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Total Content of Phenolics and Antioxidant Activity in Crispbreads with Plant By-product addition

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Abstract. Vegetable processing in food industry results in significant amount of by-products – peel, mark, bark, seeds still rich in bioactive compounds. Apple, carrot and pumpkin peel and mark may be used for production of crispbreads as functional ingredients. The objective of this study is to investigate the stability of total phenolic content (TPC) and antioxidant activity after high temperature and short time (HTST) extrusion cooking of a wheat and rice- based crispbreads with addition of apple, carrot and pumpkin by-products obtained after juice extraxtion and dried. Raw materials for crispbread production were wheat flour, rice flour, wheat bran (72%, 24% and 4% respectively) with addition of microwave–vacuum dried by-product powder in different amount (5%, 10%, 15%, 20%). Extrusion process was performed by using a laboratory single-screw extruder GÖTTFERT 1 screw Extrusiometer L series (Germany). Total phenolic content (TPC) was determined using the Folin Ciocalteu method. Antioxidant activity was evaluated by free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant scavenging activity using a modified colorimetric method. Comparing different raw formulations, it was observed that the TPC of the apple by-product flour was significantly higher ($p < 0.05$) than in carrot and pumpkin flour. TPC in cereal-based crispbread was 36.06±1.15 before extrusion and 13.90±1.01 mg GAEg-1 DW (milligram Gallic acid equivalent per 100 g of dry weight (mg GAE 100 g^{-1} DW) after extrusion. Addition of apple BPF increased TPC in crispbreads to 106.25±2.08, carrot BPF 84.73±3.45 and pumpkin BPF to 108.82±1.04 mg GAEg^{-1} DW. Antioxidant activity of control sample was 1.07±0.01mg TE (Trolox equivalents) g^{-1} DW but in samples with addition of 20% apple by- products, it reached 3.77±0.02 TE g^{-1} DW for samples wih 20% carrot by- products reached 2.52±0.03TE g^{-1} DW and for samples wih 20% pumpkin by- products reached 3.77±0.02 TE g^{-1} DW.
Keywords: antioxidants, by-products, crispbreads, extrusion, phenolics.

Introduction

The food industry generates high amount of by-products from a variety of sources and the waste management is one of the major parts of food industries. Around 100 million tonnes of food are wasted annually in the EU. If nothing is done, food waste could rise to over 120 million tonnes by 2020. Global quantitative food losses and waste per year are roughly 30% for cereals; 40–50% for root crops, fruits and vegetables. About 38% of food wastes occur during food processing (Rizui, 2015).

Production of juice from apple, carrots and pumpkin result in such by-products as bark, mark, peel, seeds which are still rich in bioactive compounds (Figuerola & Mar, 2005; Nayak *et al*., 2011). Vegetables are one of major antioxidants sources. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. They can be synthetic or natural (Eskicioglu, Kamiloglu, & Nilufer-erdil, 2015). Several scientific evidences have shown adverse effects of synthetic antioxidants. Antioxidants act as inactivates for free radicals. Most common antioxidants in vegetables and spices are vitamin C, E, phenolic compounds, carotenoids (Haminiuk *et al*., 2012). They are compounds when added to food products can increase the shelf-life of food products during processing. In particular, phenolic compounds isolated from plants are recognised as the most promising group of molecules that help to prevent oxidation and maintain product quality (Olfe, Ianzhong, & Iu, 2003).

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The apple (*Malus domestico)* pomace of the high carbohydrate content is used as a substrate in a number of microbial processes for the production of organic acids, enzymes, and pigments (Figuerola & Mar, 2005). Apple pomace can be used for flavour compounds, oxalic acid and as a medium for bakers yeast for pigment production, radical-scavenging and antioxidant properties of apple polyphenols are frequently cited as important contributors in different models of human chronic diseases (Elleuch *et al*., 2011;Haminiuk *et al*., 2012).

The carrot (*Daucus carota*) pomace is a by-product obtained during carrot juice processing. It is rich source of β-carotene and contains other vitamins like thiamine, riboflavin and minerals. The juice yield in carrots is only 60–70%, and even up to 80% of carotene may be lost with left over carrot pomace. The consumption of carrot is increasing steadily due to its recognition as an important source of natural antioxidants with anticancer activity. Carrot is a significant source of phytochemicals, including phenolics and carotenoids. Due to their appreciable level of different compounds, carrots are considered a functional food with significant health promoting properties (Arscott & Tanumihardjo, 2010).

Pumpkin (*Cucurbita pepo*) peel is rich in pectin and used as a gelling agent and thickening agent, as well as acts as a stabilizer in food (Rakcejeva *et al*., 2011; Aziah & Komathi, 2009). Pumpkin flesh is an excellent source of β-carotene, which the body converts into the important antioxidant of vitamin A. It contains a number of biologically active components such as polysaccharides, proteins and peptides, phenolic compounds, terpenoids and sterols. Pumpkins are particularly interesting since they are rich source of fibre and bioactive substances like carotenoids and polyphenols. Besides the provitamin A activity of some carotenoids such as b carotene and a-carotene, studies have also indicated that the consumption of carotenoids lowers the risk of degenerative and cardiovascular diseases, cataracts, and certain types of carcinomas (Provesi, Dias, & Amante, 2011).

Pumpkin seeds are rich in carotenoids, including lutein, α- carotene, and β-carotene. The seed oil is rich in unsaturated fatty acids, including linoleic, oleic acids. The oil is also rich in vitamin E, including both gamma-tocopherol and alpha-tocopherol. It has been as raw material for development of functional food (Sojak, Jaros, & Głowacki, 2014). Only several studies are dealing with the influence of the extrusion on carotenoids, primarily b-carotene (Emin, Mayer-Miebach, & Schuchmann, 2012; Waramboi, Gidley, & Sopade, 2013) and lycopene (Dutta *et al.,* 2006)

Food processing waste has a potential to be converted into useful products and utilized as source of the functional ingredient for development of products with high nutritional value. One of methods is extrusion processing due to its versatility, high productivity, relative low cost, energy efficiency (Huber, 2001; Emin, Mayer-Miebach, & Schuchmann, 2012).

Extrusion is a high-temperature, short-time (HTST) process and combines several unit operations - mixing, melting, kneading which can be manipulated to provide desired product and starchy food materials are plasticised, cooked and in some cases puffed/ expanded by a combination of moisture, pressure, heating and mechanical shear. For production of extruded food products grain is used as it contains starch and protein complexes. To obtain appeelness and texture of final product several flours are mixed and by products are added (Sarawong *et al.,* 2014). Our purpose of study is to make products from cereal based matrix with addition of food processing by products.

The objective of this study is to investigate the stability of total phenolic content (TPC) and antioxidant activity after high temperature and short time (HTST) extrusion cooking of a wheat and rice-based crispbreads with addition of apple, carrot and pumpkin by-products obtained after juice extraxtion and dried.

Materials and Methods

The study was realised in the scientific laboratories of the Faculty of Food Technology at Latvia University of Agriculture.

Raw material characterisation

Raw materials for crispbread production were wheat flour, rice flour, wheat bran (72%, 24% and 4% respectively) with addition of microwave–vacuum dried by-product powder in different amount (5%, 10%, 15%, 20%) obtained from local vegetable processors JSC Terrosk and catering company Vairak Saules.

By-product moisture was 80.20 %±5.50%.

By-products were dried in a microwave – vacuum dryer "MUSON-1" (Russia) according to developed program and by- product weight separetly to receive by-product with moisture content from 3.0% to 5.5%.

Dried apple and vegetable by-products were separately ground to a powder by a grinder FOSS KNIFITECTM 1095 for 30 seconds each grind. And then they were sieved through a 2 mm sieve.

For development of crispbreads, as raw materials also 405 type wheat flour (JSC Dobeles dzirnavnieks, moisture 9.86%), rice flour obtained from rice grains

(JSC Valdo, moisture 8.4%) by grinding, 2365 type wheat bran (JSC Valdo) moisture 5.94%) were used.

Crispbread preparation

Crispbread samples were prepared according to recipes shown in Table 1.

Table 1

Raw material formulation in crispbreads

Ingredients % DW	Control	By-product 5%	By-product 10%	By-product 15%	By-product 20%
Wheat	72.0	70.0	67.5	65.0	62.5
Rice	24.0	22.0	20.0	18.0	16.0
Wheat bran	4.0	3.0	2.5	2.0	1.5
By-product powder (apple, carrot or pumpkin)	–	5	10	15	20
TOTAL	100	100	100	100	100

The moisture content of raw materials and crispbreads was determined by a standard method LVS EN ISO712:2010A in Memmert equipment, Modell-100-800.

Extrusion process was performed using a laboratory single-screw extruder GÖTTFERT 1 screw Extrusiometer L series (Germany). An extrusion screw (compression ratio 2:1) at a speed of 60-80 rpm and a rectangular die (aperture: 20 mm wide, 1.0 mm high, 100 mm long) were used.

Temperatures for extrusion process were set 78 °C/83 °C/98 °C, screw speed 800 rpm^{-1}. Obtained extrudates were cooked at 130 ºC for 20 minutes in conventional oven to receive soft and crispy product.

Chemical analysis

The total phenolic content (TPC) of the extruded product extracts was determined according to the Folin-Ciocalteu method. Extraction was performed by the method described by Paraman (Paraman *et al*., 2015). 2.5 mL of Folin- Ciocalteu reagent (diluted 10 times with water) was added to 0.5 mL of extracted sample. After 3 minutes 2.0 mL sodium carbonate ($Na_2 CO_3$) solution (7.5%) was added. The resulting solution was mixed and allowed to stand for 30 minutes at 20±1 C in dark place. Absorption was read at 765 nm with JENWAY 630 Spectrophotometer. Results were expressed as milligram Gallic acid equivalent per 100 g of dry weight (mg GAE 100 g^{-1} DW).

Antioxidant activity of the extruded product extracts was measured on the basis of scavenging activities of the stable 2, 2-diphenyl-1-picrylhydraziyl (DPPH) radical. The radical scavenging activity was expressed as Trolox equivalents (TE) 100g^{-1} DW of extruded material.

Statistical anlysis

All analysis was triplicate and results are presented as a mean value ± standard deviation (SD). Statistically significant differences between results were calculated at the level of confidence α=0.05. In order to find out if the differences in mean values estimated were statistically significant, the one way analysis of variance was applied and analyzed by Microsoft Excel 2010. To determine differences among samples, analysis of variance (ANOVA) and Pearson`s tests were used.

Results and Discussion

Antioxidants are of interest to both food scientists and health professionals, and there has been a convergence of interest among researchers in these fields as the role of antioxidants in the diet and their impact on human health has come under attention (Mala, Sathiya Mala, & EKurian, 2016).

Moisture and Total phenolic compounds (TPC)

Moisture content in samples before extrusion and after extrusion is shown in Figure 1.

TPC content was determined in dried by–product flour and other ingredients for crispbread production (Figure1).

TPC in by- product flour ranged from 132.44 in carrots to 187.79 mg of GAEg^{-1} DW in apple by-product flour. The results reported by Henri were 60–260 mg GAEg^{-1} DW in apples obtained with different drying methods (Henrı *et al*., 2013). Results about dried pumpkin reported by Priecina and Karklina in pumpkin TPC was 672.19 mg GAE 100 g^{-1} (Priecina & Karklina, 2014). For carrots some authors show results ranging from 331.00 to 366.00 mg GAE g^{-1}.

TPC was determined for both crispbreads before extrusion and for extruded samples (Table 2). The TPC of the extruded products varied from 13.90±1.01 in control sample to 108.82 mg GAE·100 g^{-1} in crispbreads with 20% addition of pumpkin by-product. This increase of polyphenol contents after heating might be responsible for the high antioxidant activity of high temperatures during HTST process and addition of different by product flours. The

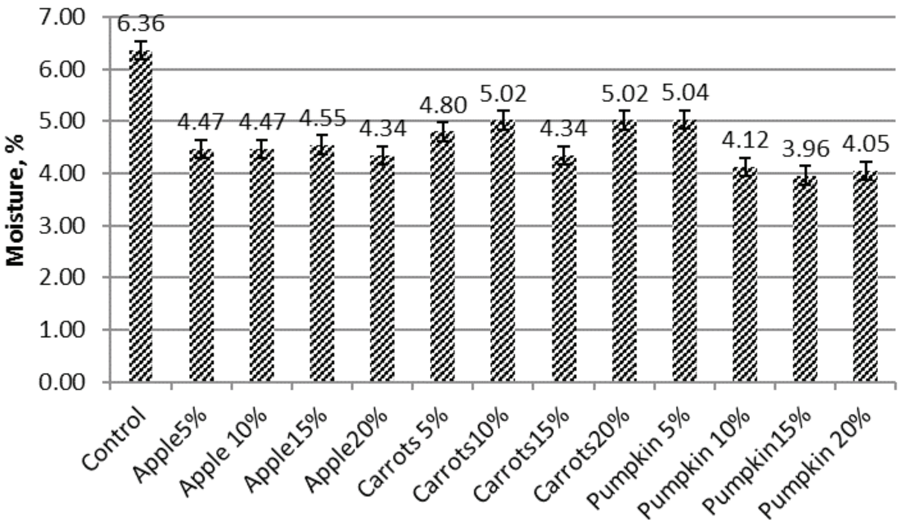

Figure 1. Moisture content in crispbread samples after extrusion.

formation of phenolic compounds might be because of the availability of precursors of phenolic molecules by non-enzymatic interconversion between phenolic molecules. Nayak *et al*., (2011) reported that the polyphenols in purple potato flour increased from 50 to 160 mg g^{-1} with an increase in heating temperature. Phenolic compounds are heatliable and can break at the exposure to high temperatures, in samples with carrot BPF (by-product flour) addition. TPC after extrusion decreased in all samples with 5-15% added BPF except TPC where it increased in the sample with 20% of carrot BPF addition. Therefore, losses in the TP content of the formulations under extrusion are expected to occur, due to break down of complex polyphenols to other phenolic or non-phenolic compounds, at a consequence of high temperature conditions (Nayak *et al*., 2011). Another explanation for the higher TPC after heating, for apple and pumpkin BPF addition at least in part is an increased formation of anthocyanins of browning compounds caused by the Maillard reaction at high temperatures. Viscidi and others (2004) reported a significant loss of total phenolics during extrusion of oat cereals. Scientists have reported losses of up to approximately 60% of phenolic compounds in extruded oat samples, compared to its respective raw sample. Camire reported higher content of soluble phenolics, as ferulic acid equivalents, in Concord grape and raspberry extrudates compared to their control samples (Camire, 2011).

Some studies indicate that levels of phenolic acids, especially ferulic acid and diferulate esters of some whole grain products, including wheat products increase after thermal treatment.

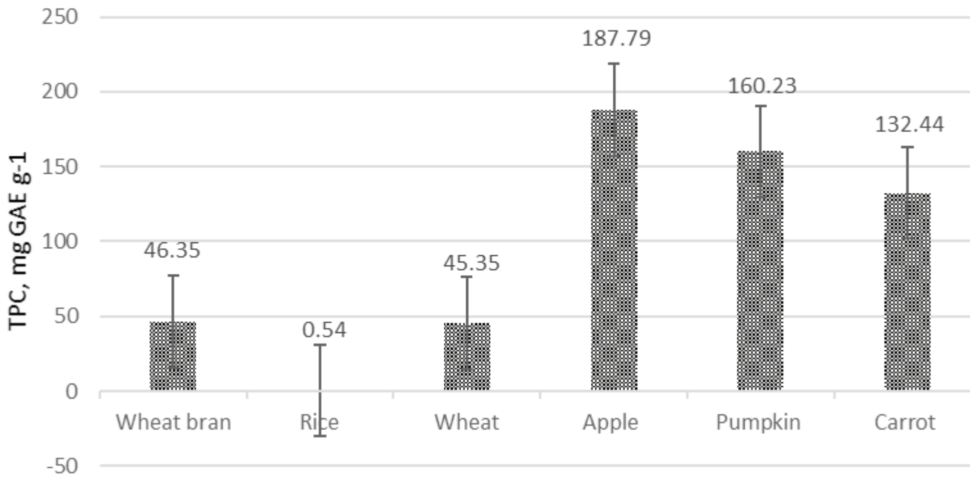

Figure 2. TPC in raw material for crispbreads.

Table 2

Total phenolic content in crispbreads before extrusion and after extrusion

Samples	TPC, mg GAE·100 g^{-1} DW	
	Before extrusion	After extrusion
Control crispbread	36.06±1.15	13.90±1.01
Apple 5%	49.26±1.57	52.40±2.14
Apple 10%	51.44±1.07	77.22±2.14
Apple 15%	53.83±0.46	92.74±3.14
Apple 20%	58.20±0.25	106.25±2.08
Carrots 5%	37.75±2.68	19.70±1.04
Carrots 10%	45.36±3.22	34.95±1.12
Carrots 15%	47.25±4.25	40.21±2.25
Carrots 20%	52.61±3.58	84.73±3.45
Pumpkin 5%	39.62±2.68	36.92±2.87
Pumpkin 10%	44.10±3.08	58.71±4.02
Pumpkin 15%	47.89±2.42	76.75±4.55
Pumpkin 20%	55.49 ±2.68	108.82±1.04

Content of phenolic compounds in apple and pumpkin by-product flour indicated the formation of phenolic substances during extrusion and for crispbread samples extrusion led to changes in TPC. The addition of 5, 10 and 15% of carrot by-product to extrudates decreased TPC during extrusion, except for samples with 20% carrot by-product addition increased TPC from 52.61±3.58 before extrusion to 84.73±3.45 mg GAE·100 g^{-1} after extrusion. Degradation of TPC during extrusion also depends upon the nature of raw materials and different composition of phenolic substances such as carotenoids (β-carotene, α-carotene, and β-cryptoxanthin).

DPPH antioxidant activity

The polarity of plant radical scavenging components is important factor defining extracts antioxidant activity.The antioxidant activity of by products, using DPPH assay, was 5.59±0, 07 TE 100g^{-1} DW in carrots and 6.24±0.05 TE 100g^{-1} DW DPPH antiradical activity in apple by- product (Figure 2).

After extrusion antioxidant activity increases and correlates (r=0.74) with total phenols increasing in samples. Some researchers show a decrease during heat treatment in extrusion while others show an increase or no change (Sojak *et al*., 2014; Zargar *et al*., 2014) for both phenols and antioxidant activity. Addition of apple, pumpkin and carrot by-product increased antioxidant activity in samples (Table 3).

Table 3

DPPH antioxidant activity

Samples	DPPH, TE 100g^{-1} DW	
	Before extrusion	After extrusion
Control crispbread	0.35±0.01	1.07±0.01
Apple 5%	0.64±0.02	2.70±0.01
Apple 10%	0.94±0.02	3.16±0.01
Apple 15%	1.24±0.01	3.44±0.02
Apple 20%	1.54±0.03	3.77±0.02
Carrots 5%	0.61±0.01	1.59±0.02
Carrots 10%	0.88±0.02	1.95±0.02
Carrots 15%	1.14±0.02	2.16±0.03
Carrots 20%	1.42±0.03	2.52±0.03
Pumpkin 5%	0.55±0.01	2.61±0.01
Pumpkin 10%	0.76±0.01	2.88±0.02
Pumpkin 15%	0.96±0.01	3.74±0.03
Pumpkin 20%	1.17±0.02	3.77±0.02

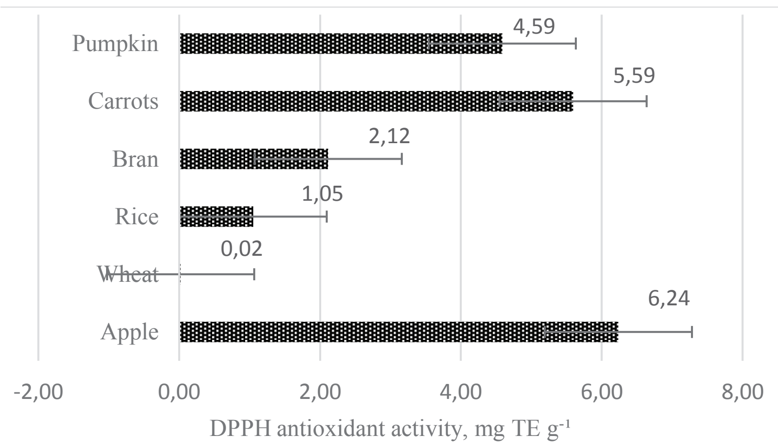

Figure 3. DPPH antioxidant activity in raw material for crispbreads.

Table 4

Pearson's coefficients between the phenolic compound levels and antioxidant activity before and after extrusion

Between group	Samples with apple BPF	Samples with carrot BPF	Samples with pumpkin BPF
TPC before extrusion / TPC after extrusion	0.95	0.91	1.00
TPC before extrusion /DPPH before extrusion	0.98	0.98	0.99
TPC after extrusion / DPPH after extrusion	0.99	0.77	0.98
DPPH before extrusion / DPPH after extrusion	0.99	0.94	0.99

There is doubtful information on the effects of extrusion processes on antioxidant and phenolic properties, and it remains a controversial subject. Reactions such as Maillard reaction (nonenzymatic browning), caramelization, and chemical oxidation of phenols could also contribute to the increase in total phenol content. Thermal processing may also change the ratio between various phenolic compounds (Guine, Raquel de Pinho Ferreira Correia, & P.M. dos R., 2013). Another study showed a significant reduction in antioxidant activity for 60–68% and for TPC 46-60% in extrusion of barley flour (Altan, Mccarthy, & Maskan, 2008). Behaviour of phenolic compounds present in selected cereals (wheat, rice) during extrusion cooking at high temperatures significantly varied among cereals (Zielinski, Kozlowska, & Lewczuk, 2001). While Camire (2011) reported no significant change ($P > 0.05$) in the antioxidant activity of the control and cranberry extruded products prepared with corn at substitution level of 1%. Kumar and Sharma investigated the action of different antioxidants and reported a significant decrease in total phenolic content during barley flour extrusion (Kumar *et al*. 2013; Sharma, Yadav, & Ritika, 2008).

Correlation between TPC, DPPH antioxidant activity

For all samples the Pearson's coefficients between the phenolic compound levels and antioxidant activity were determined (Table 4).

For all crispbread samples correlation between groups of samples before extrusion and after extrusion was very strong $0.91<r<0.99$, ($p < 0.05$). For samples with carrot BPF correlation between TPC after extrusion and DPPH after extrusion was moderate ($r=0.77$).

Conclusions

The stability of total phenolic content (TPC) and antioxidant activity after high temperature and short time (HTST) extrusion cooking of a wheat and rice-based crispbreads with addition of apple and pumpkin by-products increased for all samples. Addition of 20% apple by product flour increased TPC to 106.25±2.08 mg GAE·100 g^{-1} and 20% of pumpkin by-product flour increased TPC to 108.82±1.04 mg GAE·100 g^{-1}.The addition of carrot by- products decreased TPC in extruded samples from 37.75±2.68 before extrusion to 19.70±1.04 after extrusion with 5% carrot byproduct addition. Though addition of 20% of carrot by- product increased TPC from 52.61±3.58 before extrusion to 84.73±3.45 mg GAE·100 g^{-1} after extrusion. Therefore, we have to conclude that the difference of phenolic compounds in by- products can result in such differences.

Our study demonstrated that food products rich in antioxidants can be produced from cereals with addition of apple, carrot and pumpkin by-products using extrusion technology retaining the stability of biologically active substances.

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