African Journal of Food Science Vol. 5(6), pp. 320-323, June 2011
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2011 Academic Journals

Full Length Research paper

Pigments of guava paluma cultivar stored under environmental conditions

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Accepted 21 December, 2010

This study evaluated the levels of pigments in guavas such as chlorophyll, total carotenoids, total anthocyanins and yellow flavonoids. In the study, guava fruits (Paluma cultivar) were used, and they were stored under environmental conditions for nine days. The evaluation of guava fruit pigment levels was carried out every two days. Findings showed significant differences regarding levels of chlorophyll and carotenoid ($p<0.05$) during the storage period. Anthocyanin contents varied from 0.24 ± 0.01 to 0.37 ± 0.02 mg 100 g^{-1} guava pulp, showing no significant difference until the fifth day of storage. Values of yellow flavonoids were different from the seventh day of storage on. Levels of pigments evaluated in guava fruits (*P. cultivar*) changed during the storage period under environmental conditions, thus showing the influence of the continuous metabolic activity in the fruits during the post-harvest period.

Key words: *Psidium guajava* L., chlorophyll, carotenoids, anthocyanins, flavonóides, storage.

INTRODUCTION

Guava is a fruit with high nutritional value and with excellent acceptance for consumption “in nature”, and it is also of great economical significance in subtropical and tropical areas. The guava is a climacteric fruit, have a high respiratory rate with a clear transition between the growth and senescence, it becomes necessary to study the loss of its components during storage (Chitarra and Chitarra, 2005; Agrianual, 2003). Ribeiro et al. (2005) led to loss of chlorophyll, ascorbic acid and mass loss in the guavas, Paluma cv., stored at room temperature and under refrigeration for 12 days. Jacomino et al. (2003) in their study reported loss of firmness and color variation of the guava, Pedro Sato cv., under ambient conditions of 25°C for six days of storage. Other factors that may influence the levels of components of guava are associated with the management, soil and climatic conditions during the production (Chitarra and Chitarra, 2005). Even after crop, they present high metabolic activity which triggers their deterioration processes. The synthesis reactions of new metabolic compounds occur with countless catabolic reactions that lead to the complete degradation of the fruit (Azzolini et al., 2004). Visible signs of ripening, which make the fruit edible, are transformations in the color, texture, flavor and aroma. The color is an important attribute in judging fruit quality, and modifications in color throughout the maturation are due to rotting and synthetic processes. In addition to providing color to horticultural products, pigments have a relevant role in the human body, once they act as antioxidants. The ingestion of fruits is being associated to a decrease in the development of chronic-degenerative diseases such as cancer, inflammations, cardiovascular diseases, amaurosis, macular degeneration, among others (Kong et al., 2003).

The main pigments present in vegetable products are: carotenoids, flavonoids (anthocyanins) and chlorophyll (Chitarra, 1994). Studies show that color stability depends on the structures of anthocyanins and on the presence of colorless phenolic compounds (Malien-Aubert et al., 2001). Carotenoids (β-carotene) and flavonoids (anthocyanins) provide characteristic color to products, and have significant functions and actions on human health acting as antioxidants and free radical scavenging, which can help to reduce the risk of

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illnesses, such as cancer and cardiovascular diseases. Prior et al. (2005), found that after a rich meal containing antioxidant food, the antioxidant capacity of human plasma increases. Chlorophyll is the pigment responsible for the green color of the fruit skin. The loss of the green color is an indicative of maturity, because as the fruit ripens the chlorophyll degrades, due to factors that act separately or together (Coultate, 2004). Carotenoids are the pigments responsible for most of the yellowish, orange and red colors of vegetable products (Coultate, 2004). Such pigments become visible with the chlorophyll degradation or they can be synthesized with the progress of the fruit maturation (Chitarra and Chitarra, 2005). In addition, the carotenoids constitute the largest group of natural pigments, and some of them are precursory of vitamin A (Hiane et al., 2003). These pigments work as natural antioxidants and they protect membranes, DNA and other cellular constituents against oxidizing damages (Agostini-Costa et al., 2003). Carotenoids, such as the β-carotene, lycopene, zeaxanthin and lutein carry out antioxidant functions in lipidic phases, thus they block the free radicals that damage the lipoprotein membranes (Shami and Moreira, 2004). Besides the antioxidant activity, they are anticarcinogenic, immunogenic and they protect the body against cardiovascular diseases and diabetes (Nishino et al., 2002; Rich et al., 2003). During the ripening of fruit and vegetables, chlorophyll and carotenoids use occurs instead of suffer qualitative and quantitative changes which are indicative of quality of the useful life of these products (Roca and Mínguez-Mosquera, 2001).

Flavonoids include natural pigments frequently found in vegetables. Anthocyanins and flavonoids are compounds that belong to the group responsible for the coloration that varies from dark red to violet and from white to light yellow, respectively (Lima et al., 2000). The red characteristic coloration of ripe guava pulp is due to the presence of anthocyanins. Studies show that flavonoids (anthoxantines and anthocyanins) possess high nutritional and therapeutic value, being capable of capturing free radicals (antioxidant activity) and effects in the prevention of cardiovascular, circulatory and Alzheimer diseases, in addition to cancer and diabetes (Kuskoski et al., 2006). Flavonoids also have anti-inflammatory activity in humans and animals (Georgetti et al., 2002). Anthocyanins belong to the class of flavonoids, they are the pigments responsible for the rose, red, purple, violet and blue colors of fruit, and they occur in nature as glicosydes, the color varies according to the number of hydroxyl or metoxyl radicals in the molecule (Coultate, 2004). The variation of colors and forms of anthocyanins depend, among other factors, on the pH, structure, concentration of the pigment, and on the presence of co-pigments, such as polyphenols, organic acids, and amino acids among others. Moreover, anthocyanins can, through co-pigmentation, intensify and stabilize the color of these compounds (Lima et al., 2007). Therefore, the aim of this investigation was to evaluate chlorophyll, total carotenoids, total anthocyanins and yellow flavonoids contents in guavas, Paluma cv., stored in environmental conditions for nine days.

MATERIALS AND METHODS

The guavas, Paluma cv., were bought from a producing farm in Limoeiro do Norte, State of Ceará, from seven year-old irrigated plants. The fruits were picked in August 2008, at the maturation stage, with the color of the peel varying from dark green to light green, and transported to the Laboratory of Quality control and Food Drying of the Federal University of Ceará, where they were cleaned and conditioned in corrugated cardboard boxes. The temperature was monitored for the nine days of storage (27.0 ± 0.7°C). Every two days, during storage, a sample of 6 fruits from varied boxes was removed so that analyses could be performed. The packages used for packaging the fruits of guava were the model for export, made of corrugated cardboard, with external dimensions of 31.5 x 26.5 x 8.3 cm (length x width x height), comprising an average of 3 kg fruit, around 12 to 16 guavas. Samples used for determining chlorophyll, were taken from the fruit peel, to a depth of approximately 1 mm. The sample was macerated in mortar and the chlorophyll extraction was done with acetone at 80%. Chlorophyll was determined according to the methodology described by Engel and Poggiani (1991). The reading was done in 652 nm spectrophotometer and the results were expressed in mg 100 g^{-1} of peel. Total carotenoids in pulp were verified by using the Higby's method (1962), whose extraction happened through the agitation of 10 g of the sample in 30 ml of isopropyl alcohol and 10 ml of hexane. The content was transferred to a separation funnel, and it was allowed three 30-min-rest periods, followed by three subsequent filtrations.

Filtration was performed in cotton previously sprayed with anhydrous sodium sulphate (P.A); then 5.0 ml of acetone was added with gauging in a 50 ml round flask. The white part analysis was made by adding 5.0 ml of acetone in a 50 ml round flask, completed with hexane. Readings were done in spectrophotometer, with wavelength of 450 nm. Results were expressed in mg of total carotenoids 100 $g^{-1}$of sample and calculated multiplying the absorbance by 2. Anthocyanins and yellow flavonoids determinations were accomplished according to methodology by Francis (1982). Extraction was made with a solution of Ethanol-HCL (1.5 M) prepared in the proportion 85:15. Pulp samples were homogenized with the aid of a mortar and the content was transferred to a 50 ml round flask, without filtering, wrapped up in aluminum foil, and let to rest for one night in refrigerator. Then, the filtering was carried out and the reading in spectrophotometer in wavelength of 535 nm for anthocyanins and 374 nm for yellow flavonoids; results were expressed in mg 100 g^{-1}, calculated by the formula: absorbance x dilution factor/76.6 or 98.2 for yellow flavonoids and total anthocyanins, respectively. Readings were accomplished in spectrophotometer model B 582 – from Micronal. In the statistical analysis, the significant interactions for ANOVA F test were unfolded and the averages throughout the storage period were compared by Tukey test, at 5% probability, with the aid of computational Statistics - version 7.0, and the graphs were elaborated by the program (Statsoft, 1995).

RESULTS AND DISCUSSION

The chlorophyll contents, total carotenoids, chlorophyll/ carotenoid ratio, total anthocyanins and yellow

Table 1. Mean, standard deviation and Tukey test for chlorophyll contents, total carotenoids, anthocyanins and yellow flavonoids of guava, (*Paluma cultivar*), stored at 27°C for nine days.

Days	Chlorophyll (mg 100 g^{-1} peel)	Carotenoids (mg 100 g^{-1} pulp)	Chlorophyll/ Carotenoid Relation	Anthocyanins (mg 100 g^{-1} pulp)	Flavonoids (mg 100 g^{-1} pulp)
0	21.79 ± 8.45^{b}*	0.18 ± 0.02^{a}	123.40 ± 40.37^{c}	0.24 ± 0.01^{a}	3.38 ± 0.09^{a}
3	20.81 ± 2.56^{b}	0.30 ± 0.05^{ab}	71.00 ± 22.54^{bc}	0.24 ± 0.01^{a}	3.46 ± 0.06^{a}
5	11.30 ± 2.47^{ab}	0.41 ± 0.08^{b}	29.59 ± 13.43^{ab}	0.24 ± 0.01^{a}	3.60 ± 0.10^{a}
7	9.09 ± 1.34^{a}	0.48 ± 0.10^{b}	18.94 ± 2.01^{ab}	0.30 ± 0.04^{b}	3.95 ± 0.11^{b}
9	6.89 ± 1.36^{a}	0.82 ± 0.12^{c}	8.44 ± 1.20^{a}	0.37 ± 0.02^{c}	4.41 ± 0.10^{c}

*Different letters in the same column differ amongst themselves ($p<0.05$).

flavonoids of guava *cv Paluma* during the nine days of storage are shown in Table 1. It can be observed that the standard deviation value of chlorophyll content, in the first day of evaluation (zero), was higher than the other values. This happened because of the variation in the maturation point in the harvest day, which reduced and homogenized the color of the fruit peel during the storage period. Thus, the ratio chlorophyll/carotene also presented, in the first evaluation, the highest value for standard deviation in comparison to the values attained during storage. Both chlorophyll contents and carotenoid levels presented significant difference during the storage period ($p<0.05$). Table 1 show that chlorophyll content decreased with the ripening of fruits, since the peel color changed from dark green to yellow. The reduction in chlorophyll level was 31.62% at the end of the storage period. According to Ribeiro et al. (2005), this occurs due to chlorophyll degradation and to the synthesis of carotenoids. Total content of chlorophyll, during the nine days of storage, varied from 21.79 ± 8.45 to 6.89 ± 1.36 mg 100 g^{-1} fruit peel, and showed significant difference in a 5% -significance level, from the fifth day of storage (Table 1). Degradation of chlorophyll occurs due to the breaking of its molecule chemical structure, due to changes in pH, caused by the accumulation of organic acids, activation of chlorophyllasis enzyme or any other enzymes and other compounds in the vacuoles; and also due to the presence of enzymatic or chemical oxidizing systems (Jacomino et al., 2008). On the other hand, an increase in carotenoid contents was observed, which indicates their syntheses throughout the storage period. The values of total carotenoids (Table 1) obtained varied from 0.18 ± 0.02 to 0.82 ± 0.12 mg 100 pulp g^{-1}, presenting statistic difference at 5% significance up to the last day of storage.

Carotenoids values showed an increase of 4.6 times in relation to the initial content, at the end of storage period. Pereira (2009) found the content of such compound, for guava Paluma cv., 1.6 mg 100 pulp g^{-1}, higher than the findings of the present investigation. It can be observed a concordance in behavior, that is, as the fruits ripened (change of peel color from green to yellow) a reduction of chlorophylls occurred, and consequently, an increase in carotenoid contents. Similar values were found by Pereira et al. (2005). The development of red color in guava is also due to lycopene biosynthesis, which increases with maturation (Azzolini et al., 2004). Rodrigues-Amaya et al. (2008) in their work with guava, Paluma cv., harvest 2000/01, produced in the state of São Paulo, found the following carotenoid contents in fruit: 4.3 + 1.3 µg g^{-1} for β-carotene and of 66.5 + 5.0 µg g^{-1} for lycopene. The variation of total chlorophyll/ carotenoids ratio during the storage period can be observed in Table 1. This ratio tends to decrease during ripening process, decreasing quickly in the beginning, until the fruit acquires red pulp color and yellow color on the peel, as a result of the increase of carotenoids and anthocyanins. Similar results were found by Roca and Mínguez-Mosquera (2001) when they studied these pigments in olives, during the processing of virgin olive oil. The authors aforementioned state that the ratio between chlorophyll content and carotenoids occurs as a result of the ripening process. Throughout the storage period, anthocyanin contents varied from 0.24 ± 0.01 to 0.37 ± 0.02 mg 100 g^{-1} pulp, not showing significant difference until the fifth day of storage (Table 1). Guava pulp develops an intense red coloration as fruits ripen, as a result of an increase in these compounds. Anthocyanins had an increase of 1.54 times its initial content, compared to the last day of evaluation.

The values found were similar to those found by Pereira (2009) and Kuskoski et al. (2006) for guava Paluma cv., which were 0.34 ± 0.02 and 2.7 ± 0.2 mg 100 g^{-1}, respectively. Yellow flavonoid contents ranged from 3.38 ± 0.09 to 4.41 ± 0.10 mg 100 g^{-1} pulp, during storage period, with a variation of 1.30 times at the end of the storage (nine days) in relation to their initial contents, not presenting statistic difference until the fifth day (Table 1). Values found were lower than those found by Pereira (2009) for the same guava variety, whose content was 8.40 ± 0.34 mg 100 g^{-1}. According to Lima et al. (2000), acerolla fruits possess total flavonoid contents that vary from 9.31 to 20.22 mg 100 fruit g^{-1}. Storage conditions influenced positively the biosynthesis of pigments, thus, a longer storage period propitiated an intensification of the pulp color, represented by the increase of pigment

contents studied. Among the pigments analyzed in guavas, carotenoid contents showed higher increase at the end of the storage period. The pigment contents in guavas Paluma cv. underwent alteration during the fruit storage period at room temperature (27.0 ± 0.7°C). Chlorophyll content in guava peel, after nine days, resulted in a reduction in relation to its initial values. Carotenoid, anthocyanin and total flavonoid contents found in the fruit pulp, increased significantly at the end of the storage period. Results obtained in the present investigation show that guava fruits - Paluma cv. were affected by the temperature and by the storage period in their metabolic activity, which was observed by the change in coloration during the continuous process of fruit ripening after being harvested.

According to Azzolini et al. (2004), used at room temperature during storage positively influences the biosynthesis of pigments in guavas.

ACKNOWLEDGEMENTS

The authors are grateful to Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico - FUNCAP for granting scholarships and to CNPq, for providing financial support to the present research.

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