Frozen Storage Effects on Anthocyanins and Volatile Compounds of Raspberry Fruit

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The quantitative and qualitative evolution of the anthocyanins and volatile compounds of four raspberry cultivars (cvs. Heritage, Autumn Bliss, Zeva, and Rubi) growing in Spain were analyzed raw, just frozen, and during long-term frozen storage at −20 °C for a 1 year period. HS-SPME coupled with GC-MS and HPLC techniques were employed to study the evolution of the volatile compouds and the individual anthocyanins, respectively. The volatile aroma composition changes produced by the freezing process and long-term frozen storage were minimal. Only a significant increase in extraction capacity was obtained for α-ionone (27%) and for caryophyllene (67%) in Heritage at 12 months of storage. The stability of anthocyanins to freezing and frozen storage depends on the seasonal period of harvest. Heritage and Autumn Bliss (early cultivars) were less affected by processing and long-term frozen storage (1 year), and the total pigment extracted showed the tendency to increase 17 and 5%, respectively. Rubi and Zeva (late cultivars) suffered a decreased trend on the total anthocyanin content of 4% for Rubi and 17.5% for Zeva. Cyanidin 3-glucoside most easily suffered the degradative reactions that take place during processing and the storage period.

Keywords: *Raspberry; freezing; frozen storage effects; anthocyanin; volatile compound; color; aroma*

INTRODUCTION

Frozen fruits constitute a large and important food group in modern society. Fruit may be more extensively used if it is available during the off-season. Freezing also makes year-round further processing of fruit products, such as jams, juice, and syrups, from frozen whole fruits, slices, or pulps possible.

Sensorial fruit qualities such as color and aroma are the main factors that influence consumer's acceptance. Freezing is one of the most important methods for retaining quality during long-term storage. Factors such as variety, maturity, growing area, and seasonal variations influence frozen processing of fruit to an extent that may override the positive effect of a high freezing rate (Skrede, 1996).

For many processes it is important to know the typical pigment and volatile compound profile of a fresh product in order to identify the color and aroma changes produced during the treatment (Douillard and Guichard, 1990; Ibañez et al., 1998; de Ancos et al., 1999).

Volatile compounds forming the fruit flavor are produced through metabolic pathways during harvest, postharvest, and also storage and depend on many factors related to the species, variety, and type of technological treatment (Rizzolo et al., 1992). Changes in fruit aroma have been used to differentiate among cultivars (Borejsza-Wysocky et al., 1992) and to follow the ripening of a fruit (Macku and Jennings, 1987; Miszczak et al., 1995) as well as the evolution of the quality as a function of the processing and storing process aplied (Douillard and Guichard, 1990; Shamaila et al., 1992). Although freezing is the best way to preserve the fruit aroma (Skrede, 1996), it is well-known that freezing and thawing alter the flavor of fresh strawberries (Ueda and Iwata, 1982; Larsen and Poll, 1995; Guichard, 1982). Similar studies performed with raspberry showed no significant differences between fresh and frozen volatile compound profiles (Guichard, 1982).

Different methods have been used for flavor analysis. The most typically utilized for extraction and preconcentration are headspace techniques (Macku and Jennings, 1987), purge-and-trap (Miszczak et al., 1995), liquid−liquid extraction (Kok et al., 1987), and simultaneous distillation and extraction (Blanch et al., 1991). Methods that avoid the use of organic solvents have been developed in our laboratory for fruit flavor analysis based on headspace coupled to gas chromatography through a programmable temperature vaporizer injector (HS-PTV-GC) (Ibañez et al., 1999), off-line coupling supercritical fluid extraction gas chromatography (SFE-GC) (Ibañez et al., 1997), and headspace solid-phase microextraction (HS-SPME) (Ibañez et al., 1998). These methods have demonstrated their ability for food aroma analysis by reducing the loss of volatile components as well as minimizing sample handling. In the present study, an HS-SPME technique has been used to detect changes in the flavor of raspberry varieties due to freezing and frozen storage.

The red color of the raspberry fruit is related to its anthocyanin composition. Loss of color of raspberry products during processing and storage has been attributed to many factors such as enzymatic reactions,

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Table 1. Physical and Physicochemical Characteristics of Raspberry Fruit Cultivars Harvested in Spain[a]

characteristic	raspberry cultivars			
	Autumn Bliss	Heritage	Zeva	Rubi
fruit wt (g)	3.08 ± 0.59a	2.08 ± 0.29b	3.02 ± 1.14a	2.53 ± 0.65a
color				
L	25.89 ± 2.04a	25.80 ± 1.52a	18.29 ± 0.42b	21.26 ± 0.58ab
*a**	35.03 ± 0.70a	34.98 ± 0.58a	33.03 ± 0.50b	35.10 ± 0.40a
*b**	19.05 ± 1.87a	18.34 ± 2.42a	17.78 ± 1.40a	18.63 ± 2.73a
pH	3.65 ± 0.1a	3.87 ± 0.02b	2.88 ± 0.02c	2.65 ± 0.01d
titratable acidity (g of citric acid/100 g of fw)	1.67 ± 0.01a	1.76 ± 0.01a	1.75 ± 0.05a	2.32 ± 0.12b
soluble solids (°Brix at 20 °C)	9.26 ± 0.14a	9.50 ± 0.06b	10.54 ± 0.05c	10.00 ± 0.09b
moisture content (g/100 g of fw)	84.77 ± 0.11a	85.31 ± 0.63a	83.67 ± 1.53b	82.02 ± 3.01c
total solids (g/100 g of fw)	15.23 ± 0.02a	14.69 ± 0.11a	16.33 ± 0.30b	17.98 ± 0.66c

[a] Values are the mean of three independent determinations ± standard deviation (SD). Different letters in the same row indicate significant differences ($p \leq 0.05$).

ascorbic and other organic acids, sugar products, oxygen, fruit maturity, thawing time, metal ions, light, and temperature and also may be affected by the actual anthocyanin concentration (Wrolstad et al., 1970; Rommel et al., 1990; Boyles et al., 1993; Mazza and Miniati, 1993). Bushway et al. (1992) evaluated five raspberry cultivars harvested at the red-ripe stage at harvest and during long-term frozen storage at −20 °C. In this study the samples were rated by instrumental color analysis and by a sensory panel for color preference. The effect of freezing on instrumental hue and sensory color was not significant for any of the five varieties investigated.

Profiles of the anthocyanin composition of red raspberry juice and wine obtained from several varieties and mainly constituted by cyanidin 3-sophoroside, cyanidin 3-glucorutinoside, and cyanidin 3-glucoside determined by high-performance liquid chromatography (HPLC) have been used to study the effect of processing on the color of wine and juice (Rommel et al., 1990; Wrolstad et al., 1993). The anthocyanin and other related color compounds (vitamin C, organic acids) profile of four Spanish raspberry varieties was characterized by HPLC (de Ancos et al., 1999) to study their suitability for processing. However, the effect of freezing and long-term frozen storage on color composition, using chromatographic techniques (HPLC), has not been previously described.

The goal of the present work was to study the influence of freezing and long-term frozen storage on the anthocyanin and volatile composition, using analytical techniques (HPLC and HS-SPME coupled with GC-MS) as an objective measure of fruit quality, of the four major raspberry cultivars growing in Spain and harvested in different seasonal periods, Autumn Bliss and Heritage in the spring (May) and Rubi and Zeva in the autumn (October). The final purpose was to study their suitability for freezing and long-term frozen storage in order to select the cultivars that are less affected in terms of color and flavor, the most important fruit quality attributes from the point of view of the consumer.

MATERIALS AND METHODS

Plant Material. Raspberry fruits (*Rubus idaeus* L.) of four cultivars (cv. Autumn Bliss, Heritage, Rubi, and Zeva) were obtained from commercial orchards in the region of Valle del Jerte (Cáceres, Spain) and harvested at commercial maturity stage. Autumn Bliss and Heritage were picked in May 1996 and Rubi and Zeva in October 1996. Physicochemical characteristics of raspberries growing in Spain are shown in Table 1. They were received within 12 h after harvest. On arrival, 1 kg of fresh fruit of each cultivar was analyzed and fruits were immediatly frozen at −80 °C in a liquid nitrogen cabinet (SEO, Sociedad Española del Oxígeno, SA, Madrid, Spain) during 15 min. Frozen raspberries were packed in polyethylene bags, sealed, and stored at −20 °C for 12 months.

Samples were removed from the bags to allow semithawing and after 1 h were analyzed at 0, 30, 90, 180, 270, and 365 days of frozen storage.

HPLC Anthocyanin Analysis. Duplicates of all samples were extracted and analyzed for pigment composition using HPLC. The sample preparation, HPLC separation, and identification were carried out according to the procedure described by de Ancos et al. (1999). The identification was performed by chromatographic behavior characterized by HPLC and UV−visible absorption spectra, by comparison with authentic standards, when available, and with data found in the literature. All compounds were quantified as cyanidin 3-glucoside, previously separated by TLC for use as an external standard. A standard curve was prepared by plotting different concentrations of cyanidin 3-glucoside versus area measurements in HPLC.

Extraction and Purification of Anthocyanins. Raspberry sample (10 g) was homogenized with 100 mL of 1% HCl in methanol. The slurry was filtered, and the solids were washed with an additional 100 mL of 1% HCl in methanol. The methanol extracts were combined and concentrated to a final volume of 5 mL in a rotary evaporator (30 °C). The aqueous extract was placed in a volumetric flask and brought to 50 mL with 0.01% HCl solution. The aqueous solution of anthocyanins (4 mL) was adsorbed onto an activated C18 Sep-Pak cartridge (Water Associates, Milford, MA), previously activated with 3 mL of methanol and 3 mL of 0.01% HCl. The pigments absorbed onto the cartridge were washed with 3 mL of water and eluted with 0.01% HCl in HPLC grade methanol. The solution was evaporated to dryness in a rotary evaporator, dissolved in 4% phosphoric acid, and filtered through a 0.45 μm filter, and 20 μL was injected into the HPLC system.

Apparatus. A Hewlett-Packard 1050 quaternary solvent delivery system equipped with a Hewlett-Packard 1040A rapid scanning UV−visible photodiode array detector was employed. The data were stored and processed by means of a Hewlett-Packard model 9000/300 computing system and Colour Proplotter. The absorption spectra of the pigments were recorded between 300 and 600 nm at the rate of 12 spectra/min. The HP-9000 computer with a built-in integration program was used to evaluate the peak area and peak height.

Chromatographic Procedures. Separation was performed on a stainless steel (250 × 4 mm i.d.) ODS-Hypersil (5 μm spherical particles) column (Hewlett-Packard). The mobile phase was composed by solvent A, 4% phosphoric acid, and solvent B, 100% acetonitrile, and the program began with isocratic elution with 6% B from 0 to 10 min, then a linear gradient to 20% of B from 10 to 55 min, and finally an isocratic elution at 20% of B from 55 to 60 min. The flow rate was 1.0 mL/min, and the runs were monitored at 520 nm.

GC-MS Volatile Compound Analysis. *Material.* An SPME holder (Supelco, Bellefonte, PA) was used to perform the experiments. A fused silica fiber coated with a 100 μm layer

Table 2. Effect of Freezing and Frozen Storage on Main Anthocyanins of Spanish Raspberry Cv. Heritage, Determined by HPLC and Quantified as Milligrams of Cyanidin 3-Glucoside per 100 g of Fresh Weight[a]

anthocyanin			frozen storage				
no.	name	raw	0 days	90 days	180 days	270 days	360 days
1	Cy-3- soph	20.95 ± 1.34a	35.57 ± 2.89b	24.51 ± 1.39a	26.13 ± 0.14a	27.56 ± 7.4a	26.41 ± 4.6a
3	Pg-3-soph	2.59 ± 0.35a	2.45 ± 0.64a	1.83 ± 0.32a	1.85 ± 0.32a	1.74 ± 1.47a	2.4 ± 0.56a
4	Cy-3-glc	13.50 ± 0.68a	14.34 ± 0.48a	19.98 ± 1.01a	17.51 ± 0.16a	16.87 ± 4.30a	12.31 ± 2.38a
total		37.04 ± 2.18a	52.33 ± 3.5b	41.68 ± 6.63a	45.50 ± 1.4a	45.36 ± 1.13a	43.34 ± 3.14a

[a] Values are mean ± SD of four determinations. Different letters in the same row indicate significant differences ($p \leq 0.05$). Numbers correspond to HPLC peaks in Figure 1.

Table 3. Effect of Freezing and Frozen Storage on Main Anthocyanins of Spanish Raspberry Cv. Autumn Bliss, Determined by HPLC and Quantified as Milligrams of Cyanidin 3-Glucoside per 100 g of Fresh Weight[a]

anthocyanin			frozen storage				
no.	name	raw	0 days	90 days	180 days	270 days	360 days
1	Cy-3-soph	5.28 ± 1.5a	5.80 ± 0.3a	3.55 ± 0.66a	5.49 ± 2.39a	5.08 ± 0.02a	5.19 ± 0.15a
2	Cy-3-glc rut	6.29 ± 0.12a	7.70 ± 0.97a	5.31 ± 0.26a	4.80 ± 0.9a	6.20 ± 0.27a	6.76 ± 0.55a
4	Cy-3-glc	9.05 ± 0.28a	9.75 ± 0.35a	6.27 ± 0.32a	4.42 ± 1.91b	7.07 ± 0.54a	7.98 ± 0.32a
6	Cy-3-rut	10.53 ± 1.34a	17.62 ± 0.88b	11.79 ± 0.32a	9.44 ± 0.96	10.88 ± 0.54a	12.65 ± 0.22a
total		31.13 ± 0.13a	40.88 ± 1.20b	26.92 ± 1.57a	22.44 ± 3.80a	29.56 ± 2.05a	32.68 ± 2.1a

[a] Values are mean ± SD of four determinations. Different letters in the same row indicate significant differences ($p \leq 0.05$). Numbers correspond to HPLC peaks in Figure 1.

Table 4. Effect of Freezing and Frozen Storage on Main Anthocyanins of Spanish Raspberry Cv. Rubi, Determined by HPLC and Quantified as Milligrams of Cyanidin 3-Glucoside per 100 g of Fresh Weight[a]

anthocyanin			frozen storage				
no.	name	raw	0 days	90 days	180 days	270 days	360 days
1	Cy-3- soph	55.72 ± 2.34a	51.21 ± 2.0a	49.55 ± 2.5a	45.59 ± 1.5b	58.63 ± 2.5a	60.84 ± 3.0a
3	Pg-3-soph	8.77 ± 0.39a	6.43 ± 0.2b	8.02 ± 1.0a	4.08 ± 0.3	6.50 ± 0.6a	8.15 ± 0.3a
4	Cy-3-glc	23.67 ± 0.52a	15.69 ± 1.2b	18.02 ± 1.3a	16.46 ± 1.5b	18.75 ± 1.5a	18.65 ± 1.1a
7	Pg-3-glc	4.23 ± 0.11a	4.47 ± 0.3a	3.30 ± 0.4a	4.73 ± 0.2b	4.17 ± 1.3a	4.87 ± 0.3a
8	Mv-3-glc	3.64 ± 0.13a	nd	nd	nd	nd	nd
total		96.08 ± 2.84a	77.80 ± 2.0b	78.89 ± 3.4b	70.86 ± 2.6b	88.05 ± 2.8a	92.51 ± 3.1a

[a] Values are mean ± SD of four determinations. Different letters in the same row indicate significant differences ($p \leq 0.05$). nd, not detected. Numbers correspond to HPLC peaks in Figure 1.

of dimethylpolysiloxane was chosen to extract the volatile components of the raspberries. One gram of the fruit was cut in four pieces and placed in a 20 mL vial which was capped with plastic film. Extraction was performed by exposing the fiber to the headspace of the sample for 30 min at 30 °C. Conditions had been previously selected (Ibañez et al., 1998).

Instrumental Analysis. A Perkin-Elmer model 8500 gas chromatograph equipped with a PTV injector and an FID detector was used to perform the analysis. The system was coupled to a model 2600 chromatography software system (Perkin-Elmer Nelson Analytical). A 50 m × 0.25 mm i.d. fused-silica capillary column (Chrompack, Middelburg, The Netherlands) coated with a 0.25 μm layer of CP-Sil-5 CB was used. Helium was the carrier gas. Thermal desorption of the compounds in the fiber took place in the GC injector at 200 °C for 15 min in splitless mode for 5 min. The detector operated at 250 °C. The oven temperature was programmed from 50 °C (3 min constant temperature) to 250 °C at 5 °C/min. The final temperature was maintained for 17 min.

GC-MS analysis was carried out by coupling the gas chromatograph described above to a Perkin-Elmer ITD-50 ion trap detector (EI, 70 eV). The capillary column and chromatographic program used were as mentioned previously. Compounds were identified by comparison of the spectra with those in a general purpose library. Moreover, the identity of the components was confirmed by matching their mass spectrometric data with those obtained from the same equipment and corresponding to authentic reference compounds.

Statistical Analysis. Values are the average of two independent extractions of each sample and two HPLC injections of each extract. Data were statistically analyzed by an analysis of variance (ANOVA), and statistical significance was determined by Student's *t* test.

RESULTS AND DISCUSSION

Effects of Freezing on Raspberry Anthocyanins. Tables 2−5 show the anthocyanin compositions of fresh and frozen raspberry varieties growing in Spain, two early cultivars, Heritage and Autumn Bliss (May), and two late cultivars, Rubi and Zeva (October). The separation, characterization, and quantification by HPLC of the anthocyanin composition of the four raspberry cultivars was previously studied in our laboratory (de Ancos et al., 1999). Quantitative and qualitative differences (Tables 2−5) were found among cultivars. The anthocyanin HPLC profile was characteristic for each cultivar, as is shown in Figure 1. Cv. Heritage showed the simplest anthocyanin pattern, with only two major compounds; in contrast, cv. Zeva had the most complicated pattern, with at least nine compounds. The main pigments found in the four cultivars were cyanidin-based anthocyanins (sophoroside, glucoside, glucorutinoside, and rutinoside), and pelargonidin derivatives (sophroside and glucoside) were also detected. The highest total anthocyanin contents were found in the late cultivars, Zeva [116 mg/g of fresh weight (fw) and Rubi (96.08 mg/g of fw]; the early cultivars, Heritage and Autumn Bliss (31.13 and 37.04 mg/g of fw, respectively), showed less than half of late cultivars' concentration.

The freezing process affected in different ways the total anthocyanin content of the raspberry cultivars. In both early cultivars, Heritage and Autumn Bliss, freezing produced a better extraction of the total anthocyanin

Table 5. Effect of Freezing and Frozen Storage on Main Anthocyanins of Spanish Raspberry Cv. Zeva, Determined by HPLC and Quantified as Milligrams of Cyanidin 3-Glucoside per 100 g of Fresh Weight[a]

anthocyanin		frozen storage					
no.	name	raw	0 days	90 days	180 days	270 days	360 days
1	Cy-3-soph	61.64 ± 4.83a	51.21 ± 2.35a	50.43 ± 2.5a	49.80 ± 2.3a	44.61 ± 1.5a	54.20 ± 2.7a
2	Cy-glc rut	11.42 ± 1.13a	13.04 ± 1.11a	8.72 ± 1.5b	7.91 ± 0.4b	9.37 ± 0.5a	10.39 ± 1.8a
3	Pg-3-soph	4.51 ± 1.09a	5.56 ± 0.32a	7.72 ± 0.5b	5.02 ± 0.2a	5.05 ± 0.2a	2.47 ± 0.3c
4	Cy-3-glu	24.14 ± 1.67a	17.91 ± 1.63b	20.57 ± 1.2a	18.42 ± 1.4a	14.43 ± 1.7b	17.70 ± 1.6b
5	Pg-3-glc rut	2.44 ± 0.21a	3.85 ± 0.22a	4.22 ± 0.3a	4.11 ± 0.4a	2.83 ± 0.3b	2.88 ± 0.7b
6	Cy-3-rut	5.94 ± 0.45a	6.90 ± 0.45a	4.91 ± 0.6a	5.65 ± 0.2a	4.56 ± 0.6a	5.08 ± 0.2a
7	Pg-3-glu	2.56 ± 0.17a	3.72 ± 0.22a	2.50 ± 0.2a	2.43 ± 0.5a	2.92 ± 0.1a	3.20 ± 0.3a
8	Mav-3-glu	2.78 ± 0.25a	3.92 ± 0.11a	nd	nd	nd	nd
9	Def-3-glu	2.82 ± 0.22	nd	nd	nd	nd	nd
total		116.27 ± 5.58a	106.11 ± 2.5b	99.07 ± 3.6b	93.34 ± 2.3b	83.77 ± 2.3b	95.92 ± 3.5b

[a] Values are mean ± SD of four determinations. Different letters in the same row indicate significant differences ($p \leq 0.05$). nd, not detected. Numbers correspond to HPLC peaks in Figure 1.

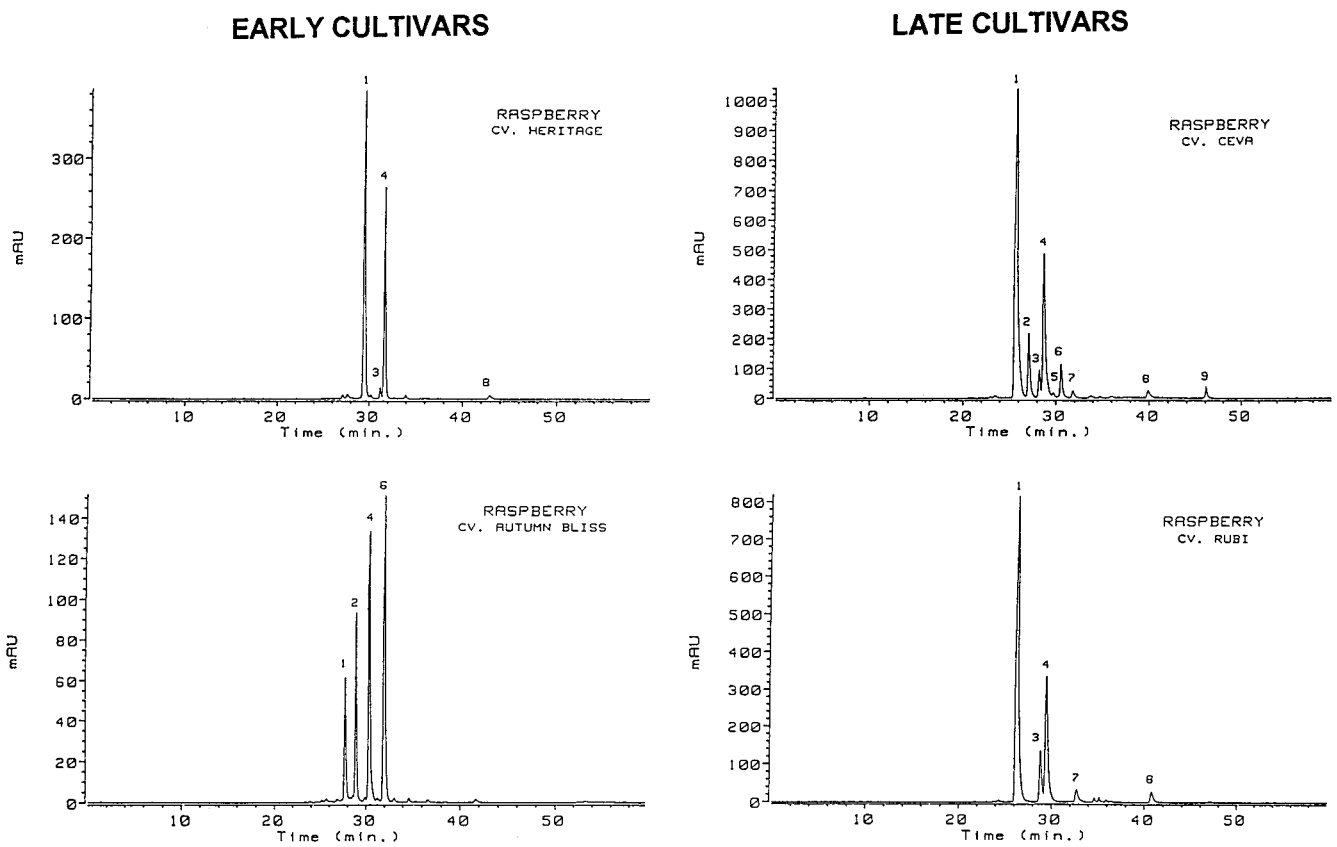

Figure 1. HPLC chromatogram of anthocyanin extracts of raspberry cultivars growing in Spain. Peaks: (1) cyanidin 3-sophoroside; (2) cyanidin 3-glucorutinoside; (3) pelargonidin 3-sophoroside; (4) cyanidin 3-glucoside; (5) pelargonidin 3-glucorutinoside; (6) cyanidin 3-rutinoside; (7) pelargonidin 3-glucoside; (8) malvidin 3-glucoside; (9) delphinidin 3-glucoside.

content, up 41% for Heritage and up 31% for Autumn Bliss (Tables 2 and 3, respectively); the total anthocyanin content was reduced 19 and 9% in Rubi and Zeva, respectively (Tables 4 and 5, respectively). The significant increase due to the freezing process on total anthocyanin content of Heritage and Autumn Bliss was related to a significant increase of individual concentration extracted of cyanidin 3-sophoroside (peak 1) (69%) in Heritage and cyanidin 3-rutinoside (peak 4) (67%) in Autumn Bliss (Figure 1), the major anthocyanin in each cultivar, respectively (Tables 2 and 3). A significant degradation of cyanidin 3-glucoside due to the freezing process was detected in the late cultivars; approximately 33 and 26% less pigment was extracted in Rubi and Zeva, respectively, according to the decrease in total pigment extracted after the freezing processing (Tables 4 and 5, respectively). This is in accordance with the literature, which reports this pigment as one of the most reactive anthocyanins during processing (Rommel et al., 1990; Boyles et al., 1993; García-Viguera et al., 1998). The freezing process produced in the raspberry fruit cultivars different effects depending on the chemical composition and biochemical activity of the tissues. The two early cultivars, Heritage and Autumn Bliss, which have low total anthocyanin and cyanidin 3-glucoside concentrations, showed no degradation and, indeed, a better extraction of the total anthocyanin content due to cellular disruption caused by the freezing process. Rubi and Zeva, the two late cultivars with high total anthocyanin and cyanidin 3-glucoside concentrations, showed a more evident degradation of total anthocyanins caused by the freezing process. This degradative effect could be due to the high content of the more reactive anthocyanin compound cyanidin 3-glucoside, or

perhaps cellular disruption caused by the freezing process produced a release of the oxidoreductase enzymatic systems (PPO). Previous work developed in our laboratory showed more polyphenol oxidase (PPO) enzyme activity in Rubi and Autumn Bliss raspberry tissues [1.21 and 1.19 ΔOD min^{-1} (g of fw)$^{-1}$, respectively] than in Zeva and Heritage tissues [0.64 and 0.83 ΔOD min^{-1} (g of fw)$^{-1}$, respectively] (Gonzalez et al., 1999). Therefore, the degradative enzymatic reactions could be one of the main reasons for the total anthocyanin concentration losses in Rubi but not in Zeva. Physicochemical characteristic differences were also found between early and late cultivars (Table 1): Rubi and Zeva showed lower pH and higher °Brix values than the early cultivars, Heritage and Autumn Bliss. These results showed that the preservation of the initial anthocyanin concentration of raspberry tissues during freezing mainly depends on the pH value, organic acids content, sugar concentration, initial anthocyanin concentration, and initial cyanidin 3-glucoside content, and no direct relationship with the initial PPO enzyme activity was found. Also, other authors reported that the stability of the anthocyanin content during raspberry fruit processing depended on different factors (pH, organic acid and sugar contents, initial anthocyanin concentration, etc.) (Wrolstad et al., 1970; Rommel et al., 1990; Boyles et al., 1993).

Frozen Storage Effect on Raspberry Anthocyanins. Frozen storage affected the anthocyanin total content and the cultivar individual anthocyanin distribution in different ways depending on the cultivar. Autumn Bliss showed a minor total anthocyanin value extracted during the frozen storage compared with that found for the raw and just frozen product (Table 3). Meanwhile, the extraction of Heritage cultivar during the frozen storage period (Table 2) showed an anthocyanin concentration value greater than in the raw product. Both raspberries, cv. Heritage and Autumn Bliss, increased slightly at 17 and 5%, respectively, the total anthocyanin content at the end of the frozen storage at 360 days (Tables 2 and 3, respectively). The relative percentage of the different anthocyanins in the early cultivars, Heritage and Autumn Bliss, suffered small changes during the frozen storage. Cyanidin 3-glucoside was the pigment that suffered a more significant degradation during the frozen storage of Autumn Bliss raspberries, so depending on the time of storage analyzed, the total anthocyanin value extracted was diminished in a range of 12−48% compared with the raw fruit value (Table 3). Cyanidin 3-glucoside was not degraded during the frozen storage period in Heritage cultivar tissue. Heritage and Autumn Bliss raspberries retained the raw concentrantion or indeed increased the extraction of their main pigments, cyanidin 3-sophoroside and cyanidin 3-rutinoside, respectively, during the frozen storage (Tables 2 and 3). These results could be explained by the different chemical compositions found between the early cultivars (Heritage and Autumn Bliss) and the late cultivars (Rubi and Zeva), as was explained previously; cultivars with low pH, high soluble solids content (°Brix), and high total anthocyanin content preserved better the initial anthocyanin concentration during raspberry processing.

The anthocyanin total value in the late cultivars was significantly decreased (∼15−27%) in both, Rubi and Zeva, during the frozen storage (Tables 4 and 5, respectively). After 365 days of storage at −20 °C, the extraction of the raspberry tissues resulted in no significant decrease of the total pigment content, down 4% for Rubi and down 17.5% for Zeva compared with the total pigment value of the raw product (Tables 4 and 5). As happened in the early cultivars, Rubi and Zeva, after frozen treatment, the relative percentage of the pigments in each storage time analyzed (90, 180, 270, and 365 days) remained almost constant, the only exception being cyanidin 3-glucoside, which was less extracted than the other pigments because it more easily undergoes the degradative reactions that take place during frozen storage. Depending on the time of storage analyzed, cyanidin 3-glucoside concentration suffered a decrease of 21−34% in Rubi (Table 4) and a decrease of 15−40% in Zeva (Table 5), compared with raw product concentration.

Table 6. Relative Percentage of the Volatile Components of the Different Fresh Spanish Raspberry Cultivars Studied

volatile compound	raspberry cultivars			
	Autumn Bliss	Heritage	Zeva	Rubi
α-pinene	2.9	13.2	2.9	17.0
citral	0.4	0.7	0.7	1.6
β-pinene	0.5	1.2	0.5	1.7
phellandrene	2.4	11.4	12.9	10.4
linalool	1.8	6.3	0.4	7.1
α-ionone	33.5	32.8	43.1	23.9
caryophyllene	37.7	15.0	25.9	20.8
β-ionone	20.7	19.3	13.3	17.3

Effects of Freezing on Raspberry Volatile Compounds. The volatile components that contribute the most to the fresh raspberry aroma of the four cultivars studied were the following: α-pinene, citral, β-pinene, phellandrene, linalool, α-ionone, caryophyllene, and β-ionone. These compounds have been previously described (Robertson et al., 1995) as important contributors to the overall aroma of mature red raspberries. The different raspberry cultivars showed qualitatively the same components but different quantitative composition. Table 6 shows the relative percentage of the abovementioned compounds corresponding to the analysis of the four different fresh raspberry cultivars. As can be seen, the different cultivars contained different relative percentages of the compounds that contribute to the overall aroma of the fruits. All of the cultivars studied presented a high content of α-ionone (ranging form 24% for Rubi to 43% for Zeva), caryophyllene (from 15% for Heritage to 38% for Autumn Bliss), β-ionone (from 13% for Zeva to 21% for Autumn Bliss), and α-pinene (from <10% for Autumn Bliss to 17% for Rubi). Ionones have been found, along with the raspberry ketone, to be the character impact compounds of the aroma of raspberries (Honkanen et al., 1980). The cultivar with the most important volatile composition, that is, the one that presented the highest amount of volatile compounds, was Heritage.

After freezing, the volatile constituents of the aroma of the four raspberry cultivars studied was unchanged. Slight changes were observed in the quantitative composition of the major volatile compounds α-ionone and caryophyllene. Cellular disruption caused by the freezing process slightly increased the release of caryophyllene in the four cultivars, ranging from a 7% increase for Heritage to a 19% increase for Zeva. Also, a greater release of α-ionone was achieved in Heritage and Rubi, increasing ∼12% their relative content in both extracts. Therefore, freezing and thawing would increase the

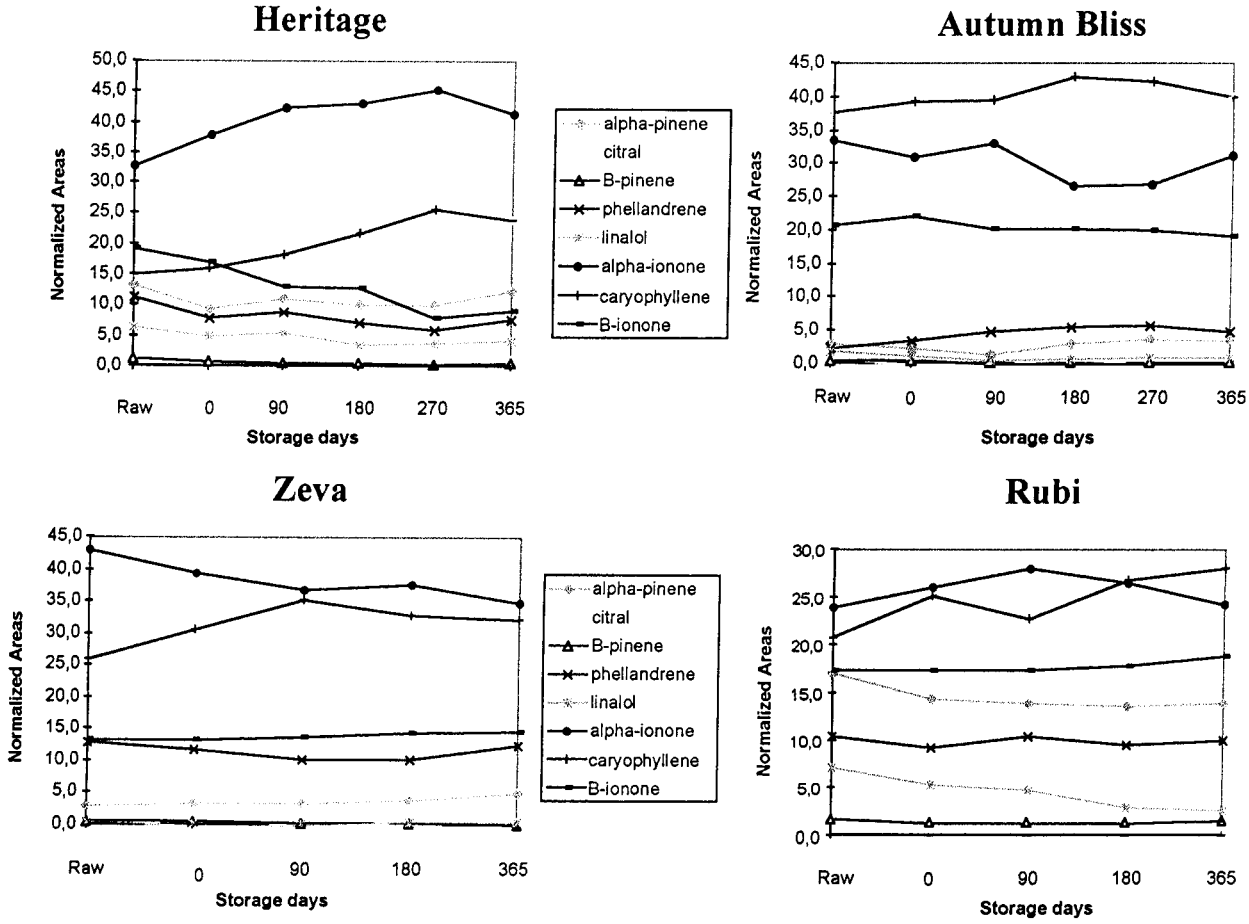

Figure 2. Evolution of the volatile components of the four raspberry cultivars growing in Spain (cv. Heritage, Autumn Bliss, Zeva, and Rubi) [normalized areas (%) as a function of the storage time].

sensory detection of raspberry aroma in Heritage and Rubi cultivars.

Effect of Frozen Storage on Raspberry Volatile Compounds. Figure 2 shows the evolution of the volatile components of the four different Spanish raspberry cultivars as a function of the frozen storage time. Also, for comparison, the initial percentages of the compounds prior to and after freezing are presented. Even though it has been previously described (Honkanen et al., 1980) that the aroma of raspberries is very sensitive to heat and storage and even deep freezing destroys the aroma of the fresh berries, in the present research it can be observed, as a general trend, that the volatile constituents of the aroma of the raspberry cultivars studied remain almost constant along the storage time (1 year period), although some of them either increase or decrease as a function of storage time. In some particular cases these percentage increases or decreases can be considered significant but, in general, differences are nonsignificant among them. This behavior was in accordance with the results found by Guichard (1982). Long-term frozen storage maintains the concentration of minor volatile constituents in the extract of α-pinene, citral, phellandrene, and linalool. More significant changes were found in the major volatile compounds. The concentration of β-ionone was unchanged at the end of the frozen storage period in Rubi, Autumn Bliss, and Zeva but suffered a significant decrease of 63% in Heritage. Also, α-ionone kept the raw concentration unchanged in Autumn Bliss and Rubi but in Heritage was better extracted, increasing by 27% from its concentration in the extract obtained after 1 year of storage at −20 °C. Caryophyllene was the better extracted volatile compound in the four cultivars studied at the end of frozen storage, ranging from a 9% increase for Autumn Bliss to a 67% increase in Heritage. From these results it can be inferred that neither the freezing process nor the storage conditions used in the present study have a negative influence in the aroma composition of the different raspberry cultivars; moreover, freezing and frozen storage would increase the perception of raspberry aroma. It is also important to consider that the changes produced in the aroma are partly due to the increase of some enzyme activities during thawing of the berries; therefore, the thawing process is also extremely important (Honkanen et al., 1980).

Conclusion. The freezing process and long-term frozen storage conditions employed in this study do not significantly affect the aroma profile and the color of the raspberry cultivar fruit studied. Moreover, the effect of frozen storage on the early cultivar tissues (Heritage and Autumn Bliss) makes easier the extraction of the total anthocyanin content. Raspberry cv. Heritage, from the standpoint of aroma and color, was the cultivar that better supported the freezing process and frozen storage. Heritage had the initial highest amount of volatile compounds, which changed only slightly during freezing and long-term frozen storage. Moreover, freezing and long-frozen storage allowed better extraction of α-ionone, one of the character impact compounds characteristic of raspberry aroma. Heritage did not have the highest anthocyanin content, but freezing and frozen storage less affected the individual anthocyanin com-

pounds and allowed a better extraction of the total anthocyanin value.

LITERATURE CITED

Blanch, G. P.; Reglero, G.; Herraiz, M.; Tabera, J. A comparison of different extraction methods for the volatile components of grape juice. *J. Chromatogr. Sci.* **1991**, *29*, 11−15.

Boresjsza-Wysocky, W.; Goers, S. K.; McArdle, R. N.; Hrazdina, G. p-Hydroxphenylbutan-2-one levels in raspberries determined by chromatographic and organoleptic methods. *J. Agric. Food Chem.* **1992**, *40*, 1176−1177.

Boyles, M. J.; Wrolstad, R. E. Anthocyanin composition of red raspberry juice: influence of cultivar, processing, and environmental factors. *J. Food Sci.* **1993**, *58*, 1135−1141.

Bushway, A. A.; Bushway, R. J.; True, R. H.; Work, T. M.; Bergeron, D.; Handley, D. T.; Perkins, L. B. Comparison of the physical, chemical and sensroy characteristics of five raspberry cultivars evaluated fresh and frozen. *Fruit Var. J.* **1992**, *46*, 229.

de Ancos, B.; Cano, M. P.; González, E. M. Differentiation of raspberry varieties according to anthocyanin composition. *Z. Lebensm.-Unters. -Forsch.* **1999**, *208*, 33−38.

Douillard, C.; Guichard, E. The aroma of strawberry (*Fragaria ananassa*): Characterization of some cultivars and influence of freezing. *J. Sci. Food Agric.* **1990**, *50*, 517−531.

García-Viguera, C.; Zafrilla, P.; Artés, F.; Romero, F.; Abellán, P.; Tomás-Barberán, F. A. Colour and anthocyanin stability of red raspberry jam. *J. Sci. Food Agric.* **1998**, *78*, 565−573.

González, E. M.; de Ancos, A.; Cano, M. P. Partial characterization of polyphenol oxidase activity in raspberry fruits. *J. Agric. Food Chem.* **1999**, in press.

Guichard, E. Identification of the flavoured volatile components of the raspberry cultivar Lloyd George. *Sci. Aliment.* **1982**, *2*, 173.

Honkanen, E.; Pyysalo, T.; Hirvi, T. The aroma of finnish wild raspberries, *Rubus idaeus*, L. *Z. Lebensm.-Unters. -Forsch.* **1980**, *180*, 180−182.

Ibañez, E.; Lopez-Sebastian, S.; Ramos, E.; Tabera, J.; Reglero, G. Analysis of highly volatile components of foods by offline SFE/GC. *J. Agric. Food Chem.* **1997**, *45*, 3940−3943.

Ibañez, E.; Lopez-Sebastian, S.; Ramos, E.; Tabera, J.; Reglero, G. Analysis of volatile fruit componets by headspace solidphase microextraction. *Food Chem.* **1998**, *63*, 281−286.

Ibañez, E.; Tabera, J.; Ramos, E.; Lopez-Sebastian, S.; Reglero, G. Analysis of volatile components of fruits by HS−PTV-GC. *J. Agric. Food Chem.* **1999**, in press.

Kok, M. F.; Yong, F. M.; Lim, G. Rapid extraction method for reproducible analysis of aroma volatiles. *J. Agric. Food Chem.* **1987**, *35*, 779−81.

Larsen, M.; Poll, L. Changes in the composition of aromatic compounds and other quality paramenters of strawberries during freezing and thawing. *Z Lebensm.-Unters. -Forsch.* **1995**, *201*, 275−277.

Macku, C.; Jennings, W. G. Production of volatiles by ripening bananas. *J. Agric. Food Chem.* **1987**, *35*, 845−848.

Mazza, G.; Miniati, E.; Introduction. In *Anthocyanins in Fruits, Vegetables and Grains*; Mazza, G., Ed.; CRC Press: Boca Raton, FL, 1993; Chapter 1.

Miszczak, A.; Forney, Ch. F.; Prange, R. K. Development of aroma volatiles and color during postharvest ripening of "Kent" strawberries. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 650−655.

Rizzolo, A.; Polesello, A.; Polesello, S. Use of headscape capillary GC to study the development of volatile compounds in fresh fruits. *J. High Resolut. Chromatogr.* **1992**, *15*, 472−477.

Robertson, G. W.; Griffths, D.; Woodford, J. A. T.; Brich, A. N. E. Changes in the chemical composition of volatiles released by the flowers and fruits of the red raspberry (*Rubus ideaeus*) cultivar glen prosen. *Phytochemistry* **1995**, *38*, 1175−1179.

Rommel, A.; Heatherbell, D. A.; Wrolstad, R. E. Red raspberrry juice and wine: effect of processing and storage on anthocyanin pigment composition, color and appearance. *J. Food Sci.* **1990**, *55*, 1011−1017.

Shamaila, M.; Powrie, W. D.; Skura, B. J. Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). *J. Food Sci.* **1992**, *5*, 1173−1176.

Skrede, G. Fruits. In *Freezing Effects on Food Quality*; Jeremiah, L. E., Ed.; Dekker: New York, 1996; p 183.

Ueda, Y.; Iwata, T. Undersirable odour of frozen strawberries. *J. Jpn. Soc. Hortic. Sci.* **1982**, *51*, 219.

Wrolstad, R. E.; Putman, T. P.; Varseveld, G. W. Color quality of frozen strawberries: effect anthocyanin, pH, total acidity and ascorbic acid variability. *J. Food Sci.* **1970**, *35*, 448−452.

Wrolstad, R. E.; McDaniel, M. R.; Durst, R. W.; Michaels, N.; Lampi, K. A.; Beaudry, E. G. Composition and sensory characterization of red raspberry juice concentrated by direct-osmosis or evaporation. *J. Food Sci.* **1993**, *58*, 633−637.

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