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Characterization of blueberry fruit powders as functional food ingredients rich in polyphenolic compounds

Charakterystyka proszków z owoców borówki amerykańskiej jako funkcjonalnych składników żywności bogatych w związki polifenolowe

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ABSTRACT

This study aimed to obtain freeze-dried highly bioactive powders from 10 blueberry fruit cultivars, which were analysed for their physicochemical and health-promoting properties. Twenty-four phenolic compounds (12.1 to 70.9 mg/g) were identified in the obtained powders, where anthocyanins were the predominant group, including malvidin 3-*O*-glucoside in the highest concentration. The richest phenolic profile was shown for the powder from the 'Star' fruit variety, which also had the strongest $OH^{\bullet}$ (107.89 µg/ml) and $O_2^{\bullet}$ (65.16 µg/ml) radical scavenging potential, as well as the highest anti-diabetic (1.87 mg/ml) and anti-obesity (1.24 mg/ml) effects. For 'Star' fruit powders, the highest antiproliferative activity was also demonstrated, including the strongest against colon cancer cells (Dld-1, 116.52 µg/ml), while showing lower activity against normal colon epithelial cells (CCD841CoN). Furthermore, the proven properties were strongly dependent on the anthocyanin content. Overall, extraction to the solid phase in combination with freeze-drying influenced the quality of the powders, leading to improved functional properties.

Keywords: blueberry, *Vaccinum*, fruits, powder, extraction SPE, health-promoting properties

ABSTRAKT

Celem pracy było otrzymanie liofilizowanych proszków o wysokiej bioaktywności z 10 odmian owoców borówki amerykańskiej, które zbadano pod kątem właściwości fizykochemicznych i prozdrowotnych. W otrzymanych proszkach zidentyfikowano dwadzieścia cztery związki fenolowe (od 12,1 do 70,9 mg/g), wśród których dominowały antocyjany, w tym w największym stężeniu 3-*O*-glukozyd malwidyny. Najbogatszy profil fenolowy wykazano dla proszku z owoców odmiany „Star", który charakteryzował się jednocześnie najsilniejszym potencjałem wymiatania rodników $OH^{\bullet}$(107,89 µg/ml) i $O_2^{\bullet}$ (65,16 µg/ml) oraz najwyższym działaniem antyoksydacyjnym, przeciwcukrzycowym (1,87 mg/ml) i przeciw otyłości (1,24 mg/ml). W przypadku proszków owocowych „Star" wykazano także najwyższą aktywność antyproliferacyjną, w tym najsilniejszą wobec komórek raka okrężnicy (Dld-1, 116,52 µg/ml), natomiast mniejszą aktywność wobec prawidłowych komórek nabłonka okrężnicy (CCD841CoN). Ponadto udowodnione właściwości były silnie zależne od zawartości antocyjanów. Ogólnie rzecz biorąc, ekstrakcja do fazy stałej w połączeniu z liofilizacją wpłynęła na jakość proszków, prowadząc do poprawy właściwości funkcjonalnych.

Słowa kluczowe: Borówka amerykańska, *Vaccinum*, owoce, proszek, ekstrakcja SPE, właściwości prozdrowotne

INTRODUCTION

The blueberry (*Vaccinum* L.) has more than 450 species belonging to the heather family (*Ericaceae*). The most common species distinguished by their organoleptic characteristics are blueberries and highbush blueberries. The highbush blueberry is a perennial plant, reaching a height of 1 to 3 meters. It bears fruit from the second year after planting. From mid-July to September, fruits ripen from the pollinated white flowers and produce 3-7 kg when fully fruited (Krishna et al., 2023).

Numerous scientific studies confirm that the regular consumption of blueberry fruit can provide numerous health benefits. A strong correlation has been proven between high fruit consumption and a reduced risk of developing heart disease, type II diabetes, osteoarthritis, improved blood lipid profile, cognitive abilities, as well as anti-obesity, anti-inflammatory and anti-bacterial effects (Carvalho et al., 2021; Du et al., 2019; Silva et al., 2020; Travica et al., 2020). Although the synergistic effect of the numerous bioactive components present in blueberry fruit is probably responsible for the aforementioned health-promoting properties, most scientific reports attribute this role primarily to the high polyphenol content (0.45 - 3.05 mg/g fresh weight). Up to 55% of the total polyphenol content of the fruit is made up of anthocyanins, of which the predominant compounds are cyanidin 3-*O*-rutinoside, peonidin 3-*O*-glucoside and malvidin 3-*O*-glucoside. Other polyphenolic compounds present in the fruit and contributing to its health-beneficial properties include: phenolic acids, flavonols and flavanols (Hui et al., 2020; Zhao et al., 2023).

The above properties of blueberry fruit make it a valuable functional component of the diet. Nevertheless, fresh blueberry fruit has a short shelf life due to its high sensitivity to environmental factors. Methods for extending their shelf life include drying, freezing, or making jams, preserves and juices. However, thermal exposure of blueberries has been shown to result in a reduction of their health-promoting properties, mainly due to a decrease in polyphenol content (Schmidt et al., 2005). An interesting method of processing blueberry fruit may therefore be to develop nutraceuticals in the form of powders with a high content of polyphenolic compounds, i.e. the compounds mainly responsible for their beneficial health-promoting properties. One alternative method for obtaining such a form of powders is extraction to the solid phase (SPE) combined with lyophilisation. The above-mentioned procedure would yield highly bioactive powders with great economic, pharmaceutical and food potential.

Therefore, this study aimed to obtain and evaluate fruit powders from 10 blueberry cultivars. Physicochemical parameters, antioxidant and anti-obesity, anti-diabetic and anti-cancer activities were evaluated, together with an assessment of the phenolic profile. Correlation and PCA analyses of the principal components were also performed to better understand the relationships between the parameters studied and the fruit varieties. The results obtained may form the basis for the development of nutraceuticals for the prevention and treatment of diseases of civilisation.

MATERIALS AND METHODS

Materials and reagents

Chemicals were purchased from Sigma-Aldrich (Steinheim, Germany) and Chempur (Piekary Śląskie, Poland).

Plant material

The research material consisted of fruits of 10 varieties of blueberries. Selected varieties are: 'Duke', 'Legacy', 'Bluecrop', 'Royal Blu Aroma', 'Sekoya Pop', 'Jewell', 'Cargo', 'Ventura', 'Star' and 'Sekoya Beauty'. The tested fruits were purchased from a local producer. The fruits were fully colored, ripe, with no signs of mold or mechanical damage. Immediately after harvesting and delivery to the laboratory, the fruits were frozen.

Preparation of powder

Blueberry fruits were combined with methanol (50% with 1% formic acid), homogenised (T18 digital, IKA, Poland) and extracted in an ultrasonic bath (30 min., 30

°C, Sonic 10, Polsonic, Poland). The extracts were then centrifuged (Centrifuge 5430, Eppendorf, Germany), and the residue re-extracted as described above, only using 80% methanol. The resulting extracts were combined, pre-evaporated (R-215 Rotavapor System, Buchi, Switzerland) and extracted to the solid phase using a LiChroprep RP-18 bed (40-63 µm). The methanolic extract was lyophilised (ALPHA 1-2 LD, Germany) and the resulting powder was used for the analyses (Żurek et al., 2020; Żurek, 2024b).

Physicochemical analysis

Equilibrium moisture content (Wr) was assessed by the moisture analyzer method (MA35, Sartorius, Kostrzyn, Poland). The pH value was assessed by the potentiometric method (CPC-411, Elmetron, Zabrze, Poland). Water solubility index (WSI) was determined by the centrifuge method (Żurek et al., 2024b).

Determination of total phenolic, flavonoid and anthocyanin content

The total phenolic content (TP), flavonoid (TF) and anthocyanin content (TA) were assessed using the method described by (Gao et al., 2000), (Chang et al., 2020) and (Żurek et al., 2024a), respectively. The results of TP, TF and TA contents were expressed in mg of gallic acid (mg GAE/g d.w.), mg of quercetin (mg QE/g d.w.) and mg of cyanidin-3-glucoside (mg C3G/g d.w.), respectively.

Determination of antioxidant activity

$ABTS^{\bullet+}$ radical scavenging activity, copper ion reduction (CUPRAC), superoxide and hydroxyl radical scavenging activity were measured using the method of (Re et al., 1999), (Apak et al., 2006), and (Halliwell et al., 1987; Robak and Gryglewski, 1988), respectively. Results were expressed as Trolox equivalent (mmol TE/g d.w.) and as IC_{50}.

Antidiabetic and -obesity activity

The antidiabetic and antiobesity activities were assessed, respectively, by the ability of the tested extracts to inhibit the activity of α-amylase (220 U/ml) and porcine type II pancreatic lipase (10 mg/ml) in accordance with the report by (Żurek et al., 2024b). The results were expressed as IC_{50}.

Cell viability assay

The human cancer cell lines Dld-1 and LS180 (colon adenocarcinoma), Mcf-7 (breast adenocarcinoma), Sk-mel-28 (melanoma) and CCD841CoN (normal colonic epithelial cells) were selected for this study. The cell lines were cultured in an incubator (CB170, Binder, Tuttlinen, Germany). The effect of the extracts on cell viability was assessed according to our previous report (Żurek et al., 2024), seeding 8.0 x 10^3 cells per well and assessing viability using the MTS test (Promega, USA). The results were expressed as the IC_{50}.

Determination of polyphenol profile

Quantitative and qualitative profiles of polyphenolic compounds were assessed using the Waters ACQUITY Ultra Performance Liquid Chromatography (UPLC-Q-TOF-MS) system (Waters, Milford, MA, USA). For the assay, the UPLC was equipped with a binary pump, sample and column manager, tandem quadrupole mass spectrometer (TQD) with electrospray ionization (ESI) source and photodiode array (PDA) detector. The results were expressed in mg/g d.w. (Żurek et al., 2024).

Statistical analysis

Statistica 13.3 (StatSoft, Krakow, Poland) was used for statistical analysis of the experimental results (n = 3, mean ± SD). Duncan's test ($P < 0.05$) and principal components analysis (PCA) were performed.

RESULTS AND DISCUSSION

Physicochemical properties

The value of pH, equilibrium moisture (Wr) and water solubility index (WSI) are among the main distinguishing factors defining the quality of the preparations available in powder form (Table 1). The physicochemical properties of the powders were significantly dependent ($P < 0.05$) on the blueberry cultivar selected.

Table 1. Physicochemical properties of blueberry fruit powders, total polyphenols, flavonoids and anthocyanins, as well as antioxidant and anticancer properties of powders from 10 varieties of blueberries

	Varieties									
	Duke	Legacy	Bluecrop	Royal Blu Aroma	Sekoya Pop	Jewel	Cargo	Ventura	Star	Sekoya Beauty
Physicochemical properties										
pH	3.64 ± 0.02^{ab}	3.77 ± 0.01^{c}	3.85 ± 0.02^{d}	3.64 ± 0.03^{ab}	3.91 ± 0.02^{e}	3.74 ± 0.01^{c}	3.66 ± 0.02^{b}	3.62 ± 0.01^{a}	3.94 ± 0.03^{f}	3.86 ± 0.02^{d}
Wr	5.37 ± 0.05^{c}	6.17 ± 0.05^{e}	4.56 ± 0.12^{a}	4.51 ± 0.08^{a}	5.64 ± 0.07^{d}	6.40 ± 0.09^{f}	6.15 ± 0.06^{e}	6.73 ± 0.10^{g}	5.45 ± 0.07^{c}	5.05 ± 0.06^{b}
WSI	94.32 ± 1.05^{cd}	91.89 ± 3.34^{bc}	87.62 ± 2.70^{a}	87.46 ± 1.80^{a}	92.01 ± 2.10^{bc}	97.39 ± 0.88^{f}	94.25 ± 1.52^{cd}	94.43 ± 2.68^{cd}	88.74 ± 2.84^{ab}	95.10 ± 1.37^{cd}
Total polyphenols (TP), flavonoid (TF) and anthocyanin (TA) content										
TP	188.12 ± 2.45^{b}	276.75 ± 4.68^{d}	307.90 ± 3.93^{e}	149.37 ± 2.00^{a}	362.01 ± 0.95^{f}	275.12 ± 4.27^{d}	200.54 ± 4.76^{c}	160.95 ± 1.25^{a}	426.40 ± 3.41^{g}	289.21 ± 2.11^{de}
TF	90.58 ± 2.47^{e}	59.98 ± 1.66^{b}	68.31 ± 1.64^{c}	50.49 ± 1.27^{a}	73.40 ± 3.61^{d}	122.90 ± 1.11^{i}	105.46 ± 2.10^{g}	47.00 ± 0.78^{a}	112.30 ± 2.95^{h}	98.23 ± 0.86^{f}
TA	131.99 ± 0.02^{g}	105.81 ± 0.05^{f}	133.99 ± 0.14^{g}	41.68 ± 0.07^{a}	145.37 ± 0.12^{h}	88.37 ± 0.10^{c}	71.66 ± 0.12^{b}	95.11 ± 0.43^{d}	148.14 ± 0.14^{h}	100.16 ± 0.21^{e}
Antioxidant activities										
ABTS	4.84 ± 0.32^{ab}	5.21 ± 0.17^{bc}	5.80 ± 0.39^{c}	4.31 ± 0.23^{a}	6.58 ± 0.41^{d}	5.55 ± 0.22^{c}	5.36 ± 0.25^{bc}	4.37 ± 0.28^{a}	7.76 ± 0.13^{e}	5.29 ± 0.13^{bc}
CUPRAC	0.43 ± 0.01^{c}	0.50 ± 0.01^{d}	0.61 ± 0.01^{fg}	0.30 ± 0.01^{a}	0.63 ± 0.01^{g}	0.60 ± 0.01^{f}	0.40 ± 0.01^{b}	0.32 ± 0.01^{a}	0.86 ± 0.01^{h}	0.54 ± 0.02^{e}
$O_2^{\cdot-}$	163.39 ± 3.95^{i}	119.53 ± 0.51^{f}	74.86 ± 0.72^{c}	148.56 ± 3.27^{h}	71.26 ± 0.50^{b}	109.89 ± 1.55^{e}	100.28 ± 3.59^{d}	130.77 ± 0.56^{g}	65.16 ± 1.07^{a}	202.29 ± 0.52^{i}
$OH^{\cdot}$	231.09 ± 2.82^{e}	279.28 ± 2.26^{f}	121.98 ± 2.00^{c}	324.59 ± 3.80^{h}	116.97 ± 2.61^{b}	143.55 ± 0.54^{d}	526.75 ± 0.63^{i}	680.50 ± 3.67^{j}	107.89 ± 2.91^{a}	311.23 ± 0.75^{g}
Antidiabetic and -obesity activities										
Amylase	2.20 ± 0.13^{bcd}	2.38 ± 0.10^{de}	2.16 ± 0.10^{bc}	3.81 ± 0.16^{f}	2.06 ± 0.11^{b}	2.48 ± 0.27^{e}	2.57 ± 0.09^{e}	2.44 ± 0.22^{e}	1.87 ± 0.18^{a}	2.39 ± 0.13^{cde}
Lipase	1.39 ± 0.22^{a}	2.06 ± 0.29^{b}	1.34 ± 0.08^{a}	3.44 ± 0.21^{d}	1.26 ± 0.05^{a}	2.56 ± 0.09^{c}	2.71 ± 0.12^{c}	2.20 ± 0.06^{b}	1.24 ± 0.05^{a}	2.22 ± 0.10^{b}
Anticancer activities										
Dld-1	605.30 ± 8.40^{f}	475.10 ± 2.19^{e}	400.45 ± 4.57^{c}	612.20 ± 8.05^{f}	355.50 ± 3.39^{b}	357.90 ± 6.59^{b}	456.38 ± 9.87^{de}	433.78 ± 4.98^{d}	116.52 ± 0.71^{a}	339.09 ± 5.44^{b}
Ls180	561.40 ± 4.12^{g}	418.74 ± 8.28^{e}	399.87 ± 2.09^{d}	547.74 ± 6.39^{g}	367.48 ± 4.65^{c}	367.18 ± 3.97^{c}	497.14 ± 5.76^{f}	229.36 ± 1.58^{b}	209.51 ± 2.71^{a}	554.85 ± 2.85^{g}
Mcf-7	457.70 ± 9.49^{f}	233.86 ± 1.74^{d}	178.19 ± 6.32^{c}	593.71 ± 1.56^{g}	125.61 ± 6.04^{a}	143.11 ± 4.83^{b}	225.98 ± 10.05^{d}	278.14 ± 11.24^{e}	123.69 ± 5.46^{a}	630.73 ± 8.63^{h}
U251mg	630.63 ± 1.86^{h}	510.75 ± 3.87^{d}	496.92 ± 3.82^{c}	712.51 ± 6.60^{j}	465.23 ± 8.03^{b}	521.10 ± 3.45^{e}	614.86 ± 3.09^{g}	460.01 ± 4.42^{b}	315.43 ± 3.27^{a}	560.14 ± 4.57^{f}
Sk-mel-28	605.87 ± 3.88^{e}	444.47 ± 3.01^{c}	394.74 ± 1.37^{b}	678.19 ± 9.43^{g}	388.63 ± 2.73^{ab}	539.65 ± 7.70^{d}	687.60 ± 4.80^{h}	601.86 ± 1.09^{e}	384.00 ± 5.04^{a}	661.04 ± 3.19^{f}
CCD841CoN	683.32 ± 5.22^{g}	676.29 ± 4.82^{g}	490.51 ± 2.44^{c}	614.11 ± 1.39^{f}	548.52 ± 3.09^{de}	563.90 ± 5.11^{e}	435.17± 4.87^{b}	453.20 ±2.48^{b}	322.67 ± 4.24^{a}	515.97 ± 4.55^{cd}

Results for Wr (Equilibrium moisture content) and WSI (Water solubility index) are expressed in %; TP in mg GAE/g; TF in mg QE/g; TA in mg C3G/g; ABTS and CUPRAC methods in mmol TE/g; $O_2^{\cdot-}$ and $OH^{\cdot}$ methods as IC_{50}, µg/ml; anticancer activities as IC_{50}, µg/ml. Values are expressed as mean ± SD. Statistically significant differences (a, b, c, ...) were assessed using the Duncan test ($P < 0.05$).

The pH value ranged from 3.62 to 3.94, Wr from 4.51 - 6.73% and WSI from 87.46 to 97.39%. The lowest values were found for the cultivars 'Ventura' and 'Royal Blu Aroma', respectively, while the highest values were found for 'Star' and 'Jewel'.

The pH value of powders determines their organoleptic properties, microbiological and phytochemical stability. It has been observed that preparations rich in polyphenolic compounds lose their properties as the value of this parameter increases. Furthermore, Gibson et al. (2013) showed that the value of this parameter for blueberry powders also depends on the degree of fruit maturity. The pH of powders from green and red fruit was significantly lower (3.2) than the pH of blue (3.3) and purple (3.63) fruit. Another parameter, Wr, determines the appropriate flowability and agglomeration of the particles, and is also important in maintaining the chemical and microbiological stability of the powders. In order to maintain these properties of the powders, the value of this parameter should be in the range of 2.5 to 7.0% (Aziz et al., 2018), which was in line with the results obtained, as well as the results of other works (Jurevičiūtė et al., 2022; Nemetz et al., 2021). In turn, the WSI determines the applicability of a given powder as a food additive, also reflecting its behaviour in the aqueous phase. The solubility of the powders in water was significantly higher compared to the range presented by other authors (11.0 - 33.5%) (Calabuig-Jiménez et al., 2022; Nemetz et al., 2021), which is mainly due to the removal of ballast substances hindering their solubility.

Total phenolic, flavonoid and anthocyanin contents

TP content ranged from 149.37 - 426.40 mg GAE/g, TF content from 47.00 - 122.90 mg QE/g and TA content from 13.89 - 49.38 mg C3G/g (Table 1). The highest and lowest TP and TA contents were found for the same cultivars, i.e. 'Star' and 'Royal Blu Aroma', respectively. In contrast, the highest TF content was found for the cultivar 'Jewell'.

The TP z content shown was significantly higher compared to previous reports. Nemetz et al. (2021), Gouw et al. (2017) and Jureviciute et al. (2022) for powders obtained from blueberry pomace showed values ranging from 6.28 - 30.0 mg GAE/g (Gouw et al., 2017; Jurevičiūtė et al., 2022; Nemetz et al., 2021). A similar trend was observed for TF and TA content. Previously published data for TF ranged from 1.62 - 5.12 mg RU/g (D. Li et al., 2017) and TA ranged from 0.11 - 25.0 mg C3G/g (M.-J. Li et al., 2023; Nemetz et al., 2021; Tatar Turan et al., 2015). The demonstrated differences in TP, TF and TA content for blueberry powders compared to the referenced works may be due to the fact that the studied preparations were obtained by SPE extraction followed by freeze-drying. This treatment scheme allowed the removal of ballast substances, proteins and carbohydrates, which translated into a higher concentration of polyphenolic compounds. In the works cited, blueberry fruit powders were usually obtained by lyophilisation or spray drying of the pomace. This can be confirmed by the study of Zhao et al. (2023), who, after extraction using a macroporous resin (HPD-100B), recorded a 175.2-fold higher TP, 209.8-fold higher TF, and 177.3-fold higher TA content in the resulting powders compared to fresh fruit (Zhao et al., 2023). The TP, TF, and TA contents of powders are also significantly influenced by the ratio of peel to pulp. Li et al. (2023) reported 6.4 times higher TP and 27.8 times higher TA concentrations in blueberry peel than in pulp (M.-J. Li et al., 2023).

Antioxidant activity

The antioxidant activity expressed by the ABTS method ranged from 4.31 - 7.76 mmol TE/g, the CUPRAC method 0.30 - 0.86 mmol TE/g, and O_2 and OH radical uptake ranged from 65.16 - 202.29 and 107.89 - 680.50 µg/ml, respectively (Table 1). The antioxidant activity of the powders was significantly dependent ($P < 0.05$) on the variety of fruit from which they were obtained, but showed the same trend. The highest activity in the four methods was shown for the blueberry fruit powder of the cultivar 'Star', and the lowest for the cultivars 'Royal Blu Aroma', 'Ventura' and 'Sekoya Beauty'.

As in the assessment of TP, TF and TA, the antioxidant activity values shown were significantly higher

(approximately 10-fold) compared to blueberry fruit powders obtained to date (Correa-Betanzo et al., 2015; Sadowska et al., 2017; Tatar Turan et al., 2015; Wilkowska et al., 2016). This indicates that polyphenolic compounds, the main component of the powders obtained, play a major role in shaping antioxidant activity. These conclusions were confirmed by correlation analysis, where a strong relationship was found between TP vs. ABTS method ($r > 0.997$; $P < 0.01$), TA vs. CUPRAC method ($r > 0.993$; $P < 0.01$) and anthocyanins vs. O_2 ($r > 0.998$; $P < 0.01$). Thus, of the compounds identified in the phenolic profile, anthocyanins are mainly responsible for the antioxidant activity of the powders. Similar findings can be found in other work evaluating the antioxidant activity of berries, where it was also proven that it is mainly anthocyanins that make a significant contribution to shaping this property of powders (Sadowska et al., 2017; Wilkowska et al., 2016).

Anti-diabetic and anti-obesity activity

A number of studies have suggested that certain phenolic compounds in berries may exhibit α-amylase and pancreatic lipase inhibitory activity, which may have beneficial effects on body weight control and blood lipid levels. This relationship was assessed in the present study by showing α-amylase inhibitory activity ranging from 1.87 to 3.81 mg/ml and lipase inhibitory activity ranging from 1.24 to 3.44 mg/ml (Table 1). The highest activity for both methods was shown for the 'Star' variety and the lowest for 'Royal Blu Aroma'. Significantly better results for α-amylase inhibition were shown in the work of Hui et al. (2020), where 3.2 times higher activity was proven. In contrast, activity against pancreatic lipase was 5.8 times lower than in the present work (Hui et al., 2020). The cited work also demonstrated that the inhibition of α-amylase and lipase by blueberry powders was driven by the hydrogen bonding of anthocyanins, of which delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside had the greatest effect. In our analyses, we also found that the inhibitory capacity of *α*-amylase was strongly dependent on TP ($r > 0.989$; $P < 0.01$). In contrast, for lipase, it was strongly dependent on TA ($r > 0.987$; $P < 0.01$). Thus, cyanidin-enriched foods may be useful in the prevention and treatment of lifestyle diseases.

Anticancer activity

The highest activity against all tested cell lines was found for 'Star' (Table 1). The IC_{50} values obtained were 116.52 µg/ml against line Dld-1 > 123.69 µg/mL against Mcf-7 > 209.51 µg/ml against Ls180 > 315.43 µg/ml against U251mg > and 384.00 µg/ml against line Sk-mel-28, respectively. In contrast, the lowest activity was found for the cultivars 'Royal Blu Aroma' (612.20 µg/ml and 712.51 µg/ml against the Dld-1 and U251mg lines, respectively), the cultivar 'Duke' (561.40 µg/ml against Ls180), 'Sekoya Beauty' (630.73 µg/ml against Mcf-7) and the cultivar 'Cargo' (687.60 µg/ml against Sk-mel-28).

To date, several experiments have been conducted on the anticancer activity of blueberry powders, mainly against colon cancer cells (Seeram et al., 2006; Yi et al., 2005). Kiernozek et al. (2022) showed that blueberry extracts inhibited the proliferation of colorectal cancer cells and induced their apoptosis (Kiernozek et al., 2022). Also, Wang et al. (2017) showed significant inhibition of melanoma cell viability (B16-F10) by blocking the cell cycle in the G0/G1 phase. In contrast, in other studies, no cytotoxic effect against acute promyelocytic leukaemia (HL-60) cells was demonstrated for the blueberry cultivars tested (Tsuda et al., 2013). These findings indicate a high selectivity of the cytotoxic activity of blueberry powders, probably largely dependent on their anthocyanin composition.

Polyphenol profile

In the blueberry fruit powders, 24 polyphenolic compounds were identified, including 13 anthocyanins and 11 other phenolic compounds (8 flavonols, 3 phenolic acids) (Table 2). The total amount of phenolic compounds identified ranged from 12.1 to 70.9 mg/g and was significantly dependent ($P < 0.05$) on fruit variety. The highest amount was identified in powders from the 'Star' cultivar and the lowest in 'Royal Blu Aroma'.

Table 2. Characterization of blueberry fruit powders as functional food ingredients rich...

	Varieties									
	Duke	Legacy	Bluecrop	Royal Blu Aroma	Sekoya Pop	Jewel	Cargo	Ventura	Star	Sekoya Beauty
Anthocyanins										
Dl-3-Glc	3.54 ± 0.05^{b}	5.17 ± 0.02^{d}	4.79 ± 0.04^{c}	0.57 ± 0.02^{a}	3.98 ± 0.02^{b}	5.18 ± 0.08^{d}	4.24 ± 0.04^{c}	0.86 ± 0.02^{a}	9.75 ± 0.02^{e}	3.05 ± 0.02^{b}
Dl-3-Gal	-	-	-	-	2.12 ± 0.08^{d}	1.31 ± 0.04^{c}	0.94 ± 0.02^{b}	0.14 ± 0.01^{a}	-	0.48 ± 0.05^{ab}
Dl-3-Ara	1.67 ± 0.03^{b}	2.33 ± 0.05^{c}	1.71 ± 0.02^{b}	0.10 ± 0.04^{a}	2.65 ± 0.20^{c}	2.21 ± 0.03^{c}	2.47 ± 0.06^{c}	0.63 ± 0.03^{a}	7.58 ± 0.15^{d}	1.85 ± 0.13^{b}
Cn-3-Glc	1.44 ± 0.03^{e}	1.21 ± 0.21^{d}	2.69 ± 0.10^{f}	1.24 ± 0.01^{d}	1.40 ± 0.04^{e}	1.11 ± 0.09^{b}	2.53 ± 0.11^{f}	0.65 ± 0.03^{a}	-	1.14 ± 0.23^{c}
Pt-3-Glc	3.42 ± 0.05^{c}	4.86 ± 0.22^{e}	5.59 ± 0.11^{f}	0.83 ± 0.02^{a}	4.47 ± 0.00^{d}	4.42 ± 0.03^{d}	3.90 ± 0.06^{c}	1.20 ± 0.02^{b}	7.77 ± 0.20^{g}	4.23 ± 0.04^{d}
Cn-3-Ara	0.35 ± 0.02^{a}	0.41 ± 0.04^{ab}	0.68 ± 0.01^{b}	0.33 ± 0.01^{a}	4.06 ± 0.10^{f}	2.14 ± 0.03^{d}	2.37 ± 0.03^{e}	0.84 ± 0.02^{c}	0.96 ± 0.05^{c}	2.22 ± 0.04^{de}
Pt-3-Ara	1.42 ± 0.02^{c}	1.96 ± 0.24^{d}	2.02 ± 0.01^{e}	0.24 ± 0.01^{a}	1.92 ± 0.01^{d}	1.43 ± 0.04^{c}	1.71 ± 0.05^{d}	0.58 ± 0.02^{b}	4.36 ± 0.35^{f}	1.88 ± 0.03^{e}
Po-3-Glc	0.82 ± 0.00^{de}	0.92 ± 0.04^{e}	1.10 ± 0.04^{f}	0.73 ± 0.02^{c}	-	0.67 ± 0.02^{b}	0.32 ± 0.07^{a}	0.64 ± 0.01^{b}	-	0.79 ± 0.06^{d}
Mv-3-Glc	11.25 ± 0.04^{e}	16.97 ± 0.32^{h}	16.78 ± 0.07^{h}	3.09 ± 0.08^{a}	16.36 ± 0.16^{g}	9.92 ± 0.09^{c}	11.14 ± 0.04^{d}	3.53 ± 0.04^{b}	20.63 ± 0.58^{i}	16.12 ± 0.05f
Mv-3-Gal	0.06 ± 0.06^{a}	0.22 ± 0.05^{b}	0.21 ± 0.05^{b}	-	11.50 ± 0.16^{h}	4.13 ± 0.12^{f}	3.73 ± 0.09^{e}	1.97 ± 0.02^{d}	0.42 ± 0.1^{1c}	6.98 ± 0.07^{g}
Mv-3-Ara	4.99 ± 0.00^{d}	7.87 ± 0.06^{g}	8.00 ± 0.02^{h}	1.90 ± 0.03^{a}	7.62 ± 0.20^{f}	3.76 ± 0.08^{c}	5.88 ± 0.30^{e}	2.20 ± 0.05^{b}	10.55 ± 0.43^{i}	7.82 ± 0.06^{g}
Po-3-Gal	-	-	-	-	0.14 ± 0.03^{a}	0.55 ± 0.01^{c}	-	0.48 ± 0.01^{b}	-	-
Cn-3-Gal	-	-	-	-	0.71 ± 0.09^{b}	0.45 ± 0.01^{a}	-	-	-	-
Sum	28.97 ± 0.09^{c}	41.93 ± 0.96^{f}	43.58 ± 0.20^{g}	9.02 ± 0.00^{a}	56.93 ± 0.22^{j}	37.28 ± 0.17^{d}	39.23 ± 0.53^{e}	13.72 ± 0.02^{b}	62.02 ± 1.90^{j}	46.57 ± 0.56^{h}
Other phenolic compounds										
5-CQA	0.26 ± 0.08^{de}	0.24 ± 0.08^{d}	0.27 ± 0.00^{e}	0.07 ± 0.01^{a}	0.12 ± 0.02^{b}	0.36 ± 0.20^{f}	0.07 ± 0.00^{a}	0.16 ± 0.01^{c}	0.56 ± 0.08^{g}	0.16 ± 0.01^{c}
3,5-CQA	0.19 ± 0.01^{d}	0.33 ± 0.01^{f}	0.14 ± 0.01^{c}	0.08 ± 0.01^{a}	0.30 ± 0.06^{e}	0.07 ± 0.01^{a}	0.07 ± 0.00^{a}	0.60 ± 0.01^{g}	0.13 ± 0.01^{b}	0.13 ± 0.02^{b}
CA	0.22 ± 0.08^{c}	0.22 ± 0.02^{c}	0.26 ± 0.01^{d}	0.12 ± 0.01^{a}	0.25 ± 0.00^{d}	0.22 ± 0.01^{c}	0.30 ± 0.07^{e}	0.41 ± 0.02^{f}	0.21 ± 0.08^{b}	0.31 ± 0.03^{e}

Table 2. Continued

	Varieties									
	Duke	Legacy	Bluecrop	Royal Blu Aroma	Sekoya Pop	Jewel	Cargo	Ventura	Star	Sekoya Beauty
M-3-Pent	0.41 ± 0.03^{c}	0.18 ± 0.00^{a}	2.29 ± 0.03^{e}	0.92 ± 0.02^{d}	5.93 ± 0.03^{h}	10.96 ± 0.04^{i}	3.20 ± 0.05^{f}	0.28 ± 0.02^{b}	0.92 ± 0.01^{d}	4.57 ± 0.03^{g}
M-3-Glc	0.17 ± 0.02^{a}	0.19 ± 0.05^{b}	0.20 ± 0.02^{b}	0.21 ± 0.00c	0.28 ± 0.01^{f}	0.33 ± 0.00^{h}	0.27 ± 0.00^{e}	0.17 ± 0.00^{a}	0.24 ± 0.03^{d}	0.31 ± 0.00^{g}
Q-3-Glc	3.62 ± 0.04^{e}	5.72 ± 0.22^{g}	7.89 ± 0.13^{h}	0.58 ± 0.00^{b}	2.35 ± 0.00^{d}	1.08 ± 0.03^{c}	3.58 ± 0.09^{e}	1.37 ± 0.02^{c}	4.38 ± 0.12^{f}	0.49 ± 0.01^{a}
Q-3-G	0.69 ± 0.05^{f}	0.82 ± 0.13^{g}	0.57 ± 0.05^{d}	0.38 ± 0.00^{b}	1.18 ± 0.03^{h}	0.26 ± 0.01^{a}	0.34 ± 0.06^{b}	0.52 ± 0.04^{c}	0.62 ± 0.07^{e}	0.60 ± 0.00^{e}
Q-3-Pent	0.94 ± 0.02^{d}	1.46 ± 0.07^{e}	1.59 ± 0.11^{f}	0.16 ± 0.01^{a}	0.44 ± 0.02^{b}	0.18 ± 0.01^{a}	0.56 ± 0.02^{c}	0.40 ± 0.05^{b}	0.91 ± 0.09^{d}	-
Q-3-Rha	0.44 ± 0.00^{b}	0.76 ± 0.02^{e}	1.13 ± 0.03^{f}	0.09 ± 0.01^{a}	0.08 ± 0.01^{a}	0.73 ± 0.04^{d}	0.65 ± 0.04^{c}	2.12 ± 0.01^{g}	0.47 ± 0.03^{b}	0.73 ± 0.00^{d}
Syrg-3-Glc	0.41 ± 0.03^{f}	0.14 ± 0.02^{b}	0.40 ± 0.01^{f}	0.10 ± 0.00^{a}	0.80 ± 0.01^{g}	0.17 ± 0.02^{c}	0.17 ± 0.05^{c}	0.29 ± 0.05^{e}	0.10 ± 0.02^{a}	0.25 ± 0.01^{d}
K-3-Rut	-	-	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}	-	0.12 ± 0.00^{b}	0.07 ± 0.00^{a}	0.35 ± 0.01^{c}	-	0.35 ± 0.01^{c}
Sum (mg/g)	7.60 ± 0.14^{b}	10.32 ± 0.54^{g}	15.13 ± 0.27^{i}	3.10 ± 0.06^{a}	12.44 ± 0.09^{h}	15.02 ± 0.26^{i}	9.36 ± 0.15^{f}	7.99 ± 0.03^{c}	8.92 ± 0.47^{e}	8.73 ± 0.10^{d}
TOTAL (mg/g)	36.57 ± 0.05^{c}	52.24 ± 1.50^{e}	58.71 ± 0.47^{g}	12.12 ± 0.06^{a}	69.38 ± 0.13^{h}	52.30 ± 0.43^{e}	48.59 ± 0.38^{d}	21.71 ± 0.01^{b}	70.94 ± 1.43^{i}	55.30 ± 0.66^{f}

Abbreviations: Dl, delphinidin; Cn, cyanidins; Pt, petunidin; Po, peonidin; Mv, malvidin; M, myricetin; Q, quercetin; Syrg, syringetin; K, kaempferol; 5-CQA, 5-caffeoylquinic acid; 3,5-CQA, 3,5-caffeoylquinic acid; CA, caffeic acid; Gal, galactoside; Glc, glucoside; Ara, arabinoside; Pent, pentoside; G, glucuronide; Rha, rhamnoside; Rut, rutinoside. Statistically significant differences (a, b, c, ...) were assessed using the Duncan test ($P < 0.05$).

Anthocyanins were among the main phenolic compounds of blueberry fruit powders (63.2 (Ventura) - 87.4% (Star) of the total identified compounds), with a quantitative and qualitative profile that differed significantly between the cultivars tested ($P < 0.05$). Their amount ranged from 9.02 (Royal Blu Aroma) to 62.02 mg/g (Star), and the main compounds included malvidin 3-*O*-glucoside, malvidin 3-*O*-pentoside and delphinidin 3-*O*-glucoside. Although the anthocyanin concentrations shown were significantly higher compared to the results presented so far (0.02 - 13.1 mg/g), malvidin 3-glucoside was identified as the dominant compound so far (Lee et al., 2016; D. Li et al., 2017; Wilkowska et al., 2016; Zhou et al., 2020). In general, it can be deduced that anthocyanins, as the main component of the powders obtained, are mainly responsible for their health-promoting properties. So far, it has been reported that the acylated groups in anthocyanins determine their activity towards α-glucosidase, antioxidant activity and antiproliferative activity of berries (Calabuig-Jiménez et al., 2022; Wu et al., 2017).

Other groups of phenolic compounds identified in the powders studied included flavonols and phenolic acids, which accounted for 11.3 (Star) - 31.4% (Ventura) and 0.9 (Cargo) - 5.4% (Ventura) of the total identified compounds, respectively. Among the flavonols, quercetin 3-*O*-glucoside was among the predominant compounds, while among the phenolic acids, 5-*O*-caffeoylquinic acid was present in the highest concentration. Wilkowska et al. out of 17 identified compounds in blueberry powders, only 3 belonged to the phenolic acid group with concentrations similar to the present work, but they did not identify any flavonols (Wilkowska et al., 2016). In contrast, Wu et al. (2017) identified citric accid, loganic acid and feruloylquinic acid in addition to the acids mentioned in this paper (Wu et al., 2017). In general, flavonols and phenolic acids are a marginal component of blueberries and no health-promoting activity has been demonstrated for these blueberry components in reports to date.

PCA

Similarity analysis between the blueberry cultivars studied and the determinations made was estimated using the PCA method (Figure 1). The PCA plot illustrated 69.41% of the total variance in the data, where the two main components, PC1 and PC2, explained 58.3% and 11.1% of the total variation, respectively. The PCA analysis showed the existence of two clusters. The first group was formed by the cultivar 'Star', which was characterised by its high anthocyanin content and antiproliferative, antidiabetic, anti-obesity, antioxidant activity, as well as physicochemical parameters such as ph. In contrast, the second group was composed of all the other varieties analysed (Duke, Legacy, Bluecrop, Royal Blu Aroma, Sekoya Pop, Jewell, Cargo, Ventura and Sekoya Beauty) and parameters such as phenolic acids, flavonols, Wr and WSI. Overall, the results obtained confirmed previous conclusions, showing that the variety of fruit studied had a significant effect on phenolic composition and health-promoting activity, as well as that anthocyanins mainly shape these properties.

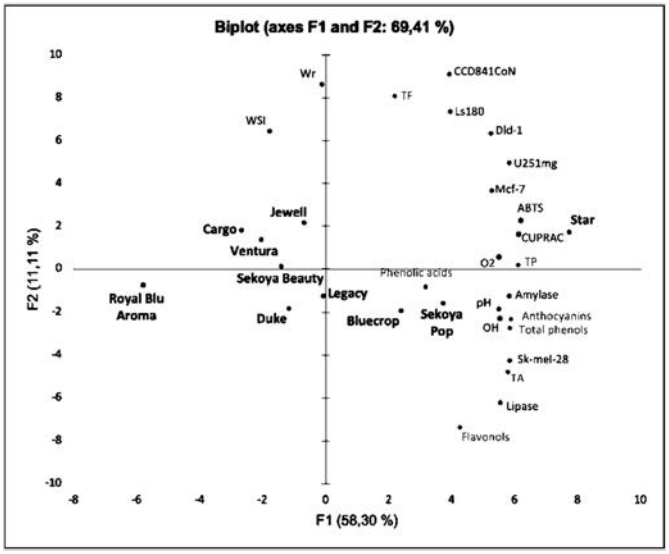

Figure 1. Principal component analysis (PCA) of data on the content of phenolic compounds and health-promoting activity of powders from 10 blueberry varieties

CONCLUSION

The powders obtained from 10 blueberry cultivars have great practical potential as nutraceuticals and as ingredients in finished food products. At the same time, the type of fruit variety significantly determines the physicochemical properties, as well as the antioxidant, antidiabetic, anti-obesity and antiproliferative activity of the powders. At the same time, the powders from the 'Star' fruit variety had the most favourable values for these parameters, as well as the richest phenolic composition in terms of quantity and quality. The phenolic composition of the powders also had a significant effect on the demonstrated health-promoting activity, where the highest proportion was shown for anthocyanins.

The results obtained offer a wide range of possibilities for the use of the powders obtained in the production of foods with preventive and therapeutic potential targeting civilisation diseases. However, further studies are necessary, including, among others, evaluation of changes in the analysed parameters of powders in the product matrix during preparation and storage. However, the data presented inspire further research into the potential beneficial health properties associated with the consumption of this type of food.

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