Ellagic Acid, Vitamin C, and Total Phenolic Contents and Radical Scavenging Capacity Affected by Freezing and Frozen Storage in Raspberry Fruit

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The ellagic acid, total phenolic, and vitamin C contents in four raspberry cultivars (Heritage, Autumn Bliss, Rubi, and Zeva) grown in Spain were detected and quantified by HPLC in fresh, just frozen, and stored fruits at −20 °C for a one year period. Ellagic acid [207−244 mg kg^{-1} of fresh weight (fw)], total phenolic (137−1776 mg kg^{-1} of fw), and vitamin C (221−312 mg kg^{-1} of fw) contents in raw material were higher in the late cultivars Zeva and Rubi than in the early cultivars Autumn Bliss and Heritage. The freezing process slightly affected the values of extracted ellagic acid, total phenolic, and vitamin C content. At the end of long-term frozen storage (12 months), no significant change of total phenolic content extracted was observed, but significant decreases of 14−21% in ellagic acid and of 33−55% in vitamin C were quantified. Free radical scavenging capacity measured as antiradical efficiency (AE) depends on the seasonal period of harvest. Late cultivars, Rubi (6.1×10^{-4}) and Zeva (10.17×10^{-4}), showed higher AE than early cultivars, Heritage (4.02×10^{-4}) and Autumn Bliss (4.36×10^{-4}). The freezing process produced a decrease of AE values in the four cultivars ranging between 4 and 26%. During the frozen storage, the AE values reached after the freezing process remained unchanged.

Keywords: *Ellagic acid; vitamin C; total phenolic; radical-scavenging capacity; frozen storage; raspberry*

INTRODUCTION

The protection provided against degenerative diseases by fruits and vegetables has been attributed to the fact that these foods may provide an optimal mix of phytochemicals, such as natural antioxidants, fiber, and other bioactive compounds (Ames et al., 1993; Steinmetz and Potter, 1991). Fruits and vegetables contain many different bioactive molecules, many of which have antioxidant properties. Strong evidence can be found in the literature supporting ascorbic acid as the most important and traditional antioxidant (Omaye and Zhang, 1998). In addition to the well-known vitamins C and E, or carotenoid compounds, fruits and vegetables have other compounds that significantly contribute to their antioxidant capacity. Recent interest in food phenolics has increased due to their antioxidant and antimutagenic properties together with their ability to react with free radicals (Robards et al., 1999), so these compounds are considered as protective micronutrients due to their intake relationship with protective effects against several degenerative diseases such as cancer and cardiovascular disorders (Hertog et al., 1993, 1994). Berry fruits are rich in flavonoids and phenolic acids that show antioxidant activity (Heinonen et al., 1998). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Rice-Evans et al., 1995). There is a particular interest in the amounts of ellagic acid, a dimeric derivative of gallic acid, in these fruits because of the increasing evidence of its anticarcinogenic and antioxidant effects (Maas and Galleta, 1991). Blackberries, strawberries, and raspberries presented high ellagic acid contents (Rommel and Wrolstad, 1993). Moreover, ellagic acid represented 88% of the total phenolic content analyzed in raspberry fruits (Häkkinen et al., 1999).

There is an increasing attention to the role of diet in human health. Due to the fact that most vegetables and fruits need to be processed for safety, quality, and economic reasons, the health-promoting capacity of fruits and vegetables depends on their processing history. The concentration of bioactive compounds may change with processing (Nicoli et al., 1999).

As is well-known, freezing is one of the most important methods to retain fruit quality during long-term storage, and factors such as variety, maturity, growing area, and seasonal variations may override the positive effects of high freezing rate (Skrede, 1996). The literature provides many studies of the effects of freezing and frozen storage on anthocyanin content in different kinds of berries and raspberries (Bushway et al., 1992: Ancos et al., 2000), but there are no references about the effect of this process in other kinds of phenolic compounds such as ellagic acid.

The objective of this study is to evaluate the effect of freezing and long-term frozen storage period on antioxidant compounds, ellagic acid, total phenolic, and vitamin C, and on the radical-scavenging activity of four raspberry cultivars grown in Spain in different seasonal periods, Autumn Bliss and Heritage (May) and Zeva and Rubi (October). The final purpose was to study their

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Table 1. Physical, Physicochemical, Chemical, and Biochemical Characteristics of Raspberry Fruit Cultivars Harvested in Spain

characteristic[a]	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 ± 0.50a	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
maturity index[b]	5.54	5.34	6.02	5.54
moisture content (g/100 g of fw)	84.77 ± 0.11a	85.31 ± 0.63a	83.67 ± 1.53a	82.02 ± 3.01a
total solids (g/100 g of fw)	15.23 ± 0.02a	14.69 ± 0.11b	16.33 ± 0.30c	17.98 ± 0.66d
total anthocyanin content[c]	31.13 ± 0.13a	37.04 ± 2.18a	116.27 ± 5.58b	96.08 ± 2.84b
PPO activity (ΔOD/min/g of fw)	1.19 ± 0.006a	0.83 ± 0.006b	0.64 ± 0.01ab	1.21 ± 0.05c

[a] Values are the mean of three independent determinations ± standard deviation. Different letters in the same row indicate significant differences ($p \leq 0.05$). [b] Soluble solids/titrable acidity. [c] Calculated by HPLC as mg of cyanidin 3-glucoside per 100 g of fw.

suitability for freezing and long-term frozen storage in order to select the cultivars that are less affected in terms of antiradical scavenging capacity and ellagic acid, total phenolic, and ascorbic acid contents.

MATERIALS AND METHODS

Plant Material. Raspberry fruits (*Rubus idaeus* L.) of four cultivars (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. They were brought to Instituto del Frío within 12 h after harvest. Physicochemical, chemical, and biochemical characteristics of raw raspberry varieties are shown in Table 1. On arrival, undamaged fruits were selected, and the plant material was 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 partial thawing under prefixed standard conditions (1 h at 7 °C) and were analyzed at 0, 30, 90, 180, 270, and 365 days of frozen storage. At the end of each storage period, a portion of the frozen berries was freeze-dried, powdered with a pestle and mortar, and stored at −20 °C. Fresh and dry weight measurements were obtained for all of the plant material.

Determination of Ellagic Acid. *Extraction and Acid Hydrolysis.* Acidic conditions for the hydrolysis of berry fruit tissues were similar to those described by Crozier et al. (1997) with minor modifications. Aliquots of 1 g of lyophilized berry were extracted with 20 mL of 60% aqueous methanol containing 125 μg of BHT (Sigma Chemical Co., St. Louis, MO) as an antioxidant. Five milliliters of 6 M HCl was added to each extract to give a 25 mL solution of 1.2 M HCl in 50% aqueous methanol. Extracts were refluxed at 90 °C for 2 h, cooled, and centrifuged for 15 min at 2000*g* in a Sorval centrifuge (model RC-5B, DuPont Instruments). Supernatant was taken and filtered through a 0.45 μm filter prior to analysis of 20 μL by reversed phase HPLC.

HPLC Analysis. A Hewlett-Packard 1050 quaternary solvent delivery equipped with a Hewlett-Packard 1040A UV−visible photodiode array detector was employed. A linear gradient separation was performed on a stainless steel (250 × 4.6 mm i.d.) ODS-Hypersil (5 μm spherical particles) column (Technochroma, Spain), starting with 15% of solvent B (acetonitrile) in solvent A (water adjusted to pH 2.5 with trifluoroacetic acid) to reach 35% B at 20 min. Flow rate was 1.0 mL/min, and the runs were monitored at 280 nm. Quantification was performed by external standard procedure employing commercial ellagic acid (Sigma Chemical Co.). To prepare stock solution, ellagic acid was first dissolved in 1 N NaOH and then in methanol/HCl 1.5% (75:25) (final ratio = 1:25), due to the low solubility of this acid.

Determination of Vitamin C. Vitamin C was analyzed by HPLC coupled with a fluorometric detector, according to the procedure described by Ancos et al. (1999). The procedure employed was the oxidation of ascorbic acid to dehydroascorbic by activated carbon and derivatization by formation of the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-*b*]quinoxalin-1-one with 1,2-phenylenediamine.

A 10 g portion of each representative sample was homogenized with 25 mL of a solution of 0.3 M of trichloroacetic acid (TCA). The solution was centrifugated at 9000*g* for 15 min at 4 °C. The supernatant was poured into a flask and made up to 50 mL with 0.3 M TCA. A 25 mL portion of this solution was made up to 50 mL with 0.3 M TCA. The mixture was allowed to stand for 30 min at room temperature and filtered. The acid ascorbic was oxidized to dehydroascorbic acid by 1 g of activated carbon. The mixture sample solution was made with 5 mL of sample filtrate diluted with 4 mL of 4.5 M sodium acetate buffer at pH 6.2, and 2 mL of 1,2-phenylenediamine solution (2.5 mg dissolved in 25 mL of deionized water) and allowed to stand at 37 °C during 30 min in the dark. After that time, the solution was cooled and injected onto the HPLC.

A stock solution was prepared with 50 mg of ascorbic acid in 50 mL of 0.3 M TCA and storage at 4 °C in the dark for 3 days. Standard solution was freshly prepared each day with 8 mL of stock solution made up to 50 mL with solution of 0.3 M TCA and treated with 1 g of activated carbon during 30 min and filtered. One milliliter of filtrate was diluted with 2 mL of 4.5 M solution of sodium acetate at pH 6.2, 4 mL of 0.3 M solution of TCA, and 2 mL of 1,2-phenylenediamine (2.5 m dissolved in 25 mL) and allowed to stand for 30 min at 37 °C in the dark; 20 μL of this solution was injected onto the HPLC system.

HPLC Vitamin C Analysis. HPLC separation was performed in the same equipment as described for ellagic acid analysis. The mobile phase was deionized water with acetic acid (0.75%) and methanol in a relative proportion of 75:25. Isocratic elution was employed with a flow rate of 0.8 mL/min, and the quinoxaline with emission at 425 nm after excitation at 355 nm was measured by fluorometric detector (HP-1047A).

Determination of Total Phenolics. The amount of total phenolics was determined according to the Folin−Ciocalteu procedure (Folin−Ciocalteu Index, 1992) and expressed as gallic acid equivalents (GAE) in milligrams per kilogram of fresh weight (fw).

Free Radical Scavenging Measurement. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams et al. (1995) that was slightly modified by Sánchez-Moreno et al. (1998).

Preparation of Sample Extracts. Ten grams of raw or partially thawed raspberry samples were homogenized for 3 min in a blender with 30 mL of 50% of aqueous methanol. After recovery of the homogenate, 15 mL of methanol was used to wash the blender and then pooled with the first homogenate.

The medium was centrifuged at 10000*g* for 15 min at 4 °C. The pellet following centrifugation was washed with 30 mL of methanol and centrifuged, and the resulting supernatant was combined with the initial extract. Triplicate supernatant extractions were made for each sample. The methanolic extract volume was reduced in the evaporator to 20 mL and then made up to 50 mL with methanol.

Determination of the Scavenging Effect on DPPH Radicals. An aliquot (0.1 mL) of the sample extracts was added to 3.9 mL of DPPH• (0.025 g L^{-1}) in methanol. Absorbances of the samples were measured using a spectrophotometer (Perkin-Elmer, model Lambda 12) at different time intervals until the reaction reached a plateau (time at steady time) at 515 nm against methanol without DPPH• as the blank reference. Raspberry sample concentrations, expressed as grams of fresh sample per gram of DPPH• in the reaction medium, ranged from 0.5 to 80.

The DPPH• concentration in the reaction medium was calculated from the following calibration curve determined by linear regresion ($r^2 = 0.999$):

$$A_{515} = 29298[\text{DPPH}^\bullet]_t + 4.54 \times 10^{-2}$$

$[\text{DPPH}\cdot]_t$ was expressed as g L^{-1}. The percentage of remaining DPPH• (%$\text{DPPH}^\bullet_{\text{rem}}$) was calculated as follows:

$$\%\text{DPPH}^\bullet_{\text{rem}} = ([\text{DPPH}^\bullet]_t/[\text{DPPH}^\bullet]_{t=0}) \times 100$$

The antiradical efficiency ($AE = 1/EC_{50}t_{EC50}$) of the raspberry extracts was calculated according to the method of Sánchez-Moreno et al. (1998).

Statistical Analysis. Results were processed by an analysis of variance (ANOVA) and statistical significance by Student's *t* test. Differences at $p < 0.05$ were considered to be significant. The computer program employed was SPSS (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Effect of Freezing and Long-Term Frozen Storage on Ellagic Acid Content. Ellagic acid content in the late cultivars (harvested in autumn) Zeva (244.38 ± 6.10 mg kg^{-1} of fw) and Rubi (233.57 ± 9.40 mg kg^{-1} of fw) was higher than in the early cultivars (harvested in spring) Autumn Bliss (207.89 ± 10.5 mg kg^{-1} of fw) and Heritage (217.03 ± 13.7 mg kg^{-1} of fw), but these differences were not statistically different ($p < 0.05$) (Table 2). The concentration of ellagic acid quantified in these raspberry cultivars was in accord with that found by other authors in this berry (Häkkinen et al., 1999).

Freezing is one the most important methods for retaining sensorial (color and aroma) and nutritional qualities of fruits (Skrede, 1996). In the present study, the freezing process affected in a similar way the extraction of the ellagic acid in the four raspberry cultivars. After freezing, the content of ellagic acid in the thawed fruits remained practically unchanged in the four cultivars. Decreases in ellagic acid content were found in Rubi (13%), Zeva (4%), and Heritage (11%), but the difference was statistically significant only in the last one (Table 2). A slight increase was detected in Autumn Bliss (4%) (Table 2). In the literature, there are no references about the effects of freezing on raspberry phenolics, with the exception of anthocyanins from different sources, pigments that belong to the flavonoid group. In general, an increase of the anthocyanin extraction due to the cellular disruption caused by the freezing process in berries was obtained. This treatment prior to pressing is employed in fruit juice production to increase juice yield (Skrede, 1996). The effect of freezing on the anthocyanin content in these Spanish raspberry cultivars showed an increase in the total anthocyanin concentration, mainly in the early cultivars Heritage and Autumn Bliss (Ancos et al., 2000). Slight changes in ellagic acid on just frozen and thawed raspberries (0 days of storage) could be related to a different effect of the freezing process, which allowed a better ellagic acid release from the cell wall. It is important to take into consideration that ellagic acid is a phenolic compound strongly linked to the cell walls (Maas et al., 1991; Rommel and Wrolstad, 1993).

Table 2. Freezing and Frozen Storage Effects on Ellagic Acid and Total Phenolics Content of Four Rasberry Cultivars

variety and frozen storage period (days)[a]	total phenolics[b] (mg of GAE/kg of fw)	ellagic acid[c] (mg/kg of fw)
Heritage		
raw	1137.25 ± 5.62a	217.03 ± 13.7a
0	995.92 ± 11.43a	193.24 ± 4.61b
90	1140.96 ± 39.72a	197.81 ± 1.92c
180	1159.24 ± 8.49a	173.62 ± 14.7c
270	1232.28 ± 66.49a	169.85 ± 3.11c
365	1014.85 ± 17.07a	170.64 ± 2.21c
Autumn Bliss		
raw	1214.42 ± 57.48a	207.89 ± 10.5a
0	1364.32 ± 80.14a	216.63 ± 8.40a
90	1138.72 ± 41.38a	162.92 ± 4.90b
180	1056.73 ± 43.73a	172.62 ± 4.50b
270	1139.45 ± 32.51a	165.32 ± 7. 60b
365	1081.94 ± 3.73a	177.80 ± 4.80b
Zeva		
raw	1776.02 ± 115.29a	244.38 ± 6.10a
0	1874.70 ± 109.91a	235.65 ± 12.7a
90	1620.39 ± 150.54a	216.23 ± 9.30ab
180	1868.29 ± 94.29a	207.71 ± 1.29b
270	2021.85 ± 23.16a	199.98 ± 7.30b
365	1358.14 ± 38.74a	205.79 ± 4.30b
Rubi		
raw	1556.67 ± 45.54a	233.57 ± 9.40a
0	1409.02 ± 93.02a	202.85 ± 13.4a
90	1423.88 ± 102.34a	219.20 ± 3.70a
180	1392.58 ± 23.67a	218.02 ± 4.00a
270	1365.27 ± 109.17a	200.67 ± 5.10a
365	1582.72 ± 8.57a	189.27 ± 12.0a

[a] Mean of four determinations ± SD (standard deviation). The same letter in the same column for each raspberry cultivar parameter analyzed indicates no significant differences ($p < 0.05$). [b] Determined according to Folin−Ciocalteu procedure and expresed as mg of gallic acid equivalents (GAE) per kg of fresh weight. [c] Determined by HPLC after acidic hydrolysis of raspberry tissue by a solution of 1.2 M HCl in 50% aqueous methanol.

Frozen storage affected in a similar way the content of ellagic acid in the extracts of the four cultivars assayed. During the long-term frozen storage period (365 days) a continuous decrease in the total ellagic acid content was achieved for the four raspberry cultivars. Greater degradation of ellagic acid was shown in Heritage and Rubi cultivars, 21 and 19%, respectively, whereas Autumn Bliss and Zeva suffered decreases of 14 and 16%, respectively, after 365 days at −20 °C (Table 2). Insignificant differences in ellagic acid content were observed in Rubi fruits during freezing and frozen storage, whereas statistically differences were found among just frozen and frozen storage Heritage, Zeva, and Autumn Bliss fruits. The greater losses of ellagic acid in raspberries observed during storage could be related to a more severe cellular disruption in these fruits, which could be produced by release of oxidoreductasic ionic forms of the enzyme polyphenol oxidase (PPO) linked to the cellular wall. Unpublished results obtained in our laboratory showed, at the end

of 365 days of storage at −20 °C, increases of PPO enzyme activity of 34% in Heritage (from 0.88 to 1.18 ΔOD/min/g of fw) and of 19% in Rubi (from 0.69 to 0.82 ΔOD/min/g of fw). PPO enzyme activity in Autumn Bliss and Zeva was less affected by long-term frozen storage, so only a slight decrease of activity of 3% in Autumn Bliss (from 1.22 to 1.18 ΔOD/min/g of fw) and an increase of 4% in Zeva (from 1.03 to 1.07 ΔOD/min/g of fw) were observed.

Effect of Freezing and Long-Term Frozen Storage on Total Phenolic Content. The amount of total phenolics in the fresh raspberries depends on the seasonal period of harvesting. Late cultivars, Zeva (1776.02 mg of GAE kg^{-1}) and Rubi (1556.67 mg of GAE kg^{-1}), showed the greater phenolic content and early cultivars, Autumn Bliss (1212.42 mg of GAE kg^{-1}) and Heritage (1137.25 mg of GAE kg^{-1}), the lowest ones (Table 2). These total phenolic values found were lower than compositional raspberry data found in previous studies (Heinonen et al., 1998). These differences could be due to environmental characteristics, period of harvesting, cultivar variability, or fruit maturity. No significant changes in total phenolic content of the four raspberries were found after freezing. There were slight increases of 12 and 5% of the total phenolic contents in Autumn Bliss and Zeva, respectively, and 13 and 5% decreases in the total phenolics in Heritage and Zeva, respectively, in just frozen and thawed fruits (Table 2).

During the long-term frozen storage at −20 °C of the four raspberry cultivars studied, the total phenolic content measured spectrometrically according to the Folin−Ciocalteu procedure was practically unchanged. In all of the sample analyses during storage, no significant increases or decreases in early cultivars were found. At the end of frozen storage (365 days), both early cultivars, Heritage and Autumn Bliss, showed a decrease of 11%; meanwhile, Zeva raspberry increased 23%. Rubi phenolic content remained unchanged after freezing and frozen storage (Table 2).

Effect of Freezing and Long-Term Frozen Storage on Vitamin C Content. The stability of vitamin C during freezing of four raspberry cultivars harvested in different seasonal periods was evaluated. Vitamin C was one of the most important parameters to be studied related to the nutritional and health-promoting quality of frozen fruits. Changes in vitamin C could be a good indicator compound for enzymatic or nonenzymatic degradative reactions taking place during processing or storage of the fruit (Skrede, 1996).

Three of the studied raspberry cultivars, Rubi (310.89 ± 16.3 mg kg^{-2}), Zeva (296.59 ± 3.8 mg kg^{-2}), and Autumn Bliss (301.89 ± 8.00 mg kg^{-2}), showed very similar initial vitamin C contents; the lowest vitamin C level was found in Heritage raspberry (220.67 ± 10.8 mg kg^{-2}). Insignificant differences in vitamin C content were found among the four raw (unprocessed) raspberry cultivars (Figure 1).

The freezing process caused small changes in the vitamin C content in the four raspberries. There was no significant increase of vitamin C in just frozen Autumn Bliss extracts, but slight increases (3%) in the late cultivars, Rubi and Zeva, and in the early cultivar, Heritage (12%), were detected (Figure 1). These results agreed with previous studies in vitamin C retention in raspberry fruit during freezing with liquid nitrogen (Fraczak and Zalewska-Korana, 1990). The slight increase of vitamin C content after freezing and thawing

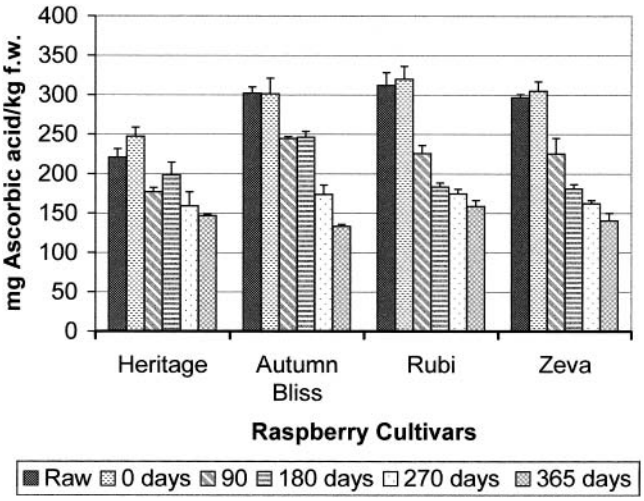

Figure 1. Freezing and frozen storage effects on vitamin C content of four raspberry cultivars.

of fruit might be explained by the same hypothesis suggested for ellagic acid increase: possible cellular disruptions caused by the freezing process.

During 365 days of frozen storage at a temperature of −20 °C, vitamin C content suffered a continuous decrease in all cultivars (Figure 1). The stability of vitamin C after 90 and 180 days seems to be related to the seasonal harvest period of the fruit. Storage vitamin C losses of 20% for Heritage and Autumn Bliss (early cultivars) were observed after 90 days and remained practically unchanged between 90 and 180 days of frozen storage. Greatest losses, ranging from 24 to 27% in Rubi and Zeva (late cultivars), were observed at 90 days of frozen storage but then increased to 40% after 180 days.

Significant changes in vitamin C content due to long-term frozen storage were observed in the four cultivars studied. Although vitamin C contents in the four cultivars studied were similar after 365 days, the percentage of loss depended on the cultivar studied: Rubi (158.62 ± 7.3 mg kg^{-2}) and Zeva (140.29 ± 9.5 mg kg^{-2}) suffered vitamin C losses of 49 and 47%, respectively, whereas Heritage (144.57 ± 1.1 mg kg^{-2}) and Autumn Bliss (133.25 ± 2.3 mg kg^{-2}) decreased 34 and 56%, respectively, in vitamin C content based on the raw fruit value. These results showed a better stability of vitamin C in Heritage than in Autumn Bliss, Rubi, and Zeva frozen raspberries. Previous studies showed that low pH values in berry tissues produce a positive effect on vitamin C stability (Skrede, 1996); however, in the present work much higher losses of vitamin C were found in the late cultivars, Rubi and Zeva, which had the lowest pH values (Table 1) and the highest vitamin C and anthocyanin contents among the studied raspberry cultivars (Table 1). Also, the Zeva and Rubi (late) cultivars, with the higher total anthocyanin content, suffered also greater anthocyanin losses than Heritage and Autumn Bliss (early) at the end of frozen storage period (Ancos et al., 2000). The anthocyanin-protecting effect on vitamin C content reported in the literature for black currant (Davies et al., 1991) was not observed for the Spanish raspberries studied. In this study, Heritage raspberry, with the lowest vitamin C content (also the lowest anthocyanin concentration) among the four cultivars, showed a smaller loss of vitamin C content at the end of the frozen storage period (365 days).

Table 3. Free Radical Scavenging Parameters of Four Raspberry Varieties and Standards: Freezing and Frozen Storage Effect on AE

variety and frozen storage period[a] (days)	EC_{50}[b] (g/g DPPH•)	t_{EC50}[c] (min)	AE × 10^{-4} [d]
Heritage			
raw	61.17 ± 1.29a	40.68 ± 0.68a	4.02 ± 0.15a
0	61.64 ± 0.73a	42.31 ± 0.27a	3.84 ± 0.07a
90	63.09 ± 0.08a	42.57 ± 0.57a	3.72 ± 0.04a
180	64.78 ± 0.03a	41.41 ± 0.37a	3.73 ± 0.03a
270	63.80 ± 0.88a	40.27 ± 0.12a	3.89 ± 0.04a
365	62.13 ± 2.62a	40.79 ± 0.76a	3.95 ± 0.09a
Autumn Bliss			
raw	59.07 ± 0.97a	38.35 ± 0.85a	4.36 ± 0.02a
0	62.19 ± 2.74a	40.69 ± 0.76a	3.96 ± 0.25a
90	60.80 ± 0.50a	40.45 ± 2.40a	4.07 ± 0.28a
180	63.97 ± 2.15a	41.20 ± 0.07a	3.80 ± 0.12a
270	61.41 ± 1.66a	40.73 ± 2.23a	4.00 ± 0.11a
365	60.65 ± 0.85a	39.87 ± 0.13a	4.14 ± 0.04a
Zeva			
raw	47.75 ± 0.35a	20.61 ± 0.72a	10.17 ± 0.43a
0	51.64 ± 2.13a	26.10 ± 1.12a	7.44 ± 0.63a
90	49.42 ± 0.11a	22.81 ± 0.98a	8.88 ± 0.40a
180	51.64 ± 0.73a	23.99 ± 0.45a	8.08 ± 0.26a
270	49.18 ± 1.45a	23.80 ± 0.99a	8.56 ± 0.61a
365	48.20 ± 0.07a	24.47 ± 0.23a	8.48 ± 0.07a
Rubi			
raw	57.85 ± 0.99a	28.39 ± 1.26a	6.10 ± 0.37a
0	60.33 ± 2.86a	30.50 ± 0.46a	5.39 ± 0.34a
90	61.81 ± 0.83a	30.88 ± 0.81a	5.24 ± 0.07a
180	61.00 ± 0.71a	30.68 ± 0.25a	5.35 ± 0.11a
270	58.51 ± 1.65a	30.44 ± 1.86a	5.62 ± 0.18a
365	58.15 ± 0.36a	30.18 ± 0.39a	5.70 ± 0.04a

[a] Raspberry extract concentration expressed as g of fresh sample/g of DPPH• in the reaction medium. [b] EC_{50} is the amount of sample needed to decrease by 50% the initial concentration. [c] t_{EC50} is the time needed to reach the steady state at EC_{50} concentration. [d] AE is the antiradical efficiency = $1/EC_{50}t_{EC50}$.

Effect of Freezing and Long-Term Frozen Storage on Radical Scavenging Activity Measure as Antiradical Efficiency (AE). The free-radical scavenging activity of aqueous methanolic extracts of four fresh raspberry cultivars was calculated as AE, a new parameter to measure the free radical scavenging of samples. It combines not only the widely used parameter EC_{50} but also the reaction time with the parameter t_{EC50} (Sanchez-Moreno et al., 1999). On the basis of t_{EC50} values of fresh Heritage and Autumn Bliss (40.68 and 38.35 min, respectively), early cultivars had a "slow" kinetic behavior, whereas late cultivars Rubi and Zeva, with t_{EC50} values of 28.39 and 20.61 min, respectively, displayed an "intermediate" kinetic behavior (Table 3) (Sanchez-Moreno et al., 1998). The EC_{50} parameter was widely used by different authors to measure the antioxidant power (Vinson et al., 1995), and according to them, the lower EC_{50} reflects the higher antioxidant power. Again, late cultivars with lower EC_{50} values showed higher antioxidant capacity than early cultivars (Table 3). Taking into account the AE parameter, fresh late cultivars, Rubi (6.1×10^{-4}) and Zeva (10.17×10^{-4}), showed higher AE values than fresh early cultivars, Autumn Bliss (4.36×10^{-4}) and Heritage (4.02×10^{-4}) (Table 3). Taking into account AE, EC_{50}, and t_{EC50} parameters, Zeva raspberry cultivar showed the highest and Heritage the lowest antioxidant capacity among the four Spanish cultivars studied. This high AE value was in accordance with the highest total phenolic (1776 mg kg^{-1} of fw), ellagic acid (244.38 mg kg^{-1} of fw), and vitamin C (295 mg kg^{-1} of fw) contents measured in Zeva; meanwhile, Heritage showed the lowest total phenolic (1137 mg kg^{-1} of fw), ellagic acid (217 mg kg^{-1} of fw), and vitamin C (232.3 mg kg^{-1} of fw) contents of the four cultivars studied (Table 2 and Figure 1). Late cultivars also showed higher contents of other kinds of phenolic compounds with possible antioxidant capacity such as anthocyanins (Saint-Cricq de Gaulejac et al., 1999). A previous study indicated that the total anthocyanin content in Zeva (116 mg/g of fw) was higher than those of the other three cultivars, whereas Heritage had the lowest total anthocyanin value (37.04 mg/g of fw) (Ancos et al., 1999).

The freezing process produced a decrease of the free radical scavenging activity in the four raspberry cultivars. AE values decreased 26 and 12% in the Zeva and Rubi extracts, respectively. Meanwhile, early raspberry cultivars suffered a minor decrease of radical-scavenging capacity with losses of 9 and 4% in Autumn Bliss and Heritage, respectively. At the end of the frozen storage period (365 days), the AE value remained practically unchanged compared with the AE values measured just after the freezing process. A low correlation coefficient between AE and total phenolic during frozen storage was found (0.482−0.5187).

In conclusion, the freezing process followed by a long-term frozen storage is a good preservative process to mantain almost unchanged the free radical scavenging capacity of raspberry fruits. Frozen storage does not significantly affect the ellagic acid and total phenolic contents or the AE value of raspberries. Vitamin C was the antioxidant most degraded after frozen storage of one year. Although freezing maintained the raw level of vitamin C, frozen storage more quickly degraded the vitamin C content in Rubi and Zeva cultivars (late) than in Heritage and Autumn Bliss cultivars (early), because a decrease of ∼20% of the raw vitamin C level was detected after 180 and 90 days of frozen storage of the early and late cultivars, respectively. Zeva raspberry, with the highest ellagic acid, total phenolic, and vitamin C contents and AE value, was more affected by the long-term frozen storage but remained as the cultivar with the highest ellacig acid content and AE value after 360 days of frozen storage at −20 °C. Therefore, Zeva raspberry could be the most suitable cultivar for freezing and long-term frozen storage with regard to the parameters studied in this work.

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