Research Article

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Influence of Production Parameters on the Content of Polyphenolic Compounds in Extruded Porridge Enriched with Chokeberry Fruit (*Aronia melanocarpa* (Michx.) Elliott)

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Abstract: Chokeberry fruit (*Aronia melanocarpa* (Michx.) Elliott) is known for its antioxidant properties and generally beneficial impact on human health. The aim of the research was to produce innovative corn porridge with a different content of chokeberry fruit percentage-wise and to test it to determine the content of polyphenolic compounds, including flavonoids and individual free phenolic acids, and determine the antioxidant properties of analysed extracts. One of the objectives was also to identify the optimum porridge production parameters, including, among other things, the rotational speed of the extruder screw during the extrusion process.

Obtained results showed that an increased content of chokeberry fruit in porridge enhances its antioxidant properties, as well the content of polyphenols, flavonoids, and free phenolic acids. The greatest free radicals scavenging activity by all extracts was reported after 10 minutes of the process. The results of the above study demonstrate that extruded porridge enriched with chokeberry fruit have a potential for becoming a good

source of natural antioxidants, and the extrusion process at 80 rpm does not degrade the tested active compounds.

Keywords: LC-MS; antioxidant tests; chokeberry fruit; phenolic compounds; functional food.

1 Introduction

In the times of more and more common diseases of affluence, to a large extent attributable to a busy and unhealthy lifestyle marked by stress, environmental pollution and improper nutrition, consumers are looking for foodstuffs that replace synthetic ingredients with the natural ones. The goal of any balanced diet is to supply enough nutrients to meet person's nutritional needs. However, there is increasing scientific evidence to confirm the hypothesis that some food can offer other beneficial effects on top of supplying the most essential nutrients. A shift towards a healthy lifestyle and focus on the prevention of diseases, just to mention cancer, diabetes, or neurodegenerative and cardiovascular conditions, contribute to the emergence of new dietary trends. Given the above, we are seeking to develop food that can bring more health benefits than mere good taste. Looking at the existing socio-economic developments and demographic trends, functional food (FF) seems to be exactly what is needed to improve the quality of life [1]. Functional food can be classified both by product ranges (snacks, pastas, porridge, breakfast cereals, etc.) and by the technological process used during production. For example, such processes may involve the addition of a bioactive ingredient or a modification of the concentration of natural ingredients to enhance the desired action [2]. Moreover, specific functional food products can be

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designed and targeted at the whole population or specific groups, for example, age groups or consumers suffering from specific diseases [3].

In order to exert a beneficial effect on human health, functional food can be enriched with biologically active secondary metabolites of plant-origin. In this context, many scientists highlight the highly positive effects of polyphenols. It is a diverse group of compounds with at least two hydroxyl groups bound to one or more aromatic rings. Structure-wise, they can be divided into flavonoids, lignans, stilbenes, coumarins, and phenolic acids [4].

Polyphenols exhibit a high antioxidant activity and a number of other pharmacological properties; besides, they reveal anti-inflammatory [5], cardioprotectively, neuroprotectively, antineoplastic, antiviral [6], immunomodulatory [7,8] antimicrobial [9], antiallergic, anti-oedematous, and antiatherosclerotic characteristics [10,11]. In addition, they work as a natural and effective food preservative [12].

The antioxidant activity of polyphenols comes from the inhibition of enzymes responsible for the formation of reactive forms of oxygen, among them: xanthine oxidase, NADPH (Nicotinamide Adenine Dinucleotide Phosphate), but they can also bind existing free radicals. Such properties are due to the presence of hydroxyl groups in the structure, and such groups reduce free radicals.

A plant known for its antioxidant properties and generally beneficial effects on human health is the chokeberry (*Aronia melanocarpa*), or, to be precise, its fruit. Chokeberry fruit contains a wide range of nutrients and health-improving substances, such as: sugars (glucose, fructose), pectins, tannins, calcium and iron compounds, vitamins (A, C, E, PP and others from the B group), flavonoids (hyperoside, quercetin, rutin), anthocyanins (cyanidin derivatives), phenolic acids (ferulic, *p*-coumaric, protocatech). Chokeberry extracts have proven effective in the prevention of stomach ulcers; besides, they help seal blood vessels, improve vessel flexibility, and reduce permeability. They are administered for colds and in periods of reduced immunity [13].

The aim of the research was to produce innovative corn porridge with a different content of chokeberry fruit percentage-wise and to test it to determine the content of polyphenolic compounds, including flavonoids and individual free phenolic acids, and determine the antioxidant properties of analysed extracts. One of the objectives was also to identify the optimum porridge production parameters, including, among other things, the rotational speed of the extruder screw during the extrusion process.

Extrusion-cooking is a process of processing starchy materials under specific thermal (120-200°C), moisture, and high pressure (20 MPa) conditions. The intense processing of mechanical shearing results in a deep transformation of individual components. This highly effective technology brings to the market new product ranges featuring attractive sensory features and texture. The outcome is microbiologically clean and durable products. Owing to the use of techniques, such as microencapsulation and on-surface application of vitamins, condiments, and colourants, the nutritional value of such products can be almost freely shaped [14,15].

2 Materials and methods

2.1 Reagents

Standards of caffeic, gallic, ferulic, isoferulic, protocatechuic, 3-OH-benzoic, 4-OH-benzoic, rosmarinic, 3,4-dihydroxyphenylacetic, vanillic, syringic, *m*-coumaric, *o*-coumaric, *p*-coumaric, salicylic, 3-OH-cinnamic, 3,4-dimethoxycinnamic acid and LC grade acetonitrile were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). Gentisic, sinapic, veratric acids were from ChromaDex (Irvine, USA). LC grade water was prepared using a Millipore Direct-Q3 purification system (Bedford, MA, USA). Ethanol and methanol (reagent grade purity) used for the preparation of extracts, Folin-Ciocalteu reagents and all analytical grade reagents were obtained from Avantor Performance Materials (Center Valley, PA, USA).

2.2 Porridge production

Chokeberry fruits purchased from a local farmer (Lublin Voivodeship located in southeastern Poland) were dried in an air oven at 40°C for 12 hours. The raw material mixes used for the tests were made from corn porridge (DASCA, distributor: Awiko, Lublin, Poland) with an addition of 0, 5, 10, 15 and 20% of dried chokeberry fruit. The mixes were moistened up to 14%. The extrudates were prepared on the single-screw extruder TS-45 (ZMCh Metalchem, Gliwice, Poland) equipped with a modified plasticising system L/D = 12:1. The extrusion was carried out at a varied screw speed of the extruder plasticizing system: 80 and 120 rpm. A single-opening die of 3 mm in diameter was used. During the extrusion process, temperature changes were

recorded in the individual extruder sections at different temperature profiles: 132-142°C. The obtained extrudates were milled in the laboratory mill LMN10 (TestChem, Radlin, Poland) to obtain material of less than 1 mm in granularity.

2.3 Preparation of extracts

2 gram-samples of porridge were transferred to ground-necked flasks, and 40 mL of ethanol was added. An ultrasonic bath with a thermostat was used for extraction (Bandelin Electronic, Sonorex RK 100H, Germany). The extraction protocol was previously optimized in terms of temperature, time, type of solvent and ultrasound power. The extraction process took 40 minutes at the temperature of 60°C, the ultrasound frequency of 33kHz and the power of 320W. The extracts were filtered into beakers; 40 mL of ethanol was added to the reminder, and the extraction was repeated. The obtained extracts were combined and put aside in beakers under a hood until the solvent evaporated. The dry residues were quantitatively transferred to volumetric flasks and supplemented with methanol up to 5 mL [11].

2.4 Determination of the total content of polyphenolic compounds

The total content of polyphenolic compounds in the studied extracts was determined with the Folin-Ciocalteu (FC) method designed earlier [16,17]. 100 µL of the tested extracts were placed in 5 mL volumetric flasks. Next, 900 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added. The solutions were mixed and put aside. After 4 minutes, 1mL of 7.7% sodium bicarbonate and 400 µL of distilled water were added. The content was mixed and placed in a 40°C water bath for 50 minutes. After that, the absorbance of the solutions was measured using a UV spectrometer (Genesys 10S VIS, Thermo Scientific, Waltham, MA, US) at a wavelength of 765 nm.

Next, a calibration curve of gallic acid (0.05, 0.15, 0.25, 0.35, 0.5, 1.0 mg/mL) was plotted. 100 µL of each of the calibration solutions were collected, 900 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added to them and processed as in the case of the extracts above. A solution without gallic acid was used for a blank experiment. Based on obtained results, a calibration curve of gallic acid ($y = 3.7995x + 0.0986$) was plotted. It was used to calculate the total polyphenol content in the analysed extracts, expressed as the equivalents of gallic acid.

2.5 Determination of the total flavonoid content

The total flavonoid content was determined by the colorimetric method with the use of aluminium chloride. 0.5 mL of the extract were put in 10 mL volumetric flasks; next, the following were added: 0.5 mL of methanol, 4 mL of distilled water and 0.3 mL of 5% NaNO₂. The flasks were sealed and put aside for 5 minutes. After 5 minutes, 0.3 mL of 10% AlCl₃ solution was added; the content was mixed and left for 6 minutes. Then, 2 mL of 1 mol NaOH solution was added and topped up with 10 mL of distilled water. The mixture was stirred and left for 10 minutes; after this time, absorbance was measured at the wave length of 510 nm.

Next, a calibration curve of quercetin (0.08, 0.40, 0.80, 1.6, 3.2, 4.8, 6.4, 8.0 and 9.6 mg/mL) was plotted. 1mL of each solution was collected, mixed with 4 mL of distilled water; 0.3 mL of 5% NaNO₂ solution was added; the entire sample was put aside for 6 minutes and then processed as in the case of the extracts discussed above. Based on the obtained quercetin calibration curve ($y = 0.1989x + 0.0908$), the total flavonoid content in the extracts was calculated (as per quercetin). For a blank experiment, a solution was prepared in which aluminium chloride was replaced with distilled water; the remainder of the process remained unchanged [18].

2.6 TLC-DPPH Tests of obtained extracts

The antioxidant properties of obtained extracts were also examined by the TLC-DPPH test. For the stationary phase, 10x10 cm silica gel plates were used while for the mobile phase, a mixture of ethyl acetate, toluene, and formic acid at the ratio of 10:10:0.5 v/v/v. A standard solution of 0.1mg/mL quercetin was prepared. 10 µL aliquots of extracts were applied with the automatic TLC applicator Desaga AS -30. The plates were developed in one direction in horizontal chambers (DS II, Chromdes, Lublin, Poland). After drying, they were sprayed with a 0.1% DPPH solution. Next, the plates were scanned with a flat-bed scanner (Lide 50, Canon) after 0, 10, and 30 minutes. The results of TLC-DPPH tests were recorded in the form of JPG documents. For further analysis, the computer software Sorbfil TLC Videodensitometr (Sorbpolymer, Russia) [11,19] was used.

2.7 LC-ESI-MS/MS analysis of phenolic acids

Phenolic acids content was determined according to modified method described by Oniszczyk et al. [11].

Table 1: LC-ESI-MS/MS analytical results of phenolic acids investigated in samples. Compounds confirmed by comparison with authentic standards.

Compound	T _R (min)	[M-H] ⁻ (m/z)	Fragment ions (m/z)	Collision energy (eV)
Protocatechuic acid	5.9	152.9	108	- 38
			81	- 26
<i>trans</i> -Caffeic acid	6.9	178.7	134.9	- 16
			88.9	- 46
<i>cis</i> -Caffeic acid	7.4	178.7	134.9	- 16
			88.9	- 46
4-OH-benzoic acid	7.29	136.8	93	- 18
			75	- 48
Gentisic acid	7.75	152.8	108	- 36
			81	- 30
<i>p</i> -Coumaric acid	9.28	162.7	119	- 14
			93	- 44
Ferulic acid	9.9	192.8	177.9	- 12
			133.9	- 16
Isoferulic acid	10.5	192.8	177.9	- 12
			133.9	- 16
Salicylic acid	14.16	136.8	93	- 16
			75	- 48

Experiments were carried out using an Agilent 1200 Series HPLC system (Agilent Technologies, USA) connected to 3200 QTRAP Mass spectrometer (AB Sciex, USA) equipped with electrospray ionisation source (ESI). Both were controlled with Analyst 1.5 software (AB Sciex, USA), which was also used for data interpretation. Separations were carried out on a Zorbax SB-C18 column (2.1 x 100 mm, 1.8-mm particle size; Agilent Technologies, USA) at 20°C. Gradient method was used with mobile phases: water with 0.1% HCOOH (A) and acetonitrile with 0.1% HCOOH (B). Injection volume was 3 µL, the flow rate was 250 ml/min and the gradient was as follows: 0-2 min – 25%B, 3-6 min – 35%B, 8-10 min – 55%B, 12-16 min - 75%B, 19-25 min - 25%B.

ESI operated in the negative-ion mode worked at the following conditions: capillary temperature 400 °C, curtain gas at 30 psi, nebulizer gas at 50 psi, negative ionisation mode source voltage –4500 V. Triplicate injections were made for each standard solution and sample. The analytes

were identified by comparing retention time and m/z values obtained by MS and MS2 (**Table 1**) with the mass spectra from corresponding standards tested under the same conditions. The calibration curves obtained in MRM mode were used for quantification of all analytes. The identified phenolic acids were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards. Linearity ranges for calibration curves were specified. The limits of detection (LOD) and quantification (LOQ) for phenolic compounds were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Samples were purified and concentrated before LC-MS analysis using SPE technique.

2.8 Solid Phase Extraction (SPE)

Solid phase extraction was carried out using SPE system (Baker spe-12G™, J.T. Baker, Deventer, Netherlands) and columns with a chemically bonded octadecyl phase (Bakerbond C18, 500 mg). 2 ml of the extract in 50% methanol was applied to each of the conditioned columns. The desired fraction was eluted with 5 mL of 50% methanol and then with 5 mL of 80% methanol. The extracts obtained were concentrated and subjected to the LC-ESI-MS/MS analysis [11].

2.9 Statistical evaluation

Statistical analysis of data was made using the MSEXcel 2013 and Statistica 12 program (StatSoft Inc., USA). All analytical measurements were repeated six times for each sample and reference compounds. The obtained results were expressed as the mean values ± SD. The significance of the obtained results was determined at p<0.05 performing t-test for the applied methods.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

3.1 Determination of the total content of polyphenolic compounds

Extrusion-cooking is a food processing procedure that arouses a keen interest in the food industry. The action of temperature, pressure and shear forces on moist raw

Table 2: Total polyphenols and total flavonoids content.

Chokeberry content	Total polyphenols (mg GAE/mL)		Total flavonoids (mg QE/mL)	
	80 rpm screw speed	120 rpm screw speed	80 rpm screw speed	120 rpm screw speed
0	0.199 ± 0.005 ^a	0.198 ± 0.004	1.159 ± 0.011	1.157 ± 0.023
5	0.362 ± 0.018	0.346 ± 0.005	1.339 ± 0.026	1.321 ± 0.065
10	0.489 ± 0.007	0.481 ± 0.010	3.372 ± 0.033	4.100 ± 0.021
15	0.752 ± 0.008	0.725 ± 0.002	5.899 ± 0.108	5.872 ± 0.132
20	0.957 ± 0.001	0.893 ± 0.003	8.695 ± 0.089	6.935 ± 0.074

^aSD - standard deviation (n=6)

material induces profound changes in the processed matter in a very short time, among them: enhanced digestibility of nutrients, inactivation of anti-nutritive factors, modified sensory characteristics. The intensity of these changes depends both on the properties of raw material and on the settings of the extrusion-cooking proper, involving the temperature or rotational speed of the extruder screw. A high degree of mixing and homogenisation leads to a decrease in diffusion barriers and the breaking down of chemical bonds, which results in a heightened reactivity of the components. As a result, this is a source of both beneficial and non-beneficial changes in the processed material [20]. Therefore, to avoid undesirable degradation of active ingredients, including one that could occur during this process, it is important to select appropriate production parameters, among which the rotational speed of the extruder screw is key.

In the opening phase of the study, the authors compared the total content of polyphenols in porridge with the different content of chokeberry fruit (0, 5, 10, 15 and 20%) obtained at 80 and 120 rpm of the extruder screw. Polyphenol compounds are popular secondary metabolites. As mentioned above, many favourable biological effects are achieved owing to their presence in functional food products. Obtained results demonstrated that the screw speed of 80 rpm results, for all the chokeberry contents used, in a higher content of polyphenols (as per gallic acid) than the speed of 120 rpm (**Table 2**).

The broadest group of phenolic compounds are flavonoids. They have a key role in protecting the human body against various diseases, mainly due to their high antioxidant activity. Therefore, the following stage of the research focused on the influence of screw rotational speed on the total content of flavonoids (as per quercetin). It was demonstrated that, in this case, 80 rpm showed superior results compared with 120 rpm in all other examined cases (**Table 2**).

According to research done by Alonso et al. [21], the main factors stimulating the transformation of input material during the extrusion-cooking process are high temperatures and mechanical aspects related to shear forces that rise along with the increase of screw rotational speed. It is possible that production at 120 rpm is too extreme and lead to the decomposition of some phenolic compounds, including flavonoids [20]. Obtained results may be interesting for consumers as the selection of appropriate extrusion-cooking parameters helps maintain adequate nutritional, sensory and, above all, health-improving properties of the tested samples. The results of the tests clearly show that a higher level of chokeberry fruit in porridge means a higher content of polyphenols and flavonoids in the samples. The highest total content of polyphenols and flavonoids was reported in porridge a 20% addition of the chokeberry while the lowest in a sample without such functional additives.

3.2 TLC-DPPH test of obtained extracts and interpretation of results using the Sorbfil TLC Videodensitometer program

Having obtained the results discussed above, the authors decided to look at the antioxidant properties of porridge produced at the speed of 80 rpm of the extruder screw. For this purpose, a TLC was performed using a stable DPPH radical. The extracts were applied to successive plates; finally, a standard quercetin solution was added and left for development. After drying, the plates were sprayed with a DPPH solution and scanned after 0, 10, 30 minutes. Next, the obtained image was converted into numerical data in the Sorbfil TLC Videodensitometer program. For this purpose, on each scan of the chromatographic plate with the visualised area of bright spots, tacks were made corresponding to

Table 3: Results of TLC-DPPH assay showing the antiradical activity of analysed extracts in relation to the activity of 0.1 mg/mL quercetin solution (activity of quercetin solution equal to “1”).

Time	Activity in relation to quercetin (Σ of areas under the common peak/area under quercetin peak) $\pm$ SD ^a				
	Corn porridge without addition	Corn porridge with 5% addition of chokeberry	Corn porridge with 10% addition of chokeberry	Corn porridge with 15% addition of chokeberry	Corn porridge with 20% addition of chokeberry
after 0 min	2.253 $\pm$ 0.003 ^a	2.335 $\pm$ 0.023	3.193 $\pm$ 0.062	3.797 $\pm$ 0.120	4.199 $\pm$ 0.088
after 10 min	2.674 $\pm$ 0.091	3.083 $\pm$ 0.111	4.223 $\pm$ 0.106	4.995 $\pm$ 0.092	5.605 $\pm$ 0.120
after 30 min	1.169 $\pm$ 0.022	1.276 $\pm$ 0.008	2.295 $\pm$ 0.077	3.526 $\pm$ 0.041	3.685 $\pm$ 0.043

^aSD - standard deviation (n=6)

Figure 1: TLC-DPPH test results for the extracts of corn porridge of the varied chokeberry content; plate scanned after 30 min. Mobile phase: ethyl acetate, toluene, and formic acid at the ratio of 10:10:0.5 v/v/. Stationary phase: plates with silica gel 10 × 10 cm. 1- a standard solution of 0.1mg/mL quercetin; 2, 3, 4, 5, 6 - extract of porridge with 20%, 15%, 10%, 5%, 0% addition of chokeberry fruit, respectively.

the area of development of each polyphenol extract. The function of result analysis permitted a precise assessment of the colour intensity of the spots and the size of the areas under peaks (and their conversion into numerical data). The area of a standard solution of quercetin at the concentration of 0.1 mg/mL was adopted as a reference point. Its activity was marked ‘1’ [22].

The TLC-DPPH test confirmed an increase in antioxidant properties of porridge along with the growing content of chokeberry fruit (**Table 3, Figure 1**). Thus, a well visible trend is that a higher amount of functional raw material causes an increase in the content of polyphenols and flavonoids and an increase in antioxidant properties. Extracts with the largest, i.e. 20%, addition of chokeberry fruit exhibited superior antioxidant properties. The top free radicals scavenging activity by all extracts was reported at the beginning after 10 minutes of the process. According to Brand et al. [13], chokeberry fruits have a tremendous effect on the human body, largely due to their antioxidant properties attributable to the presence of polyphenol compounds, which, as demonstrated by the research results, remained active after the extrusion-cooking process. Such porridge are a good source of natural antioxidants, thus being capable of preventing certain diseases and improving the quality of health and life.

3.3 LC-ESI-MS/MS analysis of phenolic acids

At the next stage of the research, the authors performed a quantitative analysis of free phenolic acids in porridge with a 0, 5, 10, 15 and 20% addition of chokeberry fruit; the porridge were produced at the optimum rotational speed of the extruder, i.e. 80 rpm. Prior to determination, all crude extracts were purified using the SPE method. In order to assess the accuracy of the SPE method, a recovery test was held. Crude extracts were enriched with known amounts of the standard solution having three different concentrations. Next, a solid phase extraction was performed with the same methods as used for the

Table 4: Content of phenolic acids .

Phenolic acid	Yield of phenolic acids (µg/g of dry weight)				
	Corn porridge without addition	Corn porridge with 5% addition of chokeberry	Corn porridge with 10% addition of chokeberry	Corn porridge with 15% addition of chokeberry	Corn porridge with 20% addition of chokeberry
Protocatechuic	0.100	13.961	24.321	29.040	32.646
SD*	0.0013	0.2611	0.3820	0.2143	0.5212
<i>trans</i> -Caffeic	ND	0.580	0.824	1.068	1.304
SD	-	0.0005	0.0151	0.0213	0.0862
<i>cis</i> -Caffeic	BQL	BQL	BQL	BQL	BQL
SD	-	-	-	-	-
4-OH-benzoic	0.290	0.320	0.344	0.364	0.520
SD	0.0042	0.0012	0.0021	0.0045	0.0093
Gentisic	ND	ND	ND	BQL	0.036
SD	-	-	-	-	-
<i>p</i> -Coumaric	ND	3.436	3.472	3.501	3.520
SD	-	0.0452	0.0087	0.0760	0.0242
Ferulic	1.150	1.352	1.472	1.532	1.668
SD	0.0012	0.0008	0.0371	0.0521	0.0065
Isoferulic	18.430	15.120	9.120	5.560	4.920
SD	0.0324	0.4237	0.2391	0.1672	0.0883
Salicylic	0.214	0.332	0.385	0.432	0.460
SD	0.0043	0.0062	0.0088	0.0210	0.0189

*SD - standard deviation (n=3), BQL - peak detected, concentration lower than the LOQ but higher than the LOD, ND – not detected

quantification of phenolic compounds in the samples. The recovered amounts ranged from 93.7% for isoferulic acid to 102.4% for 4-hydroxybenzoic acid, which means that using the SPE for extract purification proved most effective.

The LC-MS analysis exposed a wide variety of phenolic acids present in the tested samples. In the sample with the 20% content of chokeberry fruit, nine acids were identified (Table 4, Figure 2). They derived from benzoic acid and included: protocatechuic acid, 4-OH-benzoic acid, gentiic acid, salicylic acid, and cinnamic acid derivatives: *trans*- and *cis*-caffeic, *p*-coumaric, ferulic, and isoferulic. This was the only extract where gentisic acid was determined. In the porridge without any functional additive, only five free phenolic acids were detected: protocatechuic acid, 4-OH-benzoic acid, salicylic acid, ferulic acid, and isoferulic acid. The total content of these compounds grew along with the increasing amount of chokeberry fruit in the samples and reached 18.184 µg, 35.101 µg, 39.938 µg, 41.497

µg and 45.074 µg/g of dry matter, respectively, for samples enriched with 0, 5, 10, 15 and 20% of this fruit. Also, the content of individual acids increased as more chokeberry fruit was added. We observed that porridge enriched with this functional additive become an important source of free phenolic acids, especially protocatechuic acid. Its content in samples with the addition of chokeberry fruits is very high and grows even more as more of this fruit is added. This acid plays a vital role in disease prevention, especially when we speak about diseases of affluence. Recent research have shown that it has a potential as a prevention factor in cardiovascular diseases. In the 1990s, based on the results of research on chemically induced carcinogenesis, some chemopreventive properties of this compound were reported in experimental animals. Its action is based on antioxidant properties, i.e. inhibited generation of free radicals, the ability to scavenge them and enhance the catalytic activity of endogenous enzymes taking part in the neutralisation of free radicals. Of significance is also the

Figure 2: Extracted LC-MS-MRM chromatogram of phenolic acids found in sample with 20% addition of chokeberry fruit; MRM transition are given in brackets: 1 - protocatechuic (m/z 152.9 > 107.8); 2 – *trans*-caffeic (m/z 178.7 > 134.9); 3 – *cis*-caffeic (m/z 178.7 > 134.9); 4 - 4-hydroxy-benzoic (m/z 136.9 > 93); 5 - salicylic (m/z 136.9 > 93); 6 - gentisic (m/z 152.8 > 107.9); 7 - *p*-coumaric (m/z 162.8 > 119); 8 - ferulic (m/z 192.8 > 133.9); 9 - isoferulic acid (m/z 192.8 > 133.9).

Table 5: Pearson correlation coefficient; $p < 0.05$.

	Chokeberry content	Total polyphenols	Total flavonoids	Free phenolic acid	TLC-DPPH radical scavenging capacity
Chokeberry content	1	0.981	0.964	0.900	0.991
Total polyphenols		1	0.977	0.831	0.967
Total flavonoids			1	0.754	0.970
Free phenolic acids				1	0.866
TLC-DPPH radical scavenging capacity					1

Table 6: Limit of detection (LOD), limit of quantification (LOQ) and calibration curve parameters for all analytes.

Compound	LOD [ng/μL]	LOQ [ng/μL]	r^2	Linearity range [ng/μL]
Protocatechuic acid	0.017	0.034	0.9997	0.034-3.47
<i>trans</i> -Caffeic acid	0.06	0.16	0.9990	0.175-3.5
<i>cis</i> -Caffeic acid	0.06	0.16	0.9990	0.175-3.5
4-OH-benzoic acid	0.0174	0.0347	0.9993	0.0694-3.47
Gentisic acid	0.0017	0.0033	0.9997	0.0033-0.33
<i>p</i> -Coumaric acid	0.0073	0.0181	0.9996	0.0181-1.82
Ferulic acid	0.0174	0.0347	0.9994	0.0694-11.6
Isoferulic acid	0.0172	0.686	0.9997	0.343-11.4
Salicylic acid	0.0033	0.0165	0.9989	0.0165-1.65

influence of enzymes involved in the first and the second stage of carcinogen biotransformation and, most probably, the direct blocking of the locations of specific bonds of metabolised carcinogens with a DNA molecule [23,24].

A drop in the content of isoferulic acid as more of the fruit is added to porridge is interesting. It can be explained by the fact that the matrix itself, i.e. the corn porridge free of any additives, contained more of this compound than the chokeberry.

Obtained results showed that the antioxidant activity measured in the TLC-DPPH test was positively correlated with the content of all groups of compounds identified in porridge, including with free phenolic acids. Pearson's coefficients between the addition of chokeberry fruit, the content of polyphenols, flavonoids, free phenolic acids and the antioxidant activity (measured after 10 minutes) are shown in **Table 5**. Very high and positive correlations were reported between the addition of chokeberry fruit and antioxidant properties ($r = 0.991$), between the addition of chokeberry fruit and polyphenol content ($r =$

0.981) and between antioxidant properties and the content of polyphenols ($r = 0.967$). These correlations indicate that the measured antioxidant activity of the tested porridge was mainly due to the addition of polyphenol-rich chokeberry fruit. Antioxidant properties were also closely associated with the level of flavonoids ($r = 0.970$) and free phenolic acids ($r = 0.866$). Our results are aligned with those of Madhujith et al. [25,26]. They reported that it was the level of free and not bonded and conjugated phenolic acids that significantly contributed to the antioxidant properties of the tested plant samples.

The content of free phenolic acids in the examined extracts was determined by means of calibration curves designed for each model. The limit of detection (LOD) and the limit of quantification (LOQ) were determined at the signal to noise ratio (S/N) of 3 and 10, respectively. All the tested compounds showed positive linearity. The correlation coefficients for all calibration curves were $r^2 > 0.998$. The values of LOD and LOQ as well as the linear range for all analysed compounds are shown in **Table 6**.

Our research has demonstrated that innovative porridge with the addition of chokeberry fruit can become a source of antioxidants that are so important for the human body. For this reason, they can prospectively make their way into the product range known as functional foods.

4 Conclusions

The research demonstrated that porridge manufactured in the extrusion-cooking process with the addition of chokeberry fruit, especially at the level of 20%, have a potential for becoming an important source of antioxidant polyphenolic compounds. The extrusion-cooking process at 80 rpm of the extruder screw does not degrade the tested active compounds, whose content increases along with the addition of the chokeberry to samples. Very high and positive correlations are reported between the addition of fruit and antioxidant properties ($r = 0.991$), between the addition of fruit and polyphenol content ($r = 0.981$) and between antioxidant properties and the content of polyphenolic compounds ($r = 0.967$). Antioxidant properties are also closely associated with the level of flavonoids ($r = 0.970$) and free phenolic acids ($r = 0.866$).

The presented results prove to be a major step towards making the extruded porridge obtained in our experiments a much-demanded product ranked among functional foods.

Conflict of interest: Authors declare no conflict of interest.

References

- [1] Oniszczyk A., Oniszczyk T., Wójtowicz A., Wojtunik K., Kwaśniewska A., Waksmundzka-Hajnos M., Radical scavenging activity of extruded corn porridge with addition of linden inflorescence, *Open Chem.*, 2015, 13, 1101–1107.
- [2] Arvanitoyannis I.S., Van Houwelingen-Koukaliaroglou M., Functional foods: a survey of health claims, pros and cons, and current legislation, *Crit. Rev. Food Sci. Nutr.*, 2005, 45, 385–404.
- [3] European Commission, Preparatory study on food waste across EU 27, 2002, http://ec.europa.eu/environment/eussd/pdf/bio_foodwaste_report.pdf.
- [4] D'Archivio M., Filesi C., Di Benedetto R., Gargiulo R., Giovannini C., Masella R., Polyphenols, dietary sources and bioavailability, *Ann. Ist. Super. Sanita*, 2007, 43, 348–361.
- [5] Mehta J.P., Pandya C.V., Parmar P.H., Vadia, S.H., Golakiya, B.A., Determination of flavonoids, phenolic acid and polyalcohol in *Butea monosperma* and *Hedychium coronarium* by semi-preparative HPLC Photo Diode Array (PDA) Detector, *Arab. J. Chem.*, 2014, 7, 1110–1115.
- [6] Romagnolo D.F., Selmin O.I., Flavonoids and cancer prevention: a review of the evidence, *J. Nutr. Gerontol. Geriatr.*, 2012, 31, 206–238.
- [7] Peluso I., Miglio C., Morabito G., Ioannone F., Serafini M., Flavonoids and immune function in human: a systematic review, *Crit. Rev. Food Sci. Nutr.*, 2015, 55, 383–395.
- [8] Olejnik A., Olkiewicz M., Kowalska K., Rychlik J., Dembczyński R., Mysza K., et al., Gastrointestinal digested *Sambucus nigra* L. fruit extract protects in vitro cultured human colon cells against oxidative stress, *Food Chem.*, 2016, 197, 648–657.
- [9] Jindal A., Kumar P., Antimicrobial flavonoids from *Tridax procumbens*, *Nat. Prod. Res.*, 2012, 26, 2072–2077.
- [10] Kocira A., Kocira S., Złotek U., Kornas R., Świeca M., Effects of Nano-Gro preparation applications on yield components and antioxidant properties of common bean (*Phaseolus vulgaris* L.), *Fresen. Environ. Bull.*, 2015, 24, 4034–4041.
- [11] Oniszczyk A., Olech M., Oniszczyk T., Wojtunik-Kulesza K., Wójtowicz A., Extraction methods, LC-ESI-MS/MS analysis of phenolic compounds and antiradical properties of functional food enriched with elderberry flowers or fruits, *Arab. J. Chem.*, (in press), DOI: 10.1016/j.arabjc.2016.09.003.
- [12] Bouasla A., Wójtowicz A., Zidoune M.N., Olech M., Nowak R., Mitrus M., et al., Gluten-free precooked rice-yellow pea pasta: effect of extrusion-cooking conditions on phenolic acids composition, selected properties and microstructure, *J. Food Sci.*, 2016, 81, 1070–1079.
- [13] Brand M.H., Connolly B.A., Levine L.H., Richards J.T., Shine S.M., Spencer L.E., Anthocyanins, total phenolics, ORAC and moisture content of wild and cultivated dark-fruited *Aronia* species, *Sci. Hortic.*, 2017, 224, 332–342.
- [14] Wójtowicz A., Oniszczyk A., Oniszczyk T., Kocira S., Wojtunik K., Mitrus M., et al., Application of *Moldavian dragonhead* (*Dracocephalum moldavica* L.) leaves addition as a functional component of nutritionally valuable corn snacks, *J. Food Sci. Technol.*, 2017, 54, 3218–3229.
- [15] Bhat N.A., Wani I.A., Hamdani A.M., Gani A., Effect of extrusion on the physicochemical and antioxidant properties of value added snacks from whole wheat (*Triticum aestivum* L.) flour, *Food Chem.*, 2019, 276, 22–32.
- [16] Lee S.K., Mbwapo Z.H., Chung H., Luyengi L., Gamez E.J.C., Mehta R.G., et al., Evaluation of the antioxidant potential of natural products, *Comb. Chem. High Throughput Screen.*, 1998, 1, 35–46.
- [17] Kukula-Koch W., Koch W., Angelis A., Halabalaki M., Aliannidis N., Application of pH-zone refining hydrostatic countercurrent chromatography (hCCC) for the recovery of antioxidant phenolics and the isolation of alkaloids from *Siberian barberry* herb, *Food Chem.*, 2016, 203, 394–401.
- [18] Bekbolatova E., Kukula-Koch W., Baj T., Stasiak N., Ibadullayeva G., Koch W., et al., Phenolic composition and antioxidant potential of different organs of Kazakh *Crataegus altaicaensis* Pojark: A comparison with the European *Crataegus oxyacantha* L. flowers, *Open Chem.*, 2018, 16, 415–426.
- [19] Ismail H.I., Chan K.W., Mariod A.A., Ismail M., Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts, *Food Chem.*, 2010, 119, 643–647.
- [20] Zeki Berk, Extrusion, 3rd ed., Food Process Engineering and Technology, Academic Press, Elsevier, 2018.

- [21] Alonso R., Grant G., Dewey P., Marzo F., Nutritional assessment in vitro and in vivo of raw and extruded peas (*Pisum sativum* L.), J. Agric. Food Chem., 2000, 48, 2286–2290.
- [22] Cieśla Ł., Kryszewski J., Stochmal A., Oleszek W., Waksmundzka-Hajnos M., Approach to develop a standardized TLC-DPPH test for assessing free radical scavenging properties of selected phenolic compounds, J. Pharm. Biomed. Anal., 2012, 70, 126–135.
- [23] Szumiło J., Protocatechuic acid in cancer prevention, Postepy Hig Med Dosw, 2005, 59, 608–615.
- [24] Biesaga-Kościelniak J., Kościelniak J., Filek M., Marcińska I., Krekule J., Machackova I., Kuboń., The effect of plant growth regulators and their interaction with electric current on winter wheat development, Acta Physiol Plant., 2010, 5, 987–995.
- [25] Madhujith T., Izydorczyk M., Shahidi F., Antioxidant properties of pearled barley fractions, J. Agric. Food Chem., 2006, 54, 3283–3289.
- [26] Kasprzak K., Wojtunik-Kulesza K., Oniszczyk T., Kuboń M., Oniszczyk A., Secondary metabolites, dietary fiber and conjugated fatty acids as functional food ingredients against overweight and obesity, Nat. Prod. Commun. 2018, 13, 1073–1082.