Production of oat-based synbiotic beverage by two-stage fermentation with Rhizopus oryzae and Lactobacillus acidophilus

Journal of Food, Agriculture & Environment

Gao, F.; Cai, S.; Nout, M.J.R.; Wang, Y.; Xia, Y. et al

https://www.wflpublisher.com/Abstract/2931

WFL Publisher
Science and Technology
Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.10 (2): 175-179. 2012 *www.world-food.net*

Production of oat-based synbiotic beverage by two-stage fermentation with *Rhizopus oryzae* and *Lactobacillus acidophilus*

Fengyi Gao [1#], Shengbao Cai [1#], Robert Nout, M. J. [2], Yingna Wang [1], Yujie Xia [1], Yanfang Li [1] and Baoping Ji [1*]

*[1] College of Food Science & Nutritional Engineering, China Agricultural University, P.O. Box 294, No. 17 Qinghua East Road, Haidian District, Beijing 100083, P. R. China. [2] Laboratory of Food Microbiology, Wageningen University, P.O.Box 8129, 6700 EV Wageningen, The Netherlands. *e-mail: Baoping_ji@163.com*

Received 9 January 2012, accepted 26 April 2012.

Abstract

Many studies have reported that oats could effectively reduce the serum cholesterol levels in humans, and the β-glucan in oat is believed to be responsible for this physiologic effect. Probiotics are live microorganisms that can confer a healthy benefit on the host when administered in adequate amounts. There is an increasing use of these microorganisms in food, aiming to balance intestinal microflora and alleviate dysfunction of the human gastrointestinal tract. However, a number of studies have shown that only 10-30% of these probiotic bacteria could survive after passing through the gastrointestinal (GI) tract. *Lactobacillus acidophilus* is used as a probiotic bacterium in many probiotic foods. However, *L. acidophilus* shows poor growth in cereal products due to its poor hydrolytic ability of protein and macromolecule carbohydrates. The aim of the present study was to combine *Rhizopus oryzae*-fermented oat mash and *Lactobacillus acidophilus* in an oat-based synbiotic beverage. Several factors, including starter culture concentration, *R. oryzae*-fermented oat mash and skim milk powder, were investigated. The nutritional contents in *R. oryzae*-fermented oat flour were just sufficient for survival but not growth of *L. acidophilus*. Adding sucrose (1% or 2%, w/v) did not improve the proliferation of *L. acidophilus*; however, *L. acidophilus* grew quickly when skim milk powder (1% or 2%, w/v) was added. When 5.5% *R. oryzae*-fermented oat mash with 2% added skim milk powder was used, the viable cell counts reached about 9.0 log cfu/ml at the end of 10 h fermentation. The concentration of β-glucans (about 781 mg/l) was not significantly lowered during fermentation.

Key words: *Lactobacillus acidophilus*, *Rhizopus oryzae*, fermented oat, synbiotic beverage.

Introduction

Oats (*Avena sativa* L.), as a fairly important cereal, has earned increased attention in recent years due to different dietary fibre types, such as mixed-linked (1→3), (1→4)-β-glucan, arabinoxylans and cellulose, in addition to relatively high levels of protein, lipids (unsaturated fatty acids), vitamins, antioxidants and phenolic compounds [1-3]. Many studies have reported that oats could effectively reduce the serum cholesterol levels in humans [4], and the β-glucan in oat is believed to be responsible for this physiologic effect [5]. Meanwhile, several other physiologic functions of β-glucan have also been reported, such as regulating insulin levels and blood glucose, and controlling weight [6, 7]. Given these physiologic functions, many oat-based functional foods have been developed. One group of popular oat-based foods are probiotic products. Previous studies have already shown that oats are good substrate for the proliferation of probiotic bacteria and β-glucans could also be used as prebiotics [8, 9].

Probiotics are live microorganisms that can confer a healthy benefit on the host when administered in adequate amounts. There is an increasing use of these microorganisms in food, aiming to balance intestinal microflora and alleviate dysfunction of the human gastrointestinal tract [10]. In commercial probiotic products, different strains of lactobacilli were used, such as *Lactobacillus casei, L. plantarum, Streptococcus thermophilus* and *L. bulgaricus* [11]. Probiotic bacteria need to survive in the upper gastrointestinal (GI) ecosystem if they are to exert their beneficial effect. The determining factor for probiotic bacteria to survive in GI tract is the acid and bile tolerance ability [12]. However, a number of studies have shown that only 10-30% of these probiotic bacteria could survive after passing through the GI tract [13]. Therefore, it is important to guarantee a high survival level of probiotic bacteria become important.

Strains of several *Lactobacillus* species, used as probiotics, have been proven to exert health promoting activities such as immunomodulation, enhancement of resistance against pathogens and reduction of blood cholesterol levels [14, 15]. Moreover, it was reported that *L. acidophilus* can survive at low pH and in high bile concentrations unlike other *Lactobacillus* spp. [16]. Lo Curto *et al.* [12] reported that *L. acidophilus* subsp. *johnsonii* showed the highest survival rate in the upper gastrointestinal tract compared with *L. casei* subsp. *shirota* and *L. casei* subsp. *immunitas* using an *in vitro* gastric model of digestion.

However, a bottleneck in the use of *L. acidophilus* in practice is that it has poor ability to hydrolyse proteins and macromolecular carbohydrates, which in turn adversely affects its growth due to the lack of available sugars and amino acids needed for growth [16]. In order to promote the proliferation of *L. acidophilus*, mono- or oligo-saccharides, protein hydrolysis products, and other nutritional contents can be added to the substrate. Our objective is to achieve the proliferation of *L. acidophilus* by *in-situ* hydrolysis of macromolecular substrates.

In Asia, *Rhizopus oryzae* is commonly used in the preparation of fermented foods and is generally recognized as safe. It is known

#Both of Fengyi Gao and Shengbao Cai are co-first authors

for its enzymes including α-amylase and proteases [17, 18] and could achieve the targeted hydrolysis of macromolecules. In addition, *R. oryzae* can produce some secondary metabolites which also have healthy benefits on human beings, such as malic acid [17].

We combined fermentation of *Rhizopus oryzae* and *Lactobacillus acidophilus* to take advantage of the properties of these two strains to develop an oat-based synbiotic product.

Materials and Methods

Materials: The microorganisms, used in this study including *Rhizopus oryzae* 3.2751 and *Lactobacillus acidophilus* 1.2686, were obtained from the Institute of Microbiology, Chinese Academy of Sciences, and kept in lyophilized form at -80°C.

Oat (genotype, G4), harvested in 2010, was purchased from the Chinese Academy of Agricultural Sciences in November, 2010 and stored at 4°C for further use.

Preparation for the starter culture: *Rhizopus oryzae* was grown in potato dextrose agar (PDA) at 25°C for 7 days. Spore suspension was prepared in 0.9% NaCl solution. The spore suspensions were stored at 4°C for further use. The starter culture of *L. acidophilus* was obtained by overnight incubation at 37°C in MRS-broth. The culture was centrifuged (3000×g) for 10 min at 4°C, then the precipitate was washed by sterilized water and re-suspended in distilled water to its original volume. The cell count of the culture was calculated.

Preparation of substrate and fermentation: Before fermentation, oat was soaked in water for 8 h and mashed using a HK-06A high-speed grinder (Changsha, Hunan, China). The mash was then sterilized at 121°C for 15 min. The solid-state fermentation was performed by inoculating the spore suspension of *R. oryzae* in a 500 ml Erlenmeyer flask containing 100 g of oat mash (10^6 spores/g of oat). Then oat mash was incubated at 25°C for 3 days under static conditions and stirred after 24 h and 48 h of cultivation. After 72 h fermentation, fermented oat was dried at 90°C for 4 h and ground with the HK-06A high-speed grinder (Changsha, Hunan, China) again. Then the mash of fermented oat of different concentrations (4.0, 5.5 and 7.0% in sterilized water, w/v) was used as a substrate for *L. acidophilus*. Five different treatments were investigated firstly: A) *R. oryzae*-fermented oat mash, B) *R. oryzae*-fermented oat mash + 1% sucrose, C) *R. oryzae*-fermented oat mash + 2% sucrose, D) *R. oryzae*-fermented oat mash + 1% skim milk powder (wandashan ®), E) *R. oryzae*-fermented oat mash + 2% skim milk powder. The slurry of each sample was heated at 90°C for 10 min and cooled to 37°C. Then slurry was inoculated with 2%, 5% and 10% (v/v) *L. acidophilus* (7.44 log cfu/ml) respectively. All fermentations were carried out at 37°C with 12 h.

Microbiological analysis: The number of viable cells of *L. acidophilus* was estimated by counting colony-forming units on MRS-agar plates (pH 5.7) after incubation at 37°C for 48 h. The result was expressed as $\log_{10}$ value of colony-forming units per gram of sample.

Determination of pH and titratable acidity: A pH-meter (Leici, Shanghai, China) was used to measure pH of each sample during fermentation of *L. acidophilus*. Titratable acidity (TA) was determined by titrating 10 ml sample with 0.1 M NaOH, phenolphthalein was used as an indicator. Titratable acid was expressed as H+ mmol/100 ml.

Determination of β-glucan: β-Glucan content was determined using an enzymatic kit according to the instruction of the manufacturer (Megazyme International Ireland Ltd., Co., Ireland). Absorbance was measured by spectrophotometer at 510 nm.

Statistical analysis: Each experiment was performed in three separate trials. Data are expressed as the mean values ± standard deviation. The data were also analyzed by one-way ANOVA. Tukey's procedure was used to determine the significance of the differences ($p<0.05$). Analysis was done by SPSS 16.0 (SPSS, Inc., Chicago, USA).

Results and Discussion

Viable cells of L. acidophilus in different samples: Changes of reducing sugar and free amino acids in oat mash fermented by *Rhizopus oryzae* are summarized in Table 1.The reducing sugar and free amino acids increased significantly due to the fermentation. The cell populations of *L. acidophilus* in different samples are presented in Fig. 1. In treatment A, the viable cells of *L. acidophilus* didn't increase during the fermentation process. This demonstrated that the nutritional contents in *R. oryzae*-fermented oat mash were barely enough to enable the survival of *L. acidophilus* but insufficient for its growth. As shown in Fig.1, however, the viable cells of *L. acidophilus* in treatments B (1% sucrose) and C (2% sucrose) still didn't increase. This indicates that the sugar levels in fermented oat mash were adequate for *L. acidophilus*. The reason *L. acidophilus* didn't grow maybe due to the deficiency of other nutrients. In samples D and E, 1% and 2% skim milk powder was added, respectively, in which *L. acidophilus* grew rapidly during the first 10 h fermentation ($p<0.05$). The viable cells reached 8.25 and 8.79 log cfu/ml at the end of fermentation. The viable cells of *L. acidophilus* in samples D and E meet the required levels of viable cells in probiotic products

Table 1. Changes of reducing sugar and free amino acids in oat mash fermented by *Rhizopus oryzae*.

	Fermented oat	Unfermented oat
Reducing sugar (g kg^{-1})	249.4 [a]	23.7 [b]
Free amino acids (mg kg^{-1})	2177.4[a]	389.4[b]

Values within a row with the same superscript do not differ significantly ($p>0.05$).

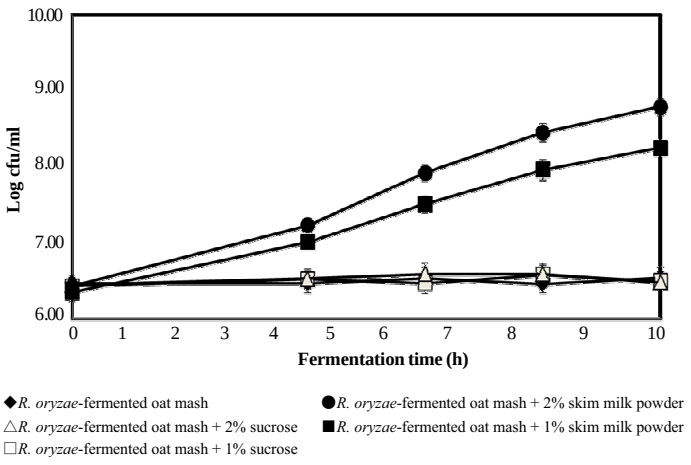

Figure 1. The viable cell counts of *L. acidophilus* in 5 different samples.

(at least 6-7 log cfu/ml) [19]. All of these results indicate that some nutritional contents in skim milk powder can trigger the proliferation of *L. acidophilus*. Future research should be directed at identifying the skim milk components that stimulate *L. acidophilus.*

Concentration of starter culture: The *R. oryzae*-fermented oat mash plus 2% skim milk powder was inoculated with 2.0, 5.0 and 10% starter culture suspension of *L. acidophilus* (7.44 log cfu/ml). All samples were fermented at 37°C for 10 h. Results from these fermentations are presented in Fig. 2. After fermentation with three inoculum concentrations of *L. acidophilus*, the level of viable cell counts reached 7.84, 8.35 and 9.01 log cfu/ml, respectively (Fig. 2 A). These levels were all above the requirement for probiotic products. Values of TA with 2.0, 5.0 and 10% inoculum reached 3.53, 3.91 and 4.62 H^+ mmol/100 ml, respectively (Fig. 2B). After 10 h fermentation, the pH of three inoculum samples dropped to 3.83-4.41 (Fig. 2C). In comparison, Màrtensson *et al.* [27] have reported that pH 3.9-4.5 was reached after 16 h fermentation using a nonspecified commercial mixed dairy starter culture, of an oat base by commercial mixed dairy cultures, and Angelov *et al.* [9] achieved pH 4.0-4.5 after about 8 and 4 h when 5.0% or 10% inoculum of *L. plantarum* (about 7×10^9 cfu/mL) was applied, respectively. The differences between our data and these previous works could be both due to the difference of strains and fermentation media. Taking all results into account, it may be more suitable to use 10.0% starter culture concentration, which can give higher amount of *Lactobacillus acidophilus*.

Concentration of fermented oat mash: The β-glucan content of *R. oryzae*-fermented oat mash was 14.2 g/kg. High concentration of fermented oat flour can obtain higher β-glucan content in the final fermented products. In order to optimize the oat mash concentration, concentrations of fermented oat mash (4.0, 5.5 and 7.0%) plus 2% skim milk powder, respectively, were tested and the results are presented in Fig. 3. The viable cell count of *L. acidophilus* in 5.5% mash was significantly higher than that in 4.0% or 7.0% mash after 10 h fermentation at 37°C ($p < 0.05$) which reached 9.11 log cfu/ml, compared to 8.13 and 8.56 log cfu/ml in 4.0% and 7.0% mash, respectively (Fig. 3A). Angelov *et al.* [9]

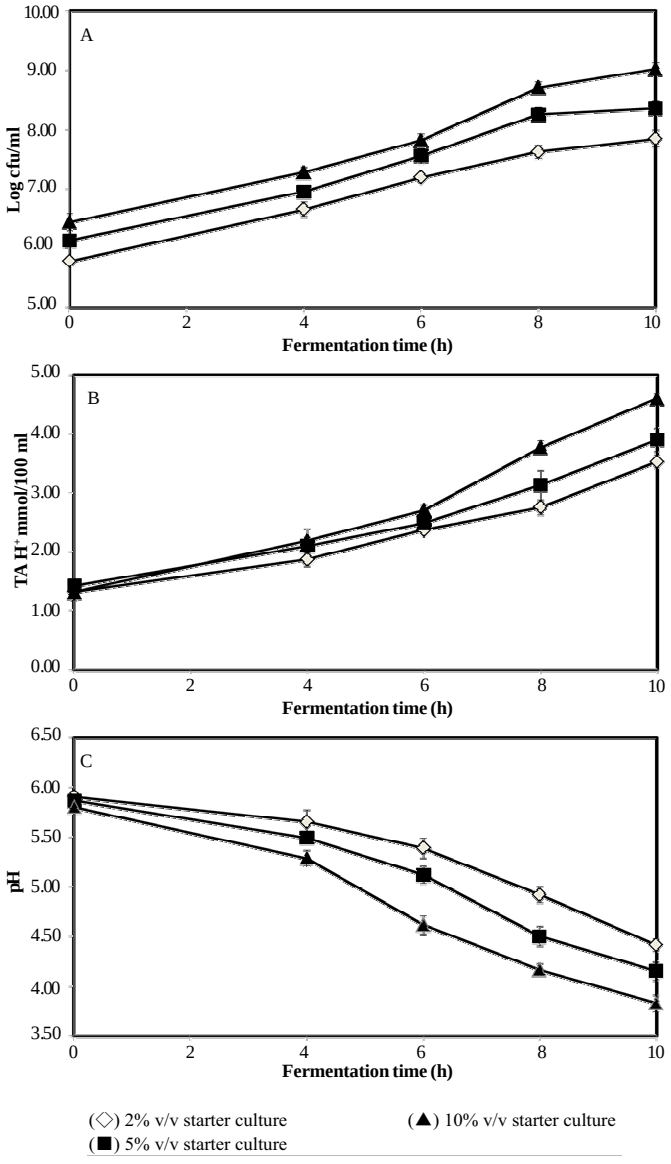

Figure 2. Effect of starter culture concentration on *R. oryzae*-fermented oat flour elaborated with skim milk powder. Viable cell counts (A), titratable acidity (B) and pH (C).

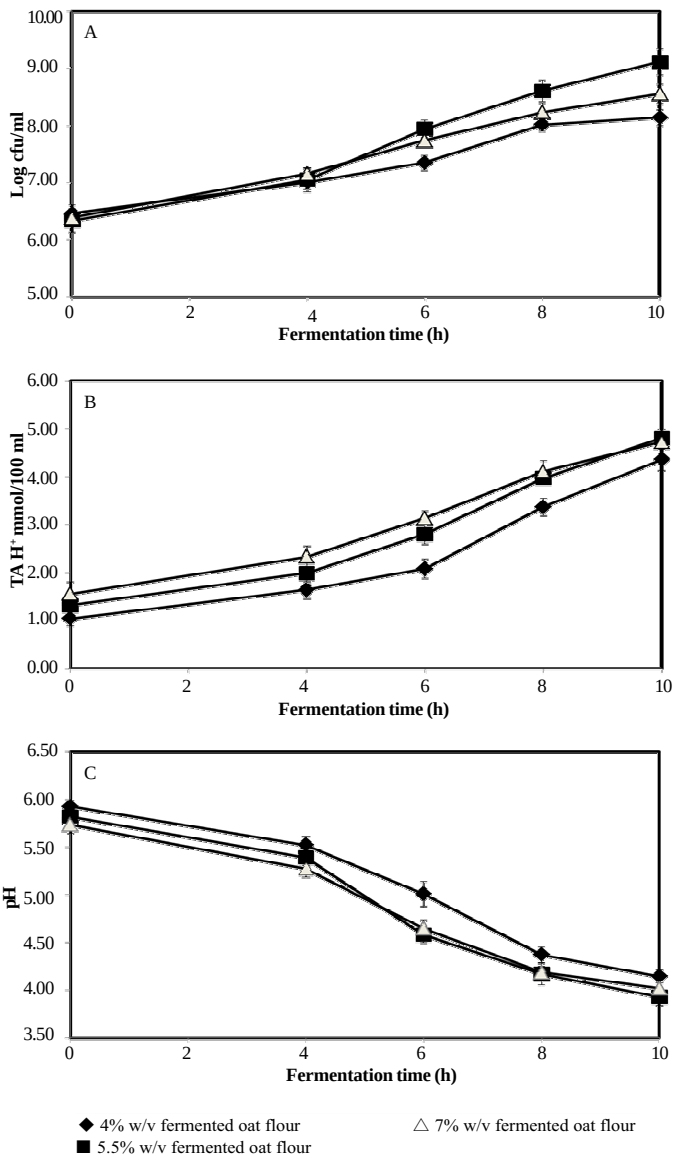

Figure 3. Effect of *R. oryzae*-fermented oat mash concentration on *L. acidophilus* fermentation. Viable cell counts (A), titratable acidity (B) and pH (C).

achieved a similar result when applying *L. plantarum* to ferment oat substrate.

In the initial stage (≤6 h), the TA value of 7.0% mash was slightly higher than that of 5.5% mash (p>0.05), but significantly higher than that of 4.0% mash (p<0.05) (Fig. 3B). This can be explained by the use of different concentrations of oat mash. The oat mash was first fermented by *R. oryzae* which is able to produce organic acids, such as malic and lactic acids [17]. The difference of TA between 5.5% and 7.0% mash has gradually disappeared during the secondary fermentation with *L. acidophilus*, which may be caused by e.g. lactic acid produced by the lactobacilli.

In the second phase of fermentation, *L. acidophilus* grew more rapidly in the 5.5% mash than in 7.0% (Fig. 3A). Thus more lactic acid would be produced in 5.5% mash than in 7.0% mash, which could gradually counteract the difference of TA caused by the different concentrations of oat mash at the start. The treatment containing 4.0% mash had the lowest value of TA throughout the entire fermentation process, which may be due to the lowest amount of *R. oryzae*-fermented oat and the lowest viable cell count of *L. acidophilus* (Fig. 3B).

The initial pH values (Fig. 3C) in the different treatments correlated with the concentrations of *R. oryzae*-fermented oat mash and ranged from 5.73 to 5.92. These subtle differences of initial pH had little effect on the growth of *L. acidophilus*, as shown (Fig. 3A) by the initial (≤4 h) counts of *L. acidophilus* obtained at the initial phase. A greater pH decrease followed within the 10 h fermentation; meanwhile, *L. acidophilus* also grew rapidly in these samples. These results demonstrated that *L. acidophilus* is resistant to low pH values. Previous studies also have reported that *L. acidophilus* may proliferate at pH below 5.0 [20] and adapt to disadvantageous environmental conditions [21].

The initial β-glucan content in the oat mashes obtained with different mash concentrations was 56.8, 78.1 and 99.4 mg/100 ml for 4.0%, 5.5% and 7.0% oat mash, respectively. The fermented treatment of 5.5% oat mash concentration had the highest viable cell count of *L. acidophilus* at the end of fermentation in comparison to the 4.0% and 7.0% oat flour concentration. Thus, 5.5% oat flour concentration was preferred because of its viable cell count and β-glucan content.

Concentration of skim milk powder: It was shown in Fig.1 that for sustaining the survival of *L. acidophilus*, the nutrient contents in the *R. oryzae*-fermented oat mash are enough, but cannot support the proliferation of *L. acidophilus*. The *L. acidophilus* grew rapidly when 1% or 2% skim milk powder was added into the *R.oryzae*-fermented oat mash. The maintenance of *L. acidophilus* requires complex nutrient including numerous amino acids, vitamins and other related growth factors besides fermentable carbohydrates [16]. Thus, it is possible that skim milk powder contains nutrients that are required for growth of *L. acidophilus*. For example, Toba *et al.* [22] have reported that lactose in milk could induce the production of an inducible enzyme -β-galactosidase, which can improve the growth of *L. acidophilus* .

Skim milk powder in concentrations of 1.0, 2.0 and 3.0% (w/v) was added to the *R. oryzae*-fermented oat mash, respectively, in order to increase fermentation rate as shown in Fig. 4. The viable cell counts of *L. acidophilus* in 1.0, 2.0 and 3.0% skim milk powder reached 8.40, 8.98 and 8.95 log cfu/ml, respectively, at the end of 10 h fermentation (Fig. 4A). The treatment containing 1.0% skim milk had the lowest TA value (Fig. 4B). A pH of 4.5 was achieved after about 6 h fermentation with 2.0% or 3.0% added skim milk powder, while this pH value was reached about at the end of 10 h fermentation in sample containing 1.0% skim milk powder. These data show that the growth of *L. acidophilus* was optimally influenced by a 2% concentration of skim milk powder.

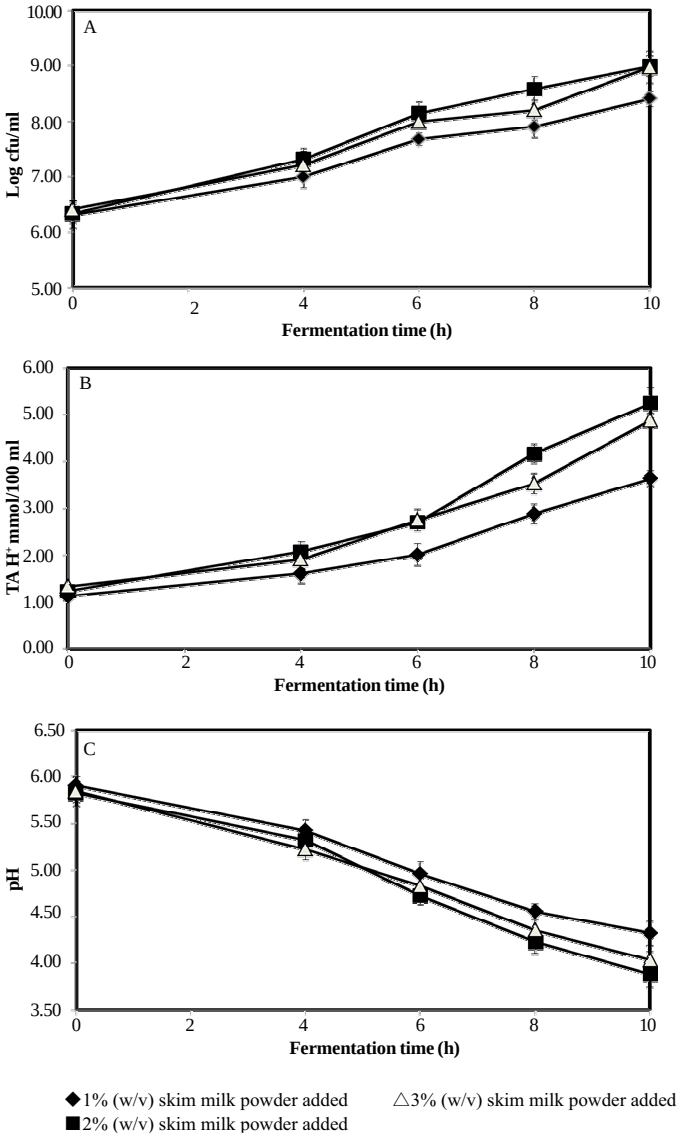

Figure 4. Effect of skim milk powder concentration on *L. acidophilus* fermentation. Viable cell counts (A), titratable acidity (B) and pH (C).

β-glucan content during fermentation: β-glucan is the main soluble fiber in oats [23]. Many physiological functions of this compound have been reported, including regulation of insulin levels, serum glucose and cholesterol [5, 6, 10]. Moreover, β-glucan is fermented by intestinal flora into low-molecular fatty acids, such as propionate, butyrate, which have potential anti-carcinogenic effect and protect against pathogenic bacteria [24]. Meanwhile, β-glucan is considered as a good kind of probiotic which can stimulate the growth of some beneficial residential colon microorganisms such as bifidobacteria [25]. Given these beneficial effects of β-glucan, a number of synbiotic functional drinks from oats have been developed.

In the present study, the initial content of β-glucan in sample was 781 mg/l. During fermentation of *L. acidophilus*, the content

of β-glucan was determined at 4, 8 and 10 h. The results are presented in Fig. 5. No statistically significant changes of β-glucans at different fermentation periods were found, which indicated that the *L. acidophilus* did not degrade β-glucan. This behaviour is similar as was reported for *L. plantarum* [9, 26].

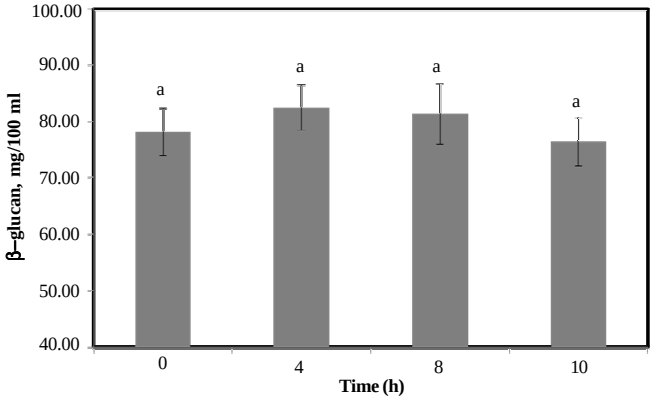

Figure 5. The change of β-glucans content at different fermentation time of *L. acidophilus*. Means (bar values) with same letter do not have significant difference (P>0.05).

Conclusions

Rhizopus oryzae-fermented oat mashes were used as fermented substrates for *L. acidophilus* with the aim to develop an oat-based synbiotic beverage. The nutrients of *R. oryzae*-fermented oat mash can sustain the survival of *L. acidophilus* but are inadequate the growth of *L. acidophilus*. Adding sucrose did not promote the proliferation of *L. acidophilus*, but adding skim milk powder facilitated rapid growth of *L. acidophilus*. β-Glucan in the beverage (about 781 mg/l) was not degraded during 10 h fermentation. In conclusion, 5.5% *R. oryzae*-fermented oat enriched with 2% skim milk powder can be used as an appropriate substrate for *L. acidophilus* fermentation. The viable cell counts in the sample reached about 9.0 log cfu/ml at the end of 10 h fermentation.

Acknowledgements

The authors are grateful for financial support from the Students Innovative Experiment Project of the People's Republic of China. The authors also thank Jianxiong Hao for reading and improving this manuscript.

References

[1]Mälkki, Y. and Virtanen, E. 2001. Gastrointestinal effects of oat bran and oat gum: A review. LWT - Food Science and Technology **34**:337-347.

[2]Viscidi, K. A., Dougherty, M. P., Briggs, J. and Camire, M. E. 2004. Complex phenolic compounds reduce lipid oxidation in extruded oat cereals. LWT - Food Science and Technology **37**:789-796.

[3]Drzikova, B., Dongowski, G., Gebhardt, E. and Habel, A. 2005. The composition of dietary fibre-rich extrudates from oat affects bile acid binding and fermentation *in vitro*. Food Chemistry **90**:181-192.

[4]Maier, S. M., Turner, N. D. and Lupton, J. R. 2000. Serum lipids in hypercholesterolemic men and women consuming oat bran and amaranth products 1. Cereal Chemistry **77**:297-302.

[5]Braaten, J., Wood, P., Scott, F., Wolynetz, M., Lowe, M., Bradley-White, P. and Collins, M. 1994. Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. European J. Clin. Nutr. **48**:465-474.

[6]Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B. and Schneeman, B. O. 1999. Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with ß-glucan. The Amer. J. Clin. Nutr. **69**:55-63.

[7]Lambo, A. M., Öste, R. and Nyman, M. E. G. L. 2005. Dietary fibre in fermented oat and barley β-glucan rich concentrates. Food Chemistry **89**:283-293.

[8]Collins, H. M., Burton, R. A., Topping, D. L., Liao, M. L., Bacic, A., and Fincher, G. B. 2010. Variability in fine structures of noncellulosic cell wall polysaccharides from cereal grains: Potential importance in human health and nutrition. Cereal Chemistry **87**:272-282.

[9]Angelov, A., Gotcheva, V., Kuncheva, R. and Hristozova, T. 2006. Development of a new oat-based probiotic drink. International Journal of Food Microbiology **112**:75-80.

[10]Kailasapathy, K. and Chin, J. 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunology and Cell Biology **78**:80-88.

[11]Siuta-Cruce, P. 2001. Improving probiotic survival rates. Food Technology **55**:36-43.

[12]Lo Curto, A., Pitino, I., Mandalari, G., Dainty, J. R., Faulks, R. M. and John Wickham, M. S. 2011. Survival of probiotic lactobacilli in the upper gastrointestinal tract using an *in vitro* gastric model of digestion. Food Microbiology **28**:1359-1366.

[13]Marteau, P., Minekus, M., Havenaar, R. and Huis In't Veld, J.H.J. 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: Validation and the effects of bile. Journal of Dairy Science **80**:1031-1037.

[14]Angelov, A., Gotcheva, V., Hristozova, T. and Gargova, S. 2005. Application of pure and mixed probiotic lactic acid bacteria and yeast cultures for oat fermentation. Journal of the Science of Food and Agriculture **85**:2134-2141.

[15]Johansson, M. L., Nobaek, S., Berggren, A., Nyman, M., Björck, I., Ahrne, S., Jeppsson, B. and Molin, G. 1998. Survival of *Lactobacillus plantarum* DSM 9843 (299v), and effect on the short-chain fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats. International Journal of Food Microbiology **42**:29-38.

[16]Zhao, R. 2007. Eosinophilic acid *Lactobacillus* and its application. (in Chinese). Science Press, BJ, China, 4 p.

[17]Oda, Y., Yajima, Y., Kinoshita, M. and Ohnishi, M. 2003. Differences of *Rhizopus oryzae* strains in organic acid synthesis and fatty acid composition. Food Microbiology **20**:371-375.

[18]Kumar, S., Sharma, N. S., Saharan, M. R. and Singh, R. 2005. Extracellular acid protease from *Rhizopus oryzae*: Purification and characterization. Process Biochemistry **40**:1701-1705.

[19]Mattila-Sandholm, T., Blum, S., Collins, J., Crittenden, R., De Vos, W., Dunne, C., Fondén, R., Grenov, G., Isolauri, E. and Kiely, B. 1999. Probiotics: Towards demonstrating efficacy. Trends in Food Science & Technology **10**:393-399.

[20]Mortazavian, A., Ehsani, M., Mousavi, S., Rezaei, K., Sohrabvandi, S. and Reinheimer, J. 2007. Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. International Journal of Dairy Technology **60**:123-127.

[21]Ostlie, H. M., Treimo, J. and Narvhus, J. A. 2005. Effect of temperature on growth and metabolism of probiotic bacteria in milk. International Dairy Journal **15**:989-997.

[22]Toba, T., Tomita, Y., Itoh, T. and Adachi, S. 1981. β-Galactosidases of lactic acid bacteria: Characterization by oligosaccharides formed during hydrolysis of lactose. Journal of Dairy Science **64**:185-192.

[23]Asp, N., Mattsson, B. and Onning, G. 1992. Variation in dietary fibre, beta-glucan, starch, protein, fat and hull content of oats grown in Sweden 1987-1989. European Journal of Clinical Nutrition **46**:31-37.

[24]Wood, P. J. 2007. Cereal β-glucans in diet and health. Journal of Cereal Science **46**:230-238.

[25]Jaskari, J., Kontula, P., Siitonen, A., Jousimies-Somer, H., Mattila-Sandholm, T. and Poutanen, K. 1998. Oat β-glucan and xylan hydrolysates as selective substrates for *Bifidobacterium* and *Lactobacillus* strains. Applied Microbiology and Botechnology **49**:175-181.

[26]McMinn, W. A. M., McKee, D. J. and Magee, T. R. A. 2007. Moisture adsorption behaviour of oatmeal biscuit and oat flakes. Journal of Food Engineering **79**:481-493.

[27]Màrtensson, O., Staaf, J., Duecas-Chasco, M., Irastorza, A., Oste, R. and Holst, O. 2002. A fermented, ropy, non-dairy oat product based on the exopolysaccharide-producing strain *Pediococcus damnosus*. Advances in Food Sciences **24**:4–11.