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Wheat Bran Carbohydrates as Substrate for *Bifidobacterium lactis* Development

V. Radenkovs, D. Klava, and K. Juhnevica

***Abstract*—**The present study addresses problems and solutions related to new functional food production. Wheat (*Triticum aestivum* L) bran obtained from industrial mill company "Dobeles dzirnavieks", was used to investigate them as raw material like nutrients for *Bifidobacterium lactis* Bb-12. Enzymatic hydrolysis of wheat bran starch was carried out by α-amylase from *Bacillus amyloliquefaciens* (Sigma Aldrich). The Viscozyme L purchased from (Sigma Aldrich) were used for reducing released sugar. *Bifidibacterium lactis* Bb-12 purchased from (Probio-Tec® CHR Hansen) was cultivated in enzymatically hydrolysed wheat bran mash. All procedures ensured the number of active *Bifidobacterium lactis* Bb-12 in the final product reached 10^5 $CFUg^{-1}$. After enzymatic and bacterial fermentations sample were freeze dried for analysis of chemical compounds. All experiments were performed at Faculty of Food Technology of Latvia University of Agriculture in January-March 2013. The obtained results show that both types of wheat bran (enzymatically treated and non-treated) influenced the fermentative activity and number of *Bifidibacterium lactis* Bb-12 viable in wheat bran mash. Amount of acidity strongly increase during the wheat bran mash fermentation. The main objective of this work was to create low-energy functional enzymatically and bacterially treated food from wheat bran using enzymatic hydrolysis of carbohydrates and following cultivation of *Bifidobacterium lactis* Bb-12.

***Keywords*—**Viscozyme L, α-amylase, *Bifidobacterium lactis*, fermented wheat bran.

I. Introduction

CEREAL and whole grain products are an important source of dietary fibre in the human diet. Wheat (*Triticum aestivum*) bran is the coarse outer layer of the wheat kernel that is separated from the cleaned and scoured kernel. It consists mainly of the large pieces of bran remaining after the flour has been extracted from the wheat.

Wheat bran is a composite material formed from different histological layers, and three different strips can be obtained from the soaked outer layers. The outer strip corresponds to outer pericarp (epidermis and hypodermis), the inner one corresponds to the aleurone layers, and the intermediate one remains a composite of several tissues (inner pericarp, testa, and nuclear tissue [11].

Wheat grain is a complex structure composed of different tissues that have distinct functions and biochemical compositions. The starchy endosperm (80–85% of the grain) is mostly composed of starch and proteins, while most of the fibre, vitamins, minerals and antioxidants are concentrated in the outer layers (12–17% of the grain) and the wheat germ (3% of the grain) [11]. Wheat endosperm is surrounded by several adhesive outer layers (including pericarp, testa, and aleurone layer). After milling, a composite material that contains all these different layers is obtained and is commonly called bran. The current wheat grain milling process aims at recovering white flour (mostly composed of starchy endosperm), with bran and germ being discarded. Wheat bran is thus mostly used for animal feeding, even though – due to its high nutritional potential – it could be used to produce ingredients to increase the nutritional quality of human foods [12].

Probiotics are defined as a microbial dietary additive that beneficially influences the host physiology by modulating mucosal and systematic immunity, as well as improving the nutritional and microbial balance in the human gastrointestinal (GI) tract [2], [8]. Probiotic bacteria are presented mostly by genera *Lactobacillus* and *Bifidobacterium* and are also claimed to prevent cancer, reduce the cholesterol level and improve the lactose utilisation [6], [14]. These bacteria must be absolutely safe for human health, able to produce bacteriocines and vitamins, and survive during passage through the upper part of the GI (gastro-resistant) to allow their entry in large amounts to the intestines, resistance to bile, and active lactic acid producers, the tolerance to lactic acid, and sustainable viability during the storage of a product [7], [19], [8].

For wheat bran based production, carbohydrates hydrolysis is necessary: to obtain monosaccharide's like D-glucose. Starch hydrolysis gives possibility to produce the maltodextrins [10], which can be used like nutrient for bacterial grow.

The main objective of this work was to create low-energy functional enzymatically and bacterially treated food from wheat bran using enzymatic hydrolysis of carbohydrates and following cultivation of *Bifidobacterium lactis* Bb-12.

II. Materials and Methods

Experiments were done at the Faculty of Food Technology of Latvia University of Agriculture in collaboration with research Laboratory of Agronomic Analysis in January-March 2013.

This research has been prepared within State Research Programme 'Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)' (2010.-2013.) Project no. 3. 'Sustainable use of local agricultural resources for development of high nutritive value food products (Food)'.

V. Radenkovs, D. Klava, and K. Juhnevica are with the Latvia University of Agriculture, Liela street 2 LV - 3001, Latvia (corresponding author, V. Radenkovs, e-mail: vitalijs.rad@inbox.lv).

A. Bran samples

Summer wheat (*Triticum aestivum* L.) bran samples were purchased from industrial mill in Latvia- SC 'Dobeles dzirnavnieks'- wheat bran (WSSD) were used as dietary fibre and carbohydrate source.

B. Enzymes

Industrial enzymes preparations produced by "Novozyme Corporation." (Bagsvaerd, Denmark) and purchased from Sigma-Aldrich. Two commercial preparations of enzymes: α-amylase from *Bacillus amyloliquefaciens* and Viscozyme L from *Aspergillus* spp. were used to hydrolyze carbohydrates. α-amylase has a declared activity ≥250 units/g, optimum conditions of enzymatic pretreatment is pH 5.0-8.0, temperature 55°C and incubation time 0.5h [5], form Viscozyme L declared activity is 100 FBG/g, optimum conditions are pH 4.6, temperature 44°C and incubation time 3.2h [23]. In this scientific work enzymes were tested both independently and in combination to quantify interaction.

C. Bacteria

Bifidobacterium lactis Bb-12 (Probio-Tec® from Chr. Hansen) was used to inoculate the samples.

D. Enzymatic Hydrolysis

Wheat bran size distribution was determined by sieving trough the sieve (sieve size ranging from 150µm to 750µm). For α-amylases treatments, wheat bran (30g) was mixed with 300mL of distillated water in 400-mL Erlenmeyer flasks with dilutions 1:10 (pH 6.65) and (45g) was mixed with 255mL of distilled water 1.5:8.5 (pH 6.65), then 300µL of α-amylase was added. Hydrolysis was carried out in a water bath at temperature 55°C, incubation time 0.5h and shaking intensity 60 rpm.

After starch hydrolysis and enzyme inactivation (10min. temperature 100°C) wheat bran mash was 3 minutes homogenized (pH 4.6) in each dilutes and Viscozyme L 0.9 µL was added. Incubation time is 3.2h, temperature 44°C and shaking intensity 60rpm.

E. Fermentation with Lactic Acid Bacteria Bifidobacterium lactis

Enzymatically hydrolysed wheat bran was used for *Bifidobacterium lactis* Bb-12 cultivation. *Bifidobacterium lactis* Bb-12 with activity 10^9CFU g^{-1} 0.1g was added. After 18h at temperature 37°C, wheat bran mash was lyophilized and stored in refrigerator at temperature -18°C. Fermentation procedure was carried out according to the recommendations specified in the company's certificate.

The amount of viable *B. lactis* Bb12 was determined on modified *Lactobacilli* MRS Broth by adding 16.4g L^{-1} of dehydrated culture media (Becton, Dickinson and Company, BD Difco$^{TM)}$ incubation in jars (Memmert incubator INB 400 L53).

Total content of bran dietary fibre was analysed using adopted methods by AOAC enzymatic / gravimetric methods 'Total Dietary Fibre in Foods' No 985.29, 991 using 'FOSS Analytical Fibertec E 1023 system'.

Starch content was determined using 'Native starch Determination of starch content. Ewers polarimetric method is used EN ISO 10520:1998.

The content of glucose, fructose and maltose of enzymatically treated wheat bran was determined by applying the method of high performance liquid chromatography (HPLC). The method is based on the fact that the chromatographic separation of glucose, fructose and maltose is based on their delayed time differences [18].

Moisture was analyzed using 'Determination of the Moisture Content of Cereals and Cereal Products method'- ICC Standard No, 110/1, by drying for 2h at 130°C.

pH was measured using 'Hydrogen-Ion Activity (pH)-Electrometric method' - AACC 02-52.01, using JENWAY 3520 (Barloworld Scientific Ltd., ESSEX, UK) pH-meter. The pH electrode was dipped into a mixture of homogenized sample and distilled water.

Fig. 1 represents the number of viable *B. lactis* Bb-12 cells in bacterially treated wheat bran samples was determined by 'Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony count technique at 30° C' - ISO 15214:1998.

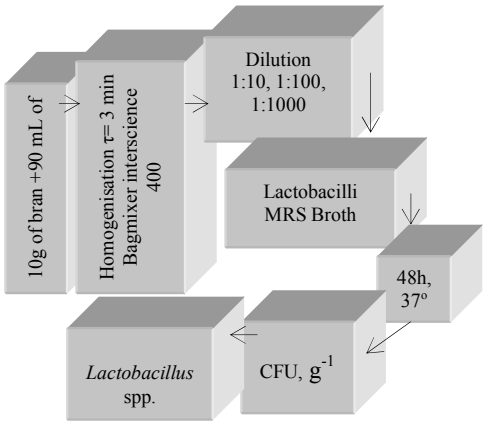

Fig. 1 Scheme of enumeration of lactic acid bacteria

Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance (Anova) where factor was bran and dependent parameters-performed analyses. Duncan's test was used for individual variety of characterization by a parameter. Statistical differences were considered significant at $p<0.05$. Microsoft office software version 2007 was used to determine significant differences between the samples.

II. Results and Discussion

Since the milling process highly influences the proportions of the different cell types in the bran, it is expected that brans originating from different genetic/agricultural backgrounds and produced by different processes have different chemical composition. The bran samples of different grains vary considerably in their chemical components including cell wall polysaccharides and bioactive compounds [17].

To select the appropriate wheat bran samples with highest starchy endosperm concentration particle size distribution of wheat bran samples was measured.

Wheat bran contained from 8.8 – 24.8% starchy endosperm, respectively. According to other scientific research particle size diameters of wheat starchy endosperm is approximately from 10 to 40μm [1]. Starchy endosperms have two types of starch granules. They vary in diameter at maturity from about 10 to >35μm [20] According to this data and data from Table I it is possible to conclude that the highest content of small particle sizes was in WSSD, it's explainable by large amount of starchy endosperm with particle size <160μm, it results in better possibility for starch hydrolysis with α-amylase as a result sugars concentration increases. Permissible losses of bran samples were occurred during bran sieving (under %).

TABLE I
RELATIVE PARTICLE SIZE DISTRIBUTION OF DIFFERENT BRAN SAMPLES

	Wheat small size(Dobele)	Wheat large size(Dobele)	Wheat large size(Riga)
Relative particle size distribution (μm)		%	
750	0.05	57.35	89.00
450	8.05	20.65	5.60
315	23.05	13.66	1.30
250	17.25	3.80	0.20
200	17.85	1.23	0.05
160	13.85	0.43	-
<160	15.1	0.25	-

Our further research will be conducted with the sample Wheat small size (Dobele), since this sample have a highest amount of starchy endosperm and it can be used like nutrient for *B. lactis* Bb-12 growing and development.

Cell wall polysaccharides hydrolysis monitored by measuring the amount of starch reducing sugars (glucose, maltose and fructose) and total dietary fibre in the samples. As expected, the concentration of starch and total dietary fibres decreased in all wheat bran samples comparing to control (non-treated). The highest hydrolysis percentage was obtained in wheat bran sample dilution 1:10 Fig. 2, starch content decreased by 44.95%, and wheat bran sample dilution 1.5:8.5 starch content decreased by 40.91%. One-way Anova showed there were significant differences ($p<0.05$) between the samples. It was reported that the controlled carbohydrates hydrolysis by combination of α-amylase with Viscozyme L results in maltodextrin, D-glucose, fructose, maltose formation, which lowers the energetic value of the product.

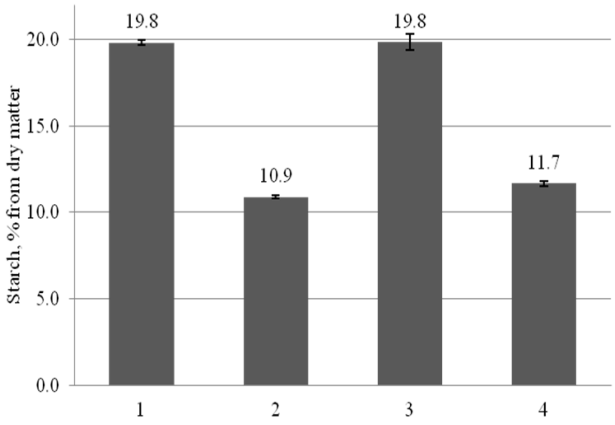

Fig. 2 Starch content of different wheat bran fractions, % from dry matter. 1 - non-treated 1:10; 2 - enzymatically treated 1:10; 3- non-treated 1.5:8.5; 4 - enzymatically treated 1.5:8.5

Dietary fibre (DF) is a group of food components which are resistant to hydrolysis by human digestive enzymes. Dietary fibre consist a polysaccharides (starch, cellulose, hemicelluloses (arabinoxylan), pectin), oligosaccharides (fructo-oligosaccharides, galacto-oligosaccharides), lignin. Specific forms of dietary fibre are readily fermentable by specific colonic bacteria, such as *bifidobacteria* and *lactobacilli* species, increasing their cell population with the concomitant production of short-chain fatty acids (SCFA) [4]. By specific enzymatic treatments, cell wall polymer properties can also be altered which can be utilized in food processing [3].

For the optimum conditions of enzyme pretreatment depended on enzyme concentration, pH, incubation time and temperature. For verification of the model, the wheat bran total dietary fibre was extracted under optimal conditions and the extracted of TDF was determined. Scientific publications and other scientific works implies the optimal conditions for cellulose, hemicelluloses and pectin hydrolysis is pH 4.6, incubation time 3.2h and amount of enzyme concentration is 900μL [23].

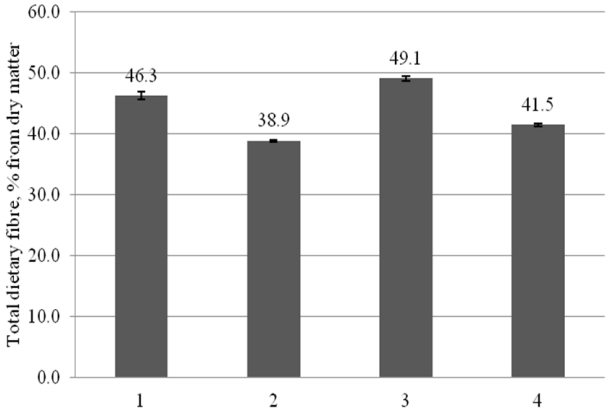

Fig. 3 Total dietary fibre content of different wheat bran fractions, % from dry matter 1 - non-treated1:10; 2 - enzymatically treated 1:10; 3 - non-treated 1.5:8.5; 4 - enzymatically treated 1.5:8.5

Fig. 3 shows results of these experiments, total dietary fibre concentration significantly decreased using Viscozyme L. The highest hydrolysis percentage was obtained in wheat bran sample dilution 1:10, total dietary fibre content decreased from 46.3±0.61% to 38.9±0.19% (19.95%), wheat bran sample dilution 1.5:8.5 total dietary fibre content decreased from 49.1 to 41.5±0.22% (15.58%) One-way Anova showed there were significant differences ($p<0.05$) between the samples (non-treated and enzymatically treated), compared dilution (1:10 to 1.5:8.5) we can conclude that there are not significant differences ($p>0.05$). Based on this data in our future work we will use only one dilution (1:10), on other hand we can assume, that the splitting of cellulose and hemicelluloses into sugars occurred.

Plant cell wall polysaccharides are the most abundant organic compounds found in nature. They make up 90% of the plant cell wall and can be divided into three groups: cellulose, hemicellulose, and pectin. Cellulose represents the major constituent of cell wall polysaccharides and consists of a linear polymer of β-1.4-linked d-glucose residues. The cellulose polymers are present as ordered structures (fibres), and their main function is to ensure the rigidity of the plant cell wall [21].

In this scientific work we don't realize investigation for all of maltodextrins, and all of sugars were represented approximately. Our current research was conducted to investigate differences between enzymatically treated and not treated wheat bran samples. To release maltodextrins by starch hydrolysis α-amylase from *Bacillus amyloliqueafaciens* was used, for degradation of plant cell wall polysaccharides like crystal structure of cellulose as well as hemicelluloses and pectin Viscozyme L were used which contains a large variety of carbohydrates (cellulase, Q-glucanase, hemicellulase and xylanase). To release sugars from crystal structure of cellulose and hemicelluloses three types of enzymes are necessary: Endo-1.4-β-clucanase, exo-1.4-β-glucanase and β-glucosidases. Endo-1.4-β-glucanases randomly cleave internal bonds in the cellulose chain. These enzymes may be non-processive or processive (in processive enzymes, enzyme-substrate association is followed by several consecutive cuts in a single polysaccharide chain that is threaded through the active site [22].

Exo-1.4-β-glucanases attack the reducing or non-reducing end of the cellulose polymer. Processive exo-1,4-β-glucanases are referred to as cellobiohydrolases; they are among the most abundant components in natural and commercial cellulase mixtures and a subject of intense study.

β-glucosidases convert cellobiose, the major product of the endo- and exo-glucanase mixture, to glucose.

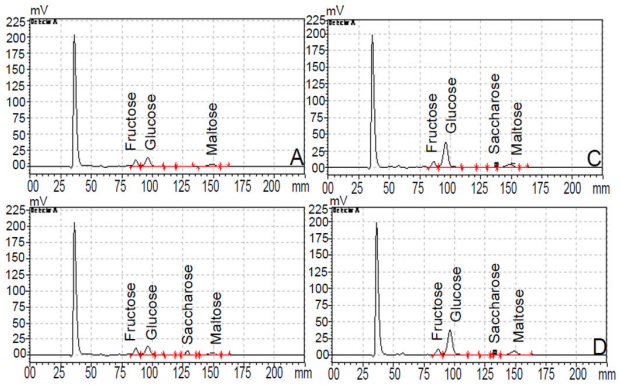

Fig. 4 Sugar content of wheat bran samples, g L^{-1}: A-non-treated 1.5:8.5; B-non-treated 1:10; C-enzymatically treated 1.5:8.5; D-enzymatically treated 1:10

Quantitative analysis of sugar content Fig. 4 shows significant differences between the non-treated and enzymatically treated wheat bran samples.

Obtained results of sugars argue that the use of enzymes increased the content of glucose in wheat bran, amount of glucose ranged from 7.7g L^{-1} (A) 7.8g L^{-1} (B) (non-treated) to 22.3g L^{-1} (C), 22.6g L^{-1} (D) (enzymatically treated), there were significant differences between the glucoses amount in the wheat bran samples ($p<0.05$). On the other hand we have similar increases of maltose when the wheat bran samples are treated with enzymes, amount of sugar ranged from 3.5 (A), 3.6 (B) g L^{-1} (non-treated), to 5.2 (C), 6.3 (D) (enzymatically treated). One-way Anova showed there were significant differences ($p<0.05$) between the samples (non-treated and enzymatically treated). Our results are similar in scientific literature found, as wheat bran samples were treated with enzymes (Depol 740 L, Ecopulp TX 200, Econase CE) glucose concentration ranged from 18g L^{-1} non-treated to 24g L^{-1} enzymatically treated [15]. Another author [16] has proved that the increase of reducing sugars in samples was associated with an increase in amount of other extractable compounds.

After the wheat bran treatment with different kind of enzymes we made sure that the enzymatical treatments allow degraded polysaccharides as well as non polysaccharides to release sugars, which is necessary for *Bifidobacterium lactis* Bb-12 growing and development.

The next step is unfractionated wheat bran mash fermentation using *Bifidobacterium lactis* Bb-12 (Probio-

Tec® from Chr. Hansen) and pH was measured during fermentation Fig. 5.

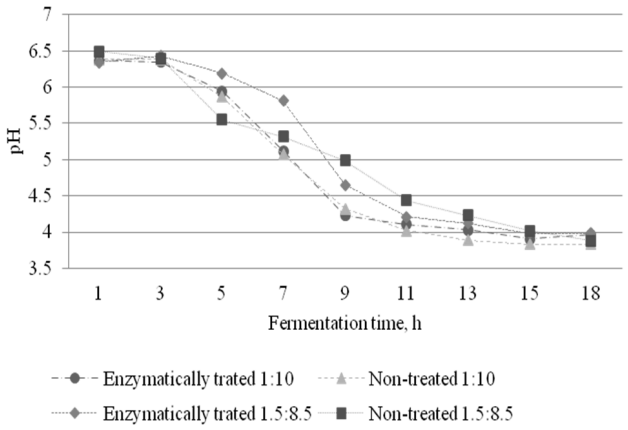

Fig. 5 Fermentation of wheat bran mash by *Bifidobacterium lactis* Bb12

Obtained data implies that during the fermentation, the pH decreases with a simultaneous increase in acidity, as lactic and other organic acids accumulate due to microbial activity. After 18 hours fermentation, in wheat bran mash, optimal pH typical for products fermented by *Bifidobacterium lactis* Bb-12 was obtained. All results were similar as compared to each other, after 18 hours fermentation pH ranged from 3.83±0.32 to 3.98 ±0.12. One-way Anova showed there were no significant differences between the four bran samples ($p>0.05$). After fermentation all wheat bran mash was lyophilized and stored in refrigerator at - 18°C.

Fig. 6 represents the number of viable *B. lactis* Bb-12 cells in non-enzymatically treated and enzymatically treated wheat bran samples.

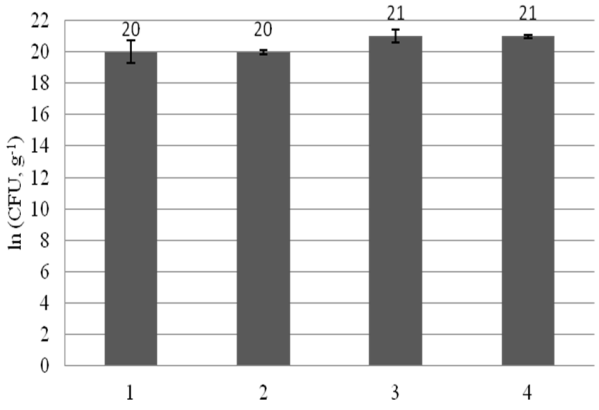

Fig. 6 Number of *B. lactis* in fermented wheat bran mash by *Bifidobacterium lactis* Bb12, ln CFU, g^{-1}. 1 - non-enzymatically treated + *B. lactis* 1.5:8.5; 2 - non-enzymatically treated + *B. lactis* 1:10; 3 - enzymatically treated + *B. lactis* 1.5:8.5; 4 - enzymatically treated + *B. lactis* 1:10

Since *bifidobacteria* are saccharolytic, they play an important role in carbohydrate fermentation in the colon, and inulin, oligofructose, and raffinose have been reported to be important prebiotics [13]. The study results indicate that the use of enzymatical treatments on wheat bran allow to increase of *B. lactis* Bb-12 acid bacteria compared to the non-enzymatically treated samples. This is due to the fact that the fermentation process increases the amount of reducing sugars that could be nutrients for the bacteria. A similar study was conducted in Latvia, obtained results were similar [9]. The number of CFU ranged from ln 20 to ln 21CFU g^{-1}. One-way ANOVA showed there were significant differences ($p<0.05$) between the samples.

IV. Conclusions

1. Wheat bran obtained from industrial mill 'Dobeles dzirnavieks' can be hydrolyzed into simple sugars by enzymatic treatment with α-amylase and Viscozyme L. After enzymatic treatment starch and total dietary fibre content strongly decreased, this confirms the fact that controlled carbohydrates hydrolysis by combination of α-amylase with Viscozyme L results in maltodextrin, D-glucose, fructose, maltose formation, lowering the energetic value of the product.
2. After enzymatic treatment glucose amount increase from 7.7g L^{-1} (B) (non-treated) to 22.6g L^{-1} (enzymatically treated), fructose amount ranged from 3.5 (A) g L^{-1} (non-treated), to 5.2 (C), 6.3 (D) (enzymatically treated). One-way Anova showed there were significant differences ($p<0.05$) between the samples (non-treated and enzymatically treated).
3. The results obtained in this study show a certain stimulatory effect achieved with enzymatical hydrolysis on the *Bifidobacterium lactis* Bb-12 growth on wheat bran. In this scientific work we proved that the use of carbohydrates to release sugars gives possibility to enrich the wheat bran with viable *B. lactis* Bb-12 with ln 21CFU g^{-1} ($1.69±0.71×10^5$) (enzymatically treated 1.5:8.5) and ln 20CFU g^{-1} ($4.04±0.40×10^4$) (non-enzymatucally treated 1.5:8.5)

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