



## Phytochemical Screening of the Fruit of *Garcinia cochinchinensis* Choisy

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### Abstract

Among the native fruits, *Garcinia* genus is composed of many fruit species used by traditional medicine. Since there are no reports in the literature about the chemical composition of the false mangosteen (*Garcinia cochinchinensis*), the present study was aimed to perform the physicochemical screening and analysis of the presence of phytochemicals in the fruit pulp of this plant. Analysis of titratable acidity, soluble solids and pH were performed in the fresh fruit and vitamin C, anthocyanins and carotenoids in fresh, dehydrated and frozen fruit after 30, 60 and 90 days. Moreover, qualitative tests to check the presence of phytochemical compounds were also performed. Fresh fruit showed average values of  $4.88 \pm 0.061$  for titratable acidity (g/100g of citric acid),  $12.3 \pm 0.265$  for soluble solids ( $^{\circ}$ Brix) and  $\text{pH } 2.52 \pm 0.067$ . The average values for Anthocyanins and total carotenoids did not differ among fresh and frozen samples, but there was significant reduction in the levels of Vitamin C. Dehydrated fruit exhibited significantly higher levels of Carotenoids and Vitamin C compared to the fresh fruit, but lower values of Anthocyanins. Colorimetric analysis revealed the presence of phenols, flavonols, flavanones, flavanonols, xanthones and alkaloids in dehydrated *G. cochinchinensis* fruit.

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### Introduction

The daily consumption of fruits and vegetables has been epidemiologically associated with a lower incidence of morbidity and mortality by so-called chronic degenerative diseases, which include cancer, type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular and cerebrovascular diseases. This association is strongly evidenced by the high content of phytochemical compounds with antioxidant and anti-inflammatory properties as carotenoids, vitamins, and

fibers in these foods (Gregoris et al., 2013; Pem and Jeewon, 2015; Oszmianski, et al., 2015; Cavallo et al., 2016).

Brazil is among the three largest producers of fruits, second only to China and India. In 2010 the country exported about 800 thousand tons (IBRAF, 2011). This high production of different varieties of native or adapted fruit is due to the extent of the territory and its different climates (Rocha et al., 2013; Virgolin, 2015). According to Lopes et al. (2012) and Sucupira et al. (2012) the

native fruits are very rich both in nutritional and functional properties. Besides they present sensory characteristics such as color, distinct and pleasant aroma and flavor, and how they are adapted to local soils, practically do not require chemical inputs, and require low cost of implementation and maintenance of the orchard.

*Garcinia* genus (Clusiaceae) is composed of about 550 species, mostly trees, distributed mainly in tropical areas of Africa, America, Polynesia and Asia. Some species are extensively used by traditional medicine in various parts of the world, and some are fruit species such as *Garcinia mangostana*, *G. buchananii*, *G. atroviridis*, *G. dulcis*, *G. xanthochymus* and *G. cochinchinensis*. Phytochemical studies have revealed that species of *Garcinia* are rich sources of secondary metabolites, including flavonoids, biflavonoids, triterpenes and xanthenes. Some of these compounds exhibit numerous biological activities, as anti-microbial, anti-cancer, anti-oxidant, anti-hyperlipidemic, and anti-inflammatory properties (Chen et al., 2010; Chang and Yang, 2012; Sukatta et al., 2013; Suttirak and Manurakchinakorn, 2014; Sharma et al., 2015; Stark et al., 2015; Kritsanawong et al., 2016).

Among the fruit species, the best known is the mangosteen (*G. mangostana*), considered an exotic and appreciable taste fruit originated from an Asia specie, that is used as fresh fruit, and is a considerable source of antioxidants, such as beta-carotene in the pulp and xanthenes in the peel (Braga et al., 2012; Carvalho et al., 2014).

*G. cochinchinensis* is known as yellow mangosteen, *Garcinia* or false mangosteen, and is commonly confused with *G. mangostana*, especially in the young fruit stage. Once there are no reports in the literature about the chemical composition of *G. cochinchinensis*, the aim of this study was to perform the physicochemical screening and analysis of the presence of phytochemicals in the fruit pulp of this plant.

## Materials and methods

### Raw material

The false mangosteen fruit used in this study was obtained directly from the orchard in School of Food Technology (FATEC- Pompeia / São Paulo) in November 2015. The plant was identified and deposited in the Herbarium of the Department of Biology of FFCLRP-USP (Faculty of Philosophy, Sciences and

Languages of Ribeirão Preto - USP). Collector and number: M. Groppo 2307, registered at the herbarium under no. SPFR 16037.

In the Food Processing Laboratory of Fatec Marília / São Paulo, the fruit was selected according to the healthy and they sanitized with sodium hypochlorite ( $\text{NaOCl}$ )  $0.2 \text{ gL}^{-1}$ , followed by rinsing. The seeds were removed and the fruit pulp and peel were submitted to analysis in the fresh form. The fruit pulp was also stored in plastic bags and frozen (ultra-rapid freezing in  $-80^{\circ}\text{C}$ ).

### Phytochemical analysis of the fresh fruit

The fresh fruit was macerated with the aid of a mixer, and underwent the analysis of titratable acidity (% (v/v) of citric acid), soluble solids ( $^{\circ}\text{Brix}$ ) and pH, according to the methodology of the Adolfo Lutz Institute (2008). After chemical analyzes the relation between soluble solids and titratable acidity was calculated (*Ratio*). Vitamin C content was expressed as mg/100g of the sample, and determined according to the reduction of 2,6-dichlorophenolindophenol indicator by ascorbic acid. Total carotenoids and anthocyanins were expressed as mg/100g and were analyzed by spectrophotometric method at wavelength of 450nm and 535 nm, respectively.

Further analyses were conducted after 30, 60 and 90 days after freezing for determination of carotenoids, vitamin C and anthocyanins. For the tests, the fruits were removed from the freezer and allowed to thaw at room temperature. All the parameters studied were performed in triplicate.

### Phytochemical analysis of the dehydrated fruit

The fruit (without the seeds) was dehydrated in air circulating oven at  $40^{\circ}\text{C}$  for 96 hrs. Quantitative analyzes were carried out for vitamin C, carotenoids and anthocyanins (IAL, 2008). For the evaluation of the presence of phytochemicals, the dehydrated fruit was prepared according to the methodology described by Matos (1997), using ethanol 30% as solvent. Tests for phenols, tannins, flavones, flavonoids, xanthenes, chalcones, flavononols, flavanones, catechins and alkaloids were performed.

### Statistical analysis

Data were presented as median and standard deviations. Data analysis was performed by analysis of variance

(ANOVA) and Tukey test using the Prism 6.0 software (GraphPad Software USA) with significance level of 5% ( $p < 0.05$ ).

## Results and discussion

### Phytochemical analysis of the fresh fruit (*G. cochinchinensis*)

An average of 4.88% of titratable acidity levels were found in the fresh pulp of the false mangosteen (Table 1).

Values between 3.85-4.42% were identified in fresh pulp of *G. xanthochymus* Hook (Cavalcante et al., 2006). Rufino (2008) in his study cites that *bacuri* also belonging to the Clusiaceae family, showed average values of 1.63% of Acidity titratable. The values for this parameter may be vary according to different kinds of processing methods, sensory and functional characteristics of certain kinds of fruit, which may be different due to genetic factors, place of production, harvest time, and the maturation stage (Batista et al., 2014).

**Table 1.** Acidity, soluble solids and pH in the fresh fruits of *G. cochinchinensis*.

	Acidity [% (v/v) of citric acid]	Soluble solids (°Brix)	pH	Ratio
Fresh pulp	4.887±0.061	12.3±0.265	2.52±0.067	2.59±0.121
Mean ± standard deviation				

The fresh fruit showed mean values for pH and soluble solids of 2.52 and 12.3%, respectively. Chavez-Cury et al. (2012) found values of pH 2.94 for the variety *G. madruno* and in the pulp of the mangosteen (*G. mangostana*). Roque et al. (2011) and Braga et al. (2012) showed mean values for pH of 2.9 and 3.25, respectively. When comparing to the more acidic fruits, such as the different lemon cultivars - Galician (1.94), Tahiti (2.23) and Rangpur (2.35), and umbu-caja fruit (2.40), the mean values of pH of the false mangosteen pulp were higher, and showed also similar to fruits like passion fruit (2.51 to 2.63) (Brighenti et al., 2011; Santos et al., 2011; Canuto et al., 2010).

The levels of soluble solids of the fresh fruit were similar to those observed in pulp *G. xanthochymus* Hook (10.8 and 12.6°Brix) (Cavalcante et al., 2006). Braga et al. (2012) showed lower values in *G. mangostana* (15.87%). When comparing to citrus fruits, according to the study Couto and Canniatti-Brazaca (2010), the tangerine Ponkan possess values of soluble solids near to the false

mangosteen this study. Murcott mandarin possess higher values (14.33%), however, different varieties of oranges may exhibit levels ranging from 9.11% to 10.89%.

Our results for the *Ratio* (SS / TA) were similar to those observed in the fresh pulp of the *G. xanthochymus* Hook (2.60 and 3.06) (Cavalcante et al., 2006). According to Chitarra and Chitarra (2005) the *Ratio* provides an indication of the flavor of the fruit, because it relates the content of sugars and acids, and tends to increase during ripening possibly because of the increase in the sugar and reduction in the acid content. It is also influenced by environmental or physiological factors, which directly interfere with the flavor of the fruit.

### Carotenoids, anthocyanins and vitamin C during the storage of the fruit

In Table 2 we observe the results for carotenoids, anthocyanins and vitamin C found in the fresh fruit, and in the frozen samples.

**Table 2.** Levels of carotenoids, anthocyanins and vitamin C in the *G. cochinchinensis*, during the storage at -80°C.

	Fresh fruit	30 days	60 days	90 days	p-value*
Carotenoids (µg/g)	0.333±0.022	0.395±0.141	0.390±0.017	0.383±0.187	0.6849
Anthocyanins (mg/100g)	1.710±0.460	1.113±0.196	1.423±1.260	1.503±0.206	0.5515
Vitamin C (mg/100g)	11.62±0.0	8.840±0.03	8.060±0.11	7.800±0.33	0.0014
Mean ± standard deviation / *p-value < 0.05.					

Fresh fruit presented average values of 0.33 mg/g to carotenoids, and during the storage period, significant losses were not observed in frozen samples. The values were lower when compared to other fruit as different mango cultivars that have, according to Silveira et al. (2009) values ranging from 11.0 to 28.2 g/g in the

carotenoids content. In papayas, fruit with significant concentrations of carotenoids, Reis et al. (2015) found values between 41.11 and 42.84 mg/g. Sena et al. (2014) found values of 4.2 µg, 4.3 µg and 3.0 µg/g of acerola, soursop and tangerine samples, respectively. Matias et al. (2014) found values of 3.3 and 14.0 g/g in yellow peach

pulp. Lopes et al. (2005) observed a significant decrease in the total Carotenoids content in the first 30 days of storage in cherry pulps frozen at  $-18^{\circ}\text{C}$ , however, in 45, 60 and 90 days a significant reduction was not verified. Carotenoid levels may suffer significant losses in fruit and vegetables when they are cut or disintegrated due to exposure to oxygen and light, and contact with catalytic enzymes of the oxidation process (Rodriguez-Amaya, 1997; Niizu and Rodriguez-Amaya, 2005).

Vitamin C levels were lower than those observed in *G. indica* pulp indicates (60 mg/100g) in a study of Krishnamurthy et al. (1982) and in the pulp of *G. madruno* (24.74 mg/100g) as showed by Chavez-Cury et al. (2012). However, the values found in this work is higher when compared to other fruit as red plum (6.8 mg/100g), apple (8.0 mg/100g), white grape (4.6 mg/100g) and watermelon (9.0 mg/100g) (Zeraik et al., 2010; Chitarra and Chitarra, 2005; Harain et al., 2004).

The levels of Vitamin C decreased significantly during the storage period (p-value 0.0014). Reduction of 32.87% in the content of vitamin C was demonstrated after 90 days under freezing conditions. According to Silva et al. (2008), vitamin C is vulnerable to processing conditions and its degradation is directly related to many factors such as temperature, humidity, pH, presence of antioxidants, oxygen and others. In a study by Silva et al. (2008) the concentration of vitamin C in frozen pulps of the fruit *Cagaita* reduced approximately 30% in the first month, and 50% in the third month of storage. Brunini et al. (2002) found that the content of vitamin C in mango frozen at  $-18^{\circ}\text{C}$ , reduced from 56.11 mg/100g to 23.85 mg/100g after 18 weeks.

In the fresh samples of *G. cochinchinensis* we found average values of 1.71mg/100g of anthocyanins, with concentrations did not vary significantly with the storage. These components are water-soluble pigments (tones from the red to blue color). However, its color is directly influenced by substitution of hydroxyl and methoxyl groups in the molecule, besides the existence of an intermolecular co-pigmentation with other compounds such as organic acids, flavonoids and alkaloids (Mazza and Brouillard, 1987; Lopes et al., 2007).

Some species of *Garcinia* with purple or red color possess higher contents of Anthocyanins when compared to yellow species such as *G. indica* Choisy and *G. mangostana*, whose concentration of Anthocyanins is very expressive in the bark of the fruit (Nayak et al.,

2010; Palapol et al., 2009). Kuskoski et al. (2006) did not identify the presence of these components in other yellow fruit as mango, pineapple and passion fruit but found values of 2.7 and 16mg/100g in guava and acerola pulp, respectively.

### Carotenoids, anthocyanins and vitamin C in the dehydrated fruit

The dehydrated fruit presented 80.46mg/100g of vitamin C; 1.013mg/100g of Carotenoids and 1.71mg/100g of anthocyanins. In pulps of dehydrated apples were observed average values of 22.33mg/100g for vitamin C (Santos et al., 2013). In different types of dehydrated mango authors observed 76.57 and 36.22mg/100g of Vitamin C (Bezerra et al., 2011).

The dehydration process promotes the reduction of the free water under mild conditions with a reduction of the speed on the chemical, enzymatic and biochemical reactions that are responsible for the deterioration of the fruit. For this reason, this process can be a viable alternative to obtain products with high levels of nutrients and increased shelf life, besides preventing undesirable changes in color, aroma texture and flavor that occur during storage (Chong et al., 2013; Zhang et al., 2015).

Values for carotenoids were similar to those observed in atomized cashew pulp (0.19mg/100g) studied by Moraes (2014). The anthocyanins levels were lower than those described by Teixeira et al. (2011) in the evaluation of *Caryocar brasiliense* pulp dried in an oven (4.25 to 6.06mg/100g). In a study by Silva et al. (2010) were observed levels of 51.57mg/100g and 52.90mg/100g of anthocyanins extracted from the bark of mangosteen (*G. mangostana*) at  $40^{\circ}\text{C}$  and  $24^{\circ}\text{C}$  respectively.

Comparatively, Carotenoids content and Vitamin C were significantly higher in samples of the dehydrated fruit. In contrast, Anthocyanins levels were lower in these samples (Table 3).

**Table 3.** Levels of carotenoids, anthocyanins and vitamin C in fresh and dehydrated fruit.

	Carotenoids ( $\mu\text{g/g}$ )	Anthocyanins (mg/100g)	Vitamin C (mg/100g)
Fresh fruit	0.333 $\pm$ 0.022	1.711 $\pm$ 0.102	11.62 $\pm$ 0.0
Dehydrated fruit	1.013 $\pm$ 0.118	0.096 $\pm$ 0.025	80.45 $\pm$ 0.010
p-value*	0.0074	0.0288	0.0001
Mean $\pm$ standard deviation / *p-value < 0.05			



Studies have shown that consumption of fruits such as natural juices or dehydrated fruits have contributed to maintaining the balance of the antioxidant system, the improvement in plasma levels of vitamins and other compounds of this nature (Samman et al., 2003; Tonin et al., 2015; Järvi et al., 2016).

The carotenoids have been related to beneficial effects on human health as enhancement of the immune response, reducing the risk of degenerative diseases such as cancer, cardiovascular disease, cataracts and macular degeneration associated with age (Kadian and Garg, 2012; Rodriguez-Bernaldo and Costa, 2006).

Ascorbic acid is an essential vitamin in humans and may exhibit antioxidant and anti-inflammatory activity protecting against the development of innumerable chronic diseases. It is associated with the production of collagen, noradrenalin and serotonin, and steroid hormones. It may act as a reducer component, preventing the oxidative and degenerative effects of the free radicals and may regenerate vitamin E. It is also related to the modulation of the immunity system (Rutkowski and Grzegorzczak, 2012; Miles et al., 2015; Li et al., 2015; Sato et al., 2015).

Anthocyanins also play relevant role as antioxidant. They consist in phenolic compounds with several important biological effects such as, anti-inflammatory and vasodilation. As they possess antioxidant and anti-inflammatory properties, they are related to the prevention of inflammation process, diabetes, dyslipidemia, obesity, metabolic syndrome, cardiovascular diseases, and cancer. These pigments are very promising in the food technology due to the possibility to use them as functional foods components (Min et al., 2015; Skrovankova et al., 2015).

#### Evaluation of the presence of phytochemicals in the dehydrated fruit

The colorimetric analysis revealed the presence of phenols, flavonols, flavanones, flavanonols, xanthenes and alkaloids in the dehydrated Fruit of *G. cochinchinensis* (Table 4).

Colorimetric analyzes with *G. mangostana* revealed the presence of phenols, flavonoids and triterpenoids in extracts with hexane and chloroform in the fruit epicarp and endocarp (Sivakumari et al., 2016). Abdallah et al. (2016) identified bioactive components that may prevent the formation of advanced glucose end products that are

responsible for the chronic complications of diabetes mellitus (D garcimangosone, aromadendrin-8-C-glucopyranoside, epicatechin and 2,3', 4,5', 6-pentahydroxybenzophenone).

**Table 4.** Qualitative phytochemicals in the extract of *G. cochinchinensis* fruits.

Phytochemicals	Extract of the dehydrated fruit
Phenols	+
Tannins	-
Flavones, flavonoids, xanthenes	-
Chalcones	-
Flavonoids	+
Flavanones	+
Catechins	-
Flavonols, flavanones, flavanonols, xanthone	+
Alkaloids	+
+ indicates presence of compounds / - indicates absence of compounds.	

Akao et al. (2008) found three types of xanthenes ( $\alpha$ ,  $\beta$  and  $\gamma$ -mangostin) identified in the pericarp of *G. mangostana* that showed antioxidant and anti-proliferative effects in cancer cells. Wihastuti et al (2014) observed reduction of mediators such as nuclear factor - kappa  $\beta$  (NF- $\kappa\beta$ ) and other reactive species involved in atherosclerosis when studying the effects of mangosteen in hypercholesterolemic Wistar rats.

Some studies have identified the presence of flavonoids (naringenin, binarigenin, morelloflavone and fukugiside) and other phenolic compounds (atroviridin, atrovirone, atroviridone and atroviridone B, cambogin, garcinol, camboginal and isogarcinol) in mature fruit and leaves of *G. atroviridis* (MacKeen et al., 2002; Permana et al., 2005; Al-Mansoub et al., 2014).

Authors have found bioactive benzophenones compounds such as aristophenone A, alloathyriol, amentoflavone, guttiferone E, isoxanthochymol, gambogenone, and in *G. xanthochymus* fruit, demonstrating activity against colon cancer cells (Baggett et al., 2005).

Hassan et al. (2013) identified levels of phenolic compounds in the peel and pulp of *G. parvifolia* higher than those found in Fruit *G. atroviridis* and *G. prainiana*. These authors also showed the presence of high concentrations of total carotenoids, flavonoids and

anthocyanins with significant antioxidant activity in pulp *G. parvifolia*.

Several studies have revealed extensive role of phenolic compounds in preventing disease and maintaining health of individuals. Once absorbed, they may modulate numerous pathophysiological processes such as inflammation, oxidative stress, high blood pressure, insulin resistance, mutagenic and microbiological processes by reducing the risk of development of many disorders (Rosa et al., 2016; Hügel et al., 2016; Ozkan et al., 2016). The intake of *G. cochinchinensis* may contribute with the consumption of these important components.

## Conclusion

The results of the present study show that the consumption of *G. cochinchinensis* may be a good source of antioxidants such as vitamin C, carotenoids, anthocyanins and other polyphenol compounds. The freezing of the fruit causes no interference with anthocyanin and carotenoid values and even reducing the levels of vitamin C, we observe that after 90 days of freezing, almost 70% of this vitamin is still available. Moreover, even with the reduction of the vitamin C after freezing, the levels are still higher than other fruits such as apple, white grape, plum red and melon. These results show that the use of this fruit can extend to products with shelf life greater than the fresh fruit, preserving important nutrients.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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