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Effect of thermal processing on carotenoids of some orange juices

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Abstract

Thermal processing and its effects on the properties and components of orange juices has recently been investigated. Heat treatment-related changes on carotenoid composition and some properties important for fruit juice technology of native oranges (Kozan Yerli, Dörtyol Yerli, Finike Yerli and Alanya Dilimlisi) were searched in this study. With the effect of heat treatment (120 s at 70°C and 80°C, 15 s at 90°C), the decrease in β-carotene content of Dörtyol Yerli orange was determined respectively as 45.06, 66.11 and 66.70%, in Alanya Dilimlisi orange respectively 32.10%, 33.79% and 37.76% and in Finike Yerli and Kozan Yerli oranges 10.62-28.18%. After the heat treatment (120 s at 70°C and 80°C, 15 s at 90°C) in orange juice the decrease in β-carotene amount was found respectively as 23-55%, 32-65% and 52-88%. Approximately half amount of the identified and determined carotenoids (xanthophyll, zeaxanthin, β-apo-8-carotenal, α-cryptoxanthin, β-cryptoxanthin, α-carotene and β-carotene) was β-carotene (38.08-55.06%). There was a significant decrease in amounts of all detected carotenoids.

Key words: Orange, juice, pasteurization, carotenoid, β-carotene, quality, HPLC.

Introduction

The abundance of fresh drinks based on fruit juices, especially citrus juices, and minimally processed products allow consumers to ingest a wide variety of antioxidants in the diet orange juice is an important source of carotenoids and ascorbic acid, a nutrient that, apart from its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits that are being actively investigated and reported [1, 2].

The main problems associated with carotenoids come from the instability of these pigments, because they are highly unsaturated molecules and are subject to isomerisation. During processing and storage desirable constituents such as nutrients, color, flavor, and texture are destroyed. Although these products may conform to strict guidelines which prevent unnatural changes in the juice, concern about diet and nutrition has led consumers to seek a more natural product [3].

Previously, it was assumed that neither pasteurization nor concentration of orange juice had an appreciable effect on carotenoid content [4]. However, a recent thermal processing study[5] with various fruits and vegetables, including orange juice, indicated a relatively large loss (36%) of provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) in orange juice with processing.

The present study was aimed to determine carotenoid composition and heat treatment-related changes on carotenoid composition and some properties important for fruit juice technology of native oranges (Kozan Yerli, Dörtyol Yerli, Finike Yerli and Alanya Dilimlisi).

Material and Methods

Materials: The orange juices were obtained from native oranges (Alanya Dilimlisi, Dörtyol Yerli, Finike Yerli, Kozan Yerli) in an appropriate stage of maturity, specifically when the ratio of the sugars to the titratable acidity is equal to or higher than 12. All chemicals and solvents were obtained from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany).

Determination of quality characteristics: Fruit quality attributes were assessed and analyzed immediately at the time of harvest. Ten oranges from each variety were used to determine fresh weight, juice yield, total soluble solids (TSS), pH, titratable acidity (TA) and carotenoid content.

The total titratable acidity was determined by titration with sodium hydroxide (0.1 N) to the phenolphthalein end point and expressed as % citric acid as this is the main acid in orange juices[6]. The pH value was measured with a digital pH meter (Hanna pH211). Total soluble solids were measured as °Brix using a refractometer (WYA Abbe refractometer) [7, 8].

Carotenoid analysis: Carotenoid pigments were extracted, saponified and analysed by the HPLC method, following a procedure described by Gama and Sylos [9], Cemeroğlu [10] and Sadler *et al.* [11]. Pigment extraction from orange juice and saponification is described as follows. Sample aliquots of 10 ml were gently mixed with 50 ml of the extracting solvent (hexane/methanol/acetone, 50:25:25, v/v/v, containing 0.1% butylated hydroxytoluene) and centrifuged (Hettich Universal 320-R model centrifuge) for 10 min

at 4000 g. Upon centrifugation, the upper colored layers containing the carotenoid pigments were recovered and washed with water (4 x 25 ml) to remove any trace of acetone. To obtain saponified carotenoids, the extracts were treated with 25 ml of ethanolic KOH (10% w/v) for 1 h under dim light and at room temperature, and washed with water (4 x 25 ml) to remove any trace of base. The colored hexane extracts obtained were concentrated to dryness in a rotary evaporator (Hahnvapor HS-2005S-N model rotary evaporator) at temperature below 40°C and redissolved in 2 ml of a mixture of tetrahydrofurane:methanol (1:9, v/v). The concentrated extracts were filtered through Chromafil® Xtra PET filters (Chromafil® Xtra PET-45/25 0.45 µm) prior to their injection in the HPLC system. The analyses were performed in duplicate.

The HPLC analysis was carried out on a Hitachi LaChrom Elite system consisting of a quaternary pump, a photodiode array detector, a column temperature control module, and an autosampler, which was set to draw 20 µl from the samples. The pigments were separated on an Phenomenex Luna 5u C18 column (5 µm, 250 x 4.6 mm) (Phenomenex, Inc., USA) kept at 30°C. Methanol (MeOH), acetonitrile (CH_3CN) and ethyl acetate (EtOAc) were used in the mobile phase. The linear gradient elution was the same as described elsewhere 0-25 min, 1% MeOH + 99% CH_3CN + 0% EtOAc; 25-30 min, 10% MeOH + 60% CH_3CN + 30% EtOAc; 30-55 min, 10% MeOH + 60% CH_3CN + 30% EtOAc; 55-60 min, 1% MeOH + 99% CH_3CN + 0% EtOAc. The mobile phase was pumped at 0.7 ml/min, and the chromatograms were monitored at 450 nm.

The identification of the carotenoids detected was made by comparison of their chromatographic and UV/VIS spectroscopic characteristics with those of standards. The absolute concentrations of orange juice carotenoids were worked out by external calibration, performed in compliance with calibration curves constructed with the corresponding standards. The total content of carotenoids was assessed as the sum of the detected amounts of the fresh samples [12].

Determination of colour: Fruit colour was measured with a Minolta portable chromameter (Minolta, model CR-400) which provided CIE L*, a*, and b* values. Chroma ($C= [(a^*)^2 + (b^*)^2]^{1/2}$) was calculated as described by McGuire [13] and Huyskens-Keil *et al.*[14]. Hue angle (°h), in degrees, was calculated as $h = arctangent\ (b^*/a^*)$ or $tan^{-1}\ (b^*/a^*)$.

Determination of pectin methyl esterase (PME) activity: Orange juice sample of 200 ml was mixed in a blender for 3 min and filtered through 40 mesh filter. The mix was heated to 30°C and 1% pectin solution added to slurry. The pH of the mixture was set up to 7 with 2 N NaOH and to 7.7 with 0.05 N NaOH. After pH was stable 7.7, 0.1 ml 0.05 N NaOH was added and necessary time for decreasing pH 7.7 was kept. PME activity was calculated with the formulation mentioned by Cemeroğlu[10]. Heat treatment conditions were determined according to PME activity. Polydera *et al.*[15] and Kola [16] reported that PME activity must be under 5% for orange juice stability. In this study heat treatment conditions were pre-investigated aiming the decrease of PME activity to 5%. Obtained data led us to determine heat treatment conditions at 70 and 80°C for 120 s, at 90°C for 15 s).

Statistical analysis: Results of analyses were statistically analyzed by ANOVA with random blocks test pattern using the software SAS (The SAS System for Windows v9.1.3, SAS Institute Inc., Cary, NC, USA, 2002) with the Duncan test to evaluate differences between treatments at a level of significance $P<0.05$. Each experiment was repeated 3 times.

Results and Discussion

Fruit and fruit juice properties: Fruit length, width, weight and fruit juice yield of fresh fruit and also pH, titratable acidity, total soluble solids of freshly squeezed fruit juices from native oranges were determined (Tables 1 and 2). Dörtyol Yerli oranges (236.02±0.18 g) had highest weight, on the contrary Kozan Yerli (165±0.26 g) had lowest. The highest fruit juice yield was at Dörtyol Yerli oranges (60%) (Table 1). As a result fruit juice yield is thought to be related with fruit weight. The pH values and titratable acidity of native orange juice samples changed between 3.53-3.64 and 1.03-1.26 g/100 ml, respectively. The highest titratable acidity value was at Kozan Yerli orange juice samples. Total soluble solids content of orange juice samples changed between 12.39 and 13.86; furthermore Alanya Dilimlisi had the highest Brix value (Table 2).

Carotenoid profile of native oranges: The carotenoid pigments that are responsible for the color of native oranges detected and amount determined (Fig. 1) are xanthophyll (15.99 min), zeaxanthin (16.85 min), β-apo-8-carotenal (18.60 min), α-cryptoxanthin (35.69 min), β-cryptoxanthin (37.10 min), α-carotene (47.50 min) and β-carotene (48.69 min).

Approximately half of the determined carotenoids was β-carotene (38.08-55.06%) and it was the most dominant carotenoid (10.12-21.65 ppm). Kozan Yerli orange juices had higher β-carotene amount than the other oranges and Finike Yerli, Alanya Dilimlisi and Dörtyol Yerli samples followed Kozan Yerli (Table 3).

Table 1. Some properties of fresh oranges.

	Orange samples			
	Alanya Dilimlisi	Dörtyol Yerli	Finike Yerli	Kozan Yerli
Fruit width (mm)	70.17 $^{c(1)}$	73.88 ^{b}	75.52 ^{a}	69.89 ^{c}
Fruit length (mm)	66.00 ^{b}	75.00 ^{a}	74.03 ^{a}	64.03 ^{c}
Fruit weight (g)	184.80 ^{c}	236.02 ^{a}	224.00 ^{b}	165.00 ^{d}
Fruit juice yield (%)	54.00 ^{c}	60.03 ^{a}	56.02 ^{b}	49.97 ^{d}

Different superscripts in the same column are statistically different ($P<0.05$)

Table 2. Some properties of orange juice samples.

	Orange samples			
	Alanya Dilimlisi	Dörtyol Yerli	Finike Yerli	Kozan Yerli
pH	3.53 $^{d(1)}$	3.56 ^{c}	3.64 ^{a}	3.61 ^{b}
Titratable acidity* (g/100 ml)	1.12 ^{b}	1.03 ^{c}	1.15 ^{b}	1.26 ^{a}
Total soluble solids (Brix)	13.86 ^{a}	13.75 ^{b}	12.86 ^{c}	12.39 ^{d}

Different superscripts in the same column are statistically different ($P<0.05$) *citric acid

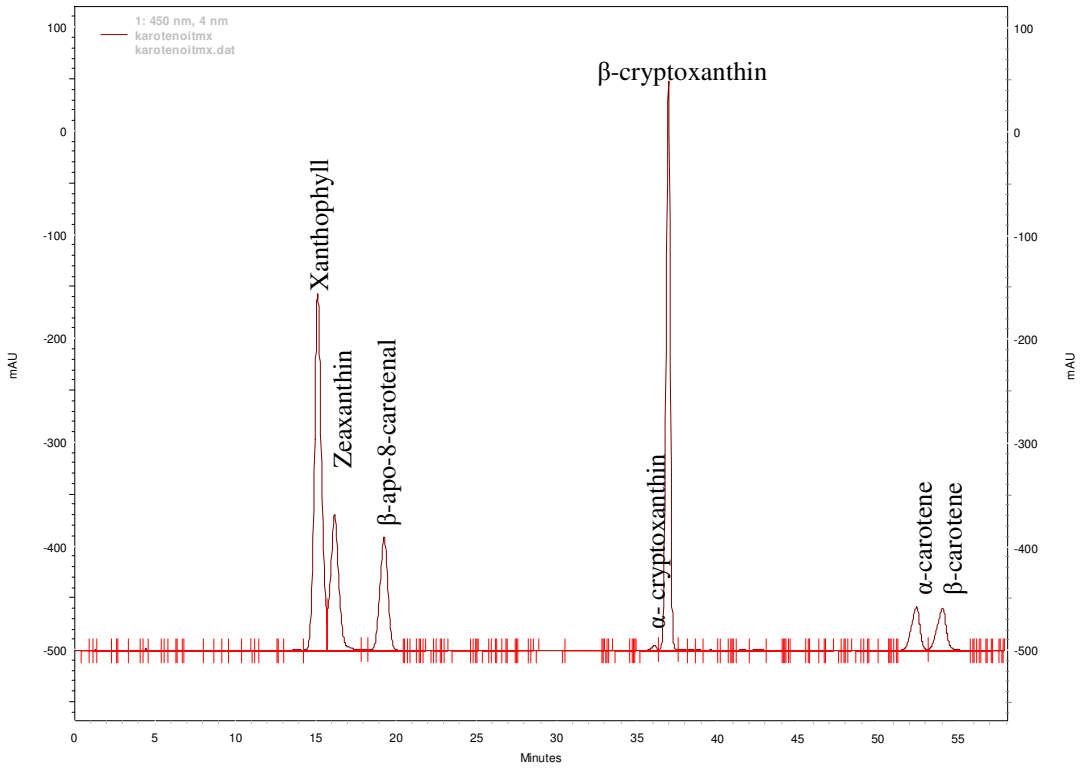

Figure 1. HPLC chromatogram of carotenoid standards.

Xanthophyll was the second abundant carotenoid (22.46-32.50%) and changed in amounts between 4.17 and 16.35 ppm in orange samples. Finike Yerli had the highest xanthophyll content but Dörtyol Yerli had the lowest one.Total carotenoid content changed between 9.40 and 3.18 ppm. Xanthophyll was most abundant in Finike Yerli (31.80 ppm) orange juice, Alanya Dilimlisi (14.30 ppm) and Dörtyol Yerli (10.00 ppm) and Kozan Yerli (9.40 ppm) were the followers (Table 3).

Effect of heat treatment on carotenoid profile: Heat treatment usually causes decrease in the carotenoid content and total carotenoid amounts. The carotenoid content and total carotenoid amount of orange juice samples after heat treatment are given in Table 3.

Gama and Sylos [17] reported that α-carotene decreased from 0.86 to 0.80 ppm in Brazilian Valencia orange juices. α-Carotene content of native oranges decreased about 52% to 80% after heat treatment. The decrease at 70ºC heat treatment was 23-56% in Alanya Dilimlisi, Dörtyol Yerli and Kozan Yerli orange juices. Furthermore the decrease was about 23% at Finike Yerli oranges. The decrease at samples treated at 80ºC was 59-65% in Dörtyol Yerli and Kozan Yerli, 32-34% in Alanya Dilimlisi and Finike Yerli orange juice samples. Furthermore the decrease at samples treated at 90ºC was highest in Finike Yerli oranges (87.65%).

β-Carotene content decrease was 45.06%, 66.11% and 66.70% at 70ºC, 80ºC and 90ºC, respectively, in Dörtyol Yerli orange juice samples (Table 3). The decrease was less in Finike Yerli and Kozan Yerli orange juices after heat treatment and changed between 10.62 and 28.18%.

On the other hand, β-apo-8-carotenal decreased most in Finike Yerli orange juice samples (61-96%). The higher temperatures the less treatment time caused less decrease in orange juice samples and that can be connected to the isomerisation of orange juice carotenoids. Dhuique-Mayer *et al.* [18] found a decrease of about 30-60% in xantophyll amounts after a heat treatment at 55ºC for 15 min and zeaxanthin and lutein were the least decreased carotenoids.

β-cryptoxanthin content of Dörtyol Yerli orange juice samples decreased 8.7%, 44% and 48% in 70ºC, 80ºC and 90ºC, respectively. Furthermore a decrease changed according to heat treatment conditions about 6-37% seen in Alanya Dilimlisi and Finike Yerli oranges. On the other hand an increase of about 12-69% was seen in Kozan Yerli samples (Table 3).

Heat treatment caused a zeaxanthin decrease especially in Dörtyol Yerli samples about 38-80% and the decrease was also similar in Alanya Dilimli and Finike Yerli orange juice samples. The conditions necessary for oxidation and isomerization of carotenoids occur during production of food. Extensive losses of carotenoids could be seen because of oxidation, relying on the availability of oxygen and is accerelated by heat, light, enzymes, metals, and co-oxidation with lipid hydroperoxides [17, 19]. Lessin *et al.* [5] reported that a decrease about 36% seen in carotenoids that had provitamin A activity (β-carotene, α-carotene and β-cryptoxanthin) after heat treatment. Also Gama and Sylos [17] introduced that heat treatment caused decrease in violaxanthin (38%), lutein (20%), ζ -carotene (14%), β-carotene (11%), α-carotene (13%) and zeaxanthin (9%) in pasteurized orange juices.

The total carotenoid content of Finike Yerli (31.80 ppm) was higher than in domestic other orange varieties. After thermal processing, the decrease in the total carotenoid content was at least in the Finike Yerli orange juice samples ($P<0.05$). Lee and Coates [3] found that total carotenoid pigment content loss was

Table 3. Carotenoid amounts in native orange juice after heat treatment (ppm).

Carotenoids	Temperature (°C)	Orange samples			
		Alanya Dilimlisi	Dörtyol Yerli	Finike Yerli	Kozan Yerli
α-carotene	Control	6.27 c(1)	2.78 f	8.99 a	5.11 d
	70	2.81 f	1.26 hi	6.99 b	2.89 f
	80	4.16 e	1.14 hi	6.10 c	1.79 g
	90	3.04 f	1.06 i	1.11 hi	1.38 h
β-carotene	Control	15.36 d	10.12 e	19.16 b	21.65 a
	70	10.43 e	5.56 f	16.65 c	19.35 b
	80	10.17 e	3.43 g	16.25 cd	18.58 b
	90	9.56 e	3.37 g	15.45 d	15.55 cd
β-Apo-8-carotenal	Control	1.03 d	0.45 f	3.02 a	1.11 c
	70	0.81 e	0.23 g	1.17 c	1.00 d
	80	0.77 e	0.20 g	0.82 e	1.11 c
	90	0.25 g	0.11 h	0.12 h	1.35 b
Xanthophyll	Control	7.38 g	4.17 i	16.35 a	11.33 c
	70	6.78 h	3.34 j	9.01 f	10.08 d
	80	6.42 h	2.10 k	6.43 h	11.12 c
	90	4.30 i	2.21 k	9.59 e	12.77 b
β-cryptoxanthin	Control	1.32 f	0.46 j	1.58 c	1.34 ef
	70	1.44 de	0.42 j	1.41 def	1.84 b
	80	1.15 g	0.26 k	1.04 h	2.27 a
	90	0.83 i	0.24 k	1.49 cd	1.50 cd
Zeaxanthin	Control	1.50 c	0.40 h	1.21 e	1.62 ab
	70	1.21 e	0.25 i	0.83 f	1.42 d
	80	1.11 e	0.08 j	0.57 g	1.57 bc
	90	0.63 g	0.12 ij	0.93 f	1.72 a
Total carotenoid	Control	14.3 d	10.0 efg	31.8 a	9.4 fgh
	70	12.1 e	8.5 gh	30.1 ab	8.8 fgh
	80	11.8 e	8.3 gh	28.9 bc	8.2 gh
	90	10.8 ef	8.2 gh	27.5 c	7.3 h

Different superscripts in the same column are statistically different ($P<0.05$)

significant in pasteurized Valencia orange juice, which is about a 10% loss (P<0.05). Gama and Sylos [17] determined that amount of total carotenoids in fresh squeezed, concentrated or thermal processed Brazilian Valencia orange juices were respectively 12.0 ± 6.7, 10.4 ± 6.9 and 9.9 ± 5.3 mg/l.

Color values of orange juices: L* values expressing the clarity and brightness of orange juice color was found the highest in Kozan Yerli orange juice samples that means Kozan Yerli orange juices are more clear than the others. a* values showed a negative and low value and led us to think orange color of orange juices resulted from a* values. Kozan Yerli orange juice samples also had the highest b* values (44.98).

As can be seen in Table 4, hue angle expressing homogenity and purity of orange juice color changed between 72.9 and 80.8, accordingly the closeness of the angle to 90° caused yellow color close to orange in orange juice samples.

Table 4. Changes in color values after heat treatment.

Color values	Temperature (ºC)	Orange samples			
		Alanya Dilimlisi	Dörtyol Yerli	Finike Yerli	Kozan Yerli
L*	Control	42.41 b(1)	38.76 efg	40.02 de	47.74 a
	70	39.47 def	38.00 fg	40.51 cd	42.05 bc
	80	47.39 a	38.82 efg	38.85 efg	42.03 bc
	90	41.88 bc	37.74 g	38.70 efg	40.72 cd
a*	Control	-8.84 i	-9.45 j	-7.96 h	-7.68 h
	70	-6.92 g	-5.97 bcde	-6.40 def	-6.59 fg
	80	-6.47 efg	-5.69 b	-5.92 bcd	-6.06 bcde
	90	-6.22 cdef	-4.81 a	-5.81 bc	-6.31 def
b*	Control	30.88 c	29.63 c	33.15 b	44.98 a
	70	26.41 e	22.68 gh	25.95 e	28.01 d
	80	18.19 i	22.71 gh	24.13 fg	27.85 d
	90	23.01 gh	22.04 h	24.17 fg	25.06 ef
Chroma	Control	30.62 c	30.33 c	33.45 b	45.65 a
	70	25.85 f	22.56 gh	27.11 e	28.81 d
	80	18.35 i	23.40 g	24.88 f	27.73 e
	90	22.99 g	21.68 h	25.42 f	27.26 e
Hue	Control	73.92 de	72.94 e	76.45 bc	80.81 a
	70	74.70 cde	75.18 cd	76.04 bcd	76.74 bc
	80	68.72 f	76.06 bcd	76.00 bcd	77.71 b
	90	75.13 cd	77.78 b	76.14 bcd	77.48 b

*Different superscripts in the same column are statistically different ($P<0.05$)

Conclusions

In this study, the effect of the heat treatment on orange juice carotenoid profile is investigated. According to obtained data, decreases approximately half amounts of detected carotenoids are seen. Approximately half amount of the determined carotenoids was β-carotene (38.08-55.06%) and β-carotene loss was about 45.06% and 66.70% in Dörtyol Yerli, 32.10% and 37.76% in Alanya Dilimlisi, 10.62% in Finike Yerli and 28.18% in Kozan Yerli. α-Carotene loss after heat treatment in orange juice samples was 23-55%, 32-65% and 52-88%, respectively, at 70ºC and 80ºC for 120 s, 90ºC for 15 s.

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