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Influence of hot water treatment on nutritional quality attributes of cold stored apple (*Malus* × *domestica*)

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ABSTRACT

The present study was carried out at ICAR-Indian Agricultural Research Institute, New Delhi during 2021–2023 to study the effect of postharvest hot water treatment (HWT) on the nutritional quality attributes of apple [*Malus × domestica* Borkh.] cv. Royal Delicious. Apple fruits were exposed to hot water at 48, 50, 52 and 54°C for 2, 3, 4 and 5 min. Following the treatment, the fruits were cold stored (2±1°C, 90–95% relative humidity) for 90 days and evaluated for quality changes at every 15 days interval. Our results revealed that HWT of apple fruits at 48°C and 50°C were best for optimum retention of nutritional quality of apple fruits. Exposure of fruits to HWT at 48°C/5 min and 50°C/2 min resulted in least (0.73%, 0.75%) loss of ascorbic acid and anthocyanin content (0.10%, 0.21%), respectively as compared to other temperature-time combinations. At the end of a three month storage period, under control treatment, fruits exhibited 9.56% reduction of antioxidant (AOX) activity in comparison to 0.17–2.21% reduction in HWT apples. The maximum loss in quality attributes was noticed at highest temperature-time exposure (54°C/5 min). Thus, it was observed that the right combination of temperature and time for HWT is crucial to maintain fruit quality attributes without compromising nutritional value.

Keywords: Anthocyanins, Antioxidants, Hot water treatment, Lipoxygenase activity, Phenolic content

Apple [*Malus × domestica* Borkh.] is popularly regarded as the king of temperate fruits owing to its popularity and nutritional composition. Clinical studies suggest that regular consumption of apples has beneficial effects on the prevention of a variety of chronic non-communicable diseases including cancer, cardiovascular diseases and age-related muscular degeneration (Jiang *et al.* 2019). The global demand for apple is expected to increase by 3.42 million tonnes by 2024 at a CAGR of more than 1% as a result of the growing consumption of fresh fruits and processed products (Raj *et al.* 2021). As per the FAOSTAT (2021) data, global apple production was around 93.14 million metric tonnes (MMT) with India producing about 2.27 MMT. Ninety per cent of commercial scale production in India is concentrated in high altitude regions of the northwestern Himalayan states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and on a limited scale in north-eastern states of Arunachal Pradesh, Sikkim and Nagaland (Anonymous 2018). Production of apple is affected by pests (woolly apple aphid, San Jose scale, root borer), diseases (scab, powdery mildew, premature leaf fall, sooty mould) and postharvest disorders (bitter pit and brown rot) (Chadha and Awasthi 2005). To tackle these adverse problems and extend the availability of apple fruit in the market, farmers follow indiscriminate use of pesticides that poses a threat to human health. Therefore, cost-effective and environment friendly green technologies are the need of the hour to control and combat these problems while retaining fruit quality traits without jeopardizing human health.

Hot water treatment (HWT) is a promising intervention that is not only effective in controlling fungal diseases and insect infestations but can also help in the reduction of pesticide residues (Spadoni *et al.* 2015). This technique is relatively inexpensive and simple to use with short process time to deliver fruits of good quality. Therefore, considering the beneficial effects of HWT, the present study was undertaken to determine its effect on functional quality attributes in apples during cold storage.

MATERIALS AND METHODS

The present study was carried out in the Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi during 2021–23. The Royal Delicious apple fruits were harvested from an orchard located in Katrain, Himachal Pradesh (32.13°N, 77.12°E, 1472 m amsl). The fruits were sorted, packed in cartons, transported to the laboratory of the Division within

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24 h and stored under low temperature [2±1°C at 90–95% relative humudity (RH)] till further study. The fruits were randomly divided into homogenous groups of 17 batches representing the number of treatments. For HWT, a digital thermostatic stainless steel water bath (Autonix®, Sanco Co., Delhi) was used. For HWT, the Royal Delicious fruits were categorized into sixteen lots, with 5 kg fruits in each lot. Fruits of each lot were immersed in hot water fixed at four different temperatures (48, 50, 52 and 54°C) for 2, 3, 4, and 5 min. Subsequently, these treated fruits were cooled by keeping them under forced air at ambient temperature (27±1°C). The last batch was not given any HWT and served as control treatment. All treated lots were cold stored at 2±1°C and 90–95% RH for three months. During the storage period of 90 days, observations on various physico-chemical parameters were recorded at 15 days interval. Six fruits from each lot of hot water treated as well as untreated (control) samples were randomly selected for further analysis.

The soluble solids content of apple was estimated using a hand refractometer (ATAGO; range: 0–93°B) and expressed as degree Brix (°B) at 20°C. The total anthocyanin content of the HWT and control samples was analyzed by using the *p*H differential method and expressed as milligram of cyanidin-3-glucoside (CG) equivalent per kilogram fresh weight (Wrolstad *et al.* 2006). Folin–Ciocalteu reagent method was used to determine the total phenolic content of apple as mg gallic acid equivalent (GAE) 100/g (Prasad *et al.* 2022). Total antioxidant (AOX) activity of the apple in cv. Royal Delicious was evaluated using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method as described by Arora *et al.* (2018). The lipoxygenase (LOX) activity was determined by the method described by Sharma *et al.* (2012) and represented in µmol/min/g fresh fruit weight.

The experiment was laid out in a factorial completely randomized design (CRD) with three replications in each treatment during 2021–23. Statistical analysis of the pooled data of two years was done by using PROC GLM of SAS software package version 9.4 (SAS Institute, Cary, North Carolina,USA). Tukey's significant difference test was used to compare differences among the treatments and storage intervals. Results were expressed as mean and significance was assessed at $P \leq 0.05$.

RESULTS AND DISCUSSION

Total soluble solid (TSS) content was significantly affected by HWT as depicted in Table 1, with the majority of the samples showing an increasing trend till 45 days of storage. The maximum total soluble solid content (14.43°B) was observed in apples treated at 54°C for 5 min after 45 days of cold storage. The gradual breakdown of starch present in fruits into simpler sugars might be the reason for increase in TSS during storage. Further progress in storage duration resulted in decline in TSS content across all treatments. The reduction in TSS after 45 days is due to the utilization of these sugars in the respiratory activity of the fruits. Similar trend was reported by Musto and Satriano (2010) in HWT treated strawberry fruits given at 45°C for 5 min. Further, it was observed that as the temperature of HWT increased from 48–54°C the average TSS content of fruits increased from 10.39–13.16°B by the end of the experiment.

A significant reduction in ascorbic acid content was recorded throughout the storage period in both control and treated apples (Table 1). About 19.12% reduction in ascorbic acid content was observed in control fruits after 3 months of cold storage. However, HWT exhibited positive influence on the retention of ascorbic acid content. Exposure of fruits to HWT at 48°C for 2 min resulted in least (0.73%) loss of ascorbic acid as compared to other temperature-time combinations. Fruits which were treated at extreme conditions of 54°C for 5 min showed maximum (~27.03%) loss of ascorbic acid. Similar to our findings, Minh (2021) also reported that the ascorbic acid content of treated pineapple fruit at 56°C/30 sec decreased slightly whereas the control treated fruits showed sharp reduction. HWT induces ascorbate peroxidase enzyme which acts as a scavenger of free radicals resulting in reduced degradation of ascorbic acid in fruit during the cold storage period (Aguayo *et al.* 2015).

Irrespective of treatment, anthocyanin content of apple peel declined gradually during cold storage with treated fruit depicting a suppressed decline (Table 2). Least reduction (0.10%) in total anthocyanin content was observed in fruits exposed to HWT at 48°C/5 min. These results are in line with the observations of Lara *et al.* (2006) who reported that greater retention of the anthocyanin content in hot air (45°C/15 min) treated strawberry cv. Pájaro as compared to untreated fruits after 10 days of storage at 3°C. Application of thermal treatment might have led to the inhibition of phenylalanine ammonia lyase (PAL) activity and thus, greater retention of the pigment over control fruits.

Till 45 days of cold storage all thermally treated samples showed a gradual increase in total phenolic content followed by decreasing trend till the end of 90 days (Table 2). Untreated (control) fruits showed elevation in values till 30 days while fruits given HWT showed increasing levels of total phenolics till 45 days. At the termination of the experiment, all HWT fruits showed slightly improved phenolic content. Average phenolic content increased with HWT but up to a certain limit of treatment, i.e. up to 50°C, beyond which, it displayed a decline. Our observation of increase in the phenolic content up to a certain time-temperature combination of hot water treatment corroborates with the findings of Prasad *et al.* (2016) who also reported an increase in phenolic content in mangoes in response to HWT but only up to a certain limit. Heat stress stimulates the fruit self defense mechanism system by stimulating the metabolic activity and therefore, it leads to improving the protective enzymes and antioxidant content in treated fruit, thus increasing the stress resistance of fruits which prolongs the storage period. Similar findings were also noticed by Schirra *et al.* (2008) in kumquat (*Fortunella japonica* Lour. Swingle cv. Ovale) fruits where the levels of total phenols slightly decreased after hot water dipping. Li *et al.* (2013)

Table 1 Changes in total soluble solids (TSS) and titratable acidity of hot water treated apple during cold storage

Treatment	TSS (°Brix)							Ascorbic acid content (mg/100 g)						
	Days of storage							Days of storage						
	0	15	30	45	60	75	90	0	15	30	45	60	75	90
Control	10.29^{mn}	10.52^{i-n}	10.71^{g-n}	10.74^{g-n}	10.39^{k-m}	10.19^{l-n}	10.32^{k-n}	61.55$^{r-z,A-G}$	61.07$^{l-z,A-H}$	59.67$^{h-z,A-G}$	58.48$^{q-z,A-H}$	52.36^{B-F}	50.73^{H}	49.78^{H}
48°C, 2 min	10.65^{g-n}	10.78^{g-n}	10.84^{g-n}	10.89^{e-n}	10.64^{h-n}	10.37^{k-m}	10.14^{mn}	79.03^{a}	78.96^{ab}	78.85^{abc}	78.69^{abc}	78.55^{abc}	78.50^{abc}	78.45^{abc}
48°C, 3 min	10.74^{g-n}	10.82^{g-n}	10.87^{g-n}	11.03^{d-n}	10.70^{h-n}	10.44^{j-n}	10.39^{k-n}	76.26^{a-d}	76.16^{a-d}	76.03^{a-e}	75.93^{a-e}	75.85^{a-e}	75.76^{a-e}	75.66^{a-f}
48°C, 4 min	10.86^{f-n}	10.91^{e-n}	10.96^{g-n}	11.06^{d-n}	11.05^{d-n}	10.98^{d-n}	10.92^{e-n}	75.38^{a-g}	74.38^{a-h}	73.94^{a-i}	71.94^{a-m}	71.83^{a-q}	69.99^{a-q}	68.16^{a-t}
48°C, 5 min	11.34^{b-n}	11.37^{a-n}	11.42^{a-n}	11.46^{a-n}	10.84^{g-n}	10.17^{m-n}	10.11^{n}	73.54^{a-i}	72.87^{a-k}	71.42^{a-m}	69.84^{a-q}	69.19^{a-r}	69.03^{a-s}	68.86$^{a-s,A-J}$
50°C, 2 min	11.39^{a-n}	11.45^{a-n}	11.87^{a-n}	12.31^{a-n}	11.89^{a-n}	11.64^{a-n}	11.52^{a-n}	72.59^{a-l}	72.46^{a-l}	72.35^{a-z}	72.29^{a-l}	72.16^{a-l}	72.10^{a-l}	72.04^{a-k}
50°C, 3 min	10.58^{h-n}	10.65^{g-n}	10.68^{g-n}	10.72^{g-n}	10.65^{h-n}	10.60^{h-n}	10.51^{i-n}	72.45^{a-l}	71.11^{a-q}	70.88^{a-p}	69.55^{a-q}	63.88$^{g-z,AB}$	63.05$^{l-z,A-D}$	62.21$^{j-z,A-G}$
50°C, 4 min	11.25^{c-n}	11.49^{a-n}	11.53^{a-n}	11.88^{a-n}	11.32^{b-n}	11.23^{d-n}	11.15^{e-n}	72.2^{1a-l}	70.55^{q-p}	69.98^{a-q}	67.31^{c-u}	64.98^{e-x}	63.65$^{h-z,A-C}$	62.32$^{j-z,A-F}$
50°C, 5 min	11.51^{a-n}	11.66^{a-n}	11.89^{a-n}	11.92^{a-n}	11.54^{a-n}	11.51^{a-n}	11.48^{a-n}	71.51^{a-m}	69.51^{a-r}	69.14^{a-r}	64.48^{e-y}	62.14$^{f-z,A-E}$	61.78$^{k-z,A-D}$	61.24$^{l-z,A-H}$
52°C, 2 min	11.59^{a-n}	11.64^{a-n}	11.66^{a-n}	11.68^{a-n}	11.58^{a-n}	11.54^{a-n}	11.49^{a-n}	71.43^{a-m}	71.33^{a-n}	71.19^{a-n}	71.12^{a-n}	71.09^{a-o}	68.49^{a-t}	65.89^{d-x}
52°C, 3 min	11.78^{a-n}	11.82^{a-n}	12.17^{a-n}	12.44^{a-n}	11.72^{a-n}	11.71^{a-n}	11.68^{a-n}	71.24^{a-n}	68.24^{a-t}	67.51^{a-u}	65.84^{e-x}	64.14^{f-z}	63.19$^{h-z,A-C}$	62.24$^{l-z,A-J}$
52°C, 4 min	12.05^{a-n}	12.23^{a-n}	12.34^{a-n}	12.42^{a-n}	11.88^{a-n}	11.81^{a-n}	11.74^{a-n}	70.74^{a-p}	70.08^{a-t}	69.68^{a-q}	64.02$^{g-z,AB}$	63.28$^{h-z,A-C}$	63.11$^{h-z,ABC}$	62.94$^{h-z,A-D}$
52°C, 5 min	12.25^{a-n}	12.36^{a-n}	12.45^{a-n}	12.53^{a-n}	12.33^{a-n}	12.27^{a-n}	12.21^{a-n}	70.59^{a-p}	70.46^{a-p}	70.35^{a-p}	60.49$^{h-z,A-H}$	59.46$^{q-z,A-H}$	59.42$^{p-z,A-H}$	59.38$^{q-z,A-H}$
54°C, 2 min	12.57^{a-n}	12.62^{a-n}	12.72^{a-n}	12.78^{a-n}	12.73^{a-n}	12.71^{a-n}	12.47^{a-n}	69.48^{a-r}	69.41^{a-r}	68.21^{a-t}	59.54$^{h-z,A-H}$	57.21t-$^{z,A-H}$	56.35$^{u-z,A-H}$	55.49$^{v-z,A-H}$
54°C, 3 min	13.09^{a-n}	13.21^{a-m}	13.27^{a-k}	13.39^{a-k}	13.19^{a-n}	13.04^{a-n}	12.88^{a-n}	68.63^{a-t}	67.96^{a-t}	67.53^{a-u}	67.43$^{h-z,BE}$	66.53^{e-v}	59.51$^{p-l,A-H}$	52.49^{A-H}
54°C, 4 min	13.59^{a-i}	13.62^{a-h}	13.64^{a-h}	13.69^{a-g}	13.59^{a-i}	13.51^{a-j}	13.39^{a-k}	68.45^{a-t}	66.48^{d-v}	64.15^{f-z}	62.55$^{i-z,AE}$	54.12$^{yz,A-H}$	52.73$^{z,A-H}$	51.34^{FG}
54°C, 5 min	14.24^{abc}	14.30^{abc}	14.34^{ab}	14.43^{a}	14.05^{a-d}	13.97^{a-e}	13.93^{a-f}	68.33^{a-t}	63.34$^{h-z,ABC}$	61.33$^{p-z,A-H}$	55.36$^{w-z,AE}$	52.08^{C-H}	50.97^{F-H}	49.86^{H}

Means with the same superscript within an attribute are not significantly different ($P < 0.05$).

Table 2 Changes of total anthocyanin and ascorbic acid contents in hot water treated apple during cold storage

Treatment	Total anthocyanin content (mg CGE/kg of peel)							Total phenolic content (mg GAE/100 g)						
	Days of storage							Days of storage						
	0	15	30	45	60	75	90	0	15	30	45	60	75	90
Control	208.03^{hi}	206.89^{i}	206.42^{i}	205.95^{i}	205.18^{i}	203.48^{i}	202.91^{i}	151.31^{jkl}	145.49^{lk}	132.98^{l}	122.29^{l}	116.61^{l}	114.47^{l}	112.34^{l}
48°C, 2 min	248.72^{a}	248.54^{a}	248.35^{a}	248.22^{a}	246.09^{a}	245.30^{abc}	244.52^{a-e}	147.49^{lk}	132.22^{l}	135.06^{l}	124.62^{l}	108.63^{l}	107.19^{l}	105.76^{l}
48°C, 3 min	245.85^{ab}	245.56^{abc}	245.35^{abc}	245.25^{abc}	245.18^{abc}	245.08^{abc}	245.05^{abc}	203.85^{l}	203.87^{ijk}	204.01^{g-k}	209.96^{e-i}	209.23^{e-k}	208.59^{f-k}	207.62^{g-k}
48°C, 4 min	246.45^{a}	246.38^{a}	246.29^{a}	246.15^{a}	246.07^{a}	245.98^{a}	245.89^{ab}	235.34^{a-i}	235.92^{a-i}	239.04^{a-i}	241.29^{a-i}	221.08^{a-i}	212.61^{c-i}	204.14^{h-k}
48°C, 5 min	245.77^{abc}	245.64^{abc}	245.57^{abc}	245.52^{abc}	245.47^{abc}	245.45^{abc}	242.19^{a-g}	247.78^{a-i}	248.66^{a-i}	249.81^{a-i}	255.62^{a-i}	252.52^{a-i}	250.48^{a-i}	248.72^{a-i}
50°C, 2 min	248.89^{a}	248.73^{a}	248.65^{a}	248.49^{d}	248.39^{a}	248.04^{a}	245.02^{abc}	261.09^{a-i}	264.93^{a-i}	268.19^{a-i}	269.93^{a-g}	265.21a-i	261.70^{a-i}	258.20^{a-i}
50°C, 3 min	245.04^{abc}	245.01^{abc}	244.91^{abc}	244.87^{abc}	244.81^{abc}	244.65^{a-e}	244.48^{a-e}	282.31^{ab}	285.79^{ab}	287.37^{a}	289.41^{a}	277.93^{abc}	272.71^{a-g}	267.49^{a-i}
50°C, 4 min	248.11^{a}	248.05^{a}	247.72^{a}	247.68^{a}	247.48^{a}	247.37^{a}	247.28^{a}	252.96^{a-i}	253.67^{a-i}	258.94^{a-i}	261.32^{a-i}	260.82^{a-i}	258.55^{a-i}	256.27^{a-i}
50°C, 5 min	248.05^{a}	247.88^{a}	247.55^{a}	247.35^{a}	247.19^{a}	247.11^{a}	247.03^{a}	265.73^{a-i}	267.88^{a-i}	271.13^{a-i}	271.62^{a-g}	259.26^{a-i}	246.04^{a-g}	232.83^{a-i}
52°C, 2 min	245.74^{abc}	245.63^{abc}	245.61^{abc}	245.48^{abc}	245.28^{abc}	245.08^{abc}	244.87^{abc}	261.53^{a-i}	263.71^{a-i}	267.98^{a-i}	268.54^{a-g}	265.01^{a-i}	263.36^{a-i}	262.38^{a-i}
52°C, 3 min	247.66^{a}	247.36^{a}	247.24^{a}	247.12^{a}	246.55^{a}	246.22^{a}	245.88^{ab}	285.28^{ab}	287.04^{a}	292.18^{a}	293.01^{a}	284.85^{ab}	280.04^{ab}	275.91^{abc}
52°C, 4 min	247.11^{a}	247.08^{a}	246.89^{a}	246.55^{a}	245.28^{abc}	245.03^{abc}	244.78^{a-d}	271.42^{a-g}	273.30^{a-f}	274.57^{a-e}	276.08^{abc}	275.11^{a-d}	272.32^{a-g}	269.53^{a-g}
52°C, 5 min	246.19^{a}	245.89^{ab}	245.69^{abc}	245.37^{abc}	244.77^{a-d}	244.46^{a-e}	244.14^{a-g}	276.98^{abc}	282.41^{ab}	282.92^{ab}	285.12^{ab}	282.12^{ab}	277.57^{abc}	273.03^{a-g}
54°C, 2 min	247.74^{a}	245.61^{abc}	244.51^{a-e}	243.97^{a-g}	242.64^{a-d}	242.29^{a-g}	241.94^{a-g}	282.31^{ab}	282.66^{a}	283.60^{ab}	284.08^{ab}	280.78^{ab}	278.64^{ab}	276.50^{abc}
54°C, 3 min	245.59^{abc}	245.52^{abc}	244.49^{a-e}	244.18^{a-g}	243.76^{a-g}	243.11^{a-g}	242.45^{a-g}	295.15^{a}	272.09^{a-g}	275.31^{a-d}	276.12^{abc}	272.12^{a-g}	268.05^{a-i}	263.98^{a-i}
54°C, 4 min	245.51^{abc}	245.46^{abc}	245.31^{abc}	244.22^{a-g}	243.22^{a-g}	242.76^{a-g}	242.29^{a-g}	233.51^{a-i}	265.60^{a}	265.43^{a-i}	267.82^{a-i}	267.72^{a-i}	267.13^{a-i}	266.55^{a-i}
54°C, 5 min	245.45^{abc}	245.42^{abc}	244.42^{a-e}	244.28^{a-f}	243.18^{a-g}	242.38^{a-g}	241.58^{a-h}	263.56^{a-i}	268.28^{a-i}	269.81^{a-i}	270.76^{a-g}	269.49^{a-i}	267.84^{a-i}	261.19^{a-i}

Means with the same superscript within an attribute are not significantly different ($P < 0.05$).

Table 3 Changes in LOX activity and total antioxidant activity in hot water treated apple during cold storage

Treatment	LOX (µmol/min/g)							Total antioxidant activity (µmol trolox/g)						
	Days of storage							Days of storage						
	0	15	30	45	60	75	90	0	15	30	45	60	75	90
Control	0.941^{edf}	0.943^{edf}	1.76^{b-e}	2.11^{a-e}	3.23^{ab}	3.32^{a}	3.41^{a}	10.88^{m}	10.68^{m}	10.53^{m}	10.35^{m}	10.22^{m}	10.03^{m}	9.84^{m}
48°C, 2 min	0.906^{edf}	0.891^{edf}	0.870^{edf}	0.821^{edf}	0.792^{edf}	0.784^{edf}	0.776^{edf}	17.78^{l}	18.05^{l}	18.11^{l}	18.12^{l}	18.09^{l}	17.86^{l}	17.64^{l}
48°C, 3 min	0.876^{edf}	0.861^{edf}	0.825^{edf}	0.783^{edf}	0.751^{edf}	0.739^{edf}	0.726^{edf}	18.08^{l}	18.13^{l}	18.15^{l}	18.34^{l}	18.24^{l}	18.22^{l}	18.21^{l}
48°C, 4 min	0.84^{1} ^{edf}	0.822^{edf}	0.793^{edf}	0.763^{edf}	0.696^{edf}	0.685^{ef}	0.673^{ef}	18.91^{kl}	18.92^{kl}	18.96^{kl}	19.08^{j-l}	19.02^{kl}	18.93^{kl}	18.85^{kl}
48°C, 5 min	0.812^{edf}	0.800^{edf}	0.781^{edf}	0.772^{edf}	0.656^{ef}	0.640^{ef}	0.624^{ef}	18.95^{kl}	19.12^{i-l}	19.16^{i-l}	19.20^{i-l}	18.93^{kl}	18.92^{kl}	18.90^{kl}
50°C, 2 min	0.901^{edf}	0.881^{edf}	0.842^{edf}	0.765^{edf}	0.746^{edf}	0.716^{edf}	0.686^{ef}	19.54^{h-l}	19.62^{g-l}	19.65^{g-l}	19.66^{g-l}	19.66^{g-l}	19.64^{g-l}	19.63^{g-l}
50°C, 3 min	0.872^{edf}	0.841^{edf}	0.805^{edf}	0.752^{edf}	0.686^{ef}	0.663^{ef}	0.653^{ef}	19.95^{e-l}	19.96^{e-l}	19.98^{d-l}	19.98^{d-l}	19.99^{d-l}	19.94^{e-l}	19.89^{e-l}
50°C, 4 min	0.821^{edf}	0.802^{edf}	0.772^{edf}	0.686^{ef}	0.651^{ef}	0.649^{ef}	0.646^{ef}	20.54^{b-l}	20.58^{b-l}	20.62^{b-l}	20.64^{b-l}	20.54^{b-l}	20.54^{e-l}	20.53^{c-l}
50°C, 5 min	0.776^{edf}	0.781^{edf}	0.742^{edf}	0.678^{ef}	0.649^{ef}	0.643^{ef}	0.636^{ef}	18.56^{kl}	18.51^{ab}	18.48^{l}	18.45^{l}	18.41^{l}	18.37^{l}	18.32^{l}
52°C, 2 min	0.876^{edf}	0.851^{edf}	0.836^{edf}	0.745^{edf}	0.716^{edf}	0.700^{edf}	0.684^{ef}	18.97^{kl}	19.09^{j-l}	19.13^{i-l}	19.14^{i-l}	18.85^{kl}	18.76^{kl}	18.68^{kl}
52°C, 3 min	0.842^{edf}	0.821^{edf}	0.791^{edf}	0.721^{edf}	0.673^{ef}	0.657^{ef}	0.642^{ef}	19.81^{g-l}	18.96^{kl}	18.99^{kl}	19.01^{kl}	18.88^{kl}	18.83^{kl}	18.77^{kl}
52°C, 4 min	0.772^{edf}	0.721^{edf}	0.705^{edf}	0.626^{ef}	0.606^{f}	0.594^{f}	0.583^{f}	19.56^{h-l}	19.62^{g-l}	19.65^{g-l}	19.67^{g-l}	19.63^{g-k}	19.61^{g-l}	19.58^{g-l}
52°C, 5 min	0.741^{edf}	0.693^{ef}	0.681^{ef}	0.641^{ef}	0.626^{ef}	0.587^{f}	0.546^{f}	23.97^{a}	23.99^{a}	24.13^{a}	24.18^{a}	24.05^{a}	23.75^{ab}	23.44^{abc}
54°C, 2 min	0.833^{edf}	0.793^{edf}	0.771^{edf}	0.734^{edf}	0.681^{ef}	0.611^{f}	0.541^{f}	24.52^{a}	24.56^{a}	24.62^{a}	24.64^{a}	24.54^{a}	24.49^{a}	24.44^{a}
54°C, 3 min	0.793^{edf}	0.753^{edf}	0.641^{ef}	0.671^{ef}	0.643^{ef}	0.590^{f}	0.53^{6f}	22.31^{a-i}	22.33^{a-i}	22.42^{a-h}	23.10^{a-e}	22.78^{a-g}	22.26^{a-j}	21.74^{a-k}
54°C, 4 min	0.761^{edf}	0.693^{ef}	0.671^{ef}	0.554^{f}	0.513^{f}	0.500^{f}	0.486^{f}	22.96^{a-f}	23.03^{a-e}	23.18^{a-d}	23.28^{abc}	23.24^{abc}	22.92^{a-f}	22.60^{a-h}
54°C, 5 min	0.706^{edf}	0.676^{ef}	0.642^{ef}	0.621^{ef}	0.571^{f}	0.508^{f}	0.445^{f}	24.84^{a}	24.88^{a}	24.92^{a}	24.94^{a}	24.90^{a}	24.77^{a}	24.65^{a}

Means with the same superscript within an attribute are not significantly different ($P < 0.05$). LOX, Lipoxygenase.

further reported that heat treatment at a lower temperature (45°C) maintained total phenolic compounds and antioxidant capacity in Red Fuji apples throughout the storage period instead of a higher temperature.

Lipoxygenase (LOX) activity gradually decreased in all the treated fruit as compared to control samples (Table 3). Among the lots, fruits treated at 54°C showed the greatest decline in LOX activity (35.45–36.96%) for a 2–5 min exposure at the end of 90 days of cold storage. This might be as a result of hot water induced inactivation of the enzyme. However, control fruits showed increasing LOX activity (26.2 fold) related to progressive senescence changes by the end of cold storage period.

With respect to AOX activity, our findings reveal gradual increase in value after 30 and 45 days of cold storage for control and HWT fruits, respectively followed by a progressive decline (Table 3). In general, HWT fruits maintained higher AOX activity as compared to control. At the end of three month storage, control fruits exhibited 9.56% reduction of AOX activity in comparison to 0.17–2.21% reduction in HWT apples. Similarly, Shen *et al.* (2013) observed that at 50°C hot water dipping treatment increased 'Satsuma' mandarin antioxidant capacity immediately after treatment and maintained it at higher levels during storage as compared to control fruits. Several studies claim that heat treatment might stimulate the protective enzymes such as catalase, superoxide dismutase against oxidative damage of fruit, thus enhancing the antioxidant activity (Ghasemnezhad *et al.* 2008). However, higher temperatures might have a negative effect on the antioxidant capacity of apple fruits as heat damage may enhance senescence metabolism. A higher antioxidant capacity is essential to help the fruit to cope up with reactive oxygen species (ROS). Excessive accumulation of ROS due to a lack of scavenging antioxidants can induce oxidative stress, which results in cell damage that can culminate in fruit senescence and cell death.

HWT is a simple technique to maintain the postharvest fruit quality. Although different studies suggest different time-temperature combinations, no systematic study had been conducted earlier to ascertain the conditions that retain the best nutritive quality of fruits. Through this study we observed the hot water treatment of apple fruits (cv. Royal Delicious) at 48°C for 5 min and 50°C for 2 min were best to maintain the nutritional quality during cold storage up to 3 months.

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