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An investigation into the effects of bioactive substances from vegetable oils on the antioxidant properties of bakery products

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Abstract. This article discusses ways in which the antioxidant capacity of bakery products (otherwise referred to as 'BPs') can be increased by adding various types of vegetable oil to the dough: chosen as test oil was unrefined rice bran oil, unrefined pumpkin seed oil, and refined and deodorised sunflower oil. The authors conducted a study of fatty acid compositions and biologically active substances to be found in vegetable oils. The antioxidant properties of vegetable oils were analysed according to the following characteristics: the formation of the primary (peroxide value) and secondary (anisidine value) oxidation products; the oxidation coefficient (IR spectroscopy) which can be determined in the process of applying thermal treatment (with five hours of heating at 120 °C), which leads to the Vitamin E being destroyed. The biochemical composition of vegetable oils affected their resistance to the thermal oxidation process in the following sequence: unrefined rice bran oil > unrefined pumpkin seed oil > refined and deodorised sunflower oil. BPs were made from wheat flour dough with the addition of 4% of the corresponding vegetable oil and 5% of sugar, and were baked at two temperature regimes: at 200 °C and at 220 °C. The antioxidant activity of the BPs was determined by means of two methods: by chemiluminescence, and by DPPH radical assay. The antioxidant activity of the BPs varies depending on the vegetable oil being used, with the differences being revealed in the following way: BPs with unrefined pumpkin seed oil > BPs with unrefined rice bran oil > BPs with refined and deodorised sunflower oil. Any increase in the baking temperature reduced the antioxidant activity of the BPs; the antioxidant properties in the crust and the crumb were reduced at differing rates.

Keywords: sunflower oil, rice bran oil, pumpkin-seed oil, bioactive substances, bakery products, antioxidant properties

INTRODUCTION

The search for natural antioxidants and their use in foodstuffs is becoming quite important in modern food technology research studies. Natural antioxidants, used as food additives, are safe for human health and can have a preventive medical effect when consumed regularly (Galkina, 2013; Nilova et al., 2016). The human habit of consuming bread and bakery products on a daily basis (Akhtar et al., 2011) makes the antioxidant properties of BPs an important object of study.

A good many research studies were devoted to the antioxidant properties of bread and bakery products. It was found that their antioxidant activity is determined by the

type of flour the particular BPs were made from, and by the flour extraction degree. Rye bread bakery products contain more antioxidants than wheat flour bakery products. The higher the degree of flour extraction, the higher the antioxidant properties of the bread (Horszwald et al., 2010; Murzahmetova et al., 2015). Bread and bakery products which are produced using wheat flour with a low degree of extraction contain the lowest volume of antioxidants (Dziki et al., 2014; Karrar, 2014). Various herbal additives are used to increase the BP antioxidant properties: pseudocereal flour (Chlopicka et al., 2012), powders, juices, extracts of berries (Meral & Doġan, 2012; Nilova et al., 2015), fruits (Belyavskaya & Rodicheva, 2013, Ummi et al., 2015), vegetables (Raba et al., 2007), seeds (Das et al., 2013; Jaisanthi et al., 2014), and other plants (Gawlik-Dziki et al., 2013). These ingredients display high antioxidant activity levels due to the water-soluble antioxidants – polyphenols and Vitamin C - but the content of these ingredients in bread and BPs is limited (Meral & Doġan, 2012; Dziki et al., 2014; Nilova et al., 2015) as they affect the sensory properties of the BPs. During the baking process, Vitamin C is almost completely destroyed, which leads to a decrease in the antioxidant properties.

It is possible to improve the antioxidant properties of BPs with the use of lipid-soluble antioxidants, which are present in vegetable oils. The use of oils and fats is essential in the production of BPs; fats carry out important technological and sensory functions (Pashchenko & Zharkova, 2006). Margarine, vegetable oils, shortenings, and other special oils and fats can be used as fatty components (Nechaev, 2013). Research on bakery products which are sold on the Italian market (Mignogna et al., 2015) showed that only in breads made with the use of vegetable oils were the required amount of tocopherols present. The use of margarine, even enriched with phytosterols, α-tocopherol and β-carotene, in BPs, increases the content of trans fatty acids up to 4.29 ± 1.48% – this exceeds the maximum allowed norms which have been adopted in the European Union (Quílez et al., 2006). Various refined deodorised oils are used in BP production in order to stabilise the sensory properties of BPs. (Nechaev, 2013; Mignogna et al., 2015). High temperature regimes used in refining and deodorising vegetable oils can decrease the levels of biologically active substances (BAS) (such as, for example, tocopherols) by as much as 30–70% (Zolochevsky, 2009). The baking process triggers the oxidation both of fatty acids and of BAS. The higher the quantity of BAS in vegetable oils, the less change occurs in them during the baking process (Caponio et al., 2013), and the lower is the level of destruction of tocoferols – the most active lipid-soluble antioxidants (Nyström et al., 2007; Nilova et al., 2013).

Rice bran or pumpkin seed oils can be used as the source of lipid-soluble antioxidants. Rice bran oil contains not only tocoferols and tocotrienols, but also γ-orysanol. The joint presence of γ-orysanol and tocopherols causes a synergistic effect in the antioxidant properties of rice bran oil, both when used in its natural form or as a food additive (Patel & Naik, 2004; Juliano et al., 2005; Lerma-Garcia et al., 2009). Pumpkin seed oil is a source of carotenoids, phospholipids, sterols, flavonoids, and tocopherols, but its chemical composition and antioxidant properties strongly depend on the raw products. Pumpkin seed oil obtained from *Cucurbita pepo* L contains the highest amounts of BAS – which increases its antioxidant properties (Rezig et al., 2012; Nawirska-Olszańska et al., 2013).

The aim of the present study is to investigate the composition of biologically active substances (BAS) and the antioxidant properties of rice bran oil and pumpkin seed oil,

and determines their role in enhancing the antioxidant properties of bakery products which are made using wheat flour.

MATERIALS AND METHODS

The materials and methods used in vegetable oil research

An unrefined rice bran oil and an unrefined pumpkin seed known as 'Dial-Export' oil, which are both produced by Butas LLC, were used as sources of lipid-soluble antioxidants.

The fatty acid composition of vegetable oil was determined by means of the gas chromatographic method, using the Agilent 6890 Series chromatograph (Agilent Technologies, Inc, USA), which was equipped with a DB-23 fused silica capillary column (60 m × 0.25 mm ID × 0.25 μm), and was used under the following conditions: the initial column temperature of 70 °C was gradually increased at a rate of 10 °C a minute^{-1} until it reached a maximum of 180 °C. This temperature was held for ten minutes, and then increased at a rate of 5 °C a minute^{-1} until it reached a final temperature of 220 °C which was held for eighteen minutes. Helium was used as the carrier gas at a flow rate of 4.6 ml per minute^{-1}.

The level of γ-orysanol was estimated by using the spectrophotometric method at a wavelength of maximum absorption of about 315 nm; the oil sample was previously dissolved in n-heptane (Srisaipet & Nuddagul, 2014); the levels of Vitamin E were estimated by means of the Emmeri-Engel spectrophotometric method with orthophenanthroline at a wavelength of maximum absorption of about 520 nm (Trineeva, 2013).

The qualitative composition of BAS was determined by means of the gas chromatography-mass-spectrometry method, using the MAESTRO 7820A gas chromatograph equipped with a 5975 mass selective detector with electron impact ionisation (70 eV), under the following conditions: a fused silica capillary column, Rxi – 5 ms (30 m × 0.25 mm × 0.25 μm); an injector temperature of 280 °C, a detector interface of 280 °C, an initial column temperature of 50 °C, a final column temperature of 280 °C, and a column heating rate of 15 °C min^{-1}. The carrier gas (Helium) was flown at a linear velocity of 1.0ml per minute^{-1}. The volume of the injected sample was 1 μl. The sample was injected with a split ratio of 1:30. Detection of the mass spectra was carried out on the full ion current in the positive ion scanning mode, in the range of masses 40–800 m z^{-1}. The mass spectra obtained from this were identified with the use of the equipment's electronic mass spectra library (library NIST11.L, DD2011.L).

The antioxidant properties of vegetable oils were determined by means of the detection of the primary (peroxide value) and secondary (anisidine value) oxidation products, and the thermal oxidation coefficient (IR-spectroscopy) in which Vitamin E is destroyed (after heating for five hours at 120 °C). The degree of thermal oxidation was recorded every hour.

The peroxide value (milliequivalents per kg) was determined by means of the potentiometric (BS EN ISO 27107:2010) method with the use of the ATP-02 automatic titrator, 'Akvilon', Russia. A test sample of vegetable oil was dissolved in isooctane and anhydrous acetic acid with the addition of potassium iodide.

The anisidine value was determined with the use of the SHIMADZU 1240 spectrophotometer (SHIMADZU, Japan) at a wavelength of 350 nm in cuvettes with an

optical path length of 10 mm in a test solution of oil in isooctane after its reaction with an acetate solution of paraanisidin (ISO 6885: 2016).

The oxidation coefficients were determined by calculating the ratio of value peaks in the IR spectra of plant oils: $K_1=A_{2850}/A_{3030}$; $K_2=A_{1465}/A_{3030}$; $K_3=A_{3450}/A_{2850}$; $K_4=A_{3450}/A_{1455}$ (Tokassado et al., 1979).

IR spectra were registered with the 'FSM 1202' FT-IR spectrometer (Monitoring LLC, Russia), with automatic calculation of the peaks in respect to the baseline. The spectra registration parameters were as follows: the spectral range was between 400–4,000 cm^{-1}; the number of scans carried out was twenty; the resolution was at 4cm^{-1}; the mode was an interferogram. The absolute accuracy of the wave-number scale calibration did not exceed ± 0.1 cm^{-1}. Deviation of the 100% transmission line (1,950–2,050 cm^{-1} at a resolution of 4 cm^{-1}, over the course of twenty scans) was less than % ± 0.5. The standard deviation of the 100% transmittance line (1,950–2,050 cm^{-1} with a resolution of 4 cm^{-1}, over the course of twenty scans), did not exceed 0.025%. The resulting interferograms were converted to the absorption spectra and peaks were identified (Silverstein, 2011).

The materials and methods used for research into bakery products

Bakery products were made from wheat flour (gluten 28.9%, ash content 0.55), supplemented with 4% vegetable oil and 5% sugar. Test samples differed only by the type of vegetable oil additive. The following types of vegetable oils were used: refined and deodorised sunflower oil produced by EFKO FOODS PLC, Russia (the control); 'Dial-Export' rice bran refined oil, and 'Dial-Export' unrefined pumpkin seed oil (Butas LLC, Russia). Sample products of 100 g were baked under two temperature regimes: at 200 °C for 25 minutes, and at 220 °C for twenty minutes.

A determination of water soluble antioxidants in bakery products

The process of determining water soluble antioxidants in bakery products was conducted with the use of aqueous extracts of crumb and crust, which were obtained by extracting crumb and crust samples that had previously dried at 40 °C to a moisture content of 6.5–7.0%. A total of 250 mg crumb and crust samples were divided into a powder, diluted with 10ml of distilled water, and centrifuged for ten minutes at 3,500 rpm. A total of 1ml of the resulting extract was used for the purposes of antioxidant determination. The ratio of each subsequent dilution was 1:2.

The antioxidant activity of aqueous extracts was determined by means of the chemiluminescence method with the use of the BCL-06M biochemiluminometer (Nizhny Novgorod, Russia) in a model system which contained riboflavin, hydrogen peroxide, and ferrous iron (Putilina et al., 2006).

The chemiluminescent reaction of the riboflavin (that of the sample substrate) was initiated in the presence of ferrous and hydrogen peroxide ions. A total of 610μL of potassium phosphate buffer (with a pH of 7.5), 40μL of 10mM riboflavin solution, 100μL of physiological saline, and 25mM of ferric sulphate solution (II) (the model system) were added to the measuring cuvette of the BCL-06M biochemiluminometer. To initiate the process, a 0.1% H_2O_2 solution was used. In determining the antioxidant activity of the test samples, physiological saline was replaced with the relevant extracts at different concentration levels. The calibration curve was built up for trolox 97%, Acros Organics, USA.

A determination was carried out of free radical scavenging activity by means of the Glevindu method (Rogozhin & Rogozhina, 2015). This method is based on the principle of scavenging the DPPH radical (1,1-diphenyl-2-picrylhydrazyl).

Hydroalcoholic extracts of crumb and crust samples were prepared in the same manner as described above, but the extraction was carried out with the use of 50% ethanol solution. The DPPH solution was prepared by dissolving a 5mg test sample in 5ml of 16.4 M (96%) ethanol heated on a bain-marie until dissolved. A total of 0.2ml 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).

Each bakery product sample has undergone researched in triplicate. The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel. The data gained through this process are presented with a confidence coefficient of 0.95.

RESULTS AND DISCUSSION

All of the vegetable oil samples belonged to the linoleic-oleic group of vegetable oils by fatty acid composition (Table 1). Similar fatty acids – linoleic, oleic, and palmitic – dominated amongst these. The levels of other fatty acids did not exceed 5%. The pumpkin seed and sunflower oils were richer in linoleic acid, while the rice bran oil showed more oleic acid. The preponderance of linoleic acid with its two double bonds, especially in sunflower oil, makes it less resistant to thermal treatment. The oxidation depends on the level of biologically active substances with antioxidant properties.

Table 1. Fatty acid composition of vegetable oils*, %

Type of oil	Fatty acid									
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{20:2}$
Rice bran	0.89	17.35	0.19	2.05	43.71	33.10	1.01	0.60	0.53	0.57
Pumpkin-seed	-	10.47	-	3.87	32.95	52.34	-	0.37	-	-
Sunflower	0.07	6.33	0.08	4.51	18.49	69.28	0.09	0.28	0.14	0.73

* The difference between the values of two determi-nations for constituents present in excess of 5% (m m^{-1}) not exceed 1.5% (relative) of the determined value; for constituents present in smaller quantities, the difference not exceed of 0.05% (m m^{-1}) of the determined value

Natural vegetable oils contain bioactive substances (BAS) such as tocopherols and tocotrienols, sterols, phospholipids, and carotenoids which display antioxidant properties. However, during the production process, especially with the use of certain refining methods, antioxidants in vegetable oils can sufficiently deteriorate (Zolochevsky, 2009). The oils being studied differed by their BAS quantitative and qualitative composition (Table 2). Refined and deodorised sunflower oil hardly contained any antioxidants, except Vitamin E, (α-tocopherol only), the amount of which was less 1.79 and 3.37 times respectively when compared to rice bran and pumpkin seed oil.

Although it contained 1.88 times less Vitamin E than pumpkin seed oil, rice bran oil is richer in antioxidant properties due to the presence of γ-orysanol and sterols. Pumpkin seed oil has the highest content of Vitamin E, represented primarily by β- and γ-tocopherols and squalene. Of phytosterols, only β- and γ-sitosterol were identified. Vegetable oil BAS do not only help to prevent oxidative processes during baking, but also enrich bakery products with antioxidants. The antioxidant properties of vegetable oils were studied by the intensity of the oxidative processes during thermal exposure at 120 °C. At this temperature, Vitamin E begins to decay. Fig. 1 shows the data taken from studies of various oil peroxide and anisidine values registered during the thermal oxidation process.

During the first hour of thermal oxidation almost no changes in peroxide values occurred in all test vegetable oil samples.

Table 2. Biologically active substances of vegetable oil samples

Bioactive substances	Type of oil		
	Rice bran	Pumpkin-seed	Sunflower
Vitamin E, mg $100g^{-1}$	98.8 ± 1.8	186.2 ± 2.9	55.3 ± 1.3
γ-orysanol, mg $100g^{-1}$	565.49 ± 9.12	-	-
Qualitative composition			
Tocopherols*:*			
α-tocopherol	+	-	+
β-tocopherol	-	+	-
γ- tocopherol	+	+	-
Sterols*:*			
β-sterol	+	+	-
γ-sterol	+	+	-
Campesterol	+	-	-
Stigmasterol	+	-	-
Squalene	-	+	-

During the next few hours, the intensive formation of peroxides occurred only in sunflower oil due to the high content of linoleic acid in which the oxidation rate is 27 times higher than in oleic acid (Nechaev, 2013). The only antioxidant present in the sunflower oil – α-tocopherol – is oxidised at this temperature at a faster rate than any other tocopherol. Its quantity is sufficient to inhibit the oxidation processes for one hour only, so after two hours of thermal oxidation the peroxide values increased by almost double. During the third hour the oxidation rate increased sharply, and the peroxide value rose beyond acceptable limits. Further changes were associated with a higher rate of peroxide destruction when compared to their formation, leading to an increase of secondary oxidation products. During the fourth hour of thermal oxidation in the sunflower oil the anisidine value exceeded three units.

In rice bran and pumpkin seed oil the oxidation rate was significantly lower due to the presence of a wide range of BAS which was rich in antioxidant properties. Changes in the values of peroxide figures were insignificant and did not exceed three milliequivalents per kg during all five hours of thermal oxidation. The oxidation processes in pumpkin seed oil were more pronounced when compared to rice bran oil,

especially in terms of the formation of secondary oxidation products. During the fourth hour their number increased dramatically.

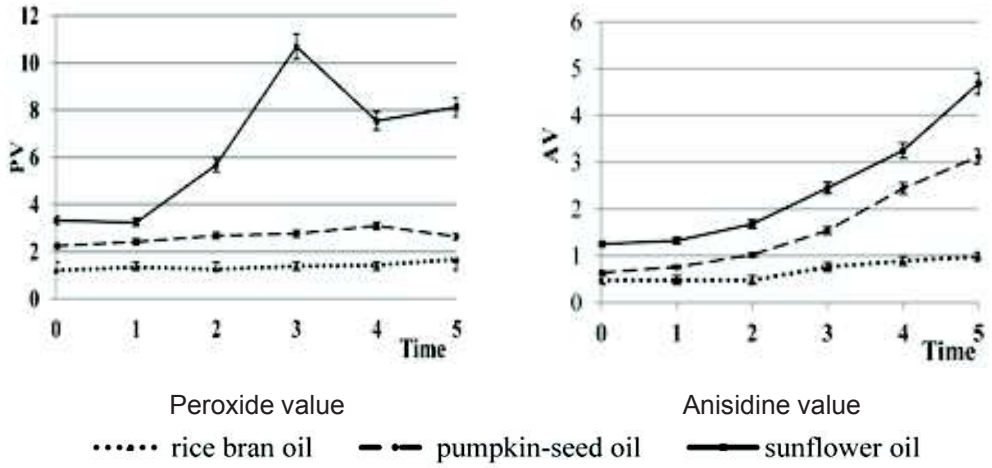

Figure 1. Peroxide (PV) and anisidine (AV) values during thermal oxidation process (hour).

The different character of the oxidative processes in the test vegetable oil samples was reflected in the change of IR absorption spectra during the five hour thermal oxidation process (Fig. 2).

Visible changes in sunflower oil occurred after two hours of thermal oxidation; in the pumpkin seed oil this took place in the fourth hour; and in the rice oil it happened only by the end of the fifth hour. In order to be able to compare the degree of oxidation in vegetable oils which were different in their original composition of fatty acids and bioactive substances, Tokassado et al. (1979) introduced oxidation coefficients which reflect the intensity of the absorption bands (in the IR spectrum) of the more oxidation-susceptible components versus the more stable ones (C-H deformation and stretching vibrations in the CH_2 group, at frequencies of 1,465 and 2,850 cm^{-1}).

Oxidation coefficients for the 3,008 cm^{-1} band (K_1 and K_2) mark the oxidative chain initiation stage, while for the 3,450 cm^{-1} band (K_3 and K_4), they mark the oxidative chain termination stage, which results in the formation of the secondary products of oxidation. Oxidation coefficients in the vegetable oil samples were calculated at the start of visible changes in the IR spectra (Table 3). Subsequent oxidation could have led to the disappearance of stretching vibrations in the 3,450 cm^{-1} band. Therefore, the pumpkin seed oil sample, in the fifth hour of thermal oxidation, demonstrated a lack of this band, probably due to polymerisation processes.

The depth and velocity of oxidation are directly dependent upon the amount of polyunsaturated fatty acids and the degree of their unsaturation. Therefore, the oxidation of linoleic acid, which is prevalent in sunflower oil, is more intense due to the opening of double bonds and the formation of oxidised molecular products that occur earlier than they do in oleic acid. This resulted in an increase of oxidation coefficient values in sunflower oil after two hours of thermal oxidation. For the 3,007 cm^{-1} band, the oxidation coefficients (K_1 and K_2) in sunflower oil after two hours of heating were greater than

they were in pumpkin seed oil after four hours of heating, or in rice bran oil after five hours of thermal oxidation.

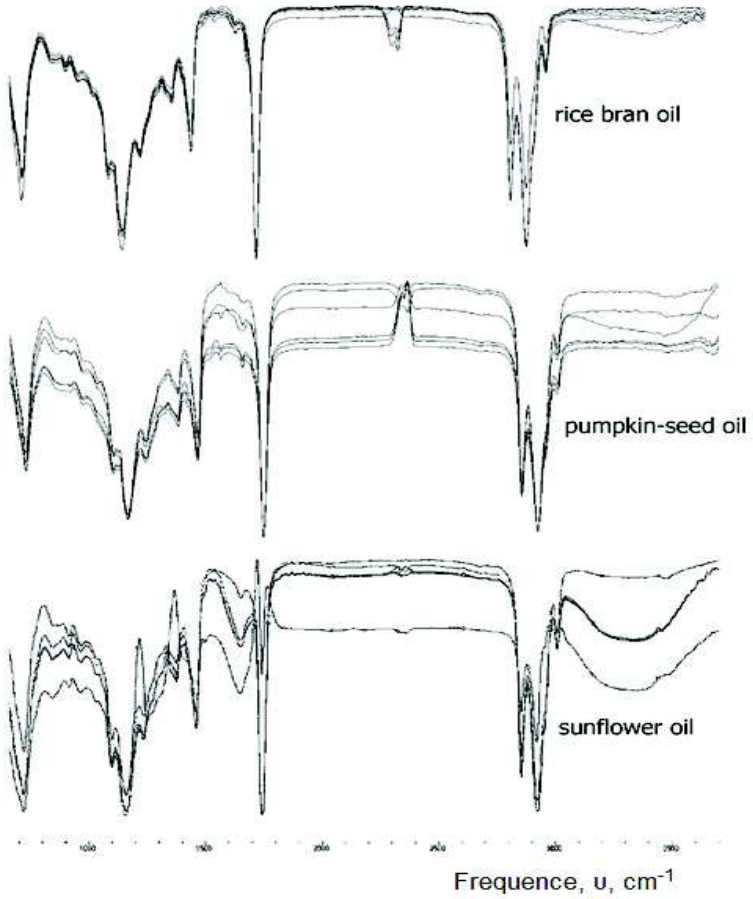

Figure 2. IR spectra of vegetable oils for 5 hours thermal oxidation.

The absorption area of 3,200–3,700 cm^{-1} is characteristic for stretching vibrations in the free OH group. The appearance of marked fluctuations in this area can be attributed to the formation of the secondary products of lipid oxidation such as, for example, hydroperoxides and hydroxy compounds (Silverstein, 2011). But stretching vibrations in this area were different for different oils. In sunflower and pumpkin seed oil, the indexes K_3 and K_4 reached almost similar values, but within different time periods – after two and four hours respectively. In rice bran oil changes occurred only after five hours of thermal oxidation, with a demonstrated velocity a hundred times less than for the 3,008 cm^{-1} band (K_1 and K_2). This shows that the formation of secondary oxidation products (K_3 and K_4) in the oil being studied occurred a hundred times less intensively than the process of oxidative chain emergence (K_1 and K_2).

Table 3. Oxidation coefficients of the vegetable oil samples at the start of visible changes in the IR spectra

Type of oil	Oxidation time, h	Oxidation coefficients			
		3,007 cm^{-1} band		3,450 cm^{-1} band	
		K_1	K_2	K_3	K_4
Rice bran	5	4.174 ± 0.090	3.275 ± 0.080	0.014 ± 0.001	0.023 ± 0.001
Pumpkin-seed	4	3.223 ± 0.078	2.202 ± 0.049	2.299 ± 0.030	3.469 ± 0.051
Sunflower	2	4.458 ± 0.105	3.812 ± 0.076	2.364 ± 0.044	3.541 ± 0.068

Thanks to their resistance to thermal treatment, the test vegetable oil samples were categorised in the following order: unrefined rice bran oil > unrefined pumpkin seed oil > refined and deodorised sunflower oil.

Biologically active substances which are present in vegetable oils affected the antioxidant properties of BPs (Table 4). The unrefined rice bran oil and pumpkin seed oil increased antioxidant properties in bakery products by almost double (by means of the DPPH method) when compared to bakery products that contained sunflower oil. By means of the BAS effect on the antioxidant activity of bakery products, the following sequence was noted: BPs with pumpkin oil > BPs with rice oil > BPs with sunflower oil.

Table 4. Antioxidant activity of bakery products, μg Trolox per g DM

Bakery products	DPPH-radical assay		Chemiluminescence	
	crust	crumb	crust	crumb
Baking at 220 °C:				
Rice bran oil	7.12 ± 0.12	8.06 ± 0.15	10.98 ± 0.21	11.77 ± 0.25
Pumpkin-seed oil	7.92 ± 0.11	9.84 ± 0.15	23.00 ± 0.24	16.14 ± 0.23
Sunflower oil	3.18 ± 0.07	4.56 ± 0.10	7.13 ± 0.17	10.51 ± 0.22
Baking at 200 °C:				
Rice bran oil	7.61 ± 0.16	8.49 ± 0.14	11.76 ± 0.19	12.40 ± 0.21
Pumpkin-seed oil	8.52 ± 0.15	10.36 ± 0.13	21.82 ± 0.22	17.01 ± 0.28
Sunflower oil	3.44 ± 0.07	5.11 ± 0.08	7.75 ± 0.17	11.13 ± 0.26

Due to the high temperature impact, the antioxidant activity in the crust was less than that of the crumb. During baking the crumb is warmed up to only 95 °C while the crust gets heated to 220 °C (Pashchenko & Zharkova, 2006). Vitamin E, contained in the crust, is destroyed more intensively at such high temperatures. Therefore the antioxidant activity of the crust which contained pumpkin seed oil was less than it was in the crumb, by a factor of 19.51%, while the crust with its rice bran oil which contained less Vitamin E, displayed a difference of only 11.66%. But despite the synergism of γ-orysanol and Vitamin E in rice bran oil (Juliano et al, 2005), the antioxidant activity of BPs which contained rice oil was lower than it was with BPs which contained pumpkin oil. Apparently, much of the BAS antioxidant activity was spent to prevent the oil's fatty acids from being oxidised.

The baking of BPs at a lower temperature (200 °C) led to an increase in the antioxidant activity values, both in the crumb and in the crust. During baking at a higher temperature, the crumb displayed better antioxidant activity than did the crust.

The antioxidant activity of aqueous crust and crumb extracts depends, firstly, on the amount of phenolic compounds in the flour (Raba et al., 2007; Horszwald et al., 2010) and, secondly, on melanoidins which are formed during baking (Belyavskaya et al., 2013; Nilova et al., 2015). Wheat flour, especially of a low extraction degree, contains the least amount of phenolics (Dziki et al., 2014). In bakery products which are made from the same flour, the antioxidant activity values of aqueous extracts with different vegetable oils depend on the amount of melanoidins formed during baking. The antioxidant activity of the crumb aqueous extracts, detected by means of the chemiluminescent method, was higher than in the crust, in BPs with rice bran and sunflower oil by 7.19 and 4.74% respectively. Contrary to this finding, in BPs with pumpkin seed oil the crust displayed a higher antioxidant activity than did the crumb. A lower baking temperature increased the antioxidant activity of aqueous extracts, more so in the crumb than in the crust.

CONCLUSIONS

Unrefined vegetable oils – rice bran oil and pumpkin seed oil – contain a wide range of biologically active substances which are rich in antioxidant properties. They differ both in terms of the quantitative content of Vitamin E, which is 1.88 times higher in the pumpkin seed oil than it is in the rice bran oil, and by their qualitative composition. Pumpkin seed oil contains β- and γ-tocopherols, while rice bran oil is rich in α- and γ-tocopherols. The rice oil contains γ-orysanol and sitosterol, while the pumpkin seed oil shows the presence of squalene and only β- and γ-sitosterol from the sterols group.

The lack of biologically active substances (with the exception of α-tocopherol), and the high content of linoleic acid in the refined and deodorised sunflower oil, led to accelerated oxidative processes during thermal treatment. The visible changes in the IR spectra occurred after two hours of thermal oxidation, and by the third hour the peroxide value exceeded ten milliequivalents per kg.

A wide range of biologically active substances in the rice bran oil and pumpkin seed oil slows down oxidative processes that occur during thermal oxidation. In the rice bran oil the oxidative processes proceeded at a slower rate, leading to changes in the IR spectra only after five hours of thermal oxidation. During thermal treatment, processes prevailed which were characteristic of the initiation stage of the oxidative chain reaction rather than of the termination stage: oxidation coefficients for the 3,008 cm^{-1} band (K_1 and K_2) exceeded those for the 3,450 cm^{-1} band (K_3 and K_4) by double the amount. In pumpkin seed oil, changes in the IR spectra occurred after four hours of thermal treatment: processes which were characteristic of the initiation stage of oxidative chain reaction and the termination stage proceeded at a similar rate. The oxidation values differed only slightly, and were of the same order.

Unrefined rice bran oil, pumpkin seed oil, and sunflower oil which is used as additives in bakery products contributed to an increase in antioxidant activity in BPs in the following order: BPs with pumpkin seed oil > BPs with rice bran oil > BPs with sunflower oil. The total antioxidant activity (as determined by means of the DPPH method) was higher in the crumb than it was in the crust, and increased with a decreasing baking temperature. The same tendency was characteristic of the antioxidant activity in aqueous extracts of crust and crumb as determined by chemiluminescence. The only

exceptions were BPs with pumpkin seed oil: the antioxidant activity of aqueous extracts in the crust was higher than that in the crumb.

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