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Changes in anthocyanins in cherries (*Prunus avium*) during osmodehydration, pasteurization and storage

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Pigment composition and colour of osmodehydrated and pasteurized cherries (cv. Vittoria) were analysed during processing and storing at ambient temperature. Pasteurization was carried out after conditioning or by direct immersion in a sugar isotonic syrup followed by hot filling and quick cooling. From thin-layer chromatography and spectrophotometric analysis the course of anthocyanin degradation and the modification of browning index were followed. Experimental results indicated the presence of cyanidin-3-glucoside, cyanidin-3-rhamnoglucoside, peonidin-3-rhamnoglucoside and peonidin-3-glucoside. Osmotic dehydration contributes slightly to the lightening of the colour in processed cherries. Even if the thermal treatments and the storage caused anthocyanin loss and formation of browning compounds, the cherry colour was judged fairly acceptable.

INTRODUCTION

Colour is one of the most important attributes of food, both for its aesthetic value and for quality judgement. The red colour of cherries is due to several water-soluble anthocyanin pigments (Shrikhande & Francis, 1973). These pigments are not very stable chemically and may change easily if not properly protected.

pH is the most important factor affecting the colour of anthocyanins, which can exist in the form of the red or yellow flavylium cation, red or blue quinoidal base, colourless carbinol pseudobase and chalcone (Mazza & Bruillard, 1987).

The stability of anthocyanins during processing is particularly dependent upon pH of the media. The carbinol base is unstable, so the oxidation of anthocyanins in food during processing or storage is proportional to the percentage of this form, which is enhanced by heat treatments (Chichester & McFeeters, 1970). Furthermore, the presence in the fruit of enzymes capable of splitting the glycosidic linkage in anthocyanins produces, during processing, the less stable aglycones, so rendering the pigments more susceptible to oxidation (Chichester & McFeeters, 1970; Harborne, 1977).

Sweet cherry (*Prunus avium*) contains the glucoside derivatives of two anthocyanidins, namely cyanidin (Cy) and peonidin (Pn): Cy-3-rhamnoglucoside (major peak), Cy-3-glucoside (Casoli *et al.*, 1967; Harborne, 1967; Van Buren, 1971; Timberlake & Bridle, 1975, 1980, 1982; Timberlake, 1981), Pn-3-glucoside and Pn-3-rhamnoglucoside (Macheix *et al.*, 1990).

Polesello and Bonzini (1977) and Pizzocaro *et al.* (1979) studied the anthocyanin composition of 13 cultivars of cherry grown in Italy, and confirmed that Cy-3-rhamnoglucoside was the major anthocyanin present. This compound accounted for 80–99% of the total anthocyanins separated by thin-layer chromatography (TLC).

The amount of anthocyanins was not exactly correlated with the external colour of fruit, but depended on the distribution of the pigments between flesh and skin. For instance, the cultivar 'Vittoria' was scored as 'dark red' on the basis of its external colour, whereas its total anthocyanin content was scored as 'medium' because its flesh was weakly coloured. Anthocyanin content is influenced by the level of ripeness, which sometimes is not visually evaluable, because the pigment development is quicker in the cell skin, which is exposed to sunlight, than in the cell flesh (Grisebach, 1982).

Osmotic dehydration has recently received attention as an intermediate step in drying, dehydrofreezing and freeze-drying of fruit (Le Maguer, 1988; Torreggiani, 1993). Maltini *et al.* (1983) proposed to use osmotic dehydration, together with vacuum packaging and pasteurization, to obtain fruit products stable at ambient temperature. The application of this process to sweet cherries was studied from a technological and chemical point of view; these researches focused on the influence of cultivars, osmosis time and storage period

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(Torreggiani *et al.*, 1987), and of pasteurization process (Torreggiani *et al.*, 1990; Forni *et al.*, 1992), on the quality and stability of processed cherries.

The chemical changes most strongly related to the acceptability of cherry products are those associated with polyphenols (flavour and colour) and pectins (texture). This paper deals with the behaviour of anthocyanins during osmodehydration and pasteurization, and pectins and textural changes will be the object of a further paper.

MATERIALS AND METHODS

Fruits and processing

Cherries of the cultivar 'Vittoria', harvested at a commercial maturity stage, were pitted and water-blanched at 70°C for 120 s; under these conditions only the thermal destruction of the surface microorganisms was obtained, but not the inactivation of enzymes. The fruits were then dehydrated using a 70° Bx syrup consisting of corn syrup (24% glucose, 29% maltose, 12% polysaccharides, 35% water)–sucrose–water (5/3/1, w/w/w), and containing ascorbic acid (1%) as an antioxidant. The dehydration was carried out at an ambient temperature of 25°C for 2 h. After osmotic concentration the fruits were drained and pasteurized following two different systems:

(a) packaging in 350-ml glass jars with an isotonic syrup filling and pasteurization at 85°C for 25 min (PC).
(b) pasteurization by direct immersion in an isotonic syrup at 85°C for 3 min and hot filling with isotonic syrup in 350-ml glass jars (Torreggiani *et al.*, 1990) (PD).

Both preserves were then cooled and stored at 25°C for 12 months.

Analytical methods

The investigations were conducted on fresh fruits (F), after osmosis (OS) and after conditioning at 0, 3, 6, 9 and 12 months of storage (T0, T3, etc.). Each sample consisted of the contents of three jars, and each analysis was repeated twice. Samples were analysed for total amount and composition of anthocyanins, for colour and for dry matter (Association of Official Analytical Chemists (AOAC), 1980). Sensory evaluations were carried out during storage.

Extraction of the pigments
Homogenized fruits (10 g) were repeatedly extracted by shaking with 1% HCl in methanol until the pulp was completely decolourized. The pooled extracts were diluted to 250 ml with 1% HCl in methanol.

Total anthocyanins
To determine the concentration of anthocyanins the method of Swain and Hills (1959) was followed, using the value of 20 200 at 528 nm as the molecular extinction coefficient of the Cy-3-glucoside (Polesello & Rampilli, 1972). The anthocyanins extracted as above were diluted 1:4 with 1% HCl in methanol at pH 0·5, to obtain the maximum absorptivity (Sondheimer & Kertesz, 1948). The absorption spectra were scanned and recorded between 400 and 600 nm against 1% HCl in methanol as a blank, using a Pye Unicam P8800 spectrophotometer. From the ratio of absorbances at 420 nm and at 528 nm the browning index was calculated (Harborne, 1958).

TLC of anthocyanins
The HCl methanol extracts were cleaned by solid-phase extraction on a Lichroprep RP 18 column (5 cm × 2·5 cm i.d.). The solid phase was previously conditioned by flushing with methanol and water successively. The extract (25 ml) was loaded onto the column and after removing sugars by flushing with water, the pigments were eluted with 0·1% HCl in methanol. Brown pigments were retained in the column. The eluate was concentrated to 1 ml in a rotatory evaporator at 40°C.

The solution (25 μl) was spotted and chromatographed on cellulose MN 300 TLC plates (20 × 20 cm size, 0·1 mm thick, Merck) with CH_3COOH–HCl–H_2O (15:3:82) in the dark (Polesello & Bonzini, 1977). After evaporation under N_2, the pigments separated were identified according to their R_F values (Dekazos, 1970; Polesello & Bonzini, 1977). The coloured bands were scraped off and extracted with 0·1% HCl in methanol and scanned from 350 to 600 nm with a Pye Unicam P8800 spectrophotometer and quantified at their absorption maximum (Polesello & Bonzini, 1977).

Colour measurements
Colour was determined on a Hunterlab Colour Difference Meter DM25. The readings were made on a double layer of fruit arranged on the transparent crystal bottom of a black-walled cylinder of 10-cm diameter; the reported data are the means of five determinations. From Hunter values of a, b and L, the hue (H), saturation (S) and colour differences (ΔE) between the colour of the products just after processing and the colour after 3, 6, 9 and 12 months storage were calculated with the formulae (Hunter, 1975)

$$H = \sqrt{a/b} \qquad S = \sqrt{a^2 + b^2},$$
$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

Sensory evaluation

The sensory evaluations were carried out by a purposely planned methodology, using an intensity test for colour and a colour uniformity and preference test for acceptability. The panel was composed of 10 trained judges, and each testing was repeated twice on subsequent days. The analysis of variance and Duncan's multiple range test were used to determine statistically significant differences ($P \leq 0{\cdot}05$) (Larmond, 1977).

Dehydration parameters

According to the method of Hawkes and Flink (1978), the following parameters were calculated:

$$\text{solid gain (SG)} = \frac{(WS - WS_0)}{(WS_0 + WW_0)} \times 100$$

where WW_0 is the weight of the water initially present, WS_0 is the initial weight of solids and WS is the weight of solids in the fruit at the time of sampling;

$$\text{material balance } (\Delta C) = \frac{C_t - C_0}{P_0} \times 100$$

where C_t is the concentration of the component in the fruit after osmosis, C_0 is the concentration before osmosis and P_0 is the initial weight of the fruit.

RESULTS AND DISCUSSION

Osmodehydration

After the 2 h osmotic process, the dry matter of the cherries increased about 30%. The weight loss was 29·29% and the solid gain was 0·07%. As previously observed by Giangiacomo *et al.* (1987) and by Torreggiani *et al.* (1987) for 'Vittoria' and other cherry cultivars, the increase of dry matter was mainly due to water loss because of low solid gain. This behaviour could be related to the small exchange surface and to the waxy peel of the cherries.

The balance, after 2 h of osmodehydration, for total anthocyanins, showed a decrease of 6 mg per 100 g of the initial fresh weight, corresponding to about 6% of the initial anthocyanin content of the cherries. As the osmodehydrated cherries were more concentrated, their anthocyanin content was higher than that of the fresh fruit (Fig. 1).

The increase of the Hunter value L and the decrease of the hue indicate a slight fading of the cherries and a shift towards a red colour, respectively (Table 1).

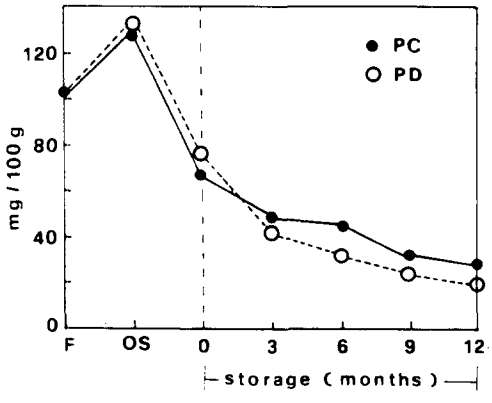

Fig. 1. Total anthocyanin contents of fresh and processed cherries.

Table 1. Hunter values of the fresh and processed cherries

	F	OS	PD	PC
L	7·44	8·02	11·71	10·39
a	10·44	12·28	13·98	15·78
b	−2·07	−1·80	−0·91	−1·42
a/b	−5·00	−6·83	−15·42	−11·16
$\sqrt{a^2 + b^2}$	10·61	12·41	14·03	15·81

F—Fresh fruit; OS—osmodehydrated fruit;
PD—pasteurization by direct immersion; PC—conventional pasteurization.

Pasteurization

As shown in Fig. 1, the pasteurization caused a decrease of total anthocyanins, which was more evident for PC cherries. Fading and shift towards redness were also observed, especially for PD, as shown in Table 1 and Fig. 2. Measurements of colour by reflectance evaluated the pigmentation intensity of the fruit peel (as the measurements were carried out on the surface of the fruit), whereas chemical analysis gave the total anthocyanin content for the whole fruit. Pasteurization by direct immersion (PD) of the cherries could therefore affect the peel colour only, whereas the conventional pasteurization (PC), because of the longer time of thermal treatment, could produce a higher degradation of anthocyanins in the whole fruit (Fig. 2).

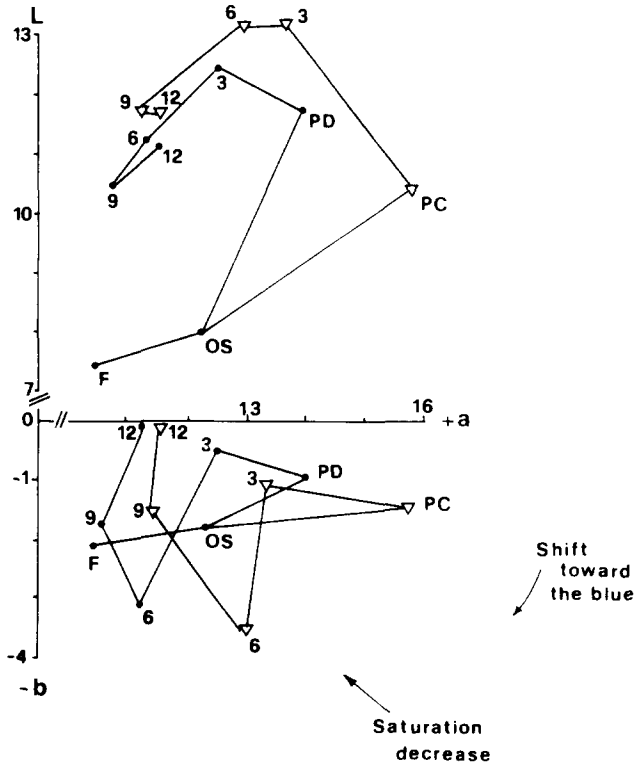

Fig. 2. L, a and b changes during processing and storage.

Table 2. Browning index

	Length of storage (months)				
	0	3	6	9	12
PC	29·0	43·5	42·5	63·2	87·6
PD	28·3	47·2	57·5	75·7	90·9

Storage

During storage, total anthocyanin content constantly decreased. Anthocyanins of PD cherries diminished by 75% in 12 months, whereas the PC cherries showed a lower decrease (65%), as shown in Fig. 1.

The objective colour data, reported in Fig. 2, illustrate that, over the storage period, both PC and PD cherries had stable lightness values and a slight and constant decrease of saturation. From 3 to 6 months storage, hue increased, indicating a shift of the colour towards the blue region. After this period, the cherry colour gradually turned back to red. Therefore, at the end of the storage, the cherries had a hue value similar to the initial one, in spite of the decreased anthocyanin content. The colour change from purple to red can be attributed to a browning process leading to yellow or brown compounds. The presence of browning compounds, which had the maximum of adsorbance at 420 nm, is confirmed by the values of browning index shown in Table 2: the index was about 26 in fresh cherries (Harborne, 1958), and increased after 6 months of storage, confirming the appearance of these compounds, especially in PD cherries. Furthermore, during the clean-up (on reverse phase) of anthocyanin extracts before TLC, a large brown band was seen on the column after eluting the red pigments.

Because of the pH stability of the fruit during processing and storage (pH = 3·8), the observed changes could not be attributed to this parameter.

The ΔE values, shown in Table 3, decrease after 6 months, confirming the return of the cherry colour to the initial red.

Sensory evaluations

As seen in Table 4, just after processing, the intensity of the cherry colour and the colour uniformity were judged high, and PC cherries showed significantly higher values than PD cherries. The colour was acceptable for both the PD and PC cherries. During the first 6 months, PC colour uniformity decreased and that of PD remained stable. Because of the change of the cherry colour from the red to the blue region, acceptability of the cherries decreased. After 6 months, judgements were stable; the cherries' colour was then judged fairly acceptable, with a preference for PC products.

Table 3. ΔE values

	Length of storage (months)			
	3	6	9	12
PC	3·1	3·8	4·0	3·6
PD	2·0	3·6	3·6	3·0

Table 4. Sensory evaluation of cherry colour

	Length of storage (months)				
	0	3	6	9	12
Intensity					
PC	6·8a	6·0a	4·9	4·5	5·0a
PD	6·1b	3·9b	4·3	4·1	4·2b
Uniformity					
PC	6·5a	6·4a	5·5	4·6	5·1
PD	4·9b	4·2b	5·4	4·9	5·0
Acceptance					
PC	5·9	5·9a	5·0a	4·4	4·5a
PD	5·5	4·0b	4·2b	4·1	4·0b

Values in a column followed by different letters are significantly different ($P \leq 0{\cdot}05$).

Anthocyanin composition

TLC of the extracted pigments showed three major bands and a minor one corresponding to the anthocyanins usually present in sweet cherry: Cy-3-glucoside, Cy-3-rhamnoglucoside, Pn-3-rhamnoglucoside and Pn-3-glucoside. The cyanidin pigments are purple, whereas peonidins are reddish. Figure 3 shows the quantitative changes of the two predominant anthocyanins, Cy-3-

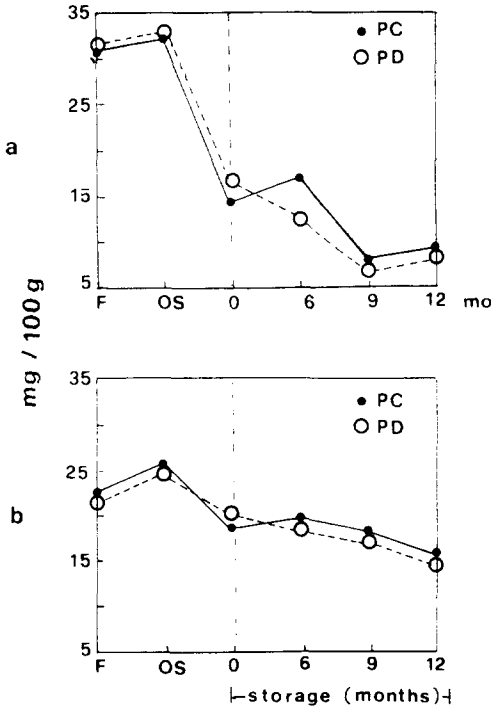

Fig. 3. Anthocyanin composition of fresh and processed cherries: (a) Cy-3-rhamnoglucoside; (b) Cy-3-glucoside.

rhamnoglucoside and Cy-3-glucoside, during processing and storage. The Cy-3-rhamnoglucoside content of fresh cherry agreed with the data reported by Pizzocaro *et al.* (1979) for the same cultivar. Pigments separately analysed showed the same behaviour as the total anthocyanin. The maximum decrease was observed during the thermal process: Cy-3-glucoside diminished about 50% and Cy-3-rhamnoglucoside about 20% with respect to the values of osmodehydrated cherry. After 12 months of storage the Cy-3-rhamnoglucoside decreased more (40%) than Cy-3-glucoside (20%). This could be due to the fact that Cy-3-rhamnoglucoside could form Cy-3-glucose during degradation through partial hydrolysis.

CONCLUSION

Results of anthocyanin content and objective colour analysis suggest that the osmotic dehydration process contributes slightly to the lightening of the red colour in processed cherries. Thermal treatments caused a loss in anthocyanin content by 50% (dry weight) and a further fading, which was more evident in the PD cherries. The higher browning index of PD cherries during storage suggests an influence of the pasteurization method on the anthocyanin degradation. Even if a decrease in anthocyanin content and formation of browning compounds occur during storage, no substantial differences in hue values are produced because of the opposite colour effect of these two phenomena. Therefore, until the end of the storage time, the cherry colour was judged fairly acceptable, with a preference for PC products.

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