chemistry proceedings

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Proceeding Paper

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

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† Presented at the 1st International Online Conference on Agriculture—Advances in Agricultural Science and Technology, 10–25 February 2022; Available online: https://iocag2022.sciforum.net/.

Abstract: This study determined the physical property variations during the traditional fermentation of *Morinda citrifolia* L. (noni) juice and their correlations to antioxidant capacity were identified. The temperature and pH of the juice, temperature, and humidity within the 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. The humidity increased rapidly from 98.95% to 99.90%. The maximum juice volume was about 1.37 L. The maximum antioxidant capacity was around 84% of the DPPH scavenging activity. Therefore, the 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

Citation: Samarasiri, M.H.; Chandrasiri, T.A.; Wijesinghe, D.B.; Gunawardena, S.P. Antioxidant Capacity with Physical Property Variations of *Morinda citrifolia* L. Juice in Traditional Fermentation. *Chem. Proc.* **2022**, *10*, 17. https://doi.org/10.3390/IOCAG2022-12177

Academic Editor: Isabel Lara

Published: 31 January 2022

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 toward the traditional fermentation (natural fermentation) of noni fruit juice and its antioxidant capacity. Several studies have shown that antioxidant capacity can be the maximum at the initial period of fermentation, and thereafter, it can be reduced drastically [3,4]. However, precise studies are essential to identify the parameters that can significantly affect the biological and chemical reactions resulting during the fermentation process and can result in reductions in 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 of the conditions that affected the antioxidant capacity of noni juice made by fermentation conducted in controlled environments [4–6]. In previous studies, the correct control of temperature improved the retention of the antioxidant capacity of noni juice, and outdoor fermentation under sunlight decreased the antioxidant capacity of the juice faster than in indoor fermentation [4]. However, the changes in the physical properties during the traditional fermentation of noni juice and their correlations with the antioxidant capacity of noni juice have 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

The S-shape fermenter airlock, 4 L air-tight polypropylene (PP) container, drip chamber, PT100 RTD Platinum Resistance Thermometer Sensor, Mercury-Glass Bulb Thermome-

ter, 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 to prepare the experimental setup. All of the sensors were purchased from the 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. The DPPH (2, 2-dipheny-l-picrylhydrazyl) and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). A UV-1800 Shimadzu Spectrophotometer (Shimadzu Cooperation, Kyoto, Japan) was used for the 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 the existing airborne particles on the surfaces of the fruits. Next, they were washed adequately with distilled water. After that, they were kept indoors for ripening for around three days. When ripening, the 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

The traditional fermentation of noni juice was carried out in the fermenter constructed with a 4 L PP container (Figure 1a) attached with sensors to continuously monitor the variation of physical properties. The S-shape fermenter airlock was fixed on the top of the fermenter to make it airtight. 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 to two days, juice forming from the noni fruits were clear in color and then turned dark with time.

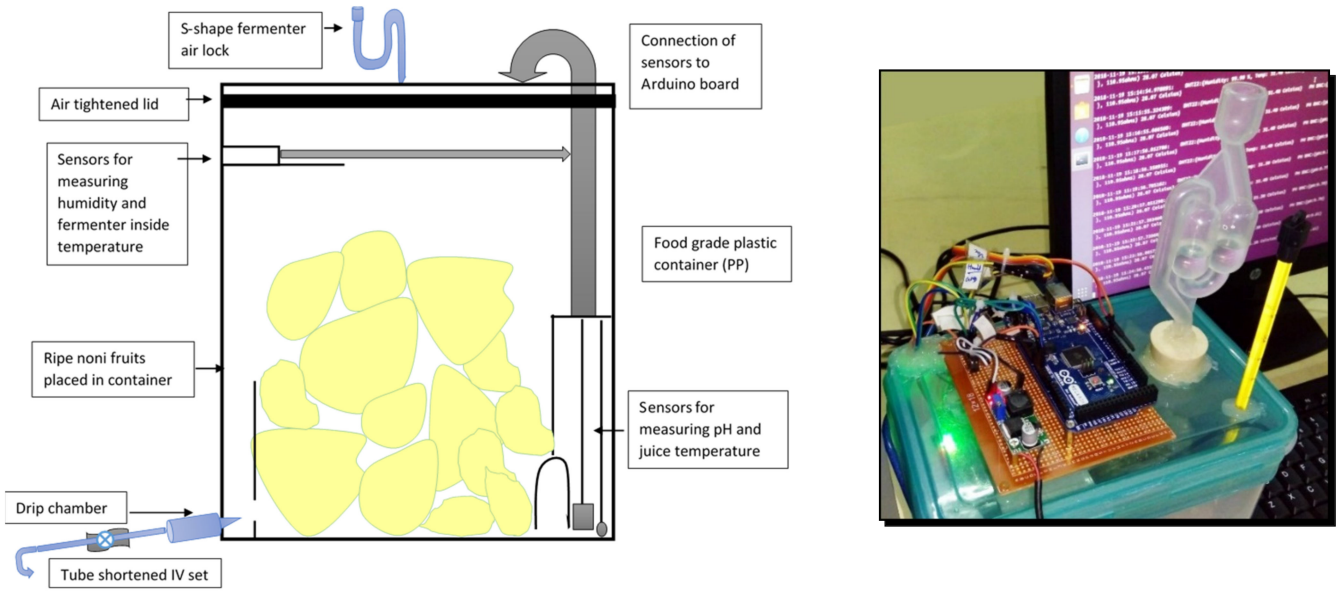


Figure 1. The measurement of the physical properties with traditional fermentation. (**a**) A schematic diagram of the experimental setup to monitor the physical property variation during the traditional fermentation of noni juice. (**b**) Arduino MEGA 2560 microcontroller board connected with sensors 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 the collected data were transmitted to a computer server. All of the connected sensors were calibrated initially by using standard calibration procedures. After two months, the 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 shown in Figure 1b. The sensor for the measurement of the humidity and fermentation temperature was physically separated from the juice and noni fruits and placed inside the air-connected upper chamber to avoid damage to the sensors. The pH and juice temperature sensors were placed at the bottom of the fermenter so that they were immersed in fruits. A measuring ruler was pasted on the fermenter wall to manually measure the juice level increment for a two month period. The 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 the traditionally fermented noni juice were determined by the 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 µL of ten-times-diluted noni juice was added to 4 mL of a solution of 0.025 g/L DPPH dissolved in methanol. After thirty minutes of reaction time, the absorbance of the solution was measured at 517 nm by a 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 the DPPH concentration vs. the absorbance [8]. The antioxidant capacity of noni juice was expressed by the DPPH scavenging activity percentage.

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

The variation of the antioxidant capacity with the variation in the physical properties of noni juice during the traditional fermentation was used to identify any significant correlation between them. The calculated daily average of the physical property values and the antioxidant capacity were considered for the correlation calculations. The distance correlation coefficients of all of the physical parameters with the antioxidant capacity of noni juice were calculated using the matrix calculations mentioned in [9]. According to the definition of the distance correlation, the 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 the 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). The juice temperature was always higher than the headspace temperature (about 1 °C difference) which was expected due to the chemical reactions and microbial actions happening in the juice that causes chemical changes in the organic substances and biomass growth while generating heat. The 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 resulted during days 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 did not show the variation in the temperature of noni juice during traditional fermentation [3,9].

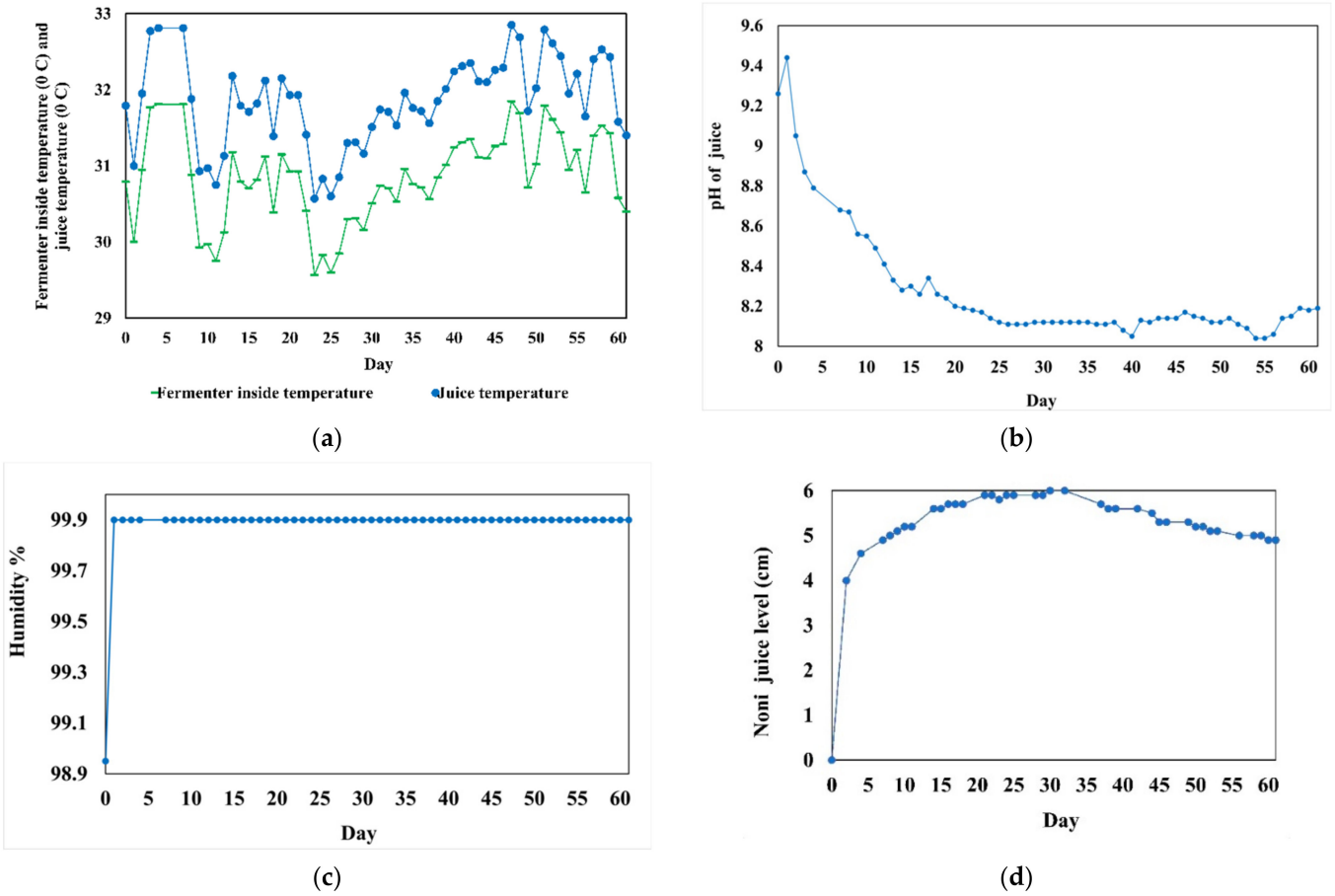


Figure 2. The variation in the physical properties during the traditional fermentation of noni juice. (**a**) Juice temperature and fermenter inside temperature; (**b**) pH; (**c**) humidity; (**d**) juice level.

3.2. pH Variation

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

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

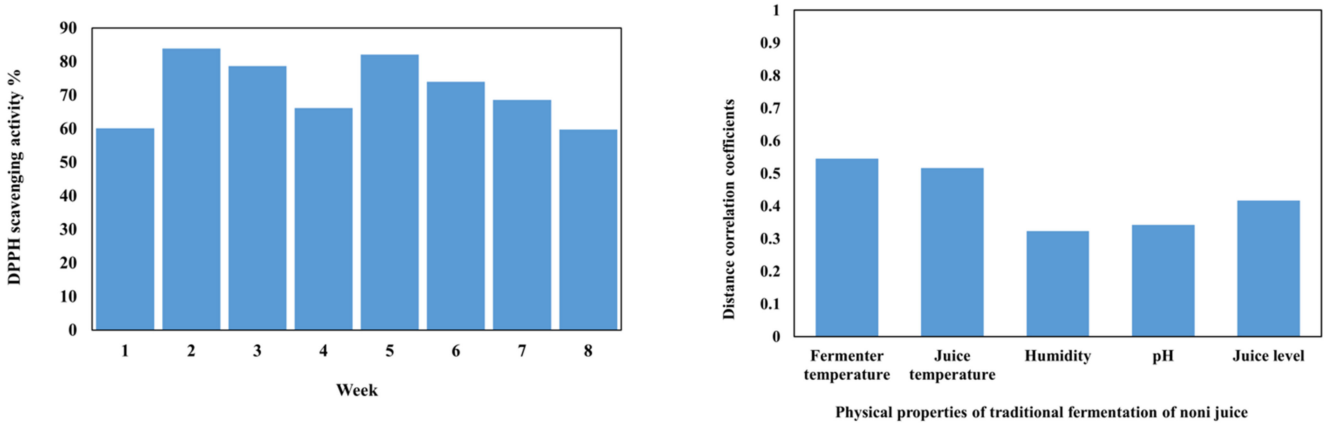
3.4. Level of Juice Formed

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 the end of the first month. The highest juice volume formed was 1.37 L. After that, it showed a gradual reduction, reaching 4.7 cm at the end of fermentation. This may be because the noni fruits started to disintegrate after a certain period of fermentation. Hence, the level of juice formed (yield) was the highest at the end of the first month of

the traditional fermentation. The volume of the noni juice sample taken each week for the measurement of the antioxidant capacity was negligible.

3.5. Antioxidant Capacity Variation

The antioxidant capacity of noni juice varied significantly during the traditional fermentation as in Figure 3a and was within the range of 59–84% of DPPH scavenging activity. The studies by Yang showed that fresh noni juice that was stored at −18 °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 in the second week, and it became the lowest in the last week of traditional fermentation. The variation in the antioxidant capacity of noni juice may be due to the reactions that occurred and the compounds that formed during traditional fermentation. With the fermentation time, antioxidants may be degraded and cause the reduction in the antioxidant capacity of noni juice. Further analysis on this variation of the antioxidant capacity of noni juice was given in a previous study [8].



(**a**) (**b**)

Figure 3. The associations of the physical properties with the antioxidant capacity of fermented noni juice. (**a**) Variation in the antioxidant capacity of noni juice during traditional fermentation. (**b**) The distance correlation coefficients of the physical properties of the 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. The fermentation temperature, juice temperature, pH, humidity, and juice level had moderate values of distance correlation, which exerted a medium strength of associations with the antioxidant capacity of noni juice. However, to have strong correlations with each other, random vectors should have large values of distance correlations that are close to 1. Since all of the calculated distance correlations were considerably low, it can be concluded that all of the physical properties did not covary with the antioxidant capacity of noni juice in a stronger manner. Thus, even though considerable variations in the physical parameters were expected during the 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 in the 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 the pH of the juice, juice

temperature, fermenter inside temperature, humidity, and the juice level with the fermentation time and then to identify any correlation between these physical properties and the 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 the variations in the physical properties during the traditional fermentation of noni juice were different from the variations in the antioxidant capacity, which was the maximum at the second week of traditional fermentation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IOCAG2022-12177/s1.

Author Contributions: Conceptualization, S.P.G.; Methodology, M.H.S., D.B.W. and T.A.C.; Software, D.B.W.; Validation, D.B.W.; Formal analysis, M.H.S.; Investigation, M.H.S. and T.A.C.; Resources, D.B.W.; Data curation, M.H.S.; Writing—original draft preparation, M.H.S.; Writing—review and editing, M.H.S., D.B.W. and S.P.G.; Visualization, M.H.S.; Supervision, S.P.G.; Project administration, S.P.G.; Funding acquisition, S.P.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data obtained/analyzed during this study are available from the corresponding author upon request.

Acknowledgments: Eng. Gunindu Jayawardhana is greatly acknowledged for their support during the fermenter setup.

Conflicts of Interest: The authors declare no conflict of interest.

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