International Journal of Biotechnology and Microbiology
www.biotechnologyjournals.com
Online ISSN: 2664-7680, Print ISSN: 2664-7672
Received: 05-06-2021; Accepted: 21-06-2021; Published: 07-07-2021
Volume 3, Issue 2, 2021, Page No. 01-04

Preliminary investigation on microflora associated with fermentation of noni (*Morinda citrifolia*) fruit and its nutritional components

Lawrence O Amadi, Hamilton Nwala
Department of Microbiology, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, P.M.B, Port Harcourt, Nigeria

Abstract
This study focused on microflora associated with the natural fermentation of noni fruit and its nutritional components. Microflora and nutritional components were determined by standard microbiological protocols and the methods of Association of Official Analytical Chemists (AOAC) respectively. The samples were subjected to three (3) weeks duration of fermentation in a prototype airtight glass fermenter/vessels at ambient temperature as traditionally practiced. The results revealed that the microbial counts increased from 0-14 d and decreased afterwards, bacterial counts (10^4CFU/mL) being apparently higher than fungal counts (10^2CFU/mL) in fermented juice. Two genera of Gram positive bacteria were identified, *Bacillus* and *Staphylococcus* whereas four were fungal genera; three moulds, *Aspergillus*, *Alternaria* and *Penicillium* and a yeast genus, *Saccharomyces*. Most of these microbes are noted for production of pectinases, amylases and lipases which functions to soften and degrade the noni fruit. Proximate composition values of the fermented juices, 0-21 d decreased in ash, protein and total titratable acidity (TTA) whereas fat increased but moisture, carbohydrate and fibre contents fluctuated. However, this investigation have been able to unravel the changes in microbiological and nutritional components of noni juice during natural fermentation. Interestingly, noni juice is nutritious, therapeutic and microbiologically safe following adequate processing protocols.

Keywords: fermentation, microbial counts, nutritious, noni juice, therapeutic

Introduction

The genus, *Morinda* covers about 80 species and *M. citrifolia* (Noni) is the most popularly known and widely cultivated in a variety of tropical environments of Polynesia, Micronesia, Australia and now pantropical. Its popularity stems from age-old application as food, therapeutic and for pharmaceutical purposes [1-3]. Linnaeus gave noni the name *Morinda citrifolia*, which is the universally recognized botanical name [4]. However, extensive research in various fields on different parts of *M. citrifolia* began after the launch of the first commercial food product in 1996 (Tahitian Noni® fruit juice), followed by its approval as a food by the European Commission in 2003 as reported in literature [5, 6].

Noni has been regarded to be the "super fruit" because of its great nutritional value, broad spectrum bioactivities and therapeutic remedy to various diseases as an antiviral, antifungal, antitumor, anti-inflammatory, antitubercular, antimicrobial, antimalarial, antihelminthic, anti-cancer effects, anti-oxidant activities, analgesic, cardiovascular, gastritis, hypertension, skin diseases, respiratory infections, menstrual and urinary tract disorders, fever, diabetes, immunostimulant, gingivitis, periodontitis and venereal diseases, etc., [6, 7, 8]. Traditionally, various parts of Noni plant were used in Polynesian island as an herbal medicine in various ailments. *Morinda citrifolia* has been used widely as a complementary and alternative therapy in many countries owing to its proven health benefits.

At present, noni juices produced over different fermentation periods are available on the market, with different formulations (like Noni beverage, Noni squash, Noni syrup) which can be grouped into short-term, month-long, and long-term fermentations [4,9]. However, the flavour profiles of fermented noni juice with different fermentation times are not clear. In addition, as a natural fermentation, the scale of fermentation may have an impact on the active substances and flavour of fermented juice depending on the scale of fermentation [10, 11]. Fermentation, the process which leads to breakdown of complex organic molecules by the action of microorganisms into simpler ones benefits consumers in terms of production of peptides, organoleptic, antimicrobial, probiotic properties and makes the product safer, etc., [12, 13, 14]. Although processing of the juice has been inconsistent and lacking in standardization, noni juice is currently marketed and consumed as a novel food ingredient or botanical nutritional supplement [15]. Consequently, juice products may vary as well due to chemical, physical and microbial properties depending on fruit maturity and processing method [16, 17].

On safety concerns, data from clinical studies, toxicity tests and chemical test have highlighted and substantiated the use of noni juice as a safe and functional food [18, 19]. More detailed examination of the few recently reported cases of adverse health effects reveals that these were likely due to factors other than noni fruit [20]. Patients with liver and kidney dysfunctions are restricted from noni juice consumption because it has substantial quantity of potassium. It produces hyperkalemia which could lead to nausea, muscle weakness and irregular heartbeat as well as delay ossification and skeleton alteration in the fetus in pregnancy [21, 22]. However, information on the

microbiological and nutritional quality of noni juice from different tropical regions of the world is limited. Lack of standardization and data regarding efficacy and safety of one commercial Noni product may not be applicable to others. So, there is an urgent need for establishment of an acceptable and applicable method of Noni preparations worldwide. Several authors have reported different microbial isolates, fermentation scale and time, and product quality [12, 16, 23, 24].

The purpose of this work was to carry out preliminary investigation on microflora associated with fermentation of noni fruit and its nutritional components.

Materials and Methods

Harvesting, Processing and Natural Fermentation

Ripe Noni fruit at yellow-white maturity stage (Plate 1) were harvested periodically during 2020 to 2021 from Dilomat farm at the Rivers State University, Nkpolu-Oroworukwo, Port Harcourt and transported in sterile polyethylene bags to the Microbiology laboratory for processing. The harvested fruits were sorted, washed and rinsed in tap water and placed into sterile wide-mouth glass vessel with lid (improvised chamber/fermenter) and stored in the dark at ambient temperature (30±2°C) for 21days. Subsequently, the juice exuded from the fruit were naturally fermented. Data for fresh Noni fruit juice represented day 0 fermentation.

Fig 1: Ripe Noni (*M. citrifolia*) fruit at Dilomat farm.

Proximate Composition and Titratable Acidity

Fruit juice were analysed for proximate composition for parameters such as moisture contents, crude carbohydrate, protein, fat, fibre, ash as well as total titratable acidity (TTA) during fermentation by standard methods [25].

Microbial Analysis of Fruit Exudes

Serial dilutions were made of the Noni fruit exudes from the vessels at weekly intervals using 5.0mL in 45.0mL of 0.85% normal physiological saline to obtain 10^{-1} homogenate for fermented and day 0 samples respectively. THBCs were determined on surface-dried nutrient agar (NA) and plates were incubated in duplicates at 37°C for 24hours. Fungal counts (FCs) were determined using an aliquot (0.1ml) portion of serially diluted samples on solidified Sabouraund' dextrose agar (SDA) and spread-plated with a sterilized bent glass rod, followed by incubated at room temperature (25±2°C) for 3-5days. Representative colonies from well-isolated plates showing (30-300) colonies were picked at random, sub-cultured for purification, stored on slants at refrigeration temperature and used for cultural, morphological and biochemical tests [26, 27, 28].

Statistical Analysis

The experimental data are expressed as means ± standard deviations were analysed using Microsoft Excel® 2016.

Results

Changes in proximate composition and total titratable acidity (TTA) values prior and during natural fermentation are presented in Table 1. The moisture contents increased during fermentation but peaked on day 7 whilst carbohydrate peaked on day 14. In contrast, protein, fat and fibre and ash values fluctuated but TTA decreased throughout the duration. Furthermore, freshly ripe Noni fruit (day 0) had more protein, ash and TTA respectively prior to and after fermentation. Such nutrient composition would likely provide a milieu for microbial community growth.

Table 1: Changes in Proximate Composition and Total titratable acidity (TTA) values of Noni fruit juice during Fermentation

	Duration (Day)			
Parameter	0	7	14	21
Moisture content	87.80±0.87	91.81±0.00	89.4±0.00	88.75±0.00
Crude carbohydrate	3.62±0.45	3.39±0.07	4.81±0.08	4.08±0.01
Crude protein	2.53±0.20	1.03±0.03	1.38±0.02	1.26±0.00
Crude fat	0.52±0.03	0.35±0.00	0.45±0.02	0.73±0.07
Crude fibre	4.87±0.02	2.83±0.06	3.70±0.01	4.93±0.19
Ash	0.68±0.00	0.60±0.25	0.63±0.02	0.28±0.00
Total titratable acidity	1.89±0.00	1.82±0.00	1.58±0.00	1.26±0.00

Each value is the means of 2 replicates.

Table 2, depicts changes in microbial counts before and during the fermentation of Noni fruit juice (NFJ). There was visible increases in microbial counts in fermenting Noni fruit juice reaching maximum values of $9.2\pm0.8x10^4$ and $3.5\pm0.0x10^3$ CFU/mL on day 14 for heterotrophic bacteria and fungi respectively. Thereafter, the counts decreased markedly, thus, indicating that the initial substrate and storage conditions during fermentation were favourable to microbial survival and proliferation.

Table 2: Changes in microbial counts of Noni fruit juice during Fermentation

	Duration (Day)			
	Microbial counts (CFU/mL)			
	0	7	14	21
THBC	$1.5\pm0.6x10^4$	$7.0\pm0.2x10^4$	$9.2\pm0.8x10^4$	$4.1\pm0.2x10^4$
FC	$2.1\pm0.0x10^3$	$2.0\pm0.1x10^3$	$3.5\pm0.0x10^3$	$8.5\pm0.0x10^2$

Legend: THBC = Total heterotrophic bacterial counts; FC = Fungal counts each value is the mean of 2 replicates

The relative abundance of microbial isolates from 0 to 21 d in the fermented juice are shown in Table 3. Bacterial isolates were predominantly Gram positive bacteria of the genera *Bacillus* and *Staphylococcus. Bacillus* species occurred from 0-21 d of fermentation with *B. cereus* being the most relatively abundant single species. There are four genera of fungi;

Aspergillus, *Alternaria*, *Penicillium* and *Saccharomyces* but the substrates were predominated by moulds especially *Aspergillus niger*, from 0-21 d, followed by *Alternaria* species, within the duration of fermentation, Table 3. The highest microbial counts/populations in NFJ were recorded at 14 d of fermentation (Table 2).

Table 3: Relative abundance of microbial isolates from Noni fruit juice during fermentation

Name of		Duration (Day)		
Microbes	0	7	14	21
Bacteria				
Bacillus species	++	+	+	+
B. subtilis	+	-		- -
B. cereus	+	++	-	++
Staphylococcus aureus	-	+	+	-
B. smithii	-	-	+	-
B. badius	-	-	+	-
B. tequilensis	-	-	-	+
B. megaterium	-	-	-	+
Fungi				
Aspergillus niger	+	++	+	+
Alternaria sp	+	-	+	+
Saccharomyces sp	+	-	-	-
Penicillium sp	-	+	-	+
Aspergillus flavus	-	-	+	+

Discussion

Ripe Noni fruit is highly susceptible to softening and degradation few days after harvest. This phenomenon has been associated with intrinsic enzymes in microorganisms such as *Mucor circinelloides*, *Gluconobacter fracteurii*, etc., [15]. Although the microbial profile differed in the fermented substrate, those isolated in this study; *Bacillus megaterium*, *B. tequilensis*, *Aspergillus niger* and others (Table 3) have been reported to produce cell wall degrading enzymes (pectinase; polygalacturonase, pectin lyase and pectinesterase, etc.) which could soften and degrade host fruit such as noni [14, 29, 30, 31]. Differences in microbial composition structure may also be influenced by chemical, physical, fruit maturity, fermentation scale, time and processing methodologies [9, 11, 16, 17], thus affecting organoleptic properties.

Microbial growth profiles increased from 0-14 d fermentation and decreased afterwards with *Bacillus subtilis* and *Saccharomyces* sp., being undetectable after 0 d. Similar findings have been reported by [15, 16]. Changes in proximate analysis (for protein, ash and moisture content), acidity and other parameters obtained from this work compared favourably with those of other regions [9, 25], thus ensuring some level of standardization of noni preparation and consumer acceptance in terms of quality and storability. Proximate, TTA and microbiological analyses indicated that the greatest changes in noni substrate occurred between 7 and 14 d which corroborates earlier reports [16] that extending fermentation period to 21 d improved juice yield, emphasizing that the traditional 2-month fermentation [32] may be reduced substantially. However, this preliminary investigation may contribute to understanding and enhancement of the microbiological and product quality of Noni fruit juice.

Conclusions

Diverse microbial community profile of noni juice produced cell wall degrading enzymes which may be linked with rapid softening of ripe fruit. *Bacillus* species and *Aspergillus niger* occurred throughout the duration of fermentation which are capable of producing cell wall degrading enzymes. The nutrient composition supported proliferation of microbes which peaked on day 14 resulting in product that was not only nutritious and palatable but microbiologically safe. Although, more physicochemical parameters and standardization of the product should be carried out.

Disclaimer

The produce used for this research are commonly used in our area of research and country. There is absolutely no conflict of interest between the authors and producers of Noni fruits because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Wang M-Y, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, Anderson G. *Morinda citrifolia* (noni): A literature review and recent advances in noni research. Acta Pharmacologica Sinica,2002:23(12):1127-1141. https://doi.org/1671-4083
2. Saminathan M, Rai RB, Dhama K, Tiwari R, Chakraborty S, Ranganath GJ, Kannan K. Systemic review on anticancer potential and other health benefits pharmacology noni. Int'l J Pharmacology,2013:9(8):462-492. https://doi.org/10.3923/ijp.2013.462.492.
3. Nascimento LCS, Rodrigues N da R, Alves MPC, Sabaa Srur AUO, Barbosa Junior JL, Barbosa MIMJ. Chemical characterization, nutritional aspects and antioxidant capacity of noni (*Morinda citrifolia* L) produced in Northeastern Brazil. International Food Research Journal,2018:25(2):870-875.
4. Nelson SC, Elevitch CR. Noni: The Complete Guide for Consumers and Growers, 1st ed.; Permanent Agriculture Resources: Holualoa, HI, USA, 2006, 67-80.
5. Deng S, West BJ, Jensen CJ. A quantitative comparison of phytochemical components in global noni fruits and their commercial products. Food Chemistry,2010:122(1):267-270. https://doi.org/10.1016/j.foodchem.2010.01.031
6. Chan-Blanco Y, Vaillant F, Mercedes Perez A, Reynes M, Brillouet JM, Brat P. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. Journal of Food Composition and Analysis,2006:19(6-7):645-654. https://doi.org/10.1016/j.jfca.2005.10.001
7. Glang J, Wolfgang F, Johannes W. Effect of *Morinda citrifolia* L. fruit juice on gingivitis/periodontitis. Modern Research in Inflammation,2013:2(2013):21-7.
8. Langbang D, Rekha, Menon T. Role of Aqueous, Methanol and Ethanol leaf Extract of *Morinda Citrifolia* (Indian Noni) in inhibiting on clinical *Pseudomonas aeruginosa* isolates. J Med Sci Clin Res,2018:06(06):984-992.
9. Ali M, Kenganora M, Manjula SN. Health Benefits of *Morinda citrifolia* (Noni): A Review. Pharmacognosy

Journal,2016:8(4):321-334.
10. Bergh AJ, Muller M, van der Rijst M, Joubert E. Optimisation and validation of high- temperature oxidation of Cyclopia intermedia (honeybush)—From laboratory to factory. S. Afr. J. Bot,2017:110:152-160.
11. Wang Z, Dou R, Yang R, Cai K, Li C, Li W. Changes in Phenols, Polysaccharides and Volatile Profiles of Noni (morinda citrifolia L) Juice during Fermentation. Molecules,2021:26:2604.
12. Nkhata SG, Ayua E, Kamau EH, Shingiro JB. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci. Nutr,2018:6:2446-2458.
13. Xiang H, Sun-Waterhouse D, Waterhouse GI, Cui C, Ruan Z. Fermentation-enabled wellness foods: A fresh perspective. Food Sci. Hum. Well,2019:8:203-243.
14. Sharma R, Garg P, Kumar P, Bhatia SK, Kulshrestha S. Microbial Fermentation and Its Role in Quality Improvement of Fermented Foods. Fermentation, 2020:6:106.
15. Wall MM, Nishijima KA, Sarnoski P, Keith L, Chang LC, Wei Y. Postharvest Ripening of Noni Fruit (*Morinda citrifolia*) and the Microbial and Chemical Properties of Its Fermented Juice. J Herbs, Spices & Medicinal Plants,2015:21:294-307.
16. Chan-Blanco Y, Vaillant F, Perez AM, Belleville M-P, Zuniga C, Brat P. The ripening and aging of noni fruits (*Morinda citrifolia* L.): Microbiological flora and antioxidant compounds. J. Sci. Food Agric,2007:87:1710-1716.
17. Pino JA, Marquez E, Quijano CE, Castro D. Volatile compounds in noni (*Morinda citrifolia* L.) at two ripening stages. *Cienc. Tecnol. Aliment*,2010:30:183-187.
18. West BJ, Leland DW, Jensen CJ, Palu AK. A double-blind clinical safety study of Noni fruit juice. Pacific Health Dialog,2009:15(2):21-32.
19. Guo M, Mao B, Ahmed Sadiq F, Hao Y, Cui S, Yi M *et al*. Effects of noni fruit and fermented noni juice against acute alcohol induced liver injury in mice. J. Functional Food,2020:70:103995.
20. West BJ, Jensen CJ, Westendorf J, White LD. A Safety Review of Noni Fruit Juice. J Food Science,2006:71(Nr. 8):101-106.
21. Mueller BA, Meri KS, Kevin MS, Kathleen AP. Noni juice (*Morinda citrifolia*): hidden potential for hyperkalemia?. Am J Kidney Dis,2000:35(2):310-2.
22. Pawlus A, Su BN, Kinghorn A. Noni (*Morinda citrifolia*). In: Coates P, Blackman M, Cragg G, *et al*., eds. Encyclopedia of dietary Supplements. New York, NY: Marcel Dekker, 2005, 1-8.
23. Liu Y, Cheng C, Yao S, Xu P, Cao Y, Li J *et al*. Composition and diversity of endophytic bacterial communities in noni (*Morinda citrifolia* L.) seeds. Int. J. Agric. Pol. Res,2014:2(3):098-104.
24. Kulathunga S, Arawwawala LDAM. Morinda citrifolia Linn Grown in Sri Lanka: Shelf Life of Fruit Juice. American J Ethnomedicine,2017:4(2):18.DOI: 10.2167/2348-9502.100018.
25. AOAC. Association of official analytical chemists, official methods of analysis. 19th edition, Washington, D.C, USA, 2012.
26. Forbes BA, Sahm DE, Weissfeld AS. Bailey and Scott's diagnostic microbiology. International edition. 12th edn. Mosby, Inc., an affiliate of Elsevier, Inc., USA, 2007.
27. Cheesbrough M. District laboratory practices in tropical countries. Volume 2 part 2 Cambridge University Press, UK, 2006.
28. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's manual of systemic bacteriology, Williams and Wilkins, Baltimore, MD, USA, 1986, 2.
29. Maldonado MC, Cáceres S, Galli E, Navarro AR. Regulation of the production of polygalacturonase by *Aspergillus niger*. Folia Microbiol (Praha),2002:47(4):409-12.
30. Shah KP, Chandok HK, Rathore P, Sharma MV, Yadav M, Nayarisseri SA. Screening, isolation and identification of polygalacturonase producing *Bacillus tequilensis* strain EMBS083 using 16S rRNA gene sequencing. Eur J Biol Sci,2013:5(1):09-13.
31. Aarti C, Khusro A. Discovery of Polygalacturonase Producing Bacillus tequilensis strain ARMATI using 16S rRNA Gene Sequencing. Asian J Pharm., Clin Res,2015:8(5):58-62.
32. Nelson SC. Noni cultivation and production in Hawai'i. Proceedings of the 2002 Hawai'i Noni Conference, 33–50. M˜anoa, HI: Agriculture and Human Resources, 2003.