The control samples were prepared without inoculum or substrate. The variations in pH, acidity, total phenolic content, and the antioxidant activity of the samples were kinetically measured by standard methods.

Results: The pH and total acidity of the samples were progressively reduced and improved when the duration of fermentation was prolonged, respectively. After 15 days of fermentation, F1 (1.190 mg GAE/ml sample) and F3 (1.265 mg GAE/ml sample) exhibited high total phenolic compound compared to other samples. Likewise, sample F3 displayed maximum antioxidant activity. The samples with cane sugar exhibited high phenolic content, free radical scavenging activity, and chelating power than samples with honey.

Conclusion: About 15 days of fermentation in sufficient to obtain the high quality (rich in phenolic compounds and antioxidant capacity) fermented M. citrifolia juice using L. paracasei, and cane sugar as starter, and carbon source, respectively.

Keywords: Morinda citrifolia L., Lactobacillus paracasei, Fermented plant beverages, Phenolic content, Antioxidant.

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INTRODUCTION

Morinda citrifolia L. is called as noni or Indian mulbery. Noni is commonly used in ancient foods and medicine. Noni fruits were comprised of carbohydrates, dietary fibers, vitamins (Vitamins C, E, B1, B2, B6, B12, biotin, pantothenic acid, folic acid, carotene, and niacin), proteins, minerals (calcium, potassium, sodium, phosphorus, iron, molybdenum, magnesium, and sodium chloride), and fat [1]. Almost all parts, such as fruits, leaves, roots, and barks, of noni plant have been used in the form of noni juice, powder, capsule, etc. The fermented noni juice is superior in terms of popularity, acceptability, and therapeutic reports [1]. Noni plant and its derivatives showed a robust therapeutic property against inflammation, infection, hypertension, cancer, ulcer, constipation, and autoimmune diseases [2-4].

The mechanism behind the health benefits of noni has been revealed in some extent. For example, limonene, quercetin, and alizarin in noni hinder the growth of the tumor cells and induce the apoptosis. Especially, quercetin suppresses the expression of PI3K/Akt/IKK-alpha/NF-kappaB pathway genes [5,6]. Citrifolinoside present in noni inhibits the ultravioleB-induced activator protein-1 activity [7]. Limonene is a monoterpene that exhibits anticancer ability [8]. The anti-inflammatory effects of noni are possibly due to the suppression of malondialdehyde by scopoletin through increased superoxide dismutase, glutathione peroxidase, and catalase activities [9].

The fermentation process positively improves the quality of fruit juice, especially lactic acid bacteria (LAB)-mediated fermentation enriched the functional property of the core ingredients [10]. The fermentation of plant materials with suitable LAB strain can supplement the functional qualities and health-enhancing abilities of the fermented beverages. For example, fermentation of mushroom with GABA producing LAB improved the quality and extended the application of the mushroom juices [11,12]. Lactobacillus spp. are a vital part of the human microbiome and are most studied bacterial probiotics. Lactobacillus brevis, Lactobacillus casei, Lactobacillus salivarius, Lactobacillus acidophilus, Lactobacillus fermentum, and Lactobacillus plantarum are often isolated from the intestinal tract of mammals. Even though live Lactobacillus are considered as safe for human consumption, they have been concerned as pathogens, particularly in the immunocompromised persons [13]. Lactobacillus spp. are commonly used for the preparation of fermented beverages because of simple growth conditions, and innocuous for the general population. The Lactobacillus-mediated fermentation improved the nutritional value of Phyllanthus emblica fruit juice [10], and Lactobacillus fermented plant juice has also been reported for cosmetic applications [14].

The present study was aimed to evaluate the impact of the fermentation process, mediated by Lactobacillus paracasei HII01, on the total polyphenol content and antioxidant capacity of M. citrifolia L. juice.

METHODS

Raw materials, strain, and experimental setup

Fresh M. citrifolia fruits and cane sugar were purchased from local market of Chiang Mai province, Chiang Mai, Thailand. Honey was bought from Agricultural Extension and Development Center, Chiang Mai. L. paracasei HII01 was obtained from Health Innovation Institute (HII), Chiang Mai. The controlled single strain fermentation of M. citrifolia was carried out with cane sugar; and honey as carbon source using L.
paracasei as starter culture. The details of fermentation setup were described as follows:
Formula 1 (F1): Morinda citrifolia:water:cane sugar (3:10:1 ratio) +10% L. paracasei
Formula 2 (F2): Morinda citrifolia:water:honey (3:10:1 ratio) +10% L. paracasei
Formula 3 (F3): Morinda citrifolia:water:cane sugar (3:10:1 ratio)
Control 1 (C1): Water:cane sugar (10:1 ratio) +10% L. paracasei
Control 2 (C2): Water:cane sugar (10:1 ratio)
Control 3 (C3): Water:honey (10:1 ratio) +10% L. paracasei

Fermentation
The preparation of starter culture, L. paracasei, and fermentation setup was done as defined earlier [10]. The fermentation was performed at 30±2°C for 6 months. Samples were collected during fermentation (day 0, 4, 7, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180). Then, they were filtered through Whatman no. 42 filter paper, 1% (v/v) of each sample was aliquoted with sterile water and stored at -70°C.

Determination of acidity, pH, and total polyphenolic content
The pH, acidity, and total polyphenolic content of fermented juice at various time points of fermentation were assessed as detailed previously [10,15,16].

Total antioxidant capacity (TAC)
TAC of fermented Morinda citrifolia juice was calculated by 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid assay as detailed previously [17,18]. Quercetin, trolox, and Vitamin C were used as standards. The results were represented as mg of quercetin equivalent antioxidant capacity (QEAC), mg of trolox equivalent antioxidant capacity (TEAC), and mg of Vitamin C equivalent antioxidant capacity (VCEAC)/ml of sample.

Ferric reducing antioxidant power (FRAP) and ferrous ion-chelating assay
The FRAP assay and chelating property of the samples were studied as detailed [10]. The values of FRAP assay were represented as mg FeSO₄ equivalents/ml sample. The chelating assay results were stated as chelating power (mg FeSO₄ equivalents/ml of sample).

Statistical analysis
Experiments were performed in triplicate. The values were denoted as mean ± standard deviation. Duncan’s new multiple range test determined the statistically significant differences, at the 95% confidential level (p<0.05) by using SPSS v.17 (Chicago, SPSS Inc, U.S.A).

RESULTS AND DISCUSSION
The fermentation process was carried out in aseptic condition, and the collected samples were analyzed. The pH of Morinda citrifolia juice (formula 1–4) was gradually reduced from 4.15–3.86 to 3.34–3.22. In the control fermentation setup also, pH of the solution was reduced (Fig 1a).

The total acidity of F1 and F2 was increased from 0.1 to 0.64, and 0.1 to 0.67 mg lactic acid equivalent/ml of sample, respectively. Likewise, the total acidity of F3 and F4 was increased from 0.08 to 0.67, and 0.08 to 0.73 mg lactic acid equivalent/ml of sample, respectively. The control fermentation (C1, C2, C3, and C4) showed slight increase in acidity (from 0.09 to 0.48, 0.08 to 0.49, 0.06 to 0.23, and 0.05 to 0.22, respectively) (Fig 1b). The changes in the pH and acidity level of the fermented samples were influenced by the carbon source (honey and cane sugar) and the presence of substrate (Morinda citrifolia). The control samples showed the shallow level of improvement in the acidity, possibly due to the lack of the substrate.

The total phenolic acid content of the fermented samples was evaluated. F1 and F3 showed high amount of phenolic content (1.198 and 1.265 mg GAE/ml sample, respectively) after 15 days of fermentation, then the concentration was slightly reduced. However, at the end of the fermentation process, after 180 days, F1 and F3 showed high phenolic content (0.713 and 0.868 mg GAE/ml sample, respectively) compared to other samples (Fig 2). The control samples (C1 and C2) exhibited relatively high phenolic content than other control samples. The sample F1, F3, C1, and C2 contains cane sugar as a carbon source, while F2, F4, C3, and C4 having honey. The results suggested that the presence of cane sugar supports the growth of inoculum which facilitates the release of phenolic compounds from the substrate Morinda citrifolia. The antimicrobial property of honey may influence the growth of inoculum and other spontaneous microbial activity. The results suggested that fermentation of Morinda citrifolia with desired microbial starter culture and cane sugar facilitates the release of more phenolic compounds in the fermented broth compared to the samples with honey as a carbon source. Furthermore, the results revealed that the fermentation of Morinda citrifolia by L. paracasei for 15 days was enough to enrich the product with phenolic compounds.

The total antioxidant capacity (TAC) of the samples has been represented as TEAC, VCEAC, and QEAC. The sample F3 showed high TEAC of 0.62, VCEAC of 0.76, and QEAC of 0.36 mg per ml of the sample after 15 days of fermentation, followed by F1 that displayed 0.47, and 0.63 mg TEAC and VCEAC per ml of sample. Whereas, F1 showed high QEAC value (0.28 mg per ml of sample) after 75 days of fermentation. All the samples exhibited its high TAC value after 15 days of the fermentation process; even the control samples displayed the similar pattern after 15 days. Primarily, the experimental and control samples with cane sugar showed high TAC compared to honey counterparts. The data suggested that 15 days of fermentation was sufficient regarding TAC of samples (Fig 3).
The naturally fermented noni aqueous extract exhibited antidiabetic, antihyperglycemic, and hepatoprotective activity in diabetic rats [27]. The probiotic, L. paracasei HII01-mediated fermented noni juice might be a potent nutraceutical candidate for the betterment of human health.

Fig. 3: The total antioxidant capacity of fermented Morinda citrifolia juice and values were represented as mg trolox equivalent antioxidant capacity (a), Vitamin C equivalent antioxidant capacity (b), and quercetin equivalent antioxidant capacity (c) per ml sample

Fig. 4: Ferric reducing antioxidant power (a) and chelating power (b) of fermented Morinda citrifolia juice

Several studies reported the health benefits of noni extracts. Noni extract has been reported for antidiabetic, antitubercular property [23,24]. The noni extract has been proved as a functional food supplement to treat infections, inflammatory diseases, and oxidative stress [25]. The noni extract has been reported for antidiabetic, antitubercular property [23,24]. The noni extract has been proved as a functional food supplement to treat infections, inflammatory diseases, and oxidative stress [25].

CONCLUSION

L. paracasei HII01-mediated fermented of Morinda citrifolia juice was enriched with phenolic compounds and antioxidants. The use of cane sugar as carbon source for the growth of L. paracasei HII01 facilitates the fermentation process compared to honey. Likewise, the study suggested that natural fermentation of noni with cane sugar was found to be relatively superior like L. paracasei HII01-mediated fermented juice. However, the use of probiotic strains as starter culture for the development of fermented juices may enhance the product quality with added benefits of probiotics.

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AUTHOR’S CONTRIBUTIONS

CC involved in the study design, experiments, review, and finalization of the manuscript. BSS and PK contributed to data analysis, manuscript...
preparation, and critical revision of the manuscript. SS, KC, RY, and SP responsible for wet lab experiments and data collection. All the authors agree with the content of the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES