FORMULATION AND IMAGE ANALYSIS OF ANTIBROWNING AGENTS FOR MUSHROOM AND PLANT PRODUCTS

A PROJECT REPORT

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BONAFIDE CERTIFICATE

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ABSTRACT

Browning of fruits and vegetables is a well-known phenomenon caused by the enzyme Polyphenol oxidase (PPO). It is a copper containing mono oxygenase enzyme that catalyses the ortho hydroxylation of mono phenols to o-diphenols (cresolase activity) and oxidation of o-diphenols to o-quinones (catecholase activity) which are molecular oxygen dependent. PPO is the major cause of enzymatic browning in higher plants. Enzyme activity of polyphenol oxidase (PPO) was compared between different apple, banana and mushroom samples. Banana pulp had showed the highest specific activity. Inhibitory effect of various antibrowning agents such as cinnamic acid, L-ascorbic acid, L-cysteine, sodium azide, sodium benzoate, benzoic acid, citric acid and potassium metabisulphate on enzyme activity were studied and found to be in a dose dependent manner. Visual Image analysis was performed in apple, banana and mushroom slices on ImageJ ver. 1.46 software platform used to calculate integrated density values (the sum of the values of the pixels in the image or selection). Analysis showed dose dependent protection against browning by the inhibitors. Inhibitors like L-ascorbic acid offered better protection compared to other inhibitors.

KEYWORDS: Image analysis, antibrowning agents, Polyphenol oxidase (PPO), Browning, Inhibition rate.

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LIST OF ABBREVIATIONS

^0C	Degree Celsius
AA	Ascorbic acid
BA	Benzoic acid
BSA	Bovine Serum Albumin
CA	Citric acid
Conc	Concentration
$CuSO_4.5H_2O$	Coppersulphate pentahydrate
Cys	Cysteine
EDTA	Ethylene diamine tetraacetic acid
EGG	Epigallocatechin Gallate
FD	Fractal Dimension
HHP	High hydrostatic pressure
HPCD	High pressure Carbon dioxide
HPP	High pressure processing
IAA	Isoascorbic acid
L	Intensity Distribution
L-DOPA	L- 3,4 Dihydroxyphenylalanine
Mins	minutes
mM	Millimolar
NaCl	Sodium chloride
$NaCO_3$	Sodium carbonate
NaOH	Sodium hydroxide
PEF	Pulsed electric field
pH	Increased acidity

POD	Peroxidase
PPO	Polyphenol Oxidase
PVPP	Polyvinylpolypyrollidone
SB	Sodium benzoate
SMB	Sodium metabisulphate
U	Enzyme activity unit
U/mg	Enzyme unit/milligram protein (specific activity unit)
UV-VIS	Ultraviolet-Visible
VIS	Visible reflectance spectra

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Browning is a major factor which occurs in certain fruits and vegetables during handling, processing operations and storage after harvest. These changes induce loss of odour, flavour and nutritional aspects in fruits and vegetables. Browning can cause economic cost, causing deterioration of products in the market. Browning results in enzymatic and non- enzymatic reaction in phenolic compounds as well as from maillard reactions that occurs when mixtures of amino acids and reducing sugars are heated (Mcevily and Iyengar, 1995).

1.2 BACKGROUND

1.2.1 ENZYMATIC BROWNING

Enzymatic browning is a chemical process takes place two reactions: polyphenoloxidases (monophenol dihyroxyphenylalanine: oxidoreductase: (E.C. 1.14.18.1; PPO), peroxydase (POD) and other enzymes create melanins and benzoquinone from natural phenols resulting in a brown pigments. There are several methods are developed to prevent enzymatic browning are chemical, physical (blanching, freezing), controlled atmosphere and coating methods. (Irina Ioannou et al., 2013). And it also beneficial for developing the flavour in tea and in dried fruits such as figs and raisins. The process of browning involves the enzyme catalysed synthesis of a dark pigment named melanin (Zhang, 2006). During melanin formation, tyrosinase catalyses the first two reactions: the conversion of tyrosine to dihydroxyphenylalanine (DOPA), and the oxidation of Dopa to o-dopaquinone (Fig. 1.1).

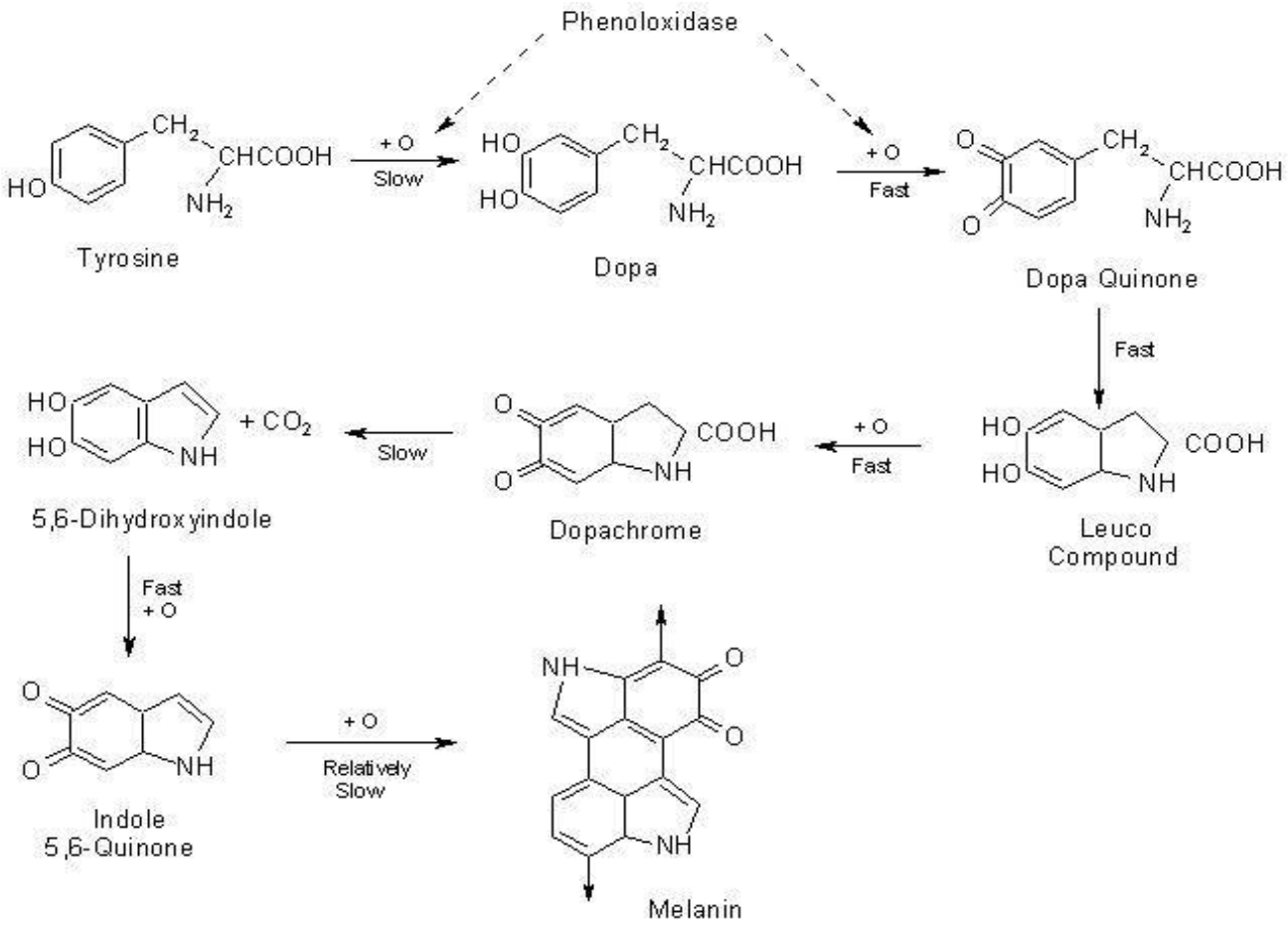


Fig 1.1: The mechanism of melanin synthesis

Combination of sodium erythorbate, cysteine and EDTA at pH 5.5 showed most effective treatment in sliced and whole mushroom. Addition of preservatives to browning inhibitor dips did not improve storage life. However, dipping in 5% hydrogen peroxide prior to application of browning inhibitors significantly increased shelf-life (Sapers, G. M., et al., 1994).

1.2.2 NON-ENZYMATIC BROWNING

Non-enzymatic browning which typically proceeds rapidly from around 140 to 165^{o}C (284 to 329^{o}F). At higher temperatures, caramelization and pyrolysis become more pronounced. The common type of non-enzymatic browning is the Maillard reaction. It is a series of chemical reactions between an

amino acids and reducing sugar that bring foods more appetizing. It is responsible for many colours and flavours in foods without using enzyme activity. The second type of non-enzymatic browning is caramelization that occurs when carbohydrates in any foods are heated. It results in light to dark brown and new flavours while roasting of coffee and commercial caramels are included in the process.

1.2.3 MECHANISM OF PPO

PPO has been widely studied in various fruits and vegetables such as apple, apricot, banana, grape, kiwi, mango, olive, potato, pear, peach and plum. Tyrosinases (monophenol, L-DOPA: oxygen oxidoreductase) are polyphenol oxidases (PPO) that belong to a group of non-blue copper proteins. Inhibition of polyphenol oxidase activity in banana (musa paradisica L.var.kanthali), apple (Malus pumila Var. ambiri kashmiri), and mushroom (Agaricus bisporous) on L-cysteine (cys), Ascorbic acid (AA), citric acid (CA) were tested at different pH Conditions (3.5, 4.0 and 4.5) (Samanta Arpita et al., 2010). The activity of PPO varies between fruits and vegetables (Nunez- Delicado et al., 2005). PPO extracted from most plants and fruits is capable of oxidising *o*-diphenol, while mushroom PPO can catalysed both mono- and *o*-diphenol oxidation (Cash et al., 1976).

PPO activity was investigated with metals and metal anti-browning agents from red poppy leaf. The effect of some known anti-browning agents which can form complexes with metals on the PPO activity was determined (Arabaci, gulnur et al., 2015). PPO mechanism is also based on its capacity to oxidize phenolic compounds. Polyphenol oxidase substrate specificity has led to many methods being to measure its activity: electrometric, radiometric,

chronometric, and especially spectrophotometric, which are used by most of the laboratories (Garcia-molina et al., 2007; Falguera et al., 2010).

1.2.4 EFFECT OF ANTIBROWNING AGENTS

Enzymatic browning may result from disruption of the fruit and vegetables by slicing, peeling and juicing. It is controlled by chemical and physical (blanching, freezing), controlled atmosphere and coating to prevent enzymatic browning. Various fruits and vegetables are treated with antioxidants (hexylresorcinol E586, erythorbic acid E315, N-acetyl cysteine E920), acidifying (citric acid E330, erythorbic acid E315, ascorbic acid E300 and glutathione), agents of firmness (calcium lactate E327, calcium propionate E282, calcium chloride E509, calcium ascorbate E302 and sodium chloride) and chelating agents (kojic acid, citric acid E330 and EDTA E385) (Irina Ioannou et al., 2013). Enzymatic browning can also limit the shelf-life and commercialization of fresh-cut fruits and vegetables.

Fresh –cut minimally processed cabbage was treated with addition of ascorbic acid, citric acid and calcium chloride to know the effect of colour, quality and texture. Citric acid helped to retain the colour and increased the overall quality at different storage conditions for fresh –cut cabbage (Manolopoulou et al., 2011). Fresh-cut ‘fuji’ apple was subjected to measure colour, browning indexes, total phenolics and PPO activity of the samples were evaluated (Jeong et al., 2008). Biochemical changes in vitamin C, sugars, soluble solids and phenols during storage of fresh-cut pineapple slices treated with anti-browning is the primary quantitative parameters of quality (Gonza´lez-Aguilar G.A, et al., 2005). It was found that ascorbic acid (AA), Isoascorbic acid (IAA) showed the better compositional quality parameters and appearance of pineapple slices during the storage period. Ascorbic acid and L-cysteine was the effective anti-browning agents used for the prevention of enzymatic browning.

1.2.5 VISUAL IMAGE ANALYSIS ON IMAGEJ SOFTWARE (Version 1.46)

ImageJ is a public domain Java image processing and analysis program. It supports ‘stacks and hyperstacks a series of images that share a single window. It is multithreaded, so time-consuming operations such as image file reading can be performed in parallel with other operations1 It can calculate area and pixel value statistics of user-defined selections. It can measure distance and angles. It can create density histograms and line profile plots. It supports standard image processing functions such as contrast manipulation, sharpening, smoothing, edge detection and median filtering. Area statistics are calculated for the complete there is no selection or for a selected sub-region defined by one of the area selection tool. With RGB images, results are calculated using brightness values. RGB pixels are converted to brightness values using the formula value = (red+green+blue) =3 or value = 0:299*red+0:587*green + 0:114 * blue.

Browning of banana and apple slices on oxalic acid was investigated using a machine vision system and it was evaluated by temporal colour spectra based on experimental variance, oxalic acid and storage time (yoruk R et al., 2003). Machine vision, a non-destructive method, can rapidly quantify the disappearance of lighter colours and the formation of darker colours in non-uniformly coloured surfaces of many foods. Three image based browning reference classes (BRC) are generated: Cluster A (corresponding to the samples), Cluster B (ti and t3 samples) and Cluster C (t7 and tg samples). An internal and external validation (n=120) was carried out: internal validation: 99.2% of samples correctly classified for both virtual images; external validation: 84% with (R - B)/(R + B) and 81% with B/R). For both validation phases a^*, b^*, BI and I_{SE} increased L^*values decreased with image based class number, thereby reflecting their browning state.

Colour measurements and analysis in fresh and processed foods has been used as an indirect measure of quality attributes such as flavour and nutrition content of pigments. Different approaches applied to model food colour are described, including reaction mechanisms, response surface methodology and others based on probabilistic and non-isothermal kinetics (Pathare, P.B., et al., 2013).

1.2.6 APPLICATION OF ENZYMATIC BROWNING

Enzymatic browning can cause considerable losses in the food and agricultural sector if necessary measurements are not implemented to prevent it. High pressure processing (HPP) is a technique widely used in the food industry to prevent the enzyme activity. This involves application of 500-700 atmospheric pressure to inactivate the enzyme and microbes. Dehydration is also one of the most effective to prevent enzymatic browning, and various methods like irradiation and pasteurization are applied to the activity of enzyme phenolase. Vacuum and pressure infiltration were investigated as means of applying ascorbate or erythorbate-based enzymatic browning inhibitors to apple and potato cut surfaces. Pressure infiltration at 108 kPa extended the life of potato plugs by 2–4 days, compared to dipping, but was ineffective with potato dice (Sapers, G.M., 1990).

1.3 OBJECTIVES

1. Formulation of chemical antibrowning agents.
2. Study of antibrowning agents in preventing browning reaction.
3. Visual image analysis by ImageJ software.

CHAPTER 2

REVIEW OF LITERATURE

2.1 General

Ioannou *et al.*, (2013) stated that the enzymatic browning in fruits and vegetables can be prevented by chemical methods (acidifying agents, chelating agents, agents of firmness, antioxidant agent) physical (blanching, freezing), controlled atmosphere and coating methods. To improve protection against fruits and vegetables against oxidation several techniques were combined. Dipping with physical (blanching, freezing and product atmosphere) and chemical methods controls the browning and control of firmness and also revealed that the optimization of the prevention by combining two techniques: alternative methods to replace thermal methods like High hydrostatic pressure (HHP), High pressure carbon dioxide (HPCD), Pulsed electric fields (PEF) but in spite of the progress in non thermal treatments, the thermal methods remain the most effective for protecting foods against oxidation.

Laurila *et al.*, (1998) reviewed that the ascorbic acid is the most effective method for preventing enzymatic browning in potatoes with different treated material. Browning measured by spectrophotometers and colorimeters depends on the L value has been considered as the best colour indices value and it is frequently used. Visual observations showed that the normalised $\Delta L/L_0*100$ value measured with a Minolta chromameter reached, 2.5 for apple slices and 3.0 for potato slices, the colour could not be distinguished from the initial colour of the slices (ΔL is the change in L value at any time and L_0 is the initial L measurement). Slight browning indicated the point of unacceptability correspond to a normalized value. Extremely brown colour slices showed that the normalized $\Delta L/L_0*100$ value showed 15.0

Chiabrando *et al.,* (2011) investigated that the fresh-cut apples during cold storage condition the browning has been inhibited. The colour (L^*), the browning and polyphenol oxidase (PPO) activity of fresh-cut apples (Golden Delicious, Scarlet Spur and Granny Smith) was evaluated. Anti-browning agents (citric acid, ascorbic acid) and calcium chloride resulted in a reduction of browning and collapse of fresh-cut apples stored at 4^0C for 5 days under normal atmosphere condition. Inhibition of browning in fresh-cut apples the use of citric acid (CA) and ascorbic acid (AA) increased the activity of PPO. These anti-browning agents helped to remain the colour of fresh-cut apples during cold storage. But the use of 1-methylcyclopropene was not effective against these anti-browning agents of fresh-cut apples from the first day of cold storage.

2.2 EFFECT OF ANTIBROWNING AGENTS

Gonzalez *et al.,* (2005) stated that the fresh-cut pineapple slices are treated with anti-browning agents. The effectiveness of ascorbic acid (AA), isoascorbic acid (IAA) and N-acetyl-cysteine (AC) of fresh-cut pineapple slices were stored up to 14 days at 10^0C was studied. Slices treated with isoascorbic acid (IAA) and ascorbic acid (AA) maintained higher levels of sugars and vitamin C than N- acetyl-cysteine (AC) and controls. Lower polyphenol oxidase activity slows down the reduction of total phenolic content in treated pineapple slices. Ascorbic acid (AA), isoascorbic acid (IAA) slowed the degradation rates of sugar, vitamin c, phenolic content where as AC was less effective in affecting these processes. Higher content of AA and IAA shows the better compositional quality parameters of pineapple slices during storage period.

Manolopoulou *et al.,* (2011) investigated the sensory quality, colour and texture of fresh-cut minimally processed cabbage with addition of ascorbic acid, citric acid and calcium chloride. Ascorbic acid showed No difference on the surface compared to the control sample at both temperatures. It was also found

that the citric acid 1% can be used for minimally processed cabbage and helped to retain the colour and increased the organoleptic quality of fresh-cut cabbage. At low temperature (0^0C) citric acid prolonged the shelf-life of minimally processed cabbage for 22 days. Calcium chloride treatment maintained the overall quality of the cut surface for 14 days at both storage temperatures.

Khan (2007) discovered and reported a large number of mild to potent inhibitors of several classes, such as phenolics, terpenes, steroids, chalcones, flavonoids, alkaloids, long-chain fatty acids, coumarins, sildenafil analogs, bipiperidines, biscoumarins, oxadiazole, tetraketones, etc. the characterization of new inhibitors are not useful for the medicinal purposes, but their potential applications in improving food quality and nutritional value, controlling insect pest, etc., are also important.

2.3 INHIBITION OF POLYPHENOL OXIDASE ACTIVITY

Akthar *et al.*, (2011) determined the antioxidant potential as well as tyrosinase inhibitory activity of citrus fruit waste. Fruit peels of 4 species of genus citrus viz. C*itrus sinensis* (Malta), *reticulate* (orange), C.*paradisi* (Grape fruit) and C.*aurantifolia* (Lemon), were analyzed for these bio-reactive properties. Tyrosinase inhibition capacity by using kojic acid as standard tyrosinase inhibitor.Alcoholic fractions of orange, malta, and lemon showed 90, 87, and 69% tyrosinase inhibition respectively, while no tyrosinase inhibition was found in any fraction of grape fruit.

Arpita *et al.*, (2010) investigated anti-browning (inhibition of polyphenol oxidase activity) effect of cysteine (cys), Ascorbic acid (AA), citric acid (CA), sodium metabisulphate (SMB) alone or in combination in apple, banana and mushroom at three different pH (3.5,4 and 4.5) in banana (Musa paradisica L. var. Kanthali), apple (Malus pumila Mill. Var. Ambri kashmir), and mushroom (Agaricus bisporous). PPO activity was analysed spectrophotometrically at

420nm (30^0C). No significant differences were observed for PPO activity concentrations of Cys and CA when combination or alone anti-browning agents were tested.

Wuyts *et al.*, (2006) stated the partial characterization and extraction of banana (Musa acuminata Grande naine) roots. Highest enzyme activities were obtained in a 0.2 M phosphate buffer at pH 7.0 with 5% insoluble polyvinylpyrollidone (PVP) and 0.25% Triton X-100. Dopamine and D-catechin showed the lowest K_m values. Banana root PPO was strongly inhibited by dithiothreitol and sodium metabisulphate. Complete inhibition was achieved with 150µM and 109µM concentrations.

Eissa *et al.*, (2014) stated the natural extracts on enzymatic browning in apple juices stored for 24 hours at room temperature (25^0C) was carried out with different extraction methods (water, ultra-filtration and alcoholic). The result was obtained at the end of 24 hour storage period with the extracts of squash, cucumber and pepper followed by mushroom in apple juices. The natural extracts derived from vegetables were active than mushroom. Alcoholic and ultra-filtration extracts showed the effective in maintaining the storage period of apple juices. Main colour change in treated apple juices were due to the decrease in chroma, hue value, absorbance at 420nm and a^*-value.

Barbagallo *et al.*, (2012) investigated the efficacy in vivo of some natural anti-browning agents in minimally processed eggplants. They were subjected with inhibitors L-ascorbic, benzoic, citric, ferulic and L-glutamic acids at three different concentrations (0.2, 0.5 and 1 %) and packed in ordinary atmosphere bags (PET) covered by a double barrier film and refrigerated for 7 days. Reduction of PPO activity was observed at higher concentrations (0.5 and 1%) of the inhibitors and conformed by brown index percentage, which was

significantly reduced by these inhibitors and also can increase the shelf-life of minimally processed egg plants.

Wang *et al.,* (2003) stated that the green tea has encountered a browning problem caused by the auto-oxidation in flavanols (catechins). One-tenth percent of the anti-browning agents were added to the freshly prepared green tea extracts processed at 121^0C for 1min and then stored in a 50C oven for 12 days. Stored tea extracts were measured for colour maintenance and epigallocatechin gallate (EGCG) content to compare the anti-browning and antioxidant effects of these agents and anti-browning activity and antioxidant effect of individual agents often did not correlate with each other, but citric acid showed both significant anti-browning and antioxidant effects on green tea extracts.

Busch *et al.,* (1999) demonstrated the enzyme catalysed reaction in the browning of potatoes. Potato slices were dipped on the chemical treatments like 100 µl 4% citric acid, (4%) 100 µl ascorbic acid, 100 µl 2% cysteine, 100 µl 1% sodium acid, pyrophosphate or 100 µl and 0.5% sodium bisulphite before the substrates is added and it can be observed visually based on the control and test samples.

Iyengar *et al.,* (1992) stated the use of sulphites in food and beverage industry. Discolouration is the action of endogenous polyphenol oxidase (enzymatic browning) followed by the polymerization of quinonoid compounds with other foods stuffs. Sulfiting agent is the most widespread chemical approach for controlling browning.

Arabaci (2015) investigated the metals and metal-antibrowning agents effect on polyphenol oxidase from red poppy leaf. Glutathione was the most potent inhibitory effect on PPO activity. Cu (II) and Fe (II) metals increased the

enzyme activities whereas the Sn (II) had the maximum inhibitory effect and Zn (II) and Pb (II) had no significant effect on the enzyme activity. EDTA and metal complexes had no significant effect on the enzyme. L-ascorbic acid and metal complexes Cu (II)-complex had no effect. Glutathione–metal complexes had the best inhibitory effect on Red poppy leaf PPO activity. Ammonium sulphate fractionation is the convenient and effective step to remove large proteins and brown pigments and the effect of pH and temperature was observed at more acidic and basic pH values due to enzyme instability of these pH values. Substrate specificity was calculated from the LineWeaver- burk graphs. The highest Km values were shown by caffeic acid, 4-methyl catechol and catechol and the lowest by pyrogallol. L-Cysteine was reported to be a strong inhibitor of apple PPO and ascorbic acid is effective inhibitor for different PPOs. EDTA showed minimum inhibition to poppy PPO activity. L-ascorbic acid and metal complexes decreased the enzyme activities but L-ascorbic acid-Cu (II)-complex had no effect on the enzyme activity.

2.4 EFFECT OF ANTIBROWNING AGENTS BY IMAGE ANANLYSIS

Lunadei *et al.*, (2011) reported a vision system to classify apple slices according to the development of enzymatic browning. It was carried out in a 'Granny Smith' apple slices stored at 7.5^0C for 9 days (n=120). Multispectral images were acquired from the samples by employing a 3-CCD camera centered at the infrared (IR, 800nm), red (R, 680nm) and blue (B, 450nm) wavelengths. Apple slices were evaluated visually according to a visual colour scale of 1-5 to obtain evaluation index (I_{SE}), visible (VIS) relative reflectance spectra (360-740 nm) were obtained.

Yoruk *et al.*, (2004) investigated the using a machine vision system for banana and apple slices. Degree of browning on fresh-cut surfaces was evaluated visually and quantitatively by observing changes of CIE L values.

Banana and apple slices are treated with oxalic concentrations of 60mM and 10Mm during 5h storage period. Oxalic acid dipped samples were slightly lighter in colour than the water dipped control. Similarly, colour spectra of the water-dipped apples (controls) were drastically changed during the storage period. 3mM concentration of oxalic acid has no difference was noticed.

Amodio (2011) determined the post-cutting quality changes of fresh-cut artichokes were evaluated by computer vision system. Different compounds like ascorbic acid, citric acid, cysteine, and their combination, ethanol, sodium chloride, 4-hexylresorcinol were tested at different concentrations. Cysteine (0.5%) was the most effective treatment to prevent browning and allowed measurement of L*, a*, and b* values from the whole quarter surface and from the browned areas. It was effectively improved by increasing the pH of the solution from the natural pH (2.1) to pH 3, resulting in L* values of browned areas about 30% higher than controls. The mean values of appearance scores for cysteine treated samples were all above the limit of marketability (score 3), significantly higher than in control samples.

Quevedo (2009) reported the enzymatic browning in apple slices by applying the fractal texture Fourier image. Absorbed irregular colour patterns emerged the colour average in the same area analysed. The images were transformed to lab space colour using a quadratic transformation function and the Fourier fractal texture image was used to calculate a fractal dimension value (FD) and complexity of lightness intensity distribution (L) over the surface. It showed the browning kinetic and sensitivity of apple slices. Enzymatic browning rates derived using the fractal kinetic method, between 14.3 and 23.2 times (in absolute values) higher than the L mean rates calculated.

2.5 APPLICATION OF ANTIBROWNING METHODS

Jose *et al.*, (2004) reviewed blanching principles and equipment, it removes trapped air (e.g., in broccoli florets) and metabolic gases within vegetable cells and replace them with water, forming a uniform crystal growth during freezing and also improving product quality (increasing retention of nutrients and other fresh like quality attributes, reducing energy consumption, and reducing waste production.

Severini *et al.*, (2003) investigated the sliced potatoes by blanching in boiling saline solutions. Response surface methodology was applied to find the two 3 factor-5 level, second order central composite designs were developed to analyse the considered variables. Low concentration of calcium chloride showed the better results than the use of sodium chloride.

Ndiaye *et al.*, (2009) investigated the steam blanching effect on polyphenol oxidase and peroxidase activity in mango (*Mangifera indica L.)* slices of relative colour was studied after different steam blanching times. There was a complete inactivation after 5 min for POD and 7 min for PPO. Steam blanching of 5 min gave residual activity than 5 min. But after the 7 min steam blanching the browning index was less than at 3 and 5-min because non-enzymatic browning had occurred.

Wang *et al.*, (2014) studied the anti-browning effect on banana by combination of Ascorbic acid, citric acid, nitrogen and carbon Dioxide. The AA+ CA treatment increased the cumulative volume of the larger particles, whereas the viscosity of the banana smoothies decreased after the different three treatments. Inclusion of AA+CA+N2 in the production of banana smoothies was the best processing method.

CHAPTER 3

MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES

Samples for screening of Polyphenol Oxidase (PPO) such as apple, banana and mushroom were purchased from the local market, Coimbatore, Tamilnadu.

3.2 CHEMICALS

Table 3.2.1: List of chemicals and its purpose

PURPOSE	CHEMICALS
For extraction buffer	Polyvinyl pyrolidine (PVP), Triton X-100, sodium phosphate buffer (0.2M, Ph 6.5) (Wutys et al., 2006)
Substrates	L-DOPA, catechol, pyrogallol, phenol, tyrosine (prepared with sodium phosphate buffer, 0.05M, Ph 7) (Wuyts et al., 2006)
For protein estimation	Folinciocalteau reagent, sodium carbonate, sodium hydroxide, potassium sodium tartarate, bovine serum albumin (BSA)
PPO inhibitors	Potassium metabisulphite, sodium azide, L-ascorbic acid, citric acid, sodium benzoate (SB), L-cysteine, benzoic acid (BA), cinnamic acid
General chemicals	Double distilled water

3.3 EQUIPMENTS

Spectrophotometer, pH meter, waterbath, microwave oven.

3.4 METHODOLOGY

3.4.1 ISOLATION OF POLYPHENOL OXIDASE (PPO)

PPO was isolated from apple, banana peel, mushroom using extraction buffer containing 0.2M phosphate buffer (PH 6.5), 0.25% triton X-100 and 1% polyvinyl pyrollidine (PVP). The samples were homogenized in 1:10 ratio (sample: extraction buffer), filtered through four layers of cheese cloth and the crude enzyme obtained was stored at 4^{o}C AS 1ml aliquots.

3.4.2 ENZYME ACTIVITY ASSAY

Presence of PPO was confirmed through enzyme assay involving its specific substrates and from the rate of quinine formation. Quinone resulted in reddish brown colour development. PPO activity was assayed by measuring the rate of increase in absorbance at 475nm wavelength (dependent on the specific quinine product of the different substrates) at 25^{o}C. The reaction mixture contained 1ml substrate in 0.05M sodium phosphate buffer (pH 7.0), lml sodium phosphate buffer (0.2M, pH 6.5) and 0.9ml double distilled water and 0.1ml enzyme. The reference contained 1ml substrate, 1ml sodium phosphate buffer (0.2M, pH 6.5) and 1ml double distilled water. The liner portion of the activity curve was used to express enzyme activity (Wuyts et al., 2006).

3.4.3 PROTEIN ESTIMATION

The protein concentration was estimated through Lowry's method. The method is as follows:

Reagent A: 2%$Naco_3$ in 0.1N NaOH

Reagent B: 0.5%$CuSO_4.5H_2O$ in 1% Potassium sodium tartarate

Reagent C: Mix 50ml reagent A with 1ml reagent B, prior to use (alkaline copper sulphate)

Reagent D: Folinciocalteau reagent

Stock: Weigh 50mg of BSA and dissolve in distilled water. Makeup to 50ml in standard flask

Working standard solution: Dilute 10ml of stock to 50ml with distilled water in a standard flask (1ml of solution contains 200ug of protein).

Procedure:

1. Measure 0.2, 0.4, 0.6, 0.8, 1ml of working standard solution and 0.1ml of test solution keeping 1ml of water as a blank.
2. Make up the volume in all tubes to 1ml with distilled water.
3. 5ml reagent C was added in all tubes and incubated for 10mins.
4. 0.5ml of reagent D was added in all tubes and incubated for 30mins.
5. Blue colour developed was read at 660nm.

3.4.4 EFFECT OF ANTIBROWNING AGENTS

Anti-browning agents such as potassium metabisulphate, sodium azide, L-ascorbic acid, citric acid, sodium benzoate (SB), L-cysteine, benzoic acid (BA), cinnamic acid were added in different concentrations and activity of PPO was studied at 475nm according to Wuyts et al., (2006).

3.4.5 FABRICATED WOODEN DEVICE

Designed wooden box with the size of 10*15cm having a 1*3cm hole used to place the slices of different plant products.

FIG.3.1 FABRICATED WOODEN DEVICE FOR IMAGE ANALYSIS

3.4.6 EFFECT OF ANTIBROWNING AGENTS- VISUAL IMAGE ANALYSIS BY IMAGEJ SOFTWARE

Freshly cut apple, banana and mushroom was dipped into the antibrowning agents with different concentrations and browning was due to polyphenol oxidase (PPO) enzyme and it was observed with time. Then, the images are analysed by ImageJ software with calculating the integrated density (number of pixels in the image).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 POLYPHENOL OXIDASE ACTIVITY IN VARIOUS PLANT FOODS

Polyphenol oxidase (PPO) activity was screened for apple, banana and mushroom using different substrates spectrophotometrically.

TABLE 4.1.1: COMPARISON OF POLYPHENOL OXIDASE (PPO) ACTIVITY FROM VARIOUS SOURCES

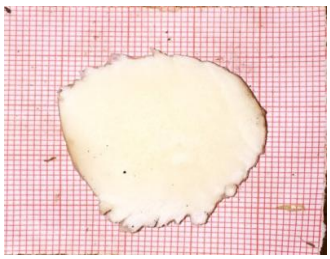
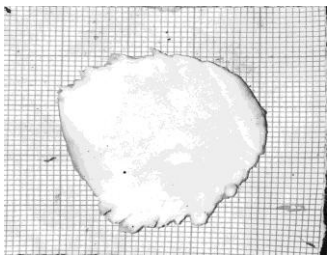
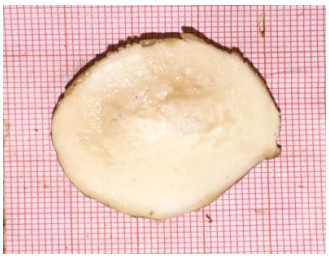
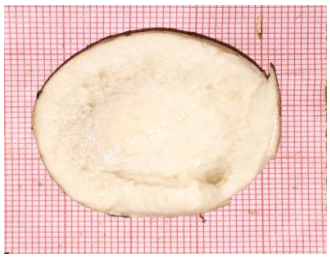
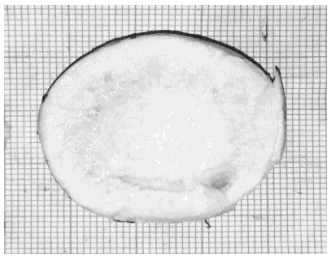
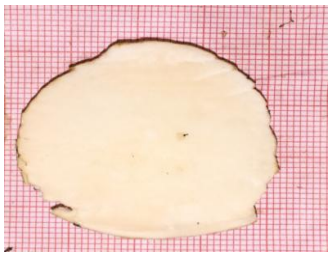
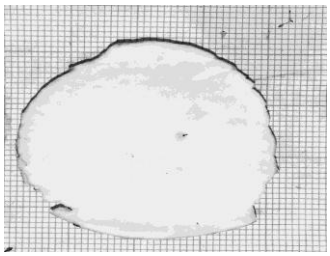
S.NO	SPECIES	TOTAL ACTIVITY (U)	SPECIFIC ACTIVITY (U/mg)
1	Apple pulp	17.11	12.22
2	Banana peel	164.55	117.95
3	Banana pulp	197.33	**140.95**
4	Mushroom pulp	33.56	23.97

Screening was done for polyphenol oxidase (PPO) enzyme in three different sources including fruits and fungi. Activity (total and specific activity) of PPO was measured in all the sources and the source which had the maximum specific activity was chosen for further studies. Wuyts et al., (2006) reported PPO activity of 285U in banana pulp with an extraction buffer of pH 6.5 and 0.3M dopamine as substrate. According to the observation it was found that banana

pulp exhibited maximum specific activity of 140.95 U/mg with an extraction buffer at pH 6.5 and 6.67mm L-dopa as substrate.

4.2 EFFECT OF ANTIBROWNING AGENTS ON MUSHROOM

TABLE 4.2.1: Representative images of the original and 8-bit grayscale images of mushroom slices treated with various inhibitors (at 120th min)

Sample (concentration)	Original image		Grayscale image used for analysis
	Control	After treatment	
L-ascorbic acid (0.6mM)			
Potassium metabisulphite (0.3mM)			
Benzoic acid (12mM)			

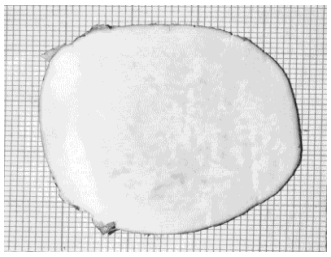
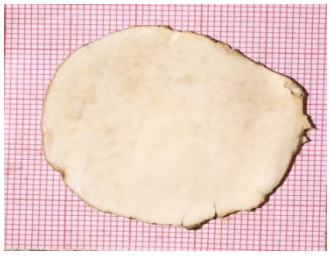
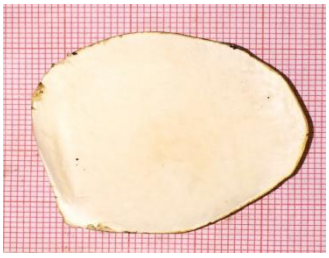
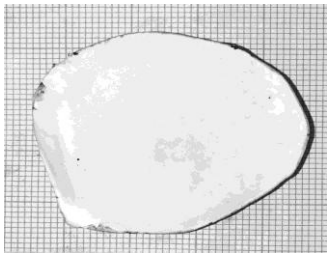
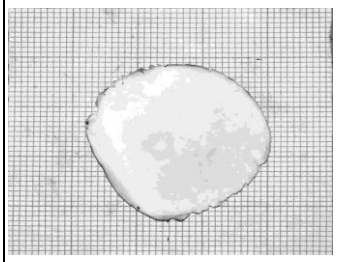
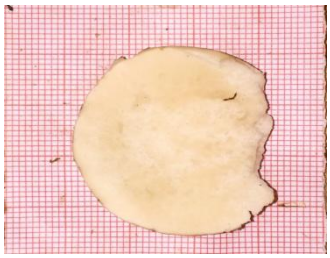
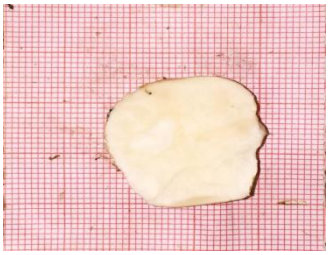
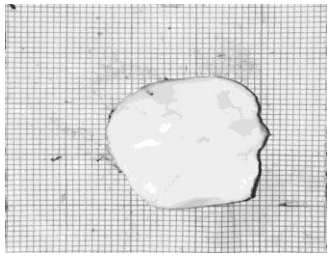
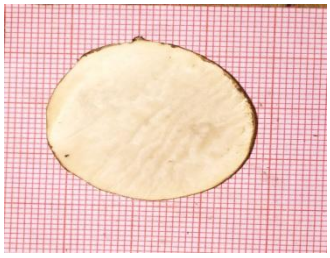
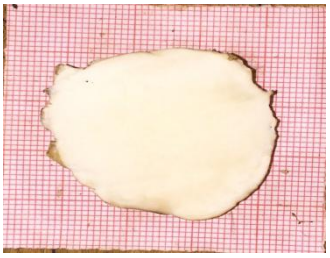
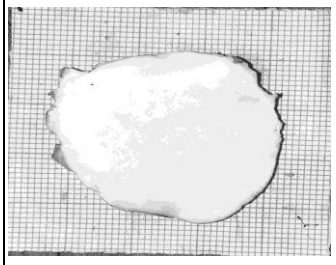
Cinnamic acid(2.5mM)			
Citric acid (25mM)			
Sodium azide (3mM)			
L-Cysteine (0.6mM)			
Sodium benzoate (20mM)			

TABLE 4.2.2: Effect of L-ascorbic acid on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.1	0.3	0.6
0	40.61	52.00	29.34
10	47.44	50.78	23.23
20	39.37	50.17	13.22
30	61.09	59.47	24.94
60	60.90	60.89	45.69
90	62.61	61.99	44.40
120	56.17	45.54	19.28

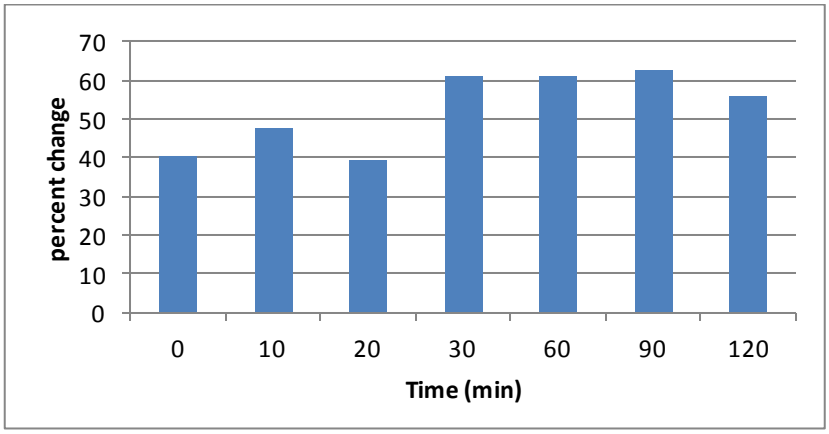


Fig 4.2.1: Effect of 0.1mM L-ascorbic acid on browning of mushroom slices

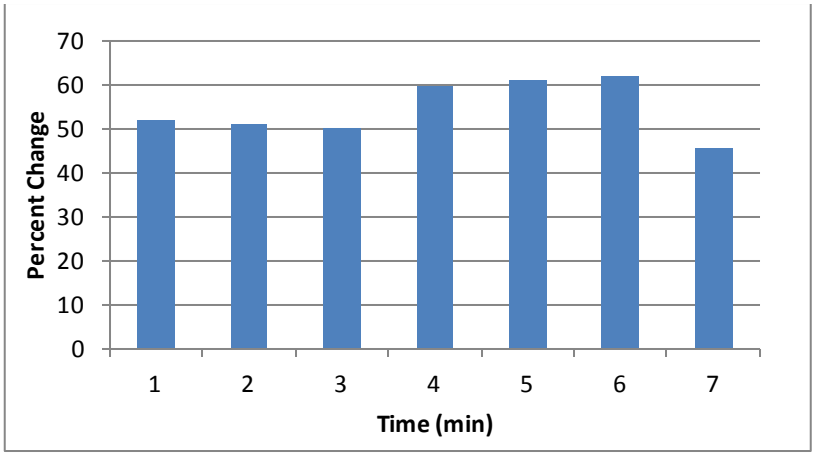


Fig 4.2.2: Effect of 0.3 mM L-ascorbic acid on browning of mushroom slices

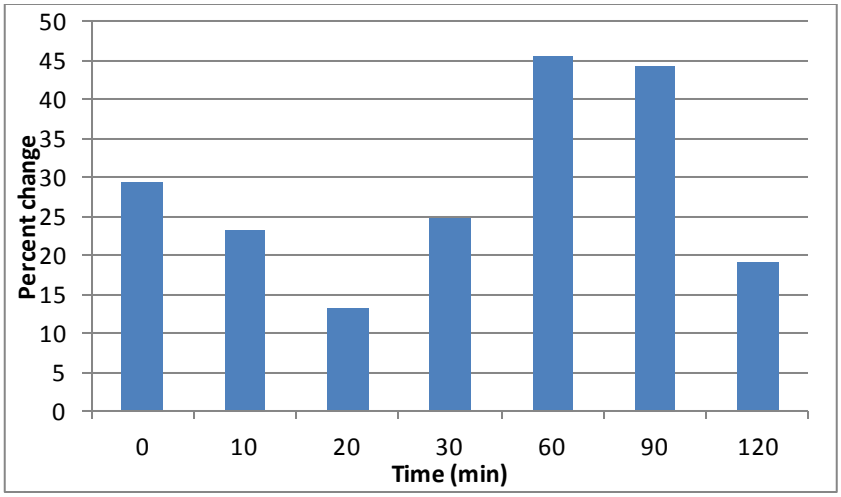


Fig 4.2.3: Effect of 0.6mM L-ascorbic acid on browning of mushroom slices

Inhibition of browning using L-ascorbic acid showed positive effect on the mushroom slices. It was observed that, mushroom slices treated with L-ascorbic acid showed lesser browning compared to that of the untreated (Control) mushroom slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentrations of L-ascorbic acid used for comparative study of anti-browning over a period of 120 minutes on the mushroom slices. Mushroom slices treated with L-ascorbic acid at the concentration of 0.3mM possessed the best effect of anti-browning in comparison to the other considered concentrations 0.1mM and 0.6mM. This was because mushroom slices treated with 0.3mM solution of L-ascorbic acid showed a degree of defence from browning consistently for the observed period.

TABLE 4.2.3: Effect of Potassium metabisulphite on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.05	0.1	0.3
0	-33.62	-30.72	-35.72
10	11.86	2.450	-8.313
20	23.62	15.54	0.249
30	-15.70	5.58	3.483
60	-7.23	-20.73	5.722
90	-6.90	37.93	12.48
120	-6.90	-13.80	39.50

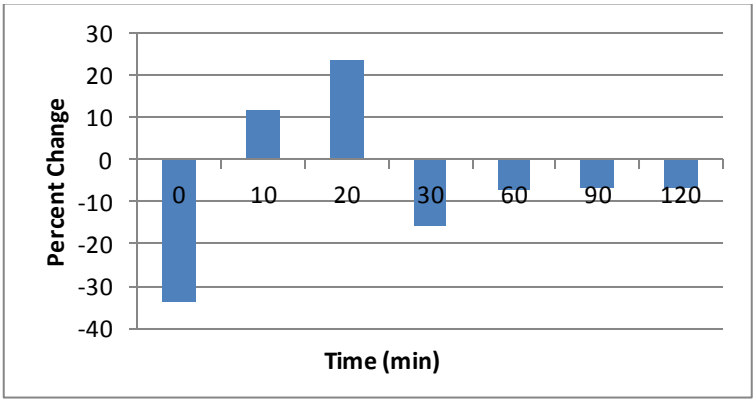


Fig.4.2.4: Effect of 0.05mM potassium metabisulphite on browning of mushroom slices

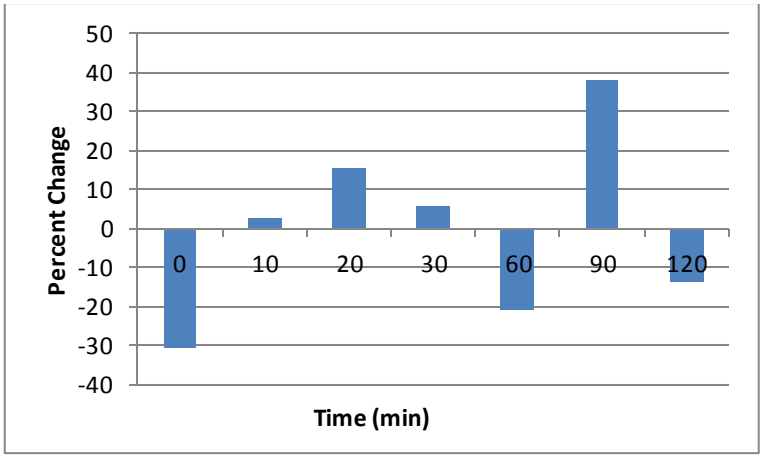


Fig.4.2.5: Effect of 0.1mM potassium metabisulphite on browning of mushroom slices

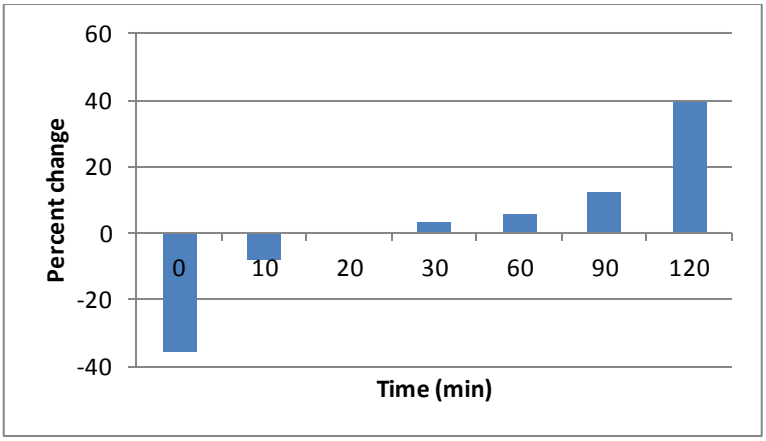


Fig.4.2.6: Effect of 0.3 mM potassium metabisulphite on browning of mushroom slices

Inhibition of browning using potassium metabisulphite also showed positive effect on the mushroom slices. It was observed that, mushroom slices treated with potassium metabisulphite showed lesser browning compared to that of the untreated (Control) mushroom slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentrations of potassium metabisulphite used for comparative study of anti-browning over a period of 120 minutes on the mushroom slices. Mushroom slices treated with potassium metabisulphite at the concentration of 0.3mM possessed the best effect of anti-browning in comparison to the other considered concentrations 0.05mM and 0.1mM. This was because mushroom slices treated with 0.05mM and 0.1mM showed fluctuation in inhibiting the browning effect over a period of 120 minutes. But use of 0.3mM concentration of potassium metabisulphite showed strong effect in preventing the browning right through the study period of 120 minutes.

TABLE 4.2.4: Effect of Benzoic acid on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	4	8	12
0	-76.54	4.24	-19.50
10	-43.10	-21.30	-40.06
20	-55.03	-23.51	-50.94
30	-24.91	-24.95	-1.00
60	-18.56	-16.24	-16.07
90	-29.56	-12.43	-0.41
120	-19.62	-28.96	10.46

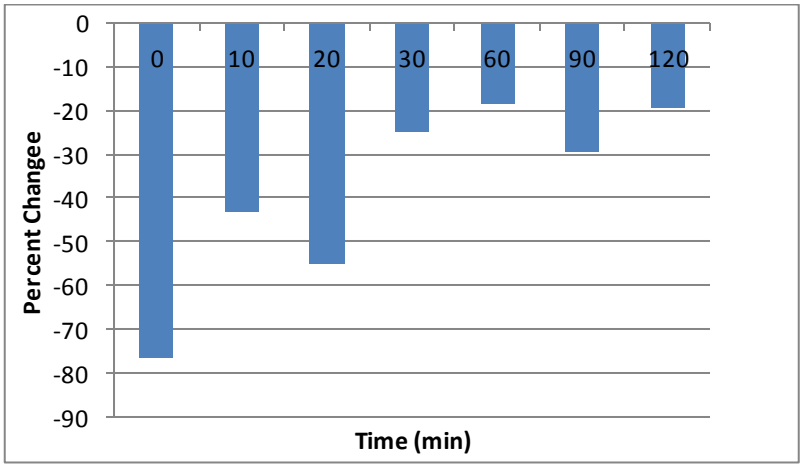


Fig.4.2.7: Effect of 4mM Benzoic acid on browning of mushroom slices

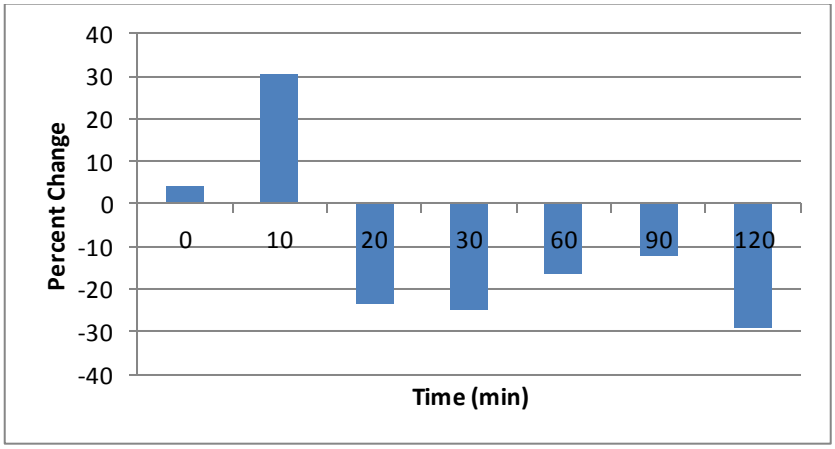


Fig.4.2.8: Effect of 8mM Benzoic acid on browning of mushroom slices

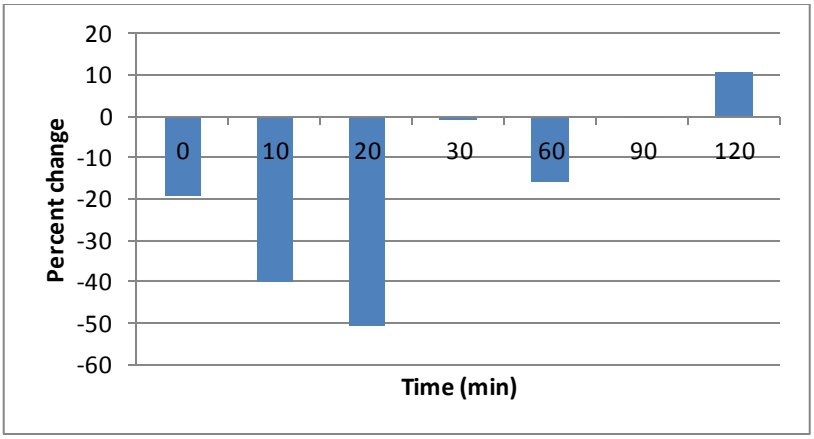


Fig.4.2.9: Effect of 12mM Benzoic acid on browning of mushroom slices

Inhibition of browning using Benzoic acid showed negative effect on the mushroom slices. It was observed that, mushroom slices treated with Benzoic acid showed no influence in preventing the browning of mushroom slices. The observation was evident from the results of analysis based on the integrated density value. All the three different concentrations (4mM, 8mM, 12mM) of Benzoic acid used for comparative study of anti-browning showed no potency as the mushrooms treated with Benzoic acid continued to brown just as the untreated ones (Control).

TABLE 4.2.5: Effect of Cinnamic acid on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.5	1.5	2.5
0	20.14	-14.78	-65.45
10	26.24	-18.54	-12.50
20	-29.84	-115.60	2.17
30	-52.76	-45.62	-47.95
60	-55.58	26.05	45.17
90	0.210	3.86	-11.06
120	57.02	65.23	44.43

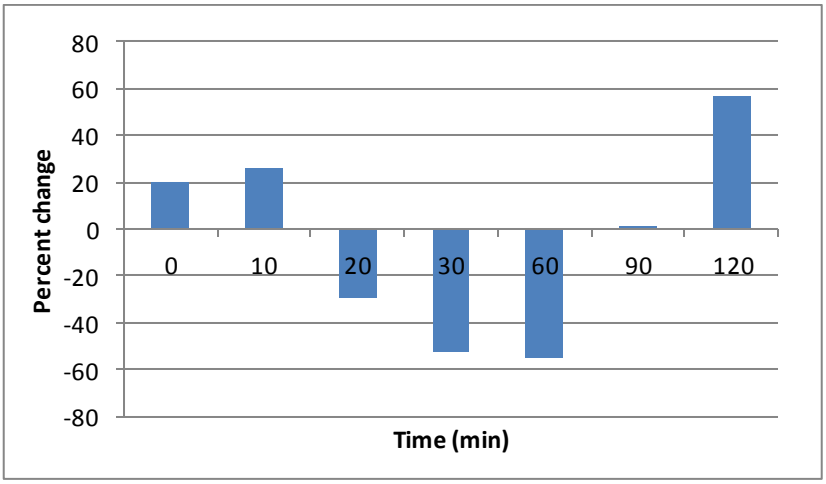


Fig.4.2.10: Effect of 0.5mM Cinnamic acid on browning of mushroom slices

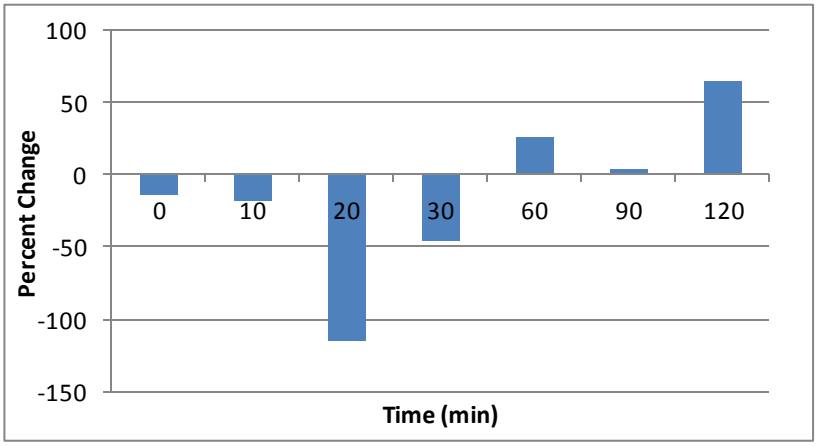


Fig.4.2.11: Effect of 1.5mM Cinnamic acid on browning of mushroom slices

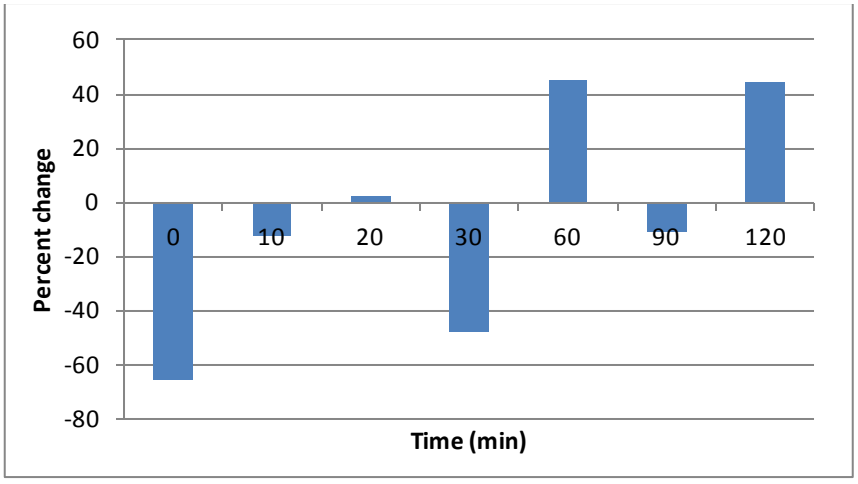


Fig.4.2.12: Effect of 2.5mM Cinnamic acid on browning of mushroom slices

Inhibition of browning using Cinnamic acid showed no significant effect on the mushroom slices. It was observed that, mushroom slices treated with Cinnamic acid continued to brown just like those of the untreated mushroom slices. The only positive change observed in the experiment was that the mushroom slices treated with 0.5mM prevented browning for a period of 10 minutes. But the effect started to deplete soon after. At the outset, Cinnamic acid showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.2.6: Effect of Citric acid on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	5	15	25
0	29.98	10.70	-6.19
10	27.41	-5.42	-7.82
20	28.75	36.92	-6.87
30	34.42	32.81	-12.04
60	32.47	30.73	-7.98
90	27.23	33.72	-10.12
120	27.27	31.02	-7.68

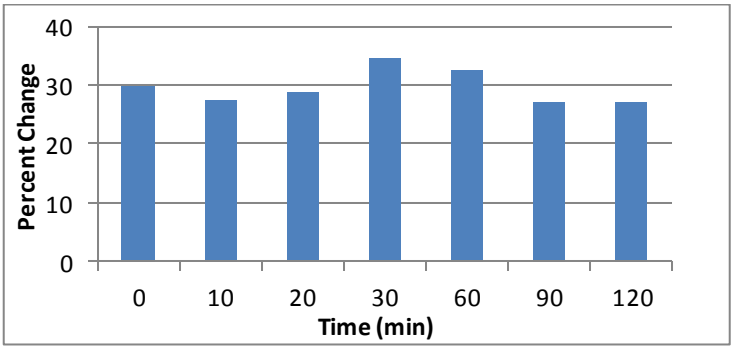


Fig.4.2.13: Effect of 5mM Citric acid on browning of mushroom slices

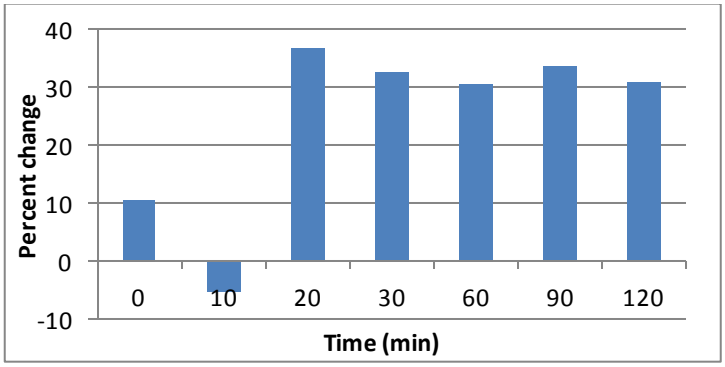


Fig.4.2.14: Effect of 15mM Citric acid on browning of mushroom slices

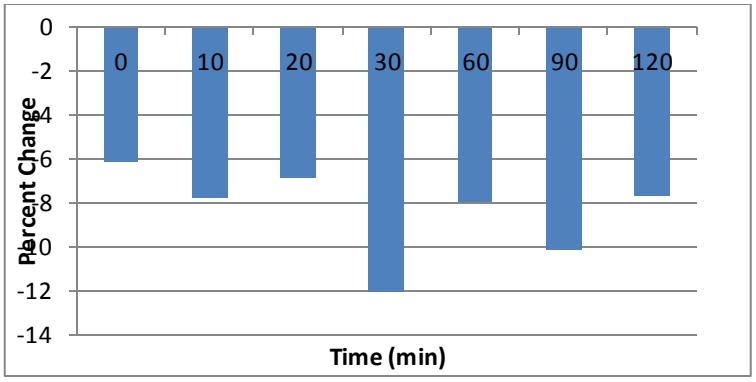


Fig.4.2.15: Effect of 25mM Citric acid on browning of mushroom slices

Inhibition of browning using Citric acid showed positive effect on the mushroom slices for the concentrations 5mM and 15mM. It was observed that, mushroom slices treated with Citric acid showed lesser browning compared to that of the untreated (Control) mushroom slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentration of Citric acid was used for comparative study of anti-browning over a period of 120 minutes on the mushroom slices. Mushroom slices treated with Citric acid at the concentration of 15mM possessed the best effect of anti-browning in comparison to the other considered concentrations 5mM and 25mM. This was because mushroom slices treated with 0.3mM solution of citric acid showed a good degree of defence from browning consistently for the observed period.

TABLE 4.2.7: Effect of Sodium azide on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	1	2	3
0	23.03	-43.66	40.24
10	25.78	-53.78	16.90
20	51.21	5.177	23.82
30	46.58	11.10	17.02
60	43.56	-1.10	17.01
90	42.29	10.68	22.19
120	43.03	11.83	19.77

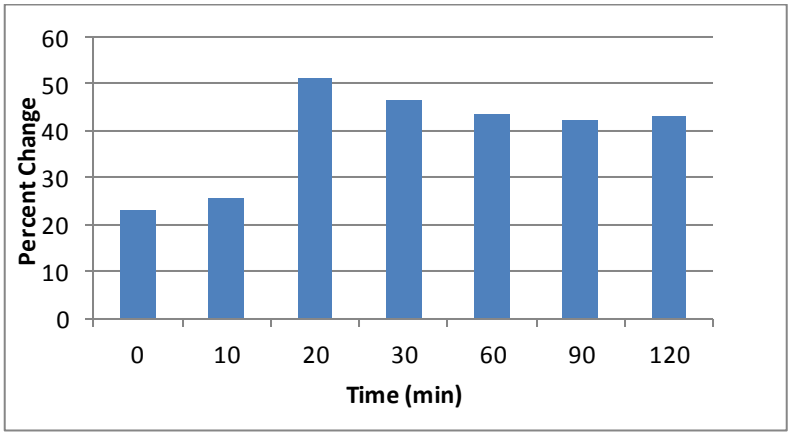


Fig.4.2.16: Effect of 1mM Sodium azide on browning of mushroom slices

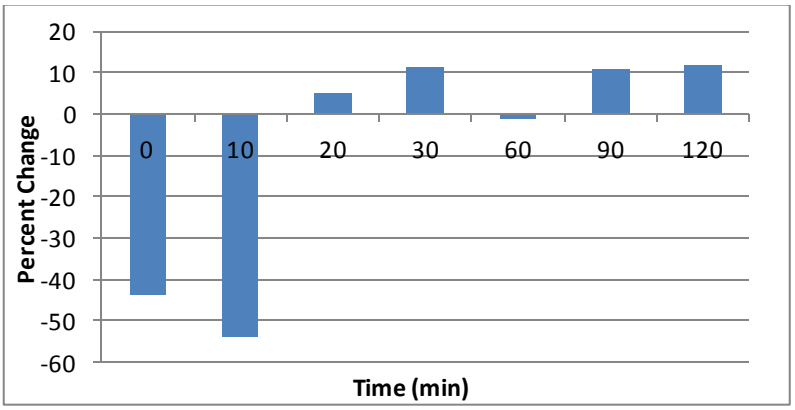


Fig.4.2.17: Effect of 2mM Sodium azide on browning of mushroom slices

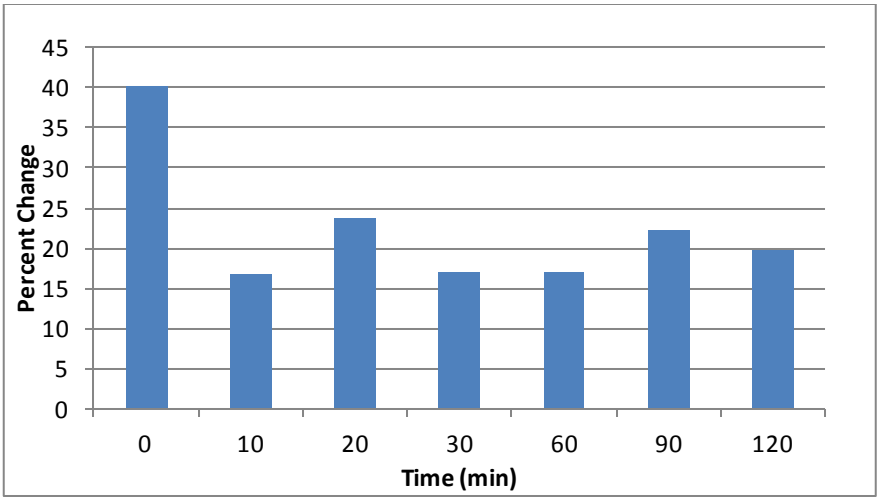


Fig.4.2.18: Effect of 3mM Sodium azide on browning of mushroom slices

Inhibition of browning using Sodium azide showed positive effect on the mushroom slices for the concentrations 1mM and 3mM. It was observed that, mushroom slices treated with Sodium azide showed lesser browning compared to that of the untreated (Control) mushroom slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentrations of Sodium azide was used for comparative study of anti-browning over a period of 120 minutes on the mushroom slices. Mushroom slices treated with Sodium azide at the concentration of 1mM and 3mM possessed the best effect of anti-browning in comparison to the other considered concentration 2mM. This was because mushroom slices treated with 1mM solution of Sodium azide showed a good degree of defence from browning consistently for the observed period.

TABLE 4.2.8: Effect of L-Cysteine on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.1	0.3	0.6
0	-61.24	80.11	-11.29
10	-10.52	-27.60	-18.27
20	31.75	13.56	48.45
30	1.30	0.478	-51.58
60	-26.80	33.64	13.06
90	-14.74	-82.11	-37.11
120	44.04	21.71	20.14

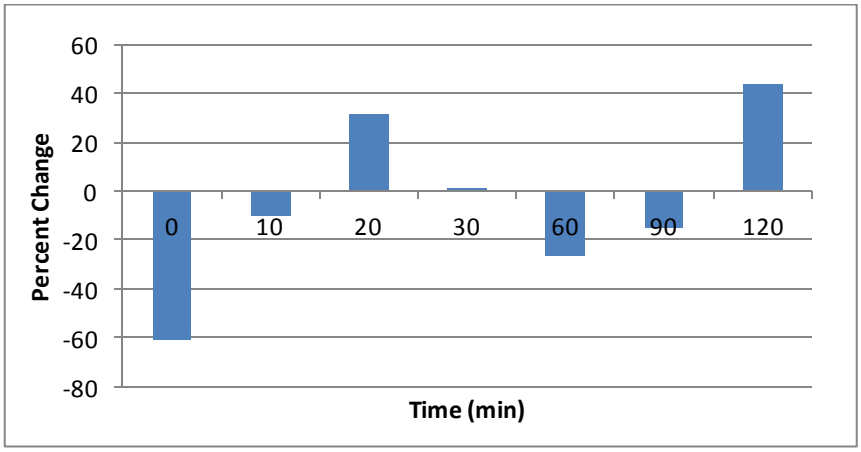


Fig.4.2.19: Effect of 0.1mM L-Cysteine on browning of mushroom slices

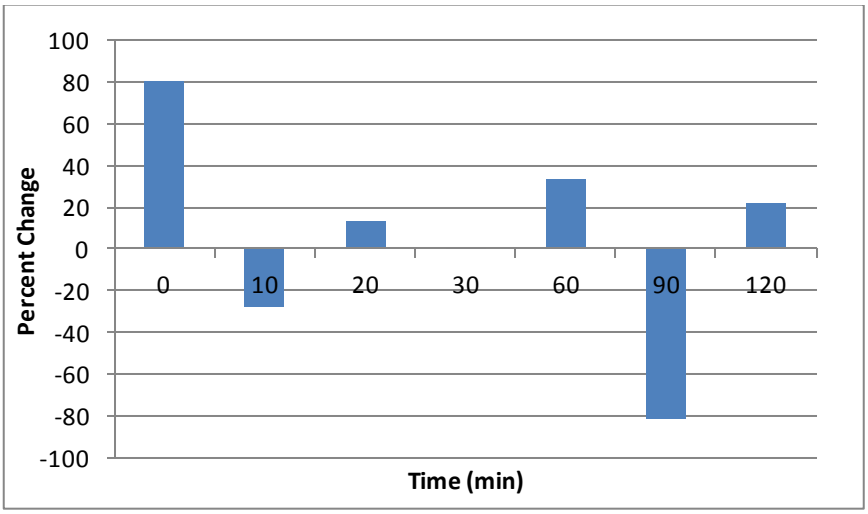


Fig.4.2.20: Effect of 0.3mM L-Cysteine on browning of mushroom slices

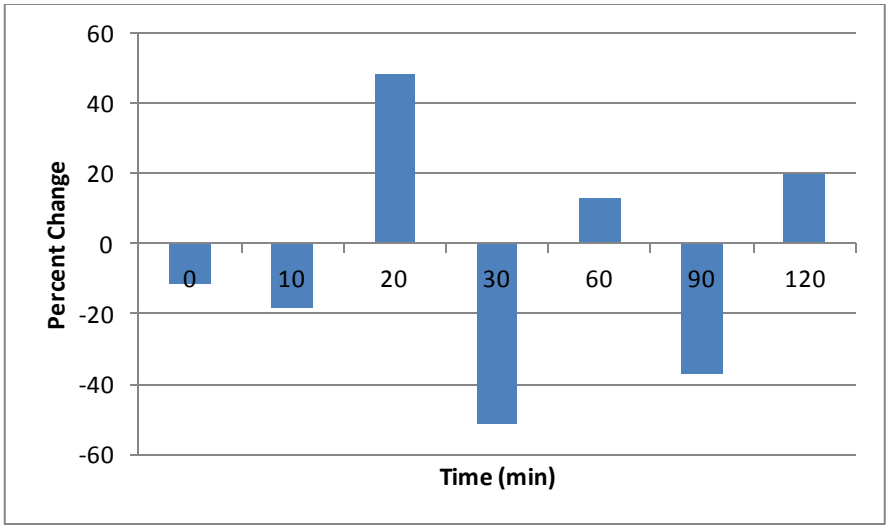


Fig.4.2.21: Effect of 0.6mM L-Cysteine on browning of mushroom slices

Inhibition of browning using L-Cysteine showed no significant effect on the mushroom slices. It was observed that, mushroom slices treated with L-Cysteine continued to brown just like those of the untreated mushroom slices. The only positive change observed in the experiment was that the mushroom slices treated with 0.3mM prevented browning for a period of 60 minutes. But the effect started to deplete soon after. At the outset, L-Cysteine showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.2.9: Effect of Sodium benzoate on browning of mushroom slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	5	10	20
0	36.71	46.21	25.86
10	26.72	36.24	-0.689
20	-96.02	22.14	35.69
30	-66.09	-68.82	18.78
60	35.64	-51.27	-34.57
90	30.98	-69.65	-63.95
120	28.95	-71.67	-50.89

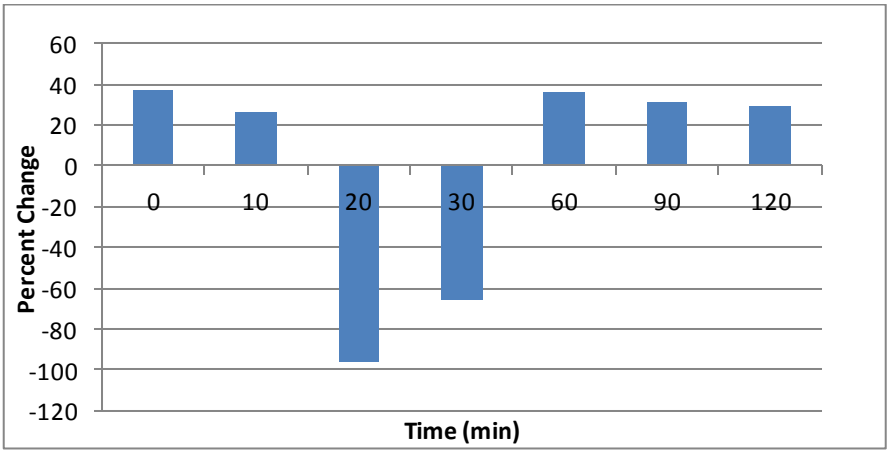


Fig.4.2.22: Effect of 5mM Sodium benzoate on browning of mushroom slices

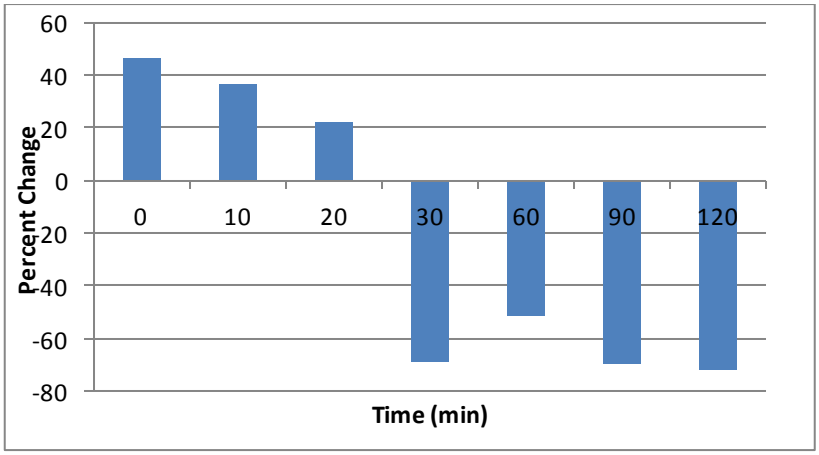


Fig.4.2.23: Effect of 10mM Sodium benzoate on browning of mushroom slices

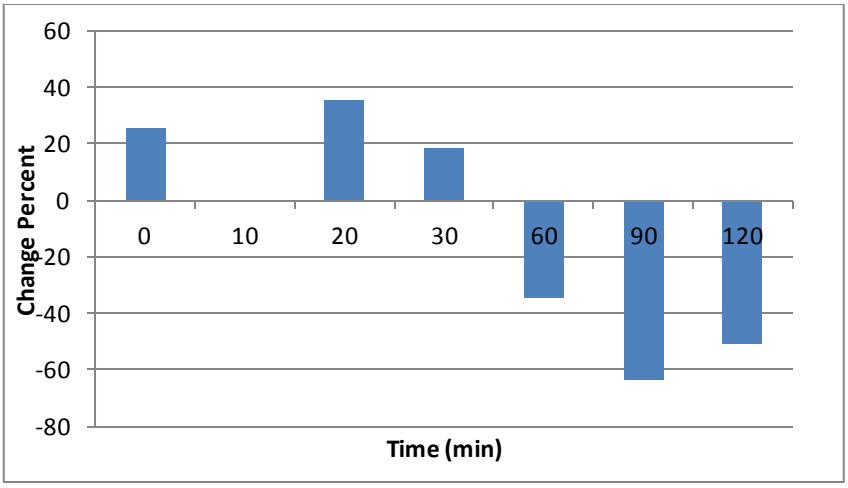
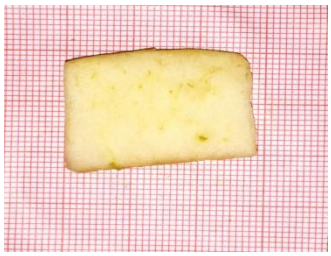
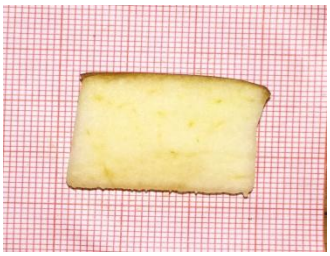
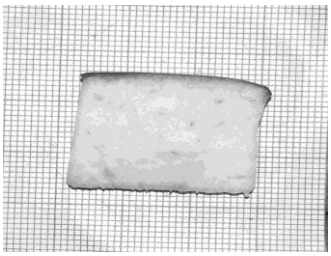
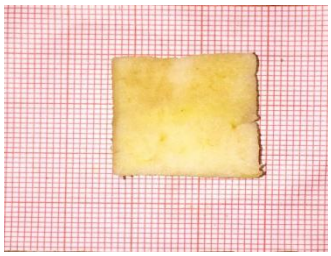
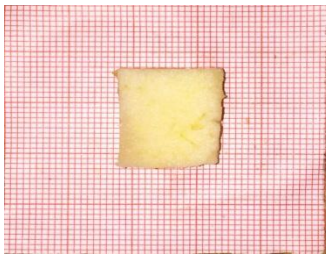
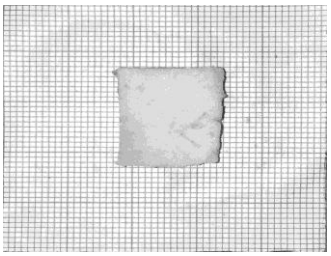
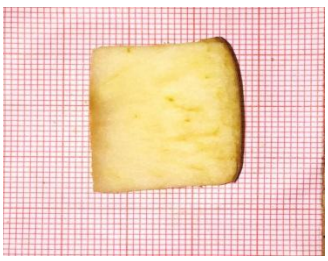
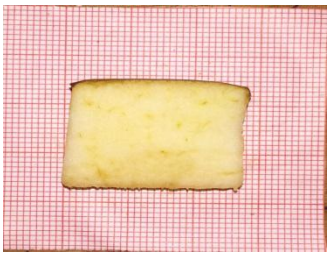
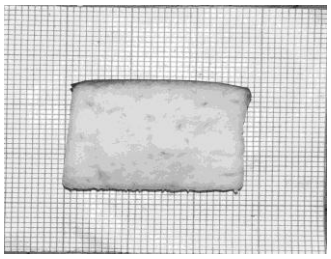


Fig.4.2.24: Effect of 20mM Sodium benzoate on browning of mushroom slices

Inhibition of browning using Sodium benzoate showed no significant effect on the mushroom slices. It was observed that, mushroom slices treated with Sodium benzoate continued to brown just like those of the untreated mushroom slices. The only positive change observed in the experiment was that the mushroom slices treated with 10mM prevented browning for a period of 20 minutes. But the effect started to deplete soon after. At the outset, Sodium benzoate showed no ability as an anti-browning agent for a duration period greater than an hour.

4.3 EFFECT OF ANTIBROWNING AGENTS ON APPLE

TABLE 4.3.1: Representative images of the original and 8-bit grayscale images of apple slices treated with various inhibitors (at 120th min)

Sample (concentration)	Original image		Grayscale image used for analysis
	Control	After treatment	
L-ascorbic acid (0.6mM)			
Potassium metabisulphite (0.3mM)			
Benzoic acid (12M			

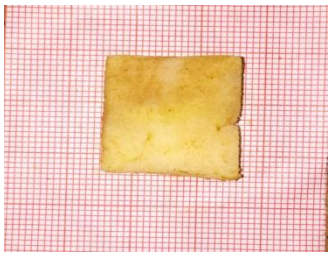
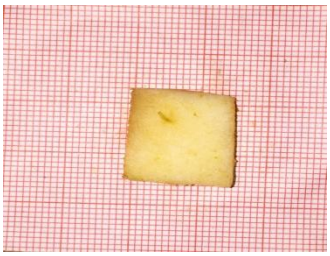
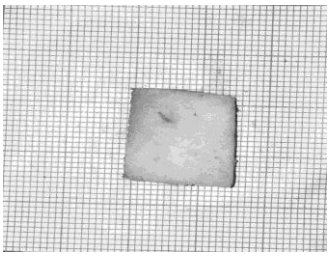
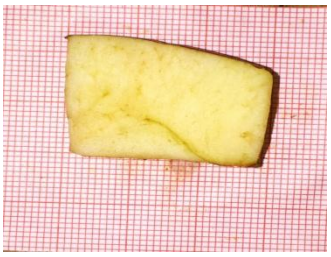
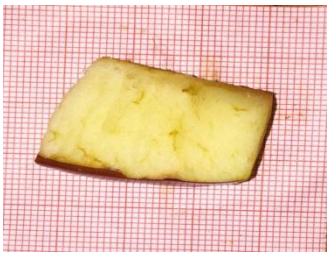
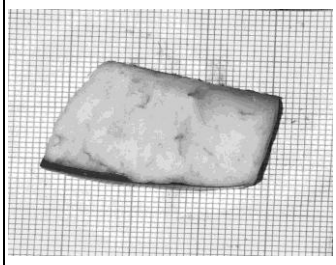
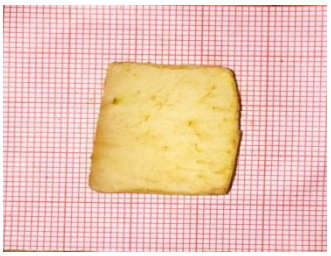
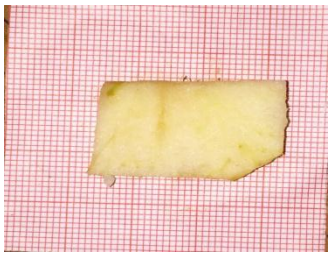
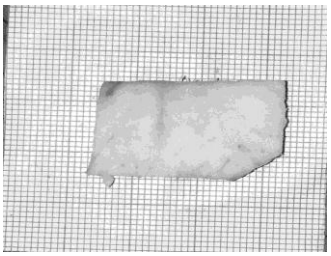
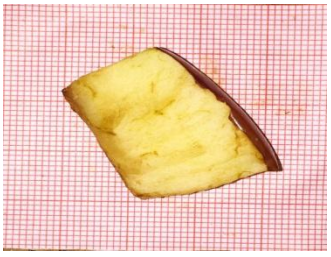
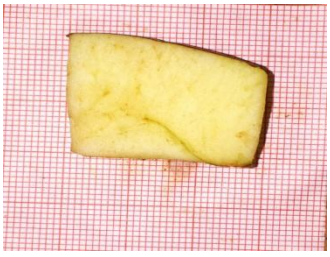
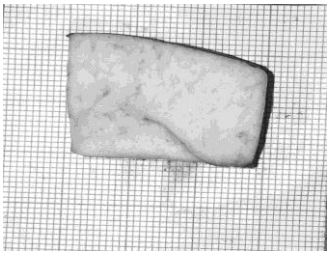
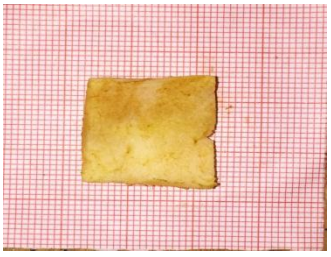
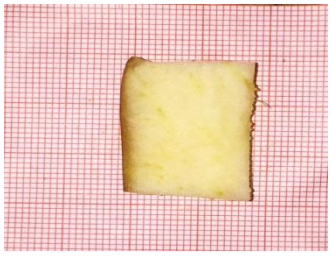
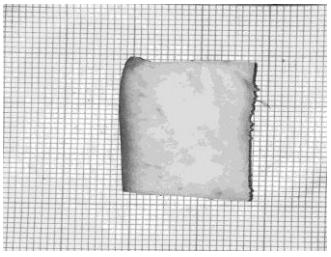
Cinnamic acid(2.5mM)			
Citric acid (25mM)			
Sodium azide (3mM)			
L-Cysteine (0.6mM)			
Sodium benzoate (20mM)			

TABLE 4.3.2: Effect of L-ascorbic acid on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.1	0.3	0.6
0	-74.44	-66.90	-4.019
10	8.212	-1.421	50.17
20	17.48	-2.497	38.70
30	-6.85	20.92	41.30
60	-2.09	8.274	56.49
90	4.708	20.92	2.007
120	-2.74	15.02	-6.455

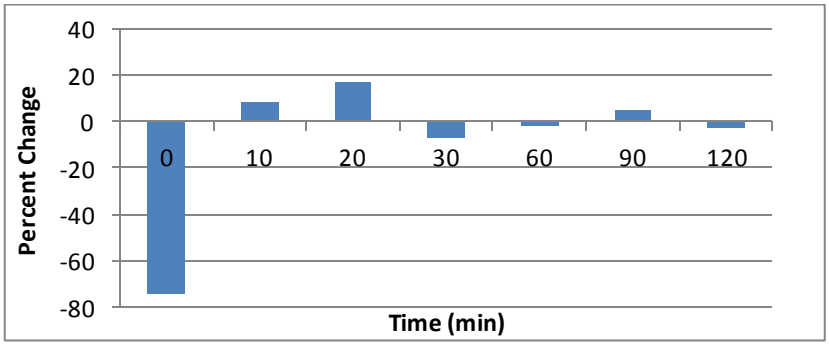


Fig.4.3.1: Effect of 0.1mM L-ascorbic acid on browning of apple slices

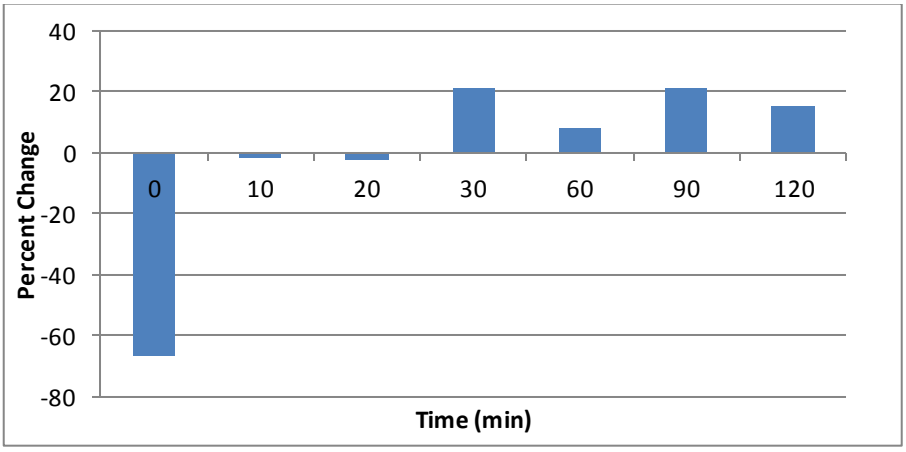


Fig.4.3.2: Effect of 0.3mM L-ascorbic acid on browning of apple slices

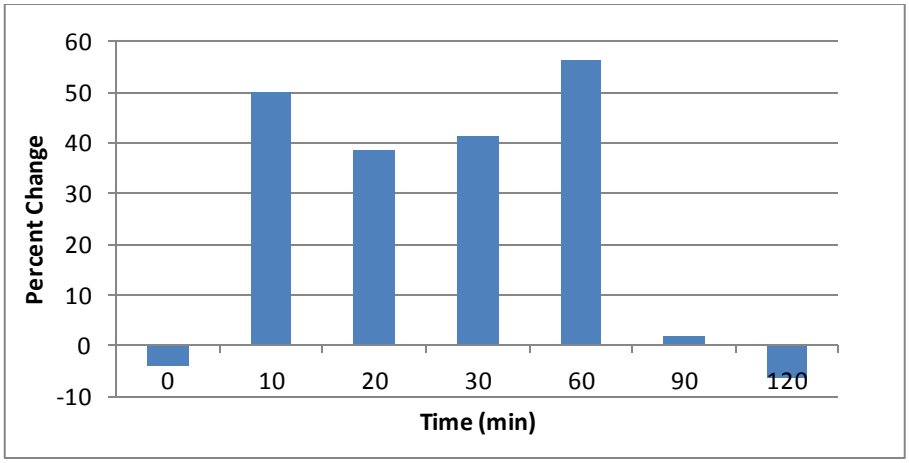


Fig.4.3.3: Effect of 0.6mM L-ascorbic acid on browning of apple slices

Inhibition of browning using L-ascorbic acid showed positive effect on the apple slices. It was observed that, apple slices treated with L-ascorbic acid showed lesser browning compared to that of the untreated (Control) apple slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentrations of L-ascorbic acid was used for comparative study of anti-browning over a period of 120 minutes on the apple slices. Apple slices treated with L-ascorbic acid at the concentration of 0.6mM possessed the best effect of anti-browning in comparison to the other considered concentrations 0.1mM and 0.3mM. This was because apple slices treated with 0.6mM solution of L-ascorbic acid showed a degree of defence from browning consistently for the observed period.

TABLE 4.3.3: Effect of Potassium metabisulphite on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.05	0.1	0.3
0	-141.211	-23.16	-3.33
10	-222.901	-45.14	-13.18
20	-119.624	-41.93	-9.55
30	-24.18	-7.749	-6.40
60	-112.532	-40.38	-21.99
90	-140.441	-47.19	7.69
120	-111.721	-66.22	18.93

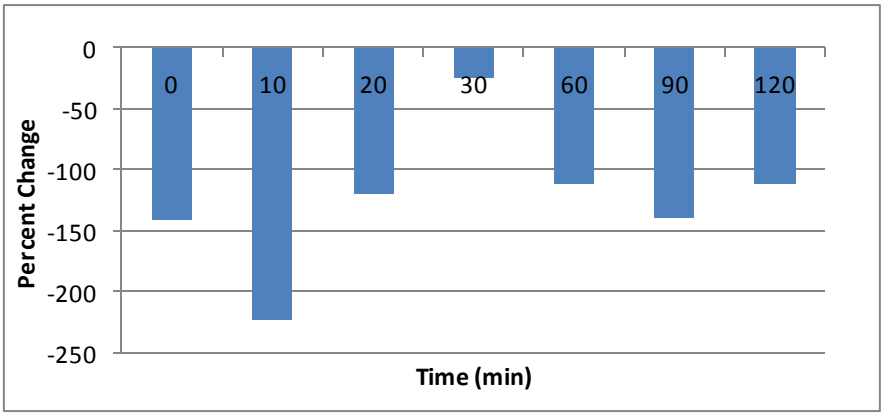


Fig.4.3.4: Effect of 0.05mM Potassium metabisulphite on browning of apple slices

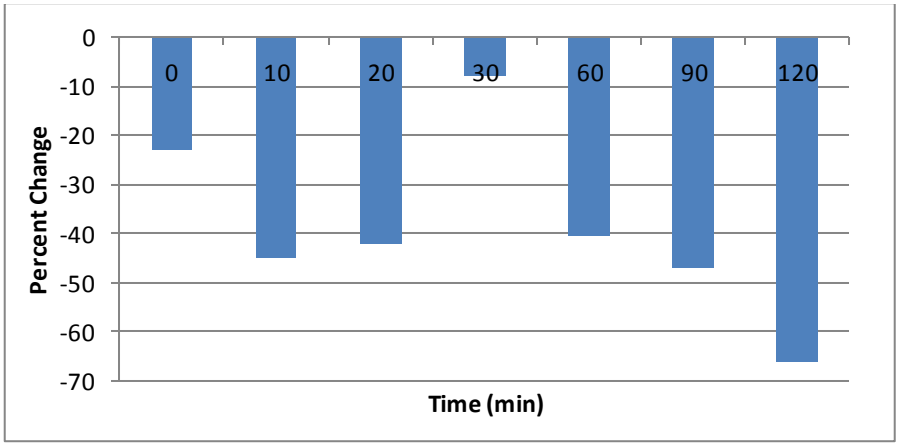


Fig.4.3.5: Effect of 0.1mM Potassium metabisulphite on browning of apple slices

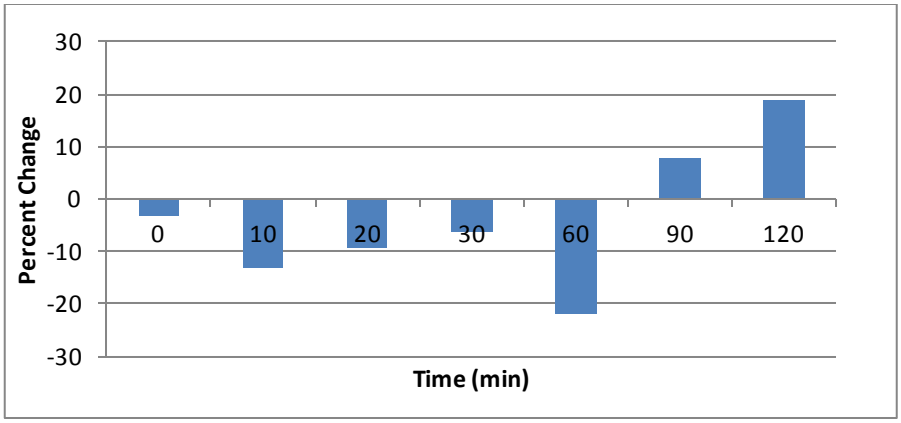


Fig.4.3.6: Effect of 0.3mM Potassium metabisulphite on browning of apple slices

Inhibition of browning using Potassium metabisulphite showed negative effect on the apple slices. It was observed that, apple slices treated with Potassium metabisulphite showed no influence in preventing the browning of apple slices. The observation was evident from the results of analysis based on the integrated density value. All the three different concentrations (0.05mM, 0.1mM, 0.3mM) of Potassium metabisulphite used for comparative study of anti-browning showed no potency as the apple treated with Potassium metabisulphite continued to brown just as the untreated ones (Control).

TABLE 4.3.4: Effect of Benzoic acid on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	4	8	12
0	-8.540	20.73	-1.847
10	6.382	18.63	1.338
20	10.46	30.90	6.745
30	20.480	23.88	9.181
60	49.528	26.21	13.18
90	17.613	14.55	-3.136
120	-14.55	36.36	62.89

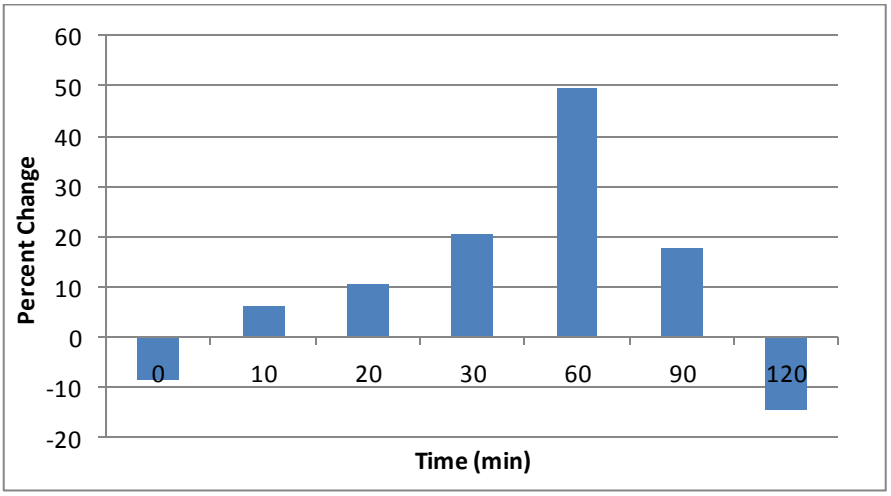


Fig.4.3.7: Effect of 4mM Benzoic acid on browning of apple slices

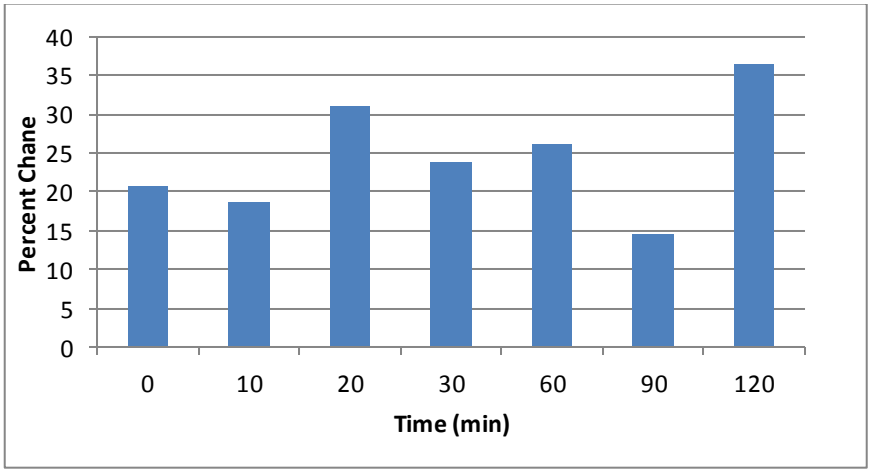


Fig.4.3.8: Effect of 8mM Benzoic acid on browning of apple slices

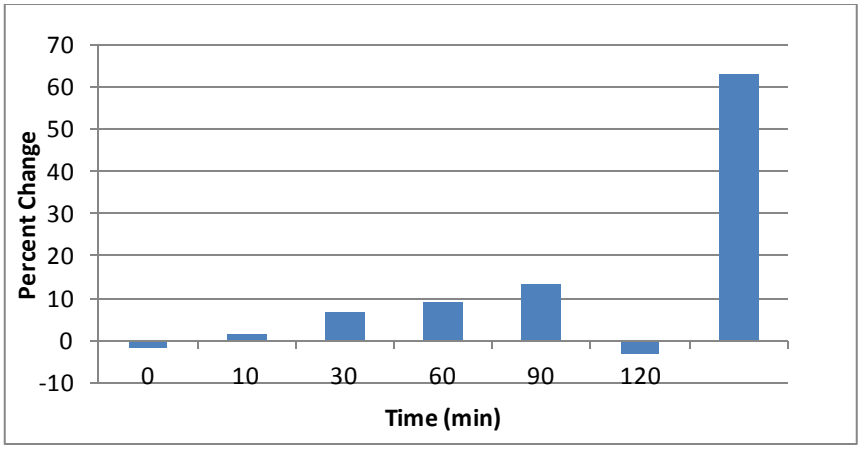


Fig.4.3.9: Effect of 12mM Benzoic acid on browning of apple slices

Inhibition of browning using Benzoic acid showed positive effect on the apple slices for the concentration 8mM. It was observed that, apple slices treated with Benzoic acid showed lesser browning compared to that of the untreated (Control) apple slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentrations of Benzoic acid was used for comparative study of anti-browning over a period of 120 minutes on the apple slices. Apple slices treated with Benzoic acid at the concentration of 8mM possessed the best effect of anti-browning in comparison to the other considered concentrations 4mM and 12mM. This was because apple slices treated with 8mM solution of Benzoic acid showed a good degree of defence from browning consistently for the observed period.

TABLE 4.3.5: Effect of Cinnamic acid on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.5	1.5	2.5
0	40.86	8.166	32.06
10	-11.92	-32.17	46.67
20	-19.35	-39.32	19.07
30	2.696	20.23	43.97
60	-29.88	-9.798	40.41
90	5.32	24.04	-5.52
120	-10.86	11.93	-30.97

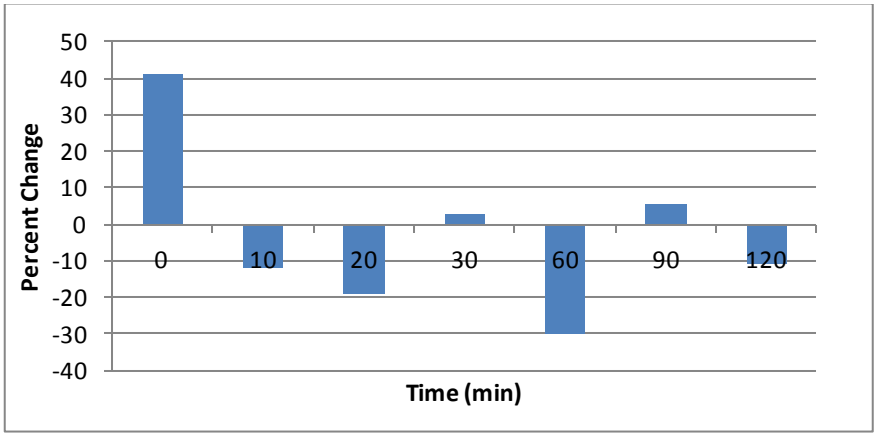


Fig.4.3.10: Effect of 0.5mM Cinnamic acid on browning of apple slices

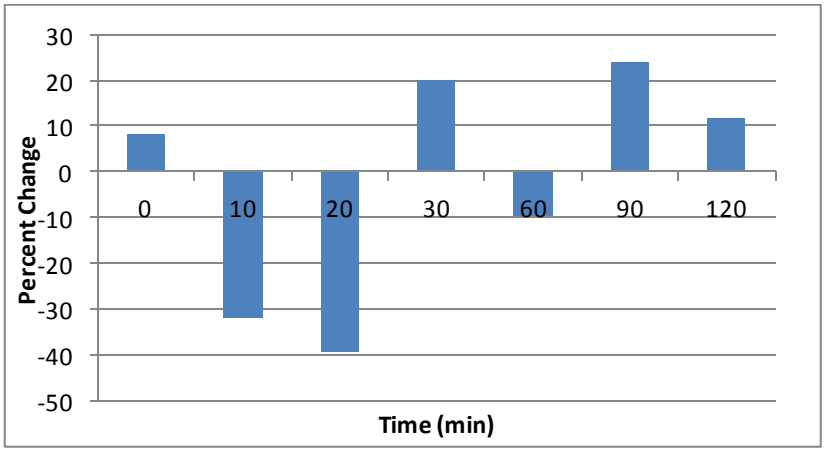


Fig.4.3.11: Effect of 1.5mM Cinnamic acid on browning of apple slices

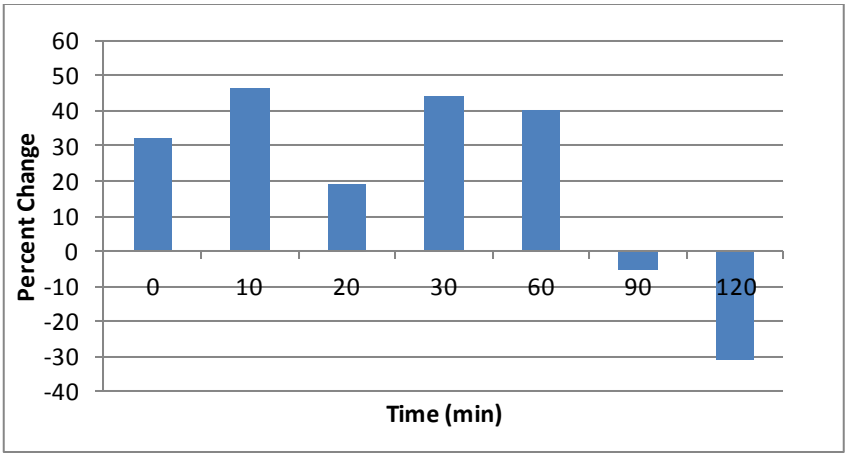


Fig.4.3.12: Effect of 2.5mM Cinnamic acid on browning of apple slices

Inhibition of browning using cinnamic acid showed no significant effect on the apple slices. It was observed that, apple slices treated with Cinnamic acid continued to brown just like those of the untreated apple slices. The only positive change observed in the experiment was that the apple slices treated with 2.5mM prevented browning for a period of 60 minutes. But the effect started to deplete soon after. At the outset, Cinnamic acid showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.3.6: Effect of Citric acid on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	5	15	25
0	-205.23	-70.02	15.08
10	-230.34	-20.35	-1.063
20	-91.134	-56.30	-11.33
30	-90.04	-34.23	13.23
60	-130.98	-8.57	-22.39
90	-99.26	-42.10	-27.51
120	26.81	50.49	16.022

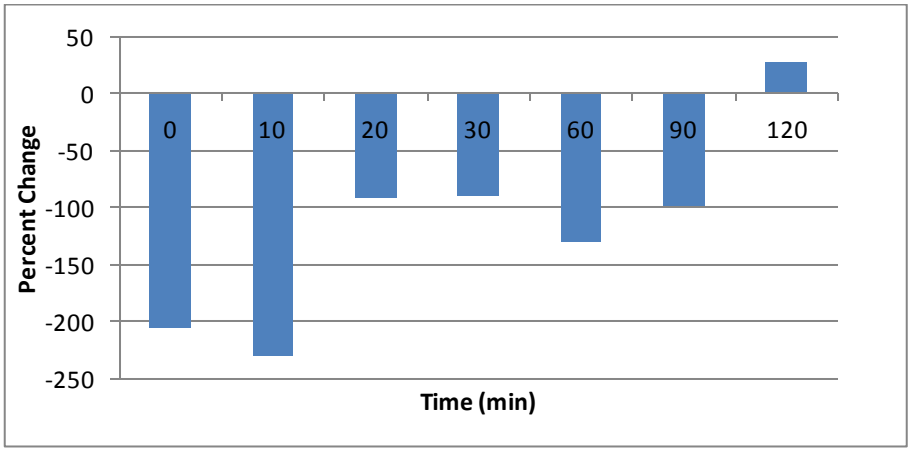


Fig.4.3.13: Effect of 5mM Citric acid on browning of apple slices

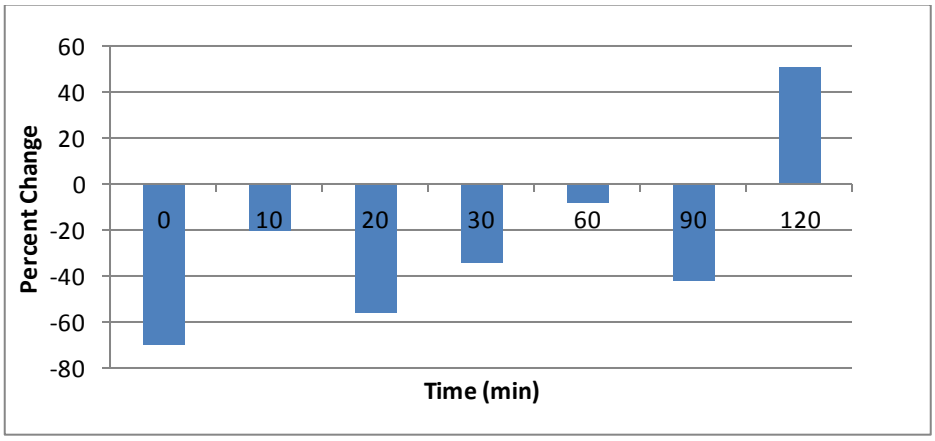


Fig.4.3.14: Effect of 15mM Citric acid on browning of apple slices

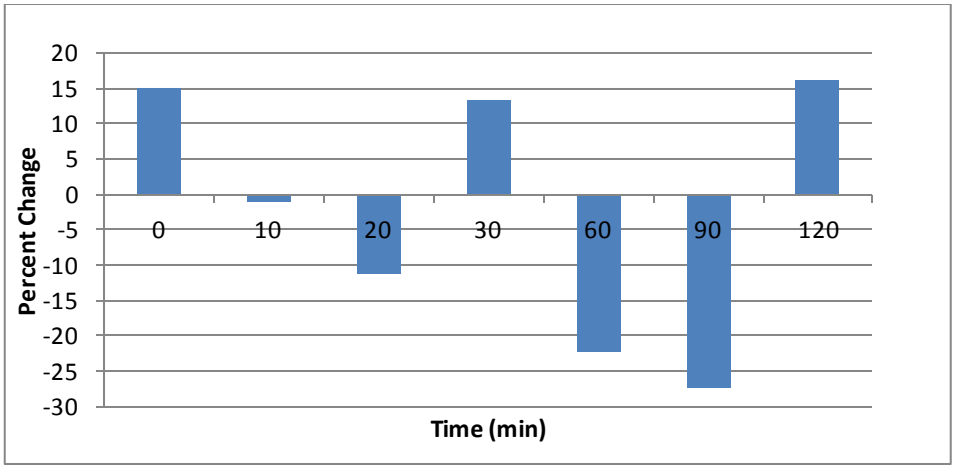


Fig.4.3.15: Effect of 25mM Citric acid on browning of apple slices

Inhibition of browning using Citric acid showed negative effect on the apple slices. It was observed that, apple slices treated with Citric acid showed no influence in preventing the browning of apple slices. The observation was evident from the results of analysis based on the integrated density value. All the three different concentrations (5mM, 15mM, 25mM) of Citric acid used for comparative study of anti-browning showed no potency as the apple slices treated with Citric acid continued to brown just as the untreated ones (Control).

TABLE 4.3.7: Effect of Sodium azide on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	1	2	3
0	-133.99	-29.77	25.34
10	-220.27	-52.28	-30.07
20	-89.85	-41.57	-10.93
30	-37.10	-11.43	-2.094
60	-132.78	-36.56	-14.46
90	-55.01	-42.42	-7.29
120	-129.59	-65.03	-35.53

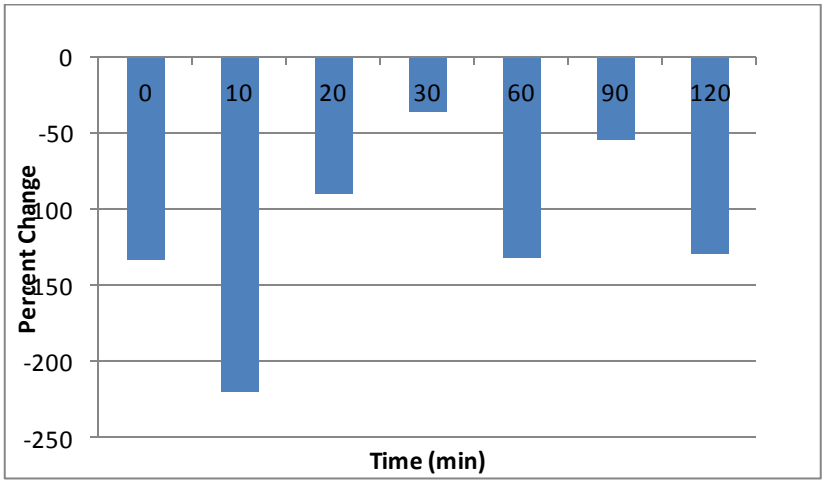


Fig.4.3.16: Effect of 1mM Sodium azide on browning of apple slices

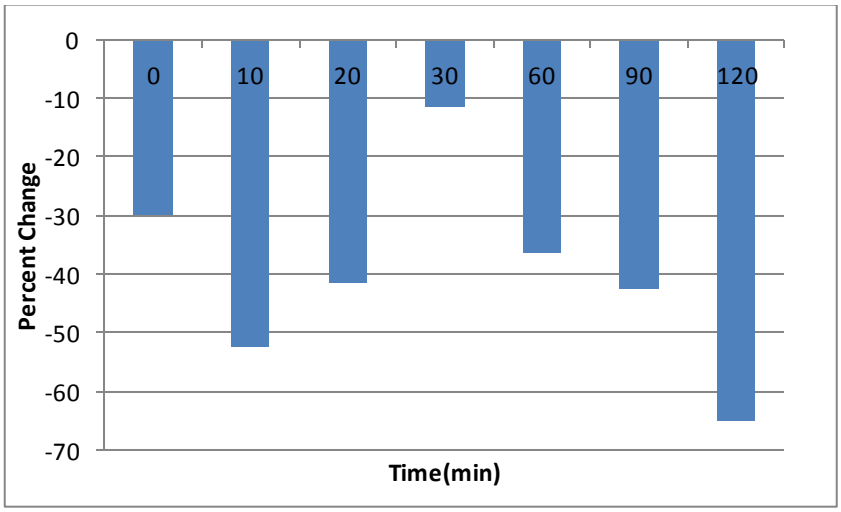


Fig.4.3.17: Effect of 2mM Sodium azide on browning of apple slices

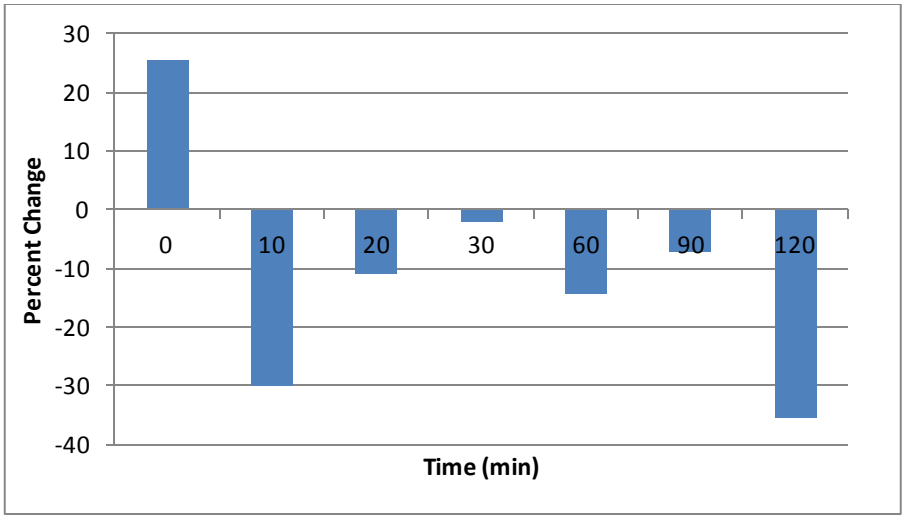


Fig.4.3.18: Effect of 3mM Sodium azide on browning of apple slices

Inhibition of browning using Sodium azide showed negative effect on the apple slices. It was observed that, apple slices treated with Sodium azide showed no influence in preventing the browning of apple slices. The observation was evident from the results of analysis based on the integrated density value. All the three different concentrations (1mM, 2mM, 3mM) of Sodium azide used for comparative study of anti-browning showed no potency as the apple slices treated with Sodium azide continued to brown just as the untreated ones (Control).

TABLE 4.3.8: Effect of L-Cysteine on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.1	0.3	0.6
0	-109.11	-21.08	29.96
10	-109.11	-21.08	29.96
20	-107.13	-44.50	-15.75
30	11.82	14.41	38.92
60	-121.13	-83.35	-37.91
90	-41.57	-9.51	5.272
120	-122.27	-33.31	27.32

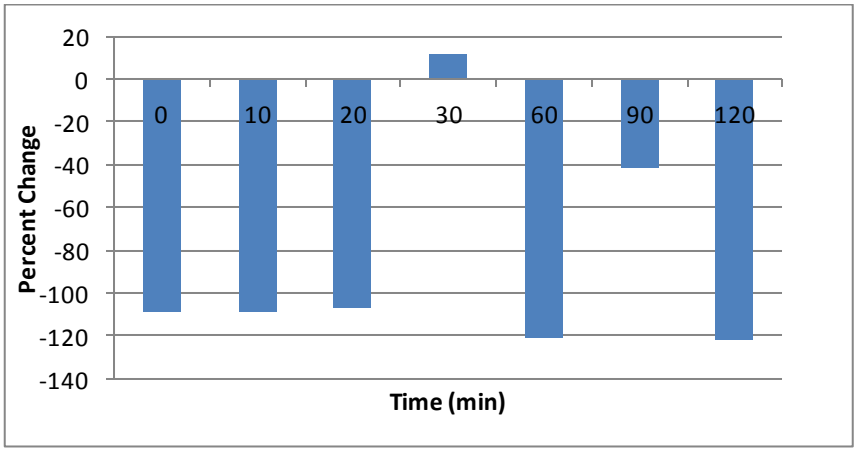


Fig.4.3.19: Effect of 0.1mM L-Cysteine on browning of apple slices

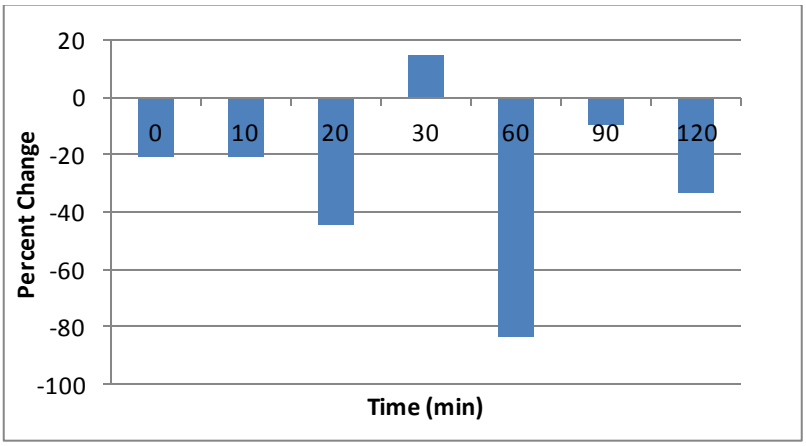


Fig.4.3.20: Effect of 0.3mM L-Cysteine on browning of apple slices

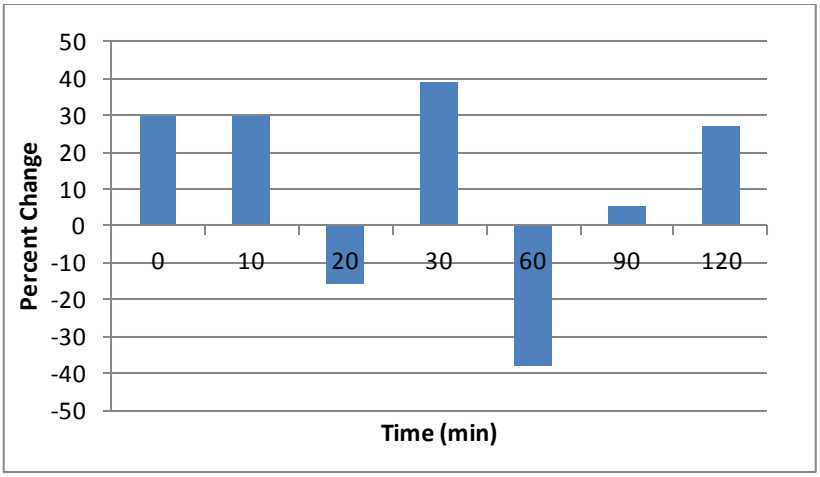


Fig.4.3.21: Effect of 0.6mM L-Cysteine on browning of apple slices

Inhibition of browning using L-Cysteine showed no significant effect on the apple slices. It was observed that, apple slices treated with L-Cysteine continued to brown just like those of the untreated apple slices. The only positive change observed in the experiment was that the apple slices treated with 0.6mM prevented browning for a period of 10 minutes. But the effect started to deplete soon after. At the outset, L-Cysteine showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.3.9: Effect of Sodium benzoate on browning of apple slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	5	10	20
0	17.34	38.85	16.16
10	-2.29	18.54	-10.35
20	-14.45	22.19	-15.73
30	14.86	21.42	-9.30
60	41.92	24.86	6.10
90	4.250	-3.88	-8.95
120	6.420	32.17	63.39

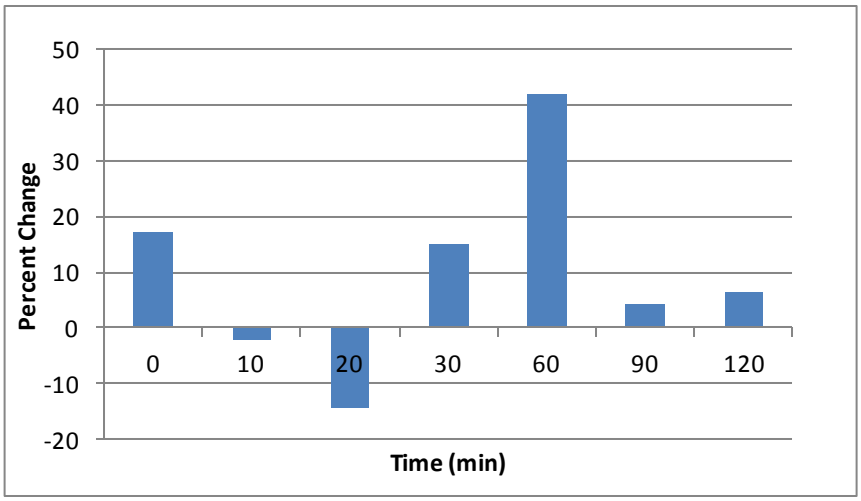


Fig.4.3.22: Effect of 5mM Sodium benzoate on browning of apple slices

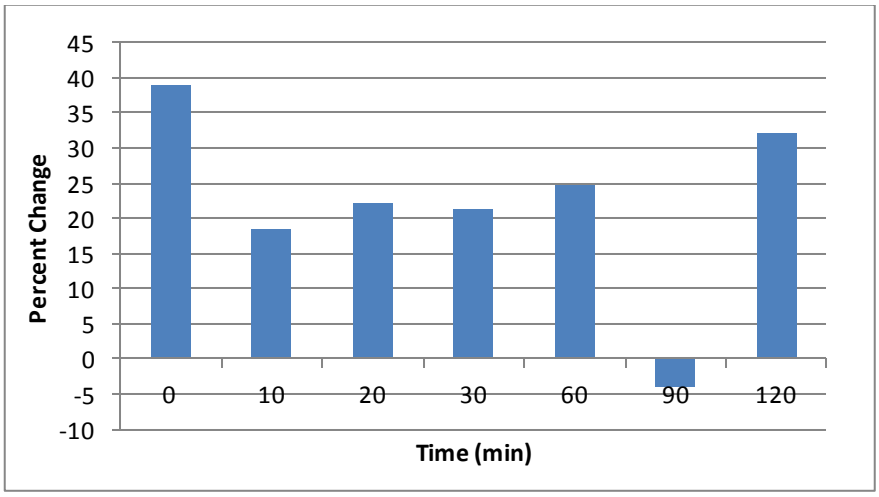


Fig.4.3.23: Effect of 10mM Sodium benzoate on browning of apple slices

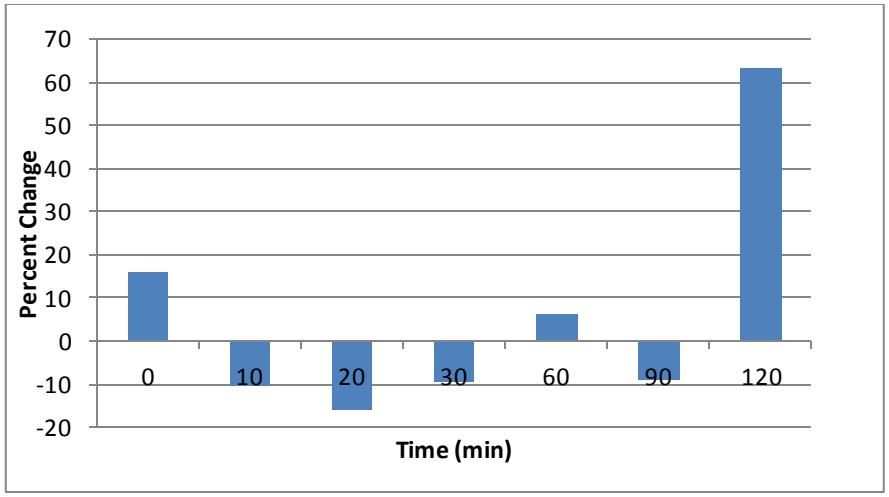
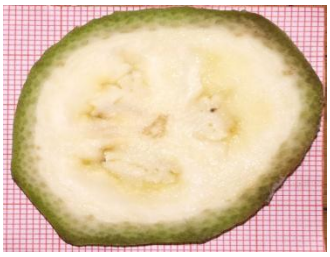
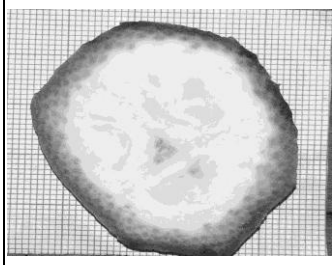
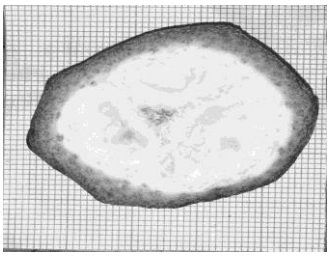
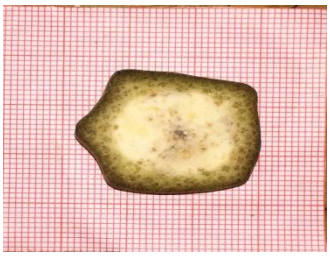
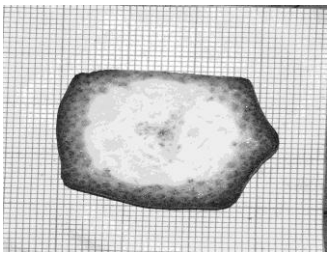
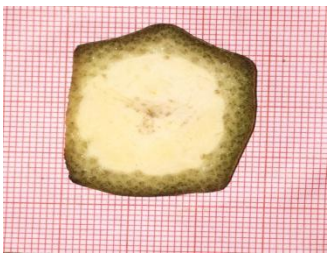
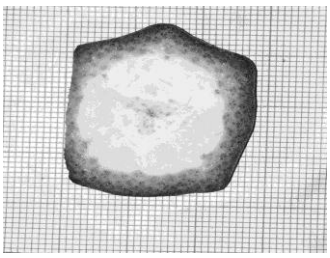


Fig.4.3.24: Effect of 20mM Sodium benzoate on browning of apple slices

Inhibition of browning using Sodium benzoate also showed positive effect on the apple slices. It was observed that, apple slices treated with Sodium benzoate showed lesser browning compared to that of the untreated (Control) mushroom slices. The observation was evident from the results of analysis based on the integrated density value. Three different concentrations of Sodium benzoate used for comparative study of anti-browning over a period of 120 minutes on the apple slices. Apple slices treated with Sodium benzoate at the concentration of 10mM possessed the best effect of anti-browning in comparison to the other considered concentrations 5mM and 20mM. This was because apple slices treated with 5mM and 20mM showed fluctuation in inhibiting the browning effect over a period of 120 minutes. But use of 10mM concentration of Sodium benzoate showed strong effect in preventing the browning right through the study period of 120 minutes.

4.4 EFFECT OF ANTIBROWNING AGENTS ON BANANA

TABLE 4.4.1: Representative images of the original and 8-bit grayscale images of banana slices treated with various inhibitors (at 120th min)

Sample (concentration)	Original image		Grayscale image used for analysis
	Control	After treatment	
L-ascorbic acid (0.6mM)			
Potassium metabisulphite (0.3mM)			
Benzoic acid (12mM)			
Cinnamic acid (2.5mM)			

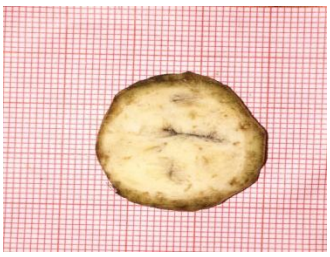
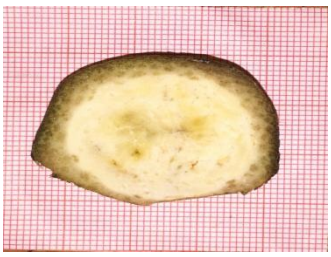
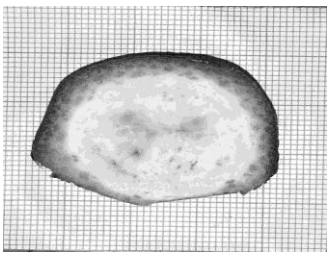
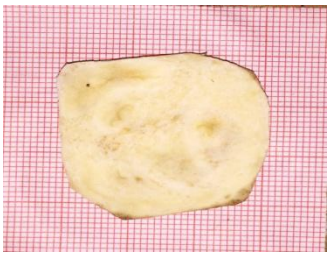
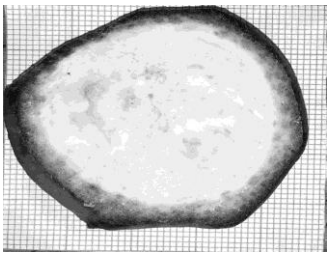
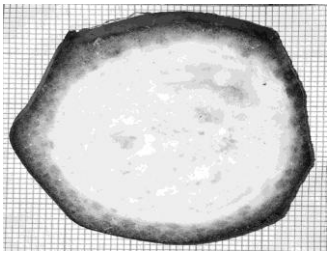
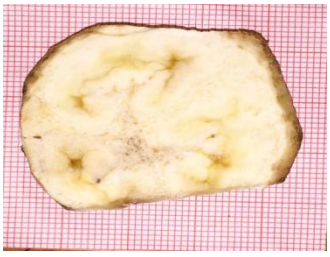
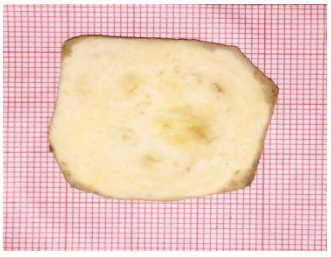
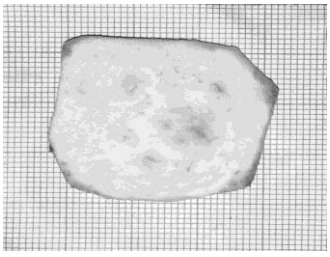
Citric acid (25mM)			
Sodium azide (3mM)			
L-Cysteine (0.6mM)			
Sodium benzoate (20mM)			

TABLE 4.4.2: Effect of L-ascorbic acid on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.1	0.3	0.6
0	-13.36	-20.98	-28.46
10	-39.66	-33.63	-26.98
20	7.46	-9.74	-26.12
30	-38.03	-28.30	18.06
60	-124.40	-139.15	-45.83
90	-12.74	-32.90	16.84
120	27.17	3.117	14.83

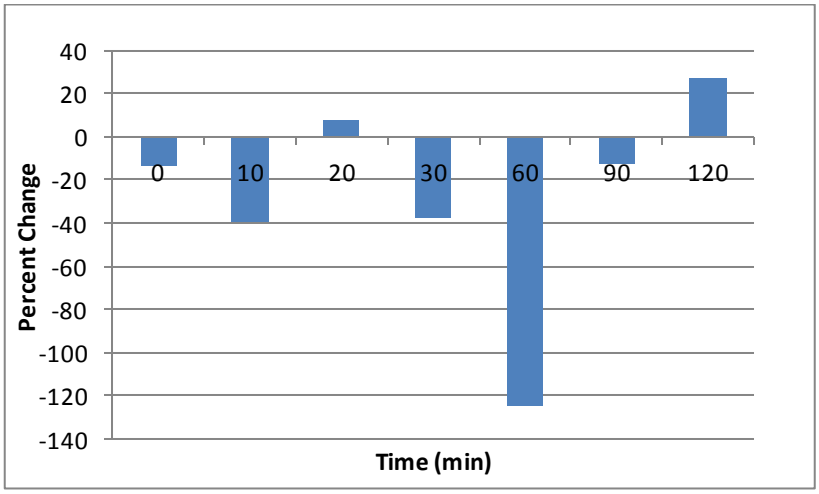


Fig.4.4.1: Effect of 0.1mM L-ascorbic acid on browning of banana slices

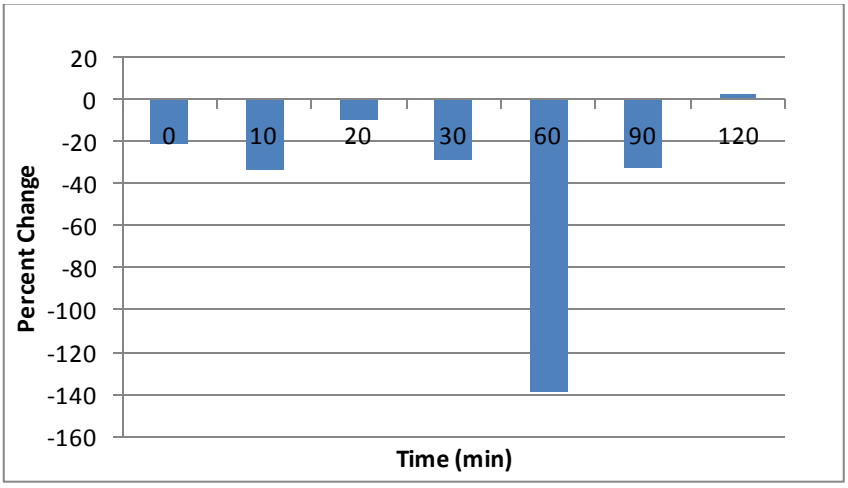


Fig.4.4.2: Effect of 0.3mM L-ascorbic acid on browning of banana slices

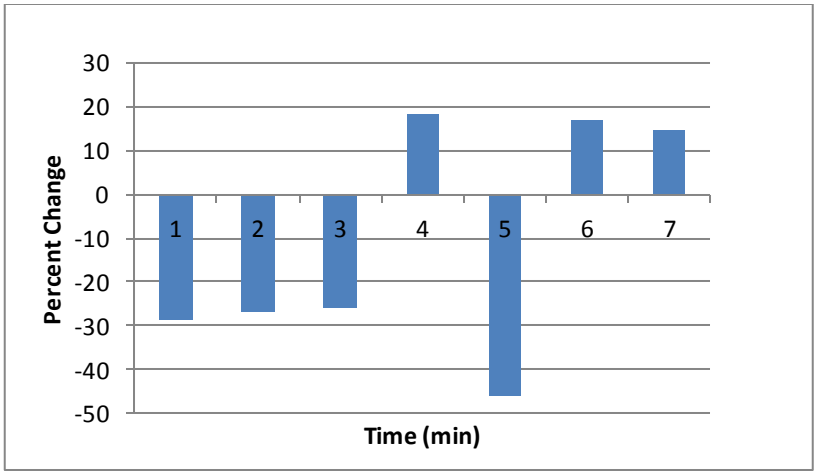


Fig.4.4.3: Effect of 0.6mM L-ascorbic acid on browning of banana slices

Inhibition of browning using L-ascorbic acid showed negative effect on the banana slices. It was observed that, banana slices treated with L-ascorbic acid showed no influence in preventing the browning of banana slices. The observation was evident from the results of analysis based on the integrated density value. All the three different concentrations (0.1mM, 0.3mM, 0.6mM) of L-ascorbic acid used for comparative study of anti-browning showed no potency as the banana slices treated with L-ascorbic acid continued to brown just as the untreated ones (Control).

TABLE 4.4.3: Effect of Potassium metabisulphite on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.05	0.1	0.3
0	38.53	-2.01	20.63
10	-40.20	-44.60	9.50
20	25.65	0.324	17.45
30	38.20	33.45	41.33
60	24.39	-13.44	20.39
90	27.93	9.35	22.07
120	-54.97	-59.18	-73.36

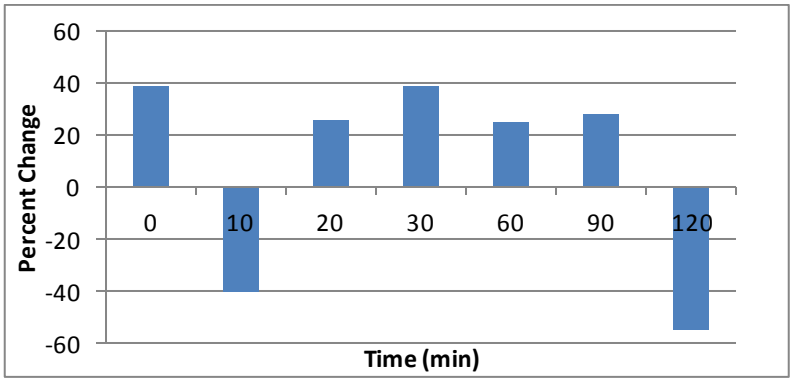


Fig.4.4.4: Effect of 0.05mM Potassium metabisulphite on browning of banana slices

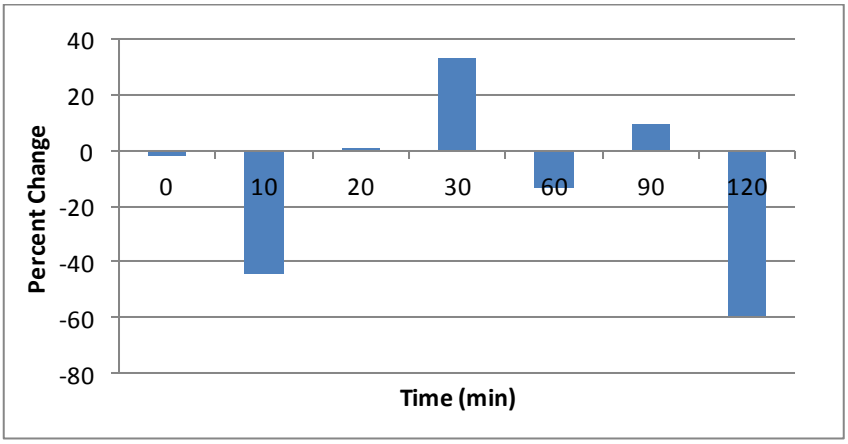


Fig.4.4.5: Effect of 0.1mM Potassium metabisulphite on browning of banana slices

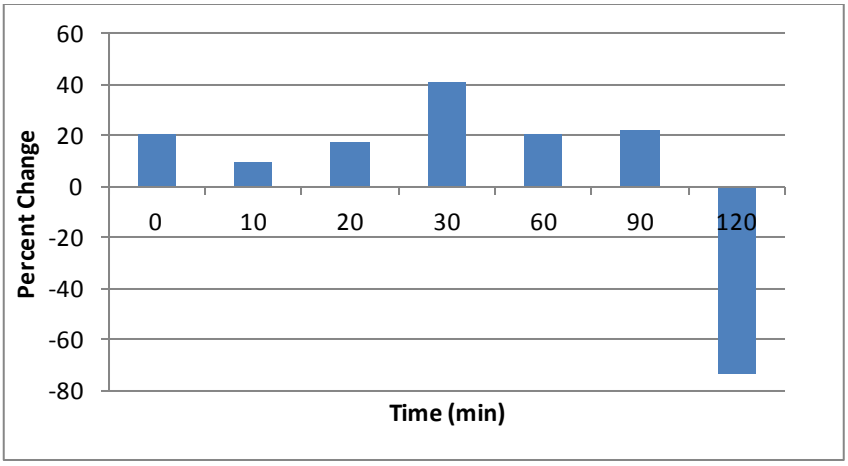


Fig.4.4.6: Effect of 0.3mM Potassium metabisulphite on browning of banana slices

Inhibition of browning using Potassium metabisulphite showed no significant effect on the banana slices. It was observed that, banana slices treated with Potassium metabisulphite continued to brown just like those of the untreated banana slices on 0.05mM and 0.1mM concentration. The only positive change observed in the experiment was that the banana slices treated with 0.3mM prevented browning for a period of 90 minutes. At 0.3mM concentration showed better treatment for the banana slices.

TABLE 4.4.4: Effect of Benzoic acid on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	4	8	12
0	2.960	-40.60	-72.27
10	-4.863	-22.15	-44.24
20	-26.08	-35.40	-86.16
30	-8.93	-14.73	-42.46
60	9.45	-31.52	-62.65
90	-25.24	-25.50	-48.77
120	5.72	-13.83	-17.83

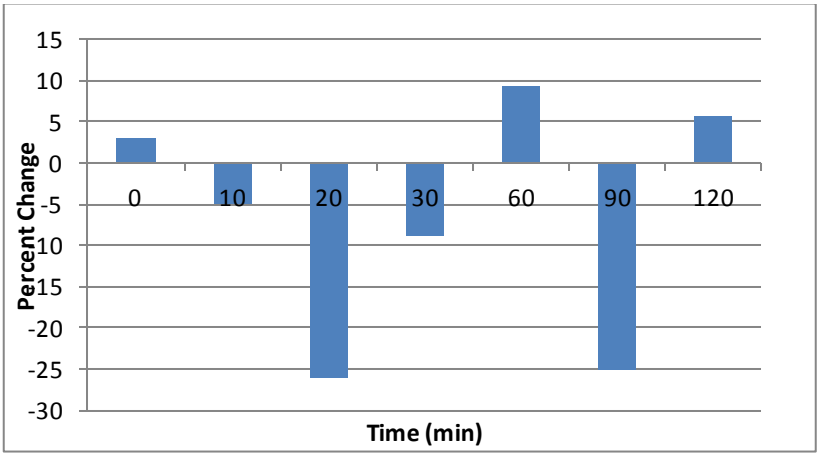


Fig.4.4.7: Effect of 4mM Benzoic acid on browning of banana slices

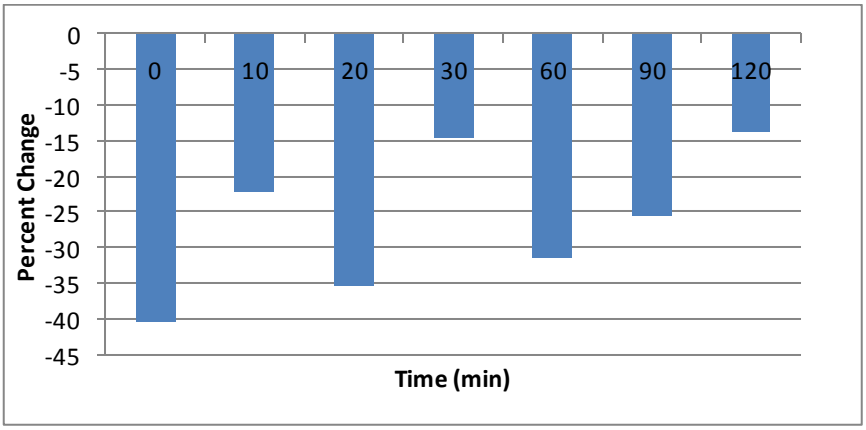


Fig.4.4.8: Effect of 8mM Benzoic acid on browning of banana slices

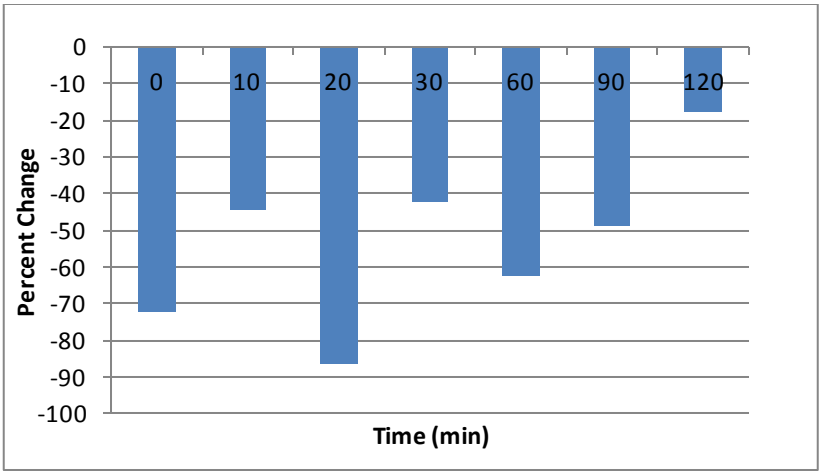


Fig.4.4.9: Effect of 12mM Benzoic acid on browning of banana slices

Inhibition of browning using benzoic acid showed no significant effect on the banana slices. It was observed that, banana slices treated with benzoic acid continued to brown just like those of the untreated banana slices (control). But the effect started to deplete soon after. At the outset, benzoic acid showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.4.5: Effect of Cinnamic acid on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.5	1.5	2.5
0	12.09	-22.60	-38.83
10	-21.89	-36.09	-39.18
20	-14.75	-22.92	-51.65
30	-18.74	-22.08	-64.10
60	-9.941	-26.96	-57.28
90	-23.86	-34.08	79.12
120	-13.31	-23.97	84.16

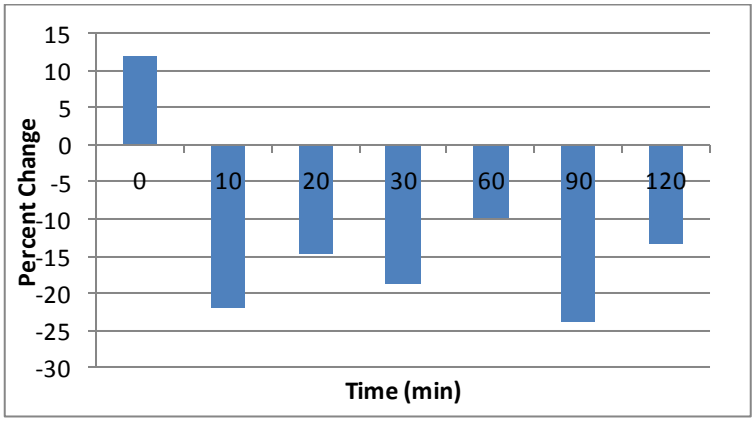


Fig.4.4.10: Effect of 0.5mM Cinnamic acid on browning of banana slices

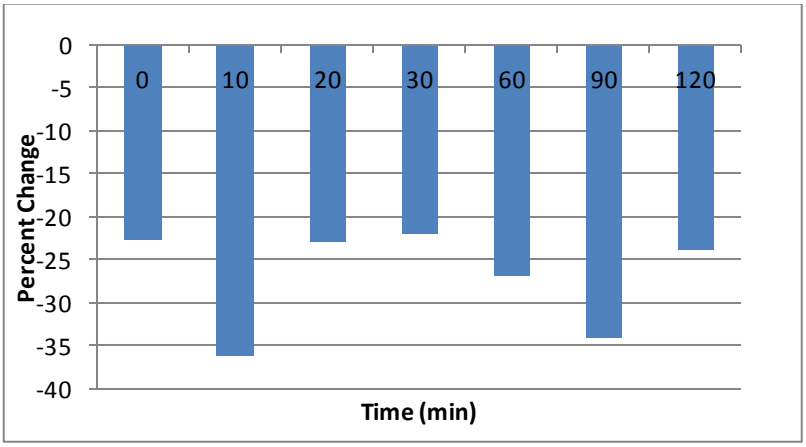


Fig.4.4.11: Effect of 1.5mM Cinnamic acid on browning of banana slices

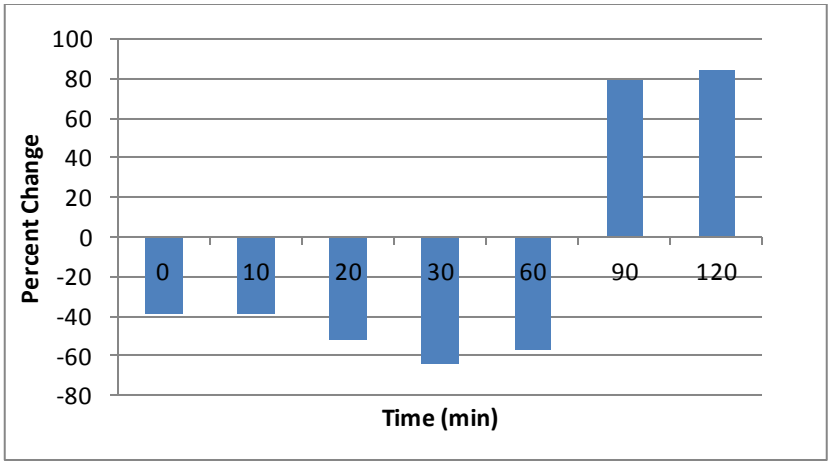


Fig.4.4.12: Effect of 2.5mM Cinnamic acid on browning of banana slices

Inhibition of browning using Cinnamic acid showed no significant effect on the banana slices. It was observed that, banana slices treated with Cinnamic acid continued to brown just like those of the untreated banana slices (control). But the effect started to deplete soon after. At the outset, Cinnamic acid showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.4.6: Effect of Citric acid on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	5	15	25
0	-31.99	-38.96	-60.80
10	-26.28	-59.15	-60.09
20	-41.89	-56.28	-71.89
30	-36.92	-44.89	-74.09
60	-51.35	-67.28	-55.50
90	-84.92	-84.92	-68.81
120	-37.03	-54.11	-40.70

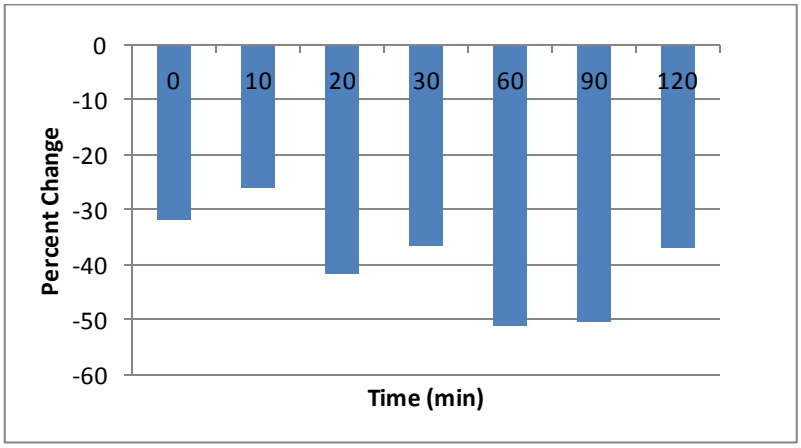


Fig.4.4.13: Effect of 5mM Citric acid on browning of banana slices

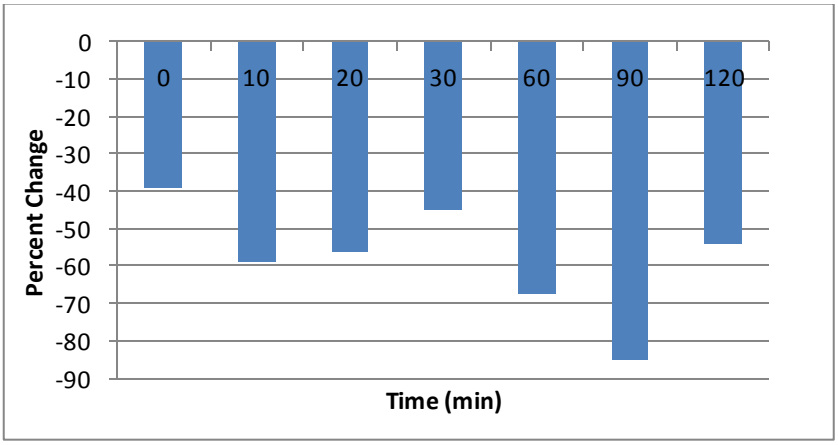


Fig.4.4.14: Effect of 15mM Citric acid on browning of banana slices

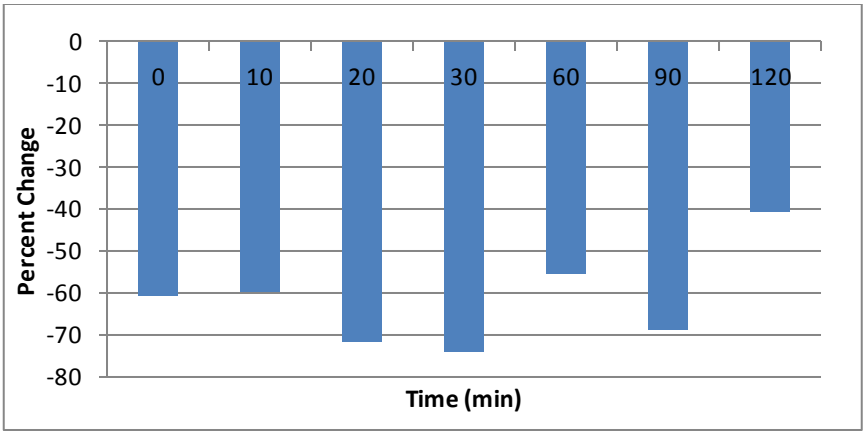


Fig.4.4.15: Effect of 25mM Citric acid on browning of banana slices

Inhibition of browning using Citric acid showed no significant effect on the banana slices. It was observed that, banana slices treated with Citric acid continued to brown just like those of the untreated banana slices (control). But the effect started to deplete soon after. At the outset, Citric acid showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.4.7: Effect of Sodium azide on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	1	2	3
0	10.08	5.60	-23.69
10	9.31	-1.69	-26.88
20	-6.20	-34.33	-45.43
30	-32.81	-17.10	22.27
60	-21.24	-23.29	1.15
90	-41.86	-34.71	-0.175
120	-31.91	-28.06	-5.188

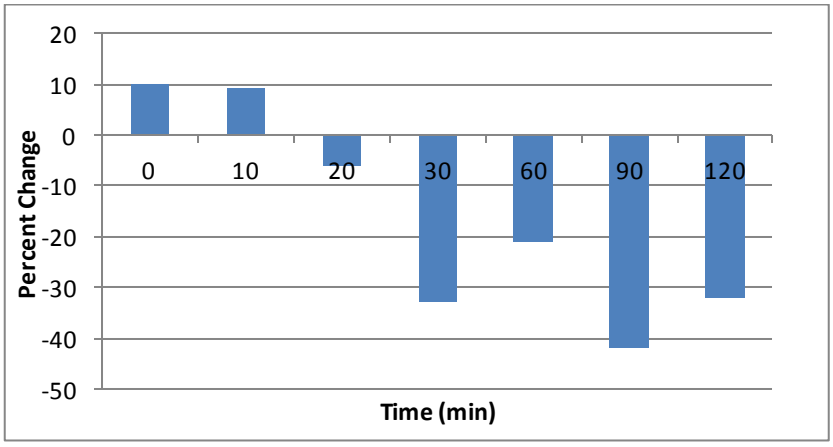


Fig.4.4.16: Effect of 1mM Sodium azide on browning of banana slices

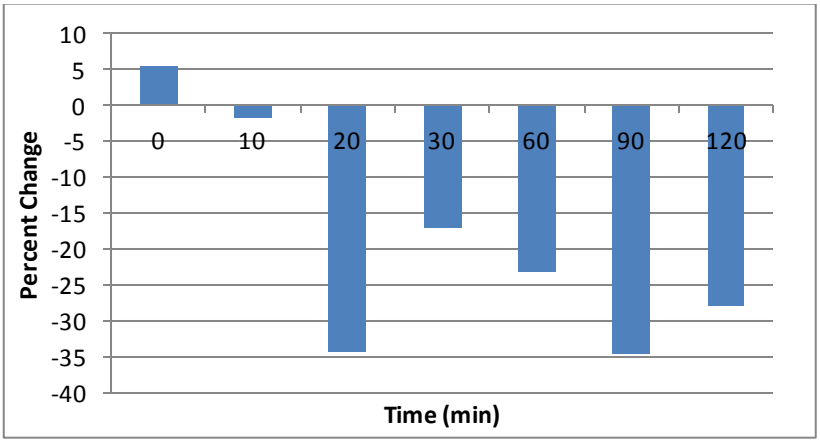


Fig.4.4.17: Effect of 2mM Sodium azide on browning of banana slices

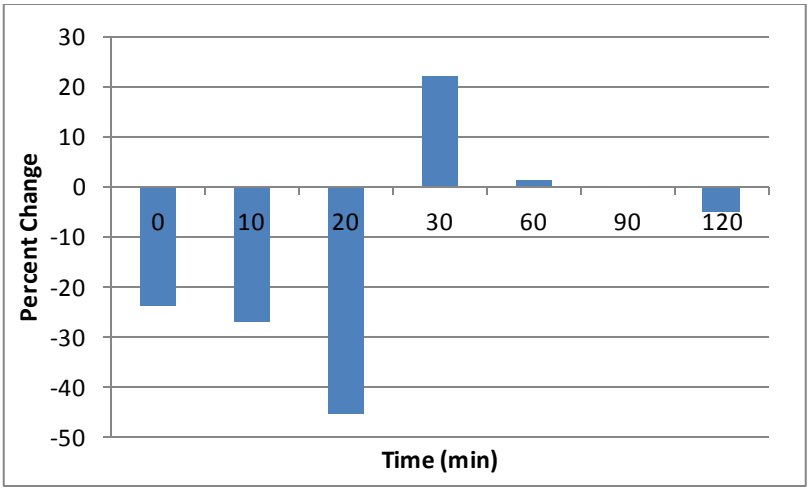


Fig.4.4.18: Effect of 3mM Sodium azide on browning of banana slices

Inhibition of browning using Sodium azide showed no significant effect on the banana slices. It was observed that, banana slices treated with Sodium azide continued to brown just like those of the untreated banana slices. The only positive change observed in the experiment was that the banana slices treated with 1mM prevented browning for a period of 10 minutes. But the effect started to deplete soon after. At the outset, Sodium azide showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.4.8: Effect of L-Cysteine on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	0.1	0.3	0.6
0	6.50	-14.67	98.71
10	10.97	-14.67	98.73
20	20.05	-10.52	98.81
30	98.60	-13.05	23.77
60	-19.93	-21.76	3.66
90	98.57	-22.07	-4.33
120	-15.67	-21.37	-3.32

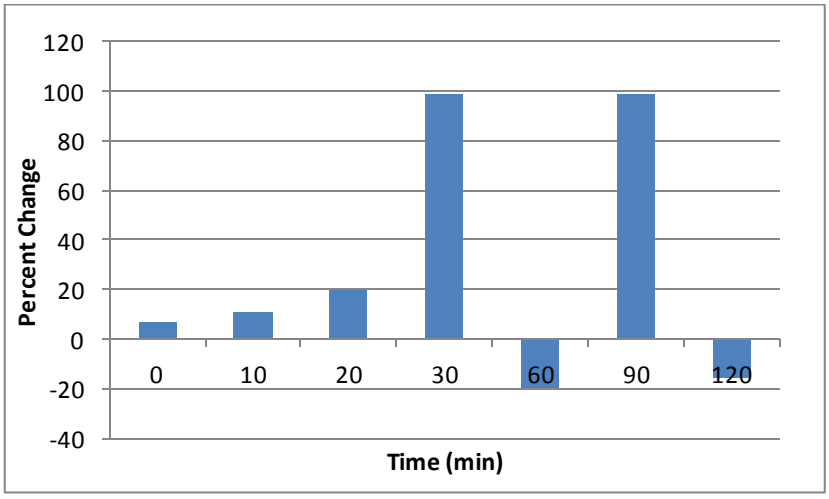


Fig.4.4.19: Effect of 0.1mM L-Cysteine on browning of banana slices

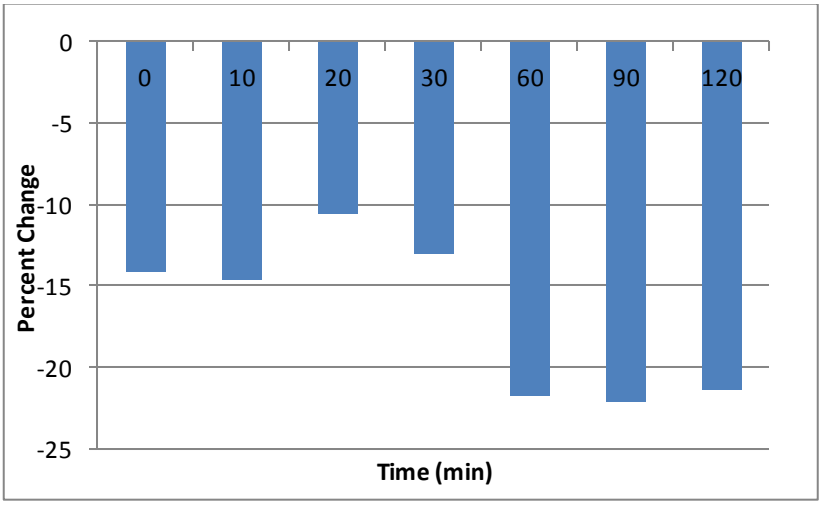


Fig.4.4.20: Effect of 0.3mM L-Cysteine on browning of banana slices

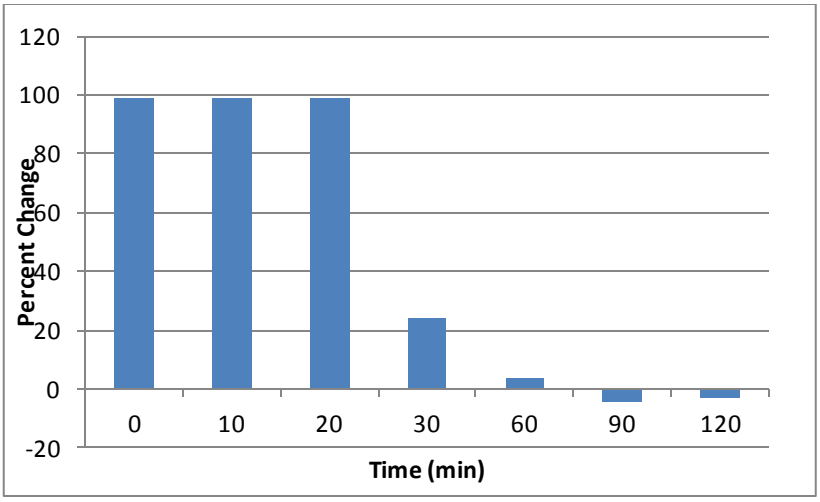


Fig.4.4.21: Effect of 0.6mM L-Cysteine on browning of banana slices

Inhibition of browning using L-Cysteine showed no significant effect on the banana slices. It was observed that, banana slices treated with L-Cysteine continued to brown just like those of the untreated banana slices. The only positive change observed in the experiment was that the banana slices treated with 0.1mM and 0.6mM prevented browning for a 10-30 minutes incubation period. But the effect started to deplete soon after. At the outset, L-Cysteine showed no ability as an anti-browning agent for a duration period greater than an hour.

TABLE 4.4.9: Effect of Sodium benzoate on browning of banana slices

Time (min)	Percent change in integrated density		
	Inhibitor concentration (mM)		
	5	10	20
0	13.70	-5.54	-28.02
10	1.24	-13.63	-36.94
20	4.76	-15.95	-25.57
30	-30.65	-28.98	27.14
60	-35.45	-24.60	-5.632
90	-51.26	-33.38	-8.885
120	-20.64	-11.64	6.156

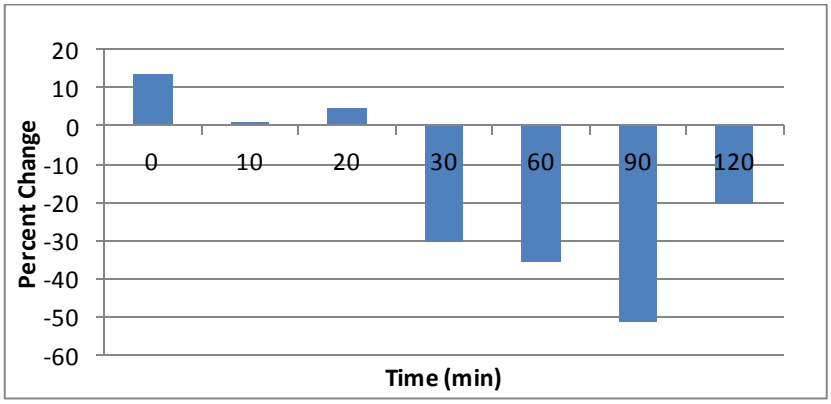


Fig.4.4.22: Effect of 5mM Sodium benzoate on browning of banana slices

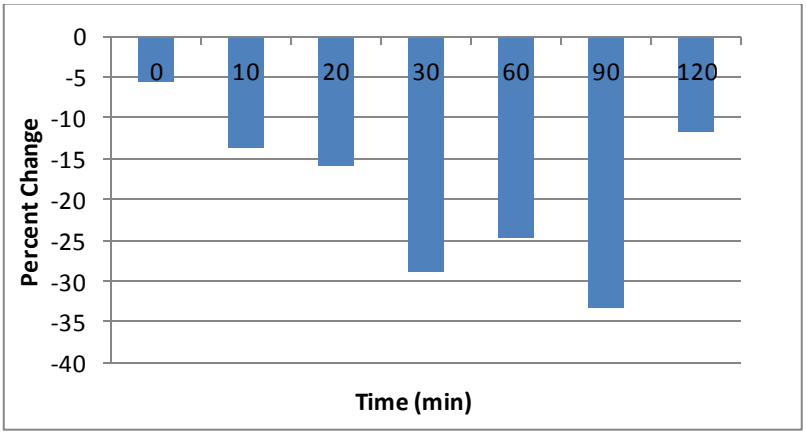


Fig.4.4.23: Effect of 10mM Sodium benzoate on browning of banana slices

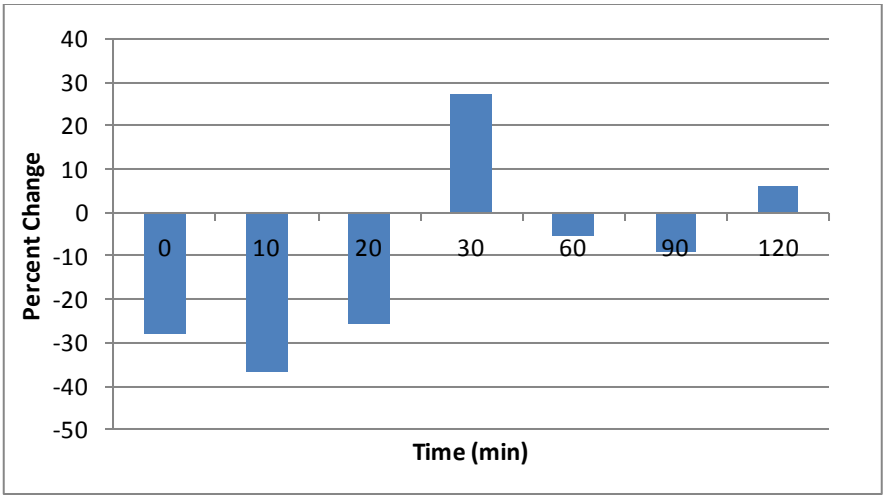


Fig.4.4.24: Effect of 20mM Sodium benzoate on browning of banana slices

Inhibition of browning using Sodium benzoate showed no significant effect on the banana slices. It was observed that, banana slices treated with Sodium benzoate continued to brown just like those of the untreated banana slices. The only positive change observed in the experiment was that the banana slices treated with 5mM prevented browning for a period of 20 minutes. But the effect started to deplete soon after. At the outset, Sodium benzoate showed no ability as an anti-browning agent for a duration period greater than an hour.

CHAPTER 5

CONCLUSIONS

Banana pulp had highest Polyphenol oxidase (PPO) activity. Banana pulp **140.95** (U/mg) had highest specific activity among apple and mushroom.

Lower concentration of ascorbic acid offered better protection against browning as determined by the image analysis. In the case of potassium metabisulphite lower concentration was less protective compared to higher concentration. However, the protection was for short duration of less than 10 minutes. 4mM benzoic acid offered best protection for mushroom reducing browning by 50%. Cinnamic acid offered up-to 30minutes after treatment. 5mM and 15mM citric acid promoted browning whereas, 25mM citric acid was very in controlling browning at all incubation times. Sodium benzoate offered protection behind 30 to 120 minutes.

Lower concentration of ascorbic acid was marginally protective whereas, higher concentration promoted browning. Potassium metabisulphite at lower concentration offered very good protective of 70% to 200%. Benzoic acid did not offered protection against browning. Citric acid offered very good protection at lower concentration. A similar trend was seen with sodium azide and L-Cysteine. Sodium benzoate did not offered any protection.

Ascorbic acid and potassium metabisulphite showed marginally protective against browning on banana slices. Benzoic acid showed dose dependent protection against browning with best results at higher concentrations. A similar was seen in Cinnamic acid and citric acid. Lower concentrations of L-Cysteine offered best protection and the opposite was true at best concentration.

CHAPTER 6

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