

## L-ASCORBIC ACID, **β**-CAROTENE AND LYCOPENE CONTENT IN PAPAYA FRUITS (*Carica papaya*) WITH OR WITHOUT PHYSIOLOGICAL SKIN FRECKLES

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**ABSTRACT:** The Skin Freckles is a papaya skin disorder that depreciates de fruit appearance and hampers its commercialization, although not lowering its nutritive value. Being the papaya a good source of ascorbic acid,  $\beta$ -carotene and lycopene this research aimed at determining L-ascorbic acid,  $\beta$ -carotene and lycopene content in papaya fruits, from 'Formosa' and 'Solo' group varieties, with and without apparent physiological skin disease (skin freckles). Fruits were harvested in the Southeast Region of Brazil. L-ascorbic acid content was determined by titration technique.  $\beta$ -carotene and lycopene contents were determined by high performance liquid chromatography technique (HPLC). L-ascorbic acid content in papaya fruits ranged from  $(59.9 \pm 3.4)$  mg 100 g<sup>-1</sup> to  $(112.4 \pm 12.6)$  mg 100 g<sup>-1</sup> in fresh papaya pulp.  $\beta$ -carotene content ranged from  $(0.19 \pm 0.07)$  mg 100 g<sup>-1</sup> to  $(0.56 \pm 0.09)$  mg 100 g<sup>-1</sup> and that of lycopene ranged from  $(1.44 \pm 0.28)$  mg 100 g<sup>-1</sup> to  $(3.39 \pm 0.32)$  mg 100 g<sup>-1</sup> in fresh papaya pulp. L-ascorbic acid contents of papaya fruits with skin disease averaged 7.0 mg 100 g<sup>-1</sup> to 10.0 mg 100 g<sup>-1</sup> higher than those of papaya fruits without skin freckles ( $P < 0.05$ ).

Key words: Brazil, fruits composition, carotenoids, vitamin C, food composition

## CONTEÚDO DE ÁCIDO L-ASCÓRBICO, **β**-CAROTENO E LYCOPENO EM FRUTOS DE MAMÃO (*Carica papaya*) COM E SEM MANCHA FISIOLÓGICA

**RESUMO:** A Mancha Fisiológica do Mamão (MFM) é uma desordem da casca do mamão, que deprecia a aparência do fruto e prejudica a sua comercialização, embora não prejudique o seu valor nutritivo. Considerando ser o mamão, uma boa fonte de ácido L-ascórbico,  $\beta$ -caroteno e licopeno, esta pesquisa visou determinar o índice destes componentes em frutas de mamão, das variedades do grupo 'Formosa' e 'Solo', com e sem Mancha Fisiológica do Mamão (MFM) aparente na pele. As frutas foram colhidas na região do sudeste de Brasil. O teor de ácido L-ascórbico foi determinado pela técnica de titulação. Os índices do  $\beta$ -caroteno e do licopeno foram determinados pela técnica de cromatografia líquida de alta eficiência (HPLC). O teor de ácido L-ascórbico variou de  $(59,9 \pm 3,4)$  mg 100 g<sup>-1</sup> a  $(112,4 \pm 12,6)$  mg 100 g<sup>-1</sup> de polpa fresca de mamão. O teor de  $\beta$ -caroteno variou de  $(0,19 \pm 0,07)$  mg 100 g<sup>-1</sup> a  $(0,56 \pm 0,09)$  mg 100 g<sup>-1</sup> e o do licopeno variou de  $(1,44 \pm 0,28)$  mg 100 g<sup>-1</sup> a  $(3,39 \pm 0,32)$  mg 100 g<sup>-1</sup> de polpa fresca de mamão. Os índices de ácidos L-ascórbico na polpa dos frutos de mamão com MFM variaram de 7,0 mg 100 g<sup>-1</sup> a 10,0 mg 100 g<sup>-1</sup> a mais do que os teores encontrados na polpa dos frutos de mamão sem a MFM ( $P < 0,05$ ).

Palavras-chave: Brasil, composição de frutas, carotenoides, vitamina C, composição de alimentos

### INTRODUCTION

Among several substances from *Carica papaya* fruits chemical composition there are those with antioxidant activity, for example, as the carotenoids and L-ascorbic acid.  $\beta$ -carotene is the main carotenoid with pro-vitamin A activity (Olson, 1996).

Multiple functions are attributable to L-ascorbic acid in humans. It is important for its buffer function in oxidation reduction processes, but also because of its molecular structure particularities in its ability for ions and hydrogen electrons transfer in reversible processes. L-ascorbic acid has an important role in corticoids and catecholamines biosynthesis and also par-

icipate in bone, teeth and blood synthesis and maintenance (Levine et al., 1997). Adult Recommended Dietary Allowance (RDA) for L-ascorbic acid is 45 mg (FAO, 2003). Physiological papaya fruit skin disease present as skin freckles, gray or brown in color, ranging from very small points to 10 mm diameter dark halos. This disease, which is reported to occur in all papaya producing countries, decreases the fruit appearance, which lower its commercial value. It has been observed in fruits over 40 days after anthesis, being more apparent as the fruits become more developed, closer to harvesting stage (Ventura et al., 2003). In South Africa, papaya skin freckles is reported to occur mainly on fruits developing in those months with soil water deficiency and lower whether temperature, from April to July. In the papaya tree, fruits more exposed to sun radiation presented higher intensity of skin freckles (Kaiser et al., 1996).

Papaya fruit skin freckles depreciate fruit appearance and lower its commercial value. In a literature review it has not been found studies on antioxidant compounds contents in papaya fruits. Thus, in this paper the objective was to evaluate L-ascorbic acid,  $\beta$ -carotene and lycopene contents in 'Formosa' and 'Solo' papaya fruits samples with and without skin freckles.

## MATERIAL AND METHODS

Papaya fruit samples from 'Formosa' group, cultivar *Tainung 01* (F1 hybrid) were harvested in January 2003 and February 2004. Papaya fruits from 'Solo' group, *Golden* cultivar, were harvested in March 2003. Thus, there were three harvesting time. Both groups were from commercial crop fields located in Linhares (19°15' S, 39°51' W), in Espirito Santo State, Brazil. The production area shows maximum temperatures ranging from 30°C to 32°C and minimum ones between 15°C and 18°C. Ten fruits with skin freckles and ten fruits without skin freckles from two ripen stage were harvested by hand, totaling 120 fruits. Ripen stage 1 were fruits with 15% skin surface in yellow color and stage 2 were 25%.

After harvesting, papaya fruits were kept under  $25.3 \pm 0.9^\circ\text{C}$  temperature and  $70.5 \pm 4.7\%$  relative humidity, until fruits presented 75% of skin surface typical yellow color. This maturation stage was reached after seven days, for the ripen stage 1, and after six days for the ripen stage two fruits. Papaya fruits had their skin manually removed with a stainless still knife and the seeds were discarded. Fruits were cut longitudinally and transversally, totaling eight pieces per fruit. Two cuts from upper half and two from lower half, each from opposed fruit side, were

grained in warring blender. Five grams exact weight of this slurry aliquots were packed in polyethylene bags, and then frozen by liquid nitrogen. After freezing, samples were kept under  $-25^\circ\text{C}$  frozen storage until time of laboratory analysis for L-ascorbic acid,  $\beta$ -carotene and lycopene content. Each fruit was analyzed separately. Preliminary essays in our laboratory had shown that these storage conditions are adequate to stabilize the substances analyzed in this research.

L-ascorbic acid content was determined by titulometric method with 2,6-dichloroindophenol (AOAC, 1998), with substitution of 10% metaphosphoric acid by 1% oxalic acid. This technique has been validated in our laboratory by titrating samples added by 0.5; 1.0 and 1.5 mg L-ascorbic acid and diluted with 10% metaphosphoric acid or in 5% or 1% oxalic acid, with 100% recovery and not finding any difference among the results, Carotenoid substances extraction in papaya fruit slurry was carried out according to procedures described by Wilberg & Rodriguez-Amaya (1995). Papaya fruit slurry was weighted and quantitatively transferred to test tube with about 20 mL of a ethanol:hexane 1:1 solvent mixture, and homogenized in a blender. Homogenate was filtered in a vacuum filter system with the help of 0.45 micra pore diameter PTFE membranes for aqueous and organic solvents.

All pulp residue retained in membrane filter were transferred to test tube, by repeating homogenization process, addition of about 20 mL methanol:hexane 1:1 and filtration running until papaya pulp lose its typical color completely (three times on average). All filtrate material was transferred to a separator funnel added by 20 mL hexane and 25 mL distilled water, and then shaken lightly for 30 to 60 seconds and left to rest for phases separation. Aqueous phase collected was transferred to a second separator funnel and subjected to two re-extraction procedures by adding 20 mL ethanol:hexane 1:1 solvent mix, collecting combined hexane phases containing all the carotenoid substances. Aqueous residue had been removed from hexane phase by adding about 1.0 g of sodium sulfate anhydride ( $\text{Na}_2\text{SO}_4$ ) and then shaken manually for 30 seconds. All hexane carotenoid extract content were removed to 250 mL flat bottom balloons and concentrated by vacuum rotary evaporator at about  $40^\circ\text{C}$ , for 25 minutes. After concentration, samples were recovered in hexane, and then transferred to brown color volumetric balloons, and the exact volume completed with hexane.

Carotenoid content was determined by High Performance Liquid Chromatography in a SPD-10 AV Shimadzu UV-VIS instrument, UV-Visible spectrophotometry detector, at 470 nm; Shimadzu injector,

equipped with 20  $\mu\text{L}$  loop; SUPERCOSIL™ LC-18 micra, 250 mm length and 4.6 mm i.d. column. Acetonitrile:chloroform (92:8) chromatographic grade at 1.5  $\text{mL min}^{-1}$  was the mobile phase. Run time for each sample was 25 min. All chemicals used in the mobile phase were vacuum filtered in a PTFE membrane system, modified for organic and aqueous solvents, with 0.45 micra diameter pores. Mobile phase chemicals and samples were degassed in an ultrasonic washer. The Standard used by HPCL of  $\beta$ -carotene or lycopene of Sigma trade. The  $\beta$ -carotene synthetic pure 95% and lycopene 90 - 95%. The pack contents lycopene (1.0 mg) and pack  $\beta$ -carotene, was transfer to balloon volumetric to the dark color and full by 30 mL of hexane HPCL of Sigma trade. A 1.0 mcg to 12.0 mcg of  $\beta$ -carotene and 10.0 mcg to 50.0 mcg of lycopene was used, divided in five points, by standard curve. Had response lineal with  $R^2$  0.99 by both  $\beta$ -carotene and lycopene. Analysis were carried out by injecting 1.0 mL of  $\beta$ -carotene or lycopene standard solutions in hexane and 1.0 mL of samples in the chromatograph, with a 3.0 mL disposable syringe. Figure 1 presents typical HPLC chromatograms of the standard  $\beta$ -carotene (a) and lycopene (b). Extraction and chromatograph injection steps were done in the same day under low light.

Experimental design was completely randomized in a factorial arrangement, with ten replications (fruits). The fruits were sampled, harvested and evaluated at two ripen stages (F2), with and without apparent physiological skin freckles (F1). Thus, there were 40 fruits from hybrid *Tainung 01* harvested in January 2003, 40 papaya fruits from the same hybrid harvested in February 2004 and 40 papaya fruits from 'Solo' group, *Golden* cultivar harvested in March 2003, totaling 120 fruits analyzed or 120 sample units. Among the 40 fruits harvested for each group, 10 were with freckles in maturation stage 1, 10 with freckles in maturation stage 2, 10 without freckles in maturation stage 1 and 10 without freckles in maturation stage 2.

The data were subjected to analysis of variance (ANOVA) at significance level  $\alpha$  of 0.05.

## RESULTS AND DISCUSSION

Papaya fruits with skin freckles had higher content in L-ascorbic acid (AA), 83.2  $\text{mg } 100 \text{ g}^{-1}$ , than fruits without skin freckles, 75.9  $\text{mg } 100 \text{ g}^{-1}$  ( $P \leq 0.05$ ) (Table 1.). Sample ranged from 7.0 to 10.0  $\text{mg } 100 \text{ g}^{-1}$  of papaya pulp, except for that from hybrid *Tainung 01* ('Formosa') at ripen stage 2, harvested in February 2004. This result can be attributed to a low incidence of skin freckles presented in this fruits.

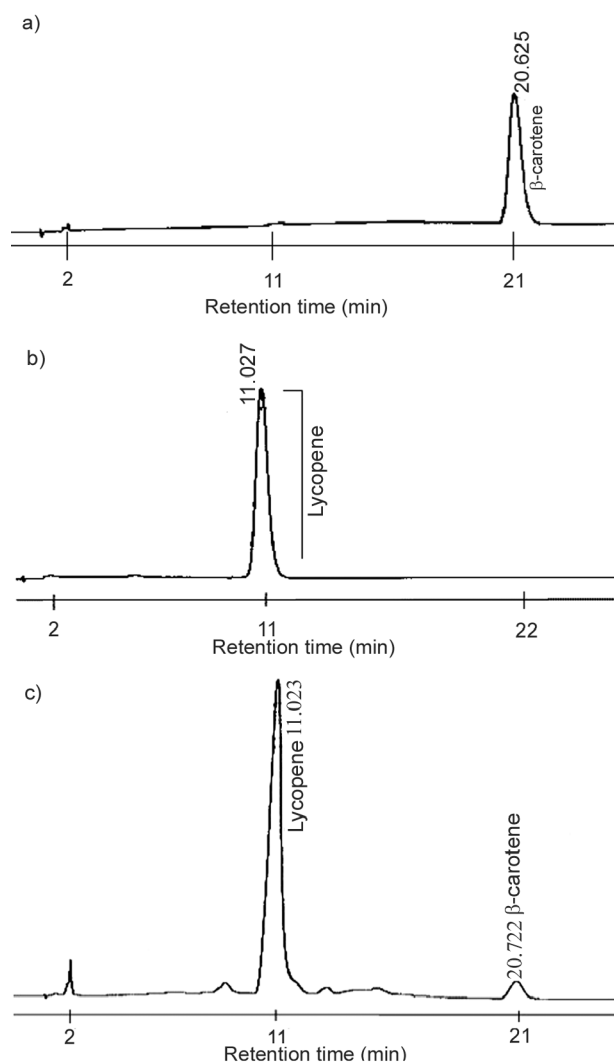


Figure 1 - Typical HPLC chromatograms of the standard  $\beta$ -carotene (a), lycopene (b), and carotenoids in papaya (c).

L-ascorbic acid levels observed in this research are similar to others reported in the literature. Oliveira (1999) reported 86.0  $\text{mg } 100 \text{ g}^{-1}$  of L-ascorbic acid in *Improved Sunrise Solo Line* papaya fruits and 73.8  $\text{mg } 100 \text{ g}^{-1}$  in *Tainung 01/781* hybrid, both determined at the seventh day after harvesting. Souza (1998) reported 90.7  $\text{mg } 100 \text{ g}^{-1}$  mean values of L-ascorbic acid in *Sunrise Solo 783* papaya fruits and 71.3  $\text{mg } 100 \text{ g}^{-1}$  in *Tainung 01* hybrid. Islam et al. (1993) analyzed papaya fruit samples from five group cultivars and reported L-ascorbic acid mean values ranging from 88  $\text{mg } 100 \text{ g}^{-1}$  to 118  $\text{mg } 100 \text{ g}^{-1}$ . Vinci et al. (1995) reported L-ascorbic acid mean values of 88  $\text{mg } 100 \text{ g}^{-1}$  for papaya fruits ripen naturally and 54  $\text{mg } 100 \text{ g}^{-1}$  for fruits artificially ripen.

Mean values and standard deviation for  $\beta$ -carotene and lycopene are presented in Table 2 and typical HPLC chromatograms of the standard carotenoids

Table 1 - L-ascorbic acid mean values in mg 100 g<sup>-1</sup> fresh papaya pulp from 'Formosa' and 'Solo' groups, with and without skin freckles (SF) harvested in two ripen stage, in January 2003, February 2004 and March 2003.

Cultivar	Ripen stage	L-ascorbic acid mg 100 g <sup>-1</sup>	
		With SF	Without SF
Tainung 01 'Formosa' harvest 01/17/2003	1	70.2 ± 8.7*	63.0 ± 10.0*
	2	77.8 ± 10.9*	68.2 ± 9.7*
Tainung 01 'Formosa' harvest 02/06/2004	1	67.9 ± 6.7*	59.9 ± 6.1*
	2	59.9 ± 3.4*	60.3 ± 4.2*
Golden 'Solo' harvest 03/21/2003	1	111.0 ± 14.6*	101.4 ± 8.1*
	2	112.4 ± 12.6*	103.1 ± 10.6*
Mean		83.2**a	75.9**b

\*Mean values for ten papaya fruits ± standard deviation. \*\*Mean values for sixty papaya fruits ± standard deviation. Different letters are different at the 5% by F test.

Table 2 - Lycopene and  $\beta$ -carotene mean values in papaya pulp from 'Formosa' and 'Solo' groups, with and without skin freckles harvested in two ripen stage, in January 2003, February 2004 and March 2003.

Cultivar	Ripen stage	Skin freckles	Lycopene and $\beta$ -carotene (mg 100 g <sup>-1</sup> ) <sup>a</sup>	
			Lycopene	$\beta$ -carotene
Tainung 01 'Formosa' harvest 01/17/03	1	With	1.57 ± 0.49	0.31 ± 0.11
		Without	2.49 ± 0.56	0.51 ± 0.06
	2	With	2.85 ± 0.47	0.56 ± 0.09
		Without	2.61 ± 0.76	0.48 ± 0.12
Tainung 01 'Formosa' harvest 02/06/04	1	With	2.51 ± 0.41	0.34 ± 0.09
		Without	2.22 ± 0.41	0.22 ± 0.09
	2	With	3.39 ± 0.32	0.23 ± 0.07
		Without	2.94 ± 0.79	0.19 ± 0.07
Golden 'Solo' harvest 03/21/03	1	With	1.44 ± 0.28	0.33 ± 0.11
		Without	1.50 ± 0.23	0.34 ± 0.13
	2	With	1.70 ± 0.30	0.48 ± 0.09
		Without	1.51 ± 0.31	0.46 ± 0.08

<sup>a</sup>Mean values for ten papaya fruits ± standard deviation.

in papaya in the Figure 1(c). Analysis of variance did not detected difference ( $P \geq 0.05$ ) in lycopene or  $\beta$ -carotene mean values between papaya fruits with and without skin freckles. Mean  $\beta$ -carotene values ranged from (0.19 ± 0.07) mg 100 g<sup>-1</sup> to (0.56 ± 0.09) mg 100 g<sup>-1</sup> fresh papaya pulp. Lower  $\beta$ -carotene values were observed in 'Formosa' *Tainung 01* papaya fruits without skin freckles, harvested in February 2004 and higher  $\beta$ -carotene values were detected in papaya fruits with physiological skin freckles harvested in January 2003. Rodriguez-Amaya (1996) observed differences in  $\beta$ -carotene content in papaya fruits produced in different Brazilian States. Papaya fruit samples from Sao Paulo State presented  $\beta$ -carotene mean values of (0.14 ± 0.05) mg 100 g<sup>-1</sup> fresh pulp and fruits from Bahia State were (0.61 ± 0.14) mg 100 g<sup>-1</sup>. Weather condi-

tions, ripen stage, variety or group cultivar, geographical area and season of the year are important for carotenoid levels in fruits (Setiawan et al., 2001).

Papaya as a fruit can be considered as a moderate source of Provitamin A, ranging from 82  $\mu$ g to 190  $\mu$ g Retinol Equivalent (RE) 100 g<sup>-1</sup> fresh pulp, when including the  $\beta$ -criptoxantine contribution Rodriguez-Amaya (1996). Overall mean value for  $\beta$ -carotene observed in our research was 0.37 mg 100 g<sup>-1</sup> fresh pulp, which is 62  $\mu$ g RE 100 g<sup>-1</sup> fresh papaya pulp. This represents 6.2% of World Health Organization Recommended Daily Allowance (RDA) for adult people, which is 1.000  $\mu$ g (FAO, 2003).

Lycopene mean value was 2.23 mg 100 g<sup>-1</sup>. Lower mean value was (1.44 ± 0.28) mg 100 g<sup>-1</sup> for cv. *Golden* ('Solo') and higher value was (3.39 ± 0.32)

mg 100 g<sup>-1</sup> for *Tainung 01* hybrid. Setiawan et al. (2001) reported 5.75 mg 100 g<sup>-1</sup> fresh pulp lycopene mean value in papaya from Indonesia. Kimura et al. (1991) reported lycopene mean value of (2.10 ± 1.60) mg 100 g<sup>-1</sup> fresh pulp in papaya fruits from 'Solo' group produced in Bahia State. She also observed lycopene mean value of (1.90 ± 0.40) mg 100 g<sup>-1</sup> in 'Formosa' group papaya fruits produced in Sao Paulo State and (2.60 ± 0.30) mg 100 g<sup>-1</sup> for those produced in Bahia State. Lycopene was the main carotenoid in papaya fruits evaluated in this research. This is in agreement with reports by Wilberg & Rodriguez-Amaya (1995), Kimura et al. (1991) and Setiawan et al. (2001).

Lycopene does not have Vitamin A activity but contribute to papaya pulp reddish color. *Golden* papaya fruit pulp reddish color was less intense than that for *Tainung 01* hybrid and this may be due to its lower content in lycopene, which ranged from (1.44 ± 0.28) mg 100 g<sup>-1</sup> to (1.70 ± 0.30) mg 100 g<sup>-1</sup> fresh papaya pulp.

Tomatoes are considered good source of lycopene, with average ranging from 3.0 to 5.0 mg 100 g<sup>-1</sup> fresh pulp. Research had shown that consumption of food products containing tomatoes reduce the possibility of developing several types of cancer, which has been associated to higher blood lycopene levels (Shi & Maguer, 2000). Lycopene levels in papaya fruit are close to that of tomatoes. Thus it can also be considered as a good source of this carotenoid.

## CONCLUSIONS

L-ascorbic acid content in papaya 'Formosa' group fruits ranged from (59.9 ± 3.4) mg 100 g<sup>-1</sup> to (70.2 ± 8.7) mg 100 g<sup>-1</sup>. β-carotene mean values ranged from (0.56 ± 0.09) mg 100 g<sup>-1</sup> to (0.19 ± 0.07) mg 100 g<sup>-1</sup> and lycopene levels ranged from (3.39 ± 0.32) mg 100 g<sup>-1</sup> to (1.57 ± 0.49) mg 100 g<sup>-1</sup>. Analysis of variance did not detect difference in lycopene or β-carotene mean values between papaya fruits with and without skin freckles, but L-ascorbic acid levels in papaya fruits with physiological skin freckles were, on average, 7.2 mg 100 g<sup>-1</sup> higher than in fruits without the disease ( $P \leq 0.05$ ).

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