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Effect of ascorbic acid treatment on some quality parameters of frozen strawberry and raspberry fruits

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Strawberry (Red Dream and Camarosa varieties) and raspberry (Nova and Killarney varieties) fruits were harvested in the middle summer in eastern part of Georgia. After harvesting average samples were immediately dipped into 0%, 1% or 2% ascorbic acid solution at 20 ± 1 °C temperature with exposure time of 2.5 min. Then, the samples were frozen at −40 °C and stored in plastic containers at −20 °C for 6 months. After 3 months storage period Total soluble solids (TSS) of strawberry and raspberry fruits decreased by 10–14% in both treated and untreated samples. TSS changes in the next three months were not statistically significant. pH values of the samples also decreased by 10–13% after 3 months regardless treatment with ascorbic acid. In the next three months pH values continued decreasing approximately with the same rate for all the samples. Due to the treatment of the fruit samples by 1% and 2% ascorbic acid, content of the last one in fruits after three months storage was increased approximately by 30% and 100% respectively and was kept practically at the same level for the next six months. In the untreated fruits of Red Dream and Camarosa of strawberry varieties during the first three months storage Total phenolic compounds (TPC) reduced by 20%. In both untreated varieties of raspberry TPC reduced by 14%. Ascorbic acid treatment increased polyphenol retaining in all frozen samples of strawberry and raspberry fruits. For Camarosa treated with 2% ascorbic acid this effect was the highest – 15%; for Red Dream the effect was the lowest – 5%. The next three months storage practically did not affect TPC in Red Dream variety of strawberry fruits neither untreated nor treated ones. In the rest varieties of berries TPC decreased maximum by 29% (Camarosa 2% treated) and minimum by 9% (Nova untreated). Antioxidant potential of the fruits was in good correlation ($R^2 = 0.93$) with TPC for all six months of storage.

Introduction

Strawberry (Fragaria x *ananassa*) cultivars Red Dream and Camarosa, Raspberry (Rubus *idaeus* L.) cultivars Nova and Killarney fruits belongs to the Rubus genus in the Rosacea family. in Georgia because of a good agro-climatic condition in Georgia for cultivation of berry fruits they are widely grown in the country [1]. Berries play important role in human nutrition and hence are very significant from the point of view of food security problems [2]. Berries are excellent sources of phytochemicals that are believed to have significant biological activity. Berry containing elevated levels of bioactive compounds, is attracting considerable attention due to their potential to lower the risk of chronic diseases and their associated huge healthcare costs [3–12]. Phenolic compounds may contribute to this protective effect. Berries are very rich in health-promoting phytochemicals [13]. Many of these phytochemicals have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals [14–18].

However, berries are also highly perishable fruits due to their soft texture, high softening rate and high sensitivity to fungal attack. Enzymes, namely polyphenoloxidase (PPO), peroxidese (POD) are involved in the fast deterioration of fruit during post-harvest handling and processing [19]. Freezing is one of the most important methods for the quality preservation of fruits and vegetables during long-term storage [20]. There is mainly antibrowning additives such as ascorbic acid, which is applied by dipping the fruit in different solution before freezing [21–23]. The freezing process reduces the rate of the degradation reactions and inhibits the microbiological and enzymatic activity [19]. Freezing processes have only a slight effect on the initial vitamin C content of fruit [24]. The destruction of vitamin C occurs during freezing and frozen

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storage and this parameter has been employed to limit the frozen storage period of frozen fruit. The main cause of loss of vitamin C is the action of the enzyme ascorbate oxidase [19].

The vitamin C content, besides being an indicator of nutrient value, is, in the case of frozen fruits and vegetables, a reliable index for estimating the quality deterioration at any point of the marketing route of a product to its final destination, the consumer [25,26].

The objective of this work was to study effect of application of ascorbic acid on the quality parameters such as content of TSS, TPC, vitamin C, also on pH and antioxidant activity of frozen strawberry and raspberry fruits during storage.

Material and methods

Chemicals

Ascorbic acid and Potassium dihydrogen phosphate were purchased from Sigma - Aldrich (Steinheim, Germany), TPTZ-2,4,6-Tris (2-pyridyl)-*s*-triazine (Sigma - Aldrich, Switzerland), Folin - Ciocalteu reagent (Appli Chem, Germany), hydrochloric acid, formic acid and phosphoric acid were provided by Merck (Darmstadt, Germany), Sodium carbonate was purchased from Chem Cruz (Chem Cruz Biochemicals, USA), Ethyl acetate and methanol (Sigma - Aldrich Steinheim, Germany) were HPLC grade. All other reagents were commercially available at the local market and were of analytical grades.

Sample collection

Berry fruits were harvested in the middle summer in eastern part of Georgia (GPS coordinates: Latitude: 41° 57′ 59.99" N, Longitude: 44° 05′ 60.00" E). After harvesting average samples of fruits were immediately dipped into 0%, 1% or 2% ascorbic acid solution at 20 ± 1 °C temperature with exposure time of 2.5 min. Then, the samples (275 g) were frozen at −40 °C and stored in plastic containers at −20 °C for 6 months.

Sample preparation for chemical analyses

Preparation of sample for ascorbic acid determination by HPLC (Varian – Prostar - 500, USA, detector - UV Varian Prostar, Auistralia, column - 250 mm × 4.6 mm, dp = 5 μm, Symmetry, Waters, Ireland) method was done according to Koyuncu et al. (2010) [27]. Briefly, sample (10 g) was extracted in 10 mL water adjusted to pH 1.5 with 10 mL phosphoric acid-water (2%, v/v). The extracts were filtered through filter paper 45 μm (Whatman, UK). Then, 1.5 mL buffer (0.01 M KH_2PO_4, pH 8.0) was added to 1.5 mL sample extract, 1 mL (vitamin C) of preferred mixtures were loaded on to C 18 cartridges (Agilent, Bond Elut, USA). After loading, 3.0 mL water adjusted to pH 1.5 with 2.0 mL phosphoric acid-water (2%, v/v) were passed through the cartridges.

Samples for antioxidant analysis were prepared according to Rodriguez - Saona et al. (2001) [28]. About 40 g of berries were cryogenically milled in liquid nitrogen. Chilled test tubes were filled with milled fruit powder and weighed (5 g), and then the powder was extracted with acetone (200 mL). The acetone was removed under vacuum in a rotary evaporator at < 30 °C and then 250 mL, 70% methanol was added to the powder. Total methanol extract was examined for antioxidant activity.

The frozen samples were defrosted during 3 h at 18 °C.

Measures

TSS

TSS was measured by digital refractometer (WYA – 2 S China) according to Brix reading [29].

Determination of pH

pH value of the berry fruits was measured using a pH-meter (PHS-3C, Shanghai Rotech Pharmaceutical Engineering Co., Ltd, China) [30].

Determination of vitamin C

Determination of vitamin C was performed by HPLC method as described by Koyuncu et al. (2010) [27]. The columns used were 250 mm × 4.6 mm, dp = 5 μm (Symmetry, Waters, Ireland), The mobile phases were water adjusted to pH 3.0 with phosphoric acid. The UV detector (Varian pro Star, Australia) was set at 215 nm. Quantitation was based on the peak area measurement. For HPLC (Varian-Prostar - 500, USA), 20 μL of the sample were injected.

Determination of total phenolic compounds (TPC)

Determination of TPC was performed by Bond et al. (2003) [31]. An aliquot of 1.0 mL of diluted sample extract was vortexed with 10 mL DI water and 1.0 mL Folin-Ciocalteau reagent, and 1.0 mL deionized water was used as control. After equilibration at room temperature for 8 min, the solutions were mixed with 4 mL of 7.5% (w/v) Na_2CO_3. The samples and standards (Gallic acid dilute working standard solutions: 10–50 μg mL^{-1}) were equilibrated at room temperature for 60 min. The absorbance of the samples and standards were measured spectrophotometrically (UV/Vis spectrophotometer, A&E Lab Co LTD, UK) at 765 nm, with a 10 mm path length cell. TPC was calculated as mg of gallic acid equivalents per 100 g fresh weight of sample.

Ferric reducing ability of plasma (FRAP) assay

The antioxidant capacity was determined following the procedure described by Benzie et al. (1996) [32]. with modifications. The FRAP reagent was freshly prepared by adding 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) (dissolved in 40 mM of HCl), 20 mM of $FeCl_3$ in water and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10. The FRAP reagent was warmed to 37 °C for 15 min. Then, 100 μL of sample was added to 3.0 mL reagent blank. The absorbance was recorded at 593 nm. The reaction was monitored for 4 min. FRAP values of samples were compared to that of ascorbic acid and expressed as vitamin C equivalents per 100 g of fresh fruits.

Statistical analysis

The data represents the mean of three replicates ± standard deviation (SD). Data were subjected to the two-way ANOVA and Tukey's HSD tests. All calculations were performed with Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA) with PHstat 2 version 3.11add-in assistance.

Results and discussion

TSS

After 3 months storage period TSS of samples of strawberry and raspberry fruits decreased by 10–14% in both treated and untreated samples, probably because of respiration process (Table 1.) But difference between the treated and untreated samples was not statistically significant ($p < 0.05$). TSS changes in the next three months were not statistically significant.

Table 1
Effect of ascorbic acid treatment on quality parameters of berries after 3 months frozen storage (at −20 ± 0.5 °C).

Berry	Variety	Treatments % with ascorbic acid	TSS %	pH	Vitamin C mg 100 g^{-1}	TPC mg 100 g^{-1}	FRAP mg equivalents vitamin C 100 g^{-1}
Strawberry	Red Dream	fresh	8.55 ± 0.15	3.50 ± 0.02	45.17 ± 0.24	150.14 ± 2.45	413.10 ± 3.52
		0	7.42 ± 0.43	3.16 ± 0.10	43.30 ± 0.75	120.81 ± 2.15[a]	290.25 ± 4.12
		1	7.67 ± 0.16	2.95 ± 0.23	45.32 ± 0.23	126.73 ± 2.05[a]	340.83 ± 2.58
		2	7.73 ± 0.32	2.82 ± 0.17	50.80 ± 0.33	128.80 ± 2.78[a]	386.35 ± 3.13
	Camarosa	fresh	7.50 ± 0.32	3.41 ± 0.02	52.14 ± 1.92	160.41 ± 3.17	584.00 ± 3.91
		0	6.15 ± 0.23	2.87 ± 0.11	35.21 ± 0.14	130.80 ± 4.28[b]	400.00 ± 3.17
		1	6.57 ± 0.35	2.79 ± 0.21	44.45 ± 0.44	147.73 ± 2.16[b]	468.12 ± 2.56
		2	6.60 ± 0.18	2.50 ± 0.13	48.63 ± 0.20	150.12 ± 3.15[b]	490.20 ± 4.18
Raspberry	Nova	fresh	11.67 ± 0.45	2.92 ± 0.01	19.24 ± 1.19	106.52 ± 2.1	190.12 ± 2.15
		0	10.12 ± 0.26	2.43 ± 0.12	12.10 ± 0.47	90.10 ± 1.95[b]	110.28 ± 3.18
		1	10.20 ± 0.09	2.21 ± 0.31	23.35 ± 0.20	102.22 ± 2.16[b]	137.18 ± 2.42
		2	10.22 ± 0.16	2.15 ± 0.16	42.18 ± 1.00	107.16 ± 3.17[b]	172.00 ± 2.88
	Killarney	fresh	11.00 ± 0.08	2.45 ± 0.02	23.87 ± 0.35	116.01 ± 1.25	220.00 ± 3.56
		0	9.56 ± 0.33	2.07 ± 0.12	13.78 ± 0.55	97.56 ± 4.16[b]	122.07 ± 2.13
		1	9.87 ± 0.35	1.95 ± 0.18	30.39 ± 1.23	103.25 ± 2.11[b]	153.18 ± 3.17
		2	9.80 ± 0.21	1.72 ± 0.19	45.52 ± 1.33	109.38 ± 3.52[b]	157.23 ± 3.12

[a] Difference between treated and untreated samples were not statistically significant ($p < 0.05$).
[b] Difference between treated and untreated samples were statistically significant ($p < 0.01$).

pH value

pH values of the samples also decreased by 10–13% after 3 months regardless treatment with ascorbic acid. The reason of reduction may be a partial hydrolyses of proteins into amino acids during storage period. Though, no statistically significant difference was observed between treated and untreated samples (Table 1.) In the next three months pH values continued decreasing approximately with the same rate for all the samples. Again, difference between the treated and untreated samples was not statistically significant ($p < 0.05$) (Table 2).

Ascorbic acid content

Ascorbic acid is an important nutrient and is very sensitive to degradation due to its oxidation compared to other nutrients during food processing and storage [33].

Ascorbic acid content in the untreated fruit samples after three months storage decreased by 37.1 and 42.3% in case of raspberry of Nova and Killarney varieties respectively, and by 32.5 and 4.1% - in case of strawberry of Camarosa and Red Dream varieties respectively (Table 1.). Reduction in ascorbic acid content of untreated fruit samples continued for the next three months as well; maximum drop (28%) was observed for untreated Red Dream strawberry, and minimum drop (24.6%) was observed for Nova raspberry (Table 2).

Due to the treatment of the fruit samples by 1% and 2% ascorbic acid, content of the last one in fruits after three months storage was increased approximately by 30% and 100% respectively for both varieties of raspberry and was kept practically at the same level for the next six months.

Strawberries seem to be much less able to absorb exogenic ascorbic acid. For instance, content of ascorbic acid in Red Dream fruits treated by 1% ascorbic acid solution did not change after three months storage and remained at the same level as in fresh fruits. 2% treatment resulted in increasing of ascorbic acid content of Red Dream fruits by 11% after three months storage.

For fruits of Camarosa variety the content of vitamin C even decreased by 15% (in case of 1% treatment) and 7% (in case of 2% treatment) after three months frozen storage.

In the next three months content of ascorbic acid in frozen strawberry fruits did not change practically.

TPC and antioxidant potential

Berry fruits are good source of polyphenolics [34]. Phenolic compounds are the main bioactive compounds that contribute to the antioxidant characteristics of fruit [19]. Hence, TPC and

Table 2
Effect of ascorbic acid treatment on quality parameters of berries after 6 months frozen storage (at −20 ± 0.5 °C).

Berry	Variety	treatments % with ascorbic acid	TSS %	pH	Vitamin C mg 100 g^{-1}	TPC mg 100 g^{-1}	FRAP mg equivalents vitamin C × 100 g^{-1}
Strawberry	Red Dream	0	7.05 ± 0.13	2.83 ± 0.18	31.12 ± 1.12	119.30 ± 2.52	260.30 ± 2.45
		1	7.21 ± 0.16	2.71 ± 0.11	43.35 ± 1.52	123.20 ± 3.18[a]	309.20 ± 4.15
		2	7.27 ± 0.52	2.53 ± 0.12	47.32 ± 2.13	126.05 ± 4.51[a]	345.27 ± 3.58
	Camarosa	0	5.83 ± 0.31	2.63 ± 0.22	28.15 ± 1.31	120.33 ± 3.72	355.50 ± 4.17
		1	5.93 ± 0.30	2.45 ± 0.17	42.43 ± 2.12	132.20 ± 2.86[b]	420.20 ± 3.16
		2	5.95 ± 0.11	2.41 ± 0.25	46.31 ± 1.18	140.02 ± 3.50[b]	445.10 ± 3.51
Raspberry	Nova	0	9.52 ± 0.18	2.18 ± 0.16	9.12 ± 0.92	82.35 ± 4.54	97.00 ± 2.50
		1	9.71 ± 0.22	2.05 ± 0.22	19.18 ± 1.12	84.51 ± 3.35[a]	105.07 ± 1.88
		2	9.78 ± 0.28	2.00 ± 0.12	39.32 ± 2.15	85.50 ± 3.35[a]	126.80 ± 2.38
	Killarney	0	9.12 ± 0.52	1.83 ± 0.20	8.05 ± 0.54	86.25 ± 2.26	103.51 ± 2.77
		1	9.27 ± 0.18	1.71 ± 0.31	27.52 ± 1.86	90.10 ± 3.30[a]	122.27 ± 2.18
		2	9.29 ± 0.25	1.62 ± 0.13	40.31 ± 1.12	95.20 ± 3.50[a]	147.18 ± 2.46

[a] Difference between treated and untreated samples were not statistically significant ($p < 0.05$).
[b] Difference between treated and untreated samples were statistically significant ($p < 0.01$).

Table 3
Two-way ANOVA analyses of effect of fruit variety and ascorbic acid treatment on the TPC in the first three months storage.

Treatment Variation	315.46	Berries varieties Variation	854.13
Within Variation	0.328	Interaction Variation	30.73
Treatment F-Statistic	958.95	P-Value	0.0005
Berries varieties F -statistic	2596.44	P-Value	0.0005
Interaction F-Statistic	93.43	P-Value	0.0005

Table 4
Two-way ANOVA analyses of effect of fruit variety and ascorbic acid treatment on the TPC in the last three months storage.

Treatment Variation	125.32	Berries varieties Variation	268.87
Within Variation	0.127	Interaction Variation	19
Treatment F-Statistic	980.27	P-Value	0.0005
Berries varieties F -statistic	2103.06	P-Value	0.0005
Interaction F-Statistic	148.64	P-Value	0.0005

antioxidant potential are well correlated in most cases [35].

In the untreated fruits of Red Dream and Camarosa of strawberry varieties during the first three months storage TPC reduced by 20%. In both untreated varieties of raspberry TPC reduced by 14% (Table 1.). In general, ascorbic acid treatment increased polyphenol retaining in all frozen samples of strawberry and raspberry fruits. For Camarosa treated with 2% ascorbic acid this effect was the higher – 15%; for Red Dream the effect was the least – 5%.

The next three months storage practically did not affect TPC in Red Dream variety of strawberry fruits neither untreated nor treated ones. In the rest varieties of berries TPC decreased maximum by 29% (Camarosa 2% treated) and minimum by 9% (Nova untreated) (Table 2).

In order to estimate an effect of fruit variety and ascorbic acid treatment on the TPC, a two-way ANOVA analyses was performed separately for the first and the last three months storage. The results are represented in Tables 3 and 4.

Two-way ANOVA analyses showed that there was a strong evidence ($p = 0.0005$) to suggest an interaction between fruit variety and ascorbic acid treatment. Also, there was a statistically significant difference ($p = 0.0005$) between mean values of TPC of the groups. Hence, Tukey's HSD analyses inside fruit variety groups were carried out to find out the groups with different TPC mean values. Such analyses showed that in the first three months there was a statistically significant difference ($p < 0.01$) between mean values of TPC in the groups of Camarosa, Nova and Killarney treated with 0, 1 and 2% ascorbic acid, whereas there was not statistically significant difference between mean values of TPC in groups of Red Dream.

In the last three months statistically significant difference between mean values of TPC of fruits treated with 0, 1 and 2% ascorbic acid was observed only in Camarosa fruits, in the all other varieties there was not statistically significant effect of ascorbic acid treatment.

Antioxidant potential was in good correlation with TPC for all six months of storage ($R^2 = 0.93$, Fig. 1). So, in general, ascorbic acid treatment increased retaining of antioxidant potential in all frozen samples of strawberry and raspberry fruits.

Fresh fruits of Camarosa characterized with the most antioxidant potential – 584.00 mg equivalents vitamin C per 100 g fruits. Fresh fruits of Nova revealed the least antioxidant potential – 190.12 mg vitamin C per 100 g fruits. This tendency was kept during storage period as well.

Conclusions

1. Ascorbic acid treatment did not affect TSS content in frozen raspberry and strawberry fruits.
2. pH values of the frozen samples of raspberry and strawberry fruits during storage decreased by the same rate regardless treatment with ascorbic acid.
3. Strawberries and raspberries absorb exogenic ascorbic acid used for treatment of the fruits.
4. Ascorbic acid treatment increases polyphenol retaining in frozen strawberry and raspberry fruits.

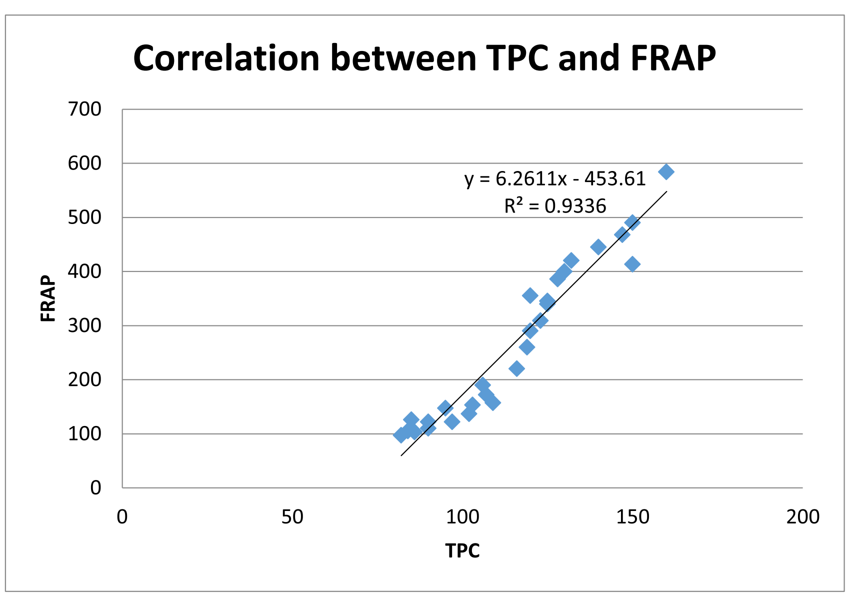

Fig. 1. Correlation between TPC and FRAP.

5. TPC and FRAP are in good correlation ($R^2 = 0.93$) in both fresh and frozen fruits of strawberry and raspberry during all time of storage period.

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