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Preservation factors and processing effects on anthocyanin pigments in plums

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The use of a combination of preservation factors has been suggested as a viable alternative to minimally process fruits and preserve them for practical periods of time. Although microbiological stability is the main goal, in the case of prunes color preservation is also of great concern. Prunes (*Prunus domestica* L.) of the 'Angeleno' variety were employed. The fruits were cut in half and the seeds removed. A treatment of 150 s under steam resulted in the permanent inactivation of browning enzymes. The treated fruit was stored for 90 days in systems ($a_w = 0.98$ with sucrose and 0.1% benzoate) at three different pH values: 2.95, 3.45 and 3.95. After 90 days of storage at room temperature, 77% of the original anthocyanin remained at pH 2.95, but only 29% at pH 3.45 and 8% at pH 3.95. There was also an increase in the degradation index with time and with increasing storage pH. Color density values decreased as a result of anthocyanin degradation and not because of browning reactions. A marmalade prepared using the pH 2.95 preserved prunes was stored at room temperature for 90 days. An additional 27% of the original anthocyanin was destroyed, but the degradation index increased only slightly and remained constant throughout the storage period. Color density values remained very low. No microbial growth occurred in the stored prunes or marmalade, and the marmalade was classified organoleptically as good. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Anthocyanins (ACY) are flavonoid phenolic compounds found naturally in anthocyanoplasts of different plant tissues that express orange, red, pink, mauve, blue, violet and black colors. More than 240 different ACY have been identified. They show differences in the degree of hydroxylation, methoxylation, glycosylation and acylation that have a direct effect on the color expressed and the stability of the pigment (Wagner, 1982; Harborne, 1988; Strack & Wray, 1989). Other factors affecting color expression and stability are the associations of the ACY molecules with other flavonoids, including itself, with hydroxycinnamic acids or with metals, as well as the pH of the system (Brouillard, 1982).

As long as the plant tissue remains intact, the ACY pigments remain stable, but their stability is strongly affected during processing and storage. The highest susceptibility is towards changes in pH, and the color-expressing flavylium ion remains more or less stable at very low pH and degrades very quickly, forming chalcones with increasing pH (Brouillard, 1982). The rate of pigment degradation increases with temperature, presence of light, hydrolysis to aglycones, use of sulfite or as a result of enzymatic or non-enzymatic browning (Markakis, 1974; Wesche-Ebeling & Montgomery, 1990). ACY are not apparently degraded directly by polyphenoloxidase (Wesche-Ebeling & Montgomery, 1990), but they seem to co-polymerize as quinoidal bases during the browning reactions and still express a red color as polymeric ACY (pACY; Somers, 1971; Little, 1977; Ribereau-Gayon *et al.*, 1983). This mechanism of color formation has been studied in red wines, but it has been suggested that similar reactions may be occurring in other ACY pigment containing processed foods such as marmalades and juices (Wesche-Ebeling & Montgomery, 1990). ACY pigment mixtures from tart cherries were found to be stable at room temperature when stored as powders with dextrin (Chandra *et al.*, 1993).

Plum is a very important ACY containing fruit in the temperate regions of the world. As with most agricultural resources, the main problem is the short harvesting period, which forces the producer to get the product to the market or storage facilities quickly. In developing countries, where transportation and storage facilities are not always adequate or affordable, a

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different strategy has to be followed in order to minimize fruit losses. The use of the hurdle or combined-methods technology has been studied as an affordable alternative for the minimum processing and preservation of fruits (CYTED, 1993–1994). This technology could be implemented on site and is based on the minimal use of a combination of several hurdles such as heat processing, storage temperature, a_w, pH, redox potential, salt, additives, preservatives, modified atmosphere, microbially derived hurdles and packaging (Bogh-Sorensen, 1994). The stabilized fruit that is obtained can be consumed as is or used as a raw material.

The aim of this study is to observe the effectiveness of the use of hurdle technology processes at different pH values for the stabilization of plums. The effects of the processes on some color characteristics and ACY pigment content will be studied in the stabilized fruit and in a marmalade obtained from it, as well as the microbial stability of the products throughout the study period.

MATERIALS AND METHODS

Fully ripe and firm plums (*Prunus domestica*) of the 'Angeleno' variety were used in this work and were purchased locally.

Scalding procedure

The fruit was washed, halved and the stones were removed manually. In order to obtain the optimum scalding time, lots of 100 g of the plum halves were placed in a sieve and held for different times (1, 2, 3, 4, 5, 6, 7 and 8 min) under steam.

Residual peroxidase activity was evaluated. Twenty-five grams of the treated fruit were homogenized in a Waring blender in 50 ml of cold 0.01 M potassium phosphate buffer pH 6.2. The homogenate was filtered, centrifuged (5000 rpm) for 5 min and the supernatant (crude enzyme extract) was kept on ice until used. For the enzyme assay a fresh substrate solution (2% guaiacol, 4.8% H_2O_2 (3% solution), 10.3% ethanol in 0.01 M potassium phosphate buffer pH 6.2) was prepared. The assay was performed by thoroughly combining 29 parts of the crude enzyme with 1 part of the substrate solution, both at 30°C, placing the cell in a spectrophotometer (Shimadzu 160) and recording the initial velocity from the change in absorbance at 470 nm; substrate solution alone was used as a blank.

Combined-methods model systems

All combined-methods model systems contained 0.1% sodium benzoate and were adjusted to an a_w of 0.98 by the addition of sucrose according to the Norrish equation (Chirife *et al.*, 1980). The a_w was measured using a hygrometer (Decagon CX-1).

Three different model systems were used: pH 3.95, 3.45 and 2.95. Lots of 2 kg of the scalded fruit were placed in double polyethylene plastic bags and immersed in the sucrose syrup in a 2:1 syrup–fruit proportion. All bags were left for equilibrium for 1 week. The systems were divided in three lots: one was left with the equilibrium pH of 3.45, the second lot was adjusted to pH 2.95 with 0.1 N HCl and the third lot adjusted to pH 3.95 with 0.1 N NaOH.

Marmalade production

The plums from the model system showing the best color characteristics and ACY retention after a 90-day storage period were used to produce the marmalade. Fruit halves without the syrup were used and enough sugar was added to increase the soluble solid content to 65° Brix and the final concentration of sodium benzoate was adjusted to 0.1%.

Measurement of ACY content and changes in color parameters

All determinations were made in duplicate and those showing a difference of > 5% were repeated. The samples (fresh plums, syrup–fruit samples and marmalade) were homogenized in appropriate amounts of distilled water. The methods employed for ACY pigment concentration measurement were those described by Wrolstad (1976). The extinction coefficient and molecular weight of cyanidin 3-glucoside were used for the calculations since this is one of the main ACY pigments reported to be present in plums (Van Buren, 1970).

The changes in color in the model systems and the marmalade were followed by measuring the color parameters (degradation index, color density, polymeric color, percentage contribution of tannin, ACY color and ACY degradation) described by Wrolstad (1976).

Microbial analysis

The enumeration of microorganisms was made using standard techniques (Association of Official Analytical Chemists, 1984) and included aerobic plate count, yeast and molds. All analyses were performed in duplicate by using two samples each time, and the averages reported. All bacteriological media were obtained from Merck.

Organoleptic analysis of the marmalade

A sensory evaluation test with a non-structured hedonic scale, from 'like a lot' (10) to 'dislike a lot' (0), was employed using 40 untrained judges. The parameters evaluated were color, smell, taste and texture.

RESULTS AND DISCUSSION

The use of the combined-methods technology process is proposed for the stabilization of plums immediately after harvesting. The main objective of the combined-methods technology is to use a combination of hurdles, each applied at a minimum, in order to sustain the least

Table 1. Residual peroxidase activity after steam-scalding at different times

Time (min)	Residual peroxidase activity (%)
0	100
1	73
2	69
3	0

loss in organoleptic quality of the raw material (Leistner, 1994). The hurdles employed in this study were a scalding heat treatment, a low pH, an a_w to 0.98 (with a sucrose syrup) and the addition of sodium benzoate as a preservative. The changes in the quality of the treated fruit were studied following the changes in ACY content and in certain color parameters.

Scalding of the plums

The plum halves were scalded under steam for different periods of time and the effectiveness of the process was evaluated by following the inactivation of peroxidase (POX). This enzyme was assayed since it has been reported to be resistant to high temperatures, and its inactivation would guarantee the destruction of polyphenol oxidase (PPO) (Richardson & Hyslop, 1985; Williams *et al.*, 1986). PPO is responsible for browning reactions and would mask the red color of ACY and could also cause co-polymerization of the pigment during the browning reactions (Wesche-Ebeling & Montgomery, 1990).

It was observed that no POX activity was detected after 3 min of steam-scalding (Table 1), and no enzyme re-activation was noticed after 24 h of storage of the extracts treated for 3 min at room temperature. The effectiveness of the scalding process was apparent visually. The samples treated for less than 3 min became completely brown after 24 h, while those treated for between 3 and 5 min showed a dark-red color. It was also observed that the longer heat treatments caused adverse effects on the texture. Since PPO is more heat-sensitive than POX, a scalding time of 2.5 min was chosen in order to sustain the least amount of texture loss due to heat.

ACY and color changes in the combined-methods model systems and the marmalade

The effect of three different pH values on the stability of the ACY pigments was studied. After an equilibration time of 1 week, the fruit–syrup systems showed a pH of 3.45. The fruit–syrup system was divided into three lots: one left at pH 3.45, the second adjusted to pH 2.95 and the third adjusted to pH 3.95.

Figures 1 and 2 show the changes in ACY concentration in these systems in 90 days. Figure 1 shows ACY concentrations determined by the differential pH method (pH_d; Wrolstad, 1976), which are lower than those determined by the single pH method (pH_s; Fig. 2). This can be explained since pH_s determines concentrations calculated from the absorbance at λ_{max} for the ACY (cyanidin 3-glucoside in this case) at pH 1, while pH_d subtracts the absorbance at this λ_{max} due to other compounds measured at pH 4.5.

The pH of the system had a great effect on the stability of the pigment, and the highest remaining ACY levels occurred at lower pH. After 90 days, almost no pigment remained in the pH 3.95 system, 9% of the original levels was present in the pH 3.45 system and 44% in the pH 2.95 system. The results are in agreement with the stability of ACY that was observed at low pH in tart cherries (Chandra *et al.*, 1993). Also not all the remaining red color in the systems originated from monomeric ACY (mACY), since the pH_s methods showed high absorbance at λ_{max} due to other red-colored compounds, such that 19% of the red color remained in the pH 3.95 system, 20% in the pH 3.45 system and 42% in the pH 2.95 system. This behavior seems to indicate that, although loss of red color due to the degradation of mACY was occurring, some of the red color was recovered, possibly due to the incorporation of ACY into the forming oligomers and polymers

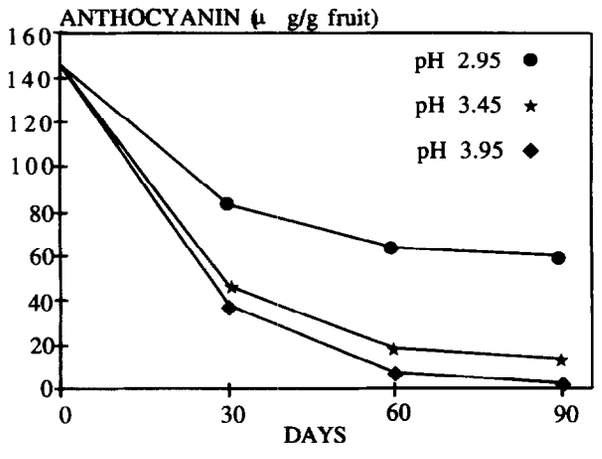

Fig. 1. Changes in ACY pigment content (single pH method) with time in different combined-methods model systems at three different pH values.

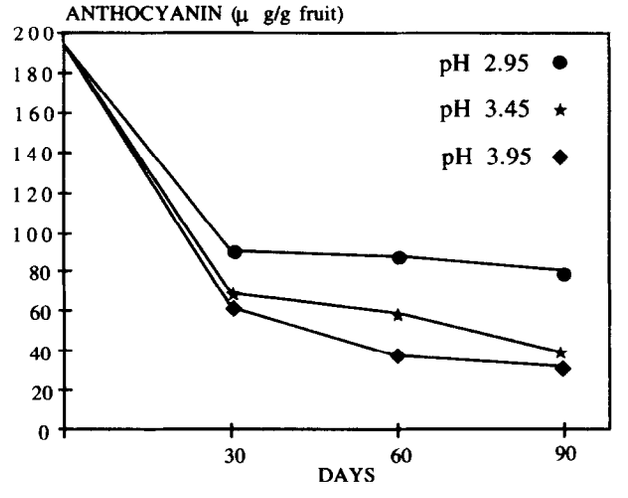

Fig. 2. Changes in ACY pigment content (differential pH method) with time in different combined-methods model systems at three different pH values.

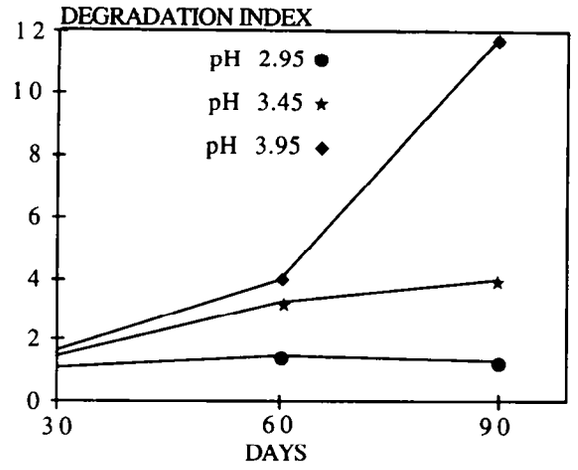

Fig. 3. Changes in the degradation index with time in different combined-methods model systems at three different pH values.

as quinoidal bases which retain a red color (Somers, 1971). Fuleki & Francis (1968) formulated a degradation index ($DI = pH_s/pH_d$) which closely follows the degradation of the ACY pigments. This can be seen in Fig. 3 for the combined-methods system, where the pH 2.95 and 3.45 systems showed a low and regular DI, while the pH 3.95 system showed a very large DI.

It is also very important to consider that another source of the pigment loss could be due to the leaching of ACY into the syrup. Visually the color loss in the fruit halves was not apparent, but if one considers the pH 2.95 as chemically stable for the ACY (Fig. 2), then approximately 41% (83 μg g^{-1} fruit out of 146 μg g^{-1} fruit) of the monomeric pigment could have been lost due to leaching, but the remainder of the pigment losses can only be attributed to pigment degradation or co-polymerization.

Since the pH 2.95 system showed the highest pigment stability and lowest color loss, the plums from this system were used for the manufacture of the marmalade. The losses of mACY pigments were very low throughout the 90-day storage period (Table 2). After 90 days the marmalade lost 73% of the ACY pigment present in the fresh fruit, but only an additional 38% of that present in the combined-methods pH 2.95 system stored for 90 days. The DI increased very slowly, but the effect of processing into marmalade did not degrade the mACY as much as the higher pH values did (Figs 2 and 3).

The marmalade was subjected to additional color parameter measurements (Wrolstad, 1976). During the processing and storage of ACY-containing fruits, several color-degrading reactions are taking place. One set of reactions could be grouped as browning reactions, and could be of enzymatic or non-enzymatic origin. These browning reactions (Maillard, ascorbic acid degradation and/or PPO activity; Abers & Wrolstad, 1979; Debicki-Pospisil *et al.*, 1983; Wesche-Ebeling & Montgomery, 1990; Brouillard, 1982) result in the formation of brown pigments with λ_{max} at 420 nm and in the ultraviolet. When ACY pigments are present during the browning reactions, they seem to be co-polymerized and form part of the polymer, but retain the ability to express red color in their quinoidal base form (Somers, 1971). Therefore, two entities with the capacity of absorbing light at 520 nm may be present at the same time: monomeric ACY (mACY) and co-polymerized or polymeric ACY (pACY). Somers (1971) reported that the mACY could be bleached with bisulfite, whereas the pACY was resistant to bleaching.

One color parameter studied was color density (CD), which expresses the total color contribution of mACY, pACY (both absorbing at 520 nm) and brown pigments (absorbing at 420 nm). Contrasting with CD, the presence of polymeric color (PC) was also determined by subtracting the color contribution of mACY by bleaching it. The ratio of PC to CD was also determined, giving as a result the percentage contribution of tannin (%T), where tannin represents all of the polymer present.

A constant decrease in CD and an increase of PC with storage time was noticed. These changes are summarized by the steep increase in %T. The results indicate that, although the quantitative losses in mACY were somewhat low (38%), most of the lost mACY seemed to have been transformed into pACY, thus having a low effect on red color.

The last color parameter measured was the ratio of λ_{max} for the ACY (520 nm) to λ_{max} of the brown polymer (420 nm), called also degradation of ACY (DA). As the brown polymer concentration increases, it starts to mask the red color due to ACY, resulting in low DA values. The results for DA indicate a slow increase during the storage period. Although a decrease in DA was expected, this increase may confirm the incorporation of the mACY into the polymer as pACY during the storage period, thus having little impact on the loss of the desired red color.

Table 2. Changes in color parameters in the marmalade obtained from the pH 2.95 combined-methods model system

Parameter	0 days	30 days	60 days	90 days
Anthocyanin (ACY) content				
μg g^{-1} fruit, single pH method	194	81	64	52
μg g^{-1} fruit, differential pH method	146	64	49	39
Degradation index	1.42	1.29	1.30	1.34
Color density	0.46	0.21	0.19	0.16
Polymeric color	0.02	0.09	0.11	0.20
Contribution of tannin (%)	0.04	0.42	0.56	1.23
ACY color	0.24	0.06	0.04	0.02
ACY degradation index	0.80	1.09	1.16	1.30

Microbiological stability

No spoilage due to microbial growth was detected during the 90-day storage periods of either the combined-methods model systems or the marmalade. Although the presence of molds and yeasts was detected in the marmalade at the end of the study, the hurdles employed (e.g. scalding, low pH and sodium benzoate) were sufficient to prevent their growth during storage.

Organoleptic analysis

The marmalade was subjected to an organoleptic analysis by 40 untrained judges using an unstructured hedonic scale from 'like it a lot' (10) to 'dislike a lot' (0). Smell received the highest score (93) followed by taste (84) and color (82). Texture received a score of 55, indicating an undesired loss in firmness, possibly due to prolonged exposure to heat during the manufacture of the marmalade.

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