The Effect of High Hydrostatic Pressure on the Strawberry Anthocyanins

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Color stability of fruit juice made from strawberries (*Fragaria* × *ananassa*, cv. Elsanta) that were subjected to high hydrostatic pressure was studied by measuring the anthocyanin content. High hysrostatic pressure is a method of preservation of food alternative to heat treatment. It is therefore essential to assess the impact of high pressure on color molecules. Samples were pressurized under 200, 400, 600, and 800 MPa for 15 min at a temperature controlled between 18 and 22 °C. After application of pressure, the anthocyanin content of the strawberry juice was analyzed by HPLC−UV using a novel isocratic elution system. The high-pressure treated samples were kept at refrigerator temperature (4 °C), room temperature (20 °C), and 30 °C. Two pigments were identified and quantified: pelargonidin 3-glucoside and pelargonidin 3-rutinoside. The highest stability of the anthocyanins was found when strawberries were stored at a temperature of 4 °C. High-pressure treatment at 800 MPa led to the lowest losses, at 4 °C.

Keywords: *Strawberry; high pressure; anthocyanins; color*

INTRODUCTION

The first characteristic of food that is noticed is its color, and this predetermines our expectation of both flavor and quality. Color will influence our appreciation of foods, our judgment on the aesthetic value, and in the case of fruits on the apparent level of sweetness as well. It is thus important not to underestimate the influence that color has on consumers (Hunt, 1991).

The color of fruits is due to the presence of anthocyanins, which are one of the most broadly distributed pigment groups in the plant world and the largest group of water-soluble pigments (Timberlake, 1981). Twenty anthocyanidins are known, but only six (pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin) are important in food. The structural unit of anthocyanins is the 2-phenylbenzopyrylium of flavylium salt. Anthocyanins exist as glucosides of polyhydroxy and/or polymethoxy derivatives of the salt. Anthocyanins differ in the number of hydroxyl and/or methoxy groups present; the types, numbers, and sites of attachment of sugars to the molecule; and the types and numbers of aliphatic or aromatic acids that are attached to the sugars in the molecule. In strawberry (*Fragaria* × *ananassa*, cv. Elsanta), the two anthocyanins present are derivatives of pelargonidin (Figure 1) (Fennema, 1996).

During this work, we have focused on cultivated strawberry, which is a quite poor source of anthocyanins, containing only 30−40 mg/100 g fruit (Sondheimer and Kertesz, 1948). In addition to the important contribution of anthocyanins and color to food, recent work has suggested the involvement of anthocyanins and their breakdown products in pain relief and cancer prevention (Nair, 1999).

HPLC with either UV or photodiode array (PDA) detection has been reported as the most suitable method for analyzing anthocyanins in fruits (Mori and Sakurai, 1994; Versari et al., 1994). In all the papers, elution of anthocyanins was performed using a gradient mobile phase (Garcia-Viguera et al., 1997). In this paper, we report the HPLC analysis isocratically. Analysis reported here is quick (less than 70 min), efficient, and easy to run.

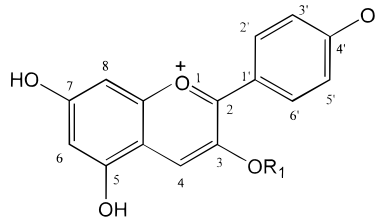

Figure 1. Structure of pelargonidin-3-glucose (R_1 = glucose) and pelargonidin-3-rutinoside (R_1 = rutinose).

High pressure is used as an alternative preservation method to heat treatment (Gould, 1995). Despite the fact that high pressure jam has been commercially available in Japan for some years (Watanabe et al., 1991), the effect of high pressure on anthocyanins has not been studied. This lack of information on the effect of high pressure on specific molecules in the food matrix prompted us to investigate the changes of the amounts of anthocyanins in strawberries after the application of different levels of high pressure.

MATERIALS AND METHODS

All solvents used were of HPLC grade and purchased from Sigma, Gillingham, U.K.

Anthocyanin extraction solvent was made by mixing methanol, acetic acid, and water in ratios of 25:1:24 (solution A). Anthocyanin dissolution solvent was made by mixing formic acid 88% (50 g) with methanol (150 mL), and then the volume was made up to 1 L with distilled water (solution B).

High-Pressure Treatment. Strawberries were subjected to four different high-pressure treatments: 200, 400, 600, and 800 MPa for 15 min at a temperature of 18−22 °C. The range

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10.1021/jf9911085 CCC: $19.00 © 2000 American Chemical Society
Published on Web 06/16/2000

Table 1. Levels of Pelargonidin 3-Glucoside As Determined in Eight Individual Analyses of the Same Sample

concn of pelargonidin 3-glucoside (mg [100 g]$^{-1}$ of strawberry)								avg concn	stand-ard devn	coeff var-iation
23.6	22.6	21.7	22.5	22.6	23.2	25.0	21.9	22.9	1.06	4.6%

of pressure and temperature treatments used commercially are 400−600 MPa at 18−22 °C. The pressure treatments in this study have, therefore, been to chosen to include and extend the commercially used range of pressures. After this treatment, samples were stored at 4 °C, room temperature (20 °C), or 30 °C. Anthocyanin content was analyzed on both untreated (blank) and high pressure treated strawberry samples. Analysis was carried out immediately after the high-pressure treatment and after 1, 2, 4, or 5 and 7 or 9 days of storage. Each time, the levels of both pelargonidin 3-glucoside and pelargonidin 3-rutinoside were analyzed in triplicate.

Anthocyanin Extraction. Strawberry fruit (5 g) was extracted by stirring with solution A (38 mL) for 5 min at room temperature. The extract was initially prefiltered through muslin and was then filtered (Whatman 1 paper filter). The filtrate was concentrated under vacuum (45 °C) and the residue redissolved in 10 mL of aqueous formic acid (50 g L^{-1}) containing methanol (150 mL L^{-1}). The resultant sample was then diluted with solution B as necessary, before HPLC analysis.

HPLC Analysis. Acetonitrile (83 mL), methanol (33 mL), and acetic acid (170 mL) were mixed with trichloroacetic acid (0.65 g) that was previously dispersed in water. Volume was completed to 1 L with distilled water. This HPLC solvent (solution C) was then degassed.

HPLC analysis was carried out using a Pye Unicam (Cambridge, U.K.) liquid chromatograph equipped with a LC3 variable-wavelength UV detector and a Hewlett-Packard 3394 integrator. A Partisil octadodecylsilane (C_{18}) (250 × 4.6 mm) with particle size 5 μm (Phenomenex, Macclesfield, U.K.) was used. The sample loop was 20 μL with an isocratic flow rate of 1 mL min^{-1}. Detection was carried out at 520 nm. All analyses were carried out in duplicate and injected at least twice; the results are expressed as mean values.

RESULTS AND DISCUSSION

Effect of Freezing on the Anthocyanin Content of Strawberries. To evaluate the effect of storage at −25 °C on the anthocyanin content of fresh strawberries, fresh fruits were frozen at that temperature, stored for 1 week, and allowed to thaw before analysis. Stored fruits were analyzed, and the pigment levels were compared to those of fresh fruits (Figure 2). There was very little difference in the levels of anthocyanin content of fruits.

Reproducibility of Extraction. To assess the reliability of the extraction method applied in this series of experiments, eight independent extractions were carried out from the same batch of fruit puree. Levels of pelargonidin 3-glucoside extracted are shown in Table 1. It can be seen that a percentage standard deviation of 4.6% from the mean was achieved. We considered this result to be acceptable repeatability for this type of biological assay.

Effect of the High-Pressure Treatment on the Anthocyanin Content of Strawberries. *Study of the Anthocyanin Content at Refrigerated Temperature.* After 9 days of storage, there is little difference between the pelargonidin 3-glucoside contents of high-pressure treated strawberries and the untreated ones (15−20% loss). However, during the first 4 days, the losses after a high-pressure treatment of 800 MPa are negligible. We can note that after a high-pressure treatment of 400 MPa

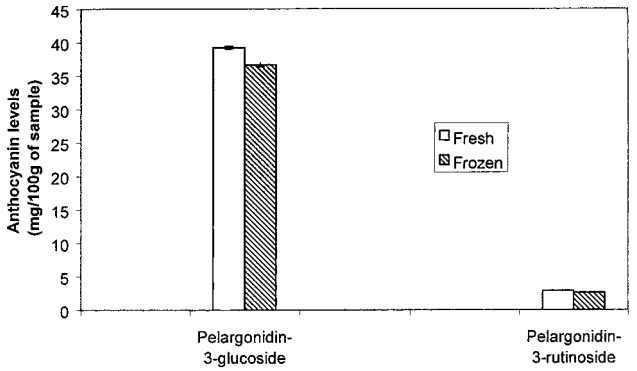

Figure 2. Anthocyanin content of fresh and frozen strawberries.

the initial losses of pelargonidin 3-glucoside are at a faster rate (20% in 2 days) compared to the other samples (5−10%, in 2 days) (Figure 3). In the case of the treatment of 800 MPa, a decrease in the loss of anthocyanin is observed between 1 and 4 days. This could be explained on the basis that the biosynthetic pathway for this color molecule could be activated following treatment at this very high pressure.

Examination of the losses of pelargonidin 3-rutinoside shows that, after 9 days of storage, they are quite similar in untreated strawberries and high-pressure treated ones at 600 MPa (25%). Smaller losses are shown in the high-pressure treated samples at 200 and 800 MPa (10%). Similar to pelargonidin 3-glucoside, with the pelargonidin 3-rutinoside both initial rate of loss and total losses after 9 days were seen to be highest when strawberries were subjected to 400 MPa (Figure 4). In the case of the treatment of 800 MPa, the observed decrease in the loss of anthocyanin that was found between 4 and 9 days could be explained as for pelargonidin 3-glucoside on the probable activation of its biosynthesis following treatment at this very high pressure.

Study of the Anthocyanin Content at Room Temperature (20 °C). When the high-pressure treated and the untreated samples were stored at room temperature, little differences were found in the pelargonidin 3-glucoside losses regardless of whether the samples were untreated or treated at any of the pressures investigated.

Pelargonidin 3-glucoside is degraded after the first day of storage. The initial rate of degradation is the highest after a 600 MPa treatment, followed by that after 400 MPa treatment. The 800 MPa samples show the slowest initial rate of losses. Total losses after 7 days, however, show little difference for any condition (all at 50−55% total loss) (Figure 5). The differences between 400, 600, and 800 MPa treatments could be explained on the basis of residual β-glucosidase activity in the fruits after the high-pressure treatment. It was found that β-glucosidase activity was higher in strawberry after a high-pressure treatment at 400 as opposed to residual enzymatic activity after treatments at 200, 600, and 800 MPa (Zabetakis et al., in press). The loss of pelargonidin 3-glucoside due to β-glucosidase activity might be the main reason for the different levels of losses of this anthocyanin in the high-pressure treated samples.

In the case of pelargonidin 3-rutinoside, during the first week of storage, the rates of losses are similar for 400 and 600 MPa treated and untreated strawberries.

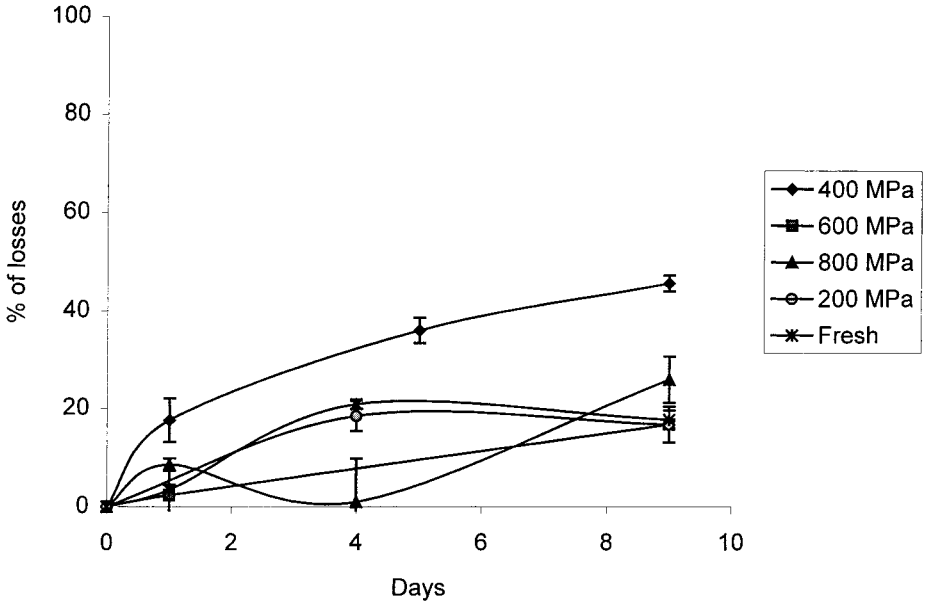

Figure 3. Profile of the losses of pelargonidin 3-glucoside with storage time at fridge temperature, at each high-pressure condition investigated.

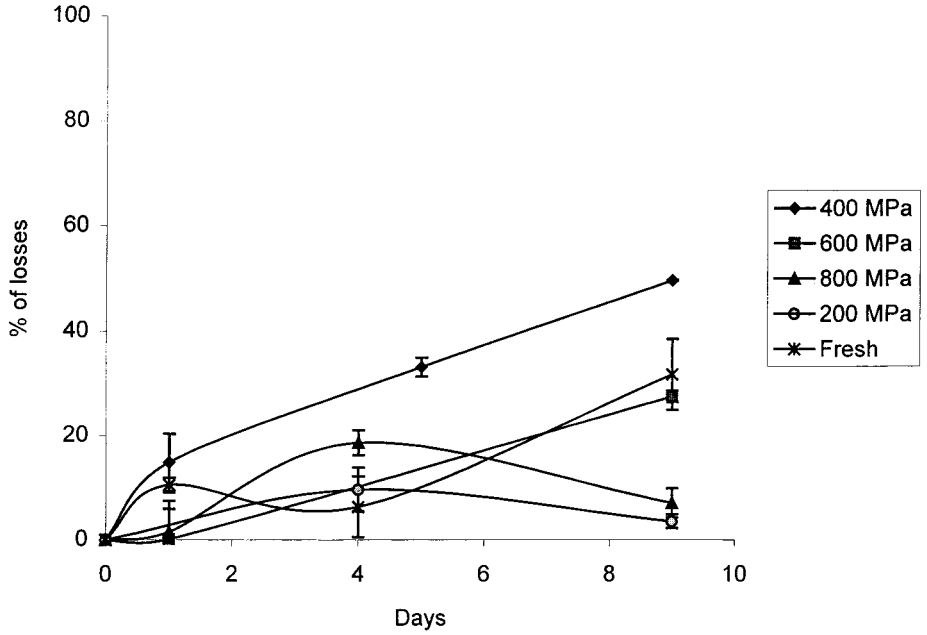

Figure 4. Profile of the losses of pelargonidin 3-rutinoside with storage time at refrigerator temperature, at each high-pressure condition investigated.

Losses are quite extensive as after 1 day of storage 20% losses were incurred, with total losses after 7 days being approximately 50%. In contrast, the 200 and 800 MPa treated strawberries exhibit only slight losses of pelargonidin 3-rutinoside during the first 2 days, and their losses are less extensive showing a total loss after 7 days of only 25% (Figure 6). The different levels of losses between pelargonidin 3-glucoside and pelargonidin 3-rutinoside could be explained in terms of substrate specificity of β-glucosidase acting on these anthocyanins. The specificity of β-glucosidase for pelargonidin 3-glucoside should be higher than that for pelargonidin 3-rutinoside, and therefore, the breakdown of the glucoside would be more extended to that of the rutinoside (Timberlake, 1981).

Study of the Anthocyanin Content at 30 °C. With storage at 30 °C, there are only slight differences in total losses of pelargonidin 3-glucoside after 7 days (70−80%). However, the initial rate of loss is the highest at 400 and 600 MPa. The 200 MPa samples can be seen to show the lowest losses of pelargonidin 3-glucoside after 7 days (Figure 7).

With pelargonidin 3-rutinoside in strawberries treated at 200 and 800 MPa, the losses are low during the first 2 days of storage with total losses of 40% after 7 days. In contrast, the 400 MPa treated strawberries exhibit the fastest initial rate of pelargonidin 3-rutinoside losses and the highest total loss after 7 days. Losses exhibited by untreated strawberries are less than those at 400 MPa (Figure 8). In the case of the treatment of 800 MPa, a decrease in the loss of pelargonidin 3-rutinoside was observed between 1 and 2 days. This result may be due to the activation of the biosynthesis of this color molecule because of either the very high-pressure treatment or the elevated storage temperature (30 °C) that could increase the rate of chemical formation.

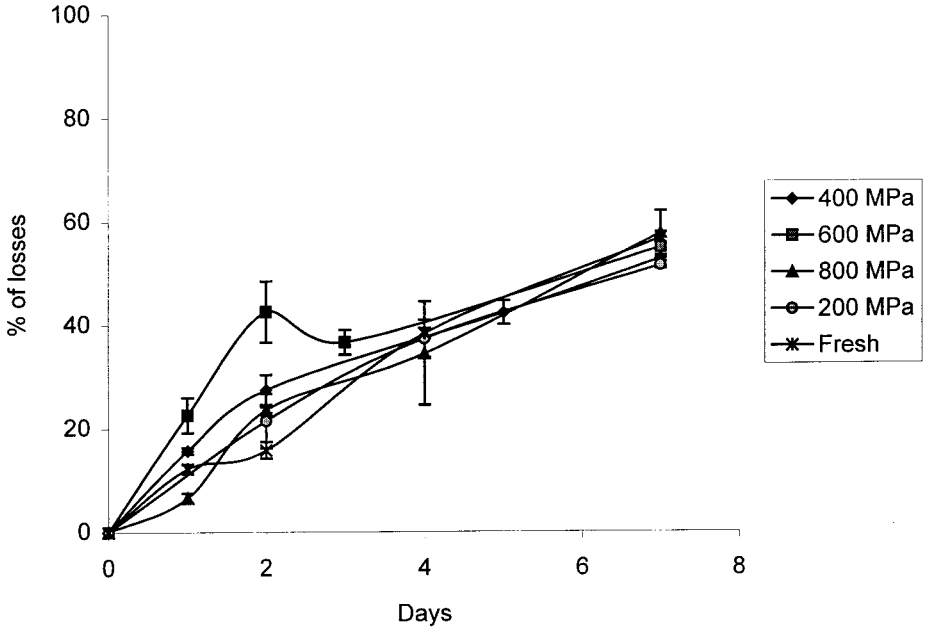

Figure 5. Profile of the losses of pelargonidin 3-glucoside with storage time at room temperature, at each high-pressure condition investigated.

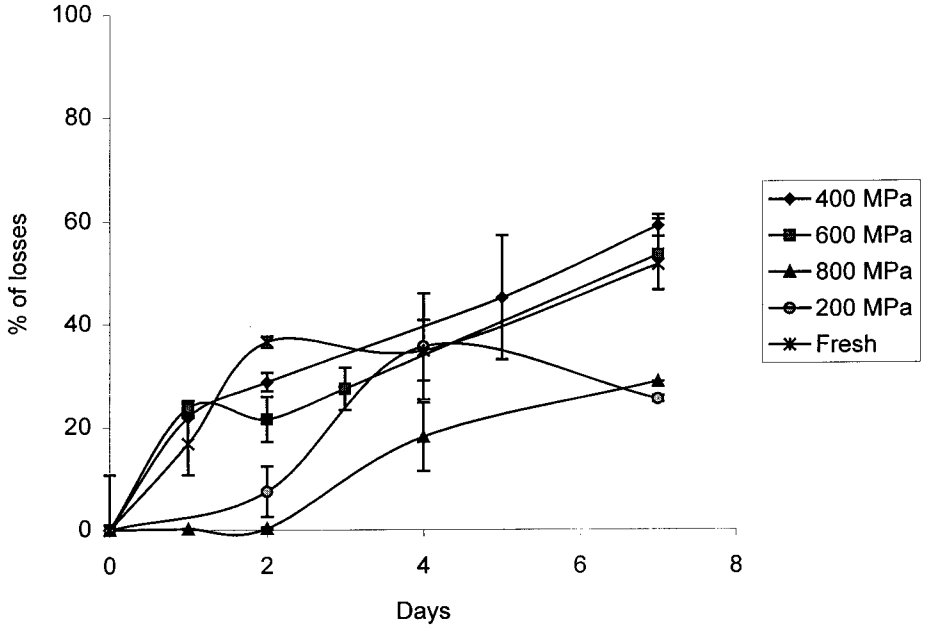

Figure 6. Profile of the losses of pelargonidin 3-rutinoside with storage time at room temperature, at each high-pressure condition investigated.

The degradation of anthocyanins can be influenced by different parameters such as temperature, enzymes, oxygen, and sugar content. In this work, the effects of the high-pressure treatments were most probably mediated because of remaining enzymatic activity. Generally, the activity of enzymes is affected by the environmental temperature. At low temperatures, an increase in the initial velocity or in the true catalytic activity is observed, whereas at higher temperature the enzymatic activity is reduced due to denaturation or degradation of the enzyme. The temperature of 30 °C used in this work is not going to have a great effect on enzyme degradation. Therefore, at 30 °C, higher rates of enzyme mediated losses of anthocyanins would be anticipated since catalytic activity would be at highest.

The enzymes peroxidase, polyphenol oxidase, and glucosidase have been implicated in the degradation of anthocyanins (Fennema, 1996; Cano et al., 1997). High-pressure treatment has a different effect on each of these enzymes, and the remaining total enzymatic activity in strawberry may be difficult to estimate. It has been reported that the peroxidase activity is decreased with high pressure up to 300 MPa for a treament carried out under the same conditions with this work (i.e., 20 °C for 15 min) (Cano et al., 1997). Treatment above 300 MPa slightly increased peroxidase activity at this temperature, whereas complete loss of enzymatic activity was achieved at 900 MPa. Polyphenol oxidase was reported to be strongly diminished with high-pressure treaments up to 400 MPa (Cano et al., 1997). On the contrary, β-glucosidase was reported to

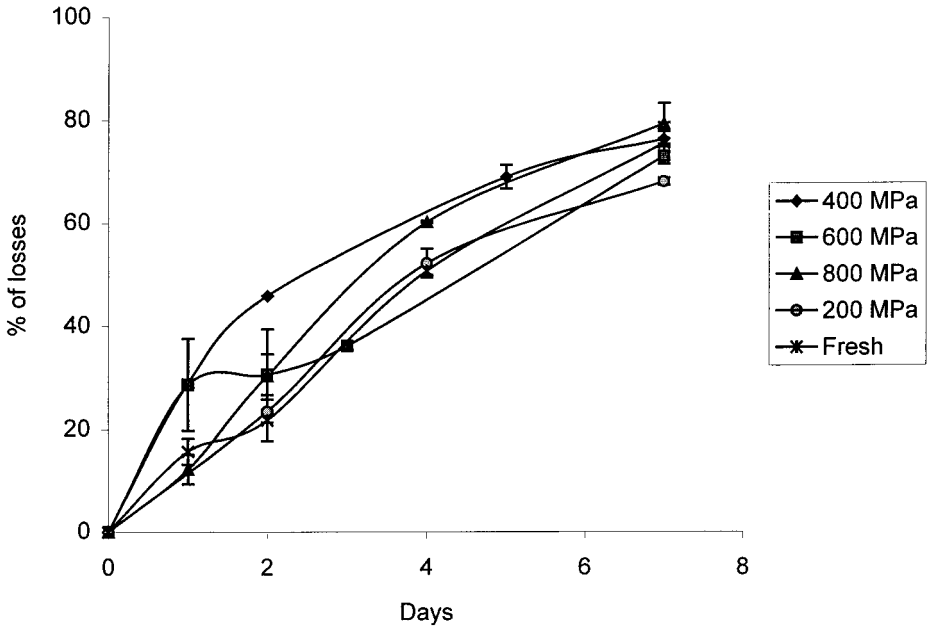

Figure 7. Profile of the losses of pelargonidin 3-glucoside with storage time at 30 °C, at each high-pressure condition investigated.

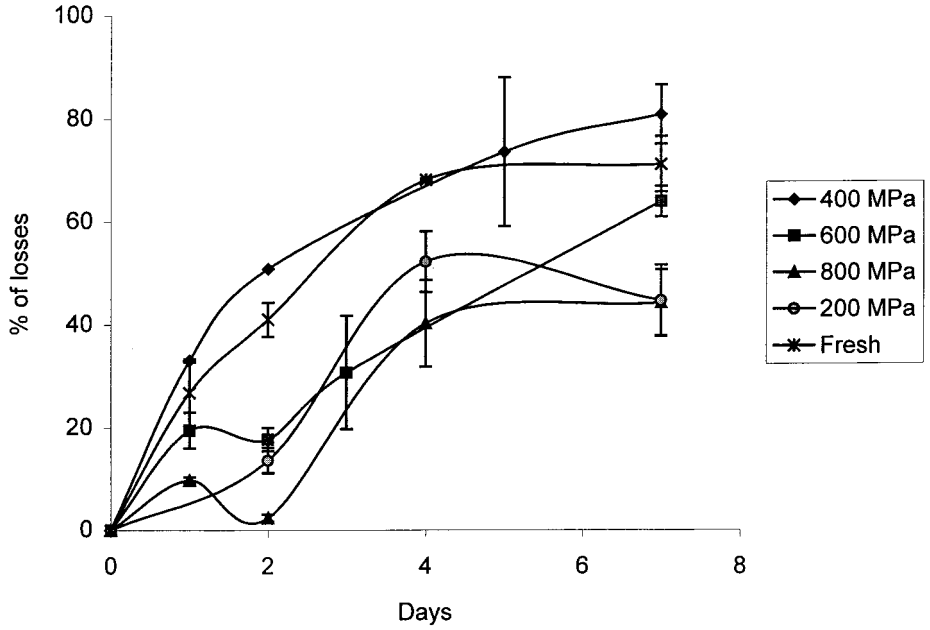

Figure 8. Profile of the losses of pelargonidin 3-rutinoside with storage time at 30 °C, at each high-pressure condition investigated.

have its highest activity after a high-pressure treatment at 400 MPa, and then the activity was decreased as the pressure of the treatment was increased up to 800 MPa (Zabetakis et al., in press).

It may be concluded that, between 400 and 600 MPa, the total enzymatic activity involved in anthocyanin degradation is reported to be at its highest and after 800 MPa at its lowest. Relating these reports to our work presented here, the loss of anthocyanins on the different storage temperatures could be explained. Storage at 4 °C always showed the lowest pigment loss due to lower enzyme activity. The losses of both pigments at 4 °C after a high-pressure treatment of 400 MPa are the highest (i.e., 45% for pelargonidin 3-glucoside and 50% for pelargonidin 3-rutinoside) due to the higher activities of peroxidase, polyphenol oxidase, and β-glucosidase at 400 MPa (Figures 3 and 4). After a high-pressure treatment at 800 MPa, β-glucosidase had a very low activity (Zabetakis et al., in press) and peroxidase had lost all its activity (Cano et al., 1997). These reduced activities could be well related to the lower losses of each of the pigments: after one week of storage at 4 °C only 25% of pelargonidin-3-glucoside and 10% of pelargonidin-3-rutinoside were lost (Figures 3 and 4). In the case of more elevated storage temperatures, the enzymatic activities may be increased. The results obtained at 20 °C and 30 °C, where the highest initial rates of degradation for both pigments were found for 400 and 600 MPa treatments (Figure 5−8) were consistent with the higher enzymatic activities anticipated in these conditions of pressure and storage temperature.

Conclusively, it could be noted that storage temperature is a very influential factor in anthocyanin degradation and loss from strawberry. Some consumers tend to store fruits, preserves, and fruit juices in refrigerators. In that way, high pressure is a factor of a great

interest because in all treated samples, except after a high-pressure treatment of 400 MPa, losses of anthocyanins were diminished when samples were kept at refrigerator temperature. Indeed, strawberries may be stored for a period of at least 4 days without degradation of anthocyanins. The most efficient experimental treatment for anthocyanin conservation in this series of experiments was 800 MPa. However, consumers may store fruits and jams at room temperature, which varies within the year. To study this variant room temperature, further experiments were carried out at 20 and 30 °C. The rate of degradation of pelargonidin 3-glucoside was only slightly changed by high-pressure treatment at storage temperatures of 20 and 30 °C. Pelargonidin 3-rutinoside was more stable after high-pressure treatments at 200 and 800 MPa.

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Received for review October 12, 1999. Revised manuscript received April 4, 2000. Accepted April 26, 2000.

JF9911085