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S Thirukkumar
Senior Research Fellow, Home Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

P Vennila
Professor (Food Science and Nutrition) and Head, Department of Home Science Extension, Home Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

T Uma Maheswari
Assistant Professor, Department of Food Science and Nutrition, Home Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

Correspondence
S Thirukkumar
Senior Research Fellow, Home Science College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

Investigation of total antioxidant activity and phenol in Indian noni fruit (*Morinda citrifolia* Linn.) juice extraction

S Thirukkumar, P Vennila and T Uma Maheswari

Abstract
Noni is called for Indian mulberry (*Morinda citrifolia* Linn.) and it has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects. The aim of this present study was to find out the total antioxidant and total phenol content for different methods of noni fruit juice extraction. The ripened fruits were subjected to hot process, where steam blanching was done at 80 ºC for 2 to 10 min, and then cooled at room condition and the juice was then extracted. In cold process, the ripened noni fruits were frozen (-18 ºC) for 6 to 30 hours, thawed and the juice was extracted. In the fermentation process, ripened fruits were kept in an airtight container at room temperature and juice was collected by seeps out from the fruits after 30, 60 and 90 days. The parameters such as total antioxidant (2, 2, diphenyl-1-picrylhydrazyl scavenging) activity and total phenolic content from extracted juice were studied. The results showed that the juice samples extracted from cold processing showed a best total antioxidant activity (4.018 to 4.001 mg/g) and total phenolic content (341.2 to 334.5 gallic acid equivalent/100g) compared with control, hot and fermented process of extraction juice. The cold extraction technique was simple and innovate extraction process with protected and higher quantity of antioxidants and phenol compounds.

Keywords: Noni fruits, Extraction, total antioxidant activity, total phenols

1. Introduction
Noni is called for Indian mulberry (*Morinda citrifolia* Linn.). It originates from Southeast Asia such as Indonesia and Australia [1]. In 2002, noni juice was accepted in the European Union as a novel food [2]. There are 160 phytochemical compounds have been identified in the noni plant the majority of the micro-nutrients are phenolic compounds, organic acids and alkaloids [3]. Particularly, the fruit juice from noni is in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems, and drug addiction [4, 3]. Mostly noni is consumed as extracts from its fruits although leaves, flowers, bark and roots were also used [5, 6]. The use of concentrates of this juice, preserved juice drinks, encapsulated freeze-dried fruit juice, concentrated extracts, powders, tinctures, and fruit leather form has become massive due to the medicinal properties attributed to noni, such as being anti-microbial, anti-carcinogenic, analgesic and anti-inflammatory. For reasons that noni is so much valued, uses and demand of noni is not only high in its producing countries but also in other countries such as United States, Japan, India and Europe [7]. Commercially noni juice was traditionally made by fermentation of ripened noni fruits in sealed containers for 2 months at ambient temperature. Some noni juice was made by boiling of noni fruits for hours and many Pacific islanders use fermentation to make noni juice at home by placing noni fruits in jars in outdoors under the sun for months [1]. In these processing, storage, light, temperature and oxygen can promote the undesirable chemical reactions that can reduce the medicinal components of the juice [8].
The aim of this study was to investigate the changes of the total antioxidant activity and phenolic content during noni juice extraction.

2. Materials and Methods
Fresh noni fruits used were obtained from Horticultural College and Research Institute, Periyakulam, Tamil Nadu. The fruits were selected at maturity stage 4 (hard white) [7]. The fruits were allowed for ripen at room temperature (30±2 ºC) for 2 days under closed condition before treatments. The weighed ripened fruits were washed using tap water and subjected to hot process technique, where on steam blanching was done at

100 ºC for 2, 4, 6, 8 and 10 min respectively, and then cooled at room condition for 5 min. Another technique was cold process method, where washed ripened noni fruits were kept on frozen at -18 ºC for 6, 12, 18, 24, 30 hours respectively and thawed at room temperature for before evaluation. After pretreatments the juice was extracted by pressing and passing in nylon net [9]. In the fermentation process, ripened fruits were kept into an airtight container at room temperature (30±2 ºC) and juice was collected by seeps out from the fruits after 30, 60 and 90 days. The changes of total antioxidant activity and total phenols were analyzed from extracted juice from noni fruits.

Total antioxidant activity (DPPH assay)

The total antioxidant activity was estimated by the 2, 2, diphenyl-1-picrylhydrazyl (DPPH) assay, according to the method [10]. Stock solution of the fruit extract was prepared in methanol from which a serial dilution was carried out to obtain concentration of 5µg/ml, 10µg/ml, 50µg/ml, 100µg/ml and 500µg/ml. Diluted solutions (2ml) were added to 2 ml solution of DPPH then mixed and allowed to stand for 30 minutes for reaction to occur. Absorption of the samples was measured at 517 nm against a blank of methanol without DPPH. Then percent inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed three times and average absorption was noted for each concentration.

Total phenol content

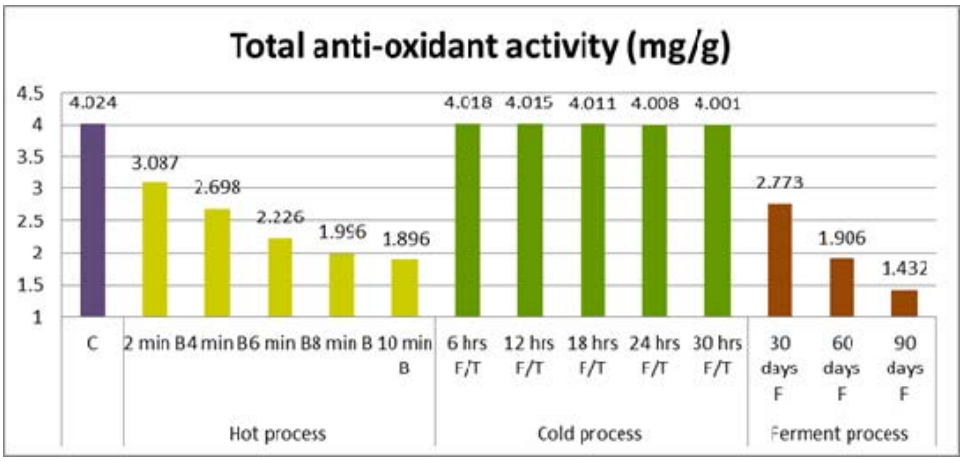
The total phenolic content was determined according to the method [11] with slight modifications. Different aliquots (0.2 - 2 ml) of methanolic extracts were made up of 3ml with distilled water. Then it was oxidized with addition of 0.5 ml of Folin-Ciocalteu reagent and neutralized by adding 2 ml of 20% Na_2CO_3 solution. The reaction mixture were placed in boiling water bath for one minute and cooled. The absorbance was measured at 650 nm using a spectrophotometer against reagent blank. A standard curve was prepared using different concentration (10 - 100 µg ml^{-1}) of gallic acid and reported as gallic acid equivalent per ml of sample.

Statistical analysis

All the experiments were conducted in triplicate and standard deviation (±SD) was calculated using MS Excel software. The significance of each sample was established by the ANOVA using Factorial Completely Randomized Design (FCRD) and it was considered to be significant at $p<0.05$.

3. Result and Discussion

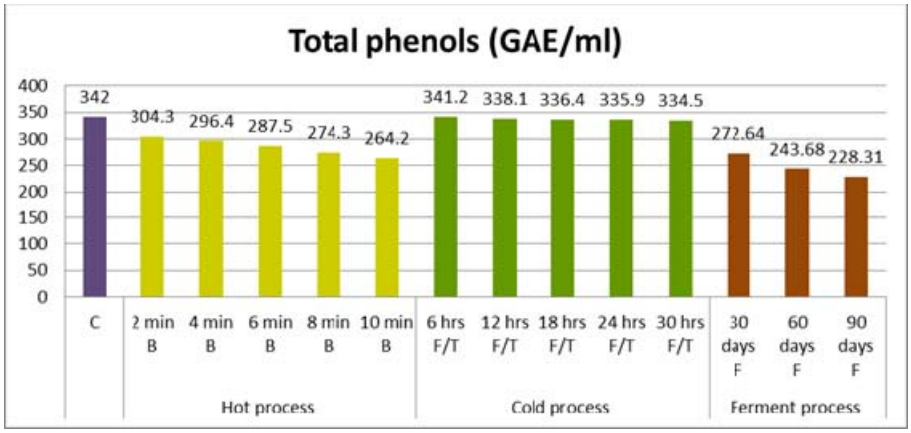
A reduction in the total antioxidant activity was observed in all the extracted noni juice (Figure -1). The antioxidant activity of the extracted noni juice in control was 4.024 mg/g, which was significantly differ with the hot and fermented extracted noni juice. Increase the blanching time in hot process of juice extraction, the antioxidant activity was systematically drawn from 3.087 to 1.896 mg/g on 2 to 10 minutes blanching. The cold extracted noni juice sample was significant with control extracted sample as it had 4.018 to 4.001 mg/g on 6 to 30 hours frozen and thawed extracted noni juice. The maximum decrease of DPPH inhibition was observed in 60 and 90 days of fermented noni extracted juice samples. During extraction of juices, the losses of certain compounds have been occurred by chemical changes due to thermal degradation, Maillard reactions or oxidation induced by heating [12, 13]. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation [14]. Ascorbic acid in noni may be a major non phenolic antioxidant contributing to the great decrease of radical scavenging activity during noni processing [8]. Thermal treatments are, generally, the main cause of depletion of natural antioxidants and phenolic content [15, 16].



C- Control B-Blanching F/T- Freezing and thawing F- Fermentation

Fig 1: Total antioxidant activity (mg/ml) of extracted noni juice

A notable quantum of total phenol losses were seen in all the hot and ferment treated extracted noni juices (Figure - 2). The total phenol content was 342.0 GAE/ml in control. The corresponding values changed from 304. 3 to 264.2 GAE/ml in 2 to 10 min blanching, 341.2 to 334.5 GAE/ml in 6 to 30 hour freezing and thawing, 272.64 to 228.31 GAE/ml in 30 to 90 days fermented extracted noni juice. The highest quantity of total phenols loses was found in 10 min blanching extracted juice and 90 days of fermented noni extracted juice which was 264.2 and 228.31 GAE/ml respectively. Higher heating temperature has affected the significant reduction of clarity, viscosity and total polyphenol content of juice during extraction [17]. The several factors including the nature, sources of raw materials, fermentation process and the extraction medium be partly responsible for causes of decreases the phenolic content [18]. The fermented extracted noni juice was lost a significant percentage of radical scavenging activity and total phenolic content [8].



C- Control B-Blanching F/T- Freezing and thawing F- Fermentation

Fig 2: Total phenol (GAE/ml) of extracted noni juice

4. Conclusion

Among the various extraction techniques experimented on noni juice, not much changes of total antioxidant activity and phenolic content was observed in cold process extracted noni juice. It was extremely on par with control, hot and fermented process of extracting noni juice was found to be with less total antioxidant activity and phenol content. So for maximum potential health benefits and commercialization of noni products to consumers, extraction of noni juice by cold processing is strongly recommended.

5. References

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