PREBIOTIC EFFECTS OF XYLANASE MODIFICATION OF β-GLUCAN FROM OAT BRAN ON *BIFIDOBACTERIUM BIFIDUM*

X.J. QIU[a,b], W.X. ZHENG[a,b], L. ZHANG[a,b], Y.L. SHI[a,b], J.H. HU[a,b], Y.L. LI[a,b], Z.Y. LIU[a,b] and M.D. ZHU*[a,b]

[a]Inner Mongolia University of Technology, Hohhot, Inner Mongolia, China
[b]Inner Mongolia Energy Conservation and Emission Reduction Engineering Technology Research Center for Fermentation Industry, Hohhot, Inner Mongolia, China
*Corresponding author: Tel.: 15904710399
Email: zhumingda2003@163.com

ABSTRACT

Oat β-glucan (BG) was isolated from oat bran, and the xylanase treatment was conducted to obtain modification of β-glucan (MBG). The relative molecular weight (M_w) of BG and MBG was determined by gel permeation chromatography (GPC). Results demonstrated that the M_w of BG was reduced from 1.66×10^4 to 5.43×10^3. We assessed the prebiotic effect of BG and MBG on human colon *Bifidobacterium bifidum*（*B. bifidum*）. Our findings suggest that the addition of BG and MBG resulted in a lower pH of the fermentation broths. Both lactic and acetic acid production increased in the fermentation broths. While BG was found to significantly promote the proliferation of *B. bifidum*, MBG had a greater effect on *B. bifidum*.

1. INTRODUCTION

Oat bran is a by-product of the oatmeal production, produced during the milling process. It is a mixture that is mainly composed of seed coat, the aleurone layer, and oat germ, which accounts for 8%~12% of the total mass of oat seeds. It contains a variety of nutrients, such as dietary fibre, fat, protein, and minerals, and a large amount of dietary fibre represents a valuable renewable resource (ZHENG *et al.*, 2017). Oat BG is a soluble dietary fibre (SDF) present in the oatmeal grain endosperm and aleurone cell wall. Its main components are (1-3) and (1-4)-β-D-glucan. After oatmeal processing, the BG is enriched in wheat bran. Following claims made by the US Food and Drug Administration (FDA), many researchers have demonstrated an association between oat BG and a reduction in the risk factors of cardio-vascular disease, in particular by lowering the blood cholesterol and glucose levels (VITAGLIONE *et al.*, 2008; YAN *et al.*, 2017), and at the same time regulating the immune system and strengthening resistance (LI *et al.*, 2018). Oat BG has also been found to relieve the immunosuppression of tumour cells and to have a good therapeutic effect on patients with early-stage cancer (MEI *et al.*, 2018).

The enzymatic method involves the use of enzymes to enzymatically decompose a raw material and to remove surface impurities to obtain insoluble dietary fibre, which is then further enzymatically modified to obtain a water-soluble dietary fibre. Studies have shown that the enzyme treatment of bran can effectively change the functional properties of dietary fibre by changing the structure or molecular rearrangement of polysaccharides (SANTALA *et al.*, 2014). The modification of bran by xylanase and cellulase can increase the soluble dietary fibre content of oat bran and reduce its water binding ability (LEBESI *et al.*, 2012). Laccase assisted by high hydrostatic pressure and cellulose bran can increase the content of soluble dietary fibre, alter the honeycomb structure of dietary fibre, and produce new polysaccharides (MA *et al.*, 2016). After the treatment of rice bran with xylanases, including amylase, glucoamylase, protease, and cellulase, the total phenolic, flavonoid, iron reducing antioxidant capacity, and oxygen free radical absorption capacity of modified dietary fibre has been found to significantly improve (LIU *et al.*, 2017). However, the precise role of BG in the enzymatic treatment of the human requires further elucidation.

The definition of prebiotics has been suggested by GIBSON *et al.* (2017) as "a substrate that is selectively utilized by host microorganisms conferring a health benefit". The catabolism of prebiotic carbohydrates by metabolic activity of the gut microbiota primarily produces short chain fatty acids (SCFA). The most abundant SCFA in the colon is acetate, which in general represents more than half of the SCFA content detected in feces (LOUIS *et al.*, 2007). Prebiotic substrates can selectively promote the growth of beneficial microorganisms and induce changes in the levels of these SCFA in healthy individuals (LECERF *et al.*, 2012). Thus, SCFA levels represent an indirect measure of the level of beneficial microorganisms in the gut and their impact on human health. In this study, Inner Mongolia oat bran was used as raw material to extract oat BG by xylanase enzymatic hydrolysis to explore the prebiotic effects on *Bifidobacterium bifidum* before and after enzymatic hydrolysis.

2. MATERIALS AND METHODS

2.1. Chemicals and media

The xylanases (1.67 millikatal (mkat)/g) used in this study were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). All other chemicals and media were of analytical grade and obtained from Haibo Biotechnology Co., Ltd. (Qingdao, China). *B. bifidum* (strain number CICC 6168) was purchased from China Center of Industrial Culture Collection. HPLC-grade water was prepared using a Milli-Qplus purification system (Millipore Corp., Bedford, MA, USA). Oat bran was produced in the Inner Mongolia Autonomous Region (Hohhot, China).

2.2. Crude BG extraction

The isolation of BG was performed as follows: fat was removed from oat bran using a 60-mesh screen with a Soxhlet extractor. The defatted oat bran (50 g) was then soaked in distilled water (0.5 L), then gelatinized at 100°C for 15 min, and finally incubated with heat-resistant a-amylase at 95°C for 1.5 h to remove starch, followed by centrifugation (2000 ×g, 15 min). Insoluble dietary fibre (IDF) was the resulting precipitation. The supernatant was isoelectrically deproteinized in a NaOH solution (0.75 mol/L, 50°C, 2 h). The pH was adjusted to 6.5 to remove protein. After centrifuging at 2000 ×g for 5 min, 200 mL of 950 g/kg ethanol solution was added to the crude BG precipitate (60°C, 2 h). The precipitation (crude BG) was then dried at 60°C for 12 h in an air-drying oven.

2.3. Enzymatic hydrolysis of BG

The enzymatic treatment was conducted as follows: the precipitation (IDF) was dried at 60°C for 12 h using an air-drying oven, then grinded and sieved through a 250 mm mesh. The crude BG and IDF were combined and treated with xylanase. The mixture (30 g) was soaked in hot water (500 mL) at 50°C for 0.5 h. Then, xylanase was added at 667 nanokatal/g (mixture) and the slurry was incubated for 2 h (50°C, pH 5.0). The treated slurry was centrifuged at 2000 ×g for 5 min after the inactivation of the enzyme in a boiling water bath for 10 min. The resulting insoluble material was washed twice with hot water (pre-heated to 50°C) and centrifuged (2000 ×g, 5 min) again. Then, 150 mL of 950 g/kg ethanol solution was added to the precipitated crude modification of β-glucan (MBG), and the residue was dried at 60°C for 12 h using a hot-air oven.

2.4. BG and MBG purification

BG crude powder (0.1 g) and MBG crude powder (0.1 g) were dissolved in 5 mL of water, respectively. After full dissolution, the solution was used for anion exchange column chromatography, using water as the eluent, at a flow rate 30 mL/h. Using 10 mL per tube, the eluent was collected from each tube. The polysaccharide distribution was determined using the phenol-sulfuric acid method, and the protein content was determined by 280 nm colorimetry. A single peak of polysaccharide was combined. The BG solution and MBG solution obtained were concentrated to 5 mL under reduced pressure, respectively, and the supernatant was centrifuged for use.

The BG and MBG concentrates treated by DEAE Sepharose CL-6B anion exchange column chromatography was respectively used for gel permeation column chromatography using

water as an eluent at a flow rate of 30 mL/h. The eluate was collected from each tube. Polysaccharide distribution and protein content were detected by the phenol-sulfuric acid method and 280 nm colorimetry, respectively. A single peak of polysaccharide was combined. BG and MBG were concentrated under reduced pressure before freeze drying.

2.5. Determination of average molecular weight (M_w) of BG and MBG

The M_w of BG and MBG was determined by GPC. The purified BG and MBG (1 mg) were analysed using a PL aquagel-OH MIXED chromatographic column at 45°C. The mobile phase consisted of 0.1 mol/L $NaNO_3$ at a flow rate of 0.9 mL/min. The quantification was performed using a VEX differential refractive index detector (PL GPC-220, Agilent Technologies Inc. California, USA).

2.6. The prebiotic effect of BG and MBG on *B. bifidum*

2.6.1. Strains activation

B. bifidum freeze-dried strains were used, such that the strains were activated and cultured before carrying out the experiments. The formulation of medium was provided by the China Center of Industrial Culture Collection, and is provided in Table 1.
Here, 30 mL of BBL medium was placed in 100 mL vial, followed by vacuum pumping and sterilization at 121°C for 30 min. The inoculation operation was carried out in an anaerobic incubator. For this, *B. bifidum* lyophilized powder was fully dissolved and inoculated into 1 mL of sterile medium. The culture period of bacteria is normally 1~2 days, however the first-generation revival culture needed to be extended appropriately. As such, the duration of this experiment was 3 days. The cells were statically cultured at 37°C in an anaerobic culture incubator. The first-generation revival cultured cells that survived were sub-cultured for two generations with 10% inoculation, with a culturing time per generation of 2 days.

Table 1. *Bifidobacterium bifidum* medium (BBL) formula (per litre, /L).

Name	Dose	Name	Dose
yeast extract	3 g	beef extract	10 g
peptone	10 g	soluble starch	1 g
glucose	5 g	L-cysteine hydrochloride	0.5 g
sodium chloride	3 g	sodium acetate	3 g
resazurin	3 mg	pH	6.8

2.6.2 Effect of BG and xylanase MBG on the growth of B. bifidum

To investigate the effect of BG and MBG on the growth of *B. bifidum*, BG and MBG were used as the sole carbon source. BG and MBG were substituted for the glucose in the BBL medium. Here, 0.5 g of BG and MBG were used to replace glucose, and 100 mL of BBL medium was prepared with an inoculation amount of 5%. After inoculation, the solution was placed in a 37°C anaerobic culture incubator for 24 h. The OD value was measured using an ultraviolet-visible spectrophotometer at a wavelength of 600 nm after 24 h. The

BBL medium not inoculated with bacteria was used to adjust the reading to zero. As can be seen in Table 1, 15 g of agar was added to BBL medium to prepare the BBL agar medium. The fermentation broth was coated and inoculated into BBL medium before culturing in an anaerobic incubator at 37° C for 24 h.

2.6.3 B. bifidum fermentation broth pH determination

The initial pH of the *B. bifidum* fermentation broth was measured before fermentation, and a sample was measured once every 6 h. For the measurement, 5 mL of the fermentation broth was sampled, centrifuged at 2000 ×g for 10 min, and the resulting supernatant was measured using a pH meter.

2.6.4 Effect of BG and MBG on the concentration of SCFA

Here, 0.5 g of BG and MBG were added to 100 mL of BBL medium with glucose. BBL medium with 5 g of glucose per litre was used as a negative control. The inoculation amount was 5%. After the inoculation was completed, it was incubated at 37°C in an anaerobic culture incubator for 24 h. The bacteria solution was filtered through a 0.22 μm filter membrane before performing high performance liquid chromatography after 24 h. The three groups of *B. bifidum* solutions were analysed using a HPX-87H chromatographic column at 50°C. The mobile phase consisted of 0.005 mol/L sulphuric acid at a flow rate of 0.5 mL/min. The quantification was performed by high performance liquid chromatography (Waters 2695, Waters Technology Co., Ltd. Milford, USA). This quantification was also carried out on a bacterial solution cultured for 0 h, that is immediately after inoculation, to determine the increased in lactic acid and acetic acid production and used as the control group.

2.7. Statistical analysis

All measurements were carried out at least in triplicate. The results presented are the mean±standard deviation (SD) of each treatment (n=3). The differences were considered significant when $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Determination of M_w of BG and MBG

The M_w distribution of untreated BG and MBG is shown in Fig.1. The M_w represents the statistical average molecular weight of the polysaccharide compared to the average weight of different molecular weights, and the average molecular weight (Mn) represents the statistical average molecular weight of the molecules in the polysaccharide with different molecular weights. According to Fig. 1(a) and Table 2, the M_w of the BG which was not subjected to the enzyme treatment was 1.49×10^4. The M_w/Mn value was 1.03, which was close to 1, indicating that the M_w distribution of BG was uniform, and the distribution was concentrated around the average molecular weight. As shown in Fig. 1(b) and Table 2, the MBG exhibited four M_w segments, 5.98×10^5, 2.68×10^4, 1.66×10^4, and 5.43×10^3, respectively. The M_w/Mn values were 1.10, 1.01, 1.02, and 1.23, respectively, that is close to 1, indicating that the M_w distribution of MBG was uniform and that the distribution was concentrated

on the average of the four molecular weight segments. Among them, the MBG of two M_w fractions, 5.98×10^5 and 2.68×10^4, accounted for 2.20% and 8.78% of the total content, respectively, and the content was low. After xylanase enzymatically cleaves the bran xylan, the macromolecular BG originally linked to xylan may be isolated. Its high molecular weight (HM_w) of 1.66×10^4 is similar to that of non-enzymatically-treated BG, and should be the same type of BG. The MBG with a low molecular weight (LM_w) of 5.43×10^3 could be the result of enzymatic hydrolysis of xylanase. Xylanase destroys the β-1,4-glycosidic linkage of connecting BG, thereby decreasing the M_w of BG, with a resulting molecular weight of 1.66×10^4. Among these, MBG with molecular weight of 5.43×10^3 accounted for 86.28% of the total content. The use of xylanase can be also a smart strategy BG to convert LM_w, the M_w of MBG showed that the M_w of BG was reduced from 1.66×10^4 to 5.43×10^3. The M_w of BG plays an important role in determining the physiological efficacy of BG in terms of health benefits. Incorporating LM_w BG may influence the palatability of food and has been shown to lower cholesterol in men (PINS *et al.*, 2005) and animals (WILSON *et al.*, 2004).
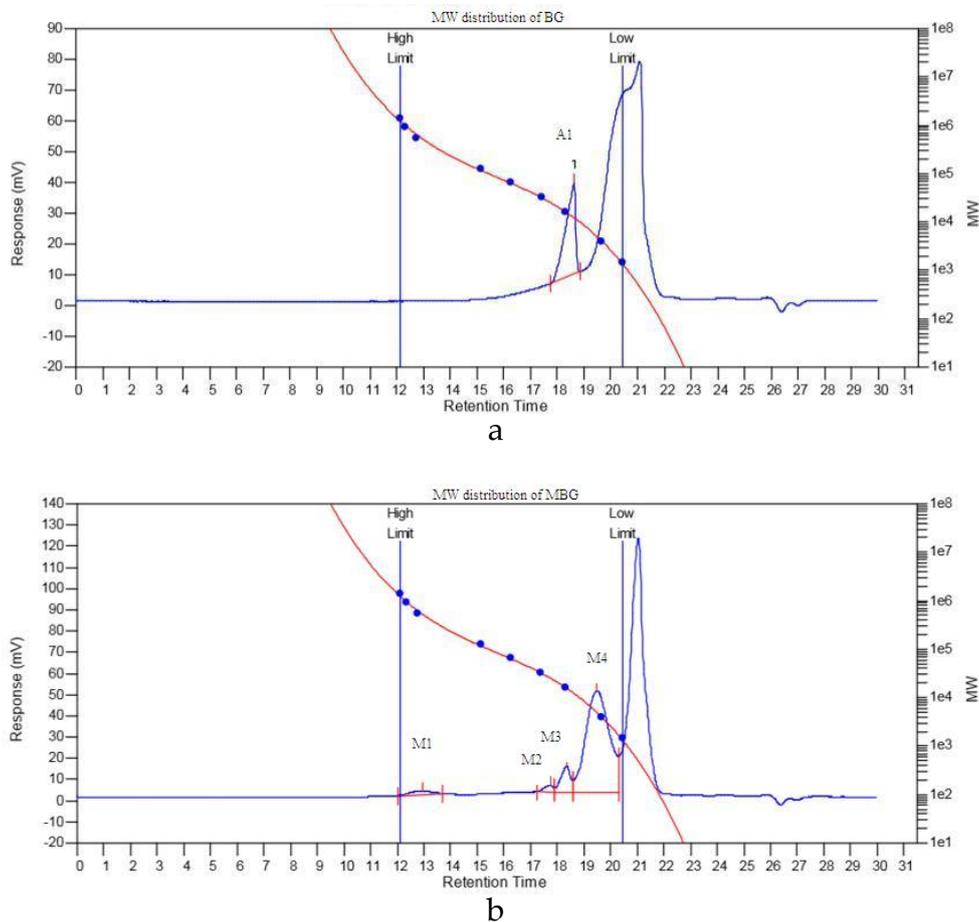

Figure 1. M_w distribution of BG and MBG.

Table 2. M_w of each component of BG and MBG.

Peak number	Mp	Mn	Mw	Mz	Area (mV.secs)	% Area
B1	12237	14501	14938	15420	777.567	100
M1	578382	544525	598568	660548	94.3102	2.75
M2	25501	26587	26868	27167	75.6232	2.20
M3	15732	16311	16600	16899	300.825	8.78
M4	5024	4415	5435	6492	2959.55	86.28

Furthermore, the action of xylanase on IDF leads to the formation of two M_w fractions, 5.98×10^5 and 2.68×10^4, which may be water soluble. As illustrated in Fig. 2, this conversion of highly polymerized IDF into SDF was achieved by performing a tailored enzymatic treatment. Polysaccharides and other polymers are cross-linked to the cell wall of the cereals with other components to form a structural network. B-(1-3,1-4)-D-glucans and arabinoxylans are the major cell wall polysaccharides in oat bran and are composed of a backbone of β-(1,4) linked D-xylopyranosyl residues. Moreover, a-L-arabinofuranoside can be present at the C (O)-3 and/or the C (O)-2 positions of the xylose moieties, and arabinoxylans can be cross-linked to ferulic acid at the C (O)-5 positions via ester linkages. Many of these polysaccharides would require enzymatic hydrolysis to be removed from the structure, formed by covalent and non-covalent cross-linking. For example, cellulose and hemicelluloses treatments can be used to improve the quality of fiber-enriched oat bran by using xylanase treatment on the fibre fraction. Enzymatic methods have been used as means for modification to improve the extractability of polysaccharides and increase yields (LAURIKAINEN et al., 1998). Similar studies have reported changes in the chemical bonds of the polysaccharide and bran dietary fibre molecular structures via various enzymatic treatments (SAULNIER et al., 2009; YA et al., 2017).
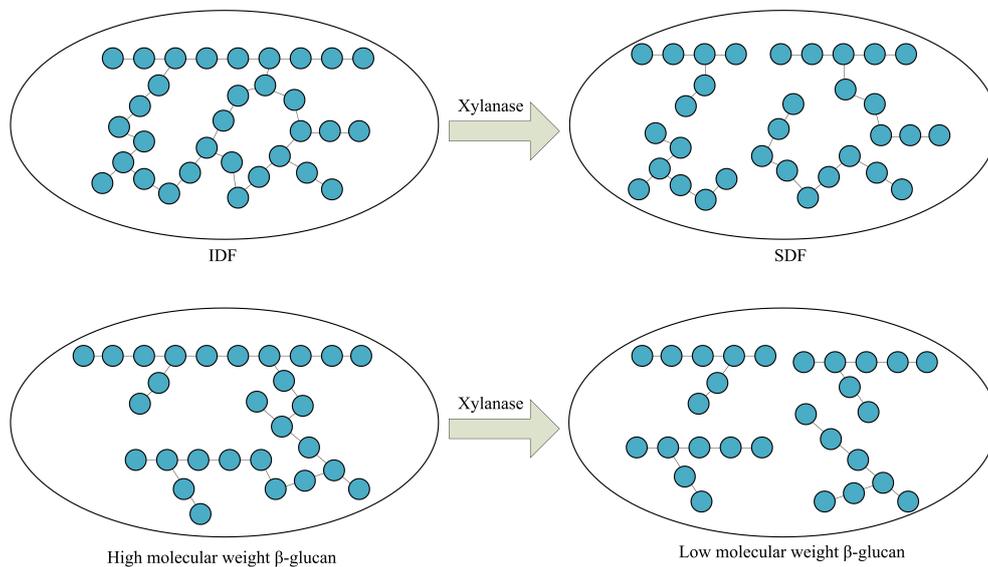
Figure 2. Schematic representation of the effects of xylanase on BG.

3.2. The prebiotic effect of BG and MBG on *B. bifidum*

3.2.1 Effect of BG and MBG on the growth of *B. bifidum*

According to the experimental methods, in which the OD_{600} of the bacterial solution of *B. bifidum* was measured *in vitro* after 24 h of anaerobic fermentation, the OD value of the bacterial solution was 0.041 cultured in BBL medium of 5 g/L glucose at 37 °C for 24 h, while the OD value of the bacterial solution were 0.089 and 0.244 cultured in BBL medium of 5 g/L BG and 5 g/L MBG, respectively. The OD value of the bacterial solution increased significantly after the addition of BG. The OD value of the BG bacterial solution then increases again after xylanase treatment. The positive correlation between the OD value of the bacterial solution and the number of bacteria suggests that BG can promote the proliferation of *B. bifidum*. However, the promotion of proliferation was greater after the addition of MBG. This may be due to *B. bifidum* being more easily oxidized in the LM_w segment than in the HM_w segment of BG during the oxidation of sugar.

Non-digestible polysaccharides cannot be degraded by mammalian enzymes. Therefore, following ingestion, these glycopolymers are delivered intact to the large intestine, where they may influence the growth or metabolic activity of members of the gut microbiota. In this context, there is growing scientific evidence of the possible prebiotic effects elicited by non-digestible polysaccharides towards various microorganisms of the mammalian gut (VITAGLIONE *et al.*, 2008; TAN *et al.*, 2006). After BG enters the large intestine as a soluble dietary fibre, probiotics such as *B. bifidum* pass through the extracellular glycosidase to promote BG degradation and utilization, thereby promoting the proliferative metabolism of probiotics.

3.2.3 Change in pH of B. bifidum fermentation broth

The decrease in the pH of the fermentation broth was mainly the result of organic acid production during fermentation. As can be seen in Fig. 3, compared to the negative control, the pH of the fermentation broth decreased rapidly after the addition of untreated BG and MBG. The environment changed from alkaline to acidic after 24 h of fermentation. The addition of MGB resulted in a more rapid decrease of the fermentation broth's pH compared to BG. Metabolically produced organic acid reduced the intestinal pH environment, resulting in an acidic environment for the intestines, thereby inhibiting the growth of harmful bacteria and promoting to intestinal health. There is also considerable evidence that supports the role of fibre in the promotion of health by its ability to modulate gut microbiota composition and metabolism (SLAVIN *et al.*, 2013). The proposed benefits of fibre on the intestinal microbiota are associated with their uptake and utilization by putative health-promoting bacteria species and the subsequent cross-species metabolism of fermentation by-products (HOLSCHER *et al.*, 2015; VERBEKE *et al.*, 2015; TAP *et al.*, 2015).
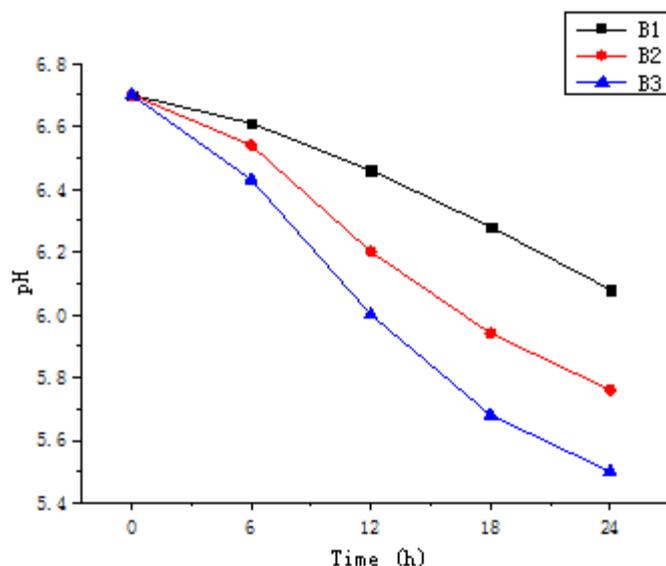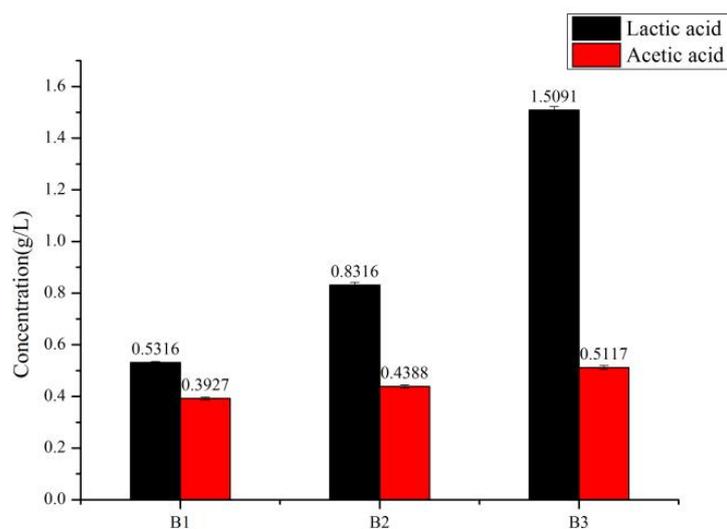
Figure 3. The effects of time on pH in fermented broth. Note: B1: BBL medium of 10 g/L glucose; B2: 5 g/L BG plus 5 g/L glucose BBL medium; B3: 5 g/L MBG plus 5 g/L glucose BBL medium.

3.2.4 Effect of BG and MBG on the concentration of SCFA

The formula of the medium (Table 1) was obtained via an optimization study based on strong metabolic and molecular studies by the China Center of Industrial Culture Collection. The production of lactic acid in the bacterial solution was measured after culturing the cells, as described in the Methods. The effect of BG and MBG on the concentration of lactic acid and acetic acid in the fermentation broth is shown in Figure 4. *B. bifidum* was cultured at 37°C for 24 h. The yields of lactic acid and acetic acid in the control (B1, 10 g/L glucose BBL medium) were 0.5316±0.0033 g/L and 0.3927±0.0043 g/L, whereas the fermentation of *B. bifidum* with 5 g/L BG plus 5 g/L glucose BBL medium and 5 g/L MBG plus 5 g/L glucose BBL medium, respectively, as a carbon source resulted in greater yields of lactic acid and acetic acid than the control. An analysis of the metabolic activity in both cultures showed that lactic acid production increased 56% during BG treatment (0.8316±0.0265 g/L) and by 184% during MBG treatment (1.5091±0.0151 g/L; $p<0.05$) compared to the control. The acetic acid production increased 12% during BG treatment (0.4388±0.0033 g/L) and by 30% during MBG treatment (0.5117±0.0046 g/L; $p<0.05$) compared to the control (Figu. 4). These results demonstrated that the addition of BG and MBG to *B. bifidum* during fermentation with glucose as a substrate can significantly promote the production of metabolic acid, since the increase in acid production of MBG was greater. The overall beneficial effects produced by MBG were higher than those induced by BG. The addition of MBG and BG to the cultures beneficially influenced the fermentation patterns of *B. bifidum,* demonstrate by the higher SCFA production and remarkably higher levels of lactic acid.

Figure 4. Effects of BG on the lactic acid and acetic acid concentration in fermentation solution (g/L). B1: BBL medium of 10 g/L glucose; B2: 5 g/L BG plus 5 g/L glucose BBL medium; B3: 5 g/L MBG plus 5 g/L glucose BBL medium.

The SCFA microbial metabolites are of particular interest, and have been suggested to promote health by regulating hormone release in the gut, as well as the cholesterol synthesis/metabolism to enhance satiety, also exerting anticancer and anti-inflammatory effects (VINOLO *et al.*, 2011; WONG *et al.*, 2006). The experiments showed that *B. bifidum* could produce acid by glycolysis with glucose as the carbon source. The MBG of the small molecular segment was more easily utilized by *B. bifidum* than the BG of the large molecular segment. The promotion of lactic acid production by probiotics was also more evident. The fermentation properties of *B. bifidum* are strongly influenced by the degree of polymerization of non-digestible polysaccharides. The increased SCFA production can be explained by the additional *B. bifidum* biomass resulting from the prebiotic effect of BG and MBG.

4. CONCLUSIONS

Oat bran dietary fibre was treated with xylanase to obtain MBG, which varied in its structure and properties compared to the original BG. We found that the M_w of MBG was reduced from 1.66×10^4 to 5.43×10^3, as determined by gel permeation chromatography. The addition of BG and MBG to the fermentation broth of *B. bifidum* significantly promoted the proliferation of *B. bifidum*, and the proliferation of *B. bifidum* in the LM_w segment of MBG was greater. The *B. bifidum* bacteria metabolites, lactic acid and acetic acid, were detected. Moreover, with glucose and BG as the carbon sources, the acid production of *B. bifidum* increased significantly. The production of lactic acid production in MBG increased significantly as a result.

ACKNOWLEDGMENTS

REFERENCES

Berecochea-Lopez A., Decorde K., Ventura E., Godard M., Bornet A., Teissedre P.L., Cristol J.P. and Rouanet JM. 2009. Fungal chitin-glucan from *Aspergillus niger* efficiently reduces aortic fatty streak accumulation in the high-fat fed hamster, an animal model of nutritionally induced atherosclerosis. J. Agr. Food Chem. 57:1093-1098.

Gibson G.R., Hutkins R., Sanders M.E., Prescott S.L., Reimer R.A., Salminen S.J. and Reid G. 2017. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastro Hepat, 14:491-502.

Holscher H.D., Caporaso J.G., Hooda S., Brulc J.M., Fahey G.C. and Swanson K.S. 2015. Fiber supplementation influences phylogenetic structure and functional capacity of the human intestinal microbiome: follow-up of a randomized controlled trial. Am. J. Clin. Nutr. 101:55-64.

Laurikainen T., Harkonen H., Autio K. and Poutanen K. 1998. Effects of enzymes in fibre-enriched baking. J Sci. Food Agric. 76:239-249.

Lebesi D.M. and Tzia C. 2012. Use of endoxylanase treated cereal brans for development of dietary fiber enriched cakes. Innov. Food Sci. Emerg. 13:207-214 .

Lecerf J.M., Dépeint F., Clerc E., Dugenet Y., Niamba C.N. and Rhazi L. 2012. Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. Brit. J. Nutr. 108:1847-1858.

Louis P., Scott K.P., Duncan S.H. and Flint H.J. 2007. Understanding the effects of diet on bacterial metabolism in the large intestine. J. Appl. Microbiol. 102:1197-1208.

Li Y., Fan Y.H., Pan H.O., Qian H.F., Qi X.G., Wu G.C., Zhang H., Xu M.J. and Wang L. 2018. Effects of functional beta-glucan on proliferation, differentiation, metabolism and its anti-fibrosis properties in muscle cells. Int. J. Biol. Macromol. 117:287-293.

Liu L., Wen W., Zhang RF., Wei ZC., Deng YY., Xiao J. and Zhang M.W. 2017. Complex enzyme hydrolysis releases antioxidative phenolics from rice bran. Food Chem. 214:1-8.

Ma M. and Mu T. 2016. Modification of deoiled cumin dietary fiber with laccase and cellulase under high hydrostatic pressure. Carbohyd Polym. 136:87-94 .

Mei K.L., Tung Y.L. and Chia C.C. 2018. Chemical identification of a sulfated glucan from *Antrodia cinnamomea* and its anti-cancer functions via inhibition of EGFR and mTOR activity. Carbohyd Polym. 202:536-544.

Neyrinck A.M., Possemiers S., Verstraete W., De Backer F., Cani P.D. and Delzenne N.M. 2012. Dietary modulation of clostridial cluster XIVa gut bacteria (*Roseburia* spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. J. Nutr. Biochem. 23:51-59.

Pins J.J., Keenan J.M., Curry L.L., Goulson M.J. and Kolberg L.W. 2005. Extracted barley beta-glucan improves CVD risk factors and other biomarkers in a population of generally healthy hypercholesterolemic men and women. Prevention and Control 1:131-136.

Santala O., Kiran A., Sozer N., Poutanen K. and Nordlund E. 2014. Enzymatic modification and particle size reduction of wheat bran improves the mechanical properties and structure of bran-enriched expanded extrudates. J. Cereal Sci. 60:448-456.

Saulnier L., Robert P., Grintchenko M., Jamme F., Bouchet B. and Guillon F. 2009. Wheat endosperm cell walls: Spatial heterogeneity of polysaccharide structure and composition using micro-scale enzymatic fingerprinting and FT-IR microspectroscopy. J. Cereal Sci. 50:312-317.

Slavin J. 2013. Fiber and prebiotics: mechanisms and health benefits. Nutrients 5:1417-1435.

Tan B. and Ren B. 2006. Current status and trends of research and development of deep processing technology for coarse grain resources. J. Chin. Cereal. 21:229-235.

Tap J., Furet J.P., Bensaada M., Philippe C., Roth H., Rabot S., Lakhdari O., Lombard V., Henrissat B., Corthier G., Fontaine E., Dore J. and Leclerc M. 2015. Gut microbiota richness promotes its stability upon increased dietary fibre intake in healthy adults. Environ. Microbiol. 17:4954-4964.

Verbeke K.A., Boobis A.R., Chiodini A., Edwards C.A., Franck A., Kleerebezem M., Nauta A., Raes J. and Tuohy K.M. 2015. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. Nutr. Res. Rev. 28:42-66.

Vinolo M.A., Rodrigues H.G., Nachbar R.T. and Curi R. 2011.Regulation of inflammation by short chain fatty acids. Nutrients 3:858-876.

Vitaglione P., Napolitano A. and Fogliano V. 2008.Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. Trends Food Sci. Tech. 19:451-463.

Wilson T.A., Nicolosi R.J., Delaney B., Chadwell K. and Moolchandani V. 2004 Reduced and high molecular weight barley beta-glucans decrease plasma total and non-HDL cholesterol in hypercholesterolemic Syrian golden hamsters. J. Nutr. 134, 2617-262.

Wong J.M., de Souza R., Kendall C.W., Emam A. and Jenkins D.J.A. 2006. Colonic health: fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40:235-243.

Wen Y., Niu M., Zhang B.J., Zhao SM. and Xiong S.B. 2017. Structural characteristics and functional properties of rice bran dietary fiber modified by enzymatic and enzyme-micronization treatments. LWT - Food Sci. Technol. 75:344-351.

Cao Y., Sun Y., Zou S.W., Li M.X. and Xu XJ. 2017. Orally administered baker's yeast beta-Glucan promotes glucose and lipid homeostasis in the livers of obesity and diabetes model mice. J. Agr. Food Chem. 65:9665-9674.

Zheng W., Liu Z. and Shi Y. 2017. Research progress on preparation of soluble dietary fiber in food processing by-products. Food and Feed Industry. 12:31-35.