

Hunter Color Dimensions, Sugar Content and Volatile Compounds in Pasteurized Yellow Passion Fruit Juice (*Passiflora edulis* var. *flavicarpa*) during Storage

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ABSTRACT

Changes in Hunter L, a and b values, glucose, fructose and sucrose contents, concentration of four volatile compounds (ethyl butyrate, ethyl caproate, hexyl butyrate and hexyl caproate) and furfural, were studied in yellow passion fruit juice (*Passiflora edulis* var. *flavicarpa*) pasteurized at 75°C/60s, 80°C/41s or 85°C/27s, during storage at room temperature (25±5°C) and refrigeration (5±1°C) for 120 days. While the sucrose content decreased, the glucose and fructose contents increased significantly over storage time. The Hunter L and b values behaved similarly, with a tendency to decrease over time, inversely to Hunter a value. Volatile compound concentrations also decreased over time, inversely to the furfural content. Pasteurization at 85°C/27s resulted minimum changes in the studied passion fruit characteristics, while that at 75°C/60s was the most harmful. Storage under refrigeration tended to keep the best quality characteristics of the juice.

Key words: Yellow passion fruit juice; passion fruit processing; storage; physical and chemical analysis, volatile compounds

INTRODUCTION

In the last ten years, there has been a great increase in the international consumption of tropical fruits. According to FAO, the market for tropical fruit juices, including passion fruit, is about US\$ 1 billion (Brasil, 2000). Passion stands out not only because of its exotic flavor but also because of its vitamin content. This consumption reflects a growing concern with more natural and healthy nutrition mainly in Europe and the United States (Bliska et al., 1994). The largest producers are Hawaii, Brazil, Colombia, Ecuador, Peru, Australia, Fiji Islands, Kenya, South Africa, Papua New Guinea, New Zealand, Venezuela, India, Sri

Lanka, Philippines and Taiwan. Brazil, Colombia, Ecuador and Peru supply the major part of the world market, and the United States and European countries are the main importers, with a growing demand for the product (Loeillet, 1995; Somagyi et al., 1996). In Brazil, the most cultivated varieties are the yellow passion fruit and the purple passion fruit. The yellow passion fruit (*Passiflora edulis* var. *flavicarpa*) is the most cultivated and has some advantages over the purple passion fruit (*edulis*); for example, larger fruits, greater yield, more attractive coloring and more acid juice. Studies have been carried out on the nutritional or microbiological characteristics of the juice but these lacks detailed information on

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the effects of processing and storage on the physical and chemical characteristics of the juice. Conservation of foodstuffs by thermal treatment aiming to increase the life, controlling the action of enzymes and microorganism activity may affect the physical and chemical characteristics of the juice, leading to variations in the sensorial and nutritional characteristics. Alterations in this sense are inevitable but the optimization of the process would decrease the problem considerably. Understanding the reasons leading the juice to modify its characteristics during thermal treatment and processing, along with the extension of these variations, may be used to limit or inhibit these processes and may be a determining factor for the success of a product on the market. The objective of the present study was to diagnose, by physical and chemical analyses, some alterations in the juice during its thermal treatment and storage.

MATERIAL AND METHODS

Juice processing

Yellow passion fruits (*Passiflora edulis* var. *flavicarpa*) from Minas Gerais State - Zona da Mata were purchased at the CEASA (wholesale fruit and vegetable market) in Belo Horizonte, and kept for four days in a cold chamber at $5\pm 1^{\circ}\text{C}$ and then processed. The experiment was carried out in a split plot design, with heat treatment in the plots carried out randomly at three levels ($85^{\circ}\text{C}/27\text{s}$, $80^{\circ}\text{C}/41\text{s}$, $75^{\circ}\text{C}/60\text{s}$) and storage time in the subplots at five levels (0, 30, 60, 90, and 120 days) kept either at room temperature (A) at $25\pm 5^{\circ}\text{C}$ or refrigerated (R) at $5\pm 1^{\circ}\text{C}$ for 120 days. There were three replications of the experiments.

Equipments used in the juice processing: a mechanical de-pulper composed by three stages (with two sieves of 0.5 and 0.3 mm mesh), a universal knife-type chopper with a cylindrical heater, a simple effect vacuum concentrator, a tubular (multitubes) pasteurizer, with 1500L/h capacity; and a semi-automatic filler, were supplied by Tecmint Company (Congonhal, Minas Gerais, Brazil).

Manually selected fruits were washed in a tank where they stayed under the action of chlorate water jets (5ppm active chloride) for at least 15 minutes. They were cut and de-pulped and sodium benzoate at a concentration of 500ppm was added to the juice before de-aeration in a vacuum concentrator at 750mmHg. The juice was treated

at three binomial time x temperature conditions, equivalent in microbial lethality to $75^{\circ}\text{C}/1.0\text{ min}$, ($z = 27.7^{\circ}\text{C}$) following the Bigelow model, based on the work of Tchango-Tchango et al. (1997). The juice was immediately packed in 500mL glass bottles, which were manually closed with metal lids, while the juice was still hot (hot-fill system). Physical and chemical analysis were carried out on the control sample (non-pasteurized juice) at zero time, and on the treatments, at zero time, 30, 60, 90 and 120 days. Microbiological analysis was carried out only at zero time.

Microbiological analysis

A commercial sterility test was performed. The bottles containing the juice were placed in a BOD chamber, model 347 (Fanem, São Paulo, Brazil) at 35°C and kept for 10 days and gas production and acidity were observed.

Reducing and non-reducing sugars

Reducing sugars (glucose and fructose) and non-reducing (sucrose) were determined by high performance liquid chromatography (HPLC). The samples were prepared diluting at the proportion of 1:10 with distilled water and centrifuged twice, at $3,833 \times g$ for 10 minutes, and at $77,600 \times g$ for 20 minutes. The samples were then frozen and kept at -30°C until analysis. After thawing, the samples were filtered in a polyethylene HV Millex syringe with a Durapore membrane (0.45 μm) (Waters-Milipore, Bedford, MA) and analyzed immediately.

Chromatographic analysis was carried out using a HP chromatograph 1050 Series with a refraction index detector HP 1047A. A 300mm x 7.8mm HP-87H column was used to separate the sugars. The mobile phase consisted of H_2SO_4 0,005N. Separations were carried at 60°C and flow rate of 0.7mL/min. Peaks were quantified by area measurement and identified by retention time of standards (Merck, Darmstadt, Germany). A 50 μL loop was used in the injection.

Color

The color was determined in a Colorquest II colorimeter, utilizing the Hunter Lab system, with direct reading of the L (luminosity), a (red intensity) and b (yellow intensity) values. The samples were diluted 1:10 with distilled water before analysis.

Volatile compound and furfural analysis

The following volatiles compounds: hexyl caproate (FEMA 2572), hexyl butyrate (FEMA 2568), ethyl caproate (FEMA 2439), ethyl butyrate (FEMA 2427) and furfural (FEMA 2489) were extracted from the samples by the solid phase microextraction technique (SPME) as described by Yang and Peppard (1994). A manual holder and fibers (100µm PDMS) were used (Supelco, Bellefonte, PA). The fibers were conditioned under helium at 290°C for 4 hours prior to use. Between uses, fibers were kept sealed by inserting the tip of the SPME needle into a small piece of septum to prevent accidental contamination. Samples were prepared by centrifugation of 30mL of diluted juice (1:10) at 3.833 x g for 10 minutes, to eliminate pulp. 20mL of liquid were transferred to a 25mL glass recipient with a silicon lid. The SPME fiber needle was inserted through the silicon lid and immersed in the juice or on headspace. The fiber was equilibrated for 2, 5, 10, 15 or 20 minutes to optimize the extraction time. The fiber was retracted, removed from the vial and placed immediately into the injector port of the GC at 200, 210, 220 or 230°C. Injection was accomplished by extending the fiber in the heated inlet port for 1, 2, 3, 4, or 5 minutes to optimize the time of desorption, while the injector operated in the splitless mode for 2 minutes. An additional time of 3 minutes of exposure in the injector port allowed the fiber to be cleaned of any compound that could not have been desorbed in the splitless mode time.

Analytical separations were performed in a Chrompack CP-SIL 88 capillary column (50m x 0.25mm) on a Varian model 3400 gas chromatograph equipped with a split/splitless injector at 230°C, splitless for 2 minutes and a flame ionization detector operating at 270°C. The oven temperature was programmed from 50°C for 4 minutes, to 220°C at 4.5°C/min, plus 18 minutes. Helium was used as carrier gas at a linear velocity of 36 cm/s. Identification of the compounds was made by the co-elution technique and by retention time of synthetic standards of the selected compounds (IFF Essências e Fragrâncias, São Paulo). The quantification was made by the external standard method. Chromatographic data were processed by the Star Chromatography Workstation program, version 4.5.

The statistical analysis of the color and sugar results was carried out by the SAS statistical

program, 6.12 version (Statistical Analysis System - SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Microbiological analysis

According to Fang et al. (1986), 75°C/40s was sufficient to ensure the microbiological quality of passion fruit juice, whether stored at room temperature or refrigerated. However, when assessing the microbiological quality of the stored samples by the commercial sterility test, the 75°C/60s treatment was enough to decrease the microbiological contamination. The bottles with this treatment, stored for 10 days in the BOD chamber, burst on the 7th or 8th days, or had an increase in acidity. By direct observation on microscope slides, yeasts and bacterial forms were observed in all the repetitions of the juice pasteurized at 75°C/60s, which indicated that the binomial used was not sufficient to guarantee the microbiological quality of the juice and that probably the problem was not caused by recontamination after pasteurization as all the replications showed contamination.

Thus, those samples treated at 75°C/60s and kept at room temperature (A) were eliminated for safety reasons. However, according to Foyet and Tchango-Tchango (1994), acidity in products such as passion fruit juice inhibited proliferation of pathogenic microorganisms. These products did not contain pathogenic *Escherichia coli*, *Streptococcus* or *Staphylococcus*, but might have non-pathogenic fungi, yeasts or lactic bacterias. Thus, the bottles treated at 75°C/60s, which were refrigerated remained in the experiment and no further microbiological alterations in the juice quality were observed during storage.

Physical and chemical analysis

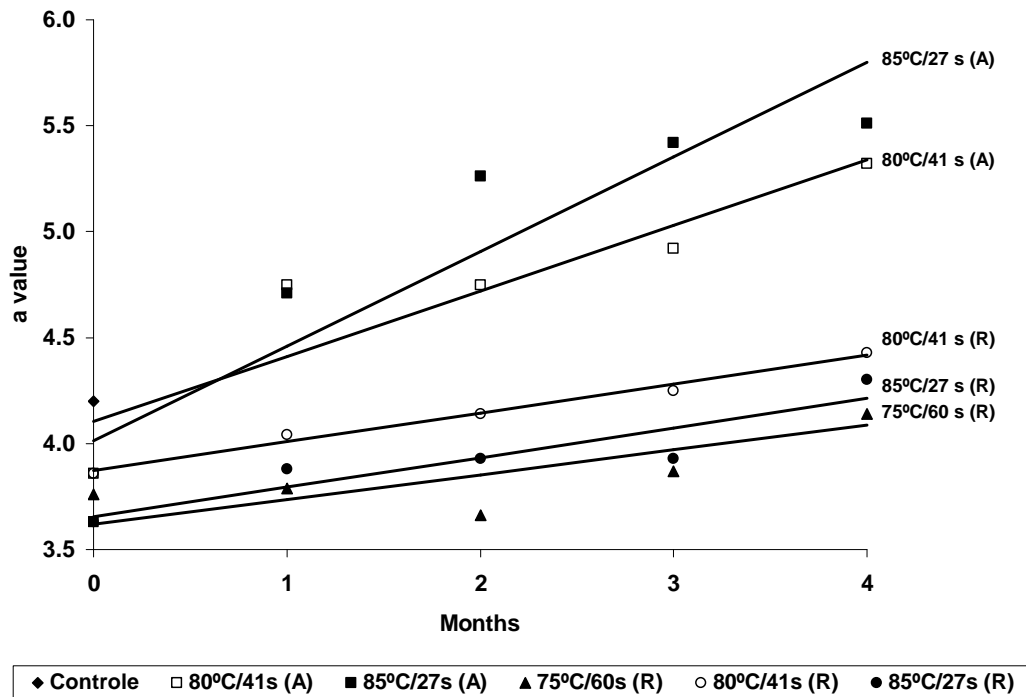
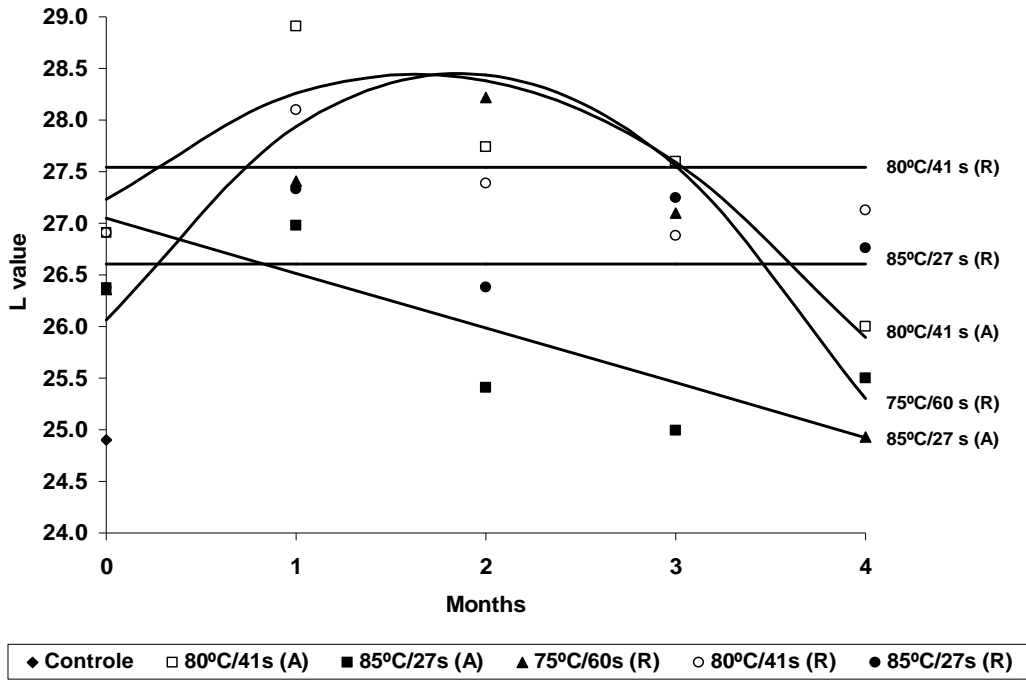
Analysis of variance was carried out to test interaction and treatments effects. Regression models were studied for all interactions

Color

Fig. 1 shows the behavior of the L (Hunter luminosity), a (red intensity) and b (yellow intensity) values in the control juice at zero time and for the processed ones during the storage period. Each treatment caused a different behavior in the juice L values during storage. However, all values were higher than that of the control. The

juice pasteurized at 80°C/41s and at 85°C/27s and kept under refrigeration did not vary significantly during storage ($P>0.05$) and the values of the later were closer to that of the control. Fang et al. (1986) working with passion fruit juice, observed that in all treatments, the juices showed L values

higher (lighter color) than the control juice, and that those values decreased to values near to that of the control during storage. Samples submitted to the same heat treatment and kept refrigerated had higher L values than those kept at room temperature, which agreed with our observations.



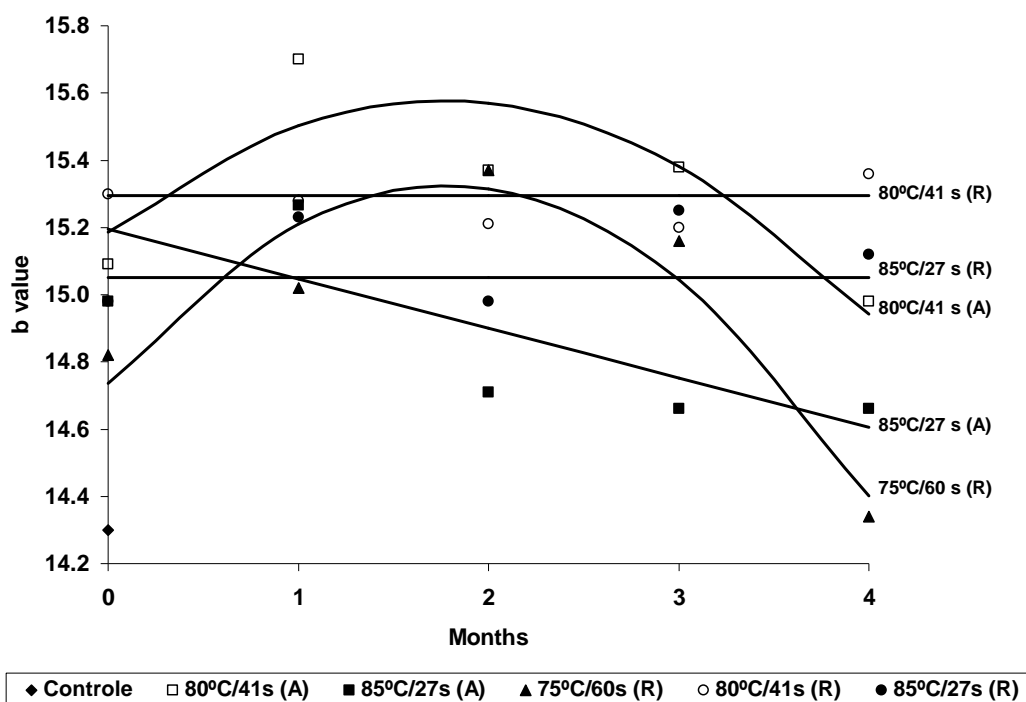


Figure 1 - Behavior of the L (luminosity) a (red intensity) and b (yellow intensity) values during storage of the juices.

According to Matsuura (1994), initial increase in luminosity could be caused by the destruction of the carotenoid structure giving a paler color. Along the time, other compounds, resulting mainly from the non-enzymatic browning reactions, like Maillard's (Remacha et al., 1992), and oxidation of ascorbic acid or precipitation of the pigments (Sistrunk and Cash, 1974), contributed to reduction of the luminosity, giving a darker appearance to the juice.

The unexpected behavior of the L value observed in the juices treated at 80°C/41s (A) and 75°C/60s (R) with increase at the beginning of storage and later decrease was observed by Marcy et al. (1989) in orange juice kept at 22°C, but it was not observed in the treatments kept at a higher temperature (30°C), where the decrease in L values was progressive over time.

The intensity of the red color (a value) was lower in the processed juices than in the control. Similar small increase was observed in all treatments during storage. However, juices treated at 80°C/41s and 85°C/27s and kept at room temperature showed the largest variation, while that treated at 80°C/41s and kept refrigerated had smallest variation than the control. According to

Fang et al. (1986), beta-carotene partially lost its red color after heat treatment, probably because it changed to the *cis* form (Coultate, 1984; Chen et al., 1995). With time, the formation of other compounds, as already discussed, increased the juice dark color, contributing to the red intensity.

The b value (yellow intensity) behaved similarly to the L value, except that there was higher difference among the treatments 75°C/60s (R) and 80°C/41s (A), which was not observed in the L value. With time, the increase in the intensity of the dark colors, such as the red one decreased the yellow intensity, contributing negatively to the maintenance of the characteristic color of the juice.

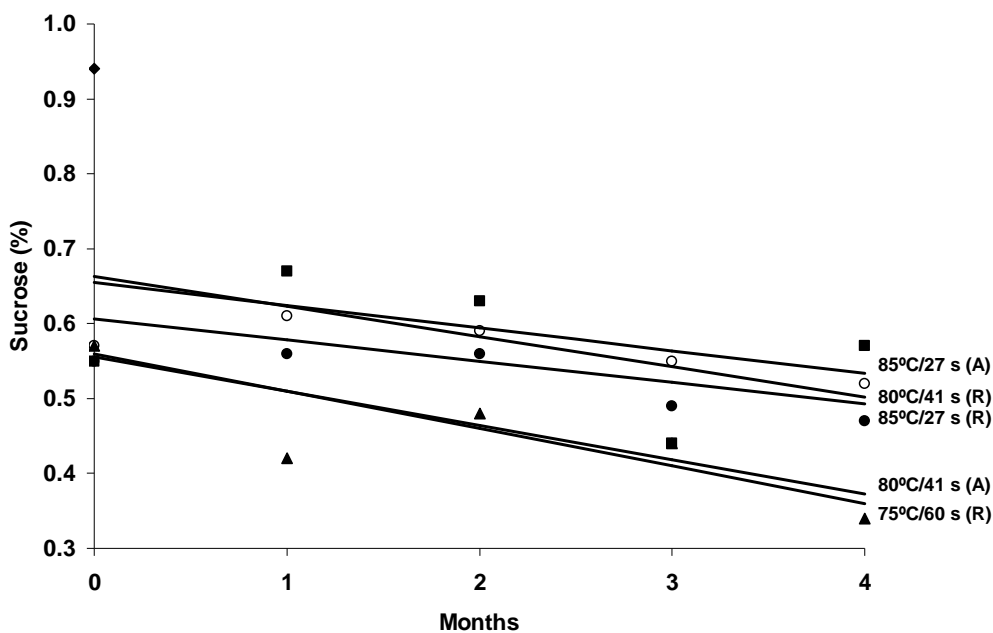
Looking at these results, it was possible to conclude that the juice pasteurized at 85°C/27s and kept under refrigeration had the smaller variation compared to the control, for the Hunter dimensions.

Reducing and non-reducing sugars

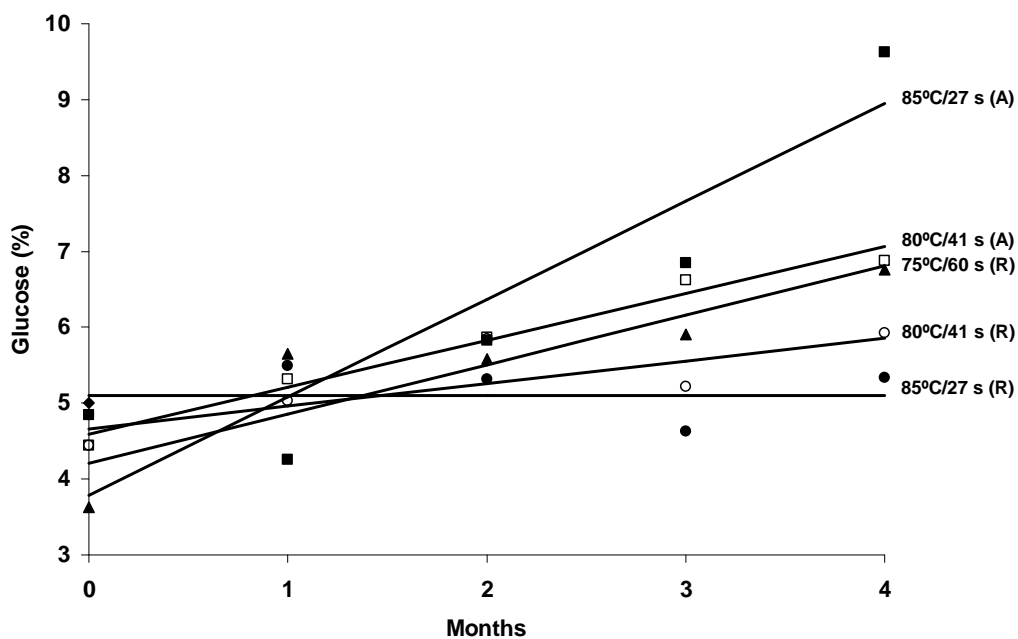
Fig. 2 shows the behavior of sucrose, glucose and fructose for the juices, compared to the control at zero time during the storage period. Sucrose was more stable in juices treated at 85°C/27s (R).

Juices treated at 80°C/41s (A) and 75°C/60s (R) showed more hydrolysis. These results were similar to those found by Fang et al. (1986), Dalal and Salunkhe (1964) and Ewaidah (1988) where the concentration of reducing sugars (fructose and glucose) increased over time while that of sucrose decreased. The increase in the reducing sugars

concentration was higher in the juices pasteurized at higher temperatures and kept at room temperature. Otherwise, for those kept refrigerated, the increase in the reducing sugars concentration was higher in the juices pasteurized at lower temperatures.



◆ Controle ■ 80°C/41s (A) ▲ 85°C/27s (A) ▲ 75°C/60s (R) ○ 80°C/41s (R) ● 85°C/27s (R)



◆ Controle ◻ 80°C/41s (A) ■ 85°C/27s (A) ▲ 75°C/60s (R) ○ 80°C/41s (R) ● 85°C/27s (R)

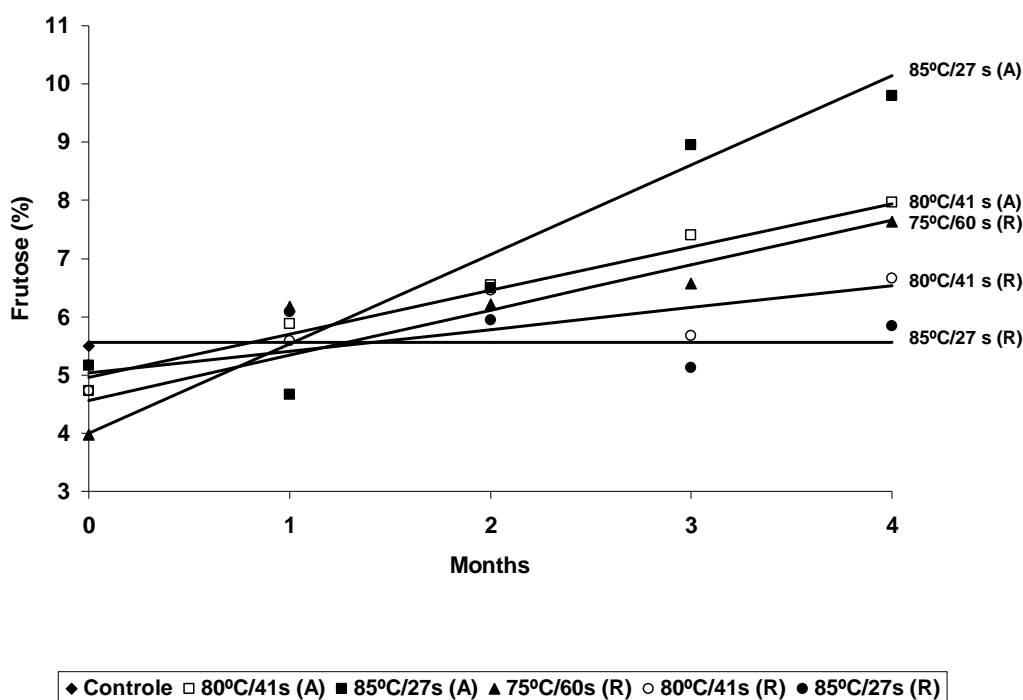


Figure 2 - Sucrose, glucose and fructose behavior during storage, in the pasteurized juices compared to the control at zero time.

According to Fang et al. (1986), as the passion fruit juice has high concentration of organic acids, storage at room temperature, over time causes higher conversion of sucrose to reducing sugars than storage at refrigeration temperature, as long as the juice has been pasteurized correctly.

It is interesting to note that for the same time/temperature binomial, the variations in the reducing sugar concentration were exactly opposite when the juices were kept at different storage temperatures. For the juice stored at room temperature, the high was the pasteurization temperature, the great the increase in the concentration for these sugars, but the effect was opposite to that stored under refrigeration. This showed that the variations depended on storage temperature and not in the pasteurization binomial.

Volatile compounds analysis

Preliminary observations indicated that the combination which best extracted the volatile compounds selected, bearing in mind the quantity extracted and the non-retention of compounds after desorption of the fiber, was: extraction time of 20 minutes in immersion; desorption time of 5 minutes; and desorption temperature of 230°C. The

procedure allowed for reproducible, quantitative transfer of the analytes into the injector port of the GC and was used for the volatile compounds extraction.

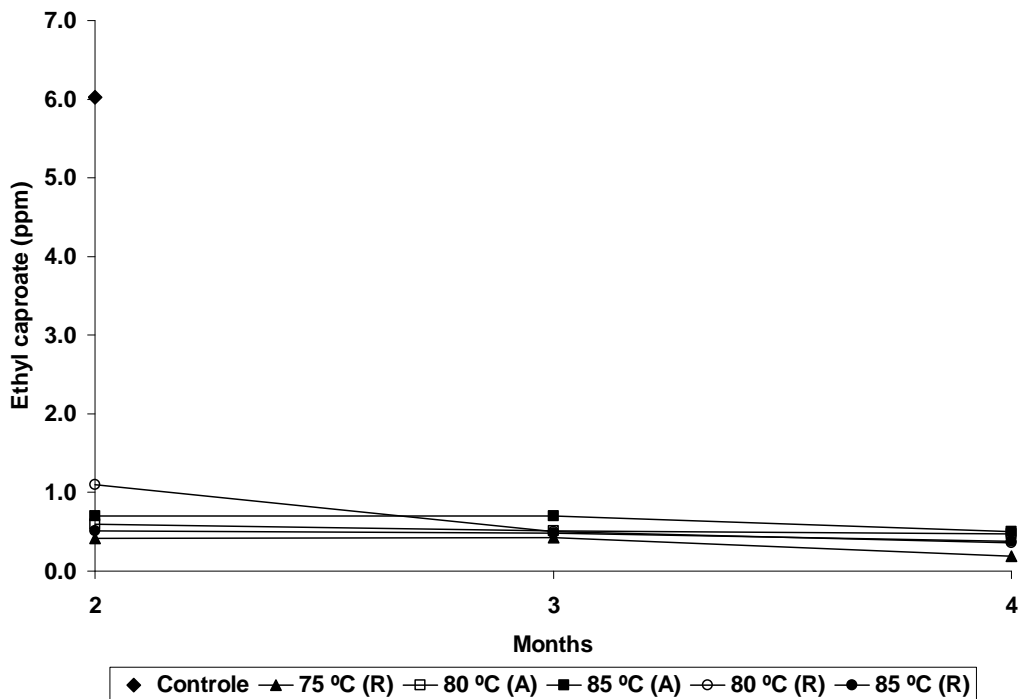
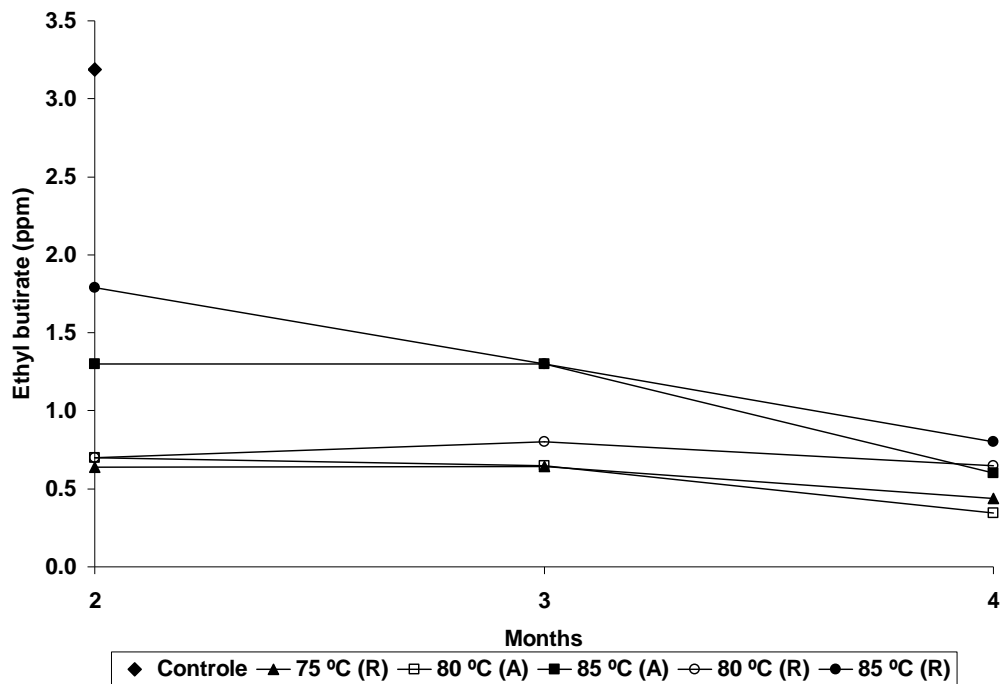
Fig. 3 shows the behavior of each of the volatile compound found at 2, 3 and 4 months of storage. The concentrations showed that the predominating compound in the passion fruit juice, found at 2 months storage, was ethyl caproate (35.0%), followed by hexyl caproate (32.6%), hexyl butirate (19.8%) and ethyl butirate (12.6%). Although the relative concentrations of these compounds was different from many authors and different varieties (Hiu and Scheuer, 1969; Winter and Kloti, 1972; Huet, 1973; Chen et al., 1982; Engel and Tressl, 1983; Narain and Bora, 1992), the results found in this study were similar to those obtained by Winter and Kloti (1972), where ethyl caproate was the constituent found at the greatest concentration.

Regarding the concentrations of volatile compounds, has been reported that juice centrifuging could cause losses of up to 30% (Kuo et al., 1985). In this study, centrifuging was used in sample preparation to analyze volatile compounds, which might have resulted in some losses and underestimating the real values.

It was found that the loss of the esters in the pasteurized passion fruit juice was less pronounced in the samples kept refrigerated, as also found by Fang et al. (1986).

Compared to the fresh juice (control), ethyl caproate was the compound that showed the

maximum decrease during storage. This indicated that this was more sensitive to heat treatment and storage, and that the losses could occur in compounds in high concentration.



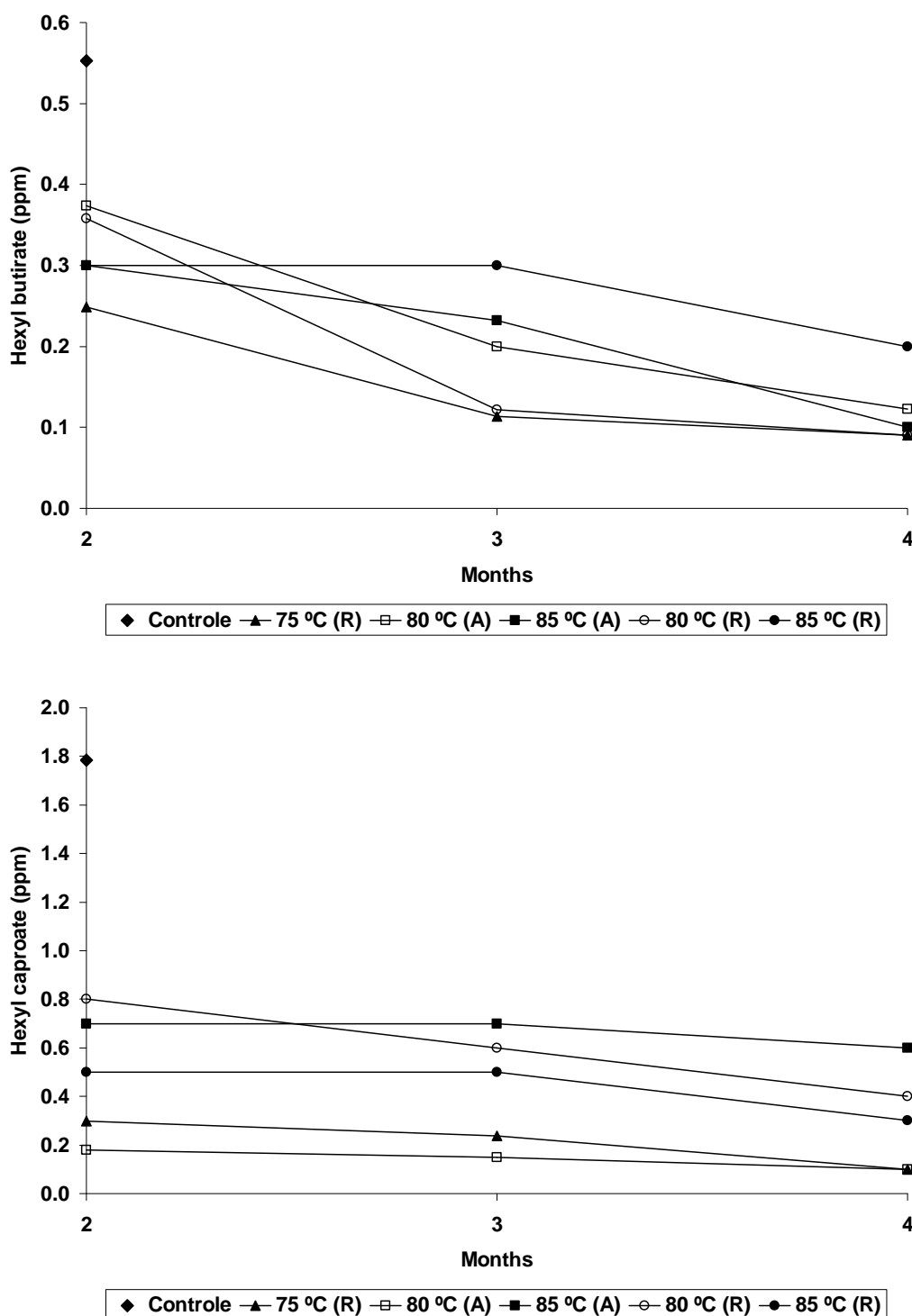


Figure 3 - Volatile compound concentration during the storage at 2, 3 and 4 months.

Results for the losses of the four volatiles compounds analyzed showed that the treatment at 85°C/27s (A) caused lowest losses, followed by treatments at 85°C/27s (R), 80°C/41s (R), 80°C/41s (A), and 75°C/60s (R) (Table 1). The losses were

higher than those observed by CUO (1982) who found that about 50% of the esters were lost in the passion fruit juice which had been pasteurized at 80°C/60s and kept frozen. According to Fang et al. (1986), the aroma of passion fruit juice easily

destroyed by heat. We found that the refrigerated storage and pasteurization at higher temperature had the lowest losses, as also observed by Fang et al. (1986). It was also noted that of the compounds studied, the smallest losses over time were observed on hexyl butirate followed by hexyl caproate, ethyl butirate and ethyl caproate (Table 1). This was not in agreement with Fang et al. (1986), who observed that the storage temperature had a more pronounced effect in the concentration of ethyl butirate and hexyl butirate. The concentration of these compounds for different treatments (Fig. 3) tended to close values during storage. This suggested that long periods of

storage were more damaging to these compounds than the pasteurization time/temperature binomial. Fang et al. (1991) observed that passion fruit juice treated with pectic enzymes suffered a 10% loss in volatile compounds.

The literature shows that higher temperatures than those used in this study could be necessary to inactivate pectin esterase. Amstalden and Montgomery (1995) noted that this enzyme showed activity after heat treatment at 60°C and 70°C for 10 minutes in orange juices. Sio et al. (1995) found residual activity after treatment at 85°C for more than 20 minutes in tomato juice.

Table 1 - Losses in the volatile concentration calculated at the final time storage (%)

	75°C (R)	80°C (A)	80°C (R)	85°C (A)	85°C (R)	Av**
EB	86.2	89.2	79.7	79.2	79.2	80.5
EC	96.9	92.2	94.1	92.6	92.6	93.9
HB	82.8	77.9	79.0	66.1	66.1	74.9
HC	91.5	95.7	71.3	60.4	60.4	80.1
Av*	90.4	88.8	81.0	74.6	79.4	

Av*: Mean variation of total volatile compounds as related to control in each treatment

Av**: Mean variation of each volatile compound as related to control in each treatment; EB: ethyl butirate; EC: ethyl caproate; HB: hexyl butirate; HC: hexyl caproate.

This indicated that the action of some enzymes could have contributed to aroma loss in the juice pasteurized at low temperatures or stored at room temperature.

Furfural behavior

The amount of furfural found in fresh juice (0.21ppm) was in agreement with other researchers who observed ranges from <0.1ppm to 0.7ppm (Winter and Kloti, 1972 and Engel and Tressl, 1983, respectively). Unlike the volatile compounds analyzed, there was an increase in furfural concentration during juice storage for all treatments (Fig. 4). This behavior was observed in orange juice by several authors (Nagy and Dinsmore, 1974; Dinsmore and Nagy, 1972; Mijares et al. 1986). In our studies, the lowest variation was noted in the juice treated at 85°C/27s (R) at (3.7% - from 0.27ppm to 0.28ppm). On the other hand, in the juice treated at 85°C/27s (R), the concentration increased from 0.29ppm to 0.37ppm, which was the highest variation observed (27.6%). The juices kept at refrigerated temperature had a less pronounced variations, (Kanner et al., 1982),

who showed that the critical storage temperature for orange juice seemed to be 12°C.

Fang et al. (1986) did not observe a significant increase in furfural in pasteurized passion fruit juice treated at temperatures varying from 75°C to 95°C and stored for four months at room temperature. However, the concentrations were always higher than those found in control juice. According to Morton and Macleod (1990), heat treatment during juice processing might produce furfural from sugars. However, ascorbic acid seemed to be one of the most important precursors of furfural in citrus juices (Nagy and Randall, 1973; Solomon and Svanberg, 1995). According to Luh (1971), passion fruit juice is an excellent source of ascorbic acid, and its stability is comparable to that of the citrus juices (Ross and Chang, 1958, quoted by Luh, 1971). Thus, the furfural might have originated from the decomposition of ascorbic acid and glucose, which had increased its concentration over time.

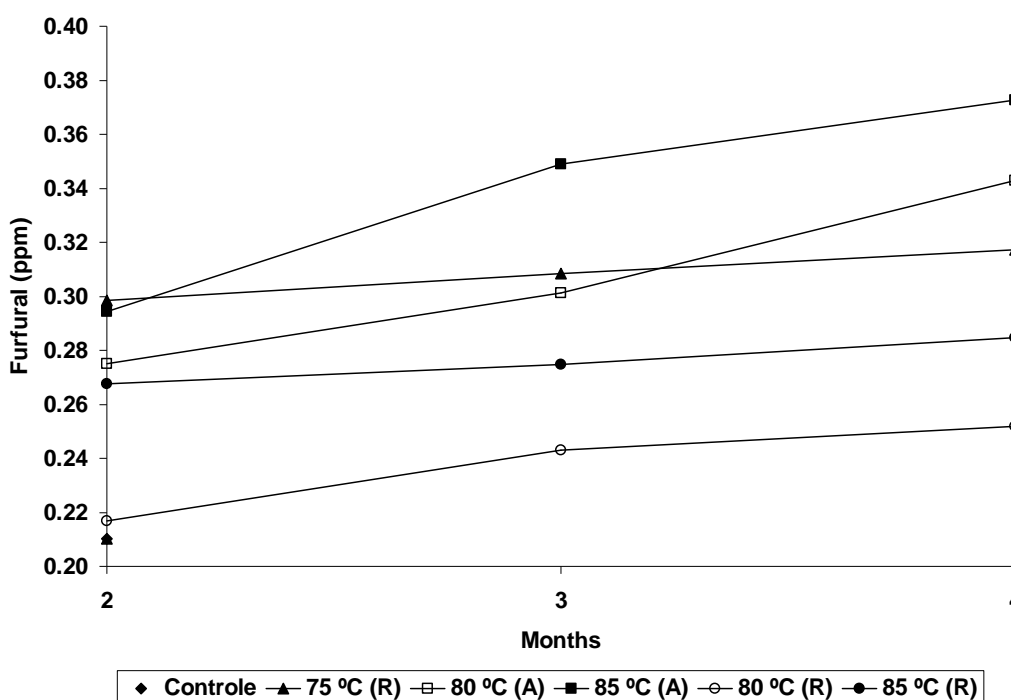


Figure 4 - Furfural concentration at 2, 3, and 5 months.

Solomon and Svanberg (1995) observed that ascorbic acid was decomposed to furfural in orange juice during processing. Furfural might polymerize and, as an active aldehyde, combine with amino acids (Maillard reactions) and contribute to darkening of orange juices. In addition, the rise in concentration of furfural in citrus juices is associated with loss of flavor (Fang et al., 1986; Kaane et al., 1988; Blair, 1964).

ACKNOWLEDGEMENTS

We thank FAPEMIG, CAPES and CNPq for scholarships/grants to the first author, and to the Escola Agrotécnica Federal de Rio Pomba, Minas Gerais, for the availability of their juice processing line.

RESUMO

Foi estudada a variação dos valores "L", "a" e "b" do sistema de Hunter, dos teores de glicose, frutose e sacarose, e da concentração de quatro compostos voláteis (butirato de etila, caproato de etila, butirato de hexila e caproato de hexila) e

furfural, em suco de maracujá-amarelo (*Passiflora edulis* var. *flavicarpa*) submetido à pasteurização (75°C/60 s, 80°C/41 s e 85°C/27 s), durante o armazenamento a temperatura ambiente (25±5°C) e refrigerada (5±1°C) por 120 dias. Enquanto os teores de sacarose diminuíram, aqueles de glicose e frutose aumentaram significativamente. Os valores "L" e "b" apresentaram comportamento semelhante, com tendência a diminuir ao longo do tempo, inversamente ao valor "a". As concentrações dos compostos voláteis também diminuíram, exceto para o furfural. A pasteurização a 85°C/27 s proporcionou as menores alterações nas características estudadas, enquanto aquela à 75°C/60 s foi a mais prejudicial. O armazenamento sob refrigeração apresentou melhor tendência na manutenção das características.

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Received: April 15, 2002;
Revised: August 19, 2002;
Accepted: November 07, 2002.