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Assessment of Some Secondary Metabolites, Minerals and Alcohol Content of Noni Juice Obtained by Fermentation of *Morinda citrifolia* L. Fruit from Senegal

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Abstract

The fruit of *Morinda citrifolia* L., commonly known as noni, has an extensive history of use as a food and traditional medicine around the world. Adding value to *Morinda citrifolia* L. products, particularly the fruit, could be one way of building resilience in vulnerable farming households. The aim of this study was to determine the secondary metabolite and mineral composition of noni juice obtained by fermenting the fruit of *Morinda citrifolia* L. Fruits were collected in August 2022 from the local field in Thiès region, West of Senegal. Extraction yields were determined and the secondary metabolites were determined using conventional analytical methods. Calcium, magnesium, iron, sodium and potassium were determined by atomic absorption spectrophotometer coupled with a CCD detector. The results show that an average fruit mass (503.2 ± 110.96 g) consists of 171.44 ± 50.01 g pulp and 34.06 ± 10.35 g seeds. The traditional extraction yield of noni juice is 16.46% after three weeks of fermentation. The contents of total polyphenols, flavonoids and tannins obtained in noni are 608.97 ± 4.53 mg EAG/100mL, 7.78 ± 0.01 mg EQ/100mL and 0.191 ± 0.01 mg EC/100mL respectively. The ethanol content of noni varies from 3.57 to 5.23 mL/100mL during extraction. Noni has a high calcium content with a concentration of 383.79 ± 33.23 mg/L. This is followed by a good concentration of magnesium, potassium and sodium, at 278.47 ± 26.30, 187.43 ± 10.7 and 155.95 ± 28.66 mg/L respectively. Noni also has an iron content of 202.15 ± 0.05 mg/L.

Keywords

Morinda citrifolia L., Juice, Alcohol, Total Phenols, Flavonoids, Tannins, Minerals

1. Introduction

The fruit of *Morinda citrifolia* L., commonly known as noni, has long been used as an functional food in all tropical regions [1] [2]. Plant-derived foods support human health by providing nutrients and phytochemicals, which are biologically active secondary plant metabolites [3]. This plant grows in almost all tropical regions of the world [4]. It is a small to medium sized tree with dark green, glossy, and elliptical leaves. *Morinda citrifolia*, commonly known as noni, is a tropical tree that produces fruit year-round [5]. The fruit is an ovoid syncarp that changes from green to pale yellow, then finally to white, during ripening and senescence. The leaves and fruit, as well as other plant parts, had significant roles in Pacific, South Asian, Southeast Asian, and Caribbean traditional medicine [5]. Various parts of the plant were used by local healers to treat inflammation, osteoarthritis, rheumatism, backache, joint problems, diabetes, ranula, skin allergies, and warts [6]. In some European island countries, all parts of the plant are used to treat a wide range of illnesses [7]. Some Pacific Islanders believe that improved skin health is one of the benefits of drinking noni juice [7]. Different parts of the plant were used by local healers in Polynesia for a broad range of health conditions [3] [8]. Since 1996, noni products, mainly fruit juice, have been available worldwide as health foods, and noni leaf tea has recently been approved by EU legislation as a novel food [9]. Different components of the plant are utilized to address inflammation, osteoarthritis, rheumatism, back pain, joint issues, abscesses, angina, diabetes, and skin allergies [10]. Moreover, different plants may have individual accumulation capacities for metals [11] [12]. The noni plants have an increased potential to accumulate minerals and trace elements [13]. Noni has been the subject of a number of research projects around the world to study its composition and health effects. However, following its recent introduction to Senegal, research on this fruit has not yet been developed at local level. Despite its sociological importance, there has been little research into the phytochemical composition of traditional fruit juices [14]. The aim of the present investigation was to determine the chemical composition of secondary metabolites and minerals in *Morinda citrifolia* L. fruit juice, which could justify their use in the traditional treatment of certain diseases.

2. Material and Methods

2.1. Plant Material

Fruits of *Morinda citrifolia* were collected in August 2022 from the local field in

Thiès region, West of Senegal. The fruit was washed in bleach and dried before juice extraction.

2.2. Noni Juice Extraction

To prepare traditional noni juice, jars are filled with ripe fruit, washed and weighed beforehand. The jars are then hermetically sealed and the fruit is left to ferment in the jars. After each week of fermentation, the juice is collected in bottles and stored in the freezer at −18˚C.

2.3. Quantitative Analysis of Polyphenols, Tannins and Flavonoids

2.3.1. Total Polyphenols

Total polyphenol content in juice was determined according to the method adopted by Georgé *et al.* (2005) [15] which uses the Folin-ciocalteu reagent and gallic acid as standard. To a test tube containing 0.5 mL of the mother solution (1000 µg/mL), 4 mL of Na_2CO_3 (7.5%) is added. After stirring, 2.5 mL of the Folin-ciocalteu solution was added. The whole is incubated in a water bath at 45˚C for 30 minutes. The absorbance is read at 765 nm against a control without extract. The total polyphenol content of the extracts was determined from the standard gallic acid curve and the results are expressed as milligram of gallic acid equivalent per hundred milliliter of juice (mg EAG/100mL).

2.3.2. Flavonoids

The flavonoid content was determined by the aluminium chloride method using quercetin as standard by Kim *et al.* (2003) [16]. 6.4 ml of distilled water and 0.3 ml of 5% sodium nitrite ($NaNO_2$) solution are added to a test tube containing 1 ml of the metered extract. Everything is well mixed. After 5 minutes, 0.3 mL of 10% aluminium trichloride solution (m/v) is added to the mixture which is incubated at room temperature for 6 minutes. To this mixture is added 2 mL of sodium carbonate (1M). The mixture is completely agitated in order to homogenize the content. After 30 minutes of incubation at room temperature, the absorbances are read using a visible UV spectrophotometer (LLG-uniSPEC 2) at 510 nm against a control (without extract). The calibration curve obtained with quercetin as standard made it possible to calculate the concentrations of flavonoids contained in our extracts (seeds, pulp, fiber, and shell of the baobab fruit). The results are expressed as milligram of quercetin equivalent per hundred milliliter of juice (mg EQ/100mL).

2.3.3. Tannins

The determination of the tannins was carried out by the method described by Rebaya *et al.* [17] using catechin as standard. 0.1 mL of the extract was mixed with 3 mL of 4% methanolic vanillin solution. Then 1.5 mL of hydrochloric acid is added, the mixture is stirred and left to stand for 20 minutes. The absorbances were measured at 500 nm. The results are expressed as milligram of catechin equivalent per hundred milliliter of juice (mg EC/100mL).

2.4. Determining Alcohol Content

The ethanol content was evaluated according to the AFNOR standard methods [18]. It is based on the separation of ethanol by distillation, followed by oxidation in a sulfuric medium by potassium dichromate. Excess dichromate is measured by ammonium iron (II) sulfate in the presence of ferrous orthophenantroline.

2.5. Color Determination

The color of the samples of the products obtained was measured using a colorimeter (type: KONICA MINOLTA. Japan) based on the CIELAB color system (L*, a*, b* and L*, C*, h, YI). The color parameters (L*, a*, b* and L*, C*, h, YI) were measured 3 times for each sample. L*, a*, b* describe the colors black-white, Green-Red and Blue-Yellow respectively: L* (0 = Black, 100 = White); a* (−a = Green, +a = Red); b* (−b = Blue, +b = Yellow) (**Figure 1**) [19].

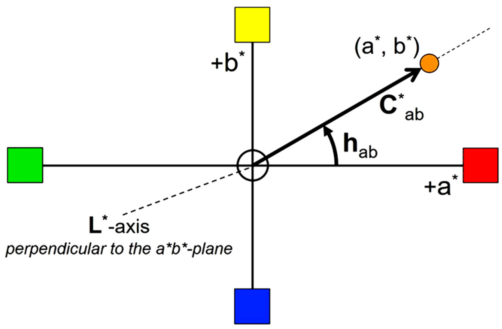

Figure 1. Color settings (a, b, L).

2.6. Minerals

The determination of minerals was carried out by atomic absorption spectrophotometer (SAA NOVAA-350, ZEENIT 700P). The results are expressed in milligrams per liter of juice.

2.7. Statistical Analysis

The results were subjected to a one-way ANOVA analysis of variance with R software version 3.2.4 Revised (2018) and Minitab-18 software. The X value of each sample is assigned a superscript letter ($X^{(i)}$ where i = a, b, c …). Samples with the same letter are not statistically different at the 5% level.

3. Results and Discussion

3.1. Yield of Different Fruit Parts

The various mass proportions and extraction yields of *Morinda citrifolia* L. fruit juice are presented in **Table 1**. The results show that an average fruit mass (503.2 ± 110.96 g) consists of 171.44 ± 50.01 g pulp and 34.06 ± 10.35 g seeds. The traditional extraction yield of noni juice is 16.46 ± 4.10% after three weeks of fermentation. Noni juice accumulates inside the containers and ferments as it oozes out. As a result, noni juice extraction yield increases with the duration of fermentation.

Table 1. *Morinda citrifolia* fruit and juice yield.

Parameters	Whole fruit	Pulp	Seed
Average value (g)	503.2 ± 110.96	171.44 ± 50.01	34.06 ± 10.35
Juice quantity (g)	82.42 ± 3.12	-	-
Yield (%)	16.46 ± 4.10	34.07 ± 6.44	6.77 ± 2.39

3.2. Quantitative Assessment of Secondary Metabolites

Total phenolics constituted one of the major groups of compounds acting as primary antioxidants, it was reasonable to determine their total amount in noni juice [20]. Quantitative analysis results show that polyphenol, flavonoid and tannin contents vary significantly with juice extraction time. Juice total polyphenol content (**Figure 2**) varies significantly from 330.13 ± 10.65 to 608.97 ± 4.53 mg EAG/100mL from the first (raw juice) to the third week of extraction. This is probably due to the micro-organisms' ability to express amylase, *β*-glucosidase, decarboxylase, phenolic acid decarboxylases, esterase, phenol reductase, tannase and glucoamylase, which have been described for their essential role in increasing polyphenol levels in fermented foods [21]. The results obtained are superior to those obtained by Wang *et al*. [21], for whom fermented noni juice increases from a concentration of 144 mg EAG/100mL for the first week of fermentation to 180 mg EAG/100mL for the third week of fermentation. The fresh juice has a lower polyphenol concentration than the fermented juice (330.13 ± 10.65 mg EAG/100mL). This concentration is nevertheless higher than those obtained by Wang *et al* in their experiment (168.35 mg EAG/100g) [22]. The differences noted between the results of the two studies may be due to experimental conditions or environmental conditions (climate, soil, growing conditions). The total polyphenol content obtained in noni juice is higher than that obtained in raw cashew apple juice (10 to 30 mg EAG/kg) [23]. These results show that noni juice is a good source of polyphenols, and therefore of antioxidants. **Figure 3** shows the evolution of flavonoid concentration in noni juice over three weeks of fermentation. The results show that the flavonoid content of noni juice increases with the duration of fermentation. Flavonoid content in fermented noni juice increases during the first week of fermentation. It rises from 5.86 ± 0.03 to 7.78 ± 0.01 mg EQ/100mL. Fresh noni juice has a low flavonoid concentration compared with fermented juice, with a concentration of 1.79 ± 0.01 mg EQ/100mL. The results obtained follow the same trend as those of Wang *et al*. [22] for whom from the first to the third week of fermentation the flavonoid concentration is 25.2 and 31 mg EQ/100mL respectively. The results show that the variation in flavonoid concentration in noni juice follow the same trend as that of total polyphenol concentration. This is because flavonoids, which are a class of polyphenols, undergo the reactions described above for these. Indeed, fermentation is one of the oldest, most economical and most useful biotechnological methods for maintaining and improving the nutritional and sensory qualities and shelf life of fruit. **Figure 4** shows the concentration of condensed tannins in noni juice during fermentation. The results show that the concentration of tannins in noni juice increases with the duration of fermentation. The fresh juice has a concentration of 0.138 ± 0.03 mg EC/100mL and the highest concentration is recorded in the second week of fermentation with 0.191 ± 0.001 mg EC/100mL. In the third week, tannin concentration drops slightly from 0.191 ± 0.001 to 0.185 ± 0.02 mg EC/100mL. Several factors, such as the polyphenol content of the food, the duration and conditions of fermentation, and microbial and enzymatic activity, can influence the condensed tannin content during fermentation. Condensed tannins, also known as proanthocyanidins, are polyphenolic compounds that contribute to astringency and other sensory characteristics of foods. This bioactive compound is known to have potential antiviral activity [24] as well as potential prophylactic and therapeutic effect against cancer cells [25]. The synergistic action of tannins, total phenols, flavonoids found would explain the antioxidant power of plant extracts [26].

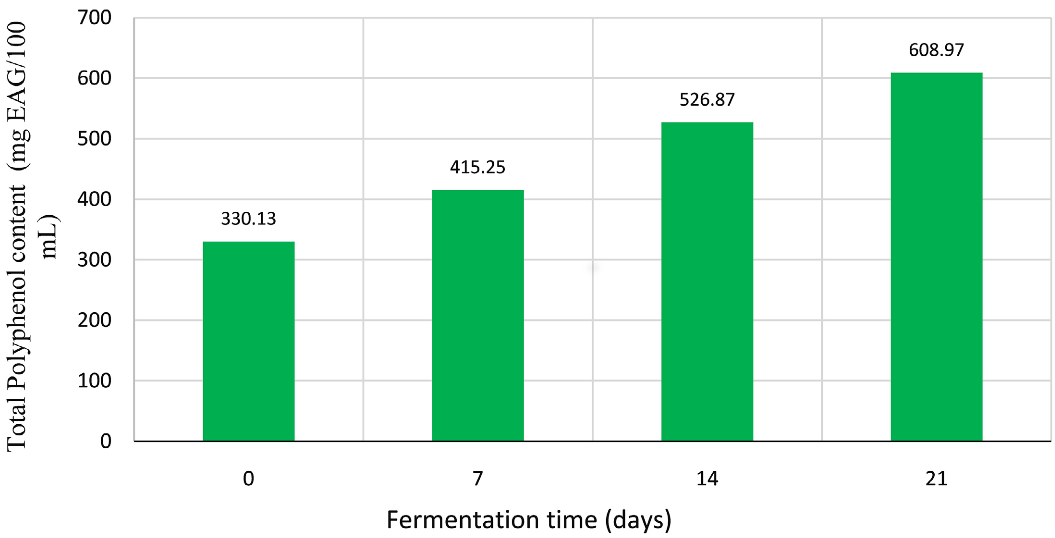

Figure 2. Change in the total polyphenol content of noni juice.

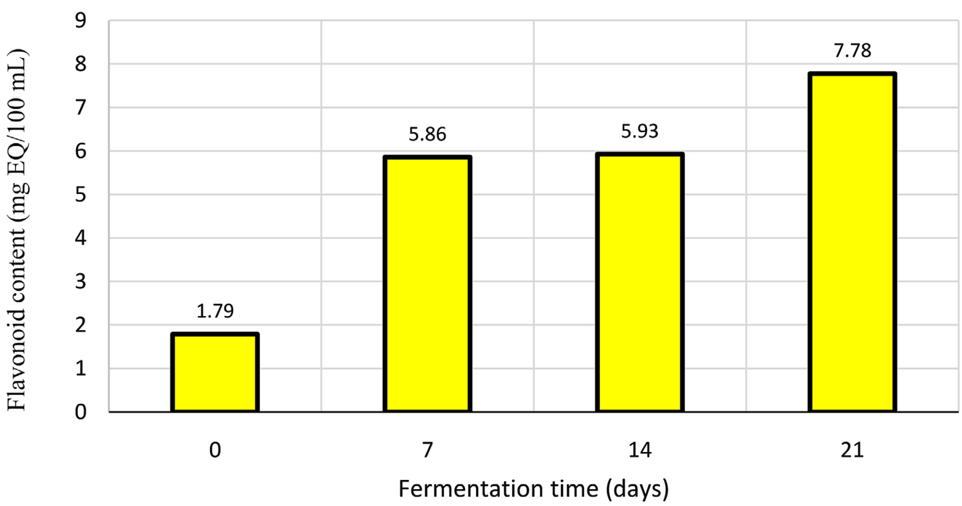

Figure 3. Change in the flavonoid content of noni juice.

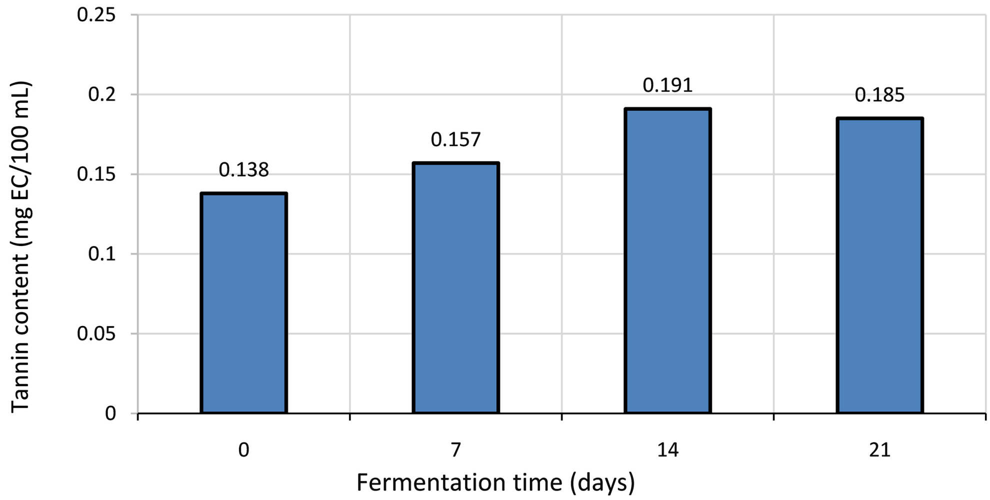

Figure 4. Change in the tannin content of noni juice.

3.3. Alcohol Content in Juice

The evolution of the ethanol content of noni juice over three weeks of fermentation is shown in **Figure 5** below. The latter shows a clear and rapid increase in the ethanol content of noni juice during fermentation. From the first to the third week of fermentation, ethanol content rises from 3.57 to 5.23 mL/100mL. The evolution of ethanol content in fermented noni juice depends on the fermentation process. Alcoholic fermentation is generally carried out by yeasts, which metabolize the sugars present in the noni juice to produce ethanol and carbon dioxide (CO_2). As fermentation progresses, the ethanol content of the noni juice increases, stabilizing at the fermentation peak. The results obtained are in line with those obtained by Wall *et al.* [27], for whom the ethanol content rises from 3.75 to 7.83 mg/mL from the first to the third week of fermentation. These results are lower than those obtained for traditional drinks, Boumkaye (6.34 ± 0.00 g/100g) in the work of Cissé *et al.* [28].

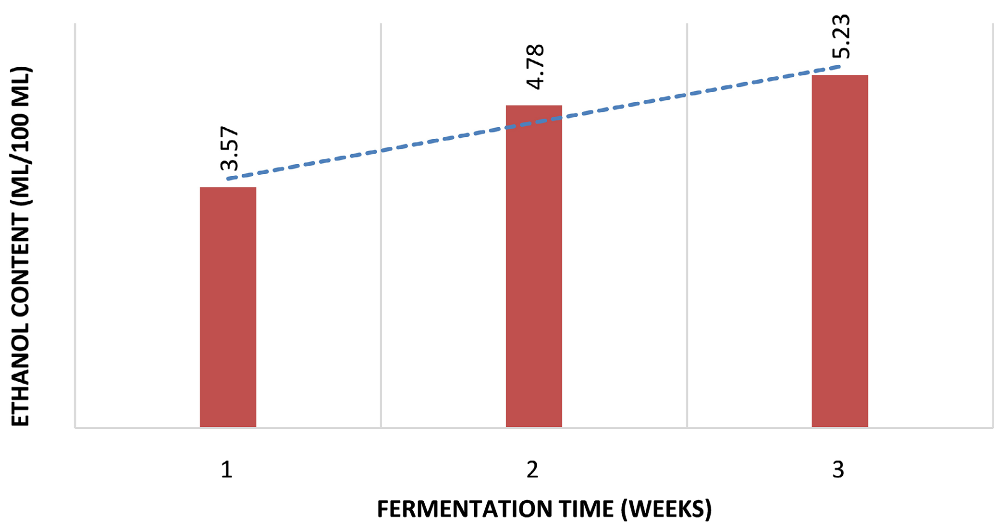

Figure 5. Changes in the ethanol content of noni juice.

3.4. Juice Color Parameters

Table 2 shows the color intensity of noni juice during fermentation. Noni juice obtained during fermentation has a brown color tending towards black. Juice clarity decreased during fermentation from 37.75 to 20.44. The intensity of red coloration given by parameter (a) increases from 26.20 to 31 from the first to the third week of fermentation. We also note that the intensity of yellow coloration is very strong during the first two weeks of fermentation, with values of 50.97 and 63.07, but decreases during the third week of fermentation, with a value of 31.25. Noni juice darkens as a result of enzymatic browning under the action of phenolase and oxygen. Noni juice has a mellow odor and a bitter taste. The changes we note in the organoleptic properties of noni juice may be due to the evolution of phenolic compounds during fermentation. The bitter taste and odor of noni juice make it difficult to drink, which is why it is often blended with other fruit juices such as grape juice and blueberry juice in the case of the best-known industrial juice, Tahitian Noni Juice.

Table 2. Color parameters (L*, a*, b*) of *Morinda citrifolia* juices.

Parameters	Fresh juice	Fermented juice *(First week)*	Fermented juice *(Second week)*	Fermented juice *(Third week)*
L	79.65 ± 0.27	37.75 ± 19.00	45.51 ± 19.09	20.44 ± 16.88
a*	0.18 ± 0.04	26.20 ± 5.76	27.98 ± 5.76	31.00 ± 4.75
b*	23.7 ± 0.10	50.97 ± 17.00	63.07 ± 18.38	31.25 ± 23.70

3.5. Mineral Elements

Certain mineral elements are involved in metabolism, notably Calcium, Magnesium, Iron and Copper [29]. These are quantified by atomic absorption spectrophotometry (AAS) and summarized in Table 3. The results show that the values are significantly different during extraction by fermentation. Indeed, the calcium concentration of the juice varies from 312.14 ± 0.01 to 383.79 ± 33.23 mg/L from the first to the third week of fermentation. The calcium content of noni juice (34.8 to 47 mg/100g) is higher than that of pome fruit (7 to 10 mg/100g) [30]. The iron concentration of noni juice increases significantly with extraction time, with values ranging from 45.76 ± 33.08 to 202.15 ± 0.05 mg/L. The iron composition of noni juice is much higher than that of raw cashew apple juice from Casamance (0.48 to 1.08 mg/kg) [23], nuts (2 to 73 mg/kg), stone fruits (2.5 to 69 mg/kg) and berries and exotic fruits (2.4 to 27 mg/kg) [30]. Iron is an essential component of hemoglobin, which is involved in oxygen transport. Magnesium content in noni juice increases with fermentation time. The highest content is obtained in the third week of fermentation (278.47 ± 26.30 mg/L) and the lowest concentration is obtained in the fresh juice (93.58 ± 0.05 mg/L). The magnesium composition of noni juice (93.58 ± 0.05 to 278.47 ± 26.30 mg/L) obtained by fermentation is higher than that of pome fruits (4 to 4.8 mg/100g), stone fruits (4.9 to 54 mg/100g) and berry and exotic fruits (2.4 to 41 mg/100g), respectively

[30]. Magnesium plays an important role in the stability of the nervous system. It is involved in muscle contraction, notably as an activator of alkaline phosphatase [31]. *Morinda citrifolia* raw juice is rich in potassium. Its potassium content (95.05 ± 0.02 to 187.43 ± 10.78 mg/L) is higher than that of nuts (265 to 1020 mg/100g), pips (65 to 183 mg/100g), stones (103 to 1370 mg/100g), berries and exotics (59 to 782 mg/100g), but lower than that of Maerua pseudopetalosa (1098 to 1342 mg/100g) [32]. Potassium concentration increases with fermentation. Fresh juice has a concentration of 95.05 ± 0.02 mg/L, while juice fermented for three weeks has a concentration of 187.43 ± 10.78 mg/L. Potassium (K) helps maintain electrolyte balance in the human body and prevents bone demineralization by preventing calcium loss in urine [33]. It is a hypotensor and is also involved in muscle contraction [34]. Potassium content and consumer preference for juice are linked [35]. Finally, we see that the trend for sodium is also upwards, with a concentration of 73.71 ± 0.01 mg/mL for fresh juice and 155.95 ± 28.66 mg/mL for fermented juice at week three. The results show that the concentration of minerals in noni juice rises steadily during fermentation. Analysis of the experimental results shows that raw noni juice is an important source of minerals compared with other fruits consumed in tropical Africa. The calcium content (312.14 to 383.79 mg/L) of noni juice is higher than that found in raw cashew apple juice (34.8 to 47 mg/100g) from Casamance [23] and in pome fruits (7 to 10 mg/100g) [30]. The organoleptic quality of juices is also an important factor in assessing the marketability of fruit juices. Mineral elements contribute to the flavour of fruit juices [36]. Fruits and vegetables regulate the body's mineral content and reduce the risk of cardiovascular disease and cancer [37].

Table 3. Some mineral content (Ca, Fe, Mg, K, Na) of noni juice.

Fermentation time	Calcium	Iron	Magnesium	Potassium	Sodium
	Concentration in mg/L				
Fresh juice	312.14 ± 0.01^a	45.76 ± 33.08^a	93.58 ± 0.05^a	95.05 ± 0.02^a	73.71 ± 0.01^a
First week	336.53 ± 43.74^b	116.88 ± 9.48^b	206.01 ± 81.36^b	134.80 ± 44.29^b	116.69 ± 33.64^b
Second week	368.34 ± 7.24^c	127.7 ± 49.31^c	236.36 ± 41.27^c	179.49 ± 6.06^c	129.73 ± 4.81^c
Third week	383.79 ± 33.23^d	202.15 ± 0.05^d	278.47 ± 26.30^d	187.43 ± 10.78^d	155.95 ± 28.66^d

a, b, c: In the same column, means followed by a different letter are significantly different at the p < 5% threshold (Newman and Keuls method).

4. Conclusion

This study is a contribution to the knowledge of little-known plant species, in order to better understand their various virtues. The study of the phytochemical composition of *Morinda citrifolia* L. fruit has provided scientific knowledge of the chemical and biochemical characteristics of noni juice grown in Senegal. The results obtained showed the presence of a high quantity of polyphenols, confirming their pharmacological qualities. Noni juice has a high vitamin C content and high ethanol potential. Characterization of the minerals in raw noni juice showed the presence of K, Mg, Na, Ca and Fe, with very high levels of calcium (K), magnesium (Mg) and iron (Fe). Adding value to *Morinda citrifolia* fruit juice will help improve the diet of vulnerable groups. Following this work, the evaluation of antioxidant activity, biological tests and biochemical characterization should provide further proof of this plant's activity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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