically significant. In the case of acidified 50% ethanol extracts, drying technique did not differentiate powders in terms of HMF content. It can therefore be speculated that due to increased stability of anthocyanins caused by acidic environment during their extraction from the pomace, there was no such significant deglycosylation at later stages, including drying, resulting in the detachment of sugar residues, which might constitute substrate for HMF formation (Ertan et al., 2019).

The average HMF content in products gained with carriers was approx. 4-times and 2-times lower in, respectively, powders obtained after 30% acetone and acidified 50% ethanol cranberry pomace extraction (**Table 4**). Thus, carrier application diminished the HMF formation in products gained. Among carriers used, the highest content of this compound was noted for powders with maltodextrin-inulin blend while application of maltodextrin exclusively resulted in the lowest average HMF content, regardless of the extraction solvent or drying method. In terms of drying technique, the highest average HMF content was found for freeze-dried powders, while vacuum drying at 60 and 90 °C reduced it by about 8 and 20%, respectively. This phenomenon may be attributed to longer time of dehydration during lyophilization (24 h) compared to vacuum drying at 60 °C (22 h) and vacuum drying at 90 °C (16 h), despite the temperature difference (Fitzpatrick et al., 2013).

Chemometric analysis

The Principal Component Analysis (PCA) was carried out to distinguish relation between (poly)phenolics content, including the flavan-3-ols, flavonols, phenolic acids anthocyanins, antioxidant properties (TEAC ABTS and FRAP) and the content of HMF in powders considering different solvent used for cranberry pomace extraction and drying techniques. For control samples (no carrier addition), two principal components (PC) that explained 81.56% of the total variance were chosen (**Figure 6**). The PCA biplot showed that control powders can be grouped into: (1) products made with application of acidified 50% ethanol and dried by freeze-drying and vacuum drying at 60 °C, (2) powders produced from 50% ethanolic extracts and dried by freeze-drying and vacuum drying at 60 °C and (3) the rest of powders gained. It can be concluded that powders belong to the first group are similar in the content of identified (poly)phenolics, whereas the second group can be characterized in similar content of flavonols and phenolic acids. Application of vacuum drying at 90 °C, in the case of 50% ethanol and its acidified counterpart, influence the powders properties that was closed to those produced with acetone.

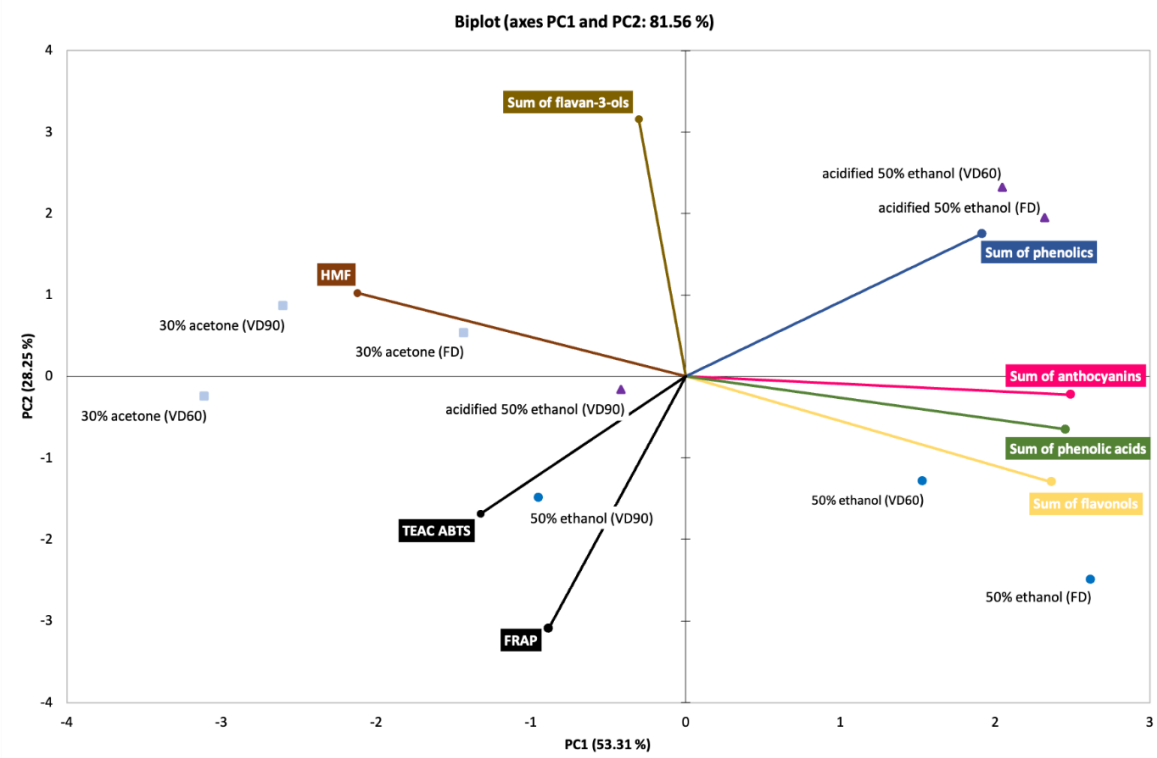
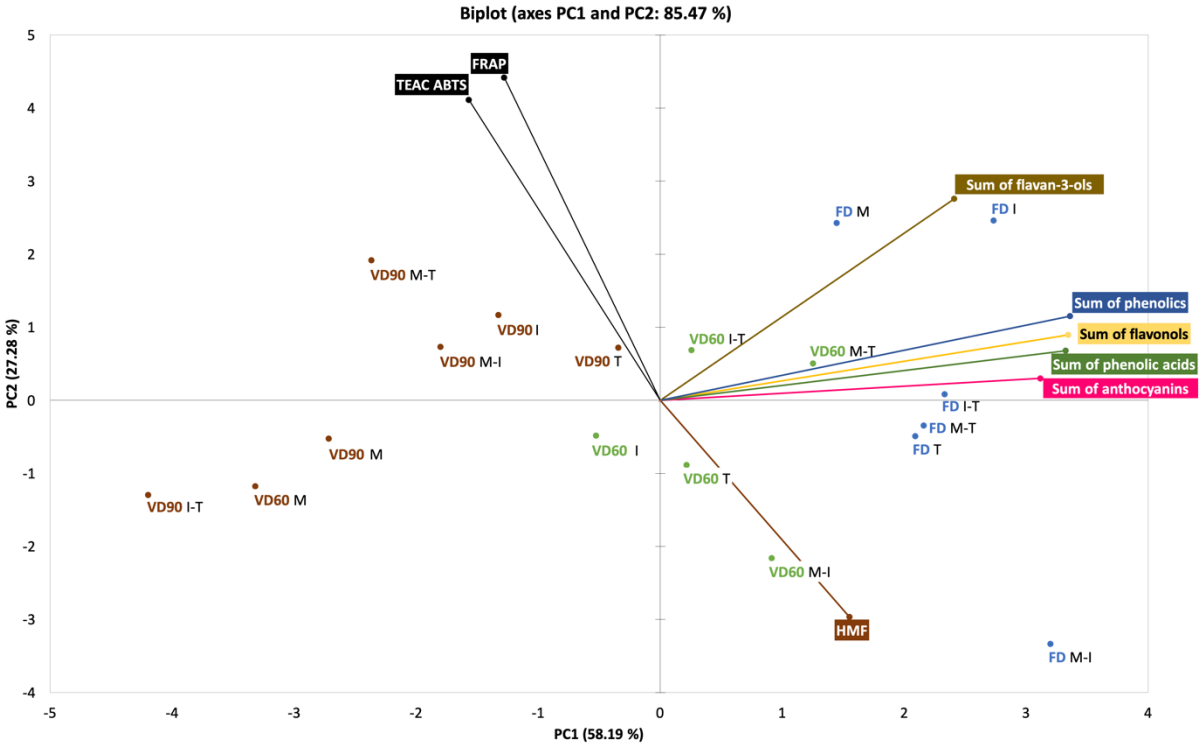


Figure 6. Principal Components Analysis (PCA) biplot that indicates principal components (PC) scores of cranberry extracts (■– 30% acetone, ● – 50% ethanol, ▲ – acidified 50% ethanol) powders gained by freeze- and vacuum drying. FD – freeze-drying, VD60 – vacuum drying at 60 °C, VD90 – vacuum drying at 90 °C.

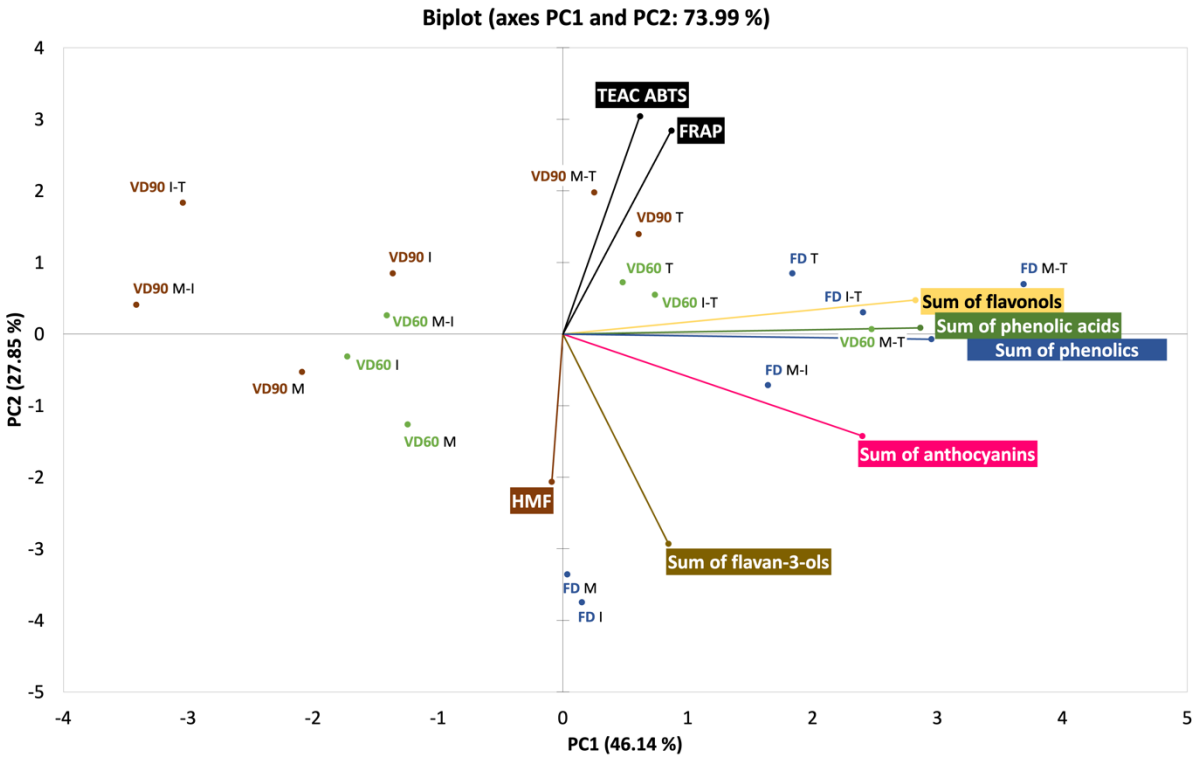
Figure 7a, 7b and **7c** showed how the type of carrier and drying techniques differentiate the cranberry pomace extracts powders. In the case of powders produced from 30% acetone extract (**Figure 7a**), the drying techniques significantly moderated the composition of powders pointing that products gained after freeze-drying and vacuum drying at 60 °C, except sample obtained after vacuum drying at 60 °C with inulin addition, were more similar to each other than powders obtained after vacuum drying at 90 °C. This confirmed that when the addition of carriers is considered, the powders can be similar in terms of (poly)phenolics after freeze-drying and vacuum drying at 60 °C that is linked to the type of carrier used for drying.

In the case of 50% ethanol cranberry pomace extracts (**Figure 7b**), drying techniques did not differentiate powders so clearly as in the case of acetone. Samples gained after freeze-drying, except those produced with addition of maltodextrin and inulin, were more similar in terms of (poly)phenolics and antioxidant capacity to powders gained after vacuum drying at 60 °C with trehalose addition as single carrier and its blend with inulin and maltodextrin and to products obtained after vacuum drying at 90 °C with trehalose and maltodextrin-trehalose blend. Vacuum drying at 60 and 90 °C produced powders with similar properties when inulin and maltodextrin (or their blends) were used.

(a)


Biplot (axes PC1 and PC2: 85.47 %)
PC2 (27.28 %)
PC1 (58.19 %)
FRAP
TEAC ABTS
Sum of flavan-3-ols
Sum of phenolics
Sum of flavonols
Sum of phenolic acids
Sum of anthocyanins
HMF
FD M
FD I
FD M-I
FD I-T
FD M-T
FD T
VD90 M-T
VD90 I
VD90 M-I
VD90 T
VD90 M
VD90 I-T
VD60 M
VD60 I-T
VD60 M-T
VD60 I
VD60 T
VD60 M-I

(b)


Biplot (axes PC1 and PC2: 73.99 %)
PC2 (27.85 %)
PC1 (46.14 %)
TEAC ABTS
FRAP
Sum of flavonols
Sum of phenolic acids
Sum of phenolics
Sum of anthocyanins
Sum of flavan-3-ols
HMF
VD90 I-T
VD90 M-T
VD90 T
VD90 I
VD90 M-I
VD90 M
VD60 T
VD60 I-T
VD60 M-I
VD60 I
VD60 M
VD60 M-T
FD T
FD M-T
FD I-T
FD M-I
FD M
FD I

(c)

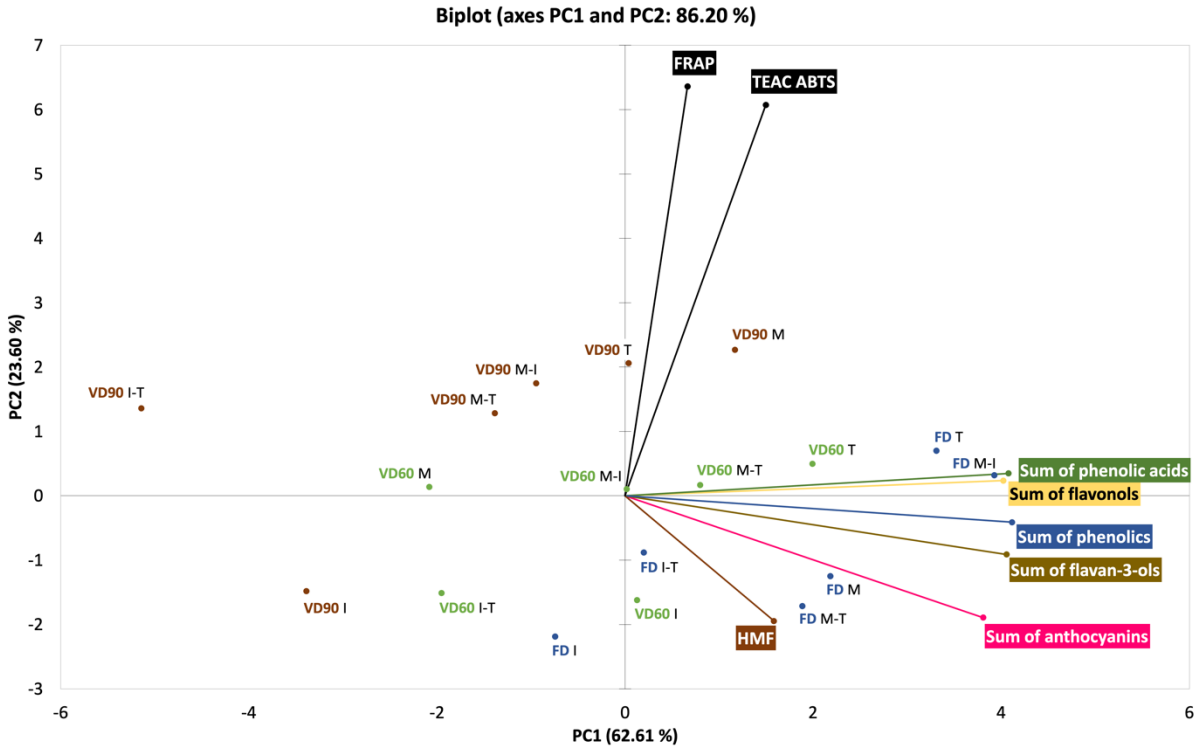


Figure 7. Principal Components Analysis (PCA) biplot that shows principal components (PC) scores of cranberry (**a**) 30% acetone, (**b**) 50% ethanol, and (**c**) 50% acidified ethanol extracts powders gained after freeze- and vacuum drying with addition of carriers. FD – freeze-drying, VD60 – vacuum drying at 60 °C, VD90 – vacuum drying at 90 °C; M – maltodextrin, I – inulin, T – trehalose, M-I – blend composed of maltodextrin and inulin, M-T – blend composed of maltodextrin and trehalose, I-T – blend composed of inulin and trehalose

Acidification of 50% ethanol moderate powders properties that was additionally affected by drying techniques and carrier type (**Figure 7c**). Changes in chemical composition caused by solvent type resulted in differences between powders quality mainly reflected in procyanidin and HMF content compared to non-acidified counterpart. Vacuum drying at 90 °C resulted in the lowest retention of (poly)phenolics, but at the same time the lowest content of HMF, while being the highest in samples after freeze-drying with maltodextrin and its blend with trehalose.

Summary

Novel approach of (poly)phenolics stabilization via extraction from cranberry pomace coupled with application of carriers and conversion by drying into powders, was proposed and successfully implemented. The most desired physical parameters were reported for vacuum-dried products, while freeze-drying yielded powders with the highest *Mc* and a_w values, and at the same time the lowest bulk density. In terms of chemical characteristic, acidified 50% ethanol was proven to be the most effective extracting solvent for cranberry pomace resulting in the highest content of (poly)phenolics in powders, while 30% acetone resulted in products with their lowest amount, and significantly higher hydroxymethyl-*L*-furfural content. Non-acidified 50% ethanol yielded powders without HMF presence, however along with only trace amounts of flavan-3-ols. The carrier application diminished HMF formation even down to 74% with regard to carrier-free samples. Compared to lyophilization, vacuum drying caused a decrease in (poly)phenolics content, however, these differences were not so distinctive when the antioxidant properties of powders were evaluated. Amongst carriers tested, the highest sum of (poly)phenolic groups was recorded for trehalose (among single carriers) and maltodextrin-trehalose (among blends). Compared to control samples, carrier addition reduced antioxidant capacity down to 70%. Overall, the feasibility of managing cranberry pomace into high-quality soluble powders that might serve as potentially functional food additives, was confirmed.

Recommendation: Acidified 50% ethanol should be considered for the extraction of cranberry pomace, as the highest retention of (poly)phenols and the lowest content of hydroxymethyl-*L*-furfural were observed for cranberry pomace extract powders when this solvent was used. Acetone can no longer be classified as an efficient extraction medium for this plant matrix. The addition of a carrier can be recommended when the minimization of HMF content is of high importance. It can be suggested that with appropriate optimization strategies, the proposed approach has promising prospects for application to the management of other types of wastes from the fruit and vegetable industry.

5.2. Stage II: *Plant-based matrix complexity vs. bioactive response and process contaminants drivers*

One of the challenges in understanding the mechanisms responsible for the bioactive properties exhibited by fruit powders is the cultivar and different content of the constituents present in the matrix. The chemical composition and structural arrangement of the food matrix determine different chemical reactivity of the components present in food products, and consequently may trigger various health-promoting properties (Capuano et al., 2018). Unfortunately, only few papers focus on powdered forms of fruit and there are scarce scientific reports devoted to comparison of such products obtained from fruit parts, namely whole fruit, pomace, juice, and sugar-free juice products, considered as different matrices. The recognition of different fruit parts properties with diverse content of selected bioactives may aid in the design of new foods or nutraceuticals in an easy-to-handle powder form.

Therefore, the **second stage** of the study was designed to recognize the effectiveness of selected components of different fruit matrices (origin and fractional differentiation) in shaping the bioactive potential of the powders obtained (**Supplementary study 2**). Moreover, in order to explore the matrix-originated drivers that influence the formation of process contaminants, model systems resembling simplified composition of selected fruit juices were used (**Supplementary study 3**) (**Figure 8**).

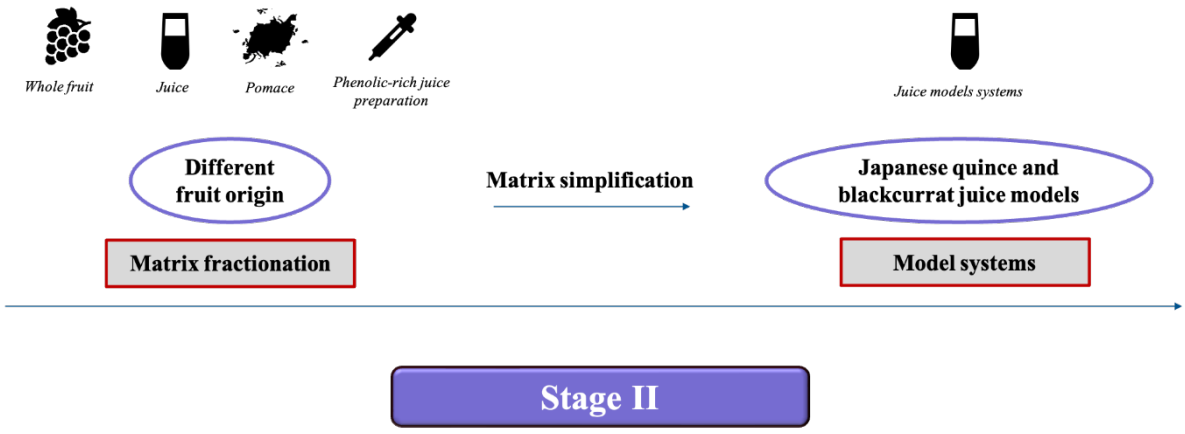


Figure 8. Schematic representation of the differentiating criteria of the matrix used for the study - stage II.

5.2.1. Supplementary study 2: *Matrix diversification and fractionation*

Obtaining fine fruit-based powders requires extensive knowledge about the physicochemical and structural properties of raw material, as well as the influence of processing (drying technique and parameters) on the possibility to produce such products (Michalska-Ciechanowska, **Brzezowska**, et al., 2021). In the **Supplementary study 2**, freeze-drying was chosen for powder preparation since due to the presence of components with a relatively low glass transition temperature, i.e., sugars and organic acids, it was the only way to produce powders from liquids without carrier addition. Freeze-drying ensured the retention of bioactives with the exclusion of the effect of a relatively high-temperature, which may cause changes in their activity. The intentional exclusion of carrier application during powder obtaining eliminated any interference caused by interactions between the carrier and bioactive compounds that may take place during processing (Michalska-Ciechanowska, **Brzezowska**, et al., 2021). The emphasis was on the type and content of the selected bioactives present in various fruit matrices, which presumably act differently depending on the composition of the surrounding matrix. Capuano et al. (2018) suggested that the chemical reactivity of particular bioactives, which induce specific potential functional properties, may differ depending on the presence of other compounds in the matrix. Previously, numerous studies ascribed specific biological properties of products to individual constituents, while the effect of the entire matrix in which these compounds are present and may interact with each other was neglected. Therefore, present study aimed to verify if depending on matrix composition of fruit-based products, effectiveness of (poly)phenolics and amino acids is heterogeneous in terms of biological properties, including antidiabetic, antiglycation and antioxidant activities of freeze-dried fruit powders.

(Poly)phenolics

For Japanese quince, among the 4 identified (poly)phenolic groups, the dominant were flavan-3-ols (monomers and dimers) followed by polymeric procyanidins, flavonols, and phenolic acids that consisted on average of 49%, 46%, 4% and 1% of all identified compounds, regardless of the fraction used (Turkiewicz et al., 2021). A significantly higher content of all (poly)phenolics was indicated for sugar-free juice products, was, on average, 14 times higher when compared to other parts (**Figure 9**). Among fruit, juice, and pomace products, the latest one had the highest content of polymeric procyanidins, while juice powders had the highest content of phenolic acids, flavonols, and flavan-3-ols. Compared to rosehip, haskap berry, and

blackcurrant, sugar-free juice product from Japanese quince had the highest content of flavan-3-ols, polymeric procyanidins, and flavonols (**Figure 10**).

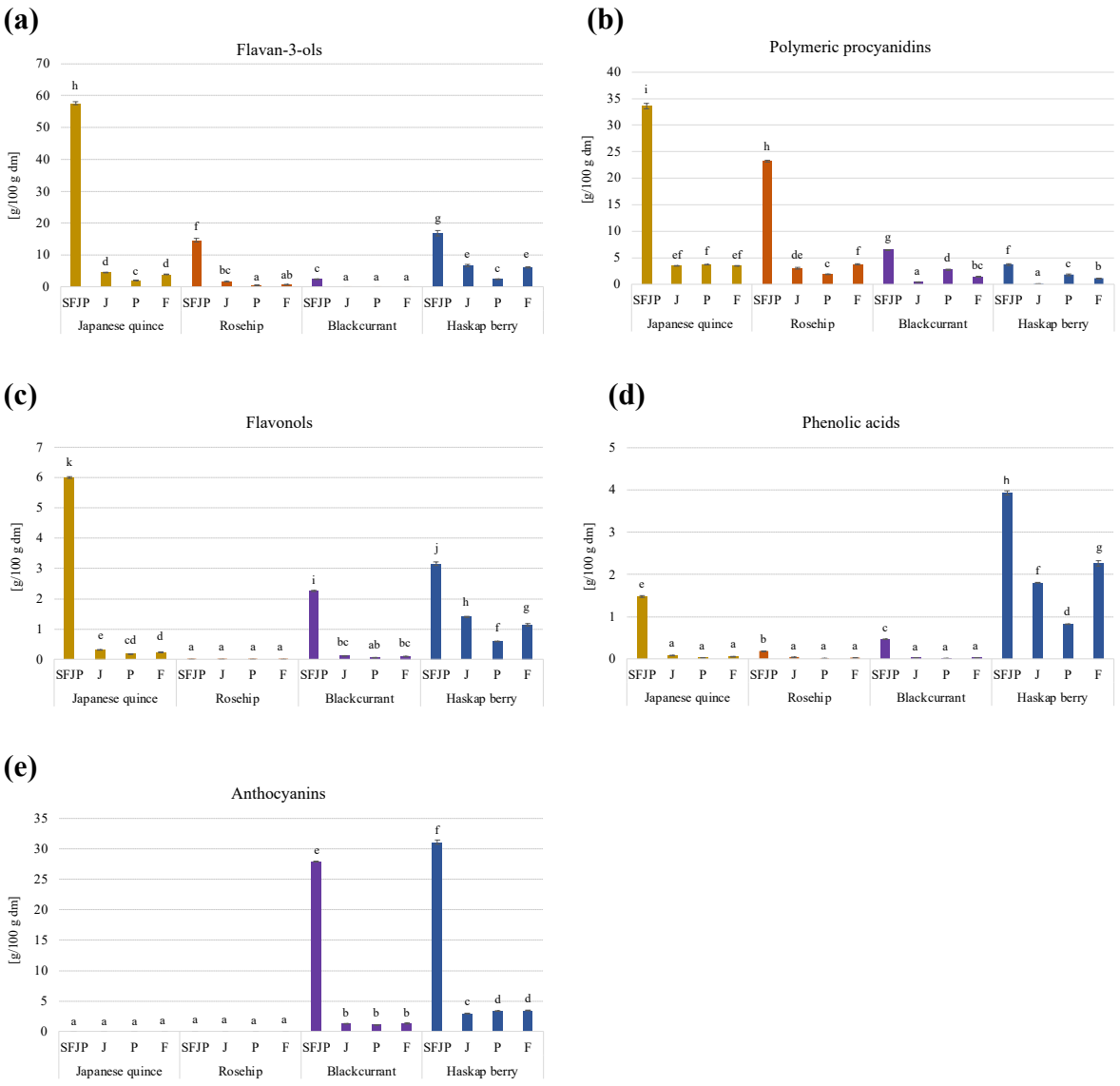
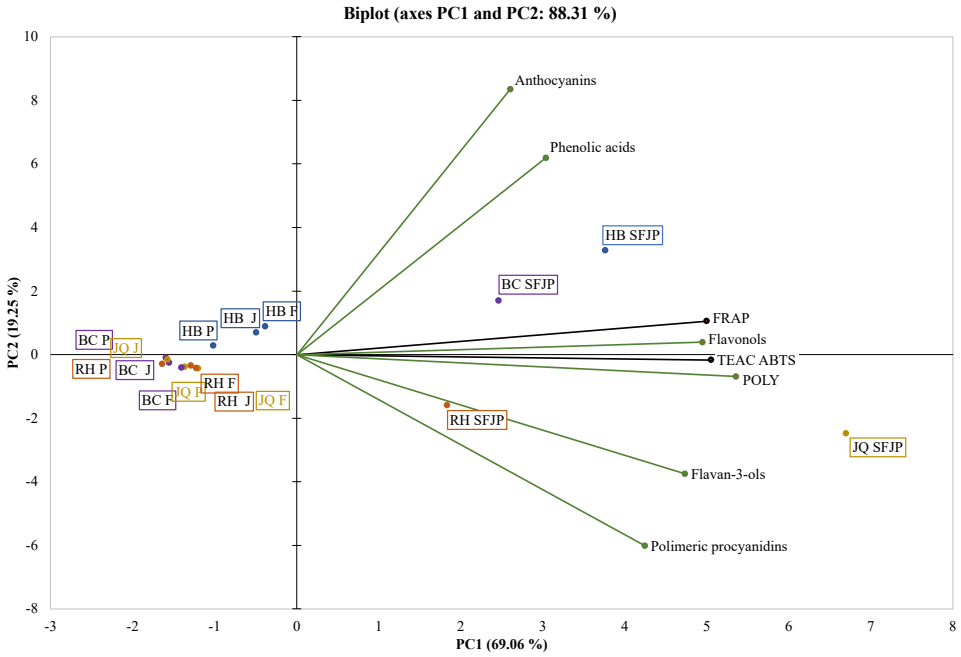


Figure 9. The content of (a) flavan-3-ols, (b) polymeric procyanidins, (c) flavonols, (d) phenolic acids, and (e) anthocyanins in freeze-dried powders gained from different parts of the Japanese quince, rosehip, blackcurrant and haskap berry [g/100 g dm]. SFJP − Sugar-free juice product; J − Juice, P – Pomace; F – Fruit

In rosehip powders, polymeric procyanidins consisted of more than 70% of the identified (poly)phenolics, regardless of the fruit fraction (**Figure 9**). Previously, (poly)phenolic characterization of rosehip fruit extract was done and flavonols were found to predominate, however, procyanidins were not analyzed in that study (Kerasioti et al., 2019). Herein, the procyanidins content was, on average, 1.6 times higher in juice and sugar-free juice product as well as 3.9 times higher for fruit and pomace compared to flavan-3-ols (**Figure 9**).

This indicates a higher content of flavan-3-ols in powdered juice and its sugar-free juice product. The phenolic acids content was less than 1% of all (poly)phenolics. Flavonols were only detected in fruit powders among rosehip products.

(a)



(b)

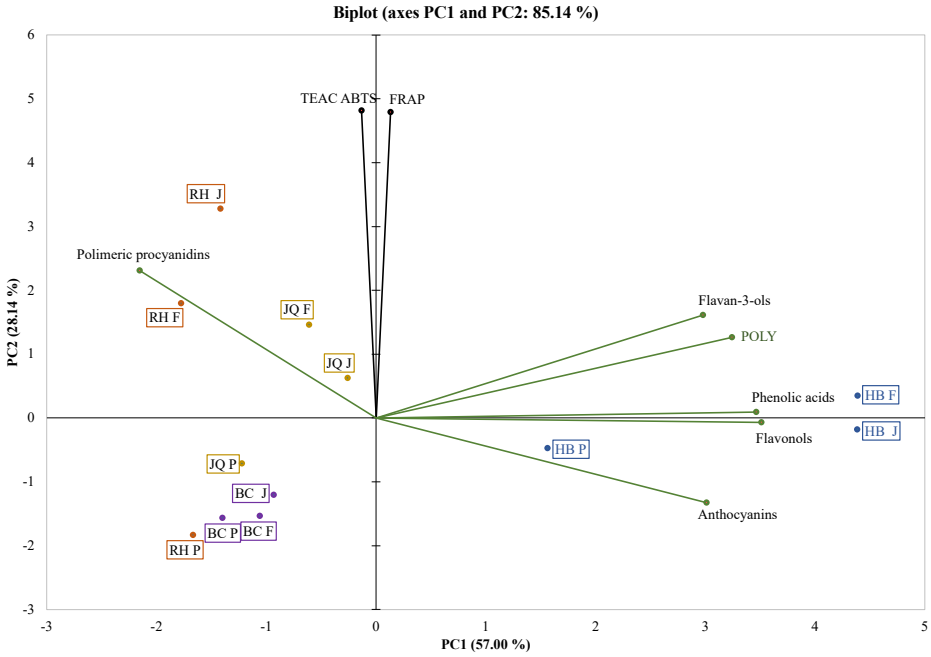


Figure 10. The two-dimensional Principal Component Analysis (PCA) biplots present PC scores of powders from (**a**) all fruit fractions (SFJP, J, P, F), and (**b**) J, P and F fractions only, which are displayed as a yellow (JQ) / orange (RH) / purple (BC) / blue (HB) color mark point, as well as loadings of explanatory variables (vectors). The length of the vectors reflects the variance of the variables, whereas inter-vector angles indicate correlations between them (e.g., small inter-vector angle signifies strong positive correlation). POLY – sum of (poly)phenolics; JQ − Japanese quince; RH − Rosehip; BC − Blackcurrant; HB − Haskap berry; SFJP − Sugar-free juice product; J – Juice; P − Pomace; F − Fruit; TEAC ABTS − Trolox Equivalent Antioxidant Capacity by ABTS; FRAP − Ferric Reducing Antioxidant Potential

For blackcurrant and haskap berry, five major groups of (poly)phenolics were quantified: anthocyanins, flavonols, flavan-3-ols (monomers and dimers), procyanidins, and phenolic acids. In blackcurrant powders, the dominant group was anthocyanins (on average, approximately 52% of all identified (poly)phenolics) which was also found previously for blackcurrant juice powders with carrier additives, followed by procyanidins (approximately 39%) (Michalska et al., 2019). Flavonols and flavan-3-ols consisted, on average, of 4% each, followed by phenolic acids whose content was below 0.9%, regardless of fraction. The anthocyanin content was dominant in juice and sugar-free preparations powders that was 1.9 times higher compared to fruit and pomace. A similar relation was observed for phenolic acids, flavonols and flavan-3-ols. For the haskap berry, fruit fractionation resulted in significant differences in the percentage share of the identified (poly)phenolics. In whole fruits and juice powders (**Figure 9**), the highest percentage share of flavan-3-ols was indicated (approximately 48% of all quantified (poly)phenolics) followed by anthocyanins (24%), phenolic acids (15%), flavonols (8%) and procyanidins (5%). A similar trend was presented for *Lonicera caerulea* L. products by Oszmiański et al. (2016). In pomace and sugar-free juice product quantities of groups were in the following order: anthocyanins > flavan-3-ols (monomers and dimers) > procyanidins > phenolic acids > flavonols. Furthermore, among all analyzed fruits and their fractions' powders, the highest content of phenolic acids was indicated for the products of haskap berries (**Figure 9 and 10**).

The two-dimensional biplot (**Figure 10a**) grouped powders produced from different types and fractions of fruits by (poly)phenolics and antioxidant capacity: (1) sugar-free juice product from Japanese quince were particularly rich in flavan-3-ols, polymeric procyanidins and flavonols, (2) haskap berry and blackcurrant sugar-free juice product that have the highest content of anthocyanins and phenolic acids, and (3) other fruit-based products, excluding sugar-free juice product (**Figure 10a**). To estimate the grouping with the exclusion of sugar-free juice product, the PCA analysis was also performed for fruit, juice and pomace powders from four types of fruits (**Figure 10b**). The score plot of PC1 versus PC2 explained 85.14% (PC1 = 57% and PC2 = 28.14%) of all variations in the experimental data set. Based on that, 3 groups of powders were distinguished: (1) haskap berry fruit, juice, and pomace, which are particularly rich in (poly)phenolics, (2) rosehip fruit and juice, Japanese quince fruit, which are characterized by high content of polymeric procyanidins, and (3) blackcurrant fruit, juice, pomace, and rosehip and Japanese quince pomace, indicating that these samples were different from others in terms of their chemical composition.

Amino acids

Previously, the amino acid profile was presented for fresh fruits of Japanese quince cultivars and rosehip (Turkiewicz, Wojdyło, Tkacz, Lech, et al., 2020; Ikhsanov et al., 2021). Here, for the first time the determination of these compounds was carried out in different parts of the fruit and 21 amino acids were identified and quantified (**Table 5**). Their content calculated as the sum of individual compounds, ranged from 135 to 1368 mg/100 g dm, for Japanese quince fruit and sugar-free juice product, respectively. Mandrioli et al. (2013) pointed that any processing, hence also mild heat treatment such as freeze-drying, can alter profile and content of amino acids, e.g., by protein and peptide hydrolysis or formation of sugar-amino acid adducts (Amadori compounds). Therefore, it is of great importance to scrutinize the amino acid profile of dehydrated fruit products and their fractions, which may differ from their fresh counterparts. Among the parts of the fruit, the lowest amino acid content (excluding Japanese quince products) was observed for the pomace powders for each type of fruit, indicating an influence of processing on their content in freeze-dried powders.

Amino acids can be divided in terms of their nutritional value as essential (13 − 685 mg/100 g dm) and non-essential (121 − 1278 mg/100 g dm) (**Table 5**). The powders obtained from sugar-free juice product had the highest ratio of essential to non-essential amino acids, whose shares decreased in the following order in products from: rosehip (65%) > Japanese quince (50%) > blackcurrant (46%) > haskap berry (26%). Within a particular raw material, one predominant amino acid cannot be distinguished in the powders of each fruit fraction. It may be linked to the structure of the amino acid side chain, which defines its affinity for water as well as the water-ethanol mixture, and thus its presence in each fruit fraction used for powder preparation (Bowden et al., 2018).

Table 5. Content of amino acids in freeze-dried fruit powders [mg/100 g dm].

Amino acid	Japanese quince				Rosehip				Blackcurrant				Haskap berry			
	SFJP	Juice	Pomace	Fruit	SFJP	Juice	Pomace	Fruit	SFJP	Juice	Pomace	Fruit	SFJP	Juice	Pomace	Fruit
Essential amino acids																
Thr	5.54 ± 0.08 ^{cd}	0.54 ± 0.02 ^{a}	3.60 ± 0.17 ^{b}	3.74 ± 0.08 ^{b}	6.91 ± 0.19 ^{d}	20.22 ± 0.70 ^{h}	4.99 ± 0.07 ^{bc}	14.76 ± 0.41 ^{f}	8.89 ± 0.19 ^{e}	22.06 ± 0.61 ^{i}	5.87 ± 0.16 ^{cd}	18.23 ± 0.63 ^{g}	4.84 ± 0.20 ^{bc}	18.74 ± 0.26 ^{g}	13.95 ± 0.20 ^{f}	20.49 ± 0.57 ^{h}
Lys	39.00 ± 0.55 ^{h}	7.29 ± 0.25 ^{cde}	7.03 ± 0.34 ^{bcd}	4.58 ± 0.10 ^{ab}	17.82 ± 0.49 ^{g}	8.74 ± 0.30 ^{de}	5.88 ± 0.08 ^{bc}	4.91 ± 0.14 ^{abc}	53.08 ± 1.11 ^{j}	6.96 ± 0.19 ^{bcd}	2.48 ± 0.07 ^{a}	6.37 ± 0.22 ^{bcd}	49.13 ± 2.02 ^{i}	11.29 ± 0.16 ^{f}	6.94 ± 0.10 ^{bcd}	9.66 ± 0.27 ^{ef}
Met	484.28 ± 6.78 ^{f}	1.09 ± 0.04 ^{a}	27.20 ± 1.30 ^{d}	12.66 ± 0.26 ^{bc}	2.59 ± 0.07 ^{a}	0.39 ± 0.01 ^{a}	2.29 ± 0.03 ^{a}	6.12 ± 0.17 ^{ab}	50.20 ± 1.05 ^{e}	0.87 ± 0.02 ^{a}	0.62 ± 0.02 ^{a}	1.66 ± 0.06 ^{a}	16.5 ± 0.68 ^{c}	0.77 ± 0.01 ^{a}	0.38 ± 0.01 ^{a}	0.68 ± 0.02 ^{a}
Val	27.68 ± 0.39 ^{f}	0.44 ± 0.02 ^{a}	7.28 ± 0.35 ^{b}	5.40 ± 0.11 ^{b}	2.28 ± 0.06 ^{a}	23.25 ± 0.80 ^{de}	5.80 ± 0.08 ^{b}	21.36 ± 0.59 ^{d}	67.07 ± 1.40 ^{h}	22.01 ± 0.61 ^{d}	7.14 ± 0.20 ^{b}	20.94 ± 0.72 ^{d}	43.58 ± 1.80 ^{g}	23.08 ± 0.32 ^{de}	16.24 ± 0.23 ^{c}	25.07 ± 0.70 ^{ef}
Ile	11.65 ± 0.16 ^{i}	1.10 ± 0.04 ^{ab}	1.76 ± 0.08 ^{ab}	0.96 ± 0.02 ^{a}	1.30 ± 0.04 ^{ab}	10.58 ± 0.36 ^{h}	3.28 ± 0.05 ^{c}	14.46 ± 0.40 ^{j}	1.88 ± 0.04 ^{b}	13.81 ± 0.38 ^{j}	4.29 ± 0.12 ^{d}	11.11 ± 0.38 ^{hi}	3.25 ± 0.13 ^{c}	5.27 ± 0.07 ^{e}	9.26 ± 0.13 ^{g}	6.67 ± 0.18 ^{f}
Leu	12.06 ± 0.17 ^{g}	0.92 ± 0.03 ^{a}	2.07 ± 0.10 ^{b}	0.86 ± 0.02 ^{a}	3.59 ± 0.10 ^{c}	13.91 ± 0.48 ^{h}	3.51 ± 0.05 ^{c}	5.90 ± 0.16 ^{d}	9.09 ± 0.19 ^{e}	6.16 ± 0.17 ^{d}	4.48 ± 0.12 ^{c}	4.29 ± 0.15 ^{c}	15.06 ± 0.62 ^{i}	14.96 ± 0.21 ^{i}	20.40 ± 0.29 ^{j}	10.59 ± 0.29 ^{f}
Phe	30.03 ± 0.42 ^{g}	0.80 ± 0.03 ^{a}	2.55 ± 0.12 ^{ab}	1.63 ± 0.03 ^{a}	106.89 ± 2.96 ^{h}	16.59 ± 0.57 ^{ef}	5.30 ± 0.07 ^{abcd}	8.38 ± 0.23 ^{bcd}	127.52 ± 2.67 ^{i}	6.39 ± 0.18 ^{abcd}	3.40 ± 0.09 ^{ab}	4.81 ± 0.17 ^{abc}	112.74 ± 4.64 ^{h}	11.48 ± 0.16 ^{de}	19.38 ± 0.27 ^{f}	9.83 ± 0.27 ^{cd}
Trp	74.42 ± 1.04 ^{f}	1.56 ± 0.05 ^{a}	2.37 ± 0.11 ^{a}	1.40 ± 0.03 ^{a}	315.22 ± 8.74 ^{h}	35.37 ± 1.22 ^{de}	17.50 ± 0.25 ^{bc}	25.00 ± 0.69 ^{cd}	252.17 ± 5.27 ^{g}	11.37 ± 0.32 ^{ab}	3.75 ± 0.10 ^{a}	10.86 ± 0.37 ^{ab}	37.55 ± 1.55 ^{e}	2.92 ± 0.04 ^{a}	6.58 ± 0.09 ^{a}	6.74 ± 0.19 ^{a}
Sum	684.66 ± 9.59 ^{h}	13.75 ± 0.47 ^{a}	53.85 ± 2.58 ^{b}	31.23 ± 0.65 ^{ab}	456.60 ± 12.66 ^{f}	129.05 ± 4.45 ^{d}	48.55 ± 0.68 ^{b}	100.88 ± 2.80 ^{c}	569.90 ± 11.91 ^{g}	89.65 ± 2.49 ^{c}	32.03 ± 0.89 ^{ab}	78.29 ± 2.70 ^{c}	282.66 ± 11.64 ^{e}	88.50 ± 1.24 ^{c}	93.13 ± 1.30 ^{c}	89.72 ± 2.49 ^{c}
Non-essential amino acids																
Asn	2.12 ± 0.03 ^{ab}	1.55 ± 0.05 ^{a}	17.07 ± 0.82 ^{d}	2.66 ± 0.06 ^{ab}	3.44 ± 0.10 ^{ab}	154.93 ± 5.34 ^{g}	24.99 ± 0.35 ^{e}	74.76 ± 2.07 ^{f}	13.04 ± 0.27 ^{cd}	7.62 ± 0.21 ^{bc}	1.88 ± 0.05 ^{ab}	6.30 ± 0.22 ^{ab}	30.07 ± 1.24 ^{e}	5.65 ± 0.08 ^{ab}	6.47 ± 0.09 ^{ab}	6.82 ± 0.19 ^{ab}
Arg	0.91 ± 0.01 ^{a}	0.51 ± 0.02 ^{a}	3.34 ± 0.16 ^{bcd}	1.37 ± 0.03 ^{ab}	5.58 ± 0.15 ^{d}	47.58 ± 1.64 ^{j}	13.46 ± 0.19 ^{f}	15.29 ± 0.42 ^{f}	9.41 ± 0.20 ^{e}	5.14 ± 0.14 ^{cd}	3.24 ± 0.09 ^{bc}	2.80 ± 0.10 ^{ab}	33.11 ± 1.36 ^{i}	10.99 ± 0.15 ^{e}	25.49 ± 0.36 ^{h}	19.47 ± 0.54 ^{g}
Ser	15.49 ± 0.22 ^{bc}	3.11 ± 0.11 ^{a}	15.89 ± 0.76 ^{bc}	6.71 ± 0.14 ^{a}	5.07 ± 0.14 ^{a}	25.89 ± 0.89 ^{d}	8.57 ± 0.12 ^{ab}	17.83 ± 0.49 ^{cd}	1.45 ± 0.03 ^{a}	85.37 ± 2.37 ^{f}	19.66 ± 0.55 ^{cd}	95.27 ± 3.29 ^{g}	24.74 ± 1.02 ^{d}	204.92 ± 2.87 ^{h}	59.12 ± 0.83 ^{e}	234.56 ± 6.50 ^{i}
Gln	15.03 ± 0.21 ^{ab}	7.61 ± 0.26 ^{a}	8.38 ± 0.40 ^{a}	13.45 ± 0.28 ^{a}	6.97 ± 0.19 ^{a}	56.24 ± 1.94 ^{d}	10.77 ± 0.15 ^{a}	34.25 ± 0.95 ^{bc}	54.37 ± 1.14 ^{d}	202.74 ± 5.62 ^{g}	45.03 ± 1.25 ^{cd}	158.46 ± 5.47 ^{f}	24.02 ± 0.99 ^{ab}	485.17 ± 6.79 ^{h}	125.70 ± 1.76 ^{c}	575.27 ± 15.95 ^{i}
Gly	2.42 ± 0.03 ^{bc}	0.99 ± 0.03 ^{a}	16.45 ± 0.79 ^{j}	5.28 ± 0.11 ^{fg}	6.41 ± 0.18 ^{gh}	19.41 ± 0.67 ^{k}	8.91 ± 0.12 ^{i}	6.84 ± 0.19 ^{h}	3.91 ± 0.08 ^{de}	7.56 ± 0.21 ^{h}	4.72 ± 0.13 ^{ef}	6.70 ± 0.23 ^{h}	1.28 ± 0.05 ^{ab}	7.04 ± 0.10 ^{h}	24.40 ± 0.34 ^{l}	3.33 ± 0.09 ^{cd}
Asp	13.04 ± 0.18 ^{abc}	5.71 ± 0.20 ^{a}	155.25 ± 7.42 ^{j}	48.95 ± 1.02 ^{f}	19.39 ± 0.54 ^{bcd}	81.08 ± 2.80 ^{h}	21.77 ± 0.30 ^{cde}	48.99 ± 1.36 ^{f}	16.37 ± 0.34 ^{bc}	30.70 ± 0.85 ^{e}	11.20 ± 0.31 ^{ab}	45.28 ± 1.56 ^{f}	110.91 ± 4.57 ^{i}	63.09 ± 0.88 ^{g}	26.92 ± 0.38 ^{de}	44.45 ± 1.23 ^{f}
Glu	14.83 ± 0.21 ^{b}	4.92 ± 0.17 ^{a}	3.82 ± 0.18 ^{a}	22.61 ± 0.47 ^{c}	13.18 ± 0.37 ^{b}	35.11 ± 1.21 ^{d}	11.62 ± 0.16 ^{b}	58.63 ± 1.63 ^{f}	19.52 ± 0.41 ^{c}	50.78 ± 1.41 ^{e}	20.32 ± 0.56 ^{c}	67.67 ± 2.33 ^{g}	6.21 ± 0.26 ^{a}	87.13 ± 1.22 ^{i}	34.73 ± 0.49 ^{d}	75.89 ± 2.10 ^{h}
Ala	12.87 ± 0.18 ^{b}	0.00 ± 0.00 ^{a}	30.19 ± 1.44 ^{d}	21.08 ± 0.44 ^{c}	16.97 ± 0.47 ^{bc}	47.78 ± 1.65 ^{e}	15.99 ± 0.22 ^{bc}	15.44 ± 0.43 ^{bc}	3.91 ± 0.08 ^{a}	127.57 ± 3.54 ^{g}	36.45 ± 1.01 ^{d}	121.81 ± 4.20 ^{g}	11.02 ± 0.45 ^{b}	80.89 ± 1.13 ^{f}	43.79 ± 0.61 ^{e}	82.47 ± 2.29 ^{f}
GABA	9.60 ± 0.13 ^{abc}	0.00 ± 0.00 ^{a}	15.07 ± 0.72 ^{c}	33.02 ± 0.69 ^{de}	9.79 ± 0.27 ^{bc}	68.49 ± 2.36 ^{f}	28.52 ± 0.40 ^{d}	18.05 ± 0.50 ^{c}	1.58 ± 0.03 ^{ab}	125.43 ± 3.48 ^{h}	38.73 ± 1.07 ^{e}	144.54 ± 4.99 ^{i}	115.06 ± 4.74 ^{g}	160.19 ± 2.24 ^{j}	60.37 ± 0.85 ^{f}	158.09 ± 4.38 ^{j}
Pro	144.63 ± 2.03 ^{i}	8.67 ± 0.30 ^{c}	3.32 ± 0.16 ^{a}	7.96 ± 0.17 ^{bc}	5.76 ± 0.16 ^{ab}	34.13 ± 1.18 ^{g}	10.24 ± 0.14 ^{c}	9.64 ± 0.27 ^{c}	37.03 ± 0.77 ^{h}	16.87 ± 0.47 ^{e}	5.49 ± 0.15 ^{ab}	16.74 ± 0.58 ^{de}	8.08 ± 0.33 ^{bc}	29.48 ± 0.41 ^{f}	14.01 ± 0.20 ^{d}	33.22 ± 0.92 ^{g}
Orn	367.45 ± 5.15 ^{i}	72.40 ± 2.50 ^{e}	33.30 ± 1.59 ^{bcd}	29.91 ± 0.63 ^{abc}	152.04 ± 4.22 ^{g}	48.29 ± 1.67 ^{d}	38.84 ± 0.54 ^{cd}	37.86 ± 1.05 ^{bcd}	390.89 ± 8.17 ^{j}	20.24 ± 0.56 ^{ab}	14.55 ± 0.40 ^{a}	122.41 ± 4.22 ^{f}	334.9 ± 13.79 ^{h}	22.65 ± 0.32 ^{abc}	38.23 ± 0.54 ^{bcd}	33.72 ± 0.94 ^{bcd}
Tyr	57.87 ± 0.81 ^{j}	14.38 ± 0.50 ^{gh}	16.04 ± 0.77 ^{h}	15.56 ± 0.33 ^{h}	1.27 ± 0.04 ^{a}	1.92 ± 0.07 ^{ab}	3.80 ± 0.05 ^{bcd}	3.96 ± 0.11 ^{cd}	54.93 ± 1.15 ^{i}	14.25 ± 0.40 ^{gh}	4.64 ± 0.13 ^{d}	12.19 ± 0.42 ^{f}	13.58 ± 0.56 ^{fg}	3.36 ± 0.05 ^{bcd}	7.65 ± 0.11 ^{e}	2.29 ± 0.06 ^{abc}
Hcys	26.71 ± 0.37 ^{d}	1.30 ± 0.04 ^{a}	0.97 ± 0.05 ^{a}	6.37 ± 0.13 ^{bc}	0.00 ± 0.00 ^{a}	1.25 ± 0.04 ^{a}	4.62 ± 0.06 ^{abc}	1.59 ± 0.04 ^{a}	50.29 ± 1.05 ^{e}	2.36 ± 0.07 ^{ab}	1.36 ± 0.04 ^{a}	2.26 ± 0.08 ^{ab}	112.30 ± 4.63 ^{f}	8.99 ± 0.13 ^{c}	3.66 ± 0.05 ^{ab}	8.79 ± 0.24 ^{c}
Sum	682.95 ± 9.56 ^{ef}	121.17 ± 4.18 ^{a}	319.10 ± 15.26 ^{c}	214.91 ± 4.49 ^{b}	245.87 ± 6.82 ^{b}	622.09 ± 21.46 ^{e}	202.11 ± 2.83 ^{b}	343.13 ± 9.51 ^{c}	656.71 ± 13.73 ^{ef}	696.64 ± 19.32 ^{f}	207.29 ± 5.75 ^{b}	802.44 ± 27.68 ^{g}	825.28 ± 33.99 ^{g}	1169.55 ± 16.38 ^{h}	470.54 ± 6.59 ^{d}	1278.37 ± 35.45 ^{i}
Sum of AA	**1367.60 ± 19.15** ^{i}	**134.92 ± 4.65** ^{a}	**372.96 ± 17.84** ^{c}	**246.14 ± 5.14** ^{b}	**702.46 ± 19.48** ^{e}	**751.14 ± 25.91** ^{e}	**250.66 ± 3.51** ^{b}	**444.01 ± 12.31** ^{c}	**1226.61 ± 25.64** ^{h}	**786.28 ± 21.80** ^{e}	**239.31 ± 6.64** ^{b}	**880.73 ± 30.38** ^{f}	**1107.93 ± 45.64** ^{g}	**1258.05 ± 17.62** ^{h}	**563.67 ± 7.89** ^{d}	**1368.10 ± 37.94** ^{i}

SFJP − Sugar-free juice product; Thr – Threonine; Lys – Lysine; Met – Methionine; Val – Valine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly –Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Ala – Alanine; GABA – γ-Aminobutyric acid; Pro – Proline; Orn – Ornithine; Tyr – Tyrosine; Hcys – Homocysteine; AA – Amino acids; a, b, c, … – different letters within groups standing for different fruit fractions i.e., Sugar-free juice products, Juice, Pomace, Fruit indicate significant differences (ANOVA, HSD Tukey, $p \geq 0.05$).

For berry powders (blackcurrant and haskap berry), sugar-free juice product had the highest ornithine content, while glycine predominated in other fruit fractions. The fact that glycine did not prevail in sugar-free juice product as well, may be due to their obtainment procedure from juice. Glycine, as the smallest amino acid, can be easily removed from the absorption resin bed with water, which is used to leach out sugars and other ballast substances. Kammerer et al. (2010) noted the effect of amino acids (and sugars) on binding of (poly)phenolics, and thus their recovery, which was reduced in most cases. This may indicate a possible interaction of amino acids (including glycine), resulting in a decrease in their content in the final fraction of sugar-free juice product. For Japanese quince juice powders, compared to other factions, no alanine and γ-aminobutyric acid (GABA) were found, which was not observed for other fruit types. It may indicate their presence mainly in the solid parts of this fruit and a trace amount in the juice, which are concentrated only after process of obtaining (poly)phenolic preparation and can be determined in this powder. Sugar-free juice product powders were the most abundant in methionine and ornithine, juice powders in ornithine, while pomace and fruit powders in aspartic acid (**Table 5**). On the contrary, Turkiewicz, Wojdyło, Tkacz, Lech, et al. (2020) found asparagine to be the predominant amino acid in fresh fruits of Japanese quince and aspartic acid as the second in terms of content. These differences may be attributed to intervarietal differences in composition, harvest time, degree of maturity, and/or processing-induced changes, as asparagine and aspartic acid share a common metabolic pathway. The same pattern can be seen for rosehip whole fruit powders, which contained glutamic acid as the predominant amino acid, while for fresh fruits, Ikhsanov et al. (2021) found glutamine as a prevailing one. This suggests that freeze-drying may evoke some alterations which eventually led to changes in amino acid profile. To date, transformation of asparagine and glutamine into aspartic and glutamic acid by acid hydrolysis and with the participation of enzymes, that is, asparaginase and glutaminase, has been reported in the literature (Orabi et al., 2019). There are no reports that discuss drying-induced transformations of this type. For the rest of rosehip fractions' powders, the predominant amino acids in the powders were asparagine in juice, ornithine in pomace, and tryptophan in sugar-free juice product. The latter product seems very attractive due to its abundant content of essential amino acids, tryptophan, which was recognized by Kumar et al. (2019) as one of the most deficient in food plants. Homocysteine was not found in sugar-free juice product; however, its content in samples from other rosehip fractions was one of the lowest, among other amino acids.

For a deeper recognition of the presence and content of essential and non-essential amino acids in each matrix of fruit-based products, the PCA analysis was performed

(**Figure 11**). PC1 and PC2 were chosen, which explained 56.83% of the total variance. The sugar-free juice product of haskap berry, blackcurrant and Japanese quince significantly differed in terms of amino acids analyzed from the rest of the powders. In these products, 5 out of 8 identified essential amino acids were recorded at higher concentrations than in other fruit fractions. However, the freeze-dried fruit and juice powders of haskap berry and blackcurrant were similar in terms of γ-aminobutyric acid, serine, glutamine, threonine, glutamic acid and alanine content. This points to that fruit processing, i.e., juicing and further modification of the matrix (preparation of sugar-free juice product), result in significant changes in the amino acid profile and content in the powders.

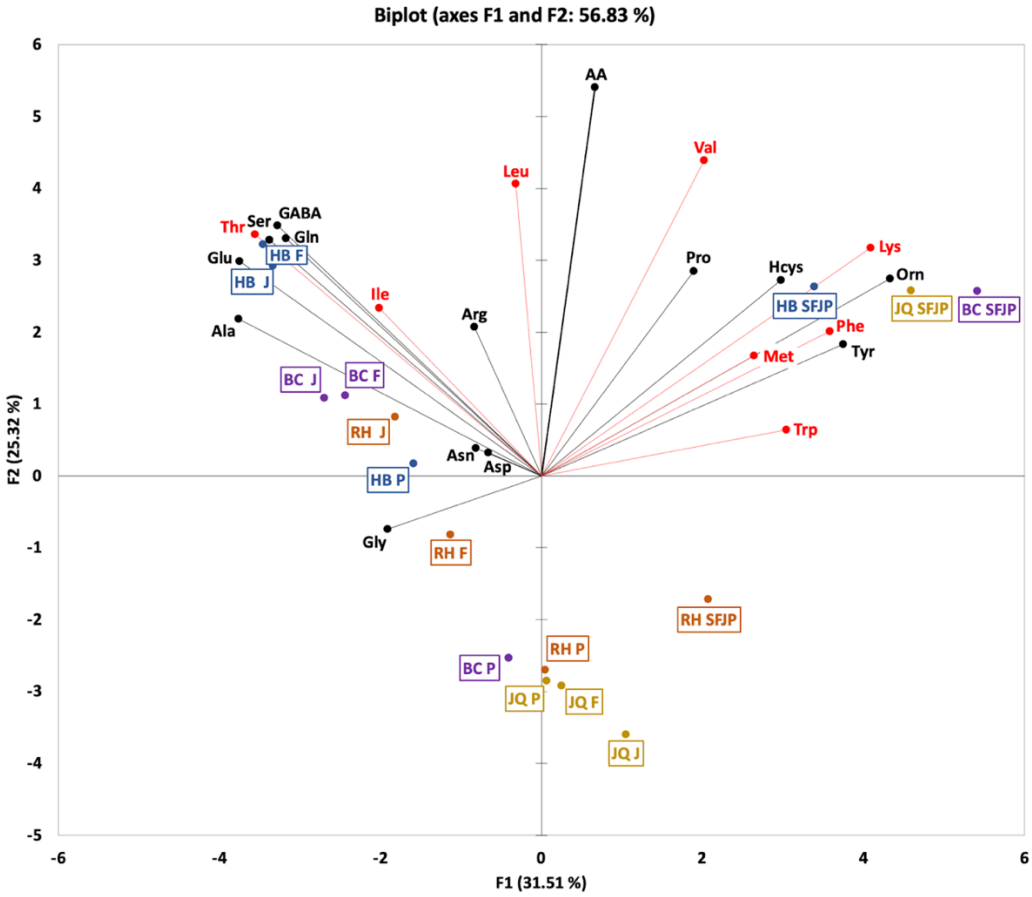


Figure 11. The two-dimensional Principal Component Analysis biplot presents PC scores of powders from all fruit fractions (SFJP, J, P, F) displayed as a yellow (JQ) / orange (RH) / purple (BC) / blue (HB) color mark point, as well as loadings of explanatory variables (vectors). The length of the vectors reflects the variance of the variables, whereas inter-vector angles indicate correlations between them (e.g., small inter-vector angle signifies strong positive correlation). JQ − Japanese quince; RH − Rosehip; BC − Blackcurrant; HB − Haskap berry; SFJP − Sugar-free juice product; J – Juice; P − Pomace; F − Fruit; Essential amino acids (marked in red): Thr – Threonine; Lys – Lysine; Met – Methionine; Val – Valine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; Non-essential amino acids (marked in black): Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly – Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Ala – Alanine; GABA – γ-Aminobutyric acid; Pro – Proline; Orn – Ornithine; Tyr – Tyrosine; Hcys – Homocysteine; AA - Sum of amino acids

Antioxidant capacity

Sugar-free juice product powders exhibited the highest antioxidant capacity values measured by the TEAC ABTS and FRAP assays, while pomace powders, excluding samples obtained from haskap berry, were the lowest (**Table 6**). This may be due to the high content of anthocyanins that are consider as strong antioxidants, being mainly distributed in the skins of haskap berry fruits, thus in the pomace fraction (Fujita et al., 2020). This was confirmed by a strong positive correlation between the TEAC ABTS and FRAP values and the content of this group of (poly)phenolics when checked only for haskap berry products (**Figure 16**). Concerning the powders of each raw material, a strong relationship was observed between antioxidant capacity and each (poly)phenolic group ($r > 0.9$), with the exception of flavonols in rosehip products due to their trace amount (**Table 6**).

The amino acid content was correlated with the antioxidant properties of the selected powders. For Japanese quince products, the positive correlation was almost linear ($r = 0.98$), while no dependence was reported for haskap berry powders (**Figure 13** and **16**). The amino acid content present in the specific fraction of the haskap berry powders was significantly higher for the juice, pomace, and whole-fruit fractions than for the Japanese quince products, while that of the sugar-free juice product powders was on a similar level. This suggests that not the content but the specific profile of amino acids determine the ability for scavenging selected free radicals of powders tested. For the first time, a strong correlation was found between ornithine content and antioxidant capacity measured by TEAC ABTS and FRAP methods ($r = 0.93$) for all samples. A similar observation was made in the case of the lysine and phenylalanine content, while, on the contrary, alanine showed a moderate negative correlation (**Figure 12**). It was proven that the antioxidant activity of specific amino acids depends on the properties of their side chains and that amino acids with (poly)phenolic groups exhibit greater antioxidant ability than others (Matsui et al., 2018).

Table 6. Antioxidant, antidiabetic and antiglycation properties of freeze-dried fruit powders.

Fruit type	Fraction	Antioxidant capacity		Antidiabetic activity		Antiglycation activity			
		TEAC ABTS	FRAP	Inhibition of α-amylase activity	Inhibition of α-glucosidase activity	BSA-fructose model system	BSA-glucose model system	BSA-MGO model system	BSA - *L*-arginine model system
		[mmol Trolox/100 g dm]		IC_{50} [mg/mL]		[% inhibition]			
Japanese quince	SFJP	430.72 ± 20.09 ^{f}	299.18 ± 7.49 ^{g}	0.73 ± 0.27 ^{h}	1.07 ± 0.58 ^{h}	94.33 ± 0.16 ^{k}	86.85 ± 0.57 ^{g}	90.69 ± 0.05 ^{l}	92.28 ± 0.60 ^{j}
	Juice	22.54 ± 0.46 ^{ab}	23.16 ± 1.48 ^{abc}	6.97 ± 0.10 ^{d}	6.01 ± 0.11 ^{ef}	92.47 ± 0.02 ^{h}	77.42 ± 0.22 ^{de}	58.89 ± 1.26 ^{i}	65.83 ± 0.32 ^{h}
	Pomace	16.34 ± 0.13 ^{a}	12.90 ± 0.21 ^{ab}	11.15 ± 0.14 ^{b}	11.08 ± 0.39 ^{cd}	89.42 ± 0.08 ^{d}	74.13 ± 0.04 ^{d}	40.45 ± 0.73 ^{de}	42.87 ± 0.20 ^{d}
	Fruit	33.86 ± 0.60 ^{ab}	26.60 ± 0.28 ^{bc}	4.63 ± 0.34 ^{f}	14.50 ± 1.49 ^{bc}	91.88 ± 0.06 ^{g}	78.54 ± 1.65 ^{ef}	52.97 ± 1.15 ^{gh}	55.12 ± 0.19 ^{e}
Rosehip	SFJP	308.31 ± 3.66 ^{d}	245.12 ± 8.60 ^{e}	2.57 ± 0.24 ^{g}	1.37 ± 0.07 ^{gh}	93.83 ± 0.11 ^{j}	80.23 ± 0.15 ^{ef}	73.75 ± 0.69 ^{j}	84.00 ± 0.56 ^{i}
	Juice	47.20 ± 0.49 ^{b}	48.60 ± 1.20 ^{d}	8.60 ± 0.09 ^{c}	14.21 ± 1.54 ^{bc}	86.58 ± 0.00 ^{b}	60.55 ± 1.14 ^{a}	48.50 ± 1.65 ^{fg}	55.60 ± 0.59 ^{ef}
	Pomace	12.81 ± 0.54 ^{a}	10.40 ± 0.03 ^{a}	9.50 ± 0.61 ^{c}	11.32 ± 0.13 ^{cd}	86.66 ± 0.06 ^{b}	68.30 ± 0.31 ^{c}	27.82 ± 0.30 ^{a}	28.84 ± 2.02 ^{ab}
	Fruit	37.63 ± 2.46 ^{ab}	33.34 ± 1.52 ^{c}	12.74 ± 0.28 ^{a}	3.92 ± 0.22 ^{fgh}	86.80 ± 0.02 ^{bc}	64.78 ± 0.29 ^{bc}	36.17 ± 0.36 ^{cd}	37.35 ± 2.09 ^{c}
Blackcurrant	SFJP	373.51 ± 10.89 ^{e}	289.22 ± 9.45 ^{g}	< 0.05 ^{h}	0.74 ± 0.01 ^{h}	93.14 ± 0.12 ^{i}	80.89 ± 0.14 ^{ef}	83.16 ± 0.63 ^{k}	91.63 ± 0.65 ^{j}
	Juice	23.21 ± 0.50 ^{ab}	19.25 ± 0.43 ^{abc}	< 0.05 ^{h}	5.75 ± 0.15 ^{ef}	87.12 ± 0.09 ^{c}	66.73 ± 0.67 ^{bc}	41.88 ± 3.21 ^{de}	38.94 ± 0.82 ^{cd}
	Pomace	14.35 ± 0.95 ^{a}	11.72 ± 0.18 ^{ab}	< 0.05 ^{h}	8.99 ± 0.54 ^{de}	85.39 ± 0.24 ^{a}	63.41 ± 0.22 ^{ab}	29.20 ± 2.33 ^{ab}	24.84 ± 1.90 ^{a}
	Fruit	17.59 ± 0.36 ^{a}	15.32 ± 0.57 ^{ab}	5.54 ± 0.33 ^{ef}	16.66 ± 1.48 ^{b}	86.94 ± 0.10 ^{bc}	66.05 ± 0.15 ^{bc}	34.34 ± 0.90 ^{bc}	30.02 ± 0.17 ^{b}
Haskap berry	SFJP	273.27 ± 13.08 ^{c}	269.10 ± 1.36 ^{f}	1.09 ± 0.23 ^{h}	0.29 ± 0.14 ^{h}	94.99 ± 0.06 ^{l}	88.63 ± 0.48 ^{g}	89.07 ± 0.04 ^{l}	93.92 ± 0.03 ^{j}
	Juice	23.47 ± 0.16 ^{ab}	22.53 ± 0.85 ^{abc}	8.79 ± 0.60 ^{c}	5.35 ± 0.15 ^{efg}	91.38 ± 0.19 ^{f}	81.20 ± 0.49 ^{f}	57.77 ± 1.91 ^{hi}	62.75 ± 0.28 ^{gh}
	Pomace	22.92 ± 0.88 ^{ab}	21.64 ± 0.24 ^{abc}	5.77 ± 0.22 ^{ef}	31.54 ± 3.23 ^{a}	90.81 ± 0.06 ^{e}	79.65 ± 0.34 ^{ef}	45.63 ± 2.57 ^{ef}	54.05 ± 1.17 ^{e}
	Fruit	25.91 ± 0.40 ^{ab}	25.3 ± 0.00 ^{abc}	6.46 ± 0.28 ^{de}	5.57 ± 0.18 ^{efg}	90.82 ± 0.11 ^{e}	79.69 ± 2.84 ^{ef}	54.74 ± 0.36 ^{hi}	59.31 ± 0.64 ^{fg}

SFJP – Sugar-free juice products; TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; BSA – Bovine Serum Albumin; MGO – Methylglyoxal; IC_{50} – Sample concentration for 50% reduction of enzyme activity; a, b, c, … – different letters within groups standing for different fruit fractions i.e., Sugar-free juice products, Juice, Pomace, Fruit indicate significant differences (ANOVA, HSD Tukey, $p \geq 0.05$)

α-Amylase and α-glucosidase inhibiting activity

The ability of the powders to inhibit α-amylase and α-glucosidase activities, varied depending on the type and fraction of the fruit. The IC_{50} values of the powders for inhibiting α- amylase activity ranged from < 0.05 to 12.5 mg/mL, and were the strongest for blackcurrant pomace powder, juice, and sugar-free juice product, while the rosehip fruit powder exhibited the weakest (**Table 6**). No recurring pattern was observed in terms of creation of α-amylase inhibition potential by type of fraction, as was observed in the case of α-glucosidase inhibiting activity for blackcurrant and Japanese quince products (described below). Previously Turkiewicz et al. (2021) examined α-amylase activity inhibition in Japanese quince (poly)phenolic products, however, in the current study a comparison of antidiabetic activity of different fruit fractions was made for the first time. Sugar-free juice product powders had the strongest α-amylase and α-glucosidase activity inhibition, which could be ascribed to the highest content of (poly)phenolics (**Figure 9**). A possible mechanism of action may be connected with a complex formation between (poly)phenolics and enzymes by means of non-covalent interactions such as van der Waals forces or hydrogen bonding (Papoutsis et al., 2021). When α-glucosidase inhibiting activity was examined both, the strongest and the weakest ability was noted for the haskap berry products, attributed to sugar-free juice product and pomace powders with IC_{50} values of 0.29 mg/mL and 31.54 mg/mL, respectively, while juice and whole fruit powders showed ability at a comparable level of 5 mg/mL. In blackcurrant and Japanese quince products, α-glucosidase inhibition ability decreased in the following order: sugar-free juice product > juice > pomace > whole fruit. However, for rosehip powders, the reverse trend was observed, with the exception of sugar-free juice product.

All blackcurrant products exhibited a stronger ability to inhibit α-amylase than α- glucosidase. Three out of four powders showed the strongest inhibitory effect against α- amylase (IC_{50} < 0.05 mg/mL), while for fruit this ability was weaker. On the contrary to α- glucosidase (r > 0.72 in the case of each (poly)phenolic group, and r = 0.63 for polymeric procyanidins), no significant correlation was found between α-amylase inhibiting activity and each specific group of (poly)phenolics in blackcurrant powders (**Figure 15**). This could be attributed to the complex and synergistic effects of other compounds present in blackcurrant powders, including, among others, water-soluble fiber (mainly pectins), some vitamins or minerals, as well as sugars and organic acids that can be expected to be present in the extracts analyzed. For instance, Xu et al. (2018) found that hypoglycemic activities can also come from basic components, i.e., polysaccharides. A divergent relationship was observed for haskap berry

products with respect to their ability to inhibit α-amylase activity, where each (poly)phenolic group appears to have a relatively significant effect ($r > 0.73$), with a strong correlation for procyanidins ($r = 0.99$) and anthocyanins ($r = 0.92$) (**Figure 16**). These powders showed a stronger ability to inhibit α-glucosidase, with the exception of pomace products, for which α- glucosidase inhibiting activity was over five times weaker than α-amylase inhibiting activity. It was previously shown that anthocyanin-rich bilberry extract exhibited α-amylase and α- glucosidase inhibiting activities by changing the structure of the enzyme, thereby reducing its stability (Ji et al., 2021). These two groups did not show such a strong effect on α-glucosidase inhibiting activity ($r = 0.12$ for procyanidins; $r = 0.48$ for anthocyanins) for haskap berry products (**Figure 16**). One likely reason may be the selectivity of action of individual (poly)phenolic compounds in a given model system applied to mimic particular antiglication stage. Zhu et al. (2020) pointed out that flavonols exhibit selective inhibition performance against enzymes depending on their origin. Different (poly)phenolic profiles depending on the fruit raw material used, which also takes into account differences in the structure of individual compounds, including anthocyanins and procyanidins, may result in contrasting mechanisms of action on a particular enzyme (Papoutsis et al., 2021). A slightly different relationship was found from that of the berry products for the Japanese quince. Powders obtained from this whole-fruit fraction exerted approximately three times greater α-amylase than α-glucosidase inhibiting activity, while for powders from other fractions ability to inhibit both enzymes was similar. Strong links were demonstrated between each specific (poly)phenolic group and both activities tested (**Figure 13**). It may be assumed that the matrix of this pome fruit was much more conducive to interactions between compounds and both enzymes compared to the matrices of the discussed berry fruit. Diez-Sánchez et al. (2021) showed that the bioaccessibility of blackcurrant (poly)phenolics from pomace was better than from (poly)phenolics pomace preparations depending on the type of matrix used (model systems: water; wheat starch; olive oil; whey protein; all ingredients). A single-nutrient-based matrix favored bioaccessibility compared to an all-nutrient matrix, with the greatest effect found for protein-based matrix composition. It was indicated that the matrix interacted with (poly)phenolics and thus determined their ability to undergo subsequent reactions. For rosehip products, sugar-free juice product and whole-fruit powders showed higher α-glucosidase inhibiting potential. However, for the juice and pomace powders, the effect was the opposite. Stronger relationships were found between the identified (poly)phenolic groups and α-amylase than α-glucosidase inhibiting activity, excluding flavonols, for which no correlation was found (**Figure 14**) that could be due to their trace amounts in the samples (**Figure 9c**).

All (poly)phenolic groups found in blackcurrant products were responsible for α- glucosidase inhibiting activity, comparably. Anthocyanins and procyanidins found in haskap berry powders were strongly correlated with α-amylase inhibiting activity, while phenolic acids affected more intensely the ability to inhibit α-glucosidase. In contrast, for rosehip powders, phenolic acids, flavan-3-ols, and procyanidins have a strong effect on inhibiting α-amylase activity. Finally, Japanese quince products were found to be comparatively effective in suppressing both α-amylase and α-glucosidase activities. Nowicka et al. (2016) proved that for *Prunus* products α-amylase inhibiting activity was affected by the anthocyanins and flavonols content, while the ability to inhibit α-glucosidase activity was created by flavan-3-ols. However, taking into account observed differences between effects of specific (poly)phenolic groups on the enzymatic activity tested, it should be emphasized that the composition of the matrix is crucial. Therefore, when creating fruit products with programmed antidiabetic potential, their (poly)phenolic-hypoglycemic property relationship should be considered in broader context, including full matrix composition.

No significant correlation was found between the two antidiabetic activities tested (**Figure 12**), indicating dependence on different constituents. Differences in demonstrated properties between powders may also be due to the complexity of the process used for their preparation, as the proportions and profile of constituents present in the matrix that exhibit favorable activities can be changed (Papoutsis et al., 2021). For example, obtaining whole-fruit powder involved merely fruit freeze-drying and grinding into powder, while preparation of the (poly)phenolic-rich extract product from juice included a number of steps, each of which carried further modification of the composition and thus the resulting properties.

The probable antidiabetic effect of fruit powders can also be ascribed to some specific amino acids (**Table 5**). Poovitha & Parani (2016) reported satisfactory *in vitro* α-amylase and α-glucosidase inhibition effects for bitter melon protein extract. Although no specific mechanism of action was proposed, the proteolytic activity of the extract toward these two enzymes was excluded. Despite the fact that plant-based proteins and peptides are abundantly described in the literature as molecules with significant antidiabetic potential unfortunately, there is little data on the hypoglycemic effect of individual amino acids, especially when it comes to dried fruit products (Patil et al., 2020). In general, considering all types of fruits and their fractions, no significant correlations were found between the sum of amino acids and the activities tested, but when considering each amino acid separately, the definitely distinctive among others was the negative correlation between glycine and α-glucosidase inhibiting activity ($r = -0.77$) (**Figure 12**). The accelerating effect of glycine on xylanase activity has

previously been reported; however, this is the first time such an effect of this amino acid has been reported on α-glucosidase activity (Deguchi & Koumoto, 2011). Due to the different content of individual amino acids in powders from selected fruits, it would be misleading to assign a noted relationship to each matrix. Therefore, to fully comprehend the influence of the fruit raw material matrix on the effects of individual amino acids on enzyme activity, it is necessary to consider them on the basis of fruit-specific raw material. Consistently, in the case of haskap berry, a high negative correlation was reported between ability to inhibit α- glucosidase activity and glycine content, although it was not the predominant amino acid (**Figure 16**). Similar observation was made for rosehip products (**Figure 14**). For Japanese quince powders, negative correlation was noted for glycine and α-amylase inhibiting activity ($r = -0.75$) (**Figure 13**), while for blackcurrant products no significant relationship was found between this amino acid content and the two antidiabetic activities tested (**Figure 15**). Some amino acids such as lysine, phenylalanine, or tryptophan were also found to show positive correlations with α-amylase/α-glucosidase or both inhibiting activities, depending on the fruit raw material (**Figure 13-16**). Ambiguous effects of selected amino acids, e.g., arginine, proline, tyrosine, on antidiabetic properties have also been observed. This strongly suggests selectivity of the action of a given amino acid on the enzymes tested depending on the fruit matrix.

Antiglycation properties

The powders analyzed were able to inhibit the formation of advanced glycation end products (AGEs) at a level greater than 60% for the BSA-glucose model and greater than 85% for the BSA-fructose model (**Table 6**). The strongest ability exerted sugar-free juice product powders and in the BSA-glucose system, these products obtained from haskap berry and Japanese quince proved to be stronger antiglycation agents than aminoguanidine (10 mM) applied as positive control, while, in the BSA-fructose system, only powders of whole fruit, juice and pomace fractions prepared from blackcurrant and rosehip raw material appeared to be weaker than aminoguanidine. Previously, greater antiglycation ability was found for three individual flavonoids (luteolin, quercetin, rutin) among ten tested, when compared to aminoguanidine (Wu & Yen, 2005). Considering that tested matrices are a powder-concentrated source of various (poly)phenolics and also other components such as polysaccharides which may exhibit strong antiglycation activity, their amplified antiglycation effect may be due to their joint actions (Xu et al., 2016). This confirmed that for the multicomponent matrix,

biological effects should be linked to composition and interactions between different components rather than to the action of individual compounds.

The second model system used (BSA-MGO) due to the negligence of sugar conversion to carbonyls, aimed to evaluate the middle step of the glycation reaction (Wang et al., 2011). The strongest ability to inhibit AGEs was reported for sugar-free juice products (74 − 91%), while pomace powders exhibited the lowest values (28 − 46%) (**Table 6**). A parallel trend was observed for the MGO-*L*-arginine model, which evaluates the specific reaction between MGO and the guanidinium group of arginine (Wang et al., 2011). A strong positive correlation was found between the results obtained from the BSA-MGO and MGO-*L*-arginine systems and the flavonol content (**Figure 12**). Previously, it was found that myricetin was efficient in trapping MGO and producing mono- and di-MGO adducts under *in vitro* and mono-MGO adducts under *in vivo* conditions (Zhang et al., 2020). It can be assumed that this group of (poly)phenolics is mainly responsible for the antiglycation capacity of the powders tested. When amino acid composition was concerned, for the same model systems high positive dependencies were found for lysine (r = 0.87 and 0.83, respectively) and ornithine (r = 0.84 and 0.79, respectively) (**Figure 12**). Although for lysine its propensity to participate in the glycation reaction has been confirmed, little is known so far about ornithine in this regard (Jia et al., 2022). Zhu & Yaylayan (2017) proved that the main product of arginine degradation is ornithine. It can be speculated that, since arginine is one of the most prone amino acids to glycation, the product of its transformation, ornithine, may exhibit a similar behavior.

Strong positive correlations were found between the BSA-MGO and MGO-*L*-arginine systems and the antioxidant capacity measured by TEAC ABTS and FRAP assays (r > 0.84) (**Figure 12**). This is consistent with the previous statement that free radicals acting as reactive intermediates may contribute to AGEs formation (Jia et al., 2022). Thus, the scavenging ability of powders against free radicals may also drive their antiglycation ability.

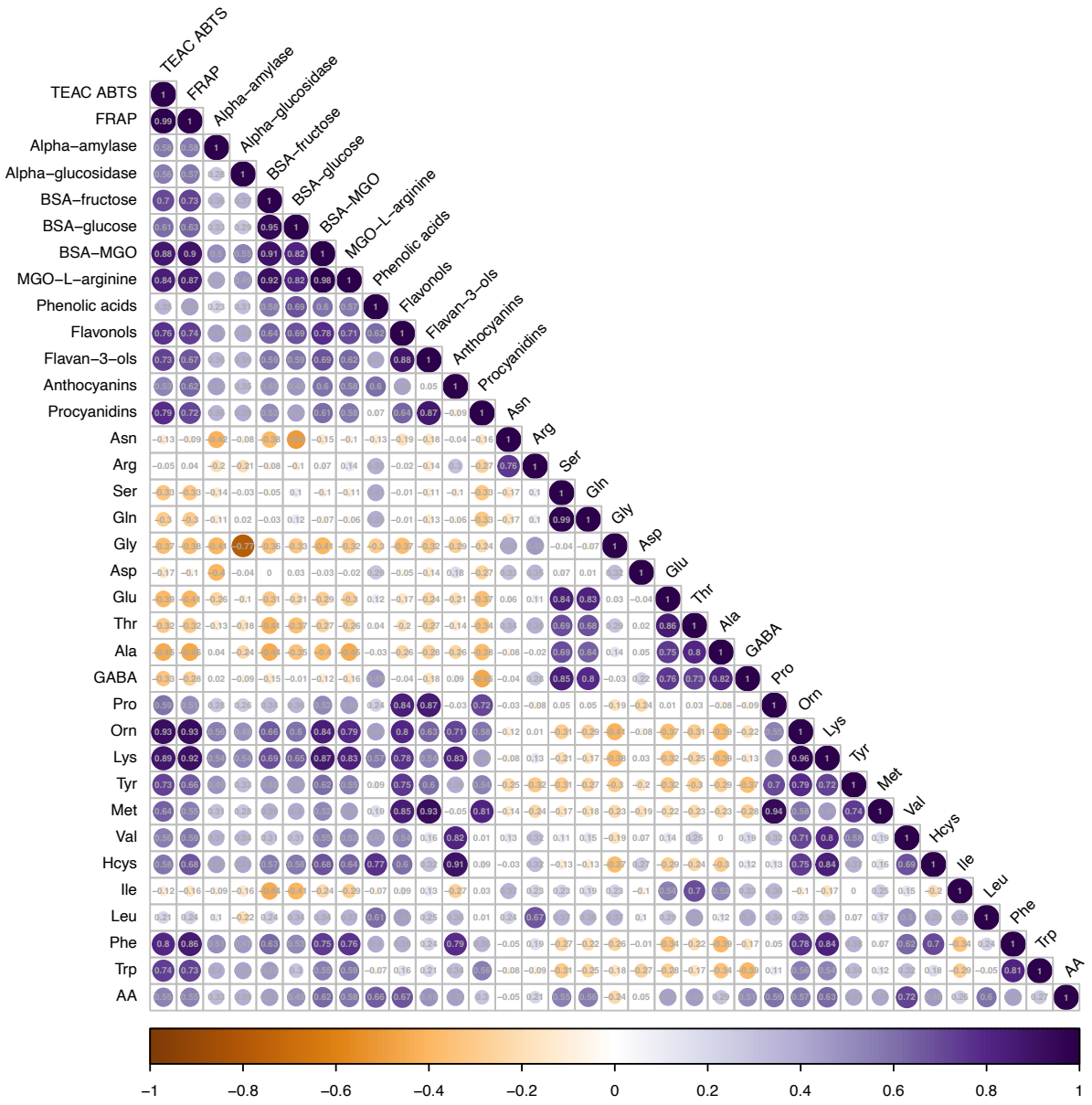


Figure 12. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antidiabetic/antiglycation activity, identified (poly)phenolic groups, amino acids) in freeze-dried fruit powders (**whole** data set). Positive and negative correlations are indicated in purple and orange, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; Alpha-amylase – inhibition of α-amylase activity; Alpha-glucosidase – inhibition of α-glucosidase activity; BSA – Bovine Serum Albumin; MGO – Methylglyoxal; Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly – Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Thr – Threonine; Ala – Alanine; GABA – γ- Aminobutyric acid; Pro – Proline; Orn – Ornithine; Lys – Lysine; Tyr – Tyrosine; Met – Methionine; Val – Valine; Hcys – Homocysteine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; AA – Sum of amino acids

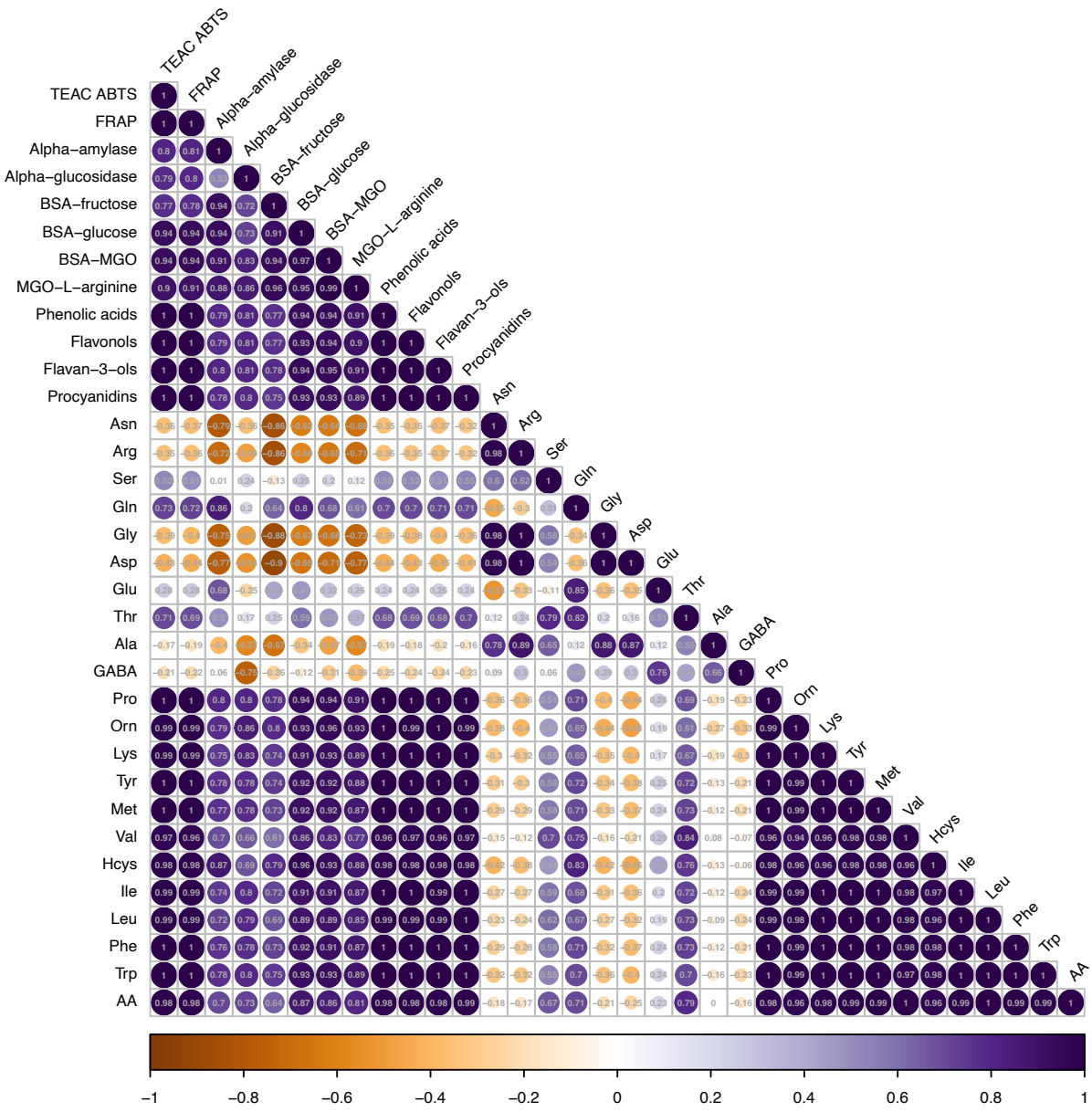


Figure 13. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antidiabetic/antiglycation activity, identified (poly)phenolic groups, amino acids) in freeze-dried fruit powders (**Japanese quince** samples data set). Positive and negative correlations are indicated in purple and orange, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; Alpha-amylase – inhibition of α-amylase activity; Alpha-glucosidase – inhibition of α- glucosidase activity; BSA – Bovine Serum Albumin; MGO – Methylglyoxal; Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly – Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Thr – Threonine; Ala – Alanine; GABA – γ- Aminobutyric acid; Pro – Proline; Orn – Ornithine; Lys – Lysine; Tyr – Tyrosine; Met – Methionine; Val – Valine; Hcys – Homocysteine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; AA – Sum of amino acids

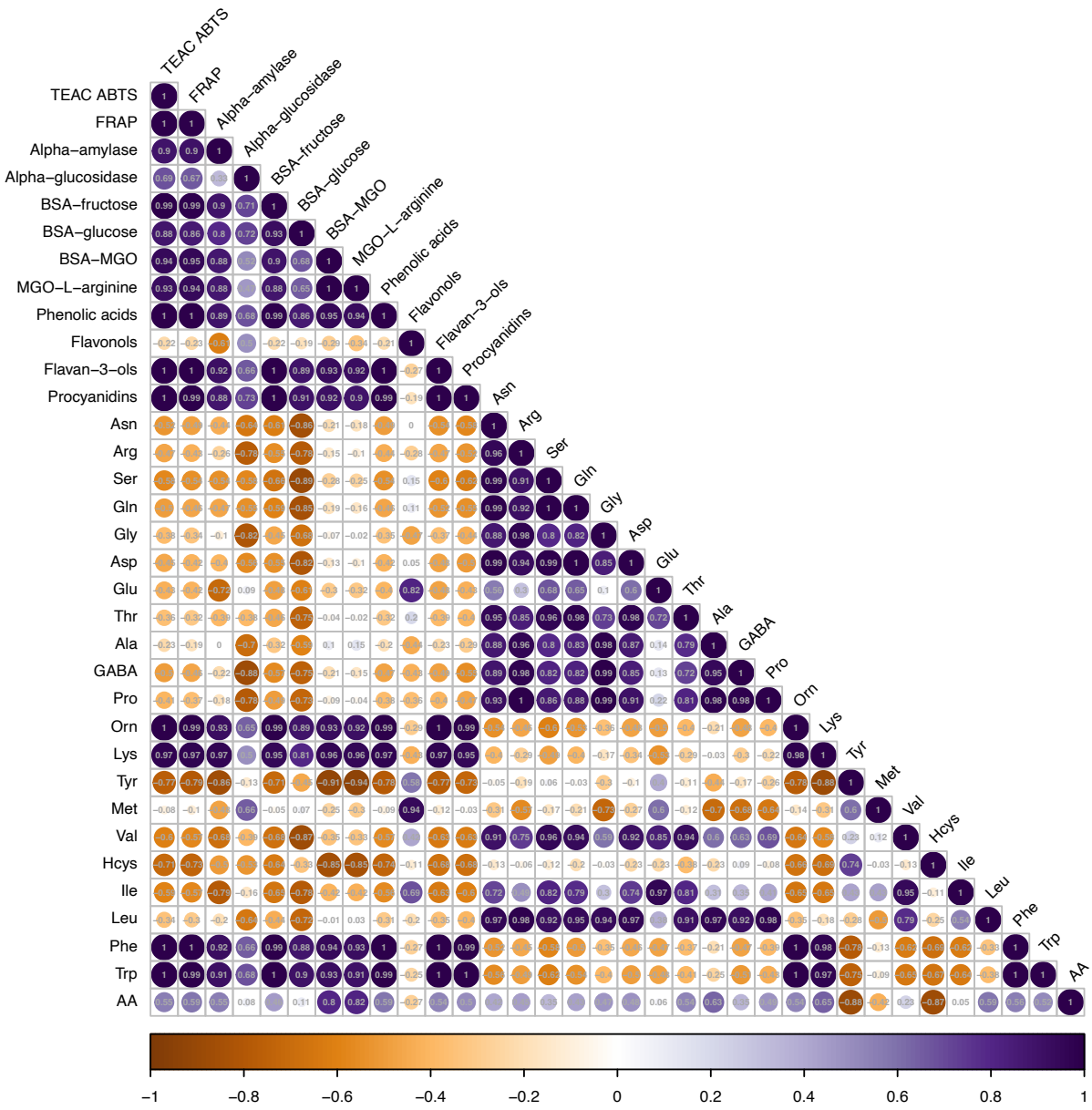


Figure 14. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antidiabetic/antiglycation activity, identified (poly)phenolic groups, amino acids) in freeze-dried fruit powders (**rosehip** samples data set). Positive and negative correlations are indicated in purple and orange, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; Alpha-amylase – inhibition of α-amylase activity; Alpha-glucosidase – inhibition of α- glucosidase activity; BSA – Bovine Serum Albumin; MGO – Methylglyoxal; Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly – Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Thr – Threonine; Ala – Alanine; GABA – γ-Aminobutyric acid; Pro – Proline; Orn – Ornithine; Lys – Lysine; Tyr – Tyrosine; Met – Methionine; Val – Valine; Hcys – Homocysteine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; AA – Sum of amino acids

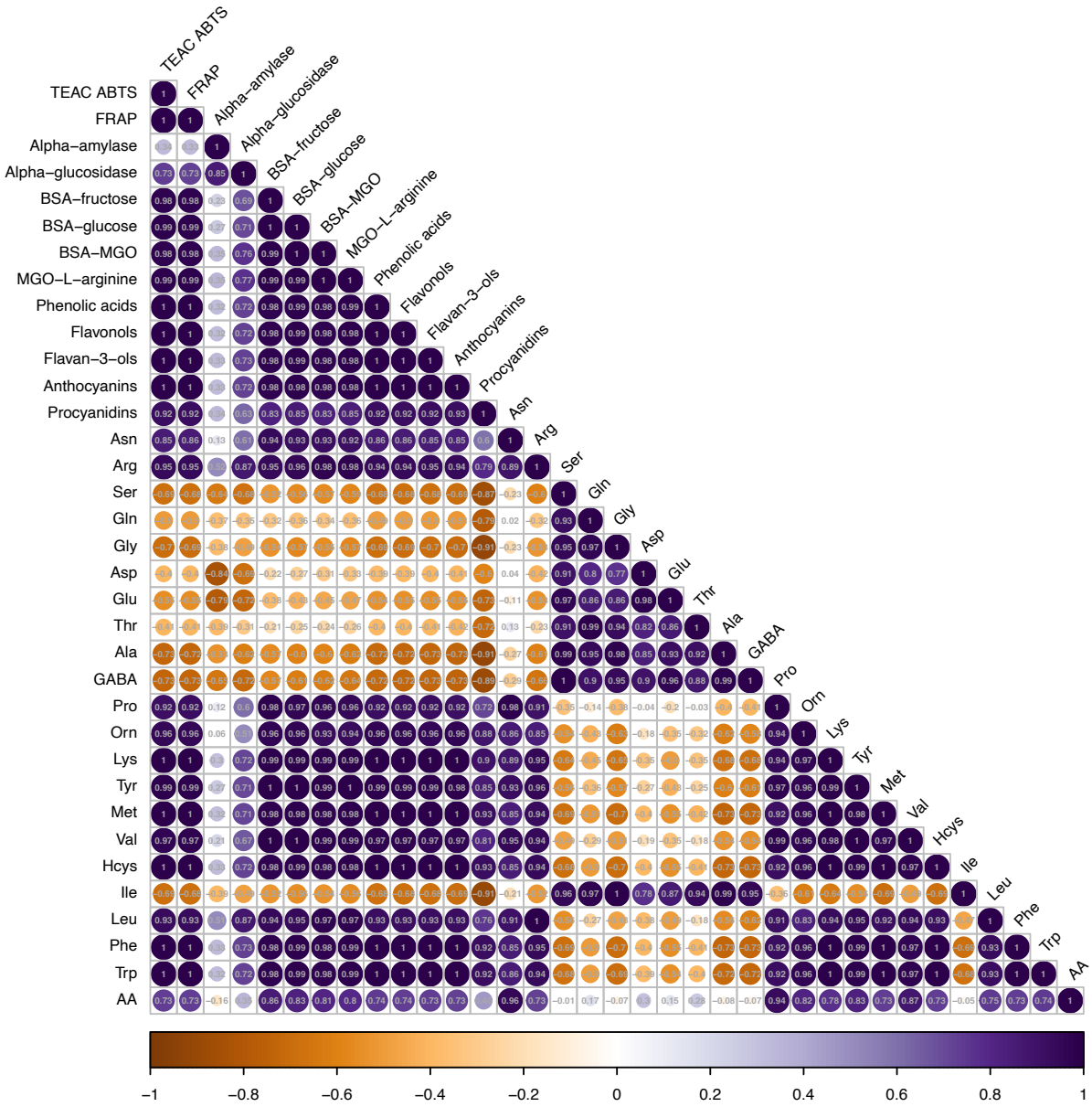


Figure 15. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antidiabetic/antiglycation activity, identified (poly)phenolic groups, amino acids) in freeze-dried fruit powders (**blackcurrant** samples data set). Positive and negative correlations are indicated in purple and orange, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; Alpha-amylase – inhibition of α-amylase activity; Alpha-glucosidase – inhibition of α- glucosidase activity; BSA – Bovine Serum Albumin; MGO – Methylglyoxal; Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly – Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Thr – Threonine; Ala – Alanine; GABA – γ-Aminobutyric acid; Pro – Proline; Orn – Ornithine; Lys – Lysine; Tyr – Tyrosine; Met – Methionine; Val – Valine; Hcys – Homocysteine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; AA – Sum of amino acids

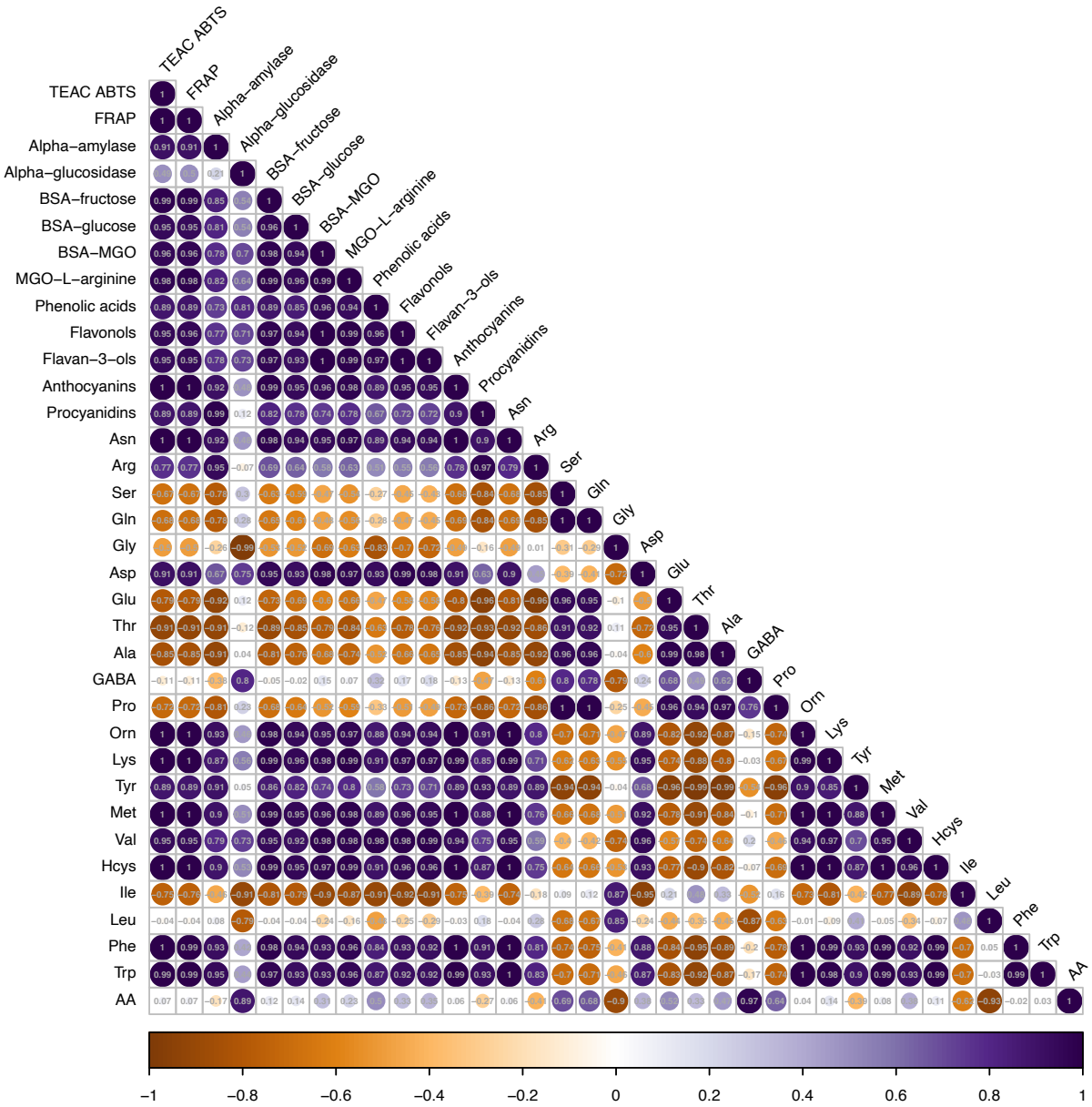


Figure 16. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antidiabetic/antiglycation activity, identified (poly)phenolic groups, amino acids) in freeze-dried fruit powders (**haskap berry** samples data set). Positive and negative correlations are indicated in purple and orange, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; Alpha-amylase – inhibition of α-amylase activity; Alpha-glucosidase – inhibition of α- glucosidase activity; BSA – Bovine Serum Albumin; MGO – Methylglyoxal; Asn – Asparagine; Arg – Arginine; Ser – Serine; Gln – Glutamine; Gly – Glycine; Asp – Aspartic acid; Glu – Glutamic acid; Thr – Threonine; Ala – Alanine; GABA – γ-Aminobutyric acid; Pro – Proline; Orn – Ornithine; Lys – Lysine; Tyr – Tyrosine; Met – Methionine; Val – Valine; Hcys – Homocysteine; Ile – Isoleucine; Leu – Leucine; Phe – Phenylalanine; Trp – Tryptophan; AA – Sum of amino acids

Summary

The contribution of the amino acid profile to the antioxidant capacity was demonstrated, with a strong indication of ornithine. Sugar-free juice products exhibited the strongest antiglycation properties, regardless of the type of fruit, which was linked to (poly)phenolics. Similar observation was made for the antidiabetic effect against α-glucosidase activity, while in case of α-amylase, additionally juice and pomace powders from blackcurrant exerted comparable ability. The differences in matrix composition between fruits tested were found to be responsible for the ambiguous behavior of the same (poly)phenolic groups towards α- amylase and α-glucosidase activity. A strong negative relationship was noted between the glycine content and α-glucosidase inhibiting activity. Flavonols played major role in inhibitory capacity against the formation of MGO- and *L*-arginine-induced advanced glycation end products. Strong positive correlations between antiglycation properties measured in BSA-MGO and MGO-*L*-arginine models and lysine and ornithine were explained by their susceptibility to glycation and thus promote the generation of AGEs, which in turn can potentially be inhibited by (poly)phenolics present in the same extract. The scavenging activity of the freeze-dried fruit powders against free radicals created their ability to inhibit the formation of AGEs *in vitro*.

Recommendation: The study provided insights about the components' influence on selected pro-health properties of freeze-dried powders depending on the fruit matrix origin and fraction that allowed preliminary to recognize the occurring tendencies. However, to fully comprehend the mechanisms that drive these dependencies, further research should include model fruit composition systems to specify the individual constituents responsible for the possible properties exerted by the resulting powders considering as a complex matrix.

5.2.2. Supplementary study 3: *Fruit juice model systems*

Given the importance of food safety, the formation of process contaminants is a critical issue that is often overlooked in the case of powdered plant-based products. Since the botanicals constitute a highly complex matrices, trapping the source of the transformations leading to the formation of these unwanted compounds poses a challenge. Although limited by their partial representation of the real food matrix, model systems make it possible to identify certain interrelationships, which are often fundamental for reactions to occur and which are almost impossible to directly identify in the complex real matrix. Therefore, the **Supplementary study 3** aimed at the recognition of the matrix-originated drivers triggering the formation of process contaminants under particular processing conditions by employing model systems resembling simplified composition of selected fruit juices.

Processing may induce formation of Maillard reaction and caramelization products in plant-based merchandises (Del Castillo et al., 1999; Sanz et al., 2000; Wellner et al., 2011). In the literature, some attempts have been made to recognize the key compounds and mechanisms responsible for their occurrence (Kavousi et al., 2015; Aktağ & Gökmen, 2021), however still little is known about their formation in the plant-based matrices (Aktağ & Gökmen, 2020). Moreover, to date, most of the studies that aimed at tracking the transformations leading to the formation of process contaminants have involved models consisting of major sugars and amino acids, as they are the main substrates in those aforementioned reactions (Agcam, 2022), especially in dairy or cereal products. Fruit juices, in turn, are relatively complex systems rich in numerous constituents such as prevailing sugars and organic acids, as well as vitamins, minerals, antioxidants, etc. in which the medium is much more conducive to interactions between the components of the matrix (Rodríguez-Roque et al., 2015) compared to solid or semi-solid formulations. Therefore, in the study, models resembling the Japanese quince and blackcurrant juices were composed of dominant sugar and organic acid. Since the powders, are the target food matrix, such prepared fruit-juice based mediums were mixed with selected carrier agents and subsequently dehydrated using different drying techniques in presence or absence of ascorbic acid (important factor for the formation of these process contaminants) (Kurata & Sakurai, 1967; Michalska, Wojdyło, Łysiak, & Figiel, 2017). The models developed in this way served as a simplified matrix for the powders, allowing the possible formation of hydroxymethyl-*L*-furfural and furfural to be tracked with inclusion of carrier effect. The powders obtained from model systems of Japanese quince and blackcurrant juices were studied in terms of ascorbic acid, furfural, and hydroxymethyl-*L*-furfural content (**Table 7**).

Table 7. The ascorbic acid, furfural and hydroxymethyl-*L*-furfural in powders obtained from Japanese quince and blackcurrant juices.

Model type	Drying technique	Carrier agent	Japanese quince juice model powders			Blackcurrant juice model powders		
			Ascorbic acid content	Furfural content	Hydroxymethyl-*L*-furfural content	Ascorbic acid content	Furfural content	Hydroxymethyl-*L*-furfural content
			[µg/mg dm]					
No vitamin C	Freeze-drying	Maltodextrin	ND	ND	ND	ND	ND	ND
		Inulin	ND	ND	ND	ND	ND	ND
		Trehalose	ND	ND	ND	ND	ND	ND
	Vacuum drying at 60 °C	Maltodextrin	ND	ND	0.24 ± 0.00 ^{ab}	ND	ND	0.25 ± 0.00 ^{ab}
		Inulin	ND	ND	0.92 ± 0.01 ^{c}	ND	ND	0.76 ± 0.02 ^{c}
		Trehalose	ND	ND	0.22 ± 0.00 ^{ab}	ND	ND	0.39 ± 0.00 ^{b}
	Vacuum drying at 90 °C	Maltodextrin	ND	0.41 ± 0.01 ^{d}	9.48 ± 0.02 ^{f}	ND	0.37 ± 0.01 ^{b}	10.03 ± 0.14 ^{d}
		Inulin	ND	0.35 ± 0.01 ^{c}	19.38 ± 0.44 ^{i}	ND	0.25 ± 0.01 ^{a}	14.07 ± 0.21 ^{g}
		Trehalose	ND	0.15 ± 0.02 ^{a}	5.96 ± 0.28 ^{e}	ND	0.46 ± 0.00 ^{c}	11.86 ± 0.06 ^{f}
	Spray drying	Maltodextrin	ND	ND	0.04 ± 0.00 ^{a}	ND	ND	ND
		Inulin	ND	ND	0.05 ± 0.00 ^{a}	ND	ND	0.07 ± 0.00 ^{ab}
		Trehalose	ND	ND	0.04 ± 0.00 ^{a}	ND	ND	0.07 ± 0.00 ^{ab}
No vitamin C	Freeze-drying	Maltodextrin	1.11 ± 0.03 ^{gh}	ND	ND	3.47 ± 0.04 ^{bc}	ND	ND
		Inulin	1.10 ± 0.00 ^{fgh}	ND	ND	3.32 ± 0.04 ^{bc}	ND	ND
		Trehalose	1.14 ± 0.01 ^{h}	ND	ND	3.51 ± 0.01 ^{c}	ND	ND
	Vacuum drying at 60 °C	Maltodextrin	0.98 ± 0.01 ^{e}	ND	0.17 ± 0.01 ^{ab}	3.34 ± 0.04 ^{bc}	ND	0.21 ± 0.01 ^{ab}
		Inulin	0.16 ± 0.02 ^{b}	ND	0.65 ± 0.01 ^{bc}	ND	ND	1.05 ± 0.02 ^{c}
		Trehalose	0.84 ± 0.01 ^{d}	ND	0.19 ± 0.01 ^{ab}	3.39 ± 0.05 ^{bc}	ND	0.29 ± 0.00 ^{ab}
	Vacuum drying at 90 °C	Maltodextrin	0.36 ± 0.00 ^{c}	0.41 ± 0.00 ^{d}	10.35 ± 0.11 ^{g}	2.70 ± 0.05 ^{a}	0.38 ± 0.02 ^{b}	9.82 ± 0.11 ^{d}
		Inulin	0.06 ± 0.02 ^{a}	0.21 ± 0.03 ^{b}	13.40 ± 0.03 ^{h}	ND	0.43 ± 0.04 ^{c}	14.08 ± 0.28 ^{g}
		Trehalose	0.14 ± 0.01 ^{b}	0.13 ± 0.01 ^{a}	5.23 ± 0.32 ^{d}	2.55 ± 0.02 ^{a}	0.36 ± 0.00 ^{b}	10.75 ± 0.10 ^{e}
	Spray drying	Maltodextrin	1.06 ± 0.01 ^{f}	ND	0.05 ± 0.00 ^{a}	3.47 ± 0.03 ^{bc}	ND	ND
		Inulin	1.08 ± 0.02 ^{fg}	ND	0.08 ± 0.00 ^{a}	3.33 ± 0.03 ^{bc}	ND	0.17 ± 0.00 ^{ab}
		Trehalose	1.07 ± 0.00 ^{fg}	ND	0.03 ± 0.00 ^{a}	3.10 ± 0.43 ^{b}	ND	0.05 ± 0.01 ^{a}

ND – not detected; a, b, c, … - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test).

In the case of Japanese quince model, the analysis of **ascorbic acid** confirmed its presence in all model powders to which this compound was added before drying (**Table 7**). The type of carrier had no influence on the retention of ascorbic acid in the case of freeze- and spray drying. Vacuum drying at 60 °C and 90 °C led to its degradation that was strongly dependent of the carrier type used. In both cases, addition of inulin resulted in the lowest retention of this constituent during drying. It has previously been noted that inulin can be hydrolyzed under acidic conditions, resulting in decomposition into short chain oligofructose units, fructose monomers and to a lesser extent glucose monomers (Jackson et al., 2022), which constitute substrates for selected process contaminants formation.

In the study, the formation of **hydroxymethyl-*L*-furfural** was confirmed only in vacuum dried samples. This proved that fructose and malic acid (presence or absence of ascorbic acid) did not solely play a part in HMF formation during freeze-drying of plant-based products (Fitzpatrick et al., 2013; **Brzezowska** et al., 2023). Application of vacuum drying resulted in the formation of HMF that was the highest in the case of vacuum drying at 90 °C (**Table 7**). Interestingly, carrier type had a strong impact on its formation as inulin added powders had the highest content of HMF that was almost 3.5 times higher for VD at 60 °C and 2.7 times higher for VD at 90 °C when compared to average value for maltodextrin and trehalose, respectively. Surprisingly, in this model system, no effect of ascorbic acid addition was noted in terms of HMF formation. Another analyzed compound in model powders was **furfural**. Its presence was confirmed only in products gained after vacuum drying at 90 °C. In this case, formation of furfural was in the following order: maltodextrin> inulin> trehalose. As the ascorbic acid degradation is a one path for furfural formation (Agcam, 2022) it was expected to observe such relationship in the models tested. However, as with HMF, ascorbic acid has not been shown to affect furfural formation. Therefore, it can be surmised, that vitamin C can trigger formation of these process contaminants in the presence of other components of fruit matrix, as it was shown in the case of cranberry product for which HMF formation was accelerated by the chlorogenic acid (Michalska et al., 2018).

In the case of blackcurrant juice model powders the freeze-drying, vacuum drying at 60 °C and spray drying techniques applied had a little influence on the ascorbic acid content when maltodextrin and trehalose was used for model powders production. It should be highlighted that usage of inulin for vacuum drying at 60 °C and 90 °C led to the degradation of this compound, while maltodextrin and trehalose ensured comparable protection during drying.

Similarly to Japanese quince model juice the presence of HMF was confirmed only in vacuum dried samples, being the highest when vacuum drying at 90 °C was used. Previously, Michalska, Wojdyło, Łysiak, Lech, et al. (2017) observed that for the blackcurrant pomace powders HMF formation increased slowly above 60 °C, while its rapid formation was noted when the temperature 80 °C and higher was applied. Moreover, also in this model, when inulin was present, the highest HMF was generated after vacuum drying. On the contrary to Nayaka et al. (2022) which proved that the ascorbic acid degradation contributed to hydroxymethyl-*L*-furfural formation in the guava fruit leather, no differences in HMF content were noted in samples with or without ascorbic acid addition.

For blackcurrant juice model powders the furfural presence was noted only after vacuum drying at 90 °C. No strict relationship could be established as, for example, powders with maltodextrin contained comparable levels of furfural regardless of the presence of ascorbic acid, whereas products with inulin contained significantly higher levels of this process contaminant when vitamin C was added. Interestingly, the highest value of FF content was reported for vacuum-dried powders at 90 °C with the addition of trehalose without ascorbic acid. Previously, as trehalose was claimed to be a non-reducing sugar incapable of Maillard reaction or caramelization, its contribution to process contaminants formation was excluded (Świąder et al., 2022). For this reason, further model research is required in order to test the behavior of this carrier agent under specific processing conditions.

Interestingly, comparing the process contaminants content in both models of selected fruit juices, it was observed, that despite the compositional differences, the furfural content was similar in powders from both models, but the HMF content was significantly higher in products made from the Japanese quince model juice. As the proportions of both glucose and ascorbic acid (and therefore the two most likely factors for HMF formation) were higher in the blackcurrant juice model, the only difference was in the origin of the organic acids. Unfortunately, there are no scientific reports that could confirm these relationships observed in the real matrix of fruit juices powders, therefore their continuation is of great importance to provide a key point for their formation.

Recommendation: Although no clear link has been found between ascorbic acid content and the formation of process contaminants, its influence should be taken into account, especially in complex plant matrices. Careful consideration should be given to the organic acid type present in the plant matrix being processed in terms of the possible excessive formation of hydroxymethyl-*L*-furfural.

5.3. Stage III: *Cross-factors affecting the biological properties of powdered plant products*

The final stage of the research (**Figure 17**) was devoted to the biological potential of the plant powders since currently, more and more stress is being placed on this aspect that dictates actual impact on human health and well-being. However, to date, little is known about the effects of the various processing steps on changes in the biological properties of plant-based powders during their production. Previously, attempts have been made to determine the impact of different processing conditions on the biological properties, including antibacterial and anti-inflammatory activities (Silvan, Michalska-Ciechanowska, et al., 2020). Nevertheless, the difficulties associated with the complexity of the plant matrix that undergoes drying, additionally magnified by plant origin, variability between cultivars within a given species, as well as specific chemical composition depending on fraction used make it impossible to develop a single, unified method of producing powders while maintaining their highest possible quality.

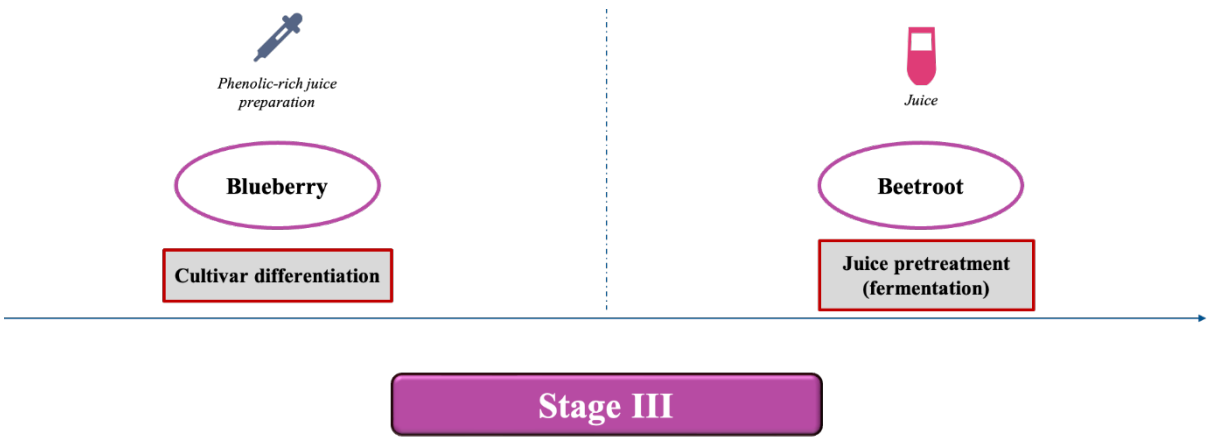


Figure 17. Schematic representation of the differentiating criteria of the matrix used for the study - stage III.

5.3.1. Supplementary study 4: *Blueberry juice preparation powders case study*

As the food matrix design was found to be a key factor in shaping the effectiveness of the antimicrobial agents in different foodstuff (Wang et al., 2023), the **Supplementary study 4** adapted this concept however, from the different perspective. Following the recent trends connected with green solutions for food preservation (Delshadi et al., 2021; Batiha et al., 2021) the present research shed a new light on that issue from the plant-based powders' point of view and stated that depending on the processing applied for (poly)phenolic-rich preparations (which may serve as an antimicrobial agents) it is possible to moderate their physico-chemical and thus biological properties of blueberry extract powders towards their improved functionality.

Therefore, the objective of the study was to evaluate how blueberry juice extract obtained from different cultivars, processed by freeze, vacuum and spray drying with or without inulin, can modify the physical attributes, (poly)phenolics composition, antioxidant capacity, antibacterial activity and anti-inflammatory properties in gastric cells.

Physical properties

The moisture content (*Mc*) of the blueberry extract powders ranged from 0.8 to 7.3% (**Table 8**). Drying technique differentiates the *Mc* to the highest extent. Samples after freeze-drying had approximately 2-times higher *Mc* compared to average values obtained using the other drying techniques, regardless of carrier addition (Silvan, Michalska-Ciechanowska, et al., 2020). Water activity (a_w) linked to the microbiological and chemical stability of food powders was the highest for products obtained by freeze-drying of cultivar 'Berkley' with and without inulin addition (**Table 8**). Similar to *Mc*, the drying technique had a stronger influence on a_w than carrier addition. Powders from 'Berkley' had the highest a_w when compared to the 'Bluejay' and 'Bluecrop' cultivars, regardless of carrier addition or the drying technique used. The inulin application resulted in powders with water activity reduced by approx. 33%, 14% and 8%, for 'Berkley', 'Bluecrop' and 'Bluejay' powders, respectively, compared to those without the carrier. No correlation between moisture content and water activity was observed.

Table 8. Moisture content (%), water activity (a_w), bulk density (g/cm^3), true density (g/cm^3), porosity (%) and color (CIE $L^*a^*b^*$) ($n = 2$; average ± standard deviation) of three cultivars of blueberry extract powders from different dried treatments with or without a carrier.

Cultivar	Drying technique	Moisture content	Water activity	Bulk density	True density	Porosity	L^*	a^*	b^*
				No carrier					
Bluejay	FD	5.24 ± 1.16 $^{d-h}$	0.210 ± 0.007 ^{jk}	0.163 ± 0.003 ^{ab}	1.13 ± 0.02 ^{ab}	85.47 ± 0.22 ^{pq}	31.36 ± 0.05 $^{b-g}$	10.43 ± 0.01 ^{hi}	0.71 ± 0.02 ^{hi}
	VD50	3.15 ± 0.23 $^{a-f}$	0.111 ± 0.007 ^{a}	0.663 ± 0.030 $^{i-k}$	1.27 ± 0.01 $^{d-f}$	47.26 ± 0.50 ^{bc}	30.06 ± 0.32 ^{a}	3.87 ± 0.24 $^{a-c}$	0.71 ± 0.02 ^{hi}
	VD70	2.23 ± 0.77 $^{a-e}$	0.130 ± 0.007 ^{ab}	0.706 ± 0.024 $^{j-l}$	1.33 ± 0.01 $^{f-j}$	46.61 ± 0.29 ^{ab}	30.47 ± 0.00 $^{a-d}$	3.24 ± 0.09 ^{ab}	0.65 ± 0.01 $^{g-i}$
	VD90	1.59 ± 0.07 $^{a-c}$	0.205 ± 0.007 ^{ij}	0.499 ± 0.016 ^{h}	1.34 ± 0.01 $^{f-k}$	62.38 ± 0.32 ^{j}	30.45 ± 0.04 $^{a-d}$	2.24 ± 0.01 ^{a}	0.84 ± 0.00 ^{i}
	SD	2.94 ± 0.19 $^{a-f}$	0.448 ± 0.007 ^{p}	0.280 ± 0.015 ^{de}	1.17 ± 0.02 ^{bc}	75.89 ± 0.40 ^{m}	30.09 ± 0.10 ^{ab}	14.83 ± 0.05 ^{lm}	1.62 ± 0.02 ^{k}
Berkley	FD	7.05 ± 0.97 ^{gh}	0.469 ± 0.007 ^{p}	0.117 ± 0.003 ^{a}	1.54 ± 0.04 ^{l}	92.35 ± 0.19 ^{s}	33.15 ± 0.01 $^{j-k}$	17.31 ± 0.06 ^{no}	1.26 ± 0.01 ^{j}
	VD50	3.80 ± 0.11 $^{a-f}$	0.146 ± 0.006 $^{b-d}$	0.690 ± 0.034 $^{j-l}$	1.35 ± 0.02 $^{g-k}$	48.42 ± 0.54 $^{c-e}$	31.86 ± 0.17 $^{e-h}$	5.98 ± 0.58 $^{d-f}$	0.18 ± 0.05 ^{cd}
	VD70	2.02 ± 0.71 $^{a-d}$	0.169 ± 0.006 $^{c-g}$	0.377 ± 0.011 ^{fg}	1.30 ± 0.02 $^{e-h}$	70.87 ± 0.45 ^{k}	31.56 ± 0.15 $^{d-g}$	4.34 ± 0.18 $^{b-d}$	0.23 ± 0.01 $^{c-e}$
	VD90	2.54 ± 1.26 $^{a-e}$	0.258 ± 0.006 ^{lm}	0.391 ± 0.012 ^{g}	1.37 ± 0.02 $^{g-k}$	71.29 ± 0.34 ^{k}	31.13 ± 0.10 $^{a-f}$	4.88 ± 0.15 $^{b-d}$	0.50 ± 0.01 ^{fg}
	SD	3.99 ± 1.92 $^{a-g}$	0.359 ± 0.007 ^{o}	0.204 ± 0.006 $^{b-d}$	1.14 ± 0.02 ^{b}	82.01 ± 0.33 ^{o}	32.58 ± 0.10 $^{g-i}$	20.08 ± 0.05 ^{p}	2.15 ± 0.00 ^{l}
Bluecrop	FD	7.33 ± 0.27 ^{h}	0.265 ± 0.007 ^{lm}	0.204 ± 0.004 $^{b-d}$	1.39 ± 0.02 $^{i-k}$	85.27 ± 0.26 ^{pq}	30.90 ± 0.72 $^{a-e}$	11.96 ± 0.87 ^{ij}	0.61 ± 0.05 ^{gh}
	VD50	3.19 ± 0.62 $^{a-f}$	0.166 ± 0.007 $^{c-f}$	0.682 ± 0.027 ^{jk}	1.39 ± 0.02 $^{i-k}$	50.63 ± 0.52 ^{fg}	30.13 ± 0.07 $^{a-c}$	5.31 ± 0.49 $^{c-e}$	0.64 ± 0.06 $^{g-i}$
	VD70	2.83 ± 0.30 $^{a-e}$	0.158 ± 0.007 $^{b-e}$	0.627 ± 0.023 ^{ij}	1.37 ± 0.02 $^{g-k}$	53.98 ± 0.49 ^{h}	30.74 ± 0.48 $^{a-e}$	4.25 ± 0.74 $^{b-d}$	0.47 ± 0.09 ^{fg}
	VD90	2.20 ± 0.08 $^{a-e}$	0.261 ± 0.007 ^{lm}	0.593 ± 0.021 ^{i}	1.38 ± 0.01 $^{h-k}$	56.62 ± 0.35 ^{i}	31.52 ± 0.14 $^{d-g}$	3.54 ± 0.47 $^{a-c}$	0.39 ± 0.02 ^{ef}
	SD	5.39 ± 0.58 $^{e-h}$	0.270 ± 0.007 ^{m}	0.262 ± 0.010 $^{c-e}$	1.23 ± 0.03 $^{c-e}$	78.53 ± 0.55 ^{n}	30.75 ± 0.02 $^{a-e}$	16.64 ± 0.25 ^{mn}	1.95 ± 0.04 ^{l}
				5% inulin					
Bluejay	FD	3.38 ± 0.99 $^{a-f}$	0.171 ± 0.000 $^{d-h}$	0.174 ± 0.006 ^{ab}	1.34 ± 0.02 $^{f-k}$	86.88 ± 0.19 ^{q}	33.20 ± 0.07 $^{i-k}$	14.29 ± 0.20 ^{kl}	-0.12 ± 0.05 ^{ab}
	VD50	4.35 ± 0.14 $^{c-h}$	0.193 ± 0.006 $^{f-j}$	0.739 ± 0.034 ^{kl}	1.41 ± 0.02 ^{jk}	47.18 ± 0.59 ^{bc}	30.88 ± 0.02 $^{a-e}$	7.60 ± 0.00 ^{fg}	0.12 ± 0.02 $^{c-d}$
	VD70	2.78 ± 0.29 $^{a-e}$	0.194 ± 0.006 $^{f-j}$	0.769 ± 0.027 ^{l}	1.41 ± 0.02 ^{jk}	45.19 ± 0.56 ^{a}	31.48 ± 0.18 $^{d-g}$	8.62 ± 0.31 ^{gh}	0.30 ± 0.05^{d-f}
	VD90	0.79 ± 0.35 ^{a}	0.213 ± 0.000 ^{jk}	0.693 ± 0.024 $^{j-l}$	1.39 ± 0.02 $^{i-k}$	49.87 ± 0.51 $^{e-g}$	31.36 ± 0.28 $^{c-g}$	7.59 ± 0.70 ^{fg}	0.26 ± 0.01 $^{c-e}$
	SD	3.86 ± 0.09 $^{a-g}$	0.249 ± 0.006 ^{lm}	0.298 ± 0.008 ^{ef}	1.19 ± 0.02 $^{b-d}$	74.80 ± 0.31 ^{lm}	31.64 ± 0.07 $^{d-g}$	17.33 ± 0.45 ^{no}	1.11 ± 0.04 ^{j}
Berkley	FD	7.07 ± 1.68 ^{gh}	0.326 ± 0.008 ^{n}	0.142 ± 0.004 ^{ab}	1.32 ± 0.04 $^{f-i}$	89.12 ± 0.33 ^{r}	35.14 ± 0.41 ^{l}	19.05 ± 0.53 ^{op}	0.72 ± 0.03 ^{hi}
	VD50	4.01 ± 0.18 $^{a-g}$	0.142 ± 0.008 ^{bc}	0.731 ± 0.024 ^{kl}	1.41 ± 0.01 ^{k}	47.96 ± 0.34 $^{b-d}$	34.03 ± 0.03 $^{j-l}$	12.46 ± 0.18 ^{jk}	-0.12 ± 0.03 ^{a}
	VD70	2.42 ± 0.15 $^{a-e}$	0.101 ± 0.008 ^{a}	0.672 ± 0.021 $^{i-k}$	1.39 ± 0.01 $^{i-k}$	51.22 ± 0.49 ^{fg}	32.19 ± 0.16 $^{f-i}$	7.91 ± 1.21 ^{g}	0.26 ± 0.08 $^{c-e}$
	VD90	1.01 ± 0.66 ^{ab}	0.177 ± 0.008 $^{e-i}$	0.689 ± 0.023 $^{j-l}$	1.38 ± 0.01 $^{g-k}$	49.52 ± 0.40 $^{d-f}$	33.09 ± 0.86 $^{h-k}$	8.66 ± 0.42 ^{gh}	0.26 ± 0.02 $^{c-e}$
	SD	4.20 ± 1.59 $^{b-h}$	0.199 ± 0.008 $^{h-j}$	0.272 ± 0.008 $^{c-e}$	1.06 ± 0.02 ^{a}	74.15 ± 0.40 ^{l}	37.02 ± 0.33 ^{m}	23.91 ± 0.27 ^{q}	1.08 ± 0.14 ^{j}
Bluecrop	FD	6.10 ± 0.40 $^{f-h}$	0.174 ± 0.006 $^{d-h}$	0.193 ± 0.006 $^{a-c}$	1.29 ± 0.03 $^{e-g}$	84.96 ± 0.29 ^{p}	34.24 ± 0.08 ^{kl}	19.04 ± 0.11 ^{op}	-0.25 ± 0.02 ^{a}
	VD50	4.99 ± 0.96 $^{d-h}$	0.195 ± 0.006 $^{f-j}$	0.671 ± 0.027 $^{i-k}$	1.50 ± 0.02 ^{l}	55.00 ± 0.59 ^{hi}	30.70 ± 0.72 $^{a-e}$	7.04 ± 0.92 $^{e-g}$	0.11 ± 0.01 ^{cd}
	VD70	2.19 ± 0.41 $^{a-e}$	0.164 ± 0.006 $^{c-e}$	0.628 ± 0.025 ^{ij}	1.39 ± 0.01 $^{i-k}$	54.36 ± 0.33 ^{h}	30.63 ± 0.15 $^{a-e}$	4.97 ± 0.04 $^{b-d}$	0.14 ± 0.01 ^{cd}
	VD90	2.18 ± 0.41 $^{a-e}$	0.195 ± 0.008 $^{g-j}$	0.676 ± 0.023 ^{jk}	1.40 ± 0.01 ^{jk}	51.32 ± 0.47 ^{g}	31.60 ± 0.20 $^{d-g}$	6.84 ± 0.37 $^{e-g}$	0.09 ± 0.11 ^{bc}
	SD	2.54 ± 0.62 $^{a-e}$	0.237 ± 0.006 $^{k-j}$	0.273 ± 0.010 $^{c-e}$	1.13 ± 0.02 ^{ab}	75.54 ± 0.35 ^{lm}	32.64 ± 0.10 $^{f-j}$	19.36 ± 0.00 ^{p}	1.24 ± 0.05 ^{j}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying; a, b, c, … - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test).

Bulk density (ρb) ranged from 0.12 to 0.77 g/cm^3 (**Table 8**). The drying techniques had the greatest impact on ρb, regardless of carrier addition and blueberry cultivar, with freeze- and spray drying resulting in the lowest values, which were, respectively, 2 and 4 times lower than for powders after vacuum drying. This is attributable to the structural differences in the powders caused by drying (Michalska & Lech, 2018) as well as *Mc* and a_w ($r = -0.6$). For powders obtained from 'Berkley' by vacuum drying at 70 and 90 °C, the addition of inulin resulted in an almost 1.8-fold increase in ρb, compared to the control sample. This relationship was not observed for the other cultivars. It may be due to differences in chemical composition between cultivars and thus different transformations of specific constituents and their interactions with inulin that may occur during vacuum drying under the aforementioned temperature conditions (Li et al., 2019).

As for true density (ρt), when considering the effect of drying techniques, the only noticeable difference was observed for spray-dried powders ($\bar{x} = 1.15$ g/cm^3), which had the lowest ρt when compared to other applied thermal treatments (comparable values) (**Table 8**). A similar relationship was previously observed for sea buckthorn (Tkacz et al., 2020) and apple powders (Michalska & Lech, 2018), which can be attributed to chemical composition changes (degradation of natively occurring constituents or probable formation of new compounds) under the influence of relatively high temperature during spray drying, which finally lead to less dense powders. The drying techniques strongly affected porosity (ε), and so the powders can be ranked by increasing values: vacuum drying at 50 °C (approx. 49%) < vacuum drying at 70 °C (approx. 54%) ≈ vacuum drying at 90 °C (approx. 57%) < spray drying (approx. 77%) < freeze-drying (approx. 87%), regardless of carrier addition or cultivar (**Table 8**). A similar trend was observed for sea buckthorn powders (Tkacz et al., 2020); however, in the study on apple powders the highest ε was observed for spray-dried products (Michalska & Lech, 2018). This may be attributed to differences in the chemical composition. The impact of the drying technique on porosity was opposite to the impact on bulk density ($r = -0.99$) since this parameter is inversely related to bulk density.

The inulin addition did not significantly affect the brightness (L^*) of powders obtained from 3 cultivars as a relatively low concentration of inulin was used (5%; *w/w*) (**Table 8**). Spray- and freeze-drying resulted in higher values of the a^* coordinate compared to vacuum drying, regardless of the blueberry cultivar and carrier application. Moreover, carrier addition caused an increase in the value of this attribute. No correlation between a^* parameter and anthocyanins content were found ($r = -0.22$). The inulin application caused a shift of the coordinate b^* values towards negative values (bluish color). When considering the impact of

the drying technique, the strongest yellowness was recorded for spray-dried powders. In most cases, the lowest values were recorded for vacuum-dried samples irrespective of carrier addition or cultivar, indicating a tendency towards bluish. However, it is worth noting that the range over which the values change is relatively small and therefore the difference may not be perceptible visually.

(Poly)phenolics and antioxidant capacity

In the blueberry powders, four major groups of (poly)phenolics were identified and quantified, namely, phenolic acids (3 compounds; on average 30.2% of all identified (poly)phenolics), anthocyanins (8 compounds; 29.9%), flavonols (11 compounds; 28.2%) and flavan-3-ols (3 compounds; 8.8%) (**Table 9a** and **9b**) (Shen et al., 2014). Their content ranged from 7.32 to 30.9 g/100 g dm. As regards the cultivar, the highest sum of (poly)phenolics was indicated for 'Bluecrop', followed by 'Bluejay' powders (approx. 15% less) and 'Berkley' (approx. 40% lower content). Products without inulin had 48% higher content of (poly)phenolics than carrier-added samples. As for the impact of the drying technique, the highest (poly)phenolic content in powders was noted after freeze-drying followed by spray drying, while the lowest was after vacuum drying at 90 °C, except for 'Bluecrop' powders with addition of inulin. This could be linked to the probable protective role of inulin during the thermal treatment of these constituents (Michalska-Ciechanowska, **Brzezowska**, et al., 2021).

Phenolic acids

The content of phenolic acids in powders without inulin was approx. 1.9-fold higher when compared to products with its addition (**Table 9a**). The highest content of phenolic acids was observed for powder from 'Bluecrop' and was, on average, 35% and 60% higher when compared to 'Bluejay' and 'Berkley'. The phenolic acid content was mainly related to the amount of chlorogenic acid. Chlorogenic acid represented 94% of total phenolic acids in 'Bluecrop' powders, while its share was on average 87% in the other cultivars. This was in line with Pico et al. (2022), who indicated that 'Bluecrop' had the highest content of chlorogenic acid among the blueberry cultivars analyzed in their study. The content of caffeoyl-glucose was the highest in powders from 'Berkley' and 'Bluejay', whereas the content of feruloyl-glucose was the highest in 'Bluejay' and 'Bluecrop' products. Among drying techniques applied, the highest retention of phenolic acids was found after freeze- and spray drying. The strongest degradation of phenolic acids was observed when vacuum drying at 90 °C was used.

Table 9a. Phenolic acids and anthocyanins identified in three cultivars of blueberry juice extract powders from different dried treatments with or without a carrier [mg/100 g dm].

Cultivar	Drying technique	Caffeoyl-glucose	Chlorogenic acid	Feruoylglucose	**Sum of phenolic acids**	Delphinidin-3-*O*-galactoside	Delphinidin-3-*O*-arabinoside	Cyanidin-3-*O*-glucoside	Petunidin-3-*O*-glucoside	Petunidin-3-*O*-arabinoside	Peonidin-3-*O*-glucoside	Malvidin-3-*O*-galactoside	Malvidin-3-*O*-arabinoside	**Sum of anthocyanins**
								No carrier						
Bluejay	FD	0.53 ± 0.02 ^{lm}	6.42 ± 0.09 ^{g}	0.30 ± 0.01 ^{h}	**7.26 ± 0.12 ^{e}**	0.85 ± 0.01 ^{lm}	0.79 ± 0.02 ^{i}	0.98 ± 0.03 ^{h}	0.46 ± 0.02 $^{h-j}$	0.64 ± 0.01 ^{j}	3.56 ± 0.08 ^{m}	0.83 ± 0.00 ^{fg}	1.69 ± 0.02 ^{kl}	**9.79 ± 0.14 ^{m}**
	VD50	0.50 ± 0.01 $^{j-m}$	5.93 ± 0.00 $^{e-g}$	0.28 ± 0.01 ^{gh}	**6.71 ± 0.02 ^{e}**	0.87 ± 0.01 ^{m}	0.80 ± 0.01 ^{i}	0.97 ± 0.01 ^{gh}	0.43 ± 0.00 $^{g-i}$	0.62 ± 0.00 ^{j}	3.43 ± 0.01 ^{lm}	0.78 ± 0.00 $^{e-g}$	1.57 ± 0.02 ^{jk}	**9.46 ± 0.01 ^{lm}**
	VD70	0.52 ± 0.02 $^{k-m}$	6.17 ± 0.17 ^{g}	0.29 ± 0.01 ^{gh}	**6.98 ± 0.21 ^{e}**	0.73 ± 0.04 ^{k}	0.68 ± 0.03 ^{h}	0.83 ± 0.01 ^{f}	0.39 ± 0.00 ^{gh}	0.52 ± 0.01 ^{i}	3.04 ± 0.09 ^{k}	0.72 ± 0.01 ^{ef}	1.40 ± 0.01 ^{j}	**8.31 ± 0.19 $^{i-k}$**
	VD90	0.50 ± 0.00 $^{j-m}$	5.99 ± 0.08 ^{fg}	0.25 ± 0.01 ^{g}	**6.74 ± 0.09 ^{e}**	0.79 ± 0.00 $^{k-m}$	0.72 ± 0.01 ^{hi}	0.87 ± 0.01 ^{fg}	0.40 ± 0.01 ^{gh}	0.54 ± 0.01 ^{i}	3.18 ± 0.12 ^{kl}	0.76 ± 0.01 $^{e-g}$	1.42 ± 0.04 ^{j}	**8.68 ± 0.22 $^{j-l}$**
	SD	0.54 ± 0.01 ^{m}	6.25 ± 0.09 ^{g}	0.30 ± 0.00 ^{h}	**7.09 ± 0.10 ^{e}**	0.76 ± 0.05 ^{kl}	0.70 ± 0.04 ^{h}	0.86 ± 0.07 ^{f}	0.39 ± 0.04 ^{g}	0.53 ± 0.03 ^{i}	3.22 ± 0.15 ^{kl}	0.75 ± 0.04 $^{e-g}$	1.46 ± 0.06 ^{j}	**8.68 ± 0.49 $^{j-l}$**
Berkley	FD	0.46 ± 0.02 $^{h-j}$	3.68 ± 0.00 ^{c}	0.13 ± 0.01 ^{bc}	**4.27 ± 0.04 ^{c}**	0.47 ± 0.06 ^{j}	0.49 ± 0.02 ^{fg}	0.54 ± 0.05 ^{e}	0.12 ± 0.00 ^{b}	0.37 ± 0.04 $^{f-h}$	2.53 ± 0.10 ^{ij}	0.14 ± 0.01 ^{ab}	1.18 ± 0.06 ^{i}	**5.84 ± 0.34 ^{h}**
	VD50	0.49 ± 0.02 $^{j-m}$	3.66 ± 0.15 ^{c}	0.13 ± 0.00 $^{b-d}$	**4.28 ± 0.17 ^{c}**	0.42 ± 0.04 ^{ij}	0.41 ± 0.00 $^{d-f}$	0.44 ± 0.01 ^{d}	0.08 ± 0.02 ^{ab}	0.30 ± 0.03 $^{d-f}$	2.16 ± 0.01 ^{gh}	0.11 ± 0.01 ^{ab}	0.98 ± 0.02 $^{f-i}$	**4.90 ± 0.09 $^{e-h}$**
	VD70	0.48 ± 0.01 $^{j-l}$	3.66 ± 0.14 ^{c}	0.13 ± 0.01 $^{b-d}$	**4.27 ± 0.16 ^{c}**	0.40 ± 0.01 $^{h-j}$	0.43 ± 0.03 $^{d-g}$	0.47 ± 0.01 ^{de}	0.10 ± 0.01 ^{ab}	0.30 ± 0.02 $^{d-f}$	2.27 ± 0.05 ^{hi}	0.12 ± 0.00 ^{ab}	1.04 ± 0.04 $^{g-i}$	**5.14 ± 0.17 $^{e-h}$**
	VD90	0.47 ± 0.01 $^{i-k}$	3.50 ± 0.08 ^{bc}	0.11 ± 0.01 ^{b}	**4.09 ± 0.10 ^{bc}**	0.36 ± 0.02 $^{g-i}$	0.37 ± 0.04 ^{de}	0.44 ± 0.04 ^{d}	0.11 ± 0.02 ^{ab}	0.27 ± 0.01 $^{c-e}$	1.89 ± 0.03 ^{g}	0.11 ± 0.00 ^{ab}	0.80 ± 0.01 $^{d-f}$	**4.36 ± 0.04 $^{d-f}$**
	SD	0.48 ± 0.01 $^{j-l}$	3.68 ± 0.05 ^{c}	0.13 ± 0.00 $^{b-d}$	**4.29 ± 0.05 ^{c}**	0.44 ± 0.02 ^{ij}	0.44 ± 0.02 $^{d-g}$	0.52 ± 0.02 ^{de}	0.13 ± 0.00 ^{bc}	0.35 ± 0.03 $^{f-h}$	2.33 ± 0.03 ^{hi}	0.15 ± 0.01 ^{ab}	1.07 ± 0.01 ^{hi}	**5.42 ± 0.07 ^{gh}**
Bluecrop	FD	0.40 ± 0.03 ^{fg}	10.51 ± 0.53 ^{ij}	0.30 ± 0.03 ^{h}	**11.20 ± 0.59 ^{g}**	0.36 ± 0.04 $^{g-i}$	0.51 ± 0.04 ^{g}	0.54 ± 0.04 ^{e}	0.57 ± 0.04 ^{k}	0.41 ± 0.04 ^{h}	2.71 ± 0.18 ^{j}	1.84 ± 0.05 ^{j}	2.20 ± 0.15 ^{n}	**9.15 ± 0.58 $^{k-m}$**
	VD50	0.40 ± 0.02 $^{f-h}$	10.05 ± 0.11 ^{i}	0.28 ± 0.00 ^{gh}	**10.74 ± 0.12 ^{g}**	0.32 ± 0.01 $^{f-h}$	0.45 ± 0.02 $^{e-g}$	0.49 ± 0.01 ^{de}	0.51 ± 0.00 ^{jk}	0.39 ± 0.01 ^{gh}	2.45 ± 0.03 $^{h-j}$	1.71 ± 0.05 ^{ij}	2.01 ± 0.01 ^{mn}	**8.33 ± 0.02 $^{i-k}$**
	VD70	0.42 ± 0.00 $^{g-i}$	10.12 ± 0.04 ^{ij}	0.29 ± 0.00 ^{h}	**10.82 ± 0.05 ^{g}**	0.29 ± 0.03 $^{d-g}$	0.41 ± 0.03 $^{d-f}$	0.46 ± 0.02 ^{de}	0.47 ± 0.03 ^{ij}	0.34 ± 0.03 $^{d-h}$	2.31 ± 0.11 ^{hi}	1.57 ± 0.04 ^{i}	1.82 ± 0.08 ^{lm}	**7.66 ± 0.39 ^{i}**
	VD90	0.36 ± 0.03 ^{ef}	9.27 ± 0.35 ^{h}	0.19 ± 0.01 ^{f}	**9.81 ± 0.40 ^{f}**	0.20 ± 0.01 $^{a-d}$	0.25 ± 0.01 ^{c}	0.28 ± 0.03 ^{c}	0.32 ± 0.02 ^{f}	0.19 ± 0.02 ^{b}	1.39 ± 0.16 ^{de}	1.05 ± 0.12 ^{h}	0.94 ± 0.11 $^{f-h}$	**4.63 ± 0.47 $^{e-g}$**
	SD	0.40 ± 0.00 $^{f-h}$	10.74 ± 0.27 ^{j}	0.31 ± 0.01 ^{h}	**11.45 ± 0.27 ^{g}**	0.30 ± 0.00 $^{e-g}$	0.44 ± 0.00 $^{d-g}$	0.48 ± 0.00 ^{de}	0.49 ± 0.02 ^{ij}	0.35 ± 0.00 $^{e-h}$	2.48 ± 0.05 $^{h-j}$	1.66 ± 0.06 ^{i}	1.96 ± 0.03 ^{m}	**8.17 ± 0.16 ^{ij}**
								5% inulin						
Bluejay	FD	0.28 ± 0.00 ^{cd}	3.34 ± 0.02 ^{bc}	0.16 ± 0.00 $^{c-f}$	**3.77 ± 0.01 ^{bc}**	0.49 ± 0.02 ^{j}	0.45 ± 0.02 $^{e-g}$	0.55 ± 0.02 ^{e}	0.25 ± 0.01 $^{d-f}$	0.35 ± 0.00 $^{f-h}$	1.95 ± 0.02 ^{g}	0.46 ± 0.01 ^{d}	0.91 ± 0.01 $^{f-h}$	**5.42 ± 0.08 ^{gh}**
	VD50	0.28 ± 0.01 ^{cd}	3.37 ± 0.10 ^{bc}	0.16 ± 0.01 $^{c-f}$	**3.81 ± 0.12 ^{bc}**	0.47 ± 0.03 ^{j}	0.43 ± 0.02 $^{d-f}$	0.52 ± 0.02 ^{de}	0.24 ± 0.00 ^{de}	0.33 ± 0.01 $^{d-g}$	1.85 ± 0.10 ^{fg}	0.43 ± 0.03 ^{d}	0.87 ± 0.03 $^{e-h}$	**5.13 ± 0.24 $^{e-h}$**
	VD70	0.28 ± 0.01 ^{cd}	3.27 ± 0.09 ^{bc}	0.15 ± 0.00 $^{b-e}$	**3.70 ± 0.11 ^{bc}**	0.41 ± 0.03 $^{h-j}$	0.36 ± 0.03 ^{d}	0.44 ± 0.03 ^{d}	0.20 ± 0.00 ^{de}	0.27 ± 0.01 ^{cd}	1.55 ± 0.06 ^{ef}	0.36 ± 0.00 ^{cd}	0.69 ± 0.02 $^{c-e}$	**4.28 ± 0.17 ^{de}**
	VD90	0.25 ± 0.01 $^{b-d}$	2.92 ± 0.07 ^{b}	0.13 ± 0.00 $^{b-d}$	**3.30 ± 0.08 ^{b}**	0.22 ± 0.01 $^{b-e}$	0.20 ± 0.01 $^{a-c}$	0.26 ± 0.01 ^{bc}	0.12 ± 0.00 ^{bc}	0.16 ± 0.00 ^{ab}	0.90 ± 0.04 ^{ab}	0.25 ± 0.02 ^{bc}	0.38 ± 0.00 ^{ab}	**2.48 ± 0.04 ^{b}**
	SD	0.30 ± 0.01 ^{de}	3.57 ± 0.02 ^{bc}	0.17 ± 0.00 ^{ef}	**4.04 ± 0.02 ^{bc}**	0.49 ± 0.01 ^{j}	0.45 ± 0.00 $^{e-g}$	0.54 ± 0.00 ^{e}	0.25 ± 0.00 ^{de}	0.33 ± 0.00 $^{d-g}$	1.92 ± 0.02 ^{g}	0.43 ± 0.02 ^{d}	0.88 ± 0.02 $^{e-h}$	**5.29 ± 0.02 $^{f-h}$**
Berkley	FD	0.24 ± 0.01 $^{a-c}$	1.93 ± 0.00 ^{a}	0.07 ± 0.00 ^{a}	**2.24 ± 0.01 ^{a}**	0.22 ± 0.01 $^{b-e}$	0.24 ± 0.00 ^{bc}	0.27 ± 0.02 ^{bc}	0.07 ± 0.00 ^{ab}	0.17 ± 0.01 ^{b}	1.25 ± 0.03 $^{c-e}$	0.07 ± 0.00 ^{a}	0.57 ± 0.00 ^{bc}	**2.86 ± 0.06 ^{bc}**
	VD50	0.25 ± 0.00 $^{b-d}$	1.96 ± 0.02 ^{a}	0.07 ± 0.00 ^{a}	**2.28 ± 0.03 ^{a}**	0.22 ± 0.00 $^{b-e}$	0.22 ± 0.00 ^{bc}	0.26 ± 0.00 ^{bc}	0.06 ± 0.01 ^{ab}	0.16 ± 0.01 ^{ab}	1.21 ± 0.00 $^{b-d}$	0.06 ± 0.01 ^{a}	0.55 ± 0.03 ^{bc}	**2.76 ± 0.02 ^{bc}**
	VD70	0.25 ± 0.00 $^{b-d}$	1.95 ± 0.01 ^{a}	0.07 ± 0.00 ^{a}	**2.26 ± 0.01 ^{a}**	0.23 ± 0.01 $^{b-f}$	0.23 ± 0.01 ^{bc}	0.26 ± 0.01 ^{bc}	0.06 ± 0.01 ^{ab}	0.15 ± 0.00 ^{ab}	1.16 ± 0.00 $^{b-d}$	0.06 ± 0.00 ^{a}	0.52 ± 0.01 ^{bc}	**2.66 ± 0.01 ^{bc}**
	VD90	0.24 ± 0.00 $^{a-c}$	1.81 ± 0.00 ^{a}	0.06 ± 0.00 ^{a}	**2.11 ± 0.01 ^{a}**	0.13 ± 0.03 ^{ab}	0.13 ± 0.01 ^{a}	0.15 ± 0.00 ^{a}	0.05 ± 0.02 ^{a}	0.09 ± 0.02 ^{a}	0.63 ± 0.04 ^{a}	0.05 ± 0.01 ^{a}	0.26 ± 0.03 ^{a}	**1.49 ± 0.11 ^{a}**
	SD	0.27 ± 0.00 ^{cd}	2.09 ± 0.04 ^{a}	0.07 ± 0.00 ^{a}	**2.43 ± 0.04 ^{a}**	0.25 ± 0.00 $^{c-f}$	0.26 ± 0.01 ^{c}	0.27 ± 0.01 ^{bc}	0.06 ± 0.01 ^{ab}	0.18 ± 0.00 ^{b}	1.28 ± 0.02 $^{c-e}$	0.07 ± 0.01 ^{a}	0.58 ± 0.02 ^{bc}	**2.95 ± 0.04 ^{bc}**
Bluecrop	FD	0.20 ± 0.00 ^{ab}	5.33 ± 0.04 ^{de}	0.15 ± 0.00 $^{c-f}$	**5.68 ± 0.03 ^{d}**	0.17 ± 0.01 $^{a-c}$	0.24 ± 0.01 ^{c}	0.26 ± 0.00 ^{bc}	0.26 ± 0.01 ^{ef}	0.20 ± 0.00 ^{bc}	1.29 ± 0.01 $^{c-e}$	0.87 ± 0.02 ^{fg}	1.02 ± 0.02 $^{g-i}$	**4.32 ± 0.05 ^{de}**
	VD50	0.21 ± 0.00 ^{ab}	5.27 ± 0.00 ^{d}	0.16 ± 0.00 $^{c-f}$	**5.64 ± 0.00 ^{d}**	0.15 ± 0.01 ^{ab}	0.22 ± 0.01 ^{bc}	0.24 ± 0.01 $^{a-c}$	0.25 ± 0.01 $^{d-f}$	0.18 ± 0.01 ^{b}	1.28 ± 0.04 $^{c-e}$	0.84 ± 0.06 ^{fg}	1.02 ± 0.04 $^{g-i}$	**4.20 ± 0.19 ^{de}**
	VD70	0.19 ± 0.00 ^{a}	4.84 ± 0.08 ^{d}	0.13 ± 0.01 $^{b-d}$	**5.16 ± 0.07 ^{d}**	0.13 ± 0.00 ^{ab}	0.19 ± 0.01 $^{a-c}$	0.21 ± 0.01 $^{a-c}$	0.22 ± 0.01 ^{de}	0.16 ± 0.01 ^{ab}	1.06 ± 0.02 ^{bc}	0.73 ± 0.01 $^{e-g}$	0.83 ± 0.03 $^{d-g}$	**3.54 ± 0.10 ^{cd}**
	VD90	0.21 ± 0.02 ^{ab}	5.44 ± 0.29 $^{d-f}$	0.16 ± 0.01 $^{d-f}$	**5.81 ± 0.33 ^{d}**	0.17 ± 0.01 $^{a-c}$	0.24 ± 0.02 ^{bc}	0.26 ± 0.02 ^{bc}	0.25 ± 0.02 $^{d-f}$	0.18 ± 0.01 ^{b}	1.30 ± 0.11 $^{c-e}$	0.89 ± 0.08 ^{g}	1.02 ± 0.08 $^{g-i}$	**4.30 ± 0.35 ^{de}**
	SD	0.20 ± 0.03 ^{ab}	4.99 ± 0.39 ^{d}	0.15 ± 0.01 $^{c-f}$	**5.35 ± 0.44 ^{d}**	0.12 ± 0.01 ^{a}	0.16 ± 0.01 ^{ab}	0.18 ± 0.02 ^{ab}	0.19 ± 0.02 ^{cd}	0.13 ± 0.02 ^{ab}	0.89 ± 0.10 ^{ab}	0.66 ± 0.08 ^{e}	0.64 ± 0.09 ^{cd}	**2.98 ± 0.35 ^{bc}**

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying; a, b, c, … - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test)

Table 9b. Flavonols and flavan-3-ols identified in three cultivars of blueberry juice extract powders from different dried treatments with or without a carrier [mg/100 g dm].

Cultivar	Drying technique	Myricetin-3-*O*-galactoside	Quercetin-3-*O*-rutinoside	Quercetin-3-*O*-galactoside	Quercetin-3-*O*-glucoside	Quercetin-3-*O*-arabinofuranoside	Quercetin-3-*O*-oxalylpentoside	Quercetin-3-*O*-rhamnoside	Quercetin-3-*O*-dimethoxyrhamnoside	Quercetin hexuronide 2	Quercetin-3-*O*-(acetyl)hexoside	Syringetin-3-*O*-rhamnoside	**Sum of flavonols**	(+)-Catechine	Procyanidin B1	Flavan-3-ol derivative	**Sum of flavan-3-ols**
No carrier																	
Bluejay	FD	0.14 ± 0.01 ^{g}	0.32 ± 0.00 ^{fg}	1.57 ± 0.02 ^{h}	0.90 ± 0.02 ^{i}	0.39 ± 0.01 ^{hi}	0.64 ± 0.00 ^{n}	0.20 ± 0.00 ^{de}	0.85 ± 0.01 ^{h}	0.46 ± 0.01 ^{g}	0.47 ± 0.03 $^{f-h}$	0.35 ± 0.03 ^{c}	**6.30 ± 0.08** ^{jk}	0.57 ± 0.00 $^{g-i}$	0.70 ± 0.01 ^{ij}	0.58 ± 0.01 $^{f-h}$	**1.85 ± 0.00** $^{e-g}$
	VD50	0.12 ± 0.01 ^{f}	0.29 ± 0.00 ^{f}	1.50 ± 0.00 $^{f-h}$	0.89 ± 0.02 ^{hi}	0.42 ± 0.01 i	0.58 ± 0.00 ^{m}	0.21 ± 0.00 ^{e}	0.81 ± 0.01 ^{h}	0.45 ± 0.01 ^{g}	0.47 ± 0.01 $^{f-h}$	0.34 ± 0.01 ^{c}	**6.07 ± 0.00** $^{i-k}$	0.52 ± 0.00 $^{f-h}$	0.63 ± 0.00 $^{g-i}$	0.51 ± 0.02 $^{d-f}$	**1.66 ± 0.02** ^{cd}
	VD70	0.12 ± 0.00 ^{f}	0.29 ± 0.02 ^{f}	1.50 ± 0.06 $^{f-h}$	0.89 ± 0.03 ^{hi}	0.40 ± 0.01 ^{hi}	0.58 ± 0.03 ^{m}	0.22 ± 0.01 ^{e}	0.83 ± 0.04 ^{h}	0.45 ± 0.01 ^{g}	0.47 ± 0.04 $^{f-h}$	0.36 ± 0.01 ^{c}	**6.11 ± 0.25** $^{i-k}$	0.53 ± 0.04 $^{f-h}$	0.66 ± 0.03 ^{hi}	0.54 ± 0.02 $^{e-g}$	**1.73 ± 0.09** $^{c-e}$
	VD90	0.12 ± 0.00 ^{f}	0.29 ± 0.01 ^{f}	1.53 ± 0.03 $^{f-h}$	0.90 ± 0.00 ^{i}	0.39 ± 0.00 ^{hi}	0.56 ± 0.00 ^{m}	0.23 ± 0.01 ^{e}	0.80 ± 0.00 ^{h}	0.45 ± 0.01 ^{g}	0.48 ± 0.01 ^{gh}	0.35 ± 0.01 ^{c}	**6.09 ± 0.08** $^{i-k}$	0.47 ± 0.01 ^{ef}	0.61 ± 0.01 $^{f-i}$	0.50 ± 0.01 $^{c-f}$	**1.58 ± 0.02** ^{c}
	SD	0.13 ± 0.01 ^{fg}	0.31 ± 0.01 ^{f}	1.53 ± 0.04 $^{f-h}$	0.89 ± 0.02 ^{hi}	0.42 ± 0.01 ^{i}	0.64 ± 0.01 ^{n}	0.22 ± 0.01 ^{e}	0.84 ± 0.01 ^{h}	0.47 ± 0.02 ^{g}	0.48 ± 0.00 $^{f-h}$	0.35 ± 0.02 ^{c}	**6.27 ± 0.11** ^{jk}	0.59 ± 0.01 $^{g-i}$	0.69 ± 0.01 ^{ij}	0.58 ± 0.01 $^{f-h}$	**1.86 ± 0.03** $^{e-g}$
Berkley	FD	0.06 ± 0.00 $^{c-}$	0.11 ± 0.01 $^{a-e}$	1.60 ± 0.01 ^{h}	0.62 ± 0.02 $^{e-f}$	0.41 ± 0.00 ^{i}	0.43 ± 0.01 ^{l}	0.06 ± 0.01 $^{a-c}$	1.08 ± 0.00 ^{j}	0.48 ± 0.03 ^{gh}	0.37 ± 0.01 $^{d-g}$	0.35 ± 0.01 ^{c}	**5.57 ± 0.07** $^{h-j}$	0.43 ± 0.02 ^{e}	0.82 ± 0.10 ^{jk}	0.64 ± 0.11 $^{f-j}$	**1.89 ± 0.23** $^{e-g}$
	VD50	0.06 ± 0.00 ^{cd}	0.11 ± 0.00 $^{a-e}$	1.50 ± 0.05 $^{f-h}$	0.58 ± 0.02 $^{c-e}$	0.37 ± 0.00 $^{g-i}$	0.37 ± 0.01 $^{j-l}$	0.05 ± 0.00 ^{ab}	1.02 ± 0.04 ^{j}	0.46 ± 0.02 ^{g}	0.32 ± 0.04 $^{b-d}$	0.34 ± 0.02 ^{c}	**5.18 ± 0.20** ^{gh}	0.47 ± 0.06 ^{ef}	0.89 ± 0.08 $^{k-m}$	0.69 ± 0.10 $^{h-j}$	**2.05 ± 0.24** $^{e-h}$
	VD70	0.06 ± 0.00 ^{cd}	0.10 ± 0.01 $^{a-e}$	1.54 ± 0.07 ^{gh}	0.58 ± 0.01 $^{c-e}$	0.39 ± 0.03 ^{hi}	0.39 ± 0.03 ^{kl}	0.06 ± 0.00 $^{a-c}$	1.06 ± 0.06 ^{j}	0.49 ± 0.02 ^{gh}	0.34 ± 0.01 $^{b-e}$	0.34 ± 0.01 ^{c}	**5.35 ± 0.23** ^{hi}	0.47 ± 0.00 ^{ef}	0.89 ± 0.00 $^{k-m}$	0.70 ± 0.00 $^{h-j}$	**2.06 ± 0.00** $^{e-i}$
	VD90	0.05 ± 0.00 ^{bc}	0.08 ± 0.00 $^{a-c}$	1.44 ± 0.02 $^{f-h}$	0.56 ± 0.00 ^{cd}	0.36 ± 0.00 $^{g-i}$	0.35 ± 0.00 $^{i-k}$	0.07 ± 0.00 $^{a-c}$	1.01 ± 0.02 ^{j}	0.46 ± 0.00 ^{g}	0.34 ± 0.01 $^{b-e}$	0.32 ± 0.01 ^{c}	**5.05 ± 0.04** ^{gh}	0.42 ± 0.02 ^{e}	0.86 ± 0.02 ^{kl}	0.69 ± 0.02 $^{h-j}$	**1.97 ± 0.06** $^{d-h}$
	SD	0.06 ± 0.01 $^{c-}$	0.10 ± 0.00 $^{a-d}$	1.59 ± 0.04 ^{h}	0.64 ± 0.04 $^{c-f}$	0.42 ± 0.01 ^{i}	0.42 ± 0.00 ^{l}	0.07 ± 0.01 $^{a-c}$	1.07 ± 0.00 ^{j}	0.50 ± 0.01 ^{gh}	0.36 ± 0.01 $^{c-f}$	0.34 ± 0.02 ^{c}	**5.56 ± 0.09** $^{h-j}$	0.50 ± 0.00 $^{e-g}$	0.91 ± 0.01 $^{k-m}$	0.75 ± 0.02 ^{j}	**2.16 ± 0.03** $^{f-i}$
Bluecrop	FD	ND	0.77 ± 0.06 ^{jk}	2.51 ± 0.16 ^{j}	1.45 ± 0.06 ^{k}	0.66 ± 0.04 ^{k}	0.35 ± 0.02 $^{i-k}$	0.69 ± 0.04 ^{gh}	0.13 ± 0.00 ^{ab}	0.69 ± 0.03 ^{i}	1.03 ± 0.06 ^{j}	ND	**8.28 ± 0.46** ^{l}	0.61 ± 0.05 ^{hi}	0.98 ± 0.08 ^{lm}	0.71 ± 0.06 $^{h-j}$	**2.30 ± 0.19** ^{hi}
	VD50	ND	0.74 ± 0.02 ^{jk}	2.44 ± 0.05 ^{j}	1.41 ± 0.02 ^{k}	0.63 ± 0.00 ^{jk}	0.32 ± 0.01 ^{hi}	0.67 ± 0.01 ^{gh}	0.12 ± 0.01 ^{ab}	0.67 ± 0.00 ^{i}	0.98 ± 0.02 ^{j}	ND	**7.97 ± 0.09** ^{l}	0.59 ± 0.02 $^{g-i}$	0.94 ± 0.01 $^{k-m}$	0.67 ± 0.00 $^{g-j}$	**2.20 ± 0.04** $^{g-i}$
	VD70	ND	0.71 ± 0.02 ^{j}	2.35 ± 0.05 ^{j}	1.34 ± 0.03 ^{k}	0.59 ± 0.01 ^{j}	0.32 ± 0.01 $^{h-j}$	0.67 ± 0.03 ^{gh}	0.13 ± 0.00 ^{ab}	0.65 ± 0.01 ^{i}	0.95 ± 0.04 ^{j}	ND	**7.71 ± 0.20** ^{l}	0.55 ± 0.01 $^{f-h}$	0.94 ± 0.00 $^{k-m}$	0.67 ± 0.00 $^{g-j}$	**2.16 ± 0.01** $^{f-i}$
	VD90	ND	0.62 ± 0.03 ^{i}	1.88 ± 0.17 ^{i}	1.12 ± 0.08 ^{j}	0.42 ± 0.02 ^{i}	0.27 ± 0.01 $^{f-h}$	0.62 ± 0.05 ^{g}	0.12 ± 0.01 ^{ab}	0.55 ± 0.04 ^{h}	0.82 ± 0.08 ^{i}	ND	**6.41 ± 0.48** ^{k}	0.40 ± 0.03 ^{de}	0.83 ± 0.05 ^{jk}	0.60 ± 0.05 $^{f-i}$	**1.83 ± 0.13** $^{c-f}$
	SD	ND	0.79 ± 0.03 ^{k}	2.53 ± 0.05 ^{j}	1.44 ± 0.01 ^{k}	0.65 ± 0.02 ^{jk}	0.36 ± 0.01 $^{i-k}$	0.71 ± 0.01 ^{h}	0.13 ± 0.00 ^{b}	0.69 ± 0.01 ^{i}	1.02 ± 0.02 ^{j}	ND	**8.32 ± 0.15** ^{l}	0.66 ± 0.04 ^{i}	1.02 ± 0.03 ^{m}	0.74 ± 0.05 ^{ij}	**2.42 ± 0.12** ^{i}
5% inulin																	
Bluejay	FD	0.07 ± 0.00 ^{de}	0.17 ± 0.00 ^{de}	0.89 ± 0.02 ^{ab}	0.55 ± 0.02 ^{cd}	0.25 ± 0.01 ^{cd}	0.36 ± 0.00 $^{i-k}$	0.12 ± 0.00 ^{bc}	0.48 ± 0.00 $^{c-e}$	0.26 ± 0.01 $^{a-c}$	0.27 ± 0.00 $^{a-d}$	0.20 ± 0.00 ^{ab}	**3.63 ± 0.01** $^{b-e}$	0.29 ± 0.00 ^{bc}	0.36 ± 0.00 $^{a-c}$	0.30 ± 0.00 ^{ab}	**0.95 ± 0.01** ^{ab}
	VD50	0.07 ± 0.00 ^{de}	0.16 ± 0.00 ^{de}	0.88 ± 0.03 ^{ab}	0.54 ± 0.02 ^{cd}	0.22 ± 0.02 $^{a-d}$	0.32 ± 0.02 ^{hi}	0.12 ± 0.01 ^{bc}	0.45 ± 0.01 ^{cd}	0.25 ± 0.01 ^{ab}	0.25 ± 0.01 $^{a-c}$	0.20 ± 0.01 ^{ab}	**3.46 ± 0.13** $^{a-e}$	0.29 ± 0.00 ^{bc}	0.36 ± 0.01 $^{a-d}$	0.29 ± 0.01 ^{ab}	**0.94 ± 0.02** ^{ab}
	VD70	0.07 ± 0.00 ^{de}	0.15 ± 0.00 $^{c-e}$	0.86 ± 0.03 ^{ab}	0.52 ± 0.02 ^{bc}	0.21 ± 0.03 $^{a-c}$	0.29 ± 0.03 ^{gh}	0.12 ± 0.02 ^{bc}	0.45 ± 0.03 ^{cd}	0.25 ± 0.02 ^{ab}	0.24 ± 0.05 $^{a-c}$	0.19 ± 0.02 ^{ab}	**3.35 ± 0.25** $^{a-d}$	0.26 ± 0.00 $^{a-c}$	0.34 ± 0.00 ^{ab}	0.27 ± 0.00 ^{ab}	**0.87 ± 0.00** ^{ab}
	VD90	0.06 ± 0.00 ^{cd}	0.13 ± 0.01 $^{b-e}$	0.68 ± 0.02 ^{a}	0.41 ± 0.01 ^{ab}	0.16 ± 0.01 ^{a}	0.25 ± 0.00 $^{e-g}$	0.12 ± 0.00 ^{bc}	0.40 ± 0.02 ^{c}	0.21 ± 0.00 ^{a}	0.22 ± 0.01 ^{ab}	0.17 ± 0.01 ^{a}	**2.81 ± 0.08** ^{a}	0.22 ± 0.01 ^{ab}	0.31 ± 0.01 ^{a}	0.24 ± 0.00 ^{a}	**0.77 ± 0.02** ^{a}
	SD	0.08 ± 0.00 ^{e}	0.17 ± 0.00 ^{e}	0.93 ± 0.03 ^{bc}	0.56 ± 0.02 $^{c-e}$	0.25 ± 0.00 $^{b-d}$	0.38 ± 0.00 ^{kl}	0.13 ± 0.00 ^{cd}	0.50 ± 0.00 $^{d-f}$	0.28 ± 0.01 $^{a-d}$	0.29 ± 0.00 $^{a-d}$	0.22 ± 0.01 ^{b}	**3.79 ± 0.06** $^{c-f}$	0.31 ± 0.00 $^{b-d}$	0.39 ± 0.00 $^{a-e}$	0.32 ± 0.01 ^{ab}	**1.02 ± 0.01** ^{ab}
Berkley	FD	0.04 ± 0.00 ^{ab}	0.05 ± 0.00 ^{a}	0.87 ± 0.00 ^{ab}	0.33 ± 0.00 ^{a}	0.23 ± 0.00 $^{b-d}$	0.24 ± 0.00 $^{d-g}$	0.04 ± 0.00 ^{a}	0.59 ± 0.00 ^{g}	0.27 ± 0.00 $^{a-c}$	0.19 ± 0.00 ^{a}	0.19 ± 0.00 ^{ab}	**3.04 ± 0.02** $^{a-c}$	0.24 ± 0.00 $^{a-c}$	0.48 ± 0.00 $^{b-f}$	0.38 ± 0.00 $^{a-d}$	**1.10 ± 0.01** ^{ab}
	VD50	0.03 ± 0.00 ^{ab}	0.05 ± 0.00 ^{a}	0.86 ± 0.01 ^{ab}	0.33 ± 0.00 ^{a}	0.23 ± 0.02 $^{b-d}$	0.23 ± 0.02 $^{c-f}$	0.04 ± 0.01 ^{a}	0.58 ± 0.02 ^{g}	0.26 ± 0.02 ^{ab}	0.19 ± 0.03 ^{a}	0.18 ± 0.00 ^{ab}	**2.96 ± 0.11** ^{ab}	0.24 ± 0.01 $^{a-c}$	0.48 ± 0.01 $^{c-f}$	0.39 ± 0.01 $^{a-d}$	**1.11 ± 0.02** ^{ab}
	VD70	0.03 ± 0.00 ^{ab}	0.05 ± 0.00 ^{a}	0.85 ± 0.00 ^{ab}	0.33 ± 0.00 ^{a}	0.21 ± 0.00 $^{a-c}$	0.22 ± 0.00 $^{b-e}$	0.03 ± 0.00 ^{a}	0.58 ± 0.00 ^{fg}	0.25 ± 0.01 ^{ab}	0.19 ± 0.01 ^{a}	0.18 ± 0.00 ^{ab}	**2.92 ± 0.03** ^{ab}	0.24 ± 0.01 $^{a-c}$	0.47 ± 0.01 $^{b-f}$	0.37 ± 0.01 $^{a-c}$	**1.07 ± 0.04** ^{ab}
	VD90	0.03 ± 0.00 ^{a}	0.04 ± 0.00 ^{a}	0.79 ± 0.00 ^{ab}	0.31 ± 0.00 ^{a}	0.18 ± 0.01 ^{ab}	0.19 ± 0.01 $^{a-d}$	0.04 ± 0.00 ^{a}	0.55 ± 0.00 $^{e-g}$	0.24 ± 0.00 ^{ab}	0.18 ± 0.01 ^{a}	0.17 ± 0.01 ^{a}	**2.74 ± 0.01** ^{a}	0.19 ± 0.02 ^{a}	0.44 ± 0.01 $^{a-c}$	0.35 ± 0.01 ^{ab}	**0.98 ± 0.04** ^{ab}
	SD	0.04 ± 0.00 ^{ab}	0.06 ± 0.00 ^{ab}	0.95 ± 0.01 $^{b-d}$	0.37 ± 0.00 ^{a}	0.24 ± 0.00 $^{b-d}$	0.25 ± 0.01 $^{e-g}$	0.04 ± 0.00 ^{a}	0.63 ± 0.01 ^{g}	0.28 ± 0.01 $^{b-e}$	0.20 ± 0.00 ^{a}	0.20 ± 0.00 ^{ab}	**3.25 ± 0.02** $^{a-d}$	0.26 ± 0.01 $^{a-c}$	0.52 ± 0.01 $^{c-h}$	0.41 ± 0.01 $^{b-e}$	**1.18 ± 0.03** ^{b}
Bluecrop	FD	ND	0.39 ± 0.01 ^{gh}	1.31 ± 0.00 $^{e-g}$	0.74 ± 0.01 ^{fg}	0.32 ± 0.00 $^{e-g}$	0.17 ± 0.00 ^{ab}	0.36 ± 0.01 ^{f}	0.06 ± 0.00 ^{ab}	0.34 ± 0.00 $^{d-f}$	0.52 ± 0.01 ^{h}	ND	**4.22 ± 0.04** ^{ef}	0.31 ± 0.00 $^{b-d}$	0.51 ± 0.01 $^{e-g}$	0.36 ± 0.00 $^{a-c}$	**1.18 ± 0.01** ^{b}
	VD50	ND	0.39 ± 0.02 ^{gh}	1.28 ± 0.04 ^{ef}	0.74 ± 0.03 ^{fg}	0.32 ± 0.00 $^{e-g}$	0.16 ± 0.00 ^{a}	0.35 ± 0.01 ^{f}	0.07 ± 0.00 ^{ab}	0.35 ± 0.01 ^{ef}	0.52 ± 0.02 ^{h}	ND	**4.19 ± 0.12** ^{ef}	0.30 ± 0.01 ^{bc}	0.50 ± 0.01 $^{d-g}$	0.36 ± 0.00 ^{ab}	**1.16 ± 0.02** ^{b}
	VD70	ND	0.35 ± 0.01 $^{f-h}$	1.15 ± 0.01 $^{c-e}$	0.65 ± 0.00 $^{d-f}$	0.27 ± 0.01 $^{c-e}$	0.14 ± 0.01 ^{a}	0.32 ± 0.01 ^{f}	0.05 ± 0.00 ^{a}	0.30 ± 0.00 $^{b-f}$	0.45 ± 0.01 $^{e-h}$	ND	**3.68 ± 0.03** $^{b-f}$	0.27 ± 0.01 $^{a-c}$	0.45 ± 0.01 $^{b-e}$	0.32 ± 0.01 ^{ab}	**1.03 ± 0.02** ^{ab}
	VD90	ND	0.41 ± 0.03 ^{h}	1.37 ± 0.10 $^{e-h}$	0.78 ± 0.05 ^{gh}	0.34 ± 0.02 $^{f-h}$	0.18 ± 0.01 $^{a-c}$	0.38 ± 0.02 ^{f}	0.07 ± 0.00 ^{ab}	0.37 ± 0.03 ^{f}	0.55 ± 0.05 ^{h}	ND	**4.44 ± 0.31** ^{fg}	0.33 ± 0.04 ^{cd}	0.52 ± 0.06 $^{e-h}$	0.36 ± 0.05 $^{a-c}$	**1.21 ± 0.15** ^{b}
	SD	ND	0.36 ± 0.03 $^{f-h}$	1.18 ± 0.13 ^{de}	0.68 ± 0.07 $^{e-g}$	0.28 ± 0.03 $^{d-f}$	0.16 ± 0.02 ^{a}	0.38 ± 0.05 ^{f}	0.07 ± 0.01 ^{ab}	0.33 ± 0.04 $^{c-f}$	0.50 ± 0.06 ^{h}	ND	**3.93 ± 0.43** $^{d-f}$	0.24 ± 0.02 $^{a-c}$	0.46 ± 0.05 $^{b-e}$	0.33 ± 0.02 ^{ab}	**1.03 ± 0.10** ^{ab}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying; ND – not detected; a, b, c, … - different letters within the column indicated statistical differences ($p < 0.05$; HSD Tukey test).

Anthocyanins

The second most abundant group of (poly)phenolics in the powders were anthocyanins, whose content ranged from 1.5 to 9.8 g/100 g dm (**Table 9a**). Considering the cultivar, 'Bluejay' powders had the highest average content of anthocyanins – approximately 56% more than 'Berkley' powders, which had the lowest anthocyanin content, regardless of the drying technique or carrier addition. Therefore, from the practical point of view, 'Bluejay' powder can be recommended whenever a high anthocyanin content is desired. This cultivar also appeared preferable when the carrier was applied to blueberry juice extract powders production, except of samples after vacuum drying at 90 °C, in which the 'Bluecrop' powders were the most attractive. The addition of inulin caused an approx. 2-fold decrease in the content of individual anthocyanins. The exceptions were powders after vacuum drying at 90 °C: 1.1-fold decrease was recorded in 'Bluecrop' products, 2.8-fold decrease in 'Berkley' products and 3.5-fold decrease in 'Bluejay' products. This confirms a strong influence of temperature (Zhu et al., 2017) and drying time (Reque et al., 2016) on anthocyanin degradation; however, the different degrees of degradation of the same anthocyanins in powders obtained from different cultivars indicate an ambiguous effect of the blueberry juice extract matrices submitted to drying (Yang et al., 2022). The drying technique influenced the average anthocyanins content in the following order: freeze-drying > vacuum drying at 50 °C > spray drying > vacuum drying at 70 °C > vacuum drying at 90 °C, regardless of carrier addition or cultivar. As for individual anthocyanins, 'Bluejay' powders had the highest content of peonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, delphinidin-3-*O*-galactoside, delphinidin-3-*O*-arabinoside, and petunidin-3-*O*-arabinoside. The exceptions were products after vacuum drying at 90 °C with inulin, while the 'Bluecrop' powders were characterized by the highest amount of malvidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside.

Among anthocyanins identified, peonidin-3-*O*-glucoside, not previously indicated as prevailing in blueberry fruit (Herrera-Balandrano et al., 2021), was predominant in all powders tested (approx. 30% higher in 'Bluejay' compared to the other cultivars). Previously, Liu et al. (2022) found that among the anthocyanins present in the fruit of greengages (*Prunus mume* Sieb. Et Zucc), only the content of peonidin-3-*O*-glucoside increased under intense UV radiation. This, in turn, may be due to the fact that meteorological parameters can induce different responses of individual anthocyanins depending on the structure (Kovinich et al., 2014), affecting their synthesis differently, and thus the final composition of anthocyanins in the fruit. Moreover, the stability of individual anthocyanin aglycones is also determined by the

technological process and their varying persistence, in particular the effects of temperature, light or the presence of oxygen (Cai et al., 2022). Some anthocyanins are more prone to technological treatment and, consequently, some will be more and others less stable, as observed in the study. Although the contents of cyanidin-3-*O*-glucoside, delphinidin-3-*O*-arabinoside, and petunidin-3-*O*-arabinoside varied, these compounds followed the same path in terms of quantitative fluctuations depending on cultivar, carrier addition, and drying technique, as in case of peonidin-3-*O*-glucoside (Michalska-Ciechanowska, Hendrysiak, et al., 2021). Slight differences deviating from this trend were noted for delphinidin-3-*O*-galactoside, the content of which, depending on the cultivar, was ranked in the following descending order: 'Bluejay' > 'Berkley' > 'Bluecrop' for all of powders, except samples after vacuum drying at 90 °C with inulin. A different trend was found for malvidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside. In the case of malvidin-3-*O*-arabinoside, the second most abundant anthocyanin in powders, the highest content was recorded for 'Bluecrop' products, while 'Bluejay' and 'Berkley' had, respectively, 16% and 47% less of this constituent. The exception were the powders after vacuum drying at 90 °C without inulin and spray dried with its application, of which 'Bluejay' powders had the highest content of this compound.

Among inulin-added samples, only in the case of 'Bluecrop' powders after vacuum drying at 90 °C, the content of malvidin-3-*O*-arabinoside, malvidin-3-*O*-galactoside, and petunidin-3-*O*-glucoside was at the similar level or even higher, compared with products obtained by other drying techniques. This stands in contrast to inulin-free powders, where vacuum drying at 90 °C resulted in 'Bluecrop' products with the lowest amount of these compounds. Two different trends in the group of anthocyanins may be due to their different stability, which depends, among others, on the anthocyanin chemical structure, the number and position of sugar moieties attached, or the degree of acylation (Ryu & Koh, 2022), as well as environmental factors, mainly pH, temperature, light, oxygen access, presence of ascorbic acid or enzymes, co-pigmentation, etc. (Enaru et al., 2021).

Flavonols

The third identified group of (poly)phenolics present in blueberry powders were flavonols, which comprised 11 compounds and their sum ranged from 2.74 to 8.32 g/100 g dm (**Table 9b**). The highest amount of flavonols was noted for 'Bluecrop' products, while 'Bluejay' and 'Berkley' were characterized by around 19% and 30% lower content, respectively. Similar to anthocyanins, inulin application resulted in an about 2-fold decrease,

regardless of the cultivar and drying technique applied. This was different for samples after vacuum drying at 90 °C (approx. 1.4-fold decrease). Moreover, in the case of 'Berkley' and 'Bluejay' powders, the fluctuation of the flavonols content depending on the drying technique used was not so noticeable as in case of 'Bluecrop' powders.

The individual flavonols followed different paths depending on the variables analyzed. The levels of quercetin-3-*O*-galactoside (predominant flavonol in all blueberry powders), quercetin hexuronide, and quercetin-3-*O*-arabinofuranoside were at the similar level in 'Berkley' and 'Bluejay' products, and were in a lower quantity than noted for 'Bluecrop' products. Additionally, in 'Bluecrop' powders obtained by vacuum drying at 90 °C the lowest content of quercetin-3-*O*-galactoside, quercetin hexuronide, and quercetin-3-*O*-arabinofuranoside was observed, while the addition of inulin resulted in powders with the highest concentration of these components compared to other carrier-added samples. For quercetin-3-*O*-glucoside, -(acetyl)hexoside, -rutinoside, and -rhamnoside the course of changes was similar; however, the content of these compounds depending on the cultivar was in the following order: 'Bluecrop' > 'Bluejay' > 'Berkley'. Interestingly, there was also a strong negative correlation between quercetin-3-*O*-rhamnoside with quercetin-3-dimethoxy-rhamnoside and syringetin-3-*O*-rhamnoside ($r = -0.71$) (**Figure 19**). This may be connected with structural changes during fruit maturing which, depending on the blueberry cultivar, occur to different degrees and ultimately result in a different compound formation, as methoxylated derivatives of quercetin glycosides (Buchner et al., 2006; Yan et al., 2020).

Quercetin-3-*O*-dimethoxyrhamnoside content varied strongly depending on the cultivar; however, it remained relatively constant regardless of the drying technique. Unlike the other flavonols, this is the only compound which was recorded as predominant in powders from 'Berkley'. Quercetin-3-*O*-oxalylpentoside represented 4%, 7% and 10% of all identified flavonols in 'Bluecrop', 'Berkley', and 'Bluejay' powders, respectively. Spray-dried 'Bluejay' products had the highest content of this compound for both inulin-supplemented and inulin-free samples. The variations in its content depending on the drying technique were considerably more noticeable than in other flavonols. Finally, syringetin-3-*O*-rhamnoside and myricetin-3-*O*-galactoside were identified and quantified only in the products obtained from 'Berkley' and 'Bluejay'. The percentage of syringetin-3-*O*-rhamnoside in total flavonols was 6% for both 'Berkley' and 'Bluejay' powders. What is more, its content oscillated on the similar level and remained unchanged independently of the applied drying technique. For myricetin-3-*O*-galactoside, the average percentage was about 1% and 2% in 'Berkley' and 'Bluejay' powders, respectively; however, 'Bluejay' powders contained about 2 times more of this constituent than

'Berkley' ones. Slight differences were observed with regard to the drying technique. As in case of the most identified flavonols, the inulin addition resulted in powders with about 45% lower content of these two constituents when compared to carrier-free products.

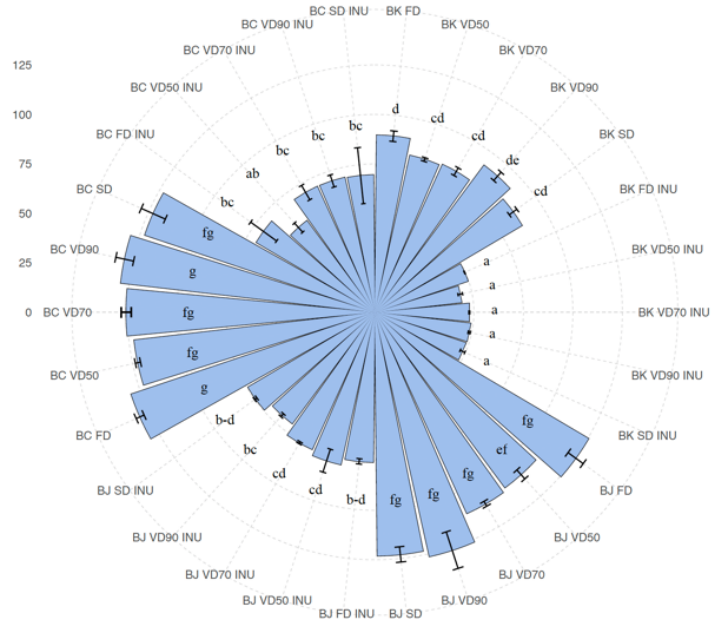
Flavan-3-ols

Flavan-3-ols were the least abundant group identified in blueberry powders, among which procyanidin B1, flavan-3-ol derivative, and (+)-catechin were identified and quantified (**Table 9b**). In most cases, the lowest content of flavan-3-ols was found in 'Bluejay' products, and the highest content in 'Bluecrop' powders. The carrier addition affected the content of flavan-3-ols in a way similar to the other groups of (poly)phenolics, while the drying technique had divergent effects depending on the cultivar, with spray drying allowing the highest retention of these compounds in most cases. Procyanidin B1 was found to be dominant among flavan-3-ols in all powders. All 'Bluejay' powders were characterized by the lowest amount of this constituent, while relatively comparable levels were identified in 'Bluecrop' and 'Berkley' products. The drying technique exerted a considerably different influence on the procyanidin B1 amount, depending on blueberry cultivar and inulin addition. The products of 'Berkley' after vacuum drying at 90 °C had the lowest, while spray-dried ones had the highest content of this compound. However, in the case of 'Bluecrop' powders, the same drying technique differently influenced inulin-free and inulin-supplemented samples, and the application of spray drying and vacuum drying at 90 °C resulted in powders with the highest procyanidin B1 content. A similar trend was observed for the flavan-3-ol derivative. The highest content of (+)-catechin was noted for 'Bluecrop' products, and the lowest for 'Berkley' ones. The only exceptions were observed among the powders after vacuum drying at 90 °C (inulin-free samples) and spray drying (inulin-added samples), of which 'Bluejay' powders were characterized by the highest amount of (+) -catechin.

Antioxidant capacity

The antioxidant capacity of powders measured by TEAC ABTS and FRAP methods indicated a strong influence of carrier addition and cultivar, while the drying technique resulted in only minor variations (**Figure 18a** and **18b**). The lowest antioxidant capacity values were noted for 'Berkley' powders, while products from the other cultivars showed approx. 30% higher TEAC ABTS and FRAP values.

(a)



(b)

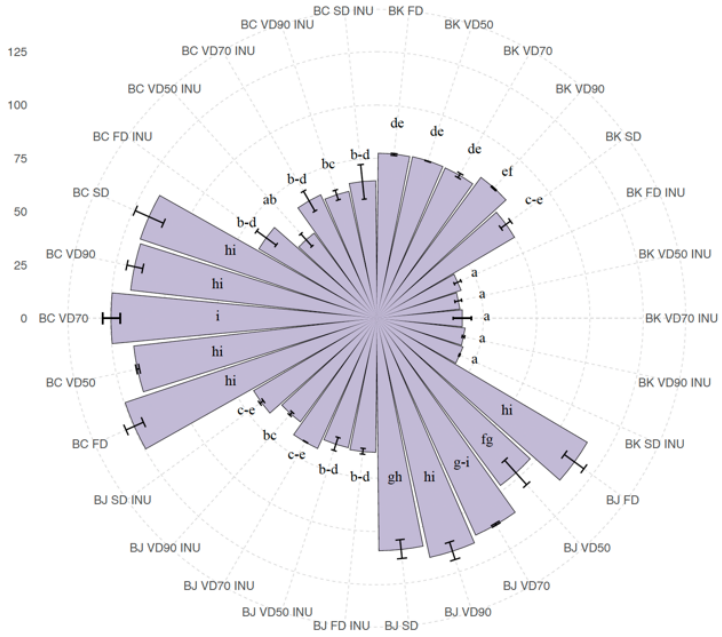


Figure 18. Antioxidant capacity of blueberry juice extract powders made from *cv*. Berkey (BK), Bluejay (BJ) and Bluecrop (BC) cultivars without and with inulin (INU) after freeze-drying (FD), vacuum drying at 50 °C (VD50), vacuum drying at 70 °C (VD70); vacuum drying at 90 °C (VD90) and spray drying (SD) measured by (a) TEAC ABTS (Trolox Equivalent Antioxidant Capacity by ABTS), and (b) FRAP (Ferric Reducing Antioxidant Potential) methods [mmol Trolox/100 g dm]. a, b, c, … - different letters indicated statistical differences ($p < 0.05$; HSD Tukey test).

The carrier addition caused an approx. 2-fold decrease in antioxidant capacity. Moreover, it was observed that 'Bluecrop' inulin-free and 'Bluejay' inulin loaded samples had the highest antioxidant capacity. An increase in temperature during vacuum drying resulted in products with antioxidant capacity similar or even higher than freeze- or spray-dried ones. This may be ascribed to the release of particular constituents from more polymerized structures or their structural changes, resulting in a presence of compounds with higher antioxidant capacity than parental ones (Liu et al., 2021; Michalska-Ciechanowska, **Brzezowska**, et al., 2021). It can also be linked to the possible formation of Maillard reaction and/or caramelization products during heat treatment, which may exhibit antioxidant properties (Nooshkam et al., 2019) that could additionally improve powders properties. After carrier application, drying did not affect the antioxidant capacity of the powders in the same way for all cultivars, which may indicate multiple interactions between inulin and the matrix components during drying, including interactions resulting in enhanced antiradical properties.

Considering all studied cultivars together, although the sum of the identified (poly)phenolics had a significant effect on the antioxidant capacity of the powders (r = 0.93 for TEAC ABTS and r = 0.95 for FRAP) (**Figure 19**), it was flavonols that were the most prominent compounds, the presence of which was correlated the most with the values measured by both the ABTS (r = 0.90) and the FRAP (r = 0.92) method, despite the fact that these constituents were not the dominant ones in the blueberry products.

Antibacterial and anti-inflammatory properties

Effect of drying technique, cultivar, and inulin addition on the antibacterial properties of blueberry powders

One of the most relevant bioactivities associated with plant products rich in (poly)phenolic compounds, such as blueberry powders, is their antibacterial capacity. In the study, none of the blueberry powders showed antibacterial activity against *E. coli*, *S. enterica*, *S. aureus*, and *L. monocytogenes* strains (data not shown). This is contrary to the previous studies describing blueberry effectiveness against these microorganisms (Shen et al., 2014; Silva et al., 2015). This confirms that moderation of fruit-based matrices affects biological properties of powdered products (Capuano et al., 2018). Although the (poly)phenolics composition may differ depending on the powder production method, involving matrix

modification, the most disputable aspect in the previous works was a relatively high concentration of experimental samples used. In this regard, it was reported that blueberry samples showed an inhibitory effect against *L. monocytogenes* and *S. aureus* strains, but this result was obtained using experimental concentrations of 300 - 900 mg/mL (Shen et al., 2014) and of 50 - 200 mg/mL (Khalifa et al., 2015; Zhou et al., 2020), respectively. The same applies to *E. coli* and *Salmonella* strains – blueberry sample concentrations of 25 - 50 mg/mL were used to obtain the inhibitory effect against *E. coli* (Khalifa et al., 2015) or even higher concentrations were used against *Salmonella* (450 - 1,800 mg/mL) (Shen et al., 2014). Considering that in most cases the strength of the inhibitory effect was dose-dependent, the importance of using experimental samples compatible with their practical application has been highlighted. In this sense, all blueberry powders with and without inulin (2 mg/mL) used in the present work significantly inhibited the growth ($p < 0.05$) of the microaerophilic strains *C. jejuni* and *H. pylori* (**Table 10**), to a different extent, compared to the experimental growth control.

For *C. jejuni* (**Table 10**), when analyzing the effect of carrier addition, all samples without inulin were significantly more active in reducing bacterial growth (from 0.7 to 2.4 log CFU/mL reduction) than those with 5% inulin (from 0.3 to 1.1 log CFU/mL reduction), irrespectively of the cultivar and drying treatment applied. This behavior seems consistent with higher content of identified (poly)phenolics in the samples without inulin compared to those with carrier addition (**Table 9a** and **9b**). Considering the influence of the blueberry cultivar, 'Bluejay' powders showed the highest antibacterial activity, independently of the drying technique, reducing *Campylobacter* growth between 1.40 and 2.38 log CFU/mL in absence of inulin, and between 0.70 and 1.09 log CFU/mL with inulin added.

Table 10. Antibacterial activity against *Campylobacter jejuni* and *Helicobacter pylori* of three cultivars of blueberry juice extract powders from different dried treatments with or without a carrier. Results are expressed as Log CFU/mL (mean ± SD).

		Cultivar	FD	VD50	VD70	VD90	SD
Campylobacter jejuni	No carrier†	Bluejay	7.82 ± 0.10* ^{aC}	7.75 ± 0.10* ^{abC}	7.31 ± 0.05* ^{aB}	6.84 ± 0.06* ^{aA}	7.69 ± 0.07* ^{aC}
		Berkley	8.12 ± 0.06* ^{bC}	7.89 ± 0.14* ^{bB}	8.00 ± 0.06* ^{cBC}	7.69 ± 0.16* ^{bA}	7.83 ± 0.17* ^{aAB}
		Bluecrop	8.55 ± 0.07* ^{cB}	7.56 ± 0.19* ^{aA}	7.62 ± 0.22* ^{bA}	7.62 ± 0.08* ^{bA}	8.46 ± 0.07* ^{bB}
	5% Inulin†	Bluejay	8.52 ± 0.07* ^{aB}	8.47 ± 0.03* ^{aB}	8.13 ± 0.07* ^{aA}	8.18 ± 0.05* ^{aA}	8.42 ± 0.09* ^{aB}
		Berkley	8.76 ± 0.04* ^{bA}	8.94 ± 0.03* ^{bB}	8.99 ± 0.05* ^{cB}	8.83 ± 0.07* ^{bA}	8.75 ± 0.08* ^{bA}
		Bluecrop	8.77 ± 0.06* ^{bB}	8.50 ± 0.17* ^{aA}	8.77 ± 0.11* ^{bB}	8.79 ± 0.08* ^{bB}	8.89 ± 0.03* ^{bB}
Helicobacter pylori	No carrier†	Bluejay	2.15 ± 0.17* ^{bB}	<1.48* ^{aA}	2.33 ± 0.21 ^{bB}	2.24 ± 0.37 ^{bB}	<1.48 ^{aA}
		Berkley	2.60 ± 0.05* ^{bB}	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}	2.57 ± 0.04* ^{bB}
		Bluecrop	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}	<1.48* ^{aA}
	5% Inulin†	Bluejay	2.67 ± 0.08* ^{aB}	2.33 ± 0.06* ^{aB}	2.68 ± 0.20 ^{aB}	2.54 ± 0.34 ^{aB}	<1.48 ^{aA}
		Berkley	5.43 ± 0.05* ^{cC}	5.80 ± 0.14* ^{bD}	4.53 ± 0.06* ^{bA}	4.87 ± 0.07* ^{cB}	4.94 ± 0.04* ^{cB}
		Bluecrop	4.70 ± 0.06* ^{bB}	5.95 ± 0.11* ^{bC}	4.69 ± 0.09* ^{bB}	3.18 ± 0.03* ^{bA}	3.26 ± 0.24* ^{bA}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying;
† All samples showed statistical difference in comparison with the control growth ($p < 0.05$, HSD Tukey test).
* Values with asterisk denote statistical difference between the same cultivar with and without inulin for each treatment ($p < 0.05$, *t*-test).
a,b,c - Values with different lowercase letters denote statistical difference within a column ($p < 0.05$, HSD Tukey test) (Effect of blueberry cultivar for each treatment).
A,B,C - Values with different capital letters denote statistical difference within a row ($p < 0.05$, HSD Tukey test) (Effect of drying treatment for each blueberry cultivar).
Control growth = 9.22 ± 0.03 Log CFU/mL
Growth detection limit = 1.48 Log CFU/mL

The highest content of (poly)phenolics was obtained from 'Bluecrop' (**Table 9a** and **9b**), which suggests that it is not the (poly)phenolic compounds in general, but the presence of some specific ones, which may determine the efficacy of the powders as antibacterial factor against *Campylobacter* (Silván et al., 2013). In this respect, particular (poly)phenolics present in different cultivars have been shown to be antibacterial agents against *Campylobacter*. In this study, a moderate correlation was found only between the inhibition of the growth of *C. jejuni* and the content of caffeoyl-glucose (r = 0.62) and identified anthocyanins (r = 0.57) (**Figure 19**), respectively, for all cultivars. However, at a closer look, a strong correlation

between caffeoyl-glucose content and *Campylobacter* growth was indicated for 'Bluejay' ($r = 0.79$) and 'Berkley' ($r = 0.93$) (**Figure 21** and **22**). As for anthocyanins, there was a significant correlation between the growth of *Campylobacter* and the anthocyanin content in 'Bluejay' ($r = 0.65$) and 'Berkley' ($r = 0.85$), but no significant correlation was found for 'Bluecrop' (**Figure 20**).

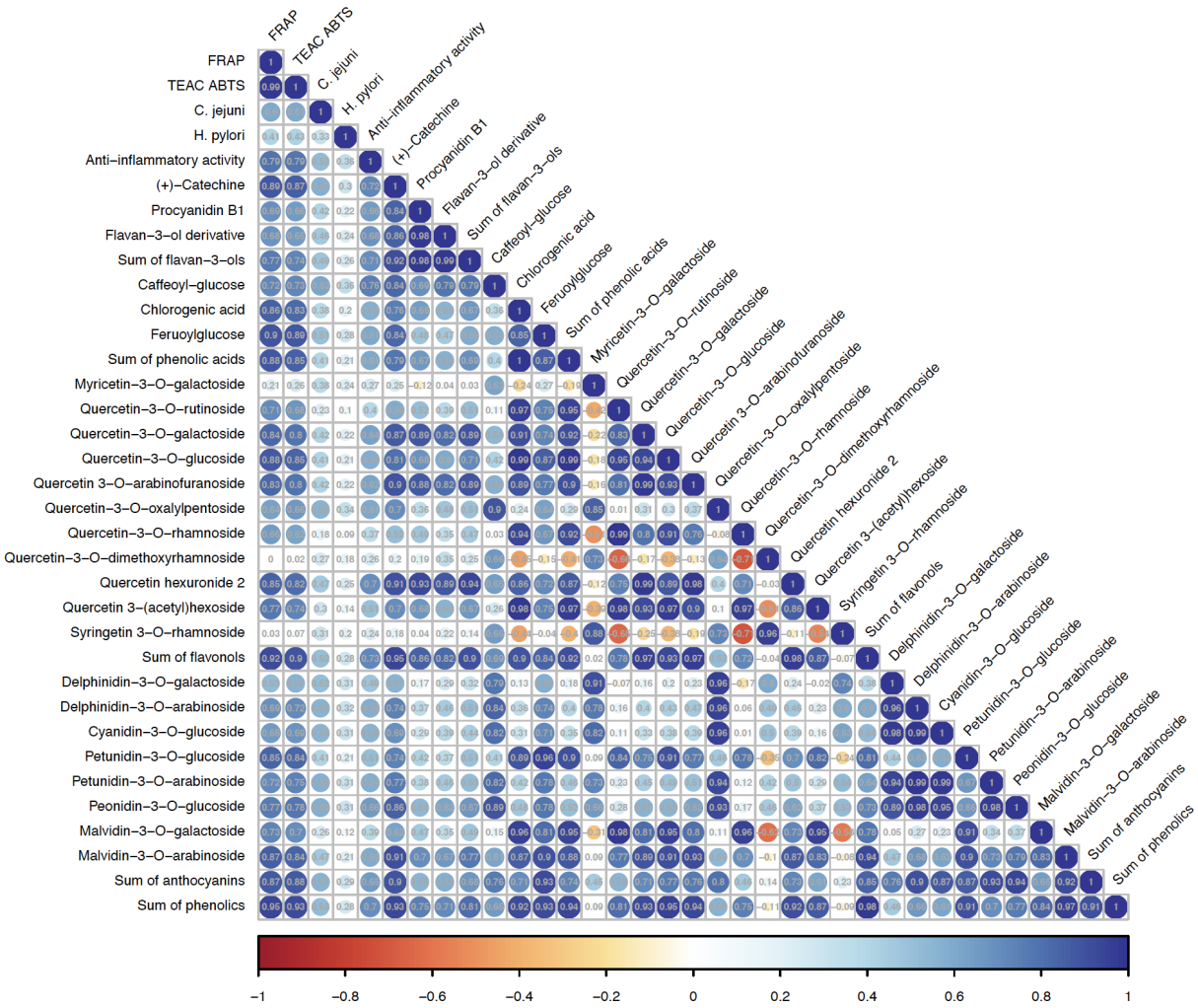


Figure 19. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antibacterial/anti-inflammatory activity, identified (poly)phenolics and their groups) in blueberry juice extract powders (**whole** samples data set). Positive and negative correlations are indicated in blue and red, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; *C. jejuni* – inhibition of *Campylobacter jejuni* growth; *H. pylori* – inhibition of *Helicobacter pylori* growth; Anti-inflammatory activity – pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori.*

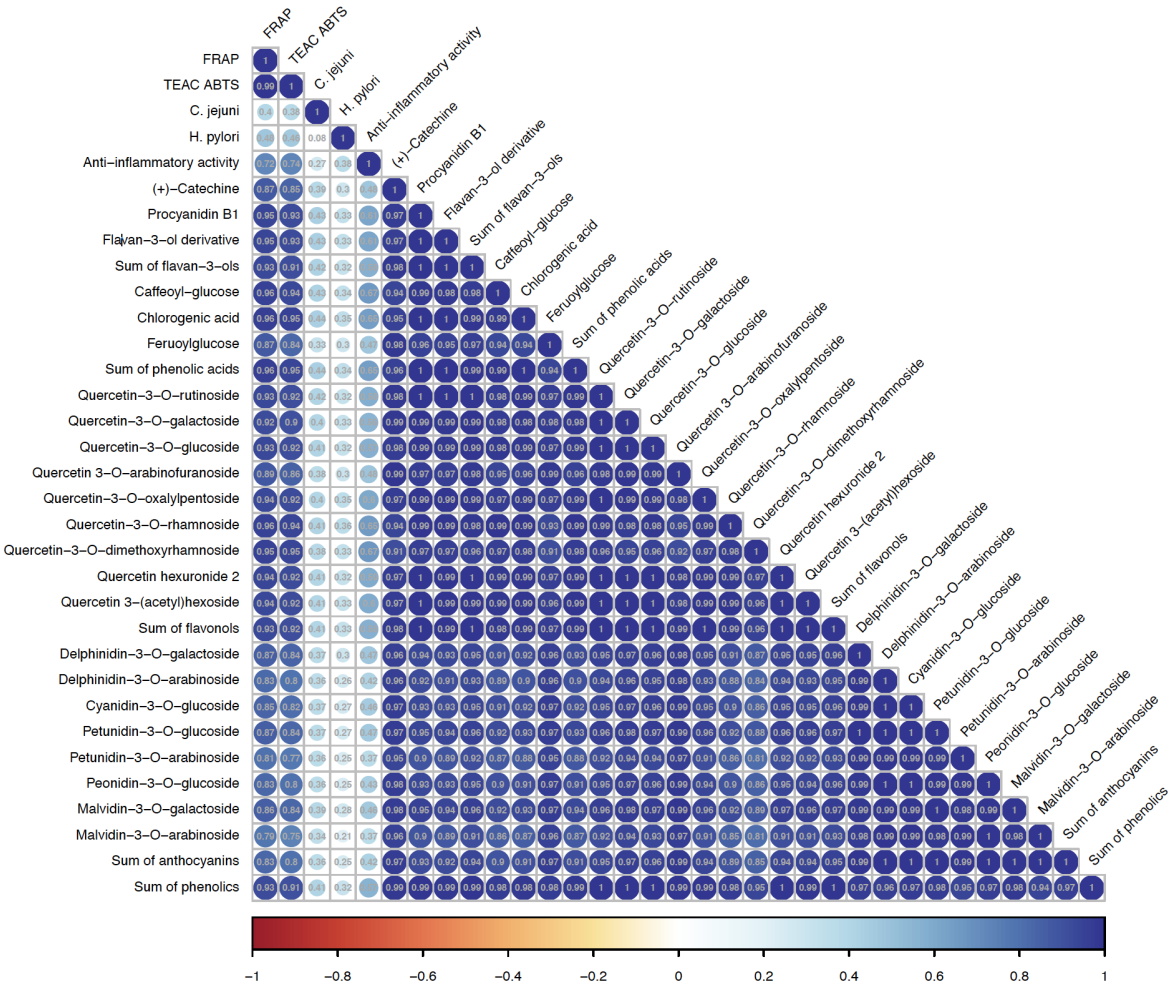


Figure 20. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antibacterial/anti-inflammatory activity, identified (poly)phenolics and their groups) in blueberry juice extract powders (*cv.* **Bluecrop samples** data set). Positive and negative correlations are indicated in blue and red, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; *C. jejuni* – inhibition of *Campylobacter jejuni* growth; *H. pylori* – inhibition of *Helicobacter pylori* growth; Anti-inflammatory activity – pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori*

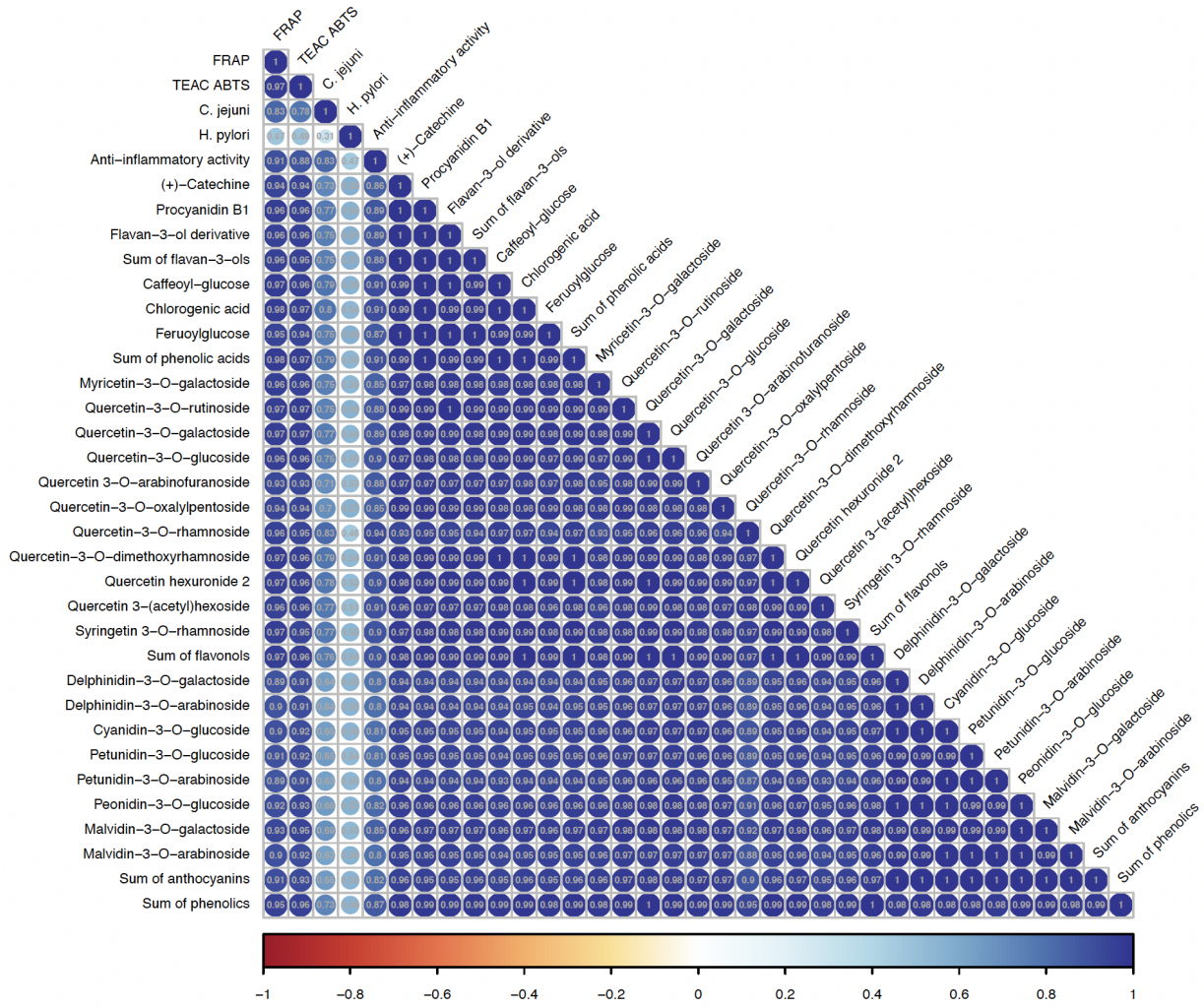


Figure 21. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antibacterial/anti-inflammatory activity, identified (poly)phenolics and their groups) in blueberry juice extract powders (*cv.* **Bluejay samples** data set). Positive and negative correlations are indicated in blue and red, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; *C. jejuni* – inhibition of *Campylobacter jejuni* growth; *H. pylori* – inhibition of *Helicobacter pylori* growth; Anti-inflammatory activity – pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori*.

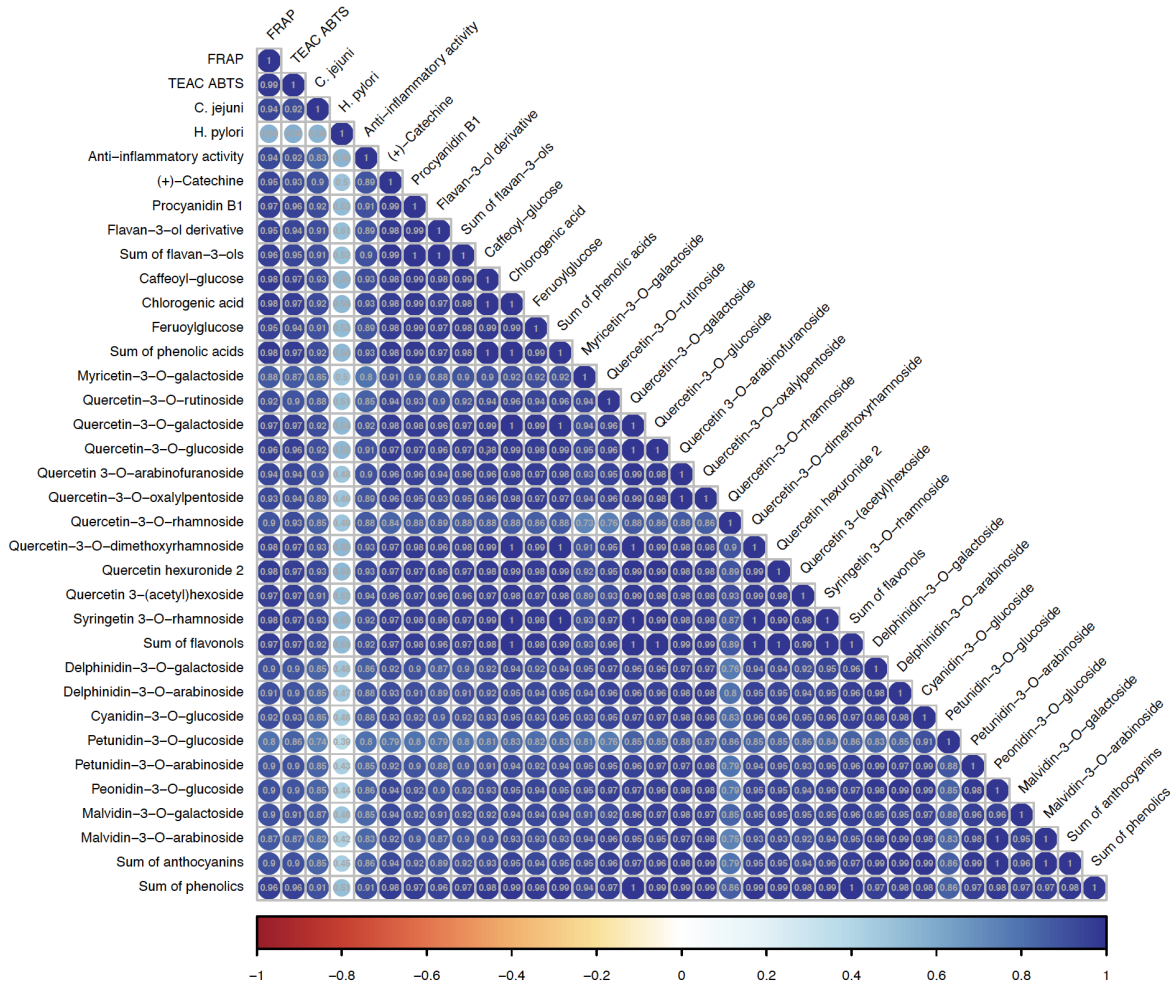


Figure 22. Correlogram depicts the strength and direction of a linear association between two variables (i.e., antioxidant/antibacterial/anti-inflammatory activity, identified (poly)phenolics and their groups) in blueberry juice extract powders (*cv.* **Berkley samples** data set). Positive and negative correlations are indicated in blue and red, accordingly, while the circle dimension and color intensity correspond proportionally to the correlation coefficients' values. Significant correlation coefficients are displayed as large circles representing a strong linear relation, and small circles indicate the smallest correlation coefficients considered insignificant. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; *C. jejuni* – inhibition of *Campylobacter jejuni* growth; *H. pylori* – inhibition of *Helicobacter pylori* growth; Anti-inflammatory activity – pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori*.

When it comes to the impact of the drying technique used, vacuum drying treatments produced powders with the strongest antibacterial activity from inulin-free extracts (**Table 10**). For 'Bluejay' and 'Berkley', the most effective treatment was vacuum drying at 90 °C, while for 'Bluecrop', the cultivar richest in (poly)phenolics, the antibacterial behavior was observed for all temperatures used (50, 70, and 90 °C). 'Bluejay' products without inulin and those obtained by vacuum drying at 90 °C achieved the greatest reduction of *Campylobacter* growth (2.4 CFU/mL log reduction). For inulin-added samples the behavior was similar. 'Bluejay' products after VD at 70 and 90 °C, 'Bluecrop' products after vacuum drying at 50 °C, and 'Berkley' products after vacuum drying at 90 °C caused the strongest reduction in

Campylobacter growth ($p < 0.05$). These results suggest that some compounds formed or released during vacuum drying could be involved in the antibacterial activity of these blueberry powders. It was reported that an increase in the temperature during vacuum drying could contribute to the enhancement of some bioactive properties, such as antioxidant capacity. This behavior has been attributed to the release of specific compounds from more polymerized structures and to the possible formation of Maillard reaction or caramelization products formed during heat treatment (Liu et al., 2021; Michalska-Ciechanowska, **Brzezowska**, et al., 2021). These newly formed products could show antibacterial activity (Chua et al., 2019) that may also contribute to improving the properties of blueberry powders. Although the antibacterial activity of blueberry products against *Campylobacter* was not higher than 3 log reduction with respect to the experimental control, the obtained results can be considered relevant in practical terms. This is because a reduction of 2 log CFU in the number of campylobacters colonizing poultry (main source of human infection by *Campylobacte*r) can have a significant impact on consumer health, reducing human infections by a median value of 42% (EFSA Panel on Biological Hazards (BIOHAZ), 2020). In the case of the poultry meat food chain, natural compounds such as blueberry powders could potentially be used at different stages of the food chain, from on-farm feed additives to packaging products.

On the other hand, the most relevant antibacterial effect was observed against *H. pylori*. Just as for *C. jejuni*, but at a higher level, all blueberry (with and without inulin) showed a significant antibacterial activity ($p < 0.05$) against *H. pylori* compared to the experimental growth control (**Table 10**). The antibacterial activity of carrier-free samples was significantly ($p < 0.05$) higher (5.5 log CFU/mL minimal growth reduction) than that of powders with inulin for each cultivar and same drying technique (2.17 log CFU/mL minimal growth reduction), except for 'Bluejay' products obtained by spray drying, vacuum drying at 70 °C, and 90 °C, which showed similar antibacterial efficacy to the samples with inulin. It is noteworthy that ten of fifteen blueberry powders without inulin showed a bactericidal effect against *H. pylori*, while only one sample with 5% inulin was bactericidal ('Bluejay' product obtained by SD), which is linked to higher (poly)phenolics content. In terms of cultivar, 'Bluecrop' powders without inulin showed the strongest antibacterial activity, exhibiting bactericidal effects regardless of the drying techniques used. As described above, this blueberry cultivar had the highest content of (poly)phenolics (**Table 9a** and **9b**). Bactericidal properties were also identified in 'Berkley' powders obtained by vacuum drying (regardless the temperature) as well as in two 'Bluejay' powders vacuum drying at 50 °C and spray drying. However, inulin-supplemented 'Bluejay'

powders were the most active in reducing bacterial growth from 5.44 log CFU/mL to a total inhibition (bactericidal effect), regardless of the drying technique used. With respect to the influence of the drying techniques, vacuum drying treatments turned out to be the most effective in samples without inulin. All blueberry juice extract powders obtained by vacuum drying at 50 °C showed a bactericidal effect. The products obtained at 70 °C and 90 °C showed the same behavior, except for 'Bluejay'. Finally, for inulin-added powders, those obtained by spray drying from 'Bluejay', vacuum drying at 70 °C from 'Berkley', and vacuum drying at 90 °C and spray drying from 'Bluecrop' showed high antibacterial activity in each case.

Effect of drying method, blueberry cultivar, and inulin addition on the anti-inflammatory activity of blueberry powders on gastric cells infected with *H. pylori*

The inflammatory response of the gastric epithelium is directly related to the progression of pathologies associated with *H. pylori* infection. For this reason, it is crucial to modulate the inflammatory process to avoid the occurrence of cell damage (Silvan & Martinez-Rodriguez, 2022). In this regard, the study evaluated the ability of the blueberry powders to reduce the inflammatory process by decreasing IL-8 production, and the impact of blueberry cultivar, inulin addition, and drying treatment (**Table 11**). All blueberry powders without inulin showed a significant ($p < 0.05$) inhibition in IL-8 production (from 11.1% to 32.7%) compared to the experimental control group (100% of IL-8 production). However, samples with inulin did not exhibit a significant anti-inflammatory activity, except for the 'Bluejay' and 'Bluecrop' powders obtained by vacuum drying at 90 °C (9.4% and 14.2% of inhibition, respectively), and the 'Bluecrop' powders obtained by vacuum drying at 70 °C (10.2% of inhibition).

Most of the (poly)phenolic compounds identified in the samples have been associated with a relevant anti-inflammatory response (Puangpraphant et al., 2022), so their reduced content related with the use of inulin would explain the decrease in anti-inflammatory activity. It was previously observed for vacuum-dried plum juice powders that a higher anti-inflammatory property was influenced by both the type of treatment used and the temperature, finding that products treated by vacuum drying at 80 °C showed the highest anti-inflammatory capacity (Silvan, Michalska-Ciechanowska, et al., 2020). This behavior may apparently be associated with the detachment of specific compounds from more complex polymers and the formation of Maillard reaction products, which have shown to be particularly effective in

inhibiting IL-8 production in intestinal epithelial cells (Kitts et al., 2012). Regarding the impact of blueberry cultivar, reduction in IL-8 production values below 80% was only obtained for all 'Bluejay' inulin-free powders. Anthocyanins, which have been predominant in 'Bluejay' powders (**Table 9a**), were positively correlated with the inhibition of IL-8 production by AGS cells infected with *H. pylori* ($r = 0.65$) (**Figure 19**), and it was in line with the anti-inflammatory activity of anthocyanins previously described for *H. pylori*-infected human gastric cells (Kim et al., 2013). However, the main reduction in IL-8 production by the infected AGS cells was observed for the 'Bluecrop' powders obtained by vacuum drying at 90 and 70 °C (30.8% and 32.7% of inhibition). In these cases, as mentioned above, other compounds produced during vacuum drying seem to be involved.

Table 11. Effect of blueberry juice extract powders on pro-inflammatory cytokine IL-8 production in AGS cells infected by *Helicobacter pylori*. Results are expressed as % production of IL-8 with respect to the untreated infected cells.

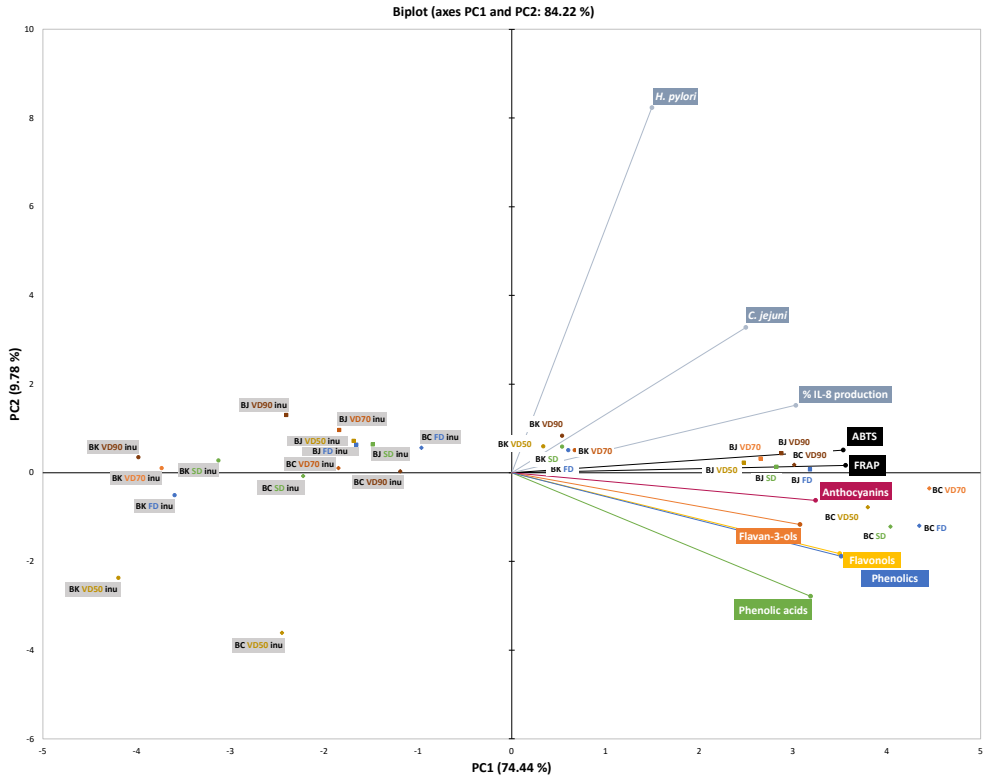
	Cultivar	FD	VD50	VD70	VD90	SD
No carrier	Bluejay	78.2 ± 1.2†* ^{aA}	76.8 ± 1.0†* ^{aA}	74.5 ± 0.7†* ^{bA}	72.9 ± 2.1†* ^{bA}	78.1 ± 4.8†* ^{aA}
	Berkley	82.9 ± 3.4†* ^{aA}	84.1 ± 2.2†* ^{bA}	80.4 ± 4.3†* ^{cA}	77.8 ± 4.3†* ^{bA}	83.0 ± 1.9†* ^{bA}
	Bluecrop	81.5 ± 4.0†* ^{aB}	88.9 ± 2.6†* ^{bB}	67.3 ± 5.0†* ^{aA}	69.2 ± 0.7†* ^{aA}	85.3 ± 0.2†* ^{bB}
5% Inulin	Bluejay	95.9 ± 5.1* ^{aB}	95.8 ± 5.9* ^{aB}	97.9 ± 2.9* ^{bB}	90.6 ± 1.9†* ^{aA}	97.2 ± 2.5* ^{aB}
	Berkley	99.2 ± 1.1* ^{aA}	98.1 ± 2.7* ^{aA}	94.1 ± 1.5* ^{bA}	99.3 ± 0.9* ^{bA}	95.0 ± 2.6* ^{aA}
	Bluecrop	95.4 ± 4.6* ^{aB}	96.8 ± 0.2* ^{aB}	89.8 ± 2.3†* ^{aA}	85.8 ± 3.1†* ^{aA}	97.2 ± 4.0* ^{aB}

FD - freeze-drying; VD - vacuum drying at 50 °C (VD50), 70 °C (VD70), 90 °C (VD90); SD - spray drying;
† - Values denoted statistical difference in comparison with control groups (untreated AGS cells; 100% IL-8 production) ($p < 0.05$, HSD Tukey test).
a,b,c - Values with different lowercase letters denote statistical difference within a column (Blueberry cultivar in presence or absence of inulin) ($p < 0.05$, HSD Tukey test).
A,B,C - Different subscript letters denote statistical difference within a row (Drying treatments) ($p < 0.05$, HSD Tukey test).
* - Values with asterisk denote statistical difference between the same cultivar with and without inulin for each treatment ($p < 0.05$, t-test).

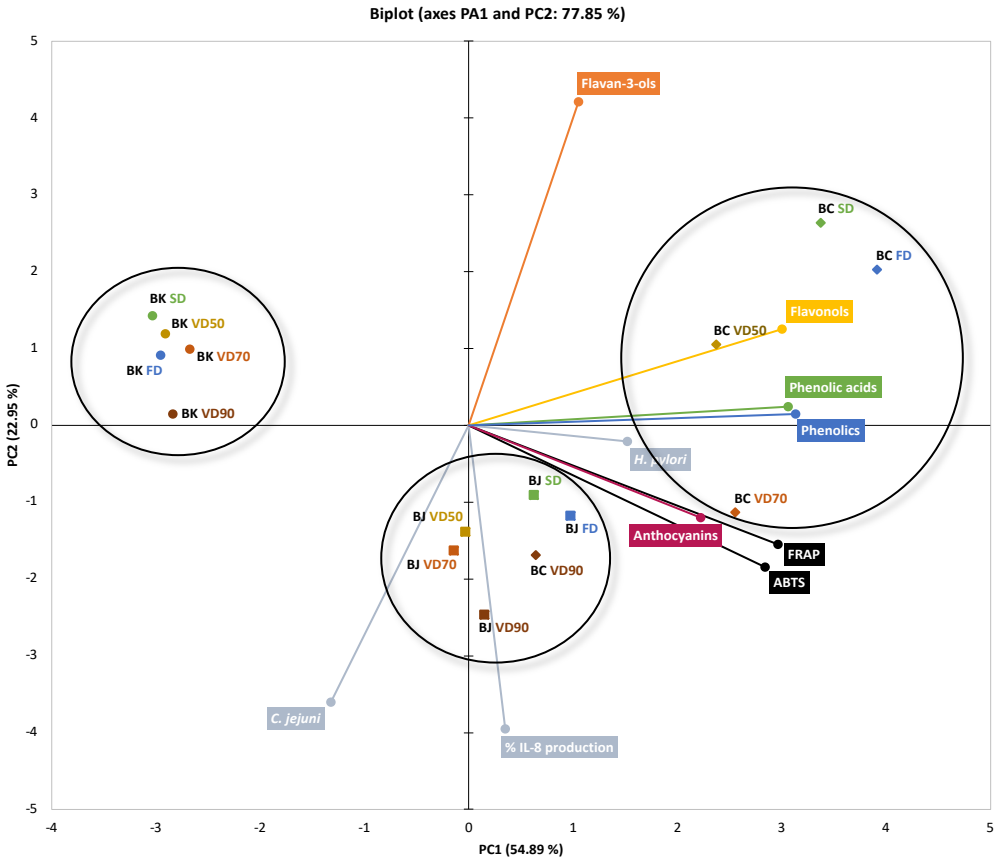
Chemometric analysis

The Principal Components Analysis (PCA) indicated the relationships between chemical composition ((poly)phenolics profile and content, antioxidant capacity) and *in vitro* biological properties of blueberry juice extract powders obtained from three different cultivars with or without the addition of inulin. In the case of whole data set (**Figure 23a**), the two main principal components identified (PC1 and PC2) explained 84.22 % of the total data variance. In **Figure 23a** two groups can be distinguished between variables and factors, i.e., powders with and without inulin addition. Moreover, among carrier-free samples, more clear distinction according to the cultivar was visible, while inulin addition partially masked these differences. When scrutinizing the data in more depth, compared to inulin-free samples (**Figure 23b**), the PCA showed how its application (**Figure 23c**) differentiates the (poly)phenolics content and bioactive properties of blueberry powders. A closer look at **Figure 23b** indicated that 3 groups of powders can be distinguished in terms of similarities of chemical and biological properties linked to the cultivar, except 'Bluecrop' powders after vacuum drying at 90 °C, which were grouped together with 'Bluejay' samples. It implied that only this product had similar quality as all 'Bluejay' samples. Drying technique had stronger influence on 'Bluecrop' samples compared to 'Berkley'. Addition of inulin grouped the samples as followed: (1) Berkley' powders and (2) 'Bluejay' and 'Bluecrop' powders (**Figure 23b** and **23c**). Moreover, in the second group, 'Bluecrop' powders were similar in terms of (poly)phenolics content and anti-inflammatory properties, while 'Bluejay' ones were comparable in terms of antioxidant capacity and inhibition of *C. jejuni* and *H. pylori* growth. This indicated that not the content, but the specific compounds are responsible for selected bioactive properties.

(a)


Biplot (axes PC1 and PC2: 84.22 %)
PC2 (9.78 %)
PC1 (74.44 %)
H. pylori
C. jejuni
% IL-8 production
ABTS
FRAP
Anthocyanins
Flavan-3-ols
Flavonols
Phenolics
Phenolic acids

(b)


Biplot (axes PA1 and PC2: 77.85 %)
PC2 (22.95 %)
PC1 (54.89 %)
Flavan-3-ols
BC SD
BC FD
Flavonols
BC VD50
Phenolic acids
Phenolics
H. pylori
BC VD70
Anthocyanins
FRAP
ABTS
BK SD
BK VD50
BK VD70
BK FD
BK VD90
BJ SD
BJ FD
BJ VD50
BJ VD70
BC VD90
BJ VD90
C. jejuni
% IL-8 production

(c)

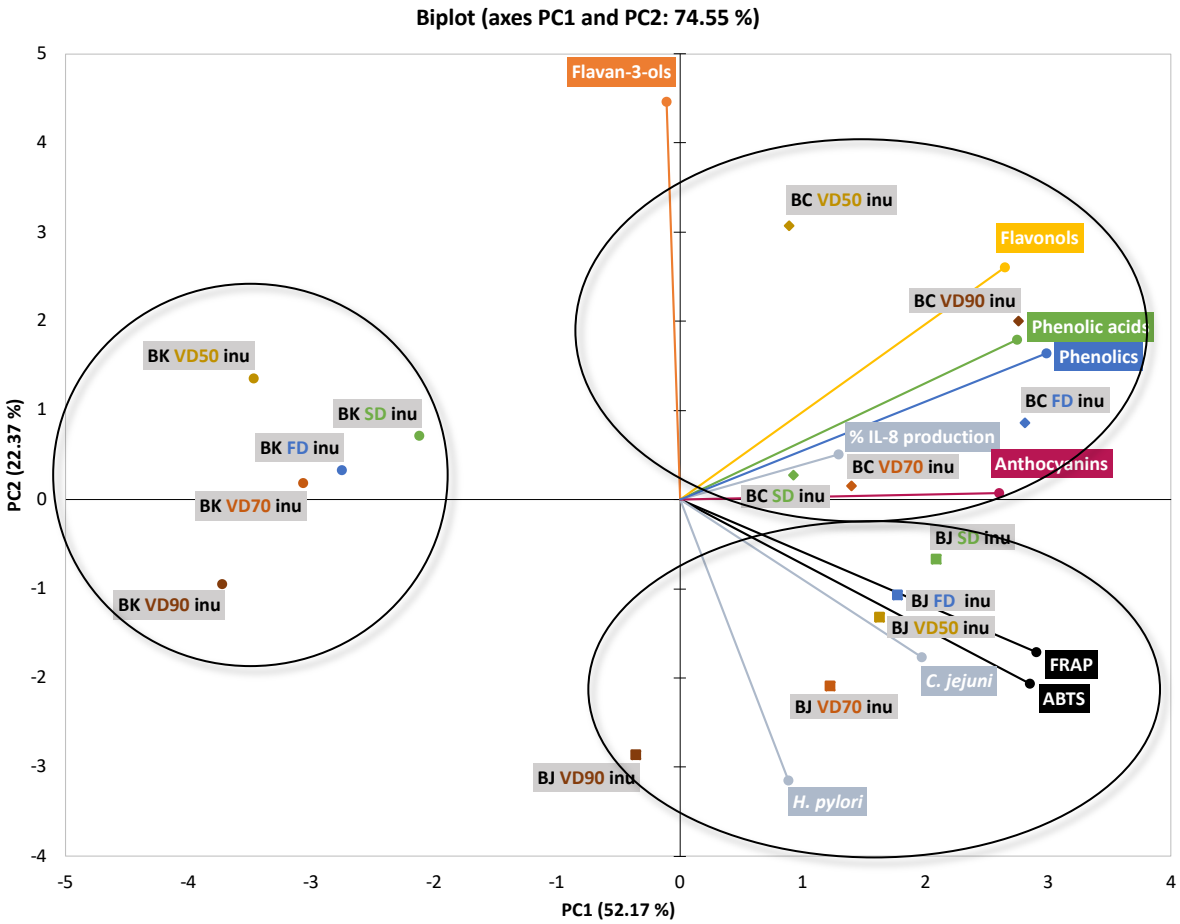


Figure 23. Principal Components Analysis (PCA) biplot that indicates principal components (PC) scores of blueberry juice extract powders with and without inulin (inu) addition gained after freeze-drying (FD), vacuum drying at 50 °C (VD50), vacuum drying at 70 °C (VD70); vacuum drying at 90 °C (VD90) and spray drying (SD) obtained from Berkley (BK; ●), Bluecrop (BC; ◆) and Bluejay (BJ; ■) for: (**a**) whole data set; (**b**) only inulin-free powders data set; (**c**) only inulin-added powders data set. TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS; FRAP – Ferric Reducing Antioxidant Potential; *C. jejuni* – inhibition of *Campylobacter jejuni* growth; *H. pylori* – inhibition of *Helicobacter pylori* growth; Anti-inflammatory activity – pro-inflammatory cytokine IL-8 production in AGS cells infected by *H. pylori.*

Summary

The study demonstrated a novel approach towards tailor-made powder manufacturing based on natural bioactives in blueberry (poly)phenolic-rich products, taking into account the influence of multistep processing.

The physical properties of the blueberry powders were strongly affected by the drying technique, with the vacuum drying being the preferred treatment resulting in the lowest moisture content and water activity as well as the highest bulk density values. Among the analyzed cultivars, 'Bluecrop' powders were the richest source of (poly)phenolics, whereas syringetin-3-*O*-rhamnoside and myricetin-3-*O*-galactoside were identified only in 'Berkley' and 'Bluejay' samples. Although the application of inulin resulted in powders with about 2-fold lower (poly)phenolic content and antioxidant capacity compared to carrier-free samples, for those with inulin, it appeared to provide protection for selected variants. In general, spray-dried powders were of similar or better quality compared to freeze-dried ones. Antibacterial activity towards *C. jejuni* was found to depend on the presence of particular bioactives, rather than on their total amount. As many as ten out of fifteen blueberry inulin-free products showed a bactericidal effect against *H. pylori*, whereas the same was true for only one inulin-supplemented product. Cultivar was shown to strongly affect the anti-inflammatory properties of blueberry products, and vacuum drying at higher temperatures was found to even enhance this effect for some powder variants, unlike the other treatments (freeze- and spray drying).

Recommendation: The study indicated that the blueberry cultivar composition and its matrix modifications through inulin addition and drying technique can serve as a tool for designing products with programmed antimicrobial and anti-inflammatory potential with possible application in customized food production. However, this approach should be adapted to specific plant-based matrix composition as numerous bioactives components (qualitative and quantitative differences) may diversely interact under specific processing conditions.

5.3.2. Publication 2: *Beetroot juice powders case study*

The results obtained from the study presented in the **Publication 2** shed a new light on the physico-chemical and functional properties of beetroot juice powders. The main concept was to evaluate the influence of the juice pre-treatment (fermentation) and carrier type (maltodextrin, inulin, oligofructose, and Nutriose®) used during freeze- and spray drying on the physical attributes, (poly)phenolics profile, HMF formation, as well as *in vitro* antioxidant, antiglycation and antiproliferative activities toward human leukemia cell lines. In addition, for the first time, the carrier type was tested in terms of the effectiveness of plant-based powders in inhibiting (or stimulating) cancer cell proliferation *in vitro*.

The juice fermentation turned out to affect powders quality starting from the physical attributes, however its impact was strongly linked to the other steps of powdering process, namely, particular carrier type addition and drying technique applied. When taking into account the moisture content, spray drying resulted in powders with its lower values compared to lyophilization. Interestingly, and in line with previous observations (Kuhn et al., 2020; Michalska-Ciechanowska, Hendrysiak, et al. 2021), inulin was an exception among the carriers used in freeze-drying that yielded the lowest *Mc* values of powdered products. This repetitive pattern across different studies has been attributed to the stability of inulin, for which exposure to different processing conditions (changing thermal and pH treatment) may affect its water-binding capacity, resulting in an ambiguous response of this carrier in a given matrix (Ozyurt & Ötles, 2016; Li et al., 2019). Based on the water activity measurement which proved all the powders obtained as microbiologically stable (Fontana, 2020), the multidirectional interplay between initial matrix composition (juice probiotically fermented or not) and applied processing was found. For instance, with respect to maltodextrin, the application of which resulted in comparable water activity after freeze- and spray drying regardless of the matrix used, oligofructose on the other hand resulted in products with the highest a_w values after lyophilization, whereas they were about 3 times lower when spray-drying was used. In the case of the application of inulin and Nutriose®, other relationships were revealed in which the pre-treatment of the matrix proved to have a decisive influence and induced an inconsistent behavior depending on the drying technique used. For example, products made from non-fermented juice using Nutriose® freeze-drying had over 2.5 times higher water activity than those prepared in the same way from fermented juice. Also, in the case of the color, it was observed that fermentation affected the yellowness of final products, which was considerably lower when fermented juice was used for their production. This was attributed to the presence of compounds

with yellow pigmentation, which could be formed as a result of Maillard reaction or caramelisation during water removal from the unfermented sugars present in higher levels in non-fermented juice than in fermented juice, as it was indicated by Hashemi et al. (2021). This reflects the scale on which the observed relationships can vary, depending not only on the processing used and its variables, but more importantly on the matrix being processed.

The (poly)phenolics identified in beetroot juice powders were classified into three main groups, i.e., betalains (the predominant group, accounting for 80% of all identified compounds), syringic acid derivatives (16%) and ferulic acid derivatives (4%). As a result of fermentation, the powder obtained from the juice treated by this process contained approximately one fifth less betalains than its unfermented counterpart. Considering the carrier type influence, based on the percentage share it was noted that oligofructose resulted in the greatest betalains retention while inulin in syringic acid derivatives. The ferulic acid derivatives proved to be the most relatively stable group during applied processing, as their proportion in the overall content was comparable regardless of the type of carrier used. In addition, the antioxidant capacity analysis together with the Total Phenolic Content assay showed a clear pattern that samples with oligofructose and inulin were characterized by significantly higher values of these parameters compared to maltodextrin and Nutriose®. When considering the drying technique employed, in line with Michalska-Ciechanowska, Brzezowska, et al. (2021), freeze- and spray drying yielded similar powders in terms of (poly)phenolic content, indicating a comparable efficiency of these two techniques in their retention.

Since, as mentioned above, processing can trigger reactions responsible for the formation of undesirable components, the analysis of hydroxymethyl-*L*-furfural, as the most widely studied food-processing-indicator, allowed the safety of the obtained beetroot powders to be assessed. In contrast to maltodextrin, inulin and Nutriose®, there is very little information available on the use of oligofructose as a carrier, especially in the context of the formation of process contaminants. Therefore, the finding of HMF formation only in powders where this substance has been applied is of great scientific and industrial importance. The phenomenon was ascribed to the compositional difference between carriers, namely sugar type and well as degree of polymerization (DP). In contrast to maltodextrin and Nutriose®, which are predominantly composed of glucose units, oligofructose is a short-chain fructan with a degree of polymerization of 2 - 9, which is significantly shorter than inulin (DP: from 2 to 60+) (Jackson et al., 2022). Consistently, oligofructose is much more susceptible to decomposition changes and is therefore the main substrate for the Maillard and caramelization reactions. Considering the effect of the drying technique, Gökmen & Morales (2014) previously stated

that the presence of HMF can be confirmed in foods for which even mild conditions (leading to water removal and thus accelerating sugar dehydration) were applied during processing. Therefore, although spray drying might be more associated with its formation due to high temperature treatment (but for a short time), freeze-drying turns out to also evoke such changes leading to HMF formation in beetroot powders. For the remaining samples, wherein HMF was not identified, two explanations were proposed including insufficient substrate content in the case of samples form fermented juice (Hashemi et al., 2021), as well as, the inherent response of a particular formulation (including type of carrier added) undergoing processing (Capuano et al., 2018).

Finally, the biological potential of each food matrix should be considered as a joint resultant of physico-chemical changes. In this study, beetroot powders were tested for their *in vitro* antiglycation properties and antiproliferative activity towards human leukemia cell lines. It was shown that only the products obtained from probiotically fermented beetroot juice exhibited a stronger effect when studied in the methylglyoxal-*L*-arginine model system, which mimics the specific reaction between these substrates in the course of the transformation that takes place during glycation. Considering initial (Bovine Serum Albumin-glucose model) and middle (Bovine Serum Albumin-methylglyoxal model) stage of this glycation, no appreciable contribution was reported.

In order to assess the potential antiproliferative activity of beetroot powdered formulations, two human leukemia cell lines were used; the J45.01 line (ATCC® CRL1990 ™), isolated from the peripheral blood of a male patient with acute T-cell leukemia, and HL-60/MX2 line (ATCC® CRL2257 ™), derived from the HL-60 cell line isolated from peripheral blood leucocytes of a patient with acute promyelocytic leukemia. Changes in cell viability occurred when powdered juices (1 mg/mL) were added to a growth medium containing cancer cells. It was proven that Nutriose® addition to the formulation resulted in reduction of the cell viability when tested on J45.01 line (regardless juice type or drying technique applied), while in the case of HL-60/MX2 line, only when non-fermented juice matrix was used for powders preparation. On the contrary, the application of oligofructose turned out to stimulate the growth of cancerous cells in the J45.01 line, regardless juice type and drying technique used. Accordingly, it is therefore possible that the anti-proliferative properties may be moderated by the type of carrier used. However, depending on the line employed for testing, the pattern of biological response was different. Therefore, the selectivity of beetroot powders towards particular leukemia cell line needs to be highlighted. Going into the details, a moderate positive correlation ($r = 0.56$) or no correlation ($r = -0.12$) was found between the betalains content and

the HL-60/MX2 and J45.01 lines, respectively, confirming the selective ability of this bioactive group to inhibit cancer cell proliferation depending on the line type, as was proven before by Lechner & Stoner, (2019). Also, the ambiguous effect of ferulic acid derivatives was confirmed by a strong negative correlation ($r = -0.83$) in the case of HL-60/MX2 human leukemia line, while no relationship was noted in case of the second line. Furthermore, not only bioactives but also the hydroxymethyl-*L*-furfural have been reported to influence the chemoprotective potential of beetroot powder, for which negative correlation in the case of J-45.01 ($r = -0.81$) as well as HL-60/MX2 ($r = -0.54$) lines were noted. And although it can be suggested that its presence may be responsible for the proliferation of cancer cells, even more so as it has only been identified in oligofructose powders, however, this effect can also be attributed to the characteristics of the formulation. This is linked to the fact that oligofructose is a short-chain fructan, consisting mainly of fructose units, which in turn contribute significantly to the development of cancer diseases (Nakagawa et al., 2020).

Conclusively, it was indicated that the initial matrix (fermented or non-fermented) combined with the addition of prebiotic carrier substance and subjected to given drying treatment moderated the quality of such products in terms of their physico-chemical and biological properties.

Recommendation: The outcomes of the study reflect a broad potential of beetroot powdered formulation, especially as a possibly functional additives to a various foodstuff, and gives an overview about pros and cons of their production form a processing point of view. Oligofructose should be carefully reconsidered for beetroot-originated matrix (and probably others) processing toward powders production as it turned out to not only drive the HMF formation, as well as, stimulate proliferation of cancerous cells *in vitro*. Nutriose® seems to be promising substance in the context of properties studied therein for beetroot juice matrix, however, each plant-based raw material constitutes an individual matrix that should be considered in light of its inherent complexity and characteristics, and therefore any processing should be carefully tailored.

6. CONCLUSIONS

The following conclusions were drawn from the results of the research carried out:

1. **The same processing conditions applied to the different fruit matrix (even from the same origin) moderated differently the same attributes of plant-based powders.** Based on the example of chokeberry and cranberry pomace extract matrices it was shown that the physical properties and chemical characteristics, including the presence of (poly)phenols, were a result of the joint interplay between matrix pre-treatment (extraction solvent), carrier addition and type, as well as the drying technique used for powders production. The incorporation of a binary carrier blend of maltodextrin and trehalose for lyophilization and vacuum drying at 90 °C resulted in the highest retention of (poly)phenols and minimized hydroxymethyl-*L*-furfural content for the preparation of chokeberry powders. In contrast to previously commonly used 30% acetone, the acidification of 50% ethanol can be considered as an effective extraction medium for the production of cranberry pomace extract powders, as the highest retention of (poly)phenolics and the lowest content of hydroxymethyl-*L*-furfural were observed for these products. Furthermore, the use of a carrier can be recommended as its addition resulted in a reduced hydroxymethyl-*L*-furfural content in the final powders. **Therefore, it has been evidenced that it is possible to control the formation of process contaminants by proper selection of matrix pretreatment as well as processing conditions, but it is directly linked to the matrix composition.**

2. **It was proven that the different bioactive response of plant-based powders is a result of given matrix composition specifically pretreated and subjected to particular processing conditions.** The antioxidant capacity of powders from different fruit fractions of selected botanical matrices was found to be influenced not only by the presence of (poly)phenolics, but also amino acids, with particular reference to the ornithine. The fractionation of the initial matrix resulted in a diverse distribution of (poly)phenolics in respective powders, however the pattern was not the same for each plant material used. The ambiguous behavior of the same (poly)phenolic groups with respect to α-amylase and α-glucosidase activity was attributed to the compositional heterogeneity of the fruit matrices tested in which the (poly)phenolics were present. Glycine was negatively correlated with α-glucosidase inhibiting activity. Flavonols have been shown to selectively inhibit glycation *in vitro* depending on the stage of the

reaction. Moreover, the interrelation between (poly)phenolics and selected amino acids were found to contribute to antiglycation potential of analysed powders.

3. **The formation of process contaminants is a result of the presence of basic components of the fruit matrices and can be additionally accelerated by the process parameters used. However, the fruit matrix complexification may lead to different responses in the formation of process contaminants.** Based on example models, i.e., the Japanese quince juice and blackcurrant juice model systems, it was proved that hydroxymethyl-*L*-furfural and furfural were formed only when vacuum drying from 60 °C and above was applied, suggesting that formation of these process contaminants after freeze-drying (demonstrated in stage I of the research) could be linked to other components than sugars and organic acids. No influence of ascorbic acid on their formation was indicated in presented models thus its presence cannot be solely responsible for process contaminants formation. Moreover, it was observed that among carriers tested, inulin resulted in powders with the highest hydroxymethyl-*L*-furfural content, confirming that its presence may trigger formation of this process contaminant under high-temperature treatment. Finally, for the first time it has been signaled that organic acids may also be involved in the formation of these unwanted constituents.

4. **Matrix heterogeneities originating from cultivar differentiation affect the biological properties of plant-based powders and can be additionally shaped (even improved) by processing conditions.** Substantial differences in the physico-chemical properties and antioxidant capacity of blueberry juice extract powders were found depending on the cultivar used. Analysed powders showed higher antibacterial activity against *Helicobacter pylori* when compared to *Campylobacter jejuni*. Products without inulin were more effective than those with its addition. Ten out of fifteen carrier-free powders showed bactericidal effect against *Helicobacter pylori*, while one among products with carrier application. The analysis of anti-inflammatory properties in gastric cell cultures infected with *Helicobacter pylori* revealed that for selected blueberry products the properties tested were enhanced by application of relatively high-temperature treatment (vacuum drying), compared to freeze- and spray drying.

5. **Matrix pre-treatment by probiotic fermentation followed by targeted processing can shape the physico-chemical properties and biological potential of plant-based powders in a multidirectional manner.** Based on the beetroot juice matrix, it was

shown that juice fermentation affected the physical attributes of resulting powders as well as reduced the betalains content in the final powders. Syringic acid derivatives were most effectively protected by inulin compared to other carriers used, while ferulic acid derivatives were relatively the most stable regardless of the processing applied compared to other (poly)phenolic groups. Oligofructose was reported to be a contributor to the formation of hydroxymethyl-*L*-furfural regardless of the processing applied, as well as the stimulator for proliferation of cancerous cells *in vitro*. A Nutriose® was found to be the most promising prebiotic carrier in the context of antiproliferative activity of beetroot powders toward human leukemia cell lines. Nevertheless, the joint interactions between influencing factors i.a. initial matrix composition, carrier type and drying technique used moderated tested properties in manifold directions, hence each variant of the powder resulted in an individual and inherent response.

Conclusively, it was confirmed that the inherent bioactive response exhibited by the final product is directly dependent on the broadly defined food matrix, which is subjected to specific processing conditions, and can be ambiguous with even the slightest change in one of the variables occurs. For this reason, **the development of plant-based powders with targeted qualities (including improved functional properties and/or decrease in formation of process contaminants) should be preceded by processing studies dedicated to the specific matrix destined for production**. In addition, the pros and cons of processing of selected botanical matrices into plant-based powders were outlined, and specific recommendations were proposed depending on the challenge identified in a given case study, Finally, the findings presented not only adds valuable insights that are lacking in the literature related to the development of high-quality powdered products based on plant matrices, but also highlights their wide potential in the context of food design with health-oriented and/or personalized properties. Accordingly, the outcome of this work provides a platform knowledge for further exploration into the development of such commodities, possibly even on an industrial scale.

The results obtained from the series of studies constituting the dissertation confirmed the research hypothesis that the adaptation of tailor-made processing for specific botanical matrices enables to shape broadly defined quality of plant powders towards their functional properties and minimized process contaminants presence.

7. REFERENCES

1. Addinsoft. (2022). XLSTAT statistical and data analysis solution. New York, USA. Retrieved from: https://www.xlstat.com/en.

2. Agcam, E. (2022). A kinetic approach to explain hydroxymethylfurfural and furfural formations induced by Maillard, caramelization, and ascorbic acid degradation reactions in fruit juice-based mediums. *Food Analytical Methods*, *15*(5), 1286–1299. https://doi.org/10.1007/s12161-021-02214-x

3. Aguilera, J. M. (2019). The food matrix: implications in processing, nutrition and health. *Critical Reviews in Food Science and Nutrition*, *59*(22), 3612–3629. https://doi.org/10.1080/10408398.2018.1502743

4. Aktağ, I. G., & Gökmen, V. (2020). A survey of the occurrence of α-dicarbonyl compounds and 5-hydroxymethylfurfural in dried fruits, fruit juices, puree and concentrates. *Journal of Food Composition and Analysis*, *91*, 103523. https://doi.org/10.1016/j.jfca.2020.103523

5. Aktağ, I. G., & Gökmen, V. (2021). Investigations on the formation of α-dicarbonyl compounds and 5-hydroxymethylfurfural in fruit products during storage: New insights into the role of Maillard reaction. *Food Chemistry*, *363*, 130280. https://doi.org/10.1016/j.foodchem.2021.130280

6. Barbosa-Cánovas, G. V., Ortega-Rivas, E., Juliano, P., Yan, H., & Barbosa-Cánovas, G. V. (2005). *Food Powders: Physical Properties, Processing, and Functionality*. Kluwer Academic/Plenum Publishers.

7. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

8. Bhandari, B. (2013). Introduction to food powders. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of Food Powders* (pp. 1–25). Woodhead Publishing.

9. Bicker, M., Hirth, J., & Vogel, H. (2003). Dehydration of fructose to 5-hydroxymethylfurfural in sub- and supercritical acetone. *Green Chemistry*, *5*(2), 280–284. https://doi.org/10.1039/b211468b

10. Bowden, N. A., Sanders, J. P. M., & Bruins, M. E. (2018). Solubility of the proteinogenic α-amino acids in water, ethanol, and ethanol–water mixtures. *Journal of Chemical & Engineering Data*, *63*(3), 488–497. https://doi.org/10.1021/acs.jced.7b00486

11. Brzezowska, J., Skrzypczak, K., Radzki, W., Turkiewicz, I. P., Ziaja-Sołtys, M., Bogucka-Kocka, A., Wojdyło, A., & Michalska-Ciechanowska, A. (2023). Comparative study of antioxidant, antiglycation and chemoprotective potential of beetroot juice powder formulations with functional carriers. *Food Bioscience*, *55*, 103049. https://doi.org/10.1016/j.fbio.2023.103049

12. Buchner, N., Krumbein, A., Rohn, S., & Kroh, L. W. (2006). Effect of thermal processing on the flavonols rutin and quercetin. *Rapid Communications in Mass Spectrometry*, *20*(21), 3229–3235. https://doi.org/10.1002/rcm.2720

13. Cai, D., Li, X., Chen, J., Jiang, X., Ma, X., Sun, J., Tian, L., Vidyarthi, S. K., Xu, J., Pan, Z., & Bai, W. (2022). A comprehensive review on innovative and advanced stabilization approaches of anthocyanin by modifying structure and controlling environmental factors. *Food Chemistry*, *366*, 130611. https://doi.org/10.1016/j.foodchem.2021.130611

14. Çalışkan Koç, G. (2020). The effect of different drying techniques and microwave finish drying on the powder properties of the red pepper powder (*Capsicum annuum* L.). *Journal of Food Science and Technology*, *57*(12), 4576–4587. https://doi.org/10.1007/s13197-020-04496-1

15. Capuano, E., Oliviero, T., & van Boekel, M. A. J. S. (2018). Modeling food matrix effects on chemical reactivity: challenges and perspectives. *Critical Reviews in Food Science and Nutrition*, *58*(16), 2814–2828. https://doi.org/10.1080/10408398.2017.1342595

16. Castagnini, J. M., Tappi, S., Tylewicz, U., Romani, S., Rocculi, P., & Dalla Rosa, M. (2021). Sustainable development of apple snack formulated with blueberry juice and trehalose. *Sustainability*, *13*(16), 9204. https://doi.org/10.3390/su13169204

17. Chua, L. Y. W., Chong, C. H., Chua, B. L., & Figiel, A. (2019). Influence of drying methods on the antibacterial, antioxidant and essential oil volatile composition of herbs: A review. *Food and Bioprocess Technology*, *12*(3), 450–476. https://doi.org/10.1007/s11947-018-2227-x

18. Crowe, J. H., Leslie, S. B., & Crowe, L. M. (1994). Is vitrification sufficient to preserve liposomes during freeze-drying? *Cryobiology*, *31*(4), 355–366. https://doi.org/10.1006/cryo.1994.1043

19. Deguchi, E., & Koumoto, K. (2011). Cellular zwitterionic metabolite analogs simultaneously enhance reaction rate, thermostability, salt tolerance, and substrate specificity of α-glucosidase. *Bioorganic & Medicinal Chemistry*, *19*(10), 3128–3134. https://doi.org/10.1016/j.bmc.2011.04.003

20. Del Castillo, M. D., Corzo, N., & Olano, A. (1999). Early stages of Maillard reaction in dehydrated orange juice. *Journal of Agricultural and Food Chemistry*, *47*(10), 4388–4390. https://doi.org/10.1021/jf990150x

21. Delshadi, R., Bahrami, A., Assadpour, E., Williams, L., & Jafari, S. M. (2021). Nano/microencapsulated natural antimicrobials to control the spoilage microorganisms and pathogens in different food products. *Food Control*, *128*, 108180. https://doi.org/10.1016/j.foodcont.2021.108180

22. Diez-Sánchez, E., Quiles, A., & Hernando, I. (2021). Interactions between blackcurrant polyphenols and food macronutrients in model systems: *In vitro* digestion studies. *Foods*, *10*(4), 847. https://doi.org/10.3390/foods10040847

23. EFSA Panel on Biological Hazards (BIOHAZ), Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Davies, R., De Cesare, A., Herman, L., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Alter, T., Crotta, M., … Chemaly, M. (2020). Update and review of control options for *Campylobacter* in broilers at primary production. *EFSA Journal*, *18*(4). https://doi.org/10.2903/j.efsa.2020.6090

24. El-Saber Batiha, G., Hussein, D. E., Algammal, A. M., George, T. T., Jeandet, P., Al-Snafi, A. E., Tiwari, A., Pagnossa, J. P., Lima, C. M., Thorat, N. D., Zahoor, M., El-Esawi, M., Dey, A., Alghamdi, S., Hetta, H. F., & Cruz-Martins, N. (2021). Application of natural antimicrobials in food preservation: Recent views. *Food Control*, *126*, 108066. https://doi.org/10.1016/j.foodcont.2021.108066

25. Enaru, B., Dreţcanu, G., Pop, T. D., Stănilă, A., & Diaconeasa, Z. (2021). Anthocyanins: factors affecting their stability and degradation. *Antioxidants*, *10*(12), 1967. https://doi.org/10.3390/antiox10121967

26. Ertan, K., Türkyılmaz, M., & Özkan, M. (2019). Effects of natural copigment sources in combination with sweeteners on the stability of anthocyanins in sour cherry nectars. *Food Chemistry*, *294*, 423–432. https://doi.org/10.1016/j.foodchem.2019.05.089

27. European Council (2019). A new strategic agenda 2019 – 2024, 20 June 2019. Retrieved from: https://www.consilium.europa.eu/media/39914/a-new-strategic-agenda-2019-2024.pdf [*Accessed 3 September 2023*].

28. Feumba Dibanda, R., Panyoo Akdowa, E., Rani P., A., Metsatedem Tongwa, Q., & Mbofung F., C. M. (2020). Effect of microwave blanching on antioxidant activity, phenolic compounds and browning behaviour of some fruit peelings. *Food Chemistry*, *302*, 125308. https://doi.org/10.1016/j.foodchem.2019.125308

29. Fitzpatrick, K., Kendrick, B., Santos, C., Green, P., Zhang, B., Hunt, D., Ronk, M., & Ying, L. (2013). Freeze-dry mediated formation of 5-(hydroxylmethyl)furfural. *Developments in Biotechnology and Bioprocessing*, 129–145. https://doi.org/10.1021/bk-2013-1125

30. Fontana, A. J. (2020). D: Minimum water activity limits for growth of microorganisms. In G. V. Barbosa-Cánovas, A. J. Fontana, S. J. Schmidt, & T. P. Labuza (Eds.), *Water Activity in Foods* (1st ed., pp. 571–572). Wiley. https://doi.org/10.1002/9781118765982.app4

31. Fujita, R., Hayasaka, T., Jin, S., Hui, S.-P., & Hoshino, Y. (2020). Comparison of anthocyanin distribution in berries of Haskap (*Lonicera caerulea* subsp. *edulis* (Turcz. Ex. Herder) Hultén), Miyama-uguisukagura (*Lonicera gracilipes* Miq.), and their interspecific hybrid using imaging mass spectrometry. *Plant Science*, *300*, 110633. https://doi.org/10.1016/j.plantsci.2020.110633

32. Gao, C., Zhao, S., Yagiz, Y., & Gu, L. (2018). Static, kinetic, and isotherm adsorption performances of macroporous adsorbent resins for recovery and enrichment of bioactive procyanidins from cranberry pomace. *Journal of Food Science*, *83*(5), 1249–1257. https://doi.org/10.1111/1750-3841.14142

33. Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry*, *48*(5), 1485–1490. https://doi.org/10.1021/jf991072g

34. Gençdağ, E., Özdemir, E. E., Demirci, K., Görgüç, A., & Yılmaz, F. M. (2022). Copigmentation and stabilization of anthocyanins using organic molecules and encapsulation techniques. *Current Plant Biology*, *29*, 100238. https://doi.org/10.1016/j.cpb.2022.100238

35. Gökmen, V., & Morales, F. (2014). Processing contaminants: hydroxymethylfurfural. In *Encyclopedia of Food Safety* (pp. 404–408). Elsevier. https://doi.org/10.1016/B978-0-12-378612-8.00209-2

36. Gościnna, K., Czapski, J., Mikołajczyk-Bator, K., & Kidoń, M. (2012). Content betalain pigments, nitrates and antioxidant capacity of beetroot juices depending on cultivars and the size of beetroot roots. *Aparatura Badawcza i Dydaktyczna*, *17*(3), 85–90.

37. Hamrouni-Sellami, I., Rahali, F. Z., Rebey, I. B., Bourgou, S., Limam, F., & Marzouk, B. (2013). Total phenolics, flavonoids, and antioxidant activity of sage (*Salvia officinalis* L.) plants as affected by different drying methods. *Food and Bioprocess Technology*, *6*(3), 806–817. https://doi.org/10.1007/s11947-012-0877-7

38. Hashemi, S. M. B., Jafarpour, D., & Jouki, M. (2021). Improving bioactive properties of peach juice using *Lactobacillus* strains fermentation: antagonistic and anti-adhesion effects, anti-inflammatory and antioxidant properties, and Maillard reaction inhibition. *Food Chemistry*, *365*, 130501. https://doi.org/10.1016/j.foodchem.2021.130501

39. Hassoun, A., Boukid, F., Pasqualone, A., Bryant, C. J., García, G. G., Parra-López, C., Jagtap, S., Trollman, H., Cropotova, J., & Barba, F. J. (2022). Emerging trends in the agri-food sector: Digitalisation and shift to plant-based diets. *Current Research in Food Science*, *5*, 2261–2269. https://doi.org/10.1016/j.crfs.2022.11.010

40. Herrera-Balandrano, D. D., Chai, Z., Beta, T., Feng, J., & Huang, W. (2021). Blueberry anthocyanins: An updated review on approaches to enhancing their bioavailability. *Trends in Food Science & Technology*, *118*, 808–821. https://doi.org/10.1016/j.tifs.2021.11.006

41. Hess, J. M., Comeau, M. E., Casperson, S., Slavin, J. L., Johnson, G. H., Messina, M., Raatz, S., Scheett, A. J., Bodensteiner, A., & Palmer, D. G. (2023). Dietary guidelines meet NOVA: Developing a menu for a healthy dietary pattern using ultra-processed foods. *The Journal of Nutrition*, *153*(8), 2472–2481. https://doi.org/10.1016/j.tjnut.2023.06.028

42. Ikhsanov, Y. S., Kusainova, K. M., Tasmagambetova, G. Y., Andasova, N. T., & Litvinenko, Y. A. (2021). Amino acid, fatty acid and vitamin composition of *Rosa canina* L. *Series chemistry and technology*, *447*(3), 39–43. https://doi.org/10.32014/2021.2518-1491.47

43. Jackson, P. P. J., Wijeyesekera, A., Theis, S., van Harsselaar, J., & Rastall, R. A. (2022). Food for thought! Inulin-type fructans: Does the food matrix matter? *Journal of Functional Foods*, *90*, 104987. https://doi.org/10.1016/j.jff.2022.104987

44. Ji, Y., Liu, D., jin, Y., Zhao, J., Zhao, J., Li, H., Li, L., Zhang, H., & Wang, H. (2021). *In vitro* and *in vivo* inhibitory effect of anthocyanin-rich bilberry extract on α-glucosidase and α-amylase. *LWT - Food Science and Technology*, *145*, 111484. https://doi.org/10.1016/j.lwt.2021.111484

45. Jia, W., Ma, R., Zhang, R., Fan, Z., & Shi, L. (2022). Synthetic-free compounds as the potential glycation inhibitors performed in in vitro chemical models: Molecular mechanisms and structure

requirements. *Trends in Food Science & Technology*, *128*, 147–159. https://doi.org/10.1016/j.tifs.2022.08.005

46. Jin, Y., Tang, J., & Sablani, S. S. (2019). Food component influence on water activity of low-moisture powders at elevated temperatures in connection with pathogen control. *LWT - Food Science and Technology*, *112*, 108257. https://doi.org/10.1016/j.lwt.2019.108257

47. Kammerer, J., Kammerer, D. R., & Carle, R. (2010). Impact of saccharides and amino acids on the interaction of apple polyphenols with ion exchange and adsorbent resins. *Journal of Food Engineering*, *98*(2), 230–239. https://doi.org/10.1016/j.jfoodeng.2010.01.001

48. Kavousi, P., Mirhosseini, H., Ghazali, H., & Ariffin, A. A. (2015). Formation and reduction of 5-hydroxymethylfurfural at frying temperature in model system as a function of amino acid and sugar composition. *Food Chemistry*, *182*, 164–170. https://doi.org/10.1016/j.foodchem.2015.02.135

49. Kerasioti, E., Apostolou, A., Kafantaris, I., Chronis, K., Kokka, E., Dimitriadou, C., Tzanetou, E. N., Priftis, A., Koulocheri, S. D., Haroutounian, S. A., Kouretas, D., & Stagos, D. (2019). Polyphenolic composition of *Rosa canina*, *Rosa sempervivens* and *Pyrocantha coccinea* extracts and assessment of their antioxidant activity in human endothelial cells. *Antioxidants*, *8*(4), 92. https://doi.org/10.3390/antiox8040092

50. Khalifa, H. O., Kamimoto, M., Shimamoto, T., & Shimamoto, T. (2015). Antimicrobial effects of blueberry, raspberry, and strawberry aqueous extracts and their effects on virulence gene expression in *Vibrio cholerae*: Antimicrobial and anti-virulence effects of berry extracts. *Phytotherapy Research*, *29*(11), 1791–1797. https://doi.org/10.1002/ptr.5436

51. Kim, J.-M., Kim, K.-M., Park, E.-H., Seo, J.-H., Song, J.-Y., Shin, S.-C., Kang, H.-L., Lee, W.-K., Cho, M.-J., Rhee, K.-H., Youn, H.-S., & Baik, S.-C. (2013). Anthocyanins from black soybean inhibit *Helicobacter pylori* -induced inflammation in human gastric epithelial AGS cells: Anthocyanins and *H. pylori* infection. *Microbiology and Immunology*, *57*(5), 366–373. https://doi.org/10.1111/1348-0421.12049

52. Kitts, D. D., Chen, X.-M., & Jing, H. (2012). Demonstration of antioxidant and anti-inflammatory bioactivities from sugar–amino acid Maillard reaction products. *Journal of Agricultural and Food Chemistry*, *60*(27), 6718–6727. https://doi.org/10.1021/jf2044636

53. Klavins, L., Perkons, I., Mezulis, M., Viksna, A., & Klavins, M. (2022). Procyanidins from cranberry press residues—extraction optimization, purification and characterization. *Plants*, *11*(24), 3517. https://doi.org/10.3390/plants11243517

54. Kovinich, N., Kayanja, G., Chanoca, A., Riedl, K., Otegui, M. S., & Grotewold, E. (2014). Not all anthocyanins are born equal: distinct patterns induced by stress in *Arabidopsis*. *Planta*, *240*(5), 931–940. https://doi.org/10.1007/s00425-014-2079-1

55. Kuhn, F., Azevedo, E. S., & Noreña, C. P. Z. (2020). Behavior of inulin, polydextrose, and egg albumin as carriers of *Bougainvillea glabra* bracts extract: rheological performance and powder characterization. *Journal of Food Processing and Preservation*, *44*(10). https://doi.org/10.1111/jfpp.14834

56. Kumar, V., Sharma, A., Kohli, S. K., Yadav, P., Bali, S., Bakshi, P., Parihar, R. D., Yuan, H., Yan, D., He, Y., Wang, J., Yang, Y., Bhardwaj, R., Thukral, A. K., & Zheng, B. (2019). Amino acids distribution in economical important plants: A review. *Biotechnology Research and Innovation*, *3*(2), 197–207. https://doi.org/10.1016/j.biori.2019.06.004

57. Kurata, T., & Sakurai, Y. (1967). Degradation of L-ascorbic acid and mechanism of nonenzymic browning reaction. *Agricultural and Biological Chemistry*, *31*(2), 170–184. https://doi.org/10.1080/00021369.1967.10858792

58. Lechner, J. F., & Stoner, G. D. (2019). Red beetroot and betalains as cancer chemopreventative agents. *Molecules*, *24*(8), 1602. https://doi.org/10.3390/molecules24081602

59. Lerbret, A., Bordat, P., Affouard, F., Guinet, Y., Hédoux, A., Paccou, L., Prévost, D., & Descamps, M. (2005). Influence of homologous disaccharides on the hydrogen-bond network of water: Complementary Raman scattering experiments and molecular dynamics simulations. *Carbohydrate Research*, *340*(5), 881–887. https://doi.org/10.1016/j.carres.2005.01.036

60. Li, Y., Ma, X., & Liu, X. (2019). Physicochemical and rheological properties of cross-linked inulin with different degree of polymerization. *Food Hydrocolloids*, *95*, 318–325. https://doi.org/10.1016/j.foodhyd.2018.11.026

61. Lin, X., Duan, N., Wu, J., Lv, Z., Wang, Z., & Wu, S. (2023). Potential food safety risk factors in plant-based foods: Source, occurrence, and detection methods. *Trends in Food Science & Technology*, *138*, 511–522. https://doi.org/10.1016/j.tifs.2023.06.032

62. Liu, C., Liu, M., Yang, L., & Zhang, X. (2022). Influence of ripening stage and meteorological parameters on the accumulation pattern of polyphenols in greengages (*Prunus mume* Sieb. Et Zucc) by widely targeted metabolomic. *Current Research in Food Science*, *5*, 1837–1844. https://doi.org/10.1016/j.crfs.2022.10.013

63. Liu, S.-C., Chang, H.-M., & Wu, J. S.-B. (2003). A study on the mechanism of browning in mei liqueur using model solutions. *Food Research International*, *36*(6), 579–585. https://doi.org/10.1016/S0963-9969(03)00005-X

64. Liu, X., Le Bourvellec, C., Guyot, S., & Renard, C. M. G. C. (2021). Reactivity of flavanols: Their fate in physical food processing and recent advances in their analysis by depolymerization. *Comprehensive Reviews in Food Science and Food Safety*, *20*(5), 4841–4880. https://doi.org/10.1111/1541-4337.12797

65. Mandrioli, R., Mercolini, L., & Raggi, M. A. (2013). Recent trends in the analysis of amino acids in fruits and derived foodstuffs. *Analytical and Bioanalytical Chemistry*, *405*(25), 7941–7956. https://doi.org/10.1007/s00216-013-7025-8

66. Matsui, R., Honda, R., Kanome, M., Hagiwara, A., Matsuda, Y., Togitani, T., Ikemoto, N., & Terashima, M. (2018). Designing antioxidant peptides based on the antioxidant properties of the amino acid side-chains. *Food Chemistry*, *245*, 750–755. https://doi.org/10.1016/j.foodchem.2017.11.119

67. Michalska, A., & Lech, K. (2018). The effect of carrier quantity and drying method on the physical properties of apple juice powders. *Beverages*, *4*(1), 2. https://doi.org/10.3390/beverages4010002

68. Michalska, A., Wojdyło, A., Brzezowska, J., Majerska, J., & Ciska, E. (2019). The influence of inulin on the retention of polyphenolic compounds during the drying of blackcurrant juice. *Molecules*, *24*(22). https://doi.org/10.3390/molecules24224167

69. Michalska, A., Wojdyło, A., Honke, J., Ciska, E., & Andlauer, W. (2018). Drying-induced physico-chemical changes in cranberry products. *Food Chemistry*, *240*, 448–455. https://doi.org/10.1016/j.foodchem.2017.07.050

70. Michalska, A., Wojdyło, A., Lech, K., Łysiak, G. P., & Figiel, A. (2016). Physicochemical properties of whole fruit plum powders obtained using different drying technologies. *Food Chemistry*, *207*, 223–232. https://doi.org/10.1016/j.foodchem.2016.03.075

71. Michalska, A., Wojdyło, A., Łysiak, G. P., & Figiel, A. (2017). Chemical composition and antioxidant properties of powders obtained from different plum juice formulations. *International Journal of Molecular Sciences*, *18*(1). https://doi.org/10.3390/ijms18010176

72. Michalska, A., Wojdyło, A., Łysiak, G. P., Lech, K., & Figiel, A. (2017). Functional relationships between phytochemicals and drying conditions during the processing of blackcurrant pomace into powders. *Advanced Powder Technology*, *28*(5), 1340–1348. https://doi.org/10.1016/j.apt.2017.03.002

73. Michalska-Ciechanowska, A., Brzezowska, J., Wojdyło, A., Gajewicz-Skretna, A., Ciska, E., & Majerska, J. (2021). Chemometric contribution for deeper understanding of thermally-induced changes of polyphenolics and the formation of hydroxymethyl-*L*-furfural in chokeberry powders. *Food Chemistry*, *342*, 128335. https://doi.org/10.1016/j.foodchem.2020.128335

74. Michalska-Ciechanowska, A., Hendrysiak, A., Brzezowska, J., Wojdyło, A., & Gajewicz-Skretna, A. (2021). How do the different types of carrier and drying techniques affect the changes in physico-chemical properties of powders from chokeberry pomace extracts? *Foods*, *10*(8), 1864. https://doi.org/10.3390/foods10081864

75. Nakagawa, T., Lanaspa, M. A., Millan, I. S., Fini, M., Rivard, C. J., Sanchez-Lozada, L. G., Andres-Hernando, A., Tolan, D. R., & Johnson, R. J. (2020). Fructose contributes to the Warburg effect for cancer growth. *Cancer & Metabolism*, *8*(1), 16. https://doi.org/10.1186/s40170-020-00222-9

76. Nayaka, V. K., Tiwari, R. B., Narayana, C. K., Ranjitha, K., Shamina, A., Vasugi, C., ... & Sujayasree, O. J. (2022). Comparative effect of different sugars instigating non-enzymatic browning and Maillard reaction products in guava fruit leather. *Journal of Horticultural Sciences*, *17*(1), 174–183. https://doi.org/10.24154/jhs.v17i1.1387

77. Nooshkam, M., Varidi, M., & Bashash, M. (2019). The Maillard reaction products as food-born antioxidant and antibrowning agents in model and real food systems. *Food Chemistry*, *275*, 644–660. https://doi.org/10.1016/j.foodchem.2018.09.083

78. Nowicka, P., Wojdyło, A., & Samoticha, J. (2016). Evaluation of phytochemicals, antioxidant capacity, and antidiabetic activity of novel smoothies from selected *Prunus* fruits. *Journal of Functional Foods*, *25*, 397–407. https://doi.org/10.1016/j.jff.2016.06.024

79. Orabi, H., M. El-Fakharany, E., S. Abdelkhalek, E., & M. Sidkey, N. (2019). *L*-asparaginase and *L*-glutaminase: sources, production, and applications in medicine and industry. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(2), 179–190. https://doi.org/10.15414/jmbfs.2019.9.2.179-190

80. Oszmiański, J., & Krzywicki, M. (1993). *Sposób otrzymywania barwników antocyjanowych* (PL-158707) (*In polish*).

81. Oszmiański, J., Wojdyło, A., & Lachowicz, S. (2016). Effect of dried powder preparation process on polyphenolic content and antioxidant activity of blue honeysuckle berries (*Lonicera caerulea* L. var. Kamtschatica). *LWT - Food Science and Technology*, *67*, 214–222. https://doi.org/10.1016/j.lwt.2015.11.051

82. Ozyurt, V. ye H., & Ötles, S. (2016). Effect of food processing on the physicochemical properties of dietary fibre. *Acta Scientiarum Polonorum Technologia Alimentaria*, *15*(3), 233–245. https://doi.org/10.17306/J.AFS.2016.3.23

83. Papoutsis, K., Zhang, J., Bowyer, M. C., Brunton, N., Gibney, E. R., & Lyng, J. (2021). Fruit, vegetables, and mushrooms for the preparation of extracts with α-amylase and α-glucosidase inhibition properties: A review. *Food Chemistry*, *338*, 128119. https://doi.org/10.1016/j.foodchem.2020.128119

84. Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour measurement and analysis in fresh and processed foods: A review. *Food and Bioprocess Technology*, *6*(1), 36–60. https://doi.org/10.1007/s11947-012-0867-9

85. Patil, S. P., Goswami, A., Kalia, K., & Kate, A. S. (2020). Plant-derived bioactive peptides: A treatment to cure diabetes. *International Journal of Peptide Research and Therapeutics*, *26*(2), 955–968. https://doi.org/10.1007/s10989-019-09899-z

86. Peighambardoust, S. H., Golshan Tafti, A., & Hesari, J. (2011). Application of spray drying for preservation of lactic acid starter cultures: a review. *Trends in Food Science & Technology*, *22*(5), 215–224. https://doi.org/10.1016/j.tifs.2011.01.009

87. Pérez-Burillo, S., Rufián-Henares, J. Á., & Pastoriza, S. (2019). Effect of home cooking on the antioxidant capacity of vegetables: Relationship with Maillard reaction indicators. *Food Research International*, *121*, 514–523. https://doi.org/10.1016/j.foodres.2018.12.007

88. Pico, J., Yan, Y., Gerbrandt, E. M., & Castellarin, S. D. (2022). Determination of free and bound phenolics in northern highbush blueberries by a validated HPLC/QTOF methodology. *Journal of Food Composition and Analysis*, *108*, 104412. https://doi.org/10.1016/j.jfca.2022.104412

89. PN-90/A-75101/02. Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczenie zawartości ekstraktu ogólnego. (*In polish*).

90. PN-90/A-75101/03. Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczenie zawartości suchej masy metoda wagową. (*In polish*).

91. PN-90/A-75101/06. Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczenie pH metodą potencjometryczną. (*In polish*).

92. PN-90/A-75101/11. Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości witaminy C. (*In polish*).

93. Poovitha, S., & Parani, M. (2016). *In vitro* and *in vivo* α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). *BMC Complementary and Alternative Medicine*, *16*(S1), 185. https://doi.org/10.1186/s12906-016-1085-1

94. Puangpraphant, S., Cuevas-Rodríguez, E.-O., & Oseguera-Toledo, M. (2022). Anti-inflammatory and antioxidant phenolic compounds. In *Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress* (pp. 165–180). Elsevier. https://doi.org/10.1016/B978-0-12-823482-2.00018-2

95. Qi, Q., Chu, M., Yu, X., Xie, Y., Li, Y., Du, Y., Liu, X., Zhang, Z., Shi, J., & Yan, N. (2022). Anthocyanins and proanthocyanidins: Chemical structures, food sources, bioactivities, and product development. *Food Reviews International*, 1–29. https://doi.org/10.1080/87559129.2022.2029479

96. R Core Team. (2016). R: A Language and Environment for Statistical Computing. Vienna, Austria. Retrieved from https://www.R-project.org/.

97. Raak, N., Symmank, C., Zahn, S., Aschemann-Witzel, J., & Rohm, H. (2017). Processing- and product-related causes for food waste and implications for the food supply chain. *Waste Management*, *61*, 461–472. https://doi.org/10.1016/j.wasman.2016.12.027

98. Radünz, M., da Trindade, M. L. M., Camargo, T. M., Radünz, A. L., Borges, C. D., Gandra, E. A., & Helbig, E. (2019). Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum* L.) essential oil. *Food Chemistry*, *276*, 180–186. https://doi.org/10.1016/j.foodchem.2018.09.173

99. Ratti, C. (2001). Hot air and freeze-drying of high-value foods: A review. *Journal of Food Engineering*, *49*(4), 311–319. https://doi.org/10.1016/S0260-8774(00)00228-4

100. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9–10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

101. Reque, P. M., Steckert, E. V., dos Santos, F. T., Danelli, D., Jablonski, A., Flôres, S. H., Rech, R., de O. Rios, A., & de Jong, E. V. (2016). Heat processing of blueberries and its effect on their physicochemical and bioactive properties: Heat processing of blueberries. *Journal of Food Process Engineering*, *39*(6), 564–572. https://doi.org/10.1111/jfpe.12249

102. Roda-Serrat, M.C., Razi Parjikolaei, B., Mohammadifakhr, M., Martin, J., Norddahl, B., Errico, M. (2022). A case study for the extraction, purification, and co-pigmentation of anthocyanins from *Aronia melanocarpa* juice pomace. *Foods* 2022, *11*, 3875. https://doi.org/10.3390/foods11233875

103. Rodríguez-Roque, M. J., de Ancos, B., Sánchez-Moreno, C., Cano, M. P., Elez-Martínez, P., & Martín-Belloso, O. (2015). Impact of food matrix and processing on the *in vitro* bioaccessibility of vitamin C, phenolic compounds, and hydrophilic antioxidant activity from fruit juice-based beverages. *Journal of Functional Foods*, *14*, 33–43. https://doi.org/10.1016/j.jff.2015.01.020

104. Roopchand, D. E., Krueger, C. G., Moskal, K., Fridlender, B., Lila, M. A., & Raskin, I. (2013). Food-compatible method for the efficient extraction and stabilization of cranberry pomace polyphenols. *Food Chemistry*, *141*(4), 3664–3669. https://doi.org/10.1016/j.foodchem.2013.06.050

105. Ryu, D., & Koh, E. (2022). Stability assessment of anthocyanins from black soybean, grape, and purple sweet potato under *in vitro* gastrointestinal digestion. *Food Science and Biotechnology*, *31*(8), 1053–1062. https://doi.org/10.1007/s10068-022-01071-6

106. Santhalakshmy, S., Don Bosco, S. J., Francis, S., & Sabeena, M. (2015). Effect of inlet temperature on physicochemical properties of spray-dried jamun fruit juice powder. *Powder Technology*, *274*, 37–43. https://doi.org/10.1016/j.powtec.2015.01.016

107. Sanz, M. L., Del Castillo, M. D., Corzo, N., & Olano, A. (2000). Presence of 2-furoylmethyl derivatives in hydrolysates of processed tomato products. *Journal of Agricultural and Food Chemistry*, *48*(2), 468–471. https://doi.org/10.1021/jf990697b

108. Shahidi, F., & Pan, Y. (2022). Influence of food matrix and food processing on the chemical interaction and bioaccessibility of dietary phytochemicals: a review. *Critical Reviews in Food Science and Nutrition*, *62*(23), 6421–6445. https://doi.org/10.1080/10408398.2021.1901650

109. Sharma, K., Ko, E. Y., Assefa, A. D., Ha, S., Nile, S. H., Lee, E. T., & Park, S. W. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *Journal of Food and Drug Analysis*, *23*(2), 243–252.

110. Shen, X., Sun, X., Xie, Q., Liu, H., Zhao, Y., Pan, Y., Hwang, C.-A., & Wu, V. C. H. (2014). Antimicrobial effect of blueberry (*Vaccinium corymbosum* L.) extracts against the growth of *Listeria monocytogenes* and *Salmonella Enteritidis*. *Food Control*, *35*(1), 159–165. https://doi.org/10.1016/j.foodcont.2013.06.040

111. Silva, S., Costa, E. M., Costa, M. R., Pereira, M. F., Pereira, J. O., Soares, J. C., & Pintado, M. M. (2015). Aqueous extracts of *Vaccinium corymbosum* as inhibitors of *Staphylococcus aureus*. *Food Control*, *51*, 314–320. https://doi.org/10.1016/j.foodcont.2014.11.040

112. Silvan, J. M., & Martinez-Rodriguez, A. J. (2022). Modulation of inflammation and oxidative stress in *Helicobacter pylori* infection by bioactive compounds from food components. In *Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress* (pp. 499–516). Elsevier. https://doi.org/10.1016/B978-0-12-823482-2.00029-7

113. Silvan, J. M., Guerrero-Hurtado, E., Gutiérrez-Docio, A., Alarcón-Cavero, T., Prodanov, M., & Martinez-Rodriguez, A. J. (2021). Olive-leaf extracts modulate inflammation and oxidative stress associated with human *H. pylori* infection. *Antioxidants*, *10*(12), 2030. https://doi.org/10.3390/antiox10122030

114. Silvan, J. M., Guerrero-Hurtado, E., Gutierrez-Docio, A., Prodanov, M., & Martinez-Rodriguez, A. J. (2023). Olive leaf as a source of antibacterial compounds active against antibiotic-resistant strains of *Campylobacter jejuni* and *Campylobacter coli*. *Antibiotics*, *12*(1), 26. https://doi.org/10.3390/antibiotics12010026

115. Silvan, J. M., Gutiérrez-Docio, A., Moreno-Fernandez, S., Alarcón-Cavero, T., Prodanov, M., & Martinez-Rodriguez, A. J. (2020). Procyanidin-rich extract from grape seeds as a putative tool against *Helicobacter pylori*. *Foods*, *9*(10), 1370. https://doi.org/10.3390/foods9101370

116. Silvan, J. M., Michalska-Ciechanowska, A., & Martinez-Rodriguez, A. J. (2020). Modulation of antibacterial, antioxidant, and anti-inflammatory properties by drying of *Prunus domestica* L. plum juice extracts. *Microorganisms*, *8*(1), 119. https://doi.org/10.3390/microorganisms8010119

117. Silván, J. M., Mingo, E., Hidalgo, M., de Pascual-Teresa, S., Carrascosa, A. V., & Martinez-Rodriguez, A. J. (2013). Antibacterial activity of a grape seed extract and its fractions against *Campylobacter* spp. *Food Control*, *29*(1), 25–31. https://doi.org/10.1016/j.foodcont.2012.05.063

118. Soto, M. L., Moure, A., Domínguez, H., & Parajó, J. C. (2011). Recovery, concentration and purification of phenolic compounds by adsorption: a review. *Journal of Food Engineering*, *105*(1), 1–27. https://doi.org/10.1016/j.jfoodeng.2011.02.010

119. Świąder, K., Lipska, A., & Boyko, N. (2022). Novel sweeteners: Isomaltulose, D-tagatose, trehalose and sucromalt – their description and properties®. *Technological Progress in Food Processing*, *1*, 186–194.

120. Szwajgier, D., Halinowski, T., Helman, E., Tylus, K., & Tymcio, A. (2014). Influence of different heat treatments on the content of phenolic acids and their derivatives in selected fruits. *Fruits*, *69*(2), 167–178. https://doi.org/10.1051/fruits/2014004

121. Teleszko, M., & Wojdyło, A. (2015). Comparison of phenolic compounds and antioxidant potential between selected edible fruits and their leaves. *Journal of Functional Foods*, *14*, 736–746. https://doi.org/10.1016/j.jff.2015.02.041

122. 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. https://doi.org/10.3390/molecules25173801

123. Tontul, I., & Topuz, A. (2017). Spray-drying of fruit and vegetable juices: Effect of drying conditions on the product yield and physical properties. *Trends in Food Science & Technology*, *63*, 91–102. https://doi.org/10.1016/j.tifs.2017.03.009

124. 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. https://doi.org/10.1016/j.lwt.2021.112247

125. 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. https://doi.org/10.1016/j.jfca.2020.103608

126. 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. https://doi.org/10.1016/j.foodchem.2020.126830

127. UN (2015). Transforming Our World: The 2030 Agenda for Sustainable Development. Resolution Adopted by the General Assembly on 25 September 2015, 42809, 1-13. [*Accessed 1 September 2023*]

128. Villalva, M., Silvan, J. M., Guerrero-Hurtado, E., Gutierrez-Docio, A., Navarro del Hierro, J., Alarcón-Cavero, T., Prodanov, M., Martin, D., & Martinez-Rodriguez, A. J. (2022). Influence of *in vitro* gastric digestion of olive leaf extracts on their bioactive properties against *H. pylori*. *Foods*, *11*(13), 1832. https://doi.org/10.3390/foods11131832

129. Wallace, T. C., & Giusti, M. M. (2010). Extraction and normal-phase HPLC-fluorescence-electrospray MS characterization and quantification of procyanidins in cranberry extracts. *Journal of Food Science*, *75*(8), C690–C696. https://doi.org/10.1111/j.1750-3841.2010.01799.x

130. Wang, L., Dekker, M., Heising, J., Zhao, L., & Fogliano, V. (2023). Food matrix design can influence the antimicrobial activity in the food systems: A narrative review. *Critical Reviews in Food Science and Nutrition*, 1–27. https://doi.org/10.1080/10408398.2023.2205937

131. Wang, W., Yagiz, Y., Buran, T. J., Nunes, C. do N., & Gu, L. (2011). Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Research International*, *44*(9), 2666–2673. https://doi.org/10.1016/j.foodres.2011.05.022

132. Wei, T., & Simko, V. (2021). *R package "corrplot": Visualization of a correlation matrix (Version 0.92).* Available from: https://github.com/taiyun/corrplot (Version 0.92) [R]. https://github.com/taiyun/corrplot (2011)

133. Wellner, A., Huettl, C., & Henle, T. (2011). Formation of Maillard reaction products during heat treatment of carrots. *Journal of Agricultural and Food Chemistry*, *59*(14), 7992–7998. https://doi.org/10.1021/jf2013293

134. White, B. L., Howard, L. R., & Prior, R. L. (2010a). Proximate and polyphenolic characterization of cranberry pomace. *Journal of Agricultural and Food Chemistry*, *58*(7), 4030–4036. https://doi.org/10.1021/jf902829g

135. White, B. L., Howard, L. R., & Prior, R. L. (2010b). Release of bound procyanidins from cranberry pomace by alkaline hydrolysis. *Journal of Agricultural and Food Chemistry*, *58*(13), 7572–7579. https://doi.org/10.1021/jf100700p

136. Wickham, H. (2016). Ggplot2: Elegant graphics for data analysis. Springer-Verlag New York. Available from: https://ggplot2.tidyverse.org.

137. Wojdyło, A., Nowicka, P., & Bąbelewski, P. (2018). Phenolic and carotenoid profile of new goji cultivars and their anti-hyperglycemic, anti-aging and antioxidant properties. *Journal of Functional Foods*, *48*, 632–642. https://doi.org/10.1016/j.jff.2018.07.061

138. Wojdyło, A., Nowicka, P., Oszmiański, J., & Golis, T. (2017). Phytochemical compounds and biological effects of *Actinidia* fruits. *Journal of Functional Foods*, *30*, 194–202. https://doi.org/10.1016/j.jff.2017.01.018

139. Wu, C.-H., & Yen, G.-C. (2005). Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation endproducts. *Journal of Agricultural and Food Chemistry*, *53*(8), 3167–3173. https://doi.org/10.1021/jf048550u

140. Xu, Y., Liu, G., Yu, Z., Song, X., Li, X., Yang, Y., Wang, L., Liu, L., & Dai, J. (2016). Purification, characterization and antiglycation activity of a novel polysaccharide from black currant. *Food Chemistry*, *199*, 694–701. https://doi.org/10.1016/j.foodchem.2015.12.078

141. Xu, Y., Niu, X., Liu, N., Gao, Y., Wang, L., Xu, G., Li, X., & Yang, Y. (2018). Characterization, antioxidant and hypoglycemic activities of degraded polysaccharides from blackcurrant (*Ribes nigrum* L.) fruits. *Food Chemistry*, *243*, 26–35. https://doi.org/10.1016/j.foodchem.2017.09.107

142. Yan, Y., Song, C., Falginella, L., & Castellarin, S. D. (2020). Day temperature has a stronger effect than night temperature on anthocyanin and flavonol accumulation in 'Merlot' (*Vitis vinifera* L.) grapes during ripening. *Frontiers in Plant Science*, *11*, 1095. https://doi.org/10.3389/fpls.2020.01095

143. Yang, W., Guo, Y., Liu, M., Chen, X., Xiao, X., Wang, S., Gong, P., Ma, Y., & Chen, F. (2022). Structure and function of blueberry anthocyanins: A review of recent advances. *Journal of Functional Foods*, *88*, 104864. https://doi.org/10.1016/j.jff.2021.104864

144. Zhang, S., Xiao, L., Lv, L., & Sang, S. (2020). Trapping methylglyoxal by myricetin and its metabolites in mice. *Journal of Agricultural and Food Chemistry*, *68*(35), 9408–9414. https://doi.org/10.1021/acs.jafc.0c03471

145. Zhou, T., Wei, C., Lan, W., Zhao, Y., Pan, Y., Sun, X., & Wu, V. C. H. (2020). The effect of Chinese wild blueberry fractions on the growth and membrane integrity of various foodborne pathogens. *Journal of Food Science*, *85*(5), 1513–1522. https://doi.org/10.1111/1750-3841.15077

146. Zhu, J., Chen, C., Zhang, B., & Huang, Q. (2020). The inhibitory effects of flavonoids on α-amylase and α-glucosidase. *Critical Reviews in Food Science and Nutrition*, *60*(4), 695–708. https://doi.org/10.1080/10408398.2018.1548428

147. Zhu, J., Wang, Y., Li, X., Li, B., Liu, S., Chang, N., Jie, D., Ning, C., Gao, H., & Meng, X. (2017). Combined effect of ultrasound, heat, and pressure on *Escherichia coli* O157:H7, polyphenol oxidase activity, and anthocyanins in blueberry (*Vaccinium corymbosum*) juice. *Ultrasonics Sonochemistry*, *37*, 251–259. https://doi.org/10.1016/j.ultsonch.2017.01.017

148. Zhu, Y., & Yaylayan, V. A. (2017). Interaction of free arginine and guanidine with glucose under thermal processing conditions and formation of Amadori-derived imidazolones. *Food Chemistry*, *220*, 87–92. https://doi.org/10.1016/j.foodchem.2016.09.173

APPENDICES

The publications included in the doctoral dissertation entitled
'*Creation of high-quality plant-based powders with targeted health-oriented properties'*

Publication 1

How do the different types of carrier and drying techniques affect the changes in physico-chemical properties of powders from chokeberry pomace extracts

foods

MDPI

Article

How Do the Different Types of Carrier and Drying Techniques Affect the Changes in Physico-Chemical Properties of Powders from Chokeberry Pomace Extracts?

Anna Michalska-Ciechanowska [1,*], Aleksandra Hendrysiak [1], Jessica Brzezowska [1], Aneta Wojdyło [1] and Agnieszka Gajewicz-Skretna [2]

[1] Department of Fruit, Vegetable and Plant Nutraceutical Technology, the Faculty of Biotechnology and Food Science, Wrocław University of Environmental and Life Sciences, Chełmońskiego 37, 51-630 Wrocław, Poland; 109211@student.upwr.edu.pl (A.H.); jessica.brzezowska@upwr.edu.pl (J.B.); aneta.wojdylo@upwr.edu.pl (A.W.)

[2] Laboratory of Environmental Chemometrics, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdansk, Poland; agnieszka.gajewicz@ug.edu.pl

* Correspondence: anna.michalska@upwr.edu.pl

Abstract: Chokeberry fruit, one of the richest plant sources of bioactives, is processed into different foodstuffs, mainly juice, which generates a considerable amount of by-products. To follow the latest trends in the food industry considering waste management, the study aimed to produce chokeberry pomace extract powders and conduct experimental and chemometric assessment of the effect of different carriers and drying techniques on the physico-chemical properties of such products. The PCA analysis showed that the examined powders were classified into two groups: freeze-dried (variation in case of moisture content, water activity, colour, and browning index) and vacuum-dried (bulk density). No clear pattern was observed for the physical properties of carrier added products. The sum of polyphenolics (phenolic acids, anthocyanins and flavonols) ranged from 3.3–22.7 g/100 g dry matter. Drying techniques had a stronger effect on the polyphenols profile than the type of carrier. Hydroxymethyl-*L*-furfural formation was enhanced by inulin addition during high-temperature treatment. Overall, the addition of maltodextrin and trehalose mixture for freeze drying and vacuum drying at 90 °C caused the highest retention of polyphenolics and the lowest formation of hydroxymethyl-*L*-furfural; however, an individual and comprehensive approach is required when the obtainment of high-quality chokeberry powders is expected.

Keywords: *Aronia melanocarpa* L.; by-products; sustainability; inulin; trehalose; polyphenols; HMF; unsupervised chemometric analysis

Citation: Michalska-Ciechanowska, A.; Hendrysiak, A.; Brzezowska, J.; Wojdyło, A.; Gajewicz-Skretna, A. How Do the Different Types of Carrier and Drying Techniques Affect the Changes in Physico-Chemical Properties of Powders from Chokeberry Pomace Extracts? *Foods* **2021**, *10*, 1864. https://doi.org/10.3390/foods10081864

Academic Editor: Adamantini Paraskevopoulou

Received: 22 June 2021
Accepted: 28 July 2021
Published: 12 August 2021

1. Introduction

As of late, a new trend has become increasingly evident in the food industry, with consumers shifting their preferences from animal to plant-based products. This is mainly driven by consumers' growing awareness of a healthy lifestyle, of which a balanced diet is an indispensable part, but also by ecological and ethical issues [1]. For this reason, the production and processing of plant products, mainly fruit and vegetables, has been increasing significantly for some time. However, this leads to the generation of an enormous amount of by-products, including pomace, the management of which is currently one of the biggest challenges for the food industry sector [2]. As it was reported earlier in the literature, fruit and vegetable pomace is a valuable source of numerous bioactive compounds [3,4]. One of the most frequently processed raw materials is black chokeberry (*Aronia melanocarpa* L.), which, due to its characteristic astringent taste, is not usually consumed as a fresh fruit. The main direction of its use is the production of juices, jams, or fruit wines, which results in significant amounts of by-products [5]. Due to its unfavourable sensory properties, it often remains wasted. However, a body of evidence has demonstrated that chokeberry pomace

is a rich source of polyphenolic compounds, which have strong antioxidant properties, but has also indicated the beneficial effects in, among others, obesity, glucose metabolic disorders, pro-inflammatory conditions, hypertension, dyslipidaemia, etc. [6]. It has to be stressed that chokeberry pomace has almost an eight times higher content of polyphenols than juice [7]. Due to the proven health-promoting properties of chokeberry pomace, it can be an excellent raw material when developing functional foods [7]. In this setting, the processing of chokeberry by-products is of great importance and the production of a powdered form is new and one of the most promising alternatives for its utilisation, while at the same time being an effective tool for introducing sustainable food management [8,9]. One of the interesting approaches is to obtain powders from chokeberry pomace extracts. This type of product, due to its easy-to-handle form, possible high solubility (in contrast to pomace), and a relatively high microbial stability, is an attractive additive to other foodstuffs as a sustainable natural colouring or functional enrichment agent [5,10]. However, in order to retain the satisfactory amount of selected polyphenolics, a correctly chosen extraction method is essential, as are the subsequent steps: solvent evaporation, purification of the extracts on a polymer-bed-type Amberlite XAD, and next solvent evaporation leading to a purified pomace polyphenolic extract obtainment [11]. Moreover, in order to acquire the powdered form, it is necessary to carry out drying, which may significantly affect the physical properties, but also can lead to alterations of the chemical composition, particularly in the profile of polyphenolic compounds. For this reason, the choice of an appropriate drying technique and its parameters is pivotal to maintain a relatively high possible content of polyphenolic compounds in the final product [12]. Freeze drying, as a low-temperature water removal process, is considered to be the least intrusive with the slightest impact on the transformation of the dried matrix [13]. On the other hand, recent studies by Michalska-Ciechanowska et al. [12] on black chokeberry juice demonstrated that the application of high temperatures during drying can positively influence the polyphenolic profile, leading to the release of significant amounts of selected bioactive constituents from more complex structures [12]. One technique that enables the use of high drying temperatures while excluding the effects of oxygen on the dried matrix is vacuum drying [14]. However, the thermolability of polyphenols, especially anthocyanins, to thermal processes, as well as the different yields for obtaining powders depending on the raw material composition, induce the necessity of using a carrier additive in the drying process [15]. Substances of carbohydrate origin, such as maltodextrin, are widely used for this purpose and have proven protective properties, allowing for an increased retention of anthocyanins at high drying temperatures compared with other carriers [14]. Further interesting additives include trehalose, which has been found to be inactive in the Maillard reaction due to its non-reducing properties, and inulin, which is well known for its functional properties [16]. Importantly, the selection of carrier is of high importance as it was previously demonstrated that the type and concentration of selected carriers may not only cause a decrease in the polyphenolics content, but may also accelerate the formation of undesirable process contaminants, especially in fruit-based matrices [12,14]. Taking the above into consideration, it is hypothesised that the drying techniques and type of carrier will simultaneously moderate the polyphenolic composition in chokeberry pomace extract powders. Thus, this study aimed to evaluate different drying techniques and parameters as well as carrier types on the alteration in the polyphenolics composition, antioxidant capacity, and formation of Maillard reaction and caramelisation products in chokeberry pomace extract powders.

2. Materials and Methods

2.1. Materials

The material used in the study was composed of chokeberry fruits (approximately 70 kg) obtained from Rolniczo-Sadownicze Gospodarstwo Doświadczalne 'Przybroda' (Rokietnica near Poznań, Poland). The fruits were ground in a Thermomix (Wuppertal,

Vorkwek, Germany) and pressed on a hydraulic press (SRSE, Warszawa, Poland). The pomace gained was frozen before the extraction process at −20 °C.

2.2. Methods

2.2.1. Extraction Procedure

The extraction of polyphenolic compounds from chokeberry pomace (initial moisture content of 44.45 ± 0.01%) was performed according to the patent Oszmiański and Krzywicki [17]. Thawed pomace (approximately 10 kg) was mixed with 30% acetone (1:4, *w*/*v*) and sonicated for 15 min. The solution was left for 24 h and sonicated again for 15 min. The acetone was evaporated (Unipan 350P, Warsaw, Poland) and the solution gained in a quantity of approximately 17 L (4.9 ± 0.07 Bx) was introduced into the Amberlite XAD-16 (Brenntag, Poland) according to the procedure of Kammerer et al. [11] in order to recover the selected polyphenolics in the extracts [18]. This resulted in approximately 2.5 L of final solution (6.9 ± 0.1 Bx), which was submitted to the formulation of the drying compositions (control, samples with carrier addition). The procedure was performed in duplicate (n = 2).

2.2.2. Preparation of Chokeberry Pomace Extract Powders

The pomace extracts were mixed with carriers, i.e., maltodextrin (M) (DE 9.3; PEPEES S.A, Poland), inulin (I) (Beneo-Orafti, Belgium), trehalose (T) (Hayashibara, Co., Okayama, Japan), and their mixtures maltodextrin-inulin (M-I), maltodextrin-trehalose (M-T), and inulin-trehalose (I-T) at the level of 10% (*w*/*w*) (chosen on the basis of experimental work). No carrier was added to the control sample. Prepared solutions were submitted to drying processes: freeze drying at −60 °C/+ 24 °C for 24 h (FreeZone freeze dryer, Labconco Corp., Kansas, MO, USA), and vacuum drying at 60 and 90 °C for, respectively, 22 and 16 h (Vacucell 111 Eco Line, MMM Medcenter Einrichtungen GmbH, Germany). The drying processes were performed in duplicate (n = 2). After the drying, the obtained powders were vacuum packed and stored at −20 °C until analysis.

2.2.3. Physical Properties

Moisture Content

The moisture content (*Mc*) was determined in duplicate (n = 2) [12] at 80 °C. The results were expressed as %.

Water Activity

The water activity (a_w) was done at 25 °C in duplicate (n = 2) using the water activity meter Dew Point Water Activity Meter 4TE (AQUA LAB, Pullman, WA, USA).

Bulk Density

The bulk density (ρ_b) of powders was performed in duplicate (n = 2) using a graduated cylinder (10 cm^3) and laboratory scale. This was calculated as follows:

$$\rho_b = \frac{m}{V_b} \quad (1)$$

m—mass of the powder, V_b—volume of the powder

Colour

The colour of the powders was measured in triplicate (n = 3) using a Minolta Chroma Meter CR-400 colorimeter (Minolta Co. Ltd., Osaka, Japan) according to the CIE *L*a*b** system. Based on the results, the browning index (BI) was calculated according to the equation described by Mexis and Kontominas [19]:

$$BI = \frac{[100(x - 0.31)]}{0.17} \quad (2)$$

where:

$$x = \frac{a^* + 1.75L^*}{5.64L^* + a^* - 3.012b^*} \quad (3)$$

2.2.4. Chemical Properties

Preparation of Extracts

The extraction of polyphenolics from the chokeberry pomace powders was performed according to the procedure described by Wojdyło et al. [20]. Samples for qualitative and quantitative determination of these compounds and hydroxymethyl-*L*-furfural (HMF) by Ultrahigh Performance Liquid Chromatography were done in duplicate (n = 2) and extracted with 1.7 mL of aqueous solution of MeOH (30%; v/v) with ascorbic acid (0.2%) and 0.1% CH_3COOH while those for antioxidant capacity analyses (n = 2) were prepared with 1.7 mL of MeOH (80%; v/v) with HCl (1 mL/L). All samples were sonicated for 15 min and refrigerated at 4 °C for 24 h. After this time, the samples were ultrasonicated again (15 min) and then centrifuged (19,515× g, 20 °C; MPW-251, MPW Med. Instruments, Poland). The obtained extracts were subjected to further analyses.

Qualitative and Quantitative Determination of Polyphenolics and Hydroxymethyl-*L*-furfural

The qualitative and quantitative analyses of polyphenolics were performed using an Acquity UPLC system (Waters, Milford, MA, USA) with a PDA detector, equipped with a binary pump system and a solvent manager. The separation of the individual compounds was done at a flow rate of 0.42 mL/min in an ACQUITY BEH C_{18} analytical column (100 mm × 1.7 µm, Waters, Milford, MA, USA). The column was conditioned with acetonitrile (100%) and an aqueous solution of acetonitrile (10%; v/v). The separation was performed by the gradient elution method using 4.5% formic acid (solvent A) and acetonitrile (solvent B): 0–10 min linear gradient, 1–15% solvent B; 10–11.5 min linear gradient, 25–100% solvent B. Anthocyanins, flavonols, phenolic acids, and HMF were detected at λ = 520 nm, 360 nm, 320 nm, and 280 nm, respectively. The determination was performed in duplicate (n = 2). The polyphenolics were identified by LC-MS QTof and assessed using the MassLynx 4.0 ChromaLynx Application Manager software [21]. Results were expressed as g/100 g dry basis (db) for individual polyphenols and as µg/100 g of db for HMF.

Antioxidant Capacity

The antioxidant capacity of polyphenolic extracts from chokeberry pomace extract powders was evaluated by $ABTS^+$ radical cation scavenging [22] and FRAP [23] by *in vitro* assays using a Synergy H1 spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). The determination was made in duplicate (n = 2) and the results were expressed as mmol Trolox equivalent (TE) per 100 g of db.

2.2.5. Statistical Analysis and Chemometrics

The results were statistically analysed using STATISTICA 13 software (StatSoft, Tulsa, OK, USA). One-way analysis of variance (ANOVA) was performed ($p < 0.05$) and the Tukey post hoc test (also referred to as the Tukey's honestly significant difference (Tukey HSD)) was applied to compare the pairwise differences between group means ($p \leq 0.05$). To investigate the dependence between the selected variables, Pearson's correlation coefficient was also calculated.

To identify the direction that maximises the variance of the projected data and to explore the trends and relations between observations and variables simultaneously, principal component analysis (PCA) [24] was performed using the 'factoextra' R package [25]. The radar plots were prepared using the 'fmsb' R package [26], allowing differences in sub-groups to be visualised.

3. Results and Discussion

3.1. Physical Properties

3.1.1. Moisture Content

The moisture content (*Mc*) of the analysed powders ranged from 0.47% to 7.59% for the products gained, respectively, after vacuum drying at 90 °C and after freeze drying with the addition of trehalose (Table 1). In general, the average moisture content of the control samples (no addition of carriers) was lower by approximately 29% when compared to the average moisture content of the products gained with the addition of carriers. The drying techniques and parameters applied for the powders' preparation had a significant effect on this parameter: the higher the drying temperature, the lower the *Mc* of the powders [27].

When the drying techniques and carrier type were concerned, it was noted that during the freeze drying process, the addition of maltodextrin resulted in the lowest *Mc*, whereas the addition of trehalose led to the highest moisture content of the powders (Table 1).

In the case of freeze and vacuum drying at 60 °C, the powders gained with the addition of inulin had a lower moisture content than the samples obtained with the addition of its mixes (M-I and I-T). However, when vacuum drying at 90 °C was considered, the powders obtained with the addition of inulin and its mixes had the highest *Mc* when compared to the rest of the applied carriers. It can be concluded that the type of carrier might influence the moisture content due to the different water-holding capacity, which could be additionally altered by the drying technique used for the powders' preparation [28]. However, taking into account the moisture content of the carriers (i.e., *Mc* of M: 10.07 ± 0.93; *Mc* of I: 3.05 ± 0.01; *Mc* of T: 1.04 ± 0.04; *Mc* of M-I: 5.52 ± 0.01; *Mc* of M-T: 3.68 ± 0.18; *Mc* of I-T: 2.66 ± 0.13), it can be observed that *Mc* of the powders was influenced more by possible interactions between the compounds present in the extracts and the constituents of the carriers (Table 1). This was also confirmed by the lack of a significant correlation between the moisture content of the carriers themselves and *Mc* of the powders gained with their addition.

3.1.2. Water Activity (a_w)

In all the analysed powders, the water activity values were below 0.45 (Table 1), indicating that these products can be considered as stable from a biochemical and microbiological point of view [29]. In the case of the controls and powders with the addition of carriers and their mixtures, the water activity values were higher after the freeze drying process than in the products gained after vacuum drying at 60 and 90 °C [27]. This might be due to the more porous structure of the products gained after freeze drying, when compared to the other drying technique used [30]. Similarly to da Silva Calvaho et al. [29], the type of carrier influenced a_w of the powders. In the products obtained with the application of maltodextrin and its mixes for freeze drying, I and I-T for vacuum drying at 60 °C and T for vacuum drying at 90 °C resulted in higher water activity in the products obtained. A positive correlation between water activity and moisture content was noted (r = 0.67) (Figure S1). A similar observation was made in case of apple juice powders [27]. In general, the application of different carriers and their mixture led to alterations in the water activity of the samples, which was also dependent on the drying technique used. There are reports suggesting that in relation to the type of carrier used for drying, the formation of a crust on the outer layer of samples was observed, which influences the water activity values in the products gained [31]. Thus, water activity may be connected with the physical changes that occur on the surface of the samples during drying that might differ in terms of their carrier properties.

Table 1. Moisture content, water activity, bulk density, colour (CIE $L^*a^*b^*$), and browning index of chokeberry powders obtained after freeze and vacuum drying (n = 2; average ± standard deviation).

Drying Technique	Process Conditions	Carrier	*Mc* (%)	a_w (−)	ρ_b (g/cm^3)	Colour			BI (AU)
						L^*	a^*	b^*	
FD	−60 °C/24 °C	(−)	4.38 ± 0.54 ^{cdef}	0.34 ± 0.02 ^{h}	0.09 ± 0.01 ^{a}	24.20 ± 0.11 ^{i}	16.14 ± 0.23 ^{i}	4.65 ± 0.02 ^{m}	0.42 ± 0.01 ^{ij}
		Maltodextrin	4.20 ± 0.42 ^{cdef}	0.41 ± 0.02 ^{i}	0.12 ± 0.01 ^{a}	25.78 ± 0.25 ^{j}	22.31 ± 0.07 ^{j}	4.09 ± 0.01 ^{l}	0.43 ± 0.01 ^{k}
		Inulin	4.74 ± 1.04 ^{def}	0.15 ± 0.01 ^{ab}	0.32 ± 0.02 ^{bc}	28.59 ± 0.06 ^{k}	25.93 ± 0.04 ^{k}	2.26 ± 0.04 ^{i}	0.42 ± 0.02 ^{i}
		Trehalose	7.59 ± 1.05 ^{g}	0.29 ± 0.02 ^{gh}	0.61 ± 0.01 ^{defg}	13.57 ± 0.16 ^{a}	12.43 ± 0.19 ^{g}	1.53 ± 0.03 ^{h}	0.43 ± 0.01 ^{jk}
		Maltodextrin-Inulin	6.15 ± 1.25 ^{efg}	0.42 ± 0.02 ^{i}	0.17 ± 0.01 ^{ab}	23.44 ± 0.71 ^{i}	22.94 ± 0.07 ^{i}	4.35 ± 0.11 ^{l}	0.45 ± 0.01 ^{l}
		Maltodextrin-Trehalose	6.53 ± 0.01 ^{fg}	0.43 ± 0.01 ^{i}	0.29 ± 0.01 ^{bc}	17.28 ± 0.03 ^{bc}	12.69 ± 0.05 ^{g}	3.32 ± 0.02 ^{k}	0.43 ± 0.01 ^{jk}
		Inulin-Trehalose	5.42 ± 0.70 ^{efg}	0.31 ± 0.01 ^{h}	0.32 ± 0.03 ^{bc}	18.27 ± 0.18 ^{cde}	12.89 ± 0.33 ^{g}	3.37 ± 0.07 ^{k}	0.42 ± 0.01 ^{ij}
VD	60 °C	(−)	1.12 ± 0.39 ^{a}	0.24 ± 0.01 ^{def}	0.64 ± 0.05 ^{defgh}	18.48 ± 0.36 ^{cdef}	9.76 ± 0.87 ^{d}	2.46 ± 0.36 ^{ij}	0.39 ± 0.01 ^{g}
		Maltodextrin	2.75 ± 0.57 ^{abcd}	0.23 ± 0.01 ^{def}	0.70 ± 0.01 ^{efghi}	16.57 ± 0.11 ^{b}	7.38 ± 0.09 ^{c}	0.49 ± 0.02 ^{cde}	0.37 ± 0.02 ^{cd}
		Inulin	2.65 ± 0.04 ^{abcd}	0.32 ± 0.01 ^{h}	0.65 ± 0.06 ^{defgh}	18.55 ± 0.54 ^{def}	15.51 ± 0.24 ^{i}	1.05 ± 0.02 ^{g}	0.41 ± 0.01 ^{h}
		Trehalose	2.74 ± 1.16 ^{abcd}	0.19 ± 0.01 ^{bcde}	0.53 ± 0.05 ^{d}	20.55 ± 0.12 ^{gh}	10.71 ± 0.03 ^{ef}	0.81 ± 0.04 ^{fg}	0.38 ± 0.01 ^{ef}
		Maltodextrin-Inulin	2.89 ± 0.45 ^{abcd}	0.21 ± 0.01 ^{cdef}	0.75 ± 0.04 ^{fghi}	17.93 ± 0.18 ^{cd}	6.34 ± 0.15 ^{ab}	0.19 ± 0.03 ^{ab}	0.35 ± 0.01 ^{ab}
		Maltodextrin-Trehalose	2.02 ± 0.42 ^{abc}	0.25 ± 0.01 ^{fg}	0.60 ± 0.01 ^{def}	19.61 ± 0.19 ^{fg}	6.12 ± 0.07 ^{ab}	0.39 ± 0.04 ^{bcd}	0.35 ± 0.01 ^{a}
		Inulin-Trehalose	3.91 ± 0.54 ^{bcde}	0.32 ± 0.01 ^{h}	0.77 ± 0.08 ^{hi}	20.29 ± 0.36 ^{gh}	13.79 ± 0.18 ^{h}	1.41 ± 0.03 ^{h}	0.40 ± 0.01 ^{g}
	90 °C	(−)	0.98 ± 0.47 ^{a}	0.24 ± 0.01 ^{efg}	0.55 ± 0.03 ^{de}	17.92 ± 0.11 ^{cd}	6.07 ± 0.21 ^{ab}	2.71 ± 0.06 ^{j}	0.38 ± 0.01 ^{f}
		Maltodextrin	0.89 ± 0.38 ^{a}	0.14 ± 0.01 ^{a}	0.65 ± 0.08 ^{defgh}	17.97 ± 0.11 ^{cd}	5.66 ± 0.01 ^{a}	0.26 ± 0.01 ^{abc}	0.35 ± 0.01 ^{a}
		Inulin	1.37 ± 0.25 ^{a}	0.15 ± 0.02 ^{ab}	0.76 ± 0.02 ^{ghi}	19.31 ± 0.12 ^{efg}	10.49 ± 0.22 ^{de}	0.02 ± 0.04 ^{a}	0.37 ± 0.01 ^{de}
		Trehalose	0.47 ± 0.02 ^{a}	0.18 ± 0.01 ^{abcd}	0.34 ± 0.02 ^{c}	21.06 ± 0.18 ^{h}	11.44 ± 0.03 ^{f}	0.70 ± 0.03 ^{ef}	0.38 ± 0.01 ^{ef}
		Maltodextrin-Inulin	1.69 ± 0.28 ^{ab}	0.14 ± 0.02 ^{a}	0.80 ± 0.04 ^{hi}	17.55 ± 0.69 ^{bcd}	7.50 ± 0.12 ^{c}	0.07 ± 0.09 ^{a}	0.36 ± 0.02 ^{bc}
		Maltodextrin-Trehalose	0.88 ± 0.12 ^{a}	0.16 ± 0.02 ^{ab}	0.57 ± 0.06 ^{de}	18.15 ± 0.13 ^{cde}	6.80 ± 0.25 ^{bc}	0.52 ± 0.04 ^{cde}	0.36 ± 0.01 ^{b}
		Inulin-Trehalose	2.62 ± 0.09 ^{abcd}	0.17 ± 0.01 ^{abc}	0.83 ± 0.01^{i}	21.75 ± 0.77 ^{h}	15.8 ± 0.27 ^{i}	0.67 ± 0.04 ^{def}	0.39 ± 0.01 ^{g}

(−)—no carrier addition, FD—freeze drying, VD—vacuum drying, M_C—moisture content, a_w—water activity, ρ_b—bulk density, AU—arbitrary units; $^{a–m}$ the same letters within a column indicate no statistically significant differences (HSD Tukey test; $p \leq 0.05$).

3.1.3. Bulk Density

The bulk density of chokeberry pomace extracts powders ranged from 0.09 g/cm^3 to 0.83 g/cm^3 for, respectively, the control sample gained after freeze drying and powders obtained after vacuum drying at 90 °C with the addition of I-T (Table 1). When the control samples and products gained with different carriers were concerned, the average value of bulk density was the lowest in the case of controls, whereas the highest bulk density was noticed for powders gained with the addition of carriers, especially when inulin and its mixtures were considered (M-I, I-T). In general, as observed by Michalska and Lech [27], freeze drying resulted in lower values of the bulk density of powders gained followed by VD at 60 °C and VD at 90 °C, except powders produced with trehalose. The addition of trehalose to pomace extract formulations caused the lowest bulk density of powders gained after VD at 60 °C and VD at 90 °C. This might be connected with the higher soluble solids content in the formulation containing trehalose [32] during the freeze drying process and/or with its different behaviour when a relatively high temperature of drying was applied. It could also be related to the fluidizing properties of this carrier [33] as trehalose, due to its chemical composition, might react differently with the compounds present in the extracts additionally moderated by the vacuum drying. What is more, the influence of the carrier type on the bulk density of powders was also noticed in the case of cranberry juice [34]. Taking the above into consideration, the selection of a carrier for powder preparation should also consider the carrier physico-chemical properties as it may moderate the physical properties of the material subjected to drying to a different extent. For the analysed powders, a negative correlation ($r = -0.60$) between bulk density and water activity (Figure S1) was noted, indicating that the specific structure of the powders gained by different drying techniques might differently influence the ability of samples to trap the water molecules [35].

3.1.4. Colour

The colour parameters measured in terms of L^*, a^*, and b^* values as well as the browning index (BI) are indicated in Table 1. In general, powders gained after the freeze drying process were the lightest, with the exception of samples prepared with trehalose, whereas vacuum drying at 60 and 90 °C resulted in slightly lower values of coordinate L^*. In the latest samples, trehalose addition caused the highest values of coordinate L^* in the analysed products. The type of carrier had a significant impact on the lightness of the powders gained [36], which was also moderated by the drying technique used. In general, when the values of coordinate a^* were concerned, it was noted that the carrier addition altered the level of red pigments in chokeberry powders. Contrary to the juice powders [37], the 10% addition of carrier led to the obtainment of products that were visually similar to the control samples. Thus, the addition of carriers, besides an improvement of the efficiency of the sustainable powder production [38], may not visually alter the colour of the products gained. In this case, the composition of chokeberry pomace extracts played a key role in colour preservation as the addition of carriers into the chokeberry juice modified the lightness to a high extent [37].

The strongest retention of red pigment was noted after freeze drying when compared to samples gained after vacuum drying, except samples prepared with the addition of trehalose and its mixes (M-T, I-T). In general, the application of vacuum drying for the powder preparation caused a decrease in the coordinate a^* values, indicating the influence of the high-temperature processing on the thermolability of the red components [39]. The strongest pigment degradation was noted in the case of powders produced with maltodextrin and its mixes (I-M; I-T). The addition of I and I-T seems to prevent deterioration of the coordinate a^* values during vacuum drying. It was observed that the b^* parameter was the highest in the control samples (no addition of carrier), regardless of the drying technique used for their preparation (Table 1). The addition of carriers led to a decrease in the coordinate b^* values, which was the strongest in the case of trehalose application for the freeze drying process. This might be connected with the presence of natural colour

compounds in the chokeberry pomace extracts (an average for FD: 3.37), as vacuum drying at 60 °C (an average: 0.97) and 90 °C (an average: 0.71) resulted in significantly lower values of parameter b^*. In the current study, the browning index was applied for determination of overall alterations in the browning colour [40]. In contrast to sea buckthorn powders [41], the highest values of BI were noted for freeze-dried samples, regardless of the type of carrier used for their preparation. It was assumed that the dominant reddish colour could mask the brown pigments present in the analysed powders [42]. Additionally, the BI values might result from the complexes formed from polyphenols [43]. The application of vacuum drying for chokeberry pomace extract powder production caused a decrease in BI when compared to freeze drying. The addition of maltodextrin and its mixes (M-I, M-T) led to lower values of BI. Additionally, a strong correlation between BI and a^* (r = 0.82) as well as b^* (r = 0.83) confirmed the masking effect of the reddish and bluish pigments (Figure S1) [44].

3.1.5. PCA Analysis

The PCA biplot (Figure 1a) shows that freeze-dried samples had a greater spread and more variance than vacuum-dried samples (at 60 and 90 °C).

The first principal component (PC1) clearly separates freeze-dried samples (positive scores) from vacuum-dried samples (negative scores). The explanatory variables (vectors) with the greatest influence on the separation of chokeberry pomace extract powders in PC1 were the colour parameters, including the browning index (BI), and coordinate a^*, b^* as well as Mc, a_w (positively correlated), and ρ_b (negatively correlated). PC2 loadings showed that negatively correlated L^* has the greatest influence on the sample distinctions (Figure 1b). Considering the locations of the samples in the space defined by the first two principal components (PCs), it can be stated that, due to low PC1 scores and positive loading values, vacuum-dried samples (at 60 and 90 °C) were characterised by a relatively low browning index (BI), and coordinate a^*, b^*, as well as Mc, a_w. At the same time, powders gained after vacuum drying had the highest values of bulk density. Interestingly, although the type of carrier was also concerned, no straightforward trends were observed for the powders gained (Figure 1c).

3.2. Chemical Properties

3.2.1. Polyphenols Content

In the powders obtained from chokeberry pomace extracts, three major groups of polyphenolic compounds were identified, i.e., phenolic acids (3), anthocyanins (4), and flavonols (8). The extraction of chokeberry pomace and usage of absorber technology led to modification of the polyphenolics composition [45] as proanthocyanidins were not identified in the powders obtained. This may be connected with the results reported by Sójka et al. [46] of major absorption of proanthocyanidins in the cell wall of chokeberry pomace. The extraction procedure applied and clarification of the polyphenols by Amberlite 16 of pomace extracts led to an absence of these constituents in the powders. As previously reported by Wang et al. [18], this could be linked to the different affinity of the particular groups of polyphenolics for the stationary phase, which might affect the elution time and thus the presence or absence of these constituents in the extract. Besides this, the presence of phenolic acids, anthocyanins, and flavonols was confirmed [46].

The sum of identified polyphenols was, on average, 3.9-fold higher for control samples (Table 2) (average 19.07 g/100 g db) when compared to powders gained with 10% (w/w) carrier addition to the extracts before drying, regardless of the drying technique applied (Figure 2; Table S1). A similar observation was previously noted in the case of cranberry juices and extracts [47]. The drying techniques influenced the sum of identified polyphenols to a high extent. In the case of control samples, the highest content of polyphenols was noted after freeze drying, followed by vacuum drying at 90 and 60 °C (Table 2).

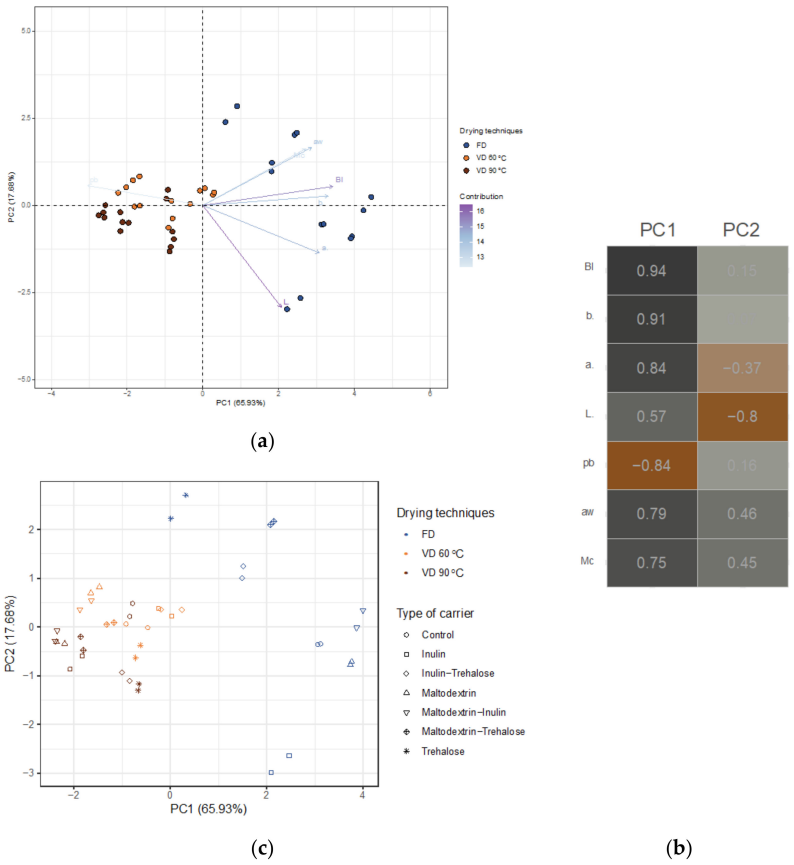


Figure 1. (**a**) The PCA biplot of the first two principal components that simultaneously shows PC scores of chokeberry pomace extract powders (points) and loadings of explanatory variables (vectors). The marker colour corresponds to the drying techniques (i.e., freeze and vacuum drying at 60 and 90 °C), while the length and the transparency of the arrows indicate the variance of the physical properties of powders from chokeberry pomace extracts and their contributions to the principal components, respectively. Together, the first two principal components explain 83.61% of the variability; (**b**) The plot of normalised factor loadings that quantify the extent to which the explanatory variable is related with a given principal component. Applying the so-called Malinowski rule (i.e., normalised factor loading cut-off of |0.70|) simplifies assigning physical meaning to each principal component; (**c**) Score plot in the space defined by the first two principal components illustrating relations and trends of chokeberry pomace extract powders gained after freeze and vacuum drying at 60 and 90 °C with the addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M—I), maltodextrin—trehalose (M—T), and inulin—trehalose (I—T).

In the case of powders produced with the addition of carriers and their mixes, no statistical differences were noted between the average content of polyphenols in the samples gained with the addition of carriers after freeze drying and vacuum drying at 60 °C (Table S1). The strongest influence was noted after the application of vacuum drying at 90 °C. Going into the details the application of the freeze drying process resulted in the highest retention of all identified polyphenols when maltodextrin and the mix of maltodextrin and trehalose (M-T) was used, whereas the usage of inulin resulted in the lowest retention of these constituents (Figure 2); however, the results were not statistically significantly different. One probable cause may be interactions between the carrier and polyphenolics as raised by Tomas et al. [48].

Table 2. The content of identified polyphenolics (g/100 g db), hydroxymethyl-*L*-furfural (µg/100 g db) and antioxidant capacity measured by TEAC ABTS and FRAP methods (mmol TE/100 g db) in chokeberry pomace extract powders (controls) obtained after freeze and vacuum drying (average ± standard deviation; $n = 2$).

	FD	VD	
	−60 °C/+24 °C	**60 °C**	**90 °C**
Total polyphenols	22.70 ± 0.12 ^{c}	15.88 ± 0.44 ^{a}	18.64 ± 0.47 ^{b}
Phenolic acids			
Neochlorogenic acid	4.86 ± 0.18 ^{b}	3.01 ± 0.09 ^{a}	4.47 ± 0.67 ^{ab}
Cryptochlorogenic acid	0.10 ± 0.01 ^{ab}	0.07 ± 0.01 ^{a}	0.14 ± 0.02 ^{b}
Chlorogenic acid	5.15 ± 0.15 ^{b}	3.43 ± 0.11 ^{a}	4.72 ± 0.37 ^{b}
Sum	10.10 ± 0.02 ^{b}	6.51 ± 0.20 ^{a}	9.33 ± 1.06 ^{b}
Anthocyanins			
Cyanidin-3-*O*-galactoside	5.90 ± 0.14 ^{b}	3.68 ± 0.09 ^{a}	3.93 ± 0.37 ^{a}
Cyanidin-3-*O*-glucoside	0.33 ± 0.05 ^{b}	0.18 ± 0.01 ^{a}	0.22 ± 0.02 ^{ab}
Cyanidin-3-*O*-arabinoside	2.56 ± 0.05 ^{b}	1.68 ± 0.05 ^{a}	1.61 ± 0.13 ^{a}
Cyanidin-3-*O*-xyloside	0.41 ± 0.01 ^{b}	0.28 ± 0.02 ^{a}	0.29 ± 0.03 ^{a}
Sum	9.19 ± 0.13 ^{b}	5.82 ± 0.12 ^{a}	6.06 ± 0.56 ^{a}
Flavonols			
Quercetin-dihexoside 1	0.26 ± 0.01 ^{b}	0.17 ± 0.01 ^{a}	0.30 ± 0.01 ^{c}
Quercetin-dihexoside 2	0.14 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.21 ± 0.04 ^{a}
Quercetin-3-*O*-vicianoside	0.14 ± 0.01 ^{b}	0.12 ± 0.01 ^{b}	0.06 ± 0.02 ^{a}
Kaempferol-3-*O*-robinobioside	0.45 ± 0.02 ^{b}	0.24 ± 0.01 ^{a}	0.43 ± 0.02 ^{b}
Kaempferol-3-*O*-rutinoside	0.53 ± 0.02 ^{b}	0.30 ± 0.01 ^{a}	0.48 ± 0.02 ^{b}
Kaempferol-3-*O*-galactoside	1.06 ± 0.02 ^{a}	1.72 ± 0.07 ^{b}	1.03 ± 0.01 ^{a}
Kaempferol-3-*O*-glucoside	0.78 ± 0.02 ^{b}	0.59 ± 0.02 ^{a}	0.72 ± 0.02 ^{b}
Derivative of quercetin	0.05 ± 0.01 ^{b}	0.20 ± 0.01 ^{c}	0.04 ± 0.01 ^{a}
Sum	3.40 ± 0.01 ^{a}	3.55 ± 0.12 ^{a}	3.26 ± 0.03 ^{a}
Hydroxymethyl-*L*-furfural	11.57 ± 0.23 ^{a}	2.62 ± 0.24 ^{b}	14.03 ± 1.59 ^{a}
TEAC ABTS	364.95 ± 11.98 ^{a}	357.33 ± 11.01 ^{a}	374.80 ± 12.92 ^{a}
FRAP	292.95 ± 7.61 ^{a}	285.14 ± 1.02 ^{a}	299.38 ± 1.01 ^{a}

FD—freeze drying, VD—vacuum drying; TE—Trolox equivalent; ^{a,b,c}—the same letters within a row indicate no statistically significant differences (HSD Tukey test, $p \leq 0.05$).

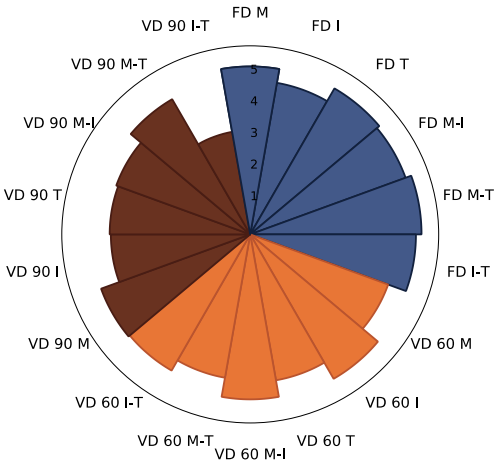


Figure 2. The radar plot of sum of identified polyphenolic compounds in chokeberry pomace extract powders gained with addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M—I), maltodextrin—trehalose (M—T) and inulin—trehalose (I—T) by freeze drying (FD) and vacuum drying at 60 °C (VD 60) and 90 °C (VD 90) ($n = 2$) (g/100 g db).

The reverse effect was noted in the case of powders gained after the application of vacuum drying at 60 °C: the addition of inulin resulted in the highest retention of these constituents. Among the powders gained after vacuum drying at 90 °C, the highest retention of the sum of polyphenolics was indicated in the samples obtained after the addition of M and M-T (Figure 2). A statistically significantly lower content of polyphenols was noted when I-T was applied for drying (Table S1). Previously, an influence on the retention of polyphenolics during application of different drying techniques and selected types of carriers was noted for blackcurrant [14] and chokeberry [12] juices.

Going into the details, the percentage share of phenolic acids, anthocyanins, and flavonols in the powders obtained by the selected drying techniques was as follows: 42.9%, 41.2%, and 15.9% for freeze-dried samples; 44%, 37.8%, and 18.2% for vacuum-dried samples at 60 °C; and 47.9%, 31.1%, and 21% for vacuum-dried samples at 90 °C. In order to follow these changes, each group of polyphenolics was examined (Table S2).

Similar to chokeberry juice powders [12], the dominant identified group of polyphenolics present in the controls and powders made with the addition of carriers consisted of phenolic acids [49], among which chlorogenic (50.9% of total phenolic acids), neochlorogenic (47.9% of total phenolic acids), and cryptochlorogenic (1.2% of total phenolic acids) acids were quantified (Table 2 and Table S2). In comparison, Sójka et al. [46] identified only chlorogenic and neochlorogenic acids in chokeberry pomace dried at 70 °C. In the current study, the content of phenolic acids was the highest in the control samples gained after freeze and vacuum drying at 90 °C, whereas the application of VD at 60 °C resulted, on average, in a 33% lower content of these constituents (Table 2). When the addition of selected carriers was concerned, the average content of the sum of phenolic acids was at a similar level, regardless of the drying technique and parameters applied (Figure 3a–d). There were no statistically significant differences noted between samples gained with the addition of M, I, T, M-I, M-T, and I-T after freeze and vacuum drying at 60 °C. Vacuum drying at 90 °C caused significant changes in the content of phenolic acids (Table S2). The lowest content of these constituents was noted when I and I-T were added for drying (Figure 3a). A similar observation was noted in the case of chokeberry juice drying, in which addition of inulin resulted in the lowest content of phenolic acids in powders [12]. When the single compounds were concerned (Figure 3b–d), the chlorogenic and neochlorogenic acids followed the comparable alterations caused by the carrier type and drying technique applied. The strongest changes were noted in the case of cryptochlorogenic acid, the content of which was the lowest. Maltodextrin and trehalose preserved the greatest content of this constituent after freeze drying and vacuum drying at 90 °C; however, the latest technique led to the strongest degradation of this compound in the powders gained. Regardless of the quantity of the selected phenolic acids present in the products gained, their thermolability, moderated by the carrier type due to their chemical structure, might be significantly different.

Overall, similar to Tkacz et al. [41], when the type of carrier was concerned, the highest retention of phenolic acids was noted in products gained with maltodextrin in this particular setting of drying techniques. It can be stated that the selection of an appropriate carrier type and drying technique used for the possible highest retention of phenolic acids in plant powders should be tested for specific products (including the initial chemical composition of raw materials) as it may differ due to the interactions between carriers and individual bioactive compounds present in plants [50].

The second group of polyphenolics identified in chokeberry pomace extract powders consisted of anthocyanins, among which the presence of cyanidin-3-*O*-galactoside, -glucoside, -arabinoside, and -xyloside was confirmed (Tables 2 and 3). Among the controls, freeze drying led to the highest retention of the sum of these constituents followed by VD at 90 and 60 °C (Table 2). A similar observation was made in the case of formulations with carriers, with some exceptions. When inulin was added to the chokeberry extracts, the application of FD and VD at 60 °C resulted in a similar content of identified anthocyanins, whereas the usage of maltodextrin and mix composed of maltodextrin and trehalose re-

sulted in a similar retention of these compounds in the products gained after vacuum drying at 60 and 90 °C. The strongest degradation of these constituents was indicated after vacuum drying at 90 °C for the formulation containing the I-T mixture.

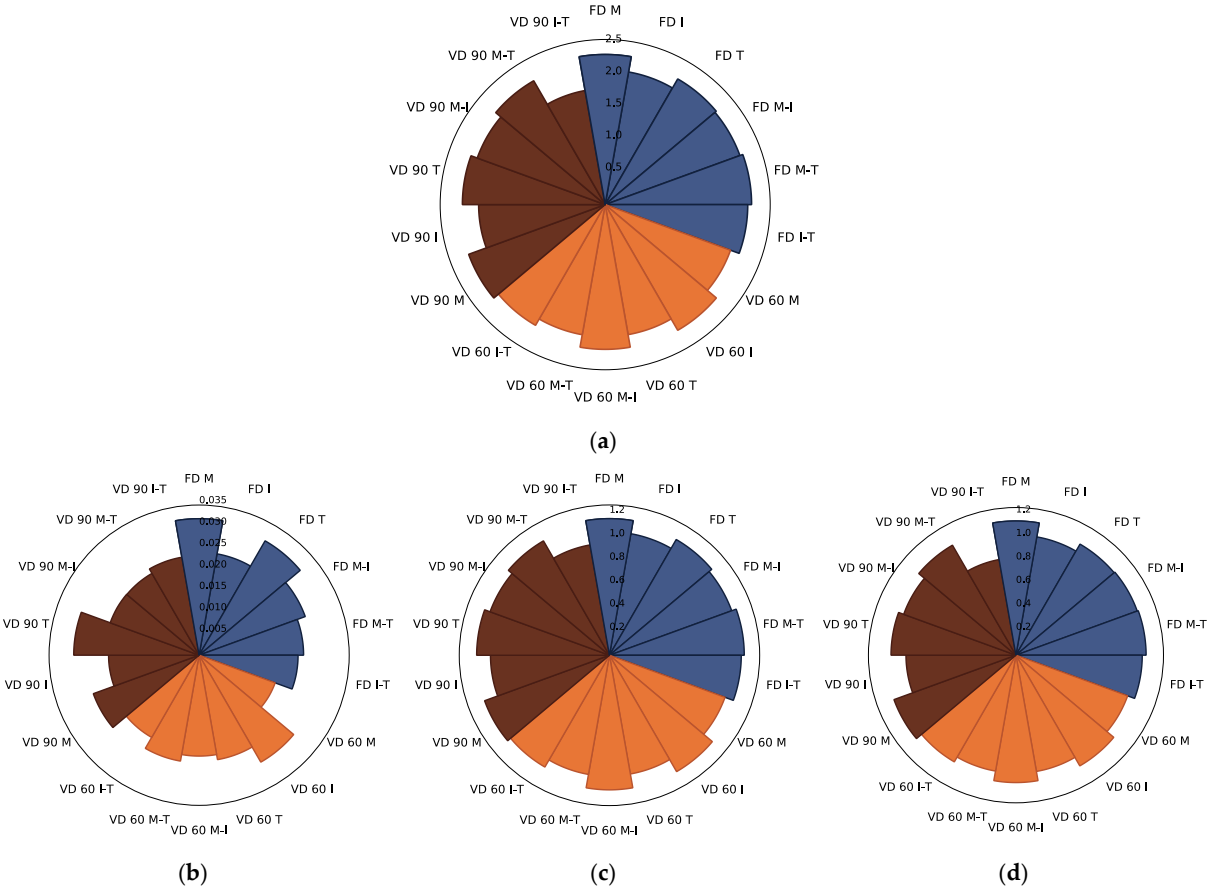


Figure 3. The radar plot of identified phenolic acids ((**a**)—sum of phenolic acids, (**b**)—chlorogenic acid, (**c**)—neochlorogenic acid, (**d**)—cryptochlorogenic acid) in chokeberry pomace extract powders gained with the addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M—I), maltodextrin—trehalose (M—T) and inulin—trehalose (I—T) by freeze drying (FD) and vacuum drying at 60 (VD 60) and 90 °C (VD 90) (n = 2) (g/100 g db).

Going into the details, the dominant anthocyanin present in chokeberry pomace extracts' powders was cyanidin-3-*O*-galactoside, which consisted, on average, of 64.5% of the sum of identified anthocyanins present in powders (controls, carrier added powders), followed by cyanidin-3-*O*-arabinoside, cyanidin-3-*O*-xyloside, and cyanidin-3-*O*-glucoside. A similar percentage share of anthocyanins was previously noted in the case of chokeberry juice powders [12]. What is more, the influence of the carriers used in the study on individual anthocyanins followed the path of the sum of anthocyanins (Table 3).

Table 3. The content of identified anthocyanins in chokeberry pomace extracts powders (g/100 g db) (n = 2; average ± standard deviation).

Drying Technique	Process Conditions	Carrier	Anthocyanins				
			Cyanidin-3-*O*-Galactoside	Cyanidin-3-*O*-Glucoside	Cyanidin-3-*O*-Arabinoside	Cyanidin-3-*O*-Xyloside	Sum of Anthocyanins
FD	−60 °C/24 °C	Maltodextrin	1.36 ± 0.04 ^{fg}	0.08 ± 0.01 ^{g}	0.59 ± 0.01 ^{fgh}	0.10 ± 0.01 ^{fg}	2.12 ± 0.06 ^{ghi}
		Inulin	1.28 ± 0.06 ^{efg}	0.07 ± 0.01 ^{defg}	0.56 ± 0.03 ^{efgh}	0.10 ± 0.01 ^{efg}	2.00 ± 0.10 ^{fghi}
		Trehalose	1.42 ± 0.08 ^{g}	0.07 ± 0.01 ^{efg}	0.62 ± 0.04 ^{gh}	0.11 ± 0.01 ^{g}	2.22 ± 0.14 ^{hi}
		Maltodextrin—Inulin	1.38 ± 0.05 ^{g}	0.06 ± 0.01 ^{cdefg}	0.59 ± 0.02 ^{fgh}	0.10 ± 0.01 ^{fg}	2.13 ± 0.07 ^{ghi}
		Maltodextrin—Trehalose	1.45 ± 0.02 ^{g}	0.07 ± 0.01 ^{fg}	0.64 ± 0.02 ^{h}	0.11 ± 0.01 ^{g}	2.27 ± 0.04 ^{i}
		Inulin—Trehalose	1.41 ± 0.04 ^{g}	0.07 ± 0.01 ^{defg}	0.62 ± 0.02 ^{gh}	0.09 ± 0.01 ^{defg}	2.18 ± 0.08 ^{hi}
VD	60 °C	Maltodextrin	1.13 ± 0.03 ^{cde}	0.05 ± 0.01 ^{bc}	0.49 ± 0.01 ^{de}	0.08 ± 0.01 ^{bcd}	1.75 ± 0.05 ^{cdef}
		Inulin	1.37 ± 0.06 ^{fg}	0.06 ± 0.01 ^{cdef}	0.60 ± 0.03 ^{fgh}	0.09 ± 0.01 ^{efg}	2.12 ± 0.09 ^{ghi}
		Trehalose	1.12 ± 0.04 ^{cde}	0.05 ± 0.01 ^{bc}	0.48 ± 0.02 ^{cde}	0.08 ± 0.01 ^{cde}	1.73 ± 0.07 ^{cdef}
		Maltodextrin—Inulin	1.27 ± 0.10 ^{efg}	0.06 ± 0.01 ^{cdef}	0.55 ± 0.05 ^{efg}	0.09 ± 0.01 ^{cdef}	1.97 ± 0.15 ^{efgh}
		Maltodextrin—Trehalose	1.10 ± 0.04 ^{cde}	0.05 ± 0.01 ^{bc}	0.47 ± 0.02 ^{cde}	0.07 ± 0.01 ^{bc}	1.70 ± 0.07 ^{cde}
		Inulin—Trehalose	1.19 ± 0.01 ^{def}	0.06 ± 0.01 ^{cde}	0.52 ± 0.01 ^{ef}	0.09 ± 0.01 ^{cdef}	1.85 ± 0.01 ^{defg}
	90 °C	Maltodextrin	1.16 ± 0.01 ^{de}	0.06 ± 0.01 ^{cdef}	0.49 ± 0.01 ^{de}	0.08 ± 0.01 ^{cdef}	1.80 ± 0.01 ^{def}
		Inulin	1.03 ± 0.02 ^{cd}	0.05 ± 0.01 ^{bc}	0.43 ± 0.01 ^{bcd}	0.07 ± 0.01 ^{bcd}	1.58 ± 0.04 ^{cd}
		Trehalose	0.82 ± 0.02 ^{b}	0.04 ± 0.01 ^{b}	0.35 ± 0.01 ^{b}	0.06 ± 0.01 ^{b}	1.27 ± 0.03 ^{b}
		Maltodextrin—Inulin	0.95 ± 0.04 ^{bc}	0.05 ± 0.01 ^{bc}	0.40 ± 0.02 ^{bc}	0.07 ± 0.01 ^{bc}	1.47 ± 0.07 ^{bc}
		Maltodextrin—Trehalose	1.15 ± 0.02 ^{de}	0.05 ± 0.01 ^{bcd}	0.48 ± 0.01 ^{cde}	0.08 ± 0.01 ^{cdef}	1.76 ± 0.02 ^{def}
		Inulin—Trehalose	0.39 ± 0.01 ^{a}	0.02 ± 0.01 ^{a}	0.16 ± 0.01 ^{a}	0.03 ± 0.01 ^{a}	0.60 ± 0.02 ^{a}

FD—freeze drying, VD—vacuum drying; $^{a–i}$—the same letters within a column indicate no statistically significant differences (HSD Tukey test, $p \leq 0.05$).

The last of the identified groups of polyphenols in all chokeberry pomace extract powders were flavonols [12] (about 18.3% of all determined compounds). Among them, eight constituents were detected: kaempferol-3-*O*-galactoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-robinobioside, quercetin-dihexoside 1, quercetin-3-*O*-vicianoside, quercetin-dihexoside 2, and a derivative of quercetin (Table S3). In comparison with the other groups of polyphenols, the sum of flavonols had the smallest fluctuations in their content depending on the drying technique or carrier type (no statistically significant differences), which confirmed their high stability during the powdering process (Figure 4a; Table S3). However, going into detail, it should be noted that their content was lower in powders obtained by freeze drying (15.9% of all identified polyphenols), while the highest content was reported in products obtained by vacuum drying at 90 °C (21% of all identified polyphenols) (Table S3). As indicated by Hamrouni-Sellami et al. [51], this may be due to the release of these compounds from more polymerised structures during heating. A similar trend was observed for quercetin and its glucoside. In this case, heating up to 120 °C resulted in an increase in their content, while further processing up to 150 °C caused their degradation [52]. However, it is worth looking at the changes in individual flavonols depending on the drying technique used and the addition of the carrier (Figure 4a–i).

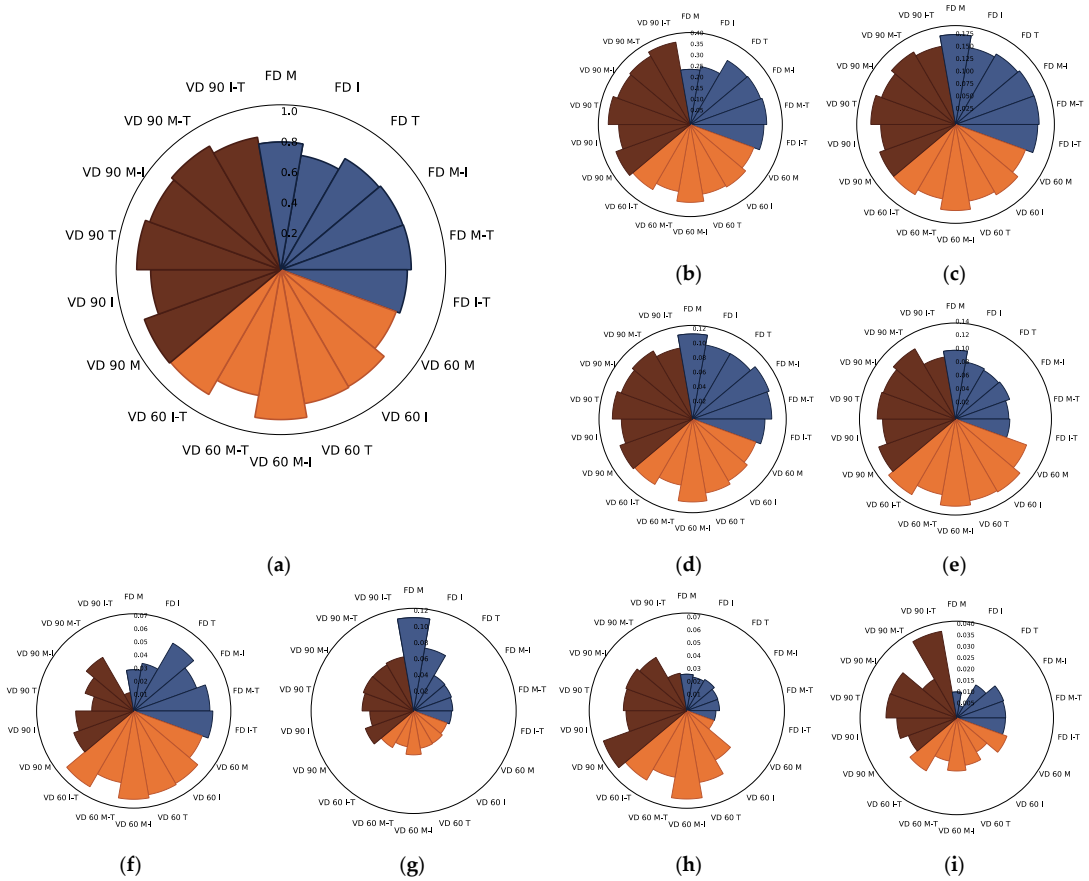


Figure 4. The radar plot of identified flavonols ((**a**)—sum of flavonols, (**b**)—kaempferol-3-*O*-galactoside, (**c**)—kaempferol-3-*O*-glucoside, (**d**)—kaempferol-3-*O*-rutinoside, (**e**)—kaempferol-3-*O*-robinobioside, (**f**)—quercetin-3-*O*-vicianoside, (**g**)—quercetin-dihexoside 1, (**h**)—quercetin-dihexoside 2, (**i**)—derivative of quercetin) in chokeberry pomace extract powders gained with addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M—I), maltodextrin—trehalose (M—T) and inulin—trehalose (I—T) by freeze drying (FD) and vacuum drying at 60 (VD 60) and 90 °C (VD 90) (n = 2) (g/100 g db).

The highest content of the dominant flavonol, i.e., kaempferol-3-*O*-galactoside, in the control powders was noted after VD at 60 °C, while the lowest when the FD and VD at 90 °C were applied (Table 2). Considering the powders with carrier addition (Figure 4b), vacuum drying at 90 °C and inulin-trehalose, trehalose, and maltodextrin application was the most suitable, while freeze-dried powders with M and I resulted in the lowest content of this compound, for which statistically significant differences were found (Table S3).

A very similar relationship was observed for the quercetin derivative in the control samples (Table 2); however, in the case of the carrier-added powders, the lowest content of this constituent was found in freeze-dried products with inulin, while an approximately 7-times higher content was determined in those obtained by the vacuum drying at 90 °C with the addition of I-T mixture (Figure 4i). Interestingly, the parameters and carriers mixture, on the one hand, allowed the highest quercetin derivative content, and on the other hand, resulted in the lowest quercetin-3-*O*-vicianoside concentration (Figure 4f). Comparing the drying techniques, VD at 60 °C was the most favourable in the context of the quercetin-3-*O*-vicianoside level in the powders analysed. Upon comparison of the content of this compound in powders without a carrier, FD and VD at 60 °C proved to be the best methods, while vacuum drying at 90 °C resulted in an approximately 2 times lower content of quercetin-3-*O*-vicianoside (Table 2). Noteworthy, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside proved to be the most stable flavonols as their content changed depending on the drying method and the type of added carrier were relatively small compared to the other compounds of the same group (Figure 4c,d). Additionally, fluctuations in the concentration of these compounds depending on the drying parameters and media type were comparable. For kaempferol-3-*O*-robinobioside in the control samples, the lowest concentration of this compound was found in vacuum-dried powders at 60 °C, while FD and VD at 90 °C yielded the highest levels (no statistically significant differences) of this flavonol (Table 2). A reverse effect was observed in the case of powders with carriers (Figure 4e), for which the lowest content of kaempferol-3-*O*-robinobioside was determined in freeze-dried products, while vacuum drying at 60 and 90 °C resulted in the highest and similar content of this constituent in the analysed powders. Taking into account the effect of the carrier agent, the highest content of this compound was found in vacuum-dried powders at 60 °C with I, M-I, and I-T, while the lowest content was found in freeze-dried products with M-T. An interesting relationship was also observed for the two quercetin-dihexosides. In case of quercetin-dihexoside 1 for no-carrier added samples, VD at 60 °C resulted in powders with the lowest concentration of this compound being obtained, while VD at 90 °C resulted in the highest concentration (Table 2). When analysing the powders with added carriers (Figure 4g), the quercetin-dihexoside 1 content was the highest in powders obtained by FD with the addition of M, while considering other drying methods, the same carrier did not have such a noticeable effect as in the case of freeze drying. Moreover, except for the samples with added maltodextrin obtained by FD, no statistically significant differences were found for all the rest of the powders (Table S3). Regarding quercetin-dihexoside 2 for the control powders, no statistically significant differences were found for the content of this compound (Table 2). Interestingly, in the case of powders with added carriers (Figure 4h), freeze drying, irrespective of the type of carrier, as well as vacuum drying at 90 °C and the addition of I-T mixture and VD at 60 °C with M resulted in the lowest content of this compound, while the other variants of powders were characterised by significantly higher quercetin-dihexoside 2 levels (Table S3). Moreover, while for quercetin-dihexoside 1, vacuum drying at 60 °C resulted in its lowest content in the powders obtained, for quercetin-dihexoside 2, the same drying allowed products with the highest level of this compound. Therefore, it can be concluded that the highest quercetin-dihexoside 1 content is possible to obtain if low-temperature drying is used, while the use of high temperatures during drying favours a high quercetin-dihexoside 2 content.

3.2.2. Hydroxymethyl-*L*-furfural

In the current study, the hydroxymethyl-*L*-furfural was identified in all analysed powders (Table 2, Figure 5). In the case of control samples, the highest content of HMF was noted in powders gained after VD at 90 °C and freeze drying (no statistically significant differences) while, interestingly, a lower content was noted after the application of vacuum drying at 60 °C (Table 2). Contrary to the expectations that freeze drying as a low-temperature treatment should result in the lowest content of HMF (or its absence), its presence in the analysed powders might be connected with the particular composition of chokeberry pomace extracts. The substrates for the HMF formation present in these extracts might additionally react with bioactives during the storage of fruit products [53] and even after freeze drying. As a confirmation of this, its occurrence was previously noted in freeze-dried products [54] and fruit juice-based foodstuffs [12,41]. Interestingly, the studies of Zhang et al. [55] showed that the formation of HMF was accelerated by the chlorogenic acid in model systems, which is in accordance with the present study as a strong correlation between HMF and phenolic acids ($r = 0.84$) was noted. What is more, Zhang and An [56] proved that its formation might be inhibited by the interactions with flavonols, which was in line with the present research where the sum of these identified compounds was concerned ($r = -0.85$) (Figure S2). However, this inhibition mechanism of single flavonols is ambiguous as between HMF and two dominant constituents, namely, kaempferol-3-*O*-galactoside and kaempferol-3-*O*-glucoside, strong negative ($r = -0.97$) and positive ($r = 0.86$) correlations, respectively, were observed at the same time (Figure S2).

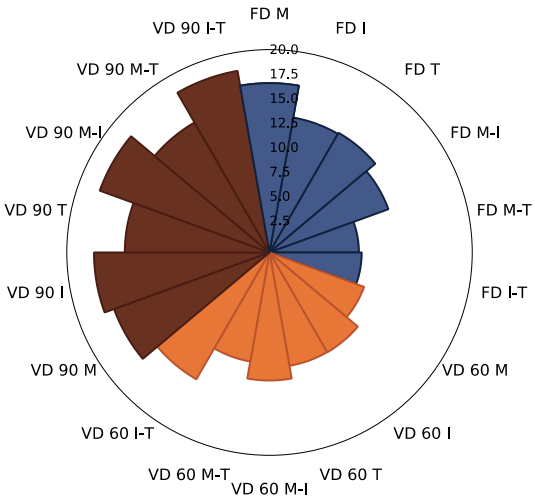


Figure 5. The radar plot of HMF in chokeberry pomace extract powders gained with the addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M-I), maltodextrin—trehalose (M-T) and inulin—trehalose (I-T) by freeze drying (FD) and vacuum drying at 60 (VD 60) and 90 °C (VD 90) ($n = 2$) (μg/100 g db).

Similar to the control samples, the strongest formation of HMF was also noted for powders obtained with carriers gained after VD at 90 °C, followed by freeze drying. Its content was the lowest when VD at 60 °C was used (Figure 5). Its slower formation at 60 °C was also reported by Olivares-Tenorio et al. [57] and Michalska et al. [58] during the drying of fruit-based products, whereas a further increase in drying temperature caused its rapid formation. As it was previously reported, such phenomena might be linked to the formation of intermediary compounds during the application of 60 °C [57]. When the type of carrier was considered, the highest content of HMF was noted when inulin and its mixes (I-T, I-M) were used for powder production at VD at 90 °C. Thus, the inulin might enhance the formation of this process contaminant during powder preparation [12].

As the different bioactives might influence the formation of HMF during drying, in the case of powders gained with the addition of carriers, no significant correlation between the sum of identified polyphenols and HMF was noted, with the exception of quercetin-3-*O*-vicianoside ($r = -0.65$) (Figure S3). Probably, as stated by Zhang and An [56], its formation might be inhibited by the interactions with some flavonols; however, until now, no particular compounds were indicated. To sum up, when the quality of chokeberry extract powders is considered, the formation of HMF could be controlled by the initial composition of material submitted for drying, process parameters, and type of carrier used for the powder preparation.

3.2.3. Antioxidant Capacity

The antioxidant capacity of chokeberry pomace extract powders determined by the TEAC ABTS and FRAP methods showed that the ability of control samples (Table 2) to scavenge free radicals was about 4.7 times (TEAC ABTS assay) and about 4.2 times higher (FRAP assay) when compared to the powders gained with the application of selected carriers. Going into detail, the phenolic acids significantly influenced the antioxidant capacity measured by TEAC ABTS ($r = 0.56$) and FRAP ($r = 0.64$) in samples produced without the addition of carriers. What is more, a negative correlation between FRAP and the sum of identified flavonols in these products was noticed ($r = -0.75$), which could be connected with the different actions of these compounds present in the analysed powders toward the reducing potential of ferric ion (Fe^{3+} to Fe^{2+}) [59]. In the case of the products with added carriers, it was observed that the antioxidant capacity varied slightly depending on the carrier used (Figure 6a,b).

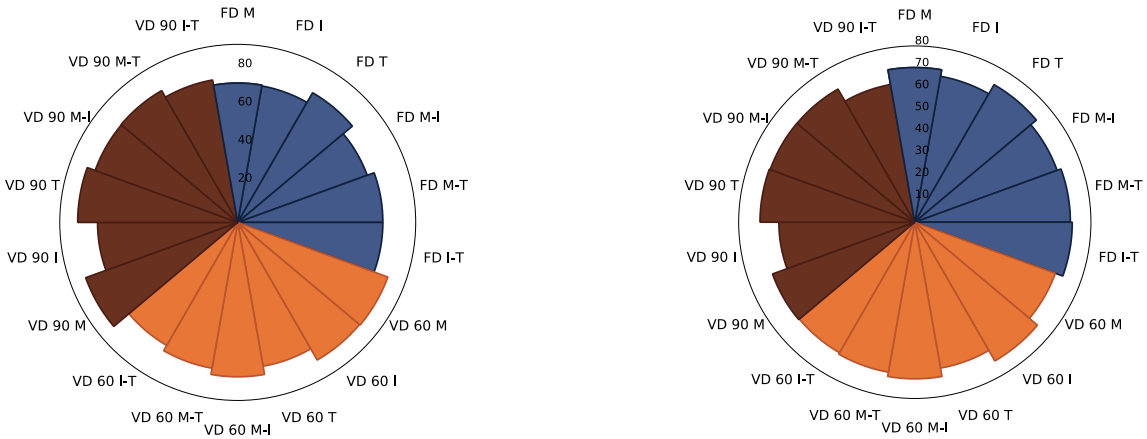


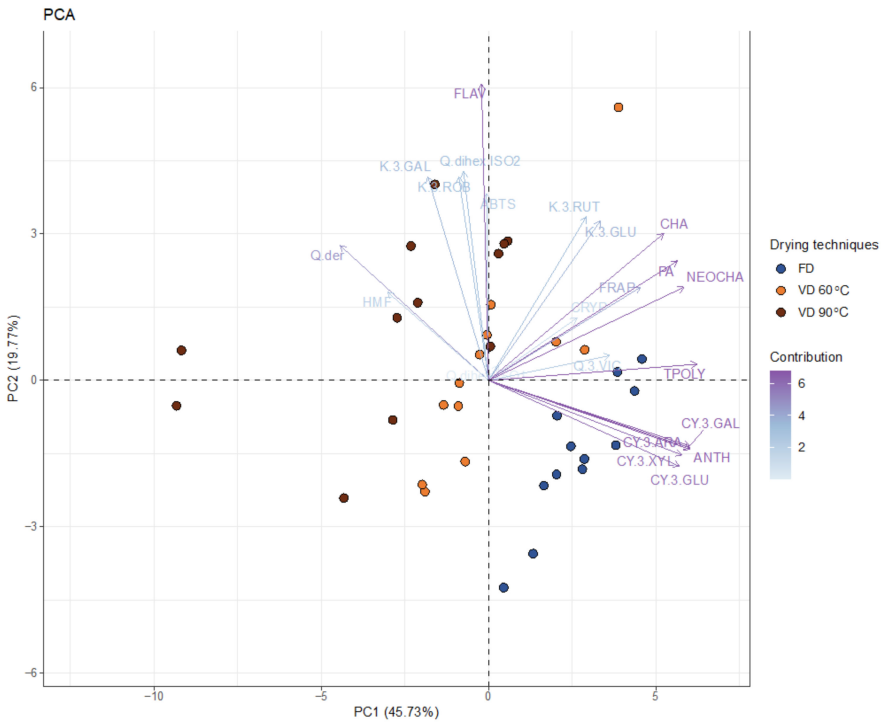
Figure 6. The radar plot of TEAC ABTS (**a**) and FRAP (**b**) of chokeberry pomace extract powders gained with the addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M—I), maltodextrin—trehalose (M—T), and inulin—trehalose (I—T) by freeze drying (FD) and vacuum drying at 60 (VD 60) and 90 °C (VD 90) ($n = 2$) (mmol Trolox/100 g db).

Interestingly, the highest average antioxidant capacity values measured by the TEAC ABTS method were observed in the powders obtained by VD at 90 °C, followed by VD at 60 °C, and freeze drying (Table S1). The reverse effect was observed in the case of FRAP. The antioxidant capacity values may be related to a significant content of certain polyphenolic compounds in those powders, such as predominant phenolic acids [60]. This was confirmed by a high positive correlation ($r = 0.78$) between the content of these constituents in the analysed powders and their antioxidant capacity measured by the FRAP method (Figure S3). Moreover, there was a moderate positive correlation between the sum of anthocyanins and antioxidant capacity measured by FRAP ($r = 0.58$) and no linear

relationship between the content of these compounds and TEAC ABTS analysis values ($r = -0.13$) (Figure S3). It might be connected with the lower content of anthocyanins than phenolic acids in the carrier-added powders; however, in the literature, there are also some reports indicating that the antioxidant capacity is related more to the total content of polyphenolic compounds than to the content of anthocyanins, which may be due to the lower free radical scavenging capacity of these compounds compared to other polyphenolics [61]. Furthermore, in the case of TEAC ABTS, and FRAP analysis, the powders obtained with the addition of trehalose and its mixes after freeze drying had the highest values. In general, during VD at 60 °C, the application of inulin resulted in higher TEAC ABTS and FRAP values of the powders analysed, whereas the addition of this carrier and its mix with trehalose lowered the antioxidant properties measured by these two methods when vacuum drying at 90 °C was applied. This proved the selectivity of the action of individual compounds and/or carrier substances [62] toward the free radical scavenging properties of products, which could be additionally moderated by the drying parameters [50].

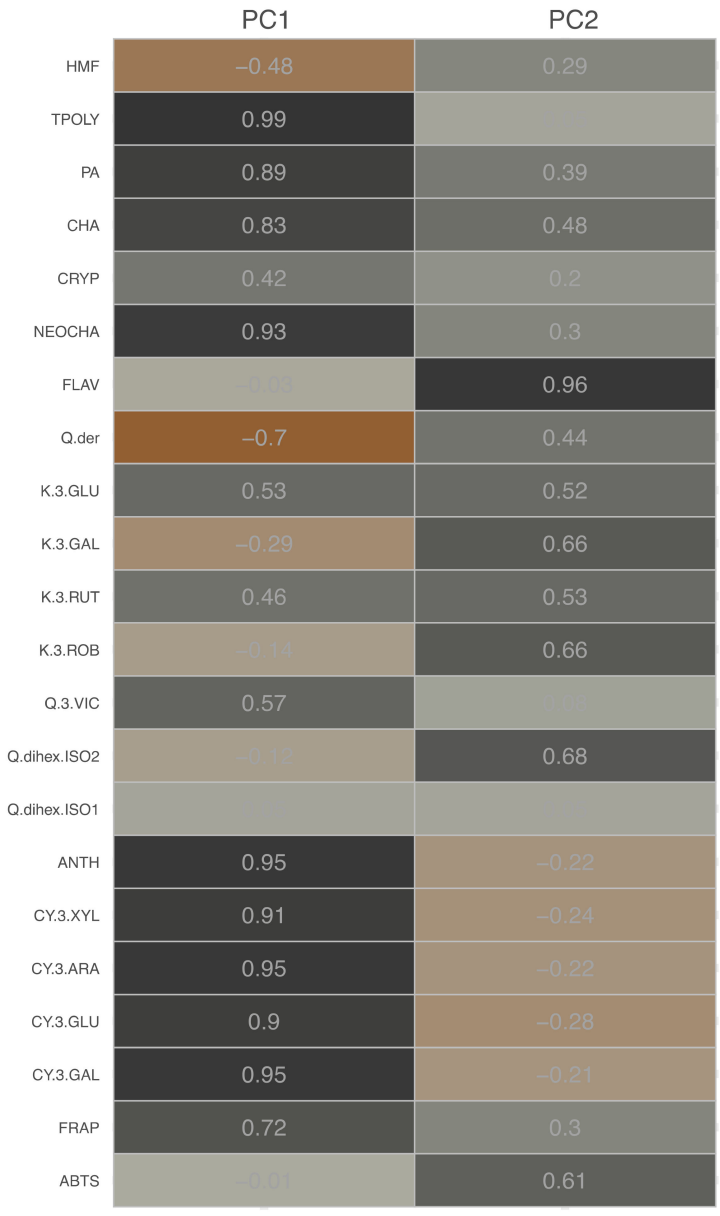
3.2.4. PCA Analysis

The PCA biplot for chemical properties (Figure 7a) showed quite the opposite results from those shown for physical properties (Figure 1a).



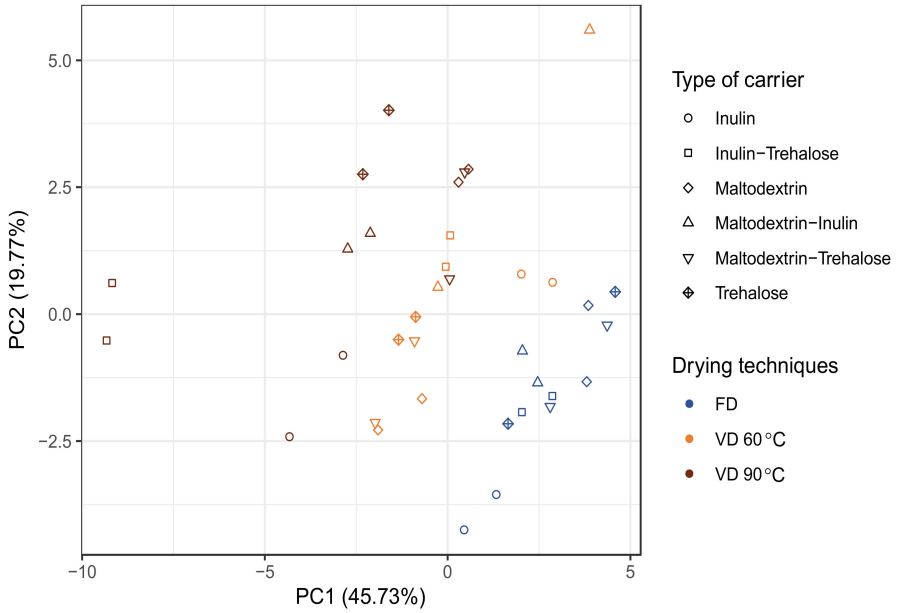
(**a**)

Figure 7. *Cont.*



(**b**)

Figure 7. *Cont.*



(**c**)

Figure 7. (**a**) The PCA biplot of the first two principal components. The marker colour corresponds to the drying techniques (i.e., freeze and vacuum drying at 60 and 90 °C), while the length and the transparency of the arrows indicate the variance of the chemical properties of powders from chokeberry pomace extracts and their contributions to the principal components, respectively. Together, the first two principal components explain 65.50% of the variability; (**b**) The plot of normalised factor loadings; (**c**) Score plot in the space defined by the first two principal components illustrating the relations and trends of the chokeberry pomace extract powders gained after freeze and vacuum drying at 60 and 90 °C with the addition of maltodextrin (M), inulin (I), trehalose (T), maltodextrin—inulin (M—I), maltodextrin—trehalose (M—T), and inulin—trehalose (I—T).

The vacuum-dried samples at 90 °C had much greater variance than the freeze-dried samples. Chokeberry pomace extract powders gained after the application of different drying techniques were split along PC1. PC1 was positively correlated with the sum of identified polyphenolics (TPOLY), phenolic acids (PA), including chlorogenic acid (CHA), neochlorogenic acid (NEOCHA), the sum of identified anthocyanins (ANTH), including cyanidin-3-*O*-xyloside (CY.3.XYL), -arabinoside (CY.3.ARA), -glucoside (CY.3.GLU), and -galactoside (CY.3.GAL) (Figure 7b). Therefore, it was straightforward to see that drying techniques changed from VD at 90 °C through freeze drying with an increasing content of anthocyanins and phenolic acids, when moving from left to right along the *X*-axis (PC1). PC1 was also negatively correlated with the derivative of quercetin (Q.der). Due to low PC1 scores and negative loading values, samples vacuum dried at 90 °C were characterised by a relatively higher value of Q.der compared to the freeze-dried samples. The most influential variable in PC2 was positively correlated with the sum of flavonols (FLAV). PC2 did not indicate variation, which clearly distinguished between the samples gained after the application of different drying techniques. Hence, only a general statement could be made that chokeberry pomace extract powders toward the top of the PCA biplot (Figure 7a) were described by the highest content of flavonols due to the positive correlations between PC2 and FLAV. A closer look at the score plot shown in Figure 7c revealed that no clear trends were observed for the powders produced with the addition of different carriers and their mixes. Nonetheless, chokeberry pomace extract powders gained after vacuum drying

at 90 °C with addition of I-T differed significantly from the rest of the samples in their low content of anthocyanins and phenolic acids.

4. Conclusions

The current study evaluated the possibility of obtaining powders from chokeberry pomace extracts by drying techniques and different carrier types as one waste management practice in the food industry. The quality of such products should consider the priorities of their potential application as the moderation of powder properties is a multifactor issue. Taking the above into consideration, the PCA analysis indicated that freeze-dried samples exhibit more variation than those produced by vacuum drying at 60 and 90 °C, especially in terms of moisture content, water activity, colour, and browning index. The bulk density was higher for products obtained after vacuum drying. No straightforward trends in physical properties were observed for products that has selected carriers added.

In the analysed powders, three groups of polyphenols were identified and quantified, i.e., phenolic acids (3), anthocyanins (4), and flavonols (8). Drying techniques significantly influenced the polyphenolics in the powders gained with the addition of selected carriers. In general, the application of freeze drying resulted in a higher content of anthocyanins and phenolic acids, while vacuum drying at 90 °C allowed for the obtainment of products with high quantities of flavonols. Where the analysed carriers were concerned, the highest retention of the sum of identified polyphenolics was noted when maltodextrin and its mixture with trehalose were applied for powder production by freeze drying and vacuum drying at 90 °C, whereas during VD at 60 °C, it was inulin and its mixes. In the case of phenolic acids and anthocyanins, a similar observation was made for FD and VD 60 in that maltodextrin and trehalose protect most of the mentioned compounds; however, in case of VD 90, trehalose caused the lowest retention of anthocyanins. Regarding flavonols, this group was characterised by the highest stability during drying, regardless of the carrier type used. A detailed analysis showed very diverse behaviour of the individual compounds with respect to the applied processing parameters, thus making it impossible to identify any specific method of powder production that results in flavonols' highest retention.

As the content of hydroxymethyl-*L*-furfural is of high importance to monitor in processed foods' quality, the lowest concentration of this compound was determined in powders gained after vacuum drying at 60 °C, while its highest level was noted after VD at 90 °C. The current study confirmed [12] that the addition of inulin and its mixes during high-temperature treatment (vacuum drying at 90 °C) should be carefully considered as this carrier may influence the formation of HMF in fruit-based products.

To sum up, the retention of polyphenolics and formation of HMF in chokeberry pomace extracts' powders was affected simultaneously by the initial composition of raw material, carrier type, drying techniques, and parameters applied. Taking all these factors into account, including interactions between the matrix composition during drying, 10% addition of maltodextrin and trehalose mixture for freeze drying and vacuum drying at 90 °C allowed the production of powders with the highest retention of polyphenolic compounds and the lowest HMF level, at the same time. The outcome of the current study supported by the chemometric analyses can provide guidance for further research as well as give directions for work on designing functional foodstuff based on powders from chokeberry pomace extracts.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods10081864/s1, Figure S1: A correlation matrix showing the Pearson's correlation coefficients between each pair of variables (i.e., physical properties) in chokeberry pomace extract powders data set (controls and powders with carrier addition), Figure S2: A correlation matrix showing the Pearson's correlation coefficients between each pair of variables (i.e., chemical properties) in chokeberry pomace extract powders data set (control samples), Figure S3: A correlation matrix showing the Pearson's correlation coefficients between each pair of variables (i.e., chemical properties) in chokeberry pomace extract powders data set (powders with addition of carriers; no controls included), Table S1: The content of sum of polyphenols, hydroxymethyl-*L*-furfural and the

antioxidant capacity measured by TEAC ABTS and FRAP methods of chokeberry pomace extracts powders made with the addition maltodextrin, inulin, trehalose and a mixture of them using different drying methods (average ± standard deviation; n = 2), Table S2: The content of identified phenolic acids in chokeberry pomace extracts powders made with the addition maltodextrin, inulin, trehalose and a mixture of them using different drying methods (g/100 g db) (n = 2; average ± standard deviation), Table S3: The content of identified flavonols in chokeberry pomace extracts powders made with the addition maltodextrin, inulin, trehalose and a mixture of them using different drying methods (g/100 g db) (n = 2; average ± standard deviation).

Author Contributions: Conceptualization, A.M.-C. and A.W.; methodology, A.W., A.M.-C., J.B., A.G.-S.; software, A.G.-S.; formal analysis, A.M.-C., J.B., A.G.-S.; investigation, A.H., A.M.-C., J.B; resources, A.M.-C., A.W., A.G.-S.; data curation, A.M.-C., J.B., A.H., A.W., A.G.-S.; writing—original draft preparation, A.M.-C., J.B., A.G.-S., A.H.; writing—review and editing, A.M.-C., J.B., A.W.; supervision, A.M.-C.; project administration, A.M.-C.; funding acquisition, A.H., A.M.-C. All authors have read and agreed to the published version of the manuscript.

Funding: The research is financed under the individual student research project 'Młode umysły—Young Minds Project' from the subsidy increased for the period 2020–2025 in the amount of 2% of the subsidy referred to Art. 387 (3) of the Law of 20 July 2018 on Higher Education and Science, obtained in 2019.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The publication is the result of the research group activity: 'Plants4FOOD' (Leading Research Group of the University of Environmental and Life Sciences).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Salehi, G.; Díaz, E.; Redondo, R. Consumers' switching to vegan, vegetarian, and plant-based (Veggan) diets: A systematic Review of literature. In Proceedings of the 19th International Congress on Public and Nonprofit Marketing Sustainability: New Challenges for Marketing and Socioeconomic Development, Madrid, Spain, 2–4 July 2020.
2. Campos, D.A.; Garcia, R.G.; Vilas-Boas, A.A.; Madureira, A.R.; Pintado, M.M. Management of fruit industrial by-products—A case study on circular economy approach. *Molecules* **2020**, *25*, 320. [CrossRef]
3. Mayer-Miebach, E.; Briviba, K.; Schiffer, C.; Geiger, L.; Behsnilian, D.; Greiner, R. Particle size of milled chokeberry pomace did not influence in vitro cellular absorption and transport efficiencies of anthocyanins, phenolic acids and flavonols. *Int. J. Food Sci. Nutr.* **2019**, *70*, 932–940. [CrossRef] [PubMed]
4. Majerska, J.; Michalska, A.; Figiel, A. A review of new directions in managing fruit and vegetable processing by-products. *Trends Food Sci. Technol.* **2019**, *88*, 207–219. [CrossRef]
5. Vagiri, M.; Jensen, M. Influence of juice processing factors on quality of black chokeberry pomace as a future resource for colour extraction. *Food Chem.* **2017**, *217*, 409–417. [CrossRef]
6. Sidor, A.; Drożdżyńska, A.; Gramza-Michałowska, A. Black chokeberry (*Aronia melanocarpa*) and its products as potential health-promoting factors—An overview. *Trends Food Sci. Technol.* **2019**, *89*, 45–60. [CrossRef]
7. Witczak, T.; Stępień, A.; Gumul, D.; Witczak, M.; Fiutak, G.; Zięba, T. The influence of the extrusion process on the nutritional composition, physical properties and storage stability of black chokeberry pomaces. *Food Chem.* **2021**, *334*, 127548. [CrossRef] [PubMed]
8. Comunian, T.A.; Silva, M.P.; Souza, C.J. The use of food by-products as a novel for functional foods: Their use as ingredients and for the encapsulation process. *Trends Food Sci. Technol.* **2021**, *108*, 269–280. [CrossRef]
9. Roda-Serrat, M.C.; Andrade, T.; Rindom, J.; Lund, P.B.; Norddahl, B.; Errico, M. Optimization of the recovery of anthocyanins from chokeberry juice pomace by homogenization in acidified water. *Waste Biomass Valorization* **2020**, *12*, 1815–1827. [CrossRef] [PubMed]
10. Bhandari, B.; Bansal, N.; Zhang, M.; Schuck, P. Woodhead Publishing Series in Food Science, Technology and Nutrition. In *Handbook of Food Powders*; Elsevier: Amsterdam, The Netherlands, 2013.
11. Kammerer, D.; Kljusuric, J.G.; Carle, R.; Schieber, A. Recovery of anthocyanins from grape pomace extracts (Vitis vinifera L. cv. Cabernet Mitos) using a polymeric adsorber resin. *Eur. Food Res. Technol.* **2005**, *220*, 431–437. [CrossRef]

12. Michalska-Ciechanowska, A.; Brzezowska, J.; Wojdyło, A.; Gajewicz-Skretna, A.; Ciska, E.; Majerska, J. Chemometric contribution for deeper understanding of thermally-induced changes of polyphenolics and the formation of hydroxymethyl-*L*-furfural in chokeberry powders. *Food Chem.* **2021**, *342*, 128335. [CrossRef]
13. Nowak, D.; Jakubczyk, E. The Freeze-Drying of Foods—The characteristic of the process course and the effect of its parameters on the physical properties of food materials. *Foods* **2020**, *9*, 1488. [CrossRef]
14. Michalska, A.; Wojdyło, A.; Brzezowska, J.; Majerska, J.; Ciska, E. The influence of inulin on the retention of polyphenolic compounds during the drying of blackcurrant juice. *Molecules* **2019**, *24*, 4167. [CrossRef]
15. Sobulska, M.; Zbicinski, I. Advances in spray drying of sugar-rich products. *Dry. Technol.* **2020**, 1–26. [CrossRef]
16. Wan, X.; Guo, H.; Liang, Y.; Zhou, C.; Liu, Z.; Li, K.; Niu, F.; Zhai, X.; Wang, L. The physiological functions and pharmaceutical applications of inulin: A review. *Carbohydr. Polym.* **2020**, *246*, 116589. [CrossRef] [PubMed]
17. Oszmiański, J. Sposób otrzymywania barwników antocyjanowych. PL-158707. 30 July 1993. Available online: https://grab.uprp.pl/sites/WynalazkiWzoryUzytkowe/Opisy/Patenty%20i%20Wzory%20uytkowe/158707_B1.pdf (accessed on 26 July 2021). (In Polish)
18. Wang, W.; Yagiz, Y.; Buran, T.J.; Nunes, C.D.N.; Gu, L. Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Res. Int.* **2011**, *44*, 2666–2673. [CrossRef]
19. Mexis, S.; Kontominas, M. Effect of oxygen absorber, nitrogen flushing, packaging material oxygen transmission rate and storage conditions on quality retention of raw whole unpeeled almond kernels (*Prunus dulcis*). *LWT* **2010**, *43*, 1–11. [CrossRef]
20. Wojdyło, A.; Oszmiański, J.; Bielicki, P. Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (*Cydonia oblonga* Miller) varieties. *J. Agric. Food Chem.* **2013**, *61*, 2762–2772. [CrossRef] [PubMed]
21. Michalska, A.; Wojdyło, A.; Łysiak, G.P.; Figiel, A. Chemical composition and antioxidant properties of powders obtained from different plum juice formulations. *Int. J. Mol. Sci.* **2017**, *18*, 176. [CrossRef]
22. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
23. Benzie, I.; Strain, J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
24. Jolliffe, I.T.; Cadima, J. Principal component analysis: A review and recent developments. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2016**, *374*, 20150202. [CrossRef]
25. Kassambara, A.; Mundt, F. Factoextra: Extract and Visualize the Results of Multivariate Data, R Package Version 1.0.7; CRAN: 2020. Available online: https://cran.r-project.org/web/packages/factoextra/index.html (accessed on 26 July 2021).
26. Nakazawa, M. Fmsb: Functions for Medical Statistics Book with Some Demographic, Data Version 0.7.1; CRAN: 2019. Available online: https://cran.r-project.org/web/packages/fmsb/index.html (accessed on 26 July 2021).
27. Michalska, A.; Lech, K. The Effect of carrier quantity and drying method on the physical properties of apple juice powders. *Beverages* **2018**, *4*, 2. [CrossRef]
28. Tontul, I.; Topuz, A. Spray-drying of fruit and vegetable juices: Effect of drying conditions on the product yield and physical properties. *Trends Food Sci. Technol.* **2017**, *63*, 91–102. [CrossRef]
29. Carvalho, A.G.D.S.; Machado, M.T.D.C.; da Silva, V.M.; Sartoratto, A.; Rodrigues, R.A.F.; Hubinger, M. Physical properties and morphology of spray dried microparticles containing anthocyanins of Jussara (*Euterpe edulis Martius*) extract. *Powder Technol.* **2016**, *294*, 421–428. [CrossRef]
30. Shafiur Rahman, M. *Handbook of Food Preservation*, 2nd ed.; Shafiur Rahman, M., Ed.; CRC Press: Boca Raton, FL, USA, 2007.
31. Adetoro, A.O.; Opara, U.L.; Fawole, O.A. Effect of carrier agents on the physicochemical and technofunctional properties and antioxidant capacity of freeze-dried pomegranate juice (*Punica granatum*) powder. *Foods* **2020**, *9*, 1388. [CrossRef]
32. Muhoza, B.; Xia, S.; Wang, X.; Zhang, X. The protection effect of trehalose on the multinuclear microcapsules based on gelatin and high methyl pectin coacervate during freeze-drying. *Food Hydrocoll.* **2020**, *105*, 105807. [CrossRef]
33. Kilburn, D.; Townrow, S.; Meunier, V.; Richardson, R.; Alam, A.; Ubbink, J. Organization and mobility of water in amorphous and crystalline trehalose. *Nat. Mater.* **2006**, *5*, 632–635. [CrossRef]
34. Michalska-Ciechanowska, A.; Majerska, J.; Brzezowska, J.; Wojdyło, A.; Figiel, A. The Influence of maltodextrin and inulin on the physico-chemical properties of cranberry juice powders. *ChemEngineering* **2020**, *4*, 12. [CrossRef]
35. Chang, K.S.; Kim, D.W.; Kim, S.S.; Jung, M.Y. Bulk flow properties of model food powder at different water activity. *Int. J. Food Prop.* **1998**, *1*, 45–55. [CrossRef]
36. Khalifa, I.; Li, M.; Mamet, T.; Li, C. Maltodextrin or gum Arabic with whey proteins as wall-material blends increased the stability and physiochemical characteristics of mulberry microparticles. *Food Biosci.* **2019**, *31*, 10445. [CrossRef]
37. Bednarska, M.A.; Janiszewska-Turak, E. The influence of spray drying parameters and carrier material on the physico-chemical properties and quality of chokeberry juice powder. *J. Food Sci. Technol.* **2019**, *57*, 564–577. [CrossRef]
38. Sarabandi, K.; Peighambardoust, S.H.; Mahoonak, A.R.S.; Samaei, S. Effect of different carriers on microstructure and physical characteristics of spray dried apple juice concentrate. *J. Food Sci. Technol.* **2018**, *55*, 3098–3109. [CrossRef] [PubMed]
39. Wojdyło, A.; Lech, K.; Nowicka, P. Effects of different drying methods on the retention of bioactive compounds, on-line antioxidant capacity and color of the novel snack from red-fleshed apples. *Molecules* **2020**, *25*, 5521. [CrossRef]
40. Pathare, P.; Opara, U.L.; Al-Said, F.A.-J. Colour measurement and analysis in fresh and processed foods: A review. *Food Bioprocess Technol.* **2012**, *6*, 36–60. [CrossRef]

41. Tkacz, K.; Wojdyło, A.; Michalska-Ciechanowska, A.; Turkiewicz, I.P.; Lech, K.; Nowicka, P. Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated sea buckthorn juice powders. *Molecules* **2020**, *25*, 3801. [CrossRef]
42. Michalska, A.; Wojdyło, A.; Lech, K.; Łysiak, G.P.; Figiel, A. Physicochemical properties of whole fruit plum powders obtained using different drying technologies. *Food Chem.* **2016**, *207*, 223–232. [CrossRef] [PubMed]
43. Liu, S.-C.; Chang, H.-M.; Wu, J.S.-B. A study on the mechanism of browning in mei liqueur using model solutions. *Food Res. Int.* **2003**, *36*, 579–585. [CrossRef]
44. Dorris, M.R.; Voss, D.M.; Bollom, M.; Krawiec-Thayer, M.P.; Bolling, B.W. Browning index of anthocyanin-rich fruit juice depends on pH and anthocyanin loss more than the gain of soluble polymeric pigments. *J. Food Sci.* **2018**, *83*, 911–921. [CrossRef]
45. Sójka, M.; Kołodziejczyk, K.; Milala, J.; Abadias, M.; Viñas, I.; Guyot, S.; Baron, A. Composition and properties of the polyphenolic extracts obtained from industrial plum pomaces. *J. Funct. Foods* **2015**, *12*, 168–178. [CrossRef]
46. Sójka, M.; Kołodziejczyk, K.; Milala, J. Polyphenolic and basic chemical composition of black chokeberry industrial by-products. *Ind. Crop. Prod.* **2013**, *51*, 77–86. [CrossRef]
47. Michalska, A.; Wojdyło, A.; Honke, J.; Ciska, E.; Andlauer, W. Drying-induced physico-chemical changes in cranberry products. *Food Chem.* **2018**, *240*, 448–455. [CrossRef] [PubMed]
48. Tomas, M.; Beekwilder, J.; Hall, R.; Simon, C.D.; Sagdic, O.; Capanoglu, E. Effect of dietary fiber (inulin) addition on phenolics and in vitro bioaccessibility of tomato sauce. *Food Res. Int.* **2018**, *106*, 129–135. [CrossRef]
49. Szopa, A.; Kokotkiewicz, A.; Kubica, P.; Banaszczak, P.; Wojtanowska-Krośniak, A.; Krosniak, M.; Marzec-Wróblewska, U.; Badura, A.; Zagrodzki, P.; Bucinski, A.; et al. Comparative analysis of different groups of phenolic compounds in fruit and leaf extracts of *Aronia* sp.: *A. melanocarpa*, *A. arbutifolia*, and *A.*×*prunifolia* and their antioxidant activities. *Eur. Food Res. Technol.* **2017**, *243*, 1645–1657. [CrossRef]
50. Turkiewicz, I.; Wojdyło, A.; Tkacz, K.; Lech, K.; Michalska-Ciechanowska, A.; Nowicka, P. The influence of different carrier agents and drying techniques on physical and chemical characterization of Japanese quince (*Chaenomeles japonica*) microencapsulation powder. *Food Chem.* **2020**, *323*, 126830. [CrossRef]
51. Hamrouni-Sellami, I.; Rahali, F.Z.; Rebey, I.B.; Bourgou, S.; Limam, F.; Marzouk, B. Total phenolics, flavonoids, and antioxidant activity of sage (*Salvia officinalis* L.) plants as affected by different drying methods. *Food Bioprocess Technol.* **2012**, *6*, 806–817. [CrossRef]
52. Sharma, K.; Ko, E.Y.; Assefa, A.; Ha, S.; Nile, S.; Lee, E.T.; Park, S.W. Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. *J. Food Drug Anal.* **2015**, *23*, 243–252. [CrossRef]
53. Aktağ, I.G.; Gökmen, V. Multiresponse kinetic modelling of α-dicarbonyl compounds formation in fruit juices during storage. *Food Chem.* **2020**, *320*, 126620. [CrossRef] [PubMed]
54. Fitzpatrick, K.; Kendrick, B.; Santos, C.; Green, P.; Zhang, B.; Hunt, D.; Ronk, M.; Luo, Y. Freeze-dry mediated formation of 5-(Hydroxylmethyl)furfural. In *Developments in Biotechnology and Bioprocessing*; ACS Symposium Series; American Chemical Society: Washington, DC, USA, 2013; Volume 1125, pp. 129–145, ISBN 978-0-8412-2910-5.
55. Zhang, Z.; Zou, Y.; Wu, T.; Huang, C.; Pei, K.; Zhang, G.; Lin, X.; Bai, W.; Ou, S. Chlorogenic acid increased 5-hydroxymethylfurfural formation when heating fructose alone or with aspartic acid at two pH levels. *Food Chem.* **2016**, *190*, 832–835. [CrossRef]
56. Zhang, Y.; An, X. Inhibitory mechanism of quercetin against the formation of 5-(hydroxymethyl)-2-furaldehyde in buckwheat flour bread by ultra-performance liquid chromatography coupled with high-resolution tandem mass spectrometry. *Food Res. Int.* **2017**, *95*, 68–81. [CrossRef] [PubMed]
57. Olivares-Tenorio, M.-L.; Verkerk, R.; van Boekel, M.A.; Dekker, M. Thermal stability of phytochemicals, HMF and antioxidant activity in cape gooseberry (*Physalis peruviana* L.). *J. Funct. Foods* **2017**, *32*, 46–57. [CrossRef]
58. Michalska-Ciechanowska, A.; Wojdyło, A.; Łysiak, G.P.; Lech, K.; Figiel, A. Functional relationships between phytochemicals and drying conditions during the processing of blackcurrant pomace into powders. *Adv. Powder Technol.* **2017**, *28*, 1340–1348. [CrossRef]
59. Firuzi, O.; Lacanna, A.; Petrucci, R.; Marrosu, G.; Saso, L. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Subj. Biochim. Biophys. Acta Gen. Subj.* **2005**, *1721*, 174–184. [CrossRef]
60. Chen, J.; Yang, J.; Ma, L.; Li, J.; Shahzad, N.; Kim, C.K. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Sci. Rep.* **2020**, *10*, 1–9. [CrossRef]
61. Wilkowska, A.; Ambroziak, W.; Czyżowska, A.; Adamiec, J. Effect of microencapsulation by spray drying and freeze drying technique on the antioxidant properties of blueberry (*Vaccinium myrtillus*) juice polyphenolic compounds. *Pol. J. Food Nutr. Sci.* **2016**, *66*, 11–16. [CrossRef]
62. Silva-Espinoza, M.A.; García-Martínez, E.; Martínez-Navarrete, N. Protective capacity of gum Arabic, maltodextrin, different starches, and fibers on the bioactive compounds and antioxidant activity of an orange puree (*Citrus sinensis* (L.) Osbeck) against freeze-drying and in vitro digestion. *Food Chem.* **2021**, *357*, 129724. [CrossRef]

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

Jessica Brzezowska
Department of Fruit, Vegetable, and Nutraceutical Plant Technology
Faculty of Biotechnology and Food Science
Wrocław University of Environmental and Life Sciences
37 Chełmońskiego Street, 51-630 Wrocław

Wrocław, 11.09.2023

OŚWIADCZENIE

Oświadczam, że w pracy:
Michalska-Ciechanowska A., Hendrysiak A., **Brzezowska J.**, Wojdyło A., Gajewicz-Skretna A. (2021). How Do the different types of carrier and drying techniques affect the changes in physico-chemical properties of powders from chokeberry pomace extracts? *Foods*, 10(8), 1864. https://doi.org/10.3390/foods10081864
mój udział polegał na tworzeniu zarysu badań, pomocy w opracowaniu technologii wytwarzania, a także otrzymaniu proszków z wytłoków aroniowych stanowiących materiał badawczy. Uczestniczyłam w wykonaniu oznaczeń parametrów fizycznych oraz analizowanych wyróżników chemicznych. Wygenerowane dane opracowałam pod katem merytorycznym oraz statystycznym. Ponadto brałam udział w przygotowaniu manuskryptu, a także jego współredagowaniu podczas procesu recenzji.

11.09.2023 Jessica Brzezowska
data i podpis

Potwierdzam treść oświadczenia.

11.09.23 Anna Michalska-Ciechanowska
data i podpis promotora

DECLARATION

I declare that in the work:
Michalska-Ciechanowska A., Hendrysiak A., **Brzezowska J.**, Wojdyło A., Gajewicz-Skretna A. (2021). How do different types of carriers and drying techniques affect changes in physicochemical properties of aronia pomace extract powders? *Food*, 10(8), 1864. https://doi.org/10.3390/foods10081864
my participation consisted of creating the outline of the research, providing assistance in developing the manufacturing technology, as well as obtaining powders from chokeberry pomace as research material. I participated in the determination of physical parameters and analyzed chemical characteristics. I processed the generated data in terms of substantive and statistical aspects. In addition, I participated in the preparation of the manuscript, as well as its co-editing during the review process.

11.09.2023 Jessica Brzezowska
date and signature

I confirm the content of the statement.

11.09.2023 Anna Michalska-Ciechanowska
date and supervisor's signature

Publication 2

Comparative study of antioxidant, antiglycation and chemoprotective potential of beetroot juice powder formulations with functional carriers

Comparative study of antioxidant, antiglycation and chemoprotective potential of beetroot juice powder formulations with functional carriers

Jessica BRZEZOWSKA[1], Katarzyna SKRZYPCZAK[2*], Wojciech RADZKI[2], Igor Piotr TURKIEWICZ[1], Marta ZIAJA-SOŁTYS[3], Anna BOGUCKA-KOCKA[3], Aneta WOJDYŁO[1], Anna MICHALSKA-CIECHANOWSKA[1]

[1] *Department of Fruit, Vegetable and Plant Nutraceutical Technology, the Faculty of Biotechnology and Food Science, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 37 Str., 51-630 Wroclaw, Poland; jessica.brzezowska@upwr.edu.pl, igor.turkiewicz@upwr.edu.pl, aneta.wojdylo@upwr.edu.pl, anna.michalska@upwr.edu.pl*

[2] *Department of Plant Food Technology and Gastronomy, Sub-department of Fruits, Vegetables and Mushrooms Technology, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland; katarzyna.skrzypczak@up.lublin.pl, wojciech.radzki@up.lublin.pl*

[3] *Department of Biology and Genetics, Medical University of Lublin, Lublin, 20-093 Poland; marta.ziaja-soltys@umlub.pl, anna.bogucka-kocka@umlub.pl*

***** *Corresponding Author: Katarzyna Skrzypczak, Ph.D.; Department of Plant Food Technology and Gastronomy, Sub-department of Fruits, Vegetables and Mushrooms Technology, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland; phone: +48 81 462 33 08, e-mail: katarzyna.skrzypczak@up.lublin.pl*

Abstract

In the present study freeze- and spray-dried powders from non-fermented and fermented beetroot juice with the addition of prebiotic carriers (inulin, oligofructose, Nutriose®) and maltodextrin were obtained and analyzed for physicochemical and biological properties. Fermentation and carrier type affected the powders' quality to a greater extent than drying techniques. Higher betalains content was noted for non-fermented juice powders, while ferulic and syringic acids' derivatives for their fermented-juice-based counterparts. Oligofructose induced hydroxymethyl-*L*-furfural formation, but also together with inulin resulted in products with the strongest antioxidant capacity. Nutriose® had the greatest *in vitro* antiproliferative activity towards human leukemia cell lines, unlike oligofructose which was shown to stimulate their growth. Overall, fermentation that led to beetroot matrix modification and carrier type affected powders' quality toward their improved potential functional properties.

Keywords:

beetroot juice powders, probiotic fermentation, prebiotic carriers, phenolics, antiproliferative activity

1. Introduction

The epidemiological basis of health disorders that currently affect society is related to the long-term oxidative stress associated with the accumulation of free radicals and reactive carbonyls that react with proteins generating advanced glycation end products (AGEs). AGEs accumulation is one of the risk factors leading to diabetic complications, aging, and cancer development (del Castillo et al., 2021). Numerous studies were devoted to finding edible plants with antiglycation activity, providing a crucial strategy for the alleviation of AGE-induced diabetic complications (Song et al., 2021). An increase in consumers' awareness about the biological activities of phytonutrients is reflected in wider interest and consumption of plant-based foodstuffs that create the need for developing new health-promoting foods. Hence, many multidirectional studies are focused on an exploration of sources of natural substances that effectively reacts against those harmful constituents.

Beetroot (*Beta vulgaris* L.) is rich in betalains that with other phenolics, exhibit health-promoting properties, including anti-inflammatory, antioxidant, immunomodulatory activities, and cancer chemopreventive effects (Fu et al., 2020). Betalains are nitrogenous pigments derived from betalamic acid (4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid). The modifications in basic structure of betalamic acid determine betalains classifications. Betacyanins (such as betanin, isobetanin) consist of betalamic acid that is condensed by cyclo-DOPA (cyclo-L-3,4-dihydroxyphenylalanine) or its glucosyl derivatives, while betaxanthins (including vulgaxanthin I) originate from the condensation of betalamic acid with amino acids, amines, or derivatives (Calva-Estrada et al., 2022). Fresh beetroot or juice are lacking in popularity due to their specific taste. An alternative for the inclusion of this vegetable in the diet is the production of fermented juice, much more palatable compared to raw material. Another solution is powder obtainment offering many advantages including easy-to-handle form.

Spray drying, a cost-effective way of powders production, especially compared to freeze-drying gives a final product of comparable or even higher quality (Michalska-Ciechanowska et al., 2021). However, the processing of juice rich in low molecular weight sugars and organic acids requires the addition of high molecular weight carriers to convert it into powder. Maltodextrin is one of the most widely used additives due to its simplicity of application and high drying efficiency. The ‘clean label’ trends urge the food industry to eliminate artificial and unnecessary additives in favor of those with functional properties. Prebiotic features are the main characteristics of substances currently being tested as carriers, of which inulin and oligofructose are the most popular ones (Verruck et al., 2019). Nutriose®, which is a sugar-free

dietary fiber derived from wheat, peas, or corn, is another example of a prebiotic ingredient that was used for i.e., honey drying (Samborska et al., 2019).

Powders belong to the processed food group, and the quality changes in these products can be induced not only by interactions between main components in their matrix during drying but also between them and carriers. Those, in turn, may lead to positive (release of bioactives from polymerized structures) and negative (formation of Maillard reaction and caramelization products, e.g., hydroxymethyl-*L*-furfural) alterations affecting the quality of powders (Michalska-Ciechanowska et al., 2021). For this reason, the composition and production of food powders should combine not only the appropriate selection of raw materials but also the knowledge about processing modulation, i.e., the addition of carriers, application of drying techniques and parameters, etc.

Despite numerous reports on the physical attributes of powders that determine their handling in industry, and chemical properties defining their nutritional value (Janiszewska-Turak et al., 2022), there is still a lack of knowledge on the biological activity of these products that reflects their beneficial properties. Therefore, the study aimed to verify if the juice treatment (fermentation) and carrier type used during freeze- and spray drying may moderate health-promoting characteristics of beetroot juice powders including alterations in phenolics profile, *in vitro* antioxidant, antiglycation and antiproliferative activities toward human leukemia cell lines.

2. Material and methods

2.1. Materials

Beta vulgaris L. *cv.* Opolski was purchased in a local store (Lublin, Poland). The probiotic strain *Lactobacillus plantarum* 299v (Sanprobi IBS, Sanum Poland) was applied as a starter culture in fermentation.

2.2. Juice obtainment

Fresh juice was made using a screw press 20K-GS (Angel, Korea). After pasteurization (80 °C; 15 min; DEST-25 696, Destiller, Poland), the juice was cooled down and portions of 5 L were inoculated (2%; *m/v*) by probiotic starter cultures (10^9 CFU/mL). Fermentation was conducted at 35 °C for 18 h in a designed fermentation vessel (Gestar, Poland) and monitored using an automatic pH control system. After fermentation juice was divided into portions (250 mL) and subjected to pasteurization (80 °C; 15 min). Non-fermented pasteurized juice was considered as a control.

2.2.1. Physicochemical analyses of beetroot juice

The total extract content (TEC) in non-fermented beetroot juice (NFJ) and fermented juice (FJ) was determined (PN-90/A-75101/02) using a refractometer (Kruss DR201-95 Merazet, Poland) and expressed as Brix degrees (°Bx). The pH was tested using a pH meter (F20 – Meter, Mettler-Toledo GmbH, Switzerland). The color parameters of NFJ and FJ were determined (CIE *L*a*b** system) using a 3Color K9000Neo spectrophotometer (3Color, Narama, Poland). The measurements were performed using a D65 light source and a standard colorimetric observer with a 10° field of view. The concentrations of betalains pigments in NFJ and FJ were determined according to Gościnna et al. (2012). Before analysis juices were properly diluted using phosphate buffer (pH 6.5) to receive absorbance values in the range of 0.2–0.8. The absorbance of diluted juices samples was measured at λ=476 nm, λ=538 nm, and λ=600 nm (Spectrophotometer UV-Vis Helios Gamma Thermo, USA). The content of betanin and vulgaxanthin-I in tested juices was calculated according to Halwni et al. (2018). All measurements were performed in triplicate (n = 3).

2.3. Powders preparation

The NFJ and FJ were divided into equal portions and mixed 4:1 (*w/w*) with carriers: maltodextrin (DE = 9.3; PEPEES S.A., Poland), inulin GR (Beneo-Orafti, Belgium), oligofructose (Beneo-Orafti, Belgium) and Nutriose® (Roquette, Lestrem, France). Freeze-drying (FD) was performed by FreeZone freeze dryer (Labconco Corp., MO, USA) for 24 h at 65 Pa (drying chamber: −60 °C; heating plates: +25 °C). Spray drying (SD) was carried out using a B290 spray dryer (Buchi, Flawil, Switzerland) at inlet and outlet temperatures of 180 and 100 °C, respectively (aspirator: 38 m^3/h; feed rate: 5 mL/min). Powders were vacuum packed (MC 2006, Tepro SA, Koszalin, Poland) and kept at −20 °C until analyses.

2.3.1. Physicochemical analyses of beetroot juice powders

The moisture content of powders was done according to Michalska-Ciechanowska et al. (2021) (24 h, 80 °C, 300 Pa) and results were expressed as %. The water activity (a_w) was measured at 25 °C (Dew Point Water Activity Meter 4TE, AQUA LAB, Pullman, WA, USA). The color of juices and powders was measured with a Minolta Chrome Meter CM-700d (Konica Minolta, Inc., Osaka, Japan) in the CIE *L*a*b** system. All measurements were performed in triplicate (n = 3).

2.3.2 Antioxidant capacity

The antioxidant capacity *in vitro* of NFJ and FJ powders was performed by TEAC $ABTS^{+\bullet}$ and FRAP assays using a Synergy H1 spectrophotometer (BioTek Instruments Inc., USA) (Michalska-Ciechanowska et al., 2021). Results were expressed as mmol Trolox equivalent per 100 g of dry matter (dm) (n = 3).

2.3.3. Phenolics and hydroxymethyl-L-furfural

Determination of phenolics and hydroxymethyl-*L*-furfural in powders (NFJP and FJP) was done according to Wojdyło et al. (2013). The extracts were prepared accordingly: 50 mg of sample were mixed with 30% methanol (methanol/water/acetic acid/ascorbic acid: 30/68/1/1, *v/v/v/w*), hand-mixed, and sonicated for 15 min. Samples were kept at 4 °C for 24 h, then sonicated again for 15 min, and centrifuged (19 000 × g, 20 °C; MPW-251, MPW Med. Instruments, Poland). Subsequently, the supernatant was filtered through a hydrophilic membrane (PTFE, 0.20 μm; Millex Samplicity™ Filter; Merck, Germany) and subjected to UPLC-PDA-Q/TOF-MS analysis. Quantification (UPLC-PDA) and identification (LC/MS Q-TOF) were performed using an Acquity UPLC system (Waters, Milford, USA) with a Q-Tof mass spectrometer (Waters, Manchester, UK). The betalains and phenolic acids were determined at the wavelengths λ=540 nm and λ=320 nm, respectively, while hydroxymethyl-*L*-furfural at λ=280. The results were elaborated with Empower 3 software and expressed as mg per 100 g of dm (n = 3).

2.3.4. Total Phenolics Content (TPC)

Determination of TPC was done based on the Folin-Ciocalteu colorimetric method (Gao et al., 2000) in beetroot powders' extracts prepared accordingly: 50 mg of sample was dissolved in 1.7 mL of 80% aqueous methanol–hydrochloric acid mixture (1 mL/L), hand-mixed and sonicated for 15 min. Samples were kept at 4 °C for 24 h, then sonicated again for 15 min, and centrifuged (19 000 × g, 20 °C; MPW-251, MPW Med. Instruments, Poland). The absorbance measurement was done with Synergy H1 spectrophotometer (BioTek Instruments Inc., USA) at λ=750 nm. The results were expressed as g of gallic acid equivalent (GAE) per 100 g of dm (n = 3).

2.3.5. Antiglycation activity

The antiglycation properties of powders were tested according to Wang et al. (2011) with slight modifications. Products were extracted using 30% methanol with 1% formic acid

(*v/v*). After 15 min of sonication, samples were stored (24 h, 4 °C) and sonicated again, followed by evaporation (Unipan 350P, Scientific Instruments, Warsaw, Poland). The residue was dissolved in 10 mL of phosphate buffer (50 mM; pH 7.4) containing 0.02% NaN_3 (*v/v*). Such prepared extracts were tested in three models reflecting different stages of protein glycation. To imitate the first stage, glucose (1.5 M) was mixed with samples, and incubated at 37 °C for 2 h, followed by the addition of BSA (30 mg/mL). The blank control was phosphate buffer without powders' extracts, while aminoguanidine (AG, 10 mM, final concentration) was a positive control. AGEs formation was assessed using a Synergy H1 spectrophotometer (BioTek Instruments Inc., USA) at excitation and emission wavelengths of λ=340 and λ=420 nm, respectively. Methylglyoxal (MGO) (60 mM) was used instead of monosaccharide to evaluate the middle step of the reaction, while *L*-arginine (60 mM) mixed with MGO constituted a model adapted to monitor major and specific AGEs formation during the last stage. In both models, the fluorescence measurement was done at λ_{ex} = 340 nm and λ_{em} = 380 nm. The results were calculated according to the following equation and expressed as % inhibition of AGEs(*n* = 3):

$$Percentage\ inhibition = \left(1 - \frac{Fluorescent\ intensity\ with\ inhibitor}{Fluorescent\ intensity\ without\ inhibitor}\right)\ x\ 100\% \quad (1)$$

2.3.6. In vitro cancer cell lines antiproliferative assay

Antiproliferative activity assay was performed on cells of human leukemia lines: HL-60/ MX2 (ATCC® CRL2257 ™) and J-45.01 (ATCC® CRL1990 ™). The cells were grown in RPMI 1640 medium (Biomed, Poland) with the addition of 10% (*v/v*) fetal bovine serum (EURx, Poland), 100 U/mL penicillin (Biological Industries, Israel), 0.1 mg/mL streptomycin (Biological Industries, Israel), 25 mg/mL antimycotic amphotericin B (Biological Industries, Israel). Both leukemia cells were grown at 37 °C in a 5% CO_2 atmosphere (95% humidity). The antiproliferative activity test was carried out using the Cell Proliferation Reagent WST-1 (Water Soluble Tetrazolium salts-1, Roche Diagnostics, Germany), which enabled the evaluation of the cytotoxic properties of dissolved powders. The principle of the WST-1 test was applied to measure the activity of a mitochondrial enzyme, i.e., succinate dehydrogenase, which converts the tetrazolium salt in living cells into water-soluble formazan. Both leukemia cell lines were seeded on 96-well plates at the density of $2 \cdot 10^4$ cells per well and incubated with beetroot juice powders (NFJP and FJP) (1 mg/mL) for 24 h at the conditions described above. Then, 10 µL of WST-1 reagent was added to each well and samples were incubated for 1.5 h. The absorbance was measured at λ=440 nm and 620 nm (reference wavelength) using a

Synergy H1 spectrophotometer (BioTek Instruments Inc., USA). The percentage of viable cells was calculated from the absorbance ($n = 4$).

2.4. Statistical analysis

The statistical analysis was performed using Statistica 13.1 (StatSoft, Poland). To determine significant differences ($p < 0.05$) among the average values of the parameters, analysis of variance (ANOVA) followed by Tukey‘s (HSD) posthoc test was used. The Shapiro-Wilk test was applied to examine the normality of the distribution of the variables. Pearson's correlation coefficient (r) was calculated to determine the relationships between selected variables. The R software was adopted for selected data visualization: the ‘corrplot’ package was used for correlation matrix preparation (Wei & Simko 2021), while ‘ggplot2’ package was for polar plots creation (Wickham, 2016).

3. Results and Discussion

3.1. Physicochemical properties of beetroot juices

The fermented beetroot juice (FJ) exhibited significantly lower pH, total extract content (TEC), values of color parameters, and a higher betacyanin concentration than non-fermented juice (NFJ) (Table 1). Juices fermented by probiotic strain (*L. plantarum* 299v) had TEC of 7.3 °Brix. A similar value was recorded by Janiszewska-Turak et al. (2022) in beetroot juices after four days of fermentation using *Lactiplantibacillus plantarum*. Sugar and organic acids (but also water-soluble components, proteins, and minerals) are the main elements influencing soluble solids content in plant juices that contribute to the level of TEC and are essential determinants affecting the overall flavor intensity (Li et al., 2021). Therefore, the lower value of TEC noted in FJ was probably caused by metabolically active bacteria that contribute to sugars and/or organic acid utilization.

FJ exhibited significantly lower values of parameters L^*, a^*, and b^* in comparison to NFJ. This corresponded to the findings of Janiszewska-Turak et al. (2022) who observed that color components (L^*, a^*, and b^*) diminished after the fermentation of beetroot juice. The juice after fermentation was greener, darker, and less red. The results were also in accordance with Ribeiro et al. (2020), who noticed that yellow mombin juice fermented by the probiotic strain *Lactobacillus acidophilus* NRRL B-4495 exhibited lower values of luminosity parameter (L^*) as well as a^* and b^* coordinates than its NFJ analogs. The color of beetroot juice depends on the concentration of naturally occurring pigments, among which water-soluble betacyanins are the most important. The stability of these pigments in solutions is limited by environmental

factors, i.e., the presence of metal cations, antioxidants, temperature, water activity, pH, and structural factors: degree of glycosylation and acylation (Calva-Estrada et al., 2022). In an acidic environment, betanin is regenerated from cyclo-DOPA and betalamic acid, as well as isobetanin is converted to its initial form, betanin (Calva-Estrada et al., 2022). Also, betacyanins are transformed during fermentation, due to the enzymatic activity of the bacteria and the lowering of the pH level to about 4.0 - 4.5 (this value was noted in the study for fermented juice). In an acidic environment, the vulgaxanthins are not stable because of the dissociation of carboxyl groups, and low pH. Therefore, the dissociation of the vulgaxanthins may induce a change in juice color toward purple (Calva-Estrada et al., 2022), which might explain a higher concentration of betacyanins in fermented juice (48.60 mg/mL) in comparison to NFJ (35.42 mg/mL) (Table 1). These findings were consistent with Jagannath et al. (2015), who indicated that the application of proper starter culture in the fermentation of beetroot-based products might contribute to enhancing the betacyanins preservation influencing the increased concentration of these components in comparison to the fresh product.

3.2. Physicochemical properties of beetroot juice powders

The moisture content (Mc) of beetroot juice powders ranged from 0.13 to 4.25% (Table 2). SD yielded powders with a comparable moisture content (<1%). In contrast, the Mc of powders obtained by FD was higher, with the exception of inulin. Lower moisture content for powders with inulin after lyophilization was previously noted for encapsulated *Bougainvillea glabra* bracts extract (Kuhn et al., 2020). The possible explanation for the ambiguous behavior of inulin in matrices tested can be linked to its stability under different thermal and pH treatment conditions, which may affect the water-binding capacity of this carrier (Li et al., 2019; Ozyurt & Ötles, 2016). Fermentation and drying techniques applied for powder preparation influenced the Mc values to a high extent. The water activity (a_w) of powders ranged between 0.049 to 0.345, thus the products can be considered microbiologically stable (Fontana, 2020). Powders after FD with oligofructose addition had the highest a_w, while SD with the same carrier yielded products with about 3 times lower values (Table 2). Maltodextrin application resulted in a comparable a_w regardless of the drying technique for both types of juice used (NFJ and FJ). When inulin and Nutriose® were applied for obtaining NFJP, FD led to products with significantly higher a_w than powders gained by SD. In the case of FJP, the reverse pattern was observed – spray-dried products had higher a_w values of approx. 43% for products with inulin and 35% for those with Nutriose®. Previously, Nutriose® was used for the production of spray-dried honey-rich powder, and when compared to maltodextrin, it resulted in products with a

higher water activity (Samborska et al., 2019). The study on fat-filled pea protein-based powders obtained by SD with, i.a., different carbohydrate carriers proved Nutriose® as a component resulting in products with the lowest a_w, compared to inulin, trehalose, and polydextrose. The latter two were characterized as yielding powders with the highest a_w and the least stability, which was attributed to the low osmolality of liquid feeds containing these carbohydrates (Domian et al., 2017). In the present study a_w was a result not only of the carrier type but also of the drying technique and matrix of beetroot juices used. For example, powders containing Nutriose® prepared from NFJ after FD had over 2.5 times higher a_w values than those from FJ. When NFJP with Nutriose® was concerned, freeze-drying led to 2-fold higher values of a_w compared to SD. Therefore, the observations made strongly indicated the validity of testing changes in a_w values depending on the above-mentioned key factors during the production of this powder type.

Spray-dried powders had higher L^* values than freeze-dried ones, regardless of juice or carrier type (Table 2). The only exception was NFJP with inulin, for which L^* values were slightly higher for lyophilized products. A similar observation was made for grapefruit powders (Egas-Astudillo et al., 2021) which may be attributed to smaller particles obtained by SD compared to FD, and consequently differences in their light reflection. For oligofructose and Nutriose®, when the NFJ was used, the brightness differences between products after FD and SD were significantly greater than in the case of using probiotically fermented juice during powdering. Since the brightness of the initial material, although statistically different, was scarcely noticeable, the probable explanation may be linked to differences in the matrix chemical composition between FJ and NFJ, their constituents' changes, and interactions during processing. The values of coordinate a^* were higher for FJP when compared to NFJP which may be due to betacyanin content in juices (Table 1). In the case of FJP, a recurring pattern was observed for each carrier, in which powders gained by SD displayed slightly more reddish pigment than those after FD. For NFJP, carrier type was more important and disturbed this tendency, thus in the case of inulin and Nutriose®, the trend was reversed. The type of juice affected the yellowness of the powders as coordinate b^* values were significantly lower for FJP. The probable reason for this may be related to a higher content of sugars in the juice that were not fermented, which under any heat treatment (low- or high-temperature) can undergo transformations such as the Maillard reaction or caramelization and thus cause the formation of products with yellow pigmentation. Previously, fermentation of peach juice with *Lactobacillus* strains was found to inhibit the Maillard reaction, due to the conversion of reducing sugars to non-reducing forms, and thus prevent browning (Hashemi et al., 2021).

3.3. Qualitative and quantitative determination of phenolics and hydroxymethyl-L-furfural

The major group of phenolics in beetroot juice powders was betalains, which consisted of approx. 80% of all identified phenolics, followed by syringic (16%) and ferulic (4%) acid derivatives (Fig. 1a, b, c). This is in line with the study on the phenolics profile of beetroot juices (Wruss et al., 2015). Powders obtained from NFJ contained about 21% more phenolics than fermented ones. This was linked to betalains content, as phenolic acids' derivatives predominated in FJP. When the carrier was concerned, the highest betalains percentage share was noted for samples with oligofructose, and syringic acid derivatives for inulin, while in the case of ferulic acid derivatives, the proportion of this phenolic group to their total content depending on the carrier type used were comparable. FD and SD did not noticeably differentiate the content of phenolics, which may indicate that both drying techniques allow the production of powders with similar properties (Michalska-Ciechanowska et al., 2021).

The presence of HMF was noted only for oligofructose-added powders (Fig. 1d). This could be connected with the composition (sugar type) and degree of polymerization (DP) of carriers. Oligofructose, unlike maltodextrins and Nutriose® which consist mainly of glucose units, is a short-chain fructan (DP: 2 – 9) composed mainly from fructose moieties, considerably shorter than inulin (DP: 2 – 60+) (Jackson et al., 2022), and therefore is the main substrate for Maillard reaction. Gökmen and Morales (2014) pointed out, even mild processing conditions that lead to lower moisture content and consequently accelerate sugar dehydration can induce HMF formation. Its absence in other samples may be connected (1) in the case of fermented juice - to insufficient substrate content due to its consumption as a result of the activity of probiotic bacteria, for which simple sugars are the main source of carbon and energy (Hashemi et al., 2021), as well as (2) in case of each sample, excluding oligofructose-added ones - to the individual response of a given formulation, which, when subjected to specific processing, may interact differently depending on the composition (Capuano et al. 2018), including carrier addition. Herein, oligofructose turned out to be the inducer of hydroxymethyl-*L*-furfural formation during the powders' production, and therefore this should be carefully reconsidered when the quality of powders is deliberated for the final product's safety.

3.5. Total Phenolics Content

Regarding the carrier type used, products containing oligofructose were distinguished by the highest total phenolics content, while those with Nutriose® by the lowest (regardless of treatment) (Table 3). Previously, Siacor et al. (2020) noticed that an increased concentration of

maltodextrin caused a decrease in TPC content in spray-dried juices which, however, was the result of the growing proportion of carrier to juice in the formulation subjected to drying. However, as the concentration for all carriers applied in the present study was the same, the observed effect may be linked to a particular carrier's ability to protect bioactives, as well as due to its specific composition in which constituents that are not phenolic compounds may be present and are able to react with Folin-Ciocalteu reagent, thus leading to an overestimation of the results. Going into the details, all spray-dried FJP demonstrated higher TPC values than NFJP.

Generally, TPC content in plant-based products subjected to SD is affected by relatively high temperatures that enhance the degradation of these compounds being responsible for the antioxidant capacity. The higher values recorded for FJP containing inulin, maltodextrin, or oligofructose (in comparison to analogs powders obtained from NFJ) might be related to the metabolic activity (and enzyme specificity) of the applied starter culture during juice fermentation (before drying). This probably contributed to the modifications in the profile of phenols (as well as other substances with antioxidant properties) (Kwaw et al., 2018). It is claimed that the adaptability of probiotics and biosynthesized enzymes strongly enhance the degradation and depolymerization of some compounds into simple phytochemicals molecules (Sharma et al., 2022). Also, higher values of TPC in fermented plant-derived beverages may be the effect of deglycosylation of glycosylated phenols in fresh juice that results in a release of insoluble bounded or soluble conjugated phenolics from the various native cells structures of the raw material subjected to fermentation (Kwaw et al., 2018). The results are in accordance with Sharma et al. (2022), who subjected the fermented pumpkin juice to powdering process indicating an increase in phenolics content after 15 h of fermentation carried on by *Lactobacillus fermentum* NCDC 141.

The possibility of incorporating beetroot powder into food products has been already indicated among others in yogurt, cakes, biscuits, bread, baked rolls, cupcakes, and cookies (Punia Bangar et al., 2022). Along with the positive impact on the nutritional properties and quality of such products, the health-promoting properties of the foods enriched with beet powder have also been confirmed. It was revealed that bread containing 10% of freeze-dried beetroot powder exhibited stronger antioxidant activity and higher lutein (zeaxanthin) content than the control (non-supplemented) products (Ranawana et al., 2016). Furthermore, cookies produced with encapsulated beetroot powder exhibited increased antioxidant activities and ACE inhibition, higher total phenolic content, and lower α-glucosidase inhibition activities (Čakarević et al., 2021). Also, tofu enriched with beetroot powder exhibited higher total

phenolic content and stronger antioxidant properties than the control (Lee et al., 2019). The results of our study also indicate the potential of applying the analyzed beetroot powders as functional food additives, however, further investigations are needed to confirm this.

3.4. Antioxidant capacity of powders

The results of TEAC ABTS$^{+\bullet}$ and FRAP showed that products with oligofructose exhibited the strongest antioxidative capacity followed by inulin, while Nutriose® resulted in the lowest values (Table 3), regardless of applied treatment (fermentation/drying). The only exception was noticed for freeze-dried FJP, wherein samples with inulin exhibited higher FRAP values than those with oligofructose. Powders with maltodextrin did not exhibit diversities between each other (no statistically significant differences). For SD products, higher antioxidant capacity values were noted for the FJP than in their unfermented counterparts, excluding samples with the addition of Nutriose® and maltodextrin which were tested using the TEAC ABTS test. In FD products this tendency was noted only for powders with inulin and oligofructose, while no statistically significant differences were noted for Nutriose® and maltodextrin-added samples. A higher antioxidant capacity observed in most FJ powders might be related to enzymatic transformations occurring during fermentation that involve the release of sugars molecules bound to phenolics. This may lead to an increase in aglycones exhibiting higher radical scavenging properties. Furthermore, some of probiotic bacteria strains exhibit a high potential to scavenge reactive oxygen species and chelate some metal ions (Curiel et al., 2015), which also may contribute to the higher values of this parameter obtained in most variants of powdered beetroot juices fermented with the probiotic strain *L. plantarum* 299v. Drying techniques differently affected the antioxidant capacity of powders depending on the type of juice (FJ / NFJ) and carrier, thus on the composition of the matrix subjected to drying. This issue was previously raised by Capuano et al. (2018) who claimed that due to the diversity and complexity of the food matrix's chemical composition, the reactivity of single components present in this matrix is different. Consequently, the response of the whole formulation after being subjected to any processing, especially those connected with heat treatment, may evoke uncontrolled reactions in the matrix. This in turn might result in imparting new properties to the final product, including antioxidant capacity which consists of the antioxidant activity of all components occurring natively, as well as those that may be formed during processing.

3.6. Antiglycation activity

The BSA-glucose model evaluated the inhibition ability of powders towards advanced glycation end (AGEs) products generated during the initial stage of glycation – a reaction between glucose and its oxidation products i.e., glyoxal, with amino groups (Wang et al., 2011). The inhibition for all powders did not exceed 40%. Products obtained from NFJ showed slightly higher values than those from fermented one (Table 3) which may be linked to a higher content of betalains present in powders from NFJ (r = 0.76) (Fig. 2a). Previously, the antiglycative effect exhibited in BSA-fructose *in vitro* model system was proven for betanin which is the predominant constituent representing 75-95% of all betalains in beetroot (Han et al., 2015). For BSA-MGO model, which mimics the middle step of the reaction as the sugars to carbonyls conversion is omitted (Wang et al., 2011), the exerted inhibition oscillated at 20% except for fermented spray-dried powder with maltodextrin, which showed about 35% inhibition toward AGEs (Table 3). This strongly suggests that beetroot juice powders are less effective inhibitors of this glycation stage. Considering the results from MGO-*L*-arginine model, which evaluated the specific reaction between these substrates resulting in AGEs formation, i.e., argpyrimidine (Wang et al., 2011), explicitly stronger were products from FJ (approx. 40% AGEs inhibition) than from non-fermented one (approx. 30% AGEs inhibition) (Table 3). Contrary to BSA-glucose model, herein a strong negative correlation was found between inhibition of AGEs and betalains content (r = −0.92), whereas a positive correlation was found between ferulic acid derivatives (r = 0.68) (Fig. 2a). It may suggest that inhibition during the final stage of glycation was more linked to those constituents (Silván et al., 2011).

3.7. In vitro cancer cell lines antiproliferative assay

Previous studies showed that beetroot products are capable of inhibiting, among others, the growth of HeLa cervical cancer cells (Romero et al., 2021) and prostate cancer cells (Mancini et al., 2021). Other authors proved that betanin-rich extract decreased the viability of MCF-7 breast cancer cells without affecting normal cells (Nowacki et al., 2015). In the study, beetroot juice powders were investigated in terms of their potential antiproliferative activity against J-45 and HL-60/MX2 leukemia cell lines (Fig. 3).

The addition of powdered juices (1 mg/mL) to a growth medium containing cancer cells caused changes in the viability of the cells. Beetroot juice contains various phytochemicals which may inhibit the growth of cancer cells *in vitro* (alone or synergistically with other bioactives) (Romero et al., 2021). Among these compounds, betanin has been proven as one of the crucial inhibiting factors (R et al., 2022). Jovanović et al. (2021) indicated the addition of

beetroot (*Beta vulgaris* L.) pomace flour into yogurt contributed to higher inhibition of colon cancer cell (Human colon cancer cell line HCT116) viability (13.0–24.5%) and exhibited antimicrobial properties against *Escherichia*. However, in the study, a moderate positive correlation was found between these constituents and HL-60/MX2 line (r = 0.56), while for J-45.1 line no relationship was noted (r = –0.12) (Fig. 2b). This observation indicated the selectivity of betalains antiproliferative ability towards specific lines. It is in accordance with Lechner and Stoner (2019) that betanin is capable of inducing apoptosis of some cancer cells, however, some lines are not affected by this compound. In the case of HL-60/MX2 human leukemia line, a strong negative correlation was proven for ferulic acid derivatives (r = –0.83) (that was not reported for the second tested line), which is in contradiction to the literature studied. However, Berdowska et al. (2018) reported that five out of six analyzed pure ellagitannins exhibit stimulatory effects on the viability of human breast cancer cells tested on two lines, instead of inhibiting them. Moreover, the ambiguous behavior of some of these compounds was observed depending on the concentration tested. Although ellagitannins, as structures much larger than phenolic acids, should not be compared, the complex matrix in which the ferulic acid derivatives identified in this study were found, additionally modified through processing, may affect their activity in different ways, and thus the cell line responses they induce (Capuano et al., 2018). Furthermore, negative relationships between HMF content and J-45.1 (r = –0.81) as well as HL-60/MX2 (r = –0.54) lines were also found (Fig. 2b). This suggested that HMF might take part in the stimulation of cancer cell growth, especially since this compound was found only in oligofructose-added powders. However, the observed effect may be even substantially more driven by the composition of this carrier, i.e., oligofructose contains mainly fructose units, which is a major contributor to cancer development (Nakagawa et al., 2020). Accordingly, in the case of J-45.1 line (Fig. 3a), for which significant differences were noted between carriers used (regardless of fermentation and drying techniques), oligofructose increased their viability. On the contrary, the addition of Nutriose® caused the greatest reduction in cell viability regardless of raw material and drying technique. A slight decrease in viability was observed when carriers, i.e., inulin or maltodextrin were applied. In the case of HL-60/MX2 line (Fig. 3b), among NFJ-derived samples, the use of inulin caused the greatest decrease in cell viability, followed by Nutriose® and maltodextrin. On the other hand, the use of oligofructose, regardless of the drying technique applied, had a negligible impact on the viability of cancer cells. No statistical differences were found between the individual carriers in SD fermented juices. Oppositely, fermented lyophilized juices containing oligofructose and Nutriose® slightly increased the viability of cancerous cells. Thus, the

antiproliferative properties may be moderated by the carrier type used for drying that may play a role in the protection of bioactives influencing this property

4. Conclusions

The obtained results are of high practical significance providing a better insight into the functional and physicochemical characteristics of beetroot juice powders. It can be concluded that the initial matrix composition and carrier type influence physico-chemical properties, including HMF formation, as well as *in vitro* antioxidant, antiglycation, and chemoprotective activities of freeze- and spray-dried beetroot powders, and proves that the quality of final products is moderated by these factors. Powders produced from probiotically fermented beetroot juice were stronger antiglycation agents in the MGO-*L*-arginine system, while no effect was observed towards AGEs' inhibition during the initial and middle stages of glycation. Carrier type proved to affect retention of identified phenolics, as the greatest betalains' share was noted for oligofructose-added powders, syringic acid derivatives for those with inulin, while in the case of ferulic acid derivatives, the proportion of this phenolic group to their total content depending on the carrier type used were comparable. In general, powders containing oligofructose as a carrier were distinguished by the strongest antioxidant capacity and TPC values, followed by inulin, maltodextrin, and Nutriose®. In the *in vitro* antiproliferative assay with human leukemia cell lines, Nutriose® turned out to reduce the cell viability regardless of raw material and drying technique in the case of J-45.1 line, and in HL-60/MX2 line when the NFJ was used for the preparation of the powder. The findings revealed a wide possibility of applications of the tested powders in the manufacture of foodstuffs, with potential bioactive characteristics. However, a further in-depth analysis regarding the influence of the incorporation of such powders into diverse food matrices on the physicochemical properties, stability, and storage safety of the final products is of high importance. A considerable challenge will be investigating the health-promoting properties *in vivo* of various food matrices fortified by such powders. Nevertheless, the obtained results are encouraging to continue the study on potential beneficial health effects related to the consumption of this type of food.

CRediT authorship contribution statement

Jessica Brzezowska: Conceptualization, Funding acquisition, Project administration, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Katarzyna Skrzypczak:** Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Wojciech Radzki**: Methodology, Investigation, Writing – reviewing & editing. **Igor Piotr Turkiewicz**: Methodology, Investigation, Writing – reviewing & editing. **Marta Ziaja-Sołtys**: Methodology, Investigation. **Anna Bogucka-Kocka:** Methodology, Verification of statistical analyses. **Aneta Wojdyło**: Methodology, Investigation, Writing – reviewing & editing. **Anna Michalska-Ciechanowska**: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – reviewing & editing.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this paper.

Acknowledgments

This work was supported by "UPWR 2.0: international and interdisciplinary programme of development of Wrocław University of Environmental and Life Sciences", co-financed by the European Social Fund under the Operational Program Knowledge Education Development, under contract No. POWR.03.05.00-00-Z062/18 of June 4, 2019.

The publication is the result of the research group activity: '**Plants4FOOD**'.

References

Berdowska, I., Zieliński, B., Saczko, J., Sopel, M., Gamian, A., & Fecka, I. (2018). Modulatory impact of selected ellagitannins on the viability of human breast cancer cells. *Journal of Functional Foods*, *42*, 122–128. https://doi.org/10.1016/j.jff.2017.12.053

Calva-Estrada, S. J., Jiménez-Fernández, M., & Lugo-Cervantes, E. (2022). Betalains and their applications in food: The current state of processing, stability and future opportunities in the industry. *Food Chemistry: Molecular Sciences*, *4*, 100089. https://doi.org/10.1016/j.fochms.2022.100089

Capuano, E., Oliviero, T., & van Boekel, M. A. J. S. (2018). Modeling food matrix effects on chemical reactivity: Challenges and perspectives. *Critical Reviews in Food Science and Nutrition*, *58*(16), 2814–2828. https://doi.org/10.1080/10408398.2017.1342595

Curiel, J. A., Pinto, D., Marzani, B., Filannino, P., Farris, G. A., Gobbetti, M., & Rizzello, C. G. (2015). Lactic acid fermentation as a tool to enhance the antioxidant properties of *Myrtus communis* berries. *Microbial Cell Factories*, *14*(1), 67. https://doi.org/10.1186/s12934-015-0250-4

Čakarević, J., Torbica, A., Belović, M., Tomić, J., Sedlar, T. & Popović, L. (2021). Pumpkin oil cake protein as a new carrier for encapsulation incorporated in food matrix: Effect of processing, storage and *in vitro* digestion on bioactivity. *International Journal of Food Science & Technology 56*(7), 3400–3408. https://doi.org/10.1111/ijfs.14964

del Castillo, M. D., Iriondo-DeHond, A., Iriondo-DeHond, M., Gonzalez, I., Medrano, A., Filip, R., & Uribarri, J. (2021). Healthy eating recommendations: Good for reducing dietary contribution to the body's advanced glycation/lipoxidation end products pool? *Nutrition Research Reviews*, *34*(1), 48–63. https://doi.org/10.1017/S0954422420000141

Domian, E., Brynda-Kopytowska, A., Cieśla, J., & Ostrowska-Ligęza, E. (2017). Effect of the type of carbohydrate on the DVS critical relative humidity in spray-dried fat-filled pea protein-based powders: Comparison with monolayer coverage and *Tg* values. *Food Hydrocolloids*, *73*, 335–343. https://doi.org/10.1016/j.foodhyd.2017.07.011

Egas-Astudillo, L. A., Martínez-Navarrete, N., & Camacho, M. del M. (2021). Quality of a powdered grapefruit product formulated with biopolymers obtained by freeze-drying and spray-drying. *Journal of Food Science*, *86*(6), 2255–2263. https://doi.org/10.1111/1750-3841.15750

Fontana, A. J. Jr. (2020). Appendix D: Minimum water activity limits for growth of microorganisms. In Water Activity in Foods. G. V. Barbosa-Cánovas, A. J. Fontana, S. J. Schmidt, & T. P. Labuza (Eds.) John Wiley & Sons, Inc. https://doi.org/10.1002/9781118765982.app4

Fu, Y., Shi, J., Xie, S.-Y., Zhang, T.-Y., Soladoye, O. P., & Aluko, R. E. (2020). Red beetroot betalains: Perspectives on extraction, processing, and potential health benefits. *Journal of Agricultural and Food Chemistry*, *68*(42), 11595–11611. https://doi.org/10.1021/acs.jafc.0c04241

Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry*, *48*(5), 1485–1490. https://doi.org/10.1021/jf991072g

Gościnna, K., J. Czapski, J. Mikołajczyk-Bator, K. & Kidoń, M. (2012). Content betalain pigments, nitrates, and antioxidant capacity of beetroot juices depending on cultivars and the size of beetroot roots. *Aparatura Badawcza i Dydaktyczna 17*(3), 85-90.

Gökmen, V., & Morales, F. (2014). Processing contaminants: Hydroxymethylfurfural. In *Encyclopedia of Food Safety* (pp. 404–408). *Food Science*. https://doi.org/10.1016/B978-0-12-378612-8.00209-2

Halwani, A.F., Sindi, H.A. & Jambi, RH.A. (2018). Characterization of physical properties of red beet pigments. *Journal Of Biochemical Technology 9*(3), 10-14.

Han, J., Tan, C., Wang, Y., Yang, S., & Tan, D. (2015). Betanin reduces the accumulation and cross-links of collagen in high-fructose-fed rat heart through inhibiting non-enzymatic glycation. *Chemico-Biological Interactions*, *227*, 37–44. https://doi.org/10.1016/j.cbi.2014.12.032

Hashemi, S. M. B., Jafarpour, D., & Jouki, M. (2021). Improving bioactive properties of peach juice using *Lactobacillus* strains fermentation: Antagonistic and anti-adhesion effects, anti-inflammatory and antioxidant properties, and Maillard reaction inhibition. *Food Chemistry*, *365*, 130501. https://doi.org/10.1016/j.foodchem.2021.130501

Jackson, P. P. J., Wijeyesekera, A., Theis, S., van Harsselaar, J., & Rastall, R. A. (2022). Food for thought! Inulin-type fructans: Does the food matrix matter? *Journal of Functional Foods*, *90*, 104987. https://doi.org/10.1016/j.jff.2022.104987

Jagannath, A., Kumar, M., & Raju, P. S. (2015). Fermentative stabilization of betanin content in beetroot and its loss during processing and refrigerated storage: Stabilization of beetroot betanin. *Journal of Food Processing and Preservation*, *39*(6), 606–613. https://doi.org/10.1111/jfpp.12267

Janiszewska-Turak, E., Walczak, M., Rybak, K., Pobiega, K., Gniewosz, M., Woźniak, Ł., & Witrowa-Rajchert, D. (2022). Influence of fermentation beetroot juice process on the physico-chemical properties of spray dried powder. *Molecules*, *27*(3), 1008. https://doi.org/10.3390/molecules27031008

Kuhn, F., Azevedo, E. S., & Noreña, C. P. Z. (2020). Behavior of inulin, polydextrose, and egg albumin as carriers of *Bougainvillea glabra* bracts extract: Rheological performance and powder characterization. *Journal of Food Processing and Preservation*, *44*(10). https://doi.org/10.1111/jfpp.14834

Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M. T., Wu, M., Sackey, A. S., Xiao, L., & Tahir, H. E. (2018). Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant

activities of lactic-acid-fermented mulberry juice. *Food Chemistry*, *250*, 148–154. https://doi.org/10.1016/j.foodchem.2018.01.009

Lechner, J. F., & Stoner, G. D. (2019). Red beetroot and betalains as cancer chemopreventative agents. *Molecules*, *24*(8), 1602. https://doi.org/10.3390/molecules24081602

Lee, K. Y., Kim, A. N., Rahman, M.S., & Choi, S. G. (2019). Effect of red beet (*Beta vulgaris* L.) powder addition on physicochemical and microbial characteristics of tofu. *Korean Journal of Food Preservation 26*(6), 659–666. https://doi.org/10.11002/kjfp.2019.26.6.659

Li, N., Wang, J., Wang, B., Huang, S., Hu, J., Yang, T., Asmutola, P., Lan, H., & Qinghui, Y. (2021). Identification of the carbohydrate and organic acid metabolism genes responsible for brix in tomato fruit by transcriptome and metabolome analysis. *Frontiers in Genetics*, *12*, 714942. https://doi.org/10.3389/fgene.2021.714942

Li, Y., Ma, X., & Liu, X. (2019). Physicochemical and rheological properties of cross-linked inulin with different degree of polymerization. *Food Hydrocolloids*, *95*, 318–325. https://doi.org/10.1016/j.foodhyd.2018.11.026

Mancini, M. C. S., Ponte, L. G. S., Silva, C. H. R., Fagundes, I., Pavan, I. C. B., Romeiro, S. A., da Silva, L. G. S., Morelli, A. P., Rostagno, M. A., Simabuco, F. M., & Bezerra, R. M. N. (2021). Beetroot and leaf extracts present protective effects against prostate cancer cells, inhibiting cell proliferation, migration, and growth signaling pathways. *Phytotherapy Research*, *35*(9), 5241–5258. https://doi.org/10.1002/ptr.7197

Michalska-Ciechanowska, A., Brzezowska, J., Wojdyło, A., Gajewicz-Skretna, A., Ciska, E., & Majerska, J. (2021). Chemometric contribution for deeper understanding of thermally-induced changes of polyphenolics and the formation of hydroxymethyl-*L*-furfural in chokeberry powders. *Food Chemistry*, *342*, 128335. https://doi.org/10.1016/j.foodchem.2020.128335

Nakagawa, T., Lanaspa, M. A., Millan, I. S., Fini, M., Rivard, C. J., Sanchez-Lozada, L. G., Andres-Hernando, A., Tolan, D. R., & Johnson, R. J. (2020). Fructose contributes to the Warburg effect for cancer growth. *Cancer & Metabolism*, *8*(1), 16. https://doi.org/10.1186/s40170-020-00222-9

Nowacki, L., Vigneron, P., Rotellini, L., Cazzola, H., Merlier, F., Prost, E., Ralanairina, R., Gadonna, J.-P., Rossi, C., & Vayssade, M. (2015). Betanin-enriched red beetroot (*Beta vulgaris* L.) extract induces apoptosis and autophagic cell death in MCF-7 cells: *In vitro* antitumor activity of betacyanins. *Phytotherapy Research*, *29*(12), 1964–1973. https://doi.org/10.1002/ptr.5491

Ozyurt, V. ye H., & Ötles, S. (2016). Effect of food processing on the physicochemical properties of dietary fibre. *Acta Scientiarum Polonorum Technologia Alimentaria*, *15*(3), 233–245. https://doi.org/10.17306/J.AFS.2016.3.23

Punia Bangar, S., Singh, A., Chaudhary, V., Sharma, N., & Lorenzo, J. M. (2022). Beetroot as a novel ingredient for its versatile food applications. *Critical Reviews in Food Science and Nutrition*, 1–25. https://doi.org/10.1080/10408398.2022.2055529

Ranawana, V., Campbell, F., Bestwick, C., Nicol, P., Milne, L., Duthie, G., & Raikos, V. (2016). Breads fortified with freeze-dried vegetables: Quality and nutritional attributes. Part II: Breads not containing oil as an ingredient. *Foods 5*(4):62. https://doi.org/10.3390/foods5030062

R, R., Shafreen, M., Kumar, N. (2022). Inhibition of proliferation in ovarian cancer cell line (PA-1) by the action of green compound "Betanin". *Applied biochemistry and biotechnology*, *194*(1), 71–83. doi: 10.1007/s12010-021-03744-0

Ribeiro, E. S. S., Damasceno, K. S. F. S. C., Dantas, L. M. D. C., Azevedo, W. M. de, Leite, P. I. P., Assis, C. F., & Sousa Junior, F. C. (2020). Fermented yellow mombin juice using *Lactobacillus acidophilus* NRRL B-4495: Chemical composition, bioactive properties and survival in simulated gastrointestinal conditions. *PLoS ONE*, *15*(9), e0239392. https://doi.org/10.1371/journal.pone.0239392

Romero, S. A., Pavan, I. C. B., Morelli, A. P., Mancini, M. C. S., da Silva, L. G. S., Fagundes, I., Silva, C. H. R., Ponte, L. G. S., Rostagno, M. A., Bezerra, R. M. N., & Simabuco, F. M. (2021). Anticancer effects of root and beet leaf extracts (*Beta vulgaris* L.) in cervical cancer cells (HeLa). *Phytotherapy Research*, *35*(11), 6191–6203. https://doi.org/10.1002/ptr.7255

Samborska, K., Wiktor, A., Jedlińska, A., Matwijczuk, A., Jamróz, W., Skwarczyńska-Maj, K., Kiełczewski, D., Tułodziecki, M., Błażowski, Ł., & Witrowa-Rajchert, D. (2019). Development and characterization of physical properties of honey-rich powder. *Food and Bioproducts Processing*, *115*, 78–86. https://doi.org/10.1016/j.fbp.2019.03.004

Sharma, P., Kashyap, P., Kehinde, B. A., & Kaur, S. (2022). *Sustainable utilization and optimization of spray dried fermented pumpkin juice* [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-1589838/v1

Siacor, F. D. C., Lim, K. J. A., Cabajar, A. A., Lobarbio, C. F. Y., Lacks, D. J., & Taboada, E. B. (2020). Physicochemical properties of spray-dried mango phenolic compounds extracts. *Journal of Agriculture and Food Research*, *2*, 100048. https://doi.org/10.1016/j.jafr.2020.100048

Silván, J. M., Assar, S. H., Srey, C., del Castillo, M. D., & Ames, J. M. (2011). Control of the Maillard reaction by ferulic acid. *Food Chemistry*, *128*(1), 208–213. https://doi.org/10.1016/j.foodchem.2011.03.047

Song, Q., Liu, J., Dong, L., Wang, X., & Zhang, X. (2021). Novel advances in inhibiting advanced glycation end product formation using natural compounds. *Biomedicine & Pharmacotherapy*, *140*, 111750. https://doi.org/10.1016/j.biopha.2021.111750

Verruck, S., de Liz, G. R., Dias, C. O., de Mello Castanho Amboni, R. D., & Prudencio, E. S. (2019). Effect of full-fat goat's milk and prebiotics use on *Bifidobacterium* BB-12 survival and on the physical properties of spray-dried powders under storage conditions. *Food Research International*, *119*, 643–652. https://doi.org/10.1016/j.foodres.2018.10.042

Wang, W., Yagiz, Y., Buran, T. J., Nunes, C. do N., & Gu, L. (2011). Phytochemicals from berries and grapes inhibited the formation of advanced glycation end-products by scavenging reactive carbonyls. *Food Research International*, *44*(9), 2666–2673. https://doi.org/10.1016/j.foodres.2011.05.022

Wei, T., & Simko, V. (2021). R package 'corrplot': Visualization of a Correlation Matrix (Version 0.92). Retrieved from: https://github.com/taiyun/corrplot

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag New York. Retrieved from: https://ggplot2.tidyverse.org.

Wojdyło, A., Oszmiański, J., & Bielicki, P. (2013). Polyphenolic composition, antioxidant activity, and polyphenol oxidase (PPO) activity of quince (*Cydonia oblonga* Miller) varieties. *Journal of Agricultural and Food Chemistry*, *61*(11), 2762–2772. https://doi.org/10.1021/jf304969b

Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., Höglinger, O., & Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, *42*, 46–55. https://doi.org/10.1016/j.jfca.2015.03.005

Tables

Table 1. Physicochemical characteristics of beetroot juice for the preparation of the powders.

Parameters		Beetroot juice variant	
		NFJ	**FJ**
pH		6.09 ± 0.02 [b]	4.00 ± 0.02 [a]
Total extract content [°Bx]		8.7 ± 0.16 [b]	7.3 ± 0.64 [a]
CIE color parameter	*L**	28.69 ± 0.33 [b]	27.73 ± 0.97 [a]
	*a**	5.35 ± 1.24 [b]	3.36 ± 0.51 [a]
	*b**	1.80 ± 0.37 [b]	0.99 ± 0.37 [a]
Betacyanin [mg/L]		35.42 ± 0.02 [a]	48.60 ± 0.02 [b]
Betaxanthin [mg/L]		26.17 ± 0.02 [a]	26.36 ± 0.02 [a]

Explanatory notes: Results as average values ($\overline{x}$ ± s/SD; n = 3) of the defined measured parameter with different lowercase (a - d) letters (in the same row) express a significant difference ($p < 0.05$) between the values noted for fermented (FJ) and nonfermented (NFJ) beetroot juices.

Table 2. Physicochemical characteristics of beetroot juice powders.

Treatment	Carrier type	Moisture content [%]	Water activity [-]	Color		
				*L**	*a**	*b**
Freeze-dried NFJP	Inulin	0.78 ± 0.10 ^{bA}	0.290 ± 0.112 ^{dC}	43.47 ± 0.72 ^{bcC}	33.95 ± 0.23 ^{aC}	4.815 ± 0.057 ^{dA}
	Oligofructose	4.25 ± 0.13 ^{cC}	0.345 ± 0.001 ^{dD}	27.32 ± 3.63 ^{aA}	26.47 ±1.19 ^{aA}	4.485 ± 0.196 ^{dA}
	Nutriose®	2.26 ± 0.02 ^{cB}	0.214 ± 0.004 ^{dB}	42.26 ± 1.27 ^{aC}	33.31 ± 0.26 ^{bC}	6.085 ± 0.150 ^{cB}
	Maltodextrin	2.34 ± 0.06 ^{cB}	0.128 ± 0.006 ^{bA}	35.32 ± 1.11 ^{aB}	31.25 ± 0.65 ^{aB}	6.335 ± 0.333 ^{cB}
Spray-dried NFJP	Inulin	0.75 ± 0.07 ^{bC}	0.086 ± 0.027 ^{bA}	40.04 ± 0.65 ^{aA}	29.11 ± 0.56 ^{aA}	3.645 ± 0.115 ^{cB}
	Oligofructose	0.39 ± 0.04 ^{aA}	0.114 ± 0.006 ^{bB}	51.96 ± 4.25 ^{cB}	30.41 ± 1.40 ^{aAB}	2.035 ± 0.106 ^{cA}
	Nutriose®	0.62 ± 0.10 ^{aB}	0.105 ± 0.001 ^{bB}	57.85 ± 1.88 ^{cB}	31.89 ± 0.91 ^{aB}	2.935 ± 0.495 ^{bB}
	Maltodextrin	0.80 ± 0.03 ^{bC}	0.138 ± 0.005 ^{bC}	52.26 ± 3.26 ^{bB}	32.32 ± 0.44 ^{aB}	3.285 ± 0.415 ^{aB}
Freeze-dried FJP	Inulin	0.13 ± 0.02 ^{aA}	0.049 ± 0.005 ^{aA}	41.67 ± 2.06 ^{abC}	37.04 ± 0.45 ^{bAB}	−1.225 ± 0.409 ^{bA}
	Oligofructose	3.95 ± 0.01 ^{bD}	0.289 ± 0.002 ^{cD}	36.66 ± 0.90 ^{bAB}	38.04 ± 0.16 ^{cC}	−1.925 ± 0.361 ^{bA}
	Nutriose®	1.40 ± 0.29 ^{bB}	0.079 ± 0.003 ^{aB}	39.29 ± 0.90 ^{bBC}	37.59 ± 0.20 ^{cBC}	2.875 ± 0.232 ^{bC}
	Maltodextrin	2.64 ± 0.03 ^{dC}	0.110 ± 0.008 ^{aC}	35.63 ± 0.78 ^{aA}	36.75 ± 0.20 ^{bA}	1.395 ± 0.162 ^{bB}
Spray-dried FJP	Inulin	0.69 ± 0.06 ^{bB}	0.107 ± 0.030 ^{cAB}	46.20 ± 0.16 ^{cA}	41.85 ± 0.40 ^{cA}	−3.145 ± 0.059 ^{aC}
	Oligofructose	0.33 ± 0.06 ^{aA}	0.086 ± 0.008 ^{aA}	48.36 ± 0.22 ^{cB}	41.38 ± 0.25 ^{bA}	−4.855 ± 0.078 ^{aA}
	Nutriose®	0.32 ± 0.11 ^{aA}	0.123 ± 0.009 ^{cB}	48.59 ± 0.74 ^{aB}	42.81 ± 0.32 ^{dB}	−3.415 ± 0.170 ^{aC}
	Maltodextrin	0.28 ± 0.04 ^{aA}	0.108 ± 0.005 ^{aAB}	50.70 ± 0.25 ^{bC}	41.89 ± 0.181 ^{cA}	−3.725 ± 0.115 ^{aB}

Explanatory notes: Results as average values ($\overline{x}$ ± s/SD; n = 3) of the defined measured parameter with different lowercase (a-d) letters express a significant difference ($p < 0.05$) between powders (FJP or NFJP) obtained through different treatments (NFJP – powder derived from non-fermented juice or FJP – powder derived from probiotically fermented juice after spray drying or freeze-drying) with using the same carrier type, while capital letters (A-D) indicate significant differences among the powders produced with applying the same treatment in the production but using a different type of carrier.

Table 3. Total phenolics content (TPC), antioxidant capacity and antiglycation activity of beetroot juice powders.

Treatment	Carrier type	Total phenolics content (TPC)	Antioxidant capacity		Antiglycation activity		
			TEAC ABTS	FRAP	BSA-glucose	BSA-MGO	MGO-*L*-arginine
		[g of GAE/100 g dm]	[mmol Trolox/100 g dm]		[% inhibition]		
Freeze-dried NFJP	**Inulin**	0.23 ± 0.03 ^{Ba}	1.02 ± 0.02 ^{Ca}	1.21 ± 0.53 ^{Ca}	38.62 ± 2.35 ^{Cb}	15.82 ± 2.37 ^{Aa}	27.49 ± 2.21 ^{Aa}
	Oligofructose	0.31 ± 0.03 ^{Cab}	1.29 ± 0.04 ^{Da}	1.50 ± 0.51 $_{Da}$	29.04 ± 3.17 ^{ABb}	20.07 ± 3.26 ^{Aa}	29.33 ± 3.06 ^{Aab}
	Nutriose®	0.11 ± 0.01 ^{Ac}	0.47 ± 0.03 ^{Ab}	0.54 ± 0.47 ^{Ab}	26.75 ± 0.54 ^{Aa}	20.25 ± 3.78 ^{Aa}	29.19 ± 0.91 ^{Aa}
	Maltodextrin	0.12 ± 0.04 ^{Aa}	0.60 ± 0.08 ^{Ba}	0.72 ± 0.05 ^{Bb}	31.82 ± 0.33 ^{Bab}	21.42 ± 3.85 ^{Aa}	26.30 ± 2.12 ^{Aa}
Spray-dried NFJP	**Inulin**	0.24 ± 0.03 ^{Ba}	1.06 ± 0.16 ^{Ba}	1.42 ± 0.56 ^{Cb}	33.88 ± 2.42 ^{Bb}	18.23 ± 1.88 ^{Aa}	25.13 ± 2.42 ^{ABa}
	Oligofructose	0.26 ± 0.03 ^{Ba}	1.24 ± 0.14 ^{Ba}	1.48 ± 0.51 ^{Ca}	29.60 ± 1.29 ^{ABb}	18.26 ± 4.02 ^{Aa}	26.07 ± 3.37 ^{ABa}
	Nutriose®	0.06 ± 0.00 ^{Aa}	0.32 ± 0.01 ^{Aa}	0.42 ± 0.53 ^{Aa}	27.29 ± 2.54 ^{Aa}	19.73 ± 3.64 ^{Aa}	29.49 ± 0.73 ^{Ba}
	Maltodextrin	0.08 ± 0.02 ^{Aa}	0.51 ± 0.03 ^{Aa}	0.59 ± 0.04 ^{Ba}	34.77 ± 4.76 ^{Bb}	18.63 ± 2.26 ^{Aa}	23.65 ± 1.56 ^{Aa}
Freeze-dried FJP	**Inulin**	0.27 ± 0.02 ^{Cab}	1.32 ± 0.10 ^{Bb}	1.78 ± 0.61 ^{Dd}	19.70 ± 9.29 ^{Aa}	17.83 ± 2.22 ^{ABa}	37.64 ± 6.46 ^{Ab}
	Oligofructose	0.32 ± 0.00 ^{Db}	1.49 ± 0.02 ^{Cb}	1.64 ± 0.66 ^{Cb}	20.66 ± 3.79 ^{Aa}	18.44 ± 5.47 ^{ABa}	39.81 ± 5.87 ^{Ab}
	Nutriose®	0.08 ± 0.02 ^{Aab}	0.42 ± 0.04 ^{Aab}	0.48 ± 0.07 ^{Aab}	26.71 ± 6.50 ^{Aa}	22.42 ± 2.20 ^{Ba}	46.84 ± 5.20 ^{Ac}
	Maltodextrin	0.11 ± 0.01 ^{Ba}	0.51 ± 0.04 ^{Aa}	0.68 ± 0.02 ^{Bab}	25.06 ± 4.71 ^{Aa}	15.47 ± 3.76 ^{Aa}	37.30 ± 7.08 ^{Ab}
Spray-dried FJP	**Inulin**	0.32 ± 0.02 ^{Bb}	1.30 ± 0.04 ^{Cb}	1.56 ± 0.05 ^{Cc}	13.98 ± 3.48 ^{Aa}	16.21 ± 1.77 ^{Aa}	44.09 ± 1.56 ^{Ab}
	Oligofructose	0.32 ± 0.02 ^{Bb}	1.47 ± 0.02 ^{Db}	1.70 ± 0.09 ^{Cb}	20.64 ± 3.28 ^{Aa}	18.53 ± 1.06 ^{Aa}	40.77 ± 9.10 ^{Ab}
	Nutriose®	0.10 ± 0.01 ^{Abc}	0.34 ± 0.07 ^{Aa}	0.47 ± 0.06 ^{Aab}	20.51 ± 4.40 ^{Aa}	18.08 ± 0.47 ^{Aa}	39.47 ± 1.48 ^{Ab}
	Maltodextrin	0.14 ± 0.03 ^{Aa}	0.59 ± 0.11 ^{Ba}	0.71 ± 0.09 ^{Bb}	34.77 ± 4.76 ^{Bb}	34.29 ± 3.61 ^{Bb}	43.82 ± 0.81 ^{Ab}

Explanatory notes: Results as average values ($\overline{x}$ ± s/SD; $n = 3$) of the defined measured parameter with different lowercase (a-d) letters express a significant difference ($p < 0.05$) between powders (FJP or NFJP) obtained through different treatments (NFJP – powder derived from non-fermented juice or FJP – powder derived from probiotically fermented juice after spray drying or freeze-drying) with using the same carrier type, while capital letters (A-D) indicate significant differences among the powders produced with applying the same treatment in the production but using a different type of carrier.

Figures

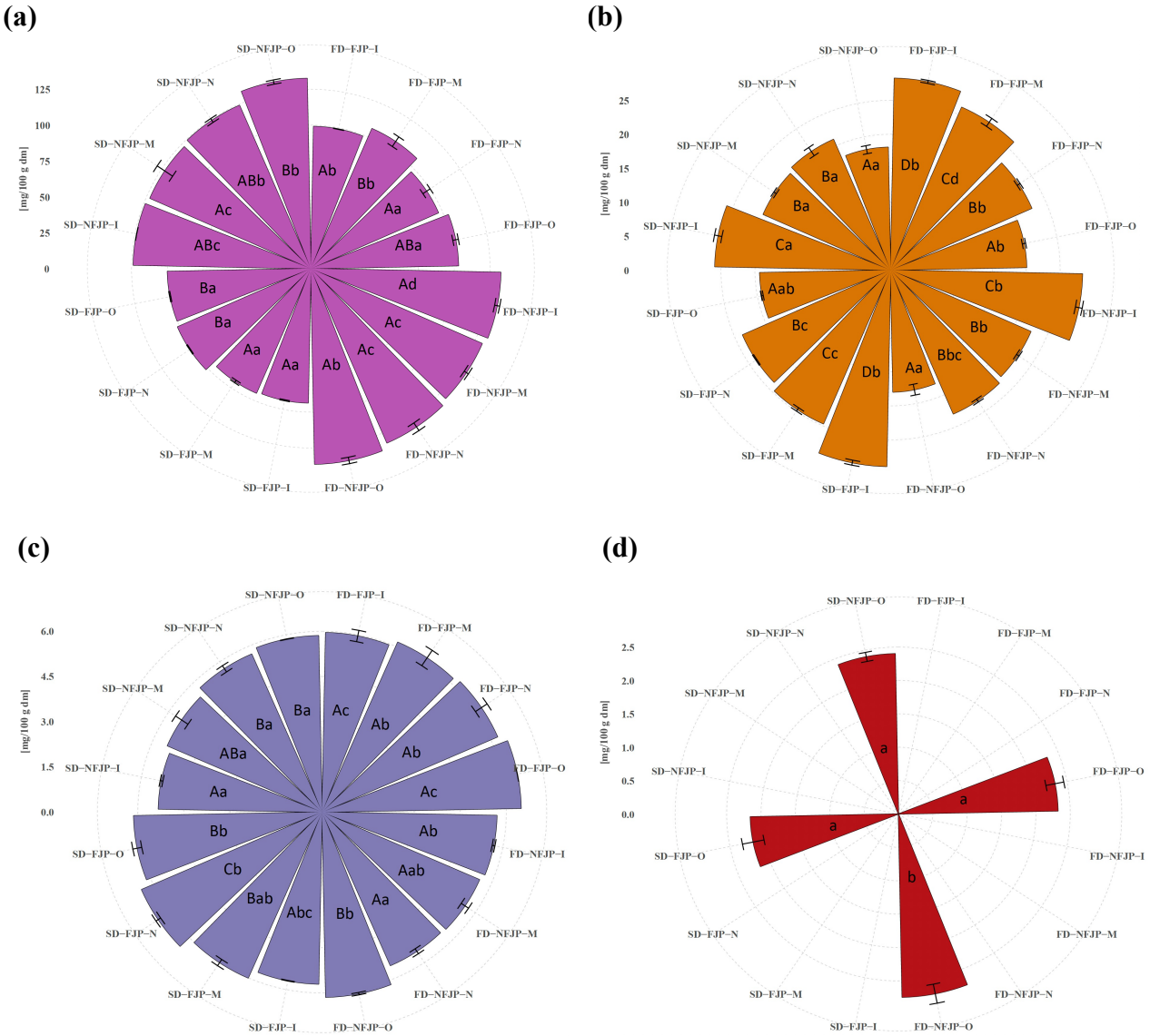
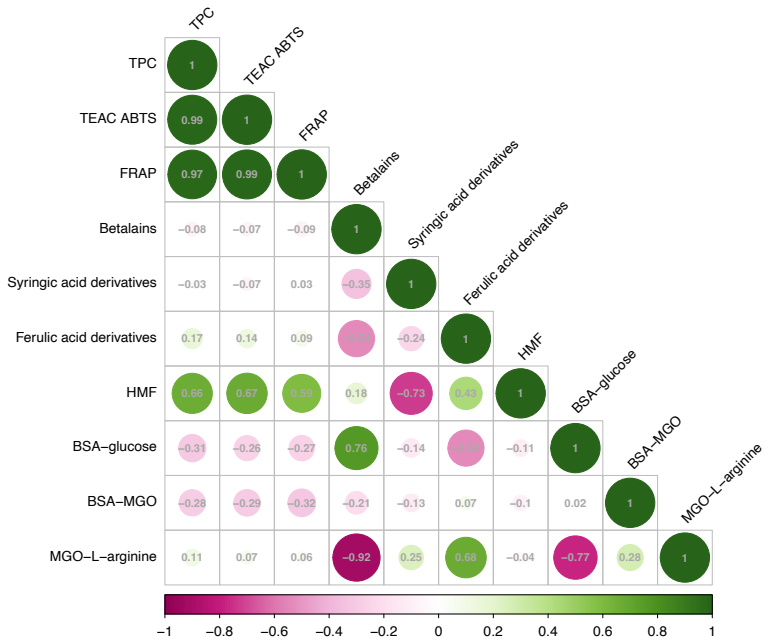


Fig. 1. The polar plots present the content of (a) betalains, (b) syringic acid derivatives, (c) ferulic acid derivatives, and (d) hydroxymethyl-*L*-furfural identified in beetroot powders [mg/100 g dm].
Explanatory notes: The results expressed as mean values ($\bar{x}$ ± s/SD; $n = 3$) of the measured parameter with different lowercase (a-d) letters expressing a significant difference ($p < 0.05$) between powders obtained through different treatments (FD – freeze-dried or SD – spray-dried and NFJP – powder derived from non-fermented juice or FJP – powder derived from probiotically fermented juice) with using the same carrier type (I – inulin; M – maltodextrin; N – Nutriose®; O – oligofructose), while capital letters (A-D) indicate significant differences ($p < 0.05$) among the powdered juices produced with applying the same treatment but with introducing a different type of carrier.

(a)



(b)

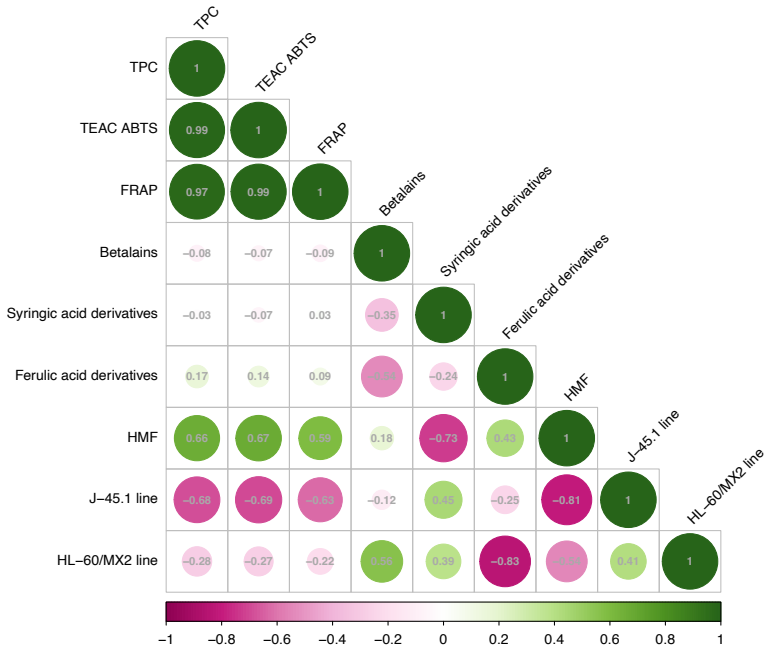
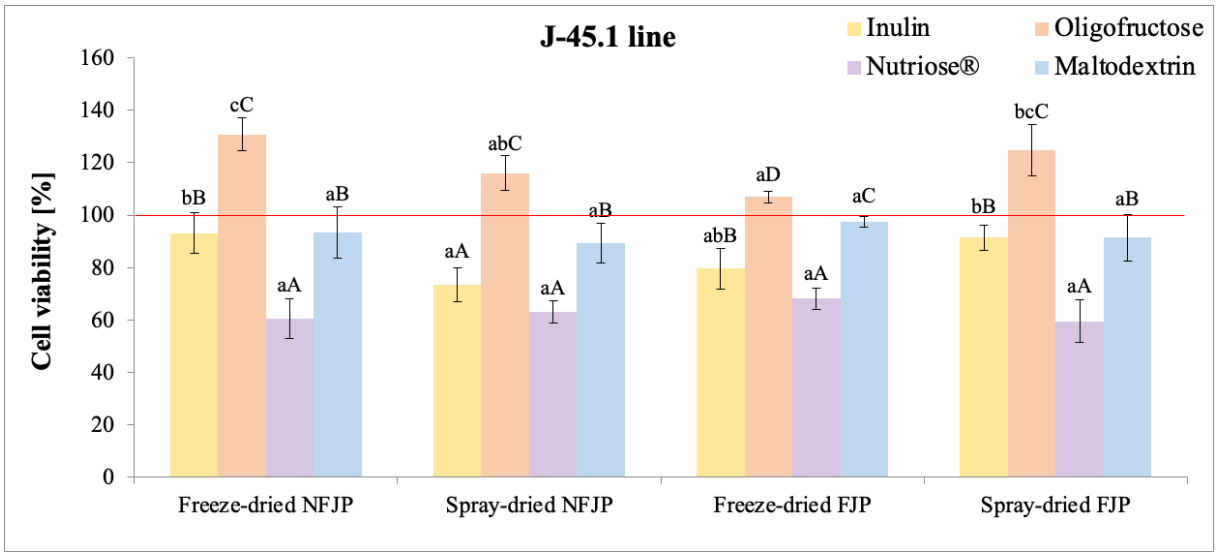


Fig. 2. Correlogram demonstrating the strength and direction of a linear association between (a) antiglycation and (b) antiproliferative activities and other variables analyzed in the obtained beetroot juice powders.
Explanatory notes: Positive and negative correlations are displayed in green and purple, respectively (the circle dimension and color intensity correspond to the values of the correlation coefficients; the large circles depict significant correlation coefficients and represent a strong linear relation, whereas small circles indicate the smallest correlation coefficients considered insignificant); HMF – Hydroxymethyl-*L*-furfural; TPC – Total Phenolics Content; TEAC ABTS – Trolox Equivalent Antioxidant Capacity by ABTS assay; FRAP – Ferric Reducing Antioxidant Potential; Antiglycation activity in models: Bovine Serum Albumin-glucose (BSA-glucose), Bovine Serum Albumin-methylglyoxal (BSA-MGO) and methylglyoxal-*L*-arginine (MGO-*L*-arginine); Antiproliferative activity assay on cells of human leukemia lines: HL-60 / MX2 and J-45.01.

(a)



(b)

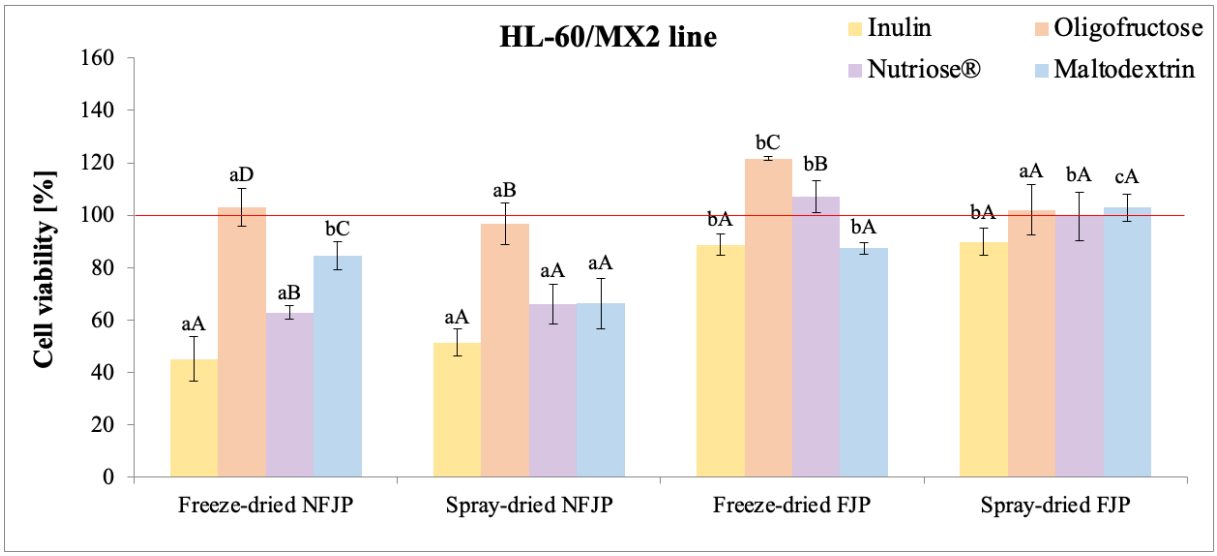


Fig.3. Antiproliferative activity of beetroot powders towards (a) J-45.1 and (b) HL-60/MX2 lines.
Explanatory notes: The average values ($\bar{x}$ ± s/SD; n = 4) of the defined measured parameter with different lowercase (a-d) letters express a significant difference ($p < 0.05$) between powders obtained through different treatments (NFJP – powder derived from non-fermented juice or FJP – powder derived from probiotycally fermented juice after spray drying or freeze-drying) with using the same carrier type, while capital letters (A-D) indicate significant differences among the powdered juices produced with applying the same treatment in the production but using a different type of carrier. The red line indicates the initial viability level (100%) of the human leukemia lines: (a) J-45.01 (ATCC® CRL1990 ™), (b) L-60/MX2 (ATCC® CRL2257 ™).

Załącznik nr 3 do Trybu postępowania w sprawie nadania stopnia naukowego doktora

Jessica Brzezowska
Department of Fruit, Vegetable, and Nutraceutical Plant Technology
Faculty of Biotechnology and Food Science
Wrocław University of Environmental and Life Sciences
37 Chełmońskiego Street, 51-630 Wrocław

Wrocław, 11.09.2023

OŚWIADCZENIE

Oświadczam, że w pracy:
Michalska-Ciechanowska A., Hendrysiak A., **Brzezowska J.**, Wojdyło A., Gajewicz-Skretna A. (2021). How Do the different types of carrier and drying techniques affect the changes in physico-chemical properties of powders from chokeberry pomace extracts? *Foods*, 10(8), 1864. https://doi.org/10.3390/foods10081864
mój udział polegał na tworzeniu zarysu badań, pomocy w opracowaniu technologii wytwarzania, a także otrzymaniu proszków z wytłoków aroniowych stanowiących materiał badawczy. Uczestniczyłam w wykonaniu oznaczeń parametrów fizycznych oraz analizowanych wyróżników chemicznych. Wygenerowane dane opracowałam pod katem merytorycznym oraz statystycznym. Ponadto brałam udział w przygotowaniu manuskryptu, a także jego współredagowaniu podczas procesu recenzji.

11.09.2023 Jessica Brzezowska
data i podpis

Potwierdzam treść oświadczenia.

11.09.23 Anna Michalska-Ciechanowska
data i podpis promotora

DECLARATION

I declare that in the work:
Michalska-Ciechanowska A., Hendrysiak A., **Brzezowska J.**, Wojdyło A., Gajewicz-Skretna A. (2021). How do different types of carriers and drying techniques affect changes in physicochemical properties of aronia pomace extract powders? *Food*, 10(8), 1864. https://doi.org/10.3390/foods10081864
my participation consisted of creating the outline of the research, providing assistance in developing the manufacturing technology, as well as obtaining powders from chokeberry pomace as research material. I participated in the determination of physical parameters and analyzed chemical characteristics. I processed the generated data in terms of substantive and statistical aspects. In addition, I participated in the preparation of the manuscript, as well as its co-editing during the review process.

11.09.2023 Jessica Brzezowska
date and signature

I confirm the content of the statement.

11.09.2023 Anna Michalska-Ciechanowska
date and supervisor's signature

SCIENTIFIC ACHIEVEMENTS

Education

2018 – 2019 **Master degree**; Food Technology and Human Nutrition
Wrocław University of Environmental and Life Sciences, the Faculty of Biotechnology and Food Sciences, Wrocław, Poland

2014 – 2018 **Engineer degree**; Food Technology and Human Nutrition
Wrocław University of Environmental and Life Sciences, the Faculty of Biotechnology and Food Sciences, Wrocław, Poland

Work experience

2018 Apprenticeship at the plant of the Fruit and Vegetable Processing "DROS" in Drobnice (16.07-10.08.2018)

2017 Apprenticeship at the plant of the "Mlekovita" Dairy Cooperative in Zakopane (3-28.07.2017)

Foreign internships

2022 – 2023 **12-month internship abroad** at the Institute of Food *Science Research* (CIAL), part of The Spanish National *Research Council* (CSIC) and the Autonomous University of Madrid (UAM) (Madrid, Spain)

Scientific projects

As a Principal Investigator (PI):

2020 – 2023 **PI** of scientific project **PRELUDIUM 18**, no. 2019/35/N/NZ9/03208; title: "*The effect of the natural food additives on the formation of Maillard reaction/caramelisation*

2020 – 2022 **PI** of the research program "**Innowacyjny Doktorat**", no. B031/0014/20; title: *„Proszki owocowo-warzywne fortyfikowane ekstraktami z roślin leczniczych o ukierunkowanych właściwościach prozdrowotnych",* (Wrocław University of Environmental and Life Sciences, Poland)

As a Contractor:

2023 – Contractor of the national scientific project **PRELUDIUM 21,** no. 2022/45/N/NZ9/02587; title: *"Formation of the stability, bioavailability and bioaccessibility of plant pigments by microencapsulation in the context of their potential anti-diabetic properties"* (National Science Centre, Poland)

2022 – Contractor of the international scientific project **FERBLEND** (ERA-NET SUSFOOD2 and CORE Organic Cofunds joint Call 2019 'Towards sustainable and organic food systems'), agreement no. DWM/SF-CO/32/2021; title: "*Fermentation-induced valorization of side stream blends from oilseed and dairy industry*" (The National Centre for Research and Development, Poland)

2021 – 2022 Contractor of the international scientific project **ALPHORN**, no. 2019/01/Y/NZ9/00051; title: "*Interactions between bioactive compounds and carrier agents during drying of fruit juices"*, (National Science Centre, Poland)

2020 – 2021 Contractor in the **research and development project,** no. POIR.01.01.01-00-1293/17 carried out in cooperation with Regis Sp. z o.o.; title: "*Naturalne substancje aromatyczne uzyskane z surowców mięsnych poddanych hydrolizie enzymatycznej*", (The National Centre for Research and Development, Poland)

Trainings and courses

2023 **SEASONED Summer School** on Basic Sensory Methods in Food Analysis at the Miguel Hernandez University of Elche (Orihuela, Spain)
2023 **Donserv**: Training in the operation of an encapsulation apparatus using the Buchi B-390 encapsulator completed with certificate (15.06.2023)
2023 **Shim-pol: VII Academy of Analytic Chemistry**: Improving quality of research in liquidity chromatography complexed with mass spectrometry - from sample preparation through method optimization to LCMS(/MS) analysis (28-31.05.2023)
2023 **SEASONED** training regarding project and metadata management as well as publishing skills (02-03.02.2023)
2022 **Santander PWr Soft Skills Academy** for doctoral students and young scientists (April, 2022)
2022 **Donserv**: Training on operation and implementation of application methods of B-290 spray dryer completed with certificate (31.03.2022)
2021 **Waters**: Training in the operation of Acquity UPLC high-performance liquid chromatograph coupled to Xevo G2-QTof high-resolution mass spectrometer (15-17.12.2021)
2021 **Shim-pol**: **VII Academy of Analytic Chemistry:** Mass spectrometry in liquid chromatography - practical applications (19-22.09.2021)
2021 **Shim-pol**: Training in liquid chromatography with mass spectrometer on LCMS-8045 (LC-MS-MS) equipment completed with certification (21-22. 04. 2021)
2020 **EIT FOOD Venture Creation School III** programme (23.10-14.11.2020)
2020 **EIT Food Summer School** on new product development for the food industry training (24-28.08.2020)
2019 **IFS and BRC network standard requirements** training (12-13.01.2019)
2018 **Internal HACCP auditor** training (15-16.12.2018)
2018 **ISO 9001internal quality auditor** training (24-25.11.2018)

Most significant awards and distinctions

2022 **Distinction for presentation** entitled "*Drying-dependent valorization of cranberry pomace extracts for natural food additives in powder form*", Nordic Baltic Drying Conference 2022 (7th – 9th September 2022, Wrocław, Poland)
2020 **Second place award** for an oral presentation ęntitled "*Wpływ suszenia na profil polifenolowy proszków z ekstraktów owocowych*", XXV Jubileuszowa Sesja Młodej Kadry Naukowej PTTŻ „Przyszłość w żywności - żywność w przyszłości" (20th – 21st May 2020, Wrocław, Poland)

2019 **Award for the Best Diploma of the Year 2019** granted by the Local Government of the Lower Silesian Voivodship (3rd October 2019, Wroclaw, Poland)

2019 **Distinction for the scientific poster** entitled "*Comparison of physicochemical properties and organoleptic assessment of smoothie products*", XXIV International Conference of Student Scientific Groups (XXXVI Sejmik SKN), (16th – 17th May 2019, Wrocław, Poland).

2015 – 2019 **Fellowship for the best students** awarded by the Rector of Wroclaw University of Environmental and Life Sciences.

Scientific publications not included in the dissertation

1. Hendrysiak A., **Brzezowska J.**, Nicolet N., Bocquel D., Andlauer W., Michalska-Ciechanowska A. Juice powders from rosehip (*Rosa canina* L.): Physical, chemical, and antiglycation properties. *Molecules* 2023, *28*, 1674. DOI: 10.3390/molecules28041674
2. Kita A., Kołodziejczyk M., Michalska-Ciechanowska A., **Brzezowska J.**, Wicha-Komsta K., Turski W. The effect of thermal treatment on selected properties and content of biologically active compounds in potato crisps. *Applied Science* 2022, *12*, 555. DOI: 10.3390/app12020555
3. Michalska-Ciechanowska A., **Brzezowska J.** Naturally all year round! New trends in the production of plant powders as natural food additives and dietary supplements. *Fermentation, Fruits and Vegetable Industry* 2021, *4*, 28-32. DOI:10.15199/64.2021.4.2
4. Michalska-Ciechanowska A., **Brzezowska J.**, Wojdyło A., Gajewicz-Skrętna A., Ciska E., Majerska J. Chemometric contribution for deeper understanding of thermally-induced changes of polyphenolics and the formation of hydroxymethyl-*L*-furfural in chokeberry powders. *Food Chemistry* 2020, 128335. DOI: 10.1016/j.foodchem.2020.128335
5. **Brzezowska J.**, Michalska-Ciechanowska A. Nowe kierunki w produkcji proszków owocowych i warzywnych. I Konferencja Naukowa Forum Inżynierów Przyszłości (materiały pokonferencyjne). Oficyna Wydawnicza Politechniki Wrocławskiej, Wrocław 2020. DOI: 10.37190/FIP2020; ISBN 978-83-7493-149-6
6. Michalska A., Majerska J., **Brzezowska J.**, Wojdyło A., Figiel A. The influence of maltodextrin and inulin on the physico-chemical properties of cranberry juice powders. *ChemEngineering* 2020, *4*, 12. DOI: 10.3390/chemengineering4010012
7. Nowak J., **Brzezowska J.**, Michalska-Ciechanowska A., Kita A. The characteristics of snacks with an addition of Jerusalem artichoke and juice powders expanded by different methods. Przyszłość w żywności – żywność w przyszłości, Wydawnictwo Uniwersytetu Przyrodniczego we Wrocławiu, Wrocław 2020. DOI: 10.30825/1.16.2020; ISBN 978-83-7717-354-1
8. Michalska A., Wojdyło A., **Brzezowska J.**, Majerska J., Ciska E. The influence of inulin on the retention of polyphenolic compounds during the drying of blackcurrant juice. *Molecules* 2019, *24*, 4167. DOI: 10.3390/molecules24224167
9. Michalska A., Wojdyło A., Majerska J., Lech K., **Brzezowska J**. Qualitative and quantitative evaluation of heat-induced changes in polyphenols and antioxidant capacity in *Prunus domestica* L. by-products. *Molecules* 2019, *24*, 3008. DOI: 10.3390/molecules24163008

Scientific conferences

The 3rd International Electronic Conference on Foods: Food, Microbiome, and Health - A Celebration of the 10th Anniversary of Foods' Impact on Our Wellbeing, 1st – 15th October 2022. Silvan J.M., Michalska-Ciechanowska A., Villalva M., **Brzezowska J.**, Díaz S., Martinez-Rodriguez A.J. Bioactive properties of blueberry extracts obtained by different drying methods against *Helicobacter pylori* (*Oral presentation*)

15th World Congress on Polyphenols Application 2022, 28th - 30th September 2022, Valencia, Spain. **Brzezowska J.**, Del Castillo M.D., Michalska-Ciechanowska A., Hendrysiak A. Enhancement of the nutritional value and antioxidant properties of blackcurrant juice powder by using protein-based carrier (*Oral presentation*)

Nordic Baltic Drying Conference 2022, 7th – 9th September 2022, Wrocław, Poland. **Brzezowska J.**, Hendrysiak A., Wojdyło A., Michalska-Ciechanowska A. Drying-dependent valorization of cranberry pomace extracts for natural food additives in powder form (*Oral presentation*)

As co-author:

- Masztalerz K., Lech K., Figiel A., **Brzezowska J.**, Michalska-Ciechanowska A. The influence of the pretreatment method on the physico-chemical properties of sunflower seeds cake products (*Oral presentation*)
- Maksimowski D., **Brzezowska J.**, Hendrysiak A. The effect of instantization process on bioactive potential of hot brewed and cold brew coffee concentrates according to different drying techniques (*Oral presentation*)
- Hendrysiak A., Michalska-Ciechanowska A., **Brzezowska J.**, Bocquel D., Maruel F., Nicolet N., Andlauer W. Effect of juice pretreatment and carrier type on the physico-chemical properties of rosehip powders (*Oral presentation*)
- Kucharska-Guzik A., **Brzezowska J.**, Kułaga M. Influence of freeze- and vacuum drying on the physical and chemical properties of fruit juice powders enriched with herbal infusions (*Poster presentation*)
- Kułaga M., **Brzezowska J.**, Kucharska-Guzik A. Moderating the physical and chemical properties of blackcurrant and chokeberry powders with the addition of vegetable juices and selected drying methods (*Poster presentation*)

IXth International Session of Young Scientific Staff Nowadays food - local vs. global? Traditional vs. innovative? 18th – 20th May 2022, Poznań, Poland

As co-author:

- Hendrysiak A., Michalska-Ciechanowska A., **Brzezowska J.**, Piotrowska N. Influence of selected herbal infusions and carrier agents on the quality of rosehip juice powders (*Oral presentation*)

2nd CiFOOD Conference 2022 "Major Challenges of Future Food Systems", 31st January - 1st February 2022, Aarhus, Denmark. **Brzezowska J.**, Michalska-Ciechanowska A., Struck S., Morejon S. Food processing side streams as a novel source of proteins (*Oral presentation*) and **Brzezowska J.**, Michalska-Ciechanowska A. Food processing side streams as a novel source of proteins (*Poster presentation*)

2nd Intonational Virtual Conference on Raw Materials to Processed Foods, Istanbul, Turkey, 3rd – 4th June 2021. **Brzezowska J.**, Michalska-Ciechanowska A., Hendrysiak A. Antiglycation potential of freeze-dried powders obtained from different fruit fractions (*Oral presentation*)

As co-author:

- Michalska-Ciechanowska A., Wojdyło A., Kramek D., **Brzezowska J.**, Hendrysiak A., Majerska J. Moderation of polyphenols composition in the cranberry extract powders by spray drying parameters and carrier addition (*Oral presentation*)
- Hendrysiak A., Michalska-Ciechanowska A., Wojdyło A., **Brzezowska J.** Influence of the carrier type and drying methods on the physico-chemical properties of sustainable powders gained from chokeberry pomace extracts (*Poster presentation*)

XXV Jubileuszowa Sesja Młodej Kadry Naukowej PTTŻ „Przyszłość w żywności - żywność w przyszłości", 20th – 21st May 2020, Wrocław, Poland. **Brzezowska J.**, Michalska-Ciechanowska A., Wojdyło A. Wpływ suszenia na profil polifenolowy proszków z ekstraktów owocowych (*Oral presentation*)

Forum Inżynierów Przyszłości, 23rd – 25th October 2020, Wrocław, Poland. **Brzezowska J.**, Michalska-Ciechanowska A. New directions in the production of fruit and vegetable powders (*Poster presentation*)

Other scientific activities

1. Member of the **Organizing Committee** of the international Nordic Baltic Drying Conference 2022 (7-9 September)
2. Member of the **Polish Society of Food Technologists** (Wroclaw Division) (PL: Oddział Wrocławski Polskiego Towarzystwa Technologów Żywności)

Bibliometric data of scientific achievements*

Hirsch index:	5 (*WoS & Scopus*), 6 (*Google Scholar*)
Impact Factor:	34.291
MSHE points:	950
Citations:	48 (*WoS*), 56 (*Scopus*), 78 (*Google Scholar*)
ORCID:	0000-0002-5127-2706
Scopus Author ID:	57210447968
ResearcherID:	DTR-4568-2022
Leading Research Group:	Plants4FOOD

* *as of 16.09.2023*