Effect of anti-browning solutions on quality of fresh-cut apple slice

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Abstract
Apple is an important fruit rich in antioxidant and nutrients. Browning is a major disorder occurring during long-term storing of apples and its adverse effect on economic loss, especially when disordered fruit affected internally. Enzymatic browning in apple fruit is related to the flesh and the presence of cavities. So, there is urgent need to inhibit the browning in an apple slices. The present study was, therefore, undertaken to study effect of various chemical, biochemical and thermal treatment for inhibit the browning in an apple slices with an utmost objective to minimize the colour and nutrient loss in present investigation different kind of chemical such as calcium lactate. KMS bio-chemical such as ascorbic acid, citric acid, cysteine, L-cystine and thermal treatment includes microwave blanching, hot water blanching, steam blanching. In addition effect of some pretreatment has also been determined on colour degradation (L, a, b) of apple slices. The study revealed that the enzymatic oxidation of phenolic and poly phenolic compounds responsible for the brown colour. The development of browning depends on many factor such as postharvest ripening duration, climate condition and etype of variety.

Keywords: anti-browning, solutions, quality, fresh-cut

Introduction
The apple is one of the most consumable, nutrient rich fruit, widely grown in the temperate regions of the world. Apples can be classified on the basis of their texture, size and colour. Some of the major problem associated with apple is its cut surface browning which adversely affect the appearance and nutritive value of the fruit. Browning is the undesirable change in flavor, colour and nutritive value of fruit and vegetable due to chemical reaction. Browning of fruits and vegetable during process and storages decreases its commercial as well as nutritive value (Billaud et al., 2004, Lu et al., 2007, López-Nicolás et al., 2007) [3, 17]. Browning is majorly classified into two categories such as enzymatic and non-enzymatic (flesh) browning (Du et al., 2012) [8]. Flesh browning is observed in whole apple at the time of harvesting and storage and sometimes it is essential in some fruit cultivars as it is related to chilling demand (Hatoum et al., 2016) [11]. Whereas the enzymatic browning is a result of disturbance of cells which occurs due to cutting, peeling and exposure of fruit with undesirable condition during postharvest operations. The enzymatic browning is caused due to chemical reaction which involved polyphenol oxidase, catechol oxidase, and other enzymes that create melanins and benzoquinone from natural phenols. Enzymatic browning is also called oxidation of foods which requires exposure to oxygen. Enzymatic browning in apple cut slices occurs due to oxidation of phenols by polyphenol oxidase (PPO) and convert into quinones (Gil et al., 1998) [10]. These quinones are further reacted and polymerized which results in the formation of brown pigments (melanosis) on the surface of the food (Tomas-Barberan and Espin, 2001 Hemachandran et al., 2017) [21, 12]. The amount of active polyphenol oxidases (PPO) affects the browning rate. Therefore extensive research is being done to investigate and study the factors affecting the browning and inhibit the browning by reducing the PPO activity (Billaud et al., 2004, Bradford, 1976, Burdurlu and Karadeniz, 2003) [3, 4, 5]. The past studies id focused on the use of anti-browning agents on apple which includes citric acid, ascorbic acid, calcium chloride and carboxymethyl cellulose (Chiabrand and Giacalone, 2012; Koushesh Saha and Sogvar, 2016) [6, 15]. Various methods are used to slow down and prevent the browning of food and inhibitors of enzymatic browning is divided into reducing agents, chelating agents, complexing agents, acidulants, enzyme inhibitors and enzyme treatment (Lu et al., 2007) [17].
The inhibition and control of enzymatic browning plays very important role in many food industry because this reaction affect the colour, taste, flavor and nutritive value. The appearance of the fruit is more important and maintaining the colour value is a big challenge during processing. Therefore considering the view the present investigation was, undertaken to study the effect of various chemical, biochemical and thermal treatment for inhibit the browning in an apple slices with an utmost objective to minimize the colour and nutrient loss.

Materials & methods
Preparation of raw material
Fresh fruits of Maharaji apple (length = 2.25 to 2.55 inch; maximum diameter = 2.24 to 2.61 inch) were procured from the local orchard in the state of Jammu and kashmir, India. Fresh apple were washed in running tap water, drained under shed to remove droplets of water present on the surface of apple and destalked manually. Washing was usually performed with 100 ppm chlorinated water (Sodium hypochlorite). The purpose of washing was not only to remove field soil and surface microorganisms but also to remove fungicides, insecticides and other pesticides from the fruit apple, and the apple slices were prepared by chopping the fruit with the help of knife or slicer in a different thickness (5-15mm). In case of Lye peeling, 2% NaOH was used as a peeling agent. These were then washed in portable water to remove adhering peel by rubbing in running tap water. These slices were then dipped in 1.0% citric acid solution for 10 minutes to remove the residual effect of alkali. Finally these fruits were rinsed with plain water for use in processing purpose. The samples thus prepared were used for preparation of apple slices by the previously described method.

Different preprocessing treatment were given to the apple slices like KMS, Ascorbic acid, Citric acid, Cysteine, L-Cystine, Calcium lactate, Orthophosphoric acid, Water blanching, Microwave blanching, Steam blanching in order to examine the their effect on browning by observing colour values, phenol and ppo activity.

Chemical preprocessing treatment
a. Ascorbic acid
In this treatment different concentration of the solution containing 0.1%, 0.5% and 1.0% of ascorbic acid were prepared. In this treatment apple slices were dipped for 5 minute and then placed outside on the paper for removing excess moisture and comparing the color, phenol and polyphenol value with control piece of fruit slice in hourly interval upto eight hours.

b. Citric acid
In this treatment different concentration of the solution containing 0.1%, 0.5% and 1.0% of citric acid were prepared. In this treatment apple slices were dipped for 5 minute and then placed outside on the paper for removing excess moisture and comparing the color, phenol and polyphenol value with control piece of fruit slice in hourly interval upto eight hours.

c. Cysteine
In this treatment different concentration of the solution containing 0.1%, 0.5% and 1.0% of cystine were prepared. In this treatment apple slices were dipped for 5 minute and then placed outside on the paper for removing excess moisture and comparing the color, phenol and polyphenol value with control piece of fruit slice in hourly interval upto eight hours.

d. Calcium lactate
In this treatment different concentration of the solution containing 0.25%, 0.5%, 1.0% of calcium lactate were prepared. In this treatment apple slices were dipped for 5 minute and then placed outside on the paper for removing excess moisture and comparing the color, phenol and polyphenol value with control piece of fruit slice in hourly interval up to eight hours.

e. L-cystine
In this treatment different concentration of the solution was made up say 5Mm, 10Mm and 25Mm of L-cystine. In this treatment apple slices were kept for 5 minute and then placed outside on the paper and comparing the colour, phenol and polyphenol value with control piece of fruit slice in hourly interval up to eight hours.

f. Orthophosphoric acid
In this treatment different concentration of the solution containing 0.5%, 1.0% and 2.0% of orthophosphoric acid were prepared. In this treatment apple slices were dipped for 5 minute and then placed outside on the paper for removing excess moisture and comparing the color, phenol and polyphenol value with control piece of fruit slice in hourly interval up to eight hours.

Lye solution
Lye solution was prepared by mixing sodium hydroxide (NaOH) and tap water. Lye solution was commonly used for peeling the fruits. e.g @ 2% Lye solution means solution containing 20g of sodium hydroxide with one liter of water.

Pre processing thermal treatment
a. Water blanching
Blanching treatment was carried out by HOT water, microwave and steaming the slice for specific period. In case of hot water blanching, processing was done at 80 to 85°C for 5 to 10 minutes. The temperatures were measured with ± 0.1°C accuracy and come-up time was less than one minutes. Water bath was used as a heating device for temperature up to 85°C. For apple slices, blanched in a blancher or water bath maintained at mentioned temperature and periodically agitated to ensure uniform temperature throughout the bulk of sample. The temperature of sample at its geometric center was monitored using a thermometer. After desired temperature was achieved, the sample was taken out at regular interval of 1 min for a period of 7 minutes for each sample. For determining the colour value, PPO and phenol values. The samples were taken out from the water bath and placed immediately in ice bath to bring down the temperature at room temperature (25-30°C). Samples were withdrawn periodically and the Hunter L, a and b values were measured using Hunter Lab DP-9000 D25A Colorimeter (Hunter Associates Laboratory, Reston, VA, USA). An overview of the thermal treatment is given.

b. Microwave Blanching treatment
Microwave Blanching was done to inactivate the enzyme by heating apple slices of thickness 10mm. Blanching is a delicate processing step. Therefore time, temperature and other conditions required for this process must be carefully monitored. The sliced material is placed carefully on a dish in
a microwave. Microwave blanching was performed at different power level say 80, 60, 40and 20. The sample was taken out at regular interval of 1 min for a period of 7 minutes for each sample. For determining the colour value, PPO and phenol values. The samples were taken out from the water bath and placed immediately in ice bath to bring down the temperature at room temperature (25-30°C). Samples were withdrawn periodically and the Hunter L, a and b values were measured using Hunter Lab DP-9000 D25A Colorimeter (Hunter Associates Laboratory, Reston, VA, USA). An overview of the thermal treatment is given
1. It should be careful about the kind of dish is used and it shouldn’t be of metal.
2. The time is a very important parameter so be careful about the time.
3. Gloves should be used to avoid burn.

c. Steaming treatment
Steaming was also carried using the microwave oven to in activate the enzymes by steaming apple slices of thickness 10 mm. in steaming process a steam pot was used. Adding water of 250ml in a pot and a cover plate is placed over the pot that has nothing. The steam pot was kept inside the microwave for 5 to 10 minutes for start steaming and then apple slices were placed on a cover plate and steam pot was kept inside in a microwave at high power. The sample was taken out at regular interval of 2 min for a period of 13 minutes for each sample. For determining the colour value, PPO and phenol values. The samples were taken out from the water bath and placed immediately in ice bath to bring down the temperature at room temperature (25-30°C). Samples were withdrawn periodically and the Hunter L, a and b values were measured using Hunter Lab DP-9000 D25A Colorimeter (Hunter Associates Laboratory, Reston, VA, USA). An overview of the thermal treatment is given

Measurement of processing parameters
a. Colour value: Colour measurement was carried out using a Hunter colorimeter D25 optical sensor (Hunter Associates Laboratory, Trestoa, VA, USA) on the basis of three variables (L, a, b value). The L value signifies the lightness (100 for white and 0 for black), the a value represents greenness and redness (-80 for green and 80 for red) while the b value signifies changes from blueness to yellowness (-80 for blue and 80 for yellow). The instrument was calibrated against a standard white reference tile. Each experiment was replicated thrice and the average values were used in the analysis. a* and b* values were used to compute values for hue angle and Chroma angle

Formula used

\[
\text{Hue Angle} = \tan^{-1}(b^*/a^*) \\
\text{Chroma} = (a^*+b^*)^{1/2}
\]

b. Physico-chemical properties:
Physical dimension of fruit in terms of length (L) and width (W) were measured by using Vernier caliper with a sensitivity of 0.01mm. The aspect ratio (Ra) of apple fruit was calculated by using the following equation (Omobuwajo et al., 1999)

\[ \text{Ra} = \frac{W}{L} \times 100 \]

Geometric mean diameter and sphericity is of apple fruit was calculated based on given equation: Geometric mean diameter is \( D_e = (LW)^{1/3} \)
And Sphericity = \( D_s / L \times 100 \)
Total surface area (S) of the apple fruit was calculated from the equation given by Baryeh (2001)

\[ \text{Surface area} (S) = \pi D_s^2 \]

c. pH of the fruit:
The most convenient and reliable method of measuring pH is by the use of pH meter which measure the e.m.f. of a concentration cell formed from a reference electrode, the test solution, and a glass electrode sensitive to hydrogen electrode.

d. Total soluble solid:
Total solid content was determined following method described by Manohar et al., (1991) for tamarind paste. The TSS was measured by using Hand Refractrometer Sugars are the major soluble solid in fruit juice and therefore, total soluble solid can be used as an estimation of sugar content, organic acid, amino acid and soluble pectin also contribute to soluble solids. Soluble solids contents can be determine in a small sample of fruit juice using hand Refractrometer. The refractometer measures the refractive index, which indicates how much a light beam will be refracted when it passes through the fruit juice, which is then correlated with TSS as degree Brix on the scale ranging from 0-50.

Results and Discussion
Data presented in table shows that physical, chemical and mechanical properties of the maharaji apple fruit. The average size of the maharaji apple fruit is comparatively larger and crispy in texture to soft apple fruit average volume and weight of the apple was 136 ml and 0.15kg respectively.

<table>
<thead>
<tr>
<th>Type of fruit</th>
<th>Storage time</th>
<th>Colour value</th>
<th>Ppo (o.d.)</th>
<th>Phenol (o.d.)</th>
<th>Fruit WT (Kg)</th>
<th>Vol. (ml)</th>
<th>TSS (B %)</th>
<th>Acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maharaji apple</td>
<td>Initial</td>
<td>71.15</td>
<td>23.75</td>
<td>18.82</td>
<td>0.073</td>
<td>0.237</td>
<td>----</td>
<td>13.33</td>
</tr>
<tr>
<td></td>
<td>After 1month</td>
<td>69.00</td>
<td>28.66</td>
<td>33.22</td>
<td>0.235</td>
<td>0.338</td>
<td>0.15</td>
<td>136.2</td>
</tr>
</tbody>
</table>

Table 2: Physico-mechanical properties of maharaji apple after 1 month storage at 10°C

<table>
<thead>
<tr>
<th>Type of fruit</th>
<th>Sphericity</th>
<th>Aspect ratio</th>
<th>Fruit density(kg/m³)</th>
<th>Bulk density(kg/m³)</th>
<th>Porosity (%)</th>
<th>Fruit dia. (cm)</th>
<th>Apex Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maharaji apple</td>
<td>1.004</td>
<td>100.67</td>
<td>1099.95</td>
<td>1034.48</td>
<td>5.90</td>
<td>6.387</td>
<td>6.375</td>
</tr>
</tbody>
</table>
This table represents the physical properties of the maharaji. The length and diameter of the maharaji was remained unaffected.

When comparing fruit properties after storing at 10°C one month

1) The colour value of fruit slice (ring form), a slight change occurred among the fresh and one month old sample. The colour value at initial was (L 71.15, a 23.75, b 18.8) and after 1 month (L 69.00, a 28.66, b 33.22) for L, a and b respectively one month of cold storage.

2) The phenol and polyphenol value shows much change in their o.d. value after keeping fruit 1 month. The PPO value decreases whereas the phenol value increases.

3) When comparing their acidity value after keeping one month, the acidity value increases.

4) When comparing their TSS value after keeping one month, the TSS value decreases.

2. Antioxidant treatment (Ascorbic acid) for enzymatic browning

Apple fruit ring slices were treated with different concentration of ascorbic acid. Table 3 presented, colour values of apple slice after treatment of different concentration of ascorbic acid. The brightness decreased slightly in case of 0.1% ascorbic acid treated fruit and some more was observed in case of 0.5% ascorbic acid treated fruit. Similar changes were also observed Chroma value of 0.5% ascorbic acid treated fruit.

Table 3: Antioxidant treatment (Ascorbic acid) for enzymatic browning

<table>
<thead>
<tr>
<th>Ascorbic acid</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>0.1%</td>
<td>76.09</td>
<td>20.64</td>
</tr>
<tr>
<td>0.5%</td>
<td>76.09</td>
<td>20.64</td>
</tr>
<tr>
<td>1.0%</td>
<td>76.09</td>
<td>20.64</td>
</tr>
</tbody>
</table>

3. Antioxidant treatment (Citric acid) for enzymatic browning

The colour values (L, a, b, chroma and hue) of the fruit ring slices in different concentration of citric acid were observed and it is presented in table 4. The brightness decreased in each case of citric acid treated fruit compared to untreated sample but in case of 1.0% citric acid it was found that the brightness was less and its L value was 64.79. There was hardly any change in Chroma and Hue angle among different treatment.

Table 4: Antioxidant treatment (Citric acid) for enzymatic browning

<table>
<thead>
<tr>
<th>Citric acid</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>0.1%</td>
<td>76.83</td>
<td>20.235</td>
</tr>
<tr>
<td>0.5%</td>
<td>76.83</td>
<td>20.235</td>
</tr>
<tr>
<td>1.0%</td>
<td>76.83</td>
<td>20.235</td>
</tr>
</tbody>
</table>

4. Antioxidant treatment (calcium lactate) for enzymatic browning

Calcium lactate were used as antioxidant treatment for observing colour value on apple fruit ring slices and it was treated with different concentration of calcium lactate.

Experimental data were presented in table 5 and was observed the brightness decreased in each case of calcium lactate treatment compared to initial sample but in case of 1.0% calcium lactate treatment, the brightness decreased to a greater extent and its L value become 70.26.

Table 5: Antioxidant treatment (calcium lactate) for enzymatic browning

<table>
<thead>
<tr>
<th>Calcium lactate</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>0.25%</td>
<td>75.18</td>
<td>23.855</td>
</tr>
<tr>
<td>0.5%</td>
<td>75.18</td>
<td>23.855</td>
</tr>
<tr>
<td>1.0%</td>
<td>75.18</td>
<td>23.855</td>
</tr>
</tbody>
</table>

5. Antioxidant treatment (L-Cysteine) for enzymatic browning

Apple slices were treated with L-Cystine solution and colour values observed that is presented in table 6. Different concentration of L-Cystine solution were used and some of the results were obtained, the brightness decreased slightly in case of L-cysteine treated sample and the colour value was nearly same with compared to fresh slice was observed in 25 mM solution of L-Cystine. There was slight variation in Chroma and hue angle was observed in each case of L-Cysteine.

Table 6: Antioxidant treatment (L-Cysteine) for enzymatic browning

<table>
<thead>
<tr>
<th>L-Cystine</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a</td>
</tr>
<tr>
<td>5mM</td>
<td>75.18</td>
<td>23.855</td>
</tr>
<tr>
<td>10mM</td>
<td>75.18</td>
<td>23.855</td>
</tr>
<tr>
<td>25mM</td>
<td>75.18</td>
<td>23.855</td>
</tr>
</tbody>
</table>

6. Antioxidant treatment (orthophosphoric acid) for enzymatic browning

The brightness was decreased slightly in case of 1.0% Orthophosphoric treated apple slice as compared to control one but in case of 0.5% and 2.0% Orthophosphoric, it was found that the brightness was much less and its L value was found to 66.86. Table 7 present data of colour values of antioxidant treated apple slices.
Table 7: Antioxidant treatment (orthophosphoric acid) for enzymatic browning

<table>
<thead>
<tr>
<th>Orthophosphoric acid</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L a b</td>
<td>Chroma</td>
</tr>
<tr>
<td>0.5%</td>
<td>75.18</td>
<td>23.855</td>
</tr>
<tr>
<td>1.0%</td>
<td>75.18</td>
<td>23.855</td>
</tr>
<tr>
<td>2.0%</td>
<td>75.18</td>
<td>23.855</td>
</tr>
</tbody>
</table>

7. Effect of pretreatment on enzymatic browning of apple
The brightness decreased slightly in case of control and KMS treated compared to initial but in case of blanched it was greatly affected with least value of 44.46. It was observed that Hue angle and Chroma values changes to greater extant in case of blanched treatment

Table 8: Effect of pretreatment on enzymatic browning of apple

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L a b</td>
<td>Chroma</td>
</tr>
<tr>
<td>Control</td>
<td>75.60</td>
<td>20.98</td>
</tr>
<tr>
<td>KMS</td>
<td>75.60</td>
<td>20.98</td>
</tr>
<tr>
<td>Blanched</td>
<td>75.60</td>
<td>20.98</td>
</tr>
</tbody>
</table>

Conclusion
Enzymatic browning of apple slice decreases during processing with antioxidant treatment. Some of the concluded points from the above study:
1. Significant difference were observed in colour value L when pretreated with blanched application on the freshly cutted slice and comparing with control.
2. A very small difference was observed in colour value L, after antioxidant (L-cystine) treatment.
3. Colour value croma was increased after antioxidant treatment in each case comparing to the initial.

References