J Food Sci Technol (December 2018) 55(12):4782–4791
https://doi.org/10.1007/s13197-018-3411-8

ORIGINAL ARTICLE

Phenolic profile, free amino acids composition and antioxidant potential of dried longan fermented by lactic acid bacteria

Sher Ali Khan[1] · Lei Liu[1] · Ting Lai[1] · Ruifen Zhang[1] · Zhencheng Wei[1] · Juan Xiao[1] · Yuanyuan Deng[1] · Mingwei Zhang[1]

Revised: 29 August 2018 / Accepted: 4 September 2018 / Published online: 21 September 2018
© Association of Food Scientists & Technologists (India) 2018

Abstract In this study, dried longan pulp (DLP) was subjected to fermentation using selected strains of lactic acid bacteria (*Lactobacillus plantarum* subsp. *Plantarum* and *Leuconostoc mesenteroides*). We then studied changes in the free and bound phytochemical profile, antioxidant activity, free amino acid, and organic acid composition. Fermentation exhibited a 17.4% and 5.7% increase in the amount of free and total phenolic contents of DLP. Phenolic composition determined by HPLC revealed significant changes due to fermentation that were primarily in the contents of gallic acid, vanillic acid, 4-methylcatechol and *p*-coumaric acid, resulting in a 37.9% and 25.7% increase in free gallic acid and 4-methylcatechol, respectively. Fermentation was also found to enhance the ferric reducing antioxidant power of both free and total and the oxygen radical absorbance capacity of free phenolic fraction by 18.3%, 11.8%, and 37.4%, respectively. In addition, fermentation was observed to reduce the contents of free amino acids with bitter taste (phenylalanine, tyrosine and leucine), and increase amino acids (taurine, aspartic acid, cysteine, cysteine thiazoline and γ-amino-butyric acid) having antioxidant potential. Therefore, this study provides basis for the production of fermented longan-based functional products with improved antioxidant activity.

Keywords Lactic fermentation · Longan · Phenolic composition · Antioxidant activity · Free amino acid · Organic acid

Sher Ali Khan and Lei Liu are contributed equally to this work.

✉ Mingwei Zhang
mwzhh@vip.tom.com

[1] Sericultural & Agri-Food Research Institute Guangdong Academy of Agricultural Sciences/Key Laboratory of Functional Foods, Ministry of Agriculture/Guangdong Key Laboratory of Agricultural Products Processing, Guangzhou 510610, People's Republic of China

Introduction

Longan (*Dimocarpus longan* Lour.) is a fruit that is widely cultivated in China, Thailand, Vietnam, and other parts of the world that have tropical and subtropical climates. The longan pulp possesses numerous nutritional and functional components, including carbohydrates, protein, fiber, fat, vitamin C, amino acids, minerals, polyphenols, and volatile compounds. In traditional Chinese medicines, longan is utilized to improve immunity, cure insomnia, neural pain and swelling, promote blood metabolism, and to enhance learning and memory (Li et al. 2015). However, it has been demonstrated that the blood glucose response of longan in healthy adults is the second highest following the banana, in comparison with glucose as a reference (Yusof et al. 2005). The high quantity of sugars present in longan arils (sucrose 38.55–150.69 mg/g, glucose 15.08–53.95 mg/g and fructose 13.6–49.23 mg/g) (Shi et al. 2015) may hinder the consumption of longan in both diabetic and obese individuals. A processing technique that is used to help minimize the sugar content of longan pulp, and improve its nutritional and quality attributes would be of significant interest to the food industry. Fermentation represents an ancient process that not only preserves foods but also reduces the excessive sugar content of the substrates. Fermentation has been shown to improve the nutritional value of food, enhance digestibility, and eliminate antinutrients.

Lactic acid bacteria (LAB) serve as industrially important microorganisms for the fermentation of food products of plant origins due to their GRAS (Generally Recognized

as Safe) status. Through the process of fermentation, LAB not only produce lactic acid, but also help to preserve nutrients and vitamins. LAB are typically utilized as starter cultures to convert sugars into lactic acid and other end products, which gives the typical flavor to fermented products (Leroy and De Vuyst 2004). In our previous study, a significant decline of reducing sugar was observed during the optimization of LAB fermentation for longan pulp (Lai et al. 2016). In addition, the health benefits that were claimed to be associated with longan consumption were primarily linked to its phytochemical constituents. Previously, our group demonstrated that longan pulp contains a high amount of phenolics (298.63 mg of GAE/100 g DW) and flavonoids (26.43 mg of CE/100 g DW), consisting of gallic acid, protocatechuic acid, vanillic acid, syringic acid, (-)-epicatechin, 4-methycatechol, *p*-coumaric acid, and isoquercitrin (Lai et al. 2015). These compounds exist in the forms of both free phenolics and bound phenolics covalently attached to cell wall material. During LAB fermentation, phenolics undergo profile changes that not only influence the antioxidant activity of fermented products, but also affect their sensory characteristics. Hervert-Hernandez and Goni (2011) demonstrated that numerous LAB strains have the potential to liberate dietary phenolic compounds that are covalently bound to fiber. Hole et al. (2012) demonstrated that LAB exhibiting feruloyl esterase activity functioned to increase the free phenolic content of barley and oat groat during fermentation. Recent studies on fruit fermentation have focused primarily on the physicochemical properties of fermented products, including evaluating the formation of the color and aroma compound in fermented substrate for the production of longan based juice, vinegar, and wine (Huang et al. 2009; Trinh et al. 2012; Chen et al. 2013). However, little attention has been paid to the phenolics of longan pulp throughout LAB fermentation. It is clear that complex biochemical reactions take place during LAB fermentation. Therefore, it is necessary that we understand the transformation of free and bound phenolics and their antioxidant capacity throughout the fermentation process. In addition, complex biochemical reactions take place during fermentation which necessitates examining the free amino acid profile of fermented products as a crucial element related to its nutritional value. Previous work demonstrated that free amino acids are the precursors of aromatic substances, including alcohols, aldehydes, and ketonic acids, and could affect the aromatic profile of the product during fermentation, while some amino acids function as antioxidants (Ardö 2006; Cerrillo et al. 2015).

Therefore, the aims of the present study were to investigate the effect of lactic acid bacteria (*L. plantarum* subsp. *Plantarum* and *L. mesenteroides*) fermentation on (1) changes in free and bound phytochemical (phenolics and flavonoids) content and their antioxidant activities, (2) compositional changes of individual phenolic compounds in both the free and bound forms and (3) changes in the composition of free amino acids and organic acids in DLP.

Methods and materials

Chemicals and reagents

MRS (*de man*, *Rogosa*, *Sharpe*) medium was procured from Guangdong Haikou Microbiology Biotech Inc., Haikou, China. Methanol (MeOH), acetone, hexane, ethyl acetate, hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), and sodium hydroxide (NaOH) of analytical grade were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). Folin–Ciocalteu reagent, acetonitrile (chromatographic grade), glacial acetic acid (HAC, chromatographic grade), and catechin hydrate, chlorogenic acid, *p*-coumaric acid, protocatechuic acid, vanillic acid, syringic acid, quercetin, isoquercetin, 4-methylcatechol and (+)-catechin were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Aluminum chloride ($AlCl_3 \cdot 6H_2O$, analytical grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Gallic acid was purchased from ICN Biomedicals, Inc. (Aurora, OH, USA). All other reagents used were of analytical grade.

Fermentation of dried longan pulp

Dried longan fruits were provided by Pomology Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, P. R. China. The substrate was prepared by subjecting the dried longan fruit to baking at 55 °C, containing 15% moisture. The hull and seeds were then removed and the pulp obtained was stored at − 18 °C. Bacterial stains *Lactobacillus plantarum* subsp. *Plantarum GIM 1.380* and *Leuconstoc mesenteroides GIM 1.473* were obtained from Centre for Microbial Culture Collection, Guangdong Province P. R. China. The starter culture inocula were prepared by subculturing the bacterial strains in MRS medium at 30 °C static incubation for 24 h. After third transfer of MRS broth, the activated strains were used for fermentation. Before inoculation, a certain amount of substrate was mixed with water (1:1.5, w/v) and thoroughly blended using food blender. A 100 g of the slurry was transferred to a 250 mL conical flask and autoclaved at 121 °C for 20 min. The starter culture of *L. plantarum* subsp. *plantarum* and *L. mesenteroides* mixed together (1:1) constituting 8.0–9.0 Lg CFU/mL were centrifuged at 6000 rpm for 10 min. The supernatant was discarded and cells were re-suspended in 1 mL sterile solution of 0.9%

NaCl. The suspension was then added to the flask containing the substrate and incubated at 30 °C for 53 h. The fermented sample was stored at − 20 °C till further analysis.

Enumeration of bacteria, pH, titratable acidity (TA) and organic acids

Enumeration of microbes was performed by pour plate method. Samples for determination of pH, TA and organic acids were directly drawn from the fermentation broth. A 10 g sample was homogenized in 70 mL deionized water and filtered through Watman No.42 paper. Measurement of pH was performed on a Metrohm744 pH meter with a glass electrode (Metrohm Co. Ltd., Switzerland), calibrated for every measurement at pH 4.00 and 6.86. For TA, an aliquot of 25 mL was titrated against 0.01 N NaOH to the end point of pH 8.1. The results were expressed as mEq of sodium hydroxide per Kg of dry longan pulp.

Analysis of organic acids were performed on Agilent 1200 high performance liquid chromatography (HPLC) equipped with a diode array detector and ZORBAX SB-C18 column (4.6 mm × 250 mm, 5 µm). Separation was carried at a flow rate of 0.8 mL/min with column temperature 30 °C using 0.1 mol/L $(NH_4)_2HPO_4$ aqueous solution (pH 2.7) as the elution. The content of organic acids were detected at 210 nm and quantified on the basis of external calibration peak area. The results were expressed as µg per gram DW. All analyses were performed in triplicate.

Extraction of free phenolics

The free phenolic extract of fermented and unfermented longan pulp was obtained by the method of Sun et al. (2002). Briefly, 50 g of longan pulp was mixed with pre cold 80% aqueous acetone (1:2, W/V). The mixture was homogenized at 10,000 rpm for 5 min in an ice-cold condition using Philips blender followed by centrifugation at 5000 rpm for 10 min. The 2 supernatants obtained were concentrated under a vacuum at 45 °C until the filtrate had been evaporated. The concentrated filtrate was reconstituted to a final volume of 50 mL with distilled water and stored at − 40 °C till further analysis.

Extraction of bound phenolics

The bound phenolic extract was obtained according to a previous method (Naczk and Shahidi 1989; Sun et al. 2007). Briefly, the residue left after free phenolic extraction was hydrolyzed by 40 mL of 4 M NaOH solution for 3 h with shaking at room temperature under the protection of nitrogen gas. The hydrolyzed solution was then adjusted to pH 1.0 with 6 M HCl and finally extracted five times with ethyl acetate. All the extracts were pooled together and evaporated at 45 °C. The resulting residue was reconstituted in 10 mL distilled water and stored at −40 °C until analysis.

Total phenolic content

Folin–Ciocalteu (FC) colorimetric method (Dewanto et al. 2002) was adapted to determine the total phenolic content of each extract. Briefly, the FC reagent (0.125 mL) was added to the test tube containing phenolic extract (0.125 mL) and distilled water (0.5 mL). After allowing the mixture to react for 6 min, 7% aqueous sodium carbonate solution (1.25 mL) and distilled water was added to make the total volume up to 3 mL. The mixture was then incubated for 90 min at room temperature and the absorbance was recorded at 760 nm using a Shimadzu UV-1800 spectrometer (Shimadzu Inc., Kyoto, Japan). The total phenolic contents were determined on the basis of standard curve obtained for gallic acid. The results were expressed as mg gallic acid equivalents (GAE) per 100 g dry weight (DW) of sample.

Total flavonoids content

The content of total flavonoids was assessed as described by Dewanto et al. (2002). Briefly, a 250 µL of each fraction or standard solution was mixed in a tube containing 1.25 mL of deionized water and 75 µL aqueous solution of $NaNO_2$ (5% mass fraction). After allowing the mixture to stand for 5 min at room temperature, an aliquot of 150 µL aqueous 10% $AlCl_3 \cdot 6H_2O$ solution was added and kept for 5 min. The solution was then mixed with 0.5 mL of aqueous 1 mol/L NaOH and diluted to 3.0 mL with deionized water. The absorption was read at 510 nm against blank. The flavonoids content was determined using (+)-catechin as standard and results were expressed as milligrammes of (+)-catechin equivalents per 100 g of DLP.

Phenolic composition

Phenolic composition were determined by Agilent 1200 HPLC system (Waldbronn, Germany) equipped with an Agilent 1200 series VWD detector and autosampler, using a 250 × 4.6 mm id, 5 µm Agilent Zorbox SB-C18 column (Palo Alto, CA, USA). The elution was performed using A: 0.4% glacial acetic acid and B: acetonitrile with gradient 0–40 min, solution B 5–25%; 40–45 min, solution B 25–35%; 45–50 min, solution B 35–50% at a flow rate 1 mL/min and column temp 30 °C. The injection volume was set to 20 µL, and total run time was 50 min. Each sample was filtered through a 0.25 µm membrane filter

(Millipore, Billerica, MA, USA) before run. The compounds were detected at a wavelength of 280 nm. Identification of individual phenolics was made primarily on comparison of their retention times with the known authentic standards. A percent recovery of 94% was achieved during analysis.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed by the method of Benzie and Strain (1996). Briefly, sample of each phenolic extract was first diluted with deionized water. The working solution of FRAP was prepared by adding 10 volume of 300 mM acetate buffer of pH 3.6 to 1 volume of 10 mM TPTZ in 40 mM HCl and 1 volume of 20 mM $FeCl_3 \cdot 6H_2O$. An aliquot of 3 mL working solution of FRAP was warmed to 37 °C. Finally 100 µL of sample and 300 µL of deionized water were added to the FRAP solution and absorbance was noted at 593 nm against blank after 4 min. The FRAP value of each extract was expressed as micromoles of Fe^{2+} equivalent per 100 g of sample using the calibration curve of Fe^{2+}. The linear range of calibration curve was 0.5–8.0 mM with r = 0.99.

Oxygen radical scavenging capacity (ORAC) assay

For determining the antioxidant activity by ORAC, a modified method of our lab was used (Zhang et al. 2010). Briefly, the dilutions of phenolic extract were prepared with 75 mM phosphate buffer (pH 7.4). Finally the reaction mixture had 20 µL of extract or 20 µL Trolox standard (range: 6.25–50 µM) and 200 µL fluorescein (final concentration 0.96 µM). The mixture was incubated at 37 °C for 20 min and 20 µL ABAP solution was quickly added to each well with the help of a multichannel pipettor. The fluorescence intensity was recorded on a Fluoroskan Ascent FL plate-reader (Thermo Labsystems, Franklin, MA, USA) at 485 nm for excitation and 538 nm for emission for 35 cycles every 4.5 min. The ORAC value was expressed as micromole Trolox equivalents per gram dried longan pulp.

Free amino acids analysis

The longan pulp was centrifuged at 4800 rpm for 10 min. The supernatant obtained was sonicated for 15 min and then deproteinized by an equal volume of 6% sulfo-salicylic acid solution. The final volume of extracted samples was 50 mL. All samples were filtered through 50 mm disposable syringe filters prior to free amino acid determination and derivatization. The analyses were carried out by Amino Acid Analyzer (L-8900 System: Hitachi Inc.) equipped with a visible detector, analytical 2622# (4.6 mm × 60 mm) and guard 2650# (4.6 mm × 40 mm) columns. The auto-sampler was used for the inline-derivatization by Ninhydrin (NIN) post-column derivatization immediately after sample injection onto the columns. The NIN-derivatized amino acids were monitored at 570 nm and at 440 nm. The external standards of each individual amino acid were used for identification and quantification. The value of each individual free amino acid was expressed as µg/g of the DLP.

Statistical analysis

Data were statistically analyzed by SPSS statistical package version 16.0 (SPSS Inc. Chicago, IL, USA). Significant differences between sample means were determined by using one way ANOVA followed by Duncan post hoc test. Results were expressed as mean ± SD. Significant differences were considered at $p < 0.05$ and $p < 0.01$. All analyses were performed in triplicate.

Results and discussion

Effect of fermentation on bacterial growth, pH, TA and organic acids

The growth kinetics of *L. plantarum* sub species *Plantarum* and *L. mesenteroides* throughout fermentation of longan pulp at 30 °C for 96 h are depicted in Fig. 1a. A gradual increase was observed in the growth of bacteria after 53 h of fermentation which indicated the ability of the selected strains to achieve optimal growth and adapt to the fermentation condition. However, a slight decline in the bacterial population was observed between 53 and 96 h of fermentation ($p < 0.05$). The growth kinetics of the microbial population revealed that the mixed culture of bacteria reached 9.85 Lg CFU/mL in 53 h. This demonstrates that both strains are capable of growing fast in longan pulp, causing a substantial decrease in pH. The effect of fermentation time on pH is depicted in Fig. 1b. The initial pH of longan pulp (unfermented substrate) was found to be slightly acidic (pH 5.4). A significant decrease in pH was observed immediately after 12 h of fermentation ($p < 0.05$), and gradually it reduced to pH 3.1 after 53 h of fermentation with no significant change observed in pH by the end of the fermentation time ($p > 0.05$). Numerous LAB cultures were found to be capable of rapidly utilizing various juicy substrates, reaching almost 5×10^9 CFU/mL and reducing the pH up to 3 or below (Yoon et al. 2005; Gupta et al. 2011).

The trend observed for the pH corresponds well with that observed for TA, as shown in Fig. 1b. TA was found to be remarkably increased after 53 h of fermentation

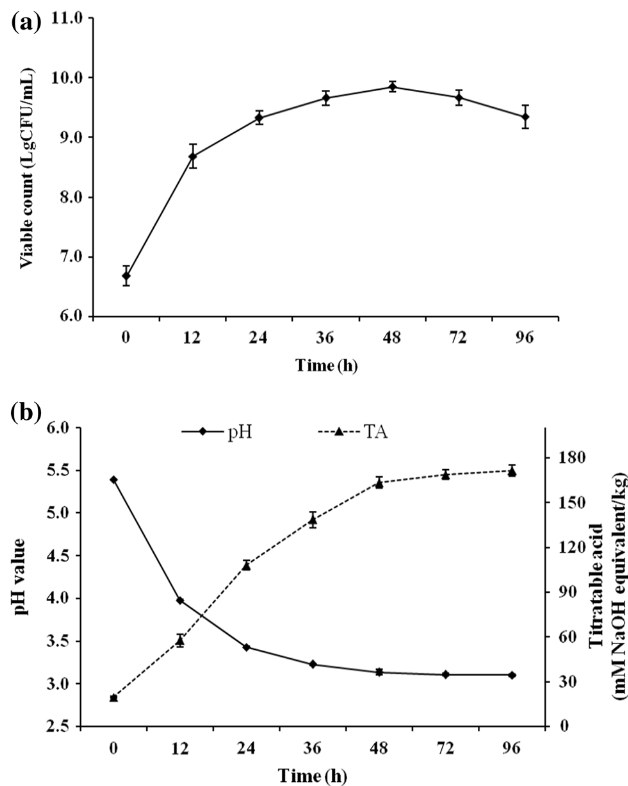

Fig. 1 Changes in the viability count of bacteria (*L. plantarum* subsp. *Plantarum* and *L. mesenteroides*) **a** and alterations in the pH and titratable acidity **b** of dried longan pulp during fermentation by lactic acid bacteria

($p < 0.05$) and then gradually increased with fermentation time ($p < 0.05$). TA represents a superior indicator of acidity compared to pH, and exhibited an increasing trend. The observed decrease in pH and increase in TA was found to be primarily due to the organic acids that were produced during the fermentation. A large variation was also observed in the content of organic acids that were determined prior to and after fermentation (Table 1). The total organic acid content in non-fermented longan pulp was

Table 1 Organic acids composition (mg/g DW) of dried longan pulp before and after fermentation by lactic acid bacteria

Organic acid	Non-fermented	Fermented
Oxalic acid	3.60 ± 0.39	1.93 ± 0.27*
Malic acid	4.67 ± 0.91	1.28 ± 0.28*
Lactic acid	2.81 ± 0.07	23.37 ± 1.86**
Acetic acid	3.34 ± 0.41	20.06 ± 1.64**
Citric acid	0.18 ± 0.01	0.16 ± 0.04
Succinic acid	6.12 ± 1.10	12.82 ± 1.27*
Total organic acid	20.72	59.62**

The data are given as mean ± SD (n = 3)

Significant differences are shown at $p < 0.05$* and $p < 0.01$**

determined to be 20.72 mg/g DW, while in fermented samples it was significantly ($p < 0.05$) increased to 59.62 mg/g DW. Lactic acid, acetic acid, and succinic acid exhibited remarkable increase after fermentation, whereas the content of both oxalic acid and malic acid were observed to decrease significantly ($p < 0.05$). Lactic and acetic acids were the primary metabolites produced by LAB during fermentation. However, when more sugar is available in the substrate, as in case of DLP, the bacteria have also been shown to generate larger quantities of acetic and succinic acids (Song and Lee 2006). The changes observed in the other organic acids could be due to the dilution of the substrate during the fermentation process.

Effect of fermentation on free, bound and total phenolics

Figure 2a represents variations in the free, bound, and total phenolics in DLP after fermentation. The free, bound, and total phenolic contents in non-fermented DLP were determined to be 122.56 ± 2.49, 24.40 ± 0.35, and 146.97 ± 2.33 mg GAE/100 g DW, respectively. After

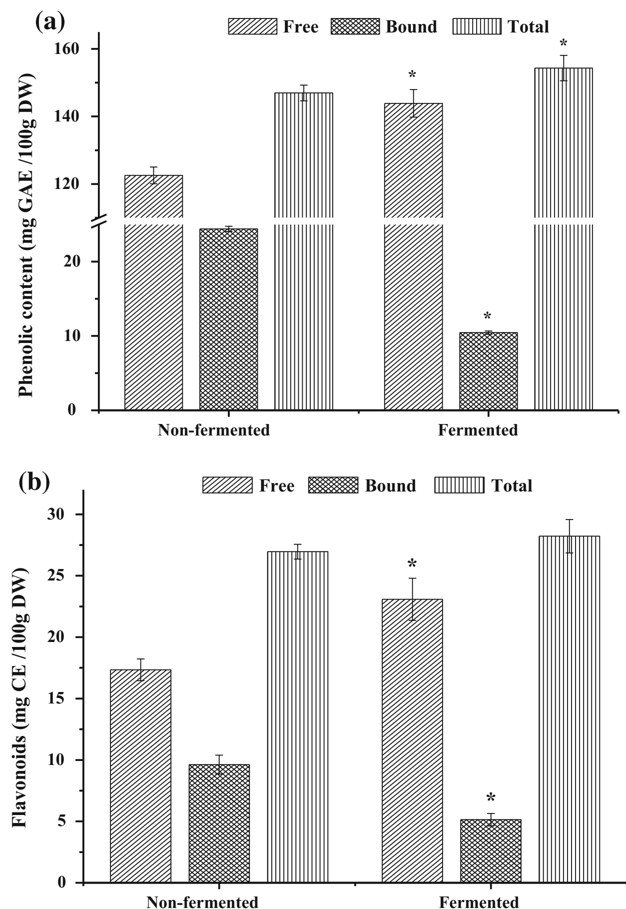

Fig. 2 Free, bound and total phenolic **a** and flavonoid **b** contents of dried longan pulp before and after fermentation by lactic acid bacteria

fermentation, the free and total phenolic contents were observed to be increased by 17.4% (143.87 mg GAE/100 g DW) and 5.7% (154.33 mg GAE/100 g DW), respectively ($p < 0.05$), while bound phenolics were observed to be decreased by 57.1% (10.45 mg GAE/100 g DW) ($p < 0.05$). The contribution of the free and bound forms to the total phenolics in the non-fermented sample was 83.4% and 16.6%, respectively. Significant variations were observed in the free and bound phenolic contents, with a net increase observed in the free phenolic content during fermentation (Fig. 2a) ($p < 0.05$). The highest total phenolic content was observed in a fermented sample with a 93.3% contribution of free phenolics. It was reported previously that feruloyl esterases from LAB strains are able to release the bound phenolics from fibrous plant materials. Most notably, *L. plantarum* was shown to release bound phenolics from cereals (Hole et al. 2012). Yu et al. (2015) also reported an increase of total phenolic contents from 130 to 160 mg GAE/kg in *Prunus mume* fruits throughout LAB fermentation.

Effect of fermentation on free, bound and total flavonoids

The amount of free, bound and total flavonoids in non-fermented and fermented DLP are shown in Fig. 2b. Fermentation was observed to cause significant changes to the free and bound flavonoids content of longan pulp ($p < 0.05$). However, it was not found to change the amount of total flavonoids ($p > 0.05$). The contents of free, bound, and total flavonoids in non-fermented samples were determined to be 17.34, 9.62, and 26.96 mg CE/100 g DW, respectively. Fermentation was observed to increase the free flavonoids content by 33.1% and reduce the bound flavonoids by 46.5% ($p < 0.05$). The fractions of free and bound flavonoids to the total flavonoid content in non-fermented DLP were determined to be 64.3 and 35.7%, respectively; while these fractions were determined to be 81.8 and 18.2%, respectively, in fermented DLP ($p < 0.05$). Dulf et al. (2016) observed a significant increase of 30% in the total phenolic content and a similar increase in the total flavonoid content of plum fruit byproduct during solid state fermentation carried out by filamentous fungi.

Effect of fermentation on phenolic composition

The composition of phenolic compounds in the free and bound forms of DLP before and after fermentation is depicted in Table 2. Analysis revealed that the composition of monomeric phenolic compounds in the free and bound forms was not changed after fermentation, with the exception of vanillic acid, whereas their contents in each form were significantly changed ($p < 0.05$). The composition further revealed that gallic acid and 4-methylcatechol were identified in both the free and bound fractions, while syringic acid and (-)-epicatechin were identified only in the free fraction, and protocatechuic acid, vanillic acid, *p*-coumaric acid, and isoquercitrin were found to exist only in the bound fraction. Interestingly, vanillic acid was observed in the free fraction after fermentation. Fermentation was found to cause significant changes to the content of gallic acid, vanillic acid, 4-methylcatechol, and *p*-coumaric acid in DLP. The free gallic acid content increased from 626.2 to 1009.1 μg/g ($p < 0.05$), while the bound gallic acid decreased from 661.3 to 105.4 μg/g after fermentation ($p < 0.05$). The percent contribution of free to total gallic acid content prior to and after fermentation was determined to be 48.6% and 90.5%, respectively. The non-fermented DLP was observed to contain a total of 3637.8 μg/g of vanillic acid in the bound form, which was changed to 2691.5 μg/g as free and 768.0 μg/g as bound vanillic acid after fermentation with a percent contribution of 88.2% and 22.5% to the total vanillic acid content in DLP, respectively. The content of 4-methylcatechol in free form increase from 1116.2 to 1502.2 μg/g after fermentation, while in bound form it decreased from 583.4 $\pm$ 65.7 to 149.4 $\pm$ 38.1 μg/g ($p < 0.05$). The contribution of free 4-methylcatechol to the total was from 86.4 to 91.9%. The amount of bound *p*-coumaric acid was found to be reduced from 4.4 to 2.1 μg/g after fermentation ($p < 0.05$).

Mehdizadeh et al. (2015) also reported variations in the phenolics composition of rambutan seed after fermentation. These changes could be attributed to the biological factors that were involved in the fermentation process, including alterations in pH, temperature, and enzymatic activity, which could affect some of the phenolic compounds. It was previously shown that LAB metabolizes phenolic compounds via strain specific activities of decarboxylase and/or reductase enzymes. It was assumed that the synthesis of these inducible enzymes by selected strains occurred due to specific chemical stress response to overcome phenolic acids toxicity (Rodríguez et al. 2009). However, the precise mechanism that is involved in protocatechuic decarboxylation and the bioconversion of hydroxycinnamic acids to the corresponding dihydro-derivatives by LAB remains unclear and is yet to be fully characterized.

Effect of fermentation on the antioxidant activity

The antioxidant activities of free, bound, and total phenolics in DLP before and after fermentation by FRAP assay are shown in Fig. 3a. The free and bound FRAP values of non-fermented DLP were determined to be 1681.71 and 245.45 μmol Fe^{2+}/100 g DW, which were changed to 1989.74 and 166.63 μmol Fe^{2+}/100 g DW, respectively,

Table 2 Free, bound and total phenolic compositions (μg/g DW) of dried longan pulp before and after fermentation by lactic acid bacteria

Phenolics		Free	Bound	Total
Gallic acid	Non-fermented	626.2 ± 7.3 (48.6)	661.3 ± 64.6 (51.4)	1287.5 ± 71.9
	Fermented	1009.1 ± 26.5* (90.5)	105.4 ± 5.9* (9.5)	1114.6 ± 32.4*
Protocatechuic acid	Non-fermented	nd	277.5 ± 2.2 (100)	277.5 ± 2.2
	Fermented	nd	251.9 ± 8.4 (100)	251.9 ± 8.4
Vanillic acid	Non-fermented	nd	3637.8 ± 110.2 (100)	3637.8 ± 110.2
	Fermented	768.0 ± 7.0 (22.0)	2691.5 ± 121.5* (88.0)	3459.5 ± 114.5
Syringic acid	Non-fermented	1297.2 ± 20.1 (100)	nd	1297.2 ± 20.1
	Fermented	1205.7 ± 87.4 (100)	nd	1205.7 ± 87.4
(-)-Epicatechin	Non-fermented	1443.4 ± 19.0 (100)	nd	1443.4 ± 19.0
	Fermented	1676.0 ± 113.6 (100)	nd	1676.0 ± 113.6
4-Methylcatechol	Non-fermented	1116.2 ± 39.0 (86.4)	583.4 ± 65.7 (13.6)	1699.6 ± 56.4
	Fermented	1502.2 ± 30.1* (91.9)	149.4 ± 381.1* (8.1)	1632.9 ± 69.4*
p-Coumaric acid	Non-fermented	nd	4.4 ± 0.7 (100)	4.4 ± 0.7
	Fermented	nd	2.1 ± 0.1* (100)	2.1 ± 0.1*
Isoquercitrin	Non-fermented	nd	174.3 ± 17.4 (100)	174.3 ± 17.4
	Fermented	nd	130.7 ± 39.3 (100)	130.7 ± 39.3

The data are given as mean ± SD (n = 3)

nd not detected

Values in parentheses show the percentage contribution of each fraction to the total

Significant differences are shown at $p < 0.05$*

causing an 18.3% increase in the free and a 32.1% decline in the bound antioxidant activity. The fermented sample exhibited a higher FRAP value (2156.37 μmol Fe^{2+}/100 g DW) for total with a 92.3% contribution of the free fraction to the total in comparison to the non-fermented free fraction which only contributed 87.3% to the total (1927.16 μmol Fe^{2+}/100 g DW).

Changes in antioxidant activities of non-fermented and fermented DLP in regard to free, bound, and total ORAC values are shown in Fig. 3b. Fermentation significantly changed both the free and bound ORAC values ($p < 0.05$). The non-fermented free, bound, and total ORAC values were 1058.20, 604.08, and 1662.28 μmol TE/100 g DW, respectively. Fermentation enhanced the antioxidant activity of the free fraction by 37.4% (1454.03 μmol TE/100 g DW) ($p < 0.05$), but did not alter the antioxidant activity of the total (1731.99 μmol TE/100 g DW) ($p > 0.05$) and decreased the antioxidant activity of the bound fraction by 53.9% (277.96 μmol TE/100 g DW) ($p < 0.05$). The free fraction was found to contribute 63.65% to the total ORAC value in non-fermented DLP, while the contribution of free fraction to the total ORAC value was increased to 83.9% after fermentation. However, we observed no significant changes in total ORAC values of the fermented and non-fermented DLP ($p > 0.05$).

FRAP and ORAC values were consistent for both the free and bound phenolic contents of DLP. These results were in agreement with those reported by Guo et al. (2003) and Rangkadilok et al. (2007). The changes in the FRAP and ORAC values of fermented DLP were also found to be consistent with the respective phenolic contents of DLP, indicating that phenolics play a critical role in antioxidant activity (Fig. 2). Previously, it was observed that the amount of bioactive compounds could be modified by the metabolic activity of microbes during fermentation (Katina et al. 2007). However, this was shown to be dependent more on the species of microbes used for fermentation (Razak et al. 2015). In our study, *L. plantarum* subsp. *Plantarum* and *L. mesenteroides* was found to be effective for the enrichment of phenolics and antioxidant activity in longan during fermentation.

Effect of fermentation on free amino acid composition

The free amino acid composition of DLP and its variability during LAB fermentation are depicted in Table 3. We identified a total of 28 different free amino acids and derivatives in DLP. The most abundant free amino acids were determined to be phenylethylamin, alanine, glutamic acid, γ-amino-butyric acid and α-aminobutyric acid in both the fermented and non-fermented samples. The contents of phenylserine, leucine, tyrosine, β-aminoisobutyric acid, and lysine were detected only in non-fermented samples,

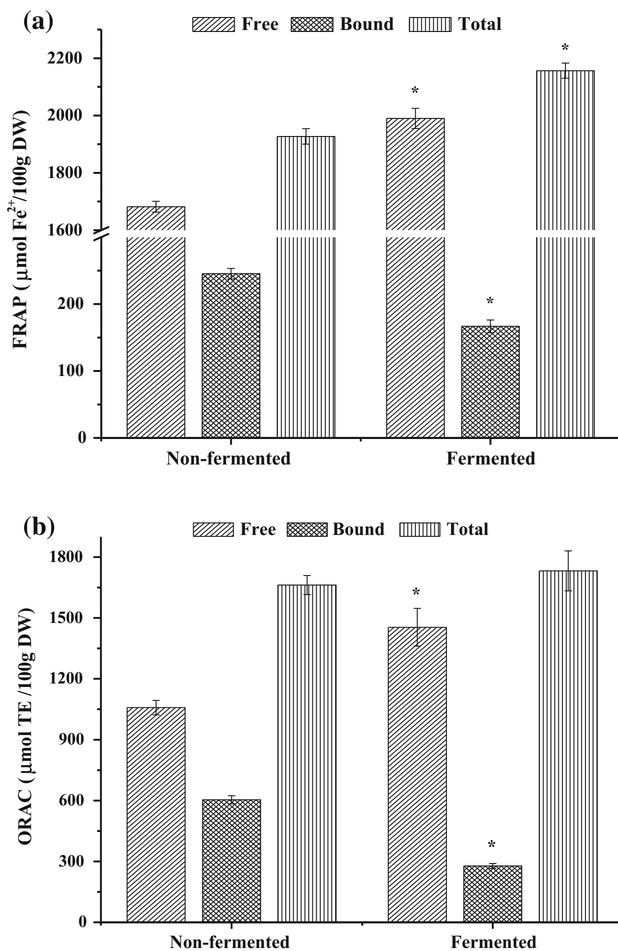

Fig. 3 Free, bound, and total antioxidant activities of dried long pulp as measured by FRAP (**a**) and ORAC (**b**) before and after fermentation by lactic acid bacteria

whereas ornithine was detected only in fermented samples. Fermentation was observed to cause a significant increase in the contents of taurine, aspartic acid, cysteine, cysteine thiazoline, and γ-amino-butyric acid ($p < 0.05$), while the free amino acids phenylethylamin, threonine, serine, α-aminoadipic acid, glycine, valine, isoleucine, phenylalanine, β-alanine, ethanol amin. Tryptophan, histidine, arginine and proline were observed to significantly decrease after fermentation ($p < 0.05$) (Table 3). In this study, we observed a decrease in the total free amino acid content, which was also reported in a previous study regarding fermentation of Pu-erh ripened tea (Zhu et al. 2016). This decrease could be attributed to the consumption of free amino acids during microbial metabolism and/or the existence of complex reactions, such as the Maillard reaction and enzymatic conversion. However, we observed significant variation in regards to different free amino acids throughout fermentation in comparison to their non-fermented counterpart ($p < 0.05$). Interestingly, amino acids characterized for bitter taste, including Phenylalanine were greatly reduced, while Tyrosine and Leucine were not detected in the fermented DLP. In our study, the contents of taurine, aspartic acid, cysteine, cysteine thiazoline, and γ-amino-butyric acid were found to be increased by 95.6%, 9.0%, 35.3%, 504.0%, and 22.2% during fermentation, respectively (Table 3). Studies have shown the antioxidant activity of certain acidic and hydrophobic amino acids in vivo and in vitro, including aspartic acid and glutamic acid (Saiga et al. 2003; Ren et al. 2008). Lee et al. (2010) demonstrated the conversion of glutamic acid to γ-amino-butyric acid by LAB, further revealing the presence of strong antioxidant activity. Cysteine and its residues were also observed to possess antioxidant potential (Elias et al. 2005). In addition, alterations in free amino acids could

Table 3 Free amino acids composition (µg/g DW) of dried longan pulp before and after fermentation by lactic acid bacteria

Free amino acid	Non-fermented	Fermented
Phenylserine	39.50 ± 1.90	nf
Taurine	92.93 ± 2.09	180.25 ± 0.78**
Phenylethylamin	658.75 ± 19.05	578.06 ± 11.64*
Aspartic acid	177.11 ± 4.34	193.33 ± 9.62*
Threonine	13.85 ± 0.22	4.77 ± 0.19**
Serine	42.76 ± 0.74	16.96 ± 0.19*
Glutamic acid	215.05 ± 3.03	171.11 ± 4.06*
α-Aminoadipic acid	15.94 ± 0.26	7.67 ± 0.16*
Glycine	21.20 ± 0.36	16.94 ± 0.01*
Alanine	399.19 ± 5.36	376.54 ± 0.79*
α-Aminobutyric acid	76.47 ± 2.72	63.57 ± 9.72
Valine	39.48 ± 0.62	18.09 ± 0.10*
Cysteine	3.77 ± 0.14	5.10 ± 1.40*
Cysteine thiazoline	1.99 ± 0.38	11.48 ± 0.77**
Isoleucine	35.41 ± 0.62	26.15 ± 0.47*
Leucine	30.48 ± 0.28	nf
Tyrosine	9.69 ± 0.19	nf
Phenylalanine	12.49 ± 0.05	3.91 ± 0.08**
β-Alanine	20.40 ± 0.41	14.56 ± 0.04*
β-Amino-isobutyric acid	7.83 ± 0.27	nf
γ-Amino-butyric acid	130.50 ± 7.16	158.91 ± 10.60*
Ethanol amin	44.89 ± 0.57	29.16 ± 0.18*
Tryptophan	7.66 ± 0.23	3.21 ± 0.16*
Lysine	5.36 ± 0.28	nf
Ornithine	nf	2.34 ± 0.02
Histidine	6.23 ± 0.31	4.98 ± 0.08*
Arginine	28.43 ± 0.79	6.40 ± 0.18**
Proline	82.43 ± 0.81	48.79 ± 0.06*
Total	2219.8	1942.27

The data are given as mean ± SD (n = 3)

nf not found

Significant differences are shown at $p < 0.05$* and $p < 0.01$**

affect the flavor and aroma of longan pulp, as alanine and arginine have been characterized to have a sweet and monosodium-like taste (Zhu et al. 2016). The phenomenal effect of the altered free amino acids profile on antioxidant activity, flavor, and aroma should be the subject of further studies.

Conclusion

This study demonstrated the effect of lactic acid bacteria fermentation on both free and bound phytochemicals, as well as on the antioxidant activity of DLP. Fermentation was observed to increase the free phenolic content, as well as corresponded antioxidant activity. The content and composition of individual phenolic compounds identified in this study confirmed a strong effect of fermentation on gallic acid, vanillic acid, 4-methylcatechol, and *p*-coumaric acid, which showed a significant increase in the contents of gallic acid and 4-methylcatechol in the free phenolic extract. In addition, fermentation was observed to result in reduced total free amino acids, specifically the content of those characterized for bitter taste (phenylalanine, tyrosine, and leucine), while it also resulted in increased contents of taurine, aspartic acid, cysteine, cysteine thiazoline, and γ-amino-butyric acid which are thought to possess antioxidant potential. Therefore, fermentation carried out by specific strains of LAB species could be utilized for the production of functional products of enhanced antioxidant potential and desirable attributes.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 31301459), and Guangdong Provincial Science and Technology Project (Grant Nos. 2017B090907022 and 2017B020232002), and Guangzhou Municipal Project of Science and Technology (Grant Nos. 201704020039, 201803020014 and 201803010079).

References

Ardö Y (2006) Flavour formation by amino acid catabolism. Biotechnol Adv 24:238–242

Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70e76

Cerrillo I, Fernández-Pachón M, Collado-González J, Escudero-López B, Berná G, Herrero-Martín G, Martín F, Ferreres F, Gil-Izquierdo A (2015) Effect of fermentation and subsequent pasteurization processes on amino acids composition of orange juice. Plant Food Hum Nutr 70:153–159

Chen C, Wu Y, Lo D, Wu M (2013) Physicochemical property changes during the fermentation of longan (*Dimocarpus longan*) mead and its aroma composition using multiple yeast inoculations. J Inst Brew 119:303–308

Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50:3010–3014

Dulf FV, Vodnar DC, Socaciu C (2016) Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (*Prunus domestica* L.) by-products. Food Chem 209:27–36

Elias RJ, McClements DJ, Decker EA (2005) Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase beta-lactoglobulin in oil-in-water emulsions. J Agric Food Chem 53:10248–10253

Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y (2003) Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res 23:1719–1726

Gupta S, Abu-Ghannam N, Scannell AG (2011) Growth and kinetics of *Lactobacillus plantarum* in the fermentation of edible Irish brown seaweeds. Food Bioprod Process 89:346–355

Hervert-Hernandez D, Goni I (2011) Dietary polyphenols and human gut microbiota: a review. Food Rev Int 27:154–169

Hole AS, Rud I, Grimmer S, Sigl S, Narvhus J, Sahlstrøm S (2012) Improved bioavailability of dietary phenolic acids in whole grain barley and oat groat following fermentation with probiotic *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri*. J Agric Food Chem 60:6369–6375

Huang Q, Lin W, Chen Z (2009) Optimization of submerged fermentation conditions for longan vinegar production. Mod Food Sci Technol 25:1434–1437

Katina K, Liukkonen KH, Kaukovirta-Norja A, Adlercreutz H, Heinonen SM, Lampi AM, Pihlava JM, Poutanen K (2007) Fermentation-induced changes in the nutritional value of native or germinated rye. J Cereal Sci 46:348–355

Lai T, Liu L, Zhang M, Zhang R, Zhang Y, Wei Z, Deng Y (2015) Effect of lactic acid bacteria fermentation on phenolic profiles and antioxidant activity of dried longan flesh. China Agric Sci 49:1979–1989

Lai T, Zhang M, Liu L, Zhang R, Deng Y, Chi J (2016) Optimization of process conditions of dry longan pulp fermented by complex lactic acid bacteria (in Chinese with English abstract). Trans Chin Soc Agric Eng 32:390–397

Lee BJ, Kim JS, Kang YM, Lim JH, Kim YM, Lee MS, Jeong MH, Ahn CB, Je JY (2010) Antioxidant activity and γ-aminobutyric acid (gaba) content in sea tangle fermented by lactobacillus brevis bj20 isolated from traditional fermented foods. Food Chem 122:271–276

Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78

Li L, Xu J, Mu Y, Han L, Liu R, Cai Y, Huang X (2015) Chemical characterization and anti-hyperglycaemic effects of polyphenol enriched longan (*Dimocarpus longan* Lour.) pericarp extracts. J Func Foods 13:314–322

Mehdizadeh S, Lasekan O, Muhammad K, Baharin B (2015) Variability in the fermentation index, polyphenols and amino acids of seeds of rambutan (*Nephelium lappaceum* L.) during fermentation. J Food Compos Anal 37:128–135

Naczk M, Shahidi F (1989) The effect of methanol–ammonia–water treatment on the content of phenolic acids of canola. Food Chem 31:159–164

Rangkadilok N, Sitthimonchai S, Worasuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J (2007) Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food Chem Toxicol 45:328–336

Razak DLA, Rashid NYA, Jamaluddin A, Sharifudin SA, Long K (2015) Enhancement of phenolic acid content and antioxidant

activity of rice bran fermented with *Rhizopus oligosporus* and *Monascus purpureus*. Biocatal Agric Biotechnol 4:33–38

Ren J, Zhao M, Shi J, Wang J, Jiang Y, Cui C, Kakuda Y, Xue SJ (2008) Purification and identification of antioxidant peptides from grass carp muscle hydrolysates by consecutive chromatography and electrospray ionization-mass spectrometry. Food Chem 108:727–736

Rodríguez H, Curiel JA, Landete JM, de las Rivas B, de Felipe FL, Gómez-Cordovés C, Mancheño JM, Muñoz R (2009) Food phenolics and lactic acid bacteria. Int J Food Microbiol 132:79–90

Saiga A, Tanabe S, Nishimura T (2003) Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. J Agric Food Chem 51:3661–3667

Shi S, Chen D, Fu J, Liu L, Wei Y, Shu B, Li W, Liang Q, Liu C, Liu C (2015) Comprehensive evaluation of fruit quality traits in longan 'Fengliduo' × 'Dawuyuan' sexual progenies. Sci Hortic 192:54–59

Song H, Lee SY (2006) Production of succinic acid by bacterial fermentation. Enzyme Microb Technol 39:352–361

Sun J, Chu Y-F, Wu X, Liu RH (2002) Antioxidant and antiproliferative activities of common fruits. J Agric Food Chem 50:7449–7454

Sun J, Shi J, Jiang Y, Xue SJ, Wei X (2007) Identification of two polyphenolic compounds with antioxidant activities in longan pericarp tissues. J Agric Food Chem 55:5864–5868

Trinh TTT, Yu B, Curran P, Liu SQ (2012) Formation of aroma compounds during Longan juice fermentation by *Williopsis saturnus* var. saturnus with the addition of selected amino acids. J Food Process Preserv 36:198–206

Yoon KY, Woodams EE, Hang YD (2005) Fermentation of beet juice by beneficial lactic acid bacteria. LWT-Food Sci Technol 38:73–75

Yu Y, Xiao G, Xu Y, Wu J, Zhang Y, Chen W (2015) Changes of quality in the fruits of *Prunus mume* during deacidification by fermentation with lactobacillus fermentium. J Food Sci 80:M405–M410

Yusof BNM, Talib RA, Karim NA (2005) A study of blood glucose response following temperate and tropical fruit ingestion in healthy adults. Malays J Nutr 11:47–57

Zhang MW, Zhang RF, Zhang FX, Liu RH (2010) Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. J Agric Food Chem 58:7580–7587

Zhu Y, Luo Y, Wang P, Zhao M, Li L, Hu X, Chen F (2016) Simultaneous determination of free amino acids in Pu-erh tea and their changes during fermentation. Food Chem 194:643–649