RESULTS AND DISCUSSION
The rapid assessment survey in a few suburbs of Mumbai indicated that most people prefer whiter chips. Amongst the home-based techniques employed to eliminate this browning 30% of the women in the household surveyed said that they dipped the chips in water before frying them, 22% of the women said that they simply sun-dried their chips before frying, 15% of the women said that they blanched and sun-dried their chips, 13.5% of the women used lime juice to eliminate browning. 14% of the women said they fried the chips directly/immediately. The rest of the women said that they did not make any chips at home. The survey indicates that keeping the chips under water thereby eliminating oxygen as well as leach cut the brown pigment into water and thus preventing browning is the most common method used at homes in India. This method is practical at home where quantities are small. For industrial usage large quantities of water would be required which may be difficult to obtain. Among the small scale / cottage industry it was seen that the most commonly used method was dipping in water if immediate frying of the commodity was not possible. Most single shop manufacturers questioned from Churchgate, Dadar, Khar and Dharavi, manufacturing both potato and banana chips sliced their commodities over hot oil i.e. allowing minimum time for browning reaction to occur. In case of excess peeled material being present it was kept under water before being fried. The survey of sachets/packets of five makes of chips (Pepsi; Lays; Ruffles; Uncle Chips and Peppy Wafers) did not indicate any usage of antioxidants to prevent browning. It was, however, observed in the case of apples that Appy Juice used a mixture of acids to inhibit enzymatic browning.
Manufacture of apple juice by the State of Himachal Pradesh did not have any added antioxidant/acid in them and the apple concentrate was extremely dark in colour.

In conclusion, one can say that there is a definite need for better processing methods in the chips and juice manufacturing industries.

**POTATOES**

Kufri Chandramukhi, a recommended chip making variety, was chosen for the study.

*Mechanism of inhibition of browning reaction in presence of sodium erythorbate:*

In order to evaluate the above mechanism a standard polyphenol oxidase system of 3.0 ml in volume, has been selected as a test system. In this assay system p-cresol and pyrocatechol at concentration 0.5 mM (1.5 μ moles in 3 ml assay mixture) were used as standard monophenolic and diphenolic substrate respectively. The development of brown colour and oxygen concentration in the assay mixture was constantly monitored by spectrophotometer and oxygraph tracings respectively.

Control samples having no antioxidant showed very rapid and intense browning (Figs. 4 and 5 for catechol and p-cresol respectively). A gradual increase in concentration of erythorbate from 0.5 mM to 15 mM in case of
Fig 4: Effect of Antioxidant Concentration on Colour Inhibition Using Catechol as Substrate in PPO Assay System in Potatoes
Fig 5: Effect of Antioxidant Concentration on Colour Inhibition using p-Cresol as Substrate in PPO Assay System in Potatoes
catechol and 0.5 mM to 2.5 mM in case of p-cresol as substrates showed proportionate suppression of brown colour development. In case of catechol the colour formation was delayed by about 10 and about 30 minutes at 1 and 1.5 mM concentration of erythorbate respectively (Fig. 4).

When the concentration of the antioxidant was increased further to 2.5, 5, 10 and 15 mM where the molar ratio of substrate to antioxidant was 1:5, 1:10, 1:20 and 1:30 there was absolutely no colour formation in the assay mixture for a period of 150 minutes, the experiment time indicating total inhibition of browning for that period (Fig. 4).

A similar overall pattern was observed with p-cresol as substrate, as shown in Fig. 5. The only difference between the two was that the effectiveness of erythorbate in inhibition of browning was much less in case of p-cresol as substrate than in the case of catechol. In catechol at molar ratio of substrate: antioxidant 1:5, there was total elimination of browning. Under the same conditions with respect to substrate: antioxidant, however, in case of cresol as substrate, the inhibition of browning was partial. For achieving the total elimination of browning much higher concentration of the antioxidant was needed.

The oxygraph (Fig. 6) illustrates the variation in oxygen concentration with time in the assay mixture containing 0.5 mM catechol as substrate, in absence (curve 1) and in presence (curve 2) of erythorbate at 2.5 mM concentration. Reaction mixture lacking erythorbate showed intense browning while that containing erythorbate remained colourless. It is clear
(1) Oxygraph in the absence of the antioxidant
(2) Oxygraph in the presence of 2.5 mM the antioxidant (Substrate: Antioxidant ratio: 1:5)
(3) Oxygraph in the presence of 2.5 mM antioxidant but lacking enzyme (Enzyme blank)
(4) Oxygraph in the presence of 2.5 mM antioxidant but lacking substrate (Substrate blank)
The dotted arrow indicates the time of addition of antioxidant while the solid arrow indicates the time of addition of substrate.

Fig. 6: Variation of Oxygen Concentration with Time
from this figure that even though there is no brown colour formation in the presence of erythorbate, oxygen was getting consumed at the same rate or slightly higher as shown by (curve 2) than that in case of control sample lacking erythorbate (curve 1) which showed intense and rapid browning. This fact clearly indicates that the presence of erythorbate in the assay mixture does not inhibit in any way the oxidation of catechol catalyst by polyphenol oxidase enzyme as judged by the change taking place in oxygen concentration in the assay mixture. In spite of this oxidation there is no brown formation in the assay mixture most probably due to reduction of the oxidized product, by the added antioxidant back to catechol at the speed at least equal or more than the formation of quinones as postulated by Friedmann (1997). Elimination of either substrate (curve 4) or enzyme (curve 3) from the assay system showed very negligible or very low consumption of oxygen indicating that the major oxygen uptake was due to the enzymatic reaction catalyzed by polyphenol oxidase.

The direct effect of erythorbate on the catalytic activity of polyphenol oxidase was investigated and the data are presented in Table 3. There was no adverse effect of presence of erythorbate on the specific activity of the enzyme. On the contrary in presence of the antioxidant specific activity doubled (Table 3).

To confirm the above hypothesis effect of erythorbate on catalytic activity of PPO was investigated (Table 3). In presence of the antioxidant specific activity was more than doubled.
Table 3: Effect of Sodium Erythorbate on Potato Polyphenolase Activity

<table>
<thead>
<tr>
<th>Concentration of sodium erythorbate in the assay mixture (mM)</th>
<th>Relative proportion of substrate and antioxidant (mole substrate:mole antioxidant)</th>
<th>Polyphenol activity expressed as rate of oxygen consumption in moles/minute/mg enzyme protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>with p-cresol as a substrate</td>
</tr>
<tr>
<td>0.00</td>
<td>1:0.0</td>
<td>19.26</td>
</tr>
<tr>
<td>0.25</td>
<td>1:0.5</td>
<td>26.22</td>
</tr>
<tr>
<td>0.50</td>
<td>1:1.0</td>
<td>40.29</td>
</tr>
<tr>
<td>1.00</td>
<td>1:2.0</td>
<td>42.04</td>
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<tr>
<td>1.50</td>
<td>1:3.0</td>
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<td>1.67</td>
<td>1:3.3</td>
<td>45.54</td>
</tr>
<tr>
<td>2.00</td>
<td>1:4.0</td>
<td>40.29</td>
</tr>
<tr>
<td>2.50</td>
<td>1:5.0</td>
<td>38.53</td>
</tr>
</tbody>
</table>
This fact supports that as soon as the oxidized product of substrates are formed due to the action of PPO (polyphenol oxidase) these oxidized products react with erythorbate and get reduced back to original state as proposed by Friedmann (1997) and thus the further chemical reactions leading to brown colour formation are eliminated.

Factors influencing the antioxidant ability of erythorbate:

The effect of citric acid and sodium singly or in combination, on erythorbate or brown colour formation with time in standard PPO assay system is seen in Fig.7.

From the data presented here a concentration dependent antibrowning effect of citric acid alone is clearly evident at fifteen minutes of reaction time from the heights of columns Nos.1, 3 and 5 (column 5 > column 1 > column 3). During rest of the time upto 120 minutes the reaction mixture having only 0.02 M citric acid represented by column No. 1 showed slightly more brown colour formation as compared with that having no citric acid, no antioxidant, represented by column No. 5 (column 5 < column 1) (Fig.7).

Addition of erythorbate to the reaction mixtures containing no citric acid 0.02 M and 0.2 M citric acid respectively showed drastic reduction in brown colour development. None of them showed any visible sign of brown colour.
Fig 7: Effect of Citric Acid and Antioxidant on Colour Inhibition in PPO Assay System in Potatoes
The spectrophotometric readings showed absorbance in decreasing order as given below.

Column 4 < column 2 < column 6 at 15, 30, 60, 90 and 120 minutes after initiation of reaction.

Although the optical density shown by assay mixture containing 0.2 M citric acid was less than that shown by the assay mixture containing 0.02 M citric acid (columns 4 and 2 respectively) the margin was very small. Hence considering the economical aspects, it is advisable to use citric acid at 0.02 M level.

The effect of pH of the medium on antioxidant property of sodium erythorbate in terms of brown colour development in reaction mixtures containing 0.5 mM catechol as a substrate along with 2.5 mM antioxidant and 0.02 M citric acid at pH 4, 5, 6 and 7 respectively has been presented in Fig. 8. A control blank at pH 7.0 without antioxidant as well as without citric acid was kept along with these samples.

Assay mixture at pH 7.0 containing no antioxidant and no citric acid showed intense brown colour. Addition of antioxidant (2.5 mM) and citric acid (0.02 M) resulted elimination of browning for 60 min and then development of mild brown tinge during next 60 min. Lowering of the pH of this mixture to 6.0 suppressed the brown colour formation in the assay mixture. Further lowering of the pH to 5 and 4 showed more suppression or total inhibition of brown colour formation.
Fig 8: Effect of pH on Colour Inhibition in Presence of Antioxidant in PPO Assay System in Potatoes
When any antioxidant is used in food products taste is a relevant factor to be considered. Very low pH such as 4.0 may introduce changes in the taste. From this point of view the pH of 6.0 appears to be the best compromise which takes care of the taste as well as the browning.

Elimination of browning of mixture of phenolic and compounds and polyphenol oxidase isolated from potato tuber:

Brown colour did develop with time in 3 ml polyphenol oxidase assay mixture at pH 7.0 containing natural phenolic compounds and polyphenol oxidase enzyme isolated from 0.3 g fresh weight of tissue (Fig. 9). The brown colour developed at 30°C in the presence of 0, 500, 1000 and 2000 ppm of sodium erythorbate can be seen from the same.

The reaction mixture without erythorbate showed rapid development of colour. However, the mixture containing 500 ppm erythorbate showed no visible brown colour upto thirty minutes but thereafter showed development of a very mild brown colour. On the contrary the samples containing 1000 and 2000 ppm erythorbate showed no brown colour at all upto the experimental period of 180 minutes.

The data indicate that 1000 ppm of sodium erythorbate can prevent browning reaction caused due to oxidation of phenolic compounds isolated from potato tuber by potato polyphenol oxidase (PPO) in the natural relative proportion which really exists in potato tuber tissue.
Fig 9: Effect of Antioxidant Concentration on Colour Inhibition using Natural Substrate from Potato Source in PPO Assay System
In vivo study

Standardization of a procedure to eliminate browning in potato chips:

The *in vitro* experiments indicated that polyphenol oxidase (PPO) assay system containing 1000 ppm of sodium erythorbate and 0.02 M citric acid at pH 6 can prevent browning caused due to oxidation of phenolic compounds of potato tuber by polyphenol oxidase (PPO) obtained from the same tubers. Hence, the same solution has been attempted *in order to* eliminate browning of diced potato chips or potato wafers.

Optimum ratio of tissue : antioxidant solution:

Table 4 shows the proportion of potato chips to volume of antibrowning solution that is needed to prevent browning of the chips. The data presented in this Table shows that 50 g chips when soaked in 12.5 and 25 ml antibrowning solution remained without any visible discolouration upto 15 and 60 minutes respectively. Thereafter, however, brown colour development was observed in these samples. The 50 g chips which were kept in 50 ml antioxidant solution remained unbrowned except for the portion which remained above the liquid level exposed to air. When the volume was raised further to 100 ml anti browning solution per 50 g chips, the chips as well as the liquid medium remained free of slightest brown tinge for period of six hours.
Table 4: Determination of Minimum Amount of Dip Solution Required to Inhibit Colour Formation in Potato Slices for a Period of 6 Hours

<table>
<thead>
<tr>
<th>Time in Minutes</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>360</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of soln. (ml)</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td>50</td>
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<tr>
<td></td>
<td>25</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key:

- No Browning

+ Area Exposed To Air Shows Browning

+ Slight to excessive browning
For application of the antibrowning solution to prevent browning, potato slices or chips were dipped in the solution. The dipping time for which the chips were kept in solution was varied from just dipping and taking out i.e. not more than 10 seconds to 15, 30 and 60 minutes after which the slices are kept over filter paper in a petridish in open atmosphere. The colour formed was observed by comparing with colour formed in the three controls (1) potato chips washed with excess of distilled water, (2) potato chips treated with 1 mM sodium diethyl dithiocarbamate, and (3) potato chips treated with 10 mM β-mercaptoethanol. The combined data of this comparison are shown in Table 5.

The chips which were dipped for less than 10 seconds showed the same colour as shown by the samples washed with excess of distilled water but was more brown as compared with the remaining two controls.

None of the experimental samples which were dipped for 15, 30 and 60 minutes showed any browning as compared to all the three controls. It appears that fifteen minutes dipping time is sufficient. To be safe, however, thirty minutes dip seems to be a better choice.

The alternative method to apply this solution containing sodium erythorbate was also studied. The data clearly shows that the antioxidant solution completely eliminates browning upto a period of six hours (Fig. 10) which is more than sufficient in food processing units. On the contrary the control
Table 5: Determination Minimum Time of Treatment of Potato Slices with Antioxidant Dip Solution to Achieve Minimum Browning

<table>
<thead>
<tr>
<th>Time of Treatment (min)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
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<td>3</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

1 D.W. Treated Potato Slices
2 DIECA Treated Potato Slices
3 MET Treated Potato Slices
4 Antioxidant Dip Treated Potato Slices

Key:

<table>
<thead>
<tr>
<th>-</th>
<th>No Browning</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Browning</td>
</tr>
</tbody>
</table>
Fig 10: Observation of Colour Development in Antioxidant Dip Solution over a Period of 6 hrs Using Potato Slices

*50 g chips were kept immersed in 100ml of the three specified solutions and colour developed was measured spectrophotometrically.
lacking antioxidant i.e. just kept under distilled water showed intense browning but the chips kept under 1 mM diethyldithiocarbamate (DIECA) did not show browning. The colour exhibited by sodium erythorbate treated chips was almost the same as DIECA treated chips where browning inhibition is 100%.

Comparative study on antibrowning ability of ascorbic acid and sodium erythorbate:

Fig. 11 gives a comparative data on the ability of equimolar solutions (from 0.00025 M to 0.005 M) of ascorbic acid and sodium erythorbate to eliminate brown colour development in standard polyphenol oxidase assay system for periods of three and six hours respectively.

At each and every molarity shown in the figure the brown colour developed is more in reaction mixtures containing sodium erythorbate as an antioxidant than those mixtures containing ascorbic acid as an antioxidant. Height of six hrs. erythorbate > six hrs ascorbate at the same time height of 3 hrs. erythorbate > height of 3 hrs ascorbate at all concentrations except at 0.0005 M.
Fig 11: Comparison of Ascorbate and Erythorbate in Inhibition of Browning in PPO Assay System in Potatoes
These results suggest that as an antioxidant ascorbic acid is more competent in preventing browning than sodium erythorbate. The difference, however, is marginal and since the latter is cheaper in cost than the former sodium erythorbate is preferable.

A photograph of the effectiveness of the antioxidant dip solution as against slices dipped in D.W. or air exposed slices. Fig. 12 shows that the colour of the antioxidant dip solution is comparable to that of the control having DIECA in it.

As amongst the various processed fruits and vegetable items potato products constitute a major portion, this commodity is used for this study. The content of phenolic compounds and phenolase enzyme which are the two factors responsible for browning, vary from variety to variety and within the same variety its content depends upon time lapsed after harvest, size of tuber and other factors. Even in a single tuber the phenolase enzyme and phenolic acids are not distributed uniformly (Pendharkar and Nair 1987).

They are least concentrated in central pulp but since most of the food products are prepared from this portion of the tuber, the enzyme as well as
Figure 12

(Photograph)

P.T.O.
E₁: Potato Slices Dipped in Antioxidant Dip Solution containing 0.1 g% Sodium Erythorbate in 0.02M Citrate Buffer at pH 6
C₁: Potato Slices Exposed to Air (control)
C₂: Potato Slices Dipped in Distilled Water (control)
C₃: Potato Slices Dipped in di-Ethyl di-Thio Carbamate Solution (DIECA) (control)

Fig. 12: Effectiveness of the Antioxidant Dip Solution as against potato slices dipped in distilled water or air exposed slices
the phenolic compounds required for in vitro study were isolated from this portion. The in vivo study also has been carried out in using the central pulp tissue only.

The in vitro study clearly showed that the inhibition is due to the reversal of oxidation process. Total elimination of browning for few hours in in vitro conditions of PPO assay system was possible when mole to mole ratio of antioxidant to substrate was 5:1 or above in the case of diphenols. In case of monophenol a much higher ratio was needed. In general the majority of the phenolic compounds of potato tubers and other plant materials are diphenolic in nature. Hence the ratio 5:1 appears to be sufficient in in vitro system under the optimum conditions namely 2.5 mM erythorbate, 0.02 M citric acid with the pH of medium adjusted to 6.0.

The in vivo study indicates that diced potato can be kept free of browning for a period of eight hours or even more which is long enough for the usual operations in food processing units. The composition of this antibrowning mixture appears to be 1000 ppm antioxidant in 0.02 M citrate adjusted to pH 6.0. 2000 ml of the solution is needed per kg of the product. Under normal condition existing in food industry two hours delay in time is more than sufficient. Hence the same solution can be used again. The experimental data suggested that the same solution can be used at least three to four times rendering the process very economical.
Based on these data following procedure is recommended for a batch of 10 kg. potato chips or slices.

Take 20 litres of water add 76.8 gm of citric acid and 20 gm sodium erythorbate. Adjust the pH of the solution using sodium citrate in the range 4 to 6, preferably to 6.0. Keep the slicing device over the tank containing this solution in such a way that the slice once cut will immediately fall down in this solution. Collect 10 kg slices under this solution without getting exposed to air until they are taken out for further processing in which PPO gets inactivated by thermal energy. Use the same solution for two or three times. The final product thus obtained remains free of brown tinge on it.

BANANA

In Mumbai the well known chip-making varieties of banana are the Nendran and the Harichal variety. The Bluggoe also called the Teendhari is mostly eaten as a vegetable (i.e. it is cooked/boiled /fried).

Thus both these varieties were studied for browning activities. It was observed that the Nendran variety show much less activity than the Harichal variety. The specific activity of PPO of Nendran banana was found to be 136.9 \( \mu \) Moles of oxygen per minute per mg protein when the substrate was dopamine hydrochloride and 46.45 \( \mu \) Moles of oxygen per minute per mg protein with catechol as substrate. However in the case of the Harichal variety the specific activity was found to be 1252 \( \mu \) Moles/mg/min when no Tween 80 was used in the extraction process and the substrate was
dopamine. When Tween 80 was used in 0.1% concentration the activity further increased to 1586 µ Moles/mg protein/minute, with the same substrate. Further increasing Tween 80 concentration to 1%, however, had no effect on enzyme activity. The use of catechol as substrate indicated lower activity of the enzyme around 230 µ Moles of oxygen/mg/minute when no Tween 80 was added in enzyme extraction process and 459 µ Moles of oxygen/mg/minute when 0.1% Tween 80 was added.

Since the Harichal variety was found to be more active even on visual observation it was chosen as the subject for further studies.

Hence after having chosen Harichal as the variety for further study all spectrophotometric estimations were performed on this variety using antioxidant.

**Determination of minimum antioxidant concentration required to inhibit browning in PPO assay system for a period of three hours:**

The polarographic results seen were similar to those observed in potato i.e. in the presence of antioxidant the activity of enzyme seemed to be enhanced. The colorimetric results indicated a lag in colour development with increasing concentrations of antioxidant. The observation was that for a period of three hours a minimum concentration 6 mM where substrate : antioxidant ratio is 1:1.5 was required to inhibit colour formation (Fig.13).
Fig 13: Effect of Antioxidant Concentration on Colour Inhibition in PPO Assay System in Bananas
Determination of minimum pH needed to inhibit browning for a period of three hours:

pH studies at predetermined concentration of antioxidant showed that any pH between 7 to 3 were equally suitable. In the absence of antioxidant at pH 7 deep brown colour was observed within the first 30 minutes of reaction. It can be observed from the colorimetric graph (Fig. 14) that in the presence of antioxidant at a molarity of 6 mM the degree of colour inhibition was almost the same from pH 7 to pH 3. Hence the pH of the fruit was taken to be ideal for the dip solution i.e. a pH of 5.7 - 6 was considered as the optimum.

Determination of minimum amount of citrate required to inhibit browning for a period of three hours:

At the predetermined molarity of antioxidant and pH, it was observed that citrate a chelating agent worked well at any concentration between 0.2M to 0.005M for a time period of three hours. As in the case of pH studies it was seen that the degree of inhibition seemed to be the same as in the case of any molar concentration. Hence a low molar concentration of 0.01M was considered the optimum (Fig. 15).

Identification of dopamine as the major substrate for banana:

Several studies have corroborated that dopamine is the only substrate present in banana for the enzyme banana PPO. The natural concentrated
Fig 14: Effect of pH on Colour Inhibition in Presence of Antioxidant PPO Assay System in Bananas
Fig 15: Effect of Citric Acid and Antioxidant on Colour Inhibition in PPO Assay System in Banana
substrate extracted from acetone spotted on chromatogram developed a single large spot clearly seen under U.V. light. The spot had the same Rf as the dopamine standard indicating that dopamine was the major substrate for banana PPO. Fig. 16 shows a simulated picture of the paper chromatogram. The absorption spectra indicated that the substrate absorption was the same as dopamine.

**Determination of minimum concentration of antioxidant required for inhibition of colour formation for a period of three hours using substrate from banana source:**

It was observed that for the natural substrate extracted from banana source, sodium erythorbate the anti oxidant gave colour inhibition at all concentrations namely 2 mM, 4 mM, 6 mM, 8 mM, 16 mM and 20 mM over a period of three hours i.e. no colour was measured spectrophotometrically. (Fig.17(a)) anti-oxidant concentration was further lowered to obtain minimum concentration level. From this it was deduced that a low concentration of 1 mM/198 ppm worked well on the natural substrate. (Fig. 17(b)). This concentration of erythorbate is just 20% of that needed in case of potatoes.
Key:
N: Natural Substrate From Banana Source
D: Standard Dopamine

Fig 16: Identification of Dopamine as Substrate in Banana using Paper Chromatography Technique
Fig 17(a): Effect of Antioxidant Concentrations (higher) on Colour Inhibition Using Natural Substrate From Banana Source in PPO Assay System
Fig 17 (b): Effect of Antioxidant Concentrations (lower) on Colour Inhibition Using Natural Substrate from Banana Source in PPO Assay System
**In vivo studies:**

*Determination of minimum time required for banana chips to be dipped in the dip solution to eliminate browning for a period of eight hours:*

The banana chips which were dipped and removed at regular intervals and compared with controls negative and positive showed that the minimum time of treatment needed for elimination of browning was thirty minutes. The records were made through visual observation. It may reduce in case of more mature bananas. (Table 6) (Photograph Fig.18).

*Determination of minimum volume required of dip solution to inhibit colour formation:*

The minimum volume determination showed that the minimum volume required for 50 grams banana chips was 100 ml i.e. a ratio of 1:2 was needed to eliminate browning completely. Any other ratio showed severe to mild browning of the chips on exposure to air, consequently showing brown to blackish pigment. Again, this was based on visual observation (Table 7).
Table 6: Determination Minimum Time of Treatment of Banana Slices with Antioxidant Dip Solution to Achieve Minimum Browning

<table>
<thead>
<tr>
<th>Time of Treatment (min)</th>
<th>0</th>
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<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation After Hours</td>
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<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
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Key:

<table>
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<th>No Browning</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Browning</td>
</tr>
</tbody>
</table>

1  D.W. Treated Banana Slices
2  MET Treated Banana Slices
3  Antioxidant Dip Treated Banana Slices
Figure 18 (photograph)

P.T.O
C1: Banana Slices Dipped in Distilled Water (control)
C2: Banana Slices Dipped β-mercaptoethanol (control)

Experimental Samples:
(7) 10 seconds, slightly darker than other two samples
(8) 15 seconds, darker than 30 seconds
(9) 30 seconds, lighter than the other two samples

Fig. 18: Minimum Time of Treatment of Banana Slices with Anti oxidant to Inhibit Colour Formation over 8 hours
Table 7: Determination of Minimum Amount of Dip Solution Required to Inhibit Colour Formation in Banana Slices for a Period of 8 Hours

<table>
<thead>
<tr>
<th>Time in Minutes</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
<th>360</th>
<th>420</th>
<th>480</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of soln. (ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key:

- No Browning
+ Area Exposed To Air Shows Browning
++ Slight to excessive browning
Comparison of colour development in banana chips in the presence and absence of the anti-browning dip solution (200 ppm erythorbate in 0.01 M citrate buffer at pH 5.7

It was observed that the dip solution maintained colour over a period of eight hours as compared to the positive control which showed extensive browning. When compared with the negative control which contained mercaptoethanol colour was seen to be similar. This indicated that the dip solution was effective for a period of eight hours (Fig. 19).

Determination of how often the dip solution can be used:

It was seen that 50 grams of freshly cut banana chips when dipped in the solution for a minimum time period of thirty minutes the dip solution could be used two times. Depending on the maturity of the banana, however, the dip solution may be used more number of times. It is recommended that after two uses the antioxidant be replenished or the whole dip solution be replaced.

Comparison of sodium ascorbate and sodium erythorbate:

A comparison of equimolar concentration of sodium erythorbate and ascorbate showed that erythorbate was highly effective in the case of banana at 1 mM, 2 mM concentrations kept for three hours and six hours. At lower concentrations, however, it was seen that the readings at six hours were higher and more browning was seen in erythorbate beaker.
Fig 19: Observation of Colour Development in Antioxidant Dip Solution over a Period of 8 Hours using Banana Slices
At the prescribed concentration of 1 mM Erythorbate is as effective as ascorbate. (Fig.20). Figure 21 shows a photograph of treatment of banana slices with dip solution.

The low concentration of the antioxidant required in the case of banana of Harichal variety could be due to the non-porous, sticky nature of the fruit which disallows further oozing of cell material thereby preventing browning.

Based on the results, it can be suggested that instead of immediately frying the chips or immersing in water as has been seen in the small scale shops the bananas can be sliced and stored in the dip solution till further use.

For a 10 kg of banana slices the following is recommended.

Take 20 litres of water add 38.4 gms of citric acid and add 4 grams of sodium erythorbate. Adjust the pH of the solution using sodium citrate to 6.0. This solution can be used two to three times. The slices can be kept in the dip solution till further processing and they will remain free of any brown tinge.

**APPLES**

A single variety of apple was chosen for the study, locally called as Deluxeson variety. This variety is widely available in all markets in Mumbai. Fresh apples showing no signs of rotting or bruising were chosen for the study.
Fig 20: Comparison of Ascorbate and Erythorbate in Inhibition of Browning in PPO Assay System in Bananas
Figure 21

P.T.O
E₁: Banana Slices Dipped in Anti oxidant Dip Solution containing 0.0198 g% Sodium Erythorbate in 0.01 M Citrate Buffer at pH 6
C₁: Banana Slices Exposed to Air (control)
C₂: Banana Slices Dipped in Distilled Water (control)
C₃: Banana Slices Dipped in 0.1% β-mercapto ethanol (control)

Fig. 21: Effectiveness of Anti oxidant Dip Solution as Compared with D.W. Dipped Banana Slices Air Exposed Slices and β-mercaptoethanol Slices
500 grams of peeled apple pulp were deseeded and used for acetone powder preparation. This yielded 18 grams acetone powder. The acetone powder was used for as a source of enzyme for spectrophotometric studies.

*In vitro* experiment

**Determination of minimum amount of antioxidant required to inhibit browning in PPO assay system for a period of three hours:**

Data of spectrophotometric observations showed a similar pattern to that of potato and banana i.e. a time lag in colour development was observed with increasing concentrations of anti-oxidant and lag period increased with increase in anti-oxidant concentrations from 0.1 mM to 0.8 mM. A concentration of 0.8 mM was chosen as optimum as it did not show any colour even after 24 hours. The motor ratio ratio of substrate : anti-oxidant was 1:4 (Fig. 22).

**Determination of minimum pH needed to inhibit browning for a period of three hours:**

The pH study showed that all pH were equally effective in inhibiting browni colour formation and the pH of apple fruit was about 4.68. Though lowering pH may eliminate browning more effectively, it may affect the taste of the commodity. Therefore, pH 5 was considered the optimum (Fig. 23).
Fig 22: Effect of Antioxidant Concentration on Colour Inhibition using Catechol as Substrate in PPO Assay System in Apples.
Fig 23: Effect of pH on Colour Inhibition in presence of Antioxidant in PPO Assay System in Apples
Determination of minimum amount of citrate required to inhibit browning for a period of three hours:

The optimum amount of citrate needed to act as an enhancer for antioxidant activity of sodium erythorbate was observed over a 3 hour period. Though all concentrations of citrate at pH 5 ranging from 0.2 M to 0.005 M showed no colour formation over a 3 hour period (Fig. 24) 0.2 M showed no colour development even after 24 hours. It appears, therefore, that this molarity is ideal from the academic point of view, practically, however, this high molarity will affect the taste of the commodity. Hence a lower concentration is desirable. Though 0.005 M citrate buffer gave satisfactory result, to be on the safer side 0.01 M citrate sodium citrate buffer appears to be optimum.

Determination of minimum amount of antioxidant required in PPO assay system using natural substrate from apple source to inhibit browning for a period of three hours:

In an attempt to inhibit browning in standard PPO assay system using natural substrate (corresponding to 1 g tissue) from apple source, it was noted that 0.8 mM was sufficient to inhibit browning for a period of three hours (Fig. 25).

In vivo studies:

Determination of optimum dipping time to inhibit browning in apple plugs:

On keeping the apple plugs for observation in the anti oxidant dip solution for eight hours, it was seen that the apple plugs softened on long exposure
Fig 24: Effect of Citric Acid and Antioxidant on Colour Inhibition in PPO Assay System in Apples
Fig 25: Effect of Antioxidant Concentration on Colour Inhibition using Natural Substrate in PPO Assay System in Apples
to liquid medium and were unable to maintain their shape. Several plugs became soggy in texture and extremely soft at times leading to breaks in the plugs due to which further exposure of substrate with enzyme was encountered resulting in browning even after several minutes of treatment. It was noted therefore that this surface treatment is good for fruits/vegetables which will maintain their nature throughout the dipping time. Tissue which remained in this dip solution longer than 30 minutes (Table 8) lost its integrity. Hence a treatment time of greater than thirty minutes is not recommended for this variety (Fig. 26). These results indicated that this kind of treatment in apple plugs was not promising and hence further trials were carried out in the alternative product - apple juice.

Prevention of browning in apple juice:

Several pilot studies suggested that a concentration of 0.75 – 1 g % of sodium erythorbate inhibited browning in apple juice. Hence using a higher concentration of 1% sodium erythorbate, an experiment to determine the optimum molarity of citrate sodium citrate buffer and pH was carried out using a range of pH from 4 to 6 and molarity from 0.2 M to 0.05 M. It was seen (Fig. 27) that all pH from 4 to 6 and having citrate molarity from 0.2 M to 0.05 M showed no development of brown colour. The high absorbance as seen in the graph is due to the inherent property of the apple itself. Though at pH 4 the colour remained stable over a six hour period with no development of brown colour, either. The juice tasted very acidic. At pH 6, however, a variation in absorbance was noticed though no brown colour was observed here, either. The juice tasted highly salty probably due to high
Table 8: Determination Minimum Time of Treatment of Apple Plugs with Antioxidant Dip Solution to Achieve Minimum Browning

<table>
<thead>
<tr>
<th>Time of Treatment (min)</th>
<th>Observation After Hours</th>
<th>0</th>
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<th>30</th>
<th>60</th>
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<td>3</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
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<td>-</td>
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<td>+</td>
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<td>6</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Key:
- No Browning
+ Browning

1 D.W. Treated Apple Plugs
2 DIECA Treated Apple Plugs
3 Antioxidant Dip Treated Apple Plugs
Figure 26

P.T.O
Untreated: Apple Plugs Dipped in D.W. and removed
Control (DDTC): Apple Plugs Dipped in DIECA Solution
< 1 min: Apple Plugs Treated with Sodium Erythorbate (0.8 mM) in 0.2 M Citrate Sodium Citrate Buffer at pH 4 for less than 1 min.
15 min: Apple Plugs Treated with Sodium Erythorbate (0.8 mM) in 0.2 M Citrate Sodium Citrate Buffer at pH 4 for 15 min.
30 min: Apple Plugs Treated with Sodium Erythorbate (0.8 mM) in 0.2 M Citrate Sodium Citrate Buffer at pH 4 for 30 min.

Fig. 26: Minimum Time of Treatment of Apple Plugs with Dip Solution to Inhibit Colour Formation Over 6 hours
Fig 27: Determination of Optimum Citrate Molarity and pH in Apple Juice at 1% Antioxidant Concentration to Inhibit Browning for a Period of 6 Hours
concentration of sodium citrate. At pH 5 all molarities 0.2 M, 0.1 M and 0.05 M gave no brown colour and also tasted like apple juice. Therefore, a low molarity of 0.05 M with anti oxidant at 1% at pH 5 seemed optimum.

Further, lowering of the antioxidant concentration to 0.75 g % and 0.5 g% with 0.05 M citrate concentration at pH 5 indicated that these were equally effective over a period of six hours. However, to be on the safe side a concentration of 0.75 g% was taken as optimum (Fig.28).

An assessment to see if the anti oxidant alone could inhibit browning was also carried out. The anti oxidant additions were of 1% 3%, 5% and 7% concentrations. It was seen that all concentrations maintained colour over a period of six hours (Fig. 29). In contrast, it was observed that mere addition of citrate at a high molar concentration of 0.2 M at a low pH of 4 was unable to retain colour and darkened within one hour and progressively darkened over a period of six hours.

Comparison of sodium ascorbate and erythorbate as antioxidants:

It was seen that in case of apple juice both three hour and six hour duplicate readings were better than that of ascorbate at 0.05M OR 1% concentration. This result is similar to what Sapers (1991) found. Hence in the case of apple juice erythorbate is definitely a better alternative to ascorbic acid. (Fig.30).
Fig 28: Determination of Minimum Antioxidant Concentration at 0.05 M Citrate at pH 5 in Apple Juice to Inhibit Enzymatic Browning for 6 Hours
Fig 29: Effect of Use of only Antioxidant at Different Concentrations and only 0.2M Citrate at pH 4 in Apple Juice to Inhibit Browning
Fig 30: Comparison of Ascorbate and Erythorbate in Inhibition of Browning in Apple Juice
A photograph of the samples studied on the effect of anti oxidants in apple juice (Fig. 31) clearly indicates that apple juice with erythorbate developed no brown colour whatsoever while control showed extensive browning.

From the above studies, the following was concluded: The nature of this variety of Indian apple is juicy and soft. As a result, the tissue becomes soft on immersing in the dip solution and breaks, leading to further browning post treatment, thereby nullifying the surface treatment.

Therefore, for commodities like jam, wherein apple pulp is often used, the above treatment may prove useful to reduce browning (It is recommended that the pulp be completely mixed with the anti oxidant to reduce the browning).

Use of antibrowning agents in juices is quite common in the Indian juice industry. The study indicated that acids like citrate, malate and ascorbate are used to reduce enzymatic browning. ‘Yo Appy Juice of Parle International Ltd. is one such company. The State of Himachal Pradesh also manufacturing apple juice, is not using any anti-browning agent / acids to reduce enzymatic browning. Hence, their products like apple juice and apple juice concentrate are highly brown in colour.

It is, therefore, recommended that for inhibition of browning in apple juice, any of the two options for 100 ml juice can be used (1) 0.75% sodium erythorbate, 0.37 g of citric acid and 0.957 g of sodium citrate (2) sodium
erythorbate alone at a concentration of 1%. This option is has a slightly higher concentration of the anti oxidant than in (1) above. Both these additions eliminate browning effectively for a period of six hours.
C1: Apple juice and apple pulp treated with DIECA (Control)
C2: Untreated apple juice and pulp (Control)
O: Distilled Water (D.W.) (Control)
E1: Apple juice and apple pulp treated with 0.75g% Sodium Erythorbate and containing 0.2M Citric Acid and Sodium Citrate at pH4

Fig. 31: Effect of Antioxidant in Apple Juice