ELUCIDATION OF THE MECHANISM OF ENZYMATIC BROWNING INHIBITION OF THE SAP FROM OIL PALM TRUNK BY CHEMICAL METHOD

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ABSTRACT

Objective of this research is to find and explain how chemical prevents enzymatic browning to occur. There are three stages in the experiment for this research, first is obtain the oil palm sap and let it react with air and observe the changes of the color of oil palm sap. Second steage is obtain the oil palm sap sample and test it with sellected chemical which is Ascobic Acid (AA), Citric Acid (CA), L-cystien (LC), Sodium Chlorite (SC) and Catechol (CAT). The last stages of this research is analysis the oil palm sap, observation in color changes of the sap for 12 hour and 24 hour, measures absorbance from the UV range of the oil palm sap and using FTIR. Since browning reaction initiated by the enzymatic oxidation of Monophenol to 0-Diphenol than Quinines, non enzymatic browning polmerization of Quinines lead to the formation of the pigmen that cause change in color of the sap. Result of the first stages of the experiment show the enzymatic browning occurs to oil palm sap with change of color of sap within 24 hour. For second stage of the experiment show AA, CA and LC show the positive result which the lowest concentration of selected chemical give change of sap color and high concentration of selected chemical does not change the original color of the sap after 24 hour. CAT and SC show the negative result which the higest concentration of CAT changes the sap color darker and highest concentration of SC change the actual sap color to other color. Result from the analysis using FTIR and Uv-Vis analysis show there are posibility all of the sample have phenolic compound that can cause the enzymatic browning . When comparing result with control mesure of FTIR with FTIR of the inhibitor, result of ascorbic acid is most prefarable to be inhibitor of browning agent for oil palm sap.

ABSTRAK

Tujuan dari kajian ini adalah untuk mencari dan menjelaskan bagaimana mencegah tindakbalas kimia enzime berlaku kepada air batang kelapa sawit. Ada tiga tahapan dalam percubaan untuk kajian ini, pertama adalah mendapatkan air batang kelapa sawit dan biarkan bertindak balas dengan udara dan mengamati perubahan warna. Peringkat kedua adalah menguji air batang kelapa sawit dengan bahan kimia yang telah dipilih antaranya asid askorbic, asid citric, L-cysteien, sodium klorit dan catechol. Peringkat terakhir ialah menganalisis berdasarkan pemerhatian perubahan warna getah selama 12 jam dan 24 jam, serapan sinaran UV dari alat UV- Vis dan menggunakan FTIR untuk dijadikan perbandingan untuk setiap bahan kimia yang diuji selepas 24 jam dengan air batang kelapa sawit yang dibiarkan dengan udara selama 24 jam. Tindak balas kimia dalam sampel dikuatkan oleh pengoksidaan enzime monophenol dari 0-Diphenol membentuk quinines, tindak balas tidak enzimatik quinines membentuk pigmen yang menyebabkan perubahan warna pada sampel. Keputusan tahap pertama percubaan menunjukkan tindak balas enzimatik berlaku untuk air batang kelapa sawit dengan perubahan warna dalam masa 24 jam. Untuk tahap kedua dari kajian ini menunjukkan asid askorbik, asid citric dan L-cysteine menunjukkan hasil positif dengan kepekatan terendah kimia yang dipilih memberikan air batang kelapa sawit berubah warna sedikit dan kepekatan tinggi bahan kimia yang dipilih tidak menukar warna asli dari getah selepas 24 jam. Catechol dan sodium klorit menunjukkan hasil negatif apabila konsentrasi tertinggi catechol merubah warna air batang kelapa sawit kepada warna gelap dan kepekatan tertinggi sodium klorit menunjukkan perubahan yang ketara iaitu semakin gelap selepas 24 jam berbanding bahan kimia yang lain. Hasil dari analisis menggunakan FTIR dan analisis Uv-Vis menunjukkan ada posibility semua sampel mempunyai sebatian fenolik yang boleh menyebabkan tindak balas enzime. Bila hasilnya dibandingkan antara FTIR sampel kawalan dendan FTIR sampel dengan bahan kimia, hasilnya asid askorbik paling dipercayai menjadi agen penghalang dari air batang kelapa sawit berubah warna.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

FTIR	-	Fourier transform infrared spectrometry
HPLC	-	*High-performance liquid chromatography*
UV- Vis	-	*Ultraviolet-visible spectroscopy*
PPO	-	Polyphenol oxideses
EDTA	-	Ethylenediaminetetraacetic acid
FDA	-	*Food and Drug Administration*
MPOB	-	Malaysian Palm Oil Board
UMP	-	University Malaysia Pahang
IR	-	Infra-red
KBr	-	*Potassium bromide*
AA	-	Ascorbic acid
CA	-	Citric acid
CAT	-	Catechol
LC	-	L-cysteine
SC	-	Sodium chlorite

LIST OF APPENDIX

NOMENCLATURE

cm	-	Centimeter
M	-	Molarity

CHAPTER 1

INTRODUCTION

1.1 Research Background

Elucidation is detail explanation of mechanism and situation. Mechanism is process of an event or reaction. Inhibition is the process of stop or retarding a chemical reaction. (Anon.2007). Browning is one of the major limitation factors that are detrimental for many fresh-cut products, such as apple, banana, potato, and lotus root. Polyphenol oxides activity is known to be the main factor involved in browning (J. Du et al 2009). Enzymatic browning is a major factor contributing to quality loss in foods and beverages (M.J Kim et al 2004). The browning phenomenon usually impairs the sensory properties of products because of the associated changes in color, flavor and softening. Browning in fruits and vegetables is caused by the enzymatic oxidation of phenolic compounds by polyphenol oxides (Martinez & Whitaker, 1995). Enzymatic browning is mechanisms that produce brown color on fruits, seafood, vegetables that expose to air (R. Lyengar et al.1992). PPO exhibits optimum activity at pH between 5 and 7, however, its activity may be inhibited by heat or removal of one of its necessary components: O2, enzyme, Cu2+ or substrate (J.A Guerrero-Beltra´n et al 2004).

Oil palm trunk is the major part of oil palm tree and it function are to give the tree structure to stand and compete with other plant to get sun ray for photosynthesis. Oil palm tree are valuable because of its fruits can produce oil for cooking, for food industry and it contribute to the cosmetic product. For an old oil palm tree usually the age of the oil palm tree 25 year and above reduce value due to their decreasing yield or because they have grown too tall which makes harvesting very difficult.

On average, 64 million to 80 million old palm trees will be felled every year. Hence it can be regarded as one of the most important biomass in Malaysia (A. Kosugi et al 2010). But in Malaysia, The oil palm tree are left to rot or burnt in the field which is not practical because after the oil palm trees are degraded, it will be ashes and it can affect untidiness in the oil palm plantation and if burn the oil palm trunk, there will be open fire and it contribute the bad air quality in the particular area. Therefore, efficient ways for utilizing oil palm trunks is desired for ideal oil palm plantation and sustainable palm oil industry (S. C. Lim et al. 2005, H. Yamada et al 2010).

Oil palm sap was reported to contain approximately 11% sugars with sucrose as a major component accounting for approximately 90% of total sugar and oil palm trunk contains large quantity of sap, which accounts for approximately 70% of the whole trunk weight, and that sugars existing in the sap increased remarkably during storage after logging which is increased from 83 mg/ml to 153 mg/ml after 30 days storage followed by the gradual decrease (H. Yamada et al 2010). The amount of fermentable sugars and glucose in the sap can be use as new energy source, bioethanol. Bioethanol is the ethanol derived from bio-mass, it is made by fermenting sugar from biomass. Its pure ethanol usually can use as additives to fuel for everyday usage. Chemically, the oil palm trunk consists of 34.5% cellulose and 31.8% hemicelluloses, which is in close association with 25.7% lignin. Enzymatic hydrolysis converts the fibers into reducing sugars which are subsequently fermented by microorganisms into bioethanol. (W.A Ibrahim et al. 2009).

Table 1.1: Free sugars contained in sap from felled oil palm trunk (A. Kosugi et al 2010).

Free Sugar Content	Inner, g/L	Middle, g/L	Outer, g/L
Sucrose	6.5 ± 1.1	3.0 ± 0.4	1.9 ± 0.1
Glucose	85.2 ± 2.5	52.2 ± 3.4	13.1 ±2 .6
Fructose	4.1 ± 1.2	3.1 ± 1.0	2.1 ± 1.7
Xylose	0.7 ± 0.1	0.8 ± 0.1	1.4 ± 1.1
Galactose	0.9 ± 0.1	0.8 ± 0.3	1.0 ± 0.8
Rhamnose	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
Other	0.3 ± 0.3	0.1 ± 0.1	0.1 ± 0.2
Total	98.1 ± 5.5	60.5 ± 3.3	20.1 ± 1.1

Sap from oil palm sap can convert to ethanol by certain reaction, the quality of the sap for ethanol production are depend from the degree its browning. The more sap have been browning, the less quality of the ethanol produce from it. Inhibitions of sap from enzymatic browning are essential to prevent sap become less valuable.

Inhibitions of enzymatic browning are compulsory especially when it's involving food industry. Enzymatic browning is unacceptable to the food industry because of effect to the food will change it color and also the taste of the food. There are list of chemical involve in inhibition of enzymatic browning from journal anti-browning agents alternative to use of sulfites in foods, the chemical are classified according to their primary mechanism of action, first enzymatic inhibitor, second is reduction agent, enzymatic treatment, chelating agents, acidulate, completing agent. (R. Lyengar et al.1992).

Table 1.2: Anti browning agent and chemical involve. (R. Lyengar et al.1992)

Anti Browning Agent	**Chemical Involve**
Enzyme Inhibitors	Aromatic Carboxyl Acids
	Aliphatic Alcohol
	Anions
	Peptides
	Substituted Resorcinol
Enzyme Treatment	Oxyanases
	0-Methyl Transfer
	Proteases
Acidulant	Citric Acid
	Phosphoric Acid
Reducing Agent	Sulphating Agent
	Ascorbic Acid and Analogs
	Cysteine
	Glutathione
Chelating Agent	Phosphate
	EDTA, Ethylenediminetetraacetic
	Organic Acid
Complexing Agent	Cyclodextrins
	Chitosans

Certain anti browning listed are suitable for liquid form of food such as aliphatic alcohols, substituted resorcinol and chitosan and it might suitable for oil palm sap. From the sentence elucidation of the mechanism of enzymatic browning inhibition of the sap from oil palm trunk by chemical method mean explanation to prevent enzymatic browning of sap from oil palm tree by using chemicals.

1.2 Problem Statement

Sap from oil palm sap can convert to ethanol by certain reaction, the quality of the sap for ethanol production are depend from the degree its browning. The more sap have been browning, the less quality of the ethanol produce from it. To inhibit the browning reaction, there are selected chemical used as inhibitor for food industry that have potential to stop the reaction since oil palm sap is same as fruits juice, but there are massive numbers of chemical used as anti-browning agent for food industry. Therefore, it is essential to find most suitable inhibitor agent of browning reaction of sap from enzymatic browning to maintain the sap qualities and to make the inhibitor practical to use.

1.3 Objective

In this research it carries an objective which is to find suitable chemical that stop the enzymatic browning for oil palm sap.

1.4 Scope Research

The scope of this research consist of

- Study the effect of enzymatic browning to oil palm sap
- Study which chemical involve to inhibit the enzymatic browning of oil palm sap

1.5 Rationale and Significance

- To stop the enzymatic browning reaction.
- Improve the quality of oil palm sap.
- Save the money for further uses of oil palm sap.

CHAPTER 2

LITERATURE REVIEW

2.1 Enzymatic Browning

Enzymatic browning is a result of the action of endogenous polyphenol oxides (enzymatic browning) followed by the spontaneous polymerization of quinonoid compounds with other food components. (Radha Lyengar et al.1992).

Browning reaction requires oxygen, phenolic compound and polyphenol oxidizes (PPO) and is usually initiated by the enzymatic oxidation of monophenol into O-diphenol and O-diphenol into quinines, which undergo further non-enzymatic polymerization lead to the formation of pigments. From journal anti-browning agent alternative to use of sulfites in foods, enzymatic browning is a result of the action of endogenous polyphenol oxides (enzymatic browning) followed by the spontaneous polymerization of quinonoid compounds with other food components. (Qiang He et al. 2008).

Enzymatic browning may occur in many fruits and vegetables. When the tissue of fruits is cut or pealed, it rapidly darkens on expose to air. As a result, conversion of phenolic compounds to brown melanin occurs. The browning phenomenon usually impairs the sensory properties of products because of the associated changes in color, flavor and softening. The enzymatic involve in browning reaction. The enzymatic involve in browning reaction are monophenol monoxyganese or tyrosinase, diphenol oxidase or catachol oxidase polyphenol oxides. For mushroom, browning occurs as a result of two distinct mechanisms of phenol oxidation which is activation of tyrosinase. Tyrosinase is an enzyme belonging to the polyphenoloxidase (PPO) family or spontaneous oxidation (Martinez & Whitaker 1995, O. Nerya et al 2006).

Polyphenolic compounds and polyphenol oxides (PPO) are directly responsible for the enzymatic browning because mainly enzymatic oxidation of endogenous phenol into quinones, which then polymerize into brown product. Enzymatic browning of the cut surfaces, leading to serious quality deterioration, has been a matter of concern for the food industry searching for efficient ways to inhibit this reaction (Ahvenainen 1996, C.K Ding et al 2001).

Mechanical or physiological injury during post-harvest storage or processing of fruit and vegetables can result browning reaction in it. Browning reaction is undesirable reaction due to unattractive appearance and development of an off-flavor, it also the limitation factor that are detrimental for many fresh-cut product such as apple, banana, potato and many food industry product and polyphenol oxides activity main factor to cause the browning reaction (Friedman 1996, J. Du et al 2009).

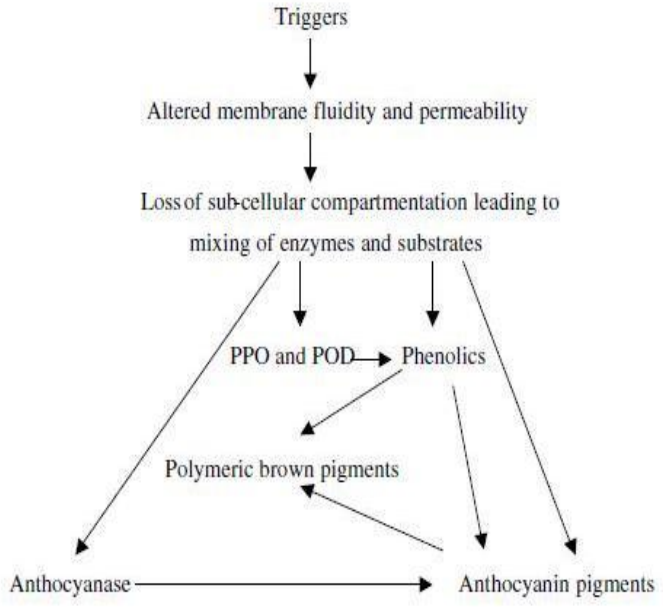

Figure 2.1: A proposed scheme for enzymatic browning in the harvested litchi fruit. (Yueming et al. 2004)

If tissue of vegetables and fruits are not treated with browning inhibitors, it can lead to the appearance of pink, grey or brown color within minutes on vegetables and fruits (Cacace et al 2002).

2.1.1 Polyphenol Oxidizes

A Polyphenol oxide which is also known as catechol oxides, catecholase, diphenol oxidase, o-diphenolase, phenolase and tyrosinase, PPO catalyzes either one or two reactions involving molecular oxygen. The first type of reaction is hydroxylation of monophenols, leading to formation of o-dihydroxy compounds. The second type of reaction is oxidation of o-dihydroxy compounds to quinines (M.J Kim et al 2004).

Polyphenol oxides catalyses two basic reactions which is hydroxylation to the *o*-position adjacent to an existing hydroxyl group of the phenolic substrate and oxidation of diphenol to *o*-benzoquinones. Both reactions utilize molecular oxygen as a co-substrate. Whether a single enzyme system exhibits both mono- and di- phenol oxides activities is still unclear. However, when both monophenol- and diphenol oxidizes are present in plants, the ratio of monophenol to diphenol oxides activity is usually 1:10 or as low as 1:40 (Marshall. 2000).

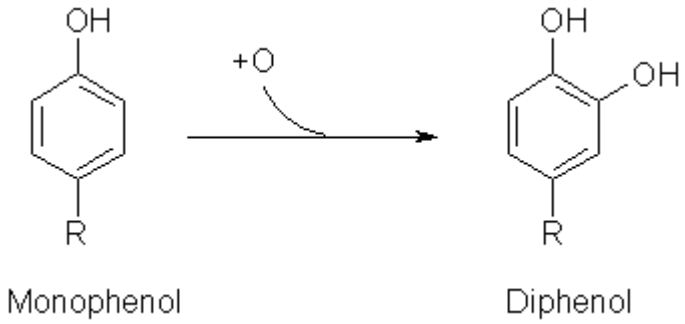

Figure 2.2: Monophenol oxides pathway producing the diphenol (Marshall 2000).

Enzymatic browning in fruit products is mainly caused by polyphenol oxides such as tyrosinase, phenol oxides, monophenol oxides or cresolase, and it can promote enzymatic browning by catalyzing the oxidation of mono- and diphenols to o-quinones. These quinones polymerize to produce melanin which is browing pigment. PPO exhibits optimum activity at pH between 5 and 7 and it can inhibit by heat or removal of one of its necessary components which is Oxygen, enzyme, Cu2+ or substrate (J.A Guerrero-Beltra´n et al 2004, A. Bayindirli et al 2001)

2.1.2 Diphenol oxides

The oxidation of diphenolic substrates to quinones in the presence of oxygen is catalysed by diphenol oxidase activity (Figure 2.3). Diphenol oxidases have received much attention owing to their high catalytic rate and their association with the formation of quinones, which lead, to production of the brown pigment, melanin (Figure 2.4). (Marshall. 2000)

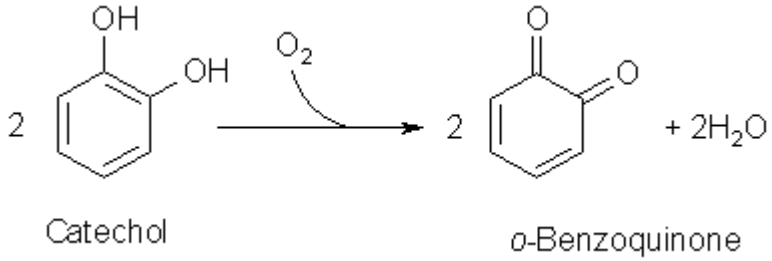

Figure 2.3: Diphenol oxidase pathway producing the quinones. (Marshall. 2000)

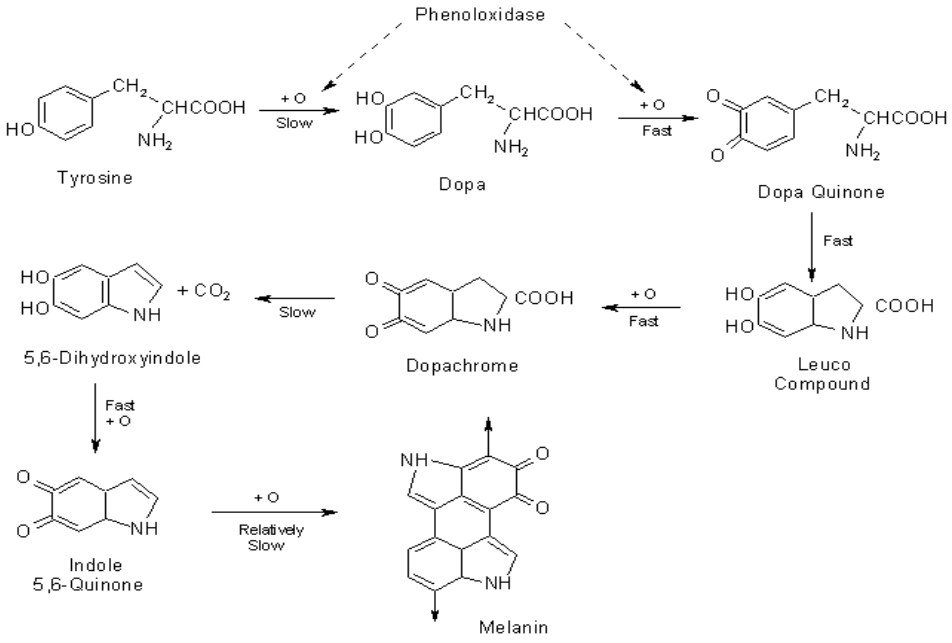

Figure 2.4: Formation of melanin from tyrosine. (Marshall 2000).

2.1.3 Quinone.

The quinone, which is an oxidation product of PPO, reacts with cysteine non-enzymatically to form colourless conjugates, PPO catalysed chlorogenic acid in fruits turn to quinone and its react with anthocyanins lead to formation of pigment. (O. Negishi and T. Ozawa 2000, F. Kader et al 2001)

Quinones are powerful electrophiles and highly reactive species involved in different reaction pathways. They are which may suffer nucleophilic attack by other polyphenols, amino acids, proteins to produce dark brown or black pigment in senescent and postharvested fruits and vegetables (L. Liu et al 2010).

Chemical control of enzymatic browning includes chelation of the copper present at the active site of the enzyme and reduction of the diquinone to its uncoloured form (O. Nerya et al 2006).

2.1.4 Tyrosinase.

Enzymatic browning in fruit products is mainly caused by polyphenol oxidises, also called tyrosinase, phenol oxidise, monophenol oxidise or cresolase. PPO can promote enzymatic browning by catalyzing the oxidation of mono- and diphenols to o-quinones. These quinones polymerize to produce malanin which is browning pigment (J. A. Guerrero-Beltra´n et al 2005).

Mechanism of ClO_2 treatment inhibiting PPO was not clear. Aromatic amino acids such as tyrosine, tryptophan and sulfur-containing amino acids (cysteine and methionine) could easily be oxidized by ClO_2. Oxidation of the amino acids or disulfide bonds that are involved in the active site in the PPO hence ClO_2 is not good inhibit agent for tyrosine (J. Du et al 2009).

A kinetic study indicated that salicylic acid is a competitive inhibitor of mushroom tyrosinase. Cinnamic acid strongly inhibited the diphenolase activity of mushroom tyrosinase by a noncompetitive mechanism (S. Lu et al 2007).

2.2 Sap from oil palm tree

Sap from oil palm tree are widely use for Indonesian food and beverage such as Indonesian sweet soya sauce (kecap manis), as well as in the Indonesian intermediate moisture meat (dendeng). Some of the volatile component are produce from the coconut sap, as well as during it's processing into coconut sugar during Mailland reaction products found in sweet soya sauce apparently originated from coconut sugar as one of the main ingredients. (Purnomo, H .2007).

From findings in develop method to utilize oil palm tree, oil palm trunk consist a large quantity of sap which is 75% to 80% of whole trunk and abundant glucose and fermented sugar in the sap. There are 10% of fermented sugars in the inner parts of the trunk. (A Kosugi. 2007).

The cellulosic material, oil palm sap can also be converted into bioethanol. Sap is extracted by peeling and squeezing the oil palm veneer. The remaining core will also be shredded and squeezed. Remaining residues from the squeezer can be hydrolyzed and fermented into bioethanol using chemical and enzymatic methods. (W.A Ibrahim et al. 2009).

2.3 Chemical Inhibition for Enzymatic Browning

Browning inhibitors can be categorized into six groups comprising first reduction agent than acid plants, next is chelating agent, complexing agent, enzyme treatment and last enzyme inhibitor. Typically ascorbic acid and its

derivatives, cysteine and glutathione have been found to be effectively in controlling browning. Calcium ascorbate based formula has been widely by the fresh cut apple industry. Acidified sodium chlorite is recently approved by FDA as sanitizing agent and sodium chlorite the major component also reported strongly inhibit enzymatic browning on fresh-cut-apple.(Qiang He et al. 2008).

Vitamin C, organic acids (oxalic acid, oxalacetic acid, citric acid), thiol-containing compounds (glutathione, cysteine, N-acetylcysteine) is the safer and cost-effective substitute, chemical compounds and agents from natural sources have been explored for their anti-browning activity (J.J Wu et. la 2008).

Table 2.1: Anti-browning agent list (Marshall 2000)

Reducing agents	Sulphiting agents, ascorbic acid and analogs, cysteine, glutathione
Chelating agents	Phosphates, EDTA, organic acids
Acidulants	Citric acid, phosphoric acid
Enzyme inhibitors	Aromatic carboxylic acids, aliphatic alcohol, anions, peptides, substituted resorcinols
Enzyme treatments	Oxygenases, *o*-methyl transferase, proteases
Complexing agents	Cyclodextrins

2.3.1 Chitosan

Chitosan, a naturally abundant polymer of N-acetylglucosamine, inhibits the enzymatic browning of apple and pear juice. The mechanism how chitosan works in inhibit enzymatic browning are un clear, but As in the case of cyclodextrins, the use of chitosan as an anti-browning agent would be limited to liquid systems (Radha Lyengar et al 1992).

Chitosan, a naturally abundant polymer of b→(1 4)-*N*-acetyl-D-glucosamine, is derived from the chitin of shellfish. Chitosan has antimicrobial properties, is soluble in dilute organic acids and is capable of forming films or membranes. Chitosan is non-toxic, biodegradable, and a naturally occurring product in our food supply. It has been shown to inhibit enzymatic browning in apple and pear juices. The addition of 200 ppm chitosan to the apple juice resulted in the inhibition of browning. Although the mechanisms by which chitosan inhibits browning are not known, its inhibitory effect is probably a consequence of the ability of the positively charged polymer to adsorb suspended polyphenol oxides, its substrates, or products. Treatment of shrimp with 2 percent chitosan resulted in a consistently reduced incidence of melanosis during storage. Chitosan also exhibited strong antimicrobial properties inhibiting several microorganisms at concentrations ranging between 0.0075 - 0.01 percent (Marshall 2000).

2.3.2 Substituted resorcinols

Novel browning inhibitors isolated from fig extracts were found to be a group of 4-substituted resorcinols Synthetic 4-substituted resorcinols have also been screened for their ability to act as PPO inhibitors. 4-hexylresorcinol may have the greatest potential for use in the food industry due to its low in the concentration necessary to result in 50% inhibition of enzyme activity in a spectrophotometric assay system, positive preliminary results from tests in actual food systems I3, and its long, safe history of human use in non-food applications. Preliminary results from laboratory studies indicate that 4 hexylresorcinol also inhibits the browning of fresh and dried apple and potato slices, avocado, and liquid systems such as apple and white grape juices. (Radha Lyengar et al.1992).