EVALUATION OF ASCORBIC ACID, L. CYSTEINE AND SODIUM METABISULPHITE AS INHIBITORS OF BROWNING IN YAM (*D.rotundata*) FLOUR PROCESSING

BY

BELLO, Tawakaltu
M.TECH./SSSE/2007/1628

DEPARTMENT OF BIOCHEMISTRY
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA,
NIGER STATE

DECEMBER, 2010.

EVALUATION OF ASCORBIC ACID, L. CYSTEINE AND SODIUM METABISULPHITE AS INHIBITORS OF BROWNING IN YAM (*D.rotundata*) FLOUR PROCESSING

BY

BELLO, Tawakaltu
M.TECH./SSSE/2007/1628

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTERS OF TECHNOLOGY (M.TECH.) IN BIOCHEMISTRY

DEPARTMENT OF BIOCHEMISTRY
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE, NIGERIA.

DECEMBER, 2010.

DECLARATION

I hereby, declare that this research was carried out by me Bello, Tawakaltu (M.Tech./SSSE/2007/1628) and no part of this work has been submitted elsewhere for the award of any degree. All the sources of information are duly acknowledged in the references.

BELLO, Tawakaltu
M.Tech/SSSE/2007/1628

21/10/10
DATE

CERTIFICATION

This thesis titled Evaluation of Ascorbic acid, L. Cysteine and sodium metabisulphite as inhibitors of browning in yam (*D. rotundata*) by Bello, Tawakaltu (M.Tech./SSSE/2007/1628) meets the regulations governing the award of the degree of Masters of Technology (M.Tech.) in Biochemistry, Federal University of Technology Minna, and is approved for its contribution to Scientific knowledge and literary presentation.

Dr. E.C. EGWIM
NAME OF SUPERVISOR

SIGNATURE AND DATE

Dr. A.A. JIGAM
NAME OF HEAD OF DEPARTMENT

02/02/11
SIGNATURE AND DATE

PROF. M. GALADIMA
NAME OF DEAN, SSSE

4/2/2011
SIGNATURE AND DATE

PROF. S.L. LAMAI
NAME OF DEAN P.G. SCHOOL

2/5/2011
SIGNATURE AND DATE

DEDICATION

This work is dedicated to my beloved husband, AbdulRasheed Adeniyi Adeleke and my daughter, Nana-Fareedat.

ACKNOWLEDGEMENTS

My sincere appreciation goes to my supervisor, Dr. E.C. Egwim whose relentless effort, supervision, corrections and suggestions made this work what it is now. My profound gratitude also goes to the Head of Department, Dr. A.A. Jigam for his contribution. Special thanks to Alh. Kabir for assisting me with one of the chemicals and, for the advice and the cool words that he used to carry me along. I am also saying a big thank you to Mal. Dauda, Mal. Sanni, Mal. Shuaibu and Mr. Isaac, of Biochemistry Department, FUT Minna for their assistance. Thank you too Mr. Bala and Mrs. Tajudeen of Chemistry Department, FUT Minna for your contribution. I would also like to express my gratitude to Mr. Bidemi of Soil Science Department, FUT Minna for assisting me with the statistical analysis. I give recognition and appreciation to my sensory panel members, staff and students of Biochemistry and Chemistry Departmet, FUT Minna. I thank you all for the knowledge shared and your assistances in various ways. Thank you too Mr. Jameelu and Ahmadu of River basin, Mr. Terry and Christy of Bida Polytechnic for your contribution. I will never forget the contribution of Tijjani Adeniyi Yahaya.

My hearty and deepest gratitude is extended to my husband, Mr. AbdurRasheed Adeniyi Adeleke and daughter, Nana-Fareedat for your love, care and support in various ways. You have really managed to go through this period with me and I appreciate it, I love you. My sincere appreciation also goes to my Parents and my siblings especially Idris for their love, Prayers and support in various ways.

I appreciate all who contributed in one way or the other to this work but whose names are too numerous to be mentioned.

Above all, all praise is due to Allah, Lord of the world. The Beneficient, The Merciful. All glory, honor and adoration to him for giving me this opportunity and all that it takes to finish it.

ABSTRACT

The possibility of inhibition of polyphenol oxidase (PPO) and browning by ascorbic acid, L-cystein and sodium metabisulphite in the processing of yam flour (elubo) was investigated. The physicochemical properties and organoleptic score of the yam flour (elubo) samples and yam flour meal (amala) samples were also evaluated using standard method of analysis. The yam samples with or without inhibitors were steeped in water for 12 hours at 30°C, 40°C and 50°C. Polyphenol oxidase activity and browning were monitored at 2 hours interval. Inhibition kinetics and browning index were measured in the yam samples. The results showed that ascorbic acid increased PPO activity significantly ($p < 0.05$) up to 2 hours and thereafter dropped while cystein and sodium metabisulphite significantly reduced ($p < 0.05$) PPO activity compared to the control samples. The kinetics of inhibition revealed that the mechanism of inhibition is uncompetitive and even though their mechanisms are the same, only sodium metabisulphite completely inhibited browning. The study also revealed that browning is temperature-dependent in all the yam samples. Sodium metabisulphite completely inhibited browning in the processed yam flour while cystein had partial inhibition and ascorbic acid on the other hand increased browning compared to the control. Polyphenoloxidase activity and browning index were positively (53.2%) correlated. Browning/PPO activity and temperature and Time were also negatively correlated. The physicochemical properties indicated that there was no significant difference ($p > 0.05$) in the moisture content, hydration capacity, swelling capacity, and solubility of the yam tissues with or without inhibitors. However, a significant increase ($P < 0.05$) in the calorific energy of yam sample processed with sodium metabisulphite was observed. The organoleptic score of *amala* produced from test samples revealed that there was no significant difference ($p > 0.05$) in the flavour, taste and texture of *amala* made from yam samples processed with or without inhibitors. However, significant differences ($P < 0.05$) in colour exist among all the *amala* samples. *Amala* made from yam samples processed with sodium metabisulphite was white throughout processing temperatures and time compared with the control samples. Those made from yam samples processed with cystein was slightly lighter in colour than the control while *amala* made from yam samples processed with ascorbic acid was darker compared to the control. This study thus, revealed that PPO and browning were effectively inhibited in processed yam flour (elubo) by sodium metabisulphite as shown by the white *amala* obtained after treatment with the inhibitor while, L-cysteine partially inhibited the enzyme. Ascorbic acid however, did not inhibit PPO in *elubo*. Polyphenol oxidase was highly correlated with browning. Also, the addition of inhibitors did not change the physicochemical properties of the processed *elubo* except that calorific energy was increased on processing with sodium metabisulphite. Of all the yam flour samples, *amala* made from yam samples processed with sodium metabisulphite was the most accepted followed by control, then *amala* made from yam samples processed with ascorbic acid and cysteine.

TABLE OF CONTENTS

CONTENT PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

Yam (*Dioscorea* spp.) is a multi-species crop that originated principally fromAfrica and Asia before spreading to other parts of the world (Hahn *et al*, 1987). It belongs to the family Dioscoreaceae within the genus *Dioscorea* (Coursey, 1983; Ayensu, 1972) and serves as a staple crop in West Africa (Asiedu *et al.*, 1992). The yam tuber, which is the most important part of the plant, can be stored longer than other root and tuber crops, ensuring food security even at times of general scarcity. It is the third most important tropical root and tuber crop after cassava and sweetpotato (Fu *et al.*, 2005). The crop is of major importance in the diet and economic life of people in West Africa, the Caribbean islands, parts of Asia, and Oceania (Ravindran and Wanasundera, 1992; Girardin *et al*, 1998). Yam is an elite crop, preferred over other root and tuber crops in West Africa and a choice during ceremonies and festivities (Coursey, 1967; Hahn *et al.*, 1987). Resource poor people, especially women, derive a good income from its production, processing, and marketing. It also has ritual and socio-cultural significance in West and Central Africa.

West Africa is the leading producer of yam and grows over 90% of the worldwide production (40 million tones fresh tubers/year), followed by the West Indies where Jamaica is the leading producer (Dipeolu *et al.*, 2002; FAOSTAT, 2004). The third most important region of yam production is East Africa where Tanzania and Sudan are the major producers. Yam is also produced in Japan, Papua New Guinea, the Philippines, and Panama. Nigeria is the world's largest producer of yams followed by Ghana, Ivory Coast and Togo (FAO, 2003). Both fresh tubers and yam flour are now exported from Ghana and Nigeria to developed countries such as

States of America. These are mainly patronised by emigrants from growing regions. According to the Nigerian Export Promotion Council (NEPC), Nigeria realized N56 billion from yam export in 2008 as against N37 billion in 2007 (Osibo, 2009). However, Ghana exports the largest quantity of yams (about 12 000t) annually and average yam consumption per capita per day is highest in Bénin (364 kcal) followed by Côte d'Ivoire (342 kcal), Ghana (296 kcal), and Nigeria (258 kcal) (IITA, 2009).

Yam, apart from providing basic food security and income, is a source of nutrition for millions of people. Information on the nutritive value of yam has been highlighted by several authors in their work on yam (Purseglove, (1976); Barquar and Oke, (1977); Bradbury and Singh, (1986); Okoli and Green, (1987); Woolfe, (1987); Bradbury and Holloway, 1988; Nair, (1989); Asemota *et al.*, (1992); Osagie, (1992); Opara, 1999; Alves, 2000; Farombi *et al.*, (2000);Afoakwa and Sefa-Dedeh, (2001); Albrecht and McCarthy, (2006); Farombi, (1998). It is a rich source of carbohydrate and also contributes to vitamins and minerals especially where it is consumed in large quantities. Yams are an excellent source of potassium, with twice the amount as found in a medium-sized banana. They are also a good source of vitamin C, B_6, folate, iron, and magnesium. Yams are high in starch and contain an enzyme, alpha amylase, which converts starches to sugars as the tuber matures, is stored, or when heated. Table 1.1 shows the nutritional composition of yam from different authors.

There are many varieties of yam species widespread throughout the humid tropics, but the edible yams are derived mainly from about ten Coursey, (1967); Opara, (1999). The most economically important of these species are: Yellow yam (*Dioscorea cayenensis* Lam.) which is also native to West Africa, Water yam (*Dioscorea alata* L.), Bitter yam (*Dioscorea dumetorum*) also called

trifoliate yam because of its leaves, White yam (*Dioscorea rotundata* Poir) White yam is the most viscous among all yam species. It originated in Africa and is the most widely grown and preferred yam species. The tuber is roughly cylindrical in shape, the skin is smooth and brown and the flesh usually white and firm Coursey, (1967); Opara, (1999).

A number of studies (Ikediobi and Oti, 1983; Ravindran and Wanasundera, 1992; Hariprakash and Nambisan, 1996; Treche and Agbor-Egbe, 1996; Afoakwa and Sefa-Dedeh; 2002 and Tschannen *et al.*, 2003) have reported on yam storage and associated physiological and biochemical changes. Traditionally, farmers store yam tubers in small quantities using simple storage techniques. The type of storage structure is influenced by climatic conditions, the purpose of the yam tubers in storage, type of building materials available and the resources of the farms (FAO, 1990). The principle involves keeping uninjured tubers in barns, usually on a raised platform, or tying the tubers singly to live poles to provide shade and allow good ventilation. Some are also stored in pits, barns and warehouses (Alabadan, 2002). Free air circulation and a low temperature are essential for good storage. There are also underground and storage housing structures, sheds, huts, silos and cribs (Ravi *et al.*, 1996). Tubers may also be left underground for several weeks as a storage method. Various modern methods, such as the use of chemicals, irradiation and low temperature or controlled atmospheric conditions, to delay or suppress sprouting of yams for longer storage, have been reported (Tschannen *et al.*, 2003; Swannell *et al.*, 2003). Even though most of these modern methods are capable of achieving long- term storage, they are expensive to maintain and not feasible in the yam producing areas due to lack of funds for required equipment and frequent interruption of electricity supply.

Table 1.1: Nutrient contents of yam species (*Dioscorea* spp.) per 100g fresh edible tuber portions

Nutrient (g/100g)	*D. alata*	*D. rotundata*	*D. cayenensis*	*D. esculenta*	*D. dumetorum*
% Moisture	65-78.6	50.0-80	60-84	67-81	67-79
% Carbohydrate	22-31	15-23	16	17-25	17-28
% Starch	16.7-28	26.8-30.2	16.0	25	18-25
% Free sugar	0.5-1.4	0.3-1	0.4	0.6	0.2
% Protein	1.1-3.1	1.1-2.3	1.1-1.5	1.3-1.9	2.8
% Crude fat	<0.1-0.6	0.05-0.1	0.06-0.2	0.04-0.3	0.3
% Fibre	1.4-3.8	1.0-1.7	0.4	0.2-1.5	0.3
% Ash	0.7-2.1	0.7-2.6	0.5	0.5-1.5	0.7
Phosphorous (mg)	28- 52	17	17	35-53	45
Calcium (mg)	28-38	36	36	12-62	52
Vitamin C (mg/100g)	2.0-8.2	6.0-12.0			
Iron (mg)	5.5 11.6	5.2	5.2	0.8	
Food energy (kcal)	140	142	71	112	122
β-carotene (µg)	5-10				
Thiamine (mg)	0.05- 0.10			0.1	
Riboflavin (mg)	0.03- 0.04			0.01	
Niacin (mg)	0.5			0.8	

Sources: Coursey (1967); Eka (1985); Bradbury and Holloway (1988); Murac-Tucker *et al.* (1993); Osagie (1992); Asiedu *et al.* (1997) and Opara (1999)

Yam tubers have been diversely utilized and still have more utilization potentials. The main use of yam in producing countries is as food (Rasper and Coursey, 1967) with little industrial involvement.

Consumer preference is highest for fresh yam, which can be pounded, fried, boiled, or roasted like potatoes (Ravindran and Wanasundera, (1992); Orkwor, (1998). After cassava and cereals, the highest source of dietary energy in Nigeria's food basket is yam (Oguntona, 1994). Yam tubers are also processed into ikokore, yam chips and pellets (Mestres *et al.*, 2002), poultry and livestock feed just as like cassava (Opara, 1999). Yam flour (elubo) is another way in which yam can be processed. To prepare *elubo*, yam tubers are peeled, sliced, and parboiled in water at about 63 ± 3°C. The slices are left in the water, well covered, for about 24 hours to ferment slightly. They are drained and dried uder the sun to reduce the moisture content. The dried slices are then ground to flour (Figure 1.1). Treatment with sodium bisulphate and other inhibitors of browing e.g. ascorbic acid, L-cystein and sodium metabisulphite, etc are often used to prevent phenolic oxidation, which darkens the colour of the product (especially with white yam, *D. rotundata*). Blanching in place of sodium bisulphate achieves similar results.

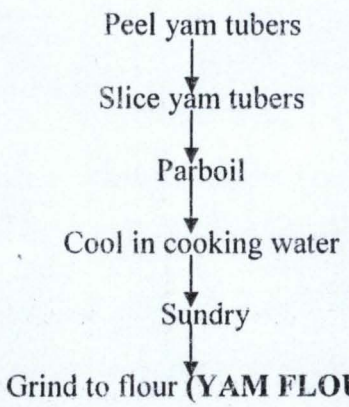


Figure 1.1: Processing of yam flour (elubo)

This *Elubo* is usually mixed with four parts of boiling water to give a smooth thick paste called *amala* (Akissoe *et al.*, 2001) which is eaten with soup. *Amala* is a delicacy for the Yoruba of western Nigerian (Osagie, 1992; Onwueme and Charles, 1994; Orkwor, 1998). The same product is popular in Benin where it is called *telibo*.

1.1 JUSTIFICATION

What makes *amala* popular all over Southern Nigeria is the taste, texture and of course, the colour, which ranges from light to dark brown and sometimes near black. However, despite its acceptability in the western part of Nigeria, its colour deters its general acceptability in other parts of Nigeria particularly, the Eastern block.

This research work was therefore designed to study the effect of some phenolic oxidase inhibitors on the reduction of browning, to improve on the texture and general appearance of the product thereby increasing the general acceptability of *amala*.

1.2 AIMS AND OBJECTIVES

The aims and objectives of this research work are:

(i) To evaluate the potentials of ascorbic acid, L-cysteine ad sodium metabisulphite to inhibit the enzyme responsible for browning in yam flour (Elubo) processing.

(ii) To correlate the extent of browning in yam flour (elubo) with inhibition of polyphenol oxidase (PPO).

(iii)To assess the pasting qualities of yam flour (elubo) produced after inhibiting PPO.

(iv)To assess the organoleptic qualities of yam flour meal (amala) produced from yam flour (elubo) processed by the inhibition of PPO.

2.2 Overall features of Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO), monophenol dihydroxy phenylalanine: oxygen oxidoreductase (E.C. 1.14.18.1), widely distributed in the plant kingdom, is the enzyme responsible for catalyzing the discoloration of fruits and vegetables (Mayer and Harel, 1979). Plant PPO is located in the chloroplast bound to thylakoid membranes (Tolbert 1973, Golbeck and Cammarata, 1981; Martinez and Whitaker, 1995). PPO is inactivated in the bound state, but when released into the cytosol such as when plant tissues undergo physical damage such as bruising, cutting, ripening, or senescence, they are activated (Whitaker, 1995).

Polyphenol oxidase (PPO) catalyzes two different oxidative reactions in combination with molecular oxygen: 1) the hydroxylation of monophenols into *o*-diphenols (monophenol oxidation) and 2) the oxidation of diphenols into *o*-quinones (diphenol oxidation). Oxidative reaction models using *p*-cresol as a monophenol substrate and catechol as a diphenol substrate are presented in Figure 1.1 (Whitaker 1994). In Figure 1.2, BH2 represents any compound that acts as a proton donor. Subsequent polymerization of *o*-quinones, amino acids, and proteins results in tissue discoloration and loss of nutrients from fruits and vegetables (Whitaker 1994).

CHAPTER TWO

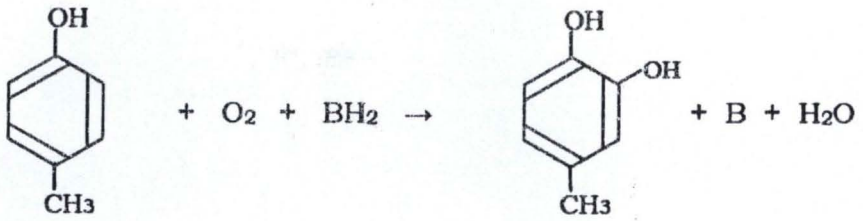
2.0 LITERATURE REVIEW

2.1 General overview of Enzymatic Browning

Appearance, flavour, texture and nutritional value are four attributes considered by consumers when making food choices. Appearance which is significantly impacted by colour is one of the first attributes used by consumers in evaluating food quality. Colour may be influenced by naturally occurring pigments such as chlorophylls, carotenoids and anthocyanins in food, or by pigments resulting from both enzymatic and non-enzymatic reactions. Enzymatic browning is one of the most important colour reactions that affect fruits, vegetables and seafoods. It is catalysed by the enzyme polyphenol oxidase (PPO), monophenol dihydroxy phenylalanine: oxygen oxidoreductase (E.C. 1.14.18.1) (Mayer and Harel, 1979).

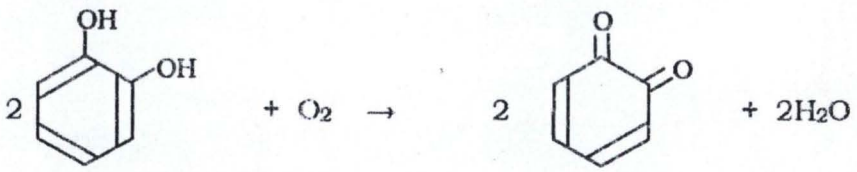
The phenomenon of enzymatic browning has long been a source of concern to food scientists as it directly affects food quality. The incidence of tissue browning is desirable in the manufacture of black tea (Takeo 1966), tobacco (Sheen and Calvert 1969) and in the processing of sultana grapes and prunes (Grncarevic and Hawker 1971). It is undesirable in the processing of fruits juices (Wolfrom *et al*, 1974), yam (Anosike and Ikediobi, 1985) plantain and grain flour (Lamkin *et al*, 1981) because of production of undesirable colour, odour and astringent flavours which reduce the commercial value of the product (Sciancalepore and Longone 1984; Lee *et al*, 1990).

1) Monophenol oxidation



p-Cresol Proton donor 4-Methyl catechol

2) Diphenol oxidation



Catechol *o*-Benzoquinone

Figure 1.2: Oxidative reactions of monophenol and diphenol by PPO (Whitaker 1994)

Active PPO molecules contain two copper atoms in the active site (Gaykema, 1984). Lerch, (1983) proposed a change in PPO molecules during the catalysis of mono- and diphenol oxidation reactions. There are three recognized molecular forms of PPO, met- (Cu2+), oxy-(Cu2+), and deoxy-(Cu1+) dependent on the charge of the copper ions and surrounding oxygen atoms (Figure 1.3). Changing of PPO molecular configurations as well as the PPO active site results in two different catalytic activities attributed to PPO. While diphenol oxidase of PPO from many plant sources were characterized in a number of literature, less intensive studies on monophenol oxidase activity of PPO were reported because of the difficulty to detect the activity in assays (Lerch, 1983).

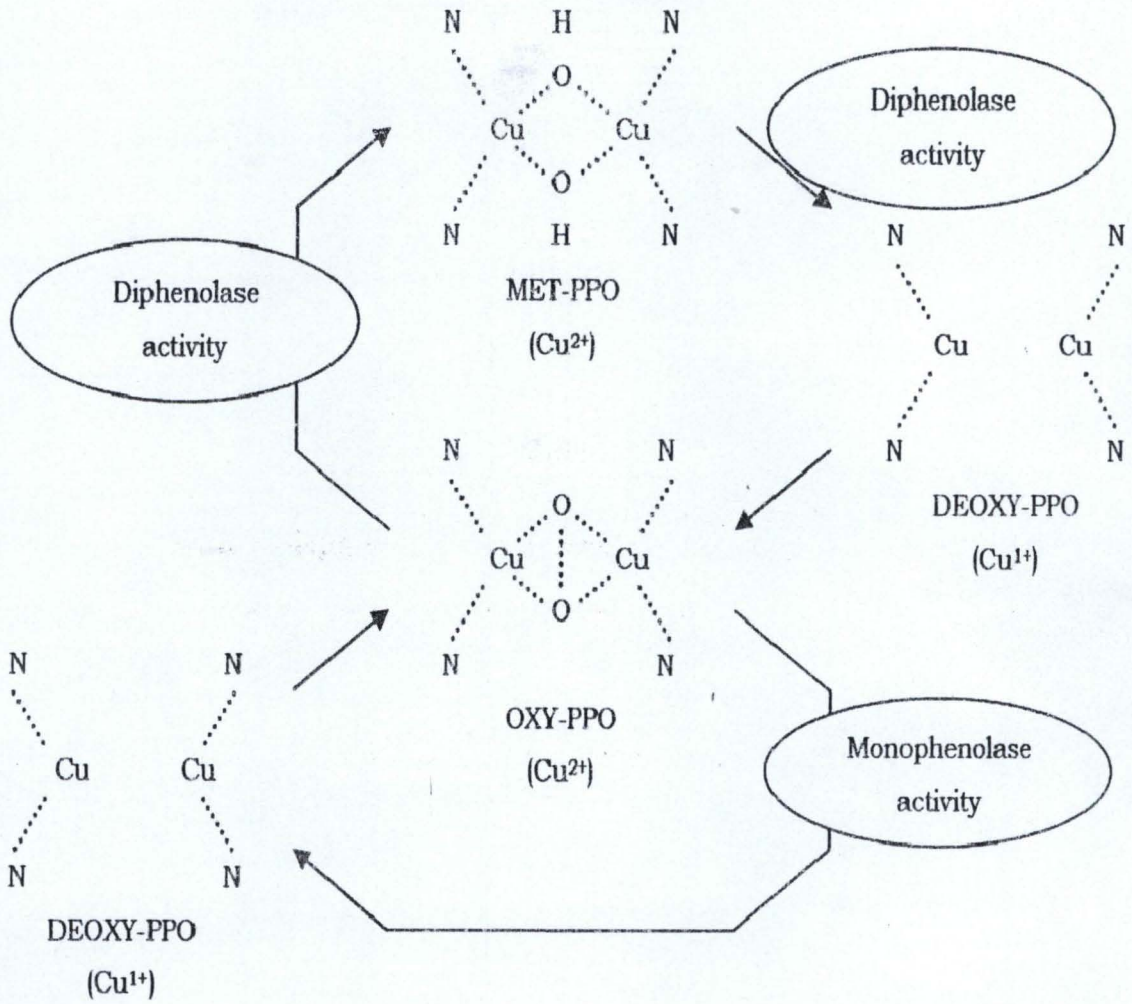


Figure 1.3: Proposed mechanisms and active sites for monophenol and diphenol oxidase activities of PPO. (Lerch, 1983)

2.3 Polyphenol oxidase (PPO) from plant sources

It is estimated that nearly 50% of harvested tropical fruits are discarded because of the quality defects resulting from tissue discoloration by the catalytic action of PPO (Whitaker, 1996). A number of intensive research studies of PPO were conducted to control PPO activity and to

prevent loss of fruit and vegetable crops for the fresh market and food processing industry. PPO has been partially purified and characterized from many plant sources.

2.4 Extraction of Polyphenol oxidase (PPO)

During PPO extraction and purification process, release of PPO from chloroplast membranes and removal of intrinsic phenolic compounds are critical to achieve high purity and yield. Detergents, such as Triton X-100 and sodium dodecyl sulfate (SDS) are added to suspensions or homogenates to solubilize PPO in extracts. Buffers containing Triton X-100 detergent are used for extraction of PPO from peaches (Wissemann and Montgomery, 1985) and bean sprouts (Nagai and Suzuki, 2003).

Triton X-100 was added to suspensions of precipitate obtained from centrifugation of homogenate for purification of PPO from strawberry (Serradell *et al,* 2000) and Chinese cabbage (Nagai and Suzuki, 2001). Acetone precipitation followed by extraction using a Triton X-100 detergent is effective for the partial purification of PPO from wild rice (Owusu-Ansah, 1989), Indian tea leaves (Halder *et al,* 1998), or mangos (Sharma *et al,* 2001). The addition of Triton X-100 to extracting buffer did not increase PPO activity from loquat fruit PPO (Ding *et al,* 1998). The combination of acetone precipitation and extraction using SDS was applied for characterization of PPO from yam tubers (Anosike and Ayaebene, 1981).

The addition of insoluble polyvinylpyrrolidone (PVP) or polyvinylpolypyrolidone (PVPP) was carried out to prevent the reaction between PPO and phenolic compounds during the extraction of litchi (Jiang *et al,* 1997), longan fruit (Jiang, 1999), field beans (Paul and Gawda, 2000), or coffee (Mazzafera and Robinson, 2000; Goulart *et al,* 2003). Gonzalez *et al,* (2000) investigated

the influence of buffer composition on extraction of PPO from blackberries, yielding high activity by adding 4% PVP and 0.5% Triton X-100 in the extracting buffer. Broothaerts *et al*, (2000) observed 44 and 74% increases in PPO activity in the extracts of tobacco and apple leaves, respectively, following the addition of Triton X- 100 during the extraction process.

Bordier, (1981) and Sanchez-Ferrer *et al*, (1989) proposed an effective extraction method as an alternative to acetone, and ammonium sulphate precipitation of PPO as well as the addition of PVP in extracting buffers. The addition of the detergent Triton X-114 to homogenates resulted in two phase partitioning between hydrophilic and hydrophobic fractions depending on temperature. PPO and endogenous phenolic compounds were separated in hydrophilic and hydrophobic solvents, respectively. This method was successfully applied to extraction of PPO from spinach (Sanchez- Ferrer *et al*, 1989), peach, plum, almond, cherry, apricot (Fraignier *et al*, 1995), iceberg lettuce (Chazarra *et al*, 1996), bananas (Sojo *et al*, 1998a), orchid aerial roots (Ho, 1999), truffles (Perez-Gilabert *et al*, 2001), medlar (Dincer *et al*, 2002), and persimmons (Nunez-Delicado *et al*, 2003).

2.5 Characterization of Polyphenol oxidase (PPO)

The examinations of substrate specificity and inhibition kinetics were assessed to characterize partially purified PPO from selected plant sources. The determinations of activity of purified PPO at selected pH and temperature were also achieved to establish adequate handling and processing methods preventing PPOcatalyzed discoloration of fruits and vegetables. Selected Properties of PPO from selected plant sources including substrate, Km, and optimum pH and temperature are summarized in Table 1.1.

The presence of both mono- and diphenol oxidase activities are well documented in kiwifruit (Park and Luh, 1985), bananas (Thomas and Janave, 1986; Sojo *et al*, 1998b), cherimoya (Martinez-Cayuela *et al*, 1988), Monastrell grapes (Sanchez-Ferrer *et al*, 1988), basil (Baritaux *et al*, 1991), olives (Stefano and Sciancalepore, 1993), potatoes (Sanchez-Ferrer *et al*, 1993), pears (Espin *et al*, 1996a; Espin *et al*, 1996b), strawberries (Espin *et al*, 1997a), avocados (Espin *et al*, 1997b), artichoke heads (Espin *et al*, 1997c), apples (Espin *et al*, 1998), raspberries (Gonzalez *et al*, 1999), lettuce (Chazarra *et al*, 1999; Hisaminato *et al*, 2001), eggplant (Perez-Gilabert and Garcia-Carmona, 2000), mangoes (Sharma *et al*, 2001), peaches (Laveda *et al*, 2001), field beans (Gowda and Paul, 2002), sorghum (Dicko *et al*, 2002), persimmons (Nunez-Delicado *et al*, 2003), and quince fruit (Orenes-Pinero *et al*, 2005). On the other hand, no monophenol oxidase activity was reported in dates (Hasegawa and Maier, 1980), Muscat grapes (Interesse *et al*, 1984), sunflower seeds (Raymond *et al*, 1993), pineapple (Das *et al*, 1997), or longan fruit (Jiang, 1999).

Table 1: Properties of partially purified PPO from selected fruits and vegetables (Miyuki Miyawaki , 2

Source	Substrate	Km (mM)	Optimum pH	Optimum T (°C)	Reference
Yam tubers	Catechol	9.1			Anosike and Ayaebene
	Pyrogallol	6.3			(1981)
	DL-DOPA	2.2			
Grape	4-MC		3.5~4.5	25~45	Valero and others (1988)
Wild rice	Catechol		7.8	25	Owusu-Ansah (1989)
Plum	Catechol	20	6.0	20	Siddiq and others (1992)
Sunflower seed	Gallic acid	1.11	7.9		Raymond and others (1993)
Mango	4-MC		5.8	30	Robinson and others (1993)
Apple	Catechol	34		18	Oktay and others (1995)
Almond	4-MC		5.0		Fraignier and others (1995)
Plum	4-MC		4.5 ~ 5.5		Fraignier and others (1995)
Peach	4-MC		5.0		Fraignier and others (1995)
Cherry	4-MC		4.5		Fraignier and others (1995)
Apricot	4-MC		5.0 ~ 5.5		Fraignier and others (1995)
Iceberg lettuce	CA	0.8 ~ 1.4			Chazarra and others (1996)
	TBC	1.0 ~ 1.2			
Litchi	4-MC	10	7.0	70	Jiang and others (1997)
Herb	Catechol	25	7.5		Arslan and others (1997)
Pineapple			6.0 ~ 7.0		Das and others (1997)
Loquat	CA	0.105	4.5	30	Ding and others (1998)
Indian tea leaf	Catechin	0.49			Halder and others (1998)
	Epicatechin	0.81			
	Catechol	12.52			
	Pyrogallol	17.81			
	Gallic acid	19.33			
Raspberry	Catechol		8.0, 5.5		Gonzalez and others (1999)
Longan	4-MC		6.6	35	Jiang (1999)
Orchid aerial	4-MC	3.7 ~4.0	7.0		Ho (1999)
root	(+)-Catechin	2.1 ~ 5.5			
Blackberry					Gonzalez and others (2000)
Wild	Catechol	17.3			
Thornless	Catechol	196.4			
Coffee					Mazzafera and Robinson
Leaves	CA	0.882	6.0 ~ 7.0	30	(2000)
Endosperm	CA	2.27	6.0 ~ 7.0	30	

4-MC; 4-methylcatechol, L-DOPA; L-3,4-dihydroxyphenylalanine, DHPPA; 3-(3,4-dihydroxyphenol) propionic acid, CA; chlorogenic acid, TBC; tert-butylcatechol

Table 1.2 Properties of partially purified PPO from selected fruits and vegetables. *(Continued)*

Source	Substrate	K_m (mM)	Optimum pH	Optimum T (°C)	Reference
Field bean	Catechol	10.5			Paul and Gowda (2000)
	4-MC	4.0			
	L-DOPA	1.18			
	Pyrogallol	12.5			
Strawberry	Catechol	11.2	7.2	50	Serradell and others (2000)
Banana					
Pulp	Dopamine	2.8	6.5	30	Yang and others (2000)
Peel	Dopamine	3.9	6.5	30	Yang and others (2001)
Chinese cabbage	Catechol	682.5	5.0	50	Nagai and Suzuki (2001)
	Pyrogallol	15.4			
	Dopamine	62.0			
Peppermint	Catechol	6.25	7.0	30	Kavrayan and Aydemir
	L-DOPA	9.00	7.5	55	(2001)
	DL-DOPA	7.93	7.5	50	
Medlar	4-MC	7.5	6.5		Dincer and others (2002)
	Catechol	88.0			
	DHPPA	7.2			
	L-DOPA	76.2			
	Epicatechin	60.6			
Persimmon	TBC		3.5		Nunez-Delicado and others (2003)
Bean sprouts	Catechol	71.0	9.0	40	Nagai and Suzuki (2003)
	Pyrogallol	84.6			
	Dopamine	4.5			
Garland	CA	2.0	4.0	30	Nkya and others (2003)
chrysanthemum	(-)-epicatechin	10.0	8.0	40	
Artichoke	Catechol	10.2	6.0		Aydemir (2004)
	4-MC	12.4	6.0		
	DL-DOPA	36.3	8.0		
	L-DOPA	37.7	8.0		
	Pyrogallol	14.3	6.5		
	Callic acid	43.6	6.0		

4-MC; 4-methylcatechol, L-DOPA; L-3,4-dihydroxyphenylalanine, DHPPA; 3-(3,4-dihydroxyphenol) propionic acid, CA; chlorogenic acid, TBC; tert-butylcatechol

2.6 Assays of PPO activity

Development of a rapid, sensitive, and accurate method to determine PPO activity is of technological importance, providing adequate handling and controlling the acceptable quality of raw plant materials for both the fresh market and processing industry. The most common quantitative assay for extracted PPO determines the rate of *o*-quinone formation by spectrophotometrically assaying the increase in absorbance at 380 - 420 nm, depending on the selected substrate. For example, *o*-quinones formed from (+)-catechin, 4-methylcatechol, and chlorogenic acid substrates exhibit λ max at 385, 400, and 420 nm, respectively (Rouet-Mayer *et al*, 1990). In the plot of absorbance over reaction time, the increase in absorbance is linear for only 30 to 90s and a decrease in absorbance is observed later. The decrease in absorbance is a result of the formaticn of insoluble dark colored polymers called melanins. While reacting with *o*-diphenol, PPO is inactivated by reacting with intermediate *o*-semibenzoquinone free radicals that act on copper atoms in the PPO active site (Whitaker, 1994).

Several other chromogenic assays coupled with the addition of amino acids such as cysteine (Gauillard *et al*, 1993; Penalver *et al*, 2002), or glutathione (Penalver *et al*, 2002) to reaction mixtures were proposed to determine diphenol oxidase activity by assaying corresponding quinone-amino acid cr quinone-thiol adducts exhibiting greater molar absorptivity (ε) than *o*-quinones.

Moreover, Rodriguez-Lopez *et al*, (1994) reported that 3-methyl-2-bezothiazoline hydrazone (MBTH) is a good nucleophile, forming MBTH-adducts with *o*-benzoquinone to increase the sensitivity of the spectrophotometric determination of PPO activity. Based on the Vmax for catechol oxidation, the assay sensitivity to determine MBTH adduct was greater by 12.5 fold than

the sensitivity to determine *o*benzoquinone attributed to the large molar absorptivity of MBTH adduct. *Espin et al*, (1996) confirmed that the method to add MBTH to reaction mixtures is also effective to determine monophenol oxidase activity of PPO extracted from pears by demonstrating large molar absorptivity of MBTH adduct as well as short lag periods.

Another approach to measurement of PPO activity is the determination of changes in oxygen concentration in reaction mixtures containing extracted PPO and selected substrate with an oxygen-sensitive electrode. Unlike the plot of absorbance of reaction mixtures containing PPO and substrates, the plot of oxygen concentration in a reaction mixture decreases over reaction time as oxygen molecules are consumed by PPO-related reactions (Mayer *et al*, 1966). The polarographic method was used for the determination of PPO from apples (Goodenough *et al*, 1983), pears (Wissemann and Montgomery, 1985) and coffee (Mazzafera and Robinson, 2000).

HPLC (high performance liquid chromatography) methods can also be applied to determine PPO activity by quantifying the amounts of degraded substrates or *o*quinones. Labuza et al (1990) determined commercial tyrosinase activity expressed as residual concentrations of tyrosine substrate.

2.7 CONTROL OF BROWNING IN FRUITS AND VEGETABLES

Enzymatic browning does not occur in intact plant cells since phenolic compounds in cell vacuoles are separated from the polyphenol oxidase which is present in the cytoplasm. Once tissue is damaged by slicing, cutting or pulping, the formation of brown pigments occurs. Both the organoleptic and biochemical characteristics of fruits and vegetables are altered by pigment

formation. The rate of enzymatic browning in fruit and vegetables is governed by the active polyphenol oxidase content of the tissues, the phenolic content of the tissue, pH, temperature and oxygen availability within the tissue (Whitaker, 1994).

As described earlier on, polyphenol oxidase catalyses the oxidation of phenols to *o*-quinones, which are highly reactive compounds. *O*-quinones thus formed undergo spontaneous polymerization to produce high-molecular-weight compounds or brown pigments (melanins). These melanins may in turn react with amino acids and proteins leading to enhancement of the brown colour produced. Many studies have focused on either inhibiting or preventing polyphenol oxidase activity in foods. Various techniques and mechanisms have been developed over the years for the control of these undesirable enzyme activities. These techniques attempt to eliminate one or more of the essential components (oxygen, enzyme, copper, or substrate) from the reaction (Lerch, 1983).

i) The **elimination of oxygen** from the cut surface of fruits or vegetables greatly retards the browning reaction. Browning however occurs rapidly upon exposure to oxygen. Exclusion of oxygen is possible by immersion in water, syrup, brine, or by vacuum treatment.

ii) The **copper prosthetic group** of polyphenol oxidases must be present for the enzymatic browning reaction to occur. Chelating agents are effective in removing copper.

iii) **Inactivation of the polyphenol oxidases** by heat treatments such as steam blanching is effectively applied for the control of browning in fruits and vegetables to be canned or frozen. Heat treatments are not however practically applicable in the storage of fresh produce.

iv) Polyphenol oxidase catalyses the oxidation of **phenolic substrates** such as caffeic acid, protocatechuic acid, chlorogenic acid, and tyrosine. Chemical modification of these substrates can however prevent oxidation.

v) Certain chemical compounds react with the **products** of polyphenol oxidase activity and inhibit the formation of the coloured compounds produced in the secondary, non-enzymatic reaction steps, which lead to the formation of melanin.

Many techniques are applied in the prevention of enzymatic browning. Relatively new techniques, such as the use of killer enzymes, naturally occurring enzyme inhibitors and ionizing radiation, have been explored and exploited as alternatives to heat treatment and the health risks associated with certain chemical treatments. Processing technologies applied in the control of enzymatic browning in fruits and vegetables are now reviewed.

2.7.1 Inhibitors of browning

Enzymatic browning can be inhibited by targeting the enzyme, the substrates (oxygen and polyphenols) or the products of the reaction.

i) Inhibition targeted toward the enzyme

Mayer and Harel, (1979) classified the inhibitors which act directly on polyphenol oxidase into two groups. The first group, which consists of metal ion chelators, such as azide, cyanide, carbon monoxide, halide ions and tropolone, is well documented for the inhibition of polyphenol oxidase from various sources. The chloride ion was shown to be noncompetitive for apple polyphenol oxidase, while other halide ions were observed to have a competitive inhibitory effect (Janovitz-Klapp *et al,* 1990). The second group of inhibitors, which consists of aromatic carboxylic acids of

the benzoic and cinnamic series, has been widely studied (Janovitz-Klapp *et al*, 1990). Compounds of this group behave as competitive inhibitors of polyphenol oxidase, owing to their structural similarity with phenolic substrates.

ii) **Inhibition targeted toward the substrate**

Enzymatic browning can be controlled by removal of either the oxygen or phenolic substrates, from the reaction medium. Elimination of oxygen is perhaps the most satisfactory methodology for preventing phenol oxidase catalysed phenolic oxidation. The removal of oxygen can however result in metabolic deviations since excessive reduction of oxygen induces anaerobic metabolism, leading to breakdown and off flavour development in foods (Ballantyne *et al*, 1988).

Vacuum packaging of pre-peeled potatoes to exclude oxygen, was observed to extend their shelf life (Langdon, 1987). Vacuum packaged products however rapidly undergo browning upon exposure to air. Anaerobic conditions created by vacuum packaging are a cause for safety concern in that they are potentially capable of supporting the growth of *Clostridium botulinum* and the production of its toxin (Tamminga *et al*, 1978).

Specific adsorbents, which undergo complexation with the phenolic substrate, may be applied in the physical elimination of phenolic compounds from food systems. The use of cyclodextrins for the removal of phenolic compounds from raw fruit and vegetable juices has been patented in the United States (Hicks *et al*, 1990). Cyclodextrins are thought to inhibit polyphenol oxidase activity through the formation of inclusion complexes with polyphenols (Sapers *et al*, 1989). Sulphated polysaccharides also have an inhibitory effect on browning (Tong and Hicks, 1991). Apart from possible complexation, sulphate groups are believed to exert their inhibitory effect

2.7.2.0 Classification of Inhibitors

The use of browning inhibitors in food processing is restricted by considerations relevant to toxicity, wholesomeness, and effect on taste, flavour, texture, and cost. Browning inhibitors may be classified in accordance with their primary mode of action. Six categories of polyphenol oxidase inhibitors are applicable in the prevention of enzymatic browning (Table 1.3). These include (1) reducing agents; (2) acidulants; (3) chelating agents; (4) complexing agents; (5) enzyme inhibitors; (6) enzyme treatments.

Each category of inhibitor is now discussed:

Table 1.3: Representative inhibitors of enzymatic browning. (McEvily *et al*, 1992)

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

2.7.2.1 Reducing agents/Antioxidants

Reducing agents play a role in the prevention of enzymatic browning either by reducing o quinones to colourless diphenols, or by reacting irreversibly with *o*-quinones to form stabl colourless products. Reducing compounds are very effective in the control of browning Sulphiting agents are the most widely applied reagents for the control of browning in the foo industry (Tong and Hicks, 1991).

a. Sulphiting agents

Sulphites are the most widely used inhibitors of enzymatic browning. Sulphiting agents includ sulphur dioxide (SO_2) and several forms of inorganic sulphite that liberate SO_2 under th conditions of their use.

SO_2: sulphur dioxide

SO_3^{2-}: sulphite

HSO_3^-: bisulphite

$S_2O_5^{2-}$: metabisulphite

SO_2 and sulphite salts form sulphurous acid (H_2SO_3) and exist as a mixture of the ionic species. bisulphite (HSO_3^-) and sulphite (SO_3^{2-}) anions in aqueous solution. The predominant ionic species varies in accordance with pH, ionic environment, water activity, presence of non-electrolytes, and concentration of the medium in which they are dissolved. Maximum HOS3 concentrations exist at pH 4, while at pH 7; both SO_3^{2-} and HSO_3^- exist in approximately equivalent concentrations (Green, 1976). Increased concentrations of sulfite at pHs of less than 5

were observed to enhance the inhibition of polyphenol oxidase-catalysed browning (Sayavedra-Soto and Montgomery, 1986). The dibasic acid undergoes ionization according to the following reaction scheme:

$SO_2.H_2O \rightarrow (H_2SO_3)^- \rightarrow HSO_3^- + H^+$

$HSO_3^- \rightarrow SO_3^{2-} + H^+$

with pKa values of 1.89 and 7.18 (25 °C, zero ionic strength) for the first and second ionizations, respectively (Figure 1.4).

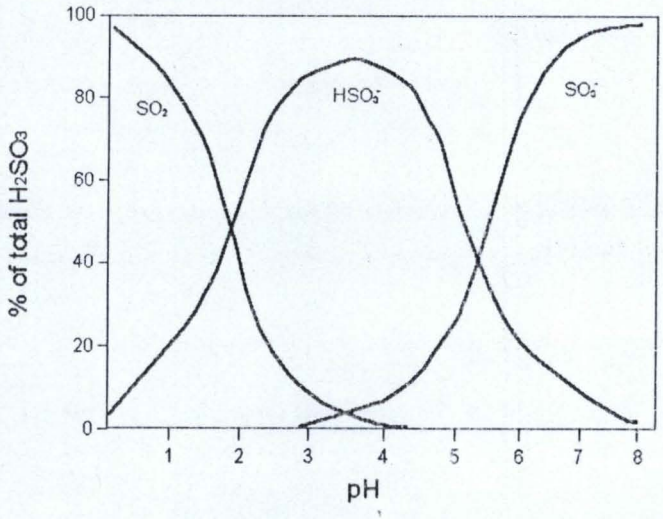


Figure 1.4: The distribution of species of sulfurous acid at various pH values. (Ough, 1984).

Sulphites serve a multifunctional role in foods. They possess antimicrobial activity and inhibit both enzymatic and non-enzymatic browning reactions. Madero and Finne, (1982) proposed that bisulphite exerted a competitive inhibitory effect on polyphenol oxidase, by binding a sulphydryl group at the active site of the enzyme. Ferrer *et al,* (1989b) on the other hand, proposed that bisulphite inhibition was due to the reaction of sulphites with intermediate quinones, resulting in

the formation of sulphoquinones, which irreversibly inhibited polyphenol oxidase, causing complete inactivation. Mechanisms involved in the control of enzymatic browning by sulphites are shown in Figure 1.5.

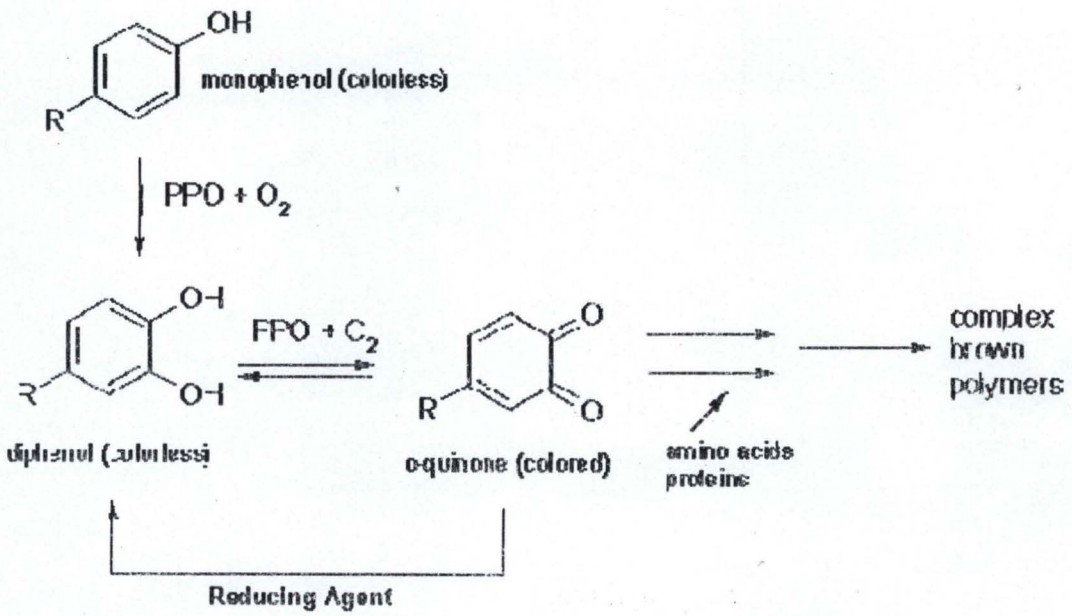


Figure 1.5: The primary role of reducing agents such as sulphiting agents in the inhibition of enzymatic browning is to reduce the pigment precursors (quinones) to colourless, less-reactive diphenols. (Walker, 1977).

Although sulphites are very effective in controlling browning, they are subject to regulatory restrictions owing to their potentially adverse effects on health. Many reports have described allergic reactions in humans, following the ingestion of sulphite-treated foods by hypersensitive asthmatics. The use of sulphiting agents in food processing is based on sulfur dioxide equivalence (Modderman, 1986). Table 1.4 gives a list of sulphiting agents and their theoretical yields of sulfur dioxide. The Joint Expert Committee on Food Additives (JECFA) of the World

Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend an acceptable sulphite daily intake of 0-0.7 mg sulphur dioxide per kg of body weight.

Table 1.4. Chemicals yielding sulfur dioxide which are currently allowed for use in food as preservatives. (Green, 1976)

Chemical	Formula	Theoretical Yield (%)	Solubility (mg/100 mL)
Sulphur dioxide	SO_2	100.0	11 at 20 °C
Sodium sulphite anhydrous	Na_2SO_3	50.8	28 at 40 °C
Sodium sulphite (heptahydrate)	$Na_2SO_3 .7H_2O$	25.4	24 at 25 °C
Sodium hydrogen sulphite	$NaHSO_3$	61.6	300 at 20 °C
Sodium metabisulphite	$Na_2S_2O_5$	67.4	54 at 20 °C
Potassium metabisulphite	$K_2S_2O_5$	57.6	25 at 0 °C

Sulphites are currently applied for the inhibition of melanosis (blackspot) in shrimp, potatoes, mushrooms, apples, and other fruits and vegetables. Sulphites are also applied in stabilizing the flavour and colour of wines. Sulphite concentrations necessary for controlling enzymatic browning vary widely in accordance with the food material and the time required for inhibition of the browning reaction (Taylor *et al.*, 1986). Where only monophenolic substrates, such as tyrosine are present, as in the case of potatoes, relatively low levels of sulphite are effective in inhibiting browning. On the other hand, where diphenols are present, as is the case in avocados, much higher sulfite concentrations are required for the control of browning.

Sulphites no longer have "Generally Required as Safe Status" (GRAS) status for use on fruits and vegetables served raw, sold raw or presented to the consumer as raw in the United States. According to the United States Federal Register, (1988) foods containing detectable levels of a sulphiting agent, at 10ppm regardless of source, must declare the sulfite and its content on the ingredient label. More regulatory restrictions are likely to be globally applied to the use of sulphites in foods since sulphite allergies pose a health risk in many populations. Regulations enacted by the United States Food and Drug Administration (FDA) in 1995 prohibit the use of sulphites in salad bars. As a result, there has been a considerable focus on identifying appropriate sulphite substitutes for use in foods. The FDA has proposed maximum residual sulphur dioxide levels for certain foods. In accordance with these proposed limits, residual sulphur dioxide levels for fruit juices, dehydrated potatoes, and dried fruit, are 300, 500, and 2000ppm respectively (Federal Register, 1988). Shrimp products having residual sulphite levels in excess of 100ppm are considered adulterated, since these levels are considered unsafe (Federal Register, 1985).

b. L-Ascorbic acid

Ascorbic acid is a moderately strong reducing compound, which is acidic in nature, forms neutral salts with bases, and is highly water-soluble. L-ascorbic acid (vitamin C) and its various neutral salts and other derivatives have been the leading GRAS antioxidants for use on fruits and vegetables and in fruit juices, for the prevention of browning and other oxidative reactions (Bauernfeind and Pinkert, 1970).

Ascorbic acid also acts as an oxygen scavenger for the removal of molecular oxygen in polyphenol oxidase reactions. Polyphenol oxidase inhibition by ascorbic acid has been attributed to the reduction of enzymatically formed *o*-quinones to their precursor diphenols (Walker, 1977).

Ascorbic acid is however irreversibly oxidized to dehydroascorbic acid during the reduction process, thus allowing browning to occur upon its depletion (Figure 1.6). More stable forms of ascorbic acid derivatives, such as erythrobic acid, 2- and 3-phosphate derivatives of ascorbic acid, phosphinate esters of ascorbic acid, and ascorbyl-6-fatty acid esters of ascorbic acid, have however been developed to overcome these problems (Sapers and Hicks, 1989). Ascorbic acid esters release ascorbic acid upon hydrolysis by acid phosphatases (Liao and Seib, 1988). Their relative effectiveness as browning inhibitors varies in accordance with the food product (Bauernfeind and Pinkert, 1970). Compounds containing reactive amino or thiol groups can greatly affect the reactivity of *o*-quinones.

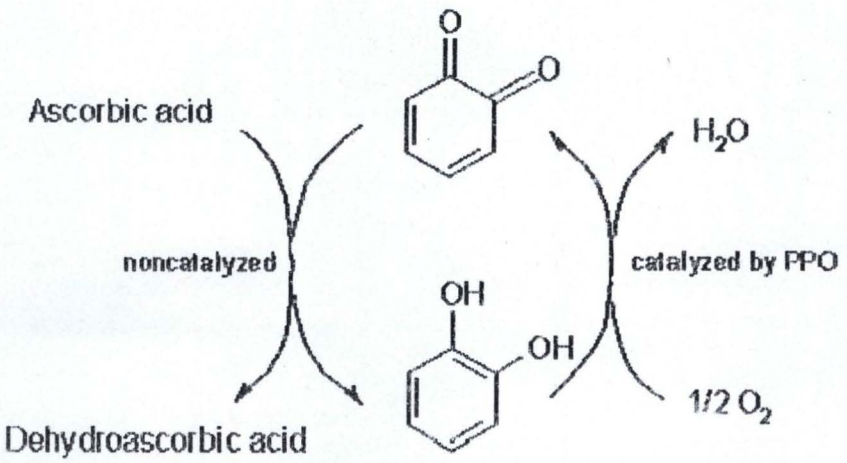


Figure 1.6: Mechanism of prevention of colour formation by ascorbic acid.

Ascorbic acid causes a distinct yellow off-colour, when used in the prevention of melanosis in shrimp (Otwell and Marshall, 1986). It is usually applied in conjunction with citric acid in order to maintain a more acidic pH level. In addition, it is also believed to have a chelating effect on the copper prosthetic group of polyphenol oxidase (Whitaker, 1972b).

c. Erythorbic acid

Erythorbic acid and its salt, sodium erythorbate, are strong reducing agents with GRAS status. They both act as oxygen scavengers, thus eliminating oxygen as a substrate for browning reactions. Erythorbic acid is the D-isomer of ascorbic acid but does not have vitamin C activity (Sapers and Hicks, 1989). Its use in conjunction with citric acid has often been suggested as a substitute for sulphites in the control of enzymatic browning. Current research suggests that L-ascorbic acid and erythorbic acid both possess equivalent antioxidant properties. A combination of both acids is applied at the retail level for inhibiting both oxidative rancidity and discolouration in vegetables, salads, apples, and frozen seafood (Sapers and Hicks, 1989). Erythobic acid or sodium erythorbate can suppress browning reactions in frozen fruits.

d. Cysteine

Cysteine is an effective inhibitor of enzymatic browning. It is reported to be more effective than sodium bisulphite as an antibrowning agent (Kahn, 1985). Concentrations of cysteine and other thiols required for the achievement of acceptable levels of browning inhibition have however been shown to have negative effects on taste. The inhibition of melanosis by cysteine is thought to be due to the formation of colourless thiol-conjugated *o*-quinones (Pierpoint, 1966). Cysteine has also been shown to reduce *o*-quinones to their phenol precursors (Walker, 1977; Cilliers and Singleton, 1990).

A mode of action for cysteine and cysteinyl addition in the control of browning proposed by Richard-Forget *et al*, (1992) is illustrated in Figure 1.7. Cysteine-quinone adducts serve as competitive inhibitors of polyphenol oxidase. Sulphydryl (thiol) compounds N-acetyl-L-cysteine

(NAC) and reduced glutathion (GSH) are also excellent inhibitors of browning of potato powder (Figure 1.8, Friedman *et al,* 1992).

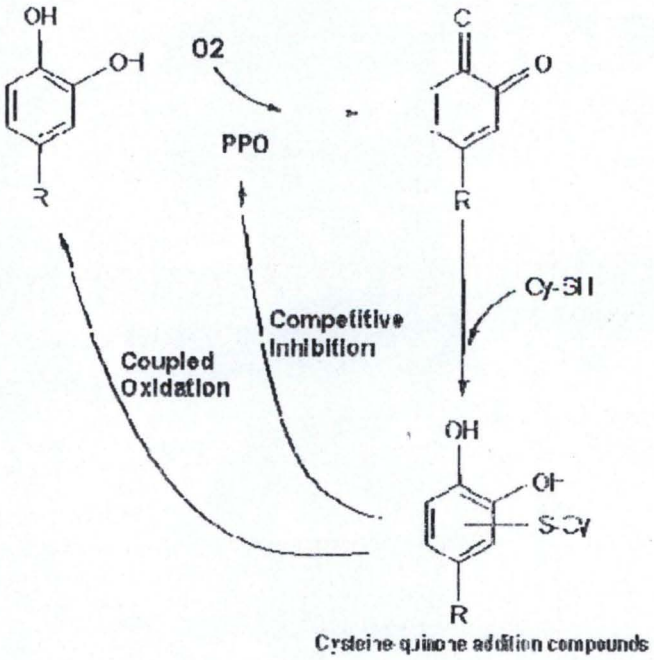


Figure 1.7: Effect of cysteine and cyteinyl addition compounds with o-quinones on the enzymatic oxidation of o-diphenols. (Richard-Forget et al., 1992).

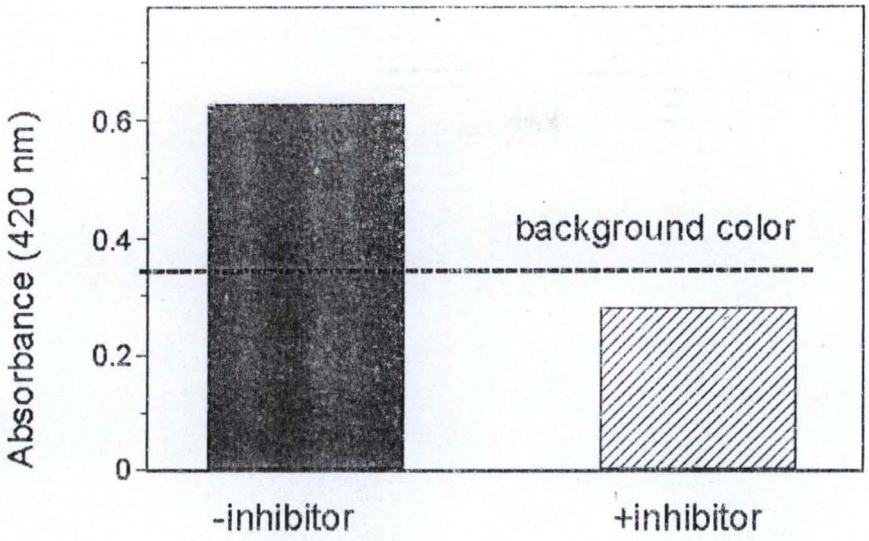


Figure 1.8: Inhibition of potato browning by N-acetyl-L-cysteine (5% potato in water; inhibitor 0.8 mM; 5h; room temperature). (Friedman et al., 1992).

e. Phenolic antioxidants

Antioxidants are defined by the United States Food and Drug Administration (FDA) as substances, which may be applied in preserving food by retarding deterioration, rancidity or discolouration due to oxidation. Antioxidants inhibit oxidative processes by reacting with free radicals, through metal chelation, and by scavenging singlet oxygen.

Both synthetic and naturally occurring phenolic antioxidants are used in food applications. Several synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiarybutyl hydroxyquinone (TBHQ) and propyl gallate (PG), are permitted for use in food. Their structures are shown in Figure 9. Plant phenolic compounds such as tocopherols, flavonoid compounds, cinnamic acid derivatives, and coumarins are naturally

occurring compounds, which have an antioxidant effect that renders them inhibitory to polyphenoloxidase, and thus browning.

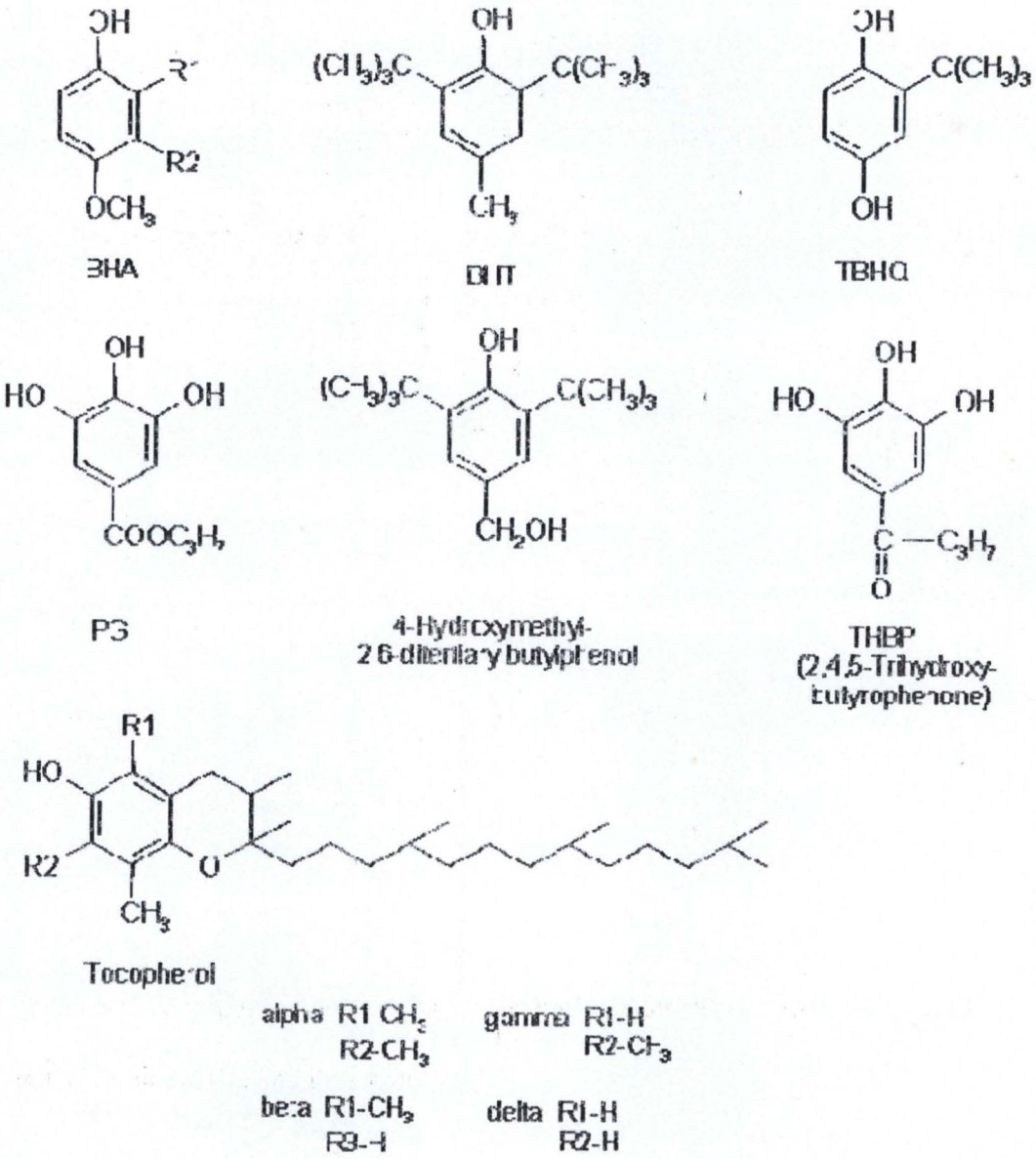


Figure 1.9: Major antioxidants used in food (Ballantyne *et al.* 1988).

2.7.3 Acidulants

Ionizable groups of the protein structure of enzymes are affected by the pH of the food medium. These groups must be in the appropriate ionic form in order to maintain the conformation of the active site, bind substrates, or catalyse the enzymatic reaction (Segel, 1976). Changes in the ionization status of enzymes are generally reversible. Irreversible denaturation can however occur under conditions of extreme pH. The stability of the substrate is also affected by changes in pH, since substrates can undergo chemical breakdown under extreme conditions of pH. Degraded substrates often behave as enzyme inhibitors, since they share the molecular features of the substrate (Tipton and Dixon, 1983).

Acidulants are generally applied in order to maintain the pH well below that required for optimum catalytic activity of an enzyme. Acidulants such as citric, malic, and phosphoric acids are capable of lowering the pH of a system, thus rendering polyphenol oxidase polyphenol oxidase inactive (Richardson and Hyslop, 1985). Acidulants are often used in combination with other antibrowning agents.

a. Citric acid

Citric acid is the one of the most widely used acidulants in the food industry. It is typically applied at levels ranging between 0.5 and 2 percent (*w/v*) for the prevention of browning in fruits and vegetables. In addition, it is often used in combination with other antibrowning agents such as ascorbic or erythorbic acids and their neutral salts, for the chelation of prooxidants and for the inactivation of polyphenol oxidase. Recommended usage levels for citric acid typically vary between 0.1 and 0.3 percent (*w/v*) with the appropriate antioxidant at levels ranging between 100

and 200 ppm (Dziezak, 1986). Citric acid exerts its inhibitory effect on polyphenol oxidase by lowering the pH as well as by chelating the copper at the active site of the enzyme.

2.7.4 Chelators

Enzymes generally possess metal ions at their active sites. Removal of these ions by chelating agents can therefore render enzymes inactive. Chelating agents complex with prooxidative agents, such as copper and iron ions, through an unshared pair of electrons in their molecular structures. Chelators have been applied in various food processing applications, for enzyme inactivation (McEvily *et al*, 1992). Chelators used in the food industry include sorbic acid, polycarboxylic acids (citric, malic, tartaric, oxalic, and succinic acids), polyphosphates (ATP and pyrophosphates), macromolecules (porphyrins, proteins), and EDTA.

Other non-GRAS chelating agents which are capable of inhibiting polyphenol oxidase include cyanide, diethyldithiocarbonate, sodium azide and 2-mercaptobenzothiazole, carbon monoxide, mercaptobenzthiazol, dimercaptopropanol, and potassium methyl xanthate.

Ascorbic acid also has a chelating effect on the prosthetic group of polyphenol oxidase.

a. Ethylene diaminetetraacetic acid (EDTA)

Ethylene diaminetetraacetic acid (EDTA) is a chelating agent permitted for use in the food industry as a chemical preservative. Calcium disodium EDTA (21 CFR 172.120) and disodium EDTA (21 CFR 172.135) have been approved for use as food additives by the United States Food and Drug Administration (Anon, 1992). Highly stable complexes are formed by the sequestering action of EDTA compounds on iron, copper, and calcium. Maximum chelating efficiency occurs at the higher pH values where carboxyl groups exist in a dissociated state (Dziezak, 1986).

Ethylene diaminetetraacetic acid (EDTA) is generally used in combination with other chemical treatments for the prevention of enzymatic browning in foods. It not very effective as an inhibitor of peach polyphenol oxidase (Wong *et al*, 1971).

A typical combination of anti-browning agents might consist of a chemical reducing agent (ascorbic acid), an acidulant (citric acid) and a chelating agent (EDTA).

b. Phosphates

Polyphosphates, sodium acid pyrophosphate, and metaphosphate are chelating agents of limited cold water solubility. They have been used as antibrowning agents for fresh-peeled fruits and vegetables at concentrations as low as 0.5 to 2 percent (final concentration in the dip solution) (McEvily *et al*, 1992). Sporix™ , an acidic polyphosphate mixture (sodium acid pyrophosphate, citric acid, ascorbic acid, and calcium chloride), has been observed to delay the onset of oxidation and enzymatic browning in fruits and vegetables (Gardner *et al*, 1991).

c. Maltol

Maltol (3-hydroxy-2-methyl-4H-pyran-4-one), a γ-pyrone derivative, is a relatively weak inhibitor of the formation of pigmented products. Structurally, it contains an α, β-unsaturated keto-enol constituent, which makes it a good chelator. Maltol does not however chelate copper at the active site of polyphenol oxidase. It prevents browning either through its ability to conjugate *o*-benzoquinones back to *o*-dihydroxyphenols or through irreversible inactivation of polyphenol oxidase (Kahn, 1995).

d. Kojic acid

Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one), a γ-pyrone derivative, is a fungal metabolite produced by many species of *Aspergillus* and *Penicillium*. It is a good chelator of transition metal ions such as Fe (III) and Cu (II) (Beélik, 1956; Wiley *et al*, 1942). Kojic acid occurs in many fermented oriental foods, and possesses both antibacterial and antifungal activities. A mixture of ascorbic acid and kojic acid has been patented for use as an anti-browning agent in foods (Fukusawa *et al,* 1982). Kojic acid has potential applicability in the prevention of melanosis in both plant and seafood products. Saruno *et al*, (1979) demonstrated that kojic acid from *Aspergillus albus* inhibited mushroom PPO activity. Kojic acid was also shown to inhibit melanosis in pink shrimp (Applewhite *et al*, 1990). Chen *et al*, (1991b) determined that kojic acid was a competitive inhibitor of the oxidation of chlorogenic acid and catechol by apple polyphenol oxidase.

Kojic acid inhibits the rate of formation of pigmented products, as well as the rate of oxygen uptake, when various *o*-dihydroxy- and trihydroxy phenols are oxidized by tyrosinase (Kahn, 1995). Tyrosinase inhibition by kojic acid was thought to be due to the ability of kojic acid to bind copper at the active site of the enzyme. Although kojic acid is a good inhibitor of polyphenol oxidase, its toxicity is of concern. Wei *et al*, (1991) reported weak mutagenic activity of kojic acid in a *Salmonella typhimurium* assay.

e. Polysaccharides

Various sulfated polysaccharides, including carrageenans, amylose sulfate, and xylan sulfate, were determined to be effective browning inhibitors in both apple juice and diced apples (Tong and Hicks, 1991). Pectin, a naturally occurring anionic polysaccharide at a concentration of 0.5 percent, gave between 5 and 10 percent inhibition of apple juice browning (Tong *et al*, 1995).

Carboxyl groups present in pectin are believed to be capable of chelating the copper moiety of polyphenol oxidase, thus preventing browning.

f. Carbon monoxide (CO)

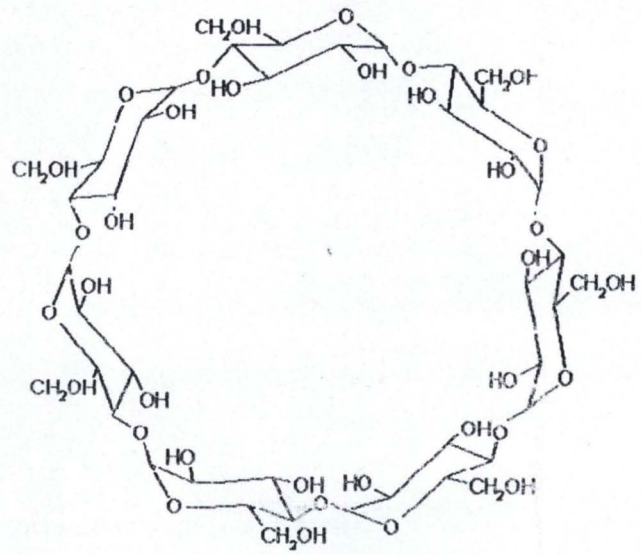
Carbon monoxide (CO) is a known inhibitor of many copper-containing oxidases and behaves as a noncompetitive inhibitor of phenolic substrates. It has been studied in preventing the discolouration of Shitake mushrooms (Fujimoto *et al*, 1972). Polyphenol oxidase activity extracted from freeze-dried mushroom powder was inhibited by CO (Albisu *et al*, 1989). This inhibition was however reversible and removal of CO led to restoration of the initial activity. A two-step gas treatment of potato strips with SO_2 followed by CO, resulted in 93.7 percent and 99.9 percent inactivation of prophenol oxidase and polyphenol oxidase respectively, after 60 days at room temperature (Kramer *et al*, 1980). A number of safety problems are however associated with the use of carbon monoxide gas.

2.7.5 Complexing agents

a. Cyclodextrins

The cyclodextrins (CDs) are a class of cyclic oligosaccharides produced by the action of cyclomaltodextrin glucanotransferase (CGTase) on liquified starches. Industrially produced CDs contain 6-8 glucose units per macrocycle, linked by α-(1,4)-glycosidic bonds and include cyclomaltohexose (α -CD, 6 units), cyclomaltoheptaose (β-CD, 7 units), and cyclomaltooctose (γ-CD, 8 units). The chemical structure and a diagrammatic representation of the functional structure of beta-CD are depicted in Figure 1.10. Structurally, all of the C-6 (primary) hydroxyl

groups project from one side of the torus of the CD, while the C-2, 3 (secondary) hydroxyl groups project from the other. The central cavity of CD is hydrophobic while the outer region of the oligosaccharide is hydrophilic due to the presence of primary and secondary hydroxyls at both the narrow and wide bases. CD's are highly insoluble. Their solubility can however be increased by the action of de-branching enzymes such as isoamylase and pullulanase (Okada *et al.*, 1988).



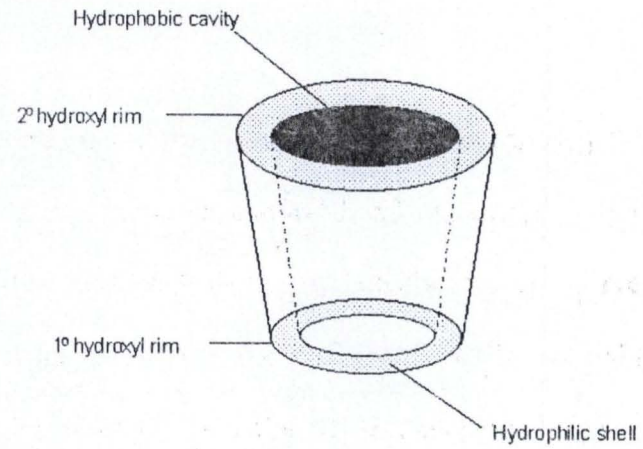


Figure 1.10: Schematic and chemical structure of a ß-cyclodextrin molecule (Okada *et al.*, 1988).

The most important functional property of cyclodextrins is their ability to behave as clathrate-like compounds in the formation of inclusion complexes with a range of guest molecules. If the guest molecule is of suitable size and conformation that allow it to bind within the hydrophobic core, complex formation takes place. Larger guest molecules form relatively weak complexes due to partial binding. Greater inclusion activity of these larger guest molecules can however be obtained by suitable chemical modification of the CD. This application is of particular interest to the food industry for the molecular encapsulation of insoluble or volatile food ingredients (Pagington, 1986).

The use of CD has been proposed for the control of enzymatic browning in apple products (Billaud *et al*, 1995; Sapers *et al*, 1989). cyclodextrins (CD) inhibits juice browning through the binding of polyphenol oxidase substrates. Polyphenols can be removed by β-CD and by insoluble polyvinyl polypyrrolidone or polyethylene glycol (Osuga *et al*, 1994). The thermodynamics of inclusion complexes of α-CD, β-CD, γ-CD, and polymerized β-CD with chlorogenic acid as a substrate of apple polyphenol oxidase were studied in order to elucidate a mechanism for the inhibition of juice browning. Alpha-CD and γ-CD were less effective than the γ-CD in the inhibition of browning in apple juice (Irwin *et al*, 1994). The internal cavity of β -CD is slightly apolar, thus allowing it to induce inclusion complex formation with guest molecules such as phenolic substrates of polyphenol oxidases, thereby preventing their oxidation to quinones and subsequent polymerization to brown pigments. The adsorption of flavour or colour compounds by cyclodextrins poses a major drawback to their use in food systems. Although the applicability of cyclodextrins in fruit and vegetable juices has been patented (Hicks *et al*, 1990), cyclodextrins have not yet been approved for food use by the United States FDA.

b. Chitosan

Chitosan, a naturally abundant polymer of β-(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 (Sapers, 1992). The addition of 200 ppm chitosan to McIntosh 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 oxidase, its substrates, or products. Treatment of shrimp with 2 percent chitosan resulted in a consistently reduced incidence of melanosis during storage (Simpson *et al*, 1997). Chitosan also exhibited strong antimicrobial properties inhibiting several microorganisms at concentrations ranging between 0.0075 - 0.01 percent (Simpson *et al*, 1997).

A research study conducted by Zhang and Quantick, (1997) indicated that chitosan coating had potential inhibitory activity on polyphenol oxidase and peroxidase activity in lychee (*Litchi chinensis* Sonn.) fruit. Chitosan has been shown to improve the storability of fruits. Its effectiveness in this respect is therefore thought to be due to the formation of a protective barrier on the surface of fruit, which reduces the supply of oxygen for the enzymatic oxidation of phenolics. Chitosan is non-toxic and is biologically safe (Hirano *et al*, 1990). Thus, the application of a chitosan coating for the control of browning and quality improvement in fruits and vegetables might be accomplished in combination with other methods such as low temperature and suitable packaging.

2.7.6 Enzyme inhibitors

a. 4-Hexylresorcinol

Substituted resorcinols, which are *m*-diphenolic compounds that are structurally related to phenolic substrates, have a competitive inhibitory effect on polyphenol oxidase activity (McEvily *et al*, 1991, 1992). Hydrophobic substitution with hexyl, dodecyl, and cyclohexyl groups at the 4-position of the aromatic resorcinol ring increases the effectiveness of their competitive inhibitory effect on polyphenol oxidase (McEvily *et al*, 1992). Studies conducted by McEvily *et al*, (1992) revealed cyclohexyl-substituted resorcinols to have the lowest $I_{50,}$ i.e. the inhibitor concentration that resulted in 50 percent inhibition of polyphenoloxidase activity, at a concentration of 0.2µM of the substituted resorcinol. Both the monophenolase and diphenolase activities of tyrosinase are inhibited by 4-hexylresorcinol (4-HR). Four-hexylresorcinol has a long history of use in pharmaceuticals and is considered to be safe and effective in use as an anti-browning agent (Frankos *et al*, 1991).

Four-hexylresorcinol has several advantages over sulphites when applied in the control of browning in foods. These include its specific mode of inhibitory action, effectiveness at low concentrations, inability to bleach preformed pigments, and chemical stability. It has a synergistic effect with ascorbic acid in the prevention of browning. Ascorbic acid reduces quinones generated by polyphenoloxidase while 4-HR specifically interacts with polyphenol oxidase, and renders it incapable of catalysing the enzymatic reaction (Kahn and Andrawis, 1985) (Figure 1.11).

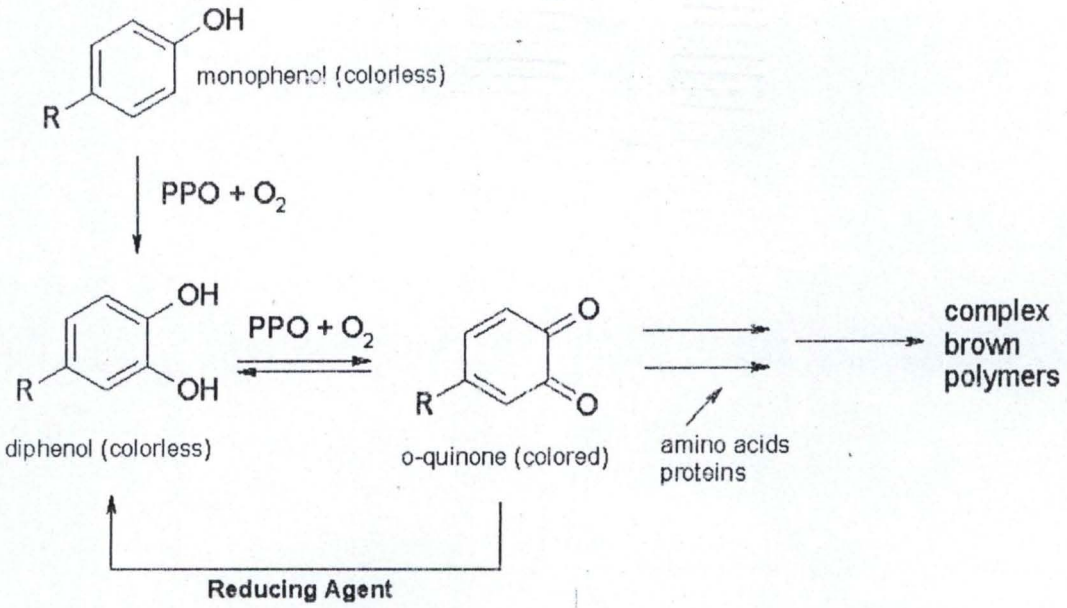


Figure 1.11: The inhibitory effect of 4-hexylresorcinol on PPO. (Kahn and Andrawis, 1985).

Four-hexylresorcinol is applicable in the control of browning in fresh and hot-air dried apple slices as well as in apple juice (McEvily *et al*, 1992). Several studies have shown the effectiveness of 4-HR in controlling enzymatic browning in shrimp (Otwell *et al,* 1992; McEvily *et al*, 1991), mushroom (Osuga *et al*, 1994) and apple slices (Monsalve-Gonzalez *et al*, 1993). EverFresh™ a patented product (United States patent # 5,049,438) which consists of 4-HR as the active ingredient and sodium chloride as the carrier agent has been studied as an alternative to sulphites in the control of enzymatic browning, or blackspot, in crustaceans (Lambrecht, 1995). Raw headless brown shrimp dipped in 4-HR for 1 min, exhibited greater stability to blackspot formation for a longer period of time than shrimp dipped in fresh water (controls) or 1.25 percent sodium metabisulphite. After 7 days of storage at 2°C, raw headless brown shrimp treated with water showed 54 percent blackspot; sulphite-treated shrimp showed 11 percent blackspot, while

4-HR-treated shrimp had only 3.6 percent blackspot (Figure 1.12). At day 14, blackspot on control and sulphite-treated shrimp increased to 75 percent and 25 percent, respectively. The 4-HR treated shrimp did not however show an increase in blackspotting. This compound has been proposed for use on various fruits and vegetables by McEvily *et al*, (1991). Figure 1.13 shows inhibition of enzymatic browning by 4-HR in star fruit.

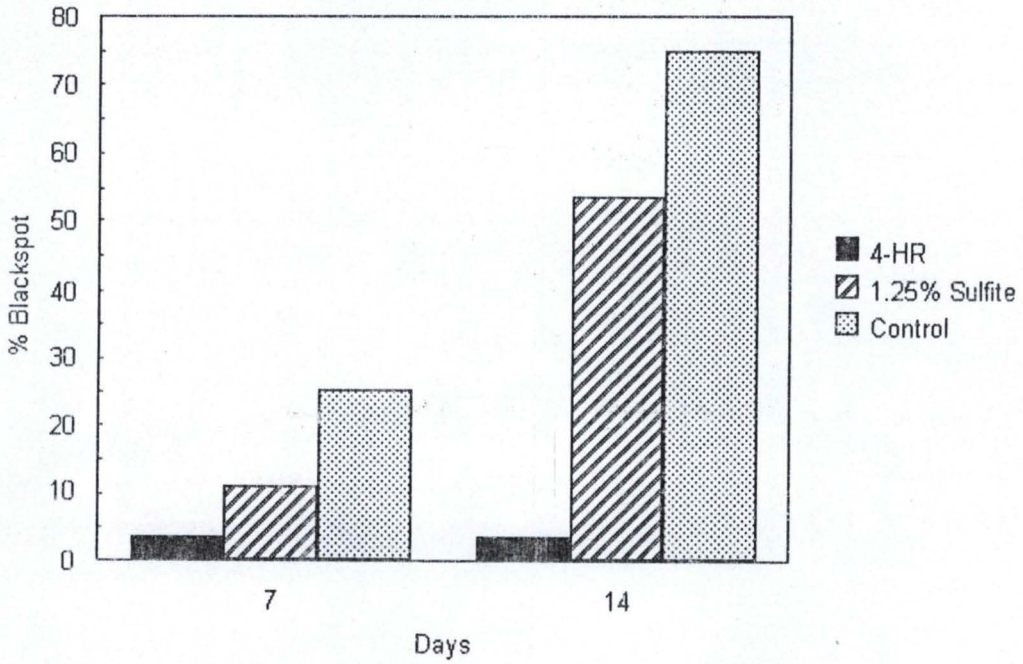


Figure 1.12: Blackspot (%) in raw head-off brown shrimp treated with 4-HR and 1.25% sulfite, and stored at 2°C. (Lambrecht, 1995).

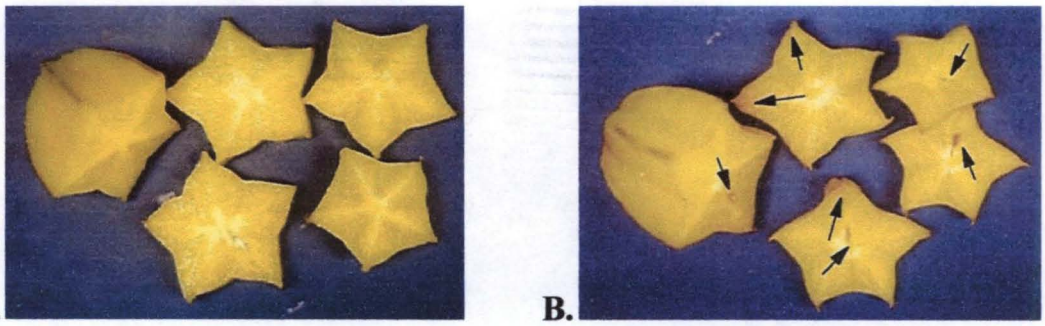


Figure 1.13: 4-HR treatment (0.02% for 2 min dip) of star fruit. A. Treated B. Non-treated McEvilv *et al.* (1991).

Four-hexylresorcinol is a chemically stable, water-soluble compound. Toxicological, mutagenic, carcinogenic, and allergenic studies have shown that there are no risks associated with the levels of 4-HR used in the treatment of shrimp (Frankos *et al*, 1991). Four-hexylresorcinol has obtained GRAS status from the United States Food and Drug Administration, for use on shrimp (Federal Register, 1992). Its use in the inhibition of shrimp melanosis has no effect on taste, texture, or colour at residual levels of less than 1 ppm (Iyengar *et al*, 1991; King *et al*, 1991).

b. Halide salts

Inorganic halides are well-known inhibitors of polyphenol oxidases (Vámos-Vigyázó, 1981). Janovitz-Klapp *et al*, (1990) determined that NaF was the most potent inhibitor of apple polyphenol oxidase, followed by NaCl, NaBr, and NaI. The inhibition of enzymatic browning by halides decreases with increasing pH. Sodium chloride and calcium chloride at concentrations of ranging between 2 and 4 percent (*w/v*) are most commonly used in the food industry for the inhibition of browning (Steiner and Rieth, 1989). Poplyphenol oxidase activity was observed to decrease with increasing concentrations of NaCl for peach (Luh and Phithakpol, 1972), eggplant

and avocado (Knapp, 1965). Sodium zinc chloride was shown to be a highly effective browning inhibitor when used in combination with calcium chloride, ascorbic acid, and citric acid (Bolin and Huxsoll, 1989).

c. Honey

Honey has been shown to inhibit enzymatic browning. The use of honey as a natural browning inhibitor is therefore of great consumer interest. Honey was shown to inhibit browning in apple slices, grape juice and in model systems (Oszmianski and Lee, 1990). The browning of apple slices was inhibited to a greater extent by 10 percent honey, than by a sucrose solution containing an equivalent sugar concentration. Purification of honey by Sephadex G-15 column chromatography revealed the compound in honey, responsible for the inhibition of polyphenol oxidase, to be a small peptide of approximately 600 Da molecular weight. Proteins, peptides, and amino acids exert an inhibitory effect on polyphenol oxidase activity by chelating the essential copper at the active site of polyphenol oxidase, thus forming stable complexes with Cu^{2+} (Kahn, 1985). The honey peptide is thought to exert its inhibitory effect through a similar mechanism.

Honey has been shown to contain antioxidants: tocopherols, alkaloids, ascorbic acid, flavonoids, and phenolics. The antioxidant content and the efficacy of honeys in inhibiting polyphenol oxidase activity vary in accordance with the type of honey (Chen *et al*, 1998). The effect of commercial browning inhibitors (ascorbate and sodium metasulphite) and various honeys were compared in a study on polyphenol oxidase activity and browning. Antioxidant content showed a positive correlation to honey colour. The addition of various honeys to fresh potato homogenates resulted in a 0-50 percent reduction in polyphenol oxidase activity and a decrease of 0-7 units in the browning index (Chen *et al*, 1998).

d. Amino acids, peptides and proteins

Amino acids, peptides or proteins can affect polyphenol oxidase-catalysed browning either through direct inhibition of the enzyme or by reacting with the quinone products of polyphenol oxidase catalysis (McEvily *et al*, 1992). Proteins, peptides and α-amino acids are capable of forming stable complexes with Cu^{2+}. In addition, they are also capable of chelating copper at the active site of polyphenol oxidase. Histidine and cysteine have particularly high affinities for Cu^{2+} since, apart from having NH_2 and COOH groups, histidine possesses an imidazole ring and cysteine, a thiol group, both of which have metal binding capacity (Bell, 1977).

Kahn, (1985) studied the effects of proteins, protein hydrolysates, and amino acids on *o*-dihydroxyphenolase activity in mushroom, avocado and banana. Mushroom PPO was weakly inhibited by mM concentrations of L-lysine, glycine, L-histidine and L-phenylalanine. L-cysteine was the most effective amino acid in inhibiting *o*-dihydroxyphenolase activity. Amino acids inhibit polyphenol oxidase activity through the formation of stable complexes with copper. In addition, thiol containing inhibitors form sulfur adducts with the *o*-quinone, thus blocking polymer formation and preventing browning (Figure 1.14). Mason and Peterson, (1965) showed that N-terminal primary amino groups, aliphatic amino groups (secondary amines in amino acids) and thiol-containing amino acids react with *o*-benzoquinones and 4-methyl-*o*-benzoquinone, while only thiol-containing compounds and aromatic amines react with oxidation products of DOPA (dihydroxyphenylalanine).

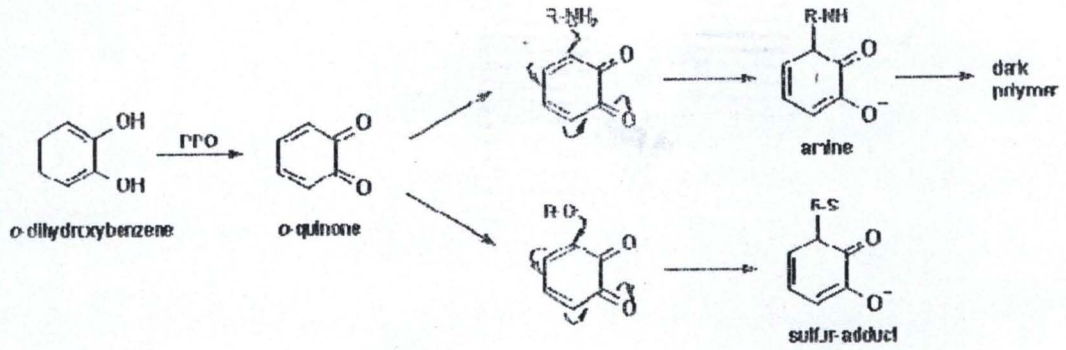


Figure 1.14: Postulated mechanism for the inhibition of PPO induced enzymatic browning by thiols. (Friedman and Molnar-Perl, 1990.

e. Aromatic carboxylic acids

Aromatic carboxylic acids of the benzoic acid and cinnamic acid series are polyphenol oxidase inhibitors, owing to their structural similarity to phenolic substrates (Krueger, 1955). Undissociated forms of these acids are capable of inhibiting polyphenoloxidase, through complexation with copper at the active site of the enzyme. The degree of polyphenol oxidase inhibition by carboxylic acids is pH dependent, and increases with a decrease in pH.

Cinnamic acid and its analogues, *p*-coumaric, ferulic, and sinapic acids were found to be potent inhibitors of potato (Macrae and Duggleby, 1968) and apple polyphenol oxidases (Pifferi *et al*, 1974; Walker and Wilson, 1975). Cinnamic acid at levels of 0.01 percent was observed to be effective in providing long-term inhibition of polyphenol oxidase in apple juice (Walker, 1976). Benzoic acid and its derivatives had an inhibitory effect on polyphenol oxidase activity in mushrooms (Kermasha *et al*, 1993) and grapes (Gunata *et al*, 1987).

f. Aliphatic alcohols

Although the inhibition of polyphenol oxidase by ethanol has been reported (Kidron *et al*, 1978), there are no extensive studies which describe the effect of aliphatic alcohols on polyphenol oxidase. Valero *et al*, (1990) studied the effects of natural aliphatic alcohols on grape polyphenoloxidase. Inhibition was observed to increase with increasing chain length of the aliphatic alcohol.

2.7.7.0 Other inhibitors

2.7.7.1 Killer enzymes

As paradoxical as it may seem, enzyme action can be exploited for the control of undesirable enzyme activities. This is achievable in three ways: (1) substrate and/or product modification by enzymes other than the target enzymes; (2) direct inactivation of the target enzyme by other enzymes and (3) inactivation by secondary reactions of highly reactive products. The activities of "killer enzymes" or "anti-enzyme enzymes" which inactivate other enzymes via direct proteolytic activity have been demonstrated Kelly and Finkle, (1969). The use of enzymes to control other enzyme-related processes has also has been reported in the control of enzymatic/nonenzymatic browning.

a. Ring-cleaving oxygenases

Kelly and Finkle, (1969) proposed irreversible modification of phenolic substrates by enzymes as one mechanism for the control of browning. Apple juice treated with the bacterial enzyme protocatechuate-3, 4-dioxygenase in combination with ascorbic acid prevented browning due to

the oxidative ring-opening reaction and ortho-fission of catechols by the enzyme. The enzyme deprived polyphenol oxidase enzymes of required substrates (Kelly and Frinkle, 1969).

b. Catechol transferase

Finkle and Nelson, (1963) proposed the use of catechol transferase (EC 2.1.1.6) for the prevention of browning in apple juice. *O*-methyl transferase, an enzyme capable of methylating the 3-position of 3,4-dihydroxy aromatic compounds was observed to cause irreversible modification of phenolic substrates, thus preventing them from serving as substrates of the browning reaction. Treatment of apple juice with *o*-methyl transferase and *s*-adenosyl methionine resulted in conversion of chlorogenic and caffeic acids, to feruloylquinic and ferulic acids respectively, both of which are polyphenol oxidase inhibitors (Finkle and Nelson, 1963).

c. Proteases

The plant proteases ficin, papain and bromelain are sulphydryl enzymes of broad specificity (Labuza *et al*, 1992; Taoukis *et al*, 1990) which are very effective browning inhibitors. Ficin was observed to be effective in preventing black spot formation in shrimp under refrigerated conditions (Taoukis *et al*, 1990). This inhibitory effect is thought to be due to either binding or hydrolysis at specific sites necessary for polyphenol oxidase activity.

Pineapple juice was found to be effective in inhibiting browning in apple rings (Lozano-De-Gonzalez *et al*, 1993). Bromelain, organic acids, sulphydryl compounds and certain metallic constituents of pineapple juice are thought to be responsible for this inhibitory effect. Polyphenol

oxidase activity in plum juice was significantly reduced when the juice was passed through a column containing immobilized proteases (Arnold *et al*, 1992).

Commercial application of enzyme treatments in the control of enzymatic browning is precluded by their high cost. Combinations of anti-browning agents of a chemical nature are however more affordable and effective in commercial use.

2.7.7.2 Edible Coatings

The use of edible coatings to minimize undesirable changes due to minimal processing has been reported for several commodities (Baldwin *et al.*, 1995). The coating of fruits and vegetables with semi-permeable films has been shown to retard ripening through modification of endogenous CO_2, O_2 and ethylene levels. Coatings are also useful as carriers of antioxidants and preservatives (Cuppett, 1994). Edible coatings have the potential to retard water loss, to form a barrier to oxygen, and to retain antioxidants, as well as preservatives, on the surface of cut tissue in order to control discolouration. A polysaccharide/lipid bilayer formulation was observed to reduce respiration in cut apples (Wong *et al*, 1994) through modification of the gas exchange between the processed tissue and the external environment. Sucrose fatty acid esters reduced browning of shredded cabbage (Sakane *et al*, 1990) which was attributed to reduction of oxygen at the cut surface. Zhang and Quantick, (1997) determined that an edible coating based on sucrose esters of fatty acids significantly delayed pericarp browning of lychee fruit. Carboxymethyl cellulose/soy protein coating formulations containing 0.5 percent ascorbic acid applied to freshly cut apples were more effective in antibrowning activity than aqueous solutions of 0.5 percent ascorbic acid

alone (Baldwin *et al*, 1996). The cellulose matrix may well have a protective effect in preventing the degradation of ascorbic acid by oxygen.

2.8 MOLECULAR BIOLOGY OF PHENOXIDASES

Polyphenol oxidases occur in the chloroplasts of almost all higher plants. Cloning of the genes which code for polyphenol oxidase offers the potentials for determining the physiological role of polyphenol oxidase within the chloroplast and for manipulating polyphenol oxidase levels within specific organs. Polyphenol oxidase genes are encoded within the nucleus and undergo translation within the cytoplasm. Once formed, propolyphenol oxidase is transported to the chloroplast where it undergoes proteolytic cleavage, to produce the active polyphenol oxidase form (Vaughn *et al.*, 1988). Predicted molecular weights for polyphenol oxidase in plants, range between 57 and 62 kDa (Hunt *et al*, 1993; Newman *et al*, 1993).

Polyphenol oxidase is generally present in low concentrations in all organisms. The enzyme is difficult to obtain in a pure form due to pigment contamination and the occurrence of multiple forms. With the advent of recombinant DNA technology, numerous amino acid sequences of polyphenol oxidases have become available. Primary structures of polyphenol oxidases from *Streptomyces glaucescens* (Huber *et al.*, 1985), *Streptomyces antibioticus* (Bernan *et al.*, 1985) and *Neurospora crassa* (Lerch, 1982), tomato (Shahar *et al.*, 1992; Newman *et al.*, 1993), broad bean (Cary *et al.*, 1992) potato (Hunt *et al.*, 1993), mice (Shibahara *et al.*, 1986) and humans (Kwon *et al.*, 1987; Giebel *et al.*, 1991) have been determined using cDNA sequencing techniques. Polyphenol oxidases of closely related plants, such as tomato and potato, show

approximately 91 percent exact homology, while those of tomato and fava bean show only 40 percent exact homology (Wong, 1995).

Polyphenol oxidases from different sources exhibit molecular weight differences. Molecular weights predicted for mature polyphenol oxidases on the basis of cDNA sequences were 58 kDa for mouse, ~63 kDa for human and 128 kDa for mushroom. Mushroom polyphenol oxidase is thought to contain four subunits having a total molecular weight of 128 kDa. Monomeric through octameric forms of mushroom polyphenol oxidases are known to exist (Whitaker and Lee, 1995). Plant polyphenol oxidases are nuclear-encoded copper metalloproteins having a molecular mass of approximately 59kDa and are localized in the membranes of plastids. Plant genes encoding polyphenol oxidase have recently been cloned and characterised. Although the sequences of plant polyphenol oxidase genes are very similar, only the putative copper binding sites are conserved when plant genes are compared to mammalian, bacterial, or fungal tyrosinases. One possible approach to lowering polyphenol oxidase activity and resultant enzymatic browning reactions is to characterize and inactivate the genes which code for polyphenol oxidase. Inactivation can be accomplished by generating antisense RNAs specific for polyphenol oxidase.

2.8.1 New approaches for the control of PPO

The involvement of polyphenol oxidase in browning has been studied for a long time. Many questions still remain about the enzyme itself, as well as the mechanism of browning. Current approaches to understanding and controlling enzymatic browning are however specifically focused on the use of antisense RNA. Antisense RNA techniques have several applications in plant research. They are applicable in studying the *in vivo* function of particular genes and their

biochemical modes of action. Antisense genes have been successfully used for the alteration of plant processes, such as flower pigmentation, fruit ripening and photosynthesis, and to determine the function of cryptic genes. They may also be put to practical use in crop improvement. Antisense RNAs were recently observed to selectively block the gene expression of other plant enzymes such as polygalacturonase and peroxidase in tomatoes.

2.8.2 Antisense RNA approach

One of the most successful methods developed in recent years for the inhibition of gene expression in plants has been the expression of introduced antisense genes. Antisense technology is based on blocking information flow from DNA via RNA to protein by the introduction of an RNA strand complementary to the sequence of the target mRNA. It is generally assumed that the antisense RNA basepairs to its target mRNA thereby forming double-stranded RNA. Duplex formation may impair mRNA maturation and/or translation or alternatively may lead to rapid mRNA degradation (Martinez and Whitaker, 1995).

Technically, it involves the insertion of a gene or a significant part of it, into the cell in a reverse orientation. Messenger RNA encoded by this antisense gene undergoes hybridization with that encoded by the endogenous gene, precluding production of the protein product (Figure 1.15). Gene silencing or the elimination of expected phenotypic characteristics, through antisense techniques has received much attention in recent years. The expression of a transgene (i.e. a gene that has been introduced into plant cells through molecular biology techniques) or an endogenous gene appears to be affected by the presence of a homologous transgene. Antisense-mediated control has been observed in bacteria, fungi, plants and mammalian systems. The biological function of naturally occurring antisense RNAs, if any, remains to be determined.

Complementary mRNA levels can be reduced in the presence of antisense genes (Martinez and Whitaker, 1995).

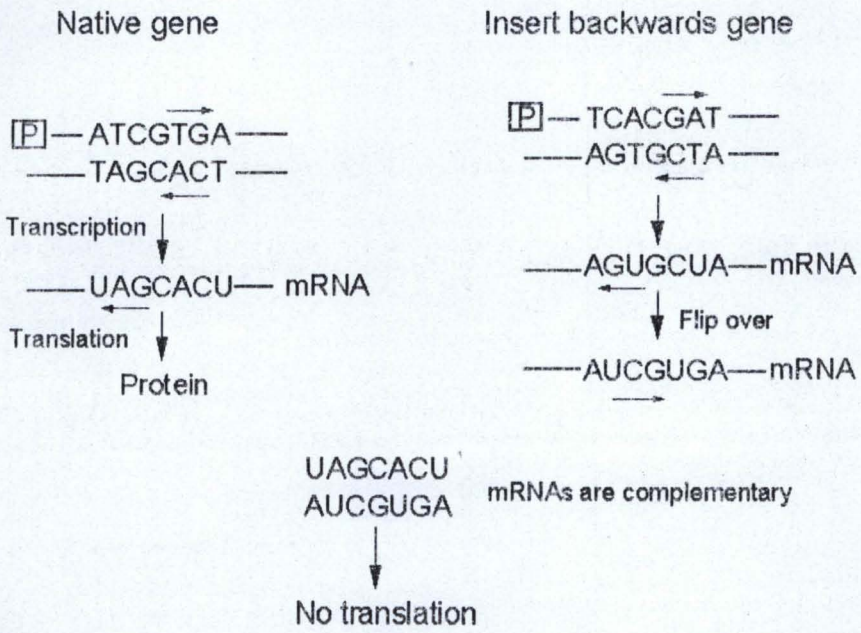


Figure 1.15: Simplified schematic showing how antisense RNA can be used to control gene expression at the translational level (P represents the promoter). (Martinez and Whitaker, 1995).

Polyphenol oxidase genes have been characterized in broad bean, tomato, potato, and grape. Seven genomic polyphenol oxidase genes were identified in tomato (Newman *et al.*, 1993). Characterization of polyphenol oxidase genes in various plant species has shown that these genes are present in the plant genome as gene families. Such transcriptional regulation offers potential for the design of antisense constructs. Antisense constructs would be required for maintenance of the enzyme if polyphenol oxidase is required for chloroplast metabolism or for resistance to pathogens or predation.

In addition to improving a fundamental knowledge of biological processes, the antisense approach has been applied, to increasing the shelf life of fruit (Fray and Grierson, 1993). Commercial applications of antisense technology now include alterations of flower colour, virus resistance and fruit ripening. The application of antisense technology also extends to improving food quality.

Bachem *et al.*, (1994) determined that the expression of polyphenol oxidase in potatoes was decreased through the use of vectors carrying antisense polyphenol oxidase cDNAs. Approximately 70 percent of the transformed plants had lower polyphenol oxidase activity than controls, and on visual scoring, a significantly low level of discolouration. Insertion of polyphenol oxidase in the sense orientation resulted in very high polyphenol oxidase activity in the lines expressing the construct.

Breeders have been working to decrease polyphenol oxidase levels in apples, bananas, mushrooms, peaches and other plants for many years. Lack of bruising sensitivity in transgenic potatoes, and the absence of any apparent detrimental side effects, opens up the possibility for preventing enzymatic browning in a wide variety of food crops, without resorting to chemical and physical treatments. Quite possibly, browning-resistant varieties may be developed in the future through the insertion of antisense genes that prevent the production of polyphenol oxidase.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES

Yam tubers (*D. rotundata*) were boug ht from the open Market in Minna.

3.2 REAGENTS

Ascorbic acid (0.02M) (analar, BDH Chemicals Ltd. Poole England), 0.02M L-cystine (Searle Company, Charwell Heath Essex England), 0.02M sodium metabisulphite (GPR, May & Baker Nigeria Ltd.), 0.05M potassium phosphate buffer (analar, BDH Chemicals Ltd. Poole England), ethanol (absolute) (analar, sigma-Aldrich laboratories Germany), folin-ciocalteau reagent, egg albumen (GPR, BDH Poole England), 0.1M sodium hydroxide (GPR, May & Baker Nigeria Ltd.), 2% sodium carbonate (GPR, May & Baker Nigeria Ltd.), 0.5% cupper sulphate (GPR, May & Baker Nigeria Ltd.), 1% sodium potassium tartarate solution (GPR, May & Baker Nigeria Ltd.), 0.02M catechol

3.3 YAM FLOUR PROCESSING

The yam tubers were sliced to a thickness of about 10 mm. The slices were then steeprd in water or inhibitors for 12 hours at 30°C, 40°C and 50°C. The steeped slices were dried uder the sun. The dried slices were then ground to flour in a blender (Crown star MC-Y44B, Trident (H.K) Limited China).

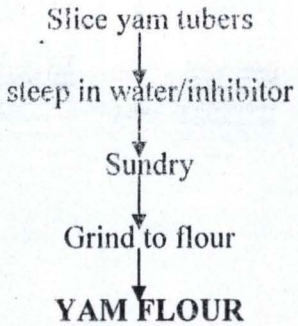


Figure 3.1: Processing of yam flour (elubo)

3.4 CRUDE ENZYME EXTRACTION

Yam samples (250g) were steeped in water for 12 hours. The samples (1g) were thereafter homogenized in 5ml of 0.05M phosphate buffer (pH 6.5) and centrifuged at 20,000xg for 15mins on a centrifuge (Gallenkamp England) to obtain crude PPO enzyme. For PPO inhibition studies, the steps above were repeated with the inclusion of 0.02M of the different inhibitors (ascorbic acid, L-cystine and sodium metabisulphite) into the steeping solution.

3.5 ASSAY OF PPO ACTIVITY

The assay procedure used was based on the method of Lee and Smith, (1979) but with slight modification. PPO activity was determined by measuring the increase in absorbance at 420nm using a spectrophotometer (Jenway 6310). The reaction mixture contained 0.2ml of the crude enzyme solution and 2.8ml of 0.02M substrate solution in 0.05M phosphate buffer (pH 6.5) at room temperature. The control sample contained only 3.0ml of substrate solution. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per minute.

Calculation

$$\text{Units/mg} = \frac{\Delta A_{420}/\text{min} \times 1000}{\text{mg protein}}$$

where, ΔA = change in absorbance,

1000 = conversion factor

3.6 ENZYME KINETICS

The tested concentrations of substrate (catechol) and anti-browning agents were 0.010, 0.015, 0.020, 0.025, and 0.030M. Two different conditions; with and without pre-incubation of enzyme in inhibitor solution for 10 minutes at room temperature were studied. The experiments were determined in 3 replicates at each concentration of each anti-browning agent.

3.7 DETERMINATION OF BROWNING INDEX

Ethanol (5ml) was added to 0.5g yam flour samples and then stoppered. The contents were mixed on a mixer for 2 minutes. The mixture was allowed to stand for 10 minutes and then centrifuged at 1000 rpm for 10 minutes on a centrifuge (Gallenkamp England) and read in spectrophotometer (Jenway 6310) at 420nm using ethanol as blank according to the method of Lee et al, (1990).

3.8 PROTEIN ESTIMATION

The protein concentration was determined by the method of Lowry, using egg albumin as the standard (Lowry et al, 1951). Aliquots of protein solutions were pipetted out and the total volume made up to 4ml with distilled water. To each tube, 5.5ml of alkaline mix was added and allowed

to stand at room temperature for 10-15mins. Folin reagent (0.5ml) was pipette into each tube, mixing rapidly after each addition. The tubes were left for 30mins and the blue colour formed was measured on a spectrophotometer (Jenway 6310) at 650nm. Blank without the protein was used and a standard graph was plotted.

3.9 SOLUBILITY

Yam flour sample (1.0g) was measured and transferred into a clean dried test tube and weighed (W_1). It was then dispersed in 50ml of distilled water using blender (Crown star MC-Y44B, Trident (H.K) Limited China). The resultant slurry was heated at 60°C, 70°C, 80°C and 90°C for 30 minutes in a regulated water bath (Griffin Britain). The mixture was cooled to room temperature and centrifuged at 500 rpm for 15 minutes on a centrifuge (Gallenkamp England). The supernatant (5ml) was withdrawn and dried to a constant weight at 110°C in an oven (Gallenkamp size 2, England). The residue was then represented as the amount of sample solubilized in water. (Leach et al, (1995); Akingbala and Rooney, 1987).

Calculation

$$\text{Solubility} = \frac{Xg}{100g \text{ of sample on dry basis.}}$$

(Leach et al, (1995); Akingbala and Rooney, 1987).

3.10 HYDRATION CAPACITY

The method of Kornblum and Stoopak, (1973) was used to determine the hydration capacity. Yam flour sample (1.0g) was placed in each of four 15ml plastic centrifuge tubes to which 10ml

of distilled water was added and then stoppered. The contents were mixed on a mixer for 2 minutes. The mixture was allowed to stand for 10 minutes and then centrifuged at 1000 rpm for 10 minutes on a centrifuge (Gallenkamp England). The supernatant was carefully decanted, the stopper replaced and the sediment weighed. The hydration capacity (Hc) was taken as the ratio of sediment weight to the dry sample weight.

3.11 SWELLING CAPACITY

This was measured at the same time as the hydration capacity and calculated as follows:

$$S = \frac{(V_2 - V_1) \times 100}{V_1}$$

Where S is the % swelling capacity, V_2 is the volume of the hydrated or swollen material and V_1 is the tapped volume of the material prior to hydration. (Kornblum and Stoopak, 1973)

3.12 MOISTURE CONTENT

Yam flour sample (5g) was dried at 110°C in an oven (Gallenkamp size 2, England) to a constant weight. The % loss in weight was calculated as the moisture content. (AOAC, 1997)

Calculation:

$$\% \text{ Moisture} = \frac{(\text{wt. of pan + fresh sample}) - (\text{wt. of pan + dry sample})}{\text{Wt. of sample}} \times 100$$

3.13 DETERMINATION OF ENERGY VALUE

Yam flour sample (1.0g) was weighed into a ballistic bomb metal iron crucible, pressed gently with a spatula to form a smooth level layer suitable for combustion. One end of a single strand

cotton thread was tied to the platinum firing wire with sample. The bomb closure ring was filled with oxygen from the steel gas cylinder to the required pressure (15atm). The firing ring was then pressed and the maximum deflection was read on the galvanometer scale (XRY – 1B Oxygen Bomb Calorimeter) (AOAC, 1997).

Calculation:

$$\frac{C_B - Q_B}{C_S - Q_S}$$

Where C_B = calorific value of standard sample (27.63kg/g)

C_S = calorific value of test sample

Q_B = peak galvanometer deflection of 1.0g of standard sample (12.50)

Q_B = peak galvanometer deflection of the test sample

$$C_S = C_B - Q_S = \frac{Kj/g}{Q_B}$$

3.14 OGANOLEPTIC SCORE OF YAM FLOUR MEALS (AMALA)

Sensory evaluation was carried out on the yam flour meal (amala) samples as described by Larmond, (1977). A 10-member taste panel made up of graduate students and lecturers in the Department of Biochemistry and Chemistry, Federal University of Technology Minna, were trained to conduct the sensory analysis. A preliminary test which served as training class for members of the panel was conducted a day before the main evaluation. The purpose was to familiarize members with both hedonic and descriptor scales. To keep the interest and morale of

the panelists for the main evaluation, they were served amala with abula soup after the preliminary test to show appreciation for their service as well as a warm invitation for the main evaluation. Every member was provided with questionnaire for both objective and subjective sensory evaluation. The objective questionnaire enabled each panelist to describe the products while the subjective questionnaire requested them to give information on the degree of like or dislike of the samples. Quality characteristics including appearance, aroma, taste and the overall acceptability of the samples were evaluated based on a seven point hedonic scale (where; 1 = like extremely, 2 = like very much, 3 = like slightly, 4 = neither like nor dislike, 5 = dislike slightly, 6 = dislike very much, 7 = dislike extremely.

3.15 STATISTICAL ANALYSIS

All experiments were done in triplicate except for organoleptic score which was an average of ten tests. Means, standard deviations, correlation coefficients, regression and analysis of variance (ANOVA) on data were performed using SPSS computer software version 15.0. Means were compared using Duncan method at a probability level of 0.05 (Duncan, 1955). Relationships among measurement variables were studied using standard correlation, r^2 being the correlation factor.

CHAPTER FOUR

4.0 RESULTS

4.1 Polyphenol oxidase (PPO) activity

Polyphenol Oxidase (PPO) activity pattern in yam flour (elubo) processed with or without inhibitors (ascorbic acid, L-cystein and sodium metabisulphite) are shown in Table 4.1 – 4.3. Ascorbic acid increased PPO activity significantly ($p < 0.05$) up to 2 hours of steeping and thereafter dropped while cystein and sodium metabisulphite significantly reduced ($p < 0.05$) PPO activity compared to the control samples.

4.2 Browning index

Table 4.1- 4.3 presents the browning index of processed yam flour (elubo) with or without inhibitors. The study revealed that browning is temperature-dependent in all the yam samples. Sodium metabisulphite completely inhibited browning in the processed yam flour while cystein had partial inhibition and ascorbic acid on the other hand, increased browning compared to the control samples.

Table 4.1: Polyphenol oxidase (PPO) Activity and Browning index of yam tissue steeped in different PPO inhibitors

Inhibitor	Activity (Units/mg protein)	Browning index (units/g)
Control	$11,618.85 \pm 15576.22^{a}$	36.47 ± 7.87^{a}
Yams + Ascorbic acid	$10,912.38 \pm 16672.51^{b}$	36.67 ± 7.84^{a}
Yams + Cysteine	$2,360.20 \pm 1415.91^{c}$	26.47 ± 2.59^{b}
Yams + Na-metabisulphite	$1,069.57 \pm 652.37^{d}$	23.07 ± 2.66^{c}

Within column, values with different letters (superscripts) are statistically different at $P < 0.05$.

Each data is mean $\pm$ SD of three replicates

Table 4.2: Influence of steeping time on PPO activity and Browning in yam flour processing

Time (hrs)	Activity (Units/mg protein)	Browning index (units/g)
2.00	13,478.68± 2084.47 [a]	34.42± 10.19 [a]
4.00	5,011.07± 10625.80[d]	30.58± 5.94[b]
6.00	5,653.95± 8559.85[c]	29.92± 5.73[b]
8.00	6,235.13± 8945.98[b]	31.33± 10.63[b]
12.00	2,072.43± 1418.22[e]	27.08± 6.72[c]

Within column, values with different letters (superscripts) are statistically different at $P<0.05$.

Each data is mean ± SD of three replicates

Table 4.3: Influence of steeping temperature on PPO activity and Browning in yam flour processing

Temperature (°C)	Activity (Units/mg protein)	Browning index (units/g)
30	13,460.93± 19344.01[a]	32.25± 10.09[a]
40	3,804.45± 2330.01[b]	30.45± 7.60[b]
50	2,205.38± 2325.23[c]	29.30± 6.99[b]

Within column, values with different letters (superscripts) are statistically different at $P<0.05$.

Each data is mean ± SD of three replicates

4.3 Correlation of PPO activity with browning

The correlation of PPO activity with browning index is presented in Table 4.4. The result showed that PPO activity and browning index were highly moderately (53.2%) correlated.

4.4 Regression

Table 4.5 – 4.6 shows the regression of PPO activity and Browning of yam flour (elubo) processed with or without inhibitors. The regression equations are:

Activity = 34624.327 – 562.78Temp – 878.59Time.

Browning index = 40.35 – 0.15Temp – 0.59Time.

;-00/pp[.=c

Table 4.4: Correlations of PPO activity with browning in yam after steeping with different inhibitors

		Activity (units/mg protein)	Browning Index (units/g)	Time (hrs)	Temperature (^{o}C)
Activity	Pearson Correlation	1	.532**	-.246**	-.374**
	Sig. (2-tailed)		.000	.001	.000
	N	18	180	180	180
Browning Index	Pearson Correlation	.532**	1	-.244**	-.144
	Sig. (2-tailed)	.000		.001	.054
	N	180	180	180	180
Time (hrs)	Pearson Correlation	-.246**	-.244**	1	0.000
	Sig. (2-tailed)	.001	.001		1.000
	N	180	180	180	180
Temperature (^{o}C)	Pearson Correlation	-.374**	-.144	0.000	1
	Sig. (2-tailed)	.000	.054	1.000	
	N	180	180	180	180

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4.5: Regression Coefficients[a] of PPO activity

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	34624.327	4404.807		7.861	.000
	Temp	-562.777	101.125	-.374	-5.565	.000
	Time	-878.590	239.959	-.246	-3.661	.000

a. Dependent Variable: Activity

Activity = 34624.327 -- 562.78Temp -- 878.59Time.

Table 4.6: Regression Coefficients[a] of Browning index

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	40.350	3.213		12.559	.000
	Temp	-.148	.074	-.144	-2.000	.047
	Time	-.591	.175	-.244	-3.378	.001

a. Dependent Variable: Browning Index

Browning index = 40.35 – 0.15Temp – 0.59Time.

4.5 Polyphenol oxidase (PPO) kinetics

The kinetics of inhibition is presented in Figure 4.1. The mechanism of inhibition is uncompetitive and even though their mechanisms are the same, only sodium metabisulphite completely inhibited browning.

4.6 Physicochemical properties

The physicochemical properties of the processed yam flour samples are presented in Table 4.7 - 4.8. The result indicated that there is no significant difference ($p > 0.05$) in the moisture content, hydration capacity, swelling capacity, and solubility of the yam tissues with or without inhibitors. However, a significant increase ($P < 0.05$) in the energy of yam tissue steeped with sodium metabisulphite was observed.

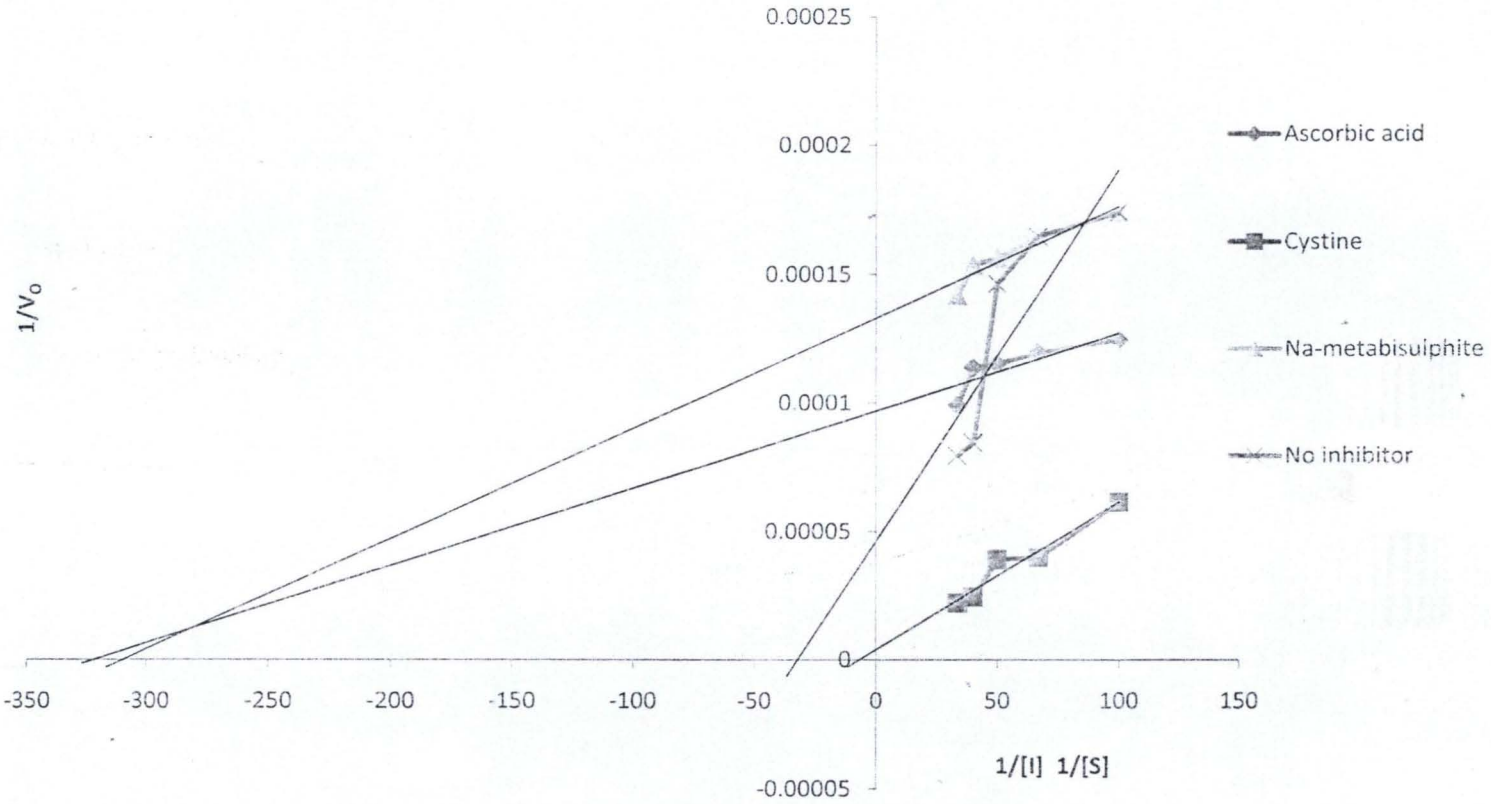


Fig 4.1: Inhibition kinetics of PPO enzyme in yam tissues

Table 4.7: Physicochemical properties of yam steeped in different PPO inhibitors

Inhibitor	Moisture Content (%)	Hydration capacity (g/g)	Swelling capacity(%)	Energy value (kj/g)
Control	6.75 ± 0.25^{a}	1.79 ± 0.26^{a}	82.60 ± 5.40^{a}	27.933 ± 2.00^{b}
Yams + Ascorbic acid	6.75 ± 1.25^{a}	1.57 ± 0.30^{a}	75.35 ± 1.85^{a}	17.605 ± 3.00^{c}
Yams + Cysteine	6.5 ± 0.00^{a}	$2 .00 \pm 0.30^{a}$	78.6 ± 1.40^{a}	15.383 ± 4.00^{d}
Yams + Na-metabisulphite	6.25 ± 0.25^{a}	1.76 ± 0.07^{a}	77.75 ± 4.25^{a}	29.058 ± 1.00^{a}

Values in a column with same letter are not significantly different ($P > 0.05$)

Each data is mean $\pm$ SD of three replicates

This implied that the addition of inhibitors did not change the physicochemical properties of yam flour except that calorific energy was increased upon processing with sodium metabisulphite.

5.7 Organoleptic score of amala

A description by Hutchings (2003) regarding expectations of people toward food is summarized as follows: "Food evaluation always starts with visual inspections. We consciously or unconsciously examine food in the first place by appearance including color, visual structure, and surface texture. Temporal factors such as climate, place, and physical condition can affect the evaluation as well as individual background. In the next step, we identify safety of the food followed by assessment of flavor and texture. The final step of food evaluation before eating is to guess pleasant and satisfaction brought to us by eating the food."

DuBose *et al* (1980) reported that colour is a very important sensory attribute of most foods since it influences the consumers' first judgement and provides sensory information, which may interact with the gustatory olfactory and textural cues to determine the overall acceptability. Francis (1980) also remarked that when the colour is unappealing, consumers are unlikely to be able to judge the flavour or texture as favourable.

In the present study, there was no significant difference ($p > 0.05$) in taste, texture and flavour between *amala* made from yam samples processed with inhibitors and the control. However, significant differences ($p < 0.05$) in colour existed between *amala* made from yam samples processed with inhibitors and the control. Statistical analysis showed that the colour scores for the control and *amala* made from yam samples processed with ascorbic acid, cystein were not significantly different from one another. In the same vain, *amala* made from yams samples

4.7 Organoleptic Score of amala

The organoleptic score of yam flour meal (amala) produced from test samples are presented in Table 4.9. The result revealed that there is no significant difference ($p > 0.05$) in the flavour, taste and texture of *amala* produced from yams samples processed with or without inhibitors. However, significant differences ($P < 0.05$) in colour exist among all the *amala* samples. That is, *amala* made from yam samples processed with sodium metabisulphite was white throughout steeping temperatures and time compared to the control samples. Those made from yam samples processed with cystein was fairly whiter than the control, while *amala* made from yam samples processed with ascorbic acid gave a golden brown colour compared to the control (even darker than the control). Generally, *amala* produced from yam steeped with sodium metabisulphite was the most acceptable of all the *amala* samples.

Table 4.9: Organoleptic evaluation of yam flour meal (amala) steeped in different PPO inhibitors

Samples	Taste	Colour	Texture	Flavour	Overall acceptability
Control	2.8 ± 1.03^{a}	2.8 ± 1.03^{a}	2.9 ± 0.88^{a}	3.3 ± 0.48^{a}	$2.3 \pm 1.16^{a,b}$
Yams + Ascorbic acid	3.7 ± 1.42^{a}	3.4 ± 2.01^{a}	3.0 ± 1.05^{a}	3.6 ± 0.70^{a}	2.9 ± 1.85^{a}
Yams + Cysteine	3.2 ± 1.14^{a}	$2.6 \pm 0.52^{a,b}$	2.9 ± 0.99^{a}	3.7 ± 0.68^{a}	2.9 ± 0.99^{a}
Yams + Na-metabisulphite	2.9 ± 0.88^{a}	1.6 ± 0.70^{b}	2.9 ± 0.88^{a}	3.5 ± 0.53^{a}	1.5 ± 0.71^{b}

Within column, values with different letters (superscripts) are statistically different at $P< 0.05$.

Each data is mean $\pm$ SD of three replicates

Plate 4.1: Yam flour meal (amala) samples

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 DISCUSSION

5.1 Polyphenol oxidase (PPO) activity

Browning is one of the most important colour reactions that affects fruits, vegetables and sea foods and has been found to be as a result of the presence of the enzyme, polyphenol oxidase (PPO). It is a surface phenomenon requiring molecular oxygen and specific phenolic substrates ((Macheix *et al.*, 1991; Nicolas *et al.,* 1994, Palmer, 1963; Sheen and Calvert 1969). The existence of physical browning process in plant tissue is well documented (Wolfrom *et al* 1974) most of which have been shown to be enzymatic.

Various techniques and mechanisms have been developed over the years for the control of these undesirable enzyme activities. These techniques attempt to eliminate one or more of the essential components (oxygen, enzyme, copper, or substrate) from the reaction. Sulfhydryl compounds such as L-cysteine and sodium metabisulphite have been investigated as inhibitors of enzymic browning. The formation of quinone-sulphite complexes prevents the quinone polymerization (Embs and Markakis, 1985). A further action of sulfhydryl compounds on PPO may directly inhibit the enzyme by combining irreversibly with copper at the active site of the enzyme (Valeroet *et al*, 1991) thus, inhibiting the enzyme.

Results in this study showed that, there were significant differences ($p < 0.05$) in PPO activities in yam samples processed with different inhibitors compared with the control irrespective of temperature and time. Statistical analysis revealed that PPO activity of the control is significantly higher than those of yam samples processed with inhibitors. This implies that the inhibitor study

were able to inhibit PPO activities in the yam tissues. Among yam samples processed with inhibitors, those processed with ascorbic acid had significantly higher PPO activity than those processed with cystein and sodium metabisulphite. The sample processed with L-cystein also had significantly higher PPO activity than those processed with sodium metabisulphite (Ascorbic acid > L-Cystein > sodium metabisulphite). This implies that sodium metabisulphite (0.02M) effectively inhibited PPO activity while L-Cystein and ascorbic acid at the same concentration partially inhibited its activity.

The result also showed that inhibition of PPO increased with time with highest inhibition rendered at 12hrs. It also showed that inhibition of PPO increased with rise in temperature with highest inhibition rendered at 50°C and optimum activity at 30°C.

Omidiji and Okpuzor, (1996) also observed that inhibition of PPO activity in yam tissues were maximal at 12hrs of incubation. They also reported that yam processing techniques at ambient temperature in the presence of PPO inhibitors should be completed within 12hrs before the onset of non-enzyme related browning.

This value (30°C) is similar to the optimum temperature of the PPO of *Dioscorea opposita Thunb* (Shuji *et al*, 2006) and another species of yam tuber (Ikediobi and Obasuyi, 1982; Omidiji and Okpuzor, 1996). This implies that rise in temperature denatured PPO enzyme.

5.2 Browning index

The browning index indicates the proportion of oxidized phenols (Jeong et al, 2008). In the present study there were significant differences ($p < 0.05$) in browning index of yam flour (elubo) made from yam samples processed with inhibitors compared with the control irrespective of temperature and time. Statistical analysis revealed that browning index of *elubo* made from yam samples processed with ascorbic acid and those of the control were not significantly different ($p > 0.05$) but were significantly higher than those of *elubo* made from yam samples processed with cystein and sodium metabisulphite. The browning index of *elubo* made from yam samples processed with cystein was significantly higher than that processed with sodium metabisulphite which had the lowest browning index. This implies that that sodium metabisulphite appears to be a potent inhibitor for preventing browning in yam flour processing, followed by L-cysteine.

Shuji *et al*, (2006) reported a complete inhibition of browning in edible yam (*D. opposite Thumb*) in the presence of L-cystein and ascorbic acid as against partial inhibition observed in this study upon steeping with L-cystein and ascorbic acid. Complete inhibition of browning by L-cystein and ascorbic acid have also been observed for PPOs of another species of yam (Ikediobi and Obasuyi, 1982,), Japanese pear (Tono *et al* 1986), head lettuce (Fujita *et al.*, 1991), and cabbage (Fujita *et al.*, 1995). The reason for this conflicting result may be as a result of low concentration of L-cystein and ascorbic acid being employed in the study.

This study also showed that the browning reaction of the enzyme was completely inhibited at 0.02M concentration of sodium metabisulphite. The reaction was also partially inhibited by 0.02M L-cysteine, which is a known quinone coupler (Shuji *et al*, 2006). However, physical observation showed that 0.02M ascorbic acid did not inhibit browning in the processed yam flour rather; it increased browning compared to the control. The study therefore, showed that ascorbic acid is undesirable in reducing tissue browning in processed yam flour (elubo) even though it is a PPO inhibitor in other tissues e.g. apples, quince, loquat, etc. Ascorbic acid is an antioxidant which is more readily oxidised than phenols and thus tends to form deep brown colour even before the phenols are used as substrates (Vamos-Vigyazo, 1981). The enhancement of browning throughout incubation in the presence of micromolar quantity of ascorbic acid therefore suggests an involvement of an *in vivo* oxidisable phenolic pool in the browning process (Omidiji and Okpuzor, 1996).

Similar effects of ascorbic acid have also been observed with yam tissues by Omidiji and Okpuzor, (1996). Their studies showed that ascorbic acid did not prevent browning in yam tissues while sodium metabisulphite completely prevented browning in yam tissues.

Among compounds that inhibit PPO activity, Sodium and potassium metabisulphite and sulfur dioxide (SO_2) are amongst the most effective and have been used in food industries for many years. However, restrictions of sulfite usage in foods associated with consumer concern about its safety generate the need for substitutes (Rocha and De Morais, 2005). Therefore, alternative chemicals without toxic effects are needed, such as sulfhydryl (SH or thiol), ascorbic acid, and citric acid. These compounds have potential to be used commercially, as substitute to sulfite as anti-browning agent, to prevent enzymatic browning in processed fruit products.

The result in this study also showed that inhibition of browning increased with time with highest inhibition rendered at 12hrs. The inhibition of browning increased with rise in temperature with highest inhibition rendered at 40°C and 50°C. Omidiji and Okpuzor, (1996) also observed that browing in *D. rotundata, D. esculenta, D. alata and D. cayenensis,* were maximal at 30°C which later declined with rise in temperature.

5.3 Correlation of PPO activity and Browning index

Polyphenol oxidase (PPO) activity and browning index were significantly, positively and moderately (53.2%) correlated. This implies that as PPO activity increases, the browning index of the yam flour (elubo) increases significantly and vice versa. PPO activity & steeping time, and; Browning index & steeping time were significantly, negatively and weakly correlated. This implies that as the steeping time increases, there will be significantly relative decrease in activity and browning index. PPO activity & steeping temperature and; Browning index & steeping temperature were also significantly, negatively and weakly correlated. This also implies that as the steeping temperature increases, there will be significantly relative decrease in activity and browning index. Jeong et al, (2008) also observed positive correlations between PPO activity and browning index for all their treatments with ascorbic acid and L-cystein.

5.4 Regression

From linear regression model; the equation for the relationship between activity, time and temperature and between browning index, time and temperature in this study were established as:

Activity = 34624.327 – 562.78Temp – 878.59Time.

Browning index = 40.35 – 0.15Temp – 0.59Time.

R^2 = 0.200 for activity and 0.080 for browning index.

Thus, if the temperature and time are known, the activity and browning index can be calculated for a particular flour using this model. This model can also be used for a scale up operation.

5.5 Polyphenol oxidase (PPO) Kinetics

The mechanism of inhibition is uncompetitive. All the samples had different V_{max} and K_m values. The Michaelis constant (K_m) for the different inhibitors (Ascorbic acid, cystein, sodium metabisulphite) and control are 0.00478M, 0.27384M, 0.00366M and 0.12387M respectively Even though their mechanisms are the same, only sodium metabisulphite completely inhibited browning.

5.6 Physicochemical characteristics

Quantifying physicochemical properties are important for food processing and quality, because they influence functional properties of flour (Moorthy, 1994; Gerard *et al.*, 2001; You and Izidorczyk, 2002) which may in turn affect the textural quality of food products.

In the present study, test samples were not significantly different ($P > 0.05$) in terms of moisture content, hydration capacity, swelling capacity and solubility when compared to that of the control sample. The moisture content of a food sample reflects the amount of solid matter in the sample. The higher the moisture content, the higher the rate of spoilage.

The data in this study indicated that moisture content of the processed yam flour (elubo) ranged between 6.25 - 6.75%. Okaka and Okechukwu, (1993) also obtained values less than 10% and stated that ≤10% moisture content is needed for prolonged shelf life (up to 6 months) for well packaged dehydrated yam products. This suggests that the processed *elubo* samples may be stored for a favourably long period of time without fear of spoilage.

High swelling powers were also observed for all the test samples. This is consistent with the work of Walter, (2002) who observed that *D. rotundata* had higher swelling power in comparison to other yam species. This high swelling power has been linked to low amylose content; due to low reinforcement of internal network by amylose molecules (Lorenz and Collins, 1990; Richardson *et al*, 2000; Hoover, 2001). Riley *et al*, (2006) also observed higher swelling power in yam varieties which had lower amylose content. According to Jane and Chen, (1992), amylopectin contributes to granule swelling while amylose and lipid contents inhibit it. Highly

associated starch granules with an extensive and strongly bonded micellar structure also exhibit resistance toward swelling (Leach *et al.*, 1959) therby exhibiting low swelling capacities.

Carbohydrate supplies energy to cells such as brain, muscles and blood. It contributes to fat metabolism and spare proteins as an energy source. It also acts as mild natural laxative for human beings and generally add to the bulk of the diet (Gordon, 2000; Gaman and Sherrington, 1996). The data in this study also showed that there were significant differences ($p < 0.05$) in calorific energy values between yam samples processed with inhibitors and the control. Statistical analysis revealed that yam samples processed with sodium metabisulphite was significantly higher in calorific energy than other inhibitors and control. In the same vein, the control was significantly higher in calorific energy than samples processed with ascorbic acid which was also significantly higher in calorific energy than yam samples processed with cystein. The high calorific energy values obtained may be attributed to the carbohydrate content (Alinnor, 2010). Alinnor, 2010 also obtained high energy values for *Colocasia esculenta* and *Dioscorea rotundata*.

Result in this study also showed that there was also no significant difference ($P > 0.05$) in solubility between yam samples processed with inhibitors and control but statistical analysis revealed that irrespective of temperature, the solubility of the control was significantly higher than those of yam flour made from yam samples processed with inhibitors and the latter were not significantly different from one another. Statistical analysis also revealed that solubility irrespective of inhibitor at 60°C, was significantly higher than at 70°C and at 70°C significantly higher than at 80 and 90°C. However, solubility at 80°C and 90°C were not significantly different from each other. Thus, solubility of *elubo* decrease with increases in temperature.

processed with cystein and sodium metabisulphite were not significantly different from each other. However, the colour scores of control and *amala* made from yam samples processed with ascorbic acid were significantly higher than those processed with sodium metabisulphite.

Also, physical observation showed that browning was partially inhibited in the presence of L-cystein thus; *amala* made from yam samples processed with L-cystein was slightly lighter than the control. It was completely inhibited in the presence of sodium metabisulphite hence; *amala* made from yam samples processed with sodium metabisulphite was completely white throughout steeping period compared to the control. However, browning was enhanced in the presence of ascorbic acid thus, yam samples processed with ascorbic acid produced *amala* that was darker than that of the control throughout steeping period.

The taste attribute of all the test samples were alike (slightly sweet) according to the panelist. This is not surprising because any trace of bitter principle in the tuber got lost in the water during steeping. The panelist also recorded similar flavor and texture for all the test samples. The test samples were very elastic in texture thus its acceptability by the panelist.

The study also showed that there was significant difference ($p < 0.05$) in acceptability between *amala* made from yam samples processed with inhibitors and the control. Statistical analysis revealed that the acceptability of the control was not significantly different from *amala* made from yam samples processed with inhibitors but among the inhibitors, *amala* made from yam samples processed with ascorbic acid and those processed with cystein were not significantly different from each other but significantly higher in acceptability score than those processed with

sodium metabisulphite. According to the seven point hedonic scale, the lower the value scored, the higher the acceptability. This therefore implies that *amala* made from yam samples processed with the inhibitors were accepted in the same manner as the control but *amala* made from yam samples processed with cystein and ascorbic acid were less preferred to *amala* made from yam processed with sodium metabisulphite which was the most preferred.

Generally speaking, the lower the values recorded for each sample, the higher the quality and its acceptability according to the seven point hedonic scale. Therefore, *amala* obtained from yam samples processed with sodium metabisulphite was the best and the most acceptable of all the amala samples.

5.8 CONCLUSION

Polyphenol oxidase (PPO) was effectively inhibited in processed yam flour (elubo) by sodium metabisulphite which also resulted in inhibition of brown and the production of white *amala* as shown by the white *amala* obtained after treatment with the inhibitor while, L-cysteine partially inhibited the enzyme. Ascorbic acid however, did not inhibit PPO in *elubo*. Also, PPO activity and browning index were significantly, positively and moderately (53.2%) correlated.

The addition of inhibitors did not change the physicochemical properties of the processed *elubo* except that calorific energy was increased on steeping with sodium metabisulphite.

Of all the yam flour samples, *amala* made from yam tissues steeped with sodium metabisulphite was the most accepted.

5.9 RECOMMENDATION

In spite of the effectiveness of sulphiting agents in the prevention of browning of foods, American laws and public health concerns surrounding the ingestion of the sulphiting agents restrict the use of sulphites in the food industry. Hence, it is advised that sodium metabisulphite be used with caution. It is also recommended that further studies be carried out using alternatives to sulphites applicable in the food industry.

REFERENCES

Adamson, I. and Abigor, R. (1980): Transformation associated with catecholase in *Dioscorea alata* during storage. *Phytochemistry* 19 1583-1595.

Afoakwa, E. O. and Sefa-Dedeh, S. (2002): Viscoelastic properties and changes in pasting characteristics of trifoliate yam (*Dioscorea dumetorum*) starch after harvesting. *Food Chemistry* 77: 203-208.

Ajayi, O. A and Madueke L. U. (1990): A study of stored yam (*Dioscorea cayenensis*) as affected by the ventilation of the storage locations. *Journal of Science, Food and Agriculture* 50: 257-260.

Akingbala, J.O. and Rooney, L.W. (1987): Paste properties of sorghum flour and starches. *Journal of food processing and preservation* 11: 13-24

Akissoe, H. N, Hounhouigan, D. J, Bricas, N., Vernier, P., Nago, C. M. and Olorunda, O. A. (2001): Physical, chemical and sensory evaluation of dried yam (*Dioscorea rotundata*) tubers, flour and *amala*, a flour-derived product. *Tropical Science.* 41:151-155.

Akoroda, M.O. and Hahn, S.K (1995): Yams in Nigeria: status and trends. *African Journal of Root and Tuber Crops*, 1(1): 38-41.

Alabadan, B. A. (2002): Assessment of storage losses in roots and tubers in Niger State, Nigeria. *African Journal of Root and Tuber Crops* 5(1): 49-52.

Albisu, I., King, R.D., Kozlov, I.A. (1989): Inhibition of the catecholase activity of mushroom tyrosinase by carbon monoxide. *Journal of Agriculture and Food Chemistry.*, 37: 775-776.

Albrecht, M. A. and McCarthy, B. C. (2006): Seed germination and dormancy in the medicinal woodland herbs *Collinsonia Canadensis* L. (Lamiaceae) and *Dioscorea villosa* L. (Dioscoreaceae). *Flora* 201:24-31.

Alinnor, I.J. and Akalezi, C.O. (2010): Proximate and Mineral Compositions of *Dioscorea rotundata* (White Yam) and *Colocasia esculenta* (White Cocoyam). *Pakistan Journal of Nutrition* 9 (10): 998-1001.

Alves, R. M. L. (2000): Caracterizacao de ingredients obtidos de cara (*Dioscorea alata)* e potencial aplicao industrial. PhD Thesis, Universidade Estadual de Londrina, Brazil. Pp 33

Anon. (1992): *Code of Federal Regulations*, Food and Drugs 21 Parts 170.199, Washington, DC, Office of the Federal Register National Archives and Records Administration.

Anosike, E.O. and Ayaebene, A.O. (1981): Purification and some properties of polyphenol oxidase from the yam tubers, *Dioscorea bulbifera. Phytochem*istry. 20: 2625-2628.

Anosike, E. 0. and Ikediobi, C.0. (1985): The biochemistry of the browning of yam tubers. In: *Advances in Yam Research* (1st edn), ed Osuji G. Biochemical Society of Nigeria, Nigeria, pp 145-160

AOAC (1997). Official Methods of Analysis of Association of Official Analytical Chemists, Washington D.C.

Applewhite, L., Otwell, W.S., Marshall, M.R. (1990): Effect of kojic acid on pink shrimp phenoloxidase. *Proceedings of the Fifteenth annual conference Tropical and Subtropical Fisheries Technological Conference of the Americas*, p. 41-146. Orlando, FL.

Arnold, J.F., Sinha, N.K., Cash, J.N. (1992): Effect of immobilized proteases on polyphenol oxidase inhibition and associated degradation of anthocyanins in plum juice. Presented at Annual Meeting, Institute of Food Technologists, New Orleans, LA. P. 185-193

Asemota, H. N., Wellington, M. A., Odutaga, A. A. and Ahmad, M. A. (1992): Effect of short-term storage on phenolic content, *o*-diphenolase and peroxidase activities of cut yam tubers (*Dioscorea* sp.). *Journal of Science, Food and Agriculture*. 60: 309-312.

Asiedu, R., Ng, S. Y. C., Vuylsteke, D., Terauchi, R. and Hahn, S. K. (1992): Analysis of the need for biotechnology research on cassava, yam and plantain. In: Biotechnology: Enhancing Research on Tropical Crops in Africa. CTA/IITA, Ibadan, Nigeria, Thottappilly, G., L. M. Monti, D. R. Mohan Raj and Moore, A. W. (eds.) pp. 70-74.

Ayensu, E. S. (1972): VI. Dioscoreales. In: Anatomy of the monocotyledons. Metcalfe, C. R. (ed.). Clarendon press, Oxford, UK. p. 329-331

Bachem, C.W.B., Speckmann, G.J., Van der Linde, P.C.G., Verheggen, F.T.M., Hunt, M.D., Steffens, J.C., Zabeau, M. (1994): Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers. *Bio/Technology*, 12: 1101-1105.

Baldwin, E.A., Nisperos-Carriedo, M.O., Baker, R.A. (1995): Edible coatings for lightly processed fruits and vegetables. *Horticultural Science*., 30: 35-38.

Baldwin, E.A., Nisperos, M.O., Chen, X., Hagenmaier, R.D. (1996): Improving storage life of cut apple and potato with edible coating. *Posthar. Bio. Tech.*, 9: 151-163.

Ballantyne, A., Stark, R. & Selman, J.D. (1988): Modified atmosphere packaging of shredded lettuce. *International Journal of Food Science and Technology* 23: 267-274.

Baritaux, O., Amiot, M.J., Richard, H., Nicolas, J. (1991): Enzymatic browning of basil (*Ocimum basillicum* L.): Studies on phenolic compounds and polyphenol oxidase. *Sci. Alim.* 11: 49-62.

Barquar, S. R. and Oke, O. L. (1977): Mineral constituents of Nigerian yams. *Nutr. Rep. Int.* 15(3): 265-272.

Bauernfeind, J.C. and Pinkert, D.M. (1970): Food processing with added ascorbic acid. *Advanced Food Research* 18: 219-315.

Beélik, A. (1956): Kojic acid. *Advaced Carbohydrate Chemistry* 11: 145-183.

Chazarra, S., Garcia-Carmona, F., Cabanes, J. (1999): Characterization of monophenolase activity of polyphenol oxidase from iceberg lettuce. *Journal of Agriculture and Food Chemistry*. 47: 1422-1426.

Chen, J.S., Wei, C.I., Rolle, R.S., Otwell, W.S., Balaban, M.O., Marshall, M.R. (1991b). Inhibititory effect of kojic acid on some plant and crustacean polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 39: 1396-1401.

Cilliers, J.J.L., Singleton, V.L. (1990): Caffeic acid autooxidation and the effects of thiols. *Journal of Agriculture and Food Chemistry*. 38: 1789-1796.

Coursey, D. G. (1967). Yams, Longman, London, UK. pp. 230.

Coursey, D.G. (1983): Yams. In: Hand Book of Tropical Foods. Chan H. T. (eds.). Marcel Dekker, New York, USA. pp 555-601.

Cuppett, S.L. (1994): Edible coatings as carriers of food additives, fungicides and natural antagonists. *In* J.M. Krochta, E.A. Baldwin & M.O. Nisperos-Carriedo, eds. *Edible Coatings and Films to Improve Food Quality*, p. 121-137. Lancaster/Basel, Technomic Publishing.

Das, J.R., Bhat, S.G., Gowda, L.R. (1997): Purification and characterization of a polyphenol oxidase from the kew cultivar of Indian pineapple fruit. *Journal of Agriculture and Food Chemistry*. 45: 2031-2035.

Dicko, M.H., Hilhorst, R., Gruppen, H., Laane, C., Berkel, W.J.H.V., Voragent, A.G.J. (2002): Zymography of monophenolase and o-diphenolase activities of polyphenol oxidase. *Analitical Biochemistry* 306: 336-339.

Dincer, B., Colak, A., Aydin, N., Kadioglu, A., Guner, S. (2002): Characterization of polyphenoloxidase from medlar fruits (*Mespillus germanica* L., Rosaceae). *Food Chemistry*. 77: 1-7.

Ding, C.K., Chachin, K., Ueda, Y., Imahori, Y. (1998): Purification and properties of polyphenol oxidase from loquat fruit. *Journal of Agriculture and Food Chemistry*. 46: 4144-4149

Dipeolu O. A., Adebayo K. and Ogundokun M. O. (2002): Consumer behaviour towards non-grain staple foods in a dynamic price and income regime in Abeokuta, Nigeria. *Agriculture and Soc Res*: 2(1):52-66.

DuBose, C. N., Cardello, A.V. and Maaller, O. (1980): Effects of colorants and flavourants on identification, perceived flavour intensity and hedonic quality of fruit flavoured beverages and cake. *Journal of Food Science* 45: 1393-1415.

Duncan, D.B. (1955). New multiple range and multiple test. *Biometrics*. 11: 1-11

Dziezak, J.D. (1986): Preservatives: Antioxidants - The ultimate answer to oxidation. *Food Technology*. 40 (9): 94-102.

Embs, R.T., and Markakis P. (1985): The mechanism of sulphite inhibition of browning caused by polyphenol oxidase, *Journal of Food Science* 30: 753-758.

Espin, J.C., Morales, M., Varon, R., Tudela, J., Garcia-canovas, F. (1996a): Continuous spectrophotometric method for determining monophenolase and diphenolase activities of pear polyphenoloxidase. *Journal of Food Science*. 61: 1177-1182.

Espin, J.C., Morales, M., Varon, R., Tudela, J., Garcia-canovas, F. (1996b): Monophenolase activity of polyphenol oxidase from Blanquilla pear. *Phytochemistry*. 44: 17-22.

Espin, J.C., Ochoa, M., Tudela, J., Garcia-Canovas, F. (1997a): Monophenolase activity of strawberry polyphenol oxidase. *Phytochemistry*. 45: 667-670.

Espin, J.C., Trujano, M.F., Tudela, J., Garcia-Canovas, F. (1997b): Monophenolase activity of polyphenol oxidase from Haas avocado. *Journal of Agriculture and Food Chemistry*. 45: 1091-1096.

Espin, J.C., Trujano, M.F., Tudela, J., Carcia-Canovas, F. (1997c): Monophenolase activity of polyphenol oxidase from artichoke heads (*Cynara scolymus* L.). Lebensm.-Wiss. u.-Technology. 30: 819-825.

Espin, J.C., Garcia-Ruiz, P.A., Tudela, J., Varon, R., Carcia-Canovas, F. (1998): Monophenolase and diphenolase reaction mechanisms of apple and pear polyphenol oxidases. J *Journal of Agriculture and Food Chemistry*. 46: 2968-2975.

FAO. (1990): Food and Agriculture Organisation. Action Programme for the Prevention of Food Losses, Improving Post-harvest Handling, Storage and Processing of Root and Tuber Crops, Rome, Italy.

FAO. (2003): FAOSTATDATA. FAO, Rome, Italy.

FAOSTAT. (2004): http://appls.fao.org (accessed on February 13th 2004)

Farombi, E. O. (1998): Modulation of hepatic xenobiotic-metabolizing enzymes by browned yam flour diet in rats fed bromobenzene. Proc. Fed Afr. Soc. Biochem. *Mol Biol*. 1:11-14.

Farombi, O. E., Britton, G. and Emerole, O. G. (2000): Evaluation of the antioxidant activity and partial characterisation of extracts from browned yam flour diet. *Food Research International* 33:493-499.

Federal Register. (1985): Notice to shippers, distributors, packers, and importers of shrimp containing sulfites. 50: 2957-2958.

Federal Register. (1988): Sulfiting agents in standardized foods; Labeling requirements. 53: 51062-51065.

Federal Register (1992): Opta Food Ingredients, Inc.; Filling of petition for affirmation of GRAS status. 57: 8461.

Ferrer, O.J., Koburger, J.A., Otwell, W.S., Gleeson, R.A., Simpson, B.K. & Marshall, M.R. (1989a): Phenoloxidase from the cuticle of Florida spiny lobster (*Panulirus argus*): mode of activation and characterization. *Journal of Food Science* .54: 63-67.

Ferrer, O.J., Otwell, W.S. & Marshall, M.R. (1989b): Effect of bisulfite on lobster shell phenoloxidase. *Journal of Food Science*. 54: 478-480.

Finkle, B.J. and Nelson, R.F. (1963): Enzyme reactions with phenolic compounds: effect of *o*-methyl-transferase on a natural substrate of fruit polyphenolxoidase. *Nature*. 197: 902-903.

Fraignier, M.P., Marques, L., Fleuriet, A., Macheix, J.J. (1995): Biochemical and immunochemical characteristics of polyphenol oxidases from different fruit of *prunus*. *Journal of Agriculture and Food Chemistry*. 43: 2375-2380.

Francis, F. J. (1980): Colour quality evaluation of horticultural crops. *Horticultural Science*. 15:14-15.

Frankos, V.H., Schmitt, D.F., Haws, L.C., McEvily, A.J., Iyengar, R., Miller, S.A., Munro, I.C., Clydesdale, F.M., Forbes, A.L., Sauer, R.M. (1991): Generally recognized as safe (GRAS) evaluation of 4-hexylresorcinol for use as a processing aid for prevention of melanosis in shrimp. *Regul. Toxicol. Pharmacol.*, 14: 202-212.

Fray, R. and Grierson, D. (1993): Molecular genetics of tomatoes fruit ripening. *Trends Genetics*, 9: 438-443.

Friedman, M. and Molnar-Perl, I. (1990): Inhibition of browning by sulfur amino acids. 1. Heated amino acid-glucose systems. *Journal of Agriculture and Food Chemistry*. 38:1642-1647.

Friedman, M., Molnar-Perl, I., Knighton, D.R. (1992): Browning prevention in fresh and dehydrated potatoes by SH-containing amino acids. *Food additives and contaminants*, 9: 499-503.

Fu,Y. C., Huang, P. Y. and Chu, C. J. (2005): Use of continuous bubble separation process of separating and recovering starch and mucilage from yam (*Dioscorea pseudojaponica* Yamamoto). LWT. 38 (7):735-744. Doi 10.1016/j.lwt.2004.09.008.

Fujita, S., Tono, T. and Kawahara, H. (1991): Purification and properties of polyphenol oxidase in head lettuce (Lactuca sativa). *Journal of Science, Food and Agriculture*. 65:643-65.

Fujita, S., Saari, N., Maegawa, M., Tetsuka, T., Hayashi, N. and Tono, T. (1995): Purification and properties of polyphenol oxidase from cabbage (Brassica oleracea L.). *Journal of Agriculture and Food Chemistry*. 43:1138-1142.

Fujimoto, K., Miyashiro, M., Kaneda, T. (1972): Enzymatic browning reaction of the Shitake mushroom and its prevention. *Mushroom Science*., 8: 861-866.

Fukusawa, R, Wakabayashi, H., Natori, T. (1982). Inhibitor of tyrosinases in foods. Japanese patent. 57-40875.

Gaillard, T. and Bowler, P. (1987): Morphology and composition of starch .In: Starch properties and potential. T. Galliard (ed) Chichester: Wiley Society of Chemical Industry. Great Britain. pp 57-58.

Gaman, P.M. and K.B. Sherrington, (1996): The Science of food. 4th Edn., Reel educational and Professional Publishers Ltd., Oxford, pp: 125-136.

Gardner, J., Manohar, S., Borisenok, W.S. (1991): Method and composition for preserving fresh peeled fruits and vegetables. U.S. Patent 4,988,523.

Gauillard, F., Richard-Forget, F., Nicolas, J. (1993): New spectrophotometric assay for polyphenol oxidase activity. *Analitical Biochemistry*. 215: 59-65.

Gaykema, W.P.J., Hol, W.G.J., Vereijken, J.M., Soeter, N.M., Bak, H.J., Beintema, J.J. (1984): A structure of the copper-containing, oxygen-carrying protein *Panulirus interruptus* haemocyanin. *Nature*. 309: 23-29.

Gérard, C., Barron, C., Colonna, P. and Planchot, V. (2001): Amylose determination in genetically modified starches. *Carbohydrate Polymers* 44:19-27.

Girardin, O., Nindjin, C., Farah, Z., Eshcher F., Stamp, P. and Otokore D. (1998): Effect of storage on system and sprout removal on post-harvest yam (*Dioscorea* spp.) fresh weight losses. *Journal of Agriculture and Science.* 130:329-336

Giebel, L.B., Strunk, K.M., Spritz, R.A. (1991): Organization and nucleotide sequences of the human tyrosinase gene and a truncated tyrosinase-related segment. *Genomics*, 9: 435-445.

Golbeck, J.H. and Cammarata, K.V. (1981): Spinach thylakoid polyphenol oxidase. *Plant Physiol*ogy. 67: 977-984.

Gonzalez, E.M., Ancos, B.D., Cano, M.P. (1999): Partial characterization of polyphenol oxidase activity in raspberry fruits. *Journal of Agriculture and Food Chemistry.* 47: 4068-4072.

Gonzalez, E.M., Ancos, B.D., Cano, M.P. (2000): Partial characterization of peroxidase and polyphenol oxidase activities in blackberry fruits. *Journal of Agriculture and Food Chemistry.* 48: 5459-5464.

Goodenough, P., Kessell, S., Lea, A.G.H., Loeffler, T. (1983): Mono- and diphenolase activity from fruit of Malus pumila apples. *Phytochemistry.* 22: 359-363.

Gordon, M.N., (2000): Contemporary nutrition; Issues and Insights. Mc-Graw Hill companies New York. 4th Edn., pp: 102-256.

Gaman, P.M. and Sherrington, K.B. (1996): The Science of food. 4th Edn., Reel educational and Professional Publishers Ltd., Oxford, pp: 125-136.

Goulart, P.F.P., Alves, J.D., Magalhaes, M.M., Lima, L.C.O., Meyer, L.E. (2003): Purification of polyphenoloxidase from coffee fruits. *Food Chemistry*. 83: 7-11.

Gowda, L.R. and Paul, B. (2002): Diphenol activation of the monophenolase and Diphenolase activities of field bean (Dolichos lablab) polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 50: 1608-1614.

Green, L.F. (1976): Sulphur dioxide and food preservation- a review. *Food Chemistry*. 1: 103-124.

Grncarevic, M. and Hawker, J.S. (1971): Browning of sultana grape berries during drying. *Journal of Science and Food Agriculture*. 22: 270-272.

Gunata, Y.Z., Sapis, J.C., Moutounet, M. (1987): Substrates and aromatic carboxylic acid inhibitors of grape phenoloxidases. *Phytochemistry*, 26: 1573-1575.

Hahn, S. K. Osiru, D. S. O. Akoroda, M. O. and Otoo, J. A. (1987): Yam production and its future prospects. *Outlook on Agriculture*, 16 (3): 105-110.

Halder, J., Tamuli, P., Bhaduri, A.N. (1998): Isolation and characterization of polyphenol oxidase from Indian tea leaf (*Camellia sinensis*). *Nutritional Biochemistry*. 9:75-80.

Hariprakash, C. S. and Nambisan, B. (1996): Carbohydrate Metabolism during Dormancy and Sprouting in Yam *(Dioscorea)* Tubers: Changes in Carbohydrate Constituentsin Yam *(Dioscorea)* Tubers during Dormancy and Sprouting. *Journal of Agriculture and Food Chemistry*. 44 (10): 3066 -3069.

Hasegawa, S. and Maier, V.P. (1980): Polyphenol oxidase of dates. *Journal of Agriculture and Food Chemistry*. 28: 891-893.

Henze, R.E. (1956): Inhibition of enzymatic browning of chlorogenic acid solutions with cysteine and glutathione. *Science*, 123: 1174-1175.

Hicks, K.B., Sapers, G.M, Seib, P.A. (1990): Process for preserving raw fruit and vegetable juices using cyclodextrins and compositions thereof. U.S.Patent 4,975,293.

Hirano, S., Itakura, C., Seino, H., Akiyama, Y., Nonaka, I., Kanbara, N., Kawakami, T. (1990): Chitosan as an ingredient for domestic animal feeds. *Journal of Agriculture and Food Chemistry*. 38: 1214-1217.

Hisaminato, H., Murata, M., Homma, S. (2001): Relationship between the enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibition of polyphenol biosynthesis. *Bioscience, Biotechnology and Biochemistry*. 65: 1016-1021.

Ho, K.K. (1999): Characterization of polyphenol oxidase from aerial roots of an orchid, Aranda 'Christine 130'. *Plant Physiology and Biochemistry*. 37: 841-848.

Hoover, R. (2001): Composition, molecular structure and physicochemical properties of 165 tuber and root starches. *Carbohydrate polymers* 45:253-267.

Hsu, A. F., Shieh, J.J., Bills, D.D. & White, K. (1988): Inhibition of mushroom polyphenol oxidase by ascorbic acid derivatives. *Journal of Food Science*., 53: 765-767, 771.

Huber, M., Hintermann, G., Lerch, K. (1985): Primary structure of tyrosinase from Streptomyces glaucescens. *Biochemistry*. 24: 6038-6044.

Hutchings, JB. (2003): Expectations, color and appearance of food. In: *Expectations and the food industry, the impact of color and appearance*. Kluwer Academic/Plenum Publishers, New York, NY. pp 141-175.

Hunt, M.D., Eannetta, N.T., Yu, H., Newman, S.M., Steffens, J.C. (1993): cDNA cloning and expression of potato polyphenol oxidase. *Plant Molecular Biology*. 21: 59-68.

IITA (2009): Yam production in Africa. International Institute of Tropical Agriculture (IITA), Nigeria. Available from

http://www.iita.org/cms/details/yam_project_details.aspx?zoneid=63&articleid=268. (Accessed on April 2009)

Ikediobi, C.0. and Obasuyi, H. N. (1982): Purification and some properties of o-diphenolase from white yam tubers. *Phytochemistry.* 21(12) 2815-2820.

Ikediobi, C. O. and Oti, E. (1983): Some biochemical changes associate with post-harvest storage of white yam (*Dioscorea rotundata*) tubers. *Journal of the Science Food and Agriculture* 34, 1123–1130.

Interesse, F.S., Alloggio, V., Lamparelli, F., D'Avella, G. (1984): Characterization of the oxidative enzymatic system of the phenolic compounds from Muscat grapes (Musts and wines). Lebensm.-Wiss. u.-*Technology.* 17: 5-10.

Irwin, P.L., Pfeffer, P.E., Doner, L.W., Sapers, G.M., Brewster, J.D., Nagahashi, G., Hicks, K.B. (1994): Binding geometry, stoichiometry, and thermodynamics of cyclomalto-oligosaccharide (cyclodextrin) inclusion complex formation with chlorogenic acid, the major substrate of apple polyphenol oxidase. *Carbohydrate Research.* 256: 13-27.

Iyengar, R., Bohmont, C.W., McEvily, A.J. (1991): 4-Hexylresorcinol and prevention of shrimp melanosis: residual analyses. *J. Food Comp. Anal.*, 4: 148-157.

Jane, J. and Chen, J. F. (1992): Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chemistry* 69: 60-65.

Janovitz-Klapp, A.H., Richard, F.C., Goupy, P.M. & Nicolas, J.J. (1990): Inhibition studies on apple polyphenol oxidase. *Journal of Agriculture and Food Chemistry.* 38: 926-931.

Jeong, H. L., Jin, W. J., Kwang, D. M. and Kee, J. P. (2008): Effects of Anti-Browning Agents on Polyphenoloxidase Activity and Total Phenolics as Related to Browning of Fresh-Cut 'Fuji' Apple. *ASEAN Food Journal* 15 (1): 79-87

Jiang, Y., Zauberman, G., Fuchs, Y. (1997): Partial purification and some properties of polyphenol oxidase extracted from litchi fruit pericarp. *Postharvest Biology and Technology* 10: 221-228.

Jiang, Y. (1999): Purification and some properties of polyphenol oxidase of longan fruit. *Food Chemistry*. 66: 75-79.

Kahn, V. and Andrawis, A. (1985): Inhibition of mushroom tyrosinase by tropolone. *Phytochemistry*, 24: 905-908.

Kahn, V. (1985): Effects of proteins, protein hydrolyzates, and amino acids on *o*-dihydroxyphenolase activity of polyphenol oxidase of mushroom, avocado and banana. *Journal of Food Science*. 50: 111-115.

Kahn, V. (1995): Multiple effects of maltol and kojic acid on enzymatic browning. *In* C.Y. Lee & J.R. Whitaker, eds. *Enzymatic Browning and Its Prevention*, p. 277-294. ACS Symposium Series 600, Washington, DC, American Chemical Society.

Kelly, S.H. and Finkle, B.J. (1969): Action of a ring-cleaving oxygenase in preventing oxidative darkening of apple juices. *Journal of the Science Food and Agriculture*., 20: 629-632.

Kermasha, S., Goetghebeur, M., Monfette, A., Metche, M., Rovel, B. (1993): Studies on inhibition of mushroom polyphenol oxidase using chlorogenic acid as substrate. *Journal of Agriculture and Food Chemistry*. 41: 526-531.

Kidron, M., Harel, E., Mayer, A.M. (1978): Catechol oxidase activity in grapes and wine. *Am. J. Enol. Vitic*., 29: 30-35.

King, J.M., McEvily, A.J., Iyengar, R. (1991): Liquid chromatographic determination of the processing aid 4-hexylresorcinol in shrimp. *J. Assoc. Off. Anal. Chem*., 74: 1003-1005.

Kornblum, S.S., Stoopak, S.B. (1973): A new tablet disintegrant agent: crosslinked polyvinylpyrollidone. *Journal of Pharmaceutical Science*. 62(1): 43-49.

Kramer, A., Solomos, T., Wheaton, F., Puri, A., Sirivichaya, S., Lotem, Y., Fowke, M., Ehrman, L. (1980): A gas exchange process for extending the shelf life of raw foods. *Food Technoogyl*, 34 (7): 65-74.

Krueger, R.C. (1955): The inhibition of tyrosinase. *Arch. Biochem. Biophys.*, 57: 52-60.

Kwon, B.S., Haq, A.K., Pomerantz, S.H., Halaban, R. (1987): Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. *Proc. Natl. Acad. Sci.*, 84: 7473-7477.

Langdon, T.T. (1987): Prevention of browning in fresh prepared potatoes without the use of sulfiting agents. *Food Technology*. 41 (5): 64-67.

Labuza, T., Lillemo, J.H., Taoukis, P.S. (1990): Inhibition of polyphenol oxidase by proteolytic enzymes, "Killer enzymes". Presented at the 20th Symposium International Federation of Fruit Juice Producers. Scientific Technical Commission. Paris, France.

Lambrecht, H.S. (1995): Sulfite substitutes for the prevention of enzymatic browning in foods. *In* C.Y. Lee & J.R. Whitaker, eds. *Enzymatic Browning and Its Prevention*, p. 313-323. ACS Symposium Series 600, Washington, DC, American Chemical Society.

Lamkin, W.M., Miller, B.S., Nelson, S.W., Traylor, D.P. and Lee, M.S.(1981): Polyphenol oxidase activities of hard red winter, soft and red winter, hard red spring, white common and durum wheat cultivars. *Cereal Chemistry* 58(11) 27-31.

Larmond, E. (1977): Laboratory methods for sensory evaluation of food. Publication No. 1637 Canada. Department of Agriculture Ottawa. Pp. 17-22

Laveda, F., Nunez-Delicado, E., Garcia-Carmona, F., Sanchez-Ferrer, A. (2001): Proteolytic activation of latent Paraguaya peach PPO: Characterization of monophenolase activity. *Journal of Agriculture and Food Chemistry*. 49: 1003-1008.

Lee, C.Y. and Smith, N.L. (1979): Blanching effect on polyphenol oxidase activity in table beets. *Journal of Food Science*, 44: 82.

Lee, C.Y., Lagon, V., Jamorski, A.W. and Brown, S.K. (1990): Enzymic browning in relation to phenolic compounds and polyphenoloxidase activity among various peach cultivars. *Journal of Agriculture and Food Chemistry. 36* 99-101.

Leach, H.W., McCowen, L.D., Schoch, J.J. (1995): Structure of the starch granule: swelling and solubility pattern of various starches cereal chemistry 36:534-44.

Leach, H. W., McCoven, L. D. and Scoch, T. J. (1959): Structure of the Starch granule. Swelling and solubility patterns of various starches. *Cereal Chemistry*. 36: 534-544.

Lerch, K. (1982): Primary structure of tyrosinase from *Neurospora crassa*. II. Complete amino acid sequence and chemical structure of a tripeptide containing an unusual thioether Fungi. *Journal of Biology and Chemistry*. 257: 6414-6419.

Lerch, K. (1983): *Neurospora* tyrosinase: structural, spectroscopic and catalytic properties. *Molecular and Cellullose Biochemistry*. 52: 125-138.

Liao, M.L. and Seib, P.A. (1988): Chemistry of L-ascorbic acid related to foods. *Food Chemistry*. 30: 289-312.

Lorenz, K. and Collins, F. (1990): Quinoa (*Chenopodium quinoa*) Starch-Physicochemical properties and functional characteristics. *Starch/stärke*. 42 (3):81-86.

Lowry, O.H., Rosenbrough, N.J., Farr, A.l. and Randall, R.J. (1951): *Journal of Biology and Chemistry. 193: 265-275.*

Lozano-De-Gonzalez, P.G., Barrett, D.M., Wrolstad, R.E., Durst, R.W. (1993): Enzymatic browning inhibited in fresh and dried apple rings by pineapple juice. *Journal of Food Science*. 58: 399-404.

Macheix, J.J., Sapis, J.C. and Fleuriet, A. (1991): Phenolic compounds and polyphenoloxidase in relation to browning in grapes and wines. *Critical Reveal Food Science and Nutrition*, 30: 441-486.

Macrae, A.R. and Duggleby, R.G. (1968): Substrates and inhibitors of potato tubers phenolase. *Phytochemistry*, 7: 855-861.

Martinez, M.V. and Whitaker, J.R. (1995): The biochemistry and control of enzymatic browning. *Trends Food Science and Technology.* June: 195-200.

Martinez-Cayuela, M., Medina, L.S., Faus, M.J., Gil, A. (1988): Cherimoya (*Annona cherimolia* Mill.) polyphenol oxidase: Monophenol oxidase and dihydroxyphenolase activities. *Journal of Food Science*. 53: 1191-1194.

Mason, H.S. and Peterson, E.W. (1965): Melanoproteins. I. Reactions between enzyme-generated quinones and amino acids. *Biochemistry, Biophysics Acta*. 111: 134-146.

Mayer, A.M., Harel, E., Ben-Shaul, R. (1966): Assay of catechol oxidase-a critical comparison of methods. *Phytochemistry*. 5: 783-789.

Mayer, A.M. and Harel, E. (1979): Polyphenol oxidases in plants. *Phytochemistry*. 18: 193-215.

Mayer, A.M. and Harel, E. (1979): Phenoloxidase in plants. *Phytochemistry*, 18: 193-215.

Mazzafera, P. and Robinson, S.P. (2000): Characterization of polyphenol oxidase in coffee. *Phytochemistry*. 55: 285-296.

McEvily, A.J., Iyengar, R., Gross, A. (1991): Compositions and methods for inhibiting browning in foods using resorcinol derivatives. U.S. patent 5,059,438. Pp. 821-826

McEvily, A.J., Iyengar, R., Otwell, W.S. (1992): Inhibition of enzymatic browning in foods and beverages. *Critical Review. Food Science and Nutrition.*, 32: 253-273.

Mestres, C., Dorthe, S., Akissoe, N. and Hounhouigan, J. (2002): Prediction of sensorial properties (color and taste) of *amala*, a paste from yam chip flour of West Africa, through flour biochemical properties. Submitted to *Plant Food for Human Nutrition* (in press). 44, 100–114.

Modderman, J.P. (1986): Technological aspects of use of sulfiting agents in food. *J. Assoc. Off. Anal.*, Chem. 69: 1-3.

Molnar-Perl, I. and Friedman, M. (1990a): Inhibition of browning by sulfur amino acids. 2. Fruit juices and protein-containing foods. *Journal of Agriculture and Food Chemistry*. 38: 1648-1651.

Molnar-Perl I, Friedman M. (1990b): Inhibition of browning by sulfur amino acids. 3. Apples and potatoes. *Journal of Agriculture and Food Chemistry*. 38: 1652-1656.

Monsalve-Gonzalez, A, Barbosa-Canovas, G.V., Cavalieri, R.P., McEvily, A.J., Iyengar, R. (1993): Control of browning during storage of apple slices preserved by combined methods. 4-hexylresorcinol as anti-browning agent. *Joural of Food Science*. 58: 797-800, 826.

Moorthy, S. N. (1994): Tuber crop starches. Technical bulletin series 18. Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. Pp. 318-325

Moorthy, S. N., and Nair, S. G. (1989): Studies on *Dioscorea rotundata* Starch properties. *Stärke*. 41:81-83.

Nagai, T. and Suzuki, N. (2001): Partial purification of polyphenol oxidase from Chinese cabbage *Brassica rapa* L. *Journal of Agriculture and Food Chemistry*. 49: 3922-3926.

Nagai, T. and Suzuki, N. (2003): Polyphenol oxidase from bean sprouts (*Glycine max* L.). Journal of Food Science. 68: 16-20.

Newman, S.M., Eannetta, N.T., Yu, H., Prince, J.P., Carmen de Vicente, M., Tanksley, S.D., Steffens, J.C. (1993): Organisation of the tomato polyphenol oxidase gene family. *Plant Molecular Biology*. 21: 1035-1051.

Nicolas, J.J., Richard-Forget, F.C., Goupy, P.M, Amiot, M.J. and Aubert, S.Y. (1994): Enzymatic browning reactions in apple and apple products. *Critical Review. Food Science and Nutrition*, 34: 109-157.

Nunez-Delicado, E., Sojo, M.M., Garcia-Carmona, F., Sanchez-Ferrer, A. (2003): Partial purification of latent persimmon fruit polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 51: 2058-2063.

Nweke, F. I., Okoroji, E. C., Njoku, J. E. and King, D. J. (1992): Elasticities of demand for major food items in a root and tuber-based food system: Emphasis on yam and cassava in South Eastern Nigeria. Research and Crop Management Program Research Monograph No. 11: International Institute of Tropical Agriculture, Ibadan, Nigeria. (IITA).

Oguntona, T. (1994): Nutritional value of Nigerian food basket. Invited keynote address for UN-FAO World Food Day, Abuja, 16 October. Pp. 1-14.

Okada, Y., Kubota, Y., Koizumi, K., Hizukuri, S., Ohfuji, T., Ogata, K. (1988): Some properties and the inclusion behavior of branched cyclodextrins. *Chem. Pharm. Bull.*, 36: 2176-2185.

Okaka JC, Okechukwu PE (1993). Yam Processing: Problems and prospects. In: Advances in Yam Research: Production and Post Harvest Technologies of the yam tubers. GO Osuji (editor), Prairie View A & M University, Prairie View, Texas. p. 78-90.

Okoli, B. E. and Green, B.O, (1987): Histochemical localisation of Calcium oxalate crystals in starch grains of yams (*Dioscorea*). *Annals of Botany*. 60(4): 391-394.

Omidiji, O. and J. Okpuzor, (1996) Time course of PPOrelated browning of yams. *Journal of the Science Food and Agriculture*., 70: 190- 196.

Onwueme, I. C. (1978): The Tropical Tuber crops. John Wiley and Sons,Chichester, pp. 234.

Onwueme, I. C. and Charles, W. B. (1994): Tropical root and tuber crops: production,

perspectives and future prospects. FAO, Rome, Italy. Pp. 288

Opara, L.U. (1999): Yam storage. In: Bakker-Arkema et al. (eds). CIGR Handbook of Agricultural Engineering Volume IV Agro Processing. pp. 182-214. The American Society of Agricultural Engineers, St. Joseph, MI

Orenes-Pinero, E., Garcia-Carmona, F., Sanchez-Ferrer, A. (2005): A kinetic study of *p*cresol oxidation by quince fruit polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 53: 1196-1200.

Orkwor, G. C. (1998): The importance of yams In: Food Yams: Advances in research. Orkwor, G. C., R. Asiedu and I. J. Ekanayake, eds. IITA, Ibadan, Nigeria and NRCRI, Umudike, Umuhahia, Abia State, Nigeria. pp. 1-12.

Osagie, A. U. (1992): The Yam in Storage. Postharvest Research Unit, University of Benin, Nigeria. pp. 11-21, 157- 173

Osagie, A. U. and Eka, O. U. (1998): Chemical Composition of Root and Tuber crops. In: Nutritional quality of plant foods. Department of biochemistry, University of Benin, Benin City Nigeria. Pp. 1-31

Osibo, O (2009): Nigeria: Country realises N56 billion from yam export. Vanguard, Nigeria. Avaialable from http://allafrica.com/stories/200903230071.html (Accessed in May 2009)

Osuga, D., van der Schaaf, A., Whitaker, J.R. (1994): Control of polyphenoloxidase activity using a catalytic mechanism. *In* R.Y. Yada, R.L. Jackman & J.L. Smith, eds. *Protein Structure-Function Relationships in Foods*, p. 62-88. New York, Blackie Academic & Professional.

Oszmianski, J. and Lee, C.Y. (1990): Inhibition of polyphenol oxidase activity and browning by honey. *Journal of Agriculture and Food Chemistry*. 38: 1892-1895.

Otwell, W.S. & Marshall, M.R. (1986): Studies on the use of sulfites to control shrimp melanosis (blackspot): Screen alternatives to sulfiting agents to control shrimp melanosis. Florida seagrant technical paper No. 46: 1-10.

Otwell, W.S., Iyengar, R., McEvily, A. (1992): Inhibition of shrimp melanosis by 4-hexylresorcinol. *Journal of Aquatic Food Production Technology*. 1: 53-65.

Ough, C.S. (1984): Sulfur Dioxide and Sulfites. *In* A.L. Branen & P.M. Davidson, eds. *Antimicrobials in Foods*, p. 177-203. New York, Marcel Dekker.

Owusu-Ansah, Y.J. (1989): Polyphenoloxidase in wild rice (*Zizania palustris*). *Journal of Agriculture and Food Chemistry*. 37: 901-904.

Pagington, J.S. (1986): Beta-cyclodextrin and its uses in the flavour industry. *In* G.G. Birch & M.G. Lindley, eds. *Developments in food flavours*. p. 131-150. London, Elsevier Applied Science Publishers.

Palmer, J.K. (1963): Banana polyphenol oxidase. Purification and properties. *Plant Physiology. 38:* 508-513.

Park, E.Y., Luh, B.S. (1985): Polyphenol oxidase of kiwifruit. *Journal of Agriculture and Food Chemistry*. 48: 3839-3846.

Paul, B. and Gowda, L.R. (2000): Purification and characterization of a polyphenol oxidase from the seeds of field bean (*Dolichos lablab*). *Journal of Agriculture and Food Chemistry*. 48: 3839-3846.

Penalver, M.J., Rodriguez-Lopez, J.N., Garcia-Molina, F., Garcia-Canovas, F,, Tudela, J. (2002): Method for the determination of molar absorptivities of thiol adducts formed from diphenolic substrates of polyphenol oxidase. *Analitical Biochemistry*. 309: 180-185.

Perez-Gilabert, M. and Garcia-Carmona, F. (2000): Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 48: 695-700.

Perez-Gilabert, M., Morte, A., Honrubia, M., Garcia-Carmona, F. (2001): Partial purification, characterization, and histochemical localization of fully latent desert truffle (*Terfezia claveryi* Chatin) polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 49: 1922-1927.

Peroni, F. H. G., Rocha, T. S. and Franco, C. M. L. (2006): Some structural and physicochemical characteristics of tuber and root starches. *Food Science Techology Int*., 12 (6); 505-513.

Pierpoint, W.S. (1966): The enzymatic oxidation of chlorogenic acid and some reactions of the quinone produced. *Biochemistry Journal*. 98: 567-580.

Pifferi, P.G, Baldassari, L, Cultrera, R. (1974): Inhibition by carboxylic acids of an *o*-diphenol oxidase from *Prunus avium* fruit. *Journal of the Science Food and Agriculture*. 25: 263-270.

Purseglove, J. W. (1976): Tropical Crops. Monocotyledons. Longman Group Ltd., London, pp 97-117.

Rasper, V. and Coursey, D. G. (1967): Properties of starches of some West African yams. *Journal of the Science Food and Agriculture*. 18: 240-244.

Ravi, V., Ked, J. and Balagopalan, C. (1996): Review on Tropical Root and Tuber. In: *Critical Reviews. Food Science and Nutrition* 36 (7): 360-730.

Ravindran, G. and Wanasundera, J. P. D. (1992): Chemical changes in Yam tubers (*Dioscorea alata and D. esculenta*) during storage. *Tropical Science*. 33: 57-62.

Raymond, J., Rakariyatham, N., Azanza, J.L. (1993): Purification and some properties of polyphenoloxidase from sunflower seeds. *Phytochemistry*.34: 927-931.

Richard-Forget, F.M., Goupy, P.M., Nicolas, J.J. (1992): Cysteine as an inhibitor of enzymatic browning. II. Kinetic studies. *Journal of Agriculture and Food Chemistry*. 40: 2108-2113.

Richardson, T. and Hyslop, D.B. (1985): Enzymes. *In* O.R. Fennema, ed. *Food Chemistry*, p. 371-476. New York, Marcel Dekker.

Richardson, P. H., Jeffcoat, R. and Shi, Y. C. (2000): High amylose starches: From biosynthesis to their use as food ingredients [online], *MRS Bulletin,* 20–24.

Riley, C. K., Wheatley, A. O. and Asemota, H. N. (2006): Isolation and characetrisation of starches from eight *Dioscorea alata* cultivars grown in Jamaica. *African Journal of Biotechnology* 5(17): 1528-1536.

Rocha, A.M.C.N. and De Morais, A.M.M.B. (2005): Polyphenol oxidase activity of minimally processed 'Jonagored' apples (Malus domestica), *Journal of Food Processing Pres.,* 29: 8-19.

Rouet-Mayer, M.A., Ralambosoa, J., Philippon, J. (1990): Roles of o-quinones and their polymers in the enzymatic browning of apples. *Phytochemistry*. 29: 435-440.

Sakane, Y., Arita, N., Shimokana, S., Ito, H., Osajima, Y. (1990): Storage of shredded cabbage in plastic films using ethylene-acetaldehyde or sucrose fatty acid esters. *Nippon Shokuhin Kogyo Gakkaishi*. 37: 281-286.

Sanchez-Ferrer, A., Bru, R., Garcia-Carmona, F. (1988): Characterization of catecholase and cresolase activities of Monastrell grape polyphenol oxidase. *Phytochem*istry. 27: 319-321.

Sanchez-Ferrer, A., Villalba, J., Garcia-Carmona, F. (1989): Triton X-114 as a tool for purifying spinach polyphenol oxidase. *Phytochemistry*. 28: 1321-1325.

Sanchez-Ferrer, A., Laveda, F., Garcia-Carmona, F. (1993): Partial purification of soluble potato polyphenol oxidase by partitioning in aqueous two-phase system. *Journal of Agriculture and Food Chemistry*. 41: 1219-1224.

Sapers, G.M., Hicks, K.B., Philips, J.G., Garzarella, L., Pondish, D.L., Matulaitis, R.M., McCormack, T.J., Sondey, S.M., Seib, P.A., El-Ataway, Y.S. (1989): Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *Journal of Food Science*. 54: 997-1002, 1012.

Sapers, G.M. and Hicks, K.B. (1989): Inhibition of enzymatic browning in fruits and vegetables. *In* J.J. Jen, ed. *Quality Factors of Fruits and Vegetables: Chemistry and Technology*, p. 29-43. Washington, DC, American Chemical Society.

Sapers, G.M., Hicks, K.B., Philips, J.G., Garzarella, L., Pondish, D.L., Matulaitis, R.M., McCormack, T.J., Sondey, S.M., Seib, P.A. & El-Ataway, Y.S. (1989): Control of enzymatic browning in apple with ascorbic acid derivatives, polyphenol oxidase inhibitors, and complexing agents. *Journal of Food Science*. 54: 997-1002, 1012.

Sapers, G.M. (1992): Chitosan enhances control of enzymatic browning in apple and pear juice by filtration. *Journal of Food Science*. 57: 1192-1193.

Saruno, R., Kato, F., Ikeno, T. (1979): Kojic acid, a tyrosinase inhibitor from *Aspergillus albus*. *Agriculture Biology and Chemistry*. 43: 1337-1339.

Sayavedra-Soto L.A. and Montgomery, M.W. (1986): Inhibition of polyphenoloxidase by sulfite. *Journal of Food Science*. 51: 1531-1536.

Sciancalepore, V. and Longone, V. (1984): Polyphenol oxidase activity and browning in green olives. *Journal of Agriculture and Food Chemistry 32:* 320-321.

Scott, G. J., Rosegrant M. and Ringler, C. (2000b): Roots and Tubers for the 21st Century: Trends, projections and policy options. Food Agriculture and the Environment Discussion 31.

International Food Policy Research Institute (IFPRI) and international Potato Centre (CIP). Washington, D.C, U. S. A.

Segel, I.H. (1976): *Biochemical calculations*. p. 273-277. New York, John Wiley and Sons.

Serradell, M.A., Rozenfeld, P.A., Martinez, G.A, Civello, P.M., Chaves, A.R., Anon, M.C. (2000): Polyphenoloxidase activity from strawberry fruit (Fragaria x ananassa, Duch., cv Selva): characterization and partial purification. *Journal of the Science Food and Agriculture*. 80: 1421-1427.

Shahar, T., Hennig, N., Gutfinger, T., Hareven, T., Lifschitz, E. (1992): The tomato 66.3-kD polyphenoloxidase gene: molecular identification and developmental expression. *Plant Cell*., 4: 135-147.

Sharma, R.R., Goswami, A.M., Singh, C.N., Chhonkar, O.P., Singh, G. (2001): Catecholase and cresolase activities and phenolic content in mango (Mangifera indica L.) at panicle initiation. *Science and Horticulture*. 87: 147-151.

Sheen, *S*.J. and Calvert, J. (1969): Studies of polyphenol content, activities and isoenzymes of polyphenol oxidase and peroxidise during air-curing in three tobacco types. *Plant Physiology*. 11 199-204.

Shibahara, S.Y., Tomita, T., Sakakura, T., Nager, C., Chaudhuri, B., Müeller, R. (1986): Cloning and expression of cDNA encoding mouse tyrosinase. *Nucleic acids Research*. 14: 2413-2427.

Shuji, F., Yun-zhe, H., Chie, K., Tomoko, M., Megumi, Y., Yasuko, H., Ying-jie, L., Nobuyuki, H. and Chang-peng, Y. (2006): Purification and Characterization of Polyphenol Oxidase from Edible Yam (*Dioscorea opposita Thunb*.) *Food Science and Technology Research*. 12 (3): 235-239

Simpson, B.K., Gagne, N., Ashie, I.N.A., Noroozi, E. (1997): Utilization of chitosan for preservation of raw shrimp (*Pandalus borealis*). *Food Biotechnology*. 11: 25-44.

Sojo, M.M., Nunez-Delicado, E., Garcia-Carmona, F., Sanchez-Ferrer, A. (1998 a): Partial purification of a banana polyphenol oxidase using triton X-114 and PEG 8000 for removal of polyphenols. *Journal of Agriculture and Food Chemistry*. 46: 4924-4930.

Sojo, M.M., Nunez-Delicado, E., Garcia-Carmona, F., Sanchez-Ferrer, A. (1998b): Monophenolase activity of latent banana pulp polyphenol oxidase. *Journal of Agriculture and Food Chemistry*. 46: 4931-4936.

Stefano, G. and Scianchalepore, V. (1993): Polyphenol oxidases and peroxidases in 5 table olive cv. Indus. Alim. 32: 731-733.

Steiner, F. and Rieth, T.E. (1989):0 Preservative method and preserved fruit or vegetable, using citric acid, sodium and calcium chloride containing preservative composition. U.S. Patent 4,818,549.

Swannell, M. C., Wheeler, T. R., Asiedu, R. and Craufurd, P. Q. (2003): Effect of harvest date on the dormancy period of yam (*Dioscorea rotundata*). *Tropical Science*. 43: 103-107

Tamminga, S.K., Beumer, R.R., Keijbets, M.J.H. & Kampelmacher, E.H. (1978): Microbial spoilage and development of food poisoning bacteria in peeled, completely or partly cooked vacuum packed potato. *Archiv fur Lebensmittel hygiene*, 29: 215-219.

Taoukis, P.S., Labuza, T.P., Lillemo, J.H., Lin, S.W. (1990): Inhibition of shrimp melanosis (black spot) by ficin. *Lebensm -Wiss u –Technology*. 23: 52-54.

Taylor, S.L., Higley, N.A., Bush, R.K. (1986): Sulfites in foods: Uses, analytical methods, residues, fate, exposure assessment, metabolism, toxicity, and hypersensitivity. *Advanced Food Research*. 30: 1-76.

Thomas, P. and Janave, M.T. (1986): Isoelectric focusing evidence for banana isoenzymes with mono and diphenolase activity. *Journal of Food Science*. 51: 384-387.

Tipton, K.F. and Dixon, H.B.F. (1983): Effect of pH on enzymes. *In* D.L. Purich, ed. *Contemporary Enzyme Kinetics and Mechanism*, p. 97-148. New York, Academic Press.

Tolbert, N.E. (1973): Activation of polyphenol oxidase of chloroplasts. *Plant Physiology.* 51: 234-244.

Tong, C.B.S. and Hicks, K.B. (1991): Sulfated polysaccharides inhibit browning of apple juice and diced apples. *Journal of Agriculture and Food Chemistry.* 39: 1719-1722.

Tong, C.B.S., Hicks, K.B., Osman, S.F., Hotchkiss, A.T., Haines, R.M. (1995): Oxalic acid in commercial pectins inhibits browning of raw apple juice. *Journal of Agriculture and Food Chemistry.* 43: 592-597.

Tono, T., Fujita, S., Kawasaki, H. and Li, Z.F. (1986): Purification and high L-epicatechin oxidase activity in polyphenol oxidase of Japanese pear. Nippon Nogeikagaku Kaishi, 60:705-712 (in Japanese).

Treche, S. and Agbor-Egbe, T. (1996): Biochemical Changes occurring during growth and storage of two yam species. *International Journal of Food Science and Nutrition.* 47 (2) 93-102.

Tschannen, A. T., Girardin, O., Nindjin, C., Dao, D., Farah, Z., Stamp, P. and Escher, F. (2003): Improving the application of gibberellic acid to prolong dormancy of yam tubers (*Dioscorea* spp.). *Journal of the Science of Food and Agriculture* 83:787-796.

Valero, E., Varon R., and Garcia-Carmona, F. (1991): A kinetic study of irreversible enzyme inhibition by an inhibitor that is rendered unstable by enzymic catalysis, *Biochemistry Journal.* 277: 869-874.

Valero, E., Varón, R., García-Carmona, F. (1990): Inhibition of grape polyphenol oxidase by several natural aliphatic alcohol. *Journal of Agriculture and Food Chemistry.* 38: 1097-1100.

Vaughn, K.C., Lax, A.R., Duke, S.O. (1988): Polyphenol oxidase: The chloroplast oxidase with no established function. *Physiology of Plants.* 72: 659-665.

Vámos-Vigyázó, L. (1981): Polyphenoloxidase and peroxidase in fruits and vegetables. *Critical Review. Food Science and Nutrition.* 15: 49-127.

Walker, J.R.L. and Wilson, E.L. (1975): Studies on the enzymatic browning of apples. Inhibition of apple *o*-diphenol oxidase by phenolic acids. *Journal of the Science Food and Agriculture.* 26: 1825-1831.

Walker, J.R.L. (1976): The control of enzymatic browning in fruit juices by cinnamic acids, *Journal of Food Technology.* 11: 341-345.

Walker, J.R.L. (1977): Enzymatic browning in foods. Its chemistry and control. *Food Technology NZ*, 12: 19-25.

Walter, W. M., Truong, V. D., Wiesenborn, D. P. and Carvajal, P. (2000): Rheological and physico-chemical properties of starches from moist and dry-type sweet potatoes. *Journal of Agriculture and Food Chemistry* 48:2937-2942.

Walter, W. M., Truong, V. D., Wiesenborn, D. P and Carvajal, P. (2000): Rheological and Physico-chemical Properties of starches from Moist and Dry-type Sweet potatoes. *Journal of Agriculture and Food Chemistry.* 48: 2937-2942.

Whitaker, J.R. (1994): Polyphenol oxidase. In: *Princeples of Enzymology for the Food Sciences. 2nd ed.* Marcel Dekker, Inc., New York. pp 543-556.

Whitaker, J.R. (1972b): Effect of pH on rates of enzyme-catalyzed reactions. *In* O.R. Fennema, ed. *Principles of enzymology for the Food Sciences,* Chapter 10, New York, Marcel Dekker.

Whitaker, J.R. and Lee, C.Y. (1995): Recent advances in chemistry of enzymatic browning. *In* C.Y. Lee & J.R.Whitaker, eds. *Enzymatic Browning and Its Prevention*, p. 2-7. ACS Symposium Series 600, Washington, DC, American Chemical Society.

Whitaker, J.R. (1996): Enzymes. In: Fennema OR, ed. *Food Chemistry.* Marcel Dekker, Inc., New York. pp 431-530.

Wei, C.I., Fernando, S.Y., Huang, T.S. (1991): Mutagenicity studies of kojic acid. Proceedings of the Fifteenth annual conference Tropical and Subtropical Fisheries Technological Conference of the Americas, p. 464-470. Orlando, FL.

Wiley, J.W., Tyson, G.N., Steller, J.S. (1942): The configuration of complex kojates formed with some transition elements as determined by magnetic susceptibility measurements. *American Chemical Society Journal.* 64: 963-964.

Wissemann, K.W. and Montgomery, M.W. (1985): Purification of d'Anjou pear (*Pyrus communis* L.) polyphenol oxidase. *Plant Physiology.* 78: 256-262.

Wolfrom, M.I., Kashimura, J. and Horton, D. (1974): Factors affecting the Maillard browning reactions between sugars and amino acids: Studies on the non-enzymic browning of the dehydrated orange juice. *Journal of Agriculture and Food Chemistry. 22(5)* 796-800.

Wong, T.C., Luh, B.S., Whitaker, J.R. (1971): Isolation and characterization of polyphenol oxidase isozymes of clingstone peach. *Plant physiology.* 48: 19-23.

Wong, W.S., Tillin, S.J., Hudson, J.S., Pavlath, A.E. (1994): Gas exchange in cut apples with bilayer coatings. *Journal of Agriculture and Food Chemistry.* 42: 2278-2285.

Wong, D.W.S. (1995): *Food Enzymes: Structure and Mechanism.* P. 284-320. New York, Chapman & Hail.

Woolfe, J.A. (1987): The potato in the human diet. Cambridge Univ. Press. Cambridge, UK, pp. 595-618

You, S. and Izidorczyk, M. S. 2002. Molecular characteristics of barley starches with variable amylose content. *Carbohydrate Polymers.* 49:33-42.

Zhang, D. and Quantick, P.C. (1997): Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit. *Postharvest Biology and Technology.* 12: 195-202.

APPENDIX 1: Polyphenol oxidase (PPO) Activity and Browning index of yam tissue steeped in different PPO inhibitors

General Linear Model

Between-Subjects Factors

		Value Label	N
Temp	30.00		60
	40.00		60
	50.00		60
Time	2.00		36
	4.00		36
	6.00		36
	8.00		36
	12.00		36
Inhibitor	1.00	Control	45
	2.00	Ascorbic acid	45
	3.00	Cystein	45
	4.00	Sodium metabisulphite	45

Descriptive Statistics

	Temp (oC)	Time (hrs)	Inhibitor	Mean	Std. Deviation	N
Activity	30.00	2.00	Control	50420.700	5.00000	3
			Ascorbic acid	66241.000	3.00000	3
			Cystein	4445.0000	3.00000	3
			Sodium metabisulphate	2312.8000	.00000	3
			Total	30854.875	29296.90143	12
		4.00	Control	39462.700	3.00000	3
			Ascorbic acid	2330.9000	4.00000	3
			Cystein	1120.3000	2.00000	3
			Sodium metabisulphate	542.1000	1.00000	3
			Total	10864.000	17258.83779	12
		6.00	Control	10407.800	4.35890	3
			Ascorbic acid	32199.000	5.00000	3
			Cystein	1517.8000	3.00000	3
			Sodium metabisulphate	1301.0000	1.00000	3
			Total	11356.400	13141.42968	12
		8.00	Control	34801.000	.17321	3
			Ascorbic acid	7010.8000	5.00000	3
			Cystein	5529.1000	2.00000	3
			Sodium metabisulphate	2023.7000	1.00000	3
			Total	12341.150	13675.23953	12
		12.00	Control	1572.0000	2.00000	3
			Ascorbic acid	3740.3000	4.00000	3
			Cystein	1626.2000	2.00000	3
			Sodium metabisulphate	614.4000	2.00749	3
			Total	1888.2250	1193.33215	12
		Total	Control	27332.840	19007.70948	15
			Ascorbic acid	22304.400	25379.07758	15
			Cystein	2847.6800	1850.82416	15
			Sodium metabisulphate	1358.8000	743.06563	15
			Total	13460.930	19344.00807	60
	40.00	2.00	Control	5420.7000	5.00000	3
			Ascorbic acid	10516.200	3.00000	3
			Cystein	2746.5000	2.00000	3
			Sodium metabisulphate	1192.6000	1.73205	3
			Total	4969.0000	3699.24530	12
		4.00	Control	4625.7000	2.64575	3
			Ascorbic acid	4716.0000	4.00000	3
			Cystein	2330.9000	2.00000	3
			Sodium metabisulphate	939.6000	1.00000	3
			Total	3153.0500	1666.80049	12
		6.00	Control	5673.7000	3.00000	3
			Ascorbic acid	5746.0000	3.00500	3
			Cystein	2927.2000	2.00000	3
			Sodium metabisulphate	2330.9000	1.00000	3
			Total	4169.4500	1624.11468	12
		8.00	Control	4390.8000	5.00000	3
			Ascorbic acid	6107.4000	3.00500	3
			Cystein	4264.3000	3.00000	3
			Sodium metabisulphate	885.4000	2.00000	3
			Total	3911.9750	1977.15255	12

Descriptive Statistics

	Temp (oC)	Time (hrs)	Inhibitor	Mean	Std. Deviation	N
Activity	40.00	12.00	Control	3252.4000	2.00000	3
			Ascorbic acid	4770.2000	5.00000	3
			Cystein	3035.6000	4.00000	3
			Sodium metabisulphate	216.8000	2.00000	3
			Total	2818.7500	1717.29781	12
		Total	Control	4672.6600	885.65718	15
			Ascorbic acid	6371.1600	2217.35479	15
			Cystein	3060.9000	670.57428	15
			Sodium metabisulphate	1113.0600	713.34258	15
			Total	3804.4450	2330.00921	60
	50.00	2.00	Control	5168.0000	4.03361	3
			Ascorbic acid	10787.200	7.00000	3
			Cystein	1626.2000	6.00000	3
			Sodium metabisulphate	867.3000	2.00000	3
			Total	4612.1750	4091.45720	12
		4.00	Control	1355.0000	5.01199	3
			Ascorbic acid	975.7000	5.00000	3
			Cystein	1029.9000	3.00000	3
			Sodium metabisulphate	704.0000	4.17971	3
			Total	1016.1500	241.66088	12
		6.00	Control	1969.0000	3.12250	3
			Ascorbic acid	2222.0000	2.17945	3
			Cystein	1011.0000	2.53574	3
			Sodium metabisulphate	542.0000	2.00749	3
			Total	1436.0000	716.38537	12
		8.00	Control	3053.0000	3.23574	3
			Ascorbic acid	4101.0000	1.57162	3
			Cystein	1517.0000	1.70880	3
			Sodium metabisulphate	1138.0000	2.06640	3
			Total	2452.2500	1244.76024	12
		12.00	Control	2710.3000	5.00300	3
			Ascorbic acid	2222.0000	3.12250	3
			Cystein	676.0000	1.57162	3
			Sodium metabisulphate	433.0000	3.23574	3
			Total	1510.3250	1018.44540	12
		Total	Control	2851.0600	1345.23661	15
			Ascorbic acid	4061.5800	3631.00764	15
			Cystein	1172.0200	363.75716	15
			Sodium metabisulphate	736.8600	257.46116	15
			Total	2205.3800	2325.23461	60
	Total	2.00	Control	20336.467	22563.44069	9
			Ascorbic acid	29181.467	27794.89801	9
			Cystein	2939.2333	1229.11074	9
			Sodium metabisulphate	1457.5667	656.71078	9
			Total	13478.683	20847.47480	36
		4.00	Control	15147.800	18291.08707	9
			Ascorbic acid	2674.2000	1639.94006	9
			Cystein	1493.7000	629.12236	9
			Sodium metabisulphate	728.5667	173.11996	9
			Total	5011.0667	10625.79896	36

Descriptive Statistics

	Temp (oC)	Time (hrs)	Inhibitor	Mean	Std. Deviation	N
Activity	Total	6.00	Control	6016.8333	3663.15994	9
			Ascorbic acid	13389.000	14189.78678	9
			Cystein	1818.6667	859.87760	9
			Sodium metabisulphate	1391.3000	777.57237	9
			Total	5653.9500	8559.84442	36
		8.00	Control	14081.600	15550.34384	9
			Ascorbic acid	5739.7333	1289.80529	9
			Cystein	3770.1333	1776.38518	9
			Sodium metabisulphate	1349.0333	517.68918	9
			Total	6235.1250	8945.97536	36
		12.00	Control	2511.5667	742.74908	9
			Ascorbic acid	3577.5000	1110.14378	9
			Cystein	1779.2667	1028.16861	9
			Sodium metabisulphate	421.4000	172.39894	9
			Total	2072.4333	1418.21692	36
		Total	Control	11618.853	15576.21778	45
			Ascorbic acid	10912.380	16672.50888	45
			Cystein	2360.2000	1415.91107	45
			Sodium metabisulphate	1069.5733	652.36781	45
			Total	6490.2517	12319.39799	180
Browning Index	30.00	2.00	Control	53.0000	2.00000	3
			Ascorbic acid	45.0000	1.00000	3
			Cystein	28.0000	1.00000	3
			Sodium metabisulphate	27.0000	.00000	3
			Total	38.2500	11.66288	12
		4.00	Control	42.0000	2.00000	3
			Ascorbic acid	35.0000	5.00000	3
			Cystein	29.0000	1.00000	3
			Sodium metabisulphate	23.0000	.00000	3
			Total	32.2500	7.72393	12
		6.00	Control	39.0000	5.19615	3
			Ascorbic acid	34.0000	2.00000	3
			Cystein	27.0000	3.46410	3
			Sodium metabisulphate	25.0000	3.46410	3
			Total	31.2500	6.63496	12
		8.00	Control	37.0000	4.00000	3
			Ascorbic acid	47.0000	22.60531	3
			Cystein	28.0000	2.64575	3
			Sodium metabisulphate	22.0000	5.29150	3
			Total	33.5000	14.12606	12
		12.00	Control	26.0000	.00000	3
			Ascorbic acid	33.0000	2.00000	3
			Cystein	24.0000	1.00000	3
			Sodium metabisulphate	21.0000	.00000	3
			Total	26.0000	4.70976	12
		Total	Control	39.4000	9.38692	15
			Ascorbic acid	38.8000	10.75839	15
			Cystein	27.2000	2.51282	15
			Sodium metabisulphate	23.6000	3.26890	15
			Total	32.2500	10.09048	60

Descriptive Statistics

	Temp (oC)	Time (hrs)	Inhibitor	Mean	Std. Deviation	N
Browning Index	40.00	2.00	Control	51.0000	.00000	3
			Ascorbic acid	28.0000	2.00000	3
			Cystein	28.0000	2.00000	3
			Sodium metabisulphate	25.0000	.00000	3
			Total	33.0000	10.99587	12
		4.00	Control	36.0000	4.00000	3
			Ascorbic acid	32.0000	2.00000	3
			Cystein	28.0000	2.00000	3
			Sodium metabisulphate	26.0000	.00000	3
			Total	30.5000	4.52267	12
		6.00	Control	34.0000	4.00000	3
			Ascorbic acid	30.0000	4.00000	3
			Cystein	27.0000	2.00000	3
			Sodium metabisulphate	24.0000	2.00000	3
			Total	28.7500	4.71217	12
		8.00	Control	30.0000	7.21110	3
			Ascorbic acid	41.0000	1.00000	3
			Cystein	25.0000	1.73205	3
			Sodium metabisulphate	22.0000	1.73205	3
			Total	29.5000	8.22966	12
		12.00	Control	34.0000	4.00000	3
			Ascorbic acid	41.0000	2.00000	3
			Cystein	26.0000	.00000	3
			Sodium metabisulphate	21.0000	.00000	3
			Total	30.5000	8.19645	12
		Total	Control	37.0000	8.41767	15
			Ascorbic acid	34.4000	6.08041	15
			Cystein	26.8000	1.89737	15
			Sodium metabisulphate	23.6000	2.16465	15
			Total	30.4500	7.59890	60
	50.00	2.00	Control	37.0000	.00000	3
			Ascorbic acid	40.0000	.00000	3
			Cystein	27.0000	1.00000	3
			Sodium metabisulphate	24.0000	1.00000	3
			Total	32.0000	6.99350	12
		4.00	Control	36.0000	2.00000	3
			Ascorbic acid	31.0000	1.00000	3
			Cystein	25.0000	.00000	3
			Sodium metabisulphate	24.0000	.00000	3
			Total	29.0000	5.15223	12
		6.00	Control	31.0000	1.00000	3
			Ascorbic acid	38.0000	2.00000	3
			Cystein	27.0000	1.00000	3
			Sodium metabisulphate	23.0000	1.00000	3
			Total	29.7500	5.89491	12
		8.00	Control	32.0000	2.00000	3
			Ascorbic acid	44.0000	2.00000	3
			Cystein	28.0000	.00000	3
			Sodium metabisulphate	20.0000	.00000	3
			Total	31.0000	9.12539	12

Descriptive Statistics

	Temp (oC)	Time (hrs)	Inhibitor	Mean	Std. Deviation	N
Browning Index	50.00	12.00	Control	29.0000	4.00000	3
			Ascorbic acid	31.0000	2.00000	3
			Cystein	20.0000	1.00000	3
			Sodium metabisulphate	19.0000	.00000	3
			Total	24.7500	5.87947	12
		Total	Control	33.0000	3.66450	15
			Ascorbic acid	36.8000	5.46678	15
			Cystein	25.4000	3.04256	15
			Sodium metabisulphate	22.0000	2.23607	15
			Total	29.3000	6.98982	60
	Total	2.00	Control	47.0000	7.61577	9
			Ascorbic acid	37.6667	7.64853	9
			Cystein	27.6667	1.32288	9
			Sodium metabisulphate	25.3333	1.41421	9
			Total	34.4167	10.19068	36
		4.00	Control	38.0000	3.87298	9
			Ascorbic acid	32.6667	3.27872	9
			Cystein	27.3333	2.12132	9
			Sodium metabisulphate	24.3333	1.32288	9
			Total	30.5833	5.94439	36
		6.00	Control	34.6667	4.82183	9
			Ascorbic acid	34.0000	4.24264	9
			Cystein	27.0000	2.06155	9
			Sodium metabisulphate	24.0000	2.23607	9
			Total	29.9167	5.72900	36
		8.00	Control	33.0000	5.26783	9
			Ascorbic acid	44.0000	11.65118	9
			Cystein	27.0000	2.17945	9
			Sodium metabisulphate	21.3333	2.95804	9
			Total	31.3333	10.62880	36
		12.00	Control	29.6667	4.50000	9
			Ascorbic acid	35.0000	4.89898	9
			Cystein	23.3333	2.73861	9
			Sodium metabisulphate	20.3333	1.00000	9
			Total	27.0833	6.72469	36
		Total	Control	36.4667	7.87285	45
			Ascorbic acid	36.6667	7.83640	45
			Cystein	26.4667	2.59019	45
			Sodium metabisulphate	23.0667	2.65775	45
			Total	30.6667	8.37728	180

Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	1.000	3E+008[a]	2.000	119.000	.000
	Wilks' Lambda	.000	3E+008[a]	2.000	119.000	.000
	Hotelling's Trace	5876032	3E+008[a]	2.000	119.000	.000
	Roy's Largest Root	5876032	3E+008[a]	2.000	119.000	.000
Temp	Pillai's Trace	1.011	61.285	4.000	240.000	.000
	Wilks' Lambda	.000	110996.6[a]	4.000	238.000	.000
	Hotelling's Trace	3446863	1E+008	4.000	236.000	.000
	Roy's Largest Root	3446863	2E+008[b]	2.000	120.000	.000
Time	Pillai's Trace	1.049	33.100	8.000	240.000	.000
	Wilks' Lambda	.000	42992.696[a]	8.000	238.000	.000
	Hotelling's Trace	1988544	3E+007	8.000	236.000	.000
	Roy's Largest Root	1988544	6E+007[b]	4.000	120.000	.000
Inhibitor	Pillai's Trace	1.055	44.644	6.000	240.000	.000
	Wilks' Lambda	.000	73149.475[a]	6.000	238.000	.000
	Hotelling's Trace	3217615	6E+007	6.000	236.000	.000
	Roy's Largest Root	3217615	1E+008[b]	3.000	120.000	.000
Temp * Time	Pillai's Trace	1.123	19.191	16.000	240.000	.000
	Wilks' Lambda	.000	24009.097[a]	16.000	238.000	.000
	Hotelling's Trace	2288681	2E+007	16.000	236.000	.000
	Roy's Largest Root	2288681	3E+007[b]	8.000	120.000	.000
Temp * Inhibitor	Pillai's Trace	1.088	23.839	12.000	240.000	.000
	Wilks' Lambda	.000	37099.291[a]	12.000	238.000	.000
	Hotelling's Trace	3195997	3E+007	12.000	236.000	.000
	Roy's Largest Root	3195997	6E+007[b]	6.000	120.000	.000
Time * Inhibitor	Pillai's Trace	1.479	28.384	24.000	240.000	.000
	Wilks' Lambda	.000	23399.432[a]	24.000	238.000	.000
	Hotelling's Trace	2903543	1E+007	24.000	236.000	.000
	Roy's Largest Root	2903543	3E+007[b]	12.000	120.000	.000
Temp * Time * Inhibitor	Pillai's Trace	1.303	9.349	48.000	240.000	.000
	Wilks' Lambda	.000	11877.156[a]	48.000	238.000	.000
	Hotelling's Trace	4002219	9838788	48.000	236.000	.000
	Roy's Largest Root	4002218	2E+007[b]	24.000	120.000	.000

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept+Temp+Time+Inhibitor+Temp * Time+Temp * Inhibitor+Time * Inhibitor+Temp * Time * Inhibitor

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Activity	2.717E+010[a]	59	460447342.0	4E+007	.000
	Browning Index	10858.000[b]	59	184.034	12.960	.000
Intercept	Activity	7582206005	1	7582206005	7E+008	.000
	Browning Index	169280.000	1	169280.000	11921.127	.000
Temp	Activity	4449842345	2	2224921172	2E+008	.000
	Browning Index	265.300	2	132.650	9.342	.000
Time	Activity	2567079876	4	641769969.1	6E+007	.000
	Browning Index	1005.000	4	251.250	17.694	.000
Inhibitor	Activity	4153448449	3	1384482816	1E+008	.000
	Browning Index	6526.800	3	2175.600	153.211	.000
Temp * Time	Activity	2954643739	8	369330467.4	3E+007	.000
	Browning Index	424.200	8	53.025	3.734	.001
Temp * Inhibitor	Activity	4126018681	6	687669780.2	6E+007	.000
	Browning Index	246.300	6	41.050	2.891	.011
Time * Inhibitor	Activity	3748503653	12	312375304.5	3E+007	.000
	Browning Index	1570.200	12	130.850	9.215	.000
Temp * Time * Inhibitor	Activity	5166856433	24	215285684.7	2E+007	.000
	Browning Index	820.200	24	34.175	2.407	.001
Error	Activity	1295.580	120	10.796		
	Browning Index	1704.000	120	14.200		
Total	Activity	3.475E+010	180			
	Browning Index	181842.000	180			
Corrected Total	Activity	2.717E+010	179			
	Browning Index	12562.000	179			

a. R Squared = 1.000 (Adjusted R Squared = 1.000)

b. R Squared = .864 (Adjusted R Squared = .798)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Activity	6490.252	.245	6489.767	6490.737
Browning Index	30.667	.281	30.111	31.223

Post Hoc Tests

Temp

Homogeneous Subsets

Activity

Duncan[a,b,c]

Temp	N	Subset 1	Subset 2	Subset 3
50.00	60	2205.3800		
40.00	60		3804.4450	
30.00	60			13460.93
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 10.796.

a. Uses Harmonic Mean Sample Size = 60.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Browning Index

Duncan[a,b,c]

Temp	N	Subset 1	Subset 2
50.00	60	29.3000	
40.00	60	30.4500	
30.00	60		32.2500
Sig.		.097	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 14.200.

a. Uses Harmonic Mean Sample Size = 60.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Time

Homogeneous Subsets

Activity

Duncan[a,b,c]

Time	N	Subset 1	2	3	4	5
12.00	36	2072.4333				
4.00	36		5011.0667			
6.00	36			5653.9500		
8.00	36				6235.1250	
2.00	36					13478.68
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 10.796.

a. Uses Harmonic Mean Sample Size = 36.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Browning Index

Duncan[a,b,c]

Time	N	Subset 1	2	3
12.00	36	27.0833		
6.00	36		29.9167	
4.00	36		30.5833	
8.00	36		31.3333	
2.00	36			34.4167
Sig.		1.000	.135	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 14.200.

a. Uses Harmonic Mean Sample Size = 36.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Inhibitor

Homogeneous Subsets

Activity

Duncan[a,b,c]

Inhibitor	N	Subset 1	2	3	4
Sodium metabisulphite	45	1069.5733			
Cystein	45		2360.2000		
Ascorbic acid	45			10912.38	
Control	45				11618.85
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 10.796.

a. Uses Harmonic Mean Sample Size = 45.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Browning Index

Duncan[a,b,c]

Inhibitor	N	Subset 1	2	3
Sodium metabisulphite	45	23.0667		
Cystein	45		26.4667	
Control	45			36.4667
Ascorbic acid	45			36.6667
Sig.		1.000	1.000	.802

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 14.200.

a. Uses Harmonic Mean Sample Size = 45.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

BROWNING

Duncan[a,b]

INHIBITOR	N	Subset 1
4.00	45	
3.00	45	
1.00	45	36.4667
2.00	45	36.6667
Sig.		.871

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 45.000.

b. Alpha = .05.

General Linear Model

Warnings

The DESIGN subcommand is empty, so a saturated design will be generated.

Between-Subjects Factors

		Value Label
Inhibitors	1.00	Control
	2.00	Ascorbic acid
	3.00	L-Cystein
	4.00	Sodium metabisulphite

Descriptive Statistics

	Inhibitors	Mean	Std. Deviation	N
ACTIVITY	Control	11618.8533	15576.2178	45
	Ascorbic acid	10912.3800	16672.5089	45
	L-Cystein	2360.2000	1415.9111	45
	Sodium metabisulphite	1069.5733	652.3678	45
	Total	6490.2517	12319.3980	180
BROWNING	Control	36.47	7.87	45
	Ascorbic acid	36.67	7.84	45
	L-Cystein	26.47	2.59	45
	Sodium metabisulphite	23.07	2.66	45
	Total	30.67	8.38	180

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.969	2695.379[c]	2.000	175.000	.000
	Wilks' Lambda	.031	2695.379[c]	2.000	175.000	.000
	Hotelling's Trace	30.804	2695.379[c]	2.000	175.000	.000
	Roy's Largest Root	30.804	2695.379[c]	2.000	175.000	.000
INHIBITOR	Pillai's Trace	.523	20.764	6.000	352.000	.000
	Wilks' Lambda	.479	25.964[c]	6.000	350.000	.000
	Hotelling's Trace	1.085	31.459	6.000	348.000	.000
	Roy's Largest Root	1.082	63.452	3.000	176.000	.000

Multivariate Tests[a]

Effect		Noncent. Parameter	Observed Power[b]
Intercept	Pillai's Trace	5390.758	1.000
	Wilks' Lambda	5390.758	1.000
	Hotelling's Trace	5390.758	1.000
	Roy's Largest Root	5390.758	1.000
INHIBITOR	Pillai's Trace	124.586	1.000
	Wilks' Lambda	155.786	1.000
	Hotelling's Trace	188.757	1.000
	Roy's Largest Root	190.356	1.000

a. Design: Intercept+INHIBITO

b. Computed using alpha = .05

c. Exact statistic

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	ACTIVITY	4153448448.657[b]	3	1384482816.219	10.588	.000
	BROWNING	6526.800[c]	3	2175.600	63.445	.000
Intercept	ACTIVITY	7582206005.401	1	7582206005.401	57.988	.000
	BROWNING	169280.000	1	169280.000	4936.585	.000
INHIBITOR	ACTIVITY	4153448448.658	3	1384482816.219	10.588	.000
	BROWNING	6526.800	3	2175.600	63.445	.000
Error	ACTIVITY	23012946023.492	176	130755375.133		
	BROWNING	6035.200	176	34.291		
Total	ACTIVITY	34748600477.550	180			
	BROWNING	181842.000	180			
Corrected Total	ACTIVITY	27166394472.149	179			
	BROWNING	12562.000	179			

Tests of Between-Subjects Effects

Source	Dependent Variable	Noncent. Parameter	Observed Power[a]
Corrected Model	ACTIVITY	31.765	.999
	BROWNING	190.336	1.000
Intercept	ACTIVITY	57.988	1.000
	BROWNING	4936.585	1.000
INHIBITOR	ACTIVITY	31.765	.999
	BROWNING	190.336	1.000
Error	ACTIVITY		
	BROWNING		
Total	ACTIVITY		
	BROWNING		
Corrected Total	ACTIVITY		
	BROWNING		

a. Computed using alpha = .05

b. R Squared = .153 (Adjusted R Squared = .138)

c. R Squared = .520 (Adjusted R Squared = .511)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error
ACTIVITY	6490.2517	852.302
BROWNING	30.67	.436

Post Hoc Tests

nhibitors

Homogeneous Subsets

ACTIVITY

Duncan[a,b]

Inhibitors	N	Subset 1	Subset 2
Sodium metabisulphite	45	1069.5733	
L-Cystein	45	2360.2000	
Ascorbic acid	45		10912.3800
Control	45		11618.8533
Sig.		.592	.769

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 45.000.

b. Alpha = .05.

BROWNING

Duncan[a,b]

Inhibitors	N	Subset 1
Sodium metabisulphite	45	
L-Cystein	45	
Control	45	36.47
Ascorbic acid	45	36.67
Sig.		.871

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 45.000.

b. Alpha = .05.

```
>Error # 2085
>The temporary period for running SPSS for Windows without a license has
>expired.  Use the License Authorization Wizard to contact SPSS for a
>license code.
>This command not executed.

>Specific symptom number: 37


End of job:  0 command lines  1 errors  0 warnings  4 CPU seconds
```

General Linear Model

Warnings

The DESIGN subcommand is empty, so a saturated design will be generated.

Between-Subjects Factors

		Value Label
TEMP	30	no label defined
	40	no label defined
	50	no label defined

Descriptive Statistics

	TEMP	Mean	Std. Deviation	N
ACTIVITY	30	13460.9300	19344.0081	60
	40	3804.4450	2330.0092	60
	50	2205.3800	2325.2346	60
	Total	6490.2517	12319.3980	180
BROWNING	30	32.25	10.09	60
	40	30.45	7.60	60
	50	29.30	6.99	60
	Total	30.67	8.38	180

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.942	1440.711[c]	2.000	176.000	.000
	Wilks' Lambda	.058	1440.711[c]	2.000	176.000	.000
	Hotelling's Trace	16.372	1440.711[c]	2.000	176.000	.000
	Roy's Largest Root	16.372	1440.711[c]	2.000	176.000	.000
TEMP	Pillai's Trace	.174	8.419	4.000	354.000	.000
	Wilks' Lambda	.827	8.791[c]	4.000	352.000	.000
	Hotelling's Trace	.209	9.160	4.000	350.000	.000
	Roy's Largest Root	.207	18.358	2.000	177.000	.000

Multivariate Tests[a]

Effect		Noncent. Parameter	Observed Power[b]
Intercept	Pillai's Trace	2881.421	1.000
	Wilks' Lambda	2881.421	1.000
	Hotelling's Trace	2881.421	1.000
	Roy's Largest Root	2881.421	1.000
TEMP	Pillai's Trace	33.676	.999
	Wilks' Lambda	35.164	.999
	Hotelling's Trace	36.640	.999
	Roy's Largest Root	36.716	1.000

a. Design: Intercept+TEMP

b. Computed using alpha = .05

c. Exact statistic

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	ACTIVITY	4449842344.639[b]	2	2224921172.319	17.336	.000
	BROWNING	265.300[c]	2	132.650	1.909	.151
Intercept	ACTIVITY	7582206005.401	1	7582206005.401	59.078	.000
	BROWNING	169280.000	1	169280.000	2436.634	.000
TEMP	ACTIVITY	4449842344.639	2	2224921172.320	17.336	.000
	BROWNING	265.300	2	132.650	1.909	.151
Error	ACTIVITY	22716552127.511	177	128342102.415		
	BROWNING	12296.700	177	69.473		
Total	ACTIVITY	34748600477.550	180			
	BROWNING	181842.000	180			
Corrected Total	ACTIVITY	27166394472.149	179			
	BROWNING	12562.000	179			

Tests of Between-Subjects Effects

Source	Dependent Variable	Noncent. Parameter	Observed Power[a]
Corrected Model	ACTIVITY	34.672	1.000
	BROWNING	3.819	.393
Intercept	ACTIVITY	59.078	1.000
	BROWNING	2436.634	1.000
TEMP	ACTIVITY	34.672	1.000
	BROWNING	3.819	.393
Error	ACTIVITY		
	BROWNING		
Total	ACTIVITY		
	BROWNING		
Corrected Total	ACTIVITY		
	BROWNING		

a. Computed using alpha = .05

b. R Squared = .164 (Adjusted R Squared = .154)

c. R Squared = .021 (Adjusted R Squared = .010)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error
ACTIVITY	6490.2517	844.400
BROWNING	30.67	.621

Post Hoc Tests

TEMP

Homogeneous Subsets

ACTIVITY

Duncan[a,b]

TEMP	N	Subset 1
50	60	2205.3800
40	60	3804.4450
30	60	
Sig.		.439

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 60.000.

b. Alpha = .05.

BROWNING

Duncan[a,b]

TEMP	N	Subset 1
50	60	29.30
40	60	30.45
30	60	32.25
Sig.		.066

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 60.000.

b. Alpha = .05.

General Linear Model

Warnings

The DESIGN subcommand is empty, so a saturated design will be generated.

Between-Subjects Factors

		Value Label
TIME	2	no label defined
	4	no label defined
	6	no label defined
	8	no label defined
	12	no label defined

Descriptive Statistics

	TIME	Mean	Std. Deviation	N
ACTIVITY	2	13478.6833	20847.4748	36
	4	5011.0667	10625.7990	36
	6	5653.9500	8559.8444	36
	8	6235.1250	8945.9754	36
	12	2072.4333	1418.2169	36
	Total	6490.2517	12319.3980	180
BROWNING	2	34.42	10.19	36
	4	30.58	5.94	36
	6	29.92	5.73	36
	8	31.33	10.63	36
	12	27.08	6.72	36
	Total	30.67	8.38	180

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.944	1477.206[c]	2.000	174.000	.000
	Wilks' Lambda	.056	1477.206[c]	2.000	174.000	.000
	Hotelling's Trace	16.979	1477.206[c]	2.000	174.000	.000
	Roy's Largest Root	16.979	1477.206[c]	2.000	174.000	.000
TIME	Pillai's Trace	.120	2.795	8.000	350.000	.005
	Wilks' Lambda	.881	2.850[c]	8.000	348.000	.004
	Hotelling's Trace	.134	2.903	8.000	346.000	.004
	Roy's Largest Root	.126	5.509	4.000	175.000	.000

Multivariate Tests[a]

Effect		Noncent. Parameter	Observed Power[b]
Intercept	Pillai's Trace	2954.413	1.000
	Wilks' Lambda	2954.413	1.000
	Hotelling's Trace	2954.413	1.000
	Roy's Largest Root	2954.413	1.000
TIME	Pillai's Trace	22.362	.941
	Wilks' Lambda	22.797	.945
	Hotelling's Trace	23.227	.950
	Roy's Largest Root	22.037	.974

a. Design: Intercept+TIME

b. Computed using alpha = .05

c. Exact statistic

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	ACTIVITY	2567079876.382[b]	4	641769969.095	4.566	.002
	BROWNING	1005.000[c]	4	251.250	3.805	.005
Intercept	ACTIVITY	7582206005.401	1	7582206005.401	53.940	.000
	BROWNING	169280.000	1	169280.000	2563.295	.000
TIME	ACTIVITY	2567079876.382	4	641769969.095	4.566	.002
	BROWNING	1005.000	4	251.250	3.805	.005
Error	ACTIVITY	24599314595.768	175	140567511.976		
	BROWNING	11557.000	175	66.040		
Total	ACTIVITY	34748600477.550	180			
	BROWNING	181842.000	180			
Corrected Total	ACTIVITY	27166394472.149	179			
	BROWNING	12562.000	179			

Tests of Between-Subjects Effects

Source	Dependent Variable	Noncent. Parameter	Observed Power[a]
Corrected Model	ACTIVITY	18.262	.940
	BROWNING	15.218	.887
Intercept	ACTIVITY	53.940	1.000
	BROWNING	2563.295	1.000
TIME	ACTIVITY	18.262	.940
	BROWNING	15.218	.887
Error	ACTIVITY		
	BROWNING		
Total	ACTIVITY		
	BROWNING		
Corrected Total	ACTIVITY		
	BROWNING		

a. Computed using alpha = .05

b. R Squared = .094 (Adjusted R Squared = .074)

c. R Squared = .080 (Adjusted R Squared = .059)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error
ACTIVITY	6490.2517	883.703
BROWNING	30.67	.606

Post Hoc Tests

TIME

Homogeneous Subsets

ACTIVITY

Duncan[a,b]

TIME	N	Subset 1
12	36	2072.4333
4	36	5011.0667
6	36	5653.9500
8	36	6235.1250
2	36	
Sig.		.178

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 36.000.

b. Alpha = .05.

BROWNING

Duncan[a,b]

TIME	N	Subset 1	Subset 2	Subset 3
12	36	27.08		
6	36	29.92	29.92	
4	36	30.58	30.58	30.58
8	36		31.33	31.33
2	36			34.42
Sig.		.084	.490	.058

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

a. Uses Harmonic Mean Sample Size = 36.000.

b. Alpha = .05.

APPENDIX 2: Physicochemical properties of yam steeped in different PPO inhibitors

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Energy	Control	3	27933.00	2.00000	1.15470	27928.0317	27937.9683	27931.00	27935.00
	Ascorbic acid	3	17605.00	3.00000	1.73205	17597.5476	17612.4524	17602.00	17608.00
	Cystein	3	15383.00	4.00000	2.30940	15373.0634	15392.9366	15379.00	15387.00
	Sodium metabisulphate	3	29058.00	1.00000	.57735	29055.5159	29060.4841	29057.00	29059.00
	Total	12	22494.75	6334.69832	1828.670	18469.8747	26519.6253	15379.00	29059.00
Hydration	Control	3	1.7900	.25515	.14731	1.1562	2.4238	1.53	2.04
	Ascorbic acid	3	1.5700	.29513	.17039	.8369	2.3031	1.27	1.86
	Cystein	3	2.0000	.30000	.17321	1.2548	2.7452	1.70	2.30
	Sodium metabisulphate	3	1.7600	.07000	.04041	1.5861	1.9339	1.69	1.83
	Total	12	1.7800	.26512	.07653	1.6115	1.9485	1.27	2.30
Moisture	Control	3	6.7500	.25000	.14434	6.1290	7.3710	6.50	7.00
	Ascorbic acid	3	6.7500	1.25000	.72169	3.6448	9.8552	5.50	8.00
	Cystein	3	6.5000	.00000	.00000	6.5000	6.5000	6.50	6.50
	Sodium metabisulphate	3	6.2500	.25000	.14434	5.6290	6.8710	6.00	6.50
	Total	12	6.5625	.59472	.17168	6.1846	6.9404	5.50	8.00
Swelling	Control	3	82.6000	5.40000	3.11769	69.1857	96.0143	77.20	88.00
	Ascorbic acid	3	75.3500	1.85000	1.06810	70.7543	79.9457	73.50	77.20
	Cystein	3	78.6000	1.40000	.80829	75.1222	82.0778	77.20	80.00
	Sodium metabisulphate	3	77.7500	4.25000	2.45374	67.1924	88.3076	73.50	82.00
	Total	12	78.5750	4.12374	1.19042	75.9549	81.1951	73.50	88.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Energy	Between Groups	4.4E+008	3	147137456.8	2E+007	.000
	Within Groups	60.000	8	7.500		
	Total	4.4E+008	11			
Hydration	Between Groups	.279	3	.093	1.505	.286
	Within Groups	.494	8	.062		
	Total	.773	11			
Moisture	Between Groups	.516	3	.172	.407	.752
	Within Groups	3.375	8	.422		
	Total	3.891	11			
Swelling	Between Groups	81.847	3	27.282	2.075	.182
	Within Groups	105.210	8	13.151		
	Total	187.057	11			

Moisture

Duncan[a]

Inhibitor	N	Subset for alpha = .05
		1
Sodium metabisulphate	3	6.2500
Cystein	3	6.5000
Control	3	6.7500
Ascorbic acid	3	6.7500
Sig.		.400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Swelling

Duncan[a]

Inhibitor	N	Subset for alpha = .05
		1
Ascorbic acid	3	75.3500
Sodium metabisulphate	3	77.7500
Cystein	3	78.6000
Control	3	82.6000
Sig.		.051

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX 3: Solubility of yam steeped in different PPO inhibitors at different temperatures

Univariate Analysis of Variance

Between-Subjects Factors

		Value Label	N
Inhibitor	1.00	Control	12
	2.00	Ascorbic acid	12
	3.00	Cystein	12
	4.00	Sodium metabisulphite	12
Temperature (°C)	60.00		12
	70.00		12
	80.00		12
	90.00		12

APPENDIX 3: Solubility of yam steeped in different PPO inhibitors at different temperatures

Univariate Analysis of Variance

Between-Subjects Factors

		Value Label	N
Inhibitor	1.00	Control	12
	2.00	Ascorbic acid	12
	3.00	Cystein	12
	4.00	Sodium metabisulphite	12
Temperature (°C)	60.00		12
	70.00		12
	80.00		12
	90.00		12

Descriptive Statistics

Dependent Variable: Solubility

Inhibitor	Temperature (°C)	Mean	Std. Deviation	N
Control	60.00	1.0333	.00577	3
	70.00	.5200	.52000	3
	80.00	.0000	.00000	3
	90.00	.0000	.00000	3
	Total	.3883	.49962	12
Ascorbic acid	60.00	1.0400	.00000	3
	70.00	.0000	.00000	3
	80.00	.0000	.00000	3
	90.00	.0000	.03000	3
	Total	.2600	.47053	12
Cystein	60.00	1.0300	.00000	3
	70.00	.0000	.00000	3
	80.00	.0167	.01528	3
	90.00	.0367	.03512	3
	Total	.2708	.45829	12
Sodium metabisulphite	60.00	1.0567	.02517	3
	70.00	.0000	.00000	3
	80.00	.0000	.00000	3
	90.00	.0167	.01528	3
	Total	.2683	.47560	12
Total	60.00	1.0400	.01537	12
	70.00	.1300	.32322	12
	80.00	.0042	.00996	12
	90.00	.0133	.02605	12
	Total	.2969	.46389	48

Tests of Between-Subjects Effects

Dependent Variable: Solubility

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.567[a]	15	.638	37.289	.000
Intercept	4.230	1	4.230	247.336	.000
Inhibitor	.135	3	.045	2.623	.067
Temp	8.954	3	2.985	174.497	.000
Inhibitor * Temp	.478	9	.053	3.108	.008
Error	.547	32	.017		
Total	14.345	48			
Corrected Total	10.114	47			

a. R Squared = .946 (Adjusted R Squared = .921)

Estimated Marginal Means

Grand Mean

Dependent Variable: Solubility

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
.297	.019	.258	.335

Post Hoc Tests

Inhibitor

Homogeneous Subsets

Solubility

Duncan[a,b]

Inhibitor	N	Subset	
		1	2
Ascorbic acid	12	.2600	
Sodium metabisulphite	12	.2683	
Cystein	12	.2708	
Control	12		.3883
Sig.		.850	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .017.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = .05.

Temperature (°C)

Homogeneous Subsets

Solubility

Duncan[a,b]

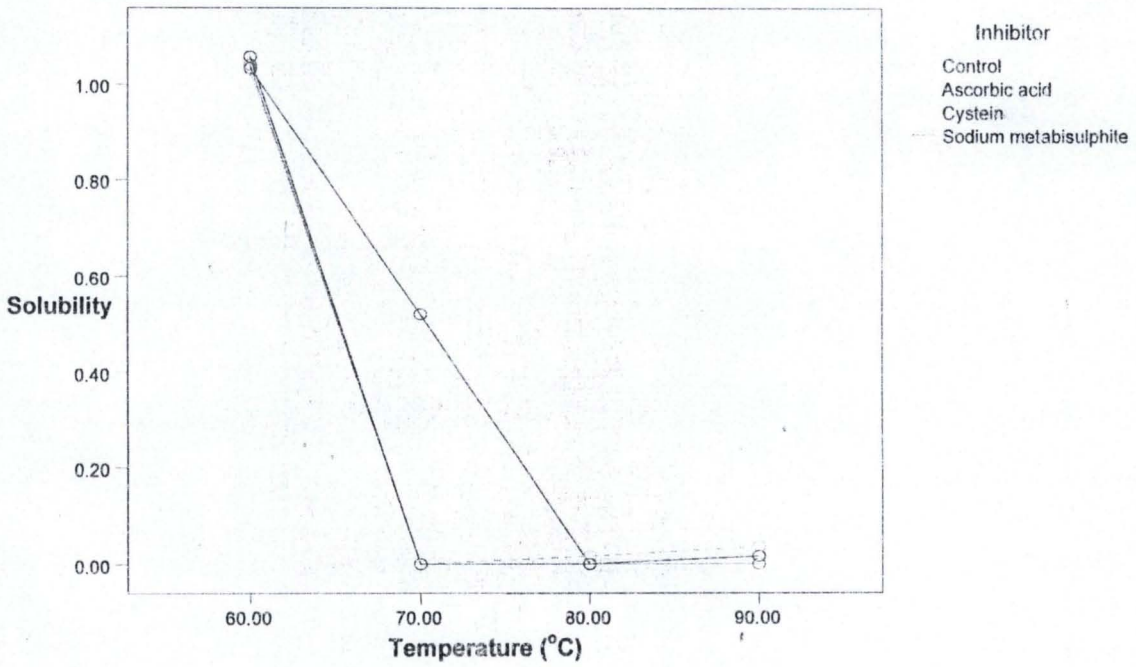
Temperature (°C)	N	Subset 1	Subset 2	Subset 3
80.00	12	.0042		
90.00	12	.0133		
70.00	12		.1300	
60.00	12			1.0400
Sig.		.865	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square (Error) = .017.

a. Uses Harmonic Mean Sample Size = 12.000.

b. Alpha = .05.

Profile Plots



>Warning # 108
>The command journal file cannot be opened.

>Warning # 1216. Command name: _SET
>A file cannot be opened. Probable causes are an attempt to open a
>read-only file for output, a directory which is too full, an invalid file
>specification, the specification of a non-existent disk, etc.

>The error involves file C:\Program Files\SPSS\SPSS.JNL

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error
SOLUBILITY Inhibitor	Control	12	.3883	.4996	.1442
	Ascorbic acid	12	.2600	.4705	.1358
	L-Cystein	12	.2708	.4583	.1323
	Sodium metabisulphite	12	.2683	.4756	.1373
	Total	48	.2969	.4639	6.696E-02

Descriptives

			95% Confidence Interval for Mean		
		Lower Bound	Upper Bound	Minimum	Maximum
SOLUBILITY	Inhibitor				
	Control	7.089E-02	.7058	.00	1.04
	Ascorbic acid	-3.8961E-02	.5590	-.03	1.04
	L-Cystein	-2.0348E-02	.5620	.00	1.03
	Sodium metabisulphite	-3.3850E-02	.5705	.00	1.08
	Total	.1622	.4316	-.03	1.08

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SOLUBILITY	Between Groups	.135	3	4.487E-02	.198	.897
	Within Groups	9.980	44	.227		
	Total	10.114	47			

Post Hoc Tests

Homogeneous Subsets

SOLUBILITY

Duncan[a]

Inhibitor	N	Subset for alpha = .05
		1
Ascorbic acid	12	.2600
Sodium metabisulphite	12	.2683
L-Cystein	12	.2708
Control	12	.3883
Sig.		.554

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000

Oneway

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
SOLUBILITY	TEMP 60	12	1.0400	1.537E-02	4.438E-03	1.0302	1.0498
	70	12	.1300	.3232	9.331E-02	-7.5366E-02	.3354
	80	12	4.167E-03	9.962E-03	2.876E-03	-2.1629E-03	1.050E-02
	90	12	1.333E-02	2.605E-02	7.521E-03	-3.2203E-03	2.989E-02
	Total	48	.2969	.4639	6.696E-02	.1622	.4316

Descriptives

		Minimum	Maximum
SOLUBILITY TEMP	60	1.03	1.08
	70	.00	1.04
	80	.00	.03
	90	-.03	.07
	Total	-.03	1.08

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SOLUBILITY	Between Groups	8.954	3	2.985	113.175	.000
	Within Groups	1.160	44	2.637E-02		
	Total	10.114	47			

Post Hoc Tests

Homogeneous Subsets

SOLUBILITY

Duncan[a]

TEMP	N	Subset for alpha = .05
		1
80	12	4.167E-03
90	12	1.333E-02
70	12	.1300
60	12	
Sig.		.079

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000

APPENDIX 4 : Organoleptic evaluation of yam flour meal (amala) steeped in different PPO inhibitors

General Linear Model

Between-Subjects Factors

		Value Label	N
Inhibitor	1.00	Control	10
	2.00	Ascorbic acid	10
	3.00	Cystein	10
	4.00	Sodium metabisulphate	10

Descriptive Statistics

	Inhibitor	Mean	Std. Deviation	N
Taste	Control	2.8000	1.03280	10
	Ascorbic acid	3.7000	1.41814	10
	Cystein	3.2000	1.13529	10
	Sodium metabisulphate	2.9000	.87560	10
	Total	3.1500	1.14466	40
Colour	Control	2.8000	1.03280	10
	Ascorbic acid	3.4000	2.01108	10
	Cystein	2.6000	.51640	10
	Sodium metabisulphate	1.6000	.69921	10
	Total	2.6000	1.33589	40
Texture	Control	2.9000	.87560	10
	Ascorbic acid	3.0000	1.05409	10
	Cystein	2.9000	.99443	10
	Sodium metabisulphate	2.9000	.87560	10
	Total	2.9250	.91672	40
Flavour	Control	3.3000	.48305	10
	Ascorbic acid	3.6000	.69921	10
	Cystein	3.7000	.67495	10
	Sodium metabisulphate	3.5000	.52705	10
	Total	3.5250	.59861	40
Acceptability	Control	2.3000	1.15950	10
	Ascorbic acid	2.9000	1.85293	10
	Cystein	2.9000	.99443	10
	Sodium metabisulphate	1.5000	.70711	10
	Total	2.4000	1.33589	40

Multivariate Tests[c]

Effect		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	.984	386.621[a]	5.000	32.000	.000
	Wilks' Lambda	.016	386.621[a]	5.000	32.000	.000
	Hotelling's Trace	60.409	386.621[a]	5.000	32.000	.000
	Roy's Largest Root	60.409	386.621[a]	5.000	32.000	.000
Inhibitor	Pillai's Trace	.438	1.164	15.000	102.000	.312
	Wilks' Lambda	.611	1.157	15.000	88.739	.320
	Hotelling's Trace	.560	1.145	15.000	92.000	.329
	Roy's Largest Root	.385	2.617[b]	5.000	34.000	.042

a. Exact statistic

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

c. Design: Intercept+Inhibitor

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Taste	4.900[a]	3	1.633	1.273	.298
	Colour	16.800[b]	3	5.600	3.818	.018
	Texture	.075[c]	3	.025	.028	.994
	Flavour	.875[d]	3	.292	.802	.501
	Acceptability	13.200[e]	3	4.400	2.809	.053
Intercept	Taste	396.900	1	396.900	309.273	.000
	Colour	270.400	1	270.400	184.364	.000
	Texture	342.225	1	342.225	376.761	.000
	Flavour	497.025	1	497.025	1365.870	.000
	Acceptability	230.400	1	230.400	147.064	.000
Inhibitor	Taste	4.900	3	1.633	1.273	.298
	Colour	16.800	3	5.600	3.818	.018
	Texture	.075	3	.025	.028	.994
	Flavour	.875	3	.292	.802	.501
	Acceptability	13.200	3	4.400	2.809	.053
Error	Taste	46.200	36	1.283		
	Colour	52.800	36	1.467		
	Texture	32.700	36	.908		
	Flavour	13.100	36	.364		
	Acceptability	56.400	36	1.567		
Total	Taste	448.000	40			
	Colour	340.000	40			
	Texture	375.000	40			
	Flavour	511.000	40			
	Acceptability	300.000	40			
Corrected Total	Taste	51.100	39			
	Colour	69.600	39			
	Texture	32.775	39			
	Flavour	13.975	39			
	Acceptability	69.600	39			

a. R Squared = .096 (Adjusted R Squared = .021)

b. R Squared = .241 (Adjusted R Squared = .178)

c. R Squared = .002 (Adjusted R Squared = -.081)

d. R Squared = .063 (Adjusted R Squared = -.016)

e. R Squared = .190 (Adjusted R Squared = .122)

Estimated Marginal Means

Grand Mean

Dependent Variable	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Taste	3.150	.179	2.787	3.513
Colour	2.600	.191	2.212	2.988
Texture	2.925	.151	2.619	3.231
Flavour	3.525	.095	3.332	3.718
Acceptability	2.400	.198	1.999	2.801

Post Hoc Tests

Inhibitor

Homogeneous Subsets

Taste

Duncan[a,b,c]

Inhibitor	N	Subset
		1
Control	10	2.8000
Sodium metabisulphate	10	2.9000
Cystein	10	3.2000
Ascorbic acid	10	3.7000
Sig.		.113

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.283.

a. Uses Harmonic Mean Sample Size = 10.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Colour

Duncan[a,b,c]

Inhibitor	N	Subset 1	Subset 2
Sodium metabisulphate	10	1.6000	
Cystein	10	2.6000	2.6000
Control	10		2.8000
Ascorbic acid	10		3.4000
Sig.		.073	.172

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.467.

a. Uses Harmonic Mean Sample Size = 10.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Texture

Duncan[a,b,c]

Inhibitor	N	Subset 1
Control	10	2.9000
Cystein	10	2.9000
Sodium metabisulphate	10	2.9000
Ascorbic acid	10	3.0000
Sig.		.833

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .908.

a. Uses Harmonic Mean Sample Size = 10.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Flavour

Duncan[a,b,c]

Inhibitor	N	Subset 1
Control	10	3.3000
Sodium metabisulphate	10	3.5000
Ascorbic acid	10	3.6000
Cystein	10	3.7000
Sig.		.185

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .364.

a. Uses Harmonic Mean Sample Size = 10.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

Acceptability

Duncan[a,b,c]

Inhibitor	N	Subset 1	2
Sodium metabisulphate	10	1.5000	
Control	10	2.3000	2.3000
Ascorbic acid	10		2.9000
Cystein	10		2.9000
Sig.		.162	.320

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 1.567.

a. Uses Harmonic Mean Sample Size = 10.000.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.