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Effects of Grape Polyphenols on the Quality and Antioxidant Activity of Yoghurt

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Grape polyphenols yoghurt (GPY) was prepared from the mixture of grape polyphenols (GP) and the reconstituted milk. The final product was subjected to the analysis of lactic acid bacteria number, physicochemical property. It was found that the counts of *Lactobacillus delbrueckii* subsp. *bulgaricus* declined gradually whereas the counts of *Streptococcus thermophilus* increased in the range of 0.1-0.4 g/L (GP), meanwhile the reducing power, DPPH· scavenging activity and Fe^{2+} chelating ability of the GPY increased significantly. These results indicated that GP can significantly enhance the antioxidant activity of yoghurt. The pH, viscosity and susceptibility to syneresis increased gradually in the range of 0.1-0.7 g/L (GP). During the 0-18th days, the total counts of lactic acid bacteria and the pH of the GPY had significantly slower decrease than that of the control yoghurt (CY). The above results indicated that addition of GP can significantly enhance the antioxidant activity of yoghurt, and GP has a protective effects on the survival of the lactic acid bacteria.

Key words: Grape polyphenols yogurt (GPY), Counts of lactic acid bacteria, Quality, Antioxidant activity, Preservation period.

Yoghurt is a kind of fermented dairy being rich in probiotics,, which is mainly obtained by fermention of fresh milk or reconstituted milk with lactic acid bacteria[1]. In recent years, yoghurt has drawn much attention due to addition of some functional compounds[2,3,4] .

It has been found that fruit polyphenols can improve health and prevent disease[5,6]. Fruit polyphenols yoghurt can be obtained by addition of fruit polyphenols to the fermentated fresh milk[7]. GP is a kind of plant polyphenols that consists of phenolic acids, flavanols, anthocyanins, flavonols and condensed tannins, among which anthocyanidins are the most abundant[8]. Research showed that the antioxidant capacity of GP was stronger than that of V_E and V_C. In addition, GP also has various activities like anti-mutation, anti-atherosclerosis, anti-gastric ulcer, anti-inflammation, anti-tumor, reduction of blood lipid, prevention of diabetic chronic complications and hypertension , etc.[5,9].

The purpose of this study was to prepare GPY, examine the change of the lactic acid bacteria counts and pH during storage, and disclosed the difference of physicochemical property.

MATERIALS AND METHODS

GP was purchased from Tianjin Jianfeng Natural Product R&D Co., Ltd. Skimmed milk powder (Bright), full cream milk powder (Nestle) and sucrose were purchased from Carrefour in Hefei, China. Yoghurt starter (*Streptococcus*

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thermophilus and *Lactobacillus bulgaricus*) was provided by the Laboratory of Microbial Resources and Application of Hefei University of Technology (Hefei, China).

Methods

Preparation of GPY

Freeze-dried powders of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were inoculated into 12% skimmed reconstituted milks respectively and activated three times, then *S. thermophilus* and *L.bulgaricus* were inoculated into 12% skimmed reconstituted milk (St:Lb=1:1), which preserved at 4°C for later use.

The full cream milk powder and the skimmed milk powder were mixed in the proportion of 5:1, added water (42°C), and stirred continuously till completed dissolution, obtained reconstituted milk 12 g/100 g total solids . Then 6 g sucrose was add to 100 mL reconstituted milk as a sweetener, homogenized at 25 MPa (Homogenizer, (JHG-Q954)-P(60), Shanghai, China) for 10 min, and mixed with GP of 0.1 g/L, 0.2 g/L, 0.3 g/L, 0.4 g/L, 0.5 g/L, 0.6 g/L, 0.7 g/L and 1 g/L, respectively, followed by sterilization at 85°C for 30 min, cooled to 42°C. The samples were filtered with sterile cotton to wipe off the residues, and the raw milk containing GP was obtained. Then the samples were inoculated with the yoghurt starter at 3 mL/100 mL, cultured at 42°C for about 6 h and preserved at 4°C, to obtain GPY of different concentration gradients. Yoghurt without GP was used as CY.

Counts of lactic acid bacteria and pH of GPY

The counts of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in GPY of different concentrations were determined according to the method of Ye and others[4] with slightly modification of dilution spread on M17-lactose and MRS-fructose agar plates. The plates were put in Forma 1029 Anaerobic System (Thermo Electron Corp., Waltham, MA, USA), and cultured at 37°C for 48 h. The colony counts on each plate were recorded.

PHS-3C pH meter (Shanghai Rex Instruments Factory) was used to determine the pH of the sample.

Susceptibility to syneresis (STS) and viscosity

Yoghurt (100 g) was placed at the top of a funnel with 6 layers of gauzes. After dripping water for 2 h, the whey was collected in a measuring cylinder. The volume (V) of the collected whey was used as the indicator for STS (mL/100 g).

Brookûeld DV I + viscometer was used to measure the viscosity of the samples at 10°C (with the rotary speed of 10 rpm).

Determination of antioxidant activity

Determination of reducing power

The reducing power of samples with different concentrations (1 mL of each) was determinated by added with 2.5 mL of 0.2 mol/L phosphate buffer with pH 6.6, l mL l% $K_3[Fe(CN)_6]$. The mixture was incubated and put at 50°C for 20 min, and l mL of l0% TCA solution was then added to the mixture, which was then centrifuged at 3000 rpm for l0 min. The supernatant was drawn off and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The OD value of the final mixture was measured at 700 nm using a cuvette. The higher the absorbance is, the stronger the reducing power is. Distilled water was used as the control.

DPPH· scavenging activity

The method as reported[10,11] with minor modifications was adopted. DPPH ethanol solution of 8 ml (1.0×10^{-4} mol/L) was mixed with 2 ml sample or 95% ethanol (blank control), fully shaken and kept in the dark at room temperature for 30 min, followed by centrifugation at 1400 rpm for 10 min. The supernatant was collected to determine the absorbance at 517 nm. DPPH· (%) scavenging rate:

$$\text{DPPH scavenging rate (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0=Blank absorbance, A_1=Sample absorbance.

Fe^{2+} chelating ability

Method of Wang and others[12] with minor modifications was adopted. The sample solutions of different concentrations (10 mL) were evenly mixed with 0.5 mL $FeCl_2$ (2 mmol/L) and 2 mL ferrozine solution (5 mmol/L). After being left for 20 min, the absorbance was measured at 562 nm. Using the distilled water instead of the sample solution as control, the absorbance was measured by the same method. The chelating rate can be obtained by the following equation:

$$Fe^{2+} \text{ chelating ability (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0=Blank absorbance; A_1=Sample absorbance.

Change of total counts of lactic acid bacteria and pH of GPY during the preservation period

During storage comprehensively considering the effects of grape polyphenols on the yoghurt quality and antioxidant activity, GP of 0.4 g/L was chosen to prepare the GPY by method in section "Preparation of OPC yoghurt". The method in section "Counts of lactic acid bacteria and pH of GPY"was used to determine the counts and pH value of the GPY and the CY on the 0th, 3rd, 6th, 9th, 12th, 15th, 18th days. MRS agar medium was adopted to determine the counts of lactic acid bacteria.

Statistical analysis

All the above experiments were repeated three times. ANOVA in SAS 9.1 software was employed for analysis of variance, and $P < 0.05$ indicated a significant difference. Data were preseted as the mean ± standard deviation.

RESULTS AND DISCUSSION

Counts of lactic acid bacteria and pH value of GPY

According to the report of Robinson[13], although the initial ratio of *Streptococcus thermophilus* to *Lactobacillus delbrueckii* subsp. *bulgaricus* for yoghurt fermentation and inoculation was 1:1, the counts of *Streptococcus thermophilus* after fermentation actually accounted for 75-85% of the total counts of live bacteria. Fig.1a showed that the counts of *Streptococcus thermophilus* in the yoghurt were significantly higher than those of in *Lactobacillus delbrueckii* subsp. *bulgaricus*. When mixing with GP in the concentration range of 0.1-0.4 g/L, the counts of *Streptococcus thermophilus* in the GPY were slightly higher than that without GP. When the GP concentration was in 0.4-0.7 g/L, the counts of *Streptococcus thermophilus* and *Lactobacillus*

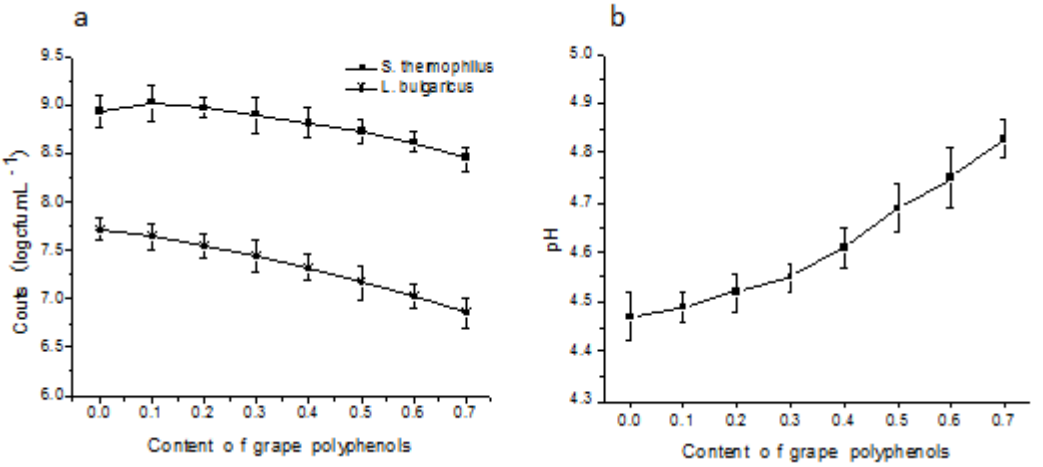

Fig. 1. Counts of lactic acid bacteria (a) and pH (b) of yogurts with different content of grape polyphenols

delbrueckii subsp. *bulgaricus* both decreased gradually, but the counts of the former were higher than that of the latter. Fig. 1b showed that pH value of the GPY increased with the increase of the GP concentration.

Viscosity and susceptibility to syneresis of GPY

Peng and others[14]reported that the total solid content (especially the protein content) in yoghurt was related to the apparent viscosity, and there are also reports claimed that the yoghurt viscosity was relevanted to the protein arrangement and the crosslinking between proteins[15]. In this study, the yoghurt viscosity was found to be related to GP and its concentration. When mixing with GP in 0.1-0.3 g/L, the yoghurt viscosity slightly changed, whereas the GP concentration was in 0.4-0.7 g/L, the yoghurt viscosity significantly increased. It has been reported that the increase of the fat content in yoghurt can enhance the water retention ability of yoghurt and decrease the yoghurt's susceptibility to syneresis[16]. Fig.2 showed that when the GP concentration was in 0.4-0.7 g/L, the syneresis value also had a significant increase, indicated that GP

can enhance the susceptibility to syneresis of yoghurt.

Antioxidant activity of GPY

Jayaprakasha and others[17] reported that the extracts from different kinds of grape seeds had significantly different reducing power, and the reducing power was related to reductone. Gordon and others[18]showed that destruction of hydrogen atoms in the free radical reaction chain caused the antioxidant capacity of reductone, which indicated that the antioxidant activity was generated along with the reducing power. Therefore the antioxidant capacity of GP and GPY was related to their reducing power. When the GP concentration was in 0.1-0.4 g/L, the reducing power of the GPY increased significantly, and was higher than those of GP and the V_C (Fig. 3a). Then, the reducing power of the yoghurt increased slowly with the increase of the GP concentration, being weaker than those of GP and the control V_C of the same concentration. This may due to lactic acid bacteria can absorb and decompose a certain amount of GP, and the decomposed products have a stronger reducing power.

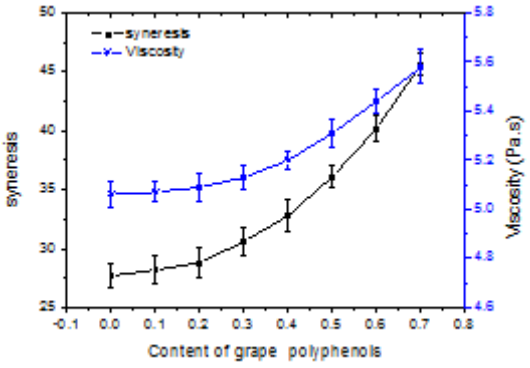

Fig. 2. Syneresis and viscosity of viscosity of yoghurts with different content of grape polyphenols

Even though metal chelators are not antioxidants, they play a very important role in rancidity of oily food. Owing to ferrozine and Fe^{2+} form complex compounds during the iron-catalyzed lipid peroxidation, and the number and red color of the compounds are decreased in the presence of other chelators, the absorbance can be determined by the change of color. The decline of the absorbance can be estimated by the effects of the additional ferrozine on the chelator[19,20]. The Fe^{2+} chelating rate of the GPY was significantly lower than that of GP but higher than that of the control EDTA-Na_2. When the GP concentration was in 0.1-0.3 g/L, the Fe^{2+} chelating rate of the yoghurt had a significant increase (Fig. 3b).

When the GP concentration was in 0.1-0.4 g/L, the DPPH· scavenging activity of GPY was significantly higher than that of GP and the control Vc. And the DPPH· scavenging rates of the three tended to be close to each other with the increase of the GP concentration (Fig. 3c).

It has been reported that the lactic acid bacteria in yoghurt have free radical scavenging effects[21], and the polypeptide (< 1000Da), free amino acids produced during the fermentation process[22], and which reported that polypeptides chain that consist of proline, histidine or tyrosine, with valine and leucine have antioxidant activity, and free amino acids in the yoghurt such as

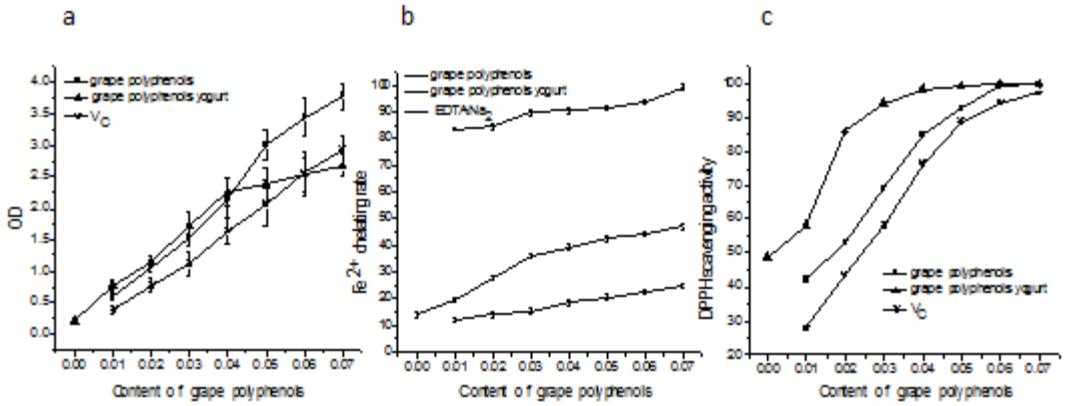

Fig. 3. Reducing powers (a), Fe^{2+} chelating ability (b) and DPPH scavenging activity (c) of yogurts of yoghurts with different content of grape polyphenols

histidine, tyrosine, threonine and lysine have antioxidant activities. Our work showed that the antioxidant activity of GPY was stronger than that of CY. But the ultimate antioxidant activity was a result of synergistic effect rather than a simple summation of them. In a certain range of concentration, the antioxidant activity was increased significantly.

At the beginning of the storage, the total counts of lactic acid bacteria in GPY was lower than that in the control group, whereas pH value was higher than that of the CY (Fig. 4b). During 0-18th days, the total counts of lactic acid bacteria and pH value of the GPY both declined. And compared to the CY, the total counts of lactic acid bacteria in the GPY had a significantly slower decrease. On 9th day, the total counts of lactic acid bacteria in GPY was close to that of CY. During the 9-18th days, the total counts of lactic acid bacteria in the GPY was higher than that of CY (Fig. 4a). It indicated that, during the preservation period, GP had a certain protective effects on the survival of its lactic acid bacteria.

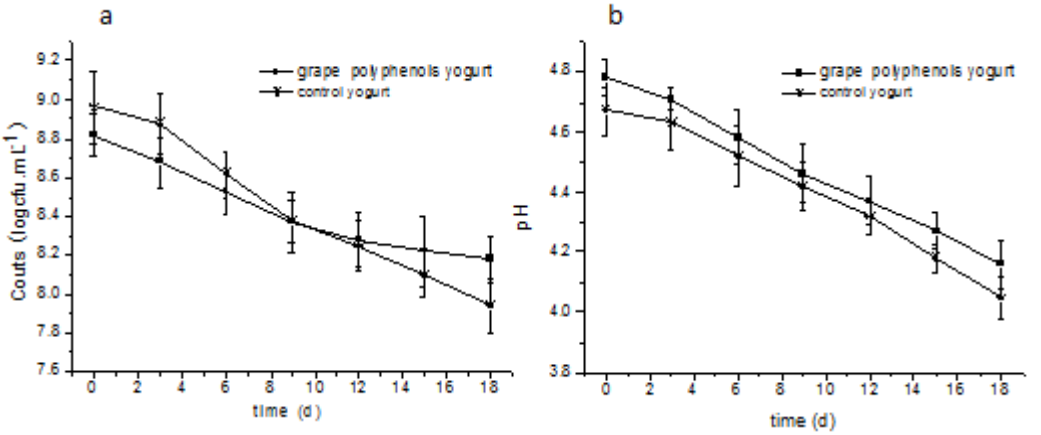

Fig . 4. Changes of lactic bacteria numbers (a) and pH (b) of grape polyphenols yoghurt during storage priod

CONCLUSIONS

When the GP concentration in the range of 0.1-0.7 g/L, the pH value, viscosity, and susceptibility to syneresis increased gradually. When the GP concentration was in 0.1-0.4 g/L, the counts of *Lactobacillus delbrueckii* subsp. *bulgaricus* declined gradually whereas the counts of *Streptococcus thermophilus* increased, the reducing power, DPPH· scavenging activity and Fe^{2+} chelating ability of the yoghurt increased significantly. During the 0-18th days, the total counts of lactic acid bacteria in the GPY had a significantly slower decrease compared to the CY. The above results indicated that mixing with GP can significantly enhance the antioxidant activity of yoghurt, and GP has a protective effect on the survival of the lactic acid bacteria in yoghurt during storage.

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