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Technology of wheat and rye bran biotransformation into functional ingredients

Kapreliants, L. and *Zhurlova, O.

Odessa National Academy of Food Technology, Kanatnaya str. 112, Odessa, 65039, Ukraine

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Abstract

Such by-products of grain processing, as bran, middling, and germ much better satisfy requirements for being functional products than flour. New biotechnological approaches are proposed for the secondary processing of the by-products into new food products and ingredients, such as additives and enriched functional foods concentrates: the carbohydrate-protein, the dietary fiber, the biologically active substances (BAS), the polyphenols and the xylooligosaccharides (XOS). It is proposed in the article is to show the developed complex biotechnology of biotransformation of wheat and rye bran by hydrolases enzymes into functional ingredients with antioxidant and prebiotic activities.

Introduction

The modern concept of a healthy diet assumes increasing of the food products biological value by introducing natural physiologically functional ingredients (biocorrectors), which become sources of important BAS. Balanced functional food products included in the diet not only provide human body with plastic material and energy, but also control specific physiological functions and contribute to the maintain of health by reducing the risk of disease (Ivanova and Uliyachenko, 2005; Venketeshwer, 2012). One of the priorities of the world politics in the field of the healthy lifestyle is the necessity to eliminate the deficit of micro- and macronutrients in the diet, the most important factors that optimize nutrition and health of planet's population.

Secondary products of grain processing are rich sources of physiologically functional ingredients, biotransformation of which made it possible to obtain a number of biologically active substances of different chemical nature with a wide range of physiological effects (Kaprelyants *et al*., 2013; Kaprelyants and Zhurlova, 2014). Dietary fibers, the functional ingredients for enriching foods, have a whole set of useful properties and can have a beneficial effect on the human body.

Wheat and rye bran are not only a rich source of the dietary fibers, but also contain the phenolic acids (Dykes and Rooney, 2007; Kaprelyants *et al.*, 2013; Kaprelyants and Zhurlova, 2014). The antioxidant components in wheat and rye bran are mainly phenolic compounds including ferulic, protocatechuic, sinapinic, vanillic, p-hydroxybenzoic, and p-coumaric acids (Dykes and Rooney, 2007; Liangly, 2008; Kaprelyants and Zhurlova, 2013) distributed in the bran fractions.

As studies show, the ferulic acid prevailing in the bran is the main contributor to the antioxidant activity. The most of phenolics in bran are insoluble and are bound by ester and ether linkages with polysaccharides, such as arabinoxylan and lignin, in the cell wall (Dykes and Rooney, 2007; Liangly, 2008; Kaprelyants and Zhurlova, 2013).

Phenolic acids level varies among cereals and their bran: 1300 - 1450 µg/g in wheat, 1360 - 1380 µg/g in rye, 4500 - 4900µg/g in wheat bran, and 4150 - 4200 µg/g in rye bran. Preparation of phenolic compounds from cereals has several advantages. Comparing with fruits and vegetables, the cereals are dry, so they are easily stored for a long time. This property contributes to simplification of stable phytonutrients concentrates producing during a year. Among the leaders in terms of polyphenols content in grains are wheat, rice, and rye (Dykes and Rooney, 2007; Liangly, 2008; Kaprelyants, L., *et al.*, 2013).

Cereals bran contains xylan polysaccharides that can be converted into the xylooligosaccharides (XOS) by using the biocatalysis. XOS selectively stimulate the growth of probiotic microorganisms. Numerous studies of XOS have shown the diversity of their biological properties, such as

*Corresponding author.
Email: *lyasya89@mail.ru*
Tel: +380666870815

prebiotic, mitogenic, antioxidant, antiinflammatory, and antihyperlipidemic activities (Chithra and Muralikrishna, 2009; Ramkrishna *et al*., 2014; Kaprelyants, 2015). Immunity creates the high resistance of human body's to adverse factors of external and internal environments. Grains' phenolic antioxidants possess the immunostimulating activity, they protect biomembranes against oxidative cells' damage, prevent formation of adducts with DNA, thus preventing the activation of mutagenic processes (Dykes and Rooney, 2007; Liangly, 2008; Belobrajdic and Bird, 2013; Kaprelyants and Zhurlova, 2013).

The problem of biocatalytic processing of secondary grain resources is urgent and requires scientifically based technological solutions. The present study refers to the development of the complex technology of secondary raw materials processing by biotransformation of the grain polymeric complex bran cell walls using hydrolases enzymes, as the most efficient method of producing functional ingredients and expanding the market range of functional foods. Therefore, the aim of this article was the development of the functional ingredients biotechnology using the processing of the secondary grain products.

Materials and Methods

Raw materials and composite components used during the experimental studies were selected from the following sources: wheat bran of 2011-2013 harvest obtained from State Enterprise "Kulindorovsky KHP" and of 2011-2012 harvest from Closed Joint Stock Company "Kievmlyn"; rye bran of 2011-2013 harvest obtained from Closed Joint Stock Company "Kievmlyn" and of 2011-2012 harvest from the Public company "Luganskmlyn"; industrial enzyme preparations produced by "Enzyme" Ladyzhyn plant biotechnology and enzyme preparations: α-amylase from *Bacillus subtilis* (2000 U/g), glucoamylase from *Aspergillus awamori* (6000 U/g) and a protease from *Bacillus subtilis* (70 U/g) and Viscozyme L multienzyme preparation from *Aspergillus aculeatus*, having a number of activities (β-glucanase – 100 U/g, xylanase – 50 U/g, cellulase – 70 U/g, pektinesterase – 40 U/g, and feruloesterase) produced by the «Novozymes A/S»company, Denmark; pure cultures of bifidobacteria: *Bifidobacterium bifidum* - drug "Bifidumbacterin" produced by "Biopharma" and lactic acid bacteria *Lactobacillus acidophilus*-Ep-317/402 from museum's Department of Biochemistry, Microbiology and Physiology of Nutrition of the Odessa National Academy of Food Technologies.

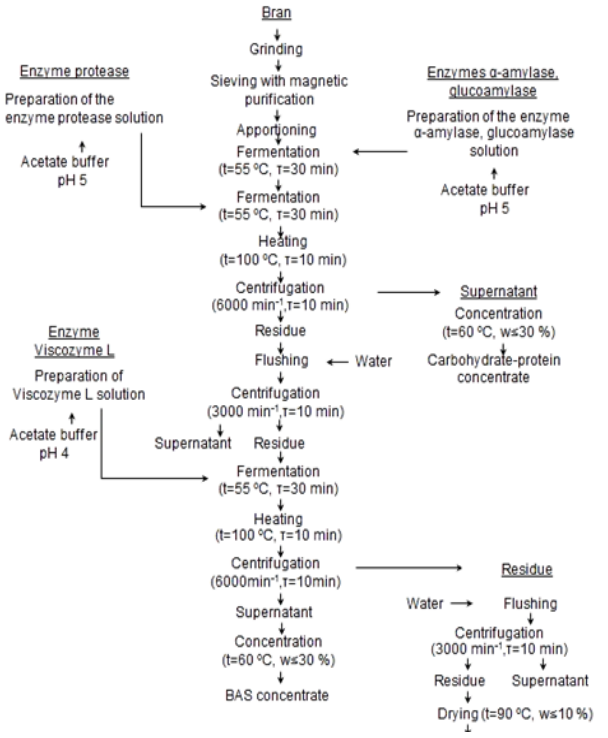

Figure 1. Technological scheme of the functional components from the grain raw materials

Results and Discussion

The biomodification of bran according to the developed technology is the thermal and mechanical pretreatment of bran with the following stepwise enzymatic hydrolysis. After the first stage of bran hydrolysis by amylase and protease we obtained the carbohydrate-protein concentrate. Further processing of the bran modified matrix by multienzyme Viscozyme L preparation having cellulase, hemicelulase, feruloesterase activities, allowed to obtain the number of functional ingredients, such as xylo-oligosaccharides, polyphenols and dietary fiber. Application of the stepwise enzymatic treatment allowed to maximally separating all resulting products, thus greatly facilitating the technological processing of of wheat and rye bran.

On the basis of experimental studies (Kapreliants and Zhurlova, 2015a; 2015b), the biotechnology of functional ingredients obtaining has been developed from grain raw materials by their hydrolytic enzymes biotransforming. The technological scheme of the functional ingredients obtaining from raw grain materials is shown in Figure 1. This is a milling of bran to obtain the particles size of 750 μm (W = 12%) pretreated by air at the temperature of (120 ± 1)°C for 5min to destroy microorganisms. The milled bran was treated with enzyme preparations: α-amylase (c = 0.001%) and glucoamylase (c = 0.0006%) at a ratio of 1:10 between bran and solution of enzyme

Table 1. The chemical composition of carbohydrate-protein concentrates from fermented bran

Components, %	Carbohydrate-protein concentrate	
	Wheat bran	Rye bran
Moisture	30.00	30.00
Ash	2.50	3.00
Carbohydrates:	43.40	35.60
Glucose	34.70	27.40
Maltodextrins	8.70	8.20
Raw protein	24.10	31.40
Essential amino acids:		
Valine	1.14	1.67
Isoleucine	0.76	1.16
Leucine	1.44	2.12
Lysine	0.93	1.27
Methionine	0.35	0.14
Threonine	0.77	1.04
Tryptophan	0.43	–
Phenylalanine	0.92	1.43
Arginine	1.69	1.95
Histidine	0.67	0.65
Nonessential amino acids	15.00	19.97
Polyphenols	0.05	0.06

The significant mean difference at 95%. n =3.

Table 2. The chemical composition of BAS concentrates

Bran's residue	Moisture	Protein	Ash	Carbohydrates:		Polyphenols
				XOS	other carbohydrates	
Wheat	30.0	4.4	7.3	49.4	8.4	0.5
Rye	30.0	4.6	7.5	51.4	5.9	0.6

The significant mean difference at 95%. n =3.

preparations, at 55°C, pH = 5 for 30min followed by the protease enzymatic preparation (c = 0.005%) at a ratio bran / solution of enzyme preparations of 1:10 at 55°C, pH = 5 for 30min in the reactor.

After one hour of the hydrolysis, enzymes were inactivated by keeping the mixture at the temperature of 100°C for 10min, then it was cooled to 25°C and the residue was separated from the supernatant by centrifugation at 6000 min^{-1} for 10min. The residue was washed three times with water and again subjected to centrifugation at 3000 min^{-1} for 10min. The supernatant containing the hydrolysis products of starch and proteins was concentrated in a vacuum evaporator at the temperature of 60°C to the final 30% of moisture content in the concentrate. The resulting carbohydrate-protein concentrate contained predominantly starch hydrolysates and protein. It was a sweet odorless paste of light gray color. The chemical composition of the carbohydrate-protein concentrate is presented in Table 1.

Expediency of the proteins hydrolyzate and starch concentrating under vacuum at 60°C is justified by the fact that protein amino groups during the heating of react with reducing sugars keto groups (the Maillard reaction). Consequently, the formation of melanoidins takes place giving a dark color to the finished product. The process of heat treatment under vacuum significantly reduces the boiling point of the carbohydrate-protein mass providing for the high quality finished product.

The residue (bran modified matrix) was subjected to enzymatic hydrolysis by multienzyme Viscozyme L preparation (c = 0.001%) at a ratio residue/solution of the enzyme preparation of 1: 10 at a temperature (50 ± 1)°C, pH=4 for 4 hours in the reactor. The hydrolyzate was maintained at the temperature of 100oC for 10min to inactivate the enzymes and then it was cooled to (25 ± 1)°C; and the supernatant was separated by centrifugation at 6000 min^{-1} for 10min. The supernatant was concentrated in the vacuum dryer at the temperature of 60°C to the final moisture content 30% of the BAS concentrate. The BAS concentrate contains polyphenols and XOS, it is an odorless light brown syrup. The BAS concentrate chemical composition is shown in Table 2.

The residue, which is the dietary fiber concentrate, was washed with water, centrifuged at 3000 min^{-1} for 10min and dried in a single-chamber fluidized-bed drier at the temperature of 90°C to the final 10% moisture content. The dietary fiber concentrate is brown powder without taste and odor. The quantity of the main component reached 79.9% (Table 3). Technological scheme of the functional food ingredients and the preparation of polyphenols and XOS from BAS concentrate is shown in Figure 2.

For separating polyphenols and XOS concentrates from the BAS concentrate, it was treated with ethanol (96%) in a ratio of concentrate / alcohol 1:3. The

Table 3. The chemical composition of Dietary Fiber concentrates

Components, %	Dietary Fiber concentrate from wheat bran	Dietary Fiber concentrate from rye bran
Moisture	10.0	10.0
Ash	3.2	3.5
Protein	3.6	3.9
Starch	4.1	2.7
Dietary Fiber:	79.1	79.9
Lignin	23.2	21.2
Monosaccharides:		
Glucose	64.9	69.0
Xylose	19.8	17.5
Arabinose	3.5	3.3

The significant mean difference at 95%. n =3.

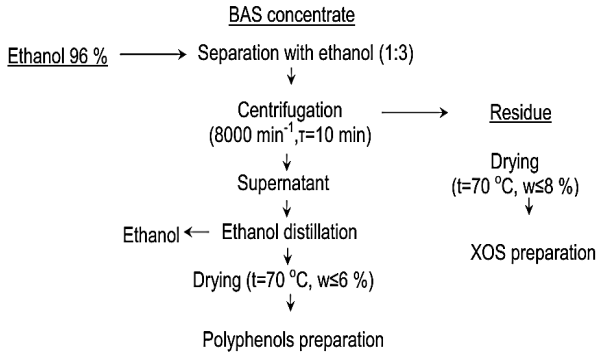

Figure 2. Technological scheme of the functional food ingredients: polyphenols and XOS preparation

residue of the XOS was separated by centrifugation at 8000 min^{-1} for 10min. Alcohol was removed from the supernatant (polyphenols alcoholic solution) by distillation and polyphenol's solution was vacuum-dried in the vacuum-circulation system to the moisture content of 6%. The polyphenols preparation was the powder of the light yellow color with the vanillin odor and bitter taste. It contained 90 and 91% of polyphenols, 7.9 and 7.1% of carbohydrates, 2.1 and 1.9% of protein in wheat and rye bran, respectively. The main component of polyphenols preparation is the ferulic acid (78 and 52% of the total polyphenol content from the wheat and rye bran, respectively). Application of the vacuum-circulation system allows to unite two operations: the distilling alcohol from the extract and the drying of the polyphenol preparation.

In the first stage of the apparatus work, alcohol evaporates to the upper part of the separation chamber where it is discharged through the pipe to the condenser. After distilling off the alcohol, the vacuum is connected and the non-alcoholic extract is dried to a crystalline state. The polyphenol preparation has a high antioxidant activity (1116.3 - 1270u.e.a./cm^3). The residue of XOS is dried in a double-drum vacuum-drier at 70°C to a final moisture content of 8%. The XOS preparation is odorless powder of the light brown color with the sweet taste. It contains 65.4 and 68% of XOS, 11.1 and 8.0% of other carbohydrates, 9.7 and 10% of ash, 5.8 and 5.9% of protein in wheat and rye bran, respectively. The XOS preparation has a stimulating effect on growing and developing the probiotic cultures. It provides for accumulation of *L. acidophilus* $1.4 \cdot 10^{10}$ and $1.9 \cdot 10^{9}$CFU/cm^3, and *B. bifidum* $9.2 \cdot 10^{10}$ and $1.1 \cdot 10^{10}$CFU/cm^3, and it can be used as a prebiotic.

Conclusion

According to the developed technology of the step-by-step biotransformation of the grain processing secondary products, it is possible to obtain a number of functional ingredients, such as concentrates of the carbohydrate-protein, the BAS, the XOS preparation, the polyphenols preparation and the dietary fiber from wheat and rye bran. The technology scheme of the functional components from the grain raw materials includes the following steps: milling and preparation of raw materials, enzymatic hydrolysis, centrifugation, concentrating, and drying. The finished products are the carbohydrate-protein concentrate and the BAS concentrate. If the further processing of the the BAS concentrate to the functional ingredients the polyphenol preparation and the XOS preparation is performed, the technological scheme becomes more complicated by adding fermentative hydrolysis and ethanol fractionation operations.

The functional ingredients produced by application of the developed technology expand the functional ingredients range market and can be used as separate dietary supplements and food ingredients with physiological and functional capacity of promoting reduction of the oxidative stress and correction of the intestinal microflora of the human body.

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