Research Article

Influence of Lactic Acid Bacteria Fermentation on Physicochemical Properties and Antioxidant Activity of Chickpea Yam Milk

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This study aimed to evaluate the influence of lactic acid bacteria fermentation on the physicochemical properties and antioxidant activity of chickpea yam milk. Four groups of chickpea milk were prepared through fermentation with lactic acid bacteria for quality and functionality improvement. Results indicate that the polysaccharide content of four samples declines during the fermentation process, and their infrared spectrums are similar with a slight difference in the transmittances of some characteristic bands. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of four samples with 24 h fermentation showed a band disappearance in the range of 94.3 KDa and generation of many small-molecule peptides, revealing the protein degradation during fermentation. The moderate enzymatic hydrolysis had no adverse effect on the texture and color of the samples. A substantial increase in DPPH, ABTS radical scavenging rates, and FRAP value was observed after 12 h fermentation, especially the CPBY sample. The present results indicate that lactic acid bacteria fermentation can be used to improve the physicochemical properties of samples and enhance their antioxidant activity.

1. Introduction

With increasing concern for health (lactose intolerance and animal milk allergies), environment protection, and sustainability, the interest and demand for plant-based milk are growing [1]. Plant-derived milk is made from legumes, cereals, or edible plant seeds [2, 3]. Applying lactic acid bacteria fermentation to plant-based milk is a subtrend of innovative plant-derived products. This process provides a natural method to increase the safety, nutrition, and shelf life of plant-derived products. The biological activities of plant-based products can be improved by fermentation because microbial proteases promote protein degradation and generate bioactive peptides during fermentation. Researchers demonstrated that lactic acid bacteria possess the ability to hydrolyze proteins [4]. Studies have proven that the concentrations of bioactive components, such as small-size peptides and free amino acids, are improved by fermentation

[5]. Thus, the microbial modification of lactic acid bacteria fermented beans is an effective method to increase their antioxidant and/or other biological activities.

In recent years, relevant studies have been conducted on the process optimization and antioxidant properties of plant-based fermented milk. Traditional fermented soybean products have been increasingly recognized to have powerful antioxidant effects. Soymilk fermented with *Lactobacillus paracasei* KUMB B005 has a credible antioxidative potential [6]. The contents of antioxidant compounds in fermented soymilk are closely related to reducing power and antiradical ability. A previous study reported that the antioxidant capacity is highly correlated with proteolytic activity [7]. Glucosidase produced by lactobacilli in the fermentation process can hydrolyze soybean isoflavone from glycoside to aglycone, causing them to exhibit strong biological activity [8]. Fermented soymilk can enrich B group vitamins, promote gut health, and enrich anticancer effect [9].

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in production, next to dry beans and field peas [10]. Compared with other legumes, chickpea is a good source of protein and carbohydrates, accounting for approximately 80% of the total dry seed mass [11]. Chickpea is also rich in minerals, dietary fiber, and vitamins [12]. The demand for chickpea milk as a good substitute for soymilk is increasing due to its nonallergenic and nutritional value [13, 14]. Yam, a famous food material, has a sweet taste and contains a variety of substances with antioxidant activity, which is good for the spleen. A previous study found that Chinese yam extract induces an improvement in digestive capability and affects the conversion of some intestinal flora to beneficial gut bacteria [15]. Therefore, adding yam to chickpea fermented milk may help to improve the flavor, function, and nutrition of chickpea fermented milk. Previous studies illustrated the effect of fermentation technology on the quality of chickpea milk [14, 16]. However, whether the physicochemical properties and antioxidant activity of chickpea yam milk can be positively influenced by lactic acid bacteria fermentation remains unknown. Therefore, this study aimed to evaluate the influence of lactic acid bacteria fermentation on the physicochemical characteristics and antioxidant activity of chickpea yam milk before and after fermentation. The findings are expected to provide theoretical support for developing new products of yam and chickpea milk.

2. Materials and Methods

2.1. Raw Materials and Chemicals. Untoasted chickpeas (Desi), yam, and sugar were purchased from a local supermarket (Zhengzhou, China). Papain (10^4 U/g) was purchased from Nanning Pangbo Biological Engineering Co., Ltd. (Nanning, China). Cellulase was purchased from Ningxia Heshibi Biotechnology Co., Ltd. (Ningxia, China). *Lactocaseibacillus rhamnosus* CICC 20257 was purchased from the China Center of Industrial Culture Collection (Beijing, China). Chemicals required for the analysis including 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonate) (ABTS⁺) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, USA). All other chemicals used were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of Fermented Chickpea Yam Milk. Chinese yam was pulverized into powder on a 200-mesh sieve and was mixed with deionized water (ratio 2:7 w/v). The yam milk was heated at 100°C for 2 min to inactivate the enzyme.

Fermented chickpea milk was prepared as follows: whole chickpeas were washed and soaked for 12 h in distilled water. A total of 200 g of swollen chickpeas was ground in 1.6 L water (ratio 1:8 w/v). The slurry was cooked at 85°C for 15 min and then filtered with double-layer gauze to separate insoluble residues. The filtrate was transferred into glass bottles and sterilized at 100°C for 15 min. After being cooled

to room temperature, chickpea milk was evenly divided into four groups. The first group consisted of chickpea milk and water at a ratio of 8:3 without adding enzyme and denoted as CP. The second group was made by mixing chickpea milk and water at a ratio of 8:3 with addition of 80 U papain per chickpea protein gram and denoted as CPB. This group was kept at 50°C for 30 min and then boiled to inactivate the enzyme. The third group was prepared by mixing chickpea milk and yam milk at a ratio of 8:3 without adding enzyme and marked as CPY. The fourth group was made by mixing chickpea milk and yam milk at a ratio of 8:3 with addition of 80 U papain per chickpea protein gram and marked as CPBY. This group was kept at 50°C for 30 min and then boiled to inactivate the enzyme. All samples were added with sucrose (previously sterilized by filtration) with a final concentration of 5% (v/v) and then stored at 4°C.

Lactocaseibacillus rhamnosus CICC 20257 was used as a starter for the production of fermented chickpea milk. The microorganism was propagated (1%, v/v) twice in MRS broth [17] and incubated at 37°C for 24 h without stirring in microaerophilic condition.

Four hundred milliliters of each chickpea milk sample was inoculated with 2% (v/v) (more than 1×10^8 cfu/mL) of an active culture of *Lactocaseibacillus rhamnosus* CICC 20257, and the mixture was allowed to ferment at 37°C for 24 h. The sample was aseptically collected at 0, 3, 6, 9, 12, and 24 h, immediately cooled on ice, and stored at 4°C until analysis.

2.3. Measurements of Polysaccharides from Chickpea Samples before and after Fermentation

2.3.1. Extraction of Polysaccharides. The extraction of polysaccharides was determined in accordance with the previous method [18] with some modifications. Samples (20 mL) of 0, 3, 6, 9, 12, and 24 h were mixed with deionized water at a ratio of 1:10. The pH of the mixture was adjusted to 5.0 by adding sodium acetate-acetic acid buffer, and then 1% cellulase was added. Subsequently, polysaccharide was extracted by ultrasonic method (300 W) at 50°C for 30 min. The slurry was filtered through vacuum filtration after the extraction. The filtrate was stirred with medium-speed magnetic for 10 min and then centrifuged at 6,000 g for 10 min to remove the protein. The supernatant was collected and was mixed with 95% ethanol at a ratio of 1:3 for 12 h at 4°C until white precipitation appeared. The residue was washed with 85% ethanol twice and dried at 60°C for 2 h after centrifugation. The content of polysaccharides was determined by the phenol-sulfuric acid method.

2.3.2. FTIR Spectra Interpretation. Dry polysaccharide (approximately 2 mg/sample) from fermented samples of 0, 12, and 24 h was mixed with 100 mg of KBr. The spectrum was collected with a Fourier transform infrared spectroscopy (FTIR) spectrometer (iS™10 FTIR Persee, Beijing, China) at 4 cm^{-1} resolution for 32 scans in the range of $4,000\text{--}400\text{ cm}^{-1}$.

2.4. Measurements of SDS-PAGE. In accordance with the modified method [19], sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to characterize the changes in protein during the fermentation process. The samples (20 μ L/sample) collected at 0 and 24 h were mixed with loading buffer (20 μ L) at 1:1 ratio. The mixture was boiled at 100°C for 5 min to induce protein denaturation, kept in an ice bath for 3 min, and centrifuged at 6,000 g for 5 min. A 10 μ L aliquot of each supernatant solution was loaded on a 10% polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA, USA). At a constant voltage of 110 V, the gels were run on a Bio-Rad Mini-Protean system (Bio-Rad) for 3 h. The proteins were then stained with Coomassie Blue R-250 for 30 min and destained for 24 h until the bands were clear. The gels were scanned (Tanon-2500; Bio-Tanon, Shanghai, China) at a resolution of 500 dpi.

2.5. Physical Properties of the Fermented Chickpea Milk

2.5.1. Texture Profile Analysis (TPA). The TPA of each sample was determined by CT3 (Brookfield Engineering Laboratories, Inc., USA). The intermediate layer of each sample was selected for texture analysis. The hardness (g), consistency (g-s), viscosity index (g-s), and cohesion (g) of the samples were measured with a cylindrical TA4/1000 probe under 1 mm/s test speed, 0.5 mm/s pretest speed, 1 mm/s posttest speed, 30% compressed strain, and 5 s compression interval.

The color of each fermented sample for 24 h was determined with a Konica Minolta CR-400 spectrophotometer (Konica Minolta Holdings Inc., Japan). The fermented sample was filled in a glass cuvette, and the CIELAB tristimulus color values of lightness/darkness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) were measured at 25°C.

2.6. Determination of Antioxidant Capacity

2.6.1. DPPH Radical Scavenging Assay. The fermented samples were analyzed for the scavenging effect on DPPH radical as described by Soison et al. [20] with minor modification. Each sample (1 mL) of 0, 3, 6, 9, 12, and 24 h was mixed with 3 mL of 0.2 mmol/L DPPH ethanol solution. The absorbance of the reaction mixture was measured at 517 nm after reaction in darkness at room temperature for 30 min. The blank control consisted of 95% ethanol and DPPH ethanol solution. The DPPH free radical scavenging rate (%) of each sample was calculated in accordance with the following equation:

$$\text{DPPH scavenging rate (\%)} = \left[\frac{(A_0 - A_s)}{A_0} \right] \times 100\%, \quad (1)$$

where A_0 is the absorbance of the blank and A_s is the absorbance of the samples.

2.6.2. Determination of Ferric Reducing Antioxidant Power (FRAP). The FRAP antioxidant capacity of fermented samples was determined in accordance with the method of

Oyaizu [21] and Marazza [22] with some modifications. An aliquot (2.0 mL) of the fermented sample was mixed with 2.0 mL of pH 6.6 phosphate buffer (0.2 M) and 2.0 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 10% trichloroacetic acid (2.0 mL) was added. The mixture was centrifuged at 6,000 g at 4°C for 10 min. The supernatant was mixed with 0.1% ferric chloride (1.0 mL) and deionized water (5.0 mL). The absorbance was measured at 700 nm after 10 min.

2.6.3. ABTS Radical Scavenging Assay. ABTS radical scavenging activity was determined, as described by Raquel [23] with some modifications. ABTS⁺ (7 mmol/L) and potassium persulfate (140 mmol/L) were prepared separately. Under the dark condition, 1.76 mL potassium persulfate solution and 100 mL ABTS⁺ were mixed evenly and reacted overnight. The prepared liquor with 95% ethanol was diluted. The absorbance at 734 nm is 0.700 ± 0.02 . Subsequently, 1.0 mL of the sample solution was mixed with 3 mL of ABTS radical solution, and the absorbance was recorded at 734 nm.

2.7. Statistical Analysis. Each experiment was conducted thrice, and results are presented as the mean values ($n = 3$) $\pm$ standard deviation (SD). Differences among different groups were analyzed by using analysis of variance and Duncan's multiple range test. Differences were considered significant at $p < 0.05$ level.

3. Results and Discussion

3.1. Polysaccharide Contents of Different Samples during Fermentation. As presented in Figure 1, the polysaccharide contents of samples in the four groups declined gradually from 0 h to 12 h. This phenomenon was because the polysaccharide molecules were hydrolyzed by lactic acid bacteria to provide a carbon source for their proliferation during the fermentation process. Therefore, the polysaccharide content gradually decreased, which was consistent with Hong's report [24].

As shown in Figure 1, the chickpea yam fermented milk without hydrolyzed enzyme in the four samples had higher polysaccharide content than those of enzymatically fermented chickpea yam milk. This condition was due to the enzymatic hydrolysis of polysaccharide molecules, resulting in a decrease in its content. The content of polysaccharides in fermented milk with yam addition was high. This condition was because the total polysaccharide content was increased by polysaccharide contained in yam itself.

3.2. FTIR Analysis. The FTIR spectra of fermented samples at 0, 12, and 24 h are presented in Figure 2. Results showed that the bands at 1019 and 1153 cm^{-1} of four samples fermented for 0 h indicated the O-H absorption peak and the C-O bending vibration, which were the characteristic bands of polysaccharides. Similar results were obtained by Yan [25]. The absorption peak at 1641 cm^{-1} indicated the

FIGURE 1: Polysaccharide contents of four samples at different fermentation times. All the values are expressed as mean $\pm$ SD ($n=3$). Means with different uppercase letters indicate significant differences of the same sample at the different fermentation time ($p < 0.05$). Means with different lowercase letters indicate a significant difference of four group samples at the same fermentation time ($p < 0.05$). CP: chickpea fermentation milk; CPB: chickpea fermentation milk with enzyme hydrolysis; CPY: chickpea yam fermentation milk; CPBY: chickpea yam fermentation milk with enzyme hydrolysis.

stretching of carbonyl ($C=O$) [26]. The weak band at 2902 cm^{-1} was attributed to the stretching vibration of methylene ($-CH$), and the hydroxyl group was identified by the broad band at 3423 cm^{-1} .

Compared with the samples at 0 h, the four samples fermented for 12 h had the band at 485 cm^{-1} , indicating the deformation vibration of $C-C-C$. The band at 1019 and 1041 cm^{-1} indicated the stretching vibration of $C-O$. The former was the ether group ($C-O-C$) of the pyran ring, whereas the latter was $C-O-H$ [25]. The peaks near 1078 cm^{-1} were ascribed to the ether group, the peak at 1651 cm^{-1} was the carboxyl group, and the band at 2923 cm^{-1} indicated the stretching vibration of saturated $C-H$. The hydroxyl absorption peak was at 3427 cm^{-1} , and the antioxidant activity of polysaccharides was connected with the number of hydroxyl groups [27].

Compared with the samples at 0 h, the four samples fermented for 24 h contained the peaks at 436 cm^{-1} , indicating the deformation vibration of $C-C-C$. The band at 759 cm^{-1} was ascribed to degraded D-glucose of the polysaccharides in four samples during fermentation, which was consistent with Hong's results [24]. The two absorption peaks observed in the spectra at 929 and 1022 cm^{-1} were the $C-O$ stretching vibration, indicating a β -pyranose ring structure. The region around $1154\text{--}1079\text{ cm}^{-1}$ can be used to infer the position of the ether groups in polysaccharides [28]. The peak near $1660\text{--}1206\text{ cm}^{-1}$ was assigned to the carboxyl group, and the peak at 2924 cm^{-1} was ascribed to the alkyl group. The peak located at 3404 cm^{-1} was the $O-H$ stretching vibration in the associated state. However, all the four

samples had characteristic bands of polysaccharides, which were β -pyranose ring structures.

As shown in Figure 2, the infrared spectrum characteristics of the four samples CP, CPB, CPY, and CPBY fermented at 0, 12, and 24 h were similar. However, the transmittance of some specific wavelength characteristic bands was different.

3.3. Effect of Fermentation on Protein Subunit Composition. SDS-PAGE (Figure 3) of four samples reflected the effect of fermentation treatment on the composition of chickpea milk protein subunits. 7S protein and 11S protein are the main components of chickpea storage proteins. The 7S protein contained three subunits: α , α' , and β . The 11S protein was divided into acidic subunit A and basic subunit B. The 94.3 KDa subunit band of the CPBY sample at 0 h disappeared, and the four groups of samples at 0 h demonstrated an intensification of bands in the range of 20.1–14.3 KDa by comparing Figures 3(a) and 3(b) of the SDS-PAGE profiles of protein. The SDS-PAGE profiles of the CPB sample treated with papain at 0 h illustrated the lightening of 7S α' band.

In accordance with Figure 3(b), the SDS-PAGE profiles of four samples fermented for 24 h showed a band disappearance in the range of 94.3 KDa and the lightening of bands in the range of 44.3–94.3. The bands in the range of 29.0–44.3 KDa of the CPB sample fermented for 24 h lightened. The bands in the range of 44.3–94.3 KDa of the CP, CPY, and CPBY fermented for 24 h lightened, and more small-molecule protein peptides were generated. In particular, the SDS-PAGE profiles of four samples showed an intensification of bands in the range of 29.0–20.1 KDa. These proteins were identical to the polypeptide chains A and B of the 11S protein [29]. The extracellular enzymes produced by lactic acid bacteria fermentation had certain hydrolysis power. The result indicated that the 11S protein of chickpea milk was less prone to hydrolysis than the 7S protein.

3.4. Physical Characteristics of the Samples. The physical characteristics of fermented samples were the vital aspect influencing customers' assessment on the quality of fermented chickpea milk. As shown in Table 1, a difference was observed in terms of firmness among the four samples, revealing that $CP > CPY > CPB > CPBY$. Some previous reports found correlations between the structure of chickpea globulin and the gel structure, where chickpea globulin was relatively compact and had weak flexibility, thereby forming a hard and brittle gel structure [30]. Therefore, the firmness of the CP sample was the highest. The firmness of CPB and CPBY samples after enzymatic hydrolysis and yam milk addition decreased, and the consistency increased. In accordance with the viscosity index, the viscosity of the CP sample was low ($p < 0.05$), and no significant difference was found in the viscosity of CPB, CPY, and CPBY samples ($p > 0.05$). The cohesion of the four samples was not significantly different ($p > 0.05$). The addition of yam milk and protease promoted the proliferation of lactic acid bacteria in

FIGURE 2: FTIR spectrum analysis of polysaccharides of four samples at 0 h (a), 12 h (b), and 24 h (c). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

FIGURE 3: SDS-PAGE diagram of four samples at 0 h (a) and 24 h (b). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

TABLE 1: The TPA of four samples of fermented milk.

Samples	Firmness (g)	Consistency (g-s)	Cohesion	Viscosity index (g-s)
CP	28.34 ± 0.06 ^c	425.01 ± 1.89 ^a	0.52 ± 0.05 ^a	19.41 ± 0.47 ^a
CPB	27.13 ± 0.02 ^b	457.32 ± 4.13 ^b	0.53 ± 0.07 ^a	21.6 ± 1.35 ^b
CPY	27.58 ± 0.03 ^b	461.86 ± 2.37 ^b	0.55 ± 0.08 ^a	21.8 ± 0.26 ^b
CPBY	26.67 ± 0.01 ^a	488.49 ± 3.52 ^c	0.56 ± 0.03 ^a	22.35 ± 0.71 ^b

All the values are expressed as mean ± SD ($n = 3$). Different superscript lowercase letters in the same column indicate significant differences ($p < 0.05$). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

fermented chickpea milk to produce hydrolase for degrading polysaccharides in fermented milk. This condition affected its viscosity [7] and resulted in delicate and smooth fermented chickpea milk.

As an important visual indicator, color plays a key role in influencing consumers' impression on the quality of plant-based fermented milk products. The results in Table 2 revealed that some color variation trends of fermented chickpea milk samples can be concluded after fermentation for 24 h. Among the four samples, the L^* of CPY was the highest (86.90), and the L^* of CP was the lowest (83.05) ($p < 0.05$). The a^* values of CPB and CPBY samples with enzymatic hydrolysis decreased (a^* was -3.26 and -3.23 , respectively). This condition represented that the fermented chickpea milk seemed to be brighter and greener after enzymatic hydrolysis. Previous studies on soy yogurt with enzymatic hydrolysis reported similar results [31]. The b^* value of the CPBY sample was the lowest (9.08). However, a small difference was observed in the CPY sample (9.12). A significant difference was observed for yellowness (b^*) between the CPB and CPY samples ($p < 0.05$). On the whole, moderate enzymatic hydrolysis had no adverse effect on the color of the sample, and the L^* of the sample with yam milk addition becomes bright. Hence, enzymatic hydrolysis and yam milk addition can be used for the processing of fermented chickpea milk.

3.5. Analysis of Antioxidant Activity

3.5.1. DPPH Radical Scavenging Rate Analysis. As a classic method for evaluating the free radical scavenging activity, the DPPH radical scavenging has been widely adopted [32]. As shown in Figure 4, in the fermentation progress, The DPPH radical scavenging rate of the four samples increased with the extension of fermentation time, and the increase in DPPH radical scavenging rates decreased after 12 h. As shown in Figure 4, some differences in DPPH were observed after 24 h fermentation among the four samples. A significant increase ($p < 0.05$) in DPPH radical scavenging rates was observed in the CPBY sample with enzymatic hydrolysis and yam milk addition (reaching 73.6%), followed by CPY. The CP sample had the lowest DPPH radical scavenging rate. The results of DPPH radical scavenging activity clearly revealed that enzymatic hydrolysis and yam milk addition facilitated to enhance the antioxidant activity of the CPBY sample. This condition was because the polyphenols and other active ingredients contained in yam were released during the fermentation process. This condition can be

TABLE 2: The color features of four samples of fermented milk.

Samples	Color characteristic value		
	L^*	a^*	b^*
CP	83.05 ± 0.08 ^a	-2.81 ± 0.05 ^a	10.63 ± 0.12 ^b
CPB	85.36 ± 0.12 ^b	-3.26 ± 0.18 ^b	10.27 ± 0.07 ^b
CPY	86.90 ± 0.26 ^c	-2.72 ± 0.03 ^a	9.12 ± 0.35 ^a
CPBY	86.52 ± 0.53 ^c	-3.23 ± 0.06 ^b	9.08 ± 0.16 ^a

All the values are expressed as mean ± SD ($n = 3$). Different superscript lowercase letters in the same column indicate significant differences ($p < 0.05$). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

helpful to increase the antioxidant capacity of chickpea milk. This finding was consistent with the study of Lee [33].

3.5.2. Analysis of ABTS Free Radical Scavenging Ability.

As shown in Figure 5, the change trend of the ABTS radical scavenging rate was similar to that of the DPPH radical scavenging rate. Compared with the counterpart of samples at 0 h, the ABTS radical scavenging rates of all samples gradually increased during 24 h fermentation and reached the highest at 12 h. Among the four samples, the ABTS radical scavenging rate of the CPB sample with enzymatic hydrolysis was higher than that of the CP sample without enzymolysis. This finding indicated that small-molecule peptides may be released in chickpea milk during proteolysis. This condition can be helpful to enhance its antioxidant capability. The CPBY sample with yam milk addition had the highest ABTS radical scavenging rate after 12 h fermentation, reaching 73.89% ($p < 0.05$). It can be speculated that the polysaccharide of yam and protein of chickpea emerged as the polysaccharide-protein conjugate, which contained crevasses, and hydrophobic cavities increased the antioxidant activities [34].

3.5.3. FRAP Antioxidant Capacity Analysis.

As shown in Figure 6, a significant increase in FRAP antioxidant capacity of four samples was observed in the chickpea milk during 24 h fermentation ($p < 0.05$). However, the FRAP antioxidant capacity increased slightly after fermentation for 12 h. The order of the FRAP antioxidant capacity of four samples was CPBY > CPY > CPB > CP. The CPBY sample with enzymatic hydrolysis had the highest FRAP antioxidant capacity after 24 h fermentation, reaching 398.86 mmol Trolox/mL.

FIGURE 4: DPPH free radical scavenging rate of four samples at different fermentation times. All the values are expressed as mean $\pm$ SD ($n=3$). Means with different lowercase letters indicate a significant difference of four group samples at the same fermentation time ($p < 0.05$). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

FIGURE 5: ABTS+ free radical scavenging rate of four samples at different fermentation times. All the values are expressed as mean $\pm$ SD ($n=3$). Means with different lowercase letters indicate a significant difference of four group samples at the same fermentation time ($p < 0.05$). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

We hypothesized that the enhancement of the antioxidant capacity of chickpea fermented milk might be related to the change in bioactive ingredients, such as polysaccharides, or the conversion of bioactive compounds, such as protein,

during the fermentation process. As mentioned above, the changes in polypeptide content caused by protease hydrolysis during fermentation lead to the release of antioxidant materials. The research of Sung-Mee Lim et al. [35] showed

FIGURE 6: FRAP antioxidant capacity of four samples at different fermentation times. All the values are expressed as mean $\pm$ SD ($n = 3$). Means with different lowercase letters indicate a significant difference of four group samples at the same fermentation time ($p < 0.05$). CP, chickpea fermentation milk; CPB, chickpea fermentation milk with enzyme hydrolysis; CPY, chickpea yam fermentation milk; CPBY, chickpea yam fermentation milk with enzyme hydrolysis.

that the proteins and peptides in chickpea milk are positively correlated with free radical scavenging activity. Studies showed that the biologically active peptides released through the fermentation process can increase the antioxidant activity [20]. In this study, the enhancement of antioxidant capacity was related to the changes in biologically active compounds, such as proteins. Moreover, the antioxidant capacity was highly correlated with proteolytic activity. External factors such as enzymatic hydrolysis can hydrolyze proteins and produce some hydrophobic amino acids. The antioxidant capacity depended on peptides, amino acids, and other bioactive substances in chickpea milk, such as polysaccharides and polyphenols. This finding implied that lactic acid bacteria fermentation had obvious effects on the antioxidant bioactivity of chickpea milk. This condition was probably attributed to the alteration of its physicochemical characteristics.

4. Conclusion

In this study, four groups of chickpea milk were prepared through fermentation with lactic acid bacteria for quality and function improvement. The results indicated that the polysaccharide content of four samples declined during the 24 h fermentation process. The infrared spectrums were similar, but the transmittances of some characteristic bands were different. The SDS-PAGE profiles of four samples fermented for 24 h showed a band disappearance in the

range of 94.3 kDa and generation of more small-molecule peptides, revealing the protein degradation during fermentation. The moderate enzymatic hydrolysis had no adverse effect on the texture and color of the samples. A significant increase in DPPH, ABTS radical scavenging rate, and FRAP value was observed in the 0–12 h fermentation, especially the CPBY sample, reaching 73.60%, 73.89%, and 398.86 mmol Trolox/mL, respectively. Accordingly, lactic acid bacteria fermentation can be developed to improve the physicochemical properties of chickpea milk and enhance its antioxidant activity.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that have no conflicts of interest.

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