Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

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Ciência e Tecnologia de Alimentos, vol. 32, núm. 1, abril-junio, 2012, pp. 156-162
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

Original

Sugar fractionation and pectin content during the ripening of guava cv. Pedro Sato

Fracionamento de açúcares e teor de pectina durante o amadurecimento de goiaba cv. Pedro Sato

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Abstract

Guava is one of the most complete and balanced fruits in in terms of s nutritional value. Highly perishable, due to its intense metabolism during ripening, its shelf life can reach 3 to 5 days under room temperature. The firmness of the green and mature fruits is due mainly to the pectin polymers. The loss of firmness during the guava ripening is due to hydrolytic enzyme activity, which promotes intense solubilization of the cell wall pectins. Given the above, with the purpose of trying to explain the rapid firmness decrease, the centesimal composition and sugar fraction of the guava fruit were determined during ripening at room temperature. The guavas were picked at the half-mature stage and stored for 8 days at 22 ± 1 °C and 78 ± 1% relative humidity. The analyses conducted were: centesimal composition, sugar fractionation, and infrared absorption spectrometry. The results showed that the guava sugars did not vary during ripening. The estimated pectin levels (5.7%) were higher than those mentioned in the literature (2.4%), which can better explain the role of the pectin in the fruit firmness.
Keywords: *guava; sugar; pectin.*

Resumo

A goiaba é uma das mais completas e equilibradas frutas no que diz respeito ao valor nutritivo. Altamente perecível, devido ao seu intenso metabolismo durante o amadurecimento, tem vida útil que pode chegar de 3 até 5 dias sob temperatura ambiente. A firmeza dos frutos verdes e maduros é devida principalmente aos polímeros de pectina. A perda de firmeza durante o amadurecimento da goiaba é devido à atividade de enzimas hidrolíticas, que promovem intensa solubilização das pectinas constituintes da parede celular. Diante do exposto, com a finalidade de tentar explicar a rápida diminuição da firmeza, determinou-se a composição centesimal dos frutos da goiaba e fez-se um fracionamento dos açúcares durante o amadurecimento em temperatura ambiente. Foram colhidas goiabas no estádio "de vez" e armazenadas por 8 dias à temperatura de 22 ± 1 °C e umidade relativa de 78 ± 1%. As análises realizadas foram: composição centesimal, fracionamento dos açúcares e espectrometria de absorção na região do infravermelho. Pelos resultados, verificou-se que os açúcares de goiaba não variaram durante o amadurecimento. Os teores de pectina estimados (5,7%) foram maiores que os teores citados na literatura (2,4%), o que pode explicar melhor o papel da pectina na firmeza do fruto.
Palavras-chave: *goiaba; açúcar; pectina.*

1 Introduction

In Brazil, guava culture has great socioeconomic importance given its wide and varied forms of use. It is produced mainly for juice, pulp, jam and jelly, preserve, and ice cream and other sophisticated products such as "guatchup" (similar to ketchup, made with guava pulp) (GONGATTI NETO et al., 1996).

Guava is one of the most important fruits in the tropical and subtropical areas not only due to its high nutritional value, but also to its excellent in natura consumption acceptance, to its development capacity under adverse conditions, and to its wide industrial application (MAIA et al., 2002). Guava is considered a quite attractive fruit in reason of its delicate color and pleasant aroma, besides being one of the most complete and balanced fruits as far as nutritional value is concerned, containing high levels of vitamin C, reasonable amounts of provitamin A, B-complex vitamins, and mineral salts such as calcium, phosphorus, and iron (PEREIRA, 1995; PEREIRA; MARTINEZ, 1986; SATO; CUNHA; ARGANDOÑA, 2005; MANICA et al., 2000, SIQUEIRA et al., 2006). However, a wide variation in the nutrition component level can occur due to the variety of the fruit, maturation stage at harvest, climatic conditions during the fruit development, and cultivation procedures (PEREIRA, 1995; CARVALHO, 1999; CARDOSO et al., 2002).

Highly perishable, due to its intense metabolism during ripening, guava has a shelf life that can reach 3 to 5 days under room temperature (CARVALHO, 1994; DURIGAN, 1997; GONGATTI NETO et al., 1996), in which the decrease of firmness is the most outstanding characteristic in the ripening process.

The firmness of the green and ripe fruits is due mainly to pectin polymers, which can be methylated and have different degrees of methylation (KERTESZ, 1951; FERTONANI, 2006). They can also bonded to ions, mainly Ca^{++}, which maintains adjacent chains bonded among themselves or present glycosidic chains interconnected among themselves by phenolic compounds. (TAIZ; ZEIGER, 2004, CHITARRA; CHITARRA,

Received 25/8/2010
Accepted 26/10/2011 (005008)
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DOI: http://dx.doi.org/10.1590/S0101-20612012005000029

2005. The loss of firmness during the ripening of the fruits is due to hydrolytic enzyme activity that promotes intense solubilization of the pectins present in the cell wall, mainly the pectinmethylesterase (PME) and polygalacturonases (PG) activity (TUCKER, 1993; JAIN et al., 2001; OLIVEIRA et al., 2006). Considering that the guava cv. Pedro Sato does not present polygalacturonase activity (LINHARES et al., 2007) or it is very low and decreases with the ripening (LIMA, 2004; XISTO et al., 2004), the explanation for the rapid decrease in firmness, still remains inexplicable. The existence of high esterase activity in the cell membrane/cell wall of guava kernel (LINHARES et al., 2007) can suggest that the rapid firmness decrease occurs by processes other than the polygalacturonic acid hydrolysis.

Although guava is considered a rich fruit in pectin (SILVA, 2000), http://www.paraisofrutas.com.br/frutas.php?titulo='Goiaba'; http://www.terra.com.br/culinaria/abc/g.htm; http://www.clesio.net/cn/index.php/2008/01/24/as_frutas_sao_importantes_no_cardapio_de?blog=12), the levels reported in the literature are around 2.4% (PAL; SELVARAJ, 1979; MOWLAH; ITOO, 1983; BULK; BABIKER; TINAY, 1997; CARVALHO, 1999, CARVALHO et al., 2001; GIANONNI, 2000; XISTO, 2002; LIMA, 2004; XISTO et al., 2004; VILA et al., 2007; LINHARES et al., 2007; MENDONÇA et al., 2007; OSHIRO et al., 2008), and with such low levels, the pectins cannot be the only compounds responsible for the guava firmness. Besides, the statement that total pectin levels can remain unaffected, decrease, or increase during the ripening of guava, is inconsistent with the fruit ripening process.

Given the aforementioned, with the purpose of trying to explain the rapid decrease of firmness, the objectives of this study were to determine the centesimal composition of guava fruits as well as perform sugar fractionation during ripening at room temperature.

2 Material and methods

2.1 Origin and crop of the fruits

The guavas (*Psidium guajava* L.) of the cv. Pedro Sato were picked manually in the early in the morning at the half-mature ripening stage (light green coloration) in a commercial orchard located in the municipal district of Lavras, Minas Gerais, altitude 845 m, latitude 21.15° S and longitude 45.22° W. They were placed in previously sterilized polyethylene boxes and transported to the Biochemistry Laboratory of the Department of Chemistry (DQI) of the Federal University of Lavras – MG.

For the analysis of the centesimal composition, the guavas were selected (containing representatives of all ripening stages: green, intermediate, and ripe), washed in running water, and cut in small slices. Soon afterwards they were taken to an electric homogenizer to be triturated forming a homogeneous paste, from which samples were removed for the determination of the average centesimal composition regardless of ripening stage The temperature and relative humidity was 22 ± 1 °C and 78 ± 1%, respectively. The methodology adopted for the conduction of the centesimal composition analyses (humidity, ash, lipids, fiber, protein, and carbohydrates) followed the official methods described in Association of Official Analytical Chemistry (2002).

For the other analyses, the fruits were selected, washed in running water, and immersed in 1% sodium hypochlorite solution at 20 °C for 5 minutes for disinfection. After drying the hypochlorite solution, the fruits were numbered, put on a shelf in the laboratory, and maintained for a period of 8 days at a temperature and relative humidity of 22 ± 1 °C and 78 ± 1%, respectively.

2.2 Experimental design

The experimental design used was completely random (CRD) with 8 treatments (0, 1, 2, 3, 4, 5, 6, and 8 days of ripening). The experimental portion was composed of 3 fruits with 4 repetitions for each treatment. The results were submitted to variance analysis using the software SANEST (ZONTA; MACHADO, 1991). Regression analyses were conducted and the polynomial models were selected observing the significance of the F test for each model and their respective determination coefficients.

2.3 Fractionation and quantification of the cell wall carbohydrates

The sugars of the guava fruits were fractioned on each day of ripening according to the scheme of Figure 1.

The soluble sugars were extracted boiling 1 g of guava previously homogenized with 40 mL of ethanol 80% for 5 minutes. The mixture was centrifuged at 10,000 xg for 10 minutes at room temperature, and the supernatant (denominated SOB 1) was used for the quantification of total soluble sugars (DISCHE, 1962); the sediment (denominated SED 1) was used to fraction insoluble sugars.

SED 1 was homogenized in 20 mL of 1% HCl and boiled for 5 minutes and centrifuged at 10,000 xg for 10 minutes at room temperature; SOB 2 and SED 2 were recovered.

Absolute ethanol was added to SOB 2 until reach 80% concentration. After remaining for 60 minutes at 20 °C negative, it was centrifuged at 10,000 xg 10 minutes at 4 °C. The SOB 3 was used for hemicelulose determination, and SED 3 was suspended in 10 mL of water and used for the determination of starch. SED 2 was homogenized in 20 mL of $ZnCl_2$-HCl (2:1, p/v) for 10 minutes (OSER, 1965) and centrifuged at 10,000 xg for 10 minutes at room temperature. SOB 4 was used for the cellulose quantification, and SED 4, which mainly corresponds to non-carbohydrate material, was homogenized in 10 mL of water and used to evaluate the possible presence of carbohydrates that were not previously fractioned. All of the carbohydrates were determined in the preparations using the anthrone method (DISCHE, 1962) for total sugars.

2.4 Absorption spectroscopy in the infrared region (FT-IR)

The characterization of the complex by infrared absorption spectroscopy was carried out at the Centro de Análise e

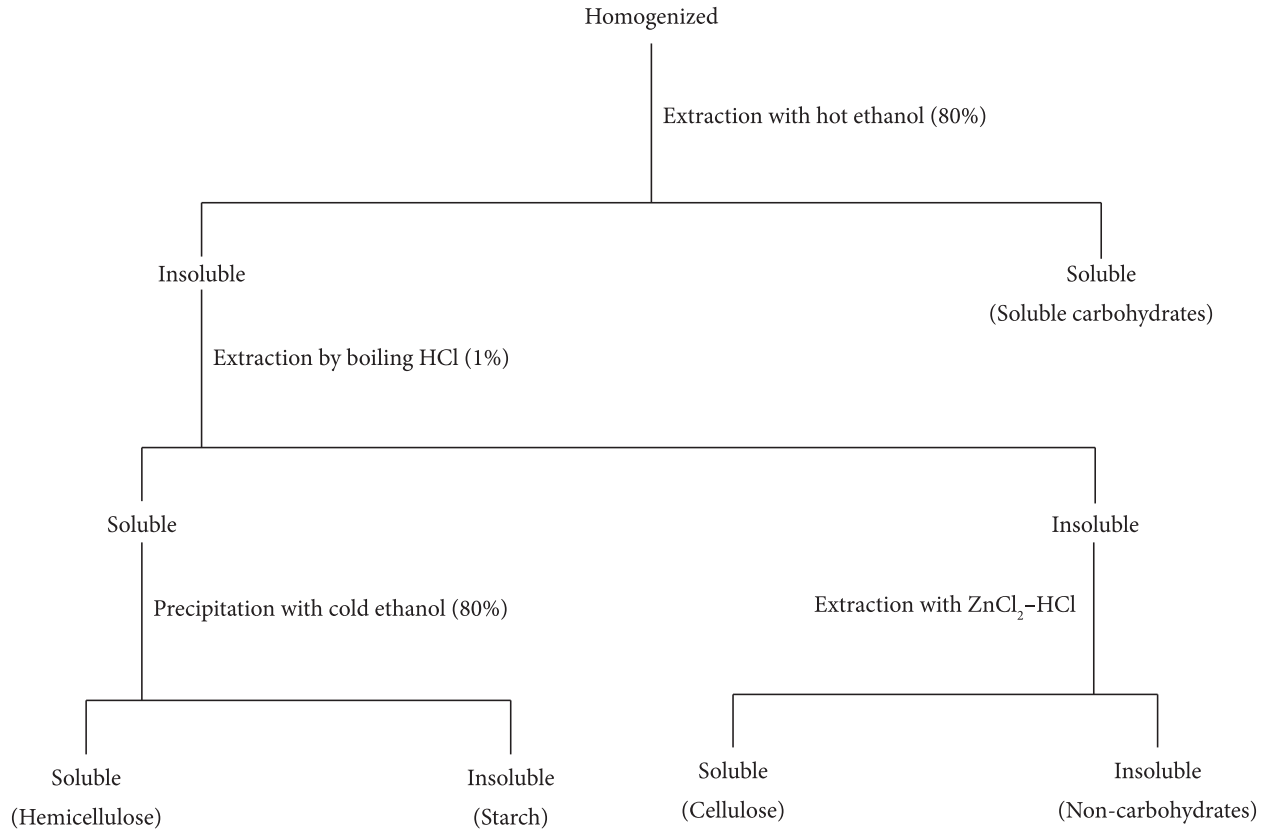

Figure 1. Fractionation of cell wall carbohydrates. Source: Terra, Valentin and Santos (1987).

Prospecção Química of DQI/UFLA using the pellet technique with 100 mg of potassium bromide (KBr) and 1 to 2 mg of dehydrated sample, which were pressed (50N/sqin) and analyzed in a FTS 3000 Excalibur Digilab spectrometer; Fourrier transform (resolution 8 cm^{-1} and 16 scans) and the spectra were registered in the range from 4,000 to 500 cm^{-1}.

3 Results and discussion

Table 1 shows the centesimal composition of guava cv. Pedro Sato. It presented high moisture, like most of the fruits, representing 83.6 g.100 g^{-1}.

Guava presented a low lipid level (0.34 g.100 g^{-1} fresh matter) because fruits rich in this are rare, as well as that of protein which was 0.77 g.100 g^{-1} fresh matter. The ash represented 0.36 g.100 g^{-1}. These results were found in the literature with some small variations (FRANCO, 2001; MENEZES; LAJOLO, 2001; PINHEIRO et al., 2002; PHILIPPI, 2002; PEREIRA et al., 2003; UNIVERSIDADE…, 2006).

The carbohydrates are the most abundant nutrients in nature, and they are the main source of energy for humans. They are found mainly in vegetables, cereals, roots, tubers, and fruits (SILVA; MURA, 2007). The glycidic fraction or non-nitrogenated extract (NNE) was determined by difference, which expresses the level of carbohydrates in the food approximately. However, the NNE level found in guava (12.38%) is within the percentage range of carbohydrates found in composition tables that vary from 9.5 to 17% (FRANCO, 2001; PINHEIRO et al., 2002; PHILIPPI, 2002; UNIVERSIDADE…, 2006).

Table 1. Centesimal composition of guava cv. Pedro Sato expressed as g.100 g^{-1} in the complete sample.*

Analysis	Average value (%)
Moisture	83.60 ± 0.17
Lipids	0.34 ± 0.05
Protein	0.77 ± 0.05
Soluble fiber	1.19 ± 0.11
Insoluble fiber	6.84 ± 0.04
Total Dietary fiber	8.03 ± 0.02
Ash	0.36 ± 0.03
Glycids (NNE)	6.90 ± 0.02

*Average of 3 repetitions ± standard deviation.

The crude fiber corresponds to the cellulose and lignin fraction of the food; therefore, it does not express the level of fiber of the foods in a complete way, representing 2.53% in this study, which is in agreement with results found by Philippi (2002).

The dietary fiber shown in Table 2 corresponds to compounds of vegetable origin that undergo hydrolysis in the human digestive tract. The fibers are divided into insoluble (cellulose, lignin, and hemicellulose) and soluble fibers (pectin, beta-glucans, gums, mucilages, etc.), according to their solubility (COZZOLINO, 2006; SILVA, 2000). The value of dietary fiber

Table 2. Fractionation and quantification of cell wall components of guava cv. Pedro Sato during ripening at room temperature.

Storage days	Soluble carbohydrates (%)	Hemicellulose (%)	Starch (%)	Cellulose (%)	Non-fractionated carbohydrates (%)	Total carbohydrates (Sum) (%)
0	7.19 ± 0.14	0.22 ± 0.02	0.14 ± 0.00	1.70 ± 0.10	0.30 ± 0.02	9.55
1	6.38 ± 0.22	0.15 ± 0.02	0.14 ± 0.00	1.80 ± 0.17	0.20 ± 0.01	8.67
2	6.74 ± 0.39	0.17 ± 0.01	0.16 ± 0.00	2.37 ± 0.04	0.23 ± 0.01	9.67
3	6.95 ± 0.26	0.22 ± 0.00	0.19 ± 0.00	1.63 ± 0.09	0.35 ± 0.01	9.34
4	7.59 ± 0.27	0.21 ± 0.00	0.19 ± 0.00	2.18 ± 0.12	0.39 ± 0.02	10.56
5	6.71 ± 0.23	0.18 ± 0.00	0.20 ± 0.01	1.87 ± 0.09	0.41 ± 0.02	9.37
6	6.96 ± 0.07	0.22 ± 0.01	0.20 ± 0.00	1.93 ± 0.09	0.37 ± 0.03	9.68
8	6.31 ± 0.33	0.18 ± 0.00	0.20 ± 0.00	1.48 ± 0.11	0.31 ± 0.00	8.48
General average	6.85	0.19	0.17	1.84	0.32	9.42

Data are an average of 3 repetitions ± standard deviation.

found was 8%, which is close to the values found in the literature (FRANCO, 2001; MENEZES; LAJOLO, 2001; PHILIPPI, 2002; UNIVERSIDADE…, 2006).

The results obtained in the physiochemical characterization of the guava cv. Pedro Sato, in a general, is close to the results of studies in the literature. Such characteristics can, however, present great variations in their components when compared with results of other studies due to the possible differences in the fruit variety, maturation stage at harvest, climatic conditions during the fruit development, and cultivation procedures (PEREIRA, 1995; CARVALHO, 1999; CARDOSO et al., 2002).

Table 2 presents the sugar fraction data. The soluble carbohydrates, hemicellulose, and total sugars did not present significant differences with ripening. Although there were no significant differences among those parameters, the fruit ripened faster. Their skin color changed (from green to yellow), and it became less firm in texture (data not shown).

The cellulose presented the best behavior, which can be explained by a quadratic equation, having an increase up to the 3rd day reaching a value of 2% and then decreasing to the end of the storage period reaching the value of 1.48%.

These results show that there are no significant differences in the sugars and carbohydrates of the guava fruits during ripening. Similar results were found by Jacomino (1999), Xisto (2002), Cardoso et al. (2002), and Linhares et al. (2007).

The fraction denominated "starch" had increasing linear behavior, rising from 0.14% at the time of harvest to 0.21% on the 8th day of ripening. However, the visual analysis of the cold ethanol precipitation, showed a transparent gel, different from the expected sediment for the starch (white sediment). A qualitative test was then conducted with Lugol's solution, and the result was negative (data not shown) suggesting the presence of another polysaccharide other than the starch. The fact of presenting a reaction with anthrone suggests the presence of sugar units different from the characteristic uronic acids of the pectins. The infrared spectroscopic analysis of that fraction (Figure 2b) showed similarities of functional groups with polygalacturonic acid (Sigma®) (Figure 2a) and with apple and commercial pectins, respectively (Figures 2c and d, FERTONANI, 2006) suggesting that this fraction is a typical pectin.

The absorption in the range between 1,000 and 2,000 cm^{-1} is the important area for identification of galacturonic acid functional groups since the free carboxyls absorb at approximately 1,620 cm^{-1} and the esterified at 1,750 cm^{-1}. The wide and strong absorption range between 2,400 and 3,600 cm^{-1} corresponds to the stretching of the hydroxyl groups related to the moisture of the pectin samples. The peaks in the range between 3,000 and 2,800 cm^{-1} are related to the C-H and CH3 bonds of the methyl ester groups (MONSOOR; KALAPATHY; PROCTOR, 2001)

The fractionation of the guava cv. Pedro Sato pulp sugars did not result in the expected value of 14.93% (total dietary fiber + glycids) presented in Table 1. The sum of the sugars in the fractions was approximately 9.42% (Table 2). That difference could be explained by the amount of pectin found in the fractions and not detected by the anthrone method although that fractionation is not the correct method for pectin extraction. Table 3 shows the amount of pectin in the fractions, which is overestimated by approximately 2.8% by the presence of sugars that interfere in the pectin analysis with carbazole (Figure 3). The same does not happen with the quantification of sugars with anthrone, which does not undrego interference by the pectin presence (Figure 4). Even with overestimated values, a total pectin level 7.77% (Table 3) was slightly higher than the expected value of 5.5%; that value is the difference between 14.93% (6.90% + 8.03% Table 1) and 9.42 (general average of the sugars total Table 2). If the correction of the pectin amount (7.77% Table 3) found in the fractions were corrected, the same value would be found (5.5%) and would be higher than the values found in the literature, which are around 2.4% (PAL; SELVARAJ, 1979; MOWLAH; ITOO, 1983; BULK; BABIKER; TINAY, 1997; CARVALHO, 1999, CARVALHO et al., 2001; GIANONNI, 2000; XISTO, 2002; LIMA, 2004; XISTO et al., 2004; VILA, 2007; LINHARES et al., 2007; MENDONÇA et al., 2007; OSHIRO et al., 2008).

Considering that the dietary fiber was 8.03% (Table 1) and that the sum of the cellulose and hemicellulose levels is 2.2% (Table 2), the pectin level among other polymers should be around 5.8%. That reasoning should be correct since the sum

Table 3. Quantification of pectin in the fracionated components of the cell wall of guava cv. Pedro Sato during ripening at room temperature.

Storage days	Soluble carbohydrates (%)	Hemicellulose (%)	Starch (%)	Cellulose (%)	Non-fractionated carbohydrates (%)	Total pectin (%)
Média geral	0.75	4.89	0.33	1.54	0.26	7.77

Data are an average of 3 repetitions ± standard deviation.

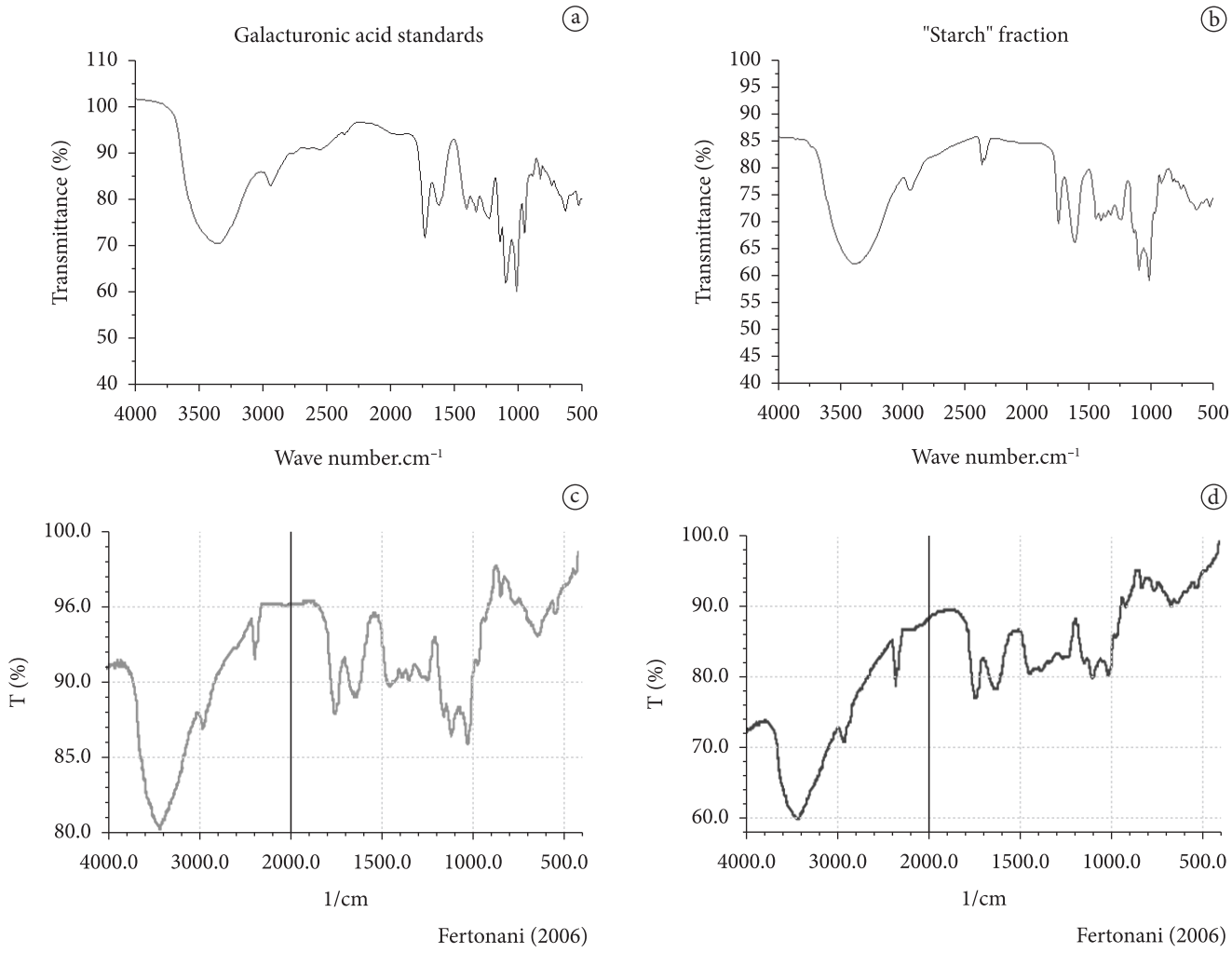

Figure 2. FT-IR Spectroscopic characterization of pectic substances. a) galacturonic acid standards; b) "Starch" fraction; c) Apple pectin; and d) Commercial pectin.

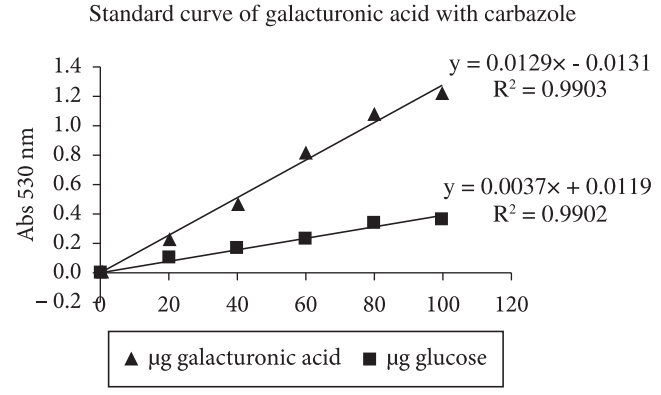

Figure 3. Standard curve of galacturonic acid with carbazole (▲) and standard curve of galacturonic acid with carbazole with substitution of galacturonic acid by glucose (▪).

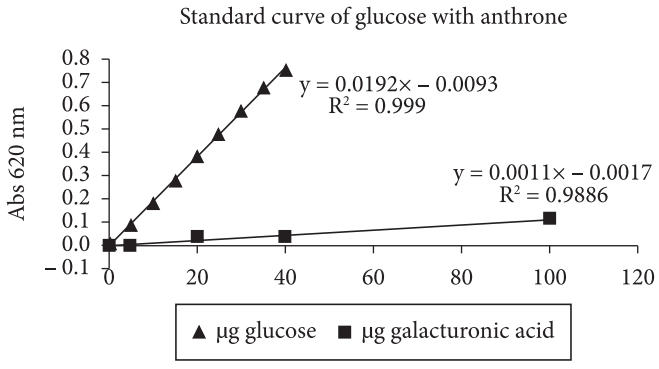

Figure 4. Standard curve of glucose with anthrone (▲) and standard curve of glucose with anthrone with substitution of glucose by galacturonic acid (▪).

of the total sugars, approximately 9% (Table 2), and the possible pectin level of 5.8% is equal to a total of 14.93% of sugars, which corresponds to the sum of 6.9% (NNE) + 8.03% (dietary fiber) (Table 1) resulting in 14.93% of total sugars.

Considering those conclusions as correct, the total pectin level determined in guava, around 2.5%, is quite underestimated, and the statements reported in the literature that the level of total pectin during the guava ripening can decrease, stay constant, or increase (PAL; SELVARAJ, 1979; MOWLAH; ITOO, 1983; BULK; BABIKER; TINAY, 1997; CARVALHO, 1999; GIANONNI, 2000; CARVALHO et al., 2001; XISTO, 2002; LIMA, 2004; XISTO et al., 2004; VILA, 2007; LINHARES et al., 2007; MENDONÇA et al., 2007; OSHIRO et al., 2008) demonstrate well the uncertainty of the results found for that substance.

4 Conclusion

There was no significant change in the sugars during guava ripening. The estimated pectin levels were more than twice those found in the literature thus explaining its possible responsibility for the firmness of the fruit.

Acknowledgements

The authors acknowledge the fellowship provided by CAPES and the Agrochemical Graduate Program.

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