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Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice

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Abstract

Changes in carotenoid pigment content of Brazilian Valencia orange juices due to thermal pasteurization and concentration were studied. Total carotenoid pigment content loss was not significant after thermal pasteurization and concentration. However, thermal effects on carotenoid pigment contents, especially violaxanthin and lutein, were clearly observed and significant ($P < 0.05$). Pasteurization reduced the content of violaxanthin by 38% and lutein by 20%. The concentration process resulted in loss of lutein (17%). With the loss of lutein, β-cryptoxanthin became the major carotenoid in the pasteurized and concentrated juices. The provitamin A content of the juice (β-carotene, α-carotene and β-cryptoxanthin) and the amount of zeaxanthin, which are considered to be active against age-related macular degeneration and cataracts, did not significantly decrease after pasteurization and concentration.

Keywords: Pasteurization; Concentration; Carotenoids; Valencia orange juice

1. Introduction

Carotenoids are one of the main classes of natural pigments and their distribution in the plant kingdom is extremely wide (Britton, 1995; Meléndez-Martinez, Vicario, & Heredia, 2003). They present structural diversity and numerous important functions for human health. Some carotenoids are provitamins A (β-carotene, α-carotene and β-cryptoxanthin), and others, such as zeaxanthin and lutein, are active against age-related macular degeneration and cataracts.

The carotenoids, when isolated from vegetable tissues, are unstable in the presence of light, heat, acids and oxygen. Depending on the severity of the thermal treatments used in the processing of foods, isomerization and oxidative degradation of carotenoids can be induced (Rodriguez-Amaya, 1999; Subagio & Morita, 2001).

Citrus fruits are a rich source of carotenoids, especially orange juice, with the largest number found in any fruit (Britton, 1997; Rodriguez-Amaya, 1999; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003). The importance of carotenoids in juice colour, along with the growing interest in these pigments due to their health benefits, has stimulated their analysis. Orange juices are probably the most recognized and globally accepted fruit juices. Among the sweet oranges, the variety Valencia is highly valued for its quality (Gross, Gabai, & Lifshitz, 1972).

Pasteurization is important to the stability of citrus juice during transportation and marketing. Carotenoids are generally stable to heat treatments, such as blanching, cooking, and canning. However, the stability of carotenoids in food varies greatly (Lee & Coates, 2003).

Brazil is the largest world producer of oranges and of concentrated orange juice. As carotenoids are unstable at high temperatures, the study of the consequences of thermal pasteurization and concentration on the carotenoid composition of orange juice becomes of great importance. This study aimed to evaluate the effect of thermal

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pasteurization and concentration on the carotenoid composition of Brazilian Valencia orange juice.

2. Materials and methods

2.1. Materials

Five lots of fresh, pasteurized and concentrated Valencia orange juices were supplied by the citrus industry in the municipal district of Araraquara (São Paulo, Brazil) from the 2002 season. Before analysis, pasteurized and concentrated orange juices were diluted to the same °Brix as the fresh juice. All samples were analysed in triplicate.

2.2. Thermal treatment

Thermal pasteurization was carried out at between 95 and 105 °C for 10 s. The concentration process was carried out until the orange juice reached 66° Brix to 20 °C, and the juices were cooled at 10 °C.

2.3. Methods

2.3.1. Chemical analysis

The total soluble solids (in °Brix) were analyzed by the official method (AOAC, 1990).

2.3.2. Extraction and saponification of carotenoids

Fresh, pasteurized and concentrated Valencia orange juices were extracted and saponified as described in our previous work (Gama & Sylos, 2005), in agreement with the method of Rodriguez-Amaya (1999). This involved extraction of the carotenoids with cold acetone, partition to petroleum ether, and saponification with methanolic potassium hydroxide (10%, w/v). All extracts were subjected to saponification, which resulted in the conversion of carotenol esters to their parent hydroxycarotenoids (Humphries & Khachik, 2003). The traces of alkali were removed by washing with water. The extracts were concentrated at less than 35 °C in a rotary evaporator and dried under nitrogen.

2.3.3. Carotenoid quantification by HPLC

The HPLC system (Shimadzu Corporation) was equipped with a solvent delivery module LC-10AT VP and a UV–Visible photodiode array detector SPD-M 10A VP. All data were processed using software Class VP (Version 5.03). Detection was at the wavelengths of maximum absorption (max plot). A C_{18} column (Shimadzu Shimpack CLC (M), 5 μm, 4.6 × 250 mm) was used with acetonitrile:methanol:ethyl acetate as the mobile phase, with the following gradient: 0–25 min (99:1:0), 30 min (60:10:30), 55 min (60:10:30) and 58 min (99:1:0) at a flow rate of 0.7 ml min^{-1}. All solvents contained 0.05% triethylamine. Immediately before injection, the samples and the standard solutions were redissolved in HPLC grade acetone and filtered with a 0.22 μm PTFE syringe filter.

Identification of the carotenoids involved the combined use of their retention times, diode array spectral characteristics, and relative elution order compared to standards and literature values.

2.3.4. Isolation and purification of standards

The isolation and purification of carotenoids standards was according to Kimura and Rodriguez-Amaya (2002). α-Carotene and β-carotene were obtained from carrot; violaxanthin, lutein and zeaxanthin from cabbage–butter, lettuce and watercress; ζ-carotene from passion fruit juice; β-cryptoxanthin from orange juice.

The extraction and saponification procedures were described previously. The pigments were separated on an MgO:Celite column (1:1, activated for 2 h at 120 °C), adjusting the mobile phase, not to separate all the carotenoids present, but to isolate the desired carotenoids as quickly and efficiently as possible. Average purity of the isolated carotenoids was 99%, 91%, 95%, 98%, 97%, 99% and 99% for violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, ζ-carotene, α-carotene and β-carotene, respectively.

2.3.5. Construction of the standard curves

The concentration ranges used in the construction of calibration curves were 0.10–1.20 μg/ml for α-carotene, 5.14–10.29 μg/ml for β-carotene, 1.04–6.14 μg/ml for ζ-carotene, 0.25–3.94 μg/ml for β-cryptoxanthin, 0.20–1.56 μg/ml for zeaxanthin, 0.24–3.16 μg/ml for lutein and 0.11–0.53 μg/ml for violaxanthin.

2.4. Statistical analysis

The results were submitted to analysis of variance and least significant differences (Tukey test), with significance defined as $P < 0.05$.

3. Results and discussion

The major carotenoids, which are responsible for the colour of fresh, pasteurized and concentrated Valencia orange juices (Lee & Castle, 2001), were measured by reversed phase HPLC (Fig. 1) and presented in Table 1. The elution order of the major carotenoids was: (i) epoxycarotenoids (auroxanthin, anteraxanthin, violaxanthin, mutatoxanthin, peaks 1, 2, 3 and 4), (ii) hydroxycarotenoids (lutein, zeaxanthin, α-cryptoxanthin and β-cryptoxanthin, peaks 6, 7, 9 and 10) and (iii) carotenes (ζ-carotene, α-carotene and β-carotene, peaks 11, 12 and 13). Peaks 5 and 8 were not identified.

Although, the qualitative carotenoid composition of fresh, pasteurized and concentrated orange juices obtained in our work was similar, the quantitative concentrations determined by HPLC were different. The major carotenoids in all juices were lutein, β-cryptoxanthin and zeaxanthin. In the fresh orange juice, the principal carotenoid was lutein (23%), β-cryptoxanthin (21%) and zeaxanthin (20%). Violaxanthin, ζ-carotene, β-carotene and α-carotene

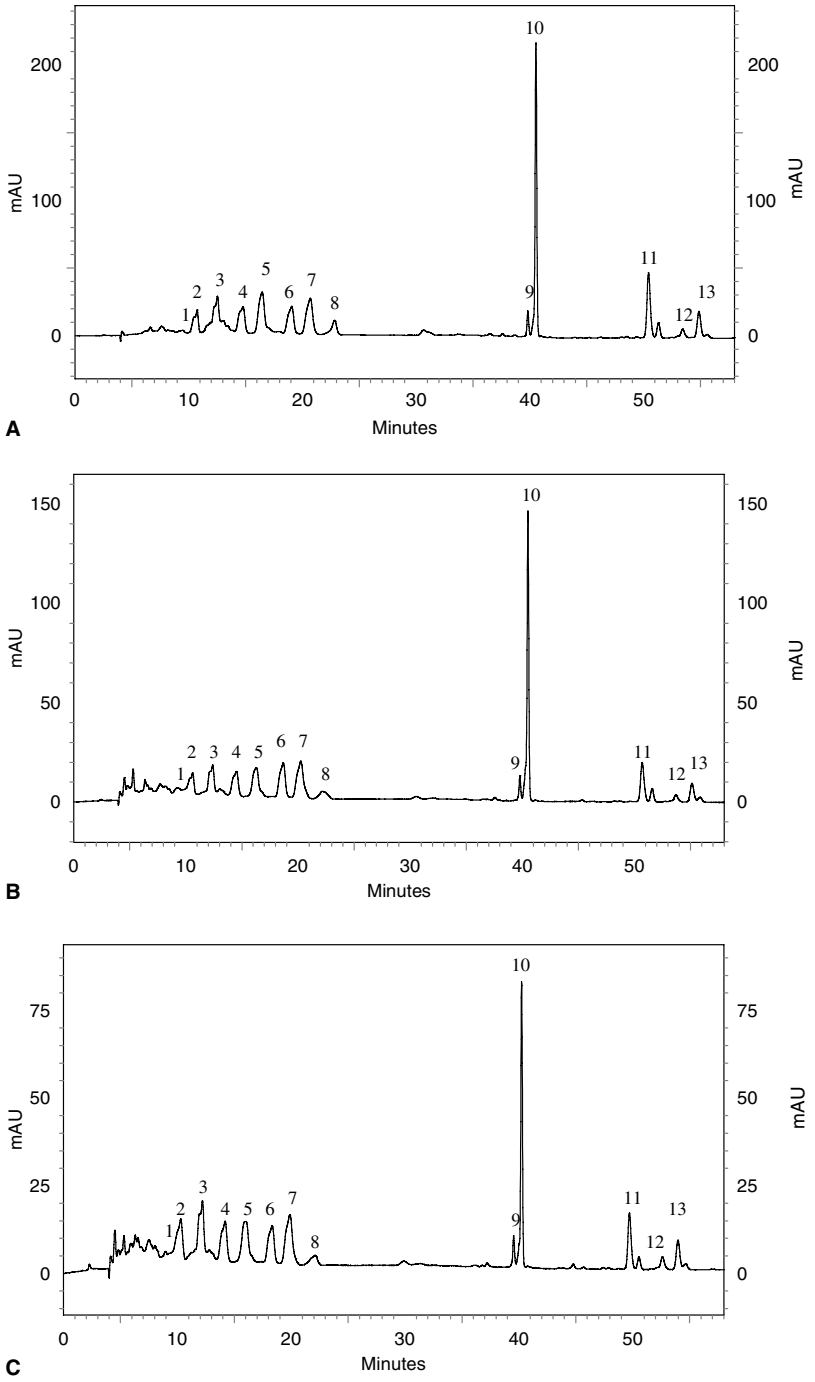

Fig. 1. HPLC chromatograms of carotenoids of fresh (A), pasteurized (B) and concentrated (C) Brazilian Valencia orange juices. Chromatographic conditions cited in the text.

represented 11%, 10%, 8% and 7%, respectively, of the total carotenoids. In pasteurized and concentrated orange juices, the major carotenoids were β-cryptoxanthin (25% and 24%), lutein (21% and 23%) and zeaxanthin (21% and 18%), respectively.

Total carotenoid content was 12.0 ± 6.7 mg/l (means $\pm$ standard deviation, $n = 5$) for fresh juices. In pasteurized juices, total pigment content decreased to 10.40 ± 6.90 mg/l, which is about 13% loss, and, in concentrated juices, total content decreased to 9.90 ± 5.30 mg/l, which is about 18% loss. However, these losses were not significant. Thermal pasteurization resulted in loss of violaxanthin (38%), lutein (20%), ζ-carotene (14%), α-carotene (13%), β-carotene (11%) and zeaxanthin (9%). However, the β-cryptoxanthin content increased slightly after pasteurization. The concentration process resulted in loss of violaxanthin (31%), ζ-carotene (29%), zeaxanthin (24%), lutein (17%), α-carotene (12%), β-cryptoxanthin (5%), and β-carotene (3%). The β-cryptoxanthin content decreased slightly after concentration.

Table 1
Chromatographic and spectral characteristics of fresh, pasteurized and concentrated Brazilian Valencia orange juice carotenoids obtained by HPLC

Peak no.	t_R (min)	λ_{max} (nm)	Literature values (nm) references	Carotenoids	Concentration (mg/l)[A]		
					Fresh	Pasteurized	Concentrated
1	9.81	381 402 427	Davies (1976)	Auroxanthin[B]	–	–	–
2	10.69	420 443 475	Britton (1995)	Anteraxanthin[B]	–	–	–
3	12.34	411 437 464	Rouseff et al. (1996)	Violaxanthin	1.40 ± 0.23a	0.84 ± 0.38b	0.90 ± 0.38b
4	14.72	400 426 453	Britton (1995)	Mutatoxanthin[B]	–	–	–
5	16.47	420 440 470	–	Unidentified	–	–	–
6	17.17	421 445 474	Britton (1995)	Lutein	2.78 ± 0.66a	2.20 ± 0.90b	2.30 ± 0.70b
7	19.45	423 450 478	Davies (1976)	Zeaxanthin	2.37 ± 0.80a	2.20 ± 0.90a	1.80 ± 0.90a
8	22.78	420 450	–	Unidentified	–	–	–
9	39.42	425 445 475	Rouseff et al. (1996)	α-Cryptoxanthin[B]	–	–	–
10	40.13	(422) 446 471	Davies (1976)	β-Cryptoxanthin	2.48 ± 2.90a	2.60 ± 3.20a	2.40 ± 2.60a
11	49.41	385 401 430	Britton (1995)	ζ-Carotene	1.20 ± 1.48a	1.00 ± 1.30a	0.90 ± 0.90a
12	52.04	424 448 476	Rouseff et al. (1996)	α-Carotene	0.86 ± 0.32a	0.80 ± 0.30a	0.80 ± 0.20a
13	53.44	(423) 448 476	Britton (1995)	β-Carotene	0.90 ± 0.60a	0.80 ± 0.50a	0.90 ± 0.50a
				Total	12.00 ± 6.70a	10.40 ± 6.90a	9.90 ± 5.30a

Different letters for each carotenoid in the different thermal treatment indicate significant differences ($P < 0.05$).
[A] The values are the averages of five lots in triplicate ± standard deviation.
[B] Tentative identification.

Among the 13 carotenoids peaks presented in Table 1, two of them showed significant changes ($P < 0.05$) after thermal pasteurization and concentration. Most pigment loss was from the 5,6-epoxide carotenoid violaxanthin and the dihydroxycarotenoid lutein. In chemical aspects of thermal processing, xanthophylls and hydrocarbon carotenoids have different stability and susceptibilities to oxidation. Lutein and violaxanthin were more labile because of the presence of oxygen in their structures when compared with carotenes. The conditions necessary for oxidation and isomerization of carotenoids exist during processing of food. Oxidative degradation is the principal cause of extensive losses of carotenoids and it depends on the availability of oxygen and is stimulated by heat, light, enzymes, metals, and co-oxidation with lipid hydroperoxides (Rodriguez-Amaya, 1999).

In pasteurized juices, the violaxanthin content decreased to 0.84 ± 0.38 mg/l ($P < 0.05$). This result is in agreement with others presented in the literature because violaxanthin is one of the most labile carotenoids and is easily isomerized in the presence of acid to luteoxanthin and then to auroxanthin (Lee & Coates, 2003; Rodriguez-Amaya, 1999).

The lutein contents decreased after thermal pasteurization and concentration to 2.20 ± 0.90 mg/l ($P < 0.05$) and 2.30 ± 0.70 mg/l ($P < 0.05$), respectively. These results were not reported in other studies on orange juice. However, Aman et al. (2005) assessed the influence of thermal treatments on degradation and isomerization of lutein from processed vegetables. Therefore, our work presents relevant information about thermal pasteurization and concentration process on lutein pigment in orange juice. Additionally, our research provided information about the effects of thermal treatments on carotenoid composition in orange juices; thus, we evaluated the production process of pasteurized and concentrated orange juices.

With the loss of the major carotenoid lutein during thermal pasteurization and concentration, the carotenoid pattern in Brazilian Valencia orange juice changed. β-Cryptoxanthin became the primary carotenoid, followed by lutein and zeaxanthin. However, changes in carotenoids with provitamin A activity (β-carotene, α-carotene and β-cryptoxanthin) and those active against age-related macular degeneration and cataracts (zeaxanthin) were relatively small, compared to violaxanthin and lutein; and not significant.

The values found in our work were higher because we saponified samples and quantified using standard curves. The results obtained were interesting because the losses of carotenoids with important functions for human health, were not significant.

4. Conclusion

Losses in carotenoids due to thermal pasteurization and concentration of Brazilian Valencia orange juices were clearly detected. Thermal pasteurization decreased ($P < 0.05$) the content of violaxanthin and lutein, and the concentration process altered significantly the lutein content ($P < 0.05$). Nevertheless, total carotenoid pigment content loss was not significant after these thermal treatments. With the loss of lutein, β-cryptoxanthin became the major carotenoid in pasteurized and concentrated Brazilian Valencia orange juices.

The provitamin A content of the juice (β-carotene, α-carotene and β-cryptoxanthin) and the amount of zeaxanthin, which is considered to be active against age-related macular degeneration and cataracts, did not significantly decrease after pasteurization and concentration.

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