ORIGINAL ARTICLE



Application of Moldavian dragonhead (*Dracocephalum moldavica* L.) leaves addition as a functional component of nutritionally valuable corn snacks

Agnieszka Wójtowicz¹ · Anna Oniszczuk² · Tomasz Oniszczuk¹ · Sławomir Kocira³ · Karolina Wojtunik² · Marcin Mitrus¹ · Anna Kocira⁴ · Jarosław Widelski⁵ · Krystyna Skalicka-Woźniak⁵

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Abstract Application of Moldavian dragonhead (Dracocephalum moldavica L.) leaves in extruded snacks was evaluated. Directly expanded corn snacks (crisps) were supplemented with 5-20% of dragonhead leaves. The supplemented snacks were characterized to have improved nutritional value and were a good source of dietary fibre. The presence of phenolic compounds, especially rosmarinic acid, showed a high antioxidant potential and a radical scavenging activity of tested snacks, especially if a high content of additive was used. The increasing amount of additive also had an impact on the physical properties of extrudates lowering the expansion ratio, water absorption and solubility, yet increasing bulk density, cutting force and the breaking index of the enriched snacks. The highest viscosity was observed at 5 and 10% addition level. The increasing amount of dragonhead leaves lowered the brightness of snacks and increased the greenness tint significantly. A sensory evaluation showed good acceptability of snacks enriched with up to 15% of dragonhead dried

Agnieszka Wójtowicz agnieszka.wojtowicz@up.lublin.pl

- ¹ Department of Food Process Engineering, University of Life Sciences in Lublin, Doświadczalna 44, 20-280 Lublin, Poland
- ² Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland
- ³ Department of Machinery Exploitation and Management of Production Processes, University of Life Sciences in Lublin, Głęboka 28, 20-950 Lublin, Poland
- ⁴ Institute of Agricultural Sciences, State School of Higher Education in Chełm, Pocztowa 54, 22-100 Chełm, Poland
- ⁵ Department of Pharmacognosy with Medical Plant Unit, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

leaves. Dried leaves of the Moldavian dragonhead seem to be a prospective functional additive for extruded crisps with a high nutritional value, especially because of dietary fibre and rosmarinic acid content, a strong antioxidant potential and acceptable sensory properties.

Keywords *Dracocephalum moldavica* · Extrusioncooking · Snacks · Nutritional characteristics · Antioxidant activity · Texture

Introduction

Extrusion-cooking is a popular food processing technique, classified as HTST high temperature-short time process, applicable to the production of a wide range of food and feed products. The thermal and mechanical treatment during the extrusion-cooking may influence starch gelatinization, protein denaturation and inactivation of enzymes, anti-nutritional factors, and microbes. It is one of effective processing methods useful for the transformation of raw materials and/or by-products into nutritionally valuable foodstuffs, due to its versatility, high productivity, relatively low costs, energy efficiency and the propensity to develop functional properties (Altan et al. 2008; Thakur et al. 2017; Wójtowicz et al. 2015). Extruded corn snacks are very popular as a source of gluten-free carbohydrates because of their specific texture and convenience of use, especially for consumers on a celiac disease diet (Wójtowicz et al. 2013).

Nowadays, consumers are looking for functional food, both tasty and having an effect on the natural body resistance; enhancing prevention or being supportive in chronic disease therapies; offering greater physical efficiency and having a favourable influence on the mental condition of the organism. The research literature on the subject shows the application of plant extracts as a source of natural antioxidants in food products. However, not only extracts, but whole plants, leaves or flowers are nutritionally valuable and could be used for food supplementation, especially as a source of flavonoids and phenolic acids (Oniszczuk et al. 2015a, b). Some previous studies showed that some herb extracts, such as rosemary, oregano or drumstick, could be good sources of natural antioxidants, phenolic compounds, especially flavonoids, in food formulations, cosmetics, pharmaceuticals and other applications (Krishnaiah et al. 2011; Molehin et al. 2014). The consumption of such products may enrich the innate antioxidative status of living cells and thus protect them against damage caused by free radicals (Shukla et al. 1997).

The Moldavian dragonhead (Dracocephalum moldavica L., Lamiaceae) is an annual, herbaceous plant with unique nutritional characteristics. It is a good source of protein, lipids and fibre. Dragonhead seeds are useful for extraction of oil rich in omega-3 fatty acids. It has been confirmed as a good source of linolenic acid (59.4% in seeds after processing to obtain volatile oils) (Domokos et al. 1994). There are some reports showing the application of Dracocephalum moldavica as tea and a herbal drug used to treat stomach and liver disorders, headaches and congestion, and it would also be used in folk medicine for the treatment of coronary heart disorders and hypertension (Yang et al. 2014). The level of essential oils in Dragonhead herbs reaches a value from 0.008 to 0.8% dry wt. The antioxidant activity of this species has been studied by some researchers (Povilaityté et al. 2001). Dastmalchi et al. (2007) suggested that the components responsible for the dragonhead activity were hydroxycinnamic acids and flavonoids, including caffeic acid, ferulic acid, rosmarinic acid, luteolin, luteolin-7-O-glucoside and apigenin. Sultan et al. (2008) found kaempferol, chrysoeriol, and quercetin 3-*O*-[α -L-rhamnopyranosyl(1 \rightarrow 6)]- β -D-glucopyranoside. The presence of terpenoids, flavonoids, alkaloids, lignans, phenols, coumarins, and cyanogenic glucosides has been reported. 31 compounds were isolated from D. moldavica extract, including flavonoids, flavonols and flavonoids glycosides. 13 phenylpropanoids compounds were also found, including phenylpropionic acid, lignans and coumarins (Zeng et al. 2010). In the dried whole plant identified were such flavonoids as apigenin, luteolin, kaempferol, isorhamnetin, tilianin, agastachoside, acacetin-glycoside, and syringaresinol. In dried leaves, apigenin and rosmarinic acid were found (Kakasy et al. 2006). It was observed that the Moldavian dragonhead extract has an antibacterial property and showed some antioxidant and cardioprotective effects (Jiang et al. 2014). However, no information about the characteristics of food products supplemented with the leaves of this plant is available.

The aim of this work was to evaluate the influence of addition of Moldavian dragonhead (*Dracocephalum mol-davica* L.) dried leaves on the nutritional characteristics and selected physical properties of a new type of directly expanded savoury corn snacks (crisps) made with extrusion-cooking. Also evaluated were the proximate chemical composition, selected phenolic acids and antioxidant activity as well as the pasting properties, expansion, water absorption, solubility, texture, colour, and the sensory attractiveness of supplemented corn snacks.

Materials and methods

Corn grit (distributor Vegetus, Lubartów, Poland) was used as the base raw material. The proximate chemical composition of corn grits was as follows: moisture content 11.8 g/100 g, protein content 9.20 g/100 g, fat content 1.66 g/100 g, ash content 0.55 g/100 g, and dietary fibre content 4.42 g/100 g.

The Moldavian dragonhead (Dracocephalum moldavica L., Lamiaceae) grown in Eastern Poland (the Lublin region) in 2014 was selected and its leaves were used in the experiment. The plant was grown on an area of 0.5 ha in Perespa (50°66'N; 23°63'E), Poland. The soil type was characterized as Brown rendzina belonging to the Rendzinas soil group. The soil was alkaline (pH in 1 M KCl was around 7.4-7.5) and rich in phosphorus, potassium, and magnesium. Tillage for the Moldavian dragonhead followed best agricultural practices. The mineral fertilization in kg of nutrient per hectare was as follows: 50 kg N/ha, 60 kg P/ha, 90 kg K/ha. No pesticides were used (the pests did not exceed the thresholds of harmfulness). Moldavian dragonhead seeds were sown in the first 10-day period of May by seed drill at a depth of 1-1.5 cm, with the spacing of 45×15 cm. The average temperature was 13.7-20.3 °C (from May to July), rainfalls from 67 to 208 mm. It spanned the period from sowing to the collection of the Dracocephalum moldavica. For drying, the plants were cut off in BBCH 61 (beginning of the flowering phase) from randomly selected 10 sites of the whole crop area. After harvesting, dragonhead leaves were cleaned and dried in natural conditions in a ventilated place protected against atmospheric precipitation and sunshine. In order to standardize the sample, dried leaves were mixed. The proximate composition of dried D. moldavica L. leaves was as follows: moisture content 9.09 g/100 g, protein content 25.57 g/100 g, fat content 1.32 g/100 g, ash content 12.86 g/100 g, and dietary fibre content 40.09 g/100 g. Dried leaves were ground in the laboratory grinder (TestChem, Poland) to a fine powder (below 300 µm) and were applied in the amounts ranging from 5 to 20% (w/w) of corn grits, moistened up to 15 g/100 g and mixed.

Snacks processing

Snacks supplemented with dragonhead leaves were manufactured using single screw extruder-cooker type TS-45 (ZMCh Metalchem, Gliwice, Poland) with the length of barrel to screw diameter ratio L:D = 12:1, the screw compression ratio was 3:1. The barrel zone temperatures were set at 125–145–135 °C throughout the experiment, and the screw speed was set at 120 rpm. Ready-to-eat snacks (crisps) were shaped with a single circular open die of 3 mm and cut directly after exiting the forming die with a rotary cutting knife (Wójtowicz et al. 2013). After cooling (moisture content of the extrudates was around 5 g/ 100 g), snacks were ground down to below 300 µm, if necessary, and stored in polyethylene bags at room temperature before the tests.

Chemical analysis

Proximate composition

The proximate chemical composition of plant materials and snacks enriched with Moldavian dragonhead leaves was analyzed according to AACC procedures (1995). The protein content was determined by the Kjeldahl method (AACC 46-10), fat content with the Soxhlet apparatus with n-hexane extraction (AACC 30-10) and ash content (AACC 08-01) by incinerating in a muffle furnace at 600 °C, in three replications. Fibre was determined with the enzymatic-gravimetric method for total dietary fibre (TDF), and soluble (SDF) and insoluble (NDF) fibre fractions were evaluated according to AOAC 991.43 (1995). The measurements were performed in duplicate. The caloric value was calculated based on the proximate composition according to Atwater's equivalent calculations of 4.0 kcal for 1 g of protein, 9.0 kcal for 1 g of fat and 4.0 kcal for 1 g of carbohydrates (Novotny et al. 2012).

Sample preparation

Extraction for the determination of phenolics and the evaluation of antioxidant activity was carried out for ground raw materials and corn snacks enriched with the leaves of the *D. moldavica*. The ultrasound assisted solvent extraction was performed 3-times as follows: 2 g of each sample were mixed with 40 mL of methanol and extracted in an ultrasonic bath (frequency 20 kHz, power 100 W) for 30 min at 60 °C (Oniszczuk et al. 2014). The extracts were filtered, combined, and evaporated to dryness. The residues were dissolved in 10 mL of methanol. The whole

procedure was repeated three times for each sample. The extracts were then filtered through a 0.45 μ m nylon syringe filter (Millex-HN, Ireland) before the chromatographic analysis.

Determination of functional components by HPLC/DAD

The HPLC analysis was performed using Shimadzu highperformance liquid chromatography (HPLC) system (Shimadzu, Japan) equipped with an automatic degasser (DGU-20A 3R), a quaternary pump (LC-20AD), an autosampler (SIL-20A HT) and a DAD diode-array detector (SPD-M20A). A Zorbax Eclipse XDB C₁₈ (Agilent) $(250 \text{ mm} \times 4.6 \text{ mm}, 5 \text{ }\mu\text{m})$ column was used. The following conditions were applied: temperature 20 °C, the flow rate of mobile phase 1.0 mL/min, injection volume 20 µL. The LC pumps, autosampler, column oven, and DAD were monitored and controlled by the LabSolutions 5.51 software (Shimadzu, Japan). The HPLC quantitative analysis was performed according to a previously published method (Skalicka-Woźniak and Głowniak 2008). The mobile phase components were acetonitrile (B) and water with 1% acetic acid (A) in a stepwise gradient as follows: 0 min-10% B in A, 10 min-10% B in A, 15 min-20% B in A, 30 min-30% B in A, 35 and 40 min-100% B. Peak identification was achieved by comparison of both the retention time and UV absorption spectrum with those obtained for the individual standards. The quantitative determination was performed using the wave length 254 and 320 nm. Each calibration curve was analyzed by three separate measurements with five different concentrations.

In order to establish the linear ranges, the determination coefficients (R^2) were considered. Methanol used for HPLC was of the chromatographic grade (J.T. Baker Inc., Netherlands). Water was purified using the SimplicityTM system (Millipore, Molsheim, France).

Antioxidant activity

Free radical-scavenging activity

The radical-scavenging activity of extracts from the tested snacks was determined spectrophotometrically against DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Kedare and Singh 2011; Marteau et al. 2013). The concentration of DPPH used for the experiment was 0.1 mM (4 mg of the free radical and 100 mL of methanol). The solution was prepared immediately before measurement. The measurement of the reference sample of the DPPH solution was prepared by mixing 2.0 mL of the solution and 1.0 mL of methanol. The measurement of snacks extracts was done after mixing 2.0 mL of the DPPH solution and 1.0 mL of the extracts. The measurement was based on absorbance

changes which corresponded to the free radical scavenging activity of the extracts. Each measurement was repeated three times at the wave length 517 nm at room temperature. The final result was the average of three replicates. The antioxidant activity was calculated with the following formula (Oniszczuk et al. 2015a):

%DPPH radical scavenging ability
$$= \frac{A_0 - A_1}{A_0} \times 100 \, [\%]$$
(1)

where A_0 —absorbance of the reference sample, A_1 —absorbance of the sample with tested extracts.

The TLC-DPPH test

The thin layer chromatography test was used for screening the antioxidant activity of the extracts and for the analysis of active compounds in prepared extracts. The first part was based on dot-blot test (Kedare and Singh 2011; Marteau et al. 2013). Selected active components were evaluated in the extracts of snacks enriched with dragonhead leaves. The material that gave positive results in this test was investigated for further research. The determination of active components in extracts and its antioxidant activity with reference to standard substance (rutin) was performed. The prepared extracts and rutin in concentration 0.5 mg/ mL were applied to HPTLC plates with the applicator Desaga S-30. The samples (10 µL of aliquots extracts) were applied spot-wise, with an 18 mm distance from the low left edge of the plate, and a distance of 8 mm between them. Silica gel was used as a solid phase and the optimized mixture of acetonitrile:water:chloroform:formic acid (60:10:10:5, v/v/v/v) was applied as a mobile phase. The plates were developed in vertical chambers pre-saturated for 5-30 min. After development, the plates were dried for 20 min. The TLC plates were immersed for 5 s in 0.1% (w/ v) DPPH-methanol solution and dried in the dark for 5 min. Afterwards, the plates were scanned every 5 min for half-hour. The test was repeated three times. The results of TLC-DPPH tests were documented by flat-bed scanning, saved in the form of JPG documents at a resolution of 40 pixels per cm. Sorbfil TLC Videodensitometer (Sorbpolymer, Russia) was used for image analysis. A suitable width of each track line was set and the evaluation of the chosen track to measure peak area was performed by the Process Track command. The software evaluated a band in each track on a TLC image on the assumption that the size and the intensity of a bright spot depended on the quantity of a substance in the band. In order to change the video scan images into chromatograms, a rectangular selection tool was used to outline the tracks. R_f (Retention Value) as a retardation factor and peak area were measured. The total areas under the peaks obtained for snacks extracts were compared with the area of peak obtained for rutin as a reference standard (Oniszczuk et al. 2015b).

Pasting properties

The pasting profile of corn snacks enriched with Moldavian dragonhead leaves was carried out in duplicate by using a Micro-Visco-Amylo-Graph (Brabender OHG, Duisburg, Germany). A ground sample (10 g) was dispersed in 100 mL of distilled water. The pasting properties were evaluated under constant conditions of measuring range (speed: 250 rpm; sensitivity: 235 cmg) by using the following time and temperature profile: heating from 30 °C up to 93 °C; holding at 93 °C for 5 min; cooling from 93 to 50 °C; holding at 50 °C for 1 min (Bouasla et al. 2016). The heating and cooling phases were carried out with a temperature gradient of 7.5 °C/min. The parameters of pasting properties were determined using the software provided with the instrument (Brabender Viscograph, version 4.1.1). The following indices were taken from the resulting curves: the beginning of gelatinization (BG, initial increase in viscosity); gelatinization temperature (GT, temperature at which the initial increase in viscosity occurs); peak viscosity (PV, maximum paste viscosity achieved during the heating cycle); breakdown (BD, index of viscosity decrease during the first holding period, corresponding to the peak viscosity minus the viscosity after the holding period at 93 °C); setback (SB, index of viscosity increase during the cooling cycle, corresponding to the difference between the final viscosity at 50 °C and the viscosity after the holding period at 93 °C); final viscosity (FV, paste viscosity achieved at the end of the cooling cycle). Results were the average of three replicates.

Quality characteristics

Physical properties

The expansion ratio (ER) index of snacks was calculated as the ratio of extrudates diameter to the diameter of the forming die measured with electronic calliper in 10 replications (Wójtowicz et al. 2013). The extrudates bulk density (BD) was calculated as the weight of extrudates divided by the equivalent volume of extrudates. The volume of extrudates was determined by substituting sand weight for extrudates volume and dividing it by sand density. Density measurements were reported as an average of ten replications.

Texture

The texture of snacks enriched with dragonhead leaves was evaluated with the universal testing machine Zwick BDO- FB0.5TH (Zwick GmbH & Co., Germany). Cutting force (F_{max}) was tested with a Warner-Bratzler steel blade 3 mm thick and 60 mm long, double-face truncated at an angle 45°. Test head speed was 500 mm/min. A force–displacement curves were recorded and analysed with *testXpertII*[®]v3.3 based on the data of 10 replications (Wójtowicz et al. 2013). The breaking index (BI) was calculated as the ratio of F_{max} to the diameter of the snacks.

Water absorption index (WAI), water solubility index (WSI)

WAI and WSI were evaluated according to method presented by Wójtowicz and Mościcki (2014). In brief, the ground extrudates in the amount of 0.7 g were suspended in 7 mL of distilled water at room temperature and mixed in plastic tubes. After 10 min of hydration, test tubes were closed and centrifuged for 10 min at 15,000 rpm (12,500×g) (Centrifuge T24, Germany). After centrifugation, the supernatant was removed and WAI was calculated as the weight of gel obtained per gram of dry ground sample. Supernatant was dried in an air oven at 105 °C to constant weight, and WSI was calculated as the percentage of dry matter recovered after evaporation of the original weight of the sample on dry basis. WAI and WSI determinations were replicated in triple.

Colour profile

The colour of snacks enriched with dragonhead leaves was evaluated using Colour and Appearance Measurements System Lovibond CAM-System 500 (The Tintometer Ltd., UK). The CIE-Lab scale was used for the evaluation of L^* for brightness, a^* for (+) redness—(-) greenness and b^* for (+) yellowness—(-) blueness, accordingly. ΔE was calculated as the total colour change index (Wójtowicz et al. 2013). Measurements were performed in 20 replications for each sample.

Acceptability

Consumer's acceptability of each sample was evaluated in relation to the sensory preferences of 15-member semi-trained panel (8 women, 7 men) who assessed the snacks with a 9-point hedonic rating scale. The panel members were instructed for acceptability evaluation according to the used rating method. For the evaluation of acceptability, the following descriptions were used for each number: 1—dislike extremely, 2—dislike very much, 3—dislike moderately, 4—dislike slightly, 5 neither like nor dislike, 6—like slightly, 7—like moderately, 8—like very much, 9—like extremely (Kaur et al. 2015). Snacks were considered acceptable if the score for acceptability was above 5.

Statistical analyses

Statistical analysis was conducted using the Statistica software version 10 (StatSoft, Poland). The data was recorded as the mean \pm SD. The results were statistically evaluated using the ANOVA analysis of variance with a level of significance set at 0.05 and 0.01. The statistically different data were compared by the LSD test for homogenous groups. A correlation matrix was built for the comparison of all the tested characteristics using linear regression.

Results and discussion

Chemical composition

The chemical composition of snacks enriched with Moldavian dragonhead leaves is presented in Table 1. The protein content of the supplemented products increased significantly (p < 0.01; r = 0.995) from 8.1 g/100 g for corn snacks up to 10.69 g/100 g for products with 20% of added leaves. Fat content did not differ significantly (p < 0.05) and was very low in enriched snacks, in the range of 0.12-0.13 g/100 g, which also resulted in a low caloric value of corn based snacks. The lower values of fat content in the processed snacks compared to raw materials composition may be the result of the formation of starchlipid complexes during the extrusion-cooking and thus reducing lipids extractability (Wójtowicz and Mościcki 2014). Ash content almost doubled when compared to corn snacks and extrudates with the highest amount of the additive. Therefore, snacks enriched with dried D. moldavica leaves are a good source of mineral components. The addition of dried dragonhead leaves to snacks recipe increased a total dietary fibre content significantly (p < 0.05; r = 0.940), especially with a high amount of the additive in the recipe. Dietary fibre content did not differ significantly for raw corn grits and processed corn snacks, whereas an increase in the fibre content in enriched snacks was of 250% compared to corn snacks. So, these products may be classified as rich-in-fibre snacks, compared to corn extrudates. The ratio of soluble SDF and non-soluble NDF fibre fractions was almost constant. Snacks processed with addition of dried leaves showed a significantly (p < 0.05; r = 0.914) higher amount of non-soluble fibres in all tested samples. These fractions have a positive effect on the improvement of peristaltic movement in the gastrointestinal tract and are responsible for the lowering of the number of digestive components and limiting calories assimilation. Soluble fibre fractions are crucial for lowering a blood cholesterol level and glycemic index (Brennan 2005) and a significant (p < 0.01; r = 0.961) increase of the soluble

Table 1 Chemical composition and caloric value of corn snacks enriched with addition of Dracocephalum moldavica

Additive level (%)	Moisture content (g/100 g)	Protein (g/100 g)	Fat (g/100 g)	Ash (g/100 g)	SDF (g/100 g)	NDF (g/100 g)	TDF (g/100 g)	Caloric value (kcal/kJ per 100 g)
0	$9.01^{a}\pm0.02$	$8.10^{\rm a}\pm0.00$	$0.12^{a}\pm0.01$	$2.04^{a}\pm0.02$	$1.84^{a}\pm0.12$	$2.40^{\rm a}\pm 0.04$	$4.24^{a}\pm0.08$	348/1477 ^a
5	$9.19^{a}\pm0.01$	$8.93^{ab}\pm0.04$	$0.13^{a}\pm0.02$	$2.24^{ab}\pm0.01$	$3.35^{\text{b}}\pm0.03$	$4.33^{\mathrm{b}}\pm0.03$	$7.68^{\rm b}\pm0.03$	344/1456 ^a
10	$9.31^{a}\pm0.02$	$9.50^{b} \pm 0.01$	$0.12^{\rm a}\pm0.02$	$2.34^{ab}\pm0.03$	$3.66^{\text{b}}\pm0.24$	$4.95^{\rm bc} \pm 0.23$	$8.61^{bc}\pm0.23$	338/1431 ^{ab}
15	$8.97^{\rm a}\pm0.02$	$10.02^{\rm bc} \pm 0.08$	$0.12^{\rm a}\pm0.03$	$2.76^{\rm b}\pm0.01$	$4.02^{\rm c}\pm0.08$	$4.80^{\rm bc}\pm0.22$	$8.82^{bc}\pm0.15$	336/1424 ^{ab}
20	$8.99^{a}\pm0.02$	$10.69^{\rm c} \pm 0.01$	$0.13^a\pm0.03$	$3.67^{\rm c}\pm0.02$	$5.02^{d}\pm0.15$	$5.91^{\rm c}\pm0.40$	$10.93^{\rm c}\pm0.27$	328/1389 ^b

Mean values (n = 3) \pm SD

SDF soluble dietary fibre, NDF not soluble dietary fibre, TDF total dietary fibre

^{a,b,c,d} Different letters in columns show significant differences at p < 0.05

fibre fraction in enriched snacks with a higher amount of dragonhead was observed. Previous results showed that the concentrations of TDF did not change greatly as a result of extrusion-cooking processing, and the values of NDF were lower for extruded ingredients compared to unprocessed materials, whereas soluble fibre concentrations were higher. It was observed that in extruded cereals the conversion of insoluble hemicelluloses into soluble fibre fractions occurred, and an increased soluble fibre concentration was noted (Dust et al. 2004).

Nutritional value of snacks

The presence of both phenolic acids and flavonoids could be an indicators of the nutritional value of food products or plants and spices. Phenolics are secondary metabolites in plants and are linked to several health benefits, including antioxidant, antibacterial, antiglycemic, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory properties (Osakabe et al. 2004). Moldavian dragonhead dried leaves haven't been used before as an alternative source of phenolic compounds for food supplementation. Corn snacks supplemented with dragonhead leaves were characterized by an improved nutritional value and a high antioxidant activity. Extruded snacks with a higher content of Moldavian dragonhead leaves addition showed a significantly (p < 0.05; r = 0.900) higher level of rosmarinic acid (Table 2), one of the most important active components with a high antioxidant potential. The amount of detected rosmarinic acid was very low in corn snacks, whereas snacks enriched with D. moldavica leaves revealed values ranging from 2.87 mg/g if 5% of the additive was applied up to 10 times more (28.84 mg/g) in snacks with 20% of Moldavian dragonhead in the recipe (Table 2). These levels of rosmarinic acids may have enhancing pro-health benefits as an effect of enriched snacks consumption. Rosmarinic acids have been found in many medicinal

plants including basil, sage, rosemary, mint and others (Lamaison et al. 1990; Osakabe et al. 2004). Povilaityté et al. (2001) have found rosmarinic acid in the dragonhead for the first time and characterized it as the major antioxidant constituent. Dastmalchi et al. (2007) reported the total phenolic content of the *Moldavian balm* was 47.659 g/ 100 g (the sum of individual extracts) evaluated with HPLC with rosmarinic acid as the most abundant component identified (24.795 g/100 g). In the case of caffeic acid, the increasing amount of the additive significantly improved (p < 0.05; r = 0.944) the amount of this compound, and twice as much caffeic acid was evaluated for snacks with 20% of the additive compared to the application of 5% of dried leaves. In the tested corn snacks, caffeic acid was not detected.

It could be noted that the extrusion-cooking treatment did not destroy detected phenolics. Heat treatment can have a deleterious effect on the micronutrient content of vegetables, but at the same time the bioavailability of some nutrients can increase. Sahlin et al. (2004) reported the reduction in the total phenolic contents, expressed as gallic acid equivalent, after boiling and frying of tomatoes when compared to the respective raw materials, but, after baking, the total phenolics amount was slightly higher. The processing of cereals with the thermoplastic extrusion-cooking may release phenolics bounds due to the breaking of conjugated moieties. On the other hand, processing temperature did not fallow any general trend on phenolics content in corn extrudates (Thakur et al. 2017). Bouasla et al. (2016) found that the conditions of extrusion have various effects on the nutritional quality of gluten-free rice-yellow pea pasta products and the application of low screw speed during processing resulted in a higher amount of phenolic acids after treatment than in the raw materials used. This phenomenon may be explained by the fact that phenolic acids in plants are bound to cell wall components, and such complexes are difficult to break down (Inglett et al. 2010).

Additive level (%)	Active component		Antioxidant activity in relation to rutin solution ^e		
	Rosmarinic acid (mg/g)	Caffeic acid (mg/g)	After 5 min	After 30 min	
0	$0.07^{\rm a}\pm 0.00001$	nd	0.14 ^a	0.57 ^a	
5	$2.87^{ab} \pm 0.0014$	$0.23^{\rm a} \pm 0.0004$	1.80 ^{ab}	2.03 ^b	
10	$4.97^{\rm b} \pm 0.0081$	$0.30^{\rm ab}\pm 0.0006$	2.84 ^b	3.17 ^{bc}	
15	$10.79^{\rm c} \pm 0.0043$	$0.41^{\rm b} \pm 0.0003$	4.82 ^{bc}	4.96 ^c	
20	$28.84^{d} \pm 0.0055$	$0.60^{\rm c} \pm 0.0001$	6.22 ^c	7.79 ^d	

 Table 2
 Content of selected phenolic acids and antioxidant activity of extracts of corn extrudates with addition of Moldavian dragonhead leaves measured as area under the common peak/area under rutin peak

Mean values (n = 3) \pm SD

nd not detected

^{a,b,c,d} Different letters in columns show significant differences at p < 0.05

^e Rutin concentration 0.5 mg mL⁻¹, activity as 1.0; measured as area under the common peak/area under rutin peak

Friction generated by the screw speed can release phenolic compounds from complexes during the extrusion-cooking. Acosta-Estrada et al. (2014) found in extruded whole maize and chickpea flours an increased antioxidant activity of free phenolic extracts while bound phenolics decreased in comparison with the unprocessed samples. Thakur et al. (2017) reported the presence in extruded normal and waxy corn various level of base-hydrolyzed bound phenolics as gallic acid, protocatechuic acid, p-coumaric acid, ferulic acid, sinapic acid and luteolin, whereas acid-hydrolyzed bound catechin and p-coumaric acid present in raw materials were not detected in extrudates. They also observed that hydrolysis method had an effect on some phenolics level; acid-hydrolysed bound gallic acid was significantly higher than base-hydrolysed, but base-hydrolyzed bound ferulic acid was higher than acid-hydrolyzed observed in all corn type extrudates. The findings of the DPPH', TLC-DPPH⁻ and HPLC analyses, presented by Oniszczuk et al. (2016), demonstrated that cichoric acid and other polyphenols susceptible to degradation (e.g. caftaric and caffeic acids, and echinacoside) do not deactivate during processing of fish feed supplemented with Echinacea purpurea and, thus, indicated that the HTST high temperature-short time extrusion-cooking used to produce extrudates does not have an effect on the deactivation of the antioxidant compounds present in raw E. purpurea.

Antioxidant activity

Polyphenols are amongst the most desirable phytochemicals due to their antioxidant activity. These substances, showcased as flagship antioxidants, play a significant role in the food industry as natural supplements capable of scavenging free radicals. Detailed analyses of commonly used spices revealed a high content of polyphenols that influence not only the flavour advantages or durability of food but are also a rich source of natural antioxidants (Durazzo et al. 2017; Pérez-Jiménez et al. 2010). A further discussion on the influence of natural antioxidants, including the commonly used addition of fruits and vegetables to food, should be considered. Is is commonly known that the aforementioned groups are an inexhaustible source of natural substances displaying various activities. Some studies have demonstrated that phytochemicals in fruits and leafy vegetables can have biologic effects, including the scavenging of oxidative agents, boosting the immune system, regulation the gene expression in cell proliferation and apoptosis, hormone metabolism, and antibacterial and antiviral properties (Björkman et al. 2011; Kennedy and Wightman 2011). Consumption of some plants may enrich the innate antioxidative status of living cells and, thus, protect them against damage caused by free radicals (Shukla et al. 1997). In the study presented by Molehin et al. (2014), the actions of extracts against ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] and DPPH indicate that vegetable species are good scavengers of radicals. Oniszczuk et al. (2015a) presented results of a radical scavenging activity of extruded instant gruels enriched with linden inflorescence, and they confirmed a high antioxidant potential of extrudates processed in similar conditions. Extruded whole maize and chickpea flours showed an increased antioxidant activity of free phenolic extracts, while bound phenolics decreased compared with the unprocessed samples (Acosta-Estrada et al. 2014). Taking into account the growing interest in functional food, the influence of dragonhead addition on the antioxidant activity of snacks was evaluated.

This study have tested the antioxidant activity of the extracts from enriched snacks as compared to a rutin solution with the concentration of 0.5 mg/mL, with an

activity taken as 1. measured as an area under the common peak/area under rutin peak. It should be mentioned that the antioxidant capacity of vegetable species, as typified by their free radical-scavenging and reductive abilities in relation to their phenolics composition, may indicate their potential roles in disease prevention and health promotion. One direction development was applied during the HPTLC assessment of extracts both for dragonhead leaves and enriched snacks with different levels of additive. The pure Moldavian dragonhead leaves extract showed a high antioxidant activity (11.536-14.096 after 5 and 30 min of test, respectively). The control sample of corn snack was not evaluated because its very limited activity was confirmed previously. The application of HTST treatment had a limited effect on antioxidant activity, and tested snacks showed high results both after 5 and 30 min of tests. The antioxidant activity of snacks with a 5% addition of Moldavian dragonhead turned out to be twice bigger in comparison with the rutin solution. The highest free radical scavenging ability was observed for snacks with the highest content of the additive. Additionally, the antioxidant activity of extruded snacks increased over the time-period of the experiment. Especially, the changes were apparent for the extrudates with 15 and 20% additive used, as shown in Table 2. In this case, activity values, obtained 30 min after the initiation of reaction, were 4.96 and 7.79, respectively. Taking this into account, it can be concluded that the processing conditions have no destructive influence on antioxidant activity. The addition of dragonhead caused a significant improvement (p < 0.01; r = 0.984) in the antioxidant activity of the snacks. This fact suggests a good antioxidant value of extruded snacks and its functional characteristics, without major losses in valuable components. Also, it should be highlighted that the effects of extrusion-cooking conditions on the changes in ingredients and composition may have been influenced by the unique chemical characteristics of individual substrates, as described previously (Dust et al. 2004; Oniszczuk et al. 2015a, b).

Foodstuffs of plant origins usually contain natural antioxidants that can scavenge free radicals. The antioxidant potential registered as changes in the absorbance of snacks enriched with dragonhead leaves (concentration of 0.4 g/mL) of methanol solutions of DPPH radical are presented in Fig. 1. The results of the antioxidant activity tested with spectrophotometrical analyses showed a significant (p < 0.01; r = 0.963) improvement of the radical scavenging activity of snacks with an increasing amount of dragonhead leaves in the snacks recipe. The highest activity (98%) was noted for the pure dragonhead extract after 30 min of test. Corn snacks were deprived of antioxidant properties (values below 50% after 30 min of test). The percentage increase in the leaves additive causes

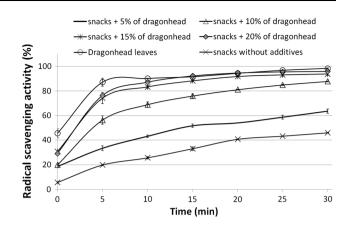


Fig. 1 Free radical scavenging activity of extract of *Dracocephalum* leaves and snacks with various addition of dragonhead leaves towards DPPH in methanol with spectrophotometric method

a proportional increase in antioxidant properties. The addition of 5% of *D. moldavica* influenced the moderate antioxidant activity of extruded snacks, but snacks with a 15 and 20% additive showed a very high activity (94–96%). Generally, these snacks showed a very good ability to scavenge free radicals, growing very fast over the test time and reached maximum values just after 5–10 min. The results of TLC-DPPH⁻, presented in Table 1, showed similar tendencies as the spectrophotometrical test results of the radical scavenging activity.

Pasting properties

The results of pasting properties of extruded corn snacks enriched with an addition of Moldavian dragonhead leaves are presented in Table 3. The highest viscosity of the tested samples was observed for snacks with a 5 and 10% additive. Paste viscosity did not show a characteristic peak viscosity of almost any extrudates. Maximum viscosity was observed at the onset of the measurements in low temperature. Temperature profile of the test was set from 30 °C up to 93 °C. In low temperature, the BG viscosity was relatively high because material had already gelatinized and water in the tested suspensions had been absorbed. A low value of BG was noted for corn snack because it had been significantly degraded during the processing and had no ability of absorbing water. Moreover, the presence of fibrous fractions in enriched snacks may increase the initial viscosity because fibre absorbs water very well. There was any gelatinization temperature observed for snacks with addition up to 15% of additive because all the starch was already gelatinized during the extrusion-cooking, so at 30-31 °C it was observed the highest viscosity, as presented in Table 3. For samples with 20% of dragonhead leaves addition, a low BG was detected as a characteristic for not completely gelatinized extrudates. Also, for these

Table 3 Pasting properties ofextruded snacks with addition ofMoldavian dragonhead leaves

Additive level (%)	BG (BU)	GT (°C)	PV (BU)	BD (BU)	SB (BU)	FV (BU)
0	$82^{ab}\pm0.1$	$30.3^{\mathrm{a}}\pm0.1$	$86^{ab}\pm0.5$	$56^{\mathrm{b}}\pm0.2$	$23^{\mathrm{b}}\pm0.1$	$53^{ab}\pm0.5$
5	$179^{\rm c} \pm 0.5$	$30.4^{\rm a}\pm0.1$	$180^{\rm c}\pm0.5$	$145^{d}\pm1.3$	$21^{ab}\pm0.1$	$55^{ab}\pm0.5$
10	$132^{bc} \pm 2.2$	$30.3^{a}\pm0.1$	$138^{bc} \pm 1.5$	$102^{\rm c} \pm 1.2$	$22^{b} \pm 0.1$	$57^{\rm b}\pm0.1$
15	$109^{b} \pm 2.5$	$31.0^{\rm a}\pm0.1$	$112^{b} \pm 1.5$	$84^{bc} \pm 1.0$	$18^{a}\pm0.2$	$46^{a}\pm0.2$
20	$42^{a} \pm 1.5$	$76.0^{b} \pm 0.2$	$46^{a} \pm 3.0$	$6^{a} \pm 3.0$	$22^{b} \pm 1.5$	$62^{\rm c}\pm0.5$

Mean values $(n = 3) \pm SD$

BG beginning of gelatinization, GT temperature at which an initial increase in viscosity occurs, PV peak viscosity, BD breakdown, SB setback, FV final viscosity

^{a,b,c,d} Different letters in columns show significant differences at p < 0.05

samples, the highest GT and FV values were observed. Probably, the high concentration of dragonhead leaves in the recipe prevented complete starch gelatinization during the processing of snacks, especially with the lower amount of total carbohydrates. In snacks supplemented with 20% of additive complete gelatinization didn't occurred so part of the starch during heating increased the viscosity when started gelatinize at 76 °C (what is characteristic for corn starch); at this temperature the highest viscosity was observed. There was a significant correlation of BG and GT parameters with WAI at p < 0.05 with r = 0.907 and r = -0.937, respectively.

Hot viscosity of the extruded snacks decreased during the heating period because these products had been already gelatinized during the extrusion-cooking. This is in agreement with the work of Ozcan and Jackson (2005). The PV is often referred as cold paste viscosity. The presence of an additive may restrict the thermal degradation of starch during the extrusion-cooking. An increased amount of the additive lowered the PV values of snacks. Structural changes with a sufficient loss of integrity and disintegration of the starch granules are observed when the thermal gelatinization and mechanical damage occurred (Thakur et al. 2015). Hence, gelatinized starch granules lose their ability to swell upon heating in water during viscographics studies, which results in low hot viscosity values referred as a final viscosity (FV). Under heating and shear stress, starch starts to rupture, and this phenomenon is referred to as the breakdown (BD). Thakur et al. (2015) defined much lower BD viscosity for both corn grit and flour with lower particle size, thus confirming the effect of shearing treatment on starchy materials breakdown and disintegration of starch granules. The lowest BD values were reported for samples with 20% of additive after the extrusion-cooking suggesting incomplete starch gelatinization occurred and resulting the presence of the highest GT and characteristic peak on Brabender viscograph. Also for these samples the highest protein, ash and TDF contents were identified (Table 1) which could cause lower total level of carbohydrates transformed during processing. Confirmation of these observations about the effect of chemical composition on pasting properties was shown by Thakur et al. (2015). The PV, as an indicator of water absorption capacity of starch (high correlation between PV and WAI at p < 0.05; r = 0.908) and the easy disintegration of starch granules due to gelatinization (high correlation between PV and BG at p < 0.01; r = 0.999), was significantly higher for samples with dragonhead leaves added than for corn snacks, except of extrudates with 20% of the additive, whereas SB values were at a similar level. The FV showed the extent of starch increase in viscosity that occurs during the cooling process. The range of FV and SB observed for extruded snacks supplemented with dragonhead addition was very low comparing to the results presented by Thakur et al. (2015) for raw normal corn grits and fours but only slightly lower than final outcomes observed for waxy corn as the result of low amount of amylose able to retrogradation and syneresis after heating and cooling stages. After the extrusion-cooking available amylose level decrease because of swelling and gelatinization occurring as a result of treatment conditions and only the extrudates with 20% of dried leaves significantly increased FV values corresponding with very low PV and BD. showed

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Physical properties of extruded snacks

The results of physical properties of Moldavian dragonhead-enriched corn snacks are presented in Table 4. The increasing level of the dragonhead leaves additive resulted in a significant lowering (p < 0.01; r = -0.968) of the ER; also, a decreased snacks dimension was noted, and the final expansion ratio of snacks enriched with 20% of dragonhead leaves decreased by around 45% compared to the control corn snack sample. The decrease in ER was highly correlated with increased dietary fibre content (p < 0.05; r = -0.963) and its SDF and NDF fractions (p < 0.05; r = -0.960 and r = -0.959, respectively), and thus with products' caloric value (p < 0.05; r = 0.956) because of limiting effect of fibrous additives on extrudates expansion.

		$DD(a 1 \dots 3)$		DI (NI/mm)		
Additive level (%)	ER (-)	BD (g/cm^3)	F _{max} (N)	BI (N/mm)	WAI (g/g)	WSI (%)
0	$4.87^{a}\pm0.06$	$41.31^{a}\pm0.85$	$12.10^{\rm a} \pm 0.50$	$0.83^{a}\pm0.18$	$5.64^{\rm b}\pm0.50$	$12.51^{ab}\pm0.25$
5	$4.09^{ab} \pm 0.15$	$40.85^{a} \pm 1.19$	$20.22^{\rm c} \pm 0.50$	$1.71^{b} \pm 0.45$	$6.11^{\rm c} \pm 0.11$	$13.88^{b} \pm 1.26$
10	$3.27^{\rm b}\pm0.08$	$53.57^{b} \pm 1.42$	$16.89^{b} \pm 0.50$	$1.80^{\rm b} \pm 0.50$	$5.74^{\rm b}\pm0.16$	$12.19^{ab} \pm 1.66$
15	$3.11^{\rm bc} \pm 0.17$	$66.57^{c} \pm 0.54$	24.74 $^{\rm cd}\pm0.50$	$2.48^{\rm c}\pm0.24$	$5.71^{b} \pm 0.10$	$11.83^{a}\pm0.88$
20	$2.72^{\rm c}\pm0.07$	$79.95^{d} \pm 3.87$	$28.41^{d} \pm 0.50$	$3.26^{\rm d}\pm0.22$	$4.71^{\rm a}\pm0.18$	$11.60^{a} \pm 0.78$

Table 4 Physical properties of extruded snacks with addition of dragonhead leaves

ER, BD, F_{max} , BI—mean values (n = 10) ± SD; WAI, WSI—mean values (n = 3) ± SD

ER expansion ratio, *BD* bulk density, F_{max} maximum cutting force, *BI* breaking index, *WAI* water absorption index, *WSI* water solubility index ^{a,b,c,d} Different letters in columns show significant differences at p < 0.05

Moreover, the lower ER was correlated with decreasing lightness (p < 0.01; r = 0.988) due to the lower amount of air cells in extrudates responsible for more bright color. These observations are similar to presented by Thakur et al. (2017) for extrudates made with African Tall variety of corn. Increased BD, cutting force and BI of enriched snacks products was observed compare to corn snacks. The negative correlations of ER were observed for BD and BI (p < 0.05; r = -0.851 and r = -0.892, respectively).Decrease in ER affected on increased extrudates density with higher dragonhead leaves incorporation in the recipe. The hardness of snacks, expressed as F_{max}, increased almost 135% compared to the control sample and was positively correlated with the fibre content (p < 0.05; r ranged from 0.835 to 0.891, depends on fibre fraction) and BD (p < 0.05; r = 0.858). It was related to the lower expansion and porosity of the extrudates with an addition of fibrous components and increased bulk density of products with high ash and fiber level (Altan et al. 2008). For BI, similar trends were observed. These results suggest that for directly expanded snacks supplemented with an addition of dragonhead leaves the recommended level of the additive should not exceed 15% of dried leaves.

With the higher level of dragonhead leaves in the recipe, the lowering of WAI was observed because of the lower amount of total carbohydrates and increased ash content (p < 0.05; r = -0.871) in the composition of snacks. These results were also correlated with the pasting properties of tested snacks as indicated by the lowering the PV and BD values. WSI of snacks, describing the starch molecules solubility, showed similar values for all the tested snacks. Samples with the addition up to 15% exhibited solubility ranged 11.83–13.88%, whereas snacks enriched with 20% of the additive characterized the lowest solubility of compounds. These may suggest less intensive treatment during snacks processing and lower macromolecular degradation because of lower content of carbohydrates, especially amylose, undergo treatment and dextrinization (Thakur et al. 2017). These results are aligned with the results of pasting properties. WSI was significantly correlated (p < 0.05; r = 0.807) only with BD of the tested products.

Colour profile and acceptance

The colour characteristic of extrudates with an addition of Moldavian dragonhead leaves is presented in Table 5. ΔE indicated significant differences in the colour change index after the addition of dragonhead leaves compared to the control sample. The increasing amount of dragonhead leaves significantly (p < 0.01; r = -0.989) lowered lightness L^* of the snacks. The sample with the highest amount of an additive characterized more than 53% darker lightness than the control sample of corn snacks. Addition of increasing amount of dragonhead leaves lowered L* and therefore it was correlated with the protein content (p < 0.05;(p < 0.01;r = -0.992), ash content r = -0.889), fibre and its SDF and NDF fractions (p < 0.01; r = -0.978, r = -0.985 and r = -0.963,respectively) as the effect of Moldavian dragonhead chemical composition. Usually, the addition of components rich in fibre, as herbs, plants or by-products, gives the lower caloric value of supplemented products, and it was directly correlated (p < 0.01; r = 0.981) with the lowering lightness of supplemented snacks, as also confirmed by the results of Altan et al. (2008). Furthermore, the lightness of snacks was correlated with the functional components and their activity; correlation coefficients -0.868 for rosmarinic acid, -0.970 for caffeic acid, -0.964 for antioxidant activity and -0.972 for the radical scavenging activity were reported as the result of increasing amount of dragonhead leaves in snacks recipes and thus the reduction of natural light and yellow colour of corn. Chromatic coordinate a^* was evaluated in the range of -6.65 for corn snacks up to -7.13 for snacks with 15% of the additive, but the differences were insignificant in these samples. A

Table 5 Colour profile andacceptability of extrudates withaddition of dragonhead leaves

Additive level (%)	Colour coordinat	ΔE	Acceptability		
	<i>L</i> *	a* b*			
0	$79.62^{a} \pm 1.75$	$-6.65^{\mathrm{a}}\pm0.91$	$35.79^{b} \pm 3.16$	Ref	7.5 ^{ab}
5	$64.81^{ab} \pm 2.34$	$-6.69^{a} \pm 3.08$	$40.53^{\mathrm{a}}\pm2.19$	15.55	8.0^{a}
10	$54.59^{b} \pm 4.22$	$-6.99^{a} \pm 2.16$	$36.15^{\text{b}}\pm2.51$	25.04	6.8 ^b
15	$48.87^{\rm bc} \pm 1.65$	$-7.13^{a} \pm 1.23$	$35.30^{\rm b} \pm 3.06$	30.76	6.6 ^b

L* (0–100 black–white); a* (–100 green, +100 red), b* (–100 blue, +100 yellow); ΔE —colour change index. Colour—mean values (n = 20) ± SD; acceptability—mean values (n = 15) ± SD

 $-13.6^{b} \pm 2.27$

^{a,b,c,d} Different letters in columns show significant differences at p < 0.05

 $37.87^{c} \pm 3.99$

significant improvement of greenness tint (more than 104% compared to the control sample) was observed along with the increase of dragonhead leaves addition to 20% of sample mass. This parameter was correlated significantly (p < 0.05; r = -0.937) with ash content and rosmarinic acid level (p < 0.05; r = -0.959) as the result of green dragonhead dried leaves addition in the tested snacks. The indicator of yellowness b^* was the most intensive for snacks enriched with 5% of the additive. The increasing amount of dragonhead leaves reduced the intensity of yellowness observed for the tested snacks, but the differences were insignificant.

20

The acceptability evaluation of snacks tested with 9-point hedonic scale showed good attractiveness of the samples enriched with dragonhead leaves up to 15% (Table 5); the snacks with a higher amount of additive were significantly less acceptable (p < 0.05; r = -0.845). Addition of 20% of Dracocephalum moldavica L. significantly reduced the expansion and increased density of the products, and consequently lowered the sensory attractiveness of snacks below an acceptable level despite their high nutritional value. The acceptability results were significantly positively correlated with the rosmarinic acid level (p < 0.05; r = 0.947) considering the intense herbal taste provided mostly by the rosmarinic acid present in Dracocephalum moldavica leaves and with snacks yellowness (p < 0.05; r = 0.940), as confirmed by colour measurements by an instrumental analysis. Also a strong negative correlation was evaluated between acceptability and bulk density (p < 0.05; r = -0.940) what was the result of lower expansion and almost double increase in bulk density with increasing amount of dragonhead leaves addition to snacks.

Conclusions

Presented results of the study have shown that dried leaves of the *Dracocephalum moldavica* L. seem to be a functional additive with high nutritional value because of the presence of rosmarinic acid, high dietary fibre content and strong antioxidant potential. Application of dragonhead leaves as an additive in amount not exceed 15% of blend was found to be recommended for the processing of directly expanded corn crisps with the extrusion-cooking process to give ready-to-eat snacks characterized improved nutritional composition compared with conventional corn snacks, proper physical features and furthermore, consumers' acceptability as a new assortment of healthy

 $33.81^{b} \pm 3.31$

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42.37

4.3°

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