Antioxidant Capacity and Total Phenolic Content Variations against *Morinda citrifolia* L. Fruit Juice Production Methods

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***Abstract*— Variations of antioxidant capacity (AC) and total phenolic content (TPC) of noni juice (*Morinda citrifolia* L) under different production methods and production time were studied. AC and TPC of noni juice were determined by DPPH (2-diphenyl-1-picrylhydrazyl) scavenging activity, and Folin-Ciocalteu reagent assay respectively. Traditional fermentation method resulted maximum AC (83.84% of DPPH scavenging activity) and maximum TPC (2698.89 mg gallic acid equivalent/L of noni juice) within two weeks of fermentation. Fresh juice had AC (77.06% of DPPH scavenging activity) and TPC (2898.89 mg gallic acid equivalent/L of noni juice). When fermenting the extracted fresh juice, AC (29% loss) and TPC (95% loss) were drastically reduced within one month of fermentation. Both traditional fermentation and fresh juice extraction had satisfactory levels of AC & TPC but allowing fresh noni juice for fermentation is not recommended.**

***Index Terms*—antioxidant capacity, fermentation, noni juice, total phenolic content**

I. Introduction

Morinda citrifolia L. which is generally known as "noni" is a traditional medicinal plant used among cultures in Southeast Asia and Australasia for over 2000 years [1], [2]. Particularly, noni fruit juice has been identified to have significant therapeutic and nutritional values which are effective for treating many diseases including cancer, hypertension, inflammation [3], diabetes [4], cardiovascular diseases, infection, arthritis, asthma, pain [5], mental depression [6], diarrhea, indigestion. Noni fruit juice also has been used as a dietary supplement, a food functional ingredient, or as a natural health enhancer throughout the world [7]. It is commercially available in fermented, unfermented and dried powder forms [8]. Noni juice is obtained by either natural fermentation process or unfermented process of compressing the fresh fruits [2]. More than 150 bioactive compounds have been identified in noni fruit and many of them are antioxidants, phenolic compounds, organic acids or alkaloids [8].

Antioxidants and phenolic compounds in noni juice play significant roles in disease controlling and preventing. Their contribution to free radical scavenging activity can prevent oxidative stress occurred in cells [2]. It has been studied about the effects of extracting [8], processing and storage methods [9], [10] for the antioxidant capacity (AC) and total phenolic content (TPC) of noni juice over the time. Yet, precise studies are necessary to specify the variance of AC and TPC of noni juice under different juice production methods and periods.

The antioxidant capacities of noni fruit juice can be determined by scavenging of DPPH free radicals while total phenolic content can be determined by Folin–Ciocalteu assay [10]. Noni juice is usually produced by placing soft, yellowing, translucent ripe fruits in sealed containers and allowing traditional fermentation for two months at ambient temperature. Fresh noni juice is manufactured by pressing fresh ripe fruit or drip extraction method [9]. Therefore, there can be variations of AC and TPC of noni juice depending on the production method and production period. In this study, we focused on evaluating variations of antioxidant capacity and total phenolic content of noni juice in the forms of traditionally fermented juice, fresh juice, fermented noni juice (Fermentation for extracted of fresh juice) derived from *Morinda citrifolia* L. grown in Sri Lanka.

According to the results of this study, traditionally fermented juice and fresh noni juice have satisfactory levels of AC and TPC with respect to the fermented noni juice produced from fresh juice. However, to maximize the effective therapeutic value in traditional fermentation method, fermentation should continue up to maximum two weeks. After that, AC and TPC reduced due to the several reactions happening and other environmental factors. Allowing the fermentation process for the fresh noni juice is not recommended since a severe loss of AC and TPC can be resulted.

This is a highly overlooked area with a smaller number of accomplished researches. It has gained a notable demand trending in the world over several decades due to its lesser side effects, despite the western medicines [11]. Optimization of the yield of noni juice production while obtaining maximum therapeutic value is one future

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research area to be studied regarding *Morinda citrifolia* L. fruit juice.

This paper is arranged into following sections: Section II described the existing related research studies. Section III consists of materials and methods used in this study. Section IV provides results and discussion of our research work and finally, the conclusions we came from this research are included in Section V.

II. Related Work

Morinda citrifolia L. has several phytochemicals containing attributes for antibacterial, antioxidant, antiviral, anti-inflammatory and improving immune system. However, noni fruit has been identified as the most valuable part in the plant which provides nutritional as well as therapeutic value. This therapeutic potential of noni juice can be varied depending on harvest location, processing methods and other physical conditions [7]. Antioxidant properties of noni juice have been highly recognized, and it can be evaluated by antioxidant capacity (AC) and total phenolic content (TPC) [10].

As Yang described, fresh noni juice derived from noni fruits from Guam had TPC of 210 mg gallic acid equivalent per 100 ml of juice. Their study reveals that 15-20% of TPC of fresh noni juice can be lost by 24 h of heat treatment at 65 ^{0}C or 75 ^{0}C [9]. The study conducted by Bramorski concludes that commercial noni juice (from a distributor of Brazil) had a higher value of TPC (91.90 mg of gallic acid equivalent per 100 ml of juice) with respect to its 5% diluted constituents (grape and blueberry) [12]. Reference [13] reveals that TPC can be increased gradually by using a membrane separator.

Reference [9] shows that traditional fermentation of noni fruit for three months caused radical scavenging activity (RSA) more than 90% while storage of fresh noni juice for three months also caused more than 90% loss of RSA. According to Liu, isolation of antioxidative compounds from fermented Xisha noni juice can be varied depending on the solvent (Ethyl acetate/petroleum ether/n-Butanol) used. Maximum scavenging activity of Xisha noni juice towards hydroxyl radical was 70.3±6.1% exhibited by Ethyl acetate extract [6]. According to a study by Krishnaiah, optimum DPPH radical scavenging activity obtained by isolated juice extract using methanol with Poly-ethersulphone membrane separator was 55.60% [13].

III. Materials and Methods

In this research study, variations of Antioxidant Capacity (AC) and Total Phenolic Content (TPC) of noni juice depending on the juice production method were studied. For that, juice was first produced from traditional fermentation, fresh juice extraction, and fermentation of freshly extracted juice. Fermentation processes were carried out in small scale air sealed fermenters. Then, these three forms of noni juice were analyzed for their AC and TPC by using DPPH free radicals scavenging method and Folin-Ciocalteu reagent assay. For DPPH assay and Folin-Ciocalteu reagent assay, standard curves should be constructed for quantification of AC and TPC. Two production methods of fermentation were also analyzed for their AC and TPC variation with fermentation time. Finally, the maximum values of AC and TPC of both fermented forms of noni juice were compared with AC and TPC of fresh noni juice.

A. Noni Fruits

Unripe noni fruits were picked from University of Moratuwa premises and they were first cleaned by raw water to remove possible dirty particles on the surface of noni fruits and then washed properly with distilled water. After that, they were ripened by three-four days while kept in open for air drying. Unripe noni fruits changed from hard white state to soft, yellowing and translucent state during ripening.

B. Chemicals and Other Equipment

2, 2-dipheny-l-picrylhydrazyl (DPPH), ascorbic acid, Folin-Ciocalteu reagent, gallic acid, and sodium carbonate were purchased from Sigma-Aldrich (St.Louis, MO, USA). Ethanol and methanol, UV-1800 Shimadzu Spectrophotometer, S-shape fermenter airlock, IV set (for sample collection), 5ml injection syringes, stainless steel strainer, 2 air-tight polypropylene (PP) containers (1 L), Nylon Syringe Filters 0.45 µm, fruit juice extractor were used for sample preparation and AC and TPC assays.

C. Traditionally Fermented Noni Juice Production

Traditional fermentation was followed. Ripe noni fruits were placed in clean polypropylene (PP) airtight container (fermenter 1-1 L) and it was sealed properly. S-shape fermenter airlock was attached to the lid of the container. Inside the fermenter 1 as in Fig. 1, Perspex (polymethyl methacrylate) board with a square of stainless-steel strainer attached to the bottom was placed inside the fermenter for the primary filtration. After one-two days, juice coming from fruits were transparent. After onwards, color changed into a darker color with time. Traditional fermentation of noni juice was carried indoors at an ambient temperature of 30 0C for about two months. Sampling for AC & TPC assays was done at various intervals for two months. Each juice sample was membrane filtered and then diluted at ten times ratio.

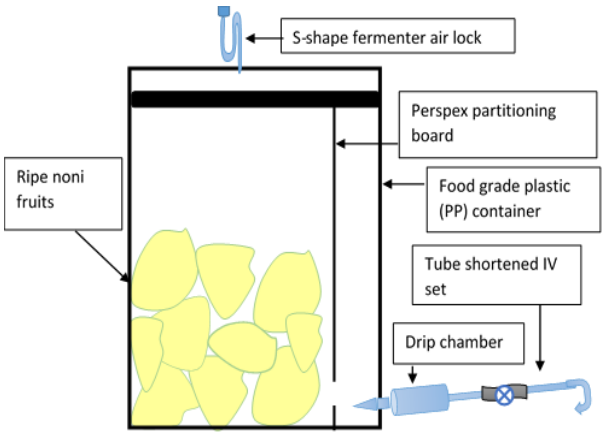

Figure 1. Schematic diagram of experimental setup for traditional fermented noni juice production (fermenter 1)

D. Fresh Noni Juice Production

Five ripe noni fruits were taken and cut into pieces by a knife. Then, pieces of noni fruits were added into previously cleaned fruit juice extractor without adding any water. The fruit juice pulp was extracted using the fruit juice extractor in the laboratory, and then it was diluted with distilled water in ten times ratio and mixed properly. Diluted fruit juice pulp was then filtered by two nylon strainers with different sizes. Finally, membrane filtered fresh juice sample was used for AC & TPC assays.

E. Fermented Noni Juice Production

Fresh noni juice was first prepared as mentioned in fresh noni juice preparation. Prepared diluted juice was then placed in clean polypropylene (PP) airtight container (fermenter 2-1 L) and it was sealed properly. Fermenter airlock was attached to the lid of the container. Inside the fermenter 2, Perspex (polymethyl methacrylate) board with a square of stainless-steel strainer attached to the bottom was placed inside the fermenter for the primary filtration. Then, extracted noni juice was allowed indoor fermentation for about one month at an ambient temperature of 30 ^{0}C. Sampling was done at various intervals for one month during fermentation for AC & TPC assays. Each juice sample was membrane filtered and then diluted with distilled water at ten times ratio.

F. DPPH Assay for AC Analysis

Antioxidant capacity of samples was determined by the methodology based on scavenging DPPH free radicals by the inherent antioxidants in noni fruit [9]. Decreased absorbance of DPPH solution is a measurement of RSA [14]. For all DPPH assays, DPPH dissolved in methanolic solutions were freshly prepared to ensure the protection from light.

The standard curve of DPPH concentration against absorbance was needed to calculate remaining DPPH concentrations in the juice medium. For that, a standard series of DPPH dissolved in methanol with different concentrations (0, 0.78125, 1.5625, 3.125, 6.25, 12.5, 25 mg/L) was prepared. Then series of freshly prepared samples were kept for thirty minutes at a dark environment and the absorbance of each sample was measured at 517 nm with the UV-1800 Shimadzu Spectrophotometer (Shimadzu Cooperation, Kyoto, Japan). Methanol was the blank used in this assay. The results obtained for DPPH standard curve were plotted as DPPH concentration vs. absorbance at 517 nm [15]. The correlation (1) obtained by the linear regression for the DPPH standard curve is shown in Fig. 2.

$$A_{517\,nm} = 57.209 \times [DPPH^{\cdot}]_t - 0.0111 \quad (1)$$

where $[DPPH\cdot]_t$ was expressed as g/L with $R^2 = 1$

Traditionally fermented juice / fresh juice / fermented juice samples were assayed for AC. 100 µl diluted noni juice sample was added to 4 ml of a solution of 0.025 g/L DPPH in methanol. After thirty minutes, the absorbance of noni juice- DPPH solution was measured at 517 nm with the UV-1800 Spectrophotometer. Remaining DPPH concentrations were calculated from the derived DPPH standard curve. Then, the remaining DPPH percentages were calculated by (2) [13].

$$DPPH_{rem} = \left(\frac{DPPH_{30}}{DPPH_0}\right) \times 100\ \% \quad (2)$$

Where $DPPH_0$ is the initial concentration of DPPH free radicals and $DPPH_{30}$ is the concentration of DPPH free radicals after thirty minutes. Finally, AC has been expressed by the DPPH scavenging activity percentage of noni juice.

G. Folin-Ciocaleu Reagent Assay for TPC Analysis

Total phenolic content of noni juice was estimated using the Folin-Ciocalteu colorimetric method described by Slinkard & Singleton [16]. Gallic acid standard curve was required to carry out the quantification of TPC. For that, a standard series of gallic acid dissolved in ethanol (~95% purity) with different concentrations (0, 50, 100, 150, 250, 500 mg/L) was prepared. 40 µL from each solution was added to 3.16 ml of distilled water and then to 200 µL of 2N Folin-Ciocalteu reagent. Then the solution was mixed properly and kept for four minutes. After that 600 µL of 20% (w/v) sodium carbonate solution (Na_2CO_3) was added to each solution. Then the series of samples was kept for two hours at a dark environment and after that absorbance of each sample was measured at 765 nm with the UV-1800 Shimadzu Spectrophotometer (Shimadzu Cooperation, Kyoto, Japan).

Then, absorbance results at 765 nm were plotted against gallic acid concentrations to obtain the standard curve required for this TPC assay. The correlation (3) obtained by the linear regression for the gallic acid standard curve shown in Fig. 3.

$$A_{765\,nm} = 0.0009 \times [\text{Gallic acid}]_t - 0.0049 \quad (3)$$

where $[\text{Gallic acid}]_t$ was expressed as mg/L with R^2 = 0.9681

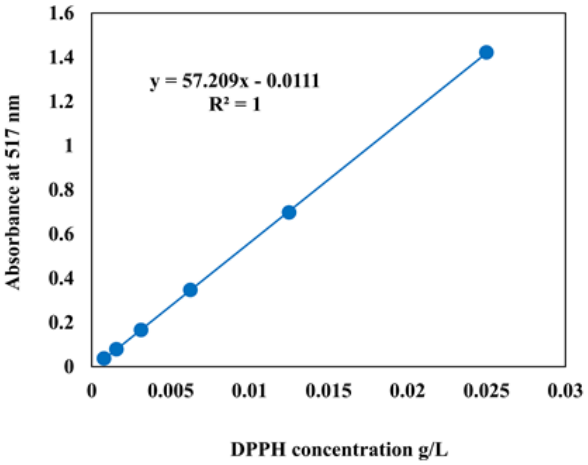

Figure 2. The standard curve of DPPH concentration against absorbance at 517 nm.

Prepared traditionally fermented juice / fresh juice / fermented juice samples were assayed for TPC. 40 µL of diluted juice sample was mixed with 3.16 ml of distilled

water and 200 µL of Folin–Ciocalteu reagent. After keeping it for four minutes, 600 µL of 20% (w/v) Na_2CO_3 was added. Then, the final solution was kept for two hours at dark and absorbance was measured at 765 nm with the UV-1800 Spectrophotometer. TPC was calculated with the standard curve and expressed as mg gallic acid equivalent per 1 L of noni juice [9].

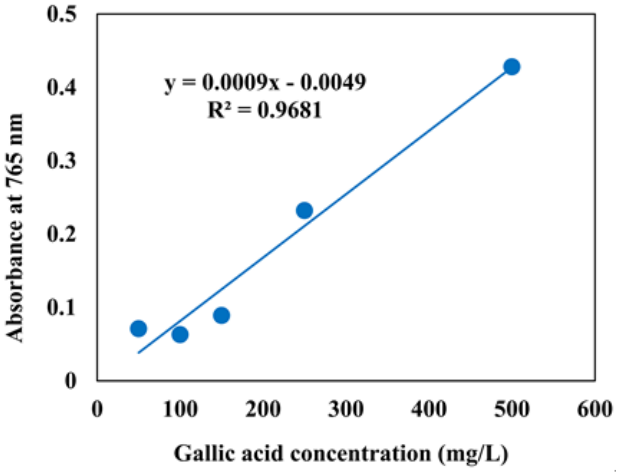

Figure 3. Gallic acid standard curve for TPC determination at 517 nm.

IV. Results and Discussion

A. Variation of AC of Noni Juice

DPPH scavenging activity is a measurement of AC of the noni juice and it is proportional to AC. The results of antioxidant capacity obtained from the traditionally fermented noni juice (two months), fresh noni juice and fermented noni juice (one month) are presented as DPPH scavenging activity percentage. DPPH scavenging activity percentage of noni juice over time was plotted.

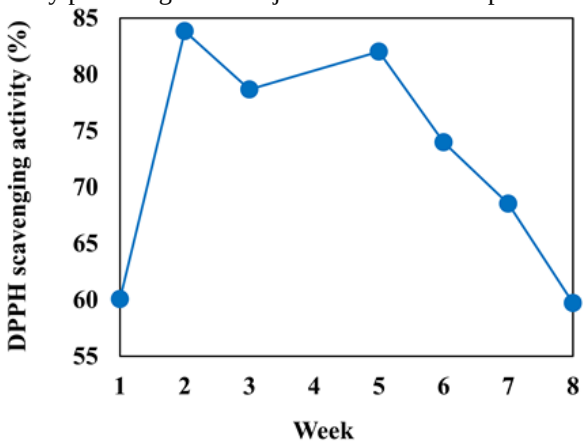

Figure 4. Variation of antioxidant capacity of traditionally fermented noni juice for two months

The DPPH scavenging activity of the traditionally fermented noni juice was maximum at the second week of the fermentation period as in Fig. 4. Therefore, after the second week of the traditional fermentation, AC decreased with the fermentation time except for the 5th week of the fermentation. In the 5th week of fermentation, a slight increase of the scavenging activity happened. Therefore, from Fig. 4, it can be observed that the AC of the noni juice was getting maximum around the second week of the fermentation and started to decrease gradually within the fermentation. Hence, it is not suitable to traditionally ferment the noni juice more than two weeks and it may be appropriate to stop the fermentation within two weeks to obtain maximum therapeutic value with respect to AC.

The maximum DPPH scavenging activity of the traditionally fermented noni juice was 83.84% and it was obtained in the second week of the fermentation. TABLE I shows that the DPPH scavenging activity of the fresh noni juice was 77.06%. Therefore, AC of the fresh noni juice was less than the maximum AC of the traditionally fermented noni juice. Therefore, it can be concluded that AC can be increased by the process of traditional fermentation of the noni juice, rather than using it as a freshly extracted juice.

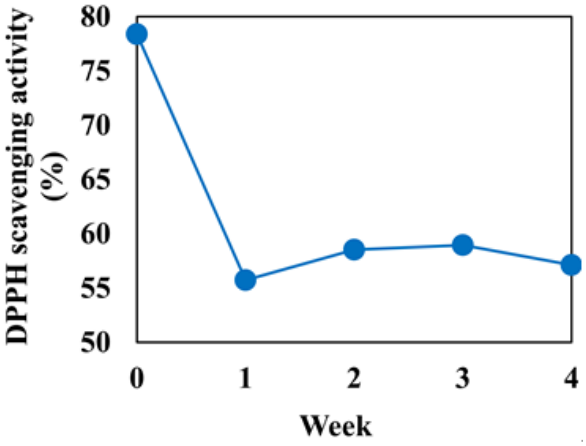

Figure 5. Variation of antioxidant capacity of fermented noni juice for one month

According to Fig. 5 allowing fermentation process for extracted fresh noni juice has caused 28% loss of AC just within one week. During one month of period, AC of noni juice was reduced, and it might be due to the increased exposure area of juice after the extraction process. Even traditional fermentation has only reduced 6% of AC for the first weeks of fermentation period.

B. Variation of TPC of Noni Juice

The results of TPC obtained from the traditionally fermented noni juice (two months) and fermented noni juice (one month) over time was plotted.

Fig. 6 shows that the maximum total phenolic content of the traditionally fermented noni juice was within the 1st week of the fermentation. After that, TPC decreased significantly until the 3rd week of the fermentation. After 3rd week, even if, it increased slightly in the 4th week, it didn't show significant difference up to the 7th week. After that, it decreased significantly. As an overall observation, it can be said that TPC of traditionally fermented noni juice decreased after 1st week of traditional fermentation. Hence, it is not suitable to continue traditional fermentation of noni juice more than one week to obtain maximum therapeutic value with respect to TPC.

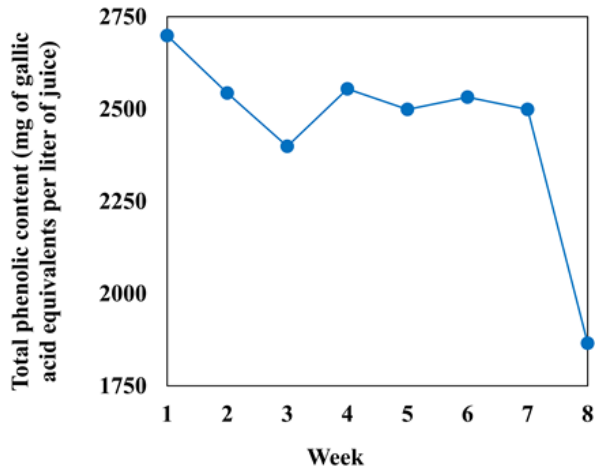

Figure 6. Variation of total phenolic content of traditionally fermented noni juice for two months

The maximum TPC of the traditionally fermented noni juice was 2698.89 mg gallic acid equivalent per 1 L of noni juice and it was within the very first week of the fermentation. Table I shows that TPC of the fresh noni juice was 2898.89 mg gallic acid equivalent per 1 L of noni juice. Therefore, the phenolic content of the fresh noni juice was higher than maximum TPC of fermented noni juice. It might be that the fresh noni juice is richer with phenolic compounds than the traditionally fermented noni juice. As TPC decreased with the fermentation time as discussed before, it can be now assumed that existing phenolic compounds within the noni fruit may be destroyed or depleted or converted into other compounds due to several reactions happening in the fermentation process. Hence, it can be possible that the fermentation process caused a reduction of TPC of noni juice.

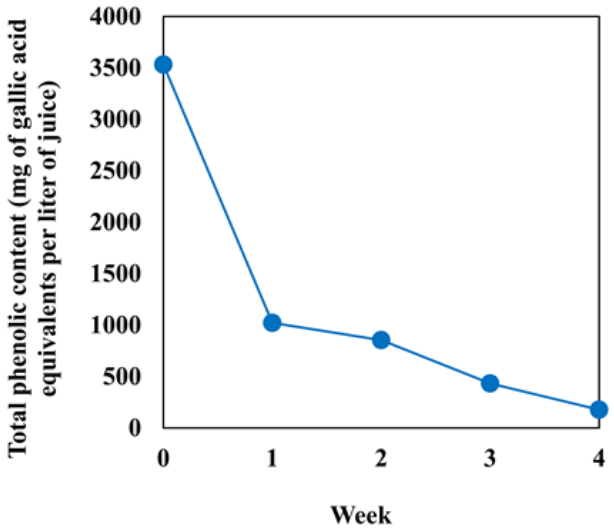

Figure 7. Variation of total phenolic content of fermented noni juice for one month

Fig. 7 shows that fermentation process has caused a drastic reduction of TPC of fresh noni juice. After extraction of fresh juice, allowing it for fermentation has made such a severe effect. Within four weeks of fermentation period, 95% of initial TPC was decreased. It might be that extraction of juice has made more exposure of phenolic compounds to the environment than when it was conducted as traditional fermentation. Therefore, degradation and destroying of phenolic compounds is highly possible due to the increased exposure area. Even the traditional fermentation has only reduced 31% of TPC for eight weeks of period. Therefore, it is not recommended to allow fermentation process for extracted noni juice.

TABLE I. ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENT OF FRESH NONI JUICE

Fresh Noni Juice	Measurement of AC/ TPC
Antioxidant Capacity (AC) (at 517 nm)	DPPH scavenging activity = 77.06%
Total Phenolic Content (TPC) (at 765 nm)	TPC = 2898.89 mg gallic acid equivalent per liter of noni juice

C. Comparison of Three Noni Juice Production Methods with Their AC and TPC

When comparing the three methods of noni juice production, it can be seen in Fig. 8 that both traditional fermentation and fresh juice extraction provides satisfactory levels of antioxidant capacity and total phenolic content. So, both methods can be used to produce noni juice with effective medicinal value. However, fermentation of fresh noni juice resulted a severe loss of antioxidants and phenolic compounds inherited by fresh noni juice. Hence, it's not recommended to allowing fermentation for extracted noni juice. Therefore, it can be concluded that production method can cause considerable variations of AC and TPC of noni juice and ultimately the therapeutic value provided by the juice.

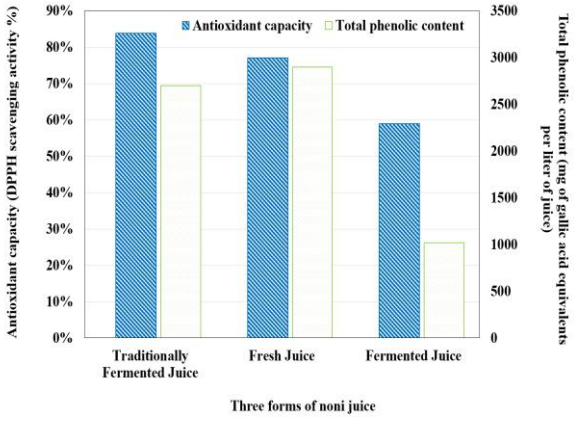

Figure 8. Comparison of three forms of noni juice with their antioxidant capacity and total phenolic content

D. Correlation between AC and TPC of Noni Juice

It is significant that it was not suitable to ferment the noni juice for a long time because AC and TPC decreased with the fermentation time. It is known that TPC is directly related to AC of noni juice. Phenolic compound is one major type of existing antioxidants of noni juice [17]. When comparing AC variation and TPC variation with fermentation time simultaneously, AC was maximum around the second week of the fermentation while TPC was maximum near the first week of the

fermentation. Perhaps, there may be other compounds as well which can contribute to the antioxidant capacity of the juice. Those compounds would have degraded after the second week of the fermentation and that may cause for the antioxidant capacity to be maximum at around the second week.

As it's mentioned before, the traditional fermentation process is generally conducted for two months of period. But both the variations of AC and TPC suggest that the fermenting noni juice for a longer period is not effective to obtain a maximum therapeutic value. Therefore, optimum fermentation time for a maximum therapeutic value of noni juice lies within the first two weeks of the fermentation and it's recommended to stop the traditional fermentation process after onwards.

However, people and the researchers are keener on the antioxidant capacity than the phenolic compounds, since the antioxidant capacity is the important chemical property that has a therapeutic value. Phenolic compounds contribute to the therapeutic value of the noni juice because they have the antioxidant capacity with them. Therefore, fermentation of noni juice is useful at the direction of the value of antioxidant capacity, because fermentation has increased antioxidant capacity.

V. Conclusion

Traditionally fermented noni juice had maximum AC in the second week of the fermentation period and the maximum TPC at the first week of the fermentation period. Therefore, it can be concluded that the therapeutic value of traditionally fermented noni juice starts to decrease after the second week of the fermentation period. Hence, it is strongly recommended not to ferment noni juice traditionally for more than two weeks. AC of the fresh noni juice was less than the maximum AC of the traditionally fermented noni juice. But, TPC of the freshly extracted juice was higher than the maximum TPC of the traditionally fermented juice. Therefore, both traditionally fermented noni juice and fresh noni juice provide AC & TPC of satisfactory levels. However, fermentation of extracted fresh noni juice has led both the AC and the TPC to decrease even within the first week of the fermentation period. Both the AC and the TPC have decreased drastically from its initial values within one month of the fermentation. Therefore, it is highly advised not to ferment the extracted fresh noni juice.

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