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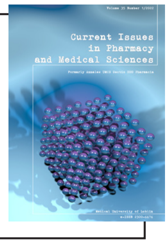

Antioxidant potential and chemical composition of new generation extruded snack pellets supplemented with fresh broccoli addition

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ABSTRACT

The therapeutic potential of broccoli has been highlighted by its function in the prevention of cancer, diabetes and other diseases. As a result, there is a great deal of interest in creating innovative functional foods supplemented with broccoli. In the extrusion-cooking process, raw materials based on potato mixes with the addition of fresh broccoli (*Brassica oleracea* L. var *italica*, *Brassicaceae*) were used to create new generation extruded snack pellets. A prototype single screw extruder-cooker with L/D=20 was utilized, and various processing conditions (screw speed 60 and 100 rpm, as well as either 32 and 36% levels of initial moisture content) were applied. Mixtures of 10% and 30% fresh broccoli contents were investigated. The final snack pellets obtained under such processing conditions were determined for their antioxidant potential, polyphenols, proximate composition and fatty acids profile. We found that in the presence of fresh broccoli in blends, higher screw speed and higher moisture content allowed obtaining (in most samples) a higher polyphenal content in the resulting pellets. Over all, the antioxidant potential of snack pellets increased with the addition of fresh broccoli. Pellets with 30% addition of the broccoli processed at screw speed 100 rpm at moisture content of 32% displayed the highest total polyphenols content and highest antioxidant activity. Moreover, such samples demonstrated notable increase in the content of total protein, crude ash, as well as MUFA and PUFA in the total amount of fatty acids.

INTRODUCTION

Today, people are becoming more and more concerned about the effects of chronic diseases such as cancer, heart disease and diabetes, and of ways of mitigation or prevention. These disorders are frequently induced by poor dietary habits, such as excessive consumption of fat, salt and sugar [1]. There is compelling evidence in the literature that taking in more vegetables and fruits lowers the risk of developing hypertension and heart disease, and reduces the incidents of stroke. There is also plausible evidence that a diet rich in vegetables and fruits is inversely connected to overall cancer risk [2,3]. Nowadays, there is a greater need for nutritious food because such functional food minimizes the risk of lifestyle disorders [4].

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The positive significance of ingesting brassica vegetables, especially broccoli (*Brassica oleracea* L. *var italica*, *Brassicaceae*), in the prevention of civilization diseases, e.g. diabetes, cardiovascular disease, and obesity, has been well demonstrated [5-7]. Broccoli's therapeutic potential has been revealed through its role in the prevention of cancer and diabetes, among other issues [8]. Furthermore, broccoli is high in vitamin C and fiber, as well as glucosinolates and flavonoids. In addition, bioactive compounds such as the isothiocyjanates (sulforaphane and indole-3-carbinol), the glucosinolates (glucorafanin and glucobrassicin), the indole derivatives (brassinin), and microelements (selenium etc.) are found in broccoli. All these biochemicals have found numerous applications in the treatment of various diseases and aliments, including cancer [6].

Novel functional foods based on or supplemented with broccoli are being developed since they contain the aforementioned crucial compounds [9-12]. For example, preliminary findings indicate that broccoli can be utilized to make a sulforaphane-rich fermented puree [13]. In other research, a unique and acceptable craft beer with broccoli addition was created, which was found to supply a valuable dietary source of sulforaphane [14]. Furthermore, a new functional soup powder high in isothiocyanates and sulforaphane has been test marketed that is derived fro the addition of broccoli florets and stems [15]. Sulforaphane via the addition of broccoli has also been incorporated in powders produced through various ways of drying, and these products possessed good antioxidant potential [16-18]. For example, a novel powdered product of very acceptable flavour, rich in bioactive and bioavailable chemicals with antioxidant and anti-inflammatory properties was created by low-temperature drying of broccoli stalks [19].

Previous research has also confirmed the utility of broccoli as a healthy ingredient in extruded snacks. However, these experiments have demonstrated that the addition of too much broccoli leads to a decrease in the physical attributes of the snacks. Moreover, process parameters were found to affect product quality [2-22]. Hence, extrusion-cooking process criterion can affect the characteristics of next generation products [23,24]. Growing customer desire for healthy snacks is critical, prompting science and industry to create new ready-to-eat enhanced products. It must be underlined, therefore, that in addition to health advantages and technological quality, customer acceptance holds paramount importance. These veggies have a distinct taste, smell and color that customers may not always tolerate; therefore, sensory tests and assessments of the other physical features of broccoli-based products are essential [25,26].

As a result, study into the production of acceptable and functional snacks including broccoli is required. Hence, it is necessary to conduct research to optimize the extrusion-cooking process and the recipe in order to obtain high-quality products with enhanced nutritional characteristics. The aim of this work was to indicate the effect of the addition of fresh broccoli on extruded pellet antioxidant activity, proximate chemical composition and fatty acid profile.

MATERIALS AND METHODS

Reagents and Chemicals

LC-MS-grade methanol, analytical-grade ethanol for extraction, and Folin–Ciocalteu reagent were purchased from J.T. Baker (Phillipsburg, PA, USA). The HPLC-grade gallic acid, Trolox and DPPH (2,2-diphenyl-1-picrylhydrazyl) were provided by Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA).

Copper II sulfate $CuSO_4$, potassium sulfate K_2SO_4, sulfuric acid (VI) H_2SO_4 96%, sodium hydroxide NaOH, boric acid H_3BO_3, hydrochloric acid 0.1N HCl, Tashiro indicator, hexane C_6H_{14}, acetone 99.5% C_3H_6O, sulfuric acid (VI) H_2SO_4 96%, sodium hydroxide NaOH were used to determine proximate compositions, while potassium hydroxide KOH, boron trifluoride BF3, hexane C_6H_{14}, sodium chloride NaCl were employed to determine fatty acid profiles.

All reagents and chemicals were provided by Alfachem Sp. z o. o. (Lublin, Poland).

Preparation of blends and extrusion-cooking process

The following raw materials were used to compose the tested blends: organically grown broccoli (*Brassica oleracea var. italica*, Monaco F1) (ANREKO Andrzej Gębka, Jakubowice Konińskie, Poland), potato starch Superior Standard (Przedsiębiorstwo Przemysłu Ziemniaczanego Bronisław S.A., Bronisław, Poland), potato flakes (Zakłady Przemysłu Ziemniaczanego w Lublinie, Poland), potato grits (Zakłady Przemysłu Ziemniaczanego w Lublinie, Poland), raffinate rapeseed oil (Zakłady Tłuszczowe ,,Kruszwica", Kruszwica, Poland), sugar and salt were purchased at a Lidl store (Lublin, Poland). Firstly, blends of dry components were prepared based on the developed recipes, the composition of which is presented in Table 1. The total weight of each recipe was 3 kg and blends were prepared in appropriate proportions depending on the amount of the vegetable additive by replacing potato starch (weight/weight) with fresh broccoli pulp.

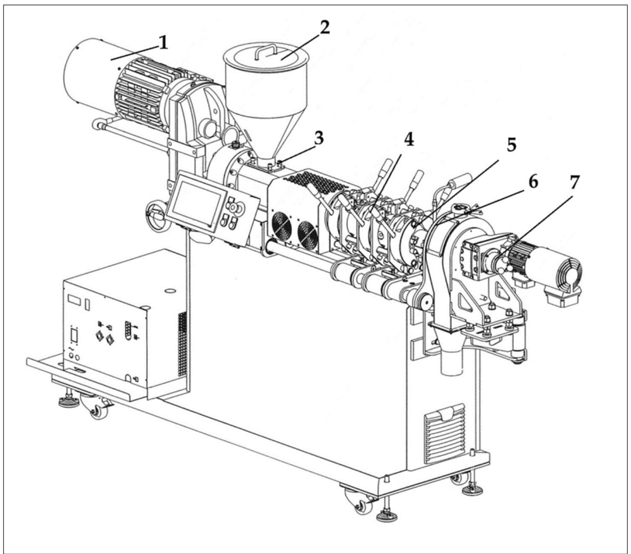
Table 1. Composition of blends for extrusion-cooking process of snack pellets with various addition of fresh broccoli

Fresh broccoli [%]	Potato starch [%]	Potato flakes [%]	Potato grits [%]	Rapeseed oil [%]	Beet sugar [%]	Table salt [%]
0	66	15.0	15.0	2	1	1
10	56	15.0	15.0	2	1	1
30	36	15.0	15.0	2	1	1

The fresh broccoli was crushed in a cup blender Germin MAX-1050-W (Germin, Berlinger, Germany). The fresh broccoli was then ground to a pulp and the resulting product was homogenized after the batch mixing so that the consistency and moisture content of the obtained pulp was uniform and constant. Mixing of fresh broccoli pulp with dry components was done with a laboratory ribbon premix mixer (Rowag, Rogoźno, Poland) until a homogenous consistency was obtained. The prepared samples were subsequently stored at a reduced temperature (in approximately 6°C) in closed containers for 24 hours to stabilize the blends and so as to ensure moisture migration between dry and moist components. These were re-mixed before the extrusion to ensure uniform moisture content. During the mixing, the appropriate amount of technological water was added to obtain definitive blend moistures of 32 and 36%. Calculation of needed water occurred after testing the moisture of the prepared samples utilizing an infrared moisture analyser (MA 50 R, Radwag, Lublin, Poland). The method described by Lisiecka and Wójtowicz [27] was applied due to differences in initial moisture content of blends when 10 or 30% of fresh broccoli pulp was added (weight/weight) (Note: the required initial moisture for snack pellets processing by extrusion must be in range of 25-36% [27]). The final blends feed to the extruder were, therefore, characterized by designed moisture content. All blends were moistened to 32% and 36% before extrusion-cooking.

The prototype single screw extruder-cooker EXP-45-35 (Zamak Mercator, Skawina, Poland) with L/D=20 was used in the tests (Figure 1).

To ensure the proper treatment of the tested recipes, an extended configuration of the barrel was applied (the screw length to diameter ratio being L/D=20). The main sections of the equipment are marked in Figure 1. Due to more intensive mixing and heating/cooling of raw materials inside the elongated barrel and appropriate selection of temperature in the various extruder zones, the tested blends were processed properly and characterized by smooth and uniform surfaces with slightly visible vegetable particles incorporated tightly in the starchy gelatinized matrix, especially with the 30% addition of fresh broccoli pulp (Figure 2). During the extrusion-cooking process, the following temperature values of each extruder-cooker sections were used: 45, 75, 90 and 95°C (extruder die).



1 – main engine, 2 – dosing unit, 3 – feeding zone, 4 – plasticizing zone, 5 – cooling zone, 6 – forming die, 7 – cutting unit (for short pellets)

Figure 1. Schematic of the prototype extruder EXP-45-35 (Zamak Mercator, Skawina, Poland)

Two screw speeds, 60 and 100 rpm, were applied in the experiment. All blends were processed through the extruder die with single flat opening (0.6 × 25 mm) into a ribbon shape with a width of approximately 25 mm. A prototype laboratory cutting machine (Konstal, Lublin, Poland) equipped with a 2-blade rotary cutting head was utilized to render the snack pellet ribbon into rectangular 25×25 mm pieces. The speeds of the feed rollers and the cutting head were adjusted to the extruder-cooker efficiency and the assumed length of the snack pellets (approx. 25 mm).

Figure 2. Snack pellets with the addition of fresh broccoli processed at 100 rpm at 36% initial moisture: from left to right – control sample, 10% of broccoli, 30% of broccoli

The new generation extrudates were placed on metal trays and positioned in a laboratory dryer to obtain a final moisture content of 8-10% (so as to obtain suitable shelf-life, snack pellets need to be dried up to max. 12% of moisture content). After drying and before testing, the snack pellets were placed in string bags (composed of a legally permitted for food contact PE supplied by Emerson Polska sp. z o.o. Sp.K., Piotrków Trybunalski, Poland) and stored in plastic boxes.

Preparation of extracts

Ultrasound-assisted extraction (UAE) via ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany) was applied to prepare the extracts. The extraction conditions were: extraction temperature 60°C, ultrasound frequency 20 kHz, ultrasound power of 100 W. Afterwards, the ground snack pellets were mixed with 80% aqueous ethanol and extracted for 60 min. (two 30-minute cycles). The extracts were filtered through a filter paper to separate the plant matter from the extract and the extraction was then repeated under the same conditions. Both portions were mixed, all of the solvent was evaporated and then methanol was added (up to 10 mL) [28].

Total Content of Polyphenolic Compounds (TPC) with Folin-Ciocalteu method

Total content of polyphenolic compounds (TPC) was measured by applying ta modified Folin-Ciocalteu (FC) method [29]. Accordingly, an appropriate amount of extract (200 mL) was mixed with distilled water (1.8 mL), after which 200 μL of FC reagent was added. The sample was then mixed and left for 5 min. In the next step, 2 mL of 7% Na_2CO_3 was added and the sample was incubated for 60 min at 40°C. Absorbance was measured at 760 nm, with the results presented as GAE (being μg gallic acid equivalents per g of dry mass (d.m.)). The study was performed in triplicate.

Free radicals scavenging activity – DPPH method

The free radicals scavenging activity of the samples was assessed by mean of application of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method (517 nm) [29]. The absorbance measurement was recorded every 5 min for 30 min. The results were presented as Trolox equivalent antioxidant activity (TEAC) calculated at 15 minutes from reaction initiation. The study was performed in triplicate.

Proximate composition

Sample contents of dry matter (method 925.09) and basic nutrients were determined according to procedures AACC [30] and AOAC [31]. The contents of fat (AACC 30–10), protein (AACC 46–10), and ash (AACC 08–01) were defined in triplicate [30]. The fiber level was obtained through applying the 993.21 method [31]. Carbohydrates were calculated as dry matter–protein–fat–ash–fiber. The study was performed in triplicate.

Fatty acid profiles

The snack pellet fatty acid composition was determined according to the AOAC method [32]. The gas chromatography technique on a Varian CP-3800 chromatograph CP-3800 (Varian Inc., Palo Alto, USA) was applied after transformation of the fats to fatty acids methyl esters (FAME). The following operating conditions of the chromatograph were established in separating out the fatty acids: capillary column (CP WAX 52CB DF) 0.25 mm of 60 m length, column temperature 120°C gradually increasing by 20°C min^{-1}, determination time – 127 min, gas carrier – helium, flow rate – 1.4 ml min^{-1}, feeder temperature – 160°C, detector temperature – 160°C and other gases: hydrogen and oxygen. All determinations were grounded on a template (Supelco 37-Component Fame Mix; Sigma-Aldrich, Poznań, Poland). The obtained results were given as the share of individual fatty acids in the total value of fatty acids, assumed as 100%. The study was performed in triplicate.

Statistical analysis – Principal component analysis (PCA)

Differences between means were tested by using ANOVA, followed by a Kruskall-Wallis post-hoc test with Statistica software (version 13.0, StatSoft Inc., Tulsa, OK, USA). Principal component analysis (PCA) and correlation determination were performed at the significance level α=0.05. Principal component analysis was applied to determine the relationship between the amount of broccoli addition, screw speed and moisture content and the physical parameters describing the obtained products. Statistica software (version 13.0, StatSoft Inc., Tulsa, OK, USA) was used for statistical analyses. The PCA data matrix for the statistical analysis of test results was composed of 14 columns and 13 rows. The input matrix was scaled automatically. The optimal number of principal components was determined on the basis of the Cattel criterion.

RESULTS AND DISCUSSION

In the course of the experiment, pellets, differed by percentage addition of broccoli (0, 10%, 30%), were produced via extrusion-cooking at various screw speeds and initial moisture contents. In the industry, dry components are preferred as additives due to easier blending and mixing, but such components are firstly heated to evaporate water from the fresh mass. In our investigation, the concept was to add fresh vegetable (as a pulp) to avoid thermal treatment during drying and to minimize the amount of technological water needed to obtain the desired initial moisture content of blend (usually 30-36% for snack pellets processing). This concept was in agreement with the work of Lisiecka and Wójtowicz [27], where the technological calculations regarding water and energy inputs were created for various snack pellet processing techniques. Thus, in the tested recipes, the use of fresh vegetable components would lead to the need to apply less thermal treatment intensity throughout the entire production chain.

Total Content of Polyphenolic Compounds (TPC) and free radical scavenging activity

Brassica oleracea group is a significant source of secondary metabolites, especially phenolic compounds. It is well known that the specific extraction technique can influence the quality of extract and content of the targeted compounds in the resulting product. Thus, the aim of our previous work was to optimise extraction method of these compounds from *Brassica oleracea* [17,28]. Recent principles indicated by green chemistry focus on the improvement of the efficiency of the extraction process and the reduction of solvent consumption. Ultrasound-assisted extraction (UAE) can offer high yield of analyzed compounds in a short time, as well as simple manipulation and reduced volume of solvent [28,29] Therefore, we decided to apply UAE in the experiment. The following parameters turned out to be the most effective for the isolation of phenolic acids: 80% aqueous ethanol and extraction time 60 min (two 30-minute cycles) at 60°C [28].

The results, presented in Table 2, revealed significant increase in polyphenolic content with increasing broccoli content. Highest total content of polyphenols was seen for pellets with 30% addition of the broccoli processed at 100 rpm screw speed at moisture content 32%, while significantly poorer results were obtained in a control sample without the plant additive extruded at 60 rpm screw speed and moisture content of 32%.

Table 2. Total phenolic content and TEAC values obtained for snack pellets supplemented with fresh broccoli (n=3; ± SD)

Fresh broccoli [%]	SS [rpm]	MC [%]	TPC [μg GAE g^{-1}]	TEAC [μg g^{-1} product]
0	60	32	19.10±0.12	73.83±3.69
		36	21.70±0.07	75.12±4.01
	100	32	23.10±0.06	74.77±2.98
		36	21.80±0.05	75.35±3.87
10	60	32	27.10±1,45	81.07±4.05
		36	32.50±1,21	112.85±6.31
	100	32	63.21±2.08	99.07±5.12
		36	67.89±1.98	151.17±7.21
30	60	32	72.81±3.25	104.21±4.99
		36	75.12±3.56	102.22±4.76
	100	32	77.20±1.56	152.80±7.54
		36	76.30±1.88	116.36±6.78

SS – screw speed; MC – moisture content; TPC – total phenolics content; TEAC – Trolox equivalent antioxidant activity; results are expressed in dry weight (d.w.)

In general, samples extruded at 100 rpm had higher TPC than those produced at 60 rpm. Moreover, a clear effect of initial moisture content on the content of polyphenols in the produced snack pellets was not evident. However, the discovered proportional increase in the total polyphenols level demonstrates that the high-temperature extrusion process did not have a destructive impact on the active compounds present in products enriched with the addition of *Brassica oleracea* var. *italica*. It should be noted that it is possible that the ingredients were not thoroughly mixed in the preparation phase (which contributed to the observed small deviations).

During the extrusion cooking, the moisture of the processed blends may have had an effect on the intensity of the thermal and mechanical treatments and thus on thermosensitive components, such as the phenolics. Higher level of raw material moistening may protect these components from loss through intensity of treatment due to the added water behaving as a lubricant, making easier and less intensive transportation of the mass inside the extruder barrel (lower shearing forces can lower destruction of heat-sensible components). A high moisture content, high degree of mixing and homogenization leads to decreasing diffusion barriers and lessens the break down of chemical bonds. This, in turn, results in heightened level and reactivity of the active components in the snack pellet product.

High moisture content and appropriate extrusion-cooking conditions (screw speed, temperature, moisture content, homogenisation) may release phenolic compounds from the chemical bonds that they create with other compounds (e.g., glycosidic bonds), without deactivating the aglycones [33]. For this reason, higher polyphenol content was recorded in our studies at higher initial moistening of processed blends. Khanal *et al.* [34] described the impact of extrusion-cooking on polyphenols composition and content in grape seed and pomace. This study demonstrated that this method can increase the levels of low-molecular-weight procyanidins and release biologically active monomers and dimers from polymer structures.

From the data presented in Table 2, it can be deduced that the high content of polyphenols in the product (especially with 30% addition of broccoli) results from the addition of *Brassica oleracea* var. *italica*. Soja *et al.* [35] confirmed that the addition of the plants from the Brassisa group (*Brassica oleracea* var. *sabellica*, kale) produced a significant increase in antioxidant activity and total phenolic content in extruded potato pellets. Herein, the range of activity was strongly dependent on the content of the kale addition. Nevertheless, even 10% of the additive led to an increase in the antioxidant ability.

According to Bisharat *et al.* [36], the phenolic content of broccoli enriched extrudates decreases with increase of feed moisture content. This variation can be explained by the fact that phenolics can be modified as their solubility and functional group properties are altered. Phenolics can also undergo decarboxylation during processing. In addition, higher moisture content presumably promotes phenolic polymerization, which affects phenol extractability and reduces antioxidant activity. Similar study results were obtained by Kasprzak *et al.* [37] for extruded corn snacks enriched with fresh kale. These results also indicated the high free-radical scavenging activity of the enriched food samples even with a minor addition of fresh kale (4, 6, and 8%).

In general, the addition of some vegetables and fruits to a base mixture raises the antioxidant properties and polyphenols level of the extruded end-product. For example, in one experiment, the addition of blackcurrant and cranberry powders to a base mixture increased the TEAC values of extruded snacks with Jerusalem artichoke (respectively, 31% and 22% for microwaved samples, and 22 % and 15 % for snacks gained after frying) [38]. Moreover, other researchers [39] have shown that the addition of fruit powders (a source of natural antioxidant compounds) inhibited oxidative changes in the fat contained in extruded snacks (the extent of oxidative changes, however, depended on the type of fruit powder).

Many studies have demonstrated that the consumption of broccoli can significantly improve human health, as well as prevent chronic diseases [2-7] These vegetables are an extraordinary source of pro-health phytochemicals, including polyphenols (flavonoids, as well as sinapic and chlorogenic acid derivatives), nitrogen-sulfur derivatives (glucosinolates), minerals (potassium, selenium, manganese), and vitamins (B6, A, C, K) [40-42]. Indeed, numerous in vitro and in vivo studies have indicated the multiple biological capacities of broccoli and broccoli sprouts (among others, antioxidant, anti-inflammatory, anticarcinogenic, antimicrobial, antidiabetic) [43-45]. Moreover, according to human-based investigations, a protective effect against a few forms of cancer and other diseases exists in consuming broccoli seedlings [46,47].

The antioxidant properties of Brassica oleracea var. italica have been comprehensively studied due to their acknowledged health-promoting effect in the human body. Therefore, studies towards free radical scavenging activity of the extracts are justified. In our work, the obtained results were presented as Trolox equivalent antioxidant activity (TEAC, Table 3). The findings confirmed that the antioxidant activity of snack pellets increases with the addition of broccoli. We particularly noted the most meaningful antiradical properties in pellets supplemented with 30% of broccoli, and produced using 100 rpm and with 32% initial moisture content.

In general, samples extruded at 100 rpm are characterized by higher TPC and TEAC values than those produced at 60 rpm. It is possible that the shear forces prevailing at 60 rpm are insufficient to break the bonds (ester, glycosidic, etc.) and release polyphenolic compounds from the bonds they form, for example, with cell wall lignins [48]. Our work did not demonstrate a clear effect of initial moisture content on the antioxidant properties of snack pellets. As reported by Bisharat *et al.* [36] who tested directly expanded extruded snacks, feed moisture content was found to decrease the antioxidant activity of broccoli enriched extrudates. Hence, antioxidant activity content may be connected with the treatment intensity induced by temperature range or moisture content during processing. Drużyńska *et al.* [49] found a higher content of polyphenolic compounds in broccoli after heat treatment, in comparison to a raw equivalent. In addition, the antioxidant capacity was significantly correlated with the total polyphenol content. This effect was mimicked in our study. According to Ellong *et al.* [50], broccoli is characterised by having a high value of antioxidant capacity (at the level of 648 μmol Trolox/ 100 g of the product) – a value higher than in the tested cereal and vegetable crisps.

Many researchers have confirmed the high TPC and TEAC values of broccoli. In a study conducted by Vega-Galvez *et al.* [51], in order to protect the bioactive ingredients, as well as to produce dried broccoli powders with high health-promoting properties, vacuum drying was used. For fresh broccoli, the total content of polyphenolic compounds was 5.38 mg of gallic acid equivalents (GAE) g^{-1} d.m. These results are very similar to those reported in the literature, namely the content of polyphenols being within the range 2.51 to 8.92 mg GAE g^{-1} d.m. [52-54]. After drying, the TPC levels were reduced and were in the range of 3.86-5.01 mg GAE g^{-1} d.m. Moreover, the antioxidant potential was also significantly lower for dried broccoli in comparison to fresh plant. The DPPH value of fresh broccoli was 23.55 µmol trolox equivalents (TE) g^{-1} d.m. and varied between 10.23 and 13.35 µmol TE g^{-1} d.m. among the dried samples. Xu *et al.* [53] presented similar results, namely, 24.06 µmol TE g^{-1} d.m. for the fresh sample. These authors also reported a decrease in the DPPH antioxidant capacity after subjecting the broccoli to drying (hot air-vacuum and microwave-vacuum methods). Based on the obtained results, it can be stated that DPPH and TPC are positively correlated (0.747). We found similar dependences in our research: the Trolox equivalent antioxidant activity of the product was positively correlated with the total content of polyphenols.

Vallejo *et al.* [55] showed large differences among four common heat treatments (microwave, conventional boiling, high pressure boiling, and steaming) in their influence on flavonoid and hydroxycinnamoyl derivative content in broccoli. Clear disadvantages were detected when broccoli was microwaved, namely high losses of flavonoids (97%), sinapic acid derivatives (74%) and caffeoyl-quinic acid derivatives (87%). Conventional boiling led to a significant loss of flavonoids (66%) from fresh raw broccoli, while high-pressure boiling caused considerable leaching (47%) of caffeoyl-quinic acid derivatives into the cooking water. On the other hand, steaming had minimal effects, in terms of loss, on both flavonoid and hydroxycinnamoyl derivative contents. Therefore they concluded that a greater quantity of phenolic compounds will be provided by consumption of steamed broccoli as compared with broccoli prepared by other cooking processes.

Proximate chemical composition

In our work, the proximate composition of the extrudates was found to differ significantly due to the varied content of the broccoli used as an ingredient for the obtaining of the new generation snack pellets. Accordingly, enhanced total protein, crude ash and fat was noted for samples with the addition of 30% fresh broccoli to base ingredient for both 60 and 100 rpm applied screw speeds (Table 3). There was also an increase in the content of raw ash and an associated probable increase in the amount of micro and macro elements [56], as well as an increase in the amount of total protein (here, ash content was 5.18-5.28 g 100 g-1 and protein content was 6.15-6.23 g 100 g-1). Both effects are desirable in the creation of food products. The visible increase in the total protein content in snacks with the highest share of broccoli undoubtedly increases their nutritional value.

The addition of broccoli as a functional ingredient to extruded snacks has many benefits, which was also confirmed in a study by Zarubin *et al.* [57], in which broccoli was one of the additives on which high-value human snacks were produced. The positive impact of adding broccoli to snacks has also been reported by other researchers [21] . However, in this case, the researchers used a lower level of broccoli addition, ranging from 4 to 10%.

Table 3. Proximate chemical composition of snack pellets with the addition of fresh broccoli

Fresh broccoli [%]	SS [rpm]	MC [%]	Component [g 100 g^{-1}]					
			Dry matter	Crude ash	Crude protein	Ether extract	Crude fibre	Carb.
0	60	32	89.94 ±0.09	3.76 ±0.03	3.64 ±0.04	0.07 ±0.01	0.31 ±0.02	82.16 ±0.11
		36	89.98 ±0.07	3.76 ±0.03	3.56 ±0.03	0.11 ±0.01	nd	82.55 ±0.08
	100	32	89.21 ±0.08	3.76 ±0.02	3.57 ±0.03	0.09 ±0.01	0.12 ±0.01	81.67 ±0.09
		36	89.14 ±0.08	3.70 ±0.03	3.46 ±0.02	0.11 ±0.02	0.04 ±0.01	81.84 ±0.09
10	60	32	87.75 ±0.09	3.79 ±0.02	4.22 ±0.04	0.08 ±0.01	0.16 ±0.04	79.49 ±0.07
		36	88.43 ±0.07	4.15 ±0.04	4.26 ±0.05	0.06 ±0.01	nd	79.96 ±0.09
	100	32	87.72 ±0.08	3.99 ±0.03	4.12 ±0.04	0.39 ±0.01	nd	79.22 ±0.08
		36	90.30 ±0.09	4.08 ±0.04	4.26 ±0.03	0.10 ±0.01	nd	81.85 ±0.09
30	60	32	91.28 ±0.08	5.27 ±0.04	6.23 ±0.06	0.12 ±0.02	nd	79.66 ±0.08
		36	91.44 ±0.09	5.18 ±0.04	6.15 ±0.06	0.09 ±0.01	nd	80.02 ±0.08
	100	32	91.38 ±0.11	5.27 ±0.04	6.15 ±0.05	0.11 ±0.01	0.27 ±0.05	79.58 ±0.08
		36	91.32 ±0.09	5.28 ±0.03	6.18 ±0.06	0.09 ±0.01	nd	79.77 ±0.08

SS – screw speed; MC – moisture content; Carb. – carbohydrates; nd – no data; results are expressed in dry weight (d.w.)

Vegetables such as broccoli are rich in protein and fibre and many phytonutrients that are health-promoting components, including polyphenols, glucosinolates and sulforaphane [58]. As demonstrated by the results of the study, the lowest protein content was observed for snack pellets without a fresh broccoli. Here, the protein content reached 3.46-3.64 g 100 g^{-1}, ash content was 3.70-3.76 g 100 g^{-1}. The addition of fresh broccoli, increased the total protein and crude ash content of the snacks to ash content 5.18-5.28 g 100 g^{-1} and protein content 6.15-6.23 g 100 g^{-1}.

The lower than expected crude fibre content of the extrudates might be caused by the degradation process of the macromolecular. This might be due to the carried out extrusion-cooking process and is connected with the applied high temperature and pressure during processing [59,60]. Interestingly and importantly, although the extrusion process may reduce the content of bioactive compounds, the same process may increase their bioavailability, which at the same time leads to an increase in the actual volume of absorbed bioactive compounds [61]. Of note, Drabińska *et al.* [62] tested the usability of broccoli by-products in durum pasta fortified with 2.5 and 5% of broccoli leaf powder. They also found an increase in ash and protein and also fat content, with slight decrease of carbohydrates content.

Although powdered broccoli was found to be a good source of protein in other work (the incorporation of broccoli has been previously reported to increase protein, fat and mineral contents in bread [63]) an insignificant increase was observed in these pasta-related experiments. This outcome can be explained by the fact that the semolina used for pasta production is itself a good source of protein. Hence, since the broccoli contribution to the pasta formulation was small in such work, it was not possible to notice its effect.

In contrast, Vázquez-Durán *et al.* [64] reported that the addition of broccoli flour significantly increased the protein (from 8.1% to 9.5%), crude fibre (from 1.9% to 3.1%), lysine (from 25.55 g·kg^{-1} protein to 35.11 g·kg^{-1} protein) and calcium contents (from 0.45 g·kg^{-1} to 0.73 g·kg^{-1}) in fortified tortilla chips. Additionally, the final oil content of tortilla chips was significantly lower (10.5%) in comparison to standard deep fat-fried products.

Fatty acid profiles

Li *et al.* [65] showed the presence of, among others, many fatty acids in various parts of broccoli, including the flowers, stems, leaves, sprouts and seeds. This parts have the potential to be developed into a range of functional food products.

The ingestion of unsaturated fatty acids positively affects cardiovascular health, via reduction of cholesterol and low-density lipoproteins in the blood [66]. In our work, MUFA and PUFA significantly increased in total amount of fatty acids which was the result of adding fresh broccoli in the amount of 30%. At the same time a simultaneous double reduction in SFA in total fatty acids was obtained, which clearly resulted in the higher nutritional value of the produced snack pellets (Table 4). Drabińska *et al.* [62] also observed that the addition of broccoli leaf powder to the pasta also resulted in a significant increase in fat content. In general, broccoli is considered a low-fat product. However, it has been reported that broccoli leaves are a rich source of polyunsaturated fatty acids, mainly α-linolenic, linoleic and palmitic acids [67]. In snack pellets supplemented with fresh broccoli, its addition increased fat content and fatty acids profile (Table 4) and unsaturated fractions were significantly higher than for control potato-based samples.

Table 4. Fatty acid profiles of snack pellets with fresh broccoli addition

Fresh broccoli [%]	10				30			
MC [%]	32		36		32		36	
SS [rpm]	60	100	60	100	60	100	60	100
C 12:0	nd	nd	nd	nd	0.761 ±0.007	nd	nd	nd
C 14:0	3.824 ±0.011	4.165 ±0.023	nd	2.115 ±0.018	1.863 ±0.016	1.353 ±0.014	nd	0.111 ±0.008
C 15:0	2.924 ±0.012	1.427 ±0.012	nd	1.788 ±0.016	nd	nd	nd	nd
C 16:0	35.549 ±0.333	32.821 ±0.298	34.414 ±0.368	28.285 ±0.242	21.021 ±0.188	13.664 ±0.134	11.912 ±0.122	11.524 ±0.108
C 16:1 n9	nd	2.706 ±0.012	nd	nd	2.633 ±0.028	nd	nd	nd
C 16:1 n-7	nd	nd	nd	2.381 ±0.016	2.500 ±0.032	6.342 ±0.048	1.062 ±0.012	6.715 ±0.042
C 17:0	1.835 ±0.008	1.405 ±0.008	nd	2.073 ±0.018	nd	0.657 ±0.006	nd	nd
C 18:0	9.472 ±0.120	12.965 ±0.162	7.992 ±0,072	7.667 ±0.046	4.321 ±0.042	5.441 ±0.038	4.490 ±0.038	5.135 ±0.048
C 18:1 n-9	27.350 ±0.225	27.934 ±0.321	29.170 ±0,262	33.303 ±0.421	39.119 ±0.368	44.303 ±0.456	52.800 ±0.522	48.221 ±0.498
C 18:2 n-6	10.524 ±0.118	8.752 ±0.088	20.915 ±0.222	14.179 ±0.152	18.870 ±0.162	17.235 ±0.177	19.548 ±0.184	18.429 ±0.192
C 18:3 n-3	7.684 ±0.077	7.167 ±0.082	7.509 ±0.077	8.209 ±0.082	8.912 ±0.084	11.005 ±0.098	10.188 ±0.088	9.865 ±0.078
C 20:0	0.838 ±0.082	0.502 ±0.006	nd	nd	nd	nd	nd	nd
C 20:1 n-9	nd	0.156 ±0.006	nd	nd	nd	nd	nd	nd
TOTAL [%]	100	100	100	100	100	100	100	100
ΣSFA [%]	54.442	53.285	42.406	41.928	27.966	21.115	16.402	16.770
ΣMUFA [%]	27.350	30.796	29.170	35.684	44.252	50.645	53.862	54.936
ΣPUFA [%]	18.208	15.919	28.424	22.388	27.782	28.240	29.736	28.294

MC – moisture content; SS – screw speed; nd – no data; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; results are expressed in dry weight (d.w.)

Table 5. Correlation matrix of the tested characteristics of snack pellets with fresh broccoli addition

	TEAC	Dry matter	Crude ash	Crude protein	Ether extract	Crude fibre	Carb.	Σ SFA	Σ MUFA	Σ PUFA
TPC	0.766*	0.607*	0.860*	0.865*	0.255	-0.269	-0.606*	0.344	0.921*	0.815*
TEAC	1.000	0.440	0.585	0.576	0.008	-0.065	-0.370	0.381	0.757*	0.759*
Dry matter		1.000	0.778*	0.726*	-0.377	0.013	0.028	-0.432	0.495	0.376
Crude ash			1.000	0.990*	-0.090	-0.121	-0.598	0.058	0.876*	0.796*
Crude protein				1.000	-0.085	-0.106	-0.662*	0.154	0.910*	0.832*
Ether extract					1.000	-0.247	-0.338	0.395	0.075	-0.020
Crude fibre						1.000	0.106	-0.282	-0.201	-0.245
Carb.							1.000	-0.665*	-0.776*	-0.780*
Σ SFA								1.000	0.490	0.590
Σ MUFA									1.000	0.957*

* Correlations significant at α=0.05

In this study, upon performing PCA analyses, 11 new variables were obtained, and the first two principal components describe 77.00% of the variability of the system. The parameters that are contained between the two red circles have the greatest impact on its volatility (Figure 3a). The correlation matrix between the tested features of snack pellets supplemented with fresh broccoli addition is presented in Table 5.

TPC content, Dry matter, Crude ash, Crude protein, ΣSFA, ΣMUFA, ΣPUFA and Carbohydrates have the greatest influence on system variability. TEAC value and Ether extract have a slightly smaller effect and Crude fiber has a weak effect. A strong positive correlation was found between TPC content, Crude ash, Crude protein, ΣMUFA and ΣPUFA. In turn, a strong negative correlation was found between ΣSFA and Carbohydrates and a slightly weaker negative correlation between TPC content, Dry matter, Crude ash, Crude protein, ΣMUFA, ΣPUFA and Carbohydrates. There was no correlation between Dry matter with Carbohydrates and ΣSFA. The PCA analysis shows that the first main component of PC1 in as much as 56.12% describes the case of using broccoli to supplement the base material (Figure 3b). Positive higher PC1 principal component values describe the results for no broccoli addition, and negative PC1 principal component values describe the results for broccoli supplementation.

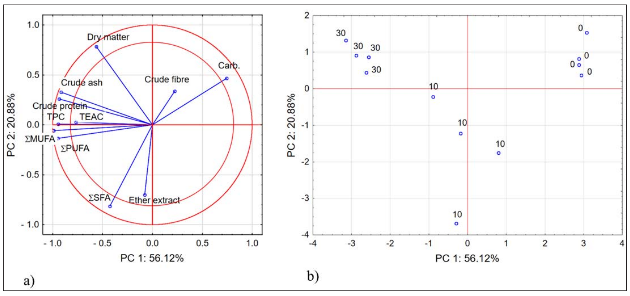


Figure 3. Loading plot (a) and score plot (b) of the principal component analysis (PC1 and PC2) carried out for addition of broccoli and tested parameters

The PCA analysis for the Screw speed and Moisture content did not show any influence on the variability of the system (Figure 4a and 4b).

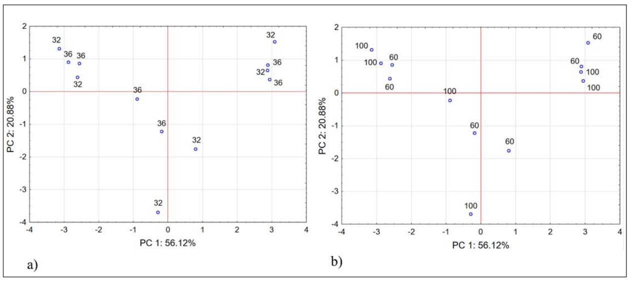


Figure 4. Score plot for Screw speed (a) and score plot for Moisture content (b) of the principal component analysis (PC1 and PC2) carried out for tested parameters

CONCLUSIONS

The addition of fresh broccoli had a significant impact on proximate composition of the new generation snack pellets. Moreover, the extrusion-cooking conditions had slight effect on chemical composition of the obtained products. In addition, fresh broccoli content at either 10 and 30% of total blend content had significant effect on snack pellets basic composition and fatty acid profiles. Furthermore, our results demonstrate that fresh broccoli addition significantly increases total polyphenols content, in comparison to the potato-based control sample. With increasing fresh broccoli content in the blend, the antioxidant activity of new generation snack pellets extracts also increased. Pellets with 30% addition of fresh broccoli processed at screw speed 100 rpm and initial moisture content 32% were found to display the highest total content of polyphenols, while the highest antioxidant activity was indicated for pellets with 30% addition of fresh broccoli processed at screw speed 100 rpm at moisture content 32%. What is more, at 36% initial moisture content, nutritional value was high. Finally, the highest values of selected chemical properties were obtained for the highest (30%) broccoli content.

Thus, these conditions can be recommended in order to obtain nutritionally valuable snack pellets with proper processing stability and appearance. It can be concluded that fresh broccoli is a vegetable component with great potential to improve the nutritional value of new generation food products and also has a positive effect on limiting the technological water requirements.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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