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REGULAR ARTICLE

CHARACTERIZATION AND SELECTION OF CEREALS FOR PREPARATION AND UTILIZATION OF FERMENTED FIBER-BETAGLUCAN PRODUCT

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ABSTRACT

Whole grains flours of diverse colored wheat species (*Triticum* sp.) and various varieties of barley (*Hordeum vulgare*) and oat (*Avena sativa*) were analysed for their nutritional composition. The highest protein values were observed in wheat yellow variety BONA DEA (14%) and also in blue variety UC 66049 (13%). Lower content of starch was determined in barley and oat varieties in compared to wheat varieties. The lowest levels of betaglucans were observed in wheat (0.3 – 0.6%) and the highest content was assessed in barley and oat (2.5 – 3.8%). Variation in dietary fiber was considerable in barley and oat varieties. Barley varieties possessed significantly the highest content of total dietary fiber among all monitored cereals (≈17%), while oat grains showed significantly the lowest values (≤7%). Knowledge of the composition of healthy substances was used to select the best variety for the development of fermented product which was developed using specially prepared oat flour and potentially probiotic lactic acid bacteria *Lactobacillus plantarum*. The series of fermentation experiments resulted in a final gelatinous product with vital bacterial cell count about 10^{10} CFU.g^{-1}, significantly reduced level of starch (1.7%) and following nutritional characteristics: dry matter of 12.91%, water activity of 0.977, pH value 4.6 and lactic acid content of 2.95 g/l. Final product was added into the dough in different quantities and served also as a starter culture. Rheological properties of dough were evaluated for the purpose of finding a suitable recipe.

Keywords: cereals, dietary fiber, betaglucan, fermentation, lactic acid bacteria

INTRODUCTION

Cereals are generally known to have a positive influence on the state of the human organism. The attention of nutritional experts is paid especially to oats and barley. Besides their accessibility, these cereals are interesting due to their relatively high content of soluble non-starch polysaccharides, out of which betaglucans have a dominant position from the aspect of providing a health benefit. The betaglucan content of barley is mostly concentrated in the endosperm while for oats it is primarily in the sub-aleurone layer of the outer endosperm of the grain. The betaglucan content ranges from 5 to 11% for barley and 3 to 7% for oats, but depends on many pre- and postharvest factors including cultivar and environmental factors (**Tiwari and Cummins, 2008; Welch *et al.,* 2000**). Hull-less or naked barley and oat cultivars are nutritionally superior to the hulled cultivars due to the higher level of betaglucans. Clinical and epidemiological studies show that the intake of betaglucan from either barley or oat based products helps to control cardio-vascular disease (CVD) in humans (**Beck *et al*., 2010; Shimizu *et al*., 2008; Karmally *et al*., 2005; Keogh *et al*., 2003**).

The multiple beneficial effects of cereals can be exploited in different ways leading to the design of novel cereal foods or cereal ingredients that can target specific populations. Cereals can be used as fermentable substrates for the growth of probiotic microorganisms (**Kocková *et al*., 2011**). The main parameters that have to be considered are the composition and processing of the cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage, the organoleptic properties and the nutritional value of the final product (**Clarke *et al.,* 2004**). Additionally, cereals can be used as sources of nondigestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of lactobacilli and bifidobacteria present in the colon and act as prebiotics (**Charalampopoulos, 2002; Duchoňová and Šturdík, 2010**). Lactic acid fermentation of cereals is a long-established processing method and is being used in Asia and Africa for the production of foods in various forms such as beverages, gruels, and porridge (**Salovaara, 2000**). The good growth of lactic acid bacteria in cereals suggests that the incorporation of a human-derived probiotic strain in a cereal substrate under controlled conditions would produce a fermented food with defined and consistent characteristics, and possibly health-promoting properties combining the probiotic and prebiotic concept (**Correia *et al.,* 2010**).

The objective of our study the nutritional composition of three main cereal types was investigated with the aim to select according to the analyses an appropriate cereal type and its variety for purposes of preparation and utilization of fermented fiber-betaglucan product.

MATERIAL AND METHODS

Materials

The evaluated set contained seed samples from wheat cultivars (*Triticum aestivum* L.) harvested in the year 2010. Colored wheats (*Triticum aestivum)*, i.e. red color grain (FEDERER), blue (UC 66049, RU440-6, 48M), purple (ABBISINSKAJA ARASAITA, KONINI, ANK 28A) yellow (CITRUS, BONA DEA, LUTEUS) were obtained from the Agricultural Research Institute (Kromeriz, Czech Republic) and four wheat varieties with brown color grain (HANA, CORVINUS) and yellow (BONA VITA, BONA DEA) obtained from Istropol Solary (Horne Myto, Slovakia).

Barley samples (SLAVEN, POPRAD, EXPRES, JUBILANT, PRIBINA) were obtained from company Hordeum, Ltd., (Sládkovičovo, Slovakia) and oat samples (naked: IZAK, HRONEC; husked: SW BETANIA, VILIAM, CDC-SOL-F1) from Plant Production Research Institute (Vígľaš-Pstruša, Slovakia) harvested in the year 2010.

Flours (white, brown, wholemeal) from oat variety SAUL were obtained from SOJAMLYN Ltd., Malé Ripňany.

Whole grains were milled using an ultra-centrifungal mill (ZM 100, Retsch GmbH&Co.KG, Haan/Germany) equipped with a 0.5 mm sieve.

Microorganisms

For the fermentation, strains *Lactobacillus plantarum* S-LAC-1, *Lactobacillus delbrueckii, Lactobacillus crispatus* (STUVITAL Ltd., SK) were used individually. The isolates were kept on the MRS Agar at 5 °C ± 1 °C. The size of lactobacilli inoculum was 10^7 CFU/g of culture.

Nutritional analysis of cereals

The analysis of nutritional components of cereals consisted of the determination of proteins (by Kjeldahl ISO 20483:2006), fat (ISO 659), starch (ISO 10520:1997), ash (ISO 2171:2007), total dietary fiber (Megazyme kit according to AOAC), total betaglucans (Megazyme kit according to ICC 166). All analyses were conducted in triplicates. Available carbohydrates (ACHO) were calculated by difference of all other basic components (total weight in grams minus water, protein, fat, ash and total dietary fiber content). Energy value (kcal) was calculated according to the formula: content of protein x 4 + ACHO x 4 + fat x 9.

Preparation of fermented fiber-betaglucan product

Wholegrain oat flour was partially fermented by *Lactobacillus plantarum*, *Lactobacillus delbrueckii, Lactobacillus crispatus* individually to improve the sensory properties and health beneficial effect of breads. Fermented fiber-betaglucan product was prepared by mixing oat flour rich in fiber (26.43%) and betaglucan (8.92%) with concentration 5, 10, 15 g to 100 ml of water. The mixture was heated up to 120°C and held there for 30 minutes and after cooling to the room temperature (23±2°C). Lyophilized bacterial culture of *Lactobacillus plantarum* S-LAC-1 with bacterial counts 10^{10} in 1 gram was added in the concentration of 0.1g to 10 ml of distilled water. The suspension was mixed and 1 ml was taken and inoculated with 100 ml sterilized oat intermediate (oat flour with water), mixed and stored in a thermostat for 24 hours at 30°C. In final fermented product titratable acidity, pH value, starch degradation and growth curve were monitored. Fermentation experiments were performed in triplicate.

Determination of organic acids

The quality and quantity of the produced organic acids (lactic and acetic) were measured by isotachophoretic analysis. A leading electrolyte containing hydrochloric acid (c = 1.0 x 10^{-2} mol.dm^{-3}) with β-alanine and 0.1% methyl hydroxyethyl cellulose (m-HEC). The solution's pH was 3.0. A terminating electrolyte containing acetic acid (c = 5 x 10^{-3} mol.dm^{-3}). It was used lithium lactate as a standard. The samples were measured by Isotachophoretic Analyser ZKI 01 (Villanova, Slovakia). The identification of organic acids was evaluated by computer software ITTPro32 according to the RSH value (Relative Step Height) which corresponds to a substance under the same conditions. The amount of substance present in the analyzed sample is proportional to the length of step isotachophoretic record. This dependence has been established by method analytical lines.

Evaluation of the lactobacilli growth in the fermented product

The amount of lactobacilli was observed during fermentation in intervals of 0, 3, 6, 15, 20, 24 and 48 hours at 30 °C after the decimal dilutions directly in tubes containing MRS broth with 0.5% share agar. At each sampling time, a number of microorganisms per gram of fermented product was determined. pH value was measured and the samples were collected for the determination of starch and lactic acid. The tubes were cultivated for 24 hours at 30 °C, and afterwards the number of lactobacilli colonies formed in the tubes was counted. This is an innovative method compared to the conventional by seeding of the dilutions in Petri dishes with followed incubation and counting colony of units.

Evaluation of the rheological properties of dough

Characteristics of the dough were studied using farinograph (Farinograph-E, Brabender, Germany). Water absorption, dough viscosity, the stability of flour under mixing and farinograph quality number (FQN) were determined using a ICC standard method 115/1. Another rheological properties were determined using extensograph (Extensograph-E, Brabender, Germany) and amylograph (Amylograph-E, Brabender, Germany). Extensogram (graphical interpretation of extensograph) includes resistance to extension, extensographic energy (cm^2), ratio number (extensibility/resistance), ratio number Db/Max (extensibility maximum/resistance) (ICC standard method 114/1). Amylograph measures gelatinization properties and enzyme activity of flour and whole meal, assessment of the flour quality, flour suitability for various applications and measurement of the baking flour characteristics (α-amylase content, gelatinization maximum, maximum gelatinization temperature) (ICC standard method 126/1).

RESULTS AND DISCUSSION

In this paper the comparison between different varieties of colored wheat, oat and barley as well as the selection of an appropriate type and variety of cereal was performed. The most suitable variety for purposes of design and utilization of fermented fiber-betaglucan product was selected to produce a new innovative health beneficial food, wheat-oat bread.

Protein, starch, betaglucans and fats in cereals

The results of the chemical analyses are presented in Tab. 1, 2, 3. In terms of technology the proteins occuring in wheat are particularly essential. The amount of proteins were in the range from 9% to 14%. The highest value was observed in wheat yellow variety BONA DEA (14%), but also in blue variety UC 66049 (13%); the lowest value was observed in red grain variety FEDERER (9%). Generally it can be stated that the highest content of proteins contained purple varieties of wheat (Tab 1). High protein contents (≥12%) were determined for the oat varieties (SAUL and VILIAM), whereas the lowest level was observed for barley SLAVEN (8%). Requirements for the quality of the cereal grain are listed in the Codex Alimentarius, where the minimum amount of protein is 11%, but this criterion did not meet thirteen of analyzed samples. The principal carbohydrate of all cereals is starch, representing 56% (oats) to 80% (maize) of the grain dry matter. Cereal starches are similar in composition, having 74-79% amylopectin, 25-30% amylose, and 1% lipids. High-amylose and high amylopectin ("waxy") cereal cultivars have also been developed. The content of the starch, which was determined in the whole set of wheat varieties, varied in the range from 65% to 71%, with the highest value recorded in purple varieties ANK 28A (71%) and yellow variety LUTEUS (70%). Lower contents of starch were determined for barley and oat cultivars compared to the wheat varieties. These results are similar to experiments reported by **Grausgruber *et al.* (2004)**.

Lipid (fats and oils) made up approximately 1-7% of a kernel, depending on the grain. Among wheat samples the highest content of fats was observed in purple wheat KONINI (1%) (Tab 1). Regarding total fat content, oats contain about twice the amount than other cereals (4-5%). The fact that concentration of betaglucans in the wheat varieties is not so high, the crop should not move into the background, but rather a challenge to examine the rich genetic resources in order to detect variability in content observed component. Average content of betaglucans in analyzed set of wheat varied is in the range from 0.25% (BONA

VITA, SK) to 0.67% (ABISSINSKAJA and LUTEUS). The lowest levels of betaglucans were observed for wheat (0.3 – 0.9%) and the significantly higher content of betaglucans was observed in barley and oat (2.5 – 3.8%, p<0.05). These results are very similar to those reported by **Wagner and Kuhn (1996)** for German-grown cereals.

Table 1 Overview of the nutritional components content in whole grain flours of different cereal varieties; results represent mean values of three measurements SD <5%. (DM-dry matter)

Sample	Proteins (g/100g DM)	Betaglucans (g/100g DM)	Starch (g/100g DM)	Fats (g/100g DM)
Wheat				
Blue UC66049	13.02 (±0.16)	0.39 (±0.07)	66.83 (±0.12)	0.89 (±0.15)
Blue RU440-6	12.32 (±0.24)	0.32 (±0.21)	70.28 (±0.11)	0.78 (±0.12)
Blue 48M	10.76 (±0.32)	0.64 (±0.12)	67.76 (±0.15)	1.12 (±0.07)
Purple Abissinskaja	12.32 (±0.08)	0.67 (±0.11)	66.56 (±0.05)	0.99 (±0.18)
Purple Konini	13.00 (±0.16)	0.43 (±0.13)	66.56 (±0.10)	1.14 (±0.03)
Purple ANK28A	11.10 (±0.04)	0,43 (±0.15)	71.15 (±0.07)	0.90 (±0.10)
Red Federer	9.02 (±0.15)	0.46 (±0.03)	68.96 (±0.08)	0.90 (±0.05)
Yellow Citrus	9.90 (±0.16)	0.58 (±0.20)	68.53 (±0.15)	0.96 (±0.06)
Yellow Bona Dea	10.58 (±0.10)	0.59 (±0.15)	69.51 (±0.10)	0.97 (±0.10)
Yellow Luteus	10.24 (±0.16)	0.67 (±0.20)	70.50 (±0.05)	0.93 (±0.12)
Brown Hana	9.36 (±0.08)	0.26 (±0.10)	65.08 (±0.01)	0.97 (±0.10)
Yellow Bona Dea	14.00 (±0.32)	0.26 (±0.10)	67.71 (±0.10)	1.00 (±0.16)
Yellow Bona Vita	11.98 (±0.08)	0.25 (±0.12)	65.68 (±0.20)	0.92 (±0.03)
Brown Corvinus	11.28 (±0.08)	0.54 (±0.13)	67.49 (±0.05)	1.00 (±0.07)
Barley				
Slaven	7.81 (±0.20)	3.80 (±0.05)	44.1 (±0.18)	1.15 (±0.12)
Poprad	9.54 (±0.11)	2.54 (±0.07)	43.1 (±0.07)	1.16 (±0.07)
Expres	9.72 (±0.12)	2.88 (±0.12)	47.6 (±0.10)	1.30 (±0.06)
Jubilant	10.19 (±0.08)	2.51 (±0.06)	48.2 (±0.11)	1.45 (±0.02)
Pribina	9.24 (±0.01)	2.89 (±0.13)	49.8 (±0.03)	1.25 (±0.6)
Oats				
Izak	10.57 (±0.02)	3.19 (±0.11)	56.1 (±0.05)	5.53 (±0.06)
Hronec	9.74 (±0.07)	2.30 (±0.04)	54.9 (±0.02)	4.94 (±0.09)
SW Betania	11.70 (±0.10)	3.38 (±0.03)	53.8 (±0.12)	5.42 (±0.11)

Viliam	12.82 (±0.03)	2.20 (±0.10)	52.3 (±0.10)	3.95 (±0.07)
Saul	12.97 (±0.02)	3.45 (±0.12)	55.5 (±0.04)	4.66 (±0.06)

Cereals are generally known to have a positive influence on the general state of the human health. The attention of the nutrition experts is paid especially to oat and barley.
Hull-les barley has been successfully used in food, feed, in industrial applications and the effect on the baking properties in bakery products and bread have been determined as well (**Anderson *et al.,* 2000**). The potential use of oat in the production of functional foods is bound to the nutritional value of the grain, in particular to the content and composition of dietary fiber, proteins, and lipids, respectively (**Demirbas, 2005**).

Polyphenols, flavonoids, antioxidant activity in cereals

Antioxidants are present in cereals in relatively small quantity, mainly in the form of polyphenols, predominantly flavonoids, but there are also other antioxidant active components such a tocotrienols, tocopherols and carotenoids. Antioxidant activity of extracts from cereals is attributed to the presence of polyphenolic compounds represented in different amounts according to the individual cereal types, such as antocyanins in purple wheat (**Abdel-Aal and Hucl, 2003**). The results from determination of polyhenols, flavonoids and antioxidant activity in cereals are listed in the Tab 2.
Variation in polyphenolic compounds was considerable. The largest content of polyphenolic compounds (134 mg GAE/l) and flavonoids (2.80 mg RE/l) was determined in blue wheat RU440-6, the lowest value was in brown wheat CORVINUS (87 mg GAE/l). The content of flavonoids was not analyzed in the four analyzed samples of wheat, whereas the absorbance was not measurable at the appropriate wavelength.
If we compare values of total polyphenolic compounds in different types of cereals, it can be concluded that content of total polyphenols in oat is smallest compared to the rest. Some types of cereals are sources of large number of different polyphenolic compounds. Number of hydroxylic groups in phenolic compounds, so as their spatial orientation are proportional to molar response of this method (**Frankel *et al.,* 1995**). This can be the reason for differences in values of total phenols in particular types of cereals.

In the last decade a number of publications have been published in which antioxidant capacity of plant material, such as antioxidant characteristics of polyphenol compounds are tested using different methods (**Brindzová *et. al.,* 2009; Halvorsen *et al.,* 2002; Javanmardi**

***et al*., 2003; Miller *et al*., 2000**). Because of this it is difficult to compare final results, even though there are the same plant species (**Zielinski and Kozlowska, 2000**). The biggest antioxidant capacity, as well as the highest polyphenolic content was found in barley varieties (SLAVEN, POPRAD, 1.0-1.13 mmol TE/l), but also in oat variety (SAUL, 1.30 mmol TE/l).

Table 2 Mean (± s.d.) of the total polyphenolic and flavonoids content and antioxidant capacity in cereal extracts prepared from different varieties

Varieties of cereals	**Polyphenols (mg GAE/L)**	**Flavonoids (mg RE/L)**	**Antioxidant capacity (mmol TE/L)**
Wheat			
Blue UC66049	113 (±0.11)	0.13 (±0.10)	0.19 (±0.08)
Blue RU440-6	134 (±0.18)	2.80 (±0.11)	0.21 (±0.11)
Blue 48M	122 (±0.10)	0.08 (±0.07)	0.27 (±0.07)
Purple Abissinskaja	100 (±0.08)	-	0.15 (±0.10)
Purple Konini	121 (±0.14)	1.33 (±0.10)	0.15 (±0.06)
Purple ANK28A	100 (±0.09)	1.02 (±0.23)	0.08 (±0.10)
Red Federer	113 (±0.10)	0.13 (±0.15)	0.19 (±0.09)
Yellow Citrus	96 (±0.07)	0.68 (±0.09)	0.16 (±0.20)
Yellow Bona Dea	100 (±0.01)	0.61 (±0.07)	0.25 (±0.10)
Yellow Luteus	91 (±0.10)	1.87 (±0.06)	0.11 (±0.06)
Brown Hana	91 (±0.09)	-	0.14 (±0.06)
Yellow Bona Dea	110 (±0.12)	-	0.12 (±0.10)
Yellow Bona Vita	111 (±0.08)	-	0.13 (±0.05)
Brown Corvinus	87 (±0.09)	0.11 (±0.11)	0.12 (±0.12)
Barley			
Slaven	201 (±0.11)	2.53 (±0.10)	1.13 (±0.08)
Poprad	237 (±0.18)	6.53 (±0.11)	1.01 (±0.11)
Expres	165 (±0.10)	2.79 (±0.07)	0.45 (±0.07)
Jubilant	189 (±0.08)	6.22 (±0.07)	0.60 (±0.10)
Pribina	148 (±0.14)	1.39 (±0.10)	0.72 (±0.06)
Oats			

Hronec	87 (±0.10)	5.37 (±0.15)	0.79 (±0.09)
SW Betania	95 (±0.07)	-	0.72 (±0.20)
Viliam	74 (±0.01)	-	0.71 (±0.10)
Saul	97 (±0.10)	-	1.30 (±0.06)

Legend: GAE - gallic acid equivalent, RE – rutin equivalent, TE - trolox equivalent.

Dry matter, ash, dietary fiber, energy value in cereals

According to experts opinions, fiber intake in the diet has an undeniable importance to our overall physical but also mental well-being and health. Between cereal grains, the content of dietary fiber varies (**Nelson, 2001**). The fiber content in the analyzed set of wheat was in the range from 2.4 to 3.2%, the highest content had exhibited sample BONA DEA (3.2%) and the lowest value was observed in sample LUTEUS (2.4%) (Tab 3). Variation in dietary fiber was notable also in barley and oat varieties.

The results revealed that barley varietes have significant higher contents of total dietary fiber (≈17%), while grains of oat showed the significantly lower values (≤ 9%) than the rest cereals. Similar results were obtained by **Grausgruber *et al.* (2004)**.

Humidity is a very important parameter in term of its behavior during storage and should not exceed 15%. The dry matter content of wheat grains was obtained by drying to the constant weight at 105°C, and its portion was 88.4% - 91.4%. Insignificantly higher ash contents showed grains of barley in comparison to oats. The highest energy values were observed for oat varieties on average. Higher ash content indicates richer mineral content and is associated with higher nutritional value. High ash content in our samples was determined in different varieties of barley (Express 2.38%) but also in a sample of oat (variety Saul 2.35%). Energy values were determined by calorimetric method (AC 500, Leco Corp., USA). The energy value of food is the amount of energy expressed in joules (J), respectively kilojoules (kJ) for a defined quantity of food (e.g. 100 g). Different results are recorded in energy values of analyzed cereals. Approximately the same results of energy values were observed for varieties of wheat (342 ± 4 kcal/100g). In regard to varieties of barley and oat, the lowest value were obtained for barley (320 ± 5 kcal/100g), higher values showed variety of oats (356 ± 9 kcal/100g).

Table 3 Mean (± s.d.) of the chemical composition and energy values for different varieties of cereals

Sample	Dry matter (%)	Ash (%)	Dietary fiber (%)	Energy value (kcal/100g)
Wheat				
Blue UC66049	88.9 (±0.08)	1.10 (±0.15)	13.80 (±0.25)	345
Blue RU440-6	89.4 (± 0.10)	1.10 (±0.18)	14.05 (±0.30)	343
Blue 48M	90.7 (±0.15)	1.09 (±0.02)	13.51 (±0.50)	345
Purple Abissinskaja	89.3 (±0.05)	1.09 (±0.05)	13.22 (±0.10)	344
Purple Konini	89.4 (±0.20)	1.11 (±0.10)	13.61 (±0.15)	346
Purple ANK28A	88.4 (±0.08)	1.12 (±0.02)	14.00 (±0.20)	343
Red Federer	90.4 (±0.10)	1.10 (±0.10)	13.03 (±0.12)	342
Yellow Citrus	89.4 (±0.08)	1.09 (±0.15)	12.74 (±0.20)	344
Yellow Bona Dea	90.2 (±0.10)	1.07 (±0.13)	12.20 (±0.10)	345
Yellow Luteus	90.6 (±0.15)	1.10 (±0.15)	13.51 (±0.20)	342
Brown Hana	90.4 (±0.08)	1.09 (±0.15)	11.22 (±0.12)	342
Yellow Bona Dea	91.4 (±0.12)	1.09 (±0.05)	13.50 (±0.13)	343
Yellow Bona Vita	90.1 (±0.05)	1.10 (±0.17)	12.70 (±0.12)	343
Brown Corvinus	90.0 (±0.09)	1.10 (±0.20)	13.50 (±0.15)	342
Barley				
Slaven	89.1 (±0.11)	1.67 (±0.08)	16.09 (±0.06)	323
Poprad	89.7 (±0.07)	2.49 (±0.02)	17.07 (±0.02)	320
Expres	89.5 (±0.12)	2.38 (±0.10)	17.32 (±0.10)	320
Jubilant	89.6 (±0.02)	2.29 (±0.09)	16.01 (±0.11)	324
Pribina	89.5 (±0.19)	2.34 (±0.09)	14.87 (±0.06)	325
Oats				
Izak	89.5 (±0.01)	1.91 (±0.13)	6.60 (±0.13)	365
Hronec	89.6(±0.10)	1.78 (±0.06)	7.82 (±0.14)	360
SW Betania	89.6 (±0.08)	2.05 (±0.08)	7.27 (±0.03)	363
Viliam	89.7 (±0.05)	1.94 (±0.09)	5.32 (±0.02)	360
Saul	90.9 (±0.09)	2.35 (±0.05)	8.66 (±0.06)	382

Selection of suitable cereal for the development of fermented product

Cereals offer another alternative for the production of functional foods. The multiple beneficial effects of cereals can be exploited in different ways leading to the design of novel cereal foods or cereal ingredients that can target specific populations. Cereals can be used as fermentable substrates for the growth of probiotic microorganisms. The main parameters that have to be considered are the composition and processing of the cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage, the organoleptic properties and the nutritional value of the final product. Additionally, cereals can be used as sources of nondigestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of lactobacilli and bifidobacteria present in the colon and act as prebiotics (**Charalampopoulos, 2002**).

By comparing the nutritional composition we selected for the further experiments variety of oat flour Saul, because in terms of healthy beneficial ingredients of all the oat varieties the highest fiber content (8.66%) was determined in this sample, as well as betaglucans (3.45%), proteins (12.97%) and antioxidant activity (1.30 mmol TE/L). This variety was milled into a white, brown and wholegrain fraction, which was subjected to nutritional analysis, focused primarily on the content of health-beneficial components, such as proteins, dietary fiber and betaglucans. After the overall evaluation and comparison of individual fractions as the most suitable for further experiments and the most acceptable in terms of health beneficial ingredients the brown flour with the highest protein content (20.97%), fiber (26.43%) and betaglucans (8.92%) was selected.

Design, preparation and analysis of fermented fibre-betaglucan product

In the next part the fermented fiber-betaglucan products which different concentrations of oat flour (5, 10, 15 g/100 ml water) and various strains of *Lactobacillus* spp. were prepared and used individually. Table data summarized the results of experiments aimed at assessing the above mentioned strains of lactic bacteria, evaluation of titratable acidity, pH and production of lactic acid after 24 hours fermentation at 30°C (Tab 4). The aim of this experiment was to choose the most suitable strain, which produces mainly lactic acid and lowers the pH of fermented product as a result of the organic acids presence.

Table 4 The titratable acidity, pH, lactic acid after 24 h fermentation of partially liquid and sugar suspensions of oat flour inoculated with various strains of *Lactobacillus* at 30 °C.

Strains of *Lactobacillus*	Concentration of oat flour (g/100ml water)	Titratable acidity (g TA/l)	Titratable acidity (g LA/l)	pH
L. plantarum	5	0.2	0.24	5.16
L. plantarum	10	0.3	0.36	4.85
L. plantarum	15	0.4	0.48	4.65
L. delbrueckii	5	0.25	0.30	5.21
L. delbrueckii	10	0.25	0.30	5.00
L. delbrueckii	15	0.28	0.33	4.78
L. crispatus	5	0.08	0.09	6.59
L. crispatus	10	0.1	0.12	6.57
L. crispatus	15	0.1	0.12	6.48

Legend: TA- tartaric acid, LA- lactic acid

From Table 4 it is clear that the lactic acid was produced after 24 hours by all strains, the highest content (0.48 g/100 g) was determined at the initial concentration of 15% oat flour in fermented suspension with *Lactobacillus plantarum*. The largest decrease of pH (4.65) was achieved with the same probiotic strain. Subsequently, this strain was evaluated as the most suitable for further experiments. Production of lactic acid by selected strain *Lactobacillus plantarum* was also in the product obtained from partially liquid and sugar suspensions of oat flour (15%) during the fermentation monitored by measuring the pH at the time (0, 3, 6, 12, 15, 20, 24, 48 hours). Results are reported in Fig 1.

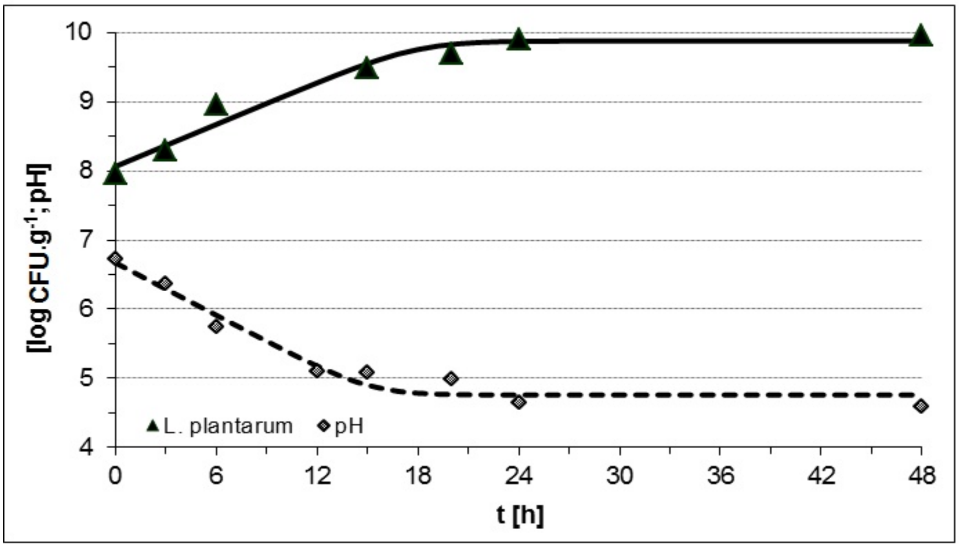

Figure 1 Changes of pH and growth of *Lactobacillus plantarum* during fermentation of partially liquid and sugar suspensions of oat flour (15%) inoculated *Lactobacilus plantarum* at 30 °C

The figure shows that the extension of the fermentation process above 24 hours didn't reduce significantly the pH value of the fermented product. A further increase in acidity that would be desirable but obviously it is not possible to achieve it in the circumstances possible to achieve, because the strain is probably not amylolytic active in order to achieve production of lactic acid in higher amounts.

Cultivation parameters of *Lactobacillus plantarum* were evaluated by the growth curve, determining the number of bacteria over time after inoculation of 15% sterilized suspension of oat flour and cultivation under static conditions at 30 °C. The initial concentration of cells at the beginning of fermentation was 9 x 10^7/g. The number of lactobacilli in fermented fiber-betaglucan product (15%) after 48 hours was 9 x 10^9/g. The numbers of lactobacilli after 48 hours of fermentation were in line with our expectations in order to obtain a product containing approximately 10^{10} CFU/g. The growth curve is shown in Fig. 1.

The lactic acid content of oat suspensions after 0, 3, 6, 15, 20 and 24 hours were determined by capillary isotachophoresis. The highest level of lactic acid was observed after 20 hour, when its concentration reached 2.3 g/l. After this time, the concentration has not significantly increased. It is important to note that the lactic acid bacteria not only contribute to the sour taste of the bread but also to the overall organoleptic quality of this type of product. A significant fact is that during the fermentation a large starch degradation was observed, which reduces the energy value of the product.

The fermented fiber-betaglucan preparation, which the design has been described above was further added to the dough in various amounts.

Rheological properties of dough

After analyzing and selecting the best oat variety we have designed baking recipes with different ratios of oat, wheat flour and addition of a fermented product into bread preparation. We suggested six bakery recipes (including 2 controls: wheat-oat bread and wheat bread, both without addition of a fermented product). Recipes were designed to be an addition of oat flour and fermented product secured the daily dose of betaglucan (3 grams per day, 75 grams of bread). Rheological properties of dough were evaluated using farinograph, extensograph and amylograph machines. For illustration we show recipe and its rheological properties which will be optimized in the next step: wheat and oat flour in ratio 70:30, 15% of a fermented fiber-betaglucan product, 2% of salt, 5% of yeast, water.

Farinographic characteristics of dough

Farinograph assesses flour as an complex in a form of dough. The farinograph output is a farinograph curve (farinogram), from which it is possible to determine water absorption, dough viscosity and the stability of flour during mixing. Water absorption of wheat flours is usually between 56 to 62%, but in our mixed wheat-oat flours was 81.4%.

Time of the dough development it's time from start adding water to flour until the farinograph curve (Fig 2) declines from the maximum consistency. It is mainly determined by the course of hydration of gluten. Weak flour have dough development between 1 to 1.5 minutes, strong flour between 5 min and more. Wheat-oat flour mixture had time of dough development 14.2 minutes, suggesting a strong flour. FQN (Farinograph quality number) is a measure of the quality of flour. Weak flour quickly weakens and has a low number of quality, while strong flour weakens later and has a high number of quality. The quality of wheat-oat mixture was 197 FQN, which is a sign of high quality.

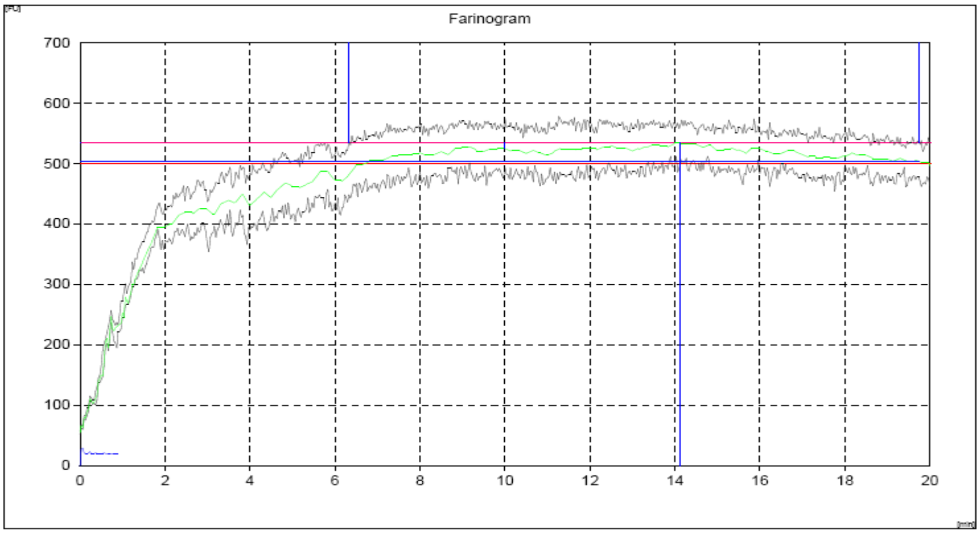

Figure 2 Farinograph curve of dough prepared from wheat-oat mixture of flours (70:30) with addition of 15% fermented fiber-glucan product. The farinograph is drawn on a curved graph with the vertical axis labeled in Brabender Units (BU) and the horizontal axis labeled as time in minutes

Extensographic characteristics of dough

Extensograph can infer whether the dough will be soft, towing, strong or elastic, but also the energy needed to tear the dough. Dough characteristics can be estimated on the basis of the shape of the curve. Smaller volume of bread can be assumed in dough with very short and low or with a very high curve. Such doughs not maintain CO_2 (low curve) or the resulting CO_2 is not enough pressured for enlarging the pores in dough (too high curve). Extensographic curve in Fig. 2 shows a too high curve. Tear energy of dough after 15 minutes was 25 cm^2, after 30 minutes reached 52 cm^2. Flexibility of dough in 15 minute acquired value 26 BU (Brabender Unit) and after 30 minute 576 BU. The value of extensibility/resistance ratio is used together with the tear energy to the preliminary estimate the volume of bread and shape (height to width ratio). Graphical representation of the extensographic curve in our mixture of flours shows Fig 3.

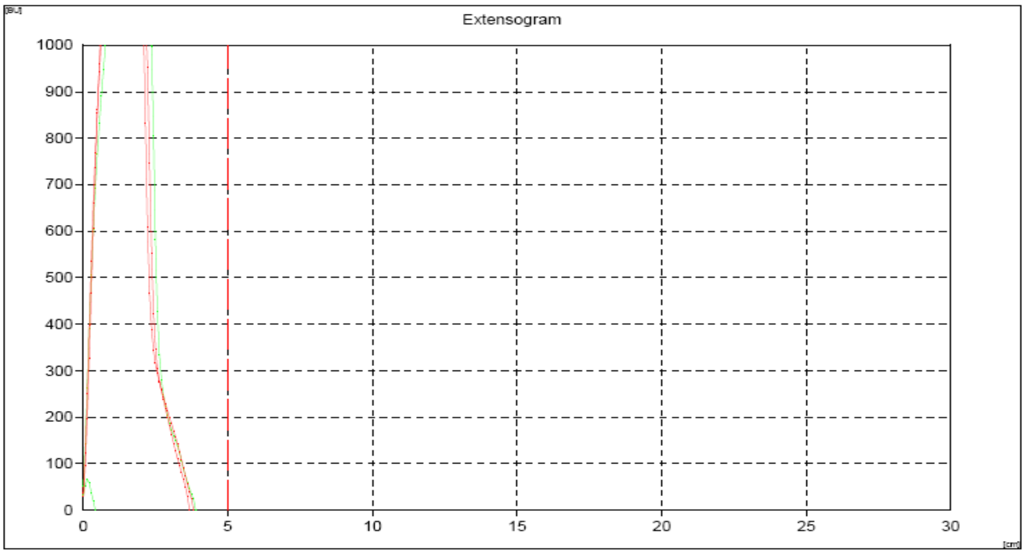

Figure 3 Extensographic curve of dough prepared from wheat-oat flour mixture (70:30) with the addition of 15% fermented fiber-betaglucan product. Horizontal axis of the graph shows the dough expansion [mm] and the vertical axis records the resistance of dough to stretch [EU] (1 EU = 12.3 ± 0.3 mN)

Amylographic characteristics of dough

Amylograph characterizes the baking properties of flour depended on the starch gelatinization and on the enzyme activity (α-amylase) in flours. It provides assessment of the flour quality, suitability of the flour for various applications and measurement of the baking characteristics of flours. Amylograph is a function of suspension consistency of flour and water measured in amylograph units (AU), depending on temperature and time. We investigated the following characteristics: beginning of gelatinization (°C), gelatinization maximum (AU) and gelatinization temperature (°C) shown on an amylographic curve (Fig 4). Value of gelatinization maximum reached 1885 AU at 91,9 °C. Temperature at the beginning of gelatinization was 60,6 °C.

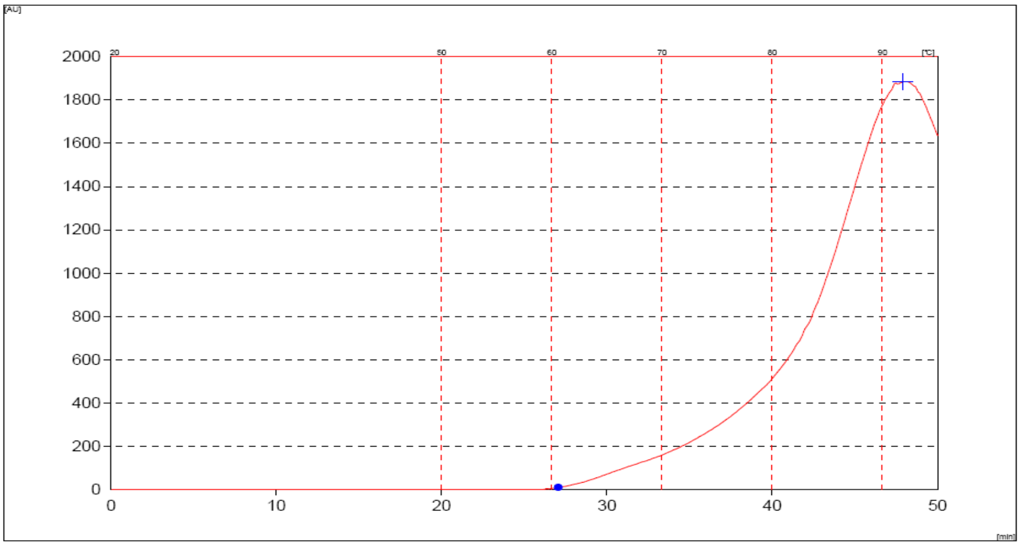

Figure 4 Amylographic curve of dough prepared from wheat-oat flour mixture (70:30) with the addition of 15% fermented fiber-glucan product. The horizontal axis shows time in minutes, in vertical axis is recorded gelatinization maximum (AU)

Observations of rheological properties of mixtures flours are further verified by bakery experiments.

CONCLUSION

To sum up, there exists a considerable variability of biologically active constituents in cereals, and especially oat has a great potential for healthy human food products. The results indicate that cereals represent a valuable source of biologically active constituents (mainly dietary fiber, betaglucans, polyphenols), which provide health-enhancing potential of a functional food. Based on the nutritional analysis which has been made, the selection, preparation and utilization of fermented fiber-betaglucan product in the bread making was conducted. Results indicate that prepared fermented fibre-betaglucan product is suitable not only for bakery products, but also for development other healthy beneficial foods and can show a positive benefit to human health.

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