

**LACTOBACILLUS PARACASEI H1101 MEDIATED FERMENTATION OF SYZYGIUM CUMINI L. FRUITS: ASSESSMENT OF CHANGES IN PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY**SASITHORN SIRILUN<sup>1</sup>, BHAGAVATHI SUNDARAM SIVAMARUTHI<sup>1</sup>, PERIYANAINA KESIKA<sup>1</sup>, SARTJIN PEERAJAN<sup>2</sup>, CHAIYAVAT CHAIYASUT<sup>1\*</sup><sup>1</sup>Innovation Center for Holistic Health, Nutraceuticals and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand. <sup>2</sup>Health Innovation Institute, Chiang Mai, 50200, Thailand. Email: [chaiyavat@gmail.com](mailto:chaiyavat@gmail.com)

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**ABSTRACT**

**Objective:** *Syzygium cumini* L. fruits are reported for several pharmacological applications and winemaking. The present study was conducted to develop and analyze the phytochemical content, and antioxidant properties of lactic acid bacteria mediated fermented *S. cumini* fruit juice (FSJ).

**Methods:** The pH of FSJ was measured by pH meter, and total acidity was calculated by titration method. The Folin-Ciocalteu colorimetric method was employed to assess the total phenolic content (TPC), and antioxidant properties were determined by 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, ferric reducing antioxidant power, and ferrous ion-chelating assays.

**Results:** The formula 1 and 2 displayed pH of 3.23, and 3.12, respectively, after 6 months of fermentation. The acidity of F1 and F2 was regularly increased from 0.23 to 0.55 and 0.29 to 0.53 mg lactic acid equivalent per ml sample, respectively. The TPC of FSJ was progressively increased. The F1 and F2 showed an increase in TPC from 0.94 to 1.61 and 1.05 to 1.95 mg Gallic acid equivalent per ml of sample, respectively. The Formula 1 and 2 exhibited antioxidant capacities of 1.90 and 2.06 mg Trolox equivalent antioxidant capacity per ml sample, 0.81 and 0.80 quercetin equivalent antioxidant capacity per ml sample, and 1.67 and 1.82 Vitamin-C equivalents antioxidant capacity per ml sample, respectively. Ferric reducing-antioxidant power of Formula 1 and 2, and Control 1 and 2 were 3.90, 4.29, 2.96, and 3.27 mg Fe<sub>2</sub>S<sub>0</sub>4 equivalents per ml of sample, respectively. Whereas, the chelating power of samples was not significantly changed at the end of fermentation.

**Conclusion:** The high-quality FSJ was prepared with enhanced antioxidant capacity and phenolic compounds. The probiotic containing FSJ is an alternative functional food supplement to prevent, cure and manage several degenerative disorders. Further, clinical studies are essential to insight the functional quality of FSJ.

**Keywords:** *Syzygium cumini* L., Phenolic compound, Lactic acid bacteria, *Lactobacillus paracasei*, Antioxidant, 2, 2'-Azino-bis-3-ethylbenzthiazoline-6-sulfonic acid, Ferric reducing antioxidant plasma.

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**INTRODUCTION**

The success of fermented plant juice (FPJ) preparation, regarding quality and safety, significantly depends on starter culture and microbial metabolic reaction on base ingredients. Thus, controlled fermentation with well-characterized starter culture is the advisable technique to produce high-quality FPJ. The starter culture with specific characters like the ability to produce antimicrobials against common foodborne pathogens, and to produce valuable secondary metabolites like  $\gamma$ -aminobutyric acid (GABA) is the best choice for the production of functional FPJs.

Lactic acid bacteria (LAB) are a group of Gram-positive, cocci or rod-shaped, non-spore forming, and acid tolerant bacteria with low guanine-cytosine content in the genetic material. *Lactobacillus* spp. are commonly used as a starter for the preparation of fermented foods. Food isolate of *Lactobacillus brevis*, *Lactobacillus fermentum* was reported to produce glutaminase and glutamate decarboxylase [1] and was used as a starter for two-stage fermentation process of *Hericium erinaceus* to produce GABA rich mushroom juice [2]. *Enterococcus faecalis*, protease producing LAB isolated from fermented food, has been used to produce GABA rich *H. erinaceus* juice [3]. Likely, LAB is used to improve the functional quality of foods. For example, LAB mediated fermentation enhanced the nutritional value and functional property of emblica juice [4].

*Syzygium cumini* L. belongs to the family Myrtaceae, generally known as jambolan or jamun inherent to the Indian subcontinent. The fruits

of jambolan are not available in all the seasons. Thus, researchers concentrating on the development of jambolan fruit based functional foods [5]. Jambolan fruit is largely used in the preparation of vinegar, alcohol beverages, and fermented foods [6]. The leaves of *S. cumini* used as a tea to manage the hyperglycemic condition. *S. cumini* fruits are rich in antioxidant compounds such as phenolic compounds, anthocyanins, and also contains fermentable sugars [7,8].

Several reports are available on the development of fermented *S. cumini* beverages, especially wine production [8], and yeast (*Saccharomyces cerevisiae*) mediated fermented *S. cumini* was reported to have hypolipidemic, hepatorenal protective, and anti-diabetic effects [9].

The reports on LAB fermented *S. cumini* beverages are very limited. Thus, the present day was conducted to develop *Lactobacillus paracasei* H11 mediated fermented *S. cumini* juice (FSJ) and evaluated the changes in pH, acidity, total phenolic content (TPC), and total antioxidant capacity (TAC) during fermentation.

**METHODS****Sample, strain, and fermentation**

Fresh *S. cumini* L. fruits and cane sugar were purchased from local market of Chiang Mai province, Thailand. *L. paracasei* H1101 was obtained from Health Innovation Institute, Chaing Mai, Thailand.

The fruits were mechanically crushed and used for the fermentation process with cane sugar, and water. The fermentation was carried out with the following experimental setup at room temperature for 6 months, and samples were collected during the fermentation and stored at  $-70^{\circ}\text{C}$  after the filtration (Whatman No. 42 filter paper). Formula 1 (F1): *S. cumini* fruit:Water:Cane sugar (3:10:1 ratio) (Initial pH 6) +10% *L. paracasei*; Formula 2 (F2): *S. cumini* fruit:water:cane sugar (3:10:1 ratio) (Initial pH 4) +10% *L. paracasei*; Control 1 (C2): *S. cumini* fruit:water:cane sugar (3:10:1 ratio) (Initial pH 6); and Control 2 (C2): *S. cumini* fruit:water:cane sugar (3:10:1 ratio) (initial pH 4).

The fermentation was conducted at room temperature for 6 months, and samples were collected at the various time point of the fermentation process to assess the changes kinetically. The collected samples were stored at  $-70^{\circ}\text{C}$  after filtration with Whatman no. 42 filter paper.

#### Determination of pH and acidity

The pH and acidity of fermented juice at the various time point of fermentation was evaluated using pH meter (Inola, pH level 2, Weilheim), and titration methods, respectively, as detailed earlier [4,10].

#### Determination of total polyphenolic content

The modified Folin-Ciocalteu colorimetric method was employed to measure the TPC of the samples. Briefly, 100  $\mu\text{l}$  of  $\times 10$  diluted Folin-Ciocalteu reagent, 1.5 ml of deionized water and 200  $\mu\text{l}$  of filtered fermented samples or Gallic acid (used as positive control), at different concentrations, were mixed and incubated at room temperature for 30 min. After incubation, 20% saturated sodium carbonate was used to stop the reaction, and the absorbance was measured at 725 nm. The phenolic content of the sample was denoted as mg Gallic acid equivalents (GAEs) per ml of the sample [11].

#### TAC

The TAC of fermented samples was measured by 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay as detailed. The results were denoted as mg Trolox equivalent antioxidant capacity (TEAC), quercetin equivalent antioxidant capacity (QEAC), and Vitamin-C equivalent antioxidant capacity (VCEAC) per ml of the sample [12-15].

#### Ferric reducing antioxidant power (FRAP) assay

The samples were subjected FRAP assay. Briefly, sample or standard solution (60  $\mu\text{l}$ ), FRAP reagent (1.8 ml) and deionized water (180  $\mu\text{l}$ ) were mixed and incubated at room temperature for 4 min. Then, the reaction mixture was examined using spectrophotometer at 539 nm. The values were denoted as mg  $\text{Fe}_2\text{SO}_4$  equivalents/ml of the sample [14].

#### Ferrous ion chelating assay

The chelating property of fermented samples was calculated. The results were stated as a chelating powder (mg  $\text{Fe}_2\text{SO}_4$  equivalents/ml of sample) [4].

#### Statistical analysis

All the investigations were performed in triplicate. The values were represented as mean $\pm$ standard deviation. Duncan's new multiple range tests determined the significant differences, at the 95% confidential level ( $p < 0.05$ ) by SPSS version 17 (Chicago, SPSS Inc., U.S.A.).

## RESULTS AND DISCUSSION

The pH of FSJ was slowly reduced from initial values. The Formula 1 (Initial pH 5.98) and 2 (Initial pH 4) showed pH of 3.23 and 3.12, respectively, after 180 days of fermentation.

Whereas the control Sample 1 (Initial pH 5.98), and 2 (Initial pH 4) showed pH of 3.67 and 4.31, respectively, after 180 days of fermentation. The acidity of F1 and F2 was regularly increased from 0.23 to 0.55 and 0.29 to 0.53 mg lactic acid equivalent per ml sample, respectively. Even control samples showed a slight increase in acidity (0.25-0.28-0.35-0.37 mg lactic acid equivalent per ml sample) (Fig. 1). The reduction in pH during fermentation process was due to the catabolism of raw

materials by *L. paracasei*, LAB knew for the release of organics acids during fermentation, which in turn reduce the pH and increase the acidity.

The TPC of FSJ, both experimental and control samples, was progressively increased. The F1 and F2 showed an increase in TPC from 0.94 to 1.61 and 1.05 to 1.95 mg GAE per ml of sample, respectively. Likewise, C1 and C2 also exhibited an increase in TPC from 0.67 to 1.30 and 0.63 to 1.46 mg GAE per ml of sample, respectively. The results suggested that the FSJ prepared with the starter (*L. paracasei*) and without starter increase the TPC content, but the concentration of TPC was high in F1 and F2 compared with C1 and C2. Moreover, the initial pH of the medium play critical role in final TPC concentration, F2 showed high TPC than F1 (Fig. 2).

The TAC of the samples was evaluated concerning the activity of Trolox, quercetin, and Vitamin-C. The Formula 1 and Formula 2 showed 1.90 and 2.06 mg TEAC per ml sample, 0.81 and 0.80 QEAC per ml sample, and 1.67 and 1.82 VCEAC per ml sample, respectively. The Control 1 and Control 2 exhibited 1.69 and 1.44 mg TEAC per ml sample, 0.71 and 0.60 QEAC per ml sample, and 1.46 and 1.23 VCEAC per ml sample, respectively (Fig. 3).

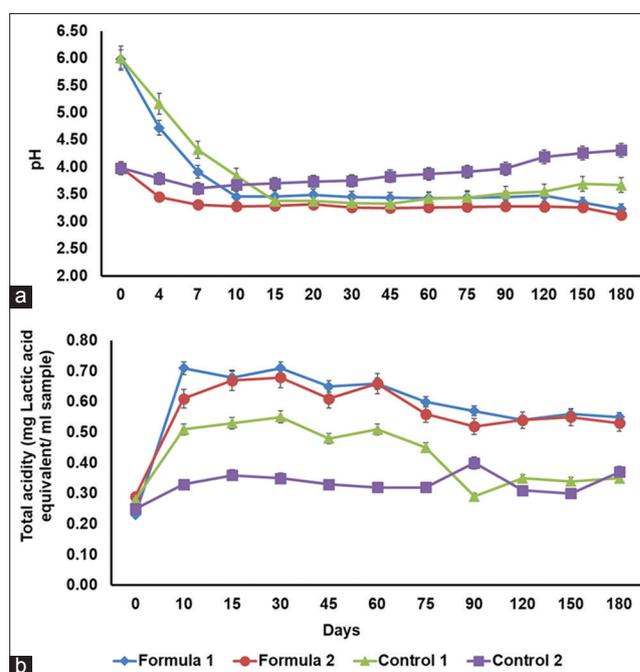


Fig. 1: pH (a) and acidity (b) profile of fermented *Syzygium cumini* fruit juice

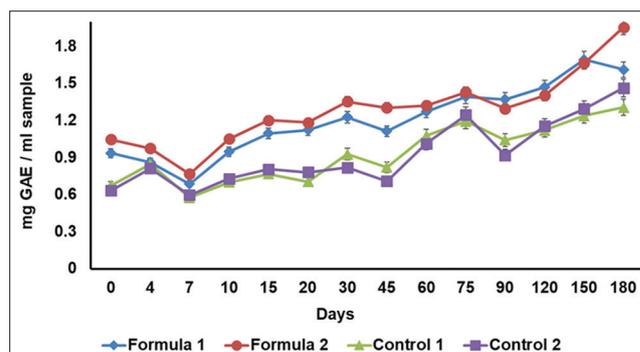


Fig. 2: The total phenolic acid content of fermented *Syzygium cumini* fruit juice. The results were represented as mg Gallic acid equivalent per ml of sample

Ferric reducing-antioxidant power of Formula 1, Formula 2, Control 1, and Control 2 was 3.90, 4.29, 2.96, and 3.27 mg Fe<sub>2</sub>SO<sub>4</sub> equivalents per ml of sample, respectively (Fig. 4a). The chelating power of Formula 1, Formula 2, Control 1, and Control 2 was 300.00, 297.08, 295.89, and 300.86 mg Fe<sub>2</sub>SO<sub>4</sub> equivalents per ml of sample, respectively (Fig. 4b). The results indicated that both experimental samples and control samples exhibited similar chelating power, and the initial pH of the medium did not significantly influence the chelating power.

All the parts of *S. cumini* fruits, seeds, leaves, and bark are reported for several beneficial applications. *S. cumini* was reported for rich antioxidant compounds such as phenolics, flavonoids, carotenoids, and vitamins. *S. cumini* was active against degenerative diseases and the pigments of *S. cumini* used as a colorant in food industries [16-18].

The methanolic seed extract of *S. cumini* has been reported for antimicrobial activity against *Bacillus subtilis*, and the activity was associated with damaging the cell wall, reduction in bacterial cell size, and leaking of cellular contents [19].

*S. cerevisiae* mediated fermented *S. cumini* stem (FSS) was evaluated for pharmacological importance. The oral supplementation of FSS for 30 days reduced the blood glucose level, improved the lipid profile, atherogenic index, renal and hepatic function in the diabetic-induced rat [9]. *S. cumini* seed kernel extract (SSKE) was evaluated for α-glucosidase activity and found that SSKE was effective inhibitors of α-glucosidase, and expands glucose tolerance in Goto-Kakizaki rats [20].

The red wine was prepared with *S. cumini* fruits and yeast, and the quality of the wine was compared with commercial red wine. The results

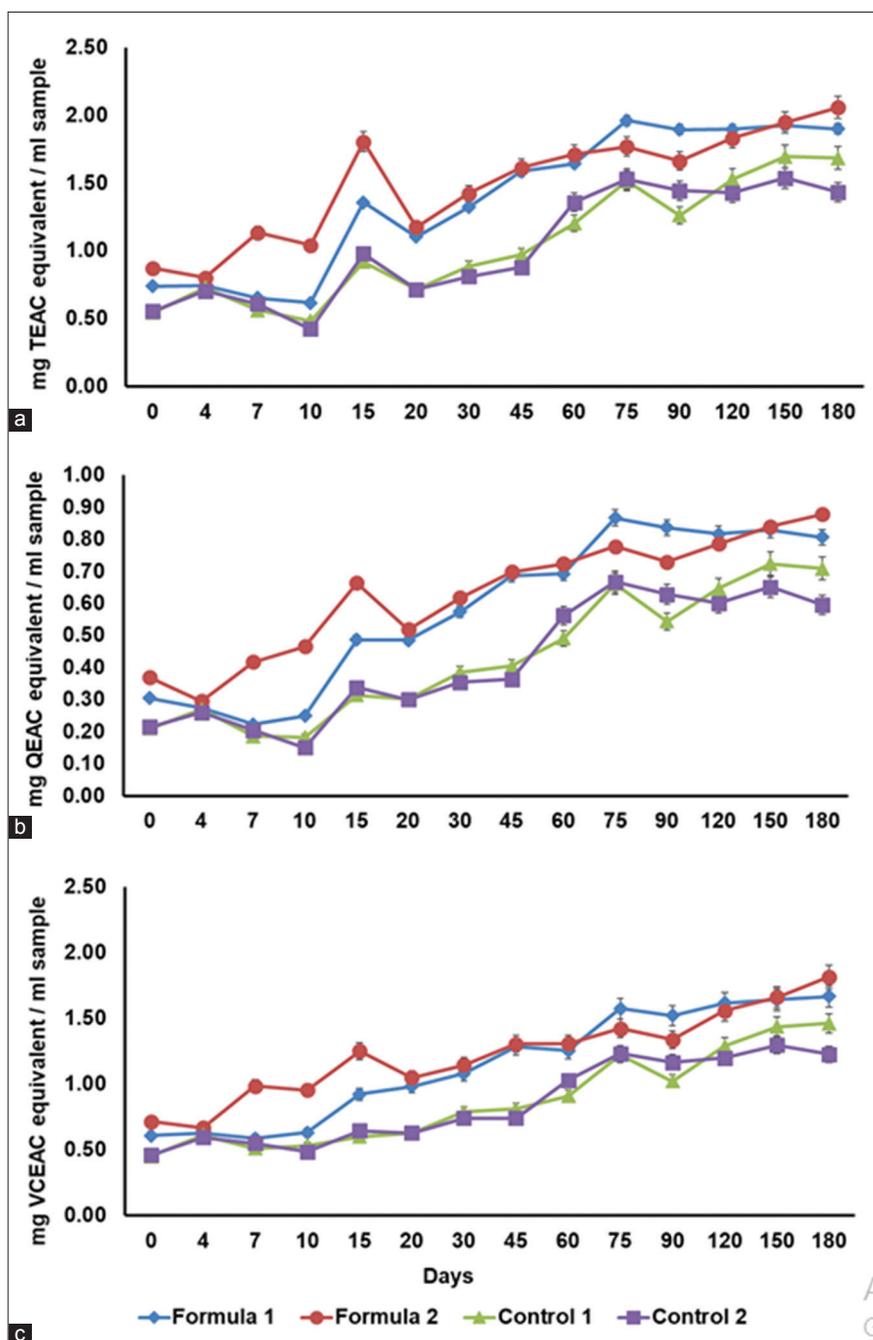


Fig. 3: The total antioxidant capacity of fermented *Syzygium cumini* fruit juice and values were represented as mg trolox equivalent antioxidant capacity (a) quercetin equivalent antioxidant capacity (b) and Vitamin-C equivalent antioxidant capacity (c) per ml sample

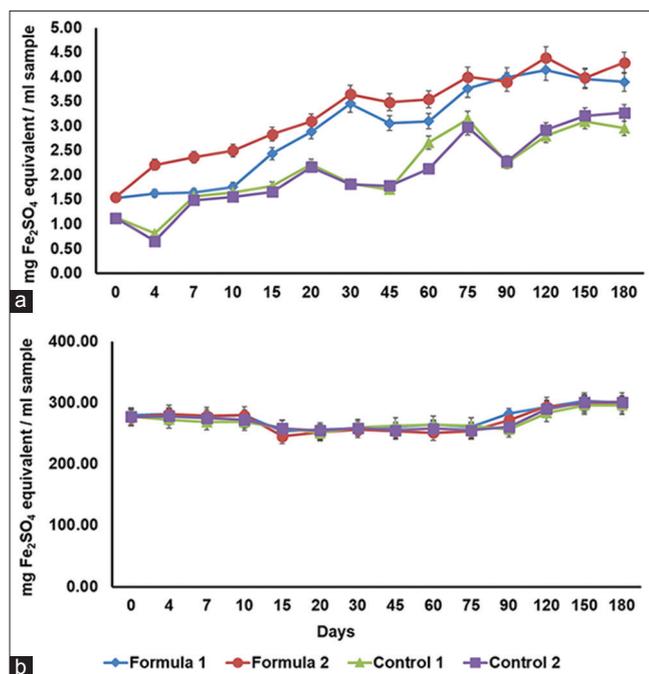


Fig. 4: Ferric reducing antioxidant plasma (a) and chelating power (b) of fermented *Syzygium cumini* fruit juice

suggested that *S. cumini* wine was rich in tannin, low alcohol content, and accepted as an alcoholic beverage by the healthy volunteers [8]. Brandao *et al.* [21] also characterized *S. cumini* fruit wine and reported that the fermentation process, carried out by *S. cerevisiae*, improved the phenolic content, and tannins up to 30, and 27.4%, respectively.

*In vitro* evaluation of *S. cumini* leaf extract (SLE) suggested that SLE reduced the acetylcholinesterase, and adenosine deaminase activity. The overall study revealed that SLE reduced the oxidative stress, inflammation, and other diabetic related consequences [22]. The essential oil extracted from leaves and fruits of *S. cumini* have reported for antioxidant and  $\alpha$ -amylase inhibitory activities [23]. The phytochemicals present in the ethanol extract of stem bark of *S. cumini* are nontoxic and have pharmacological activities [24].

There was no report on the development of LAB mediated FSJ. The present study was the initial report on *L. paracasei* FSJ. The results suggested that the TPC and antioxidant capacity of *S. cumini* fruit were significantly increased during fermentation.

## CONCLUSION

We have successfully developed the LAB mediated FSJ and found that the resulting fermented juice rich in phenolic compounds, and antioxidants. The fermentation was conducted for 6 months; the quality of the juice was increased with duration of the fermentation. The initial pH of the fermentation medium was not suggestively affected the final quality of the product. The probiotic containing FSJ is an alternative functional food supplement to prevent, cure and manage degenerative disorders especially diabetes. Further, clinical studies are necessary to figure out the functional quality of FSJ.

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## AUTHOR'S CONTRIBUTIONS

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

## CONFLICT OF INTEREST

There is no conflict of interests.

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