

Proceeding Paper

Antioxidant Capacity with Physical Property Variations of *Morinda citrifolia* L. Juice in Traditional Fermentation †

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Abstract: This study determined the physical property variations during traditional fermentation of *Morinda citrifolia* L. (noni) juice and their correlations to antioxidant capacity were identified. The temperature and pH of juice, temperature and humidity within fermenter and juice volume were monitored. Both temperatures were within the range of 29.5–33 °C. The pH of the juice decreased from 3.98 to 3.23. Humidity increased rapidly from 98.95% to 99.90%. Maximum juice volume was about 1.37 L. Maximum antioxidant capacity was around 84% of DPPH scavenging activity. Physical property variations cannot be correlated significantly to the antioxidant capacity of noni juice.

Keywords: physical properties; correlation coefficient; noni juice; antioxidant capacity; traditional fermentation

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1. Introduction

Morinda citrifolia L. which is widely known as “noni” is a medicinal fruit that has been used as a traditional folk medicine for 2000 years by Polynesians [1,2]. Significant attention has been drawn towards the traditional fermentation (natural fermentation) of noni fruit juice and its antioxidant capacity. Several studies show that antioxidant capacity can be maximum at the initial period of fermentation and thereafter it can be reduced drastically [3,4]. Yet, precise studies are essential to identify the parameters which can significantly affect biological and chemical reactions resulting during the fermentation process and can result in reductions of the antioxidant capacity of noni juice [3]. The ripeness of fruits, temperature, light intensity, fermentation time, pressure, types of bacteria and pH were identified as some conditions which affected the antioxidant capacity of noni juice made by fermentation conducted in controlled environments [4–6]. In the previous studies, correct controlling of temperature has improved the retention of antioxidant capacity of noni juice, and outdoor fermentation under the sunlight has decreased the antioxidant capacity of the juice faster than in indoor fermentation [4]. However, the changes of physical properties during traditional fermentation of noni juice and their correlations with the antioxidant capacity of noni juice has not yet been studied. Therefore, this study was conducted to study the variations of some physical properties during the traditional fermentation and to obtain their correlations with the antioxidant capacity of noni juice.

2. Materials and Methods

2.1. Materials

S-shape fermenter airlock, 4 L air-tight polypropylene (PP) container, drip chamber, PT100 RTD Platinum Resistance Thermometer Sensor, Mercury-Glass Bulb Thermometer, Liquid PH Value Detection Sensor, DHT22/AM2302 Digital Temperature and Humidity Sensor, Arduino MEGA 2560 Microcontroller Board, Multimeter, USB cables, and power supply unit were used for preparing the experimental setup. All the sensors were purchased from TxHang Electronic online store, Guangzhou Aosong Electronics Co., Ltd. and Unitech Trading (Pvt) Ltd. Laboratory-grade KOH and HCl buffer solutions were used for pH sensor calibration. DPPH (2, 2-diphenyl-1-picrylhydrazyl) and methanol, were purchased from Sigma-Aldrich (St. Louis, MO, USA). UV-1800 Shimadzu Spectrophotometer (Shimadzu Cooperation, Kyoto, Japan) was used for antioxidant capacity assay of noni juice.

2.2. Preparation of Noni Fruits

Unripe noni fruits picked from noni plants at the University of Moratuwa, Sri Lanka were cleaned initially by raw water to remove existing airborne particles on surfaces of fruits. Then, they were washed adequately with distilled water. After that, they were kept indoor for ripening for around three days. When ripening, unripe noni fruits converted from a hard white state with tinges of green to a soft, translucent, and yellowing state. Noni fruits with a total initial weight of 1.25 kg were used.

2.3. Measurement of Physical Properties with Traditional Fermentation

Traditional fermentation of noni juice was carried out in the fermenter constructed with a 4 L PP container (Figure 1a) attached with sensors to monitor the variation of physical properties continuously. S-shape fermenter airlock was fixed on the top of the fermenter to make it air sealed. Ripe and matured noni fruits were kept in the clean airtight PP container and the fermentation was carried out indoors at the ambient temperature of 30 °C for two months. After one-two days, juice forming from noni fruits were clear in color and then turned dark with time.

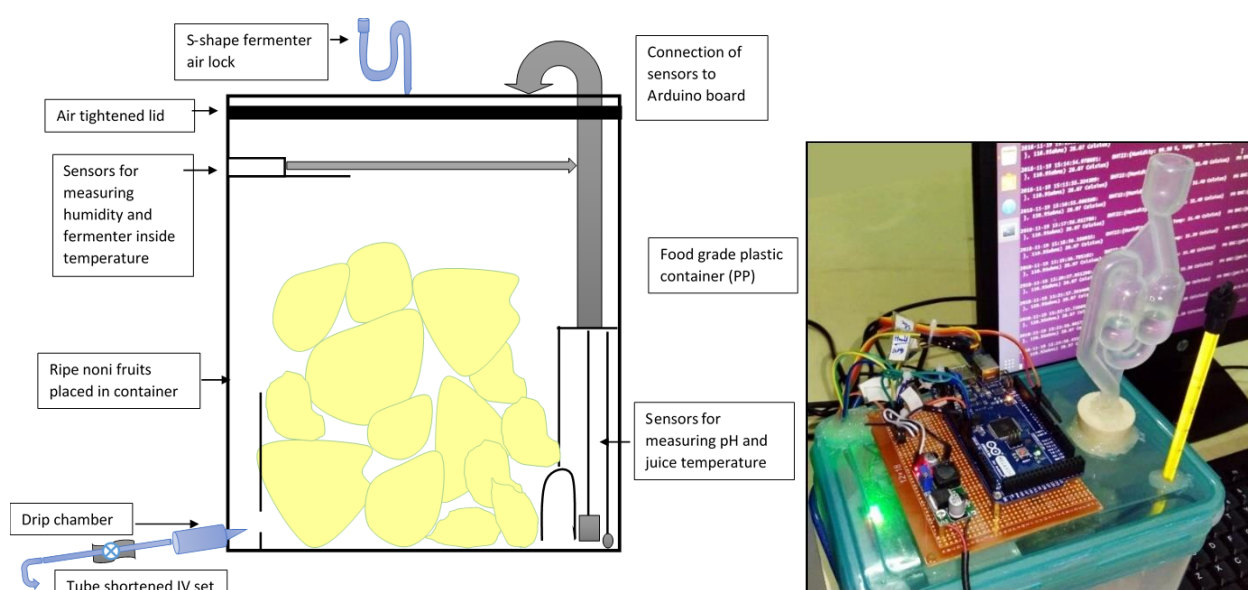


Figure 1. Measurement of physical properties with traditional fermentation: (a) Schematic diagram of the experimental setup for monitoring physical property variation during traditional fermentation of noni juice; (b) Arduino MEGA 2560 microcontroller board connected with sensors and attached to the top surface of the fermenter.

The physical parameters measured during the fermentation period were temperature and pH of the fermenting juice, temperature, and humidity within the fermenter and level of noni juice formed using a computer-controlled data acquisition unit. All sensors were controlled by a microcontroller unit and collected data were transmitted to a computer server. All the connected sensors were calibrated initially by using standard calibration procedures. After two months, sensors were calibrated again to verify the accuracy of the collected data. The data acquisition unit was fixed on the lid of the fermenter as in Figure 1b. The sensor for the measurement of humidity and fermentation temperature was physically separated from the juice and noni fruits and placed inside the air-connected upper chamber to avoid damages to sensors. pH and juice temperature sensors were placed at the bottom of the fermenter such that they were immersed in fruits. A measuring ruler was pasted on the fermenter wall to manually measure the juice level increment for two months of period. Physical properties were measured, collected, pre-processed, sampled, and stored automatically in the server computer continuously every minute for two months of the fermentation period.

2.4. Measurement of Antioxidant Capacity by DPPH Assay

The antioxidant capacities of traditionally fermented noni juice were determined by scavenging of DPPH free radicals as explained by Brand-Williams [7]. Juice samples were taken from the drip chamber once every week for two months of the fermentation period. First 100 μ L of ten-times-diluted noni juice was added to 4 mL of a solution of 0.025 g/L DPPH solved in methanol. After thirty minutes of reaction time, the absorbance of the solution was measured at 517 nm by UV-1800 Spectrophotometer. Calculations for the antioxidant capacity of noni juice were carried out as described in [8] while using an experimentally derived standard curve of DPPH concentration vs. absorbance [8]. The antioxidant capacity of noni juice was expressed by DPPH scavenging activity percentage.

2.5. Determination of Correlations of Physical Properties and Antioxidant Capacity of Noni Juice

Variation of antioxidant capacity with the variation of physical properties of noni juice during the traditional fermentation was used to identify any significant correlation between them. Calculated daily average of physical property values and antioxidant capacity were considered for the correlation calculations. Distance correlation coefficients of all physical parameters with antioxidant capacity of noni juice were calculated using matrix calculations mentioned in [9]. According to the definition of distance correlation, distance correlation is zero if and only if the random vectors are independent and range from zero to one ($0 \leq R \leq 1$). Finally, the associations of the physical properties with the antioxidant capacity of fermented noni juice were identified.

3. Results and Discussion

3.1. Temperature Variation

The temperature of the fermenter headspace (above the level of juice), as well as the juice temperature, had many fluctuations during the two months (Figure 2a). Juice temperature was always higher than the headspace temperature (about 1 °C difference) and it is expected due to the chemical reactions and microbial actions happening in the juice that causes chemical changes in organic substances and biomass growth while generating heat. Juice temperature was within the range of 30.5–33 °C and it was higher than the ambient temperature of 30 °C. Overall, the highest values of both temperatures could be seen in the period ranging from day 3 to day 7 of fermentation while the least temperatures have resulted during days from 23 to 25. Significant temperature increments in the second half of the final month were observed during the fermentation. In previous studies, traditional fermentation was conducted at room temperatures with a range of 24–32 °C. However, they have not shown the variation of temperature of noni juice during traditional fermentation [3,9].

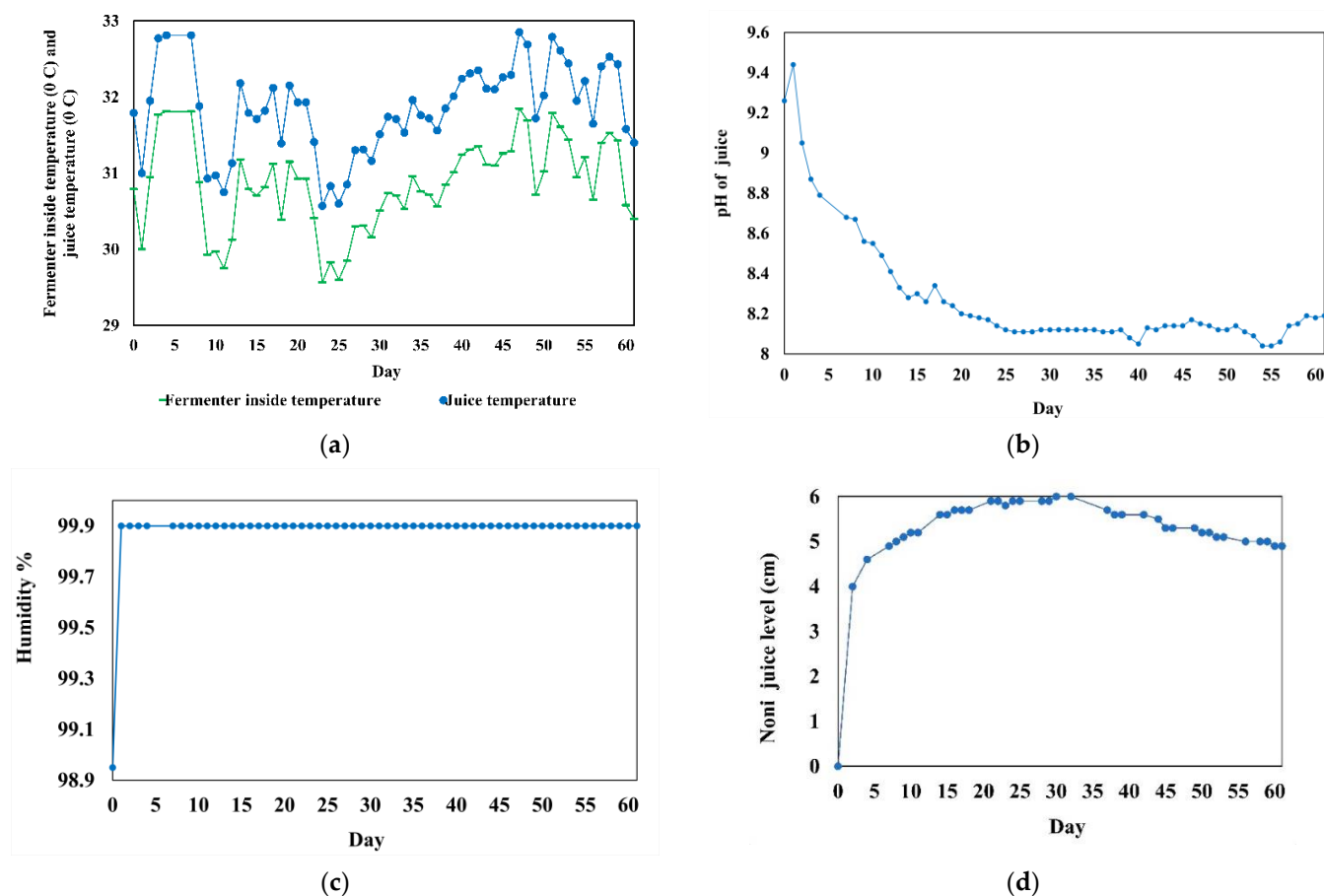


Figure 2. Variation of physical properties during traditional fermentation of noni juice: (a) Juice temperature and fermenter inside temperature; (b) pH; (c) Humidity; (d) Juice level.

3.2. pH Variation

Overall, the pH value of noni juice decreased with fermentation time as in Figure 2b. At the start of the process, it was about 3.88 and finally, it reached 3.31. pH of noni juice decreased dramatically during the first half of the first month and after that pH slightly reduced with few fluctuating points. For two months period, the highest pH was about 3.98 while the least was around 3.23. A previous study has shown that the pH of the fermenting juice reduced with fermentation time from 5 to 3 [5]. The study by Konsue has shown that fermentation led to a decrease in pH from 3.72 to less than or equal to 3.5 [10]. It was mainly caused by the formation of lactic acid during the fermentation process. For the whole period, noni juice remained in the acidic phase.

3.3. Humidity Variation

Humidity increased rapidly from 98.95% to 99.90% within one day and remained constant at 99.90% (Figure 2c).

3.4. Level of Juice Formed

At the beginning of fermentation, the juice level increased dramatically to 4 cm within two days (Figure 2d) and thereafter, the level increased gradually and reached a peak of 6 cm at end of the first month. The highest juice volume formed was 1.37 L. After that, it had a gradual reduction reaching 4.7 cm at the end of fermentation. It may be due to that noni fruits got started to disintegrate after a certain period of fermentation. Hence,

the level of juice formed (yield) was highest at the end of the first month period of traditional fermentation. The volume of the noni juice sample taken from each week for the measurement of antioxidant capacity was negligible.

3.5. Antioxidant Capacity Variation

The antioxidant capacity of noni juice varied significantly during traditional fermentation as in Figure 3a and was within the range of 59–84% of DPPH scavenging activity. The studies by Yang have shown that fresh noni juice which was stored at $-18\text{ }^{\circ}\text{C}$ for 11 weeks had 82% of DPPH scavenging activity and fermented noni juice lost 90% of free radical scavenging activity after 3 months of fermentation time [3,4]. In this study, the maximum antioxidant capacity of noni juice was at the second week, and it became the lowest at the last week of traditional fermentation. Variation of antioxidant capacity of noni juice may be due to the reactions that occurred, and compounds formed during traditional fermentation. With the fermentation time, antioxidants may be degraded and cause the reduction of the antioxidant capacity of noni juice. Further analysis about this variation of antioxidant capacity of noni juice was given in the previous study [8].

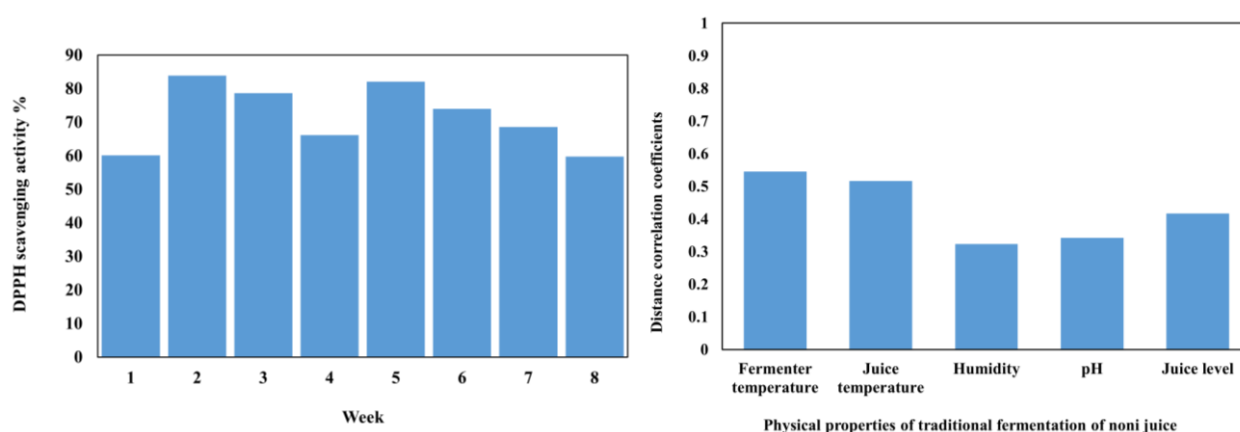


Figure 3. Associations of the physical properties with the antioxidant capacity of fermented noni juice: (a) Variation of antioxidant capacity of noni juice during traditional fermentation; (b) Distance correlation coefficients of physical properties of traditional fermentation of noni juice with its antioxidant capacity.

3.6. Correlation of Physical Properties During Traditional Fermentation of Noni Juice with Its Antioxidant Capacity

According to Figure 3b, the obtained distance correlations were comparatively small and not closer to 1. Fermentation temperature, juice temperature, pH, humidity, and juice level had moderate values of distance correlation which exerted a medium strength of associations with antioxidant capacity of noni juice. However, to have strong correlations with each other, random vectors should have large values of distance correlations which are close to 1. Since all the calculated distance correlations are considerably low, it can be concluded that all the physical properties did not covary with the antioxidant capacity of noni juice in a stronger manner. Thus, even though considerable variations of physical parameters were expected during traditional fermentation of noni juice which can be further related to its antioxidant capacity, it can be said that there were no such significant effects. Therefore, minor variations of physical conditions during traditional fermentation cannot be correlated with the antioxidant capacity of noni juice.

4. Conclusions

This study was performed to identify any significant variation patterns or critical points within the variation of physical parameters including pH of juice, juice temperature, fermenter inside temperature, humidity, and the juice level with fermentation time

and then to identify any correlation between these physical properties and antioxidant capacity of noni juice. It was seen that strong correlations did not exist in each physical parameter with the antioxidant capacity of noni juice. Patterns of variations of physical properties during traditional fermentation of noni juice were different from the variation of antioxidant capacity which was maximum at the second week of traditional fermentation.

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