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10.1515/aucft-2017-0010

EFFECTS OF PREBIOTICS ON ANTIOXIDANT ACTIVITY OF GOAT MILK FERMENTED BY *LACTOBACILLUS PLANTARUM* L60

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Abstract: The production of functional fermented goat milk with synbiotic have attracted widespread attention recently with the purpose of promoting health. The aim of present study was to investigate the effects of several prebiotics (inulin, fructo-oligosaccharide, galacto-oligosaccharide and xylo-oligosaccharide) on antioxidant activity and promote the development of functional goat milk. All the prebiotics exhibited the potential of enhancing the antioxidant activity of fermented goat milk, especially inulin and fructo-oligosaccharide showed better promotive effects. The optimum additions of inulin, xylo-oligosaccharide, galacto-oligosaccharide and fructo-oligosaccharide obtained were 0.6%, 0.6%, 0.6% and 0.4%, respectively. The DPPH radical scavenging rates reached to 75.52%, 74.12%, 69.41%, 80.28%, respectively, and the scavenging rates of superoxide radical were 21.09%, 18.20%, 27.61% and 29.92%, respectively, which were all higher than the control. This paper provides theoretical basis for the development of the functional goat milk.

Keywords: prebiotics; *Lctobacillus plantarum*; antioxidative activity; goat milk.

INTRODUCTION

Probiotics, a group of live microorganisms, can confer benefits to human beings through the interaction with host, which act by improving the growth of friendly bacteria and inhibiting potentially pathogenic bacteria in the gastrointestinal tract (Riaz & Masud, 2013). Lactic acid bacteria (LAB), kind of probiotics and widely spread in nature, confer a health benefit to body health and universally applicable to food industry, especially being incorporated into food formulations and fermented dairy product (Li et al., 2012). Numerous studies have introduced therapeutic and nutritional benefits of LAB strains, mainly included reducing certain cancers risk and intestinal disorders, modulating immunity, lowering serum cholesterol, anti-ageing and antioxidant activity, alleviating lactose-intolerance capabilities (Lee et al., 2010; Shori, 2013; Buckley et al., 2011). For example, *Lactobacillus plantarum,* a strain isolated from traditional fermented food, has been served as a health promoting agent and showed a series of biological functions (Todorov & Bdgdm, 2010; Huang et al., 2015). The term prebiotic was described as non-digestible food components and can stimulate the growth of probiotics in the digestive tract selectively in a manner, providing beneficial effect on the host (Sharma et al., 2012; Yasmin et al., 2015). Inulin, fructo-oligosaccharide, galacto-oligosaccharide, xylo-oligosaccharide, soybean oligosaccharides and lactulose are common prebiotics (Revathy et al., 2011). Prebiotics could not only show promotion on the growth of probiotic, they also exert a series of special bioactive activities such as modulating intestinal microbial balance, production of acidity, promoting mineral absorption, improving immune of host and among other effects (Moreno et al., 2014).
Reactive oxygen species (ROS) is the chemical active products produced by the partial reduction of oxygen (Duan & Kasper, 2011), which can

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contribute to various pathological processes such as aging, inflammation, cancer and atherosclerosis (Ksouri et al., 2012；Jiang et al., 2014). Increasing defenses against oxidant through food are of interest recently attribute to their ability to maintain human health and prevention of some disease (Shah et al., 2016). A novel strategy is the development of functional foods containing antioxidative peptides which can maximize benefits on the host by inhibiting the peroxidation of lipids and the production of free radicals. Antioxidative peptides are produced from milk proteins through enzymatic hydrolysis and microbial fermentation.

In our previous research, effects of five proteinases on antioxidant activity of casein hydrolysate from goat milk were studied, and the enzymolysis technology for production of antioxidative peptide from goat milk casein through single factor test and response surface methodology was optimized. (Shu et al, 2015, 2016, 2017). We have confirmed the antioxidant activity of 25 *Lactobacillus* strains during the fermentation of goat milk. *Lactobacillus plantarum* L60 showed better antioxidant activity and higher stability compared with other strains (Chen et al., 2015). The effects of inulin, fructo-oligosaccharide, galacto-oligosaccharide and xylo-oligosaccharide on the antioxidant abilities and fermented property of goat milk fermented by *Lactobacillus plantarum* L60 are investigated in this paper. Then the optimal concentration of each prebiotic is obtained.

MATERIALS AND METHODS

Microorganism: *Lactobacillus plantarum* L60, obtained from School of Food and Biological Engineering, Shaanxi University of Science and Technology, was inoculated in MRS broth (Hopebio, Qingdao, China) and cultured at 37ºC for 24h in triplicate.

Preparation of fermented milk supernate: Whole goat milk power (HongXing Dairy Co., Ltd., Shaanxi, China) dissolved in DI water was prepared. The goat milk (14%, w/w) was sterilized by water bath kettle at 90 ºC for 15min and cooled to room temperature. Then the 5% (v/v) bacterium suspensions of *L. plantarum L60* were inoculated into the sterilized milk and cultured at 41ºC for 12.3h. After shaking acutely, 1M HCL was used to adjust the pH of the fermented goat milk to 3.4-3.6. And the supernatants were harvested by centrifugation for 15 min at 8000×g. 1M NaOH was used to modify the pH of the supernatants to 8.3 and centrifuged under the same conditions. Finally, the supernatants were re-collected and the samples were obtained.

Determination of acidity and pH: The acidity (ºT) was measured by the method of neutralization titration. 3 drops phenolphthalein (1%) was added to the mixture containing 5mL fermented goat milk and 10mL distilled water in a 100mL conical flask. Then each sample was titrated with 0.1M NaOH and shocked slightly until the red does not fade in 30s. The acidity was calculated according to Eq. (1), where V (mL) means the consumption volume of sodium hydroxide standard solution.

The pH of each sample was recorded directly using a pH-meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai).

Determination of DPPH radical scavenging activity: The determination of DPPH radical scavenging activity in fermented goat milk was similar to the method of Norimasa method (Kazuko et al., 1992) with a slight modification. The mixture of 8mL DPPH radical solution (0.1mM, Sigma Chemical Co., Ltd, US) and 2mL samples was selected as experimental group. The mixture of 8mL DPPH radical solution (0.1mM) and 2mL 95% ethanol was used as control group. And 8mL 95% ethanol and 2mL samples was mixed as control group. Each mixture was kept at ambient temperature no light for 30min after oscillating acutely. Finally, the absorbance of the mixture was measured by ultraviolet spectrophotometer at 517nm and all the tests were carried out in triplicate. The scavenging activity was obtained based on Eq. (2), where A_i is the absorbance of sample with DPPH radical solution, A_j is the absorbance of the sample with anhydrous ethanol instead of DPPH solution under identical conditions as A_i. A_0 represents absorbance of ethanol containing DPPH solution without the samples.

Determination of superoxide radical scavenging activity: Superoxide scavenging activity was measured according to Jiang et al (2014) with a slight modification. EDTA-2Na (1mM, Tianjin Tianli chemical Reagent Co., Ltd) was added to adjust the pH of Tris-HCL (50mM, Xi'an LuoSenbo Technology Co., Ltd.) to 8.2, the Tris-HCL-EDTA-2Na buffer was prepared. 5.7mL buffer and 0.2mL fermented supernate were added in 20mL anaerobic tube and incubated at 25℃ for 20min. Reaction was initiated from the addition of 1mL pyrogallic acid (5mM). The antioxidant activity was recorded at 325nm by a spectrophotometer every 30s for 5min. Distilled water was added as control. The superoxide radical scavenging activity was calculated according to Eq. (3), where ΔA_0/min represents the change of absorbance of control per minute (without sample), ΔA_1/min represents the change of absorbance of mixture containing sample per minute.

$$\text{Acidity } (^{o}\text{T}) = \text{V} \times 20 \quad (1)$$

$$\text{Scavenging activity} (\%) = [1\text{-}(A_i\text{-}A_j)/A_o] \times 100\% \quad (2)$$

$$\text{Scavenging activity} (\%) = [(\Delta A_0/\text{min-}\Delta A_1/\text{min})/(\Delta A_0/\text{min})] \times 100 \quad (3)$$

RESULTS AND DISCUSSIONS

Effects of inulin on antioxidant activity of fermented goat milk

Inulin is regarded as functional food stuff with low calorie, which can promote health and reduce the risk of many diseases. (Fan et al., 2016). According to the initial fermentation conditions of reconstituted goat milk, inulin (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) was added to 14% reconstituted goat milk. The effect of inulin on pH and acidity of fermented goat milk were shown in Figure 1.

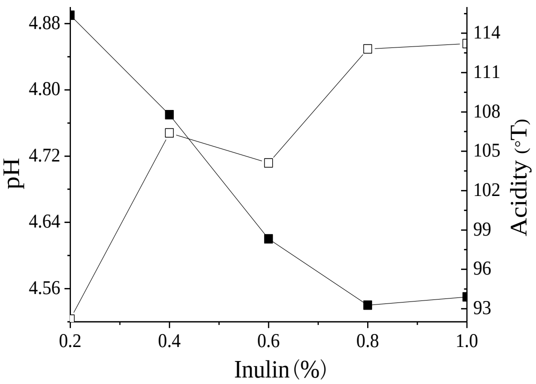

Figure 1. Effects of inulin on pH (solid symbols) and acidity (open symbols) in fermented goat milk

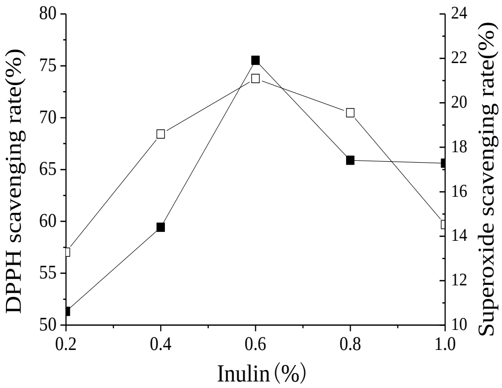

Figure 2. Effects of inulin on scavenging activities to the superoxide radical (open symbols) and DPPH radical (solid symbols) of fermented goat milk

From 0.2% to 0.8%, the pH was dropped sharply from 4.89 to 4.54 as the addition of inulin. Hauly et al (2005) manufactured soy yogurt with inulin and the final pH value was tested as 4.63. Sabir et al (2000) found that inulin addition could increase the rate of pH decrease of fermented milk products. When the inulin was 0.8%, the pH tended to be invariant at 4.85. The acidity increased obviously from 92.2ºT to 113.2 ºT as the addition of inulin from 0.2% to 1.0%, which proved that the addition of inulin had promotion effect on fermentation property of strains. Bozanic, Rogelj, and Tratnik (2002) reported that the firmness of fermented goat milk improved upon the addition of inulin.

The effect of inulin on antioxidant activities of fermented goat milk were shown in Figure 2. The scavenging DPPH and superoxide activity of fermented goat milk varied in a concentration-dependent manner, which climbed up firstly and then declined. When inulin increased from 0.2% to 0.6%, the scavenging DPPH radical activities increased from 51.32% to 75.52%. Meanwhile, the superoxide radical scavenging activity showed the same trend compared to DPPH radical. When the addition of inulin was 0.6%, the scavenging DPPH radical activities reached at maximum 75.52%, the superoxide radical scavenging activity up to 21.09%. While the scavenging rate of DPPH radical and superoxide radical of control group were 68.97% and 17.66%, respectively. The increase of antioxidant activity attributed to that inulin under a certain concentration could promote the activity of enzyme system and hydrolysis process of bacteria, while the high concentration of inulin would contribute to the death of bacteria.

Effects of xylo-oligosaccharide on pH, acidity and antioxidant activity in fermented goat milk

According to the initial fermentation conditions, xylo-oligosaccharide (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) was added to 14% reconstituted goat milk. The fermentation property and antioxidant activity were demonstrated in Figure 3 and Figure 4 respectively. As shown in Figure 3, there was a decrease in the pH and increase in the acidity in fermented goat milk as the increment of xylo-oligosaccharide, while the change of acidity was not evident. It was found that the effect of addition of xylo-oligosaccharides on the acid production of strains was not significant.

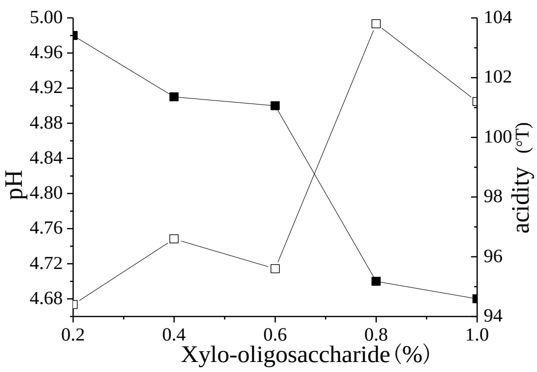

Figure 3. Effects of xylo-oligosaccharide on pH (solid symbols) and acidity (open symbols) in fermented goat milk

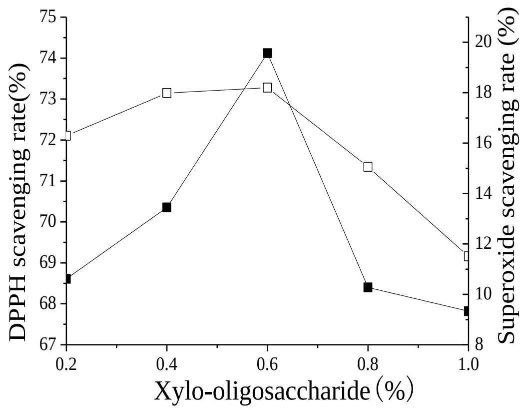

Figure 4. Effects of xylo-oligosaccharide on scavenging activities to the superoxide radical (open symbols) and DPPH radical (solid symbols) of fermented goat milk

DPPH and superoxide radical scavenging activities are shown in Figure 4. DPPH radical scavenging activity went up rapidly and peaked at the maximum, and then reduced sharply. Meanwhile, the superoxide radical scavenging activities show the similar behavior to the scavenging activity against DPPH radical. The scavenging DPPH radical activities and superoxide radical scavenging activity reach to 74.12% and 18.20% when the concentration of xylo-oligosaccharide rose to 0.6%. The effect of xylo-oligosaccharide on superoxide radical scavenging rate is insignificant compared to control group. It can be explained that the formation of metabolic end-product ascribed to the utilization of xylo-oligosaccharide and lactose by the *Lactobacillus plantarum* L60 could contribute to the increase of scavenging radical activity. Similar results were obtained by Lasrado & Gudipati (2015) that showed increase in the antioxidant activity of milk containing synbiotic combination of xylo-oligosaccharide and *Lactobacillus plantarum***.** Based on the stability of DPPH radical scavenging ability, the optimal concentration of xylo-oligosaccharide was 0.6%.

Effects of galacto-oligosaccharide on pH, acidity and antioxidant activity in fermented goat milk

According to the initial fermentation conditions, galacto-oligosaccharide (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) was added to 14% reconstituted goat milk. The effect of galacto-oligosaccharide on fermented goat milk is indicated in Figure 5. There is no significant change in pH and acidity with addition of galacto-oligosaccharide, demonstrating that the promotion of galacto-oligosaccharide on the growth of L60 is insignificant. The addition of galacto-oligosaccharide do not play an important role for the enhancement of fermentation property. The effect of galacto-oligosaccharide on antioxidant activity is shown in Figure 6. The trend of DPPH and superoxide radical scavenging activities is almost the same. When the addition of galacto-oligosaccharide is 0.6%, the scavenging DPPH radical activities and superoxide radical scavenging activity reach 69.41% and 27.61%. Galacto-oligosaccharide show not-significant effect on the increase of DPPH scavenging rate. However, the superoxide radical scavenging activity enhance almost 10% compared to the control group.

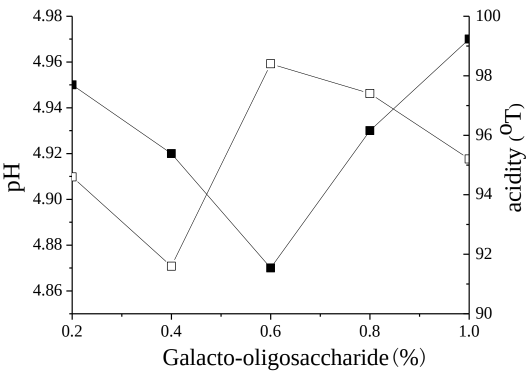

Figure 5. Effects of galacto-oligosaccharide on pH (solid symbols) and acidity (open symbols) in fermented goat milk

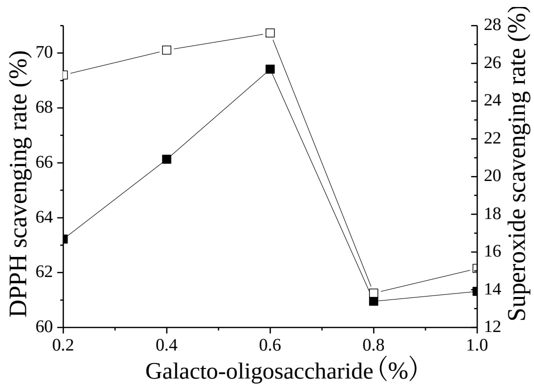

Figure 6. Effects of galacto-oligosaccharide on scavenging activities to the superoxide radical (open symbols) and DPPH radical (solid symbols) of fermented goat milk

Effects of fructo-oligosaccharide on pH, acidity and antioxidant activity in fermented goat milk

According to the initial fermentation conditions, fructo-oligosaccharide (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) was added to 14% reconstituted goat milk. The effect of fructo-oligosaccharide on fermented goat milk was indicated in Figure 7.

As Figure7 showed, with increasing of the fructo-oligosaccharide, the acidity increased at first, and then decreased. While opposite trend was displayed in pH, which decreased at first and then increased. With the increasing of fructo-oligosaccharide, the pH declined from 4.82 at 0.2% to 4.69 at 0.6%, then increased to 4.72 at 1.0%, which indicated that the promotion of fructo-oligosaccharide on the growth of *Lactobacillus plantarum* L60 was during fermentation. While the acidity increased from 99.6 °T at 0.2% to 115.4 °T at 1.0%, which proved in the acid production by L60 during fermentation of the goat milk. Thus, to some extent, the addition of fructo-oligosaccharide could promote the fermented activity of L60.

Similar tendency was displayed in DPPH radical and superoxide radical scavenging activities with the increasing of fructo-oligosaccharide (Figure 8). When the addition of galacto-oligosaccharide is 0.4%, the scavenging DPPH radical activities and superoxide radical scavenging activity reached to maximum

80.28% and 29.92%. Antioxidant activities in fermented goat milk were enhanced remarkably containing fructo-oligosaccharide compared with control group. The increase of antioxidant capacity ascribed to that active hydroxyl in molecular structure of fructo-oligosaccharide showed up the ability of scavenging radical (Li et al., 2008). Early feeding of prebiotic oligosaccharides to babies benefit a lot to breastfeeding (Arslanoglu, Moro, & Boehm, 2007). Seidel et al (2007) reported proper intake of prebiotics and natural antioxidants incorporated into a main food had an influence on several parameters of the immune system. An increasing amount of data point to a combined antioxidant and immunity-modulatory effect for prebiotics, suggesting that the underlying mechanisms might be identical. Thus, the addition of prebiotics exhibited positive effect on the antioxidant activity of fermented goat milk in this work. Their potential was considerable to be good choice to apply in functional food in the near future.

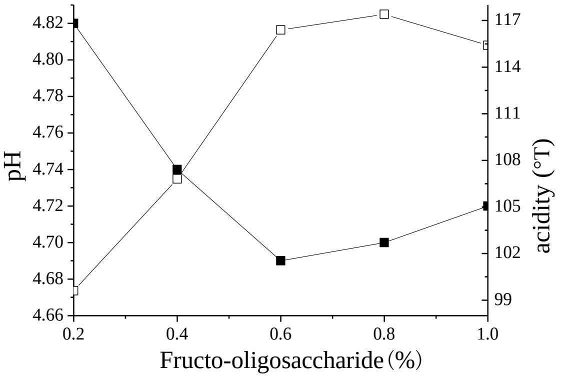

Figure 7. Effects of fructo-oligosaccharide on pH (solid symbols) and acidity (open symbols) in fermented goat milk

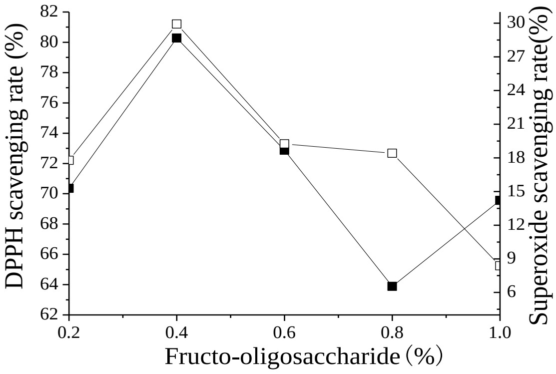

Figure 8. Effects of fructo-oligosaccharide scavenging activities to the superoxide radical (open symbols) and DPPH radical (solid symbols) of fermented goat milk

CONCLUSION

The effect of prebiotics on the antioxidant activity of goat milk fermented by *Lactobacillus plantarum* L60 had been verified in this work. Appropriate amount prebiotics could promote the fermentation of goat milk and the antioxidant activity of the fermented goat milk was enhanced in varying extent. Among all the prebiotics, inulin and fructo-oligosaccharide exhibited significant promotion on the antioxidant activity of the goat milk. The optimal addition of inulin, xylo-oligosaccharide, galacto-oligosaccharide and fructo- oligosaccharide to goat milk was 0.6%, 0.6%, 0.6% and 0.4%, respectively. The corresponding DPPH scavenging rates were 75.52%, 74.12%, 69.41%, 80.28%, and superoxide radical scavenging rate were 21.09%, 18.20%, 27.61% and 29.92%, respectively. The results provide good reference for improving the nutrition value of function food in industrial production.

ACKNOWLEDGMENTS

The project was partly supported by the Scientific Research Program Funded by Shaanxi Provincial Education Department (No.17JF005), the science and technology project of Xianyang city (No.2017K02-69), Doctoral Scientific Research Fund from Shaanxi University of Science & Technology (No.2017BJ-04) and Science and Technology Overall Planning for Innovation Engineering Project of Shaanxi Province (No.2016KTZDNY02-03).

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