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Enrichment of nutritional value of *Phyllanthus emblica* fruit juice using the probiotic bacterium, *Lactobacillus paracasei* HII01 mediated fermentation

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Abstract

The fermented herbal juices are capable of curing and preventing diseases and reducing the aging progress. The present study was performed to investigate the fermentation of *Phyllanthus emblica* fruit by *Lactobacillus paracasei* HII01 with respect to carbon sources, polyphenols, and antioxidant properties. The physical changes, for instance, color, odor, taste, turbidity and gas formation, throughout the fermentation process was manually monitored. The fermented product was rich in polyphenolic content. The acid content and pH of the product were under the norms of Thai community product standards. Antioxidant properties of the fermented product were proved using ABTS, and FRAP assays. Chelation based study suggested that fermented *P. emblica* fruit juices are healthy enough to stabilize the oxidized form of the metal ion. The optimum fermentation period was 15 days. All the results supported that studied carbon sources did not interfere with the quality of the product. This report is the prelude study on the use of probiotic starter culture for the production of *P. emblica* fruit based lactic acid bacteria fermented beverages (LAFB) enriched with bioactive compounds. Further research on the impact of different carbon sources and upstream processes on the quality of LAFB is currently in progress.

Keywords: antioxidant; lactic acid bacteria fermented beverages; *Lactobacillus paracasei*; *Phyllanthus emblica* fruit; polyphenol.

Practical Application: Development and characterization of probiotic-based fermented *P. emblica* fruit juice as health supplement.

1 Introduction

Fermented herbal juices are widely consumed all over the world especially in Thailand. Thai people believe that these herbal juices can prevent and cure disease, and are supplemented as a health promoting beverage said to have anti-aging properties. Lactic acid bacteria (LAB) fermented beverages (LAFB) such as EM-X, fermented soybean broth, and Kefir have been widely used by world population. EM-X is an antioxidant beverage prepared by fermentation of papaya, unpolished rice, and seaweeds with the help of effective microbes such as photosynthetic bacteria, LAB, and yeast (Ekpeghere et al., 2012). It is accepted in clinical practices and is recommended as a prophylactic beverage used for the cure of various infectious diseases, allergies, cancer, diabetes, hypertension, and rheumatism (Deiana et al., 2002). Fermented Soybean broth is reported to be rich in antioxidant property (Yang et al., 2000; Romero et al., 2004). Kefir is a fermented milk product and is believed to contain efficient substances (La Rivière & Kooiman, 1967; Arslan, 2015). Several LAB such as *Lactobacillus kefiri*, *L. paracasei* (Gao et al., 2015), *L. plantarum*, *L. satsumensis* (Miguel et al., 2010), *L. lactis* (Bergmann et al., 2010), *L. brevis*, *Leuconostoc mesenteroides*, and yeasts such as *Kluyveromyces marxianus*, *K. wickerhamii*, *Pichia angusta*, *P. guilliermondii* have been identified in kefir grains (Kıvanç & Yapıcı, 2015). In the kefir grains, LAB and yeast are surrounded by slimy polysaccharide matrix called kefiran (Rodrigues et al., 2005). LAB present in the kefir produces lactic acid, acetaldehyde, and several kind of bacteriocins, which delays and inhibits the pathogenic bacteria (*Bacillus cereus*, *Clostridium tyributyricum*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*) contamination (Kıvanç & Yapıcı, 2015). Many strains of Lactic acid bacteria like *L. paracasei*, *L. plantarum* (Altay et al., 2013), *L. pentosus*, *L. brevis*, *L. fermentum*, *L. casei*, *L. kimchi*, *L. fallax*, *Weissella confusa*, *W. koreenis*, *W. cibaria*, and *Pediococcus pentosaceus* are used in various fermented foods (Swain et al., 2014). *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products have been reported for its beneficial effects in preventing hyperlipidemia-induced oxidative stress and atherosclerosis, and reduces the blood pressure (Chiang & Pan, 2012). Chen et al. (2015) have revealed that *L. paracasei* 01 fermented milk beverage as a functional food in strengthening the intestinal barrier by protecting the intestinal epithelial cells. Mostly *L. paracasei* have been reported for its multiprobiotic activity (Wassenberg et al., 2011; Chiang & Pan, 2012; Pellaton et al., 2012; Chen et al., 2015). Therefore, this study have focused on *L. paracasei* as a starter culture for LAFB.

In traditional Thai medicine, several plants are used entirely or its parts as a source of antioxidants. Among which, *Phyllanthus emblica* fruit (also named as *Emblica officinalis*) is well known

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for its potent antioxidant properties. *P. emblica* fruits are pale green or yellowish brown endocarp in color from the medium sized tree. Dried *P. emblica* fruits are used in Ayurvedic Hindu traditional medicine, Unani, conventional medicine practices in middle-east and south-Asian countries, systems of medicine in various treatments for fever, liver disorders, indigestion, anemia, and urinary complications. *P. emblica* fruits are rich in vitamin-C, which is readily absorbed by the human digestive system. Thus, they are used for the treatment of scurvy and pulmonary tuberculosis. *P. emblica* fruit juice can also be used to treat diarrhea, dysentery, and some joint pains (Chevallier, 1996); and their fruits are known for its lipid peroxide inhibition property and scavenging nature of hydroxyl and superoxide radical (Jose & Kuttan, 1995). *P. emblica* extracts can inhibit lipid peroxidation induced by gamma radiation in rat liver microsomes and superoxide dismutase (SOD) mediated damages in rat liver mitochondria (Khopde et al., 2001). Chatterjee et al. (2012) revealed that the role of gallic acid includes active fraction of *P. emblica* in ulcer healing through the endothelial nitric oxide synthase-dependent pathway. Few reports have detailed the chemical constituents and pharmacogenetic properties of *P. emblica* (Habib-ur-Rehman et al., 2007; Khosla & Sharma, 2012). Colucci et al. (2015) reported that the oral supplementation of *P. emblica* fruit extracts enhances the effectiveness of treatments for vitiligo.

Fermentation of food is an ancient food processing technologies. The process, quality improvement, and duration of the fermentation are developed over the years. Even though, fermentation is an ancient bioprocess for making beverages and food preservation, the use of microorganism with known biology in bioprocess technology have emerged very recently (Ross et al., 2002). The fermented beverages prepared using medicinal plants are superior with respect to the antioxidant properties. The scientific data explaining the beneficial effect of LAFB made from *P. emblica* fruits are not adequate. Thus, the current study aimed to investigate the polyphenol content and antioxidant properties of fermented *P. emblica* fruit with *L. paracasei*, and different carbon sources. In addition, the factors affecting the preferred nature of *P. emblica* fruits based LAFB has been studied.

2 Materials and methods

2.1 Raw materials, microbes, and experimental setup

Fresh *Phyllanthus emblica* fruits were collected from amphur Maesareung, Maehongsorn province, Chiang Mai, Thailand. Cane sugar and honey were purchased from Chiang Mai market and Agricultural extension and development center, Chiang Mai, respectively. Prior to the fermentation process, fruits were cleaned with water and subjected to various treatments (data not shown) to remove resisting microbial contamination followed by mechanical crushing.

Lactobacillus paracasei HII01 was used as a starter culture, which was provided by Health Innovation Institute (HII). The fermentation was carried out as per the following batches with respective controls. Fermentation 1 (Fn-1): crushed *P. emblica* fruit + water + cane sugar and 10% (w/w) of *L. paracasei* as starter culture; Fermentation 2 (Fn-2): crushed *P. emblica* fruit + water + cane sugar; Fermentation 3 (Fn-3): crushed *P. emblica* fruit + water + honey and 10% (w/w) of *L. paracasei* as starter culture; Fermentation 4 (Fn-4): crushed *P. emblica* fruit + water + honey; Control 1 (Cl-1): water + cane sugar and 10% (w/w) *L. paracasei*; Control 2 (Cl-2): water + cane sugar; Control 3 (Cl-3): water + honey and 10% (w/w) of *L. paracasei*; Control 4 (Cl-4): water + honey. The Fn components were mixed with a ratio of 3:10:1 (w/v/w) of crushed *P. emblica* fruits, water, cane sugar or honey, respectively.

2.2 Inoculum (starter culture) preparation, fermentation, and sample collection

The probiotic strain, *L. paracasei* was used as a starter for the fermentation process in this study. Single colony of *L. paracasei* was inoculated in 180 mL of MRS broth and incubated at 35-37 °C for 12 h. Then the culture was scaled up to 1.8 liter with approximate cell concentration of 10^9 CFU/ mL. The fermentation process was carried out in polypropylene plastic reactor (18.9 liter) with airtight lock and other aseptic measures. The fermentation process was executed at 30 ± 2 °C for six months. Samples were collected on day 0, 4, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 of fermentation and the physical properties of fermentation mixture (FnM) were noted. The collected samples were immediately filtered through Whatman no. 42 filter paper, prepared 1% (v/v) of each sample with sterile water and stored at –70 °C.

2.3 Total polyphenolic content determination

Total phenolic content of the samples were determined by the modified Folin-Ciocalteu colorimetric method of Kusirisin et al. (2009) and Yang et al. (2014). The reaction mixture was prepared by adding 100 µL of Folin-Ciocalteu reagent, 1.5 mL of deionized water and 200 µL of each sample or various concentration of gallic acid or pyrogallol. Then, the reaction was deactivated with 20% saturated sodium carbonate. The absorbance was measured at 725 nm after 30 min incubation at room temperature (RT). The total phenolic content was expressed as mg gallic acid and mg pyrogallol equivalents per mL of sample.

2.4 Determination of organic acid profile and pH

The content of organic acids in the samples was assessed by high-performance liquid chromatography (HPLC). Twenty micro liter of filtered samples were used for analysis, and the separation was achieved by using Phenomenex C8 column (250 mm × 4.6 mm, 0.5 µm) (Advanced Chromatography Technologies, Scotland). Phosphate buffer (0.1 M, pH 2.1), at the flow rate of 0.8 mL/min was served as the mobile phase. The organic acids concentration was determined by comparing the peak area of the sample with the area of standards (lactic acid, and acetic acid). The values lower than the limitations of detection were reported as not detected (ND). The values equal or higher than the limitation of quantity were also reported. pH of the sample, after filtration, was measured using the standard pH meter (Metrohm 691).

2.5 ABTS assay

2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays were performed as per the modified method of Singhatong et al. (2010). Briefly, $ABTS^{\bullet+}$ radical stock solution (7 mM ABTS and 2.45 mM potassium persulfate) was prepared. The $ABTS^{\bullet+}$ working solution (2%) was prepared using the deionized water with absorbance of 0.7 ± 0.05 at 734 nm. 2 mL of $ABTS^{\bullet+}$ working solution and 100 µL of each sample or vitamin-C or trolox or quercetin was mixed and incubated at RT for 3 min. The results are expressed as VCEAC (mg of vitamin-C equivalent antioxidant capacity), TEAC (mg of trolox equivalent antioxidant capacity), and QEAC (mg of quercetin equivalent antioxidant capacity) per mL of sample.

2.6 FRAP assay

The FRAP assay was executed with slight modifications (Suwannalert et al., 2010). Briefly, 1.8 mL of FRAP reagent was mixed with 180 µL of deionized water and 60 µL of each sample or positive control. Then, the reaction mixture was incubated at RT for 4 min. After incubation, the solution was subjected to spectrophotometric analysis at 539 nm. Results were expressed as mg Fe_2SO_4 equivalents per mL of sample.

2.7 Ferrous Ion-chelating assay

The complex formation of ferrous with constituents in the samples was studied by complexometry. The buffer systems used, to test the complex in different pH conditions, were hexamine buffers with pH of 3.6, 5.0, and 6.0. The absorbance spectrum for the maximum wavelength of reagent (Tetramethylmurexide ammonium salt (TMM) solution) and TMM-Fe^{2+}complex were observed. The calibration curve for the various concentration of Fe^{2+} was measured (Decker & Welch, 1990).

2.8 Total peroxidase assay

The activity of guaiacol peroxidase in the supernatant was determined spectrophotometrically. The reaction mixture contained 15 mM guaiacol and 5 mM H_2O_2 in 0.1 M phosphate buffer, pH 3.5. The sample (500 µL) was added to the reaction mixture and incubated at 30 °C for 15 min. After the incubation period, the reaction was measured at 470 nm (Laloue et al., 1997).

2.9 β-Glucosidase activity assay

About 500 µL of each sample were mixed with 1 mM p-nitrophenyl-β-d-glucopyranoside (pNPG) and 0.1 M phosphate-citrate buffer, pH 3.5. Then, the mixtures were incubated at 30 °C for 20 min. The reaction was arrested by the addition of 0.5 M sodium carbonate and measured at 420 nm (McCue & Shetty, 2004).

2.10 Statistical analysis

All the experiments performed in this study were carried out in triplicate. Analysis of variance (ANOVA) was carried out to measure the dissimilarities in antioxidant activities. Duncan's new multiple range test determined the significant differences, at the 95% confidential level ($p < 0.05$) by SPSS v.17 (Chicago, SPSS Inc, U.S.A).

3 Results and discussion

3.1 Physical observations of the reaction

Fermentation of *P. emblica* fruits was carried out with or without *L. paracasei* as a starter culture, as detailed in materials and method section. The physical changes, such as color, odor, taste, turbidity and gas formation, during the fermentation process were observed manually and tabulated (Supplementary Material, Table 1S). Color of the fermentation mixture (FnM) in Fn-1, 2, and its respective controls Cl-1, 2 was dark brown, due to the presence of cane sugar, whereas FnM in Fn-3, 4, and Cl-3, 4 was pale yellow in color because of honey. The notable color change was not observed until 15 days. FnM in Fn-3, 4 and Cl-3, 4 turned dark yellow in color after 15 days. A possible reason for the darkened color is due to the resultant fermentation, but there was no change in color of the FnM in Fn-1, 2 and Cl-1, 2 throughout the process. The odor of the mixture was sour and pickled tamarind throughout the process. Bad odor was noticed in Cl-2 starting from day 4, which might be due to the growth of contaminated microbes. No bad odor was recognized from other reactors. Taste of the fermented *P. emblica* fruits was initially sweet and later turned into sour till the end of the process. The FnM was found as turbid throughout the process. Plenty of gas formation was observed in Fn-1, 2 and Cl-1, 2, whereas there is no noticeable level of gas formation in Fn-3, 4 and Cl-3, 4. This data indicated that during fermentation, the gas formation was due to the presence of raw cane sugar as a carbon source. So the fermentation process using cane sugar as one of the materials should follow the gas management protocol during the process. Filmy layer and bubbles were observed at the top of the FnM in Fn-2, 4 and Cl-2, 4 from 4^{th} day of the process and this filmy layer was found in the entire reactors after 60 days of the process. Alcoholic aroma was noticed in Fn-2, 4 and Cl-2, 4 after 60 days of the process. During the fermentation process, the mixture gets contaminated with yeast due to the lack of starter culture (Prachyakij et al., 2007; Kantachote & Charernjiratrakul, 2008). Furthermore, yeast contamination results in alcohol production, which might be the possible reason for the alcoholic aroma of FnM in Fn-2, 4 and its respective control (Supplementary Material, Table 1S).

3.2 Polyphenol content

The polyphenolic content of the fermented product was kinetically analyzed using both gallic acid and pyrogallol as standards. Total polyphenol level was gradually increased until day 15, and then the concentration was slowly reduced during the process. Increase in the amount of polyphenol content (10.32 ± 0.28 mg gallic acid equivalent/mL, and 10.385 ± 0.23 mg pyrogallol equivalent/mL of Fn-1, respectively) was recorded at the 15^{th} day of fermentation (Figure 1). The decrease in polyphenolic content after 15 days might be due to the enzymatic oxidation of polyphenolic content by polyphenol oxidase.

This result revealed that 15 days fermentation process is good enough to enrich the product with polyphenolic content by selected substrate and starter culture. A recent study has reported the phenolic components extraction from *P. emblica* by comparing conventional and ultrasound-assisted extractions

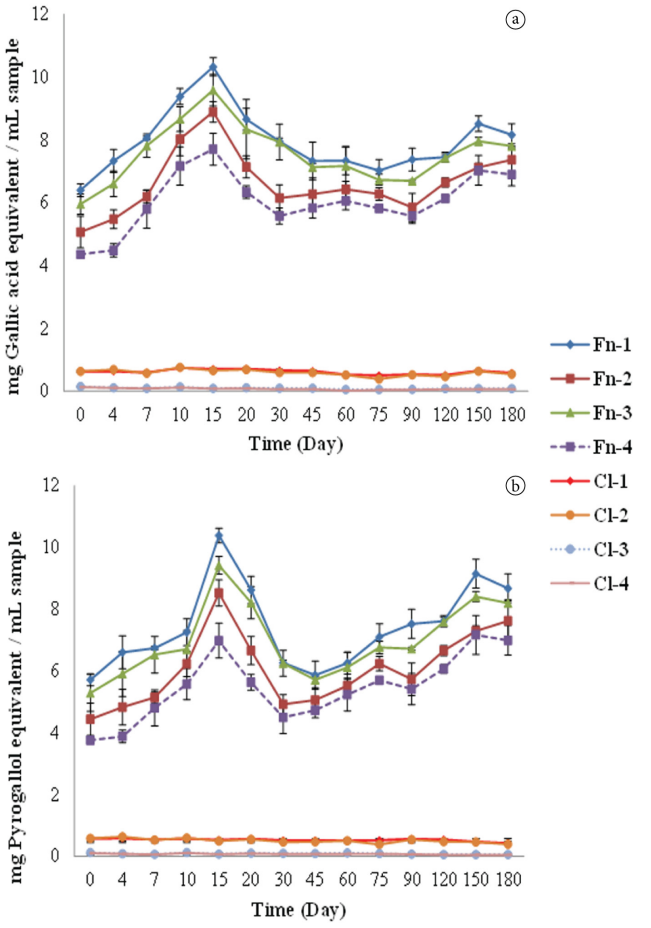

Figure 1. Polyphenolic contents of *P. emblica* juice during fermentation. Results were indicated as gallic acid (**a**) and pyrogallol (**b**) equivalents per mL of sample. The high content of phenolic acid was recorded at the 15th day of fermentation.

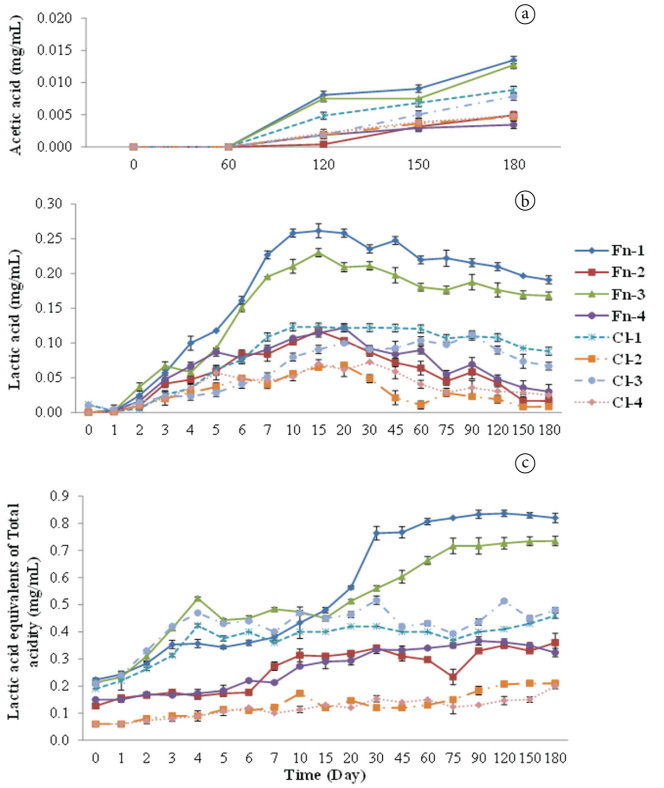

Figure 2. Acidic contents and total acidity of *P. emblica* juice during fermentation. Acetic acid content (**a**); lactic acid content (**b**); and lactic acid equivalents of total acid content (**c**) were measured as detailed in materials and methods. The gradual increase in the acid content was observed during the process.

techniques (Tsai et al., 2014). Sripanidkulchai & Junlatat (2014) also reported the antioxidant properties, and anti-inflammatory effect of phenolic content in *P. emblica* branch ethanolic extract by maceration and methanolic extract by Soxhlet apparatus. The current study revealed the release of a significant amount of phenolic content from *P. emblica* fruits by *L. paracasei* mediated fermentation.

3.3 Acidic content and pH of the FnM during fermentation process

Analysis of acid content in the consumable fermented product is necessary to ensure the quality of the food. Acetic acid, lactic acid, and the lactic acid equivalent of the total acid content were kinetically measured during the process. The presence of acetic acid was observed, i.e., detectable level, only after 60 days of fermentation. The constant increase in acetic acid content was recorded (Figure 2a). Lactic acid content was found to be higher in Fn-1 (0.26 ± 0.01 mg lactic acid/mL), Fn-3 (0.23 ± 0.005 mg lactic acid/mL) and slightly increased in its respective controls (Figure 2b). The total acid content of the ferment was gradually increased in all the reaction. Acidic content was elevated to 0.836 ± 0.1, 0.726 ± 0.02 mg of lactic acid equivalents per mL Fn-1, 3 reactions, respectively (Figure 2c). Lactic and acetic acids are the metabolites produced by the LAB, which increases acidity, thereby inhibits the growth of several pathogens (Breidt & Fleming, 1997). Thai community product standard (TCPS) for fermented herbal juice extract (TCPS 481/2547) regulates the quality of food. As per TCPS norms, the pH of the fermented food should be lower than 4.3. In the current study, the pH of the mixtures, both control and test were lower than 4.3 (Figure 3).

3.4 Antioxidant properties and other evaluation of the product

Antioxidant capacity of fermented products was assessed by ABTS and FRAP assays. Vitamin-C, trolox, and quercetin were used as standards for the evaluation. Fermented products, especially Fn-1 and Fn-3 showed high antioxidant capacity (26.55 ± 0.58, and 26 ± 1.3 mg VCEAC; 40.97 ± 0.9, and 40.16 ± 2.0 mg TEAC; 14.85 ± 0.34, and 14.54 ± 0.76 mg QEAC of Fn-1, and Fn-3, respectively) (Figure 4). Fn-1 and Fn-3, reactions consist of the same ingredients except carbon source.

This result suggested that *P. emblica* fermentation with *L. paracasei* was not extensively affected by any of the carbon

sources, cane sugar or honey, used in the current study (Figure 4). High antioxidant properties were observed in all the reaction at the 15th day of the process (Figure 4), which is correlated with polyphenol content of the product (Figure 1). There was no significant level of antioxidant abilities in controls, which indicated that nutrient nourishment was the resultant of fermentation of *P. emblica* fruits by *L. paracasei*. The polyphenol content also supported the above statement (Figure 1). This data also suggested that the nutritional content of cane sugar and honey

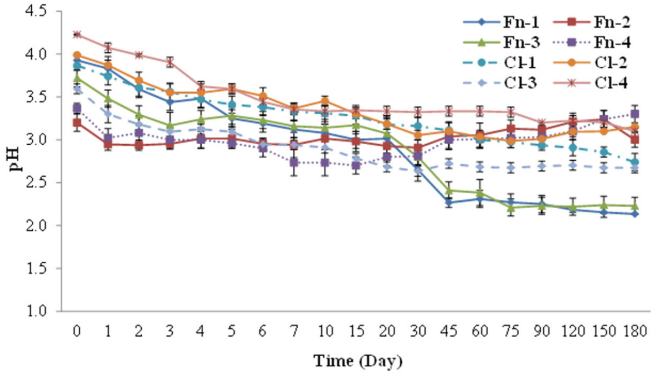

Figure 3. pH of *P. emblica* juice during fermentation.

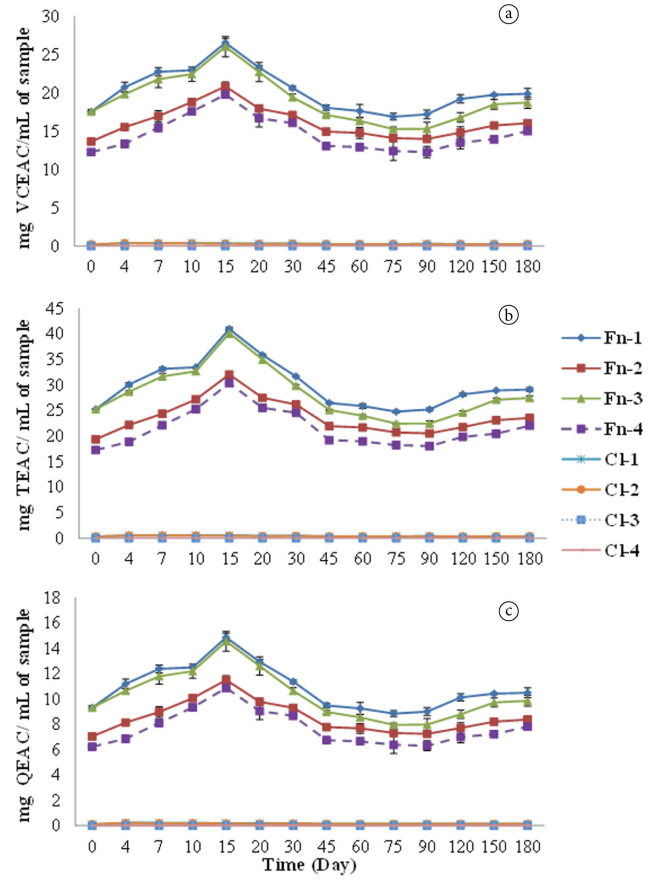

Figure 4. ABTS assay for *P. emblica* juice during fermentation. Results were represented as mg VCEAC (**a**); TEAC (**b**); and QEAC (**c**) per mL of samples. High activity was recorded at the 15th day of fermentation.

did not interfere with the results. The redox property of phenolic components positively influences the antioxidant properties (Rice-Evans et al., 1995).

Antioxidant ability of *P. emblica* extracts depends on the concentration of phenolic content. The polyphenols in *P. emblica* fruit prevents the oxidation of vitamin C and maintain its stability (Dhale, 2012). Thus reduction in polyphenols affects the stability of the vitamin C. Antioxidant activity in Fn 1-4 was decreased due to the reduction of vitamin C and phenolic content after 15th day of fermentation period. The increase in the polyphenolic content and antioxidant activity after 90 days in Fn 1-4 might be due to the completely dissolved content of fermented *P. emblica* fruit. Luo et al. (2009) have reported the ability of potent antioxidant traits of solvent extracted *P. emblica*, whereas the current study detailed the fermentation based release of active compounds from *P. emblica*. High reducing power (43.55 ± 1.45, and 41.50 ± 0.40 mg $FeSO_4$ equivalent per mL of Fn-1, and Fn-3) was recorded at 15th day of fermentation in Fn-1 and Fn-3 reactions (Figure 5a). Thus, FRAP assay results also supported the outcome of ABTS assay. Reducing power is attributed to antioxidant activity used as an important sign of the antioxidant capacity (Oktay et al., 2003).

Tsai et al. (2014) proved the reducing ability of *P. emblica* extract. A noticeable level of the ferric reducing antioxidant property was recorded in the current study with fermentation product. Thus, the *L. paracasei* assisted fermented product of *P. emblica* could be the good dietary source of antioxidants. The effect of *L. paracasei* mediated *P. emblica* fermented product with the ferrous ion chelating capacity is revealed in Figure 5b,

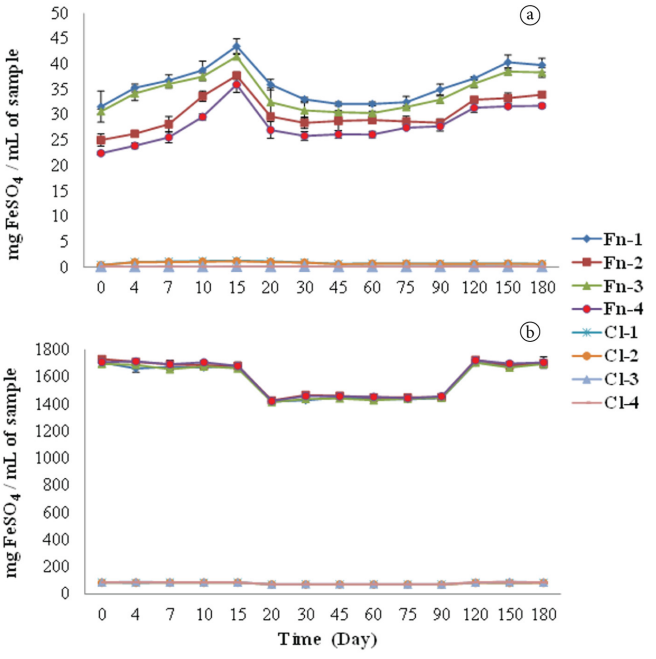

Figure 5. Representation of FRAP (**a**) and chelating ability assay (**b**) results of *P. emblica* juice during fermentation. FRAP assay result supports the antioxidant efficiency of product and chelation property of *P. emblica* juice has been proved. High activity was recorded, for both FRAP and chelating ability assay, at the 15th day of fermentation.

and the results indicated that at the 15th day of fermentation process, all the (Fn 1-4) samples scored high chelating capacity, which might be due to the presence of *P. emblica*. The chelating capacity of honey is slightly higher than that of cane sugar in the controls (Unpublished data). Present results supported the previous report by Tsai et al. (2014), with respect to the chelating property of *P. emblica*. Fermented *P. emblica* fruit juices are strong enough to stabilize the oxidized form of the metal ion.

The peroxidase enzyme plays an active role in the several metabolic process in plants such as auxins catabolism, and oxidation of cinnamyl alcohols (Quiroga et al., 2000). β-Glucosidase is an enzyme that hydrolyses a bond between glucose and an aglycone. β-glucosidase is most often associated with the cell wall of most microorganisms. They are found in a wide number of yeasts, *Saccharomyces* spp., *Candida* spp., *Aspergillus* spp., *Hanseniaspora* spp., bacteria *Oenococcus* (*Leuconostoc*) and in some strain of Lactic acid bacteria. Peroxidase and β-glucosidase are essential enzymes in human health; its deficiency lead to unpleasant impacts. Dietary supplements of peroxidase and β-glucosidase help the consumer to develop healthy life. Thus, Fn 1-4 and Cl 1-4 was assessed for quantifying the peroxidase and β-glucosidase throughout the fermentation period. But both peroxidase and β-glucosidase was not detectable in Fn 1-4 and its respective control. Overall, the results of the current study suggested that fermentation of *P. emblica* fruit by *L. paracasei* produces high quality of LAFB with independent of carbon source used in this study.

4 Conclusion

The current study revealed that LAFB produced by *L. paracasei* mediated fermentation of *P. emblica* fruit is a good dietary product with high polyphenolic content and antioxidant properties. This study also provided the impact of fermentation duration on the quality of the product. As per our knowledge, this is a preliminary study on the use of probiotic-based starter culture for the production of *P. emblica* fruit based LAFB. Further, detailed study of different carbon sources and fine tuning of upstream processes may yield the active principles and enriched high-quality products. The research concerning above is currently in progress.

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Supplementary Material

Table 1S. Color, odor, taste, turbidity and gas formation during the fermentation process of *P. emblica*. Information was collected by direct observation of fermentation and control reactors. Fn.: Fermentation; Cl.: Control.

Duration (Day)	Color	Odor	Taste	Gas	Remarks
0	DB: Fn-1, 2; Cl-1, 2 PY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind	Slightly sweet	No gas	
4	DB: Fn-1, 2; Cl-1, 2 PY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	plenty of gas in Fn-1, 2 and Cl-1, 2	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
7	DB: Fn-1, 2; Cl-1, 2 PY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	plenty of gas in Fn-1, 2 and Cl-1, 2	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
10	DB: Fn-1, 2; Cl-1, 2 PY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	plenty of gas in Fn-1, 2 and Cl-1, 2	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
15	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	plenty of gas in Fn-1, 2 and Cl-1, 2	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
20	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	plenty of gas in Fn-1, 2 and Cl-1, 2	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
30	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	plenty of gas in Fn-1, 2 and Cl-1, 2	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
45	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Strong sour	Reduced gas	Filmy and bubbles were found at top of the juice in Fn-2, 4, Cl-2, 4
60	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Strong sour	Reduced gas	Fn-2, 4, and Cl-2, 4 smell like alcohol, filmy layer was found in all Fn.
75	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	Reduced gas	Fn-2, 4, and Cl-2, 4 smell like alcohol, filmy layer was found in all Fn.
90	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	No gas	Fn-2, 4, and Cl-2, 4 smell like alcohol, filmy layer was found in all Fn.
120	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	No gas	Fn-2, 4, and Cl-2, 4 smell like alcohol, filmy layer was found in all Fn.
150	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour;*	Sour	No gas	Fn-2, 4, and Cl-2, 4 smell like alcohol, filmy layer was found in all Fn.
180	DB: Fn-1, 2; Cl-1, 2 DY: Fn-3, 4; Cl-3, 4	Similar to pickled tamarind and sour; *	Sour	No gas	Fn-2, 4, and Cl-2, 4 smell like alcohol, filmy layer was found in all Fn.

Notes: Dark brown = DB; Pale yellow = PY; Dark yellow = DY. *Bad odor in Cl–2; All the Fn and Cl samples were turbid during the fermentation process of *P. emblica*.