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Potential antioxidant retention and quality maintenance in raspberries and strawberries treated with calcium chloride and stored under refrigeration

Potencial de retenção de antioxidante e manutenção de qualidade de framboesas e morangos tratados com cloreto de cálcio e estocados sob refrigeração

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Abstract

Raspberry and strawberry fruits were stored at 0 °C and relative humidity (RH) of 95% for eight days. The fruits were treated with calcium chloride and their quality parameters and weight loss monitored and compared with those of untreated ones. A higher weight loss was observed for the untreated raspberries (16%) than for the fruits treated with 2% calcium chloride (5.3%). Similarly, untreated strawberry fruits lost more weight (8.5%) than those treated with 2% calcium chloride – only 4.1%. The application of calcium chloride did not significantly influence the total acid content of the fruits. After 8 days storage the total soluble solids (TSS) had decreased to 10.22 ± 0.06, 9.60 ± 0.05 and 9.65 ± 0.12 in the raspberry fruits treated with 0%, 1% and 2% calcium chloride, respectively, and to 7.00 ± 0.17, 6.57 ± 0.08 and 6.35 ± 0.04 in the strawberry fruits treated with 0%, 1% and 2% calcium chloride, respectively. After storage, the ascorbic acid contents were significantly ($p \leq 0.05$) higher in samples of raspberry and strawberry fruits subjected to 2% calcium chloride dips. The $CaCl_2$ treatments had a significant effect on retaining the ascorbic acid contents in these fruits. The treatment of raspberry and strawberry fruits with calcium chloride had a positive effect ($p< 0.05$) on the retention of the total phenolic contents (TPC) during the storage period. 66% and 74% of the antioxidant potentials were retained in the untreated samples of raspberries and strawberries, as against 78% and 89% in the 2% calcium chloride treated samples of these fruits.

Keywords: *Raspberry; Strawberry; Calcium chloride; Storage.*

Resumo

Frutos de framboesa e de morango foram armazenados a 0 °C e umidade relativa (UR) de 95% durante oito dias. Os frutos foram tratados com cloreto de cálcio e seus parâmetros de qualidade e perdas de peso monitorados e comparados com os valores dos frutos não tratados. Uma perda de peso maior foi observada para as framboesas não tratadas (16%) que para os frutos tratados com 2% de cloreto de cálcio (5,3%). Similarmente, os frutos de morango não tratados perderam mais peso (8,5%) que aqueles tratados com 2% de cloreto de cálcio – apenas 4,1%. A aplicação de cloreto de cálcio não influenciou significativamente no conteúdo total de ácido dos frutos. Depois de 8 dias de estocagem, o teor de sólidos totais (TST) diminuiu para 10,22 ± 0,06, 9,60 ± 0,05 e 9,65 ± 0,12 nos frutos de framboesa tratados com 0%, 1% e 2% cloreto de cálcio, respectivamente, e para 7,00 ± 0,17, 6,57 ± 0,08 e 6,35 ± 0,04 nos frutos de morango tratados com 0%, 1% e 2% de cloreto de cálcio, respectivamente. Depois da estocagem, o conteúdo de ácido ascórbico foi significativamente ($p \leq 0,05$) mais alto nas amostras de frutos de framboesa e morango sujeitos à imersão em 2% de cloreto de cálcio. Os tratamentos com $CaCl_2$ tiveram um efeito significativo na retenção de ácido ascórbico nestes frutos. O tratamento dos frutos de framboesa e morango com cloreto de cálcio teve um efeito positivo ($p < 0,05$) na retenção do conteúdo total de fenólicos (CTF) durante o período de estocagem. Do potencial de antioxidante, 66% e 74% foram retidos nas amostras não tratadas de framboesa e morango contra 78% e 89% nas amostras destes frutos tratados com 2% de cloreto de cálcio.

Palavras-chave: *Framboesa; Morango; Cloreto de cálcio; Estocagem.*

1 Introduction

Berries, especially raspberry (Rubus *Idaeus* L. cv. Killarney) and strawberry (Fragaria x *ananassa*. cv. Red Dream), members of the family Rosaceae, belong to the best dietary sources of bioactive compounds (BAC) (SKROVANKOVA et al., 2015; HALVORSEN et al., 2002; SOUZA et al., 2014; SLATNAR et al., 2012; NAMIESNIK et al., 2014; DIACONEASA et al., 2015). They have a delicious taste and flavour, economic importance, and antioxidant properties from the BAC (SKROVANKOVA et al., 2015). The BAC in berries contain mainly phenols (KATSUBE et al., 2003; GIOVANELLI; BURATTI, 2009; PRIOR et al., 1998; REMBERG et al., 2006)

Phenolic compounds may contribute to this protective effect. Berries are very rich in health-promoting phytochemicals (FORTALEZAS et al., 2010) and many of these phytochemicals have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (WADA; OU, 2002; PRIOR, 2003; ICHIYANAGI et al., 2014; ZHENG; WANG, 2003; PANICO et al., 2009). Berries can be used in the development of functional foods with the objective of enhancing the health conditions (POTTER et al., 2009). Berry antioxidants may prevent some crucial points in the genesis of carcinomas (LOBO et al., 2010; LIMBERAKI et al., 2012; KÅRLUND et al., 2014; STAINMETZ; POTTER, 1991; AMES et al., 1993). Different berries and berry phenolic compounds also possess considerable antimicrobial effects against organisms such as *Salmonella* and *Staphylococcus* (PUUPPONEN-PIMIÄ et al., 2005; BURDULIS et al., 2009).

However, raspberry and strawberry are also highly perishable fruit due to their soft texture, high softening rate and great sensitivity to fungal attack. The quality declines rapidly after harvest, which must be done at full maturity, and the storage life may be less than a week (WILLS, 1998), so to overcome this problem raspberry and strawberry fruits may be treated with calcium chloride (ASTUTI et al., 2013).

Calcium is a divalent cation that readily enters the apoplast and is bound to the cell wall and exterior surface of the plasma membrane in an exchangeable form. Calcium maintains the cell wall structure of the fruit by interacting with the pectic acid in the cell walls to form calcium pectate. Ca^{2+} forms cross-links between pairs of negatively charged homogalacturonans, thus tightening the cell wall (PICCHIONI et al., 1998). The application of calcium postharvest maintains cell turgor, membrane integrity and tissue firmness, and delays membrane lipid catabolism, extending the storage life of the fresh fruits and reducing physiological disorders (GARCIA et al., 1996; POOVAIAH, 1986).

The increase of Ca content in the fruit tissue was accompanied by reductions in the respiration rate, ascorbic acid degradation and membrane lipid peroxidation, which enhanced the total phenolic content (TPC) and total antioxidant capacity (WANG et al., 2014). Significant increases in polyphenols and anthocyanins were registered during storage, with a resulting increase in total antioxidant activity (FADDA et al., 2015). The postharvest application of $CaCl_2$ at appropriate rates imparts no detrimental effect on consumer acceptance of the treated fruit (LESTER; GRUSAK, 2001; SAFTNER et al., 1999).

The objective of the present study was to investigate the effect of the calcium treatment on berry fruits with respect to retaining the ascorbic acid and anthocyanins, TPC, antioxidant activity and also other general quality parameters during the storage period.

2 Material and methods

2.1 Chemicals

Ascorbic acid higher than 99.0% and potassium dihydrogen phosphate were purchased from Sigma-Aldrich (Steinheim, Germany); TPTZ - 2-4-6-tris (2-pyridyl)-s-triazine (Sigma-Aldrich, Switzerland), the Folin-Ciocalteau reagent (Appli Chem, Germany), hydrochloric acid, formic acid and phosphoric acid were provided by Merck (Darm-stadt, Germany); sodium carbonate was purchased from ChemCruz (ChemCruz 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 grade.

2.2 Sample collection

The strawberries and raspberries were harvested in mid-summer in the eastern part of Georgia (GPS coordinates: Latitude: 41° 57' 59.99" N, Longitude: 44° 05' 60.00" E). Quality parameters such as weight loss, vitamin C content, TPC and anthocyanin content, as well as the antioxidant potential were monitored at the start of sampling and after 8 days of storage at 0 °C. After 8-10 days most fruits were mouldy and damaged and the study was discontinued.

After harvesting, representative samples of the fruits were treated with 0%, 1% or 2% calcium chloride solution at 20 ± 1 °C with an exposure time of 2.5 min. The treated samples were stored for 8 days in a refrigerator at 0 ± 0.5 °C and 95 ± 0.5% RH.

2.3 Sampling procedure

The samples were prepared for the ascorbic acid determination by HPLC (Varian-Prostar-500, USA, detector-UV varian Prostar, Australia, column- 250 mm × 4.6 mm, dp = 5 µm (Symmetry, Waters, Ireland) (KOYUNCU; DILMAĢŰNAL, 2010) as follows: briefly, the 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 45 µm filter paper (Whatman, UK)

and 1.5 mL buffer (0.01 M KH_2PO_4, pH 8.0) then added to 1.5 mL of the sample extract. 1 mL aliquots (vitamin C) of each of the preferred mixtures were then loaded on to C 18 cartridges (Agilent, Bond Elut, USA) and 3 mL aliquots of water adjusted to pH 1.5 with 2 mL phosphoric acid-water (2%, v/v) passed through them.

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

The titratable acidity (TA) was determined by titration with 0.1 N NaOH to a pink colour using 1% phenolphthalein as indicator and expressed as g/100 g citric acid (MORRIS et al., 1985). The TSS were measured by digital refractometer (WYA -2S, China).

Vitamin C was determined by the HPLC method (KOYUNCU; DILMAĢŰNAL, 2010). The columns used were 250 mm × 4.6 mm, dp = 5 µm (Symmetry, Waters, Ireland) and the mobile phase was water adjusted to pH 3 with phosphoric acid. The UV detector (Varian pro Star, Australia) was set at 215 nm and quantification was based on the peak area measurement. For HPLC (Varian-Prostar-500, USA), 20 µL of sample were injected.

The anthocyanins were quantified by the pH differential method (GIUSTI; WROLSTAD, 2001). Samples were diluted 1:150 in pH 1.0 and pH 4.5 buffers, and the absorbance measured at 520 nm and 700 nm in a UV -Visible spectrophotometer (A & E Lab Co LTD, UK), based on a cyanidin 3-glucoside molar extinction coefficient of 26,900 ΔE/mol and a molecular weight of 449.2 g/mol. The resulting values were expressed in terms of mg of anthocyanin per 100 g of fresh fruit.

The Total phenolic compound content (BOND et al., 2003) was determined using a 1.0 mL aliquot of diluted sample extract, which was vortexed with 10 mL deionized (DI) water and 1.0 mL Folin-Ciocalteau reagent, and 1.0 mL deionized water was used as the control. After equilibrating 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 (dilute gallic acid standard working solutions: 10-50 µg/mL) were equilibrated at room temperature for 60 minutes. The absorbance values 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 and the TPC was calculated as mg of gallic acid equivalents per 100 gram fresh sample weight.

The Ferric Reducing Ability of Plasma (FRAP) assay was carried out as previously described by Benzie and Strain (1996). The experiment was carried out at 37 °C and pH 3.6 with a blank sample in parallel. In the FRAP assay, the reductants ("antioxidants") in the sample reduce the Fe (III)/tripyridyltriazine complex to the blue ferrous form, with an increase in absorbance at 593 nm. The final results were expressed as micromole ascorbic acid (AA) equivalents per 100 gram (mmol AA/ 100 g).

2.4 Statistical analysis

The data represent the mean of three replicates ± standard deviation (SD) and were subjected to ANOVA analyses. All calculations were carried out using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA) with PHstat 2 version 3.11add-in assistance.

3 Results and discussion

3.1 Weight loss

Fruit weight loss is associated with respiration and moisture evaporation through the skin. The data in Table 1 show that during storage (0 °C and 95 ± 0.5% RH) the raspberry fruits lost from 5.3 to 16% of their weight depending on the calcium treatment. The maximum loss was observed for the untreated fruits (16%) and the minimum loss for the fruits treated with 2% calcium chloride (5.3%). Similarly, untreated strawberry fruits lost a maximum weight of 8.5% and fruits treated with 2% calcium chloride only lost 4.1%, which is more than two times less than the untreated fruits.

Table 1. The effect of calcium chloride treatment on the weight loss of berries during 8 days of storage (at 0 ± 0.5 °C, RH 95 ± 0.5%).

Berry	Treatments (%,calcium chloride)	Initial weight (g)	Weight after storage (g)	Weight loss (%)
Raspberry	0	14.22 ± 0.14	11.92 ± 0.01	16.00 ± 0.04
	1	11.71 ± 0.05[a]	10.64 ± 0.07[a]	9.10 ± 0.01[a]
	2	12.48 ± 0.02[b]	11.81 ± 0.03[b]	5.30 ± 0.04[b]
Strawberry	0	18.90 ± 0.10	17.29 ± 0.08	8.50 ± 0.07
	1	21.01 ± 0.12[a]	19.74 ± 0.14[a]	6.00 ± 0.01[a]
	2	19.55 ± 0.17[b]	18.75 ± 0.10[b]	4.10 ± 0.15[b]

The data represent the mean of three replicates ± SD; [a,b] differences between test and control samples in the same column are statistically significant.

The lower weight loss in samples treated with the calcium chloride dip may be due to increased water holding by the formation of calcium pectate hydrogel, and a delay in the dehydration process (Table 1). One-way ANOVA analyses for the raspberry and strawberry fruits showed statistically significant differences between the mean values for weight loss of the 0, 1 and 2% treatment groups ($p<0.05$).

3.2 TA and TSS

The TA of the untreated raspberries and strawberries decreased from 2.34 ± 0.05 to 1.71 ± 0.02 and from 0.81 ± 0.03 to 0.70 ± 0.03, respectively during the days of storage. The application of calcium chloride did not significantly influence this parameter (Table 2).

The initial TSS content was 11.00 ± 0.08 and 8.55 ± 0.15 for raspberry and strawberry fruits, respectively.

After 8 days storage the TSS decreased to 10.22 ± 0.06, 9.60 ± 0.05 and 9.65 ± 0.12 in the raspberry fruits treated with 0%, 1% and 2% calcium chloride respectively and to 7.00 ± 0.17, 6.57 ± 0.08 and 6.35 ± 0.04 in the strawberry fruits treated with 0%, 1% and 2% calcium chloride respectively. In general, a decrease in TSS is associated with the respiration process in fruits. The slightly lower TSS in the 1% and 2% calcium chloride treated samples might be due to inhibition by the calcium chloride of the enzymatic conversion of higher polysaccharides, such as starches and pectins, into simple sugars (HUSSAIN et al., 2008).

3.3 Ascorbic acid content

Ascorbic acid is an important nutrient and is very sensitive to degradation by oxidation during food processing and storage, as compared to other nutrients (VELTMAN et al., 2000). The initial contents of ascorbic acid in the raspberry and strawberry fruits were, respectively, 23.87 ± 0.35 and 45.17 ± 0.24 mg per 100 g of fruits. Eight days of storage resulted in oxidative degradation of the ascorbic acid down to 12.98 ± 0.12 (54%) and 27.35 ± 0.35 (77%) mg per 100 g of untreated raspberry and strawberry fruits, respectively (Table 2.). After storage, the ascorbic acid contents were significantly ($p \leq 0.05$) higher in the raspberry and strawberry fruit samples subjected to a 2% calcium chloride dip.

These results showed that $CaCl_2$ treatments had a significant effect on retaining the ascorbic acid contents of these fruits. This might be because higher concentrations of $CaCl_2$ delayed the rapid oxidation of ascorbic acid in the samples.

For all 6 stored groups in Table 2, one-way ANOVA analyses showed there was strong evidence to reject the null hypothesis ($p<0.05$).

3.4 TPC and Anthocyanin content

The treatment of raspberry and strawberry fruits with calcium chloride had a positive effect ($p < 0.05$) on TPC retention during the storage period (Table 3). This might be because the addition of calcium chloride to the fruits strengthened the cell wall, enhancing the formation of an egg box structure and minimized syneresis/leaching of water soluble compounds such as polyphenols. The most important polyphenols of strawberries and raspberries are anthocyanins, and the main anthocyanin of strawberries is pelargonidin 3–O-monoglucoside and that of raspberries is cyanidin-3-O-monoglucoside (SKROVANKOVA et al., 2015). The data in Table 3 shows that the total anthocyanins in the untreated sample of raspberry were reduced by 30% during storage, whereas in the 1% and 2% calcium treated samples the total anthocyanins were only reduced by 12% and 15%, respectively. For the strawberry fruits the reduction in anthocyanins in the untreated sample was 23%, whereas in the 1% and 2% calcium treated strawberry samples the total anthocyanin reduction was only 19% and 15.5%, respectively.

3.5 Antioxidant potential

Polyphenols and ascorbic acid are the two main contributors of antioxidant activity in fruits. Since both of these were degraded during the storage period, the antioxidant potential of fruits was also reduced. Table 3 shows

Table 2. Effect of calcium chloride treatment on the quality parameters of berries during storage (at 0 ± 0.5°C, RH 95 ± 0.5%).

Berry	Storage period (days)	Treatments (%, calcium chloride)	TSS (%)	TA (%)	Vitamin C (mg/100 g)
Raspberry	0	fresh	11.00 ± 0.08	2.34 ± 0.05	23.87 ± 0.35
	8	0	10.22 ± 0.06	1.71 ± 0.08	12.98 ± 0.12
	8	1	9.60 ± 0.05[a]	1.76 ± 0.02[a]	14.96 ± 0.25[a]
	8	2	9.65 ± 0.12[b]	1.77 ± 0.02[b]	17.05 ± 0.31[b]
Strawberry	0	fresh	8.55 ± 0.15	0.81 ± 0.02	45.17 ± 0.24
	8	0	7.00 ± 0.17	0.72 ± 0.03	27.35 ± 0.35
	8	1	6.57 ± 0.08[a]	0.67 ± 0.02[a]	29.85 ± 0.17[a]
	8	2	6.35 ± 0.04[b]	0.67 ± 0.04[b]	34.68 ± 0.42[b]

The data represents the mean of three replicates ± SD; [a,b] differences between test and control samples within the columns are statistically significant.

Table 3. Effect of calcium chloride treatments on the TPC, monomeric anthocyanin contents and FRAP of berries during storage (at 0 ± 0.5 °C, RH 95 ± 0.5%).

Berry	Storage period (days)	Treatments (%, calcium chloride)	TPC (mg/100 g)	Monomeric anthocyanins (mg/100 g)	FRAP (mg equivalents vitamin C/100 g)
Raspberry	0	fresh	116.01 ± 1.25	33.34 ± 0.52	220.00 ± 3.56
	8	0	105.58 ± 2.41	23.35 ± 0.42	146.63 ± 4.23
	8	1	113.38 ± 2.20[a]	29.26 ± 0.15[a]	173.72 ± 3.65[a]
	8	2	109.98 ± 1.65[b]	27.88 ± 0.71[b]	167.70 ± 4.82[b]
Strawberry	0	fresh	152.94 ± 2.45	63.87 ± 0.51	413.10 ± 3.52
	8	0	130.60 ± 2.75	49.29 ± 1.42	306.08 ± 4.95
	8	1	136.13 ± 3.85[a]	52.17 ± 0.98[a]	357.19 ± 2.75[a]
	8	2	145.37 ± 3.15[b]	54.34 ± 1.05[b]	368.75 ± 4.32[b]

The data represents the mean of three replicates ± SD; [a,b] differences between test and control samples within the columns are statistically significant.

that the initial antioxidant potentials of the raspberry and strawberry fruits were 220.00 ± 3.56 and 413.10 ± 3.52 mg ascorbic acid equivalents per 100 g fruits, respectively. The calcium chloride treatment had a positive effect on retaining the antioxidant potential in both fruit samples. In the untreated samples of raspberry and strawberry, 66% and 74% of the antioxidant potentials were retained, respectively, whereas in the treated samples, the maximum antioxidant potentials retained were 78% and 89% in the raspberry and strawberry fruits, respectively. This was evidently due to the positive effect of the calcium chloride treatment on retaining the ascorbic acid and polyphenols in the fruits. One-way ANOVA analyses were carried out for all six stored groups shown in Table 3. The results indicated statistically significant differences between the mean values inside the groups. The differences between the test and control samples are shown in Table 3.

4 Conclusions

The application of a calcium chloride dip to fresh raspberry and strawberry fruits did not negatively influence quality factors such as TA and TSS, but the ascorbic acid content was higher in the calcium chloride treated berries. The TPC and antioxidant potential in stored fruit was higher if treated with calcium chloride. The calcium chloride treatment also had a positive effect on retaining monomeric anthocyanins during storage. A calcium chloride dip is a practical way to extend the shelf life and nutritional quality of raspberries and strawberries during chilled storage.

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