Effect of calcium and salicylic acid on quality retention in relation to antioxidative enzymes in radish stored under refrigerated conditions

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Abstract Effect of post harvest treatments with calcium chloride (CaCl₂) and salicylic acid (SA) on physiological and biochemical parameters in relation to activities of antioxidative enzymes were investigated in radish. Radish of variety Punjab Safed Mooli 2 was harvested, washed and treated with CaCl₂ (1, 1.5 and 2%) or SA (1, 1.5 and 2 mM). Treated as well as untreated radish were placed in open trays and stored under refrigerated (5 ± 1 °C, 90% RH) conditions for 42 days. Treatment of radish with CaCl₂ and SA slowed down changes in physiological weight, colour, total soluble solids, ascorbic acid, titrable acidity, total phenolics and antioxidant activity. Treated samples exhibited higher enhancement in activities of antioxidant enzymes viz. catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), peroxidase (POD), dehydroascorbate reductase (DHAR) and monodehydro-ascorbate reductase (MDHAR) than untreated samples. However SA was found to be more effective in slowing down the metabolic activities of radish as compared to CaCl₂ treatment. Among all the

treatments, 1.5 mM SA maintained the quality parameters to greater extent probably by reducing the oxidative stress to larger extent due to highest activities of antioxidative enzymes and can be used to enhance the shelf life of radish during refrigerated storage.

Keywords Antioxidative enzymes · Calcium chloride · Post-harvest · Quality parameters · Salicylic acid · Shelf life

Introduction

India is ranked second in producing fruits (81.28 million tonnes) and vegetables (162.19 million tonnes) to the total world production, each contributing 12.6 and 14.0% respectively. Radish (*Raphanus sativus* L.) is an essential cruciferous vegetable crop in India. It is a member of family *Brassicaceae*, having worldwide distribution of 7 million tonnes. It is normally seen as a short season and small rooted vegetable commonly consumed in salads. All parts of radish plant i.e. roots, leaves or seeds, have immense medicinal uses (Kim et al. 2016). The leaves of radish are good source of protein, if extracted commercially. Radish roots are very good source of minerals such as calcium, manganese and phosphorus, crude fibre, dry matter, nitrate, total soluble sugars, carotene, vitamins like thiamine, riboflavin, nicotinic acid and folic acid (Lu et al. 2008). It also contains ascorbic acid which promotes better digestion by stimulating the liver and digestive glandules and hence acts as an antiscorbutic and diuretic agent. Its roots are rich in peroxidase, an oxidoreductase, which can scavenge harmful free radicals.

Considerable quantities of vegetables produced go to waste, which average between 10 and 40% owing to the lack of proper storage and transportation. Many

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biochemical changes occur in vegetables during storage such as loss in chlorophyll, changes in colour, texture, flavour, levels of phenolics and structural breakdown of polysaccharides (Phan et al. 1993) due to post-harvest metabolic processes. These biochemical reactions result in production of reactive oxygen species (ROS) like superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) which are the major cause of oxidative damage during storage or senescence. The control of these oxidant levels is achieved by antioxidative systems. Plants have developed very efficient antioxidant scavenging system to protect themselves against destructive ROS reactions (Mittler et al. 2004) that involves non enzymatic and enzymatic components. ROS detoxifying non enzymatic components are polyphenols, vitamin C, vitamin E and glutathione. A major water-soluble antioxidant in extracellular fluids is vitamin C. Before lipid peroxidation initiation, it neutralises ROS in the aqueous phase. Vitamin C also regenerates vitamin E in cell membrane in combination with glutathione compound. Enzymatic components are superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), peroxidase (POD, EC 1.11.1.7) and glutathione reductase (GR, EC 1.6.4.2). SOD is responsible for converting superoxide ion to hydrogen peroxide and molecular oxygen. H_2O_2 produced by SOD was removed by CAT and POD. GR, DHAR and MDHAR are involved in maintaining the ascorbate levels in cells through an oxido-reductase cycle involving glutathione and ascorbate (Chanjirakul et al. 2006). Phytochemical reactions in response to storage may change the level of antioxidants and antioxidative enzymes. Therefore quantification of these reactions is critical in understanding their role in shelf life enhancement of radish.

Several treatments have been applied to improve storability of vegetables. Postharvest treatment with calcium (Ca^{2+}) plays a pivotal role in maintaining cell turgor, membrane integrity, tissue firmness, extending storage life of fresh vegetables and reduction in the physiological disorders (Mirdehghan and Ghotbi 2014). Further, $CaCl_2$ is naturally occurring, edible, inexpensive and has been approved by the US Food and Drug Administration for postharvest use (Saftner et al. 1998). Post-harvest application of calcium had been shown to inhibit ripening and senescence in tomato (Pila et al. 2010). Salicylic acid (*o*-hydroxybenzoic acid) is among a group of plant growth modulator and a well-chosen hormone-like substance of phenolic nature which participates in the regulation of many physiological processes in plants. Salicylic acid is known to slow down the process of ripening by inhibiting ethylene action and biosynthesis. Post-harvest treatments by calcium and salicylic acid have the capacity to increase

the shelf life of the perishables probably by delaying senescence and decay, but their precise role in extending the shelf life of the radish in relation of activity of antioxidative enzymes remains to be determined. So, the current experiment was designed to evaluate the effect of calcium and salicylic acid on extending the shelf life of radish in relation to changes in activities of antioxidant enzymes and associated biochemical constituents.

Materials and methods

Experimental procedure

This study was conducted in the laboratories of Department of Processing and Food Engineering, Punjab Agricultural University, Ludhiana, Punjab during 2016–2017. The variety Punjab Safed Mooli 2 was raised following the recommended agronomic and cultural practices in the fields of Vegetable Sciences, Punjab Agricultural University, Ludhiana, Punjab. The radish was harvested, sorted, washed with distilled water and dipped in aqueous solutions of 1.0, 1.5, and 2.0% calcium chloride ($CaCl_2$) or 1, 1.5 and 2.0 mM of salicylic acid (SA) for 5 min while radish dipped in distilled water served as control. After surface drying, vegetables were placed treatment wise in plastic trays in cold store at 5 ± 1 °C and 90 ± 4 % relative humidity. Five kg radish was placed in each plastic tray and for each treatment six trays were stored. After weekly interval, samples were taken from each tray, pooled for each treatment and analyzed for quality parameters and activities of antioxidative enzymes in triplicates.

Physiological quality parameters

Weight loss (%) was periodically calculated by percentage of difference between initial weight and final weight of tested radish divided by their initial weight. Changes in radish colour were recorded with the help of Miniscan XE plus Hunter lab Colorimeter (USA). Colour was measured in terms of 'L' value. Total soluble solids (TSS) content of radish was recorded with the help of hand held 'ERMA' refractometer (Znidarcic and Pozrl 2006) and was expressed as °Brix.

Biological quality parameters

Titrate acidity (TA) was determined as per Moneruzzaman et al. (2008) and expressed as percent malic acid. Ascorbic acid was determined as per AOAC (1984) and was expressed as mg/100 g FW using 0.2 mg/ml ascorbic acid as standard. Total soluble phenols were determined as described by Swain and Hillis (1959). The concentration of

total phenol was determined from standard curve prepared simultaneously using gallic acid (10–50 µg). Total soluble flavonoids were determined as described by Balabaa et al. (1974) and were determined from standard curve prepared simultaneously using rutin (40–200 µg/ml). Total antioxidant activity was determined as described by Dasgupta and De (2006).

Extraction and estimation of antioxidant enzymes

For extraction of SOD, CAT, GR and DHAR, the sample (1.0 g) was homogenized in 5 ml of cold (4 °C) 0.1 M sodium phosphate buffer (pH 7.0) containing 1%(w/v) insoluble polyvinyl pyrrolidone and 1 mM EDTA and centrifuged at 8000 rpm for 10 min at 4 °C. The supernatant was collected and analyzed for enzyme activities. SOD activity was assayed spectrophotometrically by measuring the units i.e enzyme required for 50% inhibition of the photochemical reduction of nitro blue tetrazolium according to the method described by Xing et al. (2008). CAT activity was estimated according to the method mentioned by Rao et al. (1997) and enzyme activity was expressed as µmoles of H₂O₂ decomposed /min/g FW using 0.0394 as extinction coefficient. GR activity was estimated according to the method mentioned by Esterbauer and Grill (1978). The activity was expressed as nmol NADPH oxidized /min/mg FW using 6.22 as the extinction coefficient. Dehydroascorbate reductase (DHAR) activity was analyzed using the method described by Foyer et al. (1989). The activity was expressed as nmol/min/g FW using 14 as the extinction coefficient. POD was extracted and assayed according to the method described by Hemeda and Klein (1990). The activity was expressed as nmol/min/g FW by using 26.6 as the extinction coefficient. MDHAR extracted and assayed according to the method described by Hossain et al. (1984). The activity of enzyme was expressed in nmol/min/g FW using 6.2 as the extinction coefficient.

Statistical analysis

All experiments were done in triplicates and data were expressed as mean ± standard error. Two-way analysis of variance (ANOVA) was used to determine the significance of differences. The critical difference at 5% level was analyzed by using completely randomized design in CPCS software.

Results and discussion

Changes in physiological quality parameters

Physiological loss in weight increased with increase in storage period (Table 1). Treatment of radish with CaCl₂

and SA resulted in reduction in weight loss. Post harvest weight loss is usually due to the loss of water through transpiration and respiration (De Castro et al. 2005). Reduction in the rate of respiration and transpiration by Ca²⁺ and SA (Babu et al. 2015) might resulted in lesser weight loss. Additionally, Wills et al. (1998) opined that the water loss can be reduced effectively by placing additional physical barriers between the produce and the surrounding air. Ability of CaCl₂ to strengthen the cell walls of vegetables (Jackman and Stanley 1995) and decrease in normal respiration rate and stroma closing by SA (Wolucka et al. 2005) could be another reason for weight loss reduction. CaCl₂ had the tendency to stabilize the membrane system of fruits and vegetables by the formation of Ca-Pectate which increases rigidity of the middle portion and the cell walls of the radish root there by maintaining the dry matter content by preventing the degradation of cell wall (Lester and Grusak 1999). The weight loss of untreated, 1.5% CaCl₂ and 1.5 mM SA treated radish was 34.07, 30.65 and 22.77% respectively on day 42. CaCl₂ application had also reported to slowing down the rates of weight loss in tomatoes (Pila et al. 2010). Similar results were obtained by Tsouvaltzis and Brecht (2014) for radish stored at 5 °C. With regard to colour characteristics of radish, the L* (indicates lightness) value decreased along the storage period (Table 1). The decrease was more profound in the untreated than the treated samples indicating better retention of colour in treated samples. The L* value of untreated, 1, 1.5, 2% CaCl₂, 1, 1.5 and 2 mM SA was 53.0, 64.6, 66.7, 64.2, 61.4, 63.0 and 58.4 respectively on day 42. The decrease was due to dehydration of outer tissue layers which was indicated to be a major cause of discoloration (Cisneros-Zevallos et al. 1995). Better moisture retention in the treated samples over control could have kept sample surface wet and thereby prevented surface discoloration.

Total soluble solids content is indicator of sweetness, maturity and ripening of fruits and vegetables. It is the amount of sugars and soluble minerals present in fruits and vegetables that acts as a rough index for the ripening process in vegetables and it has been correlated to the texture and composition. TSS of radish increased gradually with the advancement of storage period irrespective of the chemical treatments (Table 1). Increase in TSS content during storage was associated with hydrolytic changes in polysaccharides and their conversion to sugar. Lower increase in TSS content was found in radish treated with CaCl₂ and SA which might be due to slowing down of respiration and metabolic activities (Babu et al. 2015) by these treatments. Delayed increase in TSS by calcium treatment had also been shown by Cheour et al. (1991). SA (1.5 mM) treatment was most effective in controlling loss in weight, colour L* value and TSS content of radish.

Table 1 Effect of CaCl₂ and SA treatments on weight loss, colour L* value and TSS content of radish during storage under refrigerated conditions

Treatments (T)			Calcium chloride (%)				Salicylic acid (mM)				C.D value (<i>P</i> ≤ 0.05)
Parameter	Days of storage (S)		0	1	1.5	2	1	1.5	2		
Weight loss (%)	7		6.98 ± 0.33	6.74 ± 0.40	5.75 ± 0.36	6.45 ± 0.48	6.72 ± 0.36	5.25 ± 0.32	5.78 ± 0.35	T-0.555	
	14		15.59 ± 0.19	15.01 ± 0.46	13.88 ± 0.41	13.80 ± 0.61	14.33 ± 0.42	12.29 ± 0.75	12.81 ± 0.44	S-0.515	
	21		21.26 ± 0.21	20.77 ± 0.28	20.00 ± 0.55	18.77 ± 0.35	19.12 ± 0.74	15.68 ± 0.70	17.97 ± 0.37	TS-1.361	
	28		25.06 ± 0.24	24.90 ± 0.25	21.74 ± 0.47	22.62 ± 0.34	20.91 ± 0.56	18.31 ± 0.42	20.32 ± 0.68		
	35		30.10 ± 0.48	28.07 ± 0.73	27.31 ± 0.54	28.78 ± 0.37	26.87 ± 0.97	20.21 ± 0.37	23.45 ± 0.74		
	42		34.07 ± 0.53	32.05 ± 0.59	30.65 ± 0.32	31.84 ± 0.23	31.34 ± 0.33	22.77 ± 0.38	27.35 ± 0.43		
Color L* value	0		74.3 ± 0.08	74.3 ± 0.08	74.3 ± 0.08	74.3 ± 0.08	74.3 ± 0.08	74.3 ± 0.08	74.3 ± 0.08	T-0.113	
	7		65.1 ± 0.03	69.6 ± 0.06	71.2 ± 0.06	68.6 ± 0.06	70.1 ± 0.04	73.7 ± 0.06	66.4 ± 0.12	S-0.104	
	14		62.8 ± 0.12	69.4 ± 0.09	69.7 ± 0.06	68.5 ± 0.09	70.9 ± 0.15	70.4 ± 0.09	65.9 ± 0.06	TS-0.276	
	21		62.0 ± 0.06	69.2 ± 0.12	68.9 ± 0.18	68.3 ± 0.09	69.5 ± 0.20	69.3 ± 0.03	65.4 ± 0.12		
	28		61.3 ± 0.12	68.7 ± 0.09	67.4 ± 0.09	66.4 ± 0.09	68.8 ± 0.12	69.2 ± 0.06	65.3 ± 0.09		
	35		56.4 ± 0.12	68.4 ± 0.06	67.0 ± 0.09	65.4 ± 0.09	65.0 ± 0.06	67.3 ± 0.06	61.8 ± 0.12		
TSS (°Brix)	42		53.0 ± 0.09	64.6 ± 0.06	66.7 ± 0.12	64.2 ± 0.06	61.4 ± 0.06	63.0 ± 0.21	58.4 ± 0.12		
	0		5.0 ± 0.20	5.0 ± 0.20	5.0 ± 0.20	5.0 ± 0.20	5.0 ± 0.20	5.0 ± 0.20	5.0 ± 0.20	T-0.136	
	7		5.5 ± 0.09	5.2 ± 0.03	5.0 ± 0.03	5.1 ± 0.06	5.2 ± 0.09	5.1 ± 0.04	5.3 ± 0.12	S-0.145	
	14		6.5 ± 0.09	6.2 ± 0.09	6.3 ± 0.12	6.4 ± 0.12	5.9 ± 0.18	5.7 ± 0.12	6.3 ± 0.12	TS-NS	
	21		6.7 ± 0.06	6.5 ± 0.03	6.5 ± 0.03	6.5 ± 0.12	6.5 ± 0.15	6.1 ± 0.15	6.2 ± 0.15		
	28		7.2 ± 0.09	6.8 ± 0.09	6.7 ± 0.12	6.8 ± 0.09	6.9 ± 0.17	6.3 ± 0.18	7.0 ± 0.21		
	35		7.6 ± 0.12	7.2 ± 0.15	7.2 ± 0.09	7.3 ± 0.12	7.5 ± 0.17	7.1 ± 0.17	7.4 ± 0.20		
	42		8.5 ± 0.15	8.2 ± 0.09	8.1 ± 0.09	8.3 ± 0.15	7.9 ± 0.17	7.6 ± 0.18	8.1 ± 0.20		

Each value is the mean ± SE for three replicates

Changes in biological quality parameters

TA is a measure of the concentration of organic acids present in fruits and vegetables, which are important in maintaining quality. These acids also influence stability and keeping quality of vegetables and hence been considered as an index of maturity or spoilage. TA (% malic acid) first increased up to day 28 of storage and decreased thereafter (Table 2). The decrease in TA in all the treatments at the end of storage might be attributable to the metabolic conversion of organic acid into carbon dioxide and water during respiratory process (Echeverria and Valich 1989). CaCl_2 and SA treatments significantly decreased the change in the TA content. However, the treatment of radish with SA proved to be better in maintaining the TA as compared to CaCl_2 treated and untreated radish throughout the storage period. Slowest changes in TA was found in 1.5 mM SA treated radish followed by 1.5% CaCl_2 then 2% CaCl_2 then 1% CaCl_2 then 1 mM SA and 2 mM SA.

Ascorbic acid is considered as an indicator of nutritional and storage quality of fruits and vegetables. Irrespective of treatments in radish, the ascorbic acid content showed a gradual increase during storage period (Table 2). The slower increase in ascorbic acid content was observed in radish with CaCl_2 and SA treatment as compared to untreated samples. However the change in ascorbic acid content was slowest in 1.5 mM SA treated radish.

Phenolic compounds are the secondary metabolites of plants, characterized by having at least one aromatic ring with one or more hydroxyl group. Phenolic compounds are widely distributed and have the ability to scavenge free radicals, superoxide and hydroxyl radicals by a single electron transfer in fruits and vegetables. Phenols and flavonoids are the major phenolic contents in radish. Total phenol content increased significantly ($P \leq 0.05$) during storage in both treated and untreated radish (Table 3). Different concentration of CaCl_2 and SA were significantly effective in slowing down the enhancement in phenol content. However, among all the concentrations of CaCl_2 and SA, 1.5% CaCl_2 and 1.5 mM SA displayed slowest changes respectively. Flavonoids are the major components of total phenolics contents. These also give a high contribution to the antioxidant activity and trigger antagonistic and synergistic effects in the antioxidant power of natural mixtures. In flavonoids, a gradual increase was observed up to 21 days of storage in all the treatments except in radish treated with 1.5% CaCl_2 and 1.5 mM SA where it increased up to day 28 followed by decline (Table 3). Among CaCl_2 treated radish, slow changes in content of flavonoids was found with 1.5% CaCl_2 treatment as compared to 1 or 2% CaCl_2 treatment and with SA treatment, the slowest changes in content of flavonoids was found in

1.5 mM SA treated radish as compared to 1 or 2 mM SA treatments. Treatment of CaCl_2 and SA might slow down ethylene synthesis which slowed down the activity of phenylalanine ammonia lyase enzyme (Yao and Tian 2005) resulted in slower increase in total phenolics.

Antioxidant activity showed an increasing trend in all the treatments during storage period (Table 3). The total antioxidant activity of untreated, 1.5% CaCl_2 and 1.5 mM SA treated radish was 1.86, 1.62 and 1.55 mg/g respectively on day 42 respectively. Antioxidant activity increased at a slower rate in CaCl_2 and SA treated radish, indicating that CaCl_2 and SA treatments significantly affected the metabolic process in radish during storage. Increase in antioxidant activity might be due to the increase in phenolic contents and ascorbic acid content during storage since they are the major non enzymatic components (Ali et al. 2011). Similar results were reported by Tsouvaltzis and Brecht (2014) for antioxidant activity of radish during storage at 5 or 10 °C. Less antioxidant activity in treated samples as compared to untreated samples might be due to the slower increase in phenols, flavonoids and ascorbic acid by these treatments. Among all concentrations, minimum changes in biochemical parameters were found with 1.5 mM SA treatment followed by 1.5% CaCl_2 treatments.

Activities of antioxidant enzymes

SOD constitutes the first line of defense against ROS that converts O_2^- into H_2O_2 whereas CAT and POD play protective role in controlling the levels of H_2O_2 generated through SOD action. In both treated and untreated samples, SOD and POD activity first increased significantly ($P \leq 0.05$) up to day 28 and then decreased slowly along the storage period, however, CAT activity increased up to day 35 in all the treatments (Table 4). Elevated CAT and POD during storage would lower H_2O_2 levels, which reduced the lipid peroxidation and lessen membrane damage. The increase in POD activity might reflect greater rate of lignin biosynthesis and greater healing rate adopted by leaves in attempt to compensate for damage and protect tissue from pathogen attack, as suggested by Howard and Griffin (1993). Lower activity of POD seemed to be related to higher activity of CAT because both enzymes use hydrogen peroxide as common substrate. Activities of these three enzymes were higher in samples treated CaCl_2 and SA with as compared to untreated samples which suggested that they had stronger capacity to lessen the oxidative stress than untreated samples. SOD activity was reported to increase during storage in potato tuber (Kumar and Knowles 1993). POD activity in radish (Tsouvaltzis and Brecht 2014) and CAT activity in peach (Tareen et al.

Table 2 Effect of CaCl₂ and SA treatments on titrable acidity, and ascorbic acid of radish during storage under refrigerated conditions

Treatments (T)	Days of storage (S)	Calcium chloride (%)				Salicylic acid (mM)				C.D value ($P \leq 0.05$)
		0	1	1.5	2	1	1.5	2	2	
Titrable acidity (% malic acid)	0	0.134 ± 0.02	0.134 ± 0.02	0.134 ± 0.02	0.134 ± 0.02	0.134 ± 0.02	0.134 ± 0.02	0.134 ± 0.02	0.134 ± 0.02	T-0.020
	7	0.200 ± 0.01	0.174 ± 0.01	0.154 ± 0.01	0.167 ± 0.01	0.187 ± 0.02	0.160 ± 0.03	0.187 ± 0.01	0.187 ± 0.01	S-0.018
	14	0.281 ± 0.01	0.241 ± 0.012	0.214 ± 0.01	0.228 ± 0.01	0.241 ± 0.02	0.214 ± 0.02	0.241 ± 0.01	0.241 ± 0.01	TS-0.049
	21	0.335 ± 0.01	0.268 ± 0.01	0.255 ± 0.03	0.255 ± 0.03	0.281 ± 0.03	0.255 ± 0.01	0.268 ± 0.01	0.268 ± 0.01	
	28	0.429 ± 0.02	0.335 ± 0.03	0.308 ± 0.02	0.322 ± 0.02	0.348 ± 0.02	0.295 ± 0.02	0.362 ± 0.01	0.362 ± 0.01	
	35	0.335 ± 0.02	0.268 ± 0.01	0.241 ± 0.02	0.255 ± 0.02	0.281 ± 0.03	0.255 ± 0.02	0.268 ± 0.01	0.268 ± 0.01	
Ascorbic acid (mg/100 g FW)	42	0.268 ± 0.01	0.255 ± 0.01	0.241 ± 0.01	0.241 ± 0.02	0.268 ± 0.03	0.241 ± 0.02	0.255 ± 0.01	0.255 ± 0.01	
	0	6.2 ± 0.08	6.2 ± 0.08	6.2 ± 0.08	6.2 ± 0.08	6.2 ± 0.08	6.2 ± 0.08	6.2 ± 0.08	6.2 ± 0.08	T-0.058
	7	9.1 ± 0.90	8.3 ± 0.05	7.3 ± 0.05	8.1 ± 0.05	8.6 ± 0.05	6.8 ± 0.06	8.1 ± 0.06	8.1 ± 0.06	S-0.058
	14	10.5 ± 0.17	9.2 ± 0.12	9.0 ± 0.29	9.7 ± 0.18	10.2 ± 0.12	8.7 ± 0.09	9.5 ± 0.15	9.5 ± 0.15	TS-NS
	21	11.5 ± 0.15	10.2 ± 0.09	10.0 ± 0.21	10.7 ± 0.18	11.0 ± 0.22	9.7 ± 0.12	10.5 ± 0.15	10.5 ± 0.15	
	28	12.2 ± 0.12	11.0 ± 0.29	10.5 ± 0.17	11.7 ± 0.13	11.5 ± 0.14	10.2 ± 0.15	11.5 ± 0.15	11.5 ± 0.15	
	35	13.2 ± 0.11	12.2 ± 0.20	11.2 ± 0.08	12.2 ± 0.09	12.5 ± 0.14	11.2 ± 0.15	12.2 ± 0.3	12.2 ± 0.3	
	42	13.7 ± 0.04	12.7 ± 0.30	11.7 ± 0.15	12.7 ± 0.13	12.7 ± 0.09	11.7 ± 0.15	12.7 ± 0.12	12.7 ± 0.12	

Each value is the mean ± SE for three replicates

Table 3 Effect of CaCl₂ and SA treatments on total phenolics and antioxidant activity of radish during storage under refrigerated conditions

Treatments (T)		Calcium chloride (%)				Salicylic acid (mM)				C.D value (<i>P</i> ≤ 0.05)
Parameter	Days of storage (S)	0	1	1.5	2	1	1.5	2		
Total phenols (mg/100 g FW)	0	130.1 ± 1.50	130.1 ± 1.50	130.1 ± 1.50	130.1 ± 1.50	130.1 ± 1.50	130.1 ± 1.50	130.1 ± 1.50	130.1 ± 1.50	T-2.84
	7	143.4 ± 1.62	140.1 ± 1.32	138.4 ± 2.51	142.3 ± 2.89	140.9 ± 1.70	137.9 ± 1.69	141.9 ± 2.05	141.9 ± 2.05	S-2.63
	14	181.2 ± 2.95	169.0 ± 2.37	166.8 ± 2.18	178.1 ± 2.31	164.5 ± 2.48	162.0 ± 2.31	171.0 ± 1.30	171.0 ± 1.30	TS-6.95
	21	197.1 ± 4.33	185.6 ± 2.75	181.8 ± 3.20	190.0 ± 3.09	184.3 ± 2.83	179.5 ± 2.05	189.6 ± 1.86	189.6 ± 1.86	
	28	214.9 ± 2.72	197.9 ± 2.81	192.5 ± 2.46	205.1 ± 2.49	193.9 ± 3.47	190.0 ± 1.24	203.6 ± 1.56	203.6 ± 1.56	
	35	235.0 ± 1.91	210.9 ± 2.63	198.3 ± 2.40	218.9 ± 3.55	206.1 ± 2.72	199.7 ± 1.68	209.4 ± 2.40	209.4 ± 2.40	
	42	249.5 ± 4.04	228.6 ± 1.13	219.4 ± 1.88	235.2 ± 2.71	219.2 ± 2.30	214.6 ± 2.17	224.8 ± 1.39	224.8 ± 1.39	
Total flavonoids (mg/100 g FW)	0	11.30 ± 0.12	11.30 ± 0.12	11.30 ± 0.12	11.30 ± 0.12	11.30 ± 0.12	11.30 ± 0.12	11.30 ± 0.12	11.30 ± 0.12	T-0.199
	7	14.79 ± 0.25	13.84 ± 0.11	13.00 ± 0.08	14.41 ± 0.09	13.69 ± 0.18	13.10 ± 0.07	13.27 ± 0.07	13.27 ± 0.07	S-0.185
	14	19.54 ± 0.21	17.38 ± 0.18	16.54 ± 0.18	18.33 ± 0.17	16.54 ± 0.14	14.52 ± 0.13	15.59 ± 0.13	15.59 ± 0.13	TS-0.489
	21	21.04 ± 0.26	18.69 ± 0.22	17.21 ± 0.15	20.12 ± 0.25	18.07 ± 0.14	16.54 ± 0.14	16.90 ± 0.18	16.90 ± 0.18	
	28	18.45 ± 0.23	17.26 ± 0.18	18.15 ± 0.26	16.90 ± 0.23	15.09 ± 0.17	18.05 ± 0.16	14.78 ± 0.11	14.78 ± 0.11	
	35	16.66 ± 0.20	15.66 ± 0.18	15.71 ± 0.23	14.79 ± 0.13	14.69 ± 0.10	16.42 ± 0.10	13.95 ± 0.14	13.95 ± 0.14	
	42	12.10 ± 0.18	13.69 ± 0.15	13.53 ± 0.21	13.98 ± 0.18	12.93 ± 0.11	14.88 ± 0.14	12.46 ± 0.23	12.46 ± 0.23	
Antioxidant activity (ascorbic acid equivalent)	0	0.555 ± 0.08	0.555 ± 0.08	0.555 ± 0.08	0.555 ± 0.08	0.555 ± 0.08	0.555 ± 0.08	0.555 ± 0.08	0.555 ± 0.08	T-0.058
	7	0.755 ± 0.01	0.64 ± 0.13	0.6 ± 0.01	0.68 ± 0.01	0.615 ± 0.01	0.595 ± 0.03	0.645 ± 0.01	0.645 ± 0.01	S-0.054
	14	1.13 ± 0.03	1.05 ± 0.06	0.93 ± 0.02	1.08 ± 0.02	0.99 ± 0.02	0.88 ± 0.04	1.03 ± 0.03	1.03 ± 0.03	TS-NS
	21	1.32 ± 0.02	1.25 ± 0.02	1.19 ± 0.04	1.28 ± 0.02	1.20 ± 0.05	1.10 ± 0.05	1.26 ± 0.03	1.26 ± 0.03	
	28	1.64 ± 0.18	1.42 ± 0.03	1.38 ± 0.02	1.47 ± 0.03	1.40 ± 0.03	1.34 ± 0.02	1.46 ± 0.02	1.46 ± 0.02	
	35	1.79 ± 0.10	1.55 ± 0.04	1.45 ± 0.02	1.59 ± 0.03	1.54 ± 0.02	1.48 ± 0.04	1.59 ± 0.04	1.59 ± 0.04	
	42	1.86 ± 0.11	1.69 ± 0.06	1.62 ± 0.03	1.74 ± 0.02	1.62 ± 0.05	1.55 ± 0.03	1.63 ± 0.02	1.63 ± 0.02	

Each value is the mean ± SE for three replicates

Table 4 Effect of CaCl_2 and SA treatments on activities of enzymes SOD, CAT and POD of radish during storage under refrigerated conditions

Treatments (T)		Calcium chloride (%)				Salicylic acid (mM)				C.D value ($P \leq 0.05$)
Parameter	Days of storage (S)	0	1	1.5	2	1	1.5	2		
SOD (U/g FW)	0	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	T-0.035	
	7	0.949 ± 0.02	1.268 ± 0.03	1.298 ± 0.02	1.110 ± 0.04	1.182 ± 0.01	1.345 ± 0.02	1.126 ± 0.03	S-0.033	
	14	1.294 ± 0.04	1.455 ± 0.03	1.529 ± 0.04	1.422 ± 0.05	1.513 ± 0.03	1.549 ± 0.02	1.313 ± 0.04	TS-0.087	
	21	1.368 ± 0.04	1.717 ± 0.03	1.786 ± 0.01	1.662 ± 0.01	1.734 ± 0.02	1.850 ± 0.02	1.526 ± 0.03		
	28	1.589 ± 0.06	1.804 ± 0.03	1.834 ± 0.03	1.789 ± 0.04	1.831 ± 0.02	1.867 ± 0.04	1.693 ± 0.01		
	35	1.329 ± 0.04	1.528 ± 0.06	1.595 ± 0.02	1.429 ± 0.02	1.578 ± 0.03	1.634 ± 0.03	1.659 ± 0.02		
CAT (μmol/min/g FW)	42	1.234 ± 0.05	1.520 ± 0.02	1.548 ± 0.03	1.409 ± 0.03	1.567 ± 0.03	1.584 ± 0.02	1.537 ± 0.02		
	0	30.71 ± 0.28	30.71 ± 0.28	30.71 ± 0.28	30.71 ± 0.28	30.71 ± 0.28	30.71 ± 0.28	30.71 ± 0.28	T-0.672	
	7	63.81 ± 0.72	70.74 ± 0.45	73.15 ± 0.81	69.10 ± 0.38	70.70 ± 0.85	72.65 ± 0.78	71.50 ± 0.65	S-0.623	
	14	112.6 ± 0.84	115.1 ± 0.40	119.7 ± 0.28	114.9 ± 0.23	115.7 ± 0.42	120.2 ± 0.38	118.8 ± 0.38	TS-1.650	
	21	144.4 ± 0.57	154.2 ± 0.88	161.7 ± 0.47	151.6 ± 0.52	161.7 ± 0.54	163.2 ± 0.84	159.6 ± 0.28		
	28	175.7 ± 0.90	185.7 ± 0.60	189.2 ± 0.35	183.5 ± 0.72	189.2 ± 0.30	195.9 ± 0.52	191.1 ± 0.82		
POD (nmol/min/g FW)	35	192.2 ± 1.02	205.2 ± 0.25	208.2 ± 0.22	202.9 ± 0.79	205.2 ± 0.34	210.4 ± 0.90	208.6 ± 0.22		
	42	154.8 ± 0.23	168.5 ± 0.72	178.1 ± 0.25	166.4 ± 0.38	168.5 ± 0.35	180.2 ± 0.68	179.6 ± 0.24		
	0	338.3 ± 10.5	338.3 ± 10.5	338.3 ± 10.5	338.3 ± 10.5	338.3 ± 10.5	338.3 ± 10.5	338.3 ± 10.5	T-1.016	
	7	766.0 ± 28.0	814.0 ± 5.20	843.6 ± 4.50	871.5 ± 3.20	881.5 ± 4.70	878.4 ± 3.70	889.2 ± 3.20	S-0.941	
	14	1026.3 ± 12.5	1041.3 ± 12.0	1162.0 ± 7.20	1085.3 ± 12.0	1085.3 ± 4.20	1069.9 ± 7.20	1069.2 ± 3.50	TS-2.49	
	21	1223.3 ± 7.50	1280.2 ± 7.50	1296.2 ± 9.20	1269.0 ± 5.20	1224.0 ± 3.50	1378.4 ± 1.90	1345.9 ± 3.80		
	28	1336.8 ± 3.60	1357.3 ± 8.80	1389.5 ± 1.50	1364.8 ± 2.50	1396.2 ± 2.80	1315.0 ± 16.5	1352.3 ± 8.80		
	35	1122.3 ± 8.30	1274.1 ± 2.70	1284.8 ± 2.50	1267.9 ± 15.1	1284.2 ± 2.80	1287.7 ± 4.50	1282.0 ± 9.80		
	42	1084.2 ± 2.80	1170.5 ± 8.50	1181.2 ± 2.50	1168.6 ± 4.80	1163.6 ± 4.80	1191.2 ± 5.80	1186.0 ± 3.70		

Each value is the mean $\pm$ SE for three replicates

Table 5 Effect of CaCl₂ and SA treatments on activities of enzymes GR, DHAR and MDHAR of radish during storage under refrigerated conditions

Treatments (T)		Calcium chloride (%)				Salicylic acid (mM)				C.D value ($P \leq 0.05$)
Parameter	Days of storage (S)	0	1	1.5	2	1	1.5	2		
GR (nmol/min/g FW)	0	144.7 ± 0.35	144.7 ± 0.35	144.7 ± 0.35	144.7 ± 0.35	144.7 ± 0.35	144.7 ± 0.35	144.7 ± 0.35	T-0.545	
	7	210.3 ± 0.24	228.2 ± 0.66	230.5 ± 0.66	218.2 ± 0.37	208.5 ± 0.66	239.3 ± 0.37	220.3 ± 0.34	S-0.504	
	14	243.4 ± 0.63	243.4 ± 0.73	265.2 ± 0.37	252.1 ± 0.37	262.3 ± 0.44	273.6 ± 0.45	253.4 ± 0.73	TS-1.33	
	21	316.8 ± 0.56	328.1 ± 0.69	355.0 ± 0.89	315.5 ± 0.22	327.6 ± 0.26	362.4 ± 0.25	318.8 ± 0.40		
	28	460.5 ± 0.55	468.1 ± 0.80	513.8 ± 0.49	467.7 ± 0.30	466.8 ± 0.41	486.8 ± 0.36	457.7 ± 0.29		
	35	619.5 ± 0.25	626.8 ± 0.26	657.7 ± 0.23	624.1 ± 0.48	629.5 ± 0.22	662.3 ± 0.68	621.6 ± 0.51		
DHAR (nmol/min/g FW)	42	546.1 ± 0.79	560.1 ± 0.73	749.2 ± 0.28	559.1 ± 0.37	572.9 ± 0.11	756.4 ± 0.39	569.1 ± 0.30		
	0	70.71 ± 0.55	70.71 ± 0.55	70.71 ± 0.55	70.71 ± 0.55	70.71 ± 0.55	70.71 ± 0.55	70.71 ± 0.55	T-0.555	
	7	103.2 ± 0.42	103.5 ± 0.40	106.1 ± 0.82	102.4 ± 0.41	101.9 ± 1.1	106.7 ± 0.26	104.7 ± 0.18	S-0.515	
	14	177.1 ± 0.89	182.8 ± 0.75	190.8 ± 0.15	189.9 ± 0.24	186.5 ± 0.34	191.1 ± 0.38	187.8 ± 0.65	TS-1.34	
	21	215.7 ± 0.47	230.5 ± 0.64	237.1 ± 0.44	234.2 ± 0.42	237.8 ± 0.72	241.4 ± 0.15	238.5 ± 0.65		
	28	354.2 ± 0.25	368.5 ± 0.50	372.8 ± 0.75	369.5 ± 0.38	375.7 ± 0.31	381.9 ± 0.57	379.9 ± 0.23		
MDHAR (nmol/min/g FW)	35	469.9 ± 0.22	475.7 ± 0.35	481.4 ± 0.35	479.4 ± 0.35	477.1 ± 0.42	485.7 ± 0.28	479.7 ± 0.34		
	42	501.4 ± 0.50	511.4 ± 0.42	518.5 ± 0.62	509.9 ± 0.18	509.2 ± 0.31	558.2 ± 0.75	514.4 ± 0.31		
	0	101.6 ± 0.70	101.6 ± 0.70	101.6 ± 0.70	101.6 ± 0.70	101.6 ± 0.70	101.6 ± 0.70	101.6 ± 0.70	T-0.810	
	7	164.8 ± 0.55	169.8 ± 0.85	171.4 ± 1.25	167.8 ± 0.73	167.2 ± 1.01	173.7 ± 0.48	171.6 ± 1.23	S-0.150	
	14	264.2 ± 0.75	275.2 ± 0.47	287.2 ± 0.97	274.1 ± 0.47	274.1 ± 0.45	287.1 ± 0.28	278.2 ± 0.42	TS-1.98	
	21	336.7 ± 0.52	348.3 ± 0.67	366.1 ± 0.35	341.9 ± 1.09	366.6 ± 0.38	371.2 ± 0.38	389.3 ± 0.38		
	28	435.4 ± 0.34	486.7 ± 1.13	493.5 ± 0.75	482.5 ± 0.87	482.5 ± 0.84	496.4 ± 0.50	491.2 ± 0.32		
	35	654.8 ± 1.35	674.2 ± 0.48	680.1 ± 0.78	648.6 ± 0.45	670.9 ± 0.25	685.4 ± 0.34	682.5 ± 0.85		
	42	537.1 ± 0.94	544.2 ± 0.48	556.7 ± 1.13	545.4 ± 0.35	560.9 ± 0.47	565.9 ± 0.27	559.4 ± 0.22		

Each value is the mean ± SE for three replicates

2012) also reported to be enhanced during storage. Amongst concentrations, CaCl_2 (1.5%) and SA (1.5 mM) treated samples had higher activities of enzymes as compared to other CaCl_2 and SA concentrations respectively as well as untreated samples. The SOD catalyzed the dismutation of superoxide anions to produce hydrogen peroxide which was then removed by CAT and POD. These enzymes were reported to extend food freshness by protecting the integrity of membranes and were also related with stress tolerance gene encoding the enzyme reported to up regulate post harvest (Xing et al. 2008).

An increasing trend in GR and MDHAR activity upto day 35 was found in all samples, except the samples treated with 1.5 mM SA and 1.5% CaCl_2 where the activity of GR enhanced throughout the storage period. A continuous increase in activity of DHAR was also found with increase in storage period (Table 5). The increased GR activity could be related to the fact that, GR reduces glutathione disulphide to sulphhydryl form which is an important cellular antioxidant and protect the thiol groups on enzymes. It also regenerate ascorbate that further reduces oxidative stress (Chanjirakul et al. 2006). Meanwhile, DHAR use the GSH as the reducing agent to reduce dehydroascorbic acid to ascorbic acid while mono-dehydroascorbate (MDHA), a primary product of ascorbate peroxidase can return into the ascorbate pool by MDHAR (Chanjirakul et al. 2006). The increase in activity of DHAR (Table 5) could be responsible with the increase in activity of ascorbic acid content (Table 2). The correlation between DHAR and MDHAR activity with ascorbate was also found by Jimenez et al. (2002). The increased DHAR and MDHAR activity and lower oxidation level in fruit and vegetable ascorbate pool are correlated with decreased loss in firmness. Activities of these enzymes were higher in treated samples than the untreated samples during whole storage period. Radish treated with 1.5% CaCl_2 and 1.5 mM SA showed higher activities of enzymes as compared to their other concentrations respectively. However, highest activities of antioxidative enzymes were found in radish treated with 1.5 mM SA, thus protecting the radish from the damaging effects of ROS for longer period therefore prolonging the shelf life of the radish.

Conclusion

Treatment of radish with CaCl_2 or SA slowed down changes in quality of radish and enhanced the activities of antioxidative enzyme activities as compared to untreated sample during refrigerated storage. Among all the treatments, 1.5 mM SA maintained the quality parameters to greater extent probably by reducing the oxidative stress to larger extent due to highest activities of antioxidative

enzymes and can be used to enhance the shelf life of radish during refrigerated storage.

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