Influence of Carotenoids and Pulps on the Color Modification of Blood Orange Juice

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ABSTRACT: The causes of color modification in blood orange juice due to the heating concentration process were investigated. A Not From Concentrate (NFC) juice and a Reconstituted From Concentrated (RFC) juice, arising from the same stock of pigmented oranges, appeared very different in color although they had the same content of anthocyanins. CIE (Commision Internationale des Enclarages)-Lab measurements showed a slight decrease of L* and a* values and a very large increase of b* and hue values in RFC juice. Different distribution of carotenoids between serum and pulps and modification of pulp size appeared to be responsible for color modification.

Key Words: anthocyanins, blood orange juice, carotenoids, color, heating

Introduction

COLOR OF BLOOD ORANGE JUICE IS THE RESULT OF DIFFERENT contributes. Mainly it is due to the content of the water-soluble anthocyanins (Maccarone and others 1985, 1998) and to the amount of carotenoids and pulps present in the juice. Pasteurization and concentration of blood orange juices cause a significant modification in the distribution of original constituents and a partial transformation of some of them; in particular, a remarkable alteration of the sensory profile has been pointed out by loss of aroma components (Maccarone and others 1996) and by generation of off flavors (Fallico and others 1996). Moreover, the formation of 5-hydroxymethyl-2-furancarboxaldehyde (5-HMF) due to thermal degradation of sugars has been recently studied from a kinetic point of view (Arena and others 2000). A noteworthy change of color also occurs in the processed juices that become brick red, usually ascribed to a degradation of anthocyanins. Such a modification should be minimized as much as possible because the original bright red color is an important factor of appreciation by consumers (Sturiale 1995).

Maccarone and others (1996) compared the color of two juices arising from the same stock of pigmented oranges picked before and after the concentration process, that is, a Not From Concentrate juice (NFC) and a Reconstituted From Concentrate juice (RFC), respectively. The two juices appeared very different in color, although the anthocyanins levels were similar, showing only 7% of decrease in RFC juice with respect to NFC juice. Measurements of color by CIE-Lab parameters confirmed these results. The a* value of RFC juice decreased about 8%, but the b* value of this juice increased much more than a* value decreased. Therefore, thermal damage of color cannot be ascribed to degradation of anthocyanins because other factors interfere with the red color of these pigments.

The importance of carotenoids in blood orange juices has been pointed out (Petrus and Dougherty 1973), and a color score value, based on the carotenoids level, has been proposed (Petrus and others 1975). Some investigations on color change due to carotenoids during the heating process have been done on tomato-based products (Noble 1975; Tonucci and others 1995) and on carrot juice (Chen and others 1995). Moreover, Pesek and Warthesen (1988), studying kinetics of β-carotene photodegradation in aqueous dispersions, found that particle size largely influences the apparent color of solutions. The aim of this work is to recognize molecular and technological causes of the color modification in blood orange juices after thermal treatments.

Results and Discussion

THE CONCENTRATION OF ANTHOCYANINS IN NFC BLOOD orange juices ranges in the interval of 48 to 97 mg/L (Table 1). After the thermal concentration process and reconstitution, a remarkable change of color was observed in RFC juices that appeared yellow-reddish; however, the concentration of anthocyanins in these juices was similar to that of the corresponding NFC juices, confirming that alteration of color in processed juices does not depend on degradation of such pigments. Table 1 also reports the color CIE parameters, L*, a*, b*, C, and h. A small decrease of lightness (L*) occurs in RFC juices with respect to NFC ones (−8.1%, average), because scattering of micro-particles of pulps in the juices remained after filtration (0.45 μm). In RFC juices, no significant decrease of red component of color (a*) is observed, confirming a good stability of anthocyanins to the thermal treatment. Instead, the yellow component (b*) increases about 80%, inducing a small increase on chroma value (C) and a remarkable effect in hue angle (h) that measures the juice color change. Furthermore, the hue angle could be a useful criterion to discriminate NFC from RFC juices, as it is always lower than 30 ° for NFC juices and higher than 45 ° for RFC juices. The increase of the b* parameter in RFC juices could be ascribed to products from nonenzymatic browning reactions, but these juices had the same level of 5-HMF (0.5 mg/L) of the NFC juices (Arena and others 2000), thus suggesting an increase of yellow substances in solution due to other factors.

Figure 1 reports the visible spectra of ether extracts of NFC and RFC juices, which are characteristics of carotenoids (Belitz and Grosch 1987; Petrus and Dougherty 1973; Petrus and others 1975). Table 2 reports the content of carotenoids in the whole juices, in the corresponding clarified juices after centrifugation, and in the pulps. The concentration ranges from 10.5 mg/L to 17.5 mg/L, according to previous reports for orange juice (Di Giacomo 1970a,b; Klaui and Bauernfeind 1981). A small decrease of concentration occurs passing from NFC to the corresponding RFC juices (-7.8%), according to Petrus and Dougherty (1973). Most carotenoids are adsorbed on pulps, and a small amount is present in solution because these are xanthophylls esters containing fatty acids (Klaui and Bauernfeind 1981). Distribution of carotenoids between serum and pulps is remarkably different between NFC and RFC juices. In fact, serum of NFC juices contains about 3% of the total carotenoids, 97% being adsorbed on pulps, whereas serum of RFC juices contains about 25%, and the remainder 75% is adsorbed on pulps. The different distribution

Table 1—Anthocyanin concentrations and color CIE parameters of NFC and RFC blood orange juices

Juice No.	Type	Brix	Anthocyanins (mg/L)[a]	L*	a*	b*	C	h
1	NFC	12.1	48.3±0.3	63.1	57.9	25.9	63.4	24.1
1	RFC	60[b]	49.0±0.6	61.2	53.9	56.8	78.1	46.7
2	NFC	12.5	55.0±0.3	60.1	60.3	31.1	67.9	27.3
2	RFC	55[b]	57.1±0.1	57.6	57.4	58.9	82.3	45.7
3	NFC	11.5	72.0±0.4	57.0	70.3	35.9	79.0	27.1
3	RFC	51[b]	68.9±0.7	49.8	62.2	61.2	87.2	44.6
4	NFC	12.5	97.0±0.1	51.7	71.8	42.7	83.5	30.7
4	RFC	58[b]	104.0±1	44.6	67.2	67.7	95.1	45.0
Mean[c]	NFC	12.2 (0.5)	68.1 (21.7)	58.0 (4.9)	65.1 (7.0)	33.9 (7.1)	73.5 (9.4)	27.3 (2.7)
Mean[c]	RFC[b]	56 (4)	69.8 (24.2)	53.3 (7.5)	60.2 (5.8)	61.2 (4.7)	85.7 (7.3)	45.5 (0.9)

[a] As cyanidin-3-glucoside chloride. Each analysis was carried out in duplicate.
[b] Reconstituted to the same Brix of the corresponding NFC juice.
[c] Standard deviation in parentheses.

Table 2—Carotenoids of blood orange juices and their distribution between serum and pulps

Juice No.	Type	Carotenoids (mg/L)[a] Juice	Serum	Pulps Found	Pulps Calcd[b]	Pulps Fond/Calcd	Distribution % Serum	Distribution % Pulps
1	NFC	13.3	0.24	12.4	13.1	0.95	1.8	98.2
1	RFC	10.5	2.69	8.43	7.81	1.08	25.6	74.6
2	NFC	14.2	0.44	13.3	13.8	0.96	3.1	96.9
2	RFC	13.5	3.65	12.0	9.85	1.22	27.0	73.0
3	NFC	11.5	0.39	12.0	11.1	1.08	3.4	96.6
3	RFC	10.8	3.07	8.05	7.73	1.04	28.4	71.6
4	NFC	17.5	0.59	17.3	16.9	1.02	3.4	96.6
4	RFC	17.3	3.27	15.2	14.0	1.09	18.9	81.1
Mean	NFC	14.1	0.42	13.8	13.7	1.00	2.9	97.1
Mean	RFC	13.0	3.17	10.9	9.85	1.10	25.0	75.0

[a] Mean value from two extractions. Deviation from mean value does not exceed 8%.
[b] Difference between juice and serum.

accounts for the higher b* and h values of RFC than NFC juices.

A noteworthy difference in the structure of pulps was observed by optical microscope. Pulps appear in the form of definite particles having relatively great dimensions in NFC juices (Fig. 2a), whereas in RFC juices they appear as indistinct and diffuse micro-particles (Fig. 2b). These differences were confirmed by image analysis. In an area of 127.7 μm x 93.7 μm of NFC juice, 55 particles were counted, having an average area of 0.322 (+0.236) μm^2. In the same area of RFC juice, 663 particles were counted, with an average area of 0.166 (+0.192) μm^2. It is important to underline that we selected for counting particles in both juices an area where the largest particles were absent. Moreover, the threshold value was fixed in order to count a significant number of particles in NFC juice, thus excluding, in RFC juice, many particles smaller than the threshold value. Color of juices is corre-

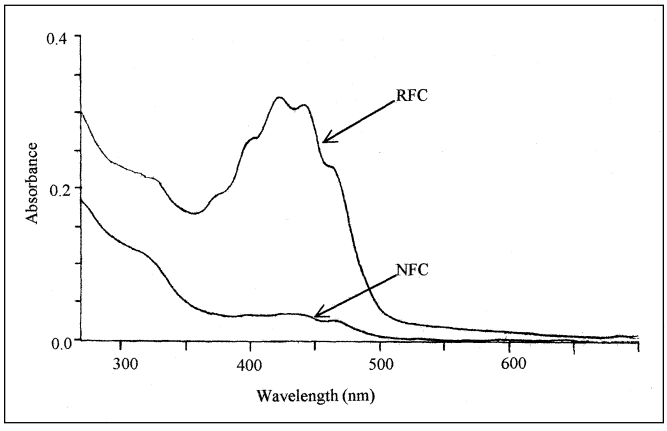

Fig. 1—Absorption spectra of carotenoids extracted from clarified RFC and NFC blood orange juices

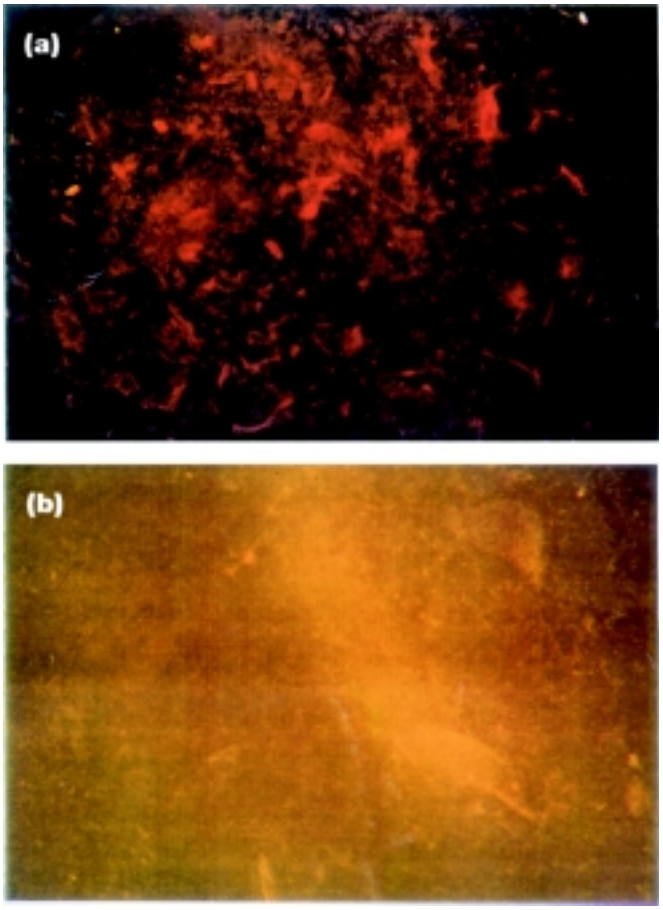

Fig. 2—Optical microscope photographs of NFC (a, x100) and RFC (b, x200) blood orange juices

spondingly different, appearing red in NFC and yellow-orange in RFC. Pesek and Warthesen (1988) demonstrated that dimension of particles influences the apparent color of β-carotene containing solutions. In fact, two model systems having the same quantity of β-carotene and different dimension of particles in suspension appear to be different in color. Systems containing smaller particles show a yellow color and those having greater particles an orange color. Therefore, the micronization effect due to thermal and mechanical stress of pulps, particularly for the high speed of juice in the nozzles of a thermal accelerated short time evaporator (TASTE), contributes to the greater dispersion of particles and, consequently, to a greater dispersion of the yellow light in the RFC juices.

Conclusions

EXPERIMENTAL RESULTS CONFIRM THAT ALTERATION OF THE original color of blood orange juices after thermal processing cannot be ascribed to degradation of anthocyanins. The different distribution of carotenoids between serum and pulps and the modification of pulp structure appear to be the more important factors of the color change in the reconstituted juices from concentrates.

Materials and Methods

FOUR NFC BLOOD ORANGE JUICES SAMPLED IN DIFFERENT periods and having a pulp content of about 8% were obtained by FMC in line extractors (Ruby Co., Catania, Italy). These juices were concentrated in a TASTE evaporator at T_{max} of 90 to 92 °C until 51 to 60 Brix and reconstituted (RFC) at the same Brix value of the corresponding NFC (11.5 to 12.5) using distilled water. Cyanidin-3-glucoside chloride and β-carotene as spectrophotometric standards were purchased from Extrasynthèses (Genay, France). Tetrahydrofuran (THF) and petroleum ether were purity-grade solvents. Spectrophotometric analyses were carried out by a Cary 1E instrument (Varian, Leinì, Italy).

Determination of anthocyanins and color CIE measurements

Two aliquots (3 mL) of centrifuged juice (3000 rpm for 15 min) were diluted up to 25 mL with aqueous solutions at pH 1 and 4.5, according to Fuleki and Francis (1968), and the absorbances of these solutions were measured at 510 nm. Concentration of anthocyanins was expressed as mg/L of cyanidin-3-glucoside chloride using the method of Rapisarda and others (1994). Visible spectra of centrifuged and then filtered (0.45 μm) orange juices were recorded in transmittance in the interval 380-780 nm using 1 cm quartz cell for determining color CIE parameters (L*, a*, b*, C, h) by "Color Calculations" software (Cary 1E, Varian, Leinì, Italy).

Extraction and determination of carotenoids

Carotenoids were extracted using the following procedure: 20 mL of juice were mixed with 100 mL THF and placed in a separator funnel together with 50 mL of petroleum ether. After shaking, the aqueous phase was separated, and the organic phase was washed with three aliquots of water (25 mL). Aqueous phases were collected and furtherly extracted with petroleum ether until they were colorless. Ether extracts were dried on anhydrous sodium sulfate and concentrated undervacuum at temperature < 20 °C. Extraction of carotenoids was also carried out in the clarified juices after centrifugation (3000 rpm for 15 min) and separation of pulps, using the above described procedure. The isolated pulps (moisture 85%) were weighed, dispersed in distilled water, and extracted as previously described. The residues were diluted with petroleum ether up to a known volume (150 mL for the whole juices, 10 mL for the clarified juices, 100 mL for the pulps). Concentration of carotenoids was spectrophotometrically determined by measuring absorbance at 450 nm and expressed as mg/L of β-carotene using the following calibration line obtained with standard solutions of β-carotene at concentrations from 0.4 mg/L to 4.0 mg/L ($r^2 = 0.9937$).

*Carotenoids (mg/L) = (Absorbance / 0.2327) * dil*

where *dil* is the dilution factor.

Recovery trials

In order to measure the recovery of carotenoids by the extraction methodology, standard solutions of β-carotene were prepared dissolving it in 1 mL of THF and diluting up to 100 mL with a citric acid (1.2 g/100 mL)/tripotassium citrate (0.6 g/100 mL) buffer solution (pH 3.38). The model solutions were extracted and analyzed as previously described. A quantitative recovery of carotenoids was obtained.

Microscope observations and image analysis

In order to verify if the heating processing had modified the pulps' size, the juices were observed at the optical microscope (Zeiss MC 80 DX STEMI SV 11, equipped with a camera) using cavity slides. Image analysis was performed using a Leitz Aristoplan microscope (40 X) equipped with an Optronics DEI-470 camera and Optimas software (5.2).

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