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# Effect of post-harvest calcium chloride dip treatment and gamma irradiation on storage quality and shelf-life extension of Red delicious apple

P. R. Hussain  $\cdot$  R. S. Meena  $\cdot$  M. A. Dar  $\cdot$  A. M. Wani

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Abstract Freshly harvested Red delicious apples were dipped in calcium chloride solution of varying concentrations  $(0.5-2.0\%$  w/v) for 1 h prior to irradiation at dose level of 0.4 kGy. Fruits after radiation treatment were stored at  $2\pm1\degree$ C, RH 90% and evaluated at intervals of 30 days for various quality parameters. Results revealed significant ( $p \leq$ 0.05) retention in firmness, juice yield and ascorbic acid content in samples treated with combination of calcium chloride at 2.0% w/v and gamma irradiation (0.4 kGy) during storage. Water soluble pectin was inversely correlated with firmness ( $r=0.88$ ) and was significantly ( $p \le 0.05$ ) lower in samples subjected to combination treatment of  $2.0\%$  w/v CaCl<sub>2</sub> and 0.4 kGy irradiation throughout the storage. The combination treatment of  $2.0\%$  CaCl<sub>2</sub> and 0.4 kGy irradiation gave about 4.3 log reduction in yeast and mold count of apple samples. Results of the post refrigeration weight loss, firmness and overall acceptability revealed that combination treatment was helpful in extending the shelf-life of Red Delicious apples by around 20– 25 days at  $17\pm2^{\circ}$ C, RH 75% following 90 days of refrigeration.

Keywords Red delicious apples. Calcium treatment . Gamma irradiation · Physico-chemical parameters · Storage quality . Shelf-life extension

The state of Jammu and Kashmir, owing to its topography and climate offers a huge potential for growing of pome and stone fruits as well as berries of temperate nature. Among the pome fruits presently grown in Kashmir valley, apple has a leading position both in terms of cultivation and

production. Apple occupies around 50% of the total area under its cultivation and constitutes about 90% of total fruit produced in the state (Anon [2007-](#page-9-0)08). Among the commercially grown varieties, Red Delicious has gained much popularity and commercial importance due to its overall sensory qualities, acceptability and market returns. Factors affecting the overall quality of apple during storage include loss of water, respiration, metabolism and microbial spoilage (Alak and Goswami [2006](#page-9-0)). Microbial contamination of fresh produce poses potential health risks besides the losses. Of the various fungal toxins which are generally associated with fresh apples, patulin is of prime concern. Patulin is a mycotoxin produced by fungal genera of Penicillium, Aspergillus and Byssochlamys (Shephard and Leggott [2000](#page-10-0); Varga et al. [2003](#page-10-0); Boonzaaijer et al. [2005\)](#page-10-0). The representative symptom of patulin is acute digestive disease, especially in infants (Lai et al. [2000;](#page-10-0) Drusch et al. [2007](#page-10-0)). Patulin also acts as a mutagen and influences the nervous and immune systems (Yun et al. [2008](#page-11-0)). Therefore, patulin should be controlled as an important quality factor in apple and processed apple products. Further, due to inappropriate post-harvest management practices, lack of proper storage and prompt transportation facilities, huge losses of the order of 20–40% occur in the fresh produce during handling, packaging, transportation, marketing and storage (Roy [1993](#page-10-0)). Therefore, post-harvest treatment of fresh apple has become necessary to maintain the quality and provide longer life to fruit. Also, in order to facilitate international trade and overcome quarantine barriers, it is needed to inactivate both food-borne pathogens as well as spoilage microorganisms in fresh produce and simultaneously extend the storage life.

Gamma irradiation is a non-thermal process in nature and has emerged as a potential alternate method of preservation of fruits and vegetables, obviating the use of

P. R. Hussain ( $\boxtimes$ ) · R. S. Meena · M. A. Dar · A. M. Wani Nuclear Research Laboratory, Bhabha Atomic Research Centre, Zakura Srinagar 190006, India e-mail: hussainpr@rediffmail.com

chemical preservatives. Gamma irradiation has proved to be effective in reducing bacterial and mold contamination, inactivating food-borne pathogens in fresh produce as well as delaying the ripening of climacteric fruits (Baskaran et al. [2007;](#page-10-0) Niemira et al. [2003](#page-10-0); Prakash et al. [2000;](#page-10-0) Bidawid et al. [2000;](#page-10-0) King and Josephson [1982](#page-10-0); Kader [1986](#page-10-0)). The use of gamma irradiation to enhance the shelf-life of fruits and vegetables and to ensure microbiological safety is increasing, as it appeared to be effective both on cells and spores (DeRuiter and Dwyer [2002\)](#page-10-0). Zegota et al. ([2001\)](#page-11-0) studied the influence of ionizing radiations on the patulin content of apple juice concentrate and reported that patulin, at an initial concentration of about 2 mg/kg disappeared after irradiation of the concentrate with doses as low as 2.5 kGy. Irradiation of the concentrate with doses sufficient for patulin disappearance did not affect the quality of concentrate. Yun et al. ([2008\)](#page-11-0) studied the radio degradation of patulin in apple model system and reported that dose of 1.0 kGy is effective for patulin degradation. The viable cell counts of the inoculated conidia in the apple showed 2 log reduction at a dose of 1.0 kGy. Our earlier study (Hussain et al. [2008\)](#page-10-0) revealed that gamma irradiation dose of 0.4 kGy was effective in extending the shelf-life of apple fruit by 30 days under ambient storage. Combinatory treatments have also been widely investigated as they often result in synergistic effects. Gamma irradiation in combination with other treatments (e.g. heat, washing, waxing, refrigeration) decreased the microbial contamination level of fresh produce, leading thus to an improvement of quality and shelf-life (Spalding and Reeder [1986](#page-10-0); Lacroix et al. [1991\)](#page-10-0).

Calcium chloride is used as an economical processing aid in the fresh-cut produce industry to minimize tissue damage during processing. Applying calcium treatment may contribute to cell wall integrity by increasing the amount of endogenous calcium available to bind with deesterified pectic residues. In addition to preserving firmness, post-harvest calcium dips reduce respiration, decrease ethylene production and delay senescence in fresh produce such as carrots (Izumi and Watada [1994\)](#page-10-0), Kiwi fruit (Agar et al. [1999](#page-9-0)), strawberries (Rosen and Kader [1989\)](#page-10-0) and tomatoes (Artes et al. [1999](#page-10-0)). The concentration of the calcium dip depends on the fruit or vegetable being treated and many studies report that 1–2% calcium salts are most effective for diced tomatoes (Castaldo et al. [1996](#page-10-0)), cantaloupes (Luna-Guzman and Barrett [2000](#page-10-0)) and pears (Dong et al. [2000\)](#page-10-0). Literature regarding the combination treatments of irradiation and calcium dip with respect to quality maintenance during storage and shelf-life extension of fresh produce are limited, although few investigations have been carried out in minimally processed apple products (Kovacs et al. [1998;](#page-10-0) Gunes et al. [2001](#page-10-0); Fan et al. [2005](#page-10-0)). Therefore, this work aims to investigate the

effects of calcium chloride dip treatment and gamma irradiation on storage quality and shelf-life extension of fresh whole "Red Delicious" apples. The assessment of the treatments is based on the evaluation of physico-chemical parameters, overall acceptability and microbial load as yeast and mold count.

## Materials and methods

Red Delicious apples of proper maturity (180 days after full bloom) and firm texture were procured from the local apple orchards of Zakura, Srinagar. Selection of apples was done from single orchard. After harvesting, the fruit was pre-cooled at 2°C for 24 h in a cold storage chamber in order to remove the field heat. The precooled fruit was graded manually for achieving uniformity in size and any blemished or diseased fruits present were discarded. The whole graded fruit was dipped for 1 h in various concentrations of calcium chloride solution  $(0.5, 1.0, 1.5, 1.0, 1.5, 2\%$  w/v). The temperature of the dip solution was  $10\pm2^{\circ}$ C. During treatment, the fruits were turned manually to facilitate uniform dipping. After dipping, the samples were drained and the adhering water was whipped out using blotting paper. Following the calcium chloride treatment, the fruit was packed in cardboard boxes of size  $(0.5 \text{ m} \times 0.3 \text{ m} \times 0.3 \text{ m})$  each containing ninety fruits.

Gamma irradiation treatment The packaged fruit was subjected to  $\gamma$ -irradiation at dose level of 0.4 kGy using PANBIT irradiator having Co-60 as  $\gamma$ -ray source. The fruit was irradiated at a mean dose rate of 195 Gy/h. The dose rate was determined by Fricke dosimetry. To ensure uniformity of dose, boxes were rotated by 180° halfway through the procedure, resulting in an over dose ratio  $(D_{\text{max}}/D_{\text{min}})$  of 1.6. After irradiation, the fruit was stored under refrigerated conditions (temp.2 $\pm$ 1°C, RH 90%) for evaluation of physico-chemical and microbiological parameters at intervals of 30 days of storage. For each treatment three boxes of fruits were used. Fruits neither dipped in calcium chloride nor gamma irradiated served as control.

Fruit quality analysis Prior to the measurement of quality attributes, fruits were allowed to attain the room temperature. Firmness of apples was determined by a hand penetrometer model "FT-327" (EFFEGI, Italy) provided with a round plunger (11.2 mm diameter). To avoid the interference of skin, fruits were peeled at the points where firmness was to be measured. Triplicate samples of ten fruits were selected randomly and evaluated for firmness measurement on three sides of each whole fruit and average

value was reported in kg. The fruits initially used for firmness measurement were subjecting to juice extraction using an Omini mixer (Philips make). Total soluble solids (TSS) were determined by hand refractometer model " SP-05A" (SIPCON, Japan) having range of 0– 32%. Titratable acidity (% malic acid) and total sugars were determined as per the methods of Ranganna ([1986](#page-10-0)). Ascorbic acid was determined by AOAC [\(1984\)](#page-10-0) method using 2, 6-dichlorophenol-indophenol dye solution. Water soluble pectin (WSP) was evaluated by modifying the method of Ranganna ([1986](#page-10-0)). A sample of 50 g was first extracted with 100 ml of 95% ethanol. The residue left after alcoholic extraction was dried at 30°C. To obtain water soluble pectin (WSP), 30 g of the leftover alcohol insoluble solids (AIS) were extracted in 250 ml boiling water for 30 min. The extract was filtered, cooled and volume made up to 250 ml with double distilled water. 100 ml aliquot of the filtrate was precipitated for 1 h with 25 ml of calcium chloride (1 N). The precipitate obtained was dried and weighed in order to calculate the amount of WSP as

Water soluble pectin $(\%$  AIS)

 $=\frac{\text{Wt. of ppt (g)} \times \text{Volume made (ml)} \times 100}{\text{Wt of sample (g)} \times \text{Aliquot taken (ml)}}$ Wt. of sample(g)  $\times$  Aliquot taken(ml)

Juice yield was determined by extracting the juice from known weight of sample. Weight loss was determined by periodical weighing of samples. Overall acceptability based on sensory attributes viz. firmness, appearance and taste was done by a trained panel of five judges using 4-point scale where, 4=excellent, 3=good, 2= fair and 1=poor. For each treatment eight fruits were selected randomly, coded and served to judges. Microbial load as yeast and mold count was determined by the serial dilution method using potato dextrose agar media (Aneja [1996\)](#page-9-0). All the analyses were done in triplicates for each treatment.

Statistical analysis The data was analyzed statistically using completely randomized design experiment (Cochran and Cox [1975](#page-10-0)). For each measurement, three replicates of samples were tested per treatment and mean±standard deviation values were reported. Analysis of variance (ANOVA) of the data was performed using MINITAB statistical analysis software package (Minitab, version 11.12, 32 bit, Minitab). Difference between means of data was compared by least significant difference (LSD) and Student's t test was applied to determine if the difference was statistically significant. Differences at  $p \le 0.05$  were considered to be statistically significant. Duncan's multiple range test was used to compare the mean values at each storage period. Coefficient of correlation was determined by Karl Pearson method.

#### Results and discussion

Red delicious apples at harvest had  $9.4 \pm 0.35$  kg firmness,  $10.6\pm0.20$  <sup>0</sup>Bx total soluble solids,  $0.38\pm0.02\%$  acidity,  $8.2\pm0.25\%$  total sugars,  $4.8\pm0.14$  mg/100 g ascorbic acid,  $0.08 \pm 0.01$  water soluble pectin (%AIS) and  $67.2 \pm 1.4$ % juice yield. Results of the change in quality parameters of Red delicious apple due to calcium chloride dip and gamma irradiation during storage are discussed as under.

Firmness Effect of calcium chloride and gamma irradiation treatments on firmness of apples is depicted in Table [1.](#page-3-0) Statistical analysis of the data revealed that after 30 days of storage, firmness of control apples and those treated with CaCl<sub>2</sub> at levels 0.5 and 1.0% w/v was marginally ( $p \ge 0.05$ ) different with respect to each other, but significantly ( $p \leq$ 0.05) lower than rest of the treatments. Among other treatments, firmness of 0.4 kGy irradiated apples and those treated with CaCl<sub>2</sub> alone at 1.5 and 2.0% w/v or combination of CaCl<sub>2</sub> (0.5 and 1.0% w/v) and gamma irradiation (0.4 kGy) was also statistically non-significant  $(p\geq 0.05)$  with respect to each other over the same storage period. Treatment of CaCl<sub>2</sub> (1.5 and 2.0% w/v) in combination with irradiation (0.4 kGy) proved effective in maintaining the significantly ( $p \leq 0.05$ ) higher firmness of apples over the same storage period. As the storage period advanced, firmness recorded a further decrease; the decrease was significantly ( $p \le 0.05$ ) higher in control, 0.5 and 1.0% w/v  $CaCl<sub>2</sub>$  treated apples. After 90 days of storage, control fruits recorded a decrease of 32.9% in firmness as against the 22.3 and 10.6% in 0.4 kGy irradiated fruits and those subjected to combination of  $CaCl<sub>2</sub>$  and irradiation  $(2.0\% \text{ w/v}, 0.4 \text{ kGy})$ . The retention of firmness in samples either calcified only or calcified and irradiated is due to fact that calcium plays an important role in maintaining cell wall structure by interaction with pectic acids in the cell walls to form calcium pectate (Poovaiah [1986;](#page-10-0) Conway and Sams [1987\)](#page-10-0). Cell wall integrity is preserved when deesterified pectic acid residues form cross-bridges between negatively charged carboxylic groups and divalent cations such as calcium, thus minimizing pectic substance solubilization (Grant et al. [1973](#page-10-0); Krall and Mc Feeters [1998\)](#page-10-0).Also calcium appears to serve as an intermolecular binding agent that stabilizes protein-pectin complexes of the middle lamella (Dey and Brinson [1984](#page-10-0)), thus plays a role in maintaining cell wall structure by interacting with pectic acids in the cell wall to form calcium pectate. Further, both irradiation as well as calcium is known to delay the natural physiological processes like respiration, ripening and senescence responsible for the solubilization and depolymerization of pectic substances and other cell wall polymers (Izumi and Watada [1994;](#page-10-0) Agar et al. [1999;](#page-9-0) Rosen and Kader [1989;](#page-10-0) Artes et al. [1999](#page-10-0); Floros et al. [1992;](#page-10-0) Kader

<span id="page-3-0"></span>

\*=Percentage decrease in firmness/Juice yield and percentage increase in water soluble pectin after 90 days of storage \*=Percentage decrease in firmness/Juice yield and percentage increase in water soluble pectin after 90 days of storage

Values with different superscript uppercase letters (A-C) within a column and superscript lowercase letters (a-f) within a row differ significantly ( $p \le 0.05$ ) Values with different superscript uppercase letters (A–C) within a column and superscript lowercase letters (a–f) within a row differ significantly (p≤0.05)

 $T_1$ =Control; T2=0.4 kGy; T3=0.5% CaCl<sub>2</sub>; T<sub>4</sub>=1.0% CaCl<sub>2</sub>; T<sub>5</sub>=1.5% CaCl<sub>2</sub>; T<sub>6</sub>=2.0% CaCl<sub>2</sub>;  $T_1$ =Control; T2=0.4 kGy; T3=0.5% CaCl<sub>2</sub>; T<sub>4</sub>=1.0% CaCl2; T<sub>5</sub>=1.5% CaCl2; T<sub>6</sub>=2.0% CaCl2;

 $T_7=0.5\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_8=1.0\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_9=1.5\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_{10}=2.0\%$  CaCl<sub>2</sub>, 0.4 kGy  $T_7=0.5\%$  CaCl2, 0.4 kGy;  $T_8=1.0\%$  CaCl2, 0.4 kGy;  $T_9=1.5\%$  CaCl2, 0.4 kGy;  $T_{10}=2.0\%$  CaCl2, 0.4 kGy

[1986](#page-10-0)). Thus, slower decrease of firmness in samples subjected either to calcification or combination of calcification and irradiation is related to delayed physiological processes, thereby resulting in reduction in the rate of increase of soluble pectic fractions. Hence the normal conversion of insoluble to soluble pectins during storage appears to have been markedly retarded both by calcification and irradiation ( El Assi et al. [1997](#page-10-0); d'Amour et al. [1993;](#page-10-0) Howard et al. [1995;](#page-10-0) Kovacs et al. [1997;](#page-10-0) Prakash et al. [2002\)](#page-10-0).

Water soluble Pectin (WSP) The presence of endogenous and exogenous calcium has stabilizing effect on the integrity of the cell wall, thus minimizing pectic substance solubilization (Buescher and Hobson [1982](#page-10-0); Krall and Mc Feeters [1998\)](#page-10-0). As calcium chloride dip concentration increased from 0.5 to 2.0% w/v, increase in WSP levels decreased ( $p \le 0.05$ ) (Table [1\)](#page-3-0). After 30 days of storage, WSP levels of control samples and those treated with CaCl<sub>2</sub> at levels 0.5 and 1.0% w/v differed non-significantly with respect to each other. During further storage WSP levels exhibited increasing trend and after 90 days of storage increase was significantly  $(p \le 0.05)$  higher in control samples than all other treatments. Among the treatments, combination of  $CaCl<sub>2</sub>$  dip (1.5 and 2.0%) and irradiation (0.4 kGy) maintained ( $p \le 0.05$ ) lowest WSP levels even after 90 days of storage. After 90 days of storage, WSP levels were increase by 88.2% for control as against the 81.4% for the combinatory treatment of CaCl<sub>2</sub> dip  $(2.0\% \text{w})$ v) and irradiation (0.4 kGy). The data also revealed that, there was no significant increase in WSP levels of apples due to irradiation at 0.4 kGy when compared with other treatments, thereby inferring that low dose irradiation does not result in significant solubilization of pectic substance. The lower levels of WSP in samples treated with combination of  $CaCl<sub>2</sub>$  dip and gamma irradiation is partly attributed to calcium absorption into the cell wall during dipping and to delaying of ripening and enzymatic activity by the synergistic effect of  $CaCl<sub>2</sub>$  dip and irradiation. This inhibitory effect of  $CaCl<sub>2</sub>$  and irradiation resulted in reduction in the rate of conversion of insoluble pectin to soluble pectin, thus maintaining the lowest levels of WSP (Hussain et al. [2008](#page-10-0); Poovaiah [1986\)](#page-10-0). Our results also revealed that WSP fraction was inversely correlated with firmness  $(r=0.88)$ . Similar significant negative correlations were also found by Gunes et al. [\(2001](#page-10-0)).

Juice yield The juice yield of apple samples showed a declining trend during storage irrespective of treatments (Table [1\)](#page-3-0). After 30 days of storage, juice yield of control and calcified (0.5% CaCl<sub>2</sub>) samples was marginally ( $p \geq$ 0.05) different with respect to each other. Juice yields of 0.4 kGy irradiated samples and those treated with  $CaCl<sub>2</sub>$ 

alone at levels  $1.0-2.0\%$  w/v or combination of CaCl<sub>2</sub> (0.5– 1.5% w/v) and irradiation (0.4 kGy) also differed nonsignificantly after 30 days of storage. Combinatory treatment of  $2.0\%$  CaCl<sub>2</sub> and 0.4 kGy irradiation proved effective in maintaining significantly  $(p \le 0.05)$  higher juice yield of apples than all other treatments throughout the whole storage. With progress of storage, juice yield decreased significantly ( $p \le 0.05$ ) when compared with the initial value. After the end of 90 days of storage, juice yield decreased by 28.9% for control, 18.7% for irradiated (0.4 kGy) and 12.4% for samples treated with combination of CaCl<sub>2</sub> dip  $(2.0\% \text{ w/v})$  and irradiation  $(0.4 \text{ kGy})$ respectively. The significantly  $(p \le 0.05)$  lower decrease in juice yield in samples treated with calcium chloride prior to irradiation can be explained due to the inhibitory effect of combinatory treatments on physiological processes responsible for loss of turgor pressure and membrane integrity (Hussain et al. [2008](#page-10-0)). Positive correlations  $(r=0.78)$  were obtained between firmness and juice yield, thus explaining the higher firmness of apples treated with combination of CaCl<sub>2</sub> dip (2.0% w/v) and gamma irradiation.

Total soluble solids (TSS) and total sugars The total soluble solids and total sugars of apple samples showed an increasing trend irrespective of treatments (Table [2\)](#page-5-0). After 30 days of storage, increase in TSS and total sugars was significantly ( $p \le 0.05$ ) lower in samples either irradiated, CaCl<sub>2</sub> treated at levels above  $0.5\%$  w/v or treated with combination of  $CaCl<sub>2</sub>$  and irradiation. Among treatments, significantly ( $p \le 0.05$ ) lower TSS (10.8) and total sugars (8.1%) were recorded in samples treated with combination of CaCl<sub>2</sub> at levels 1.5 and 2.0% w/v and 0.4 kGy irradiation over the same storage period. The data also revealed that CaCl<sub>2</sub> treatment up to  $1.0\%$  w/v had no significant effect on preventing the TSS as well as total sugar increase when compared with control. Positive correlations  $(r=0.78)$  were obtained between TSS and total sugars, indicating that total sugars increased with increase in TSS. With further advancement of storage, TSS recorded a significant increasing trend. Similar pattern was observed in total sugars during further storage. Among the treatments, increase was significantly  $(p \le 0.05)$  lower in samples treated with combination of  $2.0\%$  w/v CaCl<sub>2</sub> and 0.4 kGy irradiation even after 90 days of storage. After 90 days of storage, control samples recorded an increase of 22.1 and 29.9% in TSS and total sugars as against the 10.2 and  $17.2\%$  in samples subjected to combination of CaCl<sub>2</sub> dip  $(2.0\% \text{ w/v})$  and irradiation  $(0.4 \text{ kGy})$ . The increase in TSS and total sugars is attributed to the enzymatic conversion of higher polysaccharides such as starches and pectins in to simple sugars during ripening (Hussain et al. [2008\)](#page-10-0). Since, both  $CaCl<sub>2</sub>$  and gamma irradiation delay the natural physiological processes like ripening, senescence and

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 $V = 0.02$  ), Values are means±SD,  $n=3$ ; LSD=Least significant difference ( $p \le 0.05$ ); Least signific values are means  $\pm$  5D,  $n = 3$ ;  $\pm$  5D

\*=Percentage increase in TSS and total sugars after 90 days of storage/Percentage decrease in acidity after 90 days of storage \*=Percentage increase in TSS and total sugars after 90 days of storage/Percentage decrease in acidity after 90 days of storage

Values with different superscript uppercase letters (A-C) within a column and superscript lowercase letters (a-g) within a row differ significantly ( $p \le 0.05$ ) Values with different superscript uppercase letters (A–C) within a column and superscript lowercase letters (a–g) within a row differ significantly (p≤0.05)  $T_1$ =Control; T2=0.4 kGy; T3=0.5% CaCl<sub>2</sub>; T<sub>4</sub>=1.0% CaCl<sub>2</sub>; T<sub>5</sub>=1.5% CaCl<sub>2</sub>; T<sub>6</sub>=2.0% CaCl<sub>2</sub>;  $T_1$ =Control; T2=0.4 kGy; T3=0.5% CaCl<sub>2</sub>; T<sub>4</sub>=1.0% CaCl2; T<sub>5</sub>=1.5% CaCl2; T<sub>6</sub>=2.0% CaCl2;

 $T_7=0.5\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_8=1.0\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_9=1.5\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_{10}=2.0\%$  CaCl<sub>2</sub>, 0.4 kGy  $T_7=0.5\%$  CaCl2, 0.4 kGy;  $T_8=1.0\%$  CaCl2, 0.4 kGy;  $T_9=1.5\%$  CaCl2, 0.4 kGy;  $T_{10}=2.0\%$  CaCl2, 0.4 kGy

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respiration, responsible for increase as well as decrease of TSS and total sugars; due to inhibitory effect on the activities of enzymes involved in hydrolysis (Izumi and Watada [1994;](#page-10-0) Agar et al. [1999](#page-9-0); Rosen and Kader [1989](#page-10-0); Kader [1986](#page-10-0); Artes et al. [1999\)](#page-10-0). Therefore, the synergistic effect of  $CaCl<sub>2</sub>$  dip and gamma irradiation resulted in delaying the increase in TSS as well as total sugars in samples subjected to combination treatments of CaCl<sub>2</sub> and irradiation even after 90 days of storage.

Acidity The acidity values of apples showed a declining trend during storage irrespective of treatments (Table [2](#page-5-0)). The decrease in acidity was significantly ( $p \le 0.05$ ) lower in samples treated with CaCl<sub>2</sub> alone at levels 1.5 and 2.0% w/v and combination of CaCl<sub>2</sub> (0.5–2.0% w/v) and irradiation after 30 days of storage. After 60 day of storage, the decrease in acidity values of samples treated with combination of  $CaCl<sub>2</sub>$  (at levels above 0.5% w/v) and gamma irradiation was significantly ( $p \le 0.05$ ) lower than rest of the treatments. Towards the end of the storage, combinatory treatment of  $2.0\%$  w/v CaCl<sub>2</sub> and 0.4 kGy irradiation proved beneficial in maintaining significantly ( $p \leq 0.05$ ) higher acidity values of apple samples. After 90 days of storage, control samples recorded a loss of 52.6% in acidity compared to 23.7% in samples treated with combination of CaCl<sub>2</sub> (2.0% w/v) and irradiation. The loss of acid values is largely due to the utilization of organic acids as respiratory substrates and as carbon skeleton for the synthesis of new compounds during ripening. Also accumulation of sugars during ripening contributes to decrease of acidity as a result of increase in TSS acid ratio (Wani et al. [2008](#page-11-0)). The retention of higher values of acidity in samples subjected to combinatory treatments of  $CaCl<sub>2</sub>$  (2.0% w/v) dip and irradiation (0.4 kGy) is due to the delay in ripening process because of synergistic effect of the treatment.

Ascorbic acid Ascorbic acid also recorded decreasing trend in all the treatments (Table [3](#page-7-0)). After 30 days of storage, ascorbic acid of control,  $0.5$  and  $1.0\%$  w/v CaCl<sub>2</sub> treated samples differed marginally ( $p \ge 0.05$ ) with respect to each other. Among all other treatments, ascorbic acid was significantly ( $p \le 0.05$ ) higher in samples subjected to combination of calcium chloride dip at levels 1.5 and 2.0% w/v and irradiation at 0.4 kGy. Ascorbic acid content of 0.4 kGy irradiated samples and those treated with  $CaCl<sub>2</sub>$ alone at levels 1.5 and 2.0%w/v also differed nonsignificantly after 30 days of storage. With advancement of storage, ascorbic acid decreased significantly  $(p \le 0.05)$ . After 90 days of storage, control and  $0.5\%$  w/v CaCl<sub>2</sub> treated samples recorded a decrease of 45.8% in ascorbic acid as compared to 25% in samples treated with combination of CaCl<sub>2</sub> (1.5–2.0% w/v) and irradiation (0.4 kGy). The irradiation alone at 0.4 kGy caused a slight decrease in ascorbic acid, but the decrease was statically nonsignificant when compared with samples treated with Calcium chloride alone at levels 1.5 and 2.0% w/v. Thus, it can be inferred that main loss of ascorbic acid is due to storage rather than irradiation. The ascorbic acid loss during storage is known to be due to its antioxidant activity especially under postharvest storage conditions (Davey et al. [2000](#page-10-0)). The beneficial effect of calcium in preventing decline of ascorbic acid during storage is due to the regulation of oxidative processes is the cytosol (Faust and Shear [1972\)](#page-10-0).

Loss in weight The statistically analysis of data revealed that weight loss of control and 0.5% w/v calcium chloride treated samples differed marginally  $(p \ge 0.05)$  with respect to each other, but differed significantly  $(p \le 0.05)$  when compared with other treatments at the end of 30 days of storage (Table [3\)](#page-7-0). The weight loss of samples irradiated to 0.4 kGy and those treated with combination of Calcium chloride  $(0.5-2.0\%$  w/v) and irradiation  $(0.4 \text{ kGy})$  also differed marginally ( $p \ge 0.05$ ) with respect to each other over the same storage period. As the storage period progressed, weight loss also increased ( $p \le 0.05$ ) and was significantly higher in control samples  $(5.8 \pm 0.20)$  followed by calcium chloride treated apples at levels of 0.5% w/v  $(5.6\pm0.33\%)$  at the end of 90 days of storage. The samples treated with combination of CaCl<sub>2</sub> dip (2.0% w/v) and irradiation (0.4 kGy) recorded significantly ( $p \le 0.05$ ) lower weight loss than all other treatments over the same storage period. The lower weight loss in samples treated with combination of CaCl<sub>2</sub> dip (2.0% w/v) and irradiation  $(0.4 \text{ kGy})$  is due to the synergistic effect of CaCl<sub>2</sub> and gamma irradiation on the delaying of natural physiological processes like respiration, onset of the climacteric, ripening process and senescence (Dong et al. [1994;](#page-10-0) Wani et al. [2008](#page-11-0))

Yeast and mold count Yeast and mold count of apples was markedly reduced by irradiation alone (0.4 kGy) and in combination with CaCl<sub>2</sub> dip treatment (0.5–2.0% w/v). No yeast and mold count was recorded in samples treated with irradiation alone (0.4 kGy) and combination of  $CaCl<sub>2</sub>$  dip  $(0.5-2.0\%$  w/v) and irradiation  $(0.4 \text{ kGy})$  after 30 days of storage (Table [3](#page-7-0)). Among CaCl<sub>2</sub> treated fruits, yeast and mold count was marginally ( $p \ge 0.05$ ) different in samples treated at levels above 0.5% w/v after 30 days of storage. After 60 days of storage, yeast and mold count of samples treated with  $CaCl<sub>2</sub>$  at levels  $0.5-1.5\%$  w/v differed marginally  $(p \ge 0.05)$  with respect to each other, but differed significantly ( $p \le 0.05$ ) when compared with samples treated with  $2.0\%$  w/v CaCl<sub>2</sub>. After 90 days of storage yeast and mold count of 4.1 log cfu/g sample was recorded in control and  $0.5\%$  w/v CaCl<sub>2</sub> treated samples as against the 3.3 log cfu/g sample in 0.4 kGy irradiated samples. In fruits treated with CaCl<sub>2</sub> alone at levels above  $0.5\%$  w/v or combination

<span id="page-7-0"></span>

\*=Percentage decrease in ascorbic acid after 90 days of storage \*=Percentage decrease in ascorbic acid after 90 days of storage

Values with different superscript uppercase letters (A-C) within a column and superscript lowercase letters (a-g) within a row differ significantly ( $p \le 0.05$ ) Values with different superscript uppercase letters (A–C) within a column and superscript lowercase letters (a–g) within a row differ significantly (p≤0.05)

T<sub>1</sub>=Control; T2=0.4 kGy; T3=0.5% CaCl<sub>2</sub>; T<sub>4</sub>=1.0% CaCl<sub>2</sub>; T<sub>5</sub>=1.5% CaCl<sub>2</sub>; T<sub>6</sub>=2.0% CaCl<sub>2</sub>;  $T_1$ =Control; T2=0.4 kGy; T3=0.5% CaCl<sub>2</sub>; T<sub>4</sub>=1.0% CaCl2; T<sub>5</sub>=1.5% CaCl2; T<sub>6</sub>=2.0% CaCl<sub>2</sub>;

 $T_7=0.5\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_8=1.0\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_9=1.5\%$  CaCl<sub>2</sub>, 0.4 kGy;  $T_{10}=2.0\%$  CaCl<sub>2</sub>, 0.4 kGy  $T_7=0.5\%$  CaCl2, 0.4 kGy;  $T_8=1.0\%$  CaCl2, 0.4 kGy;  $T_9=1.5\%$  CaCl2, 0.4 kGy;  $T_{10}=2.0\%$  CaCl2, 0.4 kGy

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 $T_7=0.5\%$  CaCl2, 0.4 kGy;  $T_8=1.0\%$  CaCl2, 0.4 kGy;  $T_9=1.5\%$  CaCl2, 0.4 kGy;  $T_{10}=2.0\%$  CaCl2, 0.4 kGy

<span id="page-9-0"></span>of CaCl<sub>2</sub>  $(0.5-1.5\% \text{ w/v})$  and irradiation  $(0.4 \text{ kGv})$ , yeast and mold count was in the range of 3.1–3.9 log cfu/ g sample after 90 days of storage. No yeast and mold count was observed in samples treated with combination of 2.0%  $w/v$  CaCl<sub>2</sub> and 0.4 kGy gamma irradiation even after 90 days of storage. The combination treatment of 2.0% w/v  $CaCl<sub>2</sub>$  and 0.4 kGy irradiation gave about 4.3 log reduction in yeast and mold count of apple samples. The yeast and molds identified were Candida Sp. and Penicillium Sp.

# Post refrigeration studies under ambient conditions (temp.  $17\pm2$ °C, RH 75%)

Firmness and weight loss Effect of calcium chloride and gamma irradiation treatments on post refrigeration firmness and weight loss at 17±2°C, RH 75% is depicted in Table [4.](#page-8-0) Statistical analysis of data revealed that firmness of control, 0.5 and 1.0% w/v CaCl<sub>2</sub> treated apples differed marginally  $(p\geq 0.05)$  with respect to each other over the entire storage period and was below detection level after 20 days of storage. Firmness of 0.4 kGy irradiated and those of 1.5%  $w/v$  CaCl<sub>2</sub> treated samples was below detection level after 25 days of storage. Firmness of samples treated with 2.0% w/v CaCl<sub>2</sub> and combination of CaCl<sub>2</sub> (0.5–1.0% w/v) and irradiation (0.4 kGy) was below detection level after 30 days of storage. Among the treatments, firmness was detectable up to 30 days only in samples subjected to combination of CaCl<sub>2</sub> dip at levels  $1.5-2.0\%$  w/v and irradiation  $(0.4 \text{ kGy})$ , however the marketable and acceptable firmness was observed between 20–25 days of storage. Inverse correlations  $(r=0.76)$  were obtained between firmness and weight loss, indicating that firmness decreased with increase in weight loss. Weight loss through transpiration is a major cause of quality deterioration in fresh horticultural crops after harvest. Weight loss not only results in direct quantitative losses, but also causes losses in appearance, textural quality (softening, loss of crispness and juiciness) and nutritional quality. If weight loss is more than 10%, surface of the fruit becomes prone to quality defects like wilting and shriveling and the commodity becomes unmarketable. Data of weight loss revealed that maximum shelflife of control, 0.5 and 1.0% w/v CaCl<sub>2</sub> treated apples is about 10 days at  $17\pm2^{\circ}$ C, RH 75% following 90 days of refrigeration. Data further indicated that shelf-life of fruits irradiated at  $0.4$  kGy and those treated with CaCl<sub>2</sub> alone  $(2.0\% \text{ w/v})$  or combination of CaCl<sub>2</sub>  $(0.5 \text{ and } 1.0\% \text{ w/v})$ and irradiation (0.4 kGy) is 15 days under similar storage conditions. On the other hand, shelf-life of fruits subjected to combination treatments of 2.0% w/v CaCl<sub>2</sub> and 0.4 kGy irradiation is about 20-25 days at  $17\pm2\degree$ C, RH 75% following 90 days of refrigeration.

Overall acceptability Overall acceptability based on texture, taste and visual appearance was significantly ( $p \le 0.05$ ) lower in control, 0.5 and 1.0% w/v CaCl<sub>2</sub> treated samples after first 5 days of ambient storage following refrigeration (Table [4\)](#page-8-0). The overall acceptability of all other treatments was marginally ( $p \ge 0.05$ ) different with respect to each other over the same storage period. After 15 days of ambient storage, overall acceptability was significantly ( $p \leq$ 0.05) higher in samples treated with combination of  $CaCl<sub>2</sub>$ at levels 1.5 and 2.0% w/v and 0.4 kGy irradiation. Beyond 20 days of storage, overall acceptability of all the samples was rated poor except for those subjected to combination treatment of CaCl<sub>2</sub> at levels 1.5 and 2.0% w/v and 0.4 kGy gamma irradiation. Overall acceptability of samples treated with combination of  $2.0\%$  w/v CaCl<sub>2</sub> and 0.4 kGy irradiation was rated almost good (2.9) on a 4-point scale even after 25 days of ambient storage following 90 days of refrigeration. The higher overall acceptability of samples subjected to the above mentioned combinatory treatment is due to the synergistic effect of the treatment on delaying the processes responsible for decaying, solubilization of pectic substance and loss of volatiles. Hence the higher retention of quality attributes like firmness and appearance in sample treated with combination of CaCl<sub>2</sub> (2.0% w/v) dip and irradiation (0.4 kGy) was responsible for their higher overall acceptability values.

# Conclusion

The study reveals that combination treatment of  $CaCl<sub>2</sub>$  dip  $(2.0\%$  w/v) and gamma irradiation  $(0.4 \text{ kGy})$  proved significantly ( $p \le 0.05$ ) effective in maintaining the quality of Red Delicious apples during storage. Results of the post refrigeration weight loss and other quality parameters like firmness and overall acceptability revealed that combination treatment was helpful in extending the shelf-life of Red Delicious apples by around 20–25 days at  $17\pm2\degree$ C, RH 75% following 90 days of refrigeration.

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