REVIEW OF LITERATURE
Food processing is fast growing in India. The general trend towards easily prepared meals and fast foods is on the rise particularly in the urban sections of India (Marwaha, 1997). Hence the need for better processing techniques which would increase the market value, shelf-life and organoleptic qualities of the products are also increasing.

Among the several products that are processed into various items are potatoes, bananas and apples. These vegetables/fruits are low in L-ascorbic acid (Webb, 1972) and therefore are more prone to enzymatic browning on bruising/cutting.

**Potatoes:**

About twelve million tonnes of potatoes are produced in India annually. Only 0.5% of the annual produce is actually processed (Goenka, 1990).

**Bananas:**

Fourteen different cultivars of banana are grown in India. Several factors play a role in the yields of banana according to cultivar, region, cultural practices and pest control treatments. The yield of the dwarf Cavendish is about thirty to forty tonnes/hectare in gardens and wet lands; and hill banana fifteen to seventeen and a half tonnes/hectare (CFTRI, Mysore 1991).
Apples:

India contributes to only 2.5% to the world's total apple production. In 1992-93 the total production in tonnes in India was 1168252. Kashmir Delicious and Royal Delicious are among the marketed varieties (as cited on the Internet agroindia.org.).

The three vegetables/fruit that are mentioned above are perfect examples of fruits susceptible to enzymatic browning and this is a major problem encountered during processing (Coseteng et al, 1987).

Browning of raw fruits, vegetables and beverages is a major problem in the food industry and is believed to be one of the main causes of quality loss during post harvest handling and processing (Mathew and Parpia, 1971).

Enzymatic browning results from the oxidation of phenolic compounds by the enzyme polyphenol oxidase (PPO). Polyphenoxoxidase (PPO) is a monophenol L-DOPA oxygen oxido reductase (E.C. 1.14.18.10) also known as tyrosinase, phenol oxidase, monophenol oxidase or cresolase (McEvily et al, 1992) (Fig.3). PPO is a copper containing enzyme that in the presence of oxygen catalyzes oxidation of phenol substrates which are then polymerized to brown/red or black pigments (Janovitz et al, 1990, Mathew and Parpia 1971). Chemical reaction taking place during browning is described in Fig.3.
Localization of PPO:

Polyphenol oxidase is an ubiquitous enzyme which belongs to the group of oxido reductases. The localization of the enzyme in the plant cell depends on the species, age and in the fruit/vegetable on maturity (Vamos-Vigyazo, 1981). In potato tuber PPO is unevenly distributed (Pendharkar et al, 1987). Maximum localization is observed around the bud (eye) region followed by tissue around the skin. Least concentration was observed in pulp tissue known as parenchymatous tissue. In potato tuber nearly all subcellular fractions were found to contain PPO in amounts proportional to the protein content (Craft, 1966). In apples the enzyme could be detected in all parts of
the fruit. Some researches found the activity to be highest around the core
(Biedermann, 1966) while others found it highest in the skin (Voigt, 1966). In
freshly harvested apples, the enzyme was found to be localized in the
chloroplasts (Harel et al, 1964). In great contrast to most fruits the greatest
part of PPO was found to be localized in the pulp and only a small fraction
was found in the peel of banana fruit as well as in the leaves and bark of
the plant (Padron et al, 1959). Also in most fruits the insoluble part of the
enzyme was found to be dominant.

**Action of PPO**

Enzymatic browning is an indirect consequence of PPO action. The primary
products of the oxidative reaction catalyzed by the enzyme, the o-quinones
(a) react with each other to form higher molecular weight polymers (b) form
macro-molecular complexes with amino acids or proteins and (c) oxidize
compounds of lower oxidation reduction potentials (Mathew and Parpia,
1971). The non-enzymic reactions (a) & (b) lead to the formation of brown
pigments, the colour of which is darker the higher the molecular mass; the
products of reaction type (c) are colourless.

The copper containing enzyme catalyses two entirely different reactions
(a) hydroxylation of monophenols to corresponding o-hydroxy compounds
and (b) the oxidation of o-dihydroxy phenols to o-quinone. The reactions
PPO is itself inactivated during the reaction. The phenomenon is attributed to the action of the quinone produced which is assumed to form a covalent linkage with the enzyme in the vicinity of the active site (Whitaker, 1972, Padron et al, 1975).

**Substrates for polyphenol oxidase:**

The most important natural substrates of PPO in fruits and vegetables are catechins, cinnamic acid esters, 3,4 dihydroxyphenyl amine (DOPA) and tyrosine (Walker, 1964, Baruah et al, 1959).

In apples catechin, epicatechin and chlorogenic acid were identified as substrates (Taufel and Voigt, 1963). The principal substrate in bananas was identified as dopamine (Griffith, 1965) a substrate not commonly occurring as a phenolic constituent of plant material.

**pH and Temperature Optima:**

In most cases optimum pH has been found to be between pH 4.0 - 7.0. Several sources have reported inactivity below pH 4.0 (Aylward et al, 1969) PPO from most sources is not a heat stable enzyme and is inactivated by heat treatments at 70 - 90°C (Almeida et al, 1995).
Inhibition of PPO:

PPO being a metallo protein with copper as the prosthetic group can be chelated by metallic agents such as cyanide, carbon monooxide, diethyldithiocarbamate (DIECA), citric acid and others (Walker, 1975, Pierpoint, 1966, Robb et al, 1966, Walker, 1964, Palmer, 1963).

The inhibitors of enzymic discoloration can react with either the reaction products or the substrates and can be divided into two groups:

(1) Reducing agents acting on the quinones formed by restituting the o-dihydroxy phenols. These products are consumed in the process of inhibition and thus provide only temporary protection against discoloration unless used in high concentrations in which case reaction inactivation of the enzyme might occur prior to the depletion of the reducing agent. Some frequently used members of this group are ascorbic acid, SO$_2$, potassium metabisulfite, β-mercaptoethanol (MET), 2-mercapto benzothiazole, thioglycollate.

(2) Quinone couplers forming stable colourless compounds with the quinones thus providing a permanent protection as long as they are not entirely consumed. Cysteine, glutathione benzenesulphuric acid and such others are able to perform such reactions. These are reducing agents having sulphydryl group in them.
Methods of Inhibiting PPO:

As noted earlier, several methods have been suggested for the inhibition of browning due to PPO, to increase the market value, organoleptic properties and the shelf life of the food product. Among the several methods are traditional treatments like heat treatment (Ashie et al, 1996), the reducing agents like ascorbic acid and erythorbic acid that act as free radical scavengers thereby preventing oxidation (McEvily et al, 1992). Ascorbyl phosphate esters are esters that release ascorbic acid on hydrolysis by phosphatases. The suitability of the phosphate esters depends on the ability of the food system to absorb the compound, the acidity of the system and the activity of the endogenous acid phosphatase (Sapers et al, 1989).

As summarised in Table 1, the sulphhydryl compounds used to inhibit enzymatic browning are L-cysteine and D-L methionine the sulphur containing amino acids (Walker, 1964). The use of cysteine in the prevention of browning of apple products for over twenty four hours without introduction of off-flavour has been reported by Walker and Reddisch (1964).

The use of chelating agents that bind the copper in the enzyme thereby inhibiting action of PPO e.g. EDTA has been commonly used in the food industry (McEvily et al 1992, Wiley 1994). EDTA is a metal chelating agent. Sporix is an antibrowning chelating agent used in combination with ascorbic acid. It has been recommended for use in acidic foods such fruit based
juices, nectars and carbonated beverages (Anon, McEvily et al, 1992). Sporix is chemically an acidic polyphosphate mixture and has 3-dimensional network.

Acidulants which lower the pH of media thereby effectively inhibiting the enzyme are also used. Citric acid is the most widely used acidulant in the food industry. Citrate has a dual inhibiting effect on PPO by reducing the pH and by chelating the copper at the enzyme active site (Langdon, 1987, McEvily et al, 1992). Citric acid at pH 3.5 inhibited both enzymatic and non-enzymatic browning in processed mushroom (McCord and Kilara, 1983).

Several other PPO inhibitors are being used for food e.g. Ficin, the protease preparation from fig (McEvily et al, 1992) and aromatic carboxylic acids, that bear structural similarities to the phenolic substrate thereby causing inhibition (Ludwig 1939, Kreuger et al, 1955 and Kuttner 1953). Also inhibition of browning in apple slices by honey showed better results as compared with sucrose solutions (Lee, 1990). The honey solution (in water) was found to contain a small peptide which inhibited the PPO. Other inhibitors are cyclodextrins which inhibit browning by formation of inclusion complexes with entrapment of PPO substrates or products (Hicks et al, 1990 and Chitosan, an abundant polymer of N-acetylglucosamine (Sapers, 1991). The use of Chitosan is limited to liquid systems and the mode of action is still undefined. Other treatments include enzyme treatments like use of oxygenases (Kelly et al, 1969), an expensive treatment and proteases (Labuza, 1989, Taoukis, 1990).
Tan (1995) found that Maillard reaction products (MRP) inhibited apple PPO and that MRP synthesized under different conditions including heating time, type of amino acids, pH and concentration of both amino acid and glucose showed different levels of inhibitory effects on apple PPO. Metallothionein from Aspergillus niger was found to be an inhibitor in a model system for the enzymic activity of a commercially purified mushroom tyrosinase. The inhibitory effect of metallothionien was higher on catechin oxidation than chlorogenic acid (Goetghebeur et al, 1996). Other methods to inactivate PPO are a combination of pressure with moderate temperature which increased the degree of enzyme inactivation (Seyderhelm, 1996). Studies on antibrowning of coconut showed that treatment with heat and sodium sulphite and citric acid decreased PPO activity in coconut significantly (Jiang et al, 1995).

A useful alternative to these singular treatments is the use of combination of treatments to inhibit browning due to PPO. Erythorbic acid as mentioned before is a reducing agent and is an isomer of ascorbic acid. Its basic function is: (1) to act as a radical scavenger and thereby prevent oxidation; (2) to alter the redox potential of the system; and, (3) to reduce undesirable oxidation products. The main role of this reducing agent is its ability to reduce O-quinones to diphenol thereby inhibiting browning. (Golan-Goldhirsh et al, 1984). Sulfiting agents commonly used to control browning are no longer approved by FDA due to certain health hazards such as asthma. The most effective and safest inhibitors of enzymatic browning are L-ascorbic acid, its isomers and derivatives (Hsu et al, 1988).
Eribate is a new anti oxidant recently introduced in the market at a low cost. Eribate as compared to sodium ascorbate is much less expensive (marketing pamphlet release, by Fugisawa Pharmaceutical Company, Osaka, Japan). Chemically Eribate is sodium erythorbate, an optical isomer of sodium ascorbate identical to sodium ascorbate in antioxidant effect (Yourga, 1944; Esselein, 1945) and proven to be nontoxic. Erythorbate is generally recommended as safe (GRAS) by the FDA in 21 CFR 182. 3041. The use of erythorbate with citric acid has been recommended to retard discoulouration in vegetables.

Several studies conducted on sodium erythorbate/Erythorbic acid have shown it to be relatively effective in controlling browning in combination with other agents. Santerre et al, (1991) have reported that potatoes packed with citric acid mixtures containing erythorbic acid maintained lower microbial load and also maintained acceptable colour throughout eighteen days of storage. In another study by Santerre et al, (1988) sulfite replacement in commercial apple freezing operation was investigated. Jonathan and Spy apple slices were vacuum impregnated with 1% ascorbic acid (AA) and 1% erythorbic acid (EA). It was reported that slice colour was not significantly different between the isomers. The study indicated that erythorbic acid may be substituted for the more expensive L-ascorbic acid for treating slices prior to freezing. The comparison of per cent values for Winesap and Red Delicious apple plugs with ascorbic acid or erythorbic acid in 1% citric acid indicated that ascorbic acid was significantly more effective than erythorbic acid (Saper and Ziolkowski (1987)). But in contrast to the cut surface data
comparisons to the lag time for delicious and Granny Smith apple juice containing erythorbic acid/ascorbic acid indicated that the two were equivalent as browning inhibitors of PPO in apple. In agreement with this study, Klapp (1990) reported that the effects of increasing amount of ascorbic and erythorbic acid studied both spectrophotometrically and polarographically showed that the two isomers are equally effective for inhibition of the browning reaction in apple. Siddiq et al, 1994 reported that L-cysteine and ascorbic acid and thiourea were good inhibitors of browning in pears both Delicious red pears and Bosc pears. At 1 mM concentration the inhibition of 4 methyl catechol was 87% in Bosc pears and 82% in red pears. El-Shimi, 1991 reported that the use of ascorbic acid in apple slices at 1.5% concentration reduced PPO activity to 10% in the slices and indicated that by reducing pH of apple using ascorbic acid during processing the time of heat treatment can be reduced. Almeida et al, (1995) investigations showed that the most effective alternative to the use of SO₂ in the control of PPO was a combination of ascorbic acid, citric acid and heat treatment. In further studies by Sapers et al (1994) it was reported that the most effective treatment in controlling enzymatic browning in minimally processed mushrooms was the use of a combination of sodium erythorbate, cysteine and EDTA at a pH of 5.5. Addition of preservatives did not much improve shelf-life, however dipping in 5% hydrogen peroxide prior to application of browning significantly increased shelf-life. In another study with fresh-cut pears, Sapers et al (1998) found that dipping wedges cut from pears in 4% sodium erythorbate with 0.2% CaCl₂ and 50-100 ppm 4- hexylresorcinol in conjunction with modified atmosphere packaging controlled browning and
maintained the quality of fresh-cut Bartlett and d'Anjou pears, the Bosc pears however, did not respond to treatment or packaging. Gopalan et al. (1999) have also recommended the use of erythorbate in case of diced potatoes at a concentration of 0.1 g% at pH 6 to inhibit brown colour formation in diced potatoes.

**POTATO:**

Polyphenol oxidase in potato has been found by Thygesen et al., 1995 to be present as a small multigene family in potato and each has a specific temporal and spatial pattern of expression. The highest activity was found to be at the tuber exterior including the skin and cortex tissue to 2 mm beneath the skin.

Potato processing can be categorized into three forms viz.,

(1) Fried products such as chips and french fries

(2) Dehydrated products such as granules, flakes, dices, flour etc.

(3) Canned potatoes (Marwaha 1997)

In the quality requirements for chip making as discussed by Sukumaran et al., (1993) it is seen that enzymatic discolouration is to be avoided (Table 2).
Table 2: PROMISING POTATO CULTIVARS SHOWING PROCESSING QUALITIES AT THE TIME OF HARVEST AND DURING HIGH TEMPERATURE STORAGE.

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>0</th>
<th>75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage period</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>MP/90-83'</td>
<td>21.0</td>
<td>2</td>
</tr>
<tr>
<td>MP/90-94'</td>
<td>22.6</td>
<td>1</td>
</tr>
<tr>
<td>MP/91-1'</td>
<td>21.1</td>
<td>2</td>
</tr>
<tr>
<td>MP/91-69'</td>
<td>23.3</td>
<td>1</td>
</tr>
<tr>
<td>MP/91-76'</td>
<td>22.4</td>
<td>2</td>
</tr>
<tr>
<td>MP/91-G'</td>
<td>23.1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Chip colour score upto 5 was acceptable.

Annual Scientific Report, CPRI, Simla. (1995-96)

Source: Sukumaran et al., 1993

Enzymatic discolouration is of great significance in pre-peeled potatoes or in sun-dried potatoes (Marwaha, 1997) it has been recommended to dip peeled/cut potatoes in 0.1 - 0.2% sodium bisulphite for 5 minutes or metabisulphite solution for 10 minutes. Joshi et al (1982) recommended the use of muriate of potash to reduce browning. Mapson and Wager, (1961) reported loss of thiamine content by 32% in preserved boiled potatoes and 44% in french fries due to sulphiting. According to Burton (1989) vitamin C losses in chips are 30 - 35%. Pelletier (1977), however, reported the loss to be of 30 - 85% in ascorbic acid in the preparation of chips.
BANANA:

Banana is a fruit that is widely consumed throughout the country. Both ripe and unripe banana can be successfully processed into several products such as pulp, liquid fruit, canned slices, deep fat fried chips, toffee figs, fruit bar and brandy (CFTRI, 1989).

Browning of banana is a common problem. PPO has been shown to occur in the pulp and peel of the banana fruit (Palmer 1963). The highest concentrations of its substrate dopamine are, however, found in the peel (1-2 mg/g fr.wt) (Buckley, 1964). Unlike dopamine, the substrate, banana PPO is higher in the pulp than in the peel. Thomas and Nair (1971) reported that three Indian varieties of banana fruit showed both cresolase and catecholase activities. The pulp showed more cresolase activity than skin, whereas skin showed more catecholase activity.

APPLE:

This fruit is widely consumed in India. Several varieties are grown in Northern States of the country. Apples are processed into fruit pulp, juice, and also used in baked products. Enzymatic browning is a severe problem in apple products.