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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Effect of chemical and thermal treatments on quality parameters and antioxidant activity of apple (pulp) grown in high Himalayan regions

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Abstract: Pulp from locally grown Apple variety was given different treatments (chemical and thermal) and its various quality parameters were studied during a storage period of 90 days. All the quality parameters viz. pH, titrable acidity, total soluble solids, reducing sugars and non-reducing sugars, color and antioxidants activity were significantly affected by treatments employed as well as storage period. As a result of chemical and thermal treatments, antioxidant activity and total phenolic content of samples decreased, while color and consumer acceptability significantly improved. Color and antioxidant activity were highly correlated and the type of preservation method used affected the correlation coefficient between color and antioxidant activity. Among different treatments, a combination of pasteurization and chemical preservation was found to be most effective in increasing the shelf life of apple pulp.

Subjects: Agriculture; Food Chemistry; Food Science & Technology

Keywords: apple; preservation; physicochemical properties; antioxidants; color

1. Introduction

Apple (*Malus domestica*) is one of the most widely cultivated tree fruit (Harris, Yada, & Mitcham, 2007) and is generally believed that to have originated somewhere in Central Asia. It is the fourth most widely produced fruit in the world after banana, orange, and grapes (Ali, Raza, Khan, & Hussain, 2004). Apple is consumed not only because of their flavor, but also because of their nutritional importance due to vitamins, dietary fibers, and rich phenolic content in comparison to other fruits (Sun, Chu, Wu, & Liu, 2002). Apple contains carbohydrates (13.9 g/100 g), fiber

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PUBLIC INTEREST STATEMENT

Browning of apple pulp is one of the major obstacles during processing and storage of apple pulp. Pasteurization and chemical treatments are preferred methods in fruit preservation and processing in Asian countries that strongly affect its consumer acceptability. The results of this work reveal the individual and combined effects of various preservation techniques (thermal and chemical treatment) on color, antioxidant properties, shelf life, and consumer acceptability of apple pulp, hence establishing the comparative effectiveness of each preservation technique.

(0.8 g/100 g), proteins (0.4 g/100 g), lipid (0.3 g/100 g), ash (0.3 g/100 g), vitamin C (8 mg/100 g), sodium (0.3 mg/100 g), potassium (145 mg/100 g), calcium (7 mg/100 g), magnesium (6 mg/100 g), iron (480 µg/100 g), phosphorus (12 mg), and iodine (2 µg/100 m) (Hussain, 2001). Apples have very strong anti-oxidant activity mainly due to the polyphenols, such as quercetin, catechin, phloridzin, and chlorogenic acid present in them (Boyer & Liu, 2004; Kähkönen et al., 1999; Lu & Yeap Foo, 2000). Due to high levels of phytochemicals present, consumption of apple is associated with reduction in cardiovascular diseases (Knekt, Jarvinen, Hakkinen, Reunanen, & Maatela, 1996; Sesso, Gaziano, Liu, & Buring, 2003), anti-asthmatic effects (Knekt et al., 2002), anti-cholesterolemic effects (Leontowicz et al., 2002), and anti-diabetic effects (Knekt et al., 2002; Mueller et al., 2009). Apple consumption is also effective in treating infant intestinal disorders, such as diarrhea and dysentery (Considine, 1982) and helps in weight reduction in adults (Conceição de Oliveira, Sichier, & Sanchez Moura, 2003).

Apple is mostly consumed as fresh fruit, but due to its perishable nature, its quality deteriorates and cannot be stored for a long time. Quality of apple changes rapidly during storage that substantially affects the consumer acceptability (Vieira et al., 2009). In order to preserve the fruit for longer periods, it is processed to different products, such as juices, jams, and jellies and even stored as pulp. The preservation techniques are aimed to slow down the changes that caused by foods deterioration, due to large number of physical, chemical, enzymatic, or biological reactions (Gould, 2000). Chemical preservation is the most commonly and widely used method among several methods of preservation because it is simple and economical. The chemical preservatives are used to prevent microbial contamination and thus are effectively used in combinations with other preservative techniques for increasing the shelf life. No single chemical preservative is completely effective against all micro-organisms (Chiply, 1983). Sodium benzoate (SB) and potassium metabisulphite (KMS) are commonly used as preservatives for long-term storage of fruit pulp because of their better antimicrobial activity and prevention of browning (Lueck, 1990; Manganelli & Casolari, 1983; Sofos & Busta, 1981).

Although effect of storage on nutritional parameters has already been studied, combined effect of storage and different pretreatments on nutritional and antioxidant activity of apple pulp have not been reported so far. Hence, the present study was carried out to study the effect of different preservation techniques on quality and nutraceutical (antioxidant) parameters of apple pulp during storage.

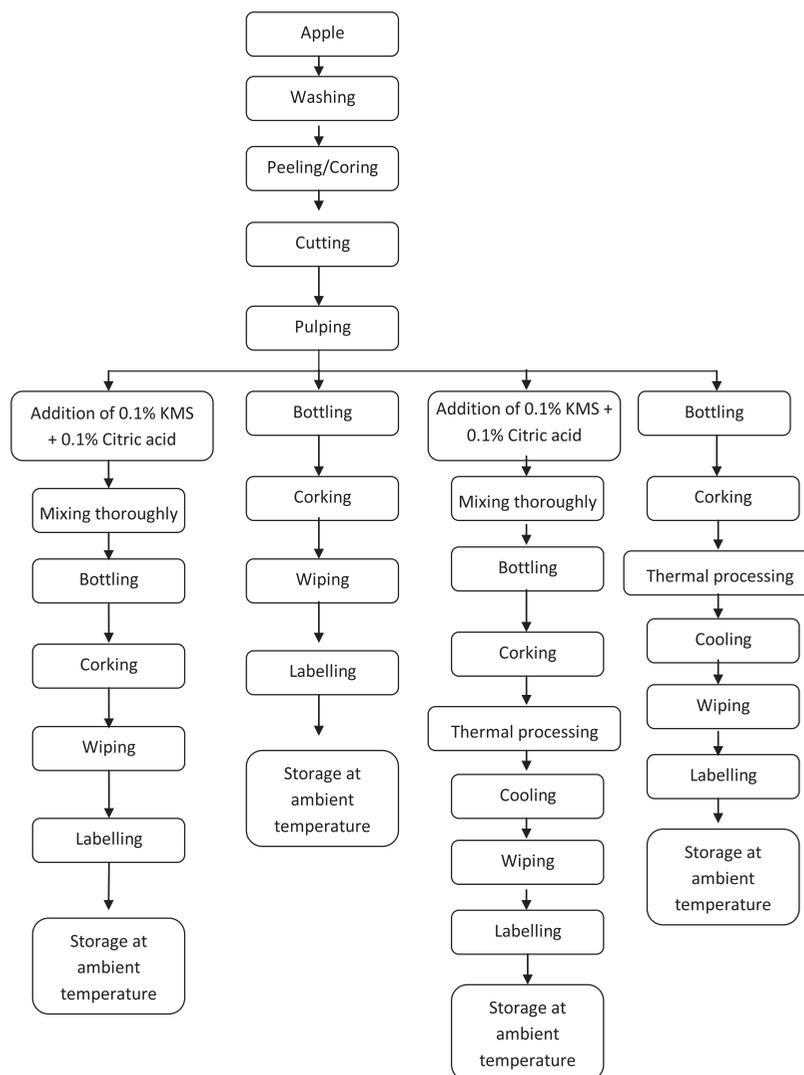
2. Materials and methods

Chemicals used were of analytical grade and were provided by HiMedia. Red Delicious variety of apples was used for production of pulp. Apple was thoroughly washed to remove dirt, dust, pesticide residues, and microflora on the surface of the fruit. It was washed, peeled, cored, and sliced. Apple was crushed with the help of home-scale mixer-cum-juicer (Sujata Powermatic +) for obtaining homogenized pulp and the pulp was given different pretreatments that include:

- (1) Treatment 1 (T_1) = storage under ambient conditions (25°C) (no preservative, no pasteurization).
- (2) Treatment 2 (T_2) = 0.1% KMS + 0.1% citric acid.
- (3) Treatment 3 (T_3) = Pasteurization at 65°C for 30 min.
- (4) Treatment 4 (T_4) = Pasteurization at 65°C for 30 min + preservative (0.1% KMS + 0.1% citric acid).

The treated pulp was transferred to sterilized glass bottles that were stored under ambient conditions (25°C) for a period of 90 days and analysis were carried out after every 15 days (Figure 1).

Figure 1. Flow chart for preparation of apple pulp.



2.1. Physicochemical analysis of the pulp

2.1.1. Determination of pH

The pH of the apple pulp was determined using digital pH meter (Inolab WTW Series 720). The pH meter was first calibrated using buffer of pH 4.0 and 7.0 at room temperature. The sample was then taken in a 100 mL beaker, stirred, and electrode of pH meter put in it and direct reading from pH meter was taken when the reading stabilized.

2.1.2. Determination of titrable acidity

Titrable acidity was done using the method of (Association of Official Analytical Chemists, 2000). Ten mL of sample was taken and homogenized with distilled water in a home-scale blender. The whole mass was then transferred to a 100 mL volumetric flask and the volume was made up to the mark with distilled water. Ten mL of this sample solution was taken in a conical flask. Two to three drops of phenolphthalein indicator were added and then the flask was shaken vigorously. It was then titrated immediately with 0.1 N NaOH solutions from a burette till pink color appeared. The volume of NaOH solution required for titration was noted and percent of titrable acidity was calculated as below:

$$\text{Acidity\%} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Volume made} \times \text{equivalent wt. of acid} \times 100}{\text{Volume of sample (taken for estimation)} \times \text{Wt. or Volume of sample} \times 1000} \times 100$$

2.1.3. Determination of total solid soluble

Total soluble solids (TSS) was done as per the method of Ahmad, Thompson, Hafiz, and Asi (2001). A hand-held refractometer (N-1E, °Brix 0–32%, ATAGO Co., Ltd, Tokyo, Japan) was used to determine the total soluble solid by calibrating with distilled water followed by dropping 1–2 drops of sample solution onto the clean surface of the refractometer.

2.1.4. Determination of sugars

Reducing and non-reducing sugar content of apple pulp was determined using the method of Lane and Eynon (1923) as described in Ranganna (1991).

2.1.5. Determination of color

The Color of the apple pulp was measured using a Hunter's Lab color analyzer (Hunter lab scan XE, Reston VA, USA). The equipment was calibrated using white and black standard ceramic tiles. In the Hunter's lab colorimeter, the color of a sample is denoted by the three dimensions, L*, a* and b*. L*, refers to lightness of the color of the diets and ranges from black = 0 to white = 100. A negative value of a* indicates a green color, whereas the positive value indicates red-purple color. A positive value of b* indicates a yellow color and the negative value a blue color. The ΔE^* is a single value which takes into account the differences between L*, a*, and b* of the apple pulp. It was calculated using the equation (Caivano, 2012):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + \Delta a^{*2} + \Delta b^{*2}}$$

where $\Delta L^* = (L_1^* - L_0^*)$; $\Delta a^* = (a_1^* - a_0^*)$; and $\Delta b^* = (b_1^* - b_0^*)$.

2.2 Antioxidant analysis

The pulp was stored in the laboratory for 90 days in glass bottles at ambient temperature (28–32°C). The antioxidant analysis of pulp was conducted on 1, 15, 30, 45, 60, 75, 90 days of storage intervals. Five gm of each sample was dissolved in 100 mL of 80:20 methanol/water solution followed by stirring for one hour. The solution was centrifuged at 800 g for 15 min. Supernatant was filtered through Whatman filter paper No. 4 and stored at –18°C for further analysis.

2.2.1. DPPH

DPPH was done as per the method of Giomaro et al. (2014) with slight modifications. The DPPH assay was conducted as follows: a 100 μ M methanolic solution of DPPH was prepared. Sample solutions of varying volumes were added to DPPH solution and the final volume was made to 3.5 mL using 80% methanol. Each solution was vortexed for 30 s and incubated for 30 min at room temperature (22°C). The decreasing absorbance at 517 nm was recorded and the percent decrease (corrected for the control, without addition of antioxidant agents) was taken as an index of the antioxidant capacity. Tests were carried out in triplicate. The IC_{50} values, defined as the amount of antioxidant necessary to decrease the initial DPPH (radical form) concentration by 50%, were calculated from the results.

2.2.2. TPC

Total phenolic content (TPC) of the samples was determined using the Folin–Ciocalteu (F–C) method as described by Carbone, Giannini, Picchi, Scalzo, and Cecchini (2011) with slight modification. Deionized water (2.5 mL) and 500 μ L of a known dilution of the extract were added to a test tube. F–C reagent (500 μ L) was added to the solution and allowed to react for 3–5 min. Then, 2 mL of 10% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted up to 20 mL with deionized water. The color developed for 90 min, and the absorbance was read at 760 nm against a blank (substituting the phenol solution in the reaction mix with water), using a UV–visible

Spectrophotometer (Model U-2900 2JI-0003, Hitachi, Japan). Quercetin was used as standard to construct a calibration curve.

2.3. Sensory analysis

Various apple pulp samples were subjected to sensory analysis, i.e. color, odor, texture, and taste by using a 9-point hedonic scale. Before the sensory evaluation was conducted, the panelists were trained by using commercial apple pulp to get familiar with the use of rating method, terminology for each attribute, and sensory characteristics. The judges rated the quality characteristics of each sample on a nine-point hedonic rating scale, where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely. The judges evaluated randomly coded apple pulp samples in terms of color, odour, texture, and taste. Assessors were instructed to cleanse their palate with cold, filtered tap water before tasting each sample. Product characterization was carried out under daylight illumination and in isolated booths within a sensory laboratory.

2.4. Statistical design

Results were expressed as mean of triplicate analyses. A two-way analysis of variance and Duncan's test were used to establish the significance of differences among the mean values at the 0.05 significance level. The statistical analyses were performed using SPSS software (Systat statistical program version 21 (SPSS Inc., USA).

3. Results and discussion

The samples were studied for Titrable acidity, TSS, pH, reducing sugars, non-reducing sugars, sensory analysis, color analysis, and anti-oxidant analysis.

3.1. Physicochemical properties

3.1.1. Titrable acidity/pH

Acidity and pH are interdependent, and lower the pH, higher is the acidity during the storage period at room temperature. Flavor promotion and preservation are mainly affected by pH and acidity of fruits, and every fruit has a specific range of pH and acidity over which consumers prefer it. A significant ($p \leq 0.05$) increase in acidity and simultaneous decrease in pH were seen in untreated as well as treated samples over 90 days of storage (Table 1). A similar trend of slight increase in titrable acidity with storage has been reported by Durrani, Ayub, Muhammad, and Ali (2010) in apple pulp during storage up to 90 days at ambient temperature, Akhtar, Riaz, Ahmad, and Nisar (2010) in mango pulp, Zewter, Woldetsadik, and Workneh (2012) in banana fruit, and Sabina, Miyan, and Hoque (2011) in strawberry juice. Acidity of fruits is attributed to the production of acids by degradation of polysaccharides, pectic substances, and uronic acid (Durrani et al., 2010; Yadav, Tripathi, & Jha, 2013). Oxidation of reducing sugars can also contribute to increase in the acidity of fruits (Hussain, Zeb, Shakir, & Sattar Shah, 2008). Among different treatments, highest value of titrable acidity was seen in sample T₂ (0.26–0.41%), while lowest value of titrable acidity was seen in sample T₃ (0.20–0.25%). A decrease in titrable acidity as a result of heat treatment was also seen by Li, Li, Wang, Jiang, and Ban (2012) in two varieties of apple fruit. Highest value of titrable acidity in sample T₂ (0.26–0.41%) may be due to the addition of citric acid. Although titrable acidity increased with storage, lesser increase was seen in heat-treated samples T₃ (0.20–0.25%). This might be due to deactivation of metabolic enzymes involved in the degradation process of polysaccharides for production of acids (Kumhar, Pareek, & Ameta, 2014). The combined effect of treatment and storage significantly ($p \leq 0.05$) affected pH of apple pulp (Table 1). From Table 1, it is observed that initial pH of apple pulp with different treatments was in range of 3.79–4.41 that varied significantly with the treatments used and after 90 days of storage, the pH decreased significantly. Highest value of pH was seen in sample T₃ (4.41), while lowest was seen in sample T₂ (3.79) in an antagonistic manner to acidity. Similar decrease in pH with storage period were reported by Muhammad et al. (2011) in apple pulp, Wisal, Ullah, Zeb, and Khan (2013) in strawberry juice, and Durrani et al. (2010) in apple pulp. Decrease in pH value and increase in total titrable acidity during the storage period of 90 days

Table 1. Effect of storage period and treatments on titrable acidity (%) and pH of apple pulp

| Treatments | | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
|------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| TA | T ₁ | 0.22 ^{oa} ± 0.02 | 0.25 ^{ob} ± 0.02 | NA | NA | NA | NA | NA |
| | T ₂ | 0.26 ^{ba} ± 0.02 | 0.29 ^{bb} ± 0.02 | 0.34 ^{oc} ± 0.02 | 0.37 ^{od} ± 0.02 | 0.41 ^{oe} ± 0.01 | NA | NA |
| | T ₃ | 0.20 ^{oa} ± 0.02 | 0.22 ^{ob} ± 0.02 | 0.25 ^{bc} ± 0.02 | NA | NA | NA | NA |
| | T ₄ | 0.24 ^{ba} ± 0.02 | 0.27 ^{db} ± 0.02 | 0.30 ^{oc} ± 0.02 | 0.35 ^{db} ± 0.02 | 0.38 ^{be} ± 0.01 | 0.42 ^{of} ± 0.01 | 0.46 ^{og} ± 0.01 |
| pH | T ₁ | 4.33 ^{oa} ± 0.01 | 4.24 ^{ob} ± 0.01 | NA | NA | NA | NA | NA |
| | T ₂ | 3.79 ^{ba} ± 0.01 | 3.62 ^{bb} ± 0.01 | 3.46 ^{oc} ± 0.01 | 3.29 ^{od} ± 0.01 | 3.18 ^{oe} ± 0.01 | NA | NA |
| | T ₃ | 4.41 ^{oa} ± 0.01 | 4.35 ^{ob} ± 0.01 | 4.21 ^{bc} ± 0.01 | NA | NA | NA | NA |
| | T ₄ | 3.83 ^{ba} ± 0.01 | 3.76 ^{db} ± 0.01 | 3.68 ^{oc} ± 0.01 | 3.21 ^{bd} ± 0.01 | 3.14 ^{be} ± 0.01 | 3.02 ^{of} ± 0.01 | 2.95 ^{og} ± 0.01 |

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column and a row vary significantly (p ≤ 0.05).

NA: Not applicable as the sample got spoiled to the extent that it could not be stored.

may also be due to activity of some acid-producing bacteria such as *Alicyclobacillus acidoterrestris* as suggested by Hussain, Zeb, & Ayub, 2011 in apple apricot juice blends.

3.1.2. Total soluble solids

Storage period and treatment had significant (p ≤ 0.05) effect on TSS of apple pulp (Table 2). TSS represent the content of soluble sugars, organic acid, and other minor constituents. TSS increase with increase in storage in both treated as well as untreated sample. This increase in TSS with storage can be due to solubilization of fruit constituents during storage (Shah, Sufi, & Zafar, 1975). Also, an increase in TSS with storage might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides (Rathod, Shakya, & Ade, 2014). Similar results were also reported by Kumhar et al. (2014) in custard apple pulp, Sharma, Vaidya, and Rana (2013) in kiwi apple juice, and Muhammad et al. (2011) in apple pulp. Treatments however affected the increase in TSS during storage period of 90 days. TSS values in T₄ increased to 14.5 (°Brix), which was highest as compared to other treatments. This might be due to the combined effect of heat treatment and addition of KMS and citric acid that increases the TSS content of apple pulp. Application of heat results in disruption of cell structure, solubilization of cell constituents, and evaporation of part of water. Kaushik, Kaur, Rao, and Mishra (2014) reported that pasteurization results in an increase in TSS content of mango pulp. Abd-Elhady (2014) reported that citric acid increases the TSS in strawberries. Increased TSS concentration with storage period (of 60 days) is in consent with the previous findings of Sirohi, Patel, Choudhary, and Sahu (2005) who observed increased TSS in fruit during storage, and attributed it to the solubilization of the insoluble portion of product.

3.1.3. Sugars

Reducing sugar is very important component for a processed product with respect to quality, shelf life, taste, and discoloration during storage. The storage period and treatments had significant (p ≤ 0.05) effect on the reducing sugar of the apple pulp (Table 3). During the storage period, reducing sugar content gradually increased. These results were in agreement with the earlier reports for apple fruit (Ali et al., 2004), mango juice (Khan et al., 2012), and cherry tomatoes (Gharezi, Joshi, & Sadeghian, 2012). According to Hakim et al. (2013) increase in reducing sugar with the progress of ripening as well as storage time was due to the degradation of starches to glucose and fructose by the activities of amylase and maltase. Ewaidah (1992) also reported that reducing sugars increased due to the hydrolysis of sucrose present in fruit pulp. The minimum value for reducing sugar was observed in untreated sample. All the treatments showed an increase in reducing sugar content during storage. The treatments T₃ (6.91–7.14%) and T₄ (6.93–7.62%) resulted in an increase in reducing sugar values than T₁ (6.83–6.94%) during storage (Table 3). This might be due to the heat treatment given to these samples that lead to an increase in starch hydrolysis (Barwal & Shrer, 2009). However, T₄ showed even higher values of reducing sugar than T₃, which can be due to increased

Table 2. Effect of storage period and treatments on TSS (°Brix) of apple pulp

| Treatments | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
|----------------|----------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| T ₁ | 10.36 ^{aA} ± 1.01 | 11.12 ^{ab} ± 1.01 | NA | NA | NA | NA | NA |
| T ₂ | 10.47 ^{aA} ± 0.50 | 11.23 ^{ab} ± 0.50 | 11.8 ^{ac} ± 0.50 | 12.5 ^{ad} ± 0.50 | 13.0 ^{ae} ± 0.50 | NA | NA |
| T ₃ | 10.77 ^{ba} ± 1.01 | 11.64 ^{cb} ± 0.50 | 12.0 ^{bc} ± 1.01 | NA | NA | NA | NA |
| T ₄ | 11.0 ^{ba} ± 1.01 | 12.23 ^{db} ± 1.01 | 12.6 ^{cc} ± 1.01 | 13.0 ^{bd} ± 1.01 | 13.5 ^{be} ± 0.50 | 14.0 ^{ef} ± 0.50 | 14.5 ^{eg} ± 1.01 |

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column and a row vary significantly (p ≤ 0.05).
 NA: Not applicable as the sample got spoiled to the extent that it could not be stored.

Table 3. Effect of storage period and treatments on reducing sugar (%) and non-reducing sugars (%) of apple pulp

| | Treatments | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
|--------------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Reducing sugars | T ₁ | 6.83 ^{aA} ± 0.05 | 6.94 ^{ab} ± 0.05 | NA | NA | NA | NA | NA |
| | T ₂ | 6.85 ^{aA} ± 0.05 | 6.96 ^{ab} ± 0.05 | 7.08 ^{ac} ± 0.05 | 7.16 ^{ad} ± 0.05 | 7.29 ^{ae} ± 0.05 | NA | NA |
| | T ₃ | 6.91 ^{ba} ± 0.05 | 7.02 ^{bb} ± 0.05 | 7.14 ^{bc} ± 0.05 | NA | NA | NA | NA |
| | T ₄ | 6.93 ^{ca} ± 0.05 | 7.05 ^{cb} ± 0.05 | 7.17 ^{cc} ± 0.05 | 7.29 ^{bd} ± 0.05 | 7.38 ^{be} ± 0.05 | 7.49 ^{ef} ± 0.05 | 7.62 ^{eg} ± 0.05 |
| Non-reducing sugar | T ₁ | 2.11 ^{aA} ± 0.05 | 2.05 ^{ab} ± 0.05 | NA | NA | NA | NA | NA |
| | T ₂ | 2.10 ^{aA} ± 0.05 | 2.03 ^{ab} ± 0.05 | 1.96 ^{ac} ± 0.05 | 1.86 ^{ad} ± 0.05 | 1.80 ^{ae} ± 0.05 | NA | NA |
| | T ₃ | 2.04 ^{ba} ± 0.05 | 1.98 ^{bb} ± 0.05 | 1.93 ^{bc} ± 0.05 | NA | NA | NA | NA |
| | T ₄ | 2.02 ^{ba} ± 0.05 | 1.96 ^{ab} ± 0.05 | 1.91 ^{cc} ± 0.05 | 1.82 ^{bd} ± 0.05 | 1.73 ^{be} ± 0.05 | 1.64 ^{ef} ± 0.05 | 1.53 ^{eg} ± 0.05 |

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column and a row vary significantly (p ≤ 0.05).
 NA: Not applicable as the sample got spoiled to the extent that it could not be stored.

hydrolysis of cellular polysaccharides under acidic conditions due to addition of citric acid (Bal, Ahmad, Senapati, & Pandit, 2014).

Results showed that non-reducing sugar content decreased in all samples significantly (p ≤ 0.05) during 90 days of storage (Table 3). Similar results were reported by Ali et al. (2004) in apple fruit, Khan et al. (2012) in mango-sea buckthorn blended juice, Chowdhury et al. (2013) in strawberry pulp during storage period of 120 days at ambient temperature. Hussain, Zeb, and Ayub (2010) also found a decrease in non-reducing sugar content during storage at low temperature in apple and apricot-blended juice preserved at refrigeration temperature during storage. This decrease is due to the conversion of non-reducing sugar to reducing sugar through the process of glucogenesis. Different treatments showed a significant (p < 0.05) effect on non-reducing sugar content of apple pulp during 90 days of storage. Non-reducing sugar of pulp treated with T₁ (2.11) varied non-significantly with T₂ treated samples (2.10), while values of T₃ and T₄ decreased significantly with respect to T₁ (Table 3). Hameed (1996) also reported that the levels of non-reducing sugar decreased and reducing sugar increased in all the preservation treatments.

3.1.4. Color

Change in color of apple pulp during storage is primarily attributed to enzymatic browning, in which polyphenols are converted into brown compounds by the action of polyphenol oxidase (Wickramarachchi & Ranamukhaarachchi, 2005). Storage period and treatment had a significant effect on total color change (ΔE), which indicates the magnitude of the color difference (Table 4). During storage, TCD increased significantly in all treatments. The increase in ΔE values can be due to Maillard condensation, and destruction of pigments. Eissa, Hassanane, and Sharaf (2014) have also reported a similar trend in commercial fruit drinks during storage up to 6 months at refrigeration temperature (Ibarz, Pagan, & Garza, 2000). Similar results were reported by Landl, Abadias, Sárraga, Viñas, and Picouet (2010) in apple puree product and Kunitake, Ditchfield, Silva, and Petrus (2014) in sugarcane beverage. Treatments also significantly affected the TCD of apple pulp. TCD decreased

Table 4. Effect of storage and pretreatment on ΔE of apple pulp

| Treatments | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
|----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------|
| T ₁ | 21.02 ^{ca} ± 0.5 | 58.80 ^{cc} ± 0.5 | NA | NA | NA | NA | NA |
| T ₂ | 19.09 ^{ba} ± 0.5 | 21.73 ^{bb} ± 0.5 | 23.37 ^{ac} ± 0.5 | 35.95 ^{ad} ± 0.5 | 58.49 ^{ae} ± 0.5 | NA | NA |
| T ₃ | 21.41 ^{aa} ± 0.5 | 27.43 ^{ab} ± 0.5 | 44.81 ^{dc} ± 0.5 | NA | NA | NA | NA |
| T ₄ | 20.23 ^{da} ± 0.5 | 25.98 ^{eb} ± 0.5 | 35.89 ^{bc} ± 0.5 | 40.75 ^{bd} ± 0.5 | 61.39 ^{be} ± 0.5 | 71.30 ^{ef} ± 0.5 | NA |

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column and a row vary significantly (p ≤ 0.05).

NA: Not applicable as the sample got spoiled to the extent that it could not be stored.

with treatment and followed the decreasing order T₁ > T₃ > T₂ > T₄. The ΔE values of sample treated with T₃ and T₄ were significantly lower than those of T₁ during the entire period of storage. This might be due to inactivation of polyphenol oxidase (Abd-Elhady, 2014). Since both heat and chemical preservatives lead to inactivation of polyphenol oxidase (Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007), hence maximum decrease in ΔE with treatments was seen in T₄ where both heat as well as chemical preservatives was added.

3.2. Antioxidant activity

3.2.1. TPC

TPC of pulp was significantly affected by storage as well as pretreatments (Table 5). TPC of treated samples was higher in comparison to TPC of untreated samples on day 1 in an increasing order of (T₂ > T₄ ≈ T₃ > T₁). KMS and citric acid prevent polyphenol degradation by inactivation of enzyme polyphenol oxidase (Saengnil, Lueangprasert, & Uthaibutra, 2006). On day 1, pretreatment with 0.1% KMS and 0.1% citric acid showed an increase in TPC of treated sample (T₂) than untreated samples. Citric acid lowers the pH below 3 that renders polyphenol oxidase inactive (Altunkaya & Gokmen, 2011; He & Luo, 2007). It also acts as a chelating agent for the copper, which is a cofactor for browning reaction. Hence, binding of citric acid to copper prevents degradation of polyphenols in comparison to untreated samples. Pasteurization leads to an increase in TPC of treated sample (T₃) than untreated samples on day 1. A possible explanation for this can be that heating leads to deactivation of polyphenol oxidase (Mizobutsi et al., 2010). However, increase in TPC due to treatment T₃ (≈11%) is lesser as compared to T₂ (34.01%) that can be because of degradation of polyphenols due to pasteurization. It has already been reported that polyphenols are stable only over a limited range of temperature. Similar results reported heat treatments decreases the concentration of polyphenols in apple juice (Aguilar-Rosas et al., 2007). There was a non-significant difference in the values of TPC between T₃ and T₄ treated samples, but was higher (≈11%) than untreated sample. Storage of pulp at ambient temperature resulted in decrease in value of total phenols in all the samples. Antioxidant activity of orange juices decreased by 45 percent after 6 months of storage at 28°C and the decrease in antioxidant activity may be linked to a decrease in TPC during storage (Klimczak, Małeczka, Szlachta, & Gliszczyńska-Świgło, 2007). However, percentage decrease in TPC was more in untreated sample than rest of the samples during storage. This is because of greater activity of polyphenol oxidase in untreated samples that resulted in conversion of greater percentage of polyphenols to brown pigments, hence decreasing the content of phenols in apple pulp. Maximum antioxidant activity was retained in samples that were treated with KMS and citric acid only.

3.2.2. DPPH

DPPH is a free radical generator to which sample is added. Antioxidants present in the sample scavenge the free radicals generated by DPPH. Scavenging of free radicals is accompanied by change in color from blue to golden yellow that is detected spectrophotometrically. Samples were analyzed for their antioxidant activity using DPPH antioxidant assay (Table 5) and then antioxidant capacity was compared using IC₅₀ values. IC₅₀ value refers to the quantity of the sample that is required for reducing the concentration of free radicals by 50% of initial concentration. Hence, lesser the value of IC₅₀, greater is the antioxidant activity. Untreated samples (highest IC₅₀) showed lowest antioxidant

Table 5. Effect of storage and pretreatment on antioxidant properties of apple pulp

| | | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
|--------------------------------|----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| DPPH IC ₅₀ µg/µL | T ₁ | 0.17 ^{aA} ± 0.02 | 0.21 ^{ab} ± 0.02 | NA | NA | NA | NA | NA |
| | T ₂ | 0.15 ^{bA} ± 0.02 | 0.16 ^{ab} ± 0.02 | 0.16 ^{ac} ± 0.02 | 0.18 ^{ad} ± 0.02 | 0.19 ^{ae} ± 0.02 | NA | NA |
| | T ₃ | 0.12 ^{cA} ± 0.02 | 0.12 ^{bA} ± 0.02 | 0.15 ^{bc} ± 0.02 | NA | NA | NA | NA |
| | T ₄ | 0.12 ^{dA} ± 0.02 | 0.17 ^{cb} ± 0.02 | 0.19 ^{cc} ± 0.02 | 0.20 ^{bd} ± 0.02 | 0.21 ^{be} ± 0.02 | 0.24 ^{df} ± 0.02 | 0.26 ^{df} ± 0.02 |
| TPC mg/g | T ₁ | 2.94 ^{dB} ± 0.02 | 1.86 ^{ea} ± 0.02 | NA | NA | NA | NA | NA |
| | T ₂ | 3.49 ^{eb} ± 0.02 | 3.16 ^{fa} ± 0.02 | 2.79 ^{dc} ± 0.02 | 2.41 ^{cd} ± 0.02 | 1.82 ^{ce} ± 0.02 | NA | NA |
| | T ₃ | 3.25 ^{fA} ± 0.02 | 2.91 ^{gb} ± 0.02 | 2.59 ^{ec} ± 0.02 | NA | NA | NA | NA |
| | T ₄ | 3.27 ^{fB} ± 0.02 | 2.94 ^{ha} ± 0.02 | 2.57 ^{ec} ± 0.02 | 2.02 ^{bd} ± 0.02 | 1.68 ^{de} ± 0.02 | 1.21 ^{bf} ± 0.02 | 0.88 ^{bf} ± 0.02 |

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column and a row vary significantly (p ≤ 0.05).

NA: Not applicable as the sample got spoiled to the extent that it could not be stored.

Table 6. Sensory analysis of apple pulp preserved by different techniques

| Parameters | Storage period (Days) | | | | | | |
|----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | Day 1 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
| Color | | | | | | | |
| T ₁ | 4.02 ^{aA} ± 0.7 | 2.12 ^{ab} ± 0.7 | NA | NA | NA | NA | NA |
| T ₂ | 8.33 ^{bA} ± 0.7 | 8.13 ^{bA} ± 0.7 | 7.93 ^{ab} ± 0.7 | 7.81 ^{ab} ± 0.7 | 7.54 ^{ac} ± 0.7 | NA | NA |
| T ₃ | 5.24 ^{cA} ± 0.7 | 2.53 ^{cb} ± 0.7 | 1.20 ^{bc} ± 0.7 | NA | NA | NA | NA |
| T ₄ | 7.22 ^{dA} ± 0.7 | 7.08 ^{dA} ± 0.7 | 6.86 ^{cb} ± 0.7 | 5.75 ^{bc} ± 0.7 | 5.48 ^{bc} ± 0.7 | 5.37 ^{ac} ± 0.7 | 4.29 ^{ad} ± 0.7 |
| Odor | | | | | | | |
| T ₁ | 8.78 ^{aA} ± 1.5 | 4.41 ^{ab} ± 1.5 | NA | NA | NA | NA | NA |
| T ₂ | 7.47 ^{bA} ± 1.5 | 6.83 ^{bb} ± 1.5 | 5.26 ^{ac} ± 1.5 | 5.01 ^{ad} ± 1.5 | 4.91 ^{ae} ± 1.5 | NA | NA |
| T ₃ | 7.80 ^{cA} ± 1.5 | 7.87 ^{cb} ± 1.5 | 6.42 ^{bc} ± 1.5 | NA | NA | NA | NA |
| T ₄ | 7.43 ^{bA} ± 1.5 | 7.28 ^{db} ± 1.5 | 7.10 ^{cb} ± 1.5 | 6.94 ^{bd} ± 1.5 | 6.65 ^{be} ± 1.5 | 6.42 ^{df} ± 1.5 | 6.21 ^{ag} ± 1.5 |
| Texture | | | | | | | |
| T ₁ | 7.38 ^{aA} ± 1.5 | 5.10 ^{ab} ± 1.5 | NA | NA | NA | NA | NA |
| T ₂ | 7.31 ^{aA} ± 1.5 | 6.05 ^{bb} ± 1.5 | 5.01 ^{ac} ± 1.5 | 4.87 ^{ad} ± 1.5 | 3.64 ^{ae} ± 1.5 | NA | NA |
| T ₃ | 7.17 ^{bA} ± 1.5 | 4.09 ^{cb} ± 1.5 | 2.65 ^{bc} ± 1.5 | NA | NA | NA | NA |
| T ₄ | 7.12 ^{bA} ± 1.5 | 6.03 ^{db} ± 1.5 | 5.62 ^{ac} ± 1.5 | 4.54 ^{ad} ± 1.5 | 3.31 ^{ae} ± 1.5 | 2.20 ^{af} ± 1.5 | 1.03 ^{ag} ± 1.5 |
| Taste | | | | | | | |
| T ₁ | 8.76 ^{aA} ± 0.5 | 5.58 ^{ab} ± 0.4 | NA | NA | NA | NA | NA |
| T ₂ | 7.21 ^{bA} ± 0.4 | 6.10 ^{bb} ± 0.2 | 5.94 ^{ac} ± 0.4 | 5.85 ^{ac} ± 0.5 | 4.61 ^{ad} ± 0.5 | NA | NA |
| T ₃ | 8.69 ^{aA} ± 0.5 | 7.19 ^{cb} ± 0.2 | 6.87 ^{bc} ± 0.5 | NA | NA | NA | NA |
| T ₄ | 7.25 ^{bA} ± 0.5 | 7.18 ^{db} ± 0.2 | 6.96 ^{cc} ± 0.4 | 5.69 ^{bd} ± 0.5 | 5.57 ^{bd} ± 0.5 | 5.33 ^{ae} ± 0.5 | 5.13 ^{af} ± 0.5 |

Notes: Values are mean ± standard deviations of three (n = 3) replications with different superscripts in a column and a row vary significantly (p ≤ 0.05).

NA: Not applicable as the sample got spoiled to the extent that it could not be stored.

activity, while treated samples (lower IC₅₀ values) showed higher antioxidant activity following an increasing order of T₂ < T₃ ≈ T₄. Although the TPC of chemically treated samples was higher than T₃ and T₄ treated samples, the IC50 value of T₂ samples was higher suggesting lesser antioxidant activity. Increase in antioxidant activity of T₃ and T₄ can be attributed to generation of browning compounds (melanoidins) by maillard reaction due to heat treatment that shows potent antioxidant activity (Baba et al., 2014).

A high correlation coefficient was seen between TPC and DPPH in T₂ (r² = 0.968^{**}) and T₄ (r² = 0.963^{**}) treated samples. However, a lower correlation coefficient was seen in T₁ and T₃ samples. In

untreated samples, TPC value decreased by $\approx 36\%$ from day 1 to day 15. However, antioxidant activity (DPPH) showed a lesser decrease ($\approx 23.5\%$) that is depicted by a lesser change in IC_{50} value from day 1 to day 15. This lesser change in IC_{50} value can be due to formation of brown-colored compounds that are reported to show antioxidant activity (Baba et al., 2014). A similar irregular trend can be seen in T_3 treated samples. In T_4 , T_3 , T_2 treated samples, a high correlation coefficient of 0.997^{**}, 0.999^{**}, 0.998^{**} was seen between color change and TPC that further confirms that loss in antioxidant activity of fruit pulp is primarily due conversion of polyphenols to brown pigments.

3.3. Sensory analysis

The analysis of our data showed that storage period and treatment had a significant effect on sensory attributes (Table 6). From Table 6, it is observed that the initial color score of apple pulp was in range of 4.02–8.33. The maximum color score of apple pulp was found in treatment T_2 (8.33) followed by T_4 (7.22) that were treated with chemical preservatives and pasteurization, respectively. The minimum color score was reported in T_1 (4.02), which is untreated sample. The sample T_1 showed mold growth at the end of 15 days due to the absence of chemical preservatives. The treatment T_4 was the best in terms of microbial stability followed by T_2 and is attributed to the combined effect of chemical and thermal treatments. The color score showed decreasing trend during storage that might be due to the action of acidity that enhances the hydrolytic reaction causes browning, and acid also enhances the Millard reaction which causes more browning in product. Polyphenolic compound present in fruit pulp gets oxidized under the influence of enzymes and causes discoloration. These findings were in accordance with the observation of Vidhya and Narain (2011) for apple fruit, Iman, Akter, Mazumder, and Rana (2011) for apple slice, Akhtar et al. (2010) in mango pulp and Kumhar et al. (2014) in custard apple. From Table 6, it is observed that the score for odor and taste decreased significantly during the storage period of 90 days. The initial taste and texture score of apple pulp, was found to be maximum in treatment T_1 and T_2 . The sensory score for taste also decreased significantly during the storage period of 90 days. The minimum decrease was observed in sample T_4 , which decreased from 7.25 to 5.13. Maximum decrease was observed in sample T_2 , which decreased from 7.21 to 4.61. The decrease in taste score may be attributed to the development of bitterness and increase in acidity in the samples during storage (Yadav et al., 2013).

4. Conclusion

From the present study it was concluded that different treatments had different effects on quality parameters and shelf life of apple pulp. Chemical preservatives and pasteurization method was most efficient in increasing the shelf life. Samples treated with both the treatments showed maximum antioxidant activity and could be consumed even after ninety days of storage under ambient conditions. Combination of both the treatments was also effective in preventing browning to a considerable extent, but chemical treatment was more effective in preventing browning.

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Competing interests

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