

Effect of Freezing and Storage on the Phenolics, Ellagitannins, Flavonoids, and Antioxidant Capacity of Red Raspberries

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Scottish-grown red raspberries are a rich source of vitamin C and phenolics, most notably, the anthocyanins cyanidin-3-sophoroside, cyanidin-3-(2^o-glucosylrutinoside), and cyanidin-3-glucoside, and two ellagitannins, sanguin H-6 and lambertianin C, which are present together with trace levels of flavonols, ellagic acid, and hydroxycinnamates. The antioxidant capacity of the fresh fruit and the levels of vitamin C and phenolics were not affected by freezing. When fruit were stored at 4 °C for 3 days and then at 18 °C for 24 h, mimicking the route fresh fruit takes after harvest to the supermarket and onto the consumer's table, anthocyanin levels were unaffected while vitamin C levels declined and those of ellagitannins increased, and overall, there was no effect on the antioxidant capacity of the fruit. It is concluded, therefore, that freshly picked, fresh commercial, and frozen raspberries all contain similar levels of phytochemicals and antioxidants per serving.

KEYWORDS: Freezing; storage; raspberries; phenolics; ellagic acid; ellagitannins; anthocyanins; vitamin C; flavonols; hydroxycinnamates; antioxidant capacity; electron spin resonance spectroscopy

INTRODUCTION

There is consistent epidemiological evidence linking consumption of a diet rich in fruit and vegetables with reduced incidences of cancer and coronary heart disease (1–4). As well as displacing dietary fat, fruits and vegetables contain several health-promoting factors, including vitamins, minerals, and high concentrations of phenolic compounds and flavonoids. These compounds, while not essential for survival, may over the long term provide protection against a number of chronic diseases (5). The phenolic compounds potentially involved in these beneficial effects include gallic acid, its dimer ellagic acid, and hydroxycinnamates, including coumaric acid, caffeic acid, and derivatives such as chlorogenic acid (6). The main flavonoids of interest are anthocyanins, flavan-3-ols, and their polymeric products, flavanones, flavonols, and flavones (7). To varying degrees, these compounds are potent antioxidants in vitro, scavenging O₂^{•-}, OH[•], and ROO[•] (8), inhibiting lipid peroxidation (9), and protecting low-density lipoproteins against oxidation (10). They can also inhibit platelet aggregation (11) and enhance vasodilation (12).

Epidemiological studies carried out in Finland between 1970 and 1990 have shown 60% declines in both heart disease and stroke over a period when multicomponent programs were successfully directed at diet and lifestyle (13). The health gains can largely be explained by major behavioral changes which resulted in a reduced intake of saturated fat, a lowering of serum cholesterol at a population level, reduced salt intake and blood pressure, a decline in smoking by men, and a 2–3-fold increase in the level of fruit and vegetable consumption nationally (14). A striking feature of the diet in Finland and other Nordic countries is the high level of consumption of antioxidant-rich berries (15), particularly wild bilberry, cloudberry, cranberry, and cowberry, which has been maintained and enhanced by the Finnish government berry project promoting the consumption of cultivated berries such as raspberries, strawberries, and black currants.

Scotland has one of the highest rates of premature deaths from chronic disease such as coronary heart disease, strokes, and colon cancer. This is ascribed, in part, to a national diet rich in saturated fats but also a habitually low level of consumption of foods rich in antioxidant micronutrients. The expense and lack of availability of fresh fruit and vegetables are cited as barriers to an improved diet, although paradoxically Scotland has an excellent climate and growing conditions for a range of popular fruits and vegetables (16).

If the Nordic model is to be followed, a potentially important source of antioxidant-rich food for the Scots could be locally

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cultivated berries, such as raspberries, which are already grown extensively on a commercial basis primarily for the export market. Most raspberries are sold fresh. Frozen berries, in pulp, are much cheaper but are widely regarded as being nutritionally inferior.

Raspberries have a high antioxidant capacity (15, 17) which can be attributed to ellagitannins, anthocyanins, and vitamin C (18). The two main ellagitannins are sanguin H-6 and lambertianin C (17–19). Although raspberries themselves contain only relatively small amounts of ellagic acid and its sugar conjugates when raspberry extracts are treated with acid, sanguin H-6 and lambertianin C are hydrolyzed and release substantial quantities of ellagic acid (6, 17, 20). The major raspberry anthocyanin is cyanidin-3-sophoroside with smaller quantities of other anthocyanins, including cyanidin-3-(2^G-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, pelargonidin-3-(2^G-glucosylrutinoside), and pelargonidin-3-glucoside (17, 21–23). Raspberries also contain quercetin and kaempferol-based flavonol conjugates (6, 17, 24) and trace levels of (–)-epicatechin and a procyanidin dimer (17).

The object of this study was to investigate the effects of freezing and storage on the antioxidant capacity and the levels of phenolics, ellagitannins, and flavonoids in Glen Ample raspberries, which are grown commercially on a wide scale in Scotland. The berries were processed in a manner that simulates the production chains for commercial raspberries from harvest to the consumer's table.

MATERIALS AND METHODS

Chemicals. Cyanidin-3-glucoside was purchased from Apin Chemicals (Abingdon, Oxford, U.K.). Methanol and acetonitrile were obtained from Rathburn Chemicals (Walkerburn, Peebleshire, U.K.). All other chemicals and reagents were purchased from Sigma-Aldrich (Poole, Dorset, U.K.).

Plant Material. Ripe field-grown raspberries (*Rubus idaeus* L.) cv. Glen Ample were hand picked at Blairgowrie, Perthshire, U.K. The fruit was divided into four lots which were treated in the following manner: fresh, extracted with methanol as described below within 3 h of picking; frozen, frozen within 3 h of picking at –30 °C in a commercial plant operated by Scottish Soft Fruit Growers plc, Blairgowrie; store, maintained at 4 °C for 3 days prior to freezing in liquid nitrogen (equivalent to arrival in the supermarket); and home, maintained at 4 °C for 3 days, then kept at 18 °C for a further 24 h before freezing in liquid nitrogen (equivalent to keeping the raspberries in the kitchen for 1 day prior to eating at home). After being frozen in liquid nitrogen, all samples were stored at –80 °C for less than 24 h before extraction.

Extraction of Raspberries. Forty grams of raspberries was macerated in an ice-cold pestle and mortar, and the resulting homogenate was centrifuged at 2000g for 30 min at 4 °C. The supernatant was decanted and the pellet vortexed in ice-cold acidified (0.1% HCl) methanol, after which the mixture was recentrifuged. The two supernatants were combined and made up to a known volume with acidified methanol. This was then subdivided into 2 mL aliquots and stored in microcentrifuge tubes at –80 °C prior to analysis.

Determination of the Total Phenol Content. The total phenol contents of raspberry extracts were determined in triplicate in gallic acid equivalents using the Folin–Ciocalteu method (25). This assay also detects vitamin C (17), but on a mole to mole basis, the response is ca. 10% of that of flavonols such as quercetin.

Colorimetric Analysis of the Total Anthocyanin Content. The free and polymeric anthocyanin contents of triplicate raspberry extracts were estimated using a pH shift method (26). Anthocyanins were quantified as cyanidin-3-glucoside equivalents, one of the three major anthocyanin in raspberries, using an extinction coefficient ϵ of 29 600.

Measurement of Antioxidant Potential by Electron Spin Resonance Spectroscopy. The antioxidant potential of triplicate raspberry

extracts was determined by their ability to reduce the Fremy's salt (potassium nitrosodisulfonate) (27). The extracts were diluted to 5% (v/v) with ethanol and water (12:88, v/v). Three 1.0 mL aliquots were reacted with an equal volume of 1 mM Fremy's radical in ethanol and water (12:88, v/v). The electron spin resonance (ESR) spectra of the low field resonance of the Fremy's radical were obtained after 20 min, by which time the reaction was complete. The signal intensity was obtained by double integration and concentration calculated by comparison with a control reaction using ethanol and water (12:88, v/v) without raspberry extract. Spectra were obtained at 21 °C on a Bruker ECS 106 spectrometer equipped with a cylindrical (TM110 mode) cavity and operating at ca. 9.5 GHz (X-band frequency). The microwave power and modulation amplitude were set at 2 mW and 0.01 mT, respectively.

Analysis of Hydroxycinnamates by HPLC with Diode Array Detection. Triplicate raspberry extracts were reduced to dryness prior to being redissolved in 0.1 M Tris-HCl (pH 7.4) and incubated with 0.3 mg of β -glucosidase for 1 h at 37 °C to cleave sugars from hydroxycinnamate conjugates. After centrifugation, the hydrolyzed and prehydrolyzed samples were analyzed by HPLC using a Waters (Milford, MA) Alliance liquid chromatograph with a photodiode array detector simultaneously monitoring wavelengths from 200 to 650 nm. Separation of chlorogenic acid, caffeic acid, and *p*-coumaric acid was achieved on a 250 mm \times 4.6 mm (inside diameter), 5 μ m ODS Hypersil column (Thermo-Finnigan, Runcorn, Cheshire, U.K.) eluted isocratically at a flow rate of 1 mL/min with 1% acetonitrile in water containing 5% acetic acid.

Quantitative Analysis of Flavonols by HPLC with Postcolumn Derivatization. Triplicate methanolic extracts of the raspberry samples (100 μ L) were placed in a 4 mL glass v-vial to which were added 1.6 mL of 60% methanol containing 20 mM sodium diethyl dithiocarbamate and 0.4 mL of 6 M HCl. The sample was incubated at 90 °C for 2 h, after which it was centrifuged at 15800g for 5 min. A 100 μ L aliquot of the supernatant was removed and added to 150 μ L of 0.5% TFA. One hundred fifty microliters of this solution was then analyzed on a Gilson (Villiers Le Bel, France) model 305 gradient HPLC system with a Shimadzu (Kyoto, Japan) 10A_{VP} autosampler, a Shimadzu SPD-10A_{VP} UV–vis absorbance monitor (28), and a fluorescence detector in series (29, 30). Separation was carried out using a RP-MAX 4 μ m, 250 mm \times 4.6 mm (inside diameter), C₁₂ reverse phase column (Phenomenex, Torrance, CA) maintained at 40 °C and eluted at a flow rate of 1.0 mL/min with a 20 min gradient from 20 to 40% acetonitrile in water containing 0.5% trifluoroacetic acid (TFA). After the column eluate passed through the absorbance monitor operating at 365 nm, 0.1 M methanolic aluminum nitrate was added at a flow rate of 1.0 mL/min by a Reeve Analytical (Glasgow, U.K.) postcolumn reaction pump. The mixture was then passed through a reaction coil at 40 °C before being directed to the fluorimetric detector (excitation at 425 nm, emission at 480 nm). Signals from the two detectors were processed by a dual-channel Reeve Analytical model 27000 data system.

Quantitative Analysis of Anthocyanins and Ellagic Acid by HPLC with Absorbance and Tandem Mass Spectrometric Detection. Anthocyanins and ellagic acid in triplicate raspberry extracts were analyzed on a P4000 liquid chromatograph fitted with an AS 3000 autosampler and with detection by a UV6000 diode array absorbance monitor scanning from 250 to 700 nm (Thermo-Finnigan, San Jose, CA). Separation was carried out using a RP-MAX 4 μ m, 250 mm \times 4.6 mm (inside diameter), C₁₂ reverse phase column (Phenomenex), maintained at 40 °C and eluted at a flow rate of 1.0 mL/min with a 30 min gradient from 8 to 18% acetonitrile in water containing 1% formic acid. Quantification of cyanidin-3-sophoroside, cyanidin-3-(2^G-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, and pelargonidin-3-(2^G-glucosylrutinoside), in cyanidin-3-glucoside equivalents, was based on absorbance at 520 nm. Ellagic acid was monitored at 365 nm and, because of the limited solubility of the reference compound, was quantified in gallic acid equivalents. After being passed through the flow cell of the diode array detector, the column eluate was split and 50% directed to an LCQ Duo mass spectrometer (Thermo-Finnigan) with an electrospray interface operating in full scan data dependent MS/MS mode from 150 to 2000 amu. Anthocyanins were analyzed in positive ion mode, and ellagic

Table 1. Effects of Freezing and Storage on the Free, Conjugated, and Total *p*-Coumaric Acid Content of Ample Raspberries^a

<i>p</i> -coumaric acid	fresh	frozen	shop	home
free	0.07 ± 0.02	0.05 ± 0.01	nd	0.19 ± 0.04
conjugated	0.28 ± 0.04	1.85 ± 0.10	0.52 ± 0.04	0.61 ± 0.05
total	0.35 ± 0.06	1.90 ± 0.08	0.52 ± 0.04	0.80 ± 0.05

^a Data expressed as nanomoles per gram fresh weight ± the standard deviation ($n = 3$). nd means not detected.

acid was analyzed by negative ionization. The mass spectral data that were obtained were used to confirm the identity of the absorbance peaks used for quantitative analysis.

Quantitative Analysis of Vitamin C by HPLC with Absorbance Detection. The vitamin C content of triplicate methanolic raspberry extracts was analyzed by HPLC (31) using a Nucleosil ODS 5 μ m, 250 mm \times 4.6 mm (inside diameter) column (Jones Chromatography, Glamorgan, U.K.) eluted isocratically at a flow rate of 0.6 mL/min using a Gilson model 305 liquid chromatograph with a cooled autoinjector and a 231 absorbance detector operating at 263 nm. Data were recorded on a Gilson 715 data system.

Quantitative Analysis of Ellagitannins by HPLC with Absorbance Detection. Methanolic raspberry extracts were analyzed in triplicate on a Shimadzu 10A ν p liquid chromatograph fitted with an autosampler and with detection by a SPD-10A ν p UV-vis absorbance monitor operating at 280 nm. Separation was carried out using a RP-MAX 4 μ m, 250 mm \times 4.6 mm (inside diameter), C₁₂ reverse phase column maintained at 40 °C and eluted at a flow rate of 1.0 mL/min with a 30 min gradient from 5 to 20% acetonitrile in water containing 0.5% TFA. The signal from the detector was processed by a Reeve Analytical 27000 data system. In the absence of reference compounds, the lambertianin C and sanguin H-6 peaks, which had retention times (t_R) of 23.9 and 25.4 min, respectively, were quantified in gallic acid equivalents.

Statistics. Data are represented as mean values ± the standard deviation (SD) ($n = 3$). Where appropriate data were subjected to statistical analysis using analysis of variance (ANOVA) to determine the significance of the treatment relationships. Statistical analyses were performed using Minitab software, version 12 (Minitab Inc., Addison-Wesley Publishing Co., Reading, MA).

RESULTS

Hydroxycinnamates and Flavonols. The hydroxycinnamate contents of fresh, frozen, shop, and home raspberries were analyzed before and after treatment with β -glucosidase under HPLC conditions that separated chlorogenic acid, caffeic acid, and *p*-coumaric acid. Only *p*-coumaric acid was detected. The free acid was present in sub-nanomole quantities per gram fresh weight with slightly larger quantities of conjugated *p*-coumaric acid (Table 1). The highest concentration of total *p*-coumaric acid was detected in frozen raspberries, but this was a mere 1.9 ± 0.4 nmol/g.

Flavonols were present at higher concentrations than hydroxycinnamates with the conjugated forms again being present in larger amounts than the aglycones. The main component was conjugated quercetin with smaller quantities of conjugated kaempferol (Table 2). The total flavonol content ranged from 22.3 to 27.0 nmol/g, and the values obtained with fresh, frozen, shop, and home raspberries were not significantly different.

Anthocyanins. The six major anthocyanins in raspberries, cyanidin-3-sophoroside, cyanidin-3-glucosylrutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside, and pelargonidin-3-glucosylrutinoside, were analyzed quantitatively by HPLC. There were no significant differences either in the levels of the individual anthocyanins or in the overall anthocyanin content of the fresh, frozen, shop, and home

Table 2. Effects of Freezing and Storage on the Free, Conjugated, and Total Flavonol Content of Ample Raspberries^a

flavonol	fresh	frozen	shop	home
free quercetin	1.0 ± 0.03	0.8 ± 0.03	1.1 ± 0.03	0.9 ± 0.07
conjugated quercetin	20.2 ± 0.5	24.8 ± 0.4	23.0 ± 0.3	21.6 ± 0.9
total quercetin	21.2 ± 0.4	25.6 ± 0.4	24.1 ± 0.3	22.5 ± 0.9
free kaempferol	0.1 ± 0.00	0.1 ± 0.00	0.1 ± 0.01	0.1 ± 0.00
conjugated kaempferol	1.0 ± 0.07	1.3 ± 0.03	1.1 ± 0.03	1.1 ± 0.03
total kaempferol	1.1 ± 0.07	1.4 ± 0.03	1.2 ± 0.03	1.2 ± 0.03
total flavonols	22.3 ± 0.4	27.0 ± 0.4	25.3 ± 0.4	23.7 ± 0.9

^a Data expressed as nanomoles per gram fresh weight ± the standard deviation ($n = 3$).

raspberries (Table 3). The anthocyanins were, however, present in the raspberries at much higher concentrations than either *p*-coumaric acid or flavonols.

Total Phenolics, Polymeric and Free Anthocyanins, Vitamin C, Lambertianin C, Sanguin H-6, Ellagic Acid, and Antioxidant Capacity. The overall levels of phenolics in extracts of the fresh, frozen, shop, and home raspberry samples were determined colorimetrically using the Folin-Ciocalteu assay, and the data that were obtained are presented in Table 4. The concentrations of phenolics in the shop and home raspberries (3510 and 3769 nmol/g⁻¹, respectively) were slightly higher than in the fresh and frozen samples (3383 and 3321 nmol/g, respectively), and this difference was statistically significant ($p = 0.004$). Anthocyanins were also measured colorimetrically which enabled a combined estimate of free and polymeric forms to be obtained (Table 4). The values for total anthocyanins ranged from 770 to 819 nmol/g, and there were no statistically significant differences in the amounts found in the fresh, frozen, shop, and home raspberry samples, in keeping with the information obtained by HPLC analysis of anthocyanins (Table 3). The vitamin C concentration, determined by HPLC, declined from 672 nmol/g for fresh to 622 nmol/g for home berries, with the levels in the shop and home raspberries being significantly lower than those in the fresh and frozen samples (Table 4). In contrast to vitamin C, the ellagitannin content rose during storage. The level of lambertianin C was significantly higher in the sample of home berries ($p = 0.002$), while sanguin H-6 exhibited a statistically significant increase from fresh and frozen (522 and 517 nmol/g, respectively) to 571 nmol/g for shop and 639 nmol/g for home ($p = 0.004$). The albeit low concentrations of ellagic acid in fresh and frozen fruit, 3.5 and 3.9 nmol/g, respectively, increased ca. 2-fold in shop and 5-fold in home raspberries. Unlike the vitamin C, ellagitannin, and ellagic acid concentrations, there were no discernible differences in the antioxidant capacity of the four raspberry samples, which ranged from 406 to 420 $\times 10^{16}$ Fremy's radicals reduced/g f.w. (Table 4).

DISCUSSION

Studies on the effects of storage and freezing on raspberries showed that the antioxidant capacity was not significantly different in fresh, frozen, shop, and home berries. Free and conjugated coumaric acid were detected in the raspberries, and although present in higher concentrations in frozen fruit, the levels were very low indeed at 1.9 ± 0.4 nmol/g (Table 1). The overall flavonol levels, comprised primarily of conjugated quercetin, ranged from 22.3 to 27.0 nmol/g and were unaffected by freezing and storage (Table 2). Anthocyanins were present at far higher concentrations with the main component cyanidin-

Table 3. Effect of Freezing and Storage on the Anthocyanin Content of Ample Raspberries^a

anthocyanin	fresh	frozen	shop	home
cyanidin-3-sophoroside	555 ± 4 (53.5)	567 ± 8 (54.1)	579 ± 7 (53.6)	536 ± 6 (53.2)
cyanidin-3-(2 ^o -glucosylrutinoside)	235 ± 2 (22.7)	232 ± 3 (22.1)	229 ± 3 (21.2)	209 ± 3 (20.7)
cyanidin-3-glucoside	156 ± 1 (15.0)	159 ± 2 (15.2)	172 ± 2 (15.9)	163 ± 2 (16.2)
cyanidin-3-rutinoside	62 ± 0.5 (6.0)	62 ± 0.8 (5.9)	71 ± 0.9 (6.6)	68 ± 0.8 (6.8)
pelargonidin-3-sophoroside	24 ± 0.2 (2.3)	23 ± 0.3 (2.2)	24 ± 0.3 (2.2)	24 ± 0.3 (2.4)
pelargonidin-3-glucose-rutinoside	5.2 ± 0.1 (0.5)	5.2 ± 0.1 (0.5)	5.4 ± 0.1 (0.5)	7.1 ± 0.1 (0.7)
total anthocyanin content	1037 ± 8	1049 ± 14	1081 ± 14	1008 ± 12

^a Data expressed as nanomoles of cyanidin-3-glucoside equivalents per gram fresh weight ± the standard deviation ($n = 3$) and in italicized parentheses as a percentage of total anthocyanin content.

Table 4. Effects of Freezing and Storage on the Total Phenolics, Anthocyanins, Vitamin C, Lambertianin C, Sanguin H-6, Ellagic Acid, and Antioxidant Capacity of Ample Raspberries^a

	fresh	frozen	shop	home
total phenolics	3383 ± 230 (100)	3321 ± 103 (98)	3510 ± 107 (104)	3769 ± 125 (111)
free anthocyanins	580 ± 25 (100)	600 ± 32 (103)	621 ± 19 (107)	621 ± 19 (107)
total anthocyanins	770 ± 21 (100)	782 ± 31 (102)	819 ± 25 (106)	794 ± 30 (103)
vitamin C	672 ± 11 (100)	671 ± 14 (100)	638 ± 12 (95)	622 ± 22 (93)
lambertianin C	110 ± 3.1 (100)	116 ± 8.7 (105)	124 ± 4.2 (113)	159 ± 4.5 (145)
sanguin H-6	407 ± 1.5 (100)	406 ± 1.5 (100)	447 ± 3.6 (110)	480 ± 4.5 (118)
ellagic acid	3.5 ± 0.1 (100)	3.9 ± 0.1 (111)	6.4 ± 0.1 (190)	18.5 ± 0.2 (528)
antioxidant capacity	406 ± 9.2 (100)	420 ± 9.1 (103)	406 ± 3.0 (100)	406 ± 6.3 (100)

^a Data presented as mean values of grams fresh weight ± the standard deviation ($n = 3$) and in italicized parentheses as a percentage of the values for fresh raspberries. Total phenolics expressed as nanomoles of gallic acid equivalents, anthocyanins as nanomoles of cyanidin-3-glucoside equivalents, lambertianin C, sanguin H-6, and ellagic acid as nanomoles of gallic acid equivalents, and antioxidant capacity as the number of Fremy's radical × 10¹⁶ reduced.

3-sophoroside being detected at concentrations of >500 nmol/g and overall anthocyanin levels being >1000 nmol/g (Table 3). Analysis of fresh, frozen, shop, and home fruit revealed no significant difference in the levels of the individual anthocyanins measured by HPLC (Table 3) or in the free and conjugated anthocyanin content that was determined colorimetrically (Table 4). The levels of total phenolics were significantly higher in the shop and home raspberries than in the fresh and frozen fruit (Table 4). The low levels of ellagic acid also rose during storage of raspberries, and there were small but significant increases in the much higher concentrations of lambertianin C in home and sanguin H-6 in shop and home fruit (Table 4). In contrast to these changes, the antioxidant capacity in fresh, frozen, shop, and home raspberries did not change significantly. Previous investigation has shown that the main contributors to the antioxidant capacity of raspberries are the ellagitannins, anthocyanins, and vitamin C (17). It is, therefore, likely that the increases in the levels of lambertianin C and sanguin H-6 during storage, and the small but significant increase in the level of total phenolics, were not accompanied by increases in antioxidant capacity because they were either relatively minor or offset by the decline in vitamin C in shop and home fruit (Table 4). The explanation for these postharvest changes lies presumably in the fact that secondary metabolism in berries remains active under home and shop conditions but not in frozen tissues.

The information obtained in this investigation is in broad agreement with studies on the effects of freezing and subsequent long-term storage of Spanish raspberries which showed that anthocyanins were more stable in spring fruiting cultivars than in autumn fruiting varieties (20). It was also demonstrated that the freezing process had little effect on the ellagic acid released by acid hydrolysis, total phenol, vitamin C content, and antioxidant capacity. However, during long-term frozen storage (12 months), the level of ellagic acid declined 14–21% and vitamin levels fell 33–55%, although the antioxidant capacity of the berries was unchanged (20).

In another study with Canadian-grown raspberries, fruit were stored at 0, 10, 20, and 30 °C for up to 8 days (32). There was an increase in the antioxidant capacity of the raspberries at storage temperatures above 0 °C which was associated with increases in the levels of anthocyanins and total phenolics and a decline in the level of vitamin C. Although the number of components analyzed was fewer than in this study, the findings are broadly similar to our own, except for the increase in the level of anthocyanins which was not observed in Glen Ample raspberries. This may well be due to the fact that the Canadian berries were, in most instances, stored for longer periods and at higher temperatures than the Glen Ample fruits where the fresh berries were stored at 4 °C for 3 days (shop) and then at 18 °C for an additional day (home).

In conclusion, this investigation has shown that Scottish-grown raspberries are a rich source of vitamin C and phenolics, most notably, anthocyanins and ellagitannins. The antioxidant capacity of the fresh fruit and the levels of vitamin C and phenolics were not affected by freezing, which is in keeping with previously published data obtained with Spanish raspberries (20). When fruit were stored at 4 °C for 3 days and then at 18 °C for 24 h, mimicking the route fresh fruit takes after harvest to the supermarket and onto the consumer's table, anthocyanin levels were unaffected while vitamin C levels declined and ellagitannin levels increased, and overall, there was no effect on the antioxidant capacity of the fruit. We conclude, therefore, that freshly picked, fresh commercial, and frozen raspberries provide similar levels of phytochemicals and antioxidants per serving.

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