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The impact of plant powders on acrylamide content in bakery products

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Abstract. This work is devoted to studying acrylamide (ACR) formation and the changes in its levels display during the storage in bakery products (BP) made of wheat flour enriched with plant powders (in the optimal amounts established earlier): blueberry – 3%; pine nut – 6%; rowan – 5%; sea buckthorn – 5%. BP were baked at two temperatures – 220 and 200 °C. ACR level was determined with the use of 'Kapel 105 M' capillary electrophoresis system in various BP parts (crust, sub-crust layer, crumb) 3 and 24 hours after baking. ACR formation differed in different BP layers. All plant powders slowed down its formation in the crust and the sub-crust layer. The process was influenced by formation of heterocyclic compounds (lactams) as a result of the Maillard reaction. In the crumb, ACR formation depended on the type of the used plant powder. In BP cooked with blueberry and rowan powders, the ACR level decreased, while in BP cooked with sea buckthorn and pine nut powders, it increased in comparison with other layers. Lowering the baking temperature helped to decrease acrylamide formation by 15–20% in the crumb and by 25–35% in the crust. After storing BP for 24 hours, a decrease in the ACR level was found, mainly in the crust and crumb. The intake of ACR in the human body of 70 kg when used with 100 g of BP enriched with plant powders will come to 0.16–0.2 µg. Lowering the baking temperature will decrease ACR level by 3–6%.

Key words: bakery products, acrylamide, crust, sub-crust layer, crumb, plant powders, storage.

INTRODUCTION

Healthy lifestyle trend has promoted the use of raw vegetable and plant supplements, rich in dietary fiber, biologically active substances and antioxidants, in production of bread and bakery products (BP) (Dziki et al., 2014; Kurek et al., 2015; Nilova & Pilipenko, 2016). The main idea is to maximize the level of plant supplements without compromising sensory properties of BP (Bagryantseva et al., 2010; Keramat et al., 2011). All BP, including the enriched ones, along with useful substances, may contain acrylamide (ACR) formed at the final production stage of baking, which has a toxic and carcinogenic effect (Bagryantseva et al., 2010; Keramat et al., 2011). The level of ACR in bread, BP and biscuits can reach up to 3,000 µg kg^{-1} (Mustafa, 2008; Bagryantseva et al., 2010; Krishnakumar & Visvanathan, 2014), with 7.5 times more in biscuits and crackers than in bread and BP (Friedman, 2003).

Formation of ACR depends on the type of flour and other prescription ingredients. Rye bread contains more ACR than wheat bread. Usage of higher yield flour, as well as multi-cereal, potato, and corn supplements in bread production stimulate formation of ACR (Claus et al., 2008; Capuano et al., 2009; Horszwald et al., 2010; Przygodzka et al., 2015). One can significantly reduce ACR levels in BP by regulating technological process of production (duration of the dough fermentation, usage of enzyme preparations and starters, regulation of temperature and steam during baking, selecting a specific type of oven) (Ahrné et al., 2007; Mustafa, 2008; Keramat et al., 2011; Kumar et al., 2014; Przygodzka et al., 2015; Bartkiene et al., 2017). Even long fermentation, typical of sourdough systems, can reduce levels of the amino-acid asparagine that is a precursor of acrylamide formation, by tens of times (Fredriksson et al., 2004).

Plant supplements have an ambiguous impact on ACR formation in BP. Fruits, berries, nuts and their products contain substances that are precursors of ACR. Fruits and berries are distinguished by a high content of glucose and fructose, the content of which varies from their type, place of growth and other factors. So, in lowbush blueberry cultivars the amount of glucose and fructose ranges from 3.69 to 10.35%, depending on the botanical variety (Kalt & McDonald, 1996). Their amount in cultivated (some varieties) and wild blueberries and can be the same (Klavins et al., 2015). But the protein content is very low and does not exceed 0.8%, so the study of the amino acid composition is usually not carried out. The content of glucose and fructose in sea buckthorn averages 3–6%, in some varieties up to 9% (Kuhkheil et al., 2017; Zemtsova, 2017), the asparagine content is 427 mg 100 g^{-1} (Oua et al., 2010, Bal et al., 2011). Nuts and their products contain мinor saccharides include glucose (0%–0.27%), fructose (0% –0.17%), maltose (0% –0.2%), but a lot of proteins, in particular pine nut (13.7–15.2%) Acidic amino acids (aspartic acid + glutamic acid) predominate in tree nuts. Aspartic acid content varies from 0.3 to 2.7%, in pine nuts - 2.2% (Nergiz & Donmez, 2004; Chang et al., 2016). Unfortunately, the data on the content of free asparagine - the predecessor of ACR is not installed. Functional groups of polyphenols in raw plant materials, having a variety of structures, can react with ACR precursors, with chemicals formed at intermediate stages of reaction, or with ACR itself, leading to a decrease of ACR levels in BP or, conversely, stimulating its formation (Liu et al., 2015). There is evidence that the formation of ACR reduces to a greater degree oxidized antioxidant than non-oxidized (Oua et al., 2010).

Plant supplements that minimize formation of ACR do not always provide optimal sensory BP properties. For example, the epicatechingallat obtained from green tea, when added to wheat bread in the amount of 3.3–9.9 g kg^{-1} (i.e. less than 1%), reduced the formation of ACR by 34–37%, while impairing the color and texture of the product (Fua et al., 2018). The use of green tea extract in bread sticks contributed to minimizing ACR formation when added in the amount of 0.1%, and in bread crisps – 0.5%. At the same time sensory properties of the products, especially their texture, significantly worsened (Zhang & Zhang, 2007; Capuano et al., 2009). The use of pomegranate flower extract together with vitamin B3 in fried donuts production reduced formation of ACR. When used in the amount of 0.07 and 1.97% respectively, the above supplements did not alter sensory properties of the donuts but in this case, the ACR level was reduced only by 11.8% (Ashkezari & Salehifar, 2018). Replacing wheat flour with date seeds powder in pita bread enriched it with common phenolic compounds, including epicatechins. A slight decrease in ACR formation (in comparison with the regular pita bread) was established only when the level of the date seeds powder was 5%. When the level of the date seeds powder reached 10–20%, ACR formation increased (Platat et al., 2015). Lack of a linear dependence between the amount of plant supplements and the formation of acrylamide is shown by examples of bamboo leaves powder, date seeds powder, green tea extract, and rosemary extract (Zhang & Zhang, 2007; Hedegaard et al., 2008; Platat et al., 2015).

One can obtain a zero ACR level in BP by adjusting the type and amount of plant supplements, baking temperature, and time of baking. For example, pita bread enriched with lyophilized mint fennel and turmeric extracts in different proportions may not contain ACR, but its organoleptic characteristics are worsened (Namir et al., 2018).

BP with plant supplements, suitable for commercial use, must have acceptable color, good porosity and taste. Thus, plant powders with high level of organic acids and ascorbic acid that strengthen gluten (i.e. made from mountain ash pomace and sea buckthorn powder) should be used in BP recipes with high sugar level (Nilova, 2012; Nilova & Malyutenkova, 2018). Blueberry and pine nut powders contain reducing sugars, so they can be used in production of BP with low sugar level. However, plant powders with high level of anthocyanins, such as blueberry powder (Nilova, 2012; Nilova et al., 2015), affect color quite strongly. Adding them in the amount of more than 3% leads to the appearance of a blue tint in BP, and this lowers its commercial value. At the same time, due to a higher level of reducing sugars in them (as well as in pine nut powder), they can be used in production of BP with low sugar levels. Therefore, when using plant powders in BP production it is very important to optimize sensory properties of BP in order to make a commercially suitable product.

This work was aimed at studying the effects of plant powders on the formation of acrylamide and its change during storage in BP of established recipes in terms of producing a valuable commercial product.

MATERIALS AND METHODS

Bakery products recipe and baking

The BP were produced by a straight dough method from wheat flour (gluten 28.9%, ash content 0.55) according to two recipes with different content of sugar and vegetable oil (i.e. control samples) and the bakery products (BP) made with the added different plant powders (in their optimal ratio, as calculated before) (Nilova, 2012; Nilova et al., 2015; Nilova & Malyutenkova, 2018). The following plant powders were used: blueberry powder, pine nut powder, rowan powder, and sea buckthorn powder. The powders of blueberry, rowan and sea buckthorn were obtained from squeezed berries, dried at 50 to 55 °C (to a moisture level of 6%) and milled into powder. The powders contained glucose and fructose in an amount, %, 11.5, 10.2, 5.6, respectively for blueberries, rowan and sea buckthorn (Shelenga et al., 2015). The content of aspartic acid was 0.62% for rowan and 2.6% for sea buckthorn. Pine nut powder produced by Specialist LLC, Russia, contained 1.2% of glucose and fructose, 34% of protein, and 3.5% of aspartic acid (Nilova, 2012; Nilova et al., 2017). BP recipes presented in Table 1. Baking of products weighing 100 g was made at two temperatures: 200 °C – 25 minutes and 220 °C – 20 minutes. After the bakery products were cooled down to the room temperature, identical samples underwent the specimen preparation procedure at once and after storage in polymer film during 24 hours. The crust, the layer under the crust with a thickness of 1 cm and the central crumb were separated from the bakery products. Such different parts of the bakery products were dried separately in a cupboard drier at a temperature of 50 °C until the constant weight was reached.

Table 1. Dough recipes

Ingredient, g	Bakery products 4% fat			Bakery products 14.5 % fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Wheat flour	1,000	970	940	1,000	950	950
Blueberry powder	-	30	-	-	-	-
Pine nut powder	-	-	60	-	-	-
Rowan powder	-	-	-	-	50	-
Sea buckthorn powder	-	-	-	-	-	50
Sugar	50	50	40	145	145	145
Sunflower oil	40	40	40	145	145	145
Yeast	20	20	20	20	20	20
Salt	15	15	15	15	15	15

Preparation of samples for the test

Acrylamide was extracted from the component parts of the bakery products: 50% (by volume) with ethanol – 5 mL per 300 mg of the grinded air-dried sample after homogenization – in the ultrasonic bath (250 Wt, 18 kHz) at 20 °C during 30 min. The extract was decanted and clarified by centrifugation (10 min., 3,000 g).

Determination of acrylamide in BP

For acrylamide identification, the 'Kapel 105 M' capillary electrophoresis system was used (NPF Lumex OJSC, Russia) with a quartz capillary having a diameter of 75 µm and total length of 60 cm, effective length of 50 cm (Kruchina-Bogdanov et al., 2018). 50 мM sodium tetraborate, pH 9.2, was used as background electrolyte (BGE). The capillary was washed before the sample was injected: 0.5 M HCl – 5 min., water – 5 min., 0.5 M NaOH – 5 min., water – 5 min., BGE – 10 min.

The capillary was thermostated at a temperature of 20 °C during 10 minutes. Detection was carried out at 202 nm. The detection threshold (signal: noise = 3:1) was 90 µg kg^{-1}. Hydrodynamic sample injection: 50 mbar 15 s.

Electrophoresis was conducted under the voltage of 10 kV.

E-O converter marker (electroosmotic flow) – benzyl alcohol (99.9%), UAHIM, Russia. Acrylamide produced by Reanal, Budapest, Hungary, was used as reference substance.

Calibration series with external standard was linear from 100 to 2,000 µg L^{-1} acrylamide (R^2 = 0.992). Lowest detection limit 30 µg L^{-1}. Slope coefficient for ACR concentration (in µg L^{-1}) vs. peak area (µV s) 0.000136. Recovery after addition of ACR to bread samples was no less than 97%.

The mass concentration of the component in the sample under analysis (X) was calculated by the formula:

$$\tilde{O} = \frac{k \cdot \tilde{N}}{m} \quad (1)$$

where k is the sample dilution coefficient; C is the acrylamide concentration determined by using the calibration graph, μg L^{-1}; m – sample weight, g.

The measurement result is represented in the following form: X±Δ μg kg^{-1} (X is the concentration of the component in the sample, μg kg^{-1}; Δ – the absolute error range in the identification, mg g^{-1}, with the confidential probability of P = 0.90). The arithmetic mean of the results from three parallel identifications was taken as the final result of the test.

Determination of cyclic amides (lactams) in BP

Cyclic amides (lactams) were explored using IR-Fourier spectroscopy in the area of 1,680–1,800 cm^{-1} (Bellami, 1971; Silverstein, 2011). The infrared spectra were determined using the IR-Fourier spectrometer 'ФСМ 1202' produced by Limited Liability Company 'Monitoring', Russia, with counting of automatic peaks relative to the baseline. Spectral registration parameters: spectral range – 400–4,000 cm^{-1}; number of scans – 20; resolution – 4 cm^{-1}; mode – interferogram. Absolute error in calibration of wavenumber scale – not more than ± 0.1 cm^{-1}. Deviation of 100% transmission line from the nominal value (1,950–2,050 cm^{-1}, resolution 4 cm^{-1}, 20 scans) – not more than % ± 0.5. Mean square deviation of the 100% transmission line (1,950–2,050 cm^{-1}, resolution 4 cm^{-1}, 20 scans) – no more than 0.025%. The obtained interferograms were transformed into transmission spectra. The samples for testing were prepared by pressing the crust or crumb of BP with potassium bromide. For the preparation of tablets, an accurate sample of potassium bromide and 2 g of BP were ground in an agate mortar. 100 mg of the mixture was selected and pressed in press moulds for 15 minutes on each side. The identification of lactams was made on the basis of the area of peaks in the region of 1,800–1,680 cm^{-1} (Bellami, 1971, Silverstein, 2011): cyclic amides (lactams), with large rings – near 1,680 cm^{-1}; monocyclic γ-lactams – in the interval of 1,700 cm^{-1}; polycyclic – in the interval of 1,700–1,750 cm^{-1}; monocyclic ß-lactams – in the interval of 1,760–1,730 cm^{-1}; polycyclic, condensed with other cycles - in the interval of 1,770–1,800 cm^{-1}.

The mean ACR level in 100g of BP was determined with account of proportions of the layers according to the following formula:

$$ACR = 0.32ACR_{ct} + 0.35ACR_{scl} + ACR0.33ACR_{cb} \qquad (2)$$

where 0.32; 0.35; 0.33 – layer proportions in BP; ACR_{ct} – ACR level in the crust per 100 g of BP; ACR_{scl} – ACR level in the sub-crust layer per 100 g of BP; ACR_{cb} – ACR level in the crumb per 100 g of BP.

The ACR was recalculated for 100 g of BP taking into account the mass fraction of moisture in the layers after their separation, which was determined by drying the layers to constant mass at 50 °C.

The research was made in triplicate. The reliability of the experimental data was evaluated by methods of mathematical statistics with the use of Microsoft Excel application for Windows 2007. All the results were expressed as means ± standard deviation and the statistical significance was assessed by Student's t test. To establish statistically significant differences between the values of the experimental BP samples compared to the control in the group, analysis of variance was used (ANOVA). Differences were considered significant at $p \pm 0.05$.

RESULTS AND DISCUSSION

Acrylamide was detected in all studied BP samples (Table 2); its amount depended on the recipe and the specific part of the product. The standard deviation from the mean did not exceed alleged ($P = 90$) and some value (*) correspond to the probabilities $P \geq 0.95$. The results did not contradict the previously published data (Mustafa, 2008; Bagryantseva et al., 2010; Krishnakumar & Visvanathan, 2014). According to the obtained results, the ACR levels in BP, in comparison with wheat flour bread, can exceed the average by 6–10 times due to the presence of sugar and vegetable oil in the recipes (Przygodzka et al., 2015; Bartkiene et al., 2017; Nachi et al., 2018).

Table 2. Content of acrylamide (ACR), μg kg^{-1} DM, in bakery products, ± standard deviation. Asterisk (*) represents statistical significance of $p \leq 0.05$ (Student's t-test).

Object	Bakery products 4% fat			Bakery products 14.5% fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Baking at 220 °C						
Crust	234.8 ± 10.3*	209.2 ± 9.3*	163.5 ± 7.3*	246.1 ± 21.0	203.0 ± 8,0*	186.8 ± 15.0
Sub-crust layer	210.0 ± 12.3	162.0 ± 7.0*	79.1 ± 6.5	213.6 ± 20.8	120.0 ± 10.5	91.4 ± 8.8
Crumb	226.4 ± 8.2*	150.5 ± 6.2*	265.2 ± 10.0*	244.5 ± 11.2*	110.0 ± 6.6	290.7 ± 23.8
Baking at 200 °C						
Crust	176.4 ± 11.0	136.8 ± 6.1*	117.2 ± 6.0	186.0 ± 14.2	140.6 ± 6.5*	122.5 ± 7.0
Sub-crust layer	166.7 ± 10.0	125,2 ± 8.0	64,5 ± 4.5	171.0 ± 15.0	94.2 ± 8.0	71.2 ± 5.0
Crumb	192.3 ± 10.0	120.0 ± 8.5	217.5 ± 12.0	212.7 ± 12.5	90.8 ± 7.0	232.5 ± 20.0[a]

a – there are no statistically significant differences between BP with plant powders and controls in the group ($p \leq 0.05$).

The highest ACR level was formed in BP which was cooked without plant powders. The amount of sunflower oil, used in the BP recipe, did not have a significant impact on the formation of ACR. The differences were minor and stayed within the error limit. The distribution of ACR in various layers of BP showed the following order: crust > crumb> sub-crust layer, despite the diverse heating temperatures they were exposed to during baking (Pashchenko & Zharkova, 2006).

Formation of ACR in BP, cooked with plant powders, showed fundamentally different results in comparison with the control samples. One of the main mechanisms of ACR formation is the Maillard reaction involving asparagine and hexoses which takes place when the temperature rises above 120 °C with maximum evaporation of water (Liu et al., 2015), which is typical for BP crust. Lacking sufficient data free asparagine content in plant powders, one can observe that ACR formation in BP correlates with free hexoses level in blueberry > rowanberry > seabuckthorn berry > pine nut. It was possible to distinguish two regularities that are associated with the presence of plant powders and do not depend on the amount of sunflower oil in the recipe. In BP cooked with blueberry and rowan powders with different amounts of sunflower oil, distribution of ACR among the layers of BP was as follows: crust > sub-crust layer > crumb. The amount of ACR in the crust was almost the same. In the crumb and the sub-crust layer the amount of ACR was less in BP cooked with rowan powder by 27.0 and 25.9%, respectively. Perhaps this was due to the fact that the rowan powder level in the recipe was by 2% higher than the level of blueberry powder.

PB enriched with pine nut and sea buckthorn powders displayed the predominant formation of ACR in the crumb, which was the highest among all BP samples and their parts. The lowest percentage of ACR was found in the sub-crust layer; it was less than in the crust and crumb by 2 and 3 times respectively, both in BP enriched with sea buckthorn powder, and with pine nut powder. The principal differences between sea buckthorn and pine nut powders from blueberry and rowan powders are the absence of anthocyanins and the fat content (18.5% in sea buckthorn powder and 20% in cedar powder). Sea buckthorn and cedar fats contain many tocopherols and carotenoids. (Chang, 2016; Kuhkheil, 2017; Zemtsova, 2017). Due to the uneven heating of the dough during the crumb formation time, the highest baking temperature does not exceed 90–95 °C (Pashchenko & Zharkova, 2006). Such temperature does not destroy the fat-soluble antioxidants. As a result, they can prevent oxidation of sunflower oil, and formation of peroxides which can interact with ACR, reducing the ACR level (Hedegaard et al., 2008). Currently, data on the effect of peroxides on reducing ACR formation are controversial. In model systems, the addition of peroxides and oxidized antioxidants led to a decrease in the formation of ACR (Oua, 2010), in oxidized oil, the formation of ACR was lower than in non-oxidized, due to its polymerizatio (Hedegaard et al., 2008). Acrylamide concentration of potato chips ranged between 525 μg kg^{-1} (fried in oxidized oil, 12 h) and 722 μg kg^{-1} (fried in unoxidized oil) Karademira et al., 2019). At the same time, a direct relationship was established between the formation of peroxides in unsaturated vegetable oils and the formation of ACR. The highest amount of ACR was formed in model systems with soybean oil, heated at 180 °C, which had the highest peroxide value. More unsaturated corn oil formed less ACR and peroxides (Daniali, 2016).

As ACR is markedly volatile with steam (Dunovská et al., 2006), one can assume that it diffuses with water vapors in every direction from the crust: into both external atmosphere and internal layers of crumb, the ratio between these two processes being controlled by penetrability of outer crust layer and kinetics of formation of the latter. So, the physical diffusion rates can contribute into ACR allocation in BP strata, and into ACR losses with storage.

For the purpose of reducing formation of ACR in BP, a lower baking temperature of 200 °C was used (Przygodzka et al., 2015), which required an increase in baking time by 5 minutes to obtain the optimal sensory properties. In all types of BP, regardless of the recipe, formation of ACR decreased at different rates depending on the part of the product (Table 2). In the crust, formation of ACR decreased by 25–35%. Plant powders contributed to reduction of ACR formation by 28–35%, whereas in the BP cooked without plant powders, formation of ACR decreased by 25% only. In the crumb and the sub-crust layer decrease of ACR formation did not exceed 20–22% due to the uneven heating of the dough layers during the crumb formation time. The temperature of the central crumb layer and the layer bordering with the crust does not exceed 100 °C; but the time it takes to reach this temperature differ, as well as the duration of the crust exposure during baking (Pashchenko & Zharkova, 2006).

One of the main mechanisms of ACR formation is the Maillard reaction which takes place when the temperature rises above 120 °C with maximum evaporation of water (Liu et al., 2015), which is typical of BP crust. Plant powders have changed the process of heterocyclic compounds (lactams) formation in different layers of BP; the results are presented in Fig. 1 as a sum of mono- and polylactams of γ- and β-forms, and lactams with large rings.

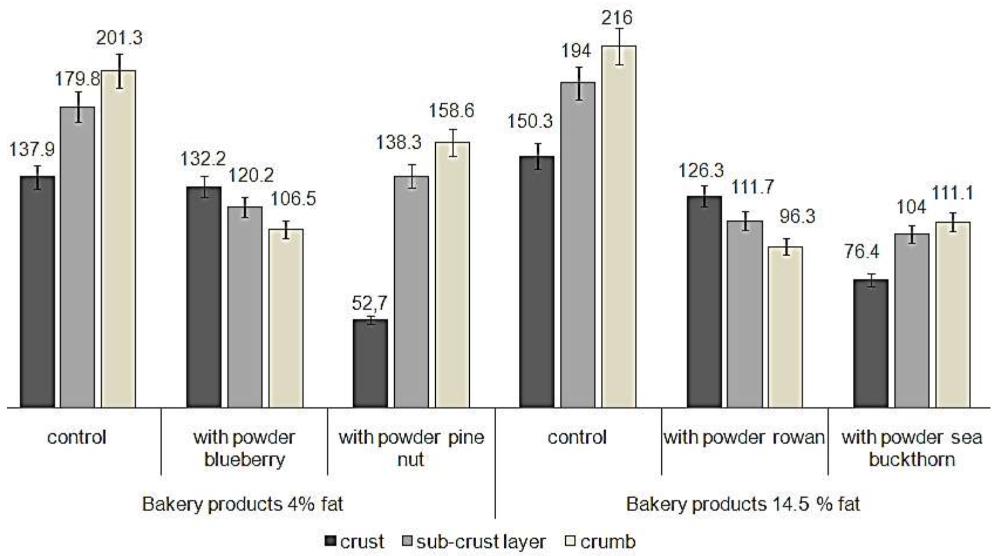

Figure 1. Content of total lactams in bakery products, relative standart units, ± standard deviation ($p \leq 0.05$, Student's t-test).

The nature of reaction of heterocyclic compounds formation depended on both in which BP part it took place, and which plant powders were used. In BP enriched with blueberry and rowan powders, which included anthocyanins, the amount of lactams in the crust was greater than in the sub-crust layer and in the crumb. BP enriched with sea buckthorn and pine nut powders as well as control samples, showed the opposite dependence: crumb > sub-crust layer > crust. In general, lactam formation in BP enriched with powders went slower in comparison with control samples. But a close relation (R^2) between the formation of lactams and ACR was established only in the crust (Table 3). We can assume that ACR formation depends on the Maillard reaction in the sub-crust layer ($R^2 > 0.6$) and does not depend on it in the crumb. However, it is impossible to firmly assert and draw conclusions, since the vegetable powders used for the production of BP are significantly different in chemical composition. When baking temperature was decreased, relation between the formation of lactams and ACR in the crust decreased, while increasing in the crumb and the sub-crust layer.

Table 3. Relation (R^2) between acrylamide content and total lactams in bakery products after baking at two temperature regimes

Baking temperatures	Part of bakery product		
	crust	sub-crust layer	crumb
220 °C	0.871	0.635	0.219
200 °C	0.725	0.671	0.296

During storage, the decrease in ACR content is dependent on humidity and storage conditions of BP, such as packaging, temperature, and relative humidity. The results of the study of the content of ACR in BP after 24 hours of storage in the polymer packaging are presented in Table 4.

Table 4. Content of acrylamide (ACR), μg kg^{-1} DM, in bakery products in 24 hours after baking, ± standard deviation. Asterisk (*) represents average results with deviations $p \leq 0.05$ (Student's t-test)

Object	Bakery products 4% fat			Bakery products 14.5 % fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Baking at 220 °C						
Crust	79.0 ± 4.0	67.6 ± 4.1	53.9 ± 4.5	85.2 ± 8.0	64.5 ± 6.1	61.1 ± 5.0
Sub-crust layer	115.3 ± 6.5	104.7 ± 6.8^{a}	73.2 ± 4.0^{b}	121.5 ± 10.8	94.8 ± 8.8	84.2 ± 8.0^{b}
Crumb	51.4 ± 4.4	39.4 ± 4.0	57.3 ± 3.9^{a}	54.9 ± 4.3	30.6 ± 3.0	65.0 ± 5.9^{a}
Baking at 200 °C						
Crust	65.3 ± 4.0	45.4 ± 4.0	39.1 ± 3.0	64.0 ± 3.0*	45.5 ± 3.9	40.8 ± 4.0
Sub-crust layer	92.5 ± 8.2	80.7 ± 7.5^{a}	60.8 ± 4.2^{b}	95.2 ± 8.6	78.5 ± 4.5	66.5 ± 4.7^{b}
Crumb	44.9 ± 3.8	32.5 ± 3.0	48.3 ± 4.1^{a}	48.0 ± 3.9	27.2 ± 2.0	50.5 ± 3.8

a – there are no statistically significant differences between BP with plant powders and controls in the group ($p \leq 0.05$); b – there are no statistically significant differences between BP before and after storage in the group ($p \leq 0.05$).

After storage, BP contained less ACR in all layers compared to freshly baked products. The absence of statistically significant differences was found in the sub-crust layer BP with blueberries and in the crumb and in the sub-crust layer BP with pine nut powder and sea buckthorn compared with the control of the corresponding group. The decrease in ACR content had a general pattern: crumb > crust > sub-crust layer. In the crumb, its amount decreased 3.6–4.5 times, depending on the formulation of BP. The smallest amount of ACR in the crumb of BP is found in products with rowan powder, despite the fact that its amount during storage has decreased only 3.6 times.

The partial redistribution of moisture during starch retrogradation (Pashchenko & Zharkova, 2006), which occurs during the storage of BP, appears to dissolve free ACR, which gradually migrates from the crumb to the crust. In the sub-crust layer, the amount of ACR was 1.3 times more than in the crumb in BP with sea buckthorn and pine nut powders, 2.2 times in BP without plant components and 2.6 and 3 times in BP with blueberry and rowan powders respectively. The overall decrease in the amount of ACR in the sub-crust layer during storage was from 8–9% in the CIB with pine nut and sea buckthorn powders to 44–45% in BP without plant components.

At the same time, the decrease in ACR content in the crust was more significant than in the sub-crust layer – from 2.7 to 3.1 times, with a predominance in BP with plant powders. ACR may have volatilized from the surface of the crust. BP with plant powders contained less ACR in the crust than control samples. The influence of the type of plant powders on the content of ACR in the crust of BP was not established, there were no statistically significant differences.

During storage, the content of ACR in BP baked at 200 °C decreased similarly in BP baked at a higher temperature. ACR prevailed in the sub-crust layer of BP irrespective of their formulation; in the crust and in the crumb its predominance depended on the plant powders used. The predominance of ACR in the crust compared to the crumb was determined in BP with blueberry and rowan powders, and in the crumb compared to the crust in all other BP.

The amount of ACR entering the human body with 100 g of BP, taking into account its content in different layers, is presented in Table 5. The proportion of the crust and sub-crust layer in BP depends on the mass of the product: the smaller the mass of BP, the greater the proportion of the crust and sub-crust layer. At the same time, the crust of freshly baked of BP has less moisture than the sub-crust layer by almost 4.5 times and 5 times than the central layer of the crumb. All this was taken into account when calculating the average content of ACR in BP.

Table 5. The amount of ACR entering the human body with 100 g of BP

ACR content	Bakery products 4% fat			Bakery products 14.5% fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Baking at 220 °C						
μg 100 g^{-1} BP	16.55	13.08	12.23	17.33	11.03	13.73
μg per kg in a 70 kg-body	0.24	0.19	0.17	0.25	0.16	0.20
Baking at 200 °C						
μg 100 g^{-1} BP	13.10	9.45	9.47	13.92	8.24	10.14
μg per kg in a 70 kg-body	0.19	0.14	0.14	0.20	0.12	0.14

In BP baked at 220 °C, the content of ACR prevailed in products without the addition of plant powders; the reduction of sunflower oil in the formulation of BP decreases the content of ACR from 17.33 to 16.55 μg 100 g^{-1}. In other BP, the content of ACR decreased depending on the used plant powders: sea buckthorn > blueberry > pine nut > rowan.

If a person with an average weight of 70 kg-body consumes 100 g of BP, then the consumption of ACR will be 16–24% of the prescribed average amount of food (Bagryantseva et al., 2010). The use of lower baking temperatures reduces the amount of ACR in BP by 20–28%, and this process is more pronounced in BP with plant powders, especially in BP with blueberry powder (27.8%) and sea buckthorn powder (26.15%). 100 g of BP with vegetable powders, baked at 200 °C, will deliver 3–6% less ACR into the human body per kg of body weight.

CONCLUSIONS

ACR formation in BP enriched with plant powders occurs unevenly in different layers, predominantly in the crust in BP cooked with blueberry and rowan powders, and in the crumb in BP cooked with buckthorn and pine nut powders. There are no statistically significant differences between ACR formation in different layers of BP cooked without plant powders with different amounts of fat in the recipes. It is established that ACR formation in the crust has a close relation ($R^2 = 0.871$) with the Maillard reaction and the formation of heterocyclic compounds (lactams). One can assume that there exists a dependence of ACR formation on the Maillard reaction in the sub-crust layer ($R^2 = 0.635$), but no such dependence was displayed in the crumb due to the use of plant powders significantly differing in chemical composition. Lowering the baking temperature leads to a decrease in ACR formation by 15–20% in the crumb and by 25–35% in the crust.

During storage of BP in polymer packaging for 24 hours, ACR is redistributed in its layers showing a tendency to reduce its level, which has a general pattern: crumb > crust > sub-crust layer. In the crumb, ACR level decreased by 3.6–4.5 times, depending on the BP recipe. Generally, ACR migration within BP can be attributed to physical diffusion with water in condensed or vaporized state.

When using 100 g of BP enriched with plant powders, the intake of ACR in the human body, calculated with account to its levels in different layers, will come to 0.16–0.20 µg per kg of the body weight, and when the baking temperature is reduced to 200 °C, it will also reduce to 0.12–0.14 µg.

The results obtained during the formation of ACR in different parts of BP using capillary electrophoresis require further research, both in model systems and using the LC-MS/MS method recommended by EFSA.

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