

The possibility of using powdered sea-buckthorn in the development of bakery products with antioxidant properties

L. Nilova* and S. Malyutenkova

Peter the Great St. Petersburg Polytechnic University, Institute of Industrial Management, Economics, and Trade, Graduate School of Commodity and Service, Novorossiyskaya street 50, U194021 St. Petersburg, Russia

*Correspondence: nilova_1_p@mai.ru

Abstract. The article examines ways of increasing the antioxidant capacity of bakery products (referred to here as BP) by adding powdered peel, powdered seeds, and powdered marc produced from sea-buckthorn berries. Three different versions of BP were developed with the maximum addition of the following powders: from the peel (3%), from the marc (5%) with a sugar content of 14.5%; and from seeds (5%) with a sugar content of 5%, and with a potato flake content of 5%. BPs with the addition of sea-buckthorn powders were baked at two temperature regimes: 200°C and 220 °C. The content of phenolic compounds, flavonoids, and ascorbic acid were determined for the sea-buckthorn powder, for the crust of BP, and in BP crumbs. The antioxidant activity of BP was determined by the use of two methods: by chemiluminescence, and by their reaction to the DPPH-radical. Cyclic amides (lactams) were determined in BP crusts and crumbs with the use of the IR spectroscopy method. The AOA of powdered sea buckthorn depended upon the volume of phenolic compounds and ascorbic acid in them: peel > marc > seeds. The antioxidant properties of BPs decreased in the following order and were aligned the following way: BP with marc > BP with peel > BP with seeds. They displayed higher AOA levels than was calculated in theoretical tests, depending upon the volume of powders in the recipe. An increase of the baking temperature led to a loss of phenolic compounds and vitamin C, as well as to the formation of poly lactams. BP baked at the temperature regime of 200 °C displayed the greatest AOA levels.

Key words: sea buckthorn, peel, seed, marc, bakery products, antioxidant activity, lactams.

INTRODUCTION

Most bread and bakery products (BP) are made of low-yield wheat flour, or what is generally known as refined flour. Such flour has good baking properties, but contains almost no vitamins, minerals, or dietary fibres (Pashchenko & Zharkova, 2006; Akhtar et al., 2011; Nechaev, 2013). In human nutrition, bread and BP which is made of low-yield wheat flour serve only as a source of energy. Besides this, melanoidins which are formed during the baking process may cause a pro-oxidative effect in the absence of natural antioxidants, thereby damaging human health (Putilina et al., 2006; Nilova & Pilipenko, 2016).

The development of alimentary products, including bread and BP which has been enriched with natural antioxidants (AO) is a popular direction when it comes to

functional nutrition. Natural raw materials containing water-soluble AO – fruit and vegetable powders, juices, and extracts (Belyavskaya & Rodicheva, 2013; Dziki et al., 2014; Jaisanthi & Banu, 2014; Karrar, 2014; Nilova et al., 2015) – or lipid-soluble AO such as vegetable oils (Caponio et al., 2013; Nilova et al., 2017) are used as the source of natural AO. A valuable source of water-soluble and lipid-soluble AO is sea-buckthorn (*Hippóphae rhamnóides L.*).

The composition of biologically active substances (BAS) in sea-buckthorn – its berries, seeds, and leaves – has been studied by a good many researchers (Bal et al., 2011; Chaman et al., 2011; Kant et al., 2012; Saikia & Handique, 2013; Fatima et al., 2015). Berries and seeds from sea-buckthorn contain phenolic compounds, ascorbic acid, tocochromanols, carotenoids, and phytosterols. They effectively combat free radicals (Zeb, 2006; Chaman et al., 2011; Kant et al., 2012; Ursache et al., 2017). It is impossible to precisely affirm which parts of sea-buckthorn contain more AO as the quantitative composition can be influenced by the botanical breed of sea-buckthorn, the area of its cultivation, or the method of research being used to study it (Jalakas et al., 2003; Bal et al., 2011). The peel and the pulp contain a lot of ascorbic acid, carotenoids, and phytosterols, while the seeds are rich in cochromanol and lignans (Li et al., 2007; Christaki, 2012; Smeds et al., 2012). Phenolic compounds can dominate in the pulp and the peel of berries or seeds (Saikia & Handique, 2013), while flavonoids (with a predominance of rutin and quercetin) are concentrated mostly in the leaves of sea-buckthorn (Fatima et al., 2015). The amount of lipid-soluble AO in processed sea-buckthorn products depends upon the amount of fat that remains in them after the extraction of sea-buckthorn oil has taken place.

Due to their valuable biochemical composition, sea-buckthorn berries are recommended for use in nutrition in their natural form or as part of food products (Bal et al., 2011). Sea-buckthorn is used mostly for making products such as oil, squash, and juices (Zeb, 2004; Cenkowski et al., 2006; Lipowski et al., 2009). The remaining marc could be used as an enriching supplement for BP, but the high percentage of ascorbic acid in the marc strengthens gluten and can reduce the quality of the BP.

This work is aimed at the development of BP which is made from wheat flour with AO properties due to the use of sea-buckthorn powders in the recipe.

MATERIALS AND METHODS

Preparing sea-buckthorn powders

Sea-buckthorn berries were harvested in the Leningrad region of Russia. The marc left over after squeezing out the juice was dried at 50–55 °C for six hours. Three types of powder were obtained: from the marc, from the seeds, and from the peel. The powder was ground down immediately before any research or BP baking took place. The ground-down powder was then sifted in order to obtain powder with a particle size less than 150 µm. The larger fractions of the powder were milled repeatedly.

The bakery product recipe and baking process

The following ingredients were used in the BP recipe: wheat flour (gluten 28.9%, ash content 0.55) produced by St Petersburg Mill Plant OJSC; refined and deodorised sunflower oil produced by EFCO FOODS PLC, Russia; sugar (99.8% sucrose) by JSC ‘Lebedyansky Sugar Plant’, Russia; pressed bakery yeast by JSC ‘Food Factory’, Russia;

and salt and potato flakes (carbohydrates 83%, proteins 7%, fats 1%) by JSC Hercules Plus, Russia.

The BP were produced by using a straight dough method according to three recipes presented in Table 1.

Table 1. Basic and experimental recipes for bakery products

Ingredient, g	Basic recipes			Experimental recipes		
	Recipe 1 (R1)	Recipe 2 (R2)	Recipe 3 (R3)	with seed powder (based on R2)	with marc powder (based on R3)	with peel powder (based on R3)
Wheat flour	1,000	1,000	1,000	900	950	970
Seed powder	-	-	-	50	-	-
Marc powder	-	-	-	-	50	-
Peel powder	-	-	-	-	-	30
Potato flakes	-	-	-	50	-	-
Sugar	-	50	145	50	145	145
Vegetable oil	-	40	145	40	145	145
Yeast	20	20	20	20	20	20
Salt	15	15	15	15	15	15
Water, mL	620	570	490	570	490	490

The amount of water in the dough was determined through calculations and depended upon the moisture and gluten content of the flour and its additional ingredients (Pashchenko & Zharkova, 2006).

To optimise the amount of sea-buckthorn powder in BP recipes, part of the wheat flour was replaced by sea-buckthorn powders: the first one made from marc; another one made from seeds; and the final one made from peel, to the amount of 1–7%.

The entire dough mass was prepared according to the recipe, from flour and water, being first kneaded for seven minutes and then left to ferment for 150 minutes at 30 °C; the dough was punched down after ninety minutes from the beginning of the fermentation period. Baking the BP which weighed 100g was done at two temperature regimes: at 200 °C for 25 minutes and at 220 °C for twenty minutes. The crust and the crumb were separated after cooling the BP and were dried separately at 40 °C, to reach a constant mass.

Methods for researching the quality of BP

The assessment of BP quality was carried out according to the following parameters:

- the sensory parameters – appearance (shape, surface, and colour of the crust), crumb condition (porosity and texture), and taste and flavour;
- the physico-chemical and physical parameters – the mass proportion of moisture was determined by drying at a temperature of 130 °C for forty minutes; the acidity by use of the titration method 0.1 with a normal NaOH solution; the porosity by determining the ratio of the volume of pores to the total product volume; the pore volume as the difference between the product volume and the volume of the non-porous mass; the specific volume by determining the ratio of product volume to that of 100 g of flour. The quality of experimental BP samples which were produced with the addition of sea-buckthorn powders was compared with control BP samples which had been produced

using the same recipe but without having any of the supplements added (Shevchenko et al., 2009).

Methods of researching individual antioxidants

The goal was to determine individual AO levels in sea-buckthorn powders, as well as in the crust and the crumb of the developed BP.

A determination of ascorbic acid was carried out by use of the titrimetric method, using a 2,6-dichlorophenolindophenolate sodium solution. The extraction of ascorbic acid from the raw materials was carried out with the use of 2% hydrochloric acid. A total of 5 g of the powder was mixed with 5 mL of 2% hydrochloric acid solution, 20 g of crust or crumb were mixed with 20 mL of a 2% solution of hydrochloric acid, infused for ten minutes and then filtered. The ascorbic acid was determined in extracts without any delay (Shevchenko et al., 2009).

Total phenol assay by Folin-Ciocalteu reagent. Ethanol extracts of sea-buckthorn powder, as well as the crust and the crumb powders from CBS, were kept in the dark with Folin-Ciocalteu reagent for thirty minutes at room temperature (1 g of powder in 50 mL of 80% ethanol) with periodic shaking. After the incubation period all samples were centrifuged at 3,500 rpm for ten minutes. The optical density was measured using a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 735 nm. The results obtained were expressed in mg of gallic acid (Rogozhin & Rogozhina, 2015).

The total content of flavonoids was determined spectrophotometrically by reaction with aluminium chloride. The extraction of flavonoids was carried out with 60% ethyl alcohol. The optical density was measured after thirty minutes on a SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 420 nm. The results obtained were expressed in mg of routine (Rogozhin & Rogozhina, 2015).

Methods for assessing overall antioxidant activity

The antioxidant activity (AOA) of aqueous extracts from sea-buckthorn powders, along with the crust and crumb of BP, was determined by use of the chemiluminescent method using the BCL-06M biochemiluminometer (Nizhny Novgorod, Russia) in a model system which contained riboflavin, hydrogen peroxide, and ferrous iron (Putilina et al., 2006). The extraction was carried out using distilled water, and was centrifuged for ten minutes at 3,500 rpm. Measurements were taken for luminescence in volts (I_{50}) at room temperature for fifty seconds. The information obtained from I_{50} of water extracts was used in making up the charts. The charts were used to determine the concentration of the substance, which reduced the intensity of chemiluminescence by 50%. Trolox 97% (Acros organics, USA) was used as the standard.

A determination of AOA of BP was carried out by use of the Glavind method (Rogozhin & Rogozhina, 2015). The hydro-alcoholic extracts of sea-buckthorn, and the crumb and the crust powder samples, were prepared in the same manner as described above, but extraction was carried out with the use of a 50% ethanol solution. The DPPH solution was prepared by dissolving a 5 mg test sample in 5 mL of 16.4M (96%) ethanol which was heated on a bain-marie until dissolved. A total of 0.2 mL of the extract was added to 2 mL of DPPH solution. The resulting solution was incubated in the dark for thirty minutes, after which the absorbance levels of the resulting solution were measured at a wavelength of 517 nm using a SHIMADZU 1240 spectrophotometer (SHIMADZU,

Japan). The results were calculated using the standard calibration curve for trolox (trolox 97%, Acros Organics, USA).

A determination of cyclic amides (lactams) in BP

Cyclic amides (lactams) were studied with the use of IR-Fourier spectroscopy in the area of 1,680–1,800 cm^{-1} (Silverstein, 2011). The infrared spectra were determined using the IR-Fourier ‘ΦCM 1202’ spectrometer produced by Monitoring LLC, Russia, with the automatic counting of peaks as compared to the baseline. Spectral registration parameters were as follows: spectral range 400–4,000 cm^{-1} ; number of scans, twenty; resolution, 4 cm^{-1} ; mode, interferogram. The absolute error in calibration for the wave number scale did not exceed $\pm 0.1\text{cm}^{-1}$. Deviation of the 100% transmission line from the nominal value (1,950–2,050 cm^{-1} , resolution 4 cm^{-1} , twenty scans) did not exceed $\pm 0.5\%$. The mean square deviation of the 100% transmission line (1,950–2,050 cm^{-1} , resolution 4 cm^{-1} , twenty scans) did not exceed 0.025%. The interferograms obtained were transformed into transmission spectra. The samples for testing were prepared by pressing the BP crust or crumb with potassium bromide. For the preparation of tablets, an exact quantity of potassium bromide was ground down using an agate mortar with 2 g of BP. A measure of 100 mg of the mixture was then pressed in press moulds for fifteen minutes on each side. The identification of lactams was carried out on the basis of the peaks area at an interval of 1,800–1,680 cm^{-1} (Bellami, 1971; Silverstein, 2011): monocyclic γ -lactams were identified at an interval of 1,700 cm^{-1} ; polycyclic at 1,700–1,750 cm^{-1} ; monocyclic β -lactams at 1,760–1,730 cm^{-1} ; and polycyclic, condensed with other cycles, at an interval of 1,770–1,800 cm^{-1} .

Statistic analysis

The research was conducted in triplicate. The reliability of the experimental data was evaluated by methods involving mathematic statistics with the use of the Microsoft Excel 2007 application for Windows. The data confidence level is 0.95.

RESULTS AND DISCUSSION

The powders had the characteristic aroma of sea-buckthorn berries of varying levels of intensity, and also by the sour taste. The main distinction of the powders lay in their colour. The peel-based powder was of a rich orange colour, the one made with seeds was grey with brown inclusions, while the marc-based powder had a pale orange colour. All of the powders contained ascorbic acid, phenolic compounds, and flavonoids in varying amounts (Table 2).

Phenolic compounds prevailed in all of the powders, followed by flavonoids. The ratio of phenolic compounds and flavonoids in different sea-buckthorn powders was different. According to their content, the powders were aligned in the following order: skin-based powders > marc-based powders > seed-based powders. The share of flavonoids in the peel-based powders came to 89.5% of all phenolic compounds, and in marc-based powders it was 79.9%. The amount of flavonoids in the seed-based powders was less: 39.6% of the total amount of phenolic compounds. The content of ascorbic acid was also higher in the peel and marc-based powders than in those made from the seeds.

The studied sea-buckthorn skin and marc-based powders contained ten times more vitamin C, five times more phenolic compounds, and four times more flavonoids when

compared to those powders that were obtained after oil extraction (Zolotareva et al., 2005; Nikulina & Ivanova 2006), but less vitamin C than in fruit juice powder (Hussain et al., 2010; Selvamuthukumaran & Khanum, 2014). The content of phenolic compounds is almost the same as in powder that was based on sea-buckthorn berries (Roidaki et al., 2015). Differences in the composition of biologically active substances are associated both with the method of obtaining the powders (Guan et al., 2005), and with the botanical variety of sea-buckthorn types and their region of growth. The content of vitamin C in various sea buckthorn types can differ from two to fifteen times, while the content of phenolic compounds and flavonoids can differ between eight to ten times (Ercisli et al., 2007; Ershova, 2009; Wani et al., 2016). The higher amount of biologically active substances increases the AOA of the berries and other sea buckthorn products (Kant et al., 2012; Zeb & Hussain, 2014; Fatima et al., 2015).

The content of individual AO had an impact on the results of studies of AOA in sea-buckthorn powders. The more AO the powder contained, the higher was its AOA: sea-buckthorn peel > sea-buckthorn marc > sea-buckthorn seeds. The AOA of water extracts was lower than that of hydro-alcoholic extracts in the peel powders. In seed powders, on the other hand, the AOA of the aqueous extract was higher than that of the hydro-alcoholic extract, probably because of the inclusion of phenolic compounds.

To assess the possibility of being able to use sea-buckthorn powders in the production of BP from wheat flour, three standard recipes were used, where a certain proportion of the flour was replaced by sea-buckthorn powder. Recipe 1 did not provide for the use of a sugar and fat component. With addition of 1% of any sea-buckthorn powder, the BP acquired a sour taste, while the fragrance of sea-buckthorn was not felt. The specific volume of BP increased by 4% when applying the marc powder, and by 3% after adding powder which was based on the peel or seeds. The further increase in concentration of powders in Recipe 1 engendered a pronounced sour taste and a decrease in the specific volume of BP. The sea-buckthorn aroma became apparent only with addition of sea-buckthorn powder to the amount of 3%. Recipe 1 cannot be used for BP which is enriched with sea-buckthorn powders.

Table 2. The content of antioxidants and the total antioxidant activity of sea-buckthorn powders, \pm standard deviation

Powdered form	The content of antioxidants, mg per g DM			AOA, μ g Trolox per g DM	
	Ascorbic acid	Total phenolic content	Total flavonoids content	DPPH-radical assay	Chemiluminescence
Marc	2.52 \pm 0.08	8.61 \pm 0.20	6.88 \pm 0.28	45.10 \pm 1.50	40.20 \pm 1.30
Seeds	0.65 \pm 0.02	6.95 \pm 0.22	2.75 \pm 0.16	12.32 \pm 0.50	15.40 \pm 0.60
Peel	3.21 \pm 0.05	9.16 \pm 0.20	8.20 \pm 0.22	58.62 \pm 1.51	51.80 \pm 1.80

Recipe 2 provided for the use of 4% vegetable oil and 5% sugar. The gradual increase in the amount of powder in the BP content led to the appearance of a sour taste, confirmed by the values of titratable acidity of BP (Fig. 1). The sour taste was noticed when applying 2% of the peel and the marc powders, and 3% of the seed powder. At the same time, the sea-buckthorn smell was practically indistinguishable.

The main factor which served to inhibit the increase in the number of sea-buckthorn powders in BP recipes was the high content of ascorbic acid in the powders. The ascorbic acid tightens gluten, thereby restraining the increase in the specific volume of BP

(Pashchenko & Zharkova, 2006). As a result, the maximum specific volume of BP was achieved only when using 2% of the peel-based powder and 3% of powder which used the marc or seeds (Fig. 1). Increasing the extent of powders in Recipe 2 resulted in a decrease in the BP-specific volume.

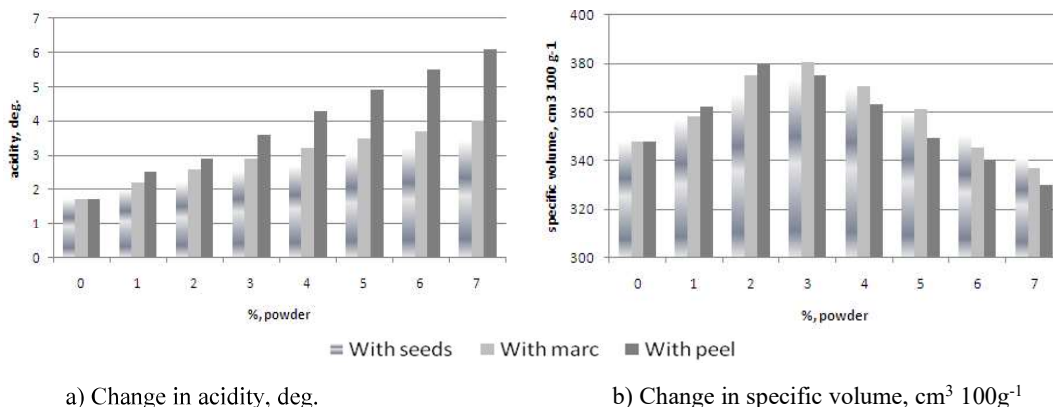


Figure 1. The effect of sea-buckthorn-based powders on the acidity (a) and specific volume (b) of BP (Recipe 2).

BP with seed-based powder acquired a sea-buckthorn aroma only with the addition of 5% powder. The crumb colour was grey. Therefore the use of powder from the seeds in Recipe 3 is not appropriate. In order to increase the BP-specific volume in Recipe 2, it was necessary to use ingredients which prevent gluten from thickening. Potato flakes were chosen as such an ingredient. Potato flakes to the amount of 3%, 5%, and 7% were added to Recipe 2 with the further addition of 5% of the seed-based powder. BP quality was controlled according to specific volume (Fig. 2).

The introduction of 3% of potato flakes increased the specific volume of BP by 1.2% with the addition of 5% of seed-based powder; while the introduction of 5% of potato flakes increased it by 2.7%, which almost reached the specific volume of BP with the addition of 3% of the seed-based powder. The values were only 1% lower. However, a further increase of the proportion of potato flakes resulted in an excessive relaxation of gluten and a decrease of the specific volume. Therefore, powder which was obtained from seeds can be used to the amount of 5% in Recipe 2 for BP, but only in combination with potato flakes to the amount of 5% (Table 1).

In order to relax gluten, one can use a significant amount of sugar in BP recipes (Pashchenko & Zharkova, 2006). For this purpose, Recipe 3 was used to increase the proportion of peel-based and marc-based powders (Fig. 3). The high content of ascorbic acid in the peel-based powder (Table 1) did not allow us to increase its quantity in BP above 3%, but the BP acquired a specific sea-buckthorn aroma, a nice orange tinged crumb colour, and a sweet-and-sour taste. Due to the reduced amount of ascorbic acid in the marc powder, its optimal amount in Recipe 3 for BP was 5%. The further increase in the amount of the peel and marc-based powders in Recipe 3 for BP led to a reduction of the specific volume of BP (Fig. 3), the appearance of a pronounced acidic taste, and also an excessive colour.

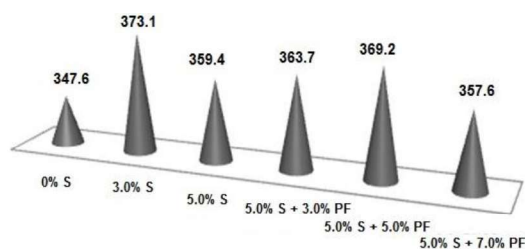


Figure 2. Changes in specific volume, $\text{cm}^3 100 \text{g}^{-1}$, BP with seed-based powder (S), with the addition of potato flakes (PF).

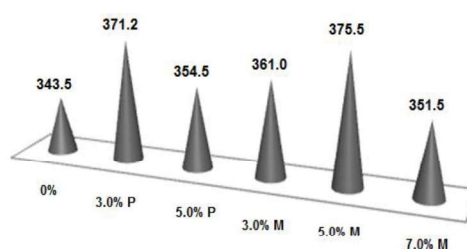


Figure 3. Changes in specific volume, $\text{cm}^3 100 \text{g}^{-1}$, BP with peel-based powder (P) and marc-based powder (M), Recipe 3 (R3).

The most optimal recipes for BP with sea-buckthorn powders are presented in Table 1. The characteristics of quality indicators for the developed BP with sea-buckthorn powders are presented in Table 3.

Table 3. Physico-chemical characteristics of enriched bakery products

Physico-chemical characteristics	Bakery products in powdered form				
	Recipe 2 (R2)		Recipe 3 (R3)		
	Control R2	Seeds	Control R3	Marc	Peel
Moisture, %	39.5 ± 1.0	39.1 ± 1.0	35.3 ± 1.0	35.2 ± 1.0	35.0 ± 1.0
Acidity, deg,	1.7 ± 0.1	3.2 ± 0.1	1.7 ± 0.1	3.4 ± 0.1	3.6 ± 0.1
Porosity, %	71.7 ± 1.3	74.5 ± 1.7	69.3 ± 2.1	72.9 ± 1.8	72.8 ± 1.8
Specific volume, $\text{cm}^3 100 \text{g}^{-1}$	347.5 ± 8.8	369.2 ± 8.8	343.5 ± 9.3	375.5 ± 8.6	371.2 ± 8.9

The sea-buckthorn powders have enriched BP with phenolic compounds that were found both in the crust and the crumb (Table 4). When compared with the control samples, the amount of phenolic compounds in BP crumb which had been produced with the use of seed-based powder increased by 26.7%, those made with marc-based powder by 42.3%, and those made with peel-based powder by 13.5%. The amount of flavonoids in BP was below the figure for phenolic compounds. In the crust, they were found only in BP with the addition of marc and peel-based powder. In the crumb, the amount of flavonoids was less than phenolic compounds by a factor of six in BP with seed-based powders and a factor of three in BP with peel and marc-based powders.

Lowering the temperature of baking increased the amount of phenolic compounds and flavonoids in the crust and crumb of all BP samples which had been produced with the addition of sea-buckthorn powders. The maximum amount of phenolic compounds and flavonoids was contained in the crumb of BP with the marc-based powder, having been baked at a temperature of 200 °C.

The actual content of phenolic compounds and flavonoids in BP with sea-buckthorn powders was below the theoretically possible extent, given their amount in the sea-buckthorn powders and the BP control samples. The loss of phenolic compounds in the crumb of BP which had been produced with the addition of sea-buckthorn powders was between 23.8–26.5%, when baked at 220 °C. Lowering the baking temperature to 200 °C reduced the loss of phenolic compounds by between 7–13%. The content of phenolic compounds in the crust decreased by between 2.2–3.0, depending upon the baking

temperature and the BP recipe. The loss of flavonoids during baking was more significant.

Table 4. The content of ascorbic acid, phenolic compounds, and flavonoids in the crust and crumb of bakery products, per g DM (mean \pm standard deviation)

Bakery products	Ascorbic acid		Total phenolic content		Total flavonoids content	
	Crust	Crumb	Crust	Crumb	Crust	Crumb
<u>Baking at 220 °C</u>						
Control R2	nd	nd	0.05 \pm 0.01	0.45 \pm 0.03	nd	nd
With seeds	nd	nd	0.08 \pm 0.01	0.57 \pm 0.02	nd	0.09 \pm 0.01
Control R3	nd	nd	0.08 \pm 0.01	0.52 \pm 0.01	nd	nd
With marc	nd	0.021 \pm 0.001	0.18 \pm 0.02	0.74 \pm 0.01	0.05 \pm 0.01	0.24 \pm 0.01
With peel	nd	0.015 \pm 0.001	0.15 \pm 0.01	0.59 \pm 0.01	0.06 \pm 0.00	0.18 \pm 0.01
<u>Baking at 200 °C</u>						
Control R2	nd	nd	0.10 \pm 0.01	0.52 \pm 0.02	nd	nd
With seeds	nd	nd	0.18 \pm 0.01	0.72 \pm 0.01	nd	0.11 \pm 0.01
Control R3	nd	nd	0.12 \pm 0.02	0.66 \pm 0.01	nd	nd
With marc	nd	0.032 \pm 0.001	0.25 \pm 0.02	0.95 \pm 0.02	0.12 \pm 0.01	0.28 \pm 0.01
With peel	nd	0.025 \pm 0.002	0.20 \pm 0.01	0.76 \pm 0.02	0.10 \pm 0.01	0.20 \pm 0.01

nd – not detected.

The high baking temperatures completely destroyed the ascorbic acid in all BP crusts. Ascorbic acid was found only in the crumb of BP which had been produced with the addition of sea-buckthorn marc and peel-based powders. Its quantity was up to 30% of the theoretically possible extent, depending upon the recipe used to produce the BP. Baking at the lower temperature of 200 °C made it possible to preserve the ascorbic acid by 50% more, when compared with baking at a temperature of 220 °C.

The presence of individual AO in BP led to an increase in their AOA (Table 5). The AOA of BP with sea-buckthorn powders depended upon the amount and type of the powder: BP with sea-buckthorn marc-based powder > BP with sea-buckthorn peel-based powder > BP with sea-buckthorn seed-based powder.

Table 5. The antioxidant activity of bakery products, μ g Trolox per g DM (mean \pm standard deviation)

Bakery products	DPPH-radical assay		Chemiluminescence	
	Crust	Crumb	Crust	Crumb
<u>Baking at 220 °C</u>				
Control R2	3.19 \pm 0.06	4.54 \pm 0.08	7.10 \pm 0.08	10.50 \pm 0.10
With seeds	4.10 \pm 0.15	6.10 \pm 0.02	8.80 \pm 0.18	12.90 \pm 0.20
Control R3	4.48 \pm 0.10	6.35 \pm 0.12	9.10 \pm 0.08	12.88 \pm 0.14
With marc	7.50 \pm 0.12	10.48 \pm 0.15	14.70 \pm 0.10	20.02 \pm 0.22
With peel	6.25 \pm 0.12	8.95 \pm 0.16	11.00 \pm 0.16	15.28 \pm 0.02
<u>Baking at 200 °C</u>				
Control R2	3.45 \pm 0.06	5.10 \pm 0.09	7.75 \pm 0.10	11.10 \pm 0.18
With seeds	4.45 \pm 0.10	6.55 \pm 0.15	9.50 \pm 0.22	12.96 \pm 0.12
Control R3	4.80 \pm 0.04	6.82 \pm 0.10	9.62 \pm 0.20	13.26 \pm 0.00
With marc	8.00 \pm 0.10	11.45 \pm 0.20	16.10 \pm 0.05	22.40 \pm 0.20
With peel	6.81 \pm 0.10	9.60 \pm 0.05	11.70 \pm 0.10	17.00 \pm 0.25

The experimentally determined AOA of BP was higher than the one that had been calculated theoretically. Therefore, the crumb of BP with a marc-based powder theoretically should have the AOA of 8.55 $\mu\text{g g}^{-1}$ DM and 8.36 $\mu\text{g g}^{-1}$ DM, as determined by DPPH and chemiluminescence methods respectively. But the experimental values of AOA of BP crumb using a marc-based powder (determined according to the DPPH and chemiluminescence methods) were higher by 22.6% and 20.02% respectively. An even greater difference in AOA was recorded for BP which had been produced with the addition of seed-based powder: 24.5% and 28.3% respectively. This tendency is traced not only in the crumb, but also in the crust, irrespective of the baking temperature. But in case of baking at a lower temperature (200 °C), the AOA figures were higher, both for the crumb and the crust. BP crumb had an AOA level that was higher than that of the crust when using two temperature profiles during baking.

Biologically active compounds such as tocopherols and carotenoids which are found in sea-buckthorn powders, something that was not considered in the present study, can also contribute to the AOA of BP, as can melanoidins that are formed during baking (Martins et al., 2001; Nilova et al., 2015).

Melanoidins include mono- and polycyclic lactams – δ -(six-membered ring), γ -(five-membered ring) and β -(four-membered ring). Their AO properties are determined by the system of conjugated double bonds in heterocyclic and quinoid chains (Selemenov et al., 2008). The IR-spectroscopy method makes it possible to identify lactams by intensity of absorption bands of the carbonyl group in the area of 1,680–1,800 cm^{-1} , which is characteristic of mono- and polycyclic γ - and β -lactams. The decrease in the number of members in the ring leads to a shift of bands towards higher frequencies. For a quantitative interpretation of the data obtained, peak areas were used which characterised oscillations in the selected space. The results are shown in Table 6.

Table 6. The characteristics of mono- and polycyclic lactams (β - and γ -forms) of the crust and the crumb of bakery products, relative standard units, \pm standard deviation

Bakery products	Lactams			
	Mono β - & γ -lactams		Poly β - & γ -lactams	
	Crust	Crumb	Crust	Crumb
Baking at 220 °C				
Control R2	49.32 \pm 0.36	73.09 \pm 0.44	77.37 \pm 0.60	110.72 \pm 0.68
With seeds	27.49 \pm 0.40	40.98 \pm 0.40	42.88 \pm 0.72	61.52 \pm 0.70
Control R3	54.18 \pm 0.70	81.20 \pm 0.35	86.82 \pm 0.80	120.55 \pm 0.70
With marc	2.35 \pm 0.45	38.25 \pm 0.60	42.72 \pm 0.82	61.46 \pm 0.65
With peel	28.19 \pm 0.50	41.50 \pm 0.40	46.01 \pm 0.45	63.72 \pm 0.49
Baking at 200 °C				
Control R2	50.08 \pm 0.30	75.10 \pm 0.55	75.85 \pm 0.58	105.40 \pm 0.60
With seeds	28.81 \pm 0.55	41.96 \pm 0.35	41.20 \pm 0.65	57.94 \pm 0.45
Control R3	55.90 \pm 0.38	81.91 \pm 0.30	83.62 \pm 0.72	116.89 \pm 0.70
With marc	28.75 \pm 0.42	42.47 \pm 0.65	39.45 \pm 0.75	57.23 \pm 0.50
With peel	29.80 \pm 0.50	44.00 \pm 0.56	43.70 \pm 0.60	61.06 \pm 0.62

The explored areas of IR spectra for the crust and the crumb of BP had a similar number of bands with different levels of intensity. The intensity of the bands depended upon the recipe and added ingredients, as well as the baking temperature. The different intensity of the bands resulted in a change in the aggregate area of peaks for mono- and

polycyclic lactams. A common pattern was noted for all infrared spectra of BP samples: the total number of lactams was greater in the crumb than in the crust by 1.5 times on average; the predominating elements in the crust and the crumb were polycyclic lactams (60–62%); the share of polycyclic lactams in the crumb was less than that in the crust by between 1–2%. The use of sea-buckthorn powders inhibited formation of cyclic lactams in BP by between 1.8–2 times. The decrease in baking temperature led to the increased intensity of infrared spectra bandwidths of BP, which were characteristic of monocyclic lactams, as concerns the crust and the crumb.

CONCLUSIONS

Powders made from various parts of sea-buckthorn berries (from the marc, seeds, or peel) display AOA that depends upon the content of particular AO. Regardless of the part that is used for preparing such powders, the AOA increased in the following order: seeds < marc < peel.

When using sea-buckthorn powders in the production of BP, it is necessary to consider the high levels of acidity in the powders, something that has an impact on the taste of the products; as well as the high content of ascorbic acid that has a tightening effect on gluten (and a decrease in the specific volume of BP). The maximum usage of powders from sea-buckthorn are best as follows: peel-based powder, 3%; marc-based powder, 5%, with a sugar content of 14.5%; seed-based powder, 5%, with a sugar content of 5%; and potato flakes with a content of 5%.

The use of sea-buckthorn powders in the production of BP increases the amount of AO in them – in other words those phenolic compounds and flavonoids which number less than the theoretically possible extent by between 23.8–26.5%. Ascorbic acid disintegrates under the influence of the high baking temperature; therefore it is absent in the crust of BP, but it is found in the crumb of BP where these have been produced with the addition of peel-based and marc-based powders, to a volume of up to 30% of the theoretical value.

It was established that BP with sea-buckthorn powders have a greater AOA than the control samples. The AOA of BP which use sea-buckthorn powders increases in the following order: BP with seed-based powder < BP with peel-based powder < BP with marc-based powder. The formation of AOA depends not only on the type and amount of sea-buckthorn powders in the BP recipe, but also on the baking temperature. A decrease in the baking temperature results in a reduction of losses specific to biologically active substances, as well as in formation of monocyclic lactams that have an impact on the AOA of BP.

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