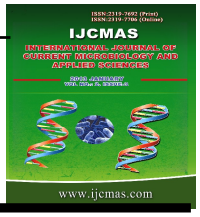
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7692 **Volume 2 Number 3 (2013) pp.65–74**
http://www.ijcmas.com



Original Research Article

Nutritive analysis of fresh and dry fruits of *Morinda tinctoria*

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K e y w o r d s

Morinda tinctoria; nutritional content; Vitamins; Minerals.

A B S T R A C T

Fruits are generally acceptable as good source of nutrient and supplement for food in a world faced with problem of food scarcity. They are known to be excellent source of nutrients such as minerals and vitamins. The present investigation aimed to assess the nutritional value of ripened fruits of a popularly known medicinal plant *Morinda tinctoria.* The present study is focused on the estimation of Ash content, Protein, carbohydrate, vitamins and mineral content of *Morinda tinctoria* fruit. The difference in nutritional content between fresh and dried fruits were also assessed. The ash content is 4% and 1.6% for fresh and dry fruits respectively. The fresh fruit is rich in protein and carbohydrate than the dry fruit. The fresh fruit is rich in Ascorbic acid and Niacin whereas the dry fruit showed the presence of Riboflavin and Thiamine in high concentration. The dry fruit is rich in calcium whereas the fresh fruit is rich in both iron and copper. Both the fresh and dry fruits showed complete absence of phosphorous. Thus, the *Morinda tinctoria* fruits could be used as a source of protein, vitamin and minerals.

Introduction

Plants are the reservoirs of a large number of imperative organic compounds and they have long been used as the sources of medicines. In order to face the problem of food scarcity, fruits can be utilized for the good source of nutrients and food supplements. Fruits are commonly well known for the excellent source of nutrients such as minerals and vitamins; and also contain carbohydrates in form of soluble sugars, cellulose and starch (Nahar *et al.*, 1990).Dependence on plants is prevalent in developing countries where the traditional herbal medicine plays a major role in health care and in the treatment of many infectious diseases. The rural population of a country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost. Noni is native from Southeast Asia to Australia and is cultivated in Polynesia, India, the Caribbean, Central and northern South America (Dixon *et al*., 1999; Ross, 2001). The Polynesians have been using the noni plant for food and medicinal purposes for more than 2000 years (Earle, 2001). In

traditional pharmacopoeia, the fruit is claimed to prevent and cure several diseases. It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections; it is also used to prevent the formation and proliferation of tumors, including malignant ones (Dixon *et al*., 1999; Earle, 2001).

The species of *Morinda* especially *M. citrifolia* has been reported to have a broad range of health benefits for cancer, infection, arthritis, asthma, hypertension, and pain (Whistler, 1992). The leaves, seeds, bark , fruits and roots of Noni have been used in various topical remedies in South Pacific Islands and South East Asia (Wang *et al*., 2002; Fygh-Berman, 2003). Noni juice is also claimed to relieve inflammation. Most noni is consumed as juice, although leaves, flowers, bark and roots can also be used (Dixon *et al*., 1999; Earle, 2001; McClatchey, 2002). It is reported to have antibacterial, anti fungal, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (Mc Clatchy, 2002; Wang *et al*., 2002; Mathivanan *et al*., 2005). Noni has recently been the object of many claims concerning its nutraceutic 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.

Two clinical studies reported a relief of arthritis and diabetes associated with noni consumption (Elkins, 1998; Solomon, 1999), the observed beneficial effects may result from certain compounds such as scopoletin, nitric oxide, alkaloids and sterols, and also to the antioxidant potential of noni. As a result of this reputation, consumption of this fruit is currently high, not only in the producing countries, but also in the United States, Japan and Europe. In response to this demand, some countries such as Costa Rica and Cambodia, have increased the fields being cultivated in noni. In these countries, the fruit is often commercialized fresh or as juice in both formal and informal markets, but it is also found as pasteurized juice, either pure or mixed with other juices (usually grape or blackberry). *Morinda citrifolia* fruit has long history of use as a food in tropical regions throughout the world. Documentation of the consumption of the fruit as a food source precedes the twentieth century. Captain James Cook of the British Navy noted in the late 1700's that the fruit was eaten in Tahiti. In 1866 publication in London explained that *M. citrifolia* fruit was consumed as a food in the Fiji Islands. Later publications described the use of this fruit throughout the Pacific Islands, Southeast Asia, Australia and India. Noni is commonly referred to the species *M. citirfolia* and is also called as Indian Mulberry. It is also known in different names locally as Cheese Fruit.

Morinda tinctoria, commonly known as Aal or Indian Mulberry is a species of flowering plant in the family Rubiaceae, native to southern Asia. It is an evergreen shrub or small tree growing to 5-10 m tall. The leaves are 15-25 cm long, oblong to lanceolate. The flowers are tubular, white, scented, about 2 cm long. The fruit is a green syncarp, 2-2.5 cm diameter. *Morinda* are distributed throughout Tamilnadu and Kerala. However, the species *M. tinctoria* is present abundantly in most parts of Tamilnadu and in some parts of Kerala. It is commercially known as *Nunaa* and is indigenous to tropical countries. MTR is considered as an important folklore medicine.

Medicinal plants provide about 80% drugs worldwide. Medicinal value of the plant is due to presence of a variety of phytochemical and elemental composition. Therefore, it is essential to investigate the phytoconstituents, elements and vitamin supplements present in the medicinal plant to assess their medicinal values. The complete physico-chemical composition of the fruit has not yet been reported and only partial information is available on noni juice. In view of its medicinal importance, the present study has been initiated to evaluate the nutritive potential of fresh and dried fruits of *Morinda tinctoria.*

Materials and Methods

The fresh fruits from wild *Morinda tinctoria* plant were collected from vacant open area at Kadambattur, Tiruvallur district, Chennai, Tamilnadu. The procedures were carried out on the same day for the analysis in fresh fruit whereas the fruits were shade dried for 10-15 days and powdered for the analysis of dry fruit. All the reagents used in this study were of analytical grade.

Determination of Ash content

According to the method 100g of each sample was weighed in a silica crussible. The crusible was heated in a muffle furnace for about 3-5 hrs at 600'C. It was cooled in a dessicator and weighed to completion of ashing. To ensure completion of ashing, it was heated again in the furnace for ½ an hour more, cooled and weighed. This was repeated consequently till the weight become constant weight of ash. The ash content was calculated by the following formula:

Ash % = weight of ashed sample /weight of sample taken × 100

Estimation of Protein

Grind 2g of sample (*Morinda tinctoria* fresh fruit and dry powder) in a pestle and mortar with 10ml of distilled water and centrifuge 4000rpm for 10mins. Then 1ml of supernatant was made upto 100ml with distilled water. The amount of protein was estimated by the method of Lowry *et al* using BSA as the standard.

Estimation of carbohydrate

1g of both samples (*Morinta tinctoria* fresh fruit and dry powder) were weighed and taken in a boiling test tube. To this 1ml of 6N hydrochloric acid was added and hydrolyzed by keeping it in a boiling water bath and cooled. The solution was neutralized by adding solid sodium carbonate until effervescence was stopped. The solution was filtered and made upto 100ml using distilled water. The total carbohydrate was estimated by Anthrone method using glucose as the standard.

Estimation of Niacin

Grind the samples (5g) in 4N H_2SO_4 (30ml) and steam it for 30 minutes. Cool and make up to 50ml with distilled water and filter through whattman No % filter paper. Add 60% basic lead acetate to 10ml of the filtrate. Adjust the pH to 9.5 with a pH meter or thymol blue indicator or using 10N sodium hydroxide. The contents were centrifuged. To the supernatant add 2ml of concentrated sulphuric acid and allowed to stand for 1 hour. Centrifuge and collect the supernatant for estimation. In to a series of test tubes pipette out 0.1 to 0.5 ml of the standard niacin solution with concentration range 10 to 50 mg. 0.5ml of test solution was pipetted out in tubes marked 'T'. Then make up the volume to 6ml with distilled water alone serves a blank. Then 3ml of

cyanogen bromide was added in all the tubes. The tubes were incubated for 10 minutes. Then 1ml of 4% aniline was added in each tube and the yellow color developed was read after 5 minutes against a reagent blank.

Extraction of sample for analysis of Thiamine and Riboflavin

5g of finely grind samples is centrifuged after adding 20ml of 0.1N sulphuric acid at 4000 rpm for 20 minutes. Decant the supernatant and extract residue again with the 10ml of the acid. Pool the collected supernatant fractions into 50ml volumetric flask and make the final volume upto the mark with 0.1N sulphuric acid.

Estimation of Thiamine

Added 3ml of potassium ferric cyanide reagent to the suitable portion of thiamine extract followed by 3ml of 15% sodium hydroxide. Ensure that the solution was alkaline and shake the contents for 30 seconds. Add 10ml of isobutanol, mix thoroughly and allow it to stand till the two phases separate out completely. Discard the lower aqueous layer by adding a small amount of solid anhydrous sodium sulphate. Thiamine is estimated using a standard solution containing 0.5 mg of thiamine hydroxide and the fluorescence intensity of the standard and a secondary filtrate is measured at 435nm. From the reference curve determine the result in terms of µg of thiamine for 1g of sample using the following formula: Concentration of unknown=U-B/s-b x Concentration of standard.

Estimation of Riboflavin

2ml of fruit sample was taken in a test tube and add 0.5ml of glacial acetic acid and 0.5ml of 15% potassium permanganate was added, shaken well and allowed to stand for 1minute.Add 0.5ml of 3% hydrogen peroxide till the color disappears completely. Then, add 10ml of isobutanol pyridine mixture, shake for 30seconds and the tubes were stand undisturbed for 10minutes and aqueous layer is discarded. Add a pinch of sodium sulphate with small dropping funnel to remove trace of moisture and keep it in dark.

Estimation of Ascorbic Acid

Ascorbic acid was estimated following titration method developed by Harris and Ray (1935). Grind 2g of sample (*Morinda tintoria* fresh fruit and dry powder) material with 10ml of distilled water. Centifuge at 4000rpm for 10mins, supernatant was taken for estimation. Into a series of clean test tubes standard ascorbic acid solution in the range of 0.1 to 0.5ml having concentrations ranging from 10 to 50mg were pipetted out. 1ml of test sample was pipetted out in 'T ' tubes. The volume in all the test tubes was made upto 1ml using 5% TCA solution. 1ml of TCA solution alone serves as blank. 0.2ml of dinitrophenyl hydrazine, thiourea and copper reagent was added to all the tubes. The contents of tubes were mixed thoroughly and placed in a boiling water bath for 10mins, and the contents were cooled at room temperature and then kept in ice.

The orange red ozone crystal formed was dissolved by adding 85% H_2SO_4 with constant stirring. The color developed was read colorimetrically at 520nm using green filter against a reagent blank. A standard calibration graph was plotted having the concentration of ascorbic acid along x axis and the corresponding optical density along y axis. From the graph, the amount

of ascorbic acid present in the given fruit sample was calculated.

Estimation of Iron

1g of the sample was weighed accurately in a previously weighed silica crucible. The crucible was placed in a muffle furnace at 600ºc for about 24 hours till all the material was completely charred. A drop of concentrated nitric acid was added and heated in a furnace for 24 hours. The ash was white in colour. The ash sample was dissolved in few drops of concentrated hydrochloric acid and the solution was made upto 25ml with the distilled water. This can be used as test sample. 0.5 to 2.5ml of standard solution with concentration range of 2-10µg were taken in five different test tubes labeled as S_1-S_5. 3ml of the sample solution was taken in a different test tubes labeled as T_1 and T_2.These tubes were made upto 3ml with distilled water. 3ml of the distilled water alone serves as blank 'B'. To all the tubes 1.5ml of sodium sulphite and 1.5ml of 2,2'-dipyridyl reagent was added. Then the standard tubes were incubated at room temperature for 5minutes and the test tubes containing the sample were heated in a boiling water bath for 5minutes.The pink colour developed was read at 540nm. A standard graph was drawn by taking the concentration on X axis and optical density on Y axis. From this graph the amount of iron present in the sample was calculated.

Estimation of Calcium

5g of each 3 samples was taken in a previously weighed silica crucible. This was heated in an incinerator at 600ºc. A drop of concentrated nitric acid was added and heated again in an incinerator. The crucible was cooled and weighed accurately. The obtained ash was dissolved in little volume of 0.1N hydrochloric acid and made upto 100ml with distilled water. This can be used for estimation of calcium by permanganametry against 0.01N potassium permanganate as standard. 5ml of each of the sample solution was taken in separate test tubes, 3ml of 4% ammonium oxalate was added drop by drop till the precipitate was formed and incubated for 1hour.Then the solution was centrifuged and the supernatant was discarded. The obtained precipitate was heated and titrated against potassium permanganate till the appearance of permanent pale pink colour. The difference in titer value gives the volume of 0.01N potassium permanganate required to neutralize the precipitated calcium in the ash solution. From the value the amount of calcium present in the given sample was calculated.

Estimation of Copper

Grind 1g of each (fresh fruit and dry powder) sample with 10ml of distilled water and filtered and used for estimation. Working standard solution of copper with the volume of 1to5ml and concentration range from 1to 5µg were taken in a five tubes, all the tubes were made upto 5ml with distilled water. 5ml of distilled water alone serves as blank. 5ml of sample supernatant was taken in another test tube. 1ml of 6% sodium pyrophosphate was added to all the tubes followed by 2ml of ammonia solution. To all the tubes, 1ml of 0.4% sodium diethyl dithio carbamate reagent was added followed by 5ml of amyl alcohol ether mixture. The contents were shaken well and the organic layer was collected. To this add a pinch of sodium sulphate crystals. The contents were mixed well;

centrifuged and golden yellow colour formed was read at 490nm colorimetrically.

Result and Discussion

The proximate composition analysed in *Morinda tintoria* fresh fruit and dry fruit powder is shown (Fig. 1). The fruit contain essential vitamins and minerals at all stages of development. The Ash content is 4% and 1.6% in *Morinda tintoria* fresh fruit and dry fruit powder respectively. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals with in a food. Analytical techniques for providing information about the total mineral content are based on the facts that the mineral can be distinguished from all other components with in a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating and that they have a low volatilabily compared to other food components. This material can include carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions and other ions.

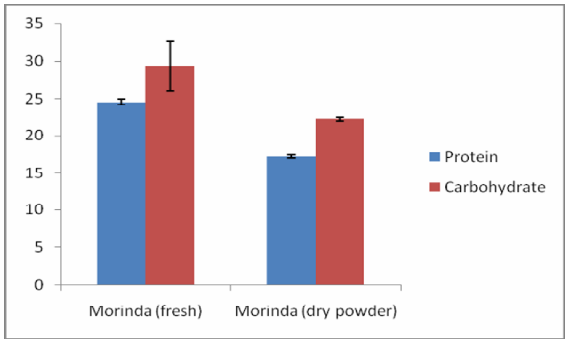
The fruit contains 90% of water and the main components of the dry matter appear to be soluble solids, dietary fibers and proteins (Chunhieng, 2003). It is evidently witnessed the fresh fruit of *Morinda tinctoria* is rich in Protein and Carbohydrate with considerable difference than the dry *Morinda tinctoria* fruit. Carbohydrates are one of the most important components in many foods. Carbohydrate may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Some carbohydrates are digestable by humans and therefore provide on important source of energy. Carbohydrates also contribute to the sweetness, appearance, and textual characteristic of many foods.

Proteins are important constituents of foods for a number of different reasons. They are a major source of energy, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g*., tenderness of meat or fish products. The fruit protein content is surprisingly high, representing 11.3% of the juice dry matter, and the main amino acids are aspartic acid, glutamic acid and isoleucine (Chunhieng, 2003).

The protein content was high in *M. citrifolia* fruit extracts obtained by two different extraction methods than the fruit extracts of *M. pubescens* (Desai *et al*., 2010). It ranged from 8.0 mg/g fresh weight (gfw) to 9.3 mg/gfw in *M. citrifolia* as against 5.3 mg/gfw to 7.0 mg/gfw in *M. pubescens* (Mathivanan *et al*., 2006). The crude protein content is twice in *M. citrifolia* fruits (8.32-9.13 mg g-1 DW) as compare to *M. pubescens* (4.87-5.09 gm g-1 DW) and these values were higher than African bush mango (0.52%) which have been recorded by Akubor, (1996), Vitex doniana (0.8%) (Egbekun *et al.*, 1996), and than cashew pulp (2.92%) (Aderiye, 1991) and pineapple pulp (1.5%) (FAO,1969). In *C. sativa* Mill 4.88 to 10.87 g/100g total protein content have been reported by Erturk *et al*., (2006). It can be observed from the results that a

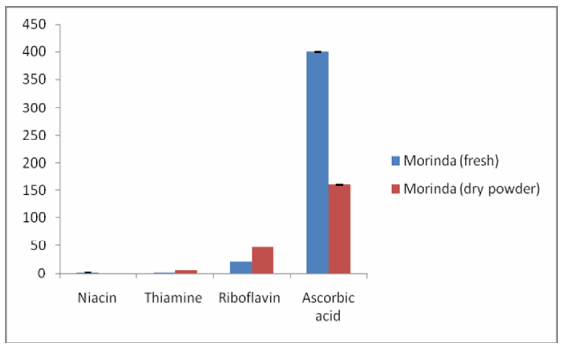
great deal of variation occur in both the protein and carbohydrate content between fresh and dry fruits.

Figure.1 Difference in protein and Carbohydrate content between Fresh and dry fruit *(Morinda tinctoria)*



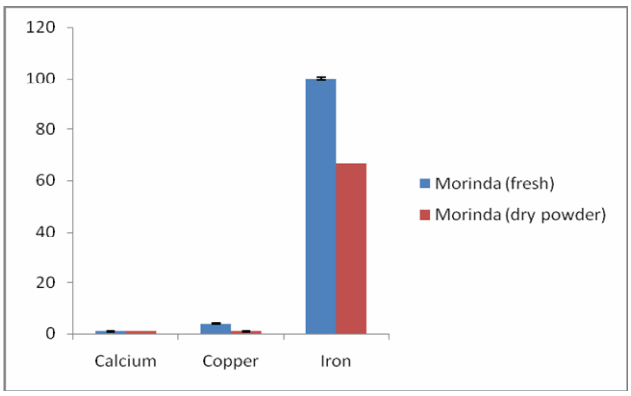
The noni fruit protein content is surprisingly high, representing 11.3% of the juice dry matter, and the main amino acids are aspartic acid, glutamic acid and isoleucine (Chunhieng, 2003). The results of the present study revealed a high amount of both protein and carbohydrate.

Figure.2 Difference in water soluble vitamins content between Fresh and dry fruit *(Morinda tinctoria)*



The vitamin content of the selected fruits reveals high content of Vitamin C (Fig 1). It is evident that the *Morinda tinctoria* fresh fruit is rich in Ascorbic acid (400mg/gm) and Niacin (2.1mg/gr). The dry fruit of *Morinda tinctoria* is rich in Riboflavin (47.12 mg/gm) and Thiamine (6.95mg/gm). Natural ascorbic acid is crucial for the body performance. It possesses anti- scorbutic activity. Ascorbic acid in the body also aids in iron absorption from the intestines. It is required for connective metabolism especially the scar tissue bones and teeth. It is necessary as an anti stress and protector against cold, chills and damp. It prevents muscle fatigue and scurvy, which is characterized by skin hemorrhages, bleeding gums, fragile bones, anemia and pains in the joints and defects in skeletal calcification. This function of ascorbic acid also accounts for its requirement for normal wound healing. It acts also as anti oxidants in the skin by scavenging and quenching free radical generated by ultra violet radiation stabilization. The production of collagen is also dependent on vitamin C. It helps in the promotion and restoration of skin tone and improvement in fine wrinkles. Vitamins have been reported in the fruit, mainly ascorbic acid (24-158 mg/ 100 g dry matter) (Morton, 1992; Shovic and Whistler, 2001), and provitamin A (Dixon *et al*., 1999).

Figure.3 Difference in mineral content between Fresh and dry fruit *(Morinda tinctoria)*



Mineral ions are of prime importance in determining the nutritional value of fruits. Potassium, calcium, and magnesium are the major ones. In the tissue of many fruits, calcium is one of the mineral believed to be an important factor governing fruit storage quality (Lechaudel *et al*., 2005). It has been reported to delay ripening and senescence (Fergusan, 1984) and to reduce storage disorder (Bangeruh, 1979). The importance of minerals such as potassium, calcium, sodium *etc.* to human health is well known. Required amounts of these elements must be in human diet to pursue good healthy life (San, 2009). The content of mineral elements in plants depends to a high degree on the soils abundance, including the intensity of fertilization (Kruczek, 2005). Minerals account for 8.4% of the dry matter, and are mainly potassium, sulfur, calcium and phosphorus; traces of selenium have been reported in the juice (Chunhieng, 2003). The mineral content analysis showed that *Morinda tinctoria* fruit is rich in iron and the fresh fruit showed a considerable increase in amount of iron and copper when compared to calcium (Fig 3).

Noomrio and Dahot (1996) studied on the evaluation of nutritive value of *Eugenia Jambosa* fruit like minerals, vitamins, free sugars and amino acids. The chromatographic analysis showed that fruit contains glucose, mannose, sucrose, alanine, arginine, asparagine, tyrosine, glutamine and cysteine. Punna and Paruchuri (2003) provided new data on total (TDF), Insoluble (IDF) and Soluble (SDF) dietary fiber contents of Jamun fruits, which play an important role in human nutrition. Anita and Malkit (2005) analyzed the Jamun seeds for proximate composition, available carbohydrates, dietary fibers and anti-nutritional factors (anti-nutrient content helps in controlling blood sugar). Protein, fat, ash, crude fiber, carbohydrate and energy contents were significantly reported. Indrayan *et al*., (2005) determined the nutritive value and analysis of mineral elements for some medicinally valued plant seeds including *E. jambolana*. Recently, (Rathi *et al*., 2002) used the plant extract of *E. jambolana* for prevention of experimental diabetic cataract.

The nutritive and energy values of some wild fruit spices in southeastern Nigerian were studied by Effiong in 2009. The *Xylopia aethiopica, Tetrapluera tetraptera and Piper guineense* fruits were studied for their nutrient values. The mineral study indicated high content of P (1215.00 + 4.90 mg/100g), Mn (52.15 + 1.02 mg/100g) zinc (9.6 + 0.71 mg/100g) and copper (14.84 + 3.90 mg/100g) for *Xylopia aethiopica* and *Piper guineense* is rich in Calcium (31.15 + 3.10 mg/1oog), Magnesium (19.65 + 0.42 mg/100g) and Pottasium (308.95 + 1.74mg/100g) while high level of Sodium (201.5 + 4.90 mg/100g) and Iron (47.49 + 1.87 mg/100g) found in *Tetrapluera tetraptera*. Minerals account for 8.4% of the dry matter, and are mainly potassium, sulfur, calcium and phosphorus; traces of selenium have been reported in the juice (Chunhieng, 2003).

The mineral nutrition is an important aspect and its pivotal role in human life provides healthy growth. The *Morinda citrifolia* plant, and especially its fruit, has been used for centuries in folk medicine. Different studies, some of them with controversial methodologies, showed that this fruit contains several nutritional and functional compounds, but most of them have not been quantified. The present study showed that the *Morinda tinctoria* fruits could also be used as a source of Protein, Vitamin and minerals. Scientific

studies have opened some interesting doors, but most have not conclusively proved the nutritional or medical value of this plant. The main proven functional properties of noni fruit are related to the control of several diseases. In vitro research and limited experiments with lab animals have shown that noni has anti-microbial, anti-cancer, antioxidant, anti-inflammatory, analgesic and cardiovascular activity. The current market, basically centering on the Polynesian noni, and more specifically the Tahitian one, has conferred upon the fruit a unique and authentic appeal. Market interest in this fruit suggests a bright future, although more studies are needed to identify the nutritional and functional compounds it contains and explain their mechanisms of action in order to determine the real potential of this fruit and the technological processes that preserve these.

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