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Research Article
Effect of Green Tea Extract During Lactic Acid Bacteria Mediated Fermentation of *Morinda citrifolia* Linn. (Noni) Fruit Juice

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Abstract

Background and Objective: Fermented noni (*Morinda citrifolia* L.) fruit juice is considered as one of the health-promoting beverage. The food industries are working on further improvement of fermented noni juice. The objective of the current study was to assess the impact of green tea (GT) extract during the lactic acid bacteria (*Lactobacillus plantarum* SK15) mediated fermentation of noni fruit juice. **Materials and Methods:** The clean-diced noni fruits were blended with sugar, water, 10% SK15 and GT extract. The mixture was kept at 30°C for 25 days. During fermentation, samples were collected. The changes in pH, acidity, alcohol, sugar, pectin content, total phenolic content (TPC), antioxidant capacity (AC), pectin methylesterase (PME) activity and microbial load were assessed. **Results:** The fermented noni fruit juice exhibited significantly low pH, sugar and pectin content. TPC and AC were increased after fermentation. The alcohol content, especially methanol volume was increased in all the samples but not exceed the lethal level. The samples with GT extract exhibited superior quality in all measured aspects. Notably, PME activity was suppressed by GT extract, which was reflected in the methanol content of the respective samples when compared to control. **Conclusion:** The results suggested that GT extract could be used in the production of fermented plant beverages to prevent the indigenous PME activity (to reduce the methanol formation) and to improve the AC of the product. Further studies are required to know the fate of other phytochemicals and volatile compounds in noni fruit juice during fermentation.

Key words: *Morinda citrifolia* L., noni, lactic acid bacteria, green tea, pectin methylesterase, antioxidant capacity, phenolic content

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the well-known Rubiaceae family plants is *Morinda citrifolia* L., commonly identified as noni. The leaves and fruits of noni have been used as food and medicine in South East Asia. The young leaves of noni and fermented noni juice are commonly consumed in Thailand[1]. Noni is deliberated as important medicinal plants among the Polynesian people[2].

The phytochemical content of noni has been reported and about >200 phytochemicals were described. The composition and concentration of the phytochemicals varied between the parts of the plants and also greatly influenced by the environmental, cultivation and harvesting time. The noni plant has been used as health-promoting phytomedicine for treating cancer, inflammation, diabetic, wound healing, tuberculosis, analgesic, immune enhancement, etc. Besides, noni juices are used as preservative and insecticide[3].

The use of noni products has been increased among people. As per the European Union authority, Noni fruit juice was popular worldwide in 2003 and is considered as potent health-promoting drink under the novel food regulation[4].

The traditional way of preparation of fermented noni juices employed natural fermentation for 4-8 weeks at the optimum temperature[5]. Most of the natural fermentation process of noni affects the quality of the product in terms of unwanted chemical content as a result of microbial contamination and uncontrolled chemical reactions[6]. Lactic acid bacteria (LAB) are commonly used as a starter for the production of several fermented products. LAB mediated fermentation process yielded a desirable product with required the quality. LAB mediated fermentation process can improve the pharmacological and cosmeceutical properties of plant juices[7-10].

Green tea (*Camellia sinensis* L.) is one of the highly consumed beverage worldwide. Green tea is rich in bioactive phytochemicals, antioxidants and minerals. The fermentation process and aging altered the phyto-composition of the tea[11]. Several health beneficial effects of green tea have been reported include cardiovascular improvement, neuroprotection, cholesterol-lowering property, antioxidant, anti-stress, anti-photoaging[12,13].

There was no detailed reported about the influence of green tea extract on noni fruit juice fermentation mediated by LAB. Thus, the objective of the current study was to study the effect of green tea extract during the fermentation of noni fruits using *Lactobacillus plantarum* SK15. The changes in pH, total acidity, sugar content, total phenolic content, antioxidant capacity, pectin content, pectin methylesterase activity, alcohol content and microbial load have been assessed kinetically to determine the impact of the addition of green tea in the noni fermentation process.

MATERIALS AND METHODS

Fresh *M. citrifolia* L. fruits were collected from the local market of Sunkampang, Chiang Mai, Thailand. The plant species was confirmed with aid of herbarium specimen (Voucher number 023238) of the Faculty of Pharmacy, Chiang Mai University. The LAB starter culture, *Lactobacillus plantarum* SK15, was obtained from Innovation Center for Holistic Health, Nutraceuticals and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Thailand. The chromatography column (Carbowax 20M polyethylene glycol capillary column) was purchased from Ohio Valley Specialty, USA. The analytical grade chemicals and reagents were purchased from Sigma-Aldrich, USA.

Starter culture preparation: *Lactobacillus plantarum* SK15 was cultured in MRS (de Man, Rogosa and Sharpe) broth at 37±2°C for 24 h or grown-up to the cell concentration of 10^9 CFU mL^{-1}. About 10% inoculum of SK15 was used for the fermentation purpose.

Green tea extraction: Green tea (*Camellia sinensis* var. *assamica*) was prepared by the infusion method. About 1 g of green tea was infused in hot distilled water (80°C) and added absolute ethanol at room temperature to reach the ethanol concentration of 40%. Green tea was infused for 30 min and the extract was filtered through Whatman No. 10 filter paper under vacuum. Samples were concentrated by using rotary evaporator (Eyela, N-1001, Tokyo Rikakikai Co., LTD. Japan). Final green tea (GT) extracts were kept at 4°C for further use[14].

HPLC analysis of EGCG: The concentration of EGCG in GT extract was determined using high-performance liquid chromatography (HPLC), Shimadzu, SPD-20A, Japan as detailed previously[15]. ACE Generix 5 C18 column (4.6 mm× 25 cm; Advanced Chromatography Technologies Ltd, Scotland) was used. 0.05% (v/v) trifluoroacetic acid: acetonitrile solution with the flow rate of 0.8 mL min^{-1} was used as the mobile phase. The sample injection volume was 1 µL. The absorption wavelength was 210 nm and experiment was conducted at 30°C.

Antimicrobial activity of GT extract: The minimal inhibitory concentration (MIC) of GT extract against *L. plantarum* SK15 was studied by broth dilution method as detailed previously[16].

Fermentation process: The washed noni fruits were cut into small pieces and blended by using blender (YC112M-4, China). The blended noni fruit was mixed with sugar and water at a ratio of 3:1:10 (Control)+10% of live active starter (ST), +3.75 mg mL^{-1} of green tea extract (ST+GT). The samples were subjected to fermentation. The fermentation processes were carried out up to 600 h at 30°C and samples were collected at different time points and stored at -20°C until use.

pH and total acidity content: The pH and total acidity content of fermented noni fruit samples were measured using pH meter (Metrohm 691) as detailed previously[17]. The total acidity of the samples was measured by titration and the values were represented as lactic acid equivalent per mL. The samples were titrated with 0.0940 N sodium hydroxide using phenolphthalein as indicator[18].

Determination of total phenolic content and antioxidant capacity: Folin-Ciocalteu colorimetric method was employed to determine the total phenolic content (TPC) of fermented noni juice samples as described previously[19]. The TPC of the samples were represented as mg gallic acid equivalent (GAE) per ml of the sample.

2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) methods were engaged to define the antioxidant capacity of noni fruit juice as detailed previously[20]. The antioxidant capacity was represented as mg trolox equivalents (TE) antioxidant capacity per mL of sample.

Determination of total and reducing sugar content: Phenol-sulfuric acid and dinitrosalicylic acid methods were employed to measure the total and reducing sugar content in fermented noni fruit juice as detailed earlier[21,22].

Determination of alcohol content: The amounts of methanol and ethanol in the samples were measured by gas chromatography (GC-14B, Shimadzu, Japan) with Carbowax 20 M polyethylene glycol capillary column (30 m×0.53 mm). Samples were filtered through a 0.22 µm nylon membrane filter and mixed with 50 ppm n-butanol (act as internal standard). The flow rate of the nitrogen carrier gas was set at 40 mL min^{-1}. The temperatures at the injector port, column oven and detector were set at 180, 38 and 260°C, respectively and splitless injection was set at 5 µL for each injection[18].

Determination of pectin content and PME activity: The pectin content and PME activity of the samples were determined as described in our previous report[23].

Enumeration of microorganisms: The total bacterial count (TBC), lactic acid bacteria (LAB), fungi and representative pathogen load (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp.) of the fermented noni fruit juices were estimated by serial dilution and plate count method as detailed previously[22]. Specific media, such as Phenol Red Egg-yolk Agar, Eosin-Methylene Blue (EMB) agar, Mannitol Salt Egg-yolk (MSEY) agar, Salmonella-Shigella (SS) agar and potato dextrose agar, were used for the culturing of specific pathogens. After the appropriate incubation period, colonies were counted and the colony-forming units (CFU) was calculated as follows:

$$\text{CFU mL}^{-1} = \frac{\text{Number of bacterial colonies counted on plate} \times \text{Dilution factor}}{\text{Volume of culture plate}}$$

Statistical analysis: The experiments were carried out in duplicate. The amount of methanol at each fermentation time was analyzed by one-way ANOVA using the statistical SPSS software version 17 (SPSS Inc., Chicago, IL, USA. The values were represented as the Mean±SD.

RESULTS

EGCG content of green tea (GT) extract was measured and about 48.02±2.66 mg g^{-1} extract of EGCG was detected in GT extract. The GT extract showed MIC (minimum inhibitory concentration) of ≥7.5 mg mL^{-1} against *L. plantarum* SK15 (Table 1).

pH and acidity of FNFJ: The pH of the samples was gradually decreased during the fermentation. The control, ST and ST+GT samples showed the pH reduction from 4.62±0.03, 4.27±0.01 and 4.51±0.02 to 3.83±0.05, 3.27±0.06 and 3.50±0.07, respectively (Fig. 1a).

The total acidity of the samples was increased. The total acidity of control, ST and ST+GT samples at the beginning of the fermentation were 0.20±0.04, 0.25±0.06 and 0.42±0.03%, respectively. After 600 h of fermentation process, acidity of the control, ST and ST+GT samples were found as 0.58±0.02, 1.08±0.04 and 0.96±0.01%, respectively (Fig. 1b).

Total phenolic content, antioxidant capacity: The TPC of control, ST and ST+GT samples at the beginning

Table 1: EGCG content and antimicrobial property of green tea extract

EGCG content	48.02±2.66 mg g^{-1} of extract
Minimum inhibitory concentration (MIC)*	≥7.5 mg mL^{-1}

*Against *L. plantarum* SK15

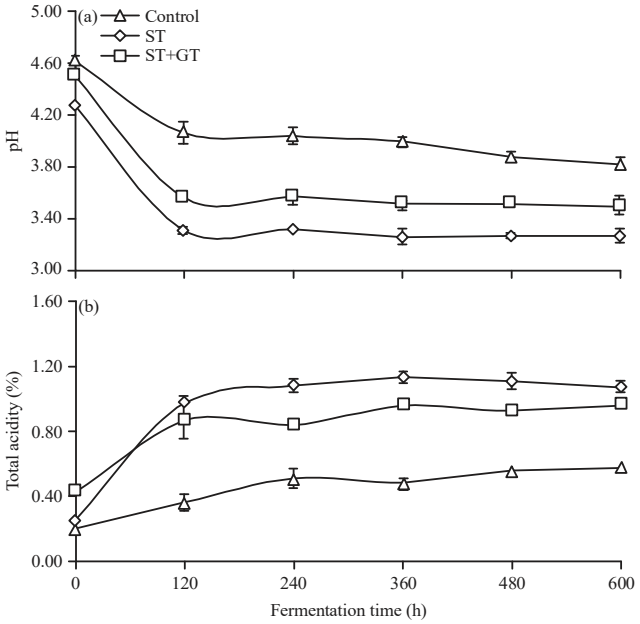

Fig. 1(a-b): Changes in (a) pH and (b) Total acidity level in noni fruit juice during fermentation

of the fermentation were 0.09±0.03, 0.13±0.02 and 0.28 mg GAE mL^{-1}, respectively. The TPC of control, ST and ST+GT samples were found as 0.20±0.02, 0.18±0.01 and 0.51 mg GAE mL^{-1}, respectively after fermentation (Fig. 2a).

The antioxidant capacity (AC) of the samples was measured by DPPH and ABTS assays. About 0.06±0.01, 0.06 and 0.81 mg TE mL^{-1} of AC were recorded in control, ST and ST+GT samples, respectively, in DPPH assay after fermentation, while ABTS assay showed 0.12±0.02, 0.11±0.01 and 1.30±0.04 mg TE mL^{-1} of AC in control, ST and ST+GT samples, respectively (Fig. 2b, c).

Sugar content: The total sugar content was gradually reduced during fermentation. The initial sugar content were 2.51±0.03, 2.52±0.13 and 2.92±0.02% in control, ST and ST+GT samples, respectively. whereas, after fermentation, the sugar content of the samples was found as 0.57±0.23, 0.51±0.04 and 0.18±0.07% in control, ST and ST+GT samples, respectively (Fig. 3a). The reducing sugar content of control and ST samples were reduced during fermentation, whereas ST+GT samples showed an increase in reducing sugar level (1.07±0.01-1.76±0.14%) (Fig. 3b).

Alcohol content: The ethanol content of the control sample was increased from 0.01 to 0.20±0.02% during fermentation. The ST and ST+GT samples do not show any change in ethanol content during fermentation (Fig. 4a).

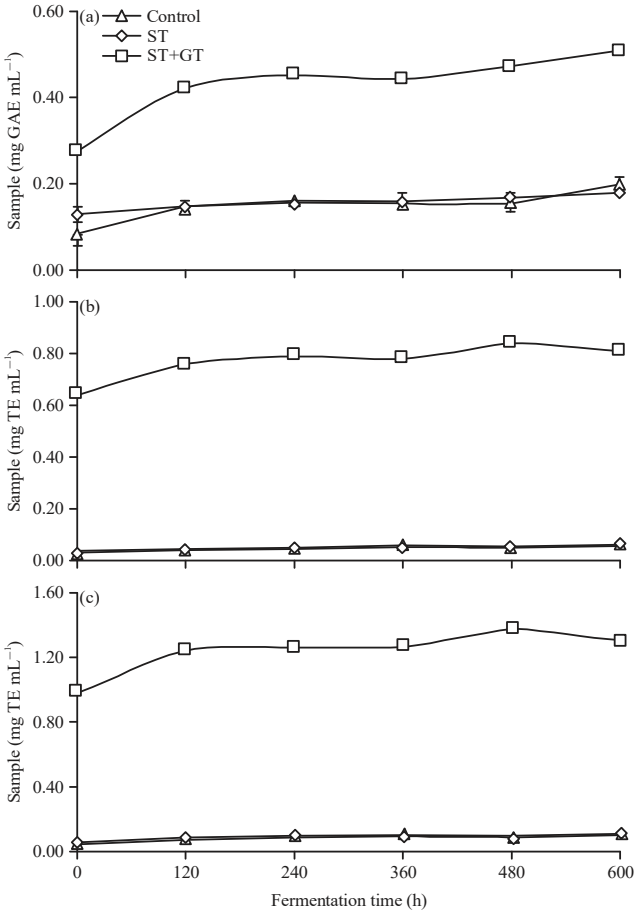

Fig. 2(a-c): Changes in (a) Total phenolic content, (b) Antioxidant capacity (DPPH) and (c) ABTS of noni fruit juice during fermentation

The methanol content of fermented noni fruit juice was increased gradually during fermentation. The control, ST and ST+GT samples showed 95.57±6.16, 83.79±3.85 and 59.54±12.37 ppm methanol at the beginning of the fermentation, respectively. After fermentation, the methanol content was increased in all the samples (Fig. 4b).

Pectin content and PME activity: The pectin content of the samples was reduced gradually during fermentation. The control, ST and ST+GT samples showed about 1.44±0.05, 1.25±0.08 and 1.85±0.31 mg mL^{-1} of pectin, respectively at the beginning of fermentation. After fermentation, the pectin concentration was found as 0.10, 0.97±0.03 and 0.75±0.03 mg mL^{-1} in control, ST and ST+GT samples, respectively (Fig. 5a).

Obviously, The PME activity was increased progressively during fermentation. After 600 h of fermentation, the control, ST and ST+GT samples

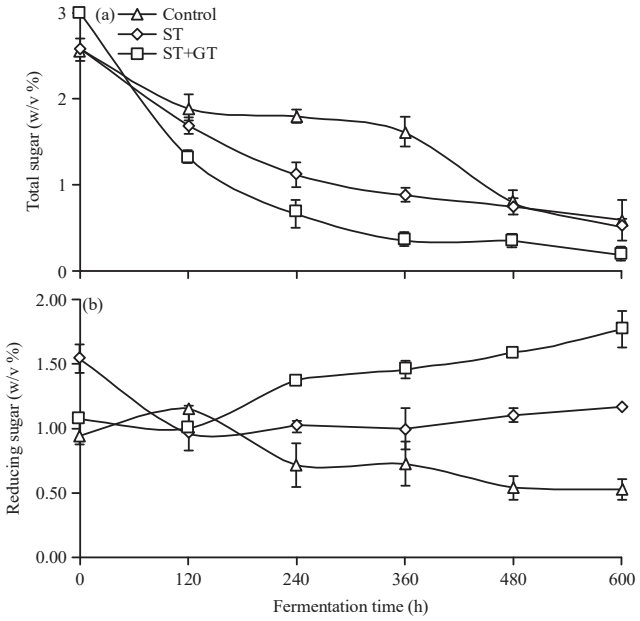

Fig. 3(a-b): (a) Total sugar and (b) Reducing sugar content of noni fruit juice during fermentation

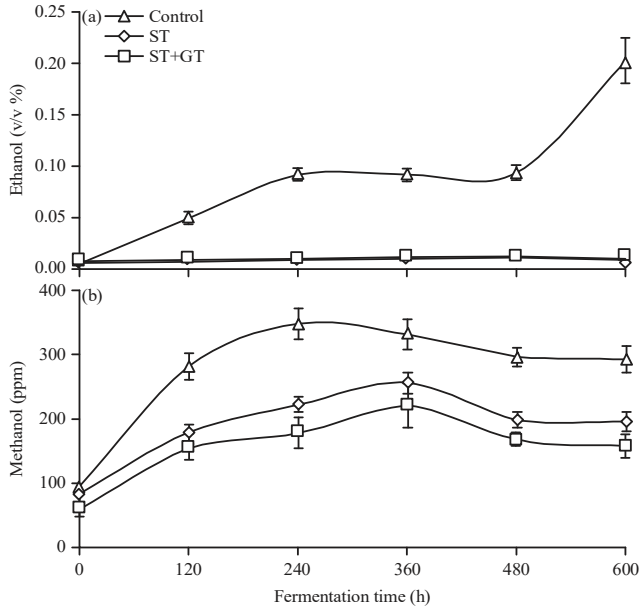

Fig. 4(a-b): Alcohol content of noni fruit juice during fermentation. The changes in (a) Ethanol and (b) Methanol content in fermented noni juice at different point of fermentation

exhibited the PME activity of 43.11±2.92, 36.45±4.47 and 24.07±3.23 mmol mL^{-1} min^{-1}, respectively, while the PME activity at the beginning of fermentation was 5.19±0.35, 4.37±0.80 and 4.98±0.25 mmol mL^{-1} min^{-1} in control, ST and ST+GT samples, respectively (Fig. 5b).

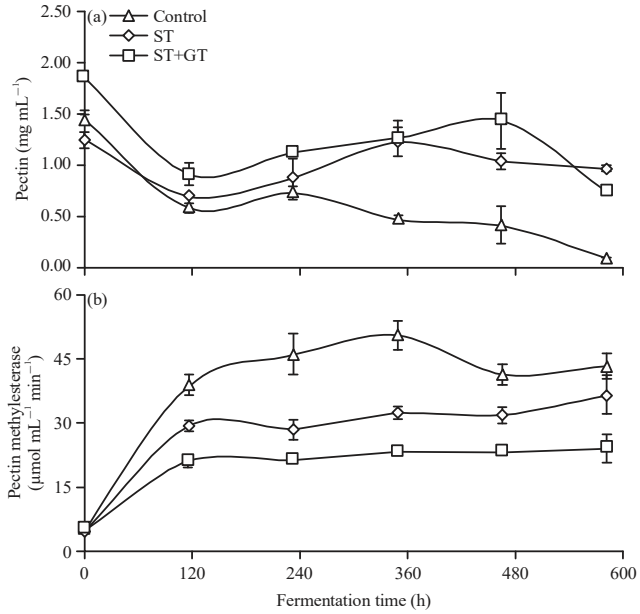

Fig. 5(a-b): (a) Pectin content and (b) Pectin methylesterase activity of fermented noni fruit juice

Microbial load: The total bacterial and LAB load was reduced after fermentation in ST and ST+GT samples. Total bacterial load of 2.31±0.06 log CFU mL^{-1} was detected in control after fermentation, while no bacterial content was noted at the beginning of the fermentation. There were no assessed pathogenic microbes were found in fermented noni fruit juice samples at the beginning and after the fermentation (Fig. 6).

DISCUSSION

Lactobacillus plantarum SK15 mediated fermentation in the presence of GT extract significantly improved the TPC, AC, reduced the PME activity and alcohol content of noni fruit juice (Fig. 2, 4, 5). The use of specific starter culture improved the microbial quality of fermented noni fruit juice (Fig. 6).

The quality improvement of fermented noni fruit juice may be attributed to the potent starter culture, which prevents the growth of contaminating microbes, reduced the pH and increase the acidity of the noni juice due to the lactic acid fermentation. Moreover, GT extract provided the antioxidant enrichment to the product and also suppressed the PME activity and formation of lethal level of methanol during fermentation. GT was not showed strong antagonistic activity against *L. plantarum* SK15 (MIC = ≥7.5 mg mL^{-1}). Thus, the fermentation was mediated by *L. plantarum* SK15 that confers positive effects to the fermented noni fruit juice.

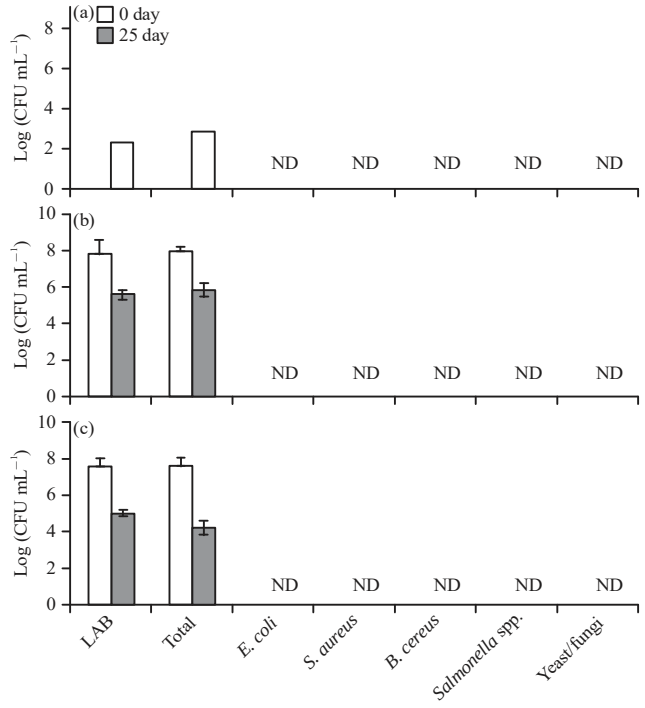

Fig. 6(a-c): Microbiological status of noni fruit juice during fermentation. The changes in the (a) Microbial load of control, (b) ST and (c) ST+GT samples at the beginning and end of the fermentation process

Generally, LAB mediated fermentation decreased the pH of the plant beverages and increased the acidity due to the production of lactic acids[24]. Noni juice exhibited reduced pH value after fermentation with LAB strains when compared to fresh juice. The slight variations were observed between the samples but *L. plantarum, L. casei* and *Bifidobacterium longum* mediated fermentation process significantly reduced the pH and sugar and increased the acidity in noni juice[10]. The reduction in pH, total sugar content and increase in acidity have been recorded in *L. plantarum* SK15 mediated fermented noni fruit juice (Fig. 1 and 3).

The TPC of *L. plantarum* and *L. casei*, fermented noni juices was reduced compared to fresh noni juice while *B. longum* fermentation was not significantly altered the TPC in noni juice. Likely, reducing power and AC of noni juice was reduced after *L. plantarum* and *L. casei* mediated fermentation[10]. In the present study, the fermentation process significantly increased the TPC of noni fruit juice (Fig. 2a). The AC of noni fruit juice was increased after 600 h of fermentation (Fig. 2b, c).

The viability of LAB in fermented plant beverage provides the additional beneficial effects to consumers and it depends on the availability of oxygen, nutrients, duration of fermentation and storage, pH, etc. The noni juices fermented for up to 72 h showed an increase in LAB content after fermentation[10]. In the present study, the level of LAB was decreased after 600 h of fermentation, but a notable amount of live LAB in the fermented noni samples. Possibly, due to the lack of sufficient nutrients in the fermentation medium, since the fermentation period was longer. Since the noni is a potent antimicrobial agent, there was no pathogenic growth was observed in fermented samples (Fig. 6).

The pasteurization of noni fruit before fermentation process inactivated the PME activity and reduced the methanol formation in fermented noni juice[23]. The green tea catechins are an inhibitor of PME[25]. In the present study, non-pasteurized surface-sterilized noni fruits were used. The addition of GT extract significantly reduced the PME activity when compared to control and ST samples (Fig. 5b). Thus, the addition of GT extracts reduced the formation of methanol in fermented noni fruit juice (Fig. 4b). Moreover, GT extract addition improved the quality of fermented noni fruit juices in terms of TPC and AC when compared to control. Additionally, the viability of *L. plantarum* was not affected by the GT extract. The results of the current study suggested that the addition of GT extract during the fermentation of noni fruit juice might improve the quality of the product with additional health benefits. Besides the use of starter culture, prevent the generation of harmful metabolites in fermented noni juice but not greatly control the PME activity.

CONCLUSION

The pH, acidity, sugar content, alcohol content, TPC, AC and microbiological quality of the noni fruit juice were improved after fermentation. The use of specific starter culture (*L. plantarum* SK15) nullified the formation of unwanted metabolites when compared to control. Moreover, the addition of GT extracts generally improved the quality of *L. plantarum* SK15-mediated fermented noni fruits. The results suggested that GT extract could be used in the production of fermented plant beverages to prevent the indigenous PME activity (to reduce the methanol formation) and to improve the AC of the product. Further studies are required to know the fate of other phytochemicals and volatile compounds in noni fruit juice during fermentation.

SIGNIFICANCE STATEMENTS

This study discovers the effect of the addition of green tea extract during the production of fermented noni fruit juice. This study will help the researcher to uncover the critical area

of methanol contamination during fermented fruit juice preparation, which provides an idea to produce enriched, in terms of bioactive phytochemicals, noni fruit juice. Thus, a new theory on production of enhanced functional fermented noni fruit juice may be arrived at.

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