

Microbiological and anti-oxidant Properties during ripening of Noni Fruits (*Morinda citrifolia*)

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Keywords: Folin / Phenols / Antioxidant capacity / ORAC / Vitamin C/Microbiology

Abstract

Morinda citrifolia has been used in traditional Polynesian medicine for over 2000 years to prevent and cure many different diseases. As a result of these uses and the market that is developing around noni juice, it has become increasingly important to confirm the plant's actual therapeutic properties. The objective of this study was to determine some functional, nutritional and microbiological changes during ripening period. The noni fruit was harvested unripe and stored in containers at room temperature for 3 days. Analyses of total mesophilic plate count, lactic acid bacteria, molds vitamin C, phenols content and antioxidant capacity were measured in unripe and ripe noni fruit. Results show that during the ripening period the pH lowered from 4,6 to 4,0 and the soluble solids are between 7,5 for unripe noni and 47,3 for ripe noni. Microbiological analysis shown that during ripening period the molds and yeasts as well as the mesophilic bacteria they are maintained relatively constant in the order of 1×10^1 and 4×10^3 UFC/g respectively. Lactic acid bacteria increase in order of 2×10^3 . Phenols content in the noni fruit change of 41,4 to 51,1 mg GAE.100g⁻¹ after ripening period; vitamin C content decrease of 391 to 316 mg 100 g⁻¹ while antioxidant activity (ORAC) oscillated between 7,4 and 8,0 $\mu\text{mol Trolox.g}^{-1}$. These results confirm that the unripe and ripe noni fruit has an important antioxidant capacity and the low pH can be due to lactic acid production.

INTRODUCTION

Noni is the common Hawaiian name for the fruit of the *Morinda citrifolia* plant, which belongs to the Rubiaceae family. Vernacular names for this fruit in the South Pacific and the Caribbean include: "Indian mulberry", "nuna" or "ach" in the Indian subcontinent, "mengkudu" in Malaysia, "nhau" in Southeast Asia, "painkiller bush" in the Caribbean and "cheese fruit" in Australia (Morton, 1992; Ross, 2001; Cardon, 2003). Noni is native from Southeast Asia to Australia, and is found from Polynesia to India. It is now cultivated throughout the tropics and is found in Mexico, Panama, Costa Rica, Colombia, Venezuela, the Florida Keys and the West Indies (Ross, 2001; Dixon et al., 1999).

The Polynesians have been using the noni plant with medicinal purposes for more than 2000 years. In traditional pharmacopoeia, the fruit is claimed to prevent and cure many different diseases. It is primarily used to stimulate the immune system and thus fight bacterial, viral, parasitic and fungal infections; while also preventing the formation and proliferation of tumors, including malignant ones (Earle, 2001; Dixon et al., 1999). Noni juice is also claimed to reduce inflammation. It is the juice of the fruit that is most often consumed, although the leaves, flowers, bark and roots are also used (Dixon et al., 1999; Earle, 2001; McClatchey, 2002).

Many claims have recently been made regarding noni's nutraceutical properties. Various publications have shown that noni can be used to relieve different diseases, and its registered uses span the Pacific and Asia, as well as Africa. In numerous clinical studies, noni has been associated with the relief of arthritis and diabetes. They report that noni's capacity to control these diseases is linked to the presence of compounds such as scopoletin, nitric oxide (Solomon, 1999), fiber, alkaloids and sterols, as well as its antioxidant potential (Elkings, 1998), all of which promote normal body function. Nevertheless, there is no scientific information to prove these medical properties.

As a result of this reputation and an efficient marketing system, consumption of this fruit is currently very high, not only in the countries where it is produced but also in the United States, Japan and Europe. As evidence of this, the company Morinda Inc., a commercial noni juice producer, has boasted record sales in recent years. In response to the high demand, growing noni is also on the rise in countries such as Costa Rica, where there are many commercial plantations. The fruit is normally commercialized unripe, ripe or as juice on both formal and informal markets. Nevertheless, despite the real market opportunities, there has been very little scientific research to determine the actual functional properties of noni products. In addition, there is no information about the phytochemical compounds responsible for their supposed functional properties. As a result, optimizing agricultural practices, post-harvest factors (harvest dates, ripening rates) and processing methods is pointless, as the functional quality of the end products cannot be assessed.

MATERIALS AND METHODS

Raw Material

The noni fruits used in this study were obtained from the noni experimental plantation, EARTH University, Limón, Costa Rica. The noni fruit was harvested unripe, washed and disinfected with 200 ppm chlorine and stored in containers at room temperature for 3 days (ripening period). The samples for the determination of polyphenols, antioxidant activity and identification of phenolic compounds were washed, chopped, frozen with liquid nitrogen, lyophilized and ground to powder. The samples for the determination of vitamin C and analyses of total mesophilic plate count, lactic acid bacteria, molds and yeasts were chopped and homogenized before use. All samples were analyzed in triplicate.

Analytical Methods

The pH was measured with a glass electrode and a Corning pH-meter (Model 530) using standard methods (AOAC, 1990). Moisture content was determined by drying the samples with a moisture determination balance (PRECISA 310). Total soluble solids (TSS) content was measured by determination of °Brix with an Abbe refractometer (Milton Roy Company LR45227).

Microbiological Analysis

Mesophilic plate count was determined using the Aerobic Plate Count methods described by AOAC (1998), the lactic acid bacteria count was determined using the method described by Downes and Ito (2001) and the mold and yeast count was determined using the method described by AOAC (1998).

Ascorbic Acid Determination

Ascorbic acid and dehydroascorbic acid contents were assessed by HPLC analysis, using the method as modified by (Kacem et al, 1986; Brause et al, 2003).

Determination of Total Phenols

A modified Folin-Ciocalteu method (Slinkard and Singleton, 1977; Georgé et al, 2005) was used to determine total phenolic compounds in noni fruits, using gallic acid as standard and expressing the results as gallic acid equivalent (GAE). Absorbance was measured at 760 nm.

Determination of Antioxidant Capacity

The antioxidant capacity of each sample was measured in terms of oxygen radical absorbing capacity (ORAC), using fluorescein as the peroxy radical damage indicator, as according to (Ou et al, 2001 and Vaillant et al, 2005). In this analysis was used 0,5 g of lyophilized sample.

RESULTS AND DISCUSSION

Main Characteristics

As seen in Table 1, during the noni fruit's ripening the pH showed a tendency to decrease. This may be attributed to microbial activity that might occur during ripening that can produce organic acids that below the pH. The moisture content and soluble solids remained similar throughout the evaluation period with no significant differences recorded in the obtained results.

Microbiologic Analysis

Table 1 shows the results of the microbiological analyses performed on the noni fruit during ripening. In this period, the total mesophilic bacteria increase slightly and this value ($1,8 \times 10^3$ UFC/g) is similar at reported by Limyati and Juniar (1998). Contamination with molds and yeasts is not changed ins the ripening period and this value is lower that reported by Limyati and Juniar (1998). Lactic acid bacteria counts increased progressively during storage and that could be attributed to contamination from air; this bacteria can be correlated with pH reduction due to the acid lactic generation.

Antioxidant Properties

The antioxidant activity of natural sources is due to the active compounds present in the plants. According to Pratt and Hudson (1992), most natural antioxidants can be found in fruits and they are normally phenolic or polyphenolic compounds.

The antioxidant properties of noni fruit during ripening is shown in Table 1. Total phenolic compound content in unripe noni fruit was lower than the ripe noni fruit, nevertheless, both values are similar or higher that reported in products such as avocado, cantaloupe, grapefruit, lemon, melon, nectarine, orange, peach, pineapple, watermelon (Vinson et al, 2001), banana, litchi, longanberry (Luximon-Ramma et al, 2003) and tomato (Georgé et al, 2005). At the same time, analysis of vitamin C shown content of 391 and 316 mg.100g⁻¹ in unripe and ripe fruits respectively and both values are higher that report by Morton (1992). The vitamin C content of the fruit depends on ripeness, seasons, climates and localities. In some fruits like guava and medlar, content is highest when the fruit is still green and lowest when ripe (Aydin and Kadioglu, 2001 and Hind and Abu-Bark, 2002). Compared to other tropical fruits, noni has more vitamin C that fruits as lemon, orange, lychee, longan and kiwi (Rodrigues et al, 2001) which makes this fruit an important source of this micronutrient.

The ORAC value of unripe and ripe noni fruits are similar and shows an important antioxidant capacity (7.4 and 8.0 $\mu\text{mol Trolox.g}^{-1}$ respectively); these values are similar to the ones found in fruits as grape fruit, banana, apple plum, orange, grape and kiwi fruit (Wang, 1996). Other studies carried by Mohd-Zin et al (2002) shows that the ethyl acetate extract of noni fruit exhibit significant antioxidant activity, which is comparable to that of both α -tocopherol and BHT. Phenolic compound has been found to be the major groups of functional micronutrients in noni; damnacanthal, scopoletin, morindone, alizarin, aucubin nordamnacanthal, rubiadin, rubiadin-1-methyl ester and other anthraquinone glycosides have

been identified in noni fruit (Dittmar, 1993; Dixon et al., 1999; Morton, 1992; Wang & Su, 2001) that confirm that this fruit is a potential source of natural antioxidants.

CONCLUSIONS

Microbiological analysis of noni shown that after ripening period the total mesophilic bacteria as well as molds and yeasts are similar. The pH decrease can be caused by lactic acid bacteria count increase observed.

The antioxidant capacity of noni in ripening period is similar and it is due of the considerable amount of vitamin C, phenolic compounds content and antioxidant activity (ORAC).

ACKNOWLEDGEMENTS

The authors acknowledge and thank the French Agency AIRE Développement, the Regional French Cooperation for Central America (CCCAC) in San José, Costa Rica, the National Council of Science and Technology (CONICIT) and the Ministry of Science and Technology of Costa Rica (MICIT) for their cooperation.

Literature Cited

- [1] AOAC Bacteriological Analytical Manual. 1998. In: *Official Methods of Analysis of the Association of Official Analytical Chemists*. 8 Ed.; Association of Official Analytical Chemists, Arlington, USA.
- [2] AOAC Fruits and fruits products. 1990. In: *Official Methods of Analysis of the Association of Official Analytical Chemists*; K. Helrich, (ed.), Association of Official Analytical Chemists, Arlington, USA, pp 910-928.
- [3] Aydin, N. and Kadioglu, A. 2001. Changes in the chemical composition, polyphenol oxidase and peroxidase activities during development and ripening of medlar fruits (*Mespilus germanica* L.)
- [4] Brause, A. R., Woollard, D.C., Indyk, R.E. 2003. Determination of total vitamin C in fruit juices and related products by liquid chromatography- Interlaboratory study. *Journal of AOAC International*, 86, 367-374.
- [5] Cardon, D. 2003. *Le Monde des teintures naturelles*. Berlin, Paris, p. 592.
- [6] Dittmar A., 1993. *Morinda citrifolia* L.- Use in indigenous Samoan medicine, *Journal of Herbs, Spices & Medicinal Plants*, 1, 77-92.
- [7] Dixon A.R., McMillen H., Etkin N.L., 1999. Ferment this: the transformation of Noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae), *Economic Botany* 53, 51-68.
- [8] Downes, F.O.; Ito, K. 2001. *Compendium of Methods for the Microbiological Examination of Foods*. 4ed. American Public Health Association, Washington DC. Chapter 19 Acid-Producing Microorganisms
- [9] Earle J.E., 2001. *Plantas medicinales en el Trópico Húmedo*, Editorial Guayacán, San José, p. 246

- [10] Elkins R., 1998. Hawaiian Noni (*Morinda citrifolia*) Prize Herb of Hawaii and the South Pacific. Woodland Publishing, Utah, p 32.
- [11] Georgé, S.; Brat, P.; Alter, P.; Amiot, M.J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agriculture and Food Chemistry*, 53, 1370-1373.
- [12] Hind A. H.A. Bashir, Abu-Bakr A. A.-B.A. Abu-Goukh. 2003. Compositional changes during guava fruit ripening. *Food Chemistry*, 80, 557 - 563
- [13] Kacem, B.; Marshall, M. R.; Mathews, R. F.; Gregory, J. F. 1986. Simultaneous analysis of ascorbic and dehydroascorbic acid by high-performance liquid chromatography with postcolumn derivatization and UV absorbance. *Journal of Agriculture and Food Chemistry*, 34, 271-274.
- [14] Limyati, D.A. and Juniar, B.L.L. 1998. Jamu Gendong, a king of traditional medicine in Indonesia: the microbial contamination of its raw materials and endproduct. *J Ethnopharmacol.* 63, 201-208.
- [15] Luximon-Ramma, A.; Bahorun, T.; Crozier, A. 2003. Antioxidant actions and vitamin C contents of common Mauritian exotic fruits. *Journal of the Science of Food and Agriculture*, 83, 496-502.
- [16] McClatchey W., 2002. From Polynesian healers to health food stores: changing perspectives of *Morinda citrifolia* (Rubiaceae), *Integrative Cancer Therapies* 1, 110-120.
- [17] Mohd Z., Abdul-Hamid A., Osman A. 2001. Antioxidative activity extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chemistry* 78, 227-231.
- [18] Morton J.F., 1992. The ocean-going Noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its "colorful" relatives, *Economic Botany* 46, 241-256.
- [19] Ou, B.; Hampsch-Woodill, M.; Prior, R. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry*, 49, 4619-4626.
- [20] Pratt, D.E. and Hudson, B.J.F. 1992. Nature antioxidants not exploited commercially. In: B.J.F. Hudson (ed.), *Food antioxidants*, London, Elsevier Applied Science. p. 171-192.
- [21] Rodrigues, R.B.; De Menezes, H.C.; Cabral, L.M.C. Dornier, M.; Reynes, M. An Amazonian fruit with a high potential as a natural sources of vitamin C: the camu-camu (*Myrciaria dubia*), *Fruit* 56(5) 345-354.
- [22] Ross I.A., 2001. *Medical Plants of the World. Chemical Constituents, Traditional and Modern Medical Uses.* Vol. 2. Humana Press, New Jersey, p. 615.
- [23] Slinkard, K. and Singleton, L. 1977. Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture.* 28, 49-55.
- [24] Solomon N., 1999. *The Noni Phenomenon*, Direct Source Publishing, Utah, p. 296.
- [25] Vaillant, F.; Perez, A.; Davila, I.; Dornier, M.; Reynes, M.. Colorant and antioxidant properties of red-purple pitahaya. *Fruit*, 60, 3-12.
- [26] Vinson, J. A.; Su, X.; Zubik, L.; Bose, P. 2001. Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry*, 49, 5315-5321.
- [27] Wang, M.Y. and Su, C. 2001. Cancer preventive effect of *Morinda citrifolia* (Noni), *Ann. NY Acad. Sci.* 952, 161-168.
- [28] Wang, H.; Cao, G. and Prior, R.L. 1996. Total Antioxidant capacity of Fruits. *Journal of Agricultural and Food Chemistry*, 44, 701-705.

Tables

Table 1. Microbiological and anti-oxidant properties of noni fruits (*Morinda citrifolia*)

Analysis	Noni	
	Unripe	Ripe
pH	4,6 (0,1) ^a	4,0 (0,1) ^b
Soluble solids (°Brix)	7,5 (0,5) ^a	7,3 (0,3) ^a
Moisture (%)	92,0 (1,6) ^a	91,8 (0,4) ^a
Phenols content ^A (mg GAE.100g ⁻¹)	41,4 (6,9) ^a	51,1 (1,8) ^a
Vitamin C ^B (mg.100g ⁻¹ pulp)	391 (22) ^a	316 (64) ^a
ORAC ^C (µmol Trolox.g ⁻¹)	7,4 (0,4) ^a	8,0 (0,8) ^a
Total mesophilic bacteria (UFC/g)	1,0x10 ³	1,8x10 ³
moulds and yeast (UFC/g)	4,0x10 ¹	3,8x10 ¹
Acid lactic bacteria (UFC/g)	<10	1,9x10 ³

^A Expressed as mg of gallic acid per 100 gram of pulp without seeds

^B Sum of ascorbic and dehydroascorbic acid

^C Expressed as µmol of Trolox per gram of fresh pulp without seeds

^{a,b} Values with the same letter in each column are not significantly different at level of $p < 0,05$

Values in parenthesis are the standard deviation

Mean and standard deviation of three samples purchased and analyzed independently