Quality Assurance and Safety of Crops & Foods, 2014; 6 (3): 327-334

SPECIAL ISSUE: *Food chemistry and safety*

Nutritionally enhanced wheat-oat bread with reduced acrylamide level

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Received: 30 November 2013 / Accepted: 18 February 2014

RESEARCH ARTICLE

Abstract

Consumption of nutritionally enhanced wheat-oat bread naturally rich in β-glucans originating from extra-wholemeal oat flour with improved digestibility by lactic fermentation provides unambiguous health benefits associated with a slower blood glucose release and a higher subjective satiety determined by a postprandial study. However, an undesirable elevated acrylamide formation in the crust of bread was observed due to a high level of a main precursor amino acid L-asparagine in oat flour. Lactic fermentation of oat flour by *Lactobacillus plantarum* and 30% substitution of extra-wholemeal oat flour with fermented oat sourdough led to more attractive organoleptic quality of wheat-oat fermented bread and to a decrease of pH value which suppressed acrylamide level up to 10%. As a more effective tool of acrylamide reduction a commercial asparaginase enzyme was used, applied on a surface of the bread loaf during proofing and before baking. This enzyme treatment resulted in a more than 46% decrease of acrylamide in the crust from 390 μg/kg to the level of 210 μg/kg without any alterations to the expected quality of the newly designed bread.

Keywords: acrylamide, β-glucans, wheat-oat bread

1. Introduction

Nowadays, an increasing demand for nutritionally enhanced foods associated with a positive impact on human health is a great challenge not only for producers, but also for food scientists and food technologists to design products, especially staple foods, with improved health-promoting attributes. Regarding this, oat-based products are considered to be a valuable part of human diet taking into account their outstanding nutritional value, resulting mainly from a large content of biologically worthwhile proteins and lipids, favourable composition of saccharides and high levels of easily soluble fibre, vitamins and minerals compared to other cereals (Wrigley *et al.*, 2004). Oats contain a large amount of indigestible polysaccharides – soluble and insoluble fibre, especially β-glucans (3-7%) (Tiwari and Cummins, 2012), which has a high satiating effect. Slow starch degradation provides slow glucose and insulin uptakes, hence providing a long-lasting source of energy and decreased feelings of hunger, especially in the case of increased physical activity (Mikušová *et al.*, 2011). Insoluble fibre helps to improve intestinal peristaltic and bowel transit time, which shortens the contact of toxins with epithelium, possibly reducing the risk of cancer of the digestive system, especially in the colon (Kaczmaryk *et al.*, 2012). Easily soluble fibre (mainly β-glucans) helps to reduce blood total and low density lipoprotein (LDL) cholesterol levels and to increase high density lipoprotein cholesterol levels, causing decreased incidence of cardiovascular diseases (Mikušová *et al*, 2012; Tiwari and Cummins, 2012). Oatmeal in the diet also improves metabolic processes in diabetics (El Khoury *et al.*, 2012; Liatis *et al.*, 2009), patients suffering from acidosis, inflammation of the mouth, stomach or intestines (Ryan *et al.*, 2011).

Bakery products prepared from other cereals than wheat, however, usually do not attain the same quality level as wheat bakery products (Peñas *et al.*, 2013) and their production in large quantities is economically non-profitable. The solution to the question of how to use the favourable dietetic characteristics of the other cereals while maintaining qualitative parameters is therefore to use 'composite flours' that contain wheat flour with the addition of other cereals to various percentages.

Despite the above-mentioned health benefits of oat rich in fibre, β-glucans, minerals and antioxidants, it is necessary to consider the health risk associated with the formation of process contaminants generated during the heat treatment of raw materials. The most investigated heat process contaminant is acrylamide, which is classified as a probable carcinogen (IARC, 1994) and has been detected in a wide range of common foods and delicacies popular especially among young people (Ciesarová, 2013). Due to the high content of acrylamide in many foods of daily consumption, international food safety authorities have recommended that food producers should put their effort into reduction of acrylamide to the lowest value reasonably achievable (JECFA, 2005). Taking into account recently updated indicative values of acrylamide content for various food commodities (EC, 2013), a development of appropriate methods to reduce acrylamide, particularly in those products which exceeded an acceptable level of acrylamide, is highly required. The main precursors of acrylamide formation are the amino acid asparagine and compounds with reactive carbonyl groups, in particular reducing sugars (Stadler *et al.*, 2004). Their content in cereals indicates a likelihood of acrylamide formation during heat treatment. Methods that can be used to minimise the acrylamide content in bakery products have been the subject of intense investigation by many research teams in recent years. From various approaches, the most effective tool seems to be an addition of L-asparaginase enzyme into the dough, what reduces asparagine concentration and therefore acrylamide formation during bread baking. This method is relatively easy to conduct and has no impact on process technology or final product quality.

The main aim of this study was to determine the impact of partial substitution of wholemeal oat flour by oat sourdough in wheat-oat bread formula on the basic bread constituents, acrylamide level, organoleptic properties, and to assess potential postprandial health benefits of the newly designed bread expressed as blood glucose release and subjective satiating perception.

2. Materials and methods

Chemicals

Sulfuric acid 98% p.a. (Mikrochem, Pezinok, Slovakia), hydrochloric acid 37% (Lachema, Brno, Czech Republic), Tashiro indicator (Sigma Aldrich, St. Louis, MO, USA), phenolphthalein (Centralchem, Bratislava, Slovakia), diethylether (Centralchem), boric acid p.a. (Mikrochem), ethanol p.a. (Mikrochem), acetone p.a. (Mikrochem), sodium hydroxide p.a. (Mikrochem), silver nitrate crystalline p.a. (Lachema), Celite 545 (Merck, Darmstadt, Germany), sodium dihydrogen phosphate (Chemapol, Praha, Czech Republic), 4-morpholineethanesulfonic acid sodium salt (Serva, Heidelberg, Germany), tris(hydroxymethyl) aminomethane (Serva), Carrez solution I and II (Lachema), glucose (Galvex, Banská Bystrica, Slovakia), analytical set Mixed-linkage β-glucan assay procedure (Megazyme International, Wicklow, Ireland), analytical set Total dietary fibre assay procedure (Megazyme International), L-asparaginase (Preventase®, 2,500 asparaginase units (ASNU)/g, complimentary delivered by DSM, Heerlen, the Netherlands).

Materials

Oat (*Avena sativa*) flour was prepared by the company Sojamlyn Ltd., Malé Ripňany, Slovakia. For experiment, the variety Saul with the extraction rate above 100% was used. Wholemeal oat flour was additionally enriched with oat bran parts producing an extra-wholemeal oat flour (4,880 mg /100 g ash content). Other ingredients were purchased from a local market such as wheat (*Triticum* spp.) flour (type T650; 650 mg/100 g ash content) produced by Penam Ltd. (Nitra, Slovakia), yeast (Old Herold Hefe Ltd., Trenčín, Slovakia) and salt (Solivary, Prešov, Slovakia). Commercial type of wheat bread was purchased from the retail.

Microorganisms

Lactobacillus plantarum S-LAC-1 purchased from Stuvital Ltd. (Bratislava, Slovakia), was used for fermentation of oat flour. The isolates were stored on MRS agar (Merck) at 5±1 °C.

Preparation of oat sourdough

The extra-wholemeal oat flour in the amount of 15 g was mixed with 100 ml of water and sterilised at 120 °C for 30 min. After cooling down to the laboratory temperature, the mash was fermented by a strain of *L. plantarum* S-LAC-1. The bacterial culture was mixed with water (0.1 g in 10 ml) and 1 ml was applied into the sterilised porridge and stored for 24 h at 30 °C in the thermostat (PollQ-Lab, Bielsko-Biala, Poland). Fermentation of oat porridge was controlled by measuring of pH value and determination of total titratable acidity according to STN 560512-9 (STN, 1993).

Bread making procedure

For one loaf of bread, 350 g of the composite flour consisting of white wheat flour (70%) and extra-wholemeal oat flour (30%), 17.5 g of yeast, 7 g of salt, and 250 ml of water (25±1 °C) was used. In newly designed bread, 15 and 30%

of extra-wholemeal oat flour, respectively, was replaced with fermented oat sourdough and addition of water was adequately diminished. Yeasts were activated for 10 min in 50 ml of water (25±1 °C). Hand bread-making was conducted in the following steps: 10 min of kneading in a household mixer (Kitchen Aid 5KSM150; Artisan, Greenville, OH, USA), 20 min of initial rising, hand-shaping, 15 min of proofing (32 °C, 85% humidity), 40 min of baking at adjusted time/temperature profile: upper heating 230 °C/30 min, 250 °C/10 min, lower heating 220 °C/40 min (Miwe Condo, Arnstein, Germany) with 140 ml of steam injection. In case of asparaginase treatment, the commercial enzyme Preventase® as a solution of 500 ASNU in 10 ml of water was spread on the surface of the bread loaf before baking. The enzyme was effective during the proofing step for 15 min at 32 °C. Baked loaves of bread were cooled down at room temperature for 2 h prior further analyses.

Chemical analyses

Unfermented and fermented wheat-oat breads were characterised by determination of moisture, proteins (N × 5.7), lipids and ash contents through AACC method 44-10.01 (AACCI, 2010), 46-13.01 (AACCI, 2010), 920.39C (AOAC, 2006) and 08-01.01 (AACCI, 2010), respectively. Starch was determined by the Ewers polarimetric method (ISO 10520; ISO, 1997), total dietary fibre, and total β-glucan content by enzymatic methods according to AOAC 985.29 and AOAC 995.16 standards using assay kits from Megazyme International.

Total and available saccharides were calculated as follows:

- Content of total saccharides was the difference of all other basic components (total weight mass in grams minus mass of water, protein, lipids, and ash content).
- Content of available saccharides (AS) was represented by the difference of total saccharides minus total dietary fibre content.
- Energy value of the product was calculated according to the formula:

$$\text{Energy value (kcal)} = (\text{protein content} \times 4) + (\text{AS content} \times 4) + (\text{lipid content} \times 9)$$

Amino acids (asparagine, aspartic acid, glutamine, glutamic acid) and acrylamide content were determined by liquid chromatography-electrospray ionisation-tandem mass spectrometry technique (Agilent Technologies, St. Clara, CA, USA) according to Ciesarová *et al.* (2009).

Sensory evaluation

A total of 20 panellists (5 men and 15 women, aged 23 to 55) were involved in the evaluation process, representing a group of consumers who were familiar with the research purpose in a form of a lecture prior to the initial assessment. The consumer preferences survey was conducted using a 5-point hedonic scale (1 = dislike very much, 2 = dislike, 3 = nor like nor dislike, 4 = like, 5 = like very much). The assessors classified overall quality of samples of bread as an appearance, a structure of crust, a structure of crumb, aroma, and taste. Profile analysis was focused on a description of properties attributed to aroma and taste, their intensity and finally on their acceptability by hedonic evaluation.

Postprandial study

Ten non-smoking women in the age of 21-32 years with body mass index 24.5 kg/m^2, 34% body fat percentage and normal blood pressure were recruited to participate in the postprandial study. They were not suffering from any chronic or acute diseases and were not taking any medication that could influence the outcomes of the study. All volunteers were fully informed of the nature and purpose of the study and a written informed consent was obtained. The experimental protocol was approved by a local ethic committee (Ethics Committee of the Bratislava Self-Governing Region, Bratislava, Slovakia; no. 104455/2011-HF). Subjects were asked to restrain from food and use of an intense physical activity for 12 h prior to the examination. An intravenous catheter (Terumo Europe N.V., Leuven, Belgium) was placed in the cubital vein for blood sampling at 8:00 a.m., 30 min before the test. In the beginning of the test with volunteers, blood samples were drawn to determine fasting glucose of the subjects. 25 g of available saccharides portions of wheat-oat bread (75.3 g of bread) and glucose, respectively, were served to each volunteer on two separate days. The study was carried out according to the ISO Standard for GI testing (ISO 26642:2010 (E); ISO, 2010). Blood samples were collected from cubital vein in selected time intervals starting from 0 min (directly before consumption) and then 15, 30, 45, 60, 90 and 120 min after consumption. Blood was drawn into tubes with EDTA as anticoagulant, immediately cooled in ice and after centrifugation (1,500 rpm for 10 min) plasma was stored at -20 °C until analysed. At the same time points, subjective satiety feeling was evaluated on a scale from -3 (extremely hungry) to +3 (totally full) (adapted from Quilez *et al.*, 2007). Pure water consumption was allowed during the whole meal test. Glucose was analysed using standard laboratory method on a Super GL ambulance analyser (Dr. Müller Gerätebau GmbH, Freital, Germany).

Statistical evaluation

All analyses were conducted in triplicates. Statistical analyses were done using Statgraphics (Statpoint Technologies Inc., Warrenton, VA, USA), and differences were evaluated using one-dimensional analysis of variance (ANOVA). A P-value less than 0.05 was considered as significant.

3. Results and discussion

A substitution of wheat flour by wholemeal oat flour can improve the nutritional characteristics of bread since oat flour contains valuable components in appropriate composition (Kulp and Ponte, 2000). Moreover, a fermentation of oat flour by *L. plantarum* and the incorporation of pre-fermented oat sourdough into the bread-making process lead to the enrichment of the resulting bread with dietetically valuable properties due to production of desirable compounds and the support of digestion. Both of these ingredients (extra-wholemeal oat flour, and *Lactobacilli*-fermented extra-wholemeal oat flour) were used in bread making.

In order to find consumer opinion on quality parameters of novel products, the acceptability and organoleptic properties of wheat-oat bread with 0, 15 and 30% substitutions of oat flour with fermented oat sourdough have been evaluated. 90% of respondents accepted all three samples of wheat-oat bread. The highest number of preferences (40%) was attributed to the 30% substituted wheat-oat fermented bread. For this reason, the bread with 30% of oat sourdough was selected as the most suitable for further experiments.

The profile sensory analysis provided the description of organoleptic attributes of aroma and taste as well as the contribution of fermented sourdough to particular perceptions. Although the aroma of fermented and unfermented wheat-oat breads were not significantly affected by the presence of oat sourdough (Figure 1), the taste attributes of fermented and unfermented wheat-oat breads were differentiated significantly, especially in attributes of sourness, bitterness, traces of yeasts, traces of lactic fermentation metabolites, and other non-specified perceptions (Figure 2). The bread with fermented oat sourdough was more intense in all of aforementioned attributes which were considered as convenient.

Wheat-oat bread with 0 and 30% substitutions of oat flour with fermented oat sourdough were analysed in order to compare their nutritional characteristics (Table 1). Total proteins, total lipids, available saccharides and dietary fibre showed higher values up to 5-13%. The increase of β-glucan content in bread with fermented oat sourdough of 29% to a level of more than 3 g/100 g of fresh weight was observed which can be considered as a significant improvement in the dietetic value of the novel product. This content of β-glucans substantiates the following health claim made for oat β-glucan: 'Oat β-glucan has been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of (coronary) heart disease' (EFSA, 2010). Based on the Scientific Opinion of the EFSA Panel on Dietetic Products, Nutrition and Allergies, the effects of oat β-glucan at doses of at least 3 g/day include a statistically significant decrease in LDL-cholesterol concentrations, with strong evidence supporting the biological plausibility of the effect (EFSA, 2011). For this reason, foods should provide at least 3 g of oat β-glucan per day, consumed as a part of a balanced diet. The recommended daily consumption of 100 g of the bread described in this study would meet this consideration.

In order to find the ability of a newly designed bread to slow glucose release of consumers and postpone the feeling of hunger, the postprandial study was conducted on the panel of ten volunteers. Results of blood glucose release and satiating ability after consumption of wheat-oat bread with fermented oat sourdough were compared with data

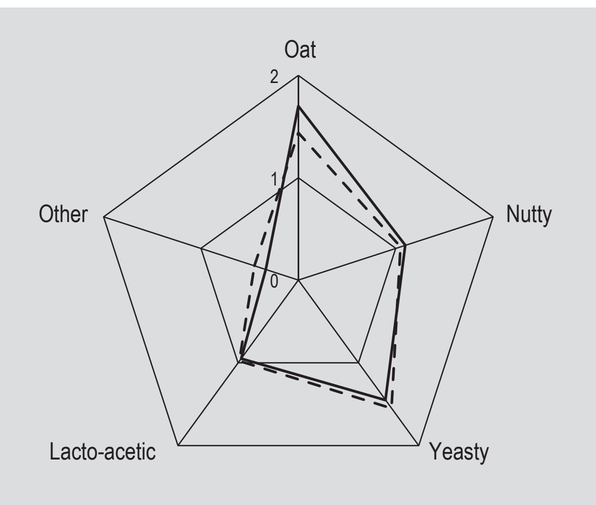

Figure 1. Aroma profile of wheat-oat bread with various substitution levels of fermented oat sourdough (solid line = 0% substitution; dashed line = 30% substitution).

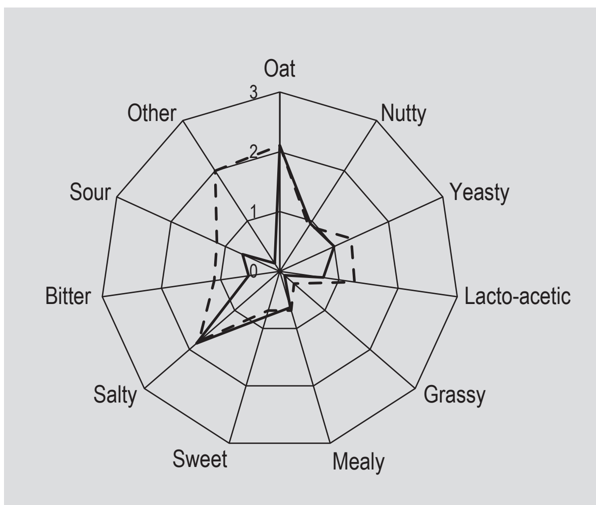

Figure 2. Taste profile of wheat-oat bread with various substitution levels of fermented oat sourdough (solid line = 0% substitution; dashed line = 30% substitution).

Table 1. Nutritional characteristics of unfermented wheat-oat bread and 30% substituted wheat-oat bread with fermented oat sourdough[1].

Nutrients (g/100 g fresh weight)	Unfermented wheat-oat bread	Fermented wheat-oat bread
Proteins	11.8±0.1[a]	13.3±0.1[b]
Lipids	2.2±0.1[a]	2.3±0.1[a]
Available saccharides	30.0±0.1[a]	33.2±0.1[b]
Dietary fibre	8.97±0.84[a]	10.15±0.92[a]
Total β-glucans	2.40±0.11[a]	3.09±0.04[b]
Ash	2.53±0.01[a]	2.97±0.01[b]
Dry matter	51.3±0.1[a]	54.4±0.1[b]
Energy value (kJ/100 g)	900±0.1[a]	844±0.5[b]

[1] Data are expressed as means ± standard deviation (n=3). Means in each row followed by different superscript letters are significantly different ($P \leq 0.05$)

achieved after a standard glucose solution uptake according to the procedure dedicated to the determination of the glycaemic index (ISO 26642:2010(E); ISO, 2010). The 30% substituted wheat-oat bread with fermented oat sourdough demonstrated significantly higher satiating ability (Figure 3) and lower glycaemia (Figure 4) during 45 min after its consumption compared to the glucose standard.

On the other hand, the formation of undesirable process contaminants, particularly acrylamide, was also checked. Observations of the higher level of the main precursor of acrylamide, the amino acid asparagine, in oat flour (Table 2) suggested that high levels of acrylamide would form during baking. Moreover, it is important to notice that extra-wholemeal flours contain more asparagine than white flours, which pre-disposes them to more intense acrylamide formation (Capuano *et al.*, 2009, 2010). Although acrylamide formation is associated directly with asparagine content, an occurrence of other structurally similar amino acids as aspartic acid, glutamine, and glutamic acid, is also important due to transamination reactions between glutamic and aspartic acids, and transamidation among aspartic acid with glutamine on one side and asparagine with glutamic acid on the other side (Ciesarová *et al.*, 2009).

Replacement of wheat flour with extra-wholemeal oat flour, which has a higher propensity to form acrylamide during baking, resulted in a doubling of the level of acrylamide measured in bread crust (390 μg/kg in wheat-oat bread crust vs. 188 μg/kg in wheat bread crust). However, if a part of the extra-wholemeal oat flour was fermented by *L. plantarum*, the acrylamide level in the crust decreased up to 10% (Figure 5). Therefore, the fermentation step was recognised as a positive tool in diminishing the acrylamide

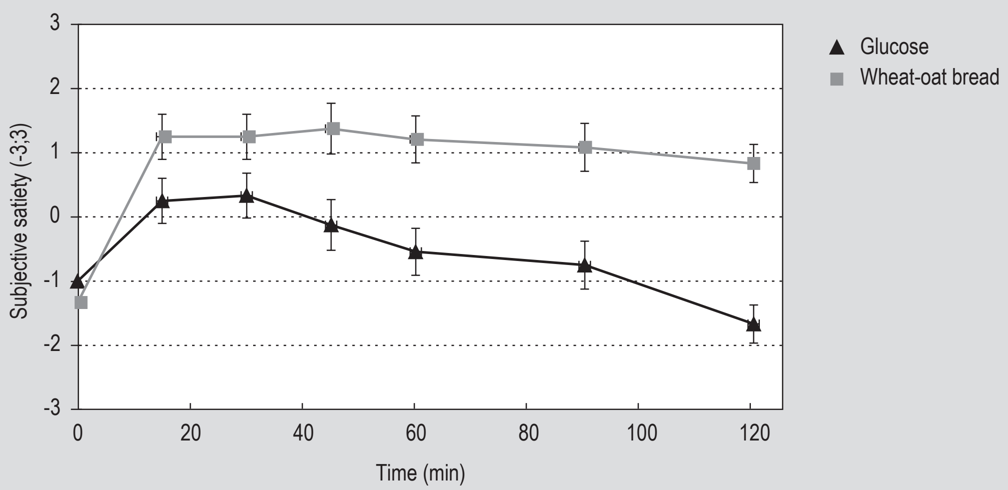

Figure 3. Feeling of satiety after consumption of glucose standard solution and 30% substituted wheat-oat bread with fermented oat sourdough.

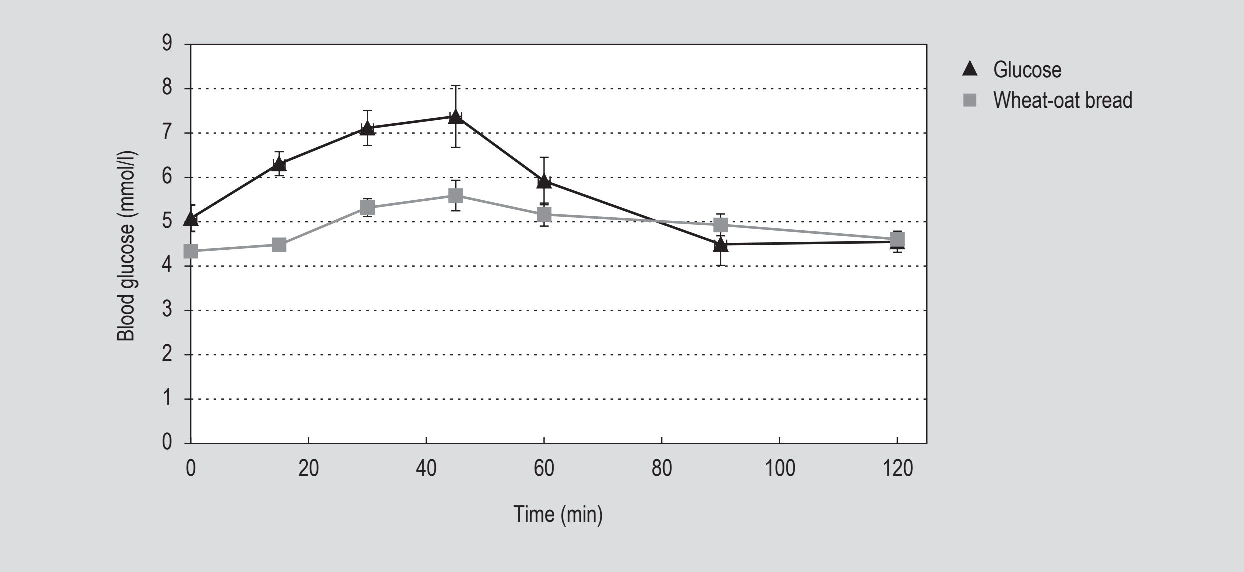

Figure 4. Blood glucose levels after ingestion of glucose standard solution and 30% substituted wheat-oat bread with fermented oat sourdough.

Table 2. Amino acids content in white wheat flour and extra-wholemeal oat flour[1].

	Asn (mg/kg)	Asp (mg/kg)	Gln (mg/kg)	Glu (mg/kg)
Wheat flour	100.38±4.18[a]	108.98±7.69[a]	129.43±7.85[a]	74.11±1.55[a]
Extra-wholemeal oat flour	787.24±9.34[b]	104.62±4.21[a]	114.72±4.65[b]	394.84±14.30[b]

[1] Data are expressed as means ± standard deviation (n=3). Means in each column followed by different superscript letters are significantly different ($P \leq 0.05$). Asn = asparagine; Asp = aspartic acid; Gln = glutamine; Glu = glutamic acid.

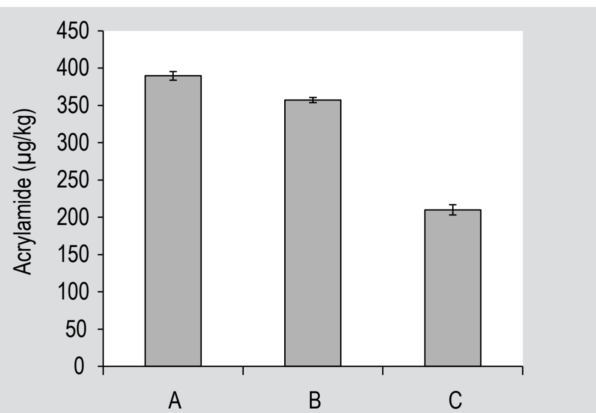

Figure 5. Acrylamide (µg/kg) in the crust of wheat-oat bread (A = unfermented wheat-oat bread; B = 30% substituted wheat-oat bread with fermented oat sourdough; C = enzymatically treated 30% substituted wheat-oat bread with fermented oat sourdough).

content in such products. This decrease can be attributed to the consumption of precursors by present microorganisms which require free amino acids and saccharides to their growth (Salmerón *et al.*, 2014). The decrease of pH from the value of 5.56 in the non-fermented wheat-oat bread to the value of 5.22 in the fermented wheat-oat bread as a consequence of organic acids presence resulting from lactic fermentation is the second reason of the limited acrylamide formation (Bartkiene *et al.*, 2013).

In order to achieve an extensive elimination of acrylamide in final products, the commercial enzyme L-asparaginase as a solution of 500 ASNU in 10 ml of water was applied to the surface of the bread loaf before baking. Application of the enzyme during the proofing step for 15 min at 32 °C resulted in a further reduction of acrylamide up to 46% (Figure 5). The final concentration of acrylamide in the crust of enzymatically treated wheat-oat fermented bread was 210 µg/kg. The big advantage of the treatment was the simplicity of application and no adverse effect on qualitative characteristics. Although there are not established any safety thresholds of acrylamide in food products so far, the

European Commission through the Recommendation of 8 November 2013 set indicative values which indicate that: 'Member States should, with the active involvement of food business operators, carry out further investigations into the production and processing methods used by food producers in case where the level of acrylamide in foodstuff, … exceeds the acrylamide indicative value set for respective food category…' (EC, 2013). The actual indicative value, released in the Annex of the Recommendation, was set for soft bread: (a) wheat based bread, 80 μg/kg; and (b) soft bread other than wheat based bread, 150 μg/kg. Taking into account this information, the acrylamide level was determined in the whole loaf of bread, not only in the crust. The observed value of acrylamide 83.5±1.9 μg/kg in the fermented 30% substituted oat sourdough wheat-oat bread is close to the indicative value dedicated for wheat based soft bread.

4. Conclusions

Novel bread with 30% substitution of wheat flour by partially fermented extra-wholemeal oat flour was successfully developed. Oat sourdough fermented by *L. plantarum* incorporated into the recipe improved the dietetic and organoleptic characteristics of the bread. Higher levels of dietary fibre and β-glucans in the novel product were positively evaluated and would be sufficient to fulfil the health claims related to oat β-glucan of lowering blood cholesterol and reducing the risk of coronary heart disease. Moreover, after consumption of a slice of this bread, the blood glucose uptake was lowered and satiety feeling was increased. The higher acrylamide level, observed as a consequence of higher asparagine level in oat flour, was reduced by both the lactic fermentation and the enzyme solution application on the bread surface.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the contract no. LPP 0310-09 and VMSP-II-0024-09 as well as by ERDF through implementation of the projects ITMS 26240220050 'Strategy of acrylamide elimination in food processing' and ITMS 26240120013 'Establishment of HiTech Centre for Research of Formation, Elimination, and Assessment of Contaminants in Food'.

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