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Quality characteristics and antioxidant activity of hickory-black soybean yogurt

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ABSTRACT

Hickory-black soybean milk and reconstituted milk (about 12 g/100 g solid content) were mixed in the proportion of 2:3 by volume to prepare hickory-black soybean yogurt (HBY). Cow milk yogurt (CMY) with no addition of hickory-black soybean milk was used as the control. Compared with CMY, HBY had higher water holding capacity, fat, crude protein and amino acids, but lower susceptibility to syneresis and ash content, and had no significant difference in the total count of lactic acid bacteria ($p > 0.05$). Sensory evaluation showed that the appearance and flavor scores of HBY had no significant difference from CMY ($p > 0.05$), but the texture score was significantly higher ($p < 0.05$). IC_{50} values of DPPH•, •OH scavenging ability, Fe^{2+} chelating ability and inhibition of lipid peroxidation of HBY were lower ($p < 0.05$) than those of CMY. These results suggested that the antioxidant activity of HBY was significantly higher than that of CMY.

1. Introduction

Yogurt, a fermented dairy product with rich nutrition, is mainly obtained by fermenting fresh milk or reconstituted milk with lactic acid bacteria, and favored by the customer because of its effects of improving the intestinal environment and enhancing the body immunity (Michael, Phebus, & Schmidt, 2010). In recent years, many researchers have made various flavor yogurts such as peanut yogurt, soybean yogurt and hawk tea yogurt (Chun, Kwon, Kim, & Kim, 2008a, 2008b; Isanga & Zhang, 2009; Ye, Liu, Zhang, Yang, & Wang, 2012), and some others have successfully prepared yogurts with antioxidant and hypoglycemic function by adding plant polyphenol and oligosaccharide (Karaaslan, Ozden, Vardin, & Turkoglu, 2011; Liu, Jiang, & Sun, 2007).

Many amino acids and small peptides (<1000 Da) are derived in the fermentation process of yogurt, which result into the strong antioxidant activity of the yogurt (Farvin, Baron, Nielsen, & Jacobsen, 2010a, 2010b). Phenolics in hickory (Arranz, Jiménez, & Calixto, 2008) and flavonoids in black soybean (Xu et al., 2007) both have strong antioxidant activity, so yogurt (hickory-black soybean yogurt) with stronger antioxidant activity can be produced by adding hickory and black soybean milk into reconstituted milk.

This study was intended to prepare hickory-black soybean yogurt using hickory kernel, black soybean and reconstituted milk, and elucidate its quality and antioxidant activity.

2. Materials and methods

2.1. Materials

Hickory kernels were provided by Zhan's Food Co., Ltd (Ningguo, China). Black soybean, whole milk powder (Nestle) and skimmed milk powder (Bright) were purchased from Carrefour in Hefei, China. *Lactobacillus delbrueckii subsp. bulgaricus 1.1480(Lb)(+)* and *Streptococcus thermophilus ys14(St)(+)* suitable for fermenting hickory-black soybean milk were isolated, domesticated and preserved in the Laboratory of Microbial Resources and Application of Hefei University of Technology.

2.2. Preparation of raw milk

The whole milk powder and the skimmed milk powder were mixed with a proportion of 5:1, dissolved in 43 °C water, and kept bath for 30 min, to obtain cow milk with about 12 g/100 g total solids.

Plump hickory kernels with no signs of fungal growth were soaked in 2 g/100 g sodium carbonate solution at 75 °C for 15 min and rinsed with running water for decortication. The decorticated hickory kernels and the soaked black soybean (at room

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temperature for 12 h) were mixed with a proportion of 1:3 by mass, added water of 6 times volume, and sieved with a 106 μm sieve after being grounded by a colloid mill, to obtain hickory-black soybean milk with the solid content of around 12 g/100 g.

2.3. Preparation of yogurt

L. delbrueckii subsp. bulgaricus 1.1480(Lb)(+) and *S. thermophilus ys14(St)(+)* were expanded cultured to prepare starter (Lb:St = 1:1) after 3 times activation.

Hickory-black soybean yogurt (HBY) was prepared with hickory-black soybean milk and reconstituted milk mixture in a proportion of 2:3 by volume, added with sucrose (7 g/100 mL), stirred, heated to 43 °C in water bath and preserved in it for 30 min, homogenized under 22 MPa by JHG-Q54-P60 Homogenizer (Shanghai Fusion Machinery Equipment Co., Ltd, Shanghai, China) for 15 min, then sterilized at 95 °C for 20 min. After being cooled to about 43 °C in water bath, the blend was inoculated with 6 mL/100 mL starter by volume ratio, cultured at 42 °C for 6 h, the pH at finishing fermentation point was about 4.3, then stored overnight at 4 °C in the fridge.

Cow milk yogurt (CMY) was prepared as the control yogurt by the same method with the reconstituted milk (12 g/100 g total solids).

2.4. Water holding capacity (WHC) and susceptibility to syneresis (STS)

The stirred yogurt of 10 mL (weight indicated by W_1) was subjected into the centrifuge tube and centrifuged at 2862× *g* for 15 min. After being left still for 10 min, the supernatant was removed. W_2 indicated the weight of the precipitate. The WHC was calculated as: $WHC = (W_2/W_1) \times 100\%$

Yogurt (50 mL) was filtered in a funnel with a single filter paper for 2 h. The collected whey volume (V) was used as the indicator for STS. The STS was calculated as: $STS(\%) = (V/50) \times 100\%$

2.5. pH and total count of lactic acid bacteria

PHS-3C pH meter (Shanghai, China) was used to determine the pH values of the samples.

Yogurt (1 mL) was accurately weighed and diluted to 10 mL with 0.9 g/100 g sterile NaCl solution (with 20–200 colonies on each plate). After being cultured for 48 ± 2 h at 36 ± 1 °C anaerobic incubator with MRS agar medium, the colony count on each plate was recorded.

2.6. Main composition and amino acid composition

The contents of ash, total solids (TS), non-fat milk solids (NFS), protein and fat were determined according to AOAC (2003) procedures.

Yogurt (1 g) was accurately weighed and put into a bottle for acidolysis, added 10 mL HCL (6 mol/L). After being aerated with nitrogen, the bottle was sealed for acidolysis at 135 °C for 6 h. The acidolysis solution was diluted to 5 mL with 0.02 mol/L HCL, and sample of 20 μL was injected into the automatic amino acid analyzer L-8900 (Hitichi, Japan) for detection of amino acids. The chromatographic conditions: sodium ion exchange column; column temperature: 60 °C; flow rate: 0.4 mL/min; flowing phase: citric acid buffer; temperature of column response column: 135 °C; flow rate: 0.35 mL/min; the detector type: VIS, detection wavelength: 570 nm and 440 nm respectively. The content of amino acid (AA) was calculated as: $AA(g) = C \times 10^{-9} \times 5$, C (ng/mL) was the concentration of amino acid.

2.7. Sensory evaluation

Four aspects (appearance/color, texture/taste, flavor and acceptability) were assessed by eight panelists (sexes were about equally and the panelists ranging in age from 20 to 40) with experience and background in scientific food knowledge. Ten points system was used for evaluation according to method of Isanga and Zhang (2009) with minor modification.

Sensory evaluation scores: Extremely unacceptable = 1; Unacceptable-barely acceptable = 2–4; Acceptable-very acceptable = 5–9; Extremely acceptable = 10.

2.8. DPPH• and •OH scavenging activity

Method of Chun et al. (2008a, 2008b) with minor modification was used to evaluate the DPPH• scavenging activity. DPPH• ethanol solution (1.0×10^{-4} mol/L) of 8 mL was mixed with 2 mL sample or 16.52 mol/L ethanol (blank control) with fully shaken, kept in the dark for 30 min reaction at room temperature, and centrifuged at 351× *g* for 10 min at room temperature. The supernatant was obtained to determine the absorption value at 517 nm. DPPH• scavenging rate (DSR) was calculated as: $DSR(\%) = (A_0 - A_1)/A_0 \times 100\%$

A modified method (Wang, Jiang, & Mu, 2007) was used to evaluate •OH scavenging activity. Phenanthroline solution (5×10^{-3} mol/L) of 1.5 mL was taken and added with 2.0 mL of phosphate buffer (pH 7.4, 0.05 mol/L), added with 1.0 mL $FeSO_4$ solution (7.5×10^{-3} mol/L), evenly mixed immediately, added with 1 mL H_2O_2 (0.0294 mol/L), and finally supplemented with distilled water to 10 mL. After being kept in 37 °C warm water for l h, $A_{damaged}$ at 536 nm was detected. The same method as mentioned above was used to assess the •OH scavenging activity of the yogurt sample. Sample solutions of different concentrations were added, followed by adding with H_2O_2, kept at 37 °C for 1 h, and centrifuged at 351× *g* for 10 min at room temperature. The supernatant was obtained to determine $A_{sampled.}$ The undamaged tubes were not added with H_2O_2 or sample solutions. •OH scavenging rate (OSR) was calculated as: $OSR(\%) = (A_{sampled} - A_{damaged})/(A_{undamaged} - A_{damaged}) \times 100\%$.

2.9. Inhibition of lipid peroxidation

Method of Ye, Xu, Chen, Yang and Lin (2010) with minor modification was used. Sample solutions of different concentrations (1 mL) and 10 g/100 mL yolk homogenate (w/v) (0.5 mL) prepared by 1.15 g/100 g KCl solution were evenly blended in the tube, added with 2 mL distilled water, 1.5 mL glacial acetic acid (20 mL/100 mL pH 3.5) and 1.5 mL TBA (0.8 g/100 mL) that contained 1.1 g/100 mL SDS, evenly mixed and then put in 95 °C water bath for 60 min. After being cooled to room temperature, the samples were added with 5 mL butanol, and centrifuged at 1610× *g* for 10 min. The supernatant was obtained to determine the absorption value A_T at 532 nm. Using the distilled water instead of the sample solution as control, A_C was measured by the same method. Inhibition of lipid peroxidation rate (ILP) was calculated as: $ILP(\%) = (1 - A_T/A_C) \times 100\%$

2.10. Fe^{2+} chelating ability

Method of Ersoy, Bagci, and Gok (2011) with minor modification was used. Sample solutions of different concentrations (10 mL) were evenly mixed with 0.5 mL $FeCl_2$ (2 mmol/L) and added with 2 mL ferrozine solution (5 mmol/L). After being left still for 20 min, the absorbance A_1 was measured at 562 nm. Using the distilled water instead of the sample solution as control, A_0 was measured with the same method. Fe^{2+} chelating rate (FCR) was calculated as: $FCR(\%) = (A_0 - A_1)/A_0 \times 100\%$