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Development of Innovative Health Beneficial Bread using a Fermented Fibre-glucan Product

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Abstract

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The impact of partial substitution of fine wholemeal oat flour by fermented oat sourdough in wheat-oat bread formula on the basic bread constituents and organoleptic properties, and to assess wheat-oat breads from the qualitative and nutritional point was find. Fermentation of sourdough from a special finewholemeal oat flour fraction was optimised using potentially probiotic lactic acid bacteria *Lactobacillus plantarum* which resulted in 10^{10} CFU/g of vital bacterial cells and 2.95 g/l of lactic acid content. Wholemeal oat sourdough was characterised by stable gel consistency suitable for its technological application as a starter with 12.91% of dry matter, pH value 4.6, and significantly reduced starch content of up to 1.7%. This type of fermented fine oat flour was incorporated into a novel bakery product in the amount of up to 30% of oat portion in wheat-oat bread according to consumer preferences. The final bread was characterised by high fibre (10.15%) and β-glucan (3.09%) contents as well as a low energy value (844 kJ/100 g) with the rate of staling comparable to that of the control sample without sourdough addition during 3 days of storage.

Keywords: wheat-oat bread; β-glucan; fibre; fermentation

Obesity is one of the most wide-spread lifestyle diseases nowadays, being an issue not only in the developed countries but also in developing societies. 500 million adults are estimated to be obese (body mass index – BMI more than 30) with 1.5 billion of them overweight (30 > BMI > 25) not including children. If nothing is done to reverse this epidemic, more than one billion of the adult population is expected to be obese by the end of 2030. The Slovak Republic is no exception to this negative trend, with every third woman and every fourth man obese. Moreover, obesity is connected with co-morbidities such as diabetes, hypertension, cardiovascular diseases, arthritis and some types of cancer. The main cause of obesity is the energy intake from food and drink in excess of expenditure through physical activity and other metabolic processes (Mikušová *et al.* 2011). Epidemiological studies suggest a strong relationship between sedentary lifestyle and diets high in calories, lipids, sugar and low in fibre leading to an increased risk of overweight and obesity (Ludwig 2000). Thus, the prevention of obesity is

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best achievable through the combination of calorie restriction, balanced nutrition, and increased physical activity (Kirk *et al.* 2000).

One of the strategies how to improve the energy intake and nutrition is to support the consumption of wholemeal cereals (Koh-Banerjee *et al*. 2003; Vijver *et al.* 2009). Whole grains, and especially dietary fibre, have received a specific attention in recent years with many studies carried out to evaluate their health beneficial effects. The wholemeal cereals intake is inversely correlated with the body-mass index and the waist-to-hip ratio or the waist circumference (Williams *et al.* 2008; Vijver *et al.* 2009). Moreover, their consumption appears to prevent weight gain (Liu *et al.* 2003) or to help lose weight (Hutkins 2006; Shepherd *et al.* 2012).

The key role in the physiological effect of the wholemeal food products is played by the dietary fibre. To define the dietary fibre, a complex approach covering nutritional and analytical concepts is required. The most common definition based on nutrition physiology is "dietary fibre is the edible parts of plants, or similar carbohydrates, that are resistant to digestion and absorption in the small intestine" (Lattimer & Haub *et al.* 2010). Fibre and resistant starch, respectively, resist upper intestinal digestion and pass into the lower intestine to be fermented by the micro flora in the colon. Short chain fatty acids are formed in this process, playing a pivotal role in the colon health (Slavin 2003). The physiological functions include improving glycaemic response and colon health, providing bulk and thus decreasing caloric intake, modulating lipid oxidation, or cholesterol metabolism (Mikušová *et al.* 2012).

One of the very interesting fibre component groups are cereal β-glucans (Wood 2007; Havrlentová *et al*. 2011). β-glucans are "a heterogeneous group of non-starch polysaccharides composed of D-glucose monomers linked by β-glycosidic bonds" and present mostly in the cell walls in the aleurone layer of oat (Hareland & Manthey 2003*)*, barley, wheat and other cereals (Wolever *et al*. 2010). The branched β-glucan structure consists of β-(1,3) and β-(1,4) glucose backbone, which can vary in length and degree of branching, molecular weight or solubility. All these factors predict β-glucans biological activity (El Khoury *et al.* 2012). A meal rich in fibre and β-glucans is digested at a slower rate and the absorption of nutrients occurs over a longer period of time. A diet providing an adequate amount of fibre is usually less energy dense and larger in volume which may limit spontaneous intake of energy (Lyly *et al.* 2003).

Cereal foods including bread and bakery products, are one of the most common components of the human diet. If we use lactic acid bacteria for the production of bread and other bakery products, particularly lactobacilli, we will achieve the above mentioned positive effects of wholemeal cereals and also many others connected with fermented food. (Kerckhoffs *et al.* 2003; Minamiyama *et al.* 2003; Kohajdová & Karovičová 2007).

MATERIAL AND METHODS

Material. Wholemeal oat flour was prepared by the company Sojamlyn Ltd., Malé Ripňany, Slovak Republic. In the experiment, the variety Saul with the extraction rate above 100% was used. Wholemeal oat flour was additionally enriched with oat bran parts producing fine flour from oat bran (4880 mg/100 g ash content). A more detailed description of the production of fine flour from oat bran is protected by the company. 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., Slovak Republic, yeast (Old Herold hefe Ltd., Trenčín, Slovak Republic), and salt (Solivary Prešov, Slovak Republic). Commercial type wheat bread was purchased from the retail.

Microorganisms. *Lactobacillus plantarum* S-LAC-1, *Lactobacillus delbrueckii, Lactobacillus crispatus,* all purchased from Stuvital Ltd., Bratislava, Slovak Republic, were used for fermentation of fine oat flour. The isolates were stored on MRS Agar at 5 ± 1°C. The strains were selected on the basis of the fact that they had been tested on other foods. The aim was to choose the most suitable strain which produces mainly lactic acid and reduces pH of the fermented product.

Preparation of oat sourdough. The fine oat flour in the amount of 5, 10, and 15 g was mixed with 100 ml of water and sterilised at 120°C for 30 minutes. The sterilisation was performed in order to prevent the access of undesirable bacteria. After cooling down to the laboratory temperature, the suspension of oat flour and water was fermented by selected strains (*Lactobacillus plantarum*, *Lactobacillus delbrueckii,* and *Lactobacillus crispatus)*. The bacterial culture was mixed with water (0.1 g in 10 ml) and 1 ml was applied into the sterile flour-water suspension and stored at 30°C for 24 h in the thermostat (PollQ-Lab, Bielsko-Biała, Poland).

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The fermentation of oat flour was followed by controlling the pH value and determining total titratable acidity. Total titratable acidity was measured by titration with 0.01 M NaOH using phenolphtalein as an indicator (AOAC 1984; STN 560512-9:1993).

Lactic acid production was analysed by capillary isotachophoresis. The leading electrolyte used conted hydrochloric acid ($c = 1.0 \times 10^{-2}$ mol/dm^3) with β-alanine and 0.1% methyl hydroxyethyl cellulose (m-HEC). The solution pH was 3.0. The terminating electrolyte contained acetic acid ($c = 5 \times 10^{-3}$ mol/dm^3). As a standard, lithium lactate was used. The measurement conditions were chosen so as to achieve optimum separation and detection in the pre-separation column in the selected stream of 250 μA. The identification of lactic acid was performed using the computer software ITP Pro32.

The starch degradation and growth curve of *Lactobacillus plantarum* S-LAC-1 during fermentation were monitored. The amount of lactobacilli was observed during fermentation in intervals of 0, 3, 6, 15, 20, 24, and 48 h at 30°C after the decimal dilutions directly in tubes containing MRS broth with 0.5% about of agar. At each sampling time, the number of microorganisms per gram of fermented product was determined. pH value was measured with a pH meter (VWR, Prague, Czech Republic) and the samples were collected for the determination of starch and lactic acid contents. The tubes were cultivated at 30°C for 24 h, and afterwards the number of lactobacilli colonies formed in the tubes were counted as an innovative method as compared to the conventional method in Petri dishes.

Preparation of bread. The basic recipe for the bread preparation consisted of 350 g of composite flour (wheat and fine flour from oat bran in ratio 70 : 30), 17.5 g of yeast (5%), 7 g of salt (2%) (the percentage based on flour weight) and 250 ml of water (25 ± 1°C). In the novel type of bread, 15 and 30% of oat flour, respectively, was replaced by the fermented oat flour. Due to the characteristic gel-like consistency and initial content of water in fermented oat flour, the original water addition into dough was lowered from 250 ml to 145 ml and 40 ml, respectively. Yeasts were activated for 10 min in 50 ml of recipe water (25 ± 1°C). All ingredients were kneaded in a household mixer (Kitchen Aid 5KSM150; Artisan, Kenmore, USA) for 5 min at a slow speed and 5 min at a fast speed. The dough was left to rest for 20 min at room temperature before hand-moulding of bread loaves. The proofing was provided directly on a tin tray for 15 min in the chamber with 85% relative humidity at 32°C before baking in a convection oven (MIWE condo, Arnstein, Germany) at 230°C for 30 min with initial steaming during 1/3 of the baking period (13 min with 140 ml of water) and final increase of the temperature up to 250°C for the last 10 min of baking. The temperature of the bottom during the whole baking process (40 min) was set at 220°C. Baked loaves of bread were cooled down at the room temperature for 2 h before analysis. The weight of bread, specific volume (AACC 10-05), moisture content (AACC 44-15A), and water activity of the crumb using an aw-meter Labmaster-aw (Novasina, Switzerland) were analysed.

The bread firmness was measured during the 3-day long storage (at room temperature) with a TA.XT Plus instrument (Stable Micro Systems; Godalming, Surrey, UK) using a cylindrical probe of 36 mm diameter, according to the AACC 74-09 Standard method. The compression tests were recorded on the slice of 25 mm cut with a commercial electric knife. The measurements from at least three bread loaves were taken for each formulation and sampling time.

Nutritional analysis. Oat flours and wheat-oat breads were characterised from the nutritional point of view by the determination of proteins by the Kjeldahl method (ISO 20483:2006), total lipid content by Soxhlet method (STN 560512:1973), starch by Ewers polarimetric method (ISO 10520:1997), ash by gravimetric method (ISO 2171:2007), total dietary fibre and total β-glucan contents by enzymatic methods according to AOAC 985.29 and AOAC 995.16 standards using assay kits from Megazyme International (Ireland).

All analyses were conducted in triplicate. Total and available saccharides were determined by calculation. The content of total saccharides (TS) was calculated as a difference of all other basic components (total weight mass in grams minus mass of water, protein, lipids, and ash content). The content of available saccharides (AS) was represented by the difference of the total saccharides content minus total dietary fibre content. Energy value of the product was calculated according to the formula:

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

Sensory evaluation. A total of 20 panellists (5 men and 15 women, aged 23–55) were involved in the evaluation process, representing a group of consumers who were familiar with the research purpose in the

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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 – neither like nor dislike, 4 – like, 5 – like very much). The assessors classified the overall quality of the bread samples such as the appearance, structure of crust, structure of crumb, aroma, and taste. Profile analysis was focused on the description of properties attributed to odour and taste and their intensity, and finally on their acceptability by hedonic evaluation.

Statistical analyses. At the beginning, a normality test was applied to all measured data. As the results were of a normal distribution, ANOVA was used to analyse all data, based on a three-way mixed design. Fisher's LSD was used to determine significant differences between the samples. A *P*-values of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Preparation of oat sourdough. Nutritional compositions of wholemeal oat and fine oat flours in terms of basic nutrients and health-beneficial ingredients were compared (Table 1). Oat flour with higher contents of proteins (20.97%), dietary fibre (26.43%), and β-glucan (8.92%) labelled as "fine flour from oat bran" was selected for the preparation of oat sourdough.

In the next step, the selected fine oat flour was mixed with water in different concentrations (5, 10, and 15 g/100 ml) and three strains of *Lactobacillus* spp. were inoculated in the suspensions individually in order to optimise the conditions of fermentation. The results of the fermentation experiments aimed to assess the most suitable strain of lactic acid bacteria in terms of the total titratable acidity expressed as the percentage of lactic acid and pH value after 24 h of fermentation at 30°C are shown in Figure 1.

Lactic acid was produced by all strains after 24 h and its highest content (0.48 g/100 g) was determined in the fermented suspension of *Lactobacillus plantarum* with the concentration of 15 g of oat flour in 100 ml of water (15 g/100 ml). The most significant decrease of pH value (4.65) was achieved with the same bacterial strain. Taking into account all these results, the mentioned strain in the concentration of 15 g/100 ml was evaluated as the most suitable for further experiments.

The lactic acid content in oat suspensions at 0, 3, 6, 15, 20, and 24 h after inoculation (Figure 2) were determined by capillary isotachophoresis. As can be seen from Figure 2, the highest level of lactic acid was observed after 20 h, when its concentration reached 2.3 g/l. After this time, the concentration did not increase significantly. It is important to note that the lactic acid bacteria contributed not only to the sour taste of the bread but also to the overall organoleptic quality of this product type (Hutkins 2006; Corsetti & Settanni 2007; Rizzelo *et al.* 2010).

The production of organic acids by *Lactobacillus plantarum* was monitored over a time period (0, 3, 6, 12, 15, 20, 24, and 48 h after the inoculation) also indirectly as the pH value decrease in a water suspension of oat flour (15 g/100 ml). Figure 3 shows that the further prolongation of the fermentation process above 24 h did not significantly reduce the pH value of the oat suspension.

The cultivation parameters of *Lactobacillus plantarum* were evaluated by the growth curve, determining the number of bacteria produced over time after the inoculation of sterilised oat flour water suspension (15 g/100 ml) and cultivation under static conditions at 30°C for 2 days. The initial concentration of cells at the beginning of fermentation was 9×10^7 CFU/g. The number of lactobacilli in fermented oat water suspension after 48 h was 9×10^9 CFU/g. The number of lactobacilli after 48 h of fermentation was in line with our expectation of obtaining approximately 10^{10} CFU/g. Lag-period in the obtained growth curve and a rapid increase in the number of lactobacilli allows us to assume that 24 h is sifficient to achieve the desired lactic acid bacteria content in the oat water suspension under current cultivation conditions.

Table 1. Nutritional characteristics (g/100 g) of wholemeal oat flour and fine flour from oat bran

Oat flour	Proteins	Total lipids	Total saccharides	Available saccharides	Starch	Dietary fibre	Total β-glucan
Wholemeal oat flour	12.78	5.52	70.45	62.26	62.90	8.19	3.45
Fine flour from oat bran	20.97	9.28	55.78	26.35	30.10	26.43	8.92

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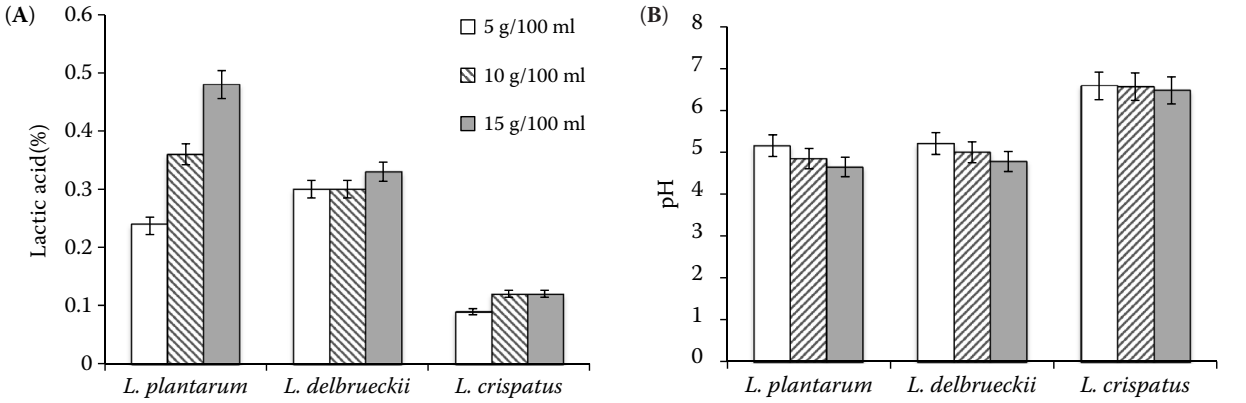

Figure 1. The total titratable acidity expressed as g/100 g of lactic acid (**A**) and pH values (**B**) after 24 h fermentation at 30°C of the fine oat flour suspension in three different concentrations inoculated with three strains of *Lactobacillus* spp.

Moreover, during the 2-day fermentation, the degradation of starch was evaluated with the aim to achieve a lower energy value of the sourdough and subsequently of the designed bread. The determination was carried out after 0, 3, 20, 24, and 48 h (Table 2). During the fermentation process, up to 77% of initial starch content was degraded, which led to the reduction of the final product energy value.

Development of new innovative bread. The main objective of the baking experiments was to develop a new type of wheat-oat bread with oat sourdough of superior organoleptic quality and potential health benefits given by the dietary fibre and β-glucan incorporation. All the baking experiments were conducted under laboratory conditions. The initial products prepared according to the general baking procedure were characterised by a very small specific volume, therefore the technological process was optimised and only the final set of the prepared bread loaves is discussed in the current contribution. It could be pointed out that several aspects are critical such as a suitable water addition in relation to the sourdough incorporation and optimisation of kneading, resting, and proofing of dough in the context with a lower quality of protein and starch of oat flour and specific rheological properties. Moreover, the proposed baking regime with steaming was critical for obtaining the final bakery products with qualitative characteristics that are shown in Table 3.

The intention of sensory evaluation in this stage of experiment was to assess and compare the acceptability of two types of novel wheat-oat bread, with 15 and 30% substitution of fine oat flour by oat sourdough, in comparison to the control type of wheat-oat bread without sourdough. It means that all three types of wheat-oat bread were evaluated: wheat-oat bread (7 : 3 based on weight) without fermented sourdough, and two types of wheat-oat bread (7 : 3) with 15 and 30% substitution, respectively, by oat sourdough. It can be summarised that 90% of respondents accepted both novel types of wheat-oat bread with sourdough and would be willing to buy

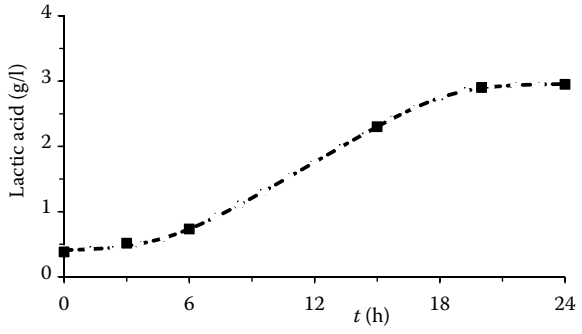

Figure 2. The production of lactic acid by *Lactobacillus plantarum* in fine oat flour water suspension (15 g/100 ml) at 30°C

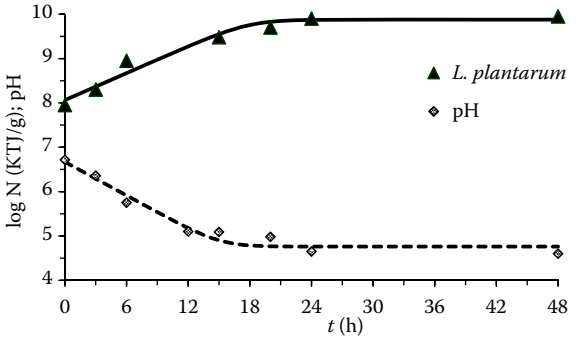

Figure 3. Changes of pH value and growth parameters of *Lactobacillus plantarum* during 2-day long fermentation of fine oat flour water suspension (15 g/100 ml) at 30°C

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Table 2. Degradation of starch during the fermentation of sterilised oat water suspension (15 g/100 ml) by *Lactobacillus plantarum* at 30°C

Time (h)	0	3	20	24	48
Starch (g/100 g)	7.6	6.7	2.9	2.6	1.7

them. Although the aroma of both the fermented and unfermented wheat-oat breads was not significantly affected by the presence of oat sourdough, the taste attributes of fermented or unfermented wheat-oat breads differed significantly, especially in the attributes of sourness, bitterness, traces of yeasts, traces of lactic fermentation metabolites, and other non-specified perceptions. The bread with fermented oat sourdough was more intense in all of the attributes mentioned which were considered as convenient. Comparing the two different substitution levels of fermented sourdough (15 and 30%), more preferences were observed for the bread with the higher sourdough substitution level. For this reason, the bread with 30% of oat sourdough was evaluated as the most suitable for further experiments.

The effect of the fermented oat sourdough on the shelf life of bread was evaluated according to their changing textural properties (Figure 4).

In the fresh product, the firmness of bread with the addition of the fermented product was up to 10% higher than that of the control wheat-oat bread. The effect of the fermented product addition on the extension of the shelf life evaluated as the firmness of bread crumb, considered to be the function of freshness, was not significantly different.

The results are partially consistent with those of other authors, concluding that the addition of oat β-glucan into wheat bread might result in a higher crumb hardness accompanied by a decreased loaf volume and height as in the current study (Brennan *et al*. 2007; Rieder *et al.* 2012). On the other hand, the incorporation of oat β-glucan into gluten-free bread led to a higher softness of bread crumb (Hager *et al.* 2011). Similar results have been reported also

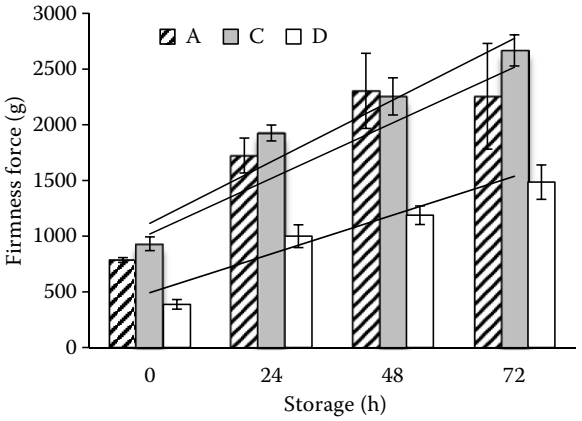

Figure 4. Firmness of bread crumb evaluated during 72 h long storage

A – control wheat-oat bread without sourdough; C – wheat-oat bread with 30% substitution of oat sourdough; D – wheat bread purchased from the retail

by Skendi *et al*. (2010) presenting a positive effect of β-glucan addition, mainly with a higher molecular weight, although the source of β-glucan was barley flour. The firmness of wheat bread crumb was also significantly lower after 24 h of storage. The aging tendency of the commercial product is comparable with that of the laboratory prepared samples of wheat-oat bread. The softness of crumb in the commercial products is generally achieved by adding additives such as L-ascorbic acid and other additives which were not added into the laboratory prepared bread samples. Hager *et al.* (2011) proposed that the mechanism through which the hardness of wheat oat bread might be increased resides in that the higher amount of water is bound to the glucans and other polysaccharides and is not available for creating the gluten network.

The content of β-glucans was determined in the designed bread loaves in order to confirm that, through the consumption of innovative bread, the recommended daily dose (RDD) of β-glucans (3.0 g/day) will be met. The RDD is the amount of active compound that should be consumed at regular basis,

Table 3. Qualitative characteristics of wheat-oat bread with 0, 15, and 30% substitution of oat flour by oat sourdough

Parameters of bread	Wheat-oat bread		
	without sourdough	with 15 % sourdough	with 30 % sourdough
Weight (g)	588 ± 29.02	596 ± 22.02	593 ± 20.39
Specific volume (ml/100 g)	249 ± 16.45	260 ± 19.20	250 ± 17.70
Moisture (%)	48.7 ± 0.05	47.0 ± 0.1	45.6 ± 0.03
Water activity	0.980 ± 0.02	0.982 ± 0.00	0.975 ± 0.01

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Table 4. Nutritional composition of bread samples: wheat oat bread and bread with 30% of oat sourdough

Nutrients (g/100 g fresh weight samples)	Wheat-oat bread	
	without sourdough	with 30% sourdough
Proteins	11.8 ± 0.04	13.3 ± 0.11
Lipids	2.2 ± 0.05	2.3 ± 0.03
Available saccharides	30.0 ± 0.09	33.2 ± 0.07
Dietary fibre	8.97 ± 0.84	10.15 ± 0.92
Total β-glucans	2.4 ± 0.11	3.09 ± 0.04
Ash	2.53 ± 0.01	2.97 ± 0.01
Moisture	48.7 ± 0.05	45.6 ± 0.03
Energy value	900 ± 0.05	844 ± 0.50

for example in two slices of bread per day, to the claimed health beneficial effects claimed (Whitehead 2008). The aforementioned wheat-oat bread met this criterion. A higher content of β-glucans was found in the bread with 30% of fermented fibre-glucan product (3.09%) compared to the white wheat bread, whose content of β-glucan is about 0.2% (based on fresh weight) (Flander *et al*. 2011). The nutritional composition of wheat oat bread compared to wheat oat bread with 30% oat sourdough addition is shown in Table 4.

The energy value of the new developed wheat-oat bread with the addition of 30% of the fermented product was 844 kJ/100 g, this value being lower compared to wheat bread commonly available in the store (1025 kJ/100 g) (Liatis *et al.* 2009). The results showed that the most suitable product, due to the higher fibre (10.15%) and β-glucan (3.09%) content, low energy value (844 kJ/100 g) as well as good organoleptic parameters, is the bread with a 30% addition of fermented sourdough.

CONCLUSIONS

It can be concluded that a wheat-oat bread was developed with a low energy value (844 kJ/100 g), a high β-glucan content, and good organoleptic parameters, in comparison to which the unfermented bread. These results have pointed but the positive benefit for the human health. The fermented oat sourdough is suitable not only for bakery wares production, but also for the development of other healthy beneficial foods.

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