LIPID STABILITY IN SOYFLOURS PRODUCED FROM RAW AND PROCESSED SOYBEANS.

A THESIS PRESENTED TO THE

DEPARTMENT OF NUTRITION AND FOODSCIENCE

UNIVERSITY OF GHANA

BY

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BSc. (GHANA) 1997

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE MASTER OF PHILOSOPHY IN FOODSCIENCE

JUNE 2001

DECLARATION

I hereby declare that with the exception of literature cited the information in this document was produced by me through research under supervision in the Department of Nutrition and Food Science, University of Ghana and the Food Research Institute, Ghana.

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DEDICATION

To Abena Tenewaa and NanaYaw Baabu.

ABSTRACT

Soybean, a high protein/oil legume is known to contain high levels of polyunsaturated fatty acids, which are susceptible to lipid oxidation and hydrolysis. However, suitable processing methods and storage conditions can enhance the stability of lipid in soyflour.

The objective of the project was to investigate the effects of some processing methods and storage conditions on the stability of lipid in soyflour and to define the parameters for the prediction of the shelf life soyflour.

Some whole commercial soybeans were milled into raw soyflour. Some of the whole beans were cooked for one hour, and milled into cooked-dried soyflour. The final portion was roasted in an open pan and then milled into roasted soyflour. The raw, cooked-dried and roasted flours produced were stored at temperatures of 5, 16, 30, 42, 68 and 80°C, and at water activities of 0.15, 0.23, 0.45, 0.68 and 0.75 for 12 weeks. Indices of lipid oxidation (peroxide value and thiobarbituric acid number) and an index of lipid hydrolysis (free fatty acids) were determined at time intervals of 0, 2, 6, 10 and 12 weeks. Sensory evaluation was performed on the samples stored at 5 and 30°C at storage times of 4, 8,10 and 12 weeks.

Results showed that the rates of lipid oxidation and hydrolysis were higher in raw soy flour than in the heat-processed flours at the same storage temperature and time and at the same water activity. The rate of lipid hydrolysis was found

to be minimal at 5°C and maximal at 30°C in both raw and heat-processed soyflours. The rate of lipid oxidation was also found to be minimal at water activity corresponding to the average monolayer value of both raw and heat-processed soyflours. The flavour of raw and heat-processed flours began to change significantly after 6 and 12 weeks of storage respectively; and this occurred at peroxide value of 4.21 meq/kg and thiobarbituric acid number of 9.76 mg/kg.

Heat-treatment of soybeans prior to processing into flours yield flours with lower rates of chemical reactions, which result in minimal lipid oxidation and hydrolysis. Storage of soyflour under cold condition or maintaining the moisture content of soyflour at or close to the monolayer value of soyflour results in lower rates of chemical reactions, which increases the stability of lipids in soyflour. The shelf life of soyflour can be predicted when the peroxide value and the TBA Number of soyflour are known. It is possible to extend the shelf life of soyflour by heat-processing the beans prior to milling into flour and storing the flour under cold conditions.

ACKNOWLEDGEMENT

I wish to thank the Lord Almighty for the sound mind and strength given me towards the successful completion of this work. I owe thanks to Professor G.S. Ayernor who supervised the execution of this work thoroughly and provided a lot of scientific advice.

I remain particularly grateful to my parents Mr. And Mrs. Bonsi and my husband Mr. Paul Omari for their prayers and moral support.

I am indebted to Dr. P.N.T. Johnson of the Food Research Institute for his academic and technical support. I continue to be indebted to all who helped in various ways during the execution of this work. I am particularly grateful to Emmanuel Afoakwa for editing this work.

Finally I wish to thank the Bank of Ghana for awarding me a Fellowship, which contributed immensely to the completion of this work.

May God bless you All.

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CHAPTER ONE

INTRODUCTION

1.1 History of soybean

Soybean (*Glycine max*) is a protein/oil legume that originated from the South-Eastern Asian countries. It grows best in warm climates and matures within five months (Scott and Aldrich, 1970). Soybean also known as the "miracle crop" or the "golden bean" is one of the oldest of all food plants, and a modern-day success with many uses (Thio, 1975). It has been used for human food in China since long before the birth of Christ (Scott and Aldrich, 1970). More recently, soybean has become the most important source of edible oil in the western world. In the Far East where animal protein remains a luxury, soybeans have been a prominent part of the diet for centuries (Scott and Aldrich, 1970). Soybean was first introduced in Ghana in 1909 for cultivation as both cash and food crop (Mercer-Quarshie and Nsowah, 1975).

1.2 Uses of soybean

Soybeans are used for the preparation of animal feed, human food and for some industrial applications. Well over 90% of soybean meal was used in livestock feeding, however, the proportion used in human food has continued to increase since 1929 when the first soyflour and grits were produced commercially (Scott and Aldrich, 1970). Soybean is also an important source of edible oil in the western world (Ferrier, 1975).

In the Orient, soybeans are traditionally used as food in two ways, that is, production of unfermented foods such as *tofu* (soymilk curd) and production of fermented foods such as *miso* and *tempeh* (Ferrier, 1975). There are three distinct categories of soy protein products and these include soyflour and grits, protein concentrates and protein isolates (Ferrier, 1975). These products can be used as ingredients in bakery products, baby foods, meat products and confections.

In recent times, in Ghana, the use of soybean in human diet is being promoted since soybean has been observed to reduce the incidence of protein deficiency. It is for instance used to fortify low protein foods such as cassava products. Soybean flour is used a lot in the preparation of infant and weaning foods, such as *Cerelac maize-soya* produced by Nestle' Ghana Limited.

1.3 Nutritional contribution of soybean

Soybean has been recognised as the best and cheapest source of food energy in terms of calories per unit cost of production provided it is consumed directly (Hammond and Call, 1970). Soy protein for human food has been shown to be of the highest nutritional value of all the well-known plant proteins (Robinson, 1971). It is particularly high in lysine, an essential amino acid, which is usually limiting in cereal-based diets, and would thus complement other plant proteins (Lin *et al.*, 1975). Properly processed soyflour generally has protein efficiency ratio (P.E.R) of 2.2-2.3 compared with 2.5 for a casein standard (Williams, 1970).

Soybean contains 36-44% proteins, 30-35% carbohydrates, 20- 25% fat, vitamins and minerals such as calcium and phosphorus (Waggle *et al.*, 1981).

1.4 Some problems associated with soy and soy products

The greatest obstacle to the general use of soybean as a source of food for human include:

i. the bitter, beany taste,
ii. objectionable odour,
iii. poor keeping qualities,
iv. presence of anti-nutritional factors such as trypsin inhibitors,
v. high tendency to undergo rancidity and
vi. presence of hard-and-intimate seed coat, which leads to prolonged cooking time (Ayernor, 1993).

The unsaturated fatty acids in soybean lipids are highly susceptible to oxidation (Hamilton and Berger, 1995). Rackis *et al.* (1979) reported that even defatted soy flour contains residual fat and a considerable amount of bound lipids, all of which are susceptible to oxidation. Lipid oxidation which occurs via autoxidation, thermal oxidation, enzymatic oxidation and photo-oxidation leads to problems such as flavour deterioration and destruction of some nutrients including unsaturated fatty acids and vitamin E (Hamilton and Berger, 1995). Kamel and

Kakuda (1995) reported that oxidation products of polyunsaturated fatty acids could be carcinogenic or co-carcinogenic.

It has also been reported that soybean lipids are susceptible to hydrolysis as a result of the presence of considerable level of lipases. The long-chain fatty acids produced from lipid hydrolysis are responsible for the development of soapy flavours in soy products (Satouchi and Matsushita, 1976).

Certain factors such as amount of oxygen present, degree of unsaturation of lipids, presence of antioxidants, presence of pro-oxidants, nature of packaging material, moisture content, light exposure and temperature of processing and storage have been shown to affect the rate of lipid oxidation (deMan, 1990). One successful way of improving lipid stability in soy products is to adequately control some of these factors (deMan, 1990).

This project focused on studying the effect of processing, storage temperature and relative humidity on lipid stability in soyflour and how these factors could be regulated to improve lipid stability and hence retard the development of flavour changes in soyflour.

1.5 Main objective

The main objectives of the study were to investigate the effects of some processing methods and storage conditions on lipid stability in soyflour and to define the parameters for the prediction of the shelf life of soyflour .

1.5.1 Specific objectives

The specific objectives of the study were as follows:

1. to process soybean into various soyflour products using different processing methods.
2. to analyse the various soyflour samples obtained in (1) above, for indices of lipid stability including:
   (a) Total lipids
   (b) Fat content
   (c) Free fatty acids
   (d) Peroxide value
   (e) Thiobarbituric acid (T.B.A.) Number
3. to study the effect of:
   (a) storage temperature and time on lipid stability, and crude fat and total lipids contents in the various soyflours.
   (b) relative humidity and time on lipid stability under storage in the various soyflours by determining the peroxide value and the TBA number as indices for lipid oxidation, and free fatty acids as an index for lipid hydrolysis.

4. to establish when flavour changes become detectable during storage of the soyflours by sensory analysis.
5. to establish the most suitable processing methods, favourable storage conditions, and shelf life for soyflour.

CHAPTER TWO

LITERATURE REVIEW

2.1 SOYBEAN

Soybean is a protein/oil legume that originated from the South-Eastern Asian countries. It grows best in warm climates and matures within five months (Scott and Aldrich, 1970). Both traditional and new soybean products are marketed extensively in Bangladesh, India and other countries in the Far East (Markley, 1951). Soybean has proved to be a versatile and highly successful food product in meeting food shortages in Europe and Asia during and since World War II (Markley, 1951). Soybean products are used as valuable ingredients in Occidental diets (Markley, 1951). Soybean has been shown to be of the highest nutritional value of all the well-known plant proteins (Robinson, 1971).

2.1.1 Advantages of soybean

a. It has higher amino acid content and better nutritive value than most vegetable proteins (Robinson, 1971). When supplemented with methionine it can be equivalent to good quality animal protein (Robinson, 1971).
b. It is relatively easy to store and transport (Scott and Aldrich, 1970).
c. Technology for processing into food is well developed (Robinson, 1971).
d. It produces good quality oil in addition to proteins (Robinson, 1971).

e. It is leguminous and fixes atmospheric nitrogen, thus requiring little or no nitrogen fertilizer (Scott and Aldrich, 1970).

f. Its indigestible carbohydrates and possibly its hull prove useful as a source of dietary fibre (Wolf, 1970).

2.1.2 Disadvantages of soybean

a. Its products have distinctive beany flavour and odour, which are objectionable in some food applications (Scott and Aldrich, 1970).

b. Proteins from soybean do not have the same desirable functional properties in food systems as do animal protein such as casein (Wolf, 1970).

c. It contains antinutritional factors, which must be inactivated before used for food (Wolf, 1970).

2.2 Components of interest in soybean

2.2.1 Protein

Soybean contains 36-44% protein on dry matter basis (Wolf, 1970). The chief protein in soybean is a globulin, glycinin, which is very similar to that of cow's milk (Wolf, 1970). Soybean proteins have high biological value and contain all the essential amino acids with exceptionally high levels of lysine but low levels of sulphur - containing amino acids: methionine and cysteine, and tryptophan (Wolf, 1970). The high level of lysine in soybean enables it to complement cereal-based diets, which are usually low in lysine. Properly processed soyflour and grits generally have a protein efficiency ratio of 2.2 – 2.3 compared with 2.5 for a

casein standard (Williams, 1970). It has been shown that soy proteins can be used to supplement and/or extend meat and fish proteins and to supplement single or mixed vegetable-based protein diets (Bressani, 1981). Soybean protein-fortified-foods have been shown to be useful in relieving malnutrition among specified segments of the population such as infants and weaned children (Williams, 1970).

2.2.2 Fat

Soybean has a high fat content between 20 and 25% depending on the soil type, environmental factors and variety (Markley, 1951). The fat is made up of 15% palmitic acid and 80% unsaturated fatty acids, that is, 70% oleic, 24% linoleic and 6% linolenic acids (Wolf, 1970). The fat contains no free fatty acids and consists most entirely of natural triglycerides (Markley, 1951).

2.2.3 Carbohydrates

Soybean contains 30-35% carbohydrates. The carbohydrates can be subdivided into soluble and insoluble fractions. The soluble carbohydrates include hexose, sucrose and oligosaccharides such as raffinose, stachyose and verbacose while the insoluble fraction include hemicellulose, celluloses, lignin, pectin, and other complex carbohydrates (Waggle *et al.*, 1981). The oligosaccharides verbacose, stachyose and raffinose are the major contributors to soybean flatulence (Rackis *et al.*, 1979). These components escape digestion and are fermented by intestinal microflora to produce excess amounts of intestinal gases.

2.2.4 Vitamins and Minerals

Green soybean has carotene content ranging from 2 - 7u/g while that of the mature bean is about 0 - 8u/g (Markley, 1951). The immature soybean is richer in riboflavin, niacin and vitamin C than the mature bean, and pyridoxin and thiamine concentrations increase with maturation (Wolf, 1970). Soybean contains 0.12% - 0.21% tocopherols (Markley, 1951). It has fairly high amount of calcium but in relation to animal requirements this is not adequate for the young animal (Markley, 1951). It has adequate amount of potassium, phosphorus and magnesium. The most notable vitamin deficiency in soy protein is the low level of vitamin B12, which is virtually absent in all vegetable proteins (Markley, 1951).

Table 2.2 Typical composition of soybean and various soybean products (%) [DMB].

	Protein *	Fat	Carbohydrates	Ash	Crude fibre	Moisture
Whole soybean	42	20	30.5	5.0	5.5	
Full-fat soyflour	40	20	30.5	5.0	5.5	
Defatted soyflour	54	1.0	35.5	6.0	3.5	6.0
Soy protein concentrate	70	1.0	21.5	5.0	3.5	6.7
Soy protein isolates	92	0.5	2.2	4.5	0.3	4.7

* N X 6.25

Source: Waggle *et al.* (1981)

2.2.5 Enzymes

Enzymes in soybean include ∝ and β amylases, lipases, lipoperoxidases, lipoxygenases, urease and proteinases (Whitaker, 1972). The lipases, lipoxygenase and lipoperoxidase catalyse the oxidation of lipids and may result in flavour changes in improperly processed soy oils and soy products (Rackis *et al.*, 1979). Lipoxygenase is the most important enzyme involved in enzymatic peroxidation of soybean lipids (Satouchi and Matsushita, 1976).

2.2.6 Phytates

Defatted soybean meal contains about 1.5% phytic acid, which is associated mainly with the germ of the intact seed (Erdman *et al.*, 1981). Processing of soybean can produce protein phytic mineral complexes that may reduce the bioavailability of minerals (Erdman *et al.*, 1981). Soaking of bean reduces phytic acid content through enzymatic hydrolysis by the action of the enzyme phytase, which is an indigenous constituent (Rackis *et al.*, 1979).

2.2.7 Protease inhibitors

Soybean contains several different factors that inhibit the action of the digestive enzymes, trypsin and chymotrypsin, thereby decreasing protein digestion. Heating of soy meal improves the nutritional value as a result of reduced protease inhibitor activity (Kakade *et al.*, 1974).

2.2.8 Hemagglutinins

Soy hemagglutinins are glyco-proteins with molecular weight of approximately 110,000 and contain about 5% carbohydrates (Anderson *et al.*, 1979). They are responsible for agglutination of red blood cells (Lin *et al.*, 1975). Soy hemagglutins do not appear to be a problem in properly processed soyflour and other soy products used for human food (Rackis *et al.*, 1979).

2.2.9 Miscellaneous components

These components include saponins, possibly sterols and triterpene alcohols, and an antithyrotoxic factor (Rackis *et al.*, 1979). Other biologically active substances are soybean allergenic compounds and lysinoalanine, which is not a constituent of raw soybean but are often found in processed soy protein products (Rackis *et al.*, 1979). Less of the population is allergic to soy-milk than cow's milk or milk from other legumes (Anderson *et al.*, 1979). Lysinoalanine is formed during alkaline treatment of soy protein (Struthers *et al.*, 1979). Addition of either mercaptoethanol or cystine to an alkaline solution or proper temperature control reduces the formation of lysinoalanine (Struthers *et al.*, 1979). Presence of lysinoalanine in food reduces the bioavailability of cystine, cysteine and lysine (Struthers *et al.*, 1979).

2.3 Chemical composition of soybean oil

Soybean oil may be defined as the composition of lipid materials extractable from ground or flaked soybeans, with organic solvents such as hexane, chloroform or

petroleum ether. Lipid can be defined as any substance that furnishes fatty acids and/or related compounds on hydrolysis.

2.3.1 Component fatty acids

Soybean oil contains 14.6% Saturated acids consisting solely of palmitic acid and 80% unsaturated fatty acid (Kamel and Kakuda, 1994). The unsaturated acids were found to comprise 56% oleic, 19% linoleic, and 4.8% linolenic acid (Markley 1951).

Table 2.3 Percentage Ranges of Component Fatty Acids of Soybean Oils

Saturated Acids (AV. 15%)		Unsaturated Acids (AV. 85%)	
Acid	Percent	Acid	Percent
Lauric	0.2	Lauroleic	-
Myristic	0.4	Myristoleic	0.64
Palmitic	11.0	Palmitoleic	1.60
Stearic	4.0	Oleic	25
Arachidic	0.9	Linoleic	51
Behenic	trace	Linolenic	9
		Arachidonic	Trace

Source: Kamel and Kakuda (1995).

2.3.2 Glycerides

Soybean oil is composed predominantly of a mixture of glycerol esters or triglycerides. In soybean oil, the five principal fatty acids, which form the various types of triglycerides are palmitic, stearic, oleic, linoleic and linolenic acids (Markley, 1951). The component glycerides of soybean oil were found to comprise 58% diunsaturated monosaturated triglycerides and 42% triunsaturated triglycerides (Markley, 1951).

2.3.3 Phospholipids

Phospholipids or phosphatides are lipids containing phosphorus and in many instances nitrogen. Soybean phospholipids include lecithin (Phosphatidylcholine), cephalin (phosphatidyl ethanolamine) and sphingomyelins (Markley, 1951). Soybean phospholipids contain about 35% lecithin and 65% cephalin (deMan, 1990). The fatty acid composition of these phospholipids is usually different from that of the oil in which they are present. The acyl groups are usually more unsaturated than those of the triglyerides (deMan, 1990). The cephalins are insoluble or slightly soluble in ethanol while the lecithins are relatively soluble in this medium.

2.3.4 Unsaponifiables

The unsaponifiable fraction of soybean oil, although representing only a small proportion of total lipids, comprises a diversity of components, which include fat-soluble pigments and vitamins, anti-and pro-oxidants, and other compounds of unknown function (deMan, 1990). The fat-soluble pigments include carotenoid

pigments and chlorophyll. The major sterol present in soybean oil is stigmasterol (deMan, 1990). Also present are sterol glycerides and other components such as tocopherols, waxes, aldehydes, ketones, alcohols, hydrocarbons, tocoquinones and dicarbonyl compounds (Markley, 1951).

Table 2.4 Component fatty acids of the phosphatides of soybean oil

Fatty Acid	Phosphatides, % (wt)	
	Alcohol Insoluble	Alcohol Soluble
Myristic		
Palmitic	11.7	17.3
Stearic	4.0	
Arachidic	1.4	
Hexadecenoic	8.6	5.5
Oleic	5.5	19.0
Linoleic	63.3	53.0
Linolenic		3.7
Unsaturated C_{20}	5.5	1.5

Source: Zadernowski *et al.* (1983)

Table 2.5 Component fatty acids of the phosphatides and glycerides of soybean oil (%)

Fatty acid	Lecithin	Glycerides
Palmitic	15.77	6.8-14.8
Stearic	6.30	2.4-5.5
Arachidic	0.00	0.3-0.9
Hexadecenoic		
Oleic	12.98	25.9-33.7
Linoleic	62.90	50.7-58.8
Linolenic	2.00	2.1-2.6
Unsaturated C_{20-22}		

Source: Zadernowski *et al.* (1983)

2.4 Problems associated with soybean and soybean products

The major problems associated with soybean and soybean products include the bitter beany taste, objectionable odour and colour, poor keeping qualities, presence of anti-nutritional factors such as trypsin inhibitors, high tendency to undergo rancidity, and presence of hard-and-intimate seed coat which leads to prolonged cooking time (Ayernor, 1993). Most of these problems can be eliminated during processing of soybean. For instance, the beany taste and objectionable odour can be removed by heat treatment to inactivate lipoxygenase, which is believed to be responsible for the objectionable flavour (Ferrier, 1975). Dehulling the beans also reduces the cooking time.

2.4.1 Oxidation of lipids in soybean and soybean products

Soybean is known to contain 20 - 25% fat which is made up of 15% saturated and 80% unsaturated acids (Markley, 1951). Soybean phospholipids, lecithin and cephalin, also contain high proportion of unsaturated fatty acids (deMan, 1990). These unsaturated acids are highly susceptible to oxidation (Hamilton and Berger, 1995). Oxidation usually takes place at the carbon atom next to the double bond (deMan, 1990).

2.4.1.1 Factors which affect lipid oxidation

(i) Amount of oxygen present

The higher the amount of oxygen present in the food sample the higher the rate of lipid oxidation (deMan 1990). Hoffman, (1995) calculated the solubility of oxygen in oil to be 22ml/kg at ambient temperature, that is, 1mmol/mg. If the oxygen reacts specifically with linolenic acids, 2 meq/kg of hydroproxide should be formed, that is, peroxide value of 2. The production of 3-Cis-hexenal from these hydroperoxides at the level of 0.1mg/kg would result in the oil being considered rancid (Hoffman, 1995). The rate of oxidation is independent of oxygen concentration at high oxygen partial pressure while it is proportional to oxygen concentration at low oxygen partial pressure (Labuza, 1975).

(ii) Degree of unsaturation of the Lipid

The unsaturated bonds present in all fats and oils represent active centers, which, among other things, may react with oxygen (deMan, 1990). Although even

saturated acids may be oxidized, the rate of oxidation greatly depends on the degree of unsturation. In the series of 18 carbon atom fatty acids 18:0, 18:1, 18:2, 18.3, the relative rate of oxidation has been reported in the ratio of 1:100:1200:2500 (deMan, 1990). In general, the greater the degree of unsaturation the more susceptible are the fatty acids to attack by oxygen. The reaction of unsaturated compounds proceeds by the abstraction of hydrogen from the ∝ carbon, and the resulting free radical is stabilised by resonance (deMan, 1990). Free fatty acids are much more susceptible to oxidation than those bound to alcohols (Zadernowski *et al.,* 1983).

(iii) Presence of pro-oxidants

Pro-oxidants are substances that facilitate lipid oxidation. They include metals such as copper and some organic compounds such as heme - containing molecules and enzymes such as lipoxygenase (deMan, 1990). Any metal with two valence states can be active in initiating autoxidation. In general metals in their higher valence state tend to be more effective as free radical initiators (Hamilton and Berger, 1995). When the metal has been converted to its lower valence state it can then react with oxygen to form complexes, which may provide singlet oxygen (Hamilton and Berger, 1995). Transition metals aid the decomposition reactions of peroxides by an oxidation mechanism and a reductive mechanism in which the metal goes from higher valence state to lower valence state in the former and from lower valence state to higher valence state in the latter (Hamilton and Berger, 1995):

$$ROOH + M^{(n+1)} \longrightarrow RO_2 + H^+ + M^{n+}$$

$$ROOH + M^{n+} \longrightarrow RO^{\cdot} + OH^- + M^{(n+1)+}$$

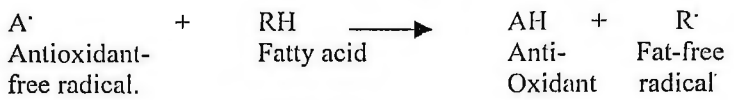
M = metal, ROOH = Fatty acid

The two most prevalent metals in food processing, storage and transport are iron and copper. Metal catalysts may enter foods via the water or spices used in food preparation (Taylor 1984).

(iv) **Presence of antioxidants**

Antioxidants are substances that inhibit lipid oxidation. They could be naturally present in food or added to food. Naturally occurring antioxidants in soybeans include tocopherols, genistein, diadzein and glycitein (Hamilton and Berger, 1995). The antioxidants interfere with lipid oxidation by interacting with the peroxy free radical and the resultant antioxidant free radical does not initiate or propagate further oxidation (deMan, 1990).

The synthetic antioxidants, which are added to food to prevent lipid oxidation are often phenolic compounds such as butylated hydroxy anisole (BHA) and butylatedhydroxytoluene (BHT). Only phenolic compounds that can easily produce quinones are active as antioxidants (Pokorny, 1971). At higher concentrations antioxidants may have a pro-oxidant effect and one of the reactions may be as follows:

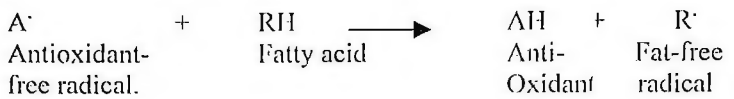


Carotenoids have been shown to have some stabilizing effect on soybean fat under certain conditions (Markley, 1951). The resistance of soybean products to oxidation can be somewhat increased by the addition of acidic compounds which apparently act synergistically with the naturally occurring antioxidants in soybean (Pokorny *et al.*, 1983). Examples are citric acid, phosphoric, ascorbic and tartaric acids. These function by chelating pro-oxidant metals in the product and to some extent by inhibiting peroxide decomposition and by regeneration or sparing of primary antioxidants (Hamilton and Berger, 1995).

Table 2.6 Tocopherol levels in soybean

Tocopherol Type	Level (mg/kg)
∝	116
β	34
γ	737
∂	275

Source: Hamilton and Berger (1995).



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Source: Hamilton and Berger (1995).

(v) Nature of packaging material

Any packaging material, which is permeable to oxygen and other factors such as proxidants favours lipid oxygen (deMan, 1990). To prevent or reduce lipid oxidation fat-containing products need to be stored in airtight packaging materials. The interaction of the food product with catalytic metals, which may be components of the packaging material should be minimised.

(vi) **Light exposure**

Light has the ability to increase the rate of lipid oxidation. Autoxidation is increased rapidly in the presence of light (deMan, 1990). Photooxidation involves reaction of an alkene with oxygen in the presene of light and a suitable sensitiser (Hamilton and Berger, 1995). Photosenstizers include riboflavin, erythrosine and methylene blue. Exposure of foods in transparent containers in supermarkets leads to a great deal of oxidation (Hamilton and Berger, 1995).

(vii) Temperature

The rate of oxidation of lipids approximately doubles for every 10^0 rise in temperature. As a general rule, it can be argued that storage temperature should be 5 - 10^0C above slip melting point of the material (Hamilton and Berger, 1995). At higher temperatures (100 - 140^0C), formic acid is produced from aldehyde decomposition and can be used to indicate the end of the induction period (Hamilton and Berger, 1995).

(vii) Moisture content

Martz *et al.* (1975) observed that as moisture content of cereal products was lowered, the products became rancid much sooner. Salwin (1959) proposed that at the B.E.T. monolayer, the water formed a protective barrier preventing the oxygen from reaching the underlying unsaturated fats. Halton and Fischer (1987) also proposed that the water retarded the diffusion of oxygen to the sites of the unsaturated double bonds. The B.E.T. monolayer value is defined as the moisture content at which each polar and ionic groups has a water molecule bound to it, to form the start of a liquidlike phase. Generally, at water activity below the monolayer value lipid oxidation rate decreases with increasing water activity (Labuza, 1975). The rate reaches a minimum around the monolayer value and increases with a further increase in the water activity (Labuza and Karel, 1980). The "antioxidant effect" of water at low water activity has been attributed to the bonding of hydroperoxides and hydration of metal catalysts whereas the "pro-oxidant effect" of water at higher water activity is due to the increased mobility of reactants (Heidelbaugh and Karel, 1970).

(ix) Other factors which affect lipid oxidation

In most foodstuffs, lipids are dispersed in a system containing various non-lipid materials such as proteins, sugars or minerals, mostly in presence of water (Pokorny *et al.*, 1983). Oxidation of the lipid fraction is affected by the non-lipidic components and interactions occur between oxidised lipids and various non-lipidic substances (Pokorny *et al.*, 1983). Among non-lipidic substances

several mineral components, especially derivatives of transition heavy metals are known as pro-oxidants, and various polyphenolic substances as antioxidants. Substituted and polyvalent acids behave as synergists of the antioxidants as they bind heavy metals into inactive complexes. Polysaccharides often protect lipids sterically from oxidation (Pokorny *et al.*, 1983).

(x) Effect of protein on the oxidation of lipids

In a mixture of lipids and proteins the reaction course depends on the water content. In dry systems the oxidation of lipids proceeds slowly during the induction period but becomes very rapid in the subsequent stages (Hamilton and Berger, 1995). Low peroxide value in mixtures of protein and lipids are caused by rapid destruction of hydroperoxides in contact with protein solution (Pokorny *et al.*, 1983). The hydroperoxidation of lipids is probably suppressed by their interaction with amine groups of protein. When amine groups of protein molecules are blocked with formaldelyde, hydroperoxides accumulate on storage (Pokorny *et al.*, 1983).

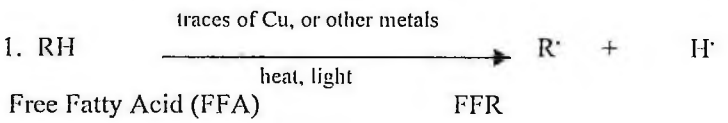
2.4.1.2 Types of lipid oxidation

Lipid oxidation occurs via autoxidation, photooxidation, enzymatic oxidation and thermal oxidation (deMan, 1990). Autoxidation is the most important route to oxidation of lipids (deMan, 1990).

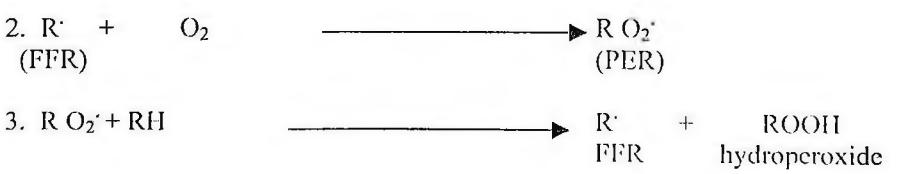
(i) **Autoxidation**

The unsaturated bonds present in all fats and oils represent active centers, which among other things, may react with oxygen. This reaction leads to the formation of primary, secondary, and tertiary oxidation products which may make the fat or fat-containing foods unsuitable for consumption (deMan, 1990). The process of autoxidation and the resulting deterioration in flavour of fats and fatty foods are often described by the term rancidity (deMan, 1990). Autoxidation reaction can be divided into three parts namely, initiation, propagation and termination. It is a free radical chain reaction.

Initiation Step: it involves the formation of fat-free radical (FFR) when loosely held hydrogen atoms are lost from the fatty acid groups.



Propagation Step: FFR combines with oxygen to form peroxy free radical (PFR). The PFR removes a hydrogen atom from unsaturated lipid to form another FFR and unstable hydroperoxide.



The FFR reacts with oxygen to form more hydroperoxides as propagation proceeds. The unstable hydroperoxides breakdown into a variety of compounds, which include hydrocarbons (such as ethane and ethene from linolenic and other n - 3 polyene acids and pentane form linoleic and other n 6 polyene acids), aldelydes, ketones, esters, lactones, alcohols and ethers all of which may be saturated or unsaturated (deMan, 1990). These compounds constitute the secondary oxidation products and are responsible for flavour deterioration.

Termination Step

This occurs when all the oxygen in the system is used up, or when two FFR reacts, or when an antioxidant radical reacts with the FFR.

4. $R^{\cdot} + R^{\cdot} \longrightarrow R_2$

Role of antioxidant in termination

The antioxidants can function in two ways:

(a) It can donate a hydrogen atom to the FFR to form the fatty acid molecule and thereby terminating the oxidation in its first step (initiation step).

$$\underset{\text{FFR}}{R^{\cdot}} + \underset{\text{antioxidant}}{AH} \longrightarrow \underset{\text{FFA}}{RH} + A^{\cdot}$$

Autoxidation is thus delayed until all the AH are used up.

(b) It can donate a hydrogen atom to PFR to from a hydroperoxide.

$$RO^{\cdot}_2 + \underset{\text{(Instead of RH)}}{AH} \longrightarrow ROOH + A^{\cdot}$$

A· is more stable than R· because of the electron resonance structure in the aromatic ring of A·. The chain reaction in the step (3) is thus terminated. Antioxidants cannot reverse the oxidation of lipids therefore they must be added as early as possible in the manufacturing process or to the finished product (deMan, 1990). The aldehydes formed are further oxidized to form fatty acids that are considered as tertiary oxidation products.

Table 2.7 Hydroperoxides and aldehydes, which may be formed in autoxidation of some unsaturated fatty acids.

Fatty acid	Methylene group involved	Isomeric hydroperoxides formed from the structures contributing to intermediate free radical resonance hybrid	Aldehydes formed by decompsition of the hydroperoxides
Oleic	11	11- hydroperoxy-9-ene	Octanal
		9-hydroperoxy-10-ene	2-decenal
	8	8-hydroperoxy-9-ene	2-undecenal
		10-hydroperoxy-8-ene	nonanal
Linoleic	11	13-hydroperoxy-9,11-diene	Hexanal
		11-hydroperoxy-9,12-diene	2-octanal
		9-hydroperoxy-10,12-diene	2,4-decadienal
Linolenic	14	16-hydroperoxy-9,12,14-triene	Propanal
		14-hydroperoxy-9,12,15-triene	2-pentenal
		12-hydroperoxy-9,13,15-triene	2,4-heptadienal
	11	13-hydroperoxy-9,11,15-triene	3-hexenal
		11-hydroperoxy-9,12,15-triene	2,5-octadienal
		9-hydroperoxy-10,12,15-triene	2,4,7-decatrienal

Source: Keeney (1962).

(ii) **Photo-Oxidation**

Photo-oxidation involves reaction of an alkene with oxygen in the presence of light and a suitable sensitizer. It involves the participation of a singlet oxygen (Hamilton and Berger, 1995). The extent of the changes in this pathway covers the transfer of energy from light to a photosensitizer, which then helps to form singlet oxygen. Photosensitizers such as erythrosine or methylene blue (the dyestuffs), flavin, porphyrin (the pigments) and anthracene, rubrene, the polycyclic (aromatic hydrocarbons) absorb the visible or near ultraviolet light to be converted to the excited state (Hamilton and Berger, 1995). The singlet, excited oxygen is converted to excited triplet state by inter-system crossing. Triplet activated sensitizer can then react with triplet oxygen to form singlet oxygen, which reacts with substrate to give hydroperoxides, which are characteristic of this mechanism (Hamilton and Berger, 1995).

Photo-oxidation is much quicker than autoxidation and the difference in reactivity between oleate, linoleate, and linoleate (1:1.3:2.3) is close to the number of double bonds in these esters (deMan, 1990). The hydroperoxides produced in this way differ from those resulting from autoxidation. It has been suggested that autoxidation of natural lipids may be initiated by photo-oxidation due to pigments remaining in the lipids after processing (Gunstone and Norris, 1983).

(iii) Enzymatic Oxidation

Some enzymes such as lipases, lipoxygenase and lipoperoxidase catalyze the oxidation of lipids. The most important agent for enzymatic peroxidation is the enzyme lipoxygenase (Satouchi and Matsushita, 1976). Chief sources of lipoxygenase (lipoxidases) are soybean, cereal grains and oil seeds, peas and beans. Soybean lipoxygenase are localized in mitochondria, plastids, chloroplasts and the cytosol of cells (Markley, 1951). When the raw tissue is broken the enzyme and substrate (oil) are liberated, and, provided some moisture is present a bitter, beany taste develops very rapidly (Baker and Mustakas (1972).

Lipoxygenase generally accelerates the addition of oxygen to the double bond of carotenoids and unsaturated fats to form peroxides, the pH for optimum activity being about 6 (Markley, 1951). The natural substrate of the enzyme is linoleic acid, but other acids such as linolenic and arachidonic acids are also oxidized (Gunstone and Norris, 1983). Lipoxygenase (Linoleate: Oxidoreductase) is highly specific and will attack the cis-cis-1, 4 -pentadiene group contained in the fatty acids linoleic, linolenic and arachidonic (deMan, 1990). The exact mechanism of the reaction is still in doubt, but initially a hydrogen atom is abstracted from the ω-8 methylene group to produce a free radical. The free radical isomerizes, causing conjugation of the double bond and isomerization of the trans-configuration. The free radical then reacts to form the ω-6 hydroperoxide. The peroxide formation by lipoxygenase is interrupted by the common lipid

antioxidants. The antioxidants are thought to react with the free radicals and thus interrupt the oxidation (Gunstone and Norris, 1983).

Lipase is a lipolytic enzyme present in soybeans (Whitaker, 1972). Lipases are capable of hydrolyzing lipids to fatty acids and glycerol. The production of long-chain fatty acids by the action of lipases leads to the development of soapy flavour in foods. The fatty acids so produced are more susceptible to oxidation (Satouchi and Matsushita, 1976). Although lipase inhibitor has been reported in soybeans (Satouchi and Matsushita, 1976) there is no inhibitor available and acceptable for food use.

(iv) Thermal Oxidation

This term refers to the oxidation of lipids at high temperature. During food preparation involving the application of heat oxidative reactions are greatly accelerated (Frankel, 1984). Prolonged exposure of lipids at elevated temperature in the presence of air and moisture leads to the formation of various oxidation products, including polymeric compounds (Chow, and Gupta, 1994). The rate of hydroperoxide formation and decomposition is markedly increased during thermal oxidation. A major pathway of thermal oxidation appears to involve a homolytic cleavage of the O-O bond of the hydroperoxides and formation of alkoxyl and hydroxyl radicals (Frankel, 1984). Alkoxyl radicals formed may undergo C-C bond scission to produce an alkyl radical and a vinyl radical (Frankel, 1984). On the other hand, the alkyl radical can react with a hydrogen radical, hydroxyl

radical or molecular oxygen to generate hydrocarbon, alcohols, and hydroperoxides, respectively. Also the vinyl radical may react with hydroxyl radical, hydrogen radical or molecular oxygen to form aldehydes and olefins (Nawar, 1984). Thus, hydroperoxides can be broken down into non-volatile oxy - and cyclic acids and volatile products such as saturated and unsaturated aldehydes, ketones, hydrocarbon, alcohols, acids and esters (Frankel, 1984).

During thermal oxidation, intra - and intermolecular reactions of alkoxyl, alkyl and peroxyl radicals may lead to the formation of dimers, trimers and large molecular weight polymers with C-O-C and C-O-O-C cross links (Nawar, 1984). Dimers are a major component of non-volatile products formed in oxidized and heated lipids (Nawar, 1984). By combining two radicals to form a non-radical dimer, the free radical chain reaction is thus terminated. At high temperature, hydroperoxides begin to decompose spontaneously, and the radical concentration becomes relatively high allowing radical - radical interaction to proceed faster (Nawar, 1984).

Heating conditions (time, temperature and aeration) and antioxidant levels in lipid-containing foods can modulate the degree of thermal oxidation and type of products generated (Hsieh and Kinsella, 1989).

2.4.1.3 Products of lipid oxidation

The products of lipid oxidation are divided into primary, secondary and tertiary oxidation products.

Primary oxidation products: These are the hydroperoxides formed. The hydroperoxides are odourless hence do not contribute to flavour themselves (deMan, 1990). They are also unstable hence decompose into other compounds. The transition metals aid the decomposition reaction of peroxides by an oxidative and reduction mechanism in which the metal goes from higher valence state to lower valence state in the former and from lower valence state to a higher valence state in the latter (Hamilton and Berger 1995).

$$ROOH + M^{(n+1)+} \longrightarrow RO_2^{\cdot} + H^+ + M^{n+}$$

$$ROOH + M^{n+} \longrightarrow RO^{\cdot} + OH^- + M^{(n+1)+}$$

If a reducing agent such as ascorbic acid is present with Fe^{2+}, the Fe^{3+} produced in the decomposition of the hydroperoxide will be reduced and the smallest quantity of metal ion catalyze very large quantities of lipid breakdown.

The decomposition of the hydroperoxide is also increased markedly during thermal oxidation (Chow and Gupta, 1994).

Secondary oxidation products: These are formed from the decomposition reaction of the hydroperoxides. Different levels of secondary oxidation products

are formed depending on the condition of heating (Chow and Gupta, 1994). Kanazawa *et al.* (1985), have shown that the secondary product fraction of peroxidized methyl linoleate consisted of about 35% polymers, 25% endoperoxide-rich components and 40% low molecular weight compounds. Approximately 16% of the total low molecular weight fraction obtained from peroxidized methyl linoleate is identified as 8 hydroxyl methyl octanoate, 41% as 4 - formyl-9-decenoate (Oarada *et al.*, 1986).

Secondary oxidation products include a variety of compounds including hydrocarbons (such as ethane and ethene), aldehydes, ketones, esters, lactones, alcohols and ethers, all of which may be saturated or unsaturated (deMan, 1990). Oganoleptic changes in fats and fatty foods are generally related to the formation of secondary oxidation products, especially aldehydic compounds. The aldehydes are strong flavour compounds and have very low flavour thresholds.

Tertiary oxidation products: These are formed from the oxidation of the secondary products, especially the carbonyls.

2.4.1.3.1 Reaction of oxidised lipids with proteins

The most important lipid oxidation products, which react with proteins are hydroperoxides and aldehydes produced by their rearrangements cleavage. Malondialdehyde (MDA) produced by cleavage of dihydroperoxide or hydroperoxy alkenals arising from polyunsaturated lipids react with thiol, amine,

and phenolic groups of protein. The reaction of a hydroperoxide molecule with 2-amino acids, either free or bound in protein, proceeds by a mechanism analogous to the strecker degradation;

$$ROOH + R^{I}CH(NH_2)\,COOH \longrightarrow ROH + R^{I}CHO + NH_3 + CO_2$$

The products of Strecker degradation are very reactive so that molecular brown pigments are the most often detected end products (Pokorny *et al.*, 1983).

Other very reactive functional groups of compounds present in oxidised lipids are various aldehydes, usually a mixture of alkanals, 2 alkenals, and 2, 4 alkadienals. When these substances interact with protein or free amino acids a Schiff base is formed by the addition of amines to aldehydes and by a subsequent dehydration reaction;

$$RCHO + H_2N\text{-}R^{I} \longrightarrow \underset{\displaystyle NH\text{-}R^{I}}{\overset{\displaystyle |}{RCHOH}} \longrightarrow RCH = N\text{-}R^{I} + H_2O$$

The Schiff base readily reacts with another molecule of aldehydes;

$$RCH_2\,CH = N\text{-}R^{I} + R^{II}\,CHO \longrightarrow \underset{\displaystyle CH\quad R^{II}}{\overset{\displaystyle |}{RCCH}} = N\text{-}R^{I} + H_2O$$

The reaction can proceed with the formation of higher oligomers (Pokorny *et al*, 1983). The reaction is very rapid at room temperature or cold storage temperatures.

The autoxidation of aldehydes is an extremely rapid reaction, more rapid than that of polyunsaturated fatty acid esters. The oxidation results in the formation of peroxy acids, which are often very stable.

Peroxy acid

They are decomposed by reaction with another molecule of aldehyde;

The resulting fatty acid belongs to the main reaction products in the system oxidised at room temperature (Pokorny *et al*, 1983).

The aldolization and oxidation reactions of aldehydes give rise to various other volatile products (Jirousova *et al*., 1975). The hydroperoxides reacts with sulphur containing amino acids such as cysteine, which is oxidised to lysine.

Cystine is further oxidized into thiosulphinate;

Methionine is attacked by lipid peroxides and oxidized into methionine sulphoxide :

$$RSCH_3 + R^{1}OOH \longrightarrow \underset{\underset{O}{||}}{R}CH_3 + R^{1}OH$$

The oxidation may proceed further with the formation of methionine sulphone at least under extreme conditions.

Table 2.8 Reactive groups taking part in the interaction of oxidized lipids with proteins.

Amino group bound in protein	Functional group present	Reacting product of lipid oxidation	Resulting functional group
Cysteine	Thiol	Hydroperoxide	Disulphide
		Aldehyde	Thioacetal
Cystine	Disulphide	Hydroperoxide	Thiosulphinal
Methionine	Sulphide	Hydroperoxide	Sulphoxide
Lysine	Amine	Hydroperoxide	Schiff base
		Aldehyde	Schiff base
		Hydroketone	Schiff base
Tryptophan	Indole	Hydroperoxide	Various products
Tyrosine	Phenol	Aldehyde	Methylene bridge
Serine,Threonine	Hydroxyl	Epoxide	Hydroxy ether
		Carboxyl	Ester
Aspartic,Glutamic acids	Carboxyl	Epoxide	Hydroxy ester
		Hydroxyl	Ester + products
		Hydroperoxide	Ester

Source: Pokorny *et al*. (1983)

2.4.1.4 Effect of lipid oxidation on the nutritive value of food

Essential fatty acids and tocopherols present in lipids and lipid-containing foods are readily oxidizable during processing, storage and usage, especially at high temperatures (Hamilton and Berger, 1995). If extensively oxidized lipids are used as the sole source of dietary lipids and vitamin E, deficiency of essential fatty acids, vitamin E or both may result (Kamel and Kakuda, 1995). Using 15% autoxidized oil as the sole source of dietary lipid, Kamel and Kakuda (1995), observed a marked growth inhibition in growing rats. However when fresh cottonseed oil was added to the diet, normal growth resumed. These findings suggest a deficiency of essential fatty acids, vitamin E or both in oxidized lipids used in the diet.

The interaction products of oxidising lipids with proteins have lower nutritional value for the following reasons:

(a) Destruction of essential factors such as essential amino acid in bound proteins particularly lysine, tryptophan and methionine, or essential fatty acids in the lipid fraction, oxylabile vitamins and various non-essential components which are important for nutrition.

(b) Decrease of digestibility due to incomplete and slower enzymic hydrolysis of bound lipids and protein.

(c) In vivo formation of precursors of various diseases (Pokorny *et al.*, 1983).

Reactions of oxidized lipids with proteins proceed in vivo very slowly producing brown insoluble deposits called ceroid (age pigment). The ceroid formation is

enhanced if diet contains excess polyunsaturated lipids while deficient in tocopherols (Horowitz and Hartroft, 1971), and may be inhibited by supplementation with tocopherols.

It has been shown that methyl linoleate has a depressing effect on carotene utilization, which can be overcome when an excess of pro-vitamin A is given. In spite of the insolubility of the water- soluble vitamins (B and C) in fats, some of them may be destroyed during lipid oxidation. Biotin and vitamin C have been found to be destroyed during lipid oxidation (Pokorny *et al.*, 1983).

2.4.1.5 Effect of lipid oxidation on sensory value of food

Since the human palate is very discriminating, it can detect odoriferous molecules at very low levels. It is believed that when as few as 1 in 1000 carbon-carbon double bonds in a fatty food reacts with oxygen, it is already too late (Hamilton and Berger, 1995). The carbonyls, especially aldehydes produced from the breakdown of hydroperoxides are responsible for flavour changes in soy products and other foods. The process of oxidation and the resulting deterioration in flavour of fats and fatty foods are often described by the term rancidity. Labuza (1975) defined rancidity as the development of off-flavour, which makes a food unacceptable for the consumer. . Usually rancidity refers to oxidative deterioration, but in the field of dairy science, rancidity refers to hydrolytic changes resulting from enzyme activity (deMan, 1990). Rancidification of soybean lipids occurs in two forms. One form involves the rapid development of

off-flavours commonly referred to as reversion and the other form involves the slow development of strong odours and flavours characteristic of lipid oxidation (deMan, 1990). Flavour reversion is a particular type of oxidized flavour that develops at comparatively low levels of oxidation. The off-flavours may develop in oils that have a peroxide value of as little as 1 or 2. Other oils may not become rancid until the peroxide value reaches 100. Linolenic acid is generally recognized as the determining factor of flavour reversion (deMan, 1990).

The interactions of oxidized lipids with proteins result in the following organoleptic changes:

(a) Changes of the colour due to browning reactions.

(b) Changes of the flavour because of binding of off-flavour compounds into neutral or less active substances or because of the formation of new flavour-active compounds.

(c) Changes of the texture caused by denaturation of proteins and by crosslinking of polypeptide chains (Pokorny *et al.*, 1983).

The activity of lipases and lipoxygenases are responsible for soapy and rancid flavours in foods. The former is due to the presence of long chain fatty acids and the latter is due to the oxidation of unsaturated fatty acids (Satouchi and Matsushita, 1976). Lipases hydrolyze fats into fatty acids and glycerol.

Table 2.9 Flavour threshold values for oxidation products in soybean

Substance	Flavour description	Threshold (ppm)
Oct-1-ene-3-one	Metallic	0.001
2-Pentyl furan	Liquorice	2.000
Oct-1-ene-3-ol	Mushroom	0.007
Pent-1-ene-3-one	Metallic	0.001

Source: Hamilton and Berger, (1995)

Table 2.10 Flavour descriptions used for crude, processed and reverted soybean oil.

State	Flavour
Crude	Grassy, beany
Freshly processed	Sweet, pleasant, nutty
Reverted	Grassy, beany, buttery, melony Tallowy, painty, fishy

Source: Hoffman (1962).

2.4.1.6 Effect of lipid oxidation on health

The biological and toxicological properties of oxidized lipids have been extensively investigated (Alexander, 1986). There is general agreement that undesirable or harmful materials are formed during storage and usage of lipids. But, there is a considerable disagreement regarding the levels of such materials formed and the nature of harmful effects caused by oxidized lipids (Alexander, 1986). This can be partly attributed to the very large variety of oxidation products

that may be formed according to the degree of lipid oxidation and the adequacy of experimental diets employed by various investigations (Chow and Gupta, 1994).

Oxidation products of polyunsaturated fatty acids may also be carcinogenic or co-carcinogenic (Kamel and Kakuda, 1995). The ability of certain anti-oxidants to protect against experimentally induced carcinogenesis (Wattenberg, 1972) had led to the suggestion that oxidation products of lipids may play a role in carcinogenesis and mutagenesis by damaging genetic materials.

Oxidation of lipids results in deficiencies of essential fatty acids and vitamin E. Animals fed on oxidized lipids containing low proteins had elevated serum glutamate -oxaloacetate transaminase and glutamate - pyruvate transaminase values, suggesting injury of the heart or liver (Huang *et al.*, 1988). Insufficiency of dietary protein has also been shown to aggravate the enhanced lipid peroxidation and decreased activities of antioxidant enzymes in rats fed on high polyunsaturated fats (Huang *et al.*, 1988).

Pure fatty acid hydroperoxides are very toxic to experimental animals when administered intravenously (i.v.) but not orally (Findlay *et al.*, 1970). The same authors have shown that the 24-h lethal dose of a high purity preparation of methyl linoleate hydroperoxides in adult male rats was about 0.07mmol/100g body weight. The major effect of the linoleate hydroperoxide was on the lungs.

Secondary oxidation products such as polymeric materials of high molecular weight are not easily absorbed (Kanazawa *et al.*, 1985) and are generally less toxic than monomeric or dimeric compounds. Consumption of large amounts of polymeric fatty acids may result in diarrhoea. Cyclic monomers, when fed at high levels to rats, have been shown to cause fatty livers (Poling *et al.*, 1970). Levels of 0.20 0.15% of cyclic monomers have produced fatty livers (Poling *et al.*, 1970).

Many secondary oxidation products of fatty acids have been shown to be more toxic to experimental animals. This is partly due to the fact that low molecular weight products have shorter carbon chain lengths and are more easily absorbed into the intestinal wall than lipid hydroperoxides or their polymeric materials (Chow and Gupta, 1994). Malondialdehyde (MDA) is a three-carbon dialdehyde and is toxic to experimental animals.

The LD_{50} levels of MDA in rats are 632 mg/kg for its enolic sodium salt and 527 mg/kg for is acetal form (Frankel, 1984), and both are more toxic than formaldehyde or glyoxal. MDA can react with mitochondrial membranes and disrupt red blood cell membranes (Frankel, 1984). MDA may be carcinogenic or co-carcinogenic under certain conditions. Mutagenicity and cytotoxicity of MDA have been demonstrated in mammalian lymphoma cells (Begin, 1987). Also, MDA may play a role in regulating tumour metastasis, host immune mechanism and the proliferation and differentiation of tumour cells (Frankel, 1984).

However the significance of MDA to human risk of cancer remains to be established (Begin, 1987).

Another group of toxic compounds formed in secondary oxidation is 4-hydroxyalkenal. An example is 4-hydroxynonenal, which has a lethal dose of 68 mg/kg in mice (Frankel, 1984). When 13-18 mg/kg of 4-hydroxynonenal or 4-hydroxyhexanal is injected intravenously as a phospholipid emulsion, severe liver damage is produced in rats (Segall *et al.*, 1985) similar to that seen after CCl_4 administration.

Components of oxidised lipids may also accelerate the turnover of vitamin E and increase the susceptibility of the red cells to hemolytic stress (Chow and Gupta, 1994). There may also be a reaction of peroxidizing lipids intracellularly with cell proteins leading to the formation of ceroids. Ceroid is deposited mainly in the brain and neuronal tissue but also in other organs such as the liver and uterus (Begin, 1987). Ceroid may also be deposited into aorta walls.

Generally oxidised lipids cause appetite and growth depression, diarrhoea, tissue enlargement, interference with reproduction, and even death in some cases (Poling *et al.*, 1970).

2.4.1.7 Laid down guidelines for avoiding rancidity

i. Maximal retention of natural antioxidants.

ii. Use as low a temperature as possible for processing and storage.

iii. Where high temperatures are unavoidable, reduce the time of exposure to a minimum.

iv. Reduce access of air (i.e., 0_2).

v. Minimise the interaction of the food with catalytic metals.

vi. Maintain good practice in terms of stock rotation and cleanliness.

vii. If these good practices are found to be insufficient use permitted chelating agents and antioxidants in the minimum amount needed (Hamilton and Berger, 1995).

2.4.1.8 Indices of lipid oxidation and rancidity

i. Lipid stability

Stability of lipids refers to resistance to the development of any off flavours, whether resulting from hydrolysis, reversion or oxidation (Hamilton and Berger, 1995). Methods such as the Swift stability test or the active oxygen method (AOM) can be used to measure stability of lipid. AOM predicts the susceptibility of lipid to oxidation and rancidity. In this test, air is continuously passed through the sample at a specified temperature and the length of time required for peroxide value to rise to a level indicative of rancidity is noted (Hamilton and Berger, 1995). For soybean oil the peroxide value at which the oil becomes rancid is between 20-40 meq/kg (Zadernowski *et al.*, 1983). The time is expressed in

hours, AOM. In general the higher the AOM the longer the shelf life of the sample.

ii. Peroxide value

The peroxide value (P.V) is a measure of active oxygen in 1000g of fat or oil and is expressed as millimoles or milliequivalents of peroxide (Ronald and Ronald, 1991). Since peroxides are the intermediate products formed in the autoxidation of oils, the test is used as measure of stability or for following course of the development of rancidity of oils. The test is also used for evaluating the effectiveness of antioxidant compounds on the keeping quality of fats and fat products. Peroxide value is an indication of the extent of rancidity; the lower the peroxide value, the less the oxidation that has occurred. Peroxide value is determined by measuring iodine liberated from potassium iodide by a given quantity of fat under prescribed conditions.

iii. Thiobarbituric acid (TBA) test

Products of lipid oxidation are apparently responsible for the colour reaction with TBA reagent (example, malonic dialdehyde and methyl oleate hydroperoxide both give colour reaction). The test is more sensitive and responsive at earlier stages of autoxidation. There is increase in the red pigment formation as oxidative rancidity advances. Zadernowski *et al.* (1983) reported that soybean oil becomes rancid at TBA Number between 15 and 20 meq/kg.

2.4.1.8.1 Other indirect indices

a. Free fatty acid and acid value

The concentration of free fatty acids in soybean oil is influenced by factors including agronomic and hydrolysis during processing and storage (Markley, 1951). Some free fatty acids could also result from the oxidation of secondary oxidation products such as the aldehydes (deMan, 1990). The free fatty acid is expressed in terms of percentage oleic acid (Ronald and Ronald, 1991). High fatty acid content indicates high instability of the soybean lipids. The term acid value is generally used to express the quantity of free fatty acid present in a food. It is defined as the number of milligrams of potassium hydroxide required to neutralise the free fatty acids in gram of a lipid sample (Ronald and Ronald, 1991). The acid value is determined by dissolving a known weight of oil or fat in hot neutral alcohol and titrating with 0.25ml alkali to a phenolphthalein end point (Ronald and Ronald, 1991). The acid value of solvent extracted soybean oil is between 0.5 and 1.92 (Zadernowski *et al.*, 1983).

b. Iodine value (I.V.)

The iodine value is defined as the number of grams of iodine absorbed by 100g of an oil or fat (Ronald and Ronald, 1991). It is an indication of the amount of unsaturation of a fat or oil but provides no information concerning the specific types or arrangement of the unsaturated bonds in the fatty acid components of the glyceride molecules. The iodine value can be used as a measure of the stability of a lipid. The higher the iodine value the more susceptible the lipid is to oxidation

hence the lower the stability. The iodine value of solvent-extracted soybean oil is between 134.1 and 135.8 (Zadernowski *et al.*, 1983).

2.5.0 Soybean processing and products

Soybeans were processed first in 1911, in the United States for oil and meals (Markley, 1951). Since 1941, soybean flour (including grits) has been the principal soybean product (Markley, 1951). Technology for processing soybean is well developed.

2.5.1 Soybean flour

There are three main types of soybean flour. These are full-fat flour, low-fat flour and defatted flour (Ferrier, 1975), which are revised below.

2.5.1.1 Full-fat flour

Full-fat flour contains all the fat originally present in the soybean seed. It is prepared by blanching or steaming the beans to debitter them and to inactivate lipid-oxidising enzymes such as lipases and lipoxygenase. The inactivation of these enzymes completely prevents the formation of any bitter, beany, or painty flavour (Ferrier, 1975). Blanching simultaneously destroys trypsin inhibitors, hemaglutinins and other known toxic factors present in the raw beans. The length of time required for these components to be destroyed decrease with increased moisture content of the whole bean.

Lipoxygenase is inactivated in rehydrated soybeans by boiling for less than five minutes (Ferrier, 1975). Boiling is also essential to produce an acceptable texture. Soaking and boiling also remove about one-third of the oligosaccharides in soybeans, some of which are responsible for the production of intestinal gas or flatus. Only a small amount of protein (1%) is lost during soaking and blanching (Ferrier, 1975).

After the soybeans have been blanched or steamed, the hulls, mainly cellulose and polysaccharides are removed and the dehulled beans are dried and then ground into full fat flour. The flour contains about 40% protein, 20% fat, 30% carbohydrate, 5% ash and 5.5% crude fibre (Waggle *et al.*, 1981).

The flour may be used as an ingredient in different bakery products to improve crumb body and resilience, as well as colour and toasting characteristics as a result of its sugar content (Waggle *et al.*, 1981). They are also used in cereal and infant foods.

2.5.1.2 Low-fat flour

This type of soybean flour has greater part of its fat removed by a continuous mechanical pressing method. It is also used as an ingredient in bakery products and has many other uses as well (Ferrier, 1975).

2.5.1.3 Defatted flour

This type of flour ordinarily contains less than 1% fat. The protein content ranges from 53 55% (Waggle *et al.*, 1981). It is prepared by cracking and dehulling the beans and tempering the cracked meats to about 11% moisture. The tempered meats are then passed through smooth rolls to form thin flakes that are extracted with hexane to remove the oil. The defatted flakes are then milled into flour and grits. These products are used in the preparation of beverages, crackers, cereal foods and infant foods. They are also used as meat extenders (Wolf, 1990).

Table 2.11 Proximate Analyses of Commercial Soybean Flour and Grits (%)

Flour or Grit Type	Moisture	Protein	Fat	Carbohydrate	Ash	Fibre
Full-Fat	5	41.5	21	25.2	5.2	2.1
Low-Fat	5.5	46.0	6.5	33.5	5.5	3.0
Defatted	5	53.0	0.9	32.3	6.0	2.9

Source: Meyer (1970)

Fat extraction constitutes one of the essential stages of the technological process of soyflour production. After the extraction flours contain small amount of free lipids and lipids bound with proteins and other hydrophilic components. Percentage bound fat in defatted soyflour is 2.2 ± 0.5. The significant quantity of bound lipids in defatted flour suggests that stability problems would be common

during storage. Polar lipid such as the predominant phospholipids, phosphatidyl choline would create an immediate flavour problem (Zadernowski *et al.*, 1983).

Table 2.12 Proportions of phospholipid fractions in soyflour as a percentage of total lipids

Phospholipid	Proportion (%)
Unknown	15.9
Phosphatidylethanolamine	23.1
Phosphatidyl glycerol	0.9
Phosphatidic acid	4.2
Phosphatidyl choline	32.7
Phosphatidyl serine	0.7
Phosphatidylinositol	20.4
Lyso Phosphatidythanolamide	0.2

Source: Zadernowski *et al.* (1983).

The ratio between neutral and polar lipids in soyflour is 1:0.9 (Zadernowski *et al.*, 1983). The stability of soyflour varies directly with the fat content. The development of a rancid off-odour always precedes the appearance of a rancid taste in the dried flour. The solvent-extracted flour having a fat content of less than 1.5% never attains appreciable concentrations of peroxides.

Table 2.13 Percentage composition of fatty acids in isolated fractions of bound lipids present in defatted soyflour

Fatty acids	Neutral lipid	Polar lipid	Glycolipid
16 : 0	29.3	30.9	28.5
18 : 0	6.9	7.6	5.7
18 : 1	8.5	10.8	8.3
18 : 2	48.0	46.3	50.8
18 : 3	7.3	4.3	5.6

Source: Zadernowski *et al.*, (1983).

2.5.2 Protein concentrates and isolates

Protein concentrates are defatted soy flake or soyflour, which has been upgraded in protein content by further fractionation to remove about one-half of the carbohydrates and some of the minor constituents (Markley, 1951). Soy protein concentrate has protein content between 60 and 70%, 1% fat, 21% carbohydrates, 5% ash and 3.5% crude fibre (Waggle *et al.*, 1981). It is mainly used as an ingredient in bakery industry and as meat additive.

Protein isolates are obtained from soyflour by an isolation process based on the differences in solubility of proteins as pH is varied. They are prepared by extracting alkaline solution at pH 7 - 8 and centrifuging out the insoluble polysaccharide residue. The clarified extract is then acidified to pH 4.5 thereby precipitating the major proteins as a white curd. The protein curd is separated

from the soluble fractions (whey) by centrifuging. The curd is washed and slurried in water and spray-dried in the isoelectric condition to give isoelectric protein isolate. The isolates have protein content between 90 and 97%, 0.5% fat, 2.5% carbohydrates, 4.5% ash and 0.3% crude fibre (Waggle *et al.*, 1981). These products are used mainly in simulated meat products, and in the bakery industry.

2.5.3 Other soy products

These include soymilk that can be used to make soy yoghurt and ice cream. Some traditional soy products include *miso*, *tempeh*, *nato*, soy sauce and *sufo* all of which are consumed extensively in the South Eastern Asian countries (Ihekoronye and Ngoddy, 1985).

2.5.4 Effect of processing on the nutritional value of soybean

Nutritive value of soybean may be improved by heat treatment or by processing into soybean products. The degree of nutritional improvement by heating depends upon, among other things, the temperature, duration of heating, and whether moist or dry heat is used (Hamilton and Berger, 1995).

Trypsin inhibitors and hemagglutinins are thermolabile hence the beneficial effect of heat treatment on the nutritive value is related to the inactivation of both components (Zademowski *et al.*, 1983). Lipoxygenase, which is responsible for the bitter, beany flavour as well as catalyzing lipid oxidation is very sensitive to heat and is destroyed at 82^0C in 15 min (Baker and Mustakas, 1972).

Excessive heating may destroy certain amino acids, such as lysine and particularly cystine, which are sensitive to heat and the loss by excessive heating may be more than 50% (Smith and Circle, 1972). Other amino acids such as arginine, tryptophan, histidine and serine may also be partially destroyed (Smith and Circle, 1972).

2.5.5 Effect of processing on flavour of soybean

Raw matured soybeans have a bitter, astringent taste and when eaten in this form will generate gas (flatulence) and cause diarrhoea (Ferrier, 1975). Raw meal is usually described as tasting beany, bitter and green but the green taste disappears after steaming for 3 minutes at atmospheric pressure (Thio, 1975). The beany, bitter flavour as well as the nutty, sweet, and toasted flavours vary in intensity with continued streaming (Thio, 1975).

2.5.6 Utilization of soybean flour in other dry foods

Soybean can be used in the fortification of cereal products. Cereals and legumes generally are individually not nutritionally adequate. For optimum utilisation, all the essential amino acids must be present in the right proportions. Soybeans are good sources of lysine but are deficient in sulphur-containing amino acids (Wolf, 1990). On the other hand, cereals such as maize, is generally deficient is lysine but have adequate amounts of sulphur-containing amino acids. These characteristics make soybean and other legumes natural complements to cereal-based diets. Studies have shown that such mixes in appropriate proportions will increase the quality of the diet above that of any single ingredient (Bressani and

Eliaz, 1983). In general a maximum supplementary effect is observed when about 50% of the legumes protein is replaced by cereal protein (Bressani and Eliaz, 1983).

Table 2.14Effect of steaming on flavour of soyflour

Steaming (Minutes)	Flavour (Score[a])	Flavour Description
0	1.5	Beany, Bitter, Green
3	4.5	Beany, Bitter, Nutty, Toasted Sweet
10	6.0	Beany, Nutty, Bitter, Toasted Sweet
20	6.3	Beany, Nutty, Bitter, Toasted Sweet
40	6.1	Beany, Nutty, Bitter, Toasted Sweet

(a) 1 = strong 10 = Bland

Source: Rackis *et al.* (1979).

Cereal and soybean can play an important role in the diet of the infant. In developing countries, most infants show satisfactory growth for the first six months of life when breast milk solely meets the nutritional needs. However with the onset of weaning, malnutrition usually sets in when protein and other nutrient requirements of infants are much higher than provided by the weaning foods used (Orraca-Tetteh, 1972). In Ghana, porridges and gruel made from maize, millet and sorghum are popular foods used during the weaning period. The net protein utilisation (N.P.U) is 44.5% for maize porridge and 50% for sorghum gruel (Orraca-Tetteh, 1972), compared with breast milk with N.P.U of about 100%.

Table 2.15 Effect of heat treatment on protein efficiency ratio (P.E.R.), biological value (B.V.), and digestibility of soybeans and soybean products.

Products	Treatment	(a) P.E.R	(b) B.V.	Digestibility
Raw, immature	None Autoclaved	1.1 2.0	49	88
Raw vine-ripened	None autoclaved	0.5 1.5	69	85
Raw, mature	None Dry heated Steamed autoclaved	0.7 0.7 1.3 1.3	58 64	82 92 90
Raw, germinated	None autoclaved	1.4 1.9		
Meal, solvent extracted	None autoclaved		52 69	74 89
Soymilk	none	2.0	79	91
Tofu	none	1.8	68	96
Tempeh	Steamed(2hrs) Deep fat fried(7min)	2.2 0.6		

a. P.E.R: Gain in body weight divided by weight of protein consumed (FAO/WHO, 1965).

b. B.V: The proportion of absorbed nitrogen retained in the body for maintenance and/or growth (FAO/WHO, 1965).

Source: Thio, 1975.

The protein quality and content of these traditional weaning foods can be improved by the addition of high-protein soybean products. The best ratio of soybean: Maize is 28:72 (Bressani *et al.*, 1974). This mixture gives a P.E.R of 2.54 whereas 100% maize results in a P.E.R of 0.69 compared with a casein standard of 2.87 (Bressani *et al.*, 1974). In Ghana, infant foods like *Brownilac*,

Cerelac and *Weanimix* have been formulated from mixture of cereals and proteins.

Table 2.16 Protein and fat contents and protein value of different maize - soy preparations

Mixture (%)		Content (%)		P.E.R
Soy bean	Maize	Protein	Fat	
0	100	9.9	4.5	0.69
21	79	16.9	8.9	2.08
28	72	17.9	10.9	2.54
38	62	18.1	11.3	2.37
100	0	40.0	25.6	2.03
casein				2.87

Source: Bressani and Eliaz (1983).

Soybean flour has been used to fortify cassava flour for the baking industry (Grace, 1977). Research done at the International Institute of Tropical Agriculture (IITA) has led to the production of nutritious bread and other baked products such as cakes and biscuits using cassava and soybean flour and other relevant ingredients (Kordylas, 1991).

In Ghana, a non-governmental organisation (NGO), the Global Farmers' Wives Association has produced soy-fortified *gari* and cassava flour on commercial scale (Industrial and Technology Fair, Ghana, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The main raw material used for the project was commercial soybeans grown in the northern savanna zone of Ghana, bought from the *Madina* market, Accra.

3.2 METHODOLOGY

The commercial soybeans were processed into flour using three different processing methods. Each of the samples prepared was stored and analyzed chemically and organoleptically over a period of twelve (12) weeks.

3.2.1 Preparation of samples

1. Raw soyflour

Sorted whole beans were milled into flour using a hammer mill (Christy and Norris Laboratory [Chelmsford, England] Mill Size 8). The Raw soyflour was packaged in high-density polythene bags obtained from the Poly-Products, Ghana Ltd., Accra and stored in the cold room for analyses.

2. Cooked-dried soyflour

The whole beans were washed thoroughly after sorting. The beans were transferred into a saucepan containing water and boiled for one (1) hour on a hot plate. The boiled beans together with the hulls were put in an air-oven set at

60°C to dry over night. The dried beans were milled into flour using the hammer mill (Christy and Norris Laboratory [Chelmsford, England] Mill Size 8). The cooked-dried soyflour was packaged in high-density polythene bags obtained from the Poly-Products, Ghana Ltd.-Accra and stored for analyses.

3. Roasted soyflour

Whole beans were first cleaned and roasted in a hot open pan for thirty (30) minutes. To get rid of burnt and broken pieces of hulls the roasted beans were sorted. The whole beans were then milled into flour using the hammer mill (Christy and Norris Laboratory [Chelmsford, England] Mill Size 8). The roasted soyflour was packaged in high-density polythene bags obtained from the Poly-Products, Ghana Ltd.-Accra and stored for analyses.

3.2.2. Chemical analyses

3.2.2.1 Total Lipids

The total lipids were determined using the method according to Bligh and Dyer (1981) in A.O.A.C. (1990). In this method, 10g of sample were accurately weighed into a 200ml-homogenizing flask. Water, chloroform and methanol were added in the volumes 10ml, 20ml and 40ml respectively. The mixture was homogenized for one (1) minute after which 20ml chloroform was added and the mixture homogenized again for 30 seconds. This was followed by the addition of 20ml water and homogenizing for 30 seconds. The homogenate was transferred into glass centrifuge tubes and centrifuged at 2000 r.p.m.for 20 minutes using the *Denley BS400* centrifuge. The aqueous layer was

Flow chart for the Preparation of roasted Soyflour

Soybeans

↓

Clean

↓

Roast

↓

(Roasted soybean)

↓

Winnow

↓

Mill

↓

(Roasted soybean flour)

↓

Package

↓

(Packaged roasted soybean flour)

Flow chart for the preparation of cooked-dried soyflour

Soybeans

↓

Clean

↓

Cook

↓

(Cooked soybeans)

↓

Oven-dry

↓

(Cooked-dried soybeans)

↓

Mill

↓

(Cooked-dried soybean flour)

↓

Package

↓

(Packaged Cooked-dried soybean flour)

Fig 3.1 Flow charts for the processing of roasted soybean flour and cooked-dried soybean flour.

removed by suction. The lipids in 20ml of chloroform was then determined after evaporation in a dried, weighed flask, initially on a steam bath and finally in an air-oven set at 105°C for 30 minutes. To get the total lipids the weight of the lipid was then multiplied by 2.

3.2.2.2 Crude Fat

The crude fat content of the samples was determined using the Soxhlets extraction method (A.O.A.C. 1990)

3.2.2.3 Free Fatty Acids

Free fatty acids were determined using the A.O.A.C. (1980) method 28.029. In this method chloroform extract of the sample was used for the analysis. The extract was prepared using the method of Bligh and Dyer (1981) in A.O.A.C. (1990).

3.2.2.4 Peroxide Value (P.V.)

The peroxide value of the sample was determined using the method according to Ronald and Ronald (1991). In this method 1g of chloroform extract of the sample was weighed into a clean dry boiling tube followed by the addition of 1g powdered potassium iodide and 20ml of solvent mixture (2 vol. glacial acetic acid = 1 vol. Chloroform). The boiling tube was then placed in boiling water so that it boiled within 30 seconds and then allowed to boil vigorously for 30 seconds. The content was quickly poured into a flask containing 20ml of 5% KI_2

solution. The boiling tube was washed out twice with 25ml water and the solution was titrated with 0.002M sodium thiosulphate solution using starch indicator. A blank was performed at the same time. The volume of sodium thiosulphate utilised in the titration was measured and this was used to calculate the peroxide value in miliequivalents per kilogram of sample.

3.2.2.5 Thiobarbituric Acid (T.B.A) Number

The T.B.A. Number was determined using the method according to Ronald and Ronald (1991). In this method 10g of soyflour and 50ml water were placed in a homogenizing flask and homogenized for 2 minutes. The homogenate was poured into a distillation flask and the homogenizing flask was washed with 47.5ml water. 2.5ml of 4M HCl was added and the pH was adjusted to 1.5. This was followed by the addition of a few glass beads. The solution was heated by means of an electric mantle and 50ml of distillate was collected 10 minutes from the time of boiling. 5ml of the distillate was pipetted into a glass–stoppered tube and TBA reagent (0.288g thiobarbituric acid/100ml of 90% glacial acetic acid) was added. The tube was stoppered, shaken and heated in boiling water for 35 minutes. A blank was prepared similarly using 5ml water with 15ml reagent. The boiling tubes were cooled in water for 10 minutes and the absorbance was measured against the blank using a spectrophotometer at 538nm. The TBA Number (mg/kg sample) was given by 7.8 x Absorbance.

3.2.2.6 Moisture Content

The moisture content of the samples was determined using an air-oven (A.O.A.C., 1990)

3.2.3 Chemical analyses of stored soyflours

The samples were stored in environment with different temperatures and relative humidity as shown in Tables 3.2 and 3.3 for a period of twelve (12) weeks. The following analyses were performed on the samples at various storage periods over the twelve- week period:

1. F.F.A. (3.2.2.3)
2. P.V (3.2.2.4).
3. T.B.A. Number (3.2.2.5)
4. Moisture content (3.2.2.6)

Total lipids and crude fat [(3.2.2.1 and 3.2.2.2) were determined only at times 0 and 12 weeks].

3.2.3.1 Investigation of the effect of storage temperature and time on lipid stability in soyflour.

Experimental design

The factors considered (temperature and time) were designed according to the Central Composite Rotatable design for K=2 as in Table 3.1.

Five levels of each of the variables were established as shown in Table 3.2.

Table 3.1 Design matrix and variable combination in experimental set up.

Coded variables	X_1	-1	-1	1	1	0	0	0	1.414	-1.414	0	0	0	0	0
	X_2	-1	1	-1	1	0	0	0	0	0	1.414	-1.414	0	0	0
		1	2	3	4	5	6	7	8	9	10	11	12	13	14

Table 3.2. Variables and their levels used in the central composite rotatable design for k=2.

Variable	Code	-1.414	-1	0	+1	+1.414
Temperature (°C)	X_1	5.0	15.9	42.2	68.5	80.0
Time (weeks)	X_2	90	2	6	10	12

The packaged samples were put in the environment with the following temperatures:

(a) 5°C (cold room),

(b)15.9°C(incubator),

(c) 30°C (laboratory temperature),

(d) 42.2°C (incubator),

(e) 68.5°C (incubator) and

(f) 80°C (incubator) for twelve weeks.

The three samples (raw, roasted and cooked-dried soyflours) were analysed chemically for indices of lipid oxidation and hydrolysis (that is, P.V., TBA

Number, FFA) at storage times of 0, 2, 6, 10 and 12 weeks. Moisture content was also determined along side the lipid stability indices (see section 3.2.2).

3.2.3.2 Investigation of the effect of relative humidity and time on lipid stability in the various stored soyflours.

A moisture sorption isotherm was constructed and the monolayer value calculated for all the samples. The minimum relative humidity/water activity used for storage of the samples was obtained using the monolayer value as the reference point.

3.2.3.2.1 Determination moisture sorption isotherm

All the samples used for the experiment were of the same of particle sizes. The uniform particle sizes were obtained using the modified form of the method of Ken-Jones *et al.* (1967). In this method 250g of each of the flours was placed in the uppermost sieve of the five sieves placed one on top of other having sieve aperture of 2mm, 1mm, 0.5mm, 0.25mm and 0.063mm respectively. The amplitude of vibration used was 50Hz and the mode of vibration was continuous. The vibration was monitored for five (5) minutes. At the end of the vibration, the weight of the flour in each sieve plus the adhering particles on the underside of each sieve was determined. The weights were expressed as a percentage of the total weight. The flours in sieves with apertures of 1mm, 0.5mm and 0.25mm were pooled together and used for the sorption isotherm

determination as well as for the storage studies. The result of the particle size analysis is shown in Fig. 4.1.

The method used for the determination of moisture sorption isotherm is as follows:

Atmospheres of different relative humidities were established using saturated solutions of different salts as described by Speiss and Wolf (1987). The saturated salt solutions and the corresponding relative humidities are shown in Table 3.1.The set-up used for exposing the samples to the various relative humidities is based on the principle of proximity equilibrium cell (Lang *et al.*, 1981). Each saturated salt solution was held in small sorption containers as described by Lang *et al.* (1981). This consisted of a small cylindrical glass jar (90mm long and 75mm diameter) with a fitted lid. Each sorption container was filled to about a third of its volume with saturated salt solution. About 2g of the flour was weighed into polypropylene weighing boats, 44 x 44mm, in size, and incubated under concentrated sulphuric acid for 24 hours so as to dry the samples to approximately zero moisture content. The samples were then suspended over the saturated salt solution, with a wire string attached to the lid of the sorption container. The sorption containers with the food samples were placed inside an incubator set at 30°C. Triplicate determinations were made at each relative humidity and the weights of samples recorded. Samples became equilibrated between eight and fourteen days and the moisture content of each

sample was determined using the air-oven method (A.O.A.C., 1990).

Table3.3 Water activity of selected saturated salt solutions at 30°C.

Salt	Water activity (a_w)
Lithium chloride	0.11
Glycerine	0.15
Potassium acetate	0.23
Potassium carbonate	0.45
Sodium bromide	0.58
Potassium iodide	0.68
Sodium chloride	0.75
Potassium chloride	0.84

Source:,Speiss,and,Wolf,(1987).

3.2.3.2.2 Determination of the effect of water activity (relative humidity) and time on lipid stability in stored soyflour.

Experimental design

The factors considered (water activity and storage time) were designed according to the Central Composite Rotatable Design for k=2 as shown in Table 3.1. Five levels of each of the variables were established as shown in Table 3.4. The minimum water activity used for storage of samples was obtained using the water activity corresponding to the B.E.T. monolayer value as the reference point. The maximum water activity used for storage of samples was chosen

based on the fact that it is the average normal relative humidity in the coastal belt of Ghana.

Table 3.4 Variables and their levels used in the central composite rotatable design for k=2.

Variable	Code	-1.414	-1	0	+1	+1.414
Water activity	X_3	0.15	0.23	0.45	0.68	0.75
Time (weeks)	X_4	0	2	6	10	12

Experimental procedure

Saturated solutions of salts corresponding to the water activities chosen were prepared using Table 3.3 as reference. Each of the solutions was poured into a desiccator and wire gauze was placed inside the desiccator about 8cm above the surface of the solution. The packaged soyflour samples were placed on the wire gauze and the desiccator covered with an airtight lid. The whole set-up was put in an incubator set at 30°C. Samples of each of the soyflours were taken at time intervals of 0, 2, 6, 10 and 12 weeks for the following chemical analysis:

(a) Moisture content

(b) Peroxide value

(c) Thiobarbituric acid number

(d) Free fatty acids

(e) Total lipids and crude fat content were determined only at times 0 and 12 weeks of storage (see section 3.2.2)

3.2.4 Sensory evaluation

The samples used for the sensory evaluation were stored at cold room temperature (5°C) and ordinary room temperature (30°C). A panel of fifteen (15) judges selected randomly from the Nutrition and Food Science Department of the University of Ghana were used for the test. The soy flour samples in storage were analysed organoleptically over a period of twelve weeks. The first two sensory evaluations were performed after four and eight weeks of storage respectively. The subsequent analyses were performed after ten and twelve weeks of storage. The questionnaire used for the sensory test is shown on Appendix 1.

3.2.5 Statistical analyses

The data obtained from the experiment were subjected to multiple regression analysis with dependent variables P.V., TBA Number and FFA. Three dimensional response surface plots were generated from the multiple regression models obtained using a stepwise multiple regression technique. The response surface plots were generated to illustrate the simultaneous triple effects of variables. The regression models were developed from *Statgraphics* software (Statgraphics, STSC Inc. version 4.2 U.S.A.).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 General observation of soyflour samples.

The raw, cooked-dried and roasted soyflours were different in terms of appearance (colour) and flavour. The raw soyflour had a cream colour; the cooked-dried had a light brown colour while the roasted flour had a brown colour. The brown colour of the processed flour might be due to browning reaction between the amino groups of proteins and the carboxyl group of carbohydrates (sugars) present in the beans (deMan, 1990). The roasted beans were subjected to prolonged dry heat than the cooked –dried hence the difference in shades of the brown colour. All the three flours had slightly different flavours. The raw flour had a beany, bitter flavour. The roasted flour however did not have a detectable beany flavour but had a strong flavour characteristic of roasted bean or cereal flour. The absence of beany flavour in the processed flour, that is, roasted and cooked-dried, might be due to heat inactivation of the enzyme lipoxygenase which is believed to be responsible for the development of the bitter, beany off flavour (Baker and Mustakas, 1972).

4.2 Particle size analysis of soyflour

The results for the particle size analysis are shown on Fig.4.1. For all the three samples, that is, raw, cooked-dried and roasted soyflours, a high proportion of the

flour was retained in the sieves with apertures between 0.25mm and 1mm, with the highest proportion at 0.5mm. Apart from the cooked-dried flour, both raw and roasted flour had more of the sample retained in the sieve with aperture 2mm (largest) than in the sieve with aperture 0.063mm (smallest). Among the three samples raw flour had the highest amount of samples retained in the largest sieve probably because of the hard seed coat, which might have been difficult to grind. The roasted flour had fewer larger particles than the raw flour probably because the roasting might have made the seed coat and the bean drier and more fragile hence grinding was easily effected.

4.3 Chemical analyses of freshly prepared soyflour samples

The results of the chemical analyses performed on the freshly prepared raw, roasted and cooked-dried soyflours are shown in Table 4.1. The raw soyflour showed a higher level of moisture than the processed flours and this could be attributed to the fact that the two processing methods (roasting and cooking-drying) involved heat drying, which might have led to some dehydration. The raw soyflour also appeared to have higher values of free fatty acids (FFA), peroxide value (P.V.) and thiobarbituric acid number (TBA no.) than the processed flours. This might be an indication that lipid-hydrolysing enzyme, lipase, and lipid-oxidizing enzyme, lipoxygenase, might have been inactivated through heat processing hence the mode of lipid oxidation might be mainly autoxidation and thermal oxidation (Hamilton and Berger, 1995).

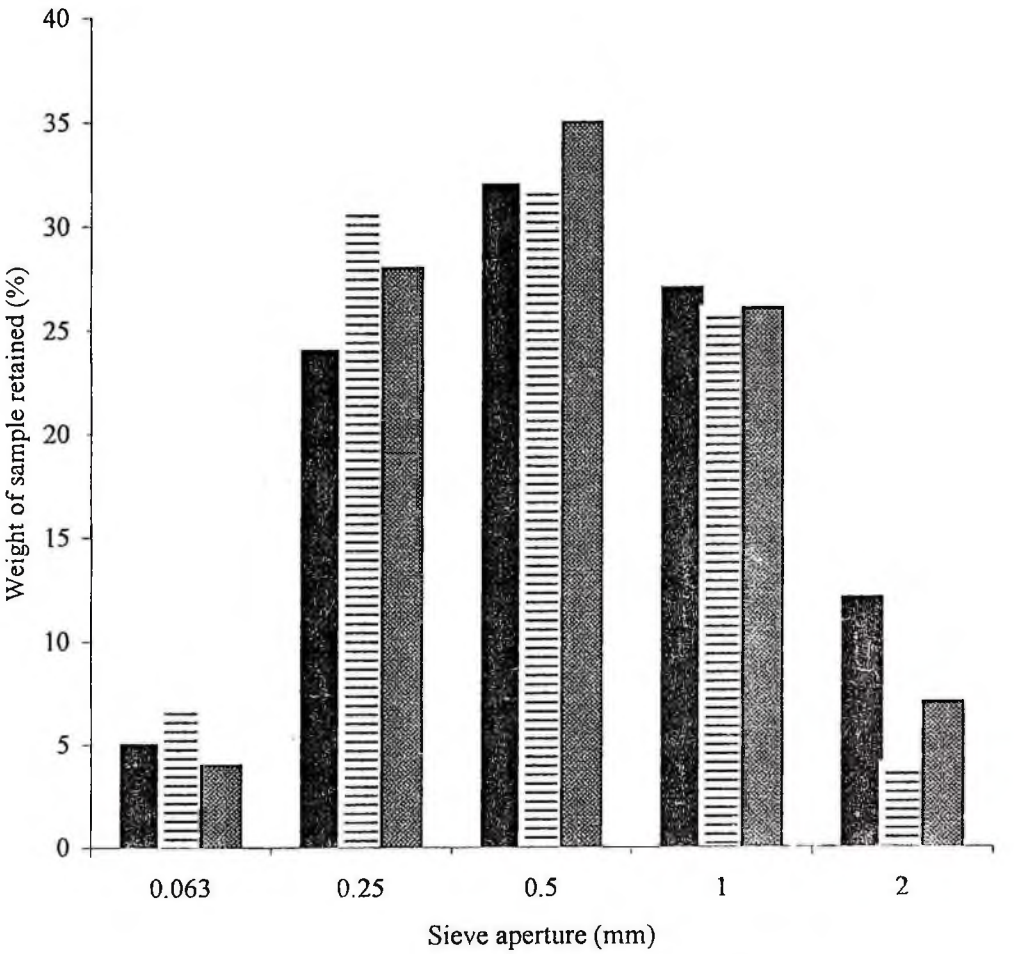


Fig.4.1 Particle size analysis of soy flours

Table 4.1 Moisture content, crude fat content, free fatty acids, hydrolytic and oxidative products in freshly prepared raw and processed soyflours.

Soyflour	Moisture content (% DMB)	Crude fat (%)	Total lipids (%)	Free fatty acids (%)	Peroxide value (meq/kg)	Thiobarbituri acid number (mg/kg)
Raw	9.78±0.52	20.16±0.16	26.29±0.09	1.76±0.10	2.56±0.21	12.50±1.03
Roasted	5.51±0.81	20.56±0.09	26.56±0.11	1.13±0.06	1.42±0.18	7.25±0.67
Cooked-dried	4.74±0.46	20.48±0.11	26.82 ±0.15	1.39±0.08	1.72±0.12	6.84±0.52

Note: Hydrolytic products include free fatty acids while oxidative products include hydroperoxides and thiobarbituric acid reactive substances.

In the case of the raw flour the mode of lipid oxidation might be mainly enzymatic and autoxidation (Hamilton and Berger, 1995). The lipoxygenase enzyme acts on the unsaturated acids as soon as the raw bean tissue is raptured and the enzyme and the substrate are released (Satouchi and Matsushita, 1976). Baker and Mustakas (1972) showed that when the raw soybean tissue is broken the enzyme and the substrate are liberated and provided some moisture is present a bitter, beany taste develops very rapidly. Lipoxygenase have been shown to generally accelerate the oxidation of lipids (Markley, 1951).

The higher moisture content and the absence of heat treatment might have been the cause of the high FFA content in the raw soyflour. Lipases are capable of hydrolysing lipids in the presence of moisture to fatty acids and glycerol (deMan, 1990). The lipases are however inactivated at temperatures above 50°C (Whitaker, 1972). It has also been reported that the fatty acids produced from the hydrolysis are even more susceptible to oxidation (Satouchi and Matsushita, 1976). This observation explains further why the raw soyflour had more oxidative products (hydroperoxides and TBA reactive substances or TBARS).

4.4 Chemical analyses of stored soyflour samples

4.4.1 Effect of storage temperature and time on lipid stability

Labuza (1975) reported that the rate of lipid oxidation in dry foods depends greatly on the moisture content. Whitaker (1972) also reported that water is an important requirement for lipid hydrolysis. With reference to these reports the moisture content in all the samples were determined along side the oxidative and hydrolytic properties. Generally the moisture contents of all the three soyflours (raw, roasted and cooked-dried) were found to decrease with increase in temperature and time. At 30°C (room temperature) however the moisture content was observed to increase slightly, that is, from 9.78 to 9.98 for raw flour, 5.51 to 5.71 for roasted flour and 4.74 to 5.09 for cooked-dried flour, at the end of the twelve weeks storage period. The slight increase in moisture content might be due to the absorption of moisture from the environment, which had a relative humidity of approximately 75% (ambient). The slight decrease in moisture

content of all the samples at 5°C (cold room) might be as a result of loss of moisture resulting from the process of cooling (Badings, 1970). Tables 4.2 and 4.3 show the variation of moisture with temperature and time in the raw and roasted soyflour samples.

Table 4.2 Effect of storage temperature and time on the moisture content of packaged roasted soyflour

Temperature (°C)	Moisture content of **Roasted** soyflour (%)				
	Week0	Week2	Week6	Week10	Week12
5	5.51	5.49	5.42	5.11	5.00
15.9	5.51	5.52	5.51	5.55	5.55
3 0	5.51	5.56	5.62	5.67	5.71
42.2	5.51	5.35	5.22	4.86	4.47
68.5	5.51	5.02	4.52	4.21	4.01
80	5.51	4.83	4.22	3.52	2.79

Table 4.3 Effect of storage temperature and time on the moisture content of packaged raw soyflour

Temperature (°C)	Moisture content of **raw** soyflour (%)				
	Week0	Week2	Week6	Week10	Week12
5	9.78	9.78	9.64	9.60	9.51
15.9	9.78	9.77	9.80	9.82	9:82
30	9.78	9.78	9.86	9.93	9.98
42.2	9.78	9.55	8.57	8.00	7.21
68.5	9.78	9.54	9.03	7.34	6.29
80	9.78	9.02	8.54	7.03	5.56

4.4.1.1 Effect of storage temperature and time on Free Fatty Acids of soyflours

The FFA for all the three samples were observed to have generally increased with time at all the selected temperatures. The increase in FFA was higher in raw soyflours at all the selected temperatures than in the processed flours. This could be attributed to the inactivation of lipases during heat processing of the soybeans. At 5°C however, the increase in FFA was very minimal, that is, from 1.39 to 1.61 for cooked-dried, 1.13 to1.35 for roasted and 1.76 to 2.13 for raw soyflour by the end of twelve weeks of storage.

The Free fatty acids in all the three soyflours increased as temperature was raised from 5°C, to reach a maximum value (that is, 2.42% in roasted flour, 2.54% in cooked-dried and 4.02% in raw soyflour) at 30°C and thereafter decreased as temperature was increase from 42.2°C to 80°C. These observations could be explained based on the observation by Whitaker (1972) that lipases have their optimum temperature around 30°C and about 50°C denaturation begins. At about 85°C the lipases are completely denatured (Whitaker, 1972). The decrease in the FFA content beyond 30°C might also be due to the accumulation of a lot of FFA resulting from the high rate of hydrolysis at 30°C, which might have acted as a competitive inhibitor of the enzyme (Whitaker, 1972). Fig.4.2 shows the relationship between the FFA and temperature for all the three soyflours after twelve weeks of storage.

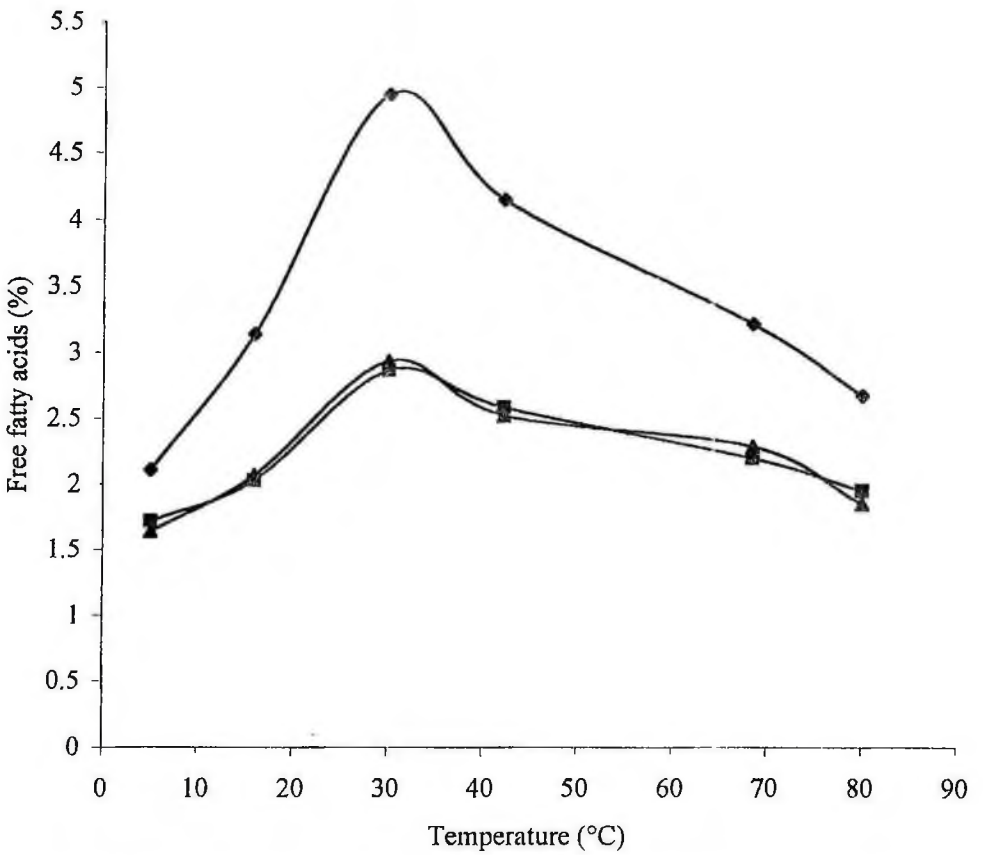


Fig 4.2 Free fatty acids in raw, cooked-dried and roasted soyflours stored at different temperatures for twelve (12) weeks

A multiple regression analysis gave a model with an R^2 of 91%, 97% and 95% for raw, cooked-dried and roasted soyflours, respectively. None of the models had a significant "lack of fit" (Appendix 2,3,4) implying that each model is sufficient for predicting the FFA in soyflour at any given storage temperature and storage time.

In all the three soyflours storage time had both linear and quadratic effects on the FFA content (Table 4.4). The linear effect of storage temperature on FFA content was significant only in the cooked-dried soyflour whereas the quadratic effect was significant in all the three soyflours. There was however a non-significant effect of the interaction between the two factors (storage temperature and time) on the FFA content in all the three soyflours.

Table 4.4 Further ANOVA for variation in the order fitted (showing only the P-values) for FFA content in soyflours stored at various temperatures over a twelve-week period

Source of variation	P-values		
	Raw	Cooked	Roasted
Time	0.0001*	0.0001*	0.0001*
Temp	0.5380	0.0257*	0.1719
$Time^2$	0.0114*	0.0004*	0.0001*
$Temp^2$	0.0107*	0.0340*	0.0056*
Time *Temp	0.7516	0.1519	0.8466

* = Significant P-values

3-D response surface plots, for FFA content in soyflour as a function of storage temperature and time were generated from the following regression equations and are shown in Figs. 4.3-4.5.

4.4.1.2 Effect of storage temperature and time on Peroxide value and the TBA number of stored soyflours

The peroxide values and the TBA numbers of the soyflours were observed to increase generally with time and temperature. These can be seen in Figs. 4.6-4.11. It was observed that, as the peroxide value increased there was a corresponding increase in the TBA number.

In all the three soyflours there was no significant change in the P.V. at storage temperatures of 5°C and 15.9°C after four weeks of storage. However beyond four weeks the P.V. of the raw soyflour began to increase gradually and by the eighth week the increase was quite pronounced. At 68.5°C and 80°C storage temperatures, the P.V. of the raw soyflour increased at an accelerated rate compared to the TBA number, reaching a maximum at 8 weeks and thereafter declined.

An inverse relationship was observed between the P.V. and the TBA Number after eight weeks of storage at 68.5°C and 80°C in raw soyflour, indicating progression of oxidation from a primary to a secondary state.

Regression equation for Fig 4.3

$Z = 0.95233 - 0.02351t + 0.0451T + 0.020692t^2 - 0.000467T^2 - 0.000476Tt$

Z = FFA

T = Storage temperature

t = storage time.

$R^2 = 91\%$

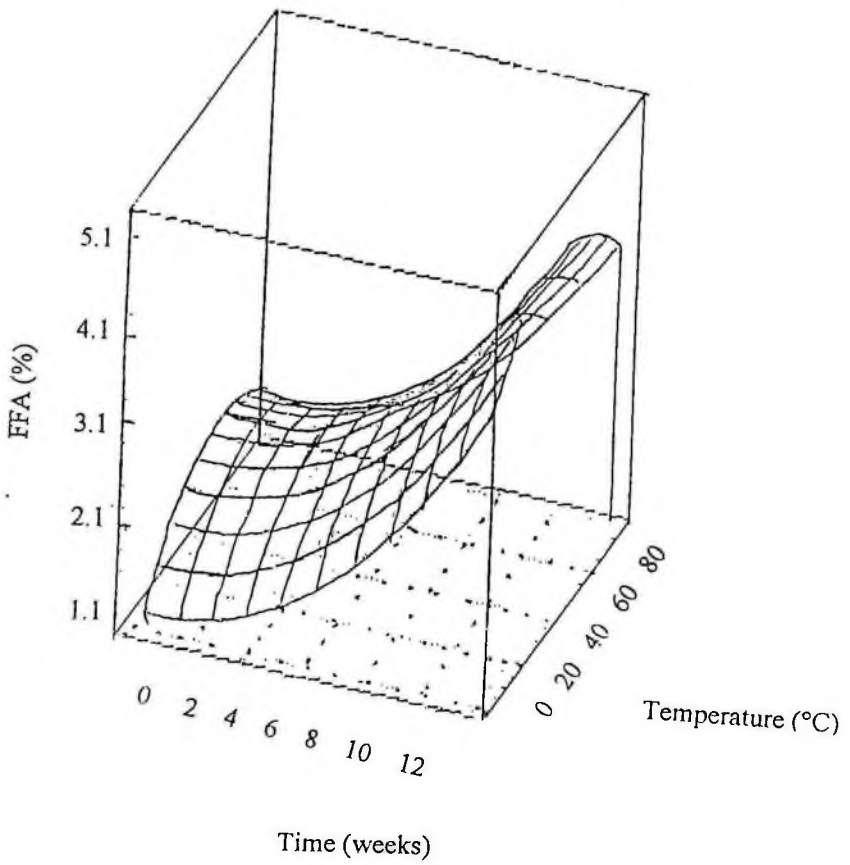


Fig. 4.3 The 3-D response surface for the free fatty acids (Z) in raw soy flour as a function of temperature (T) and time (t).

Regression equation for Fig 4.4

$Z = 1.377721 - 0.045375t + 0.005544T + 0.008994t^2 - 0.000073T^2 + 0.000546Tt$

Z = FFA

T = storage temperature

t = storage time.

$R^2 = 97\%$

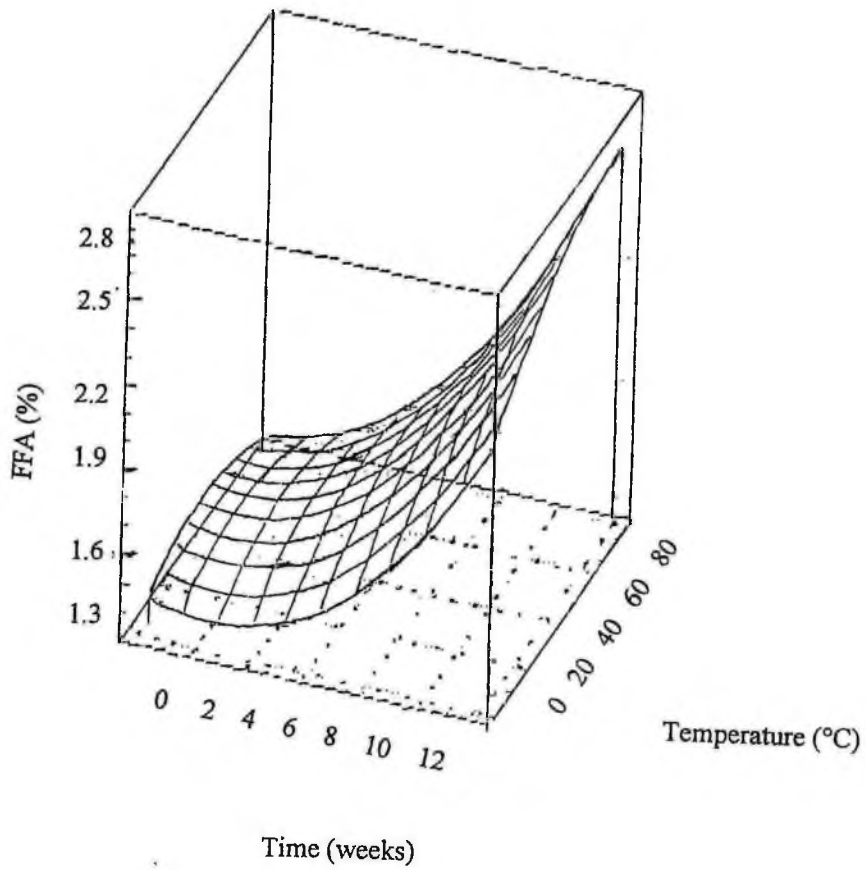


Fig 4.4 The 3-D response surface for the free fatty acids (Z) in **cooked-dried** soyflour as a function of temperature (T) and time (t)

Regression equation for Fig 4.5

$Z = 1.23069 - 0.082171t + 0.015625T + 0.014325t^2 - 0.000154T^2 - 0.000095Tt$

Z = FFA

T = storage temperature

t = storage time.

$R^2 = 95\%$

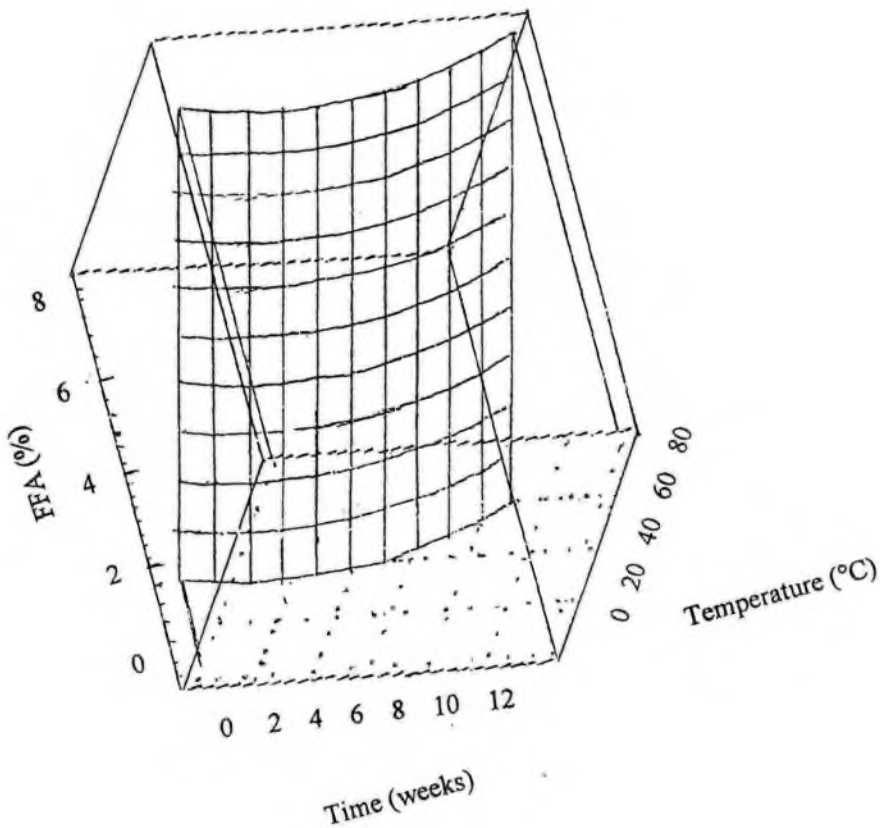


Fig 4.5 The 3-D response surface for the free fatty acids (Z) in **roasted** soyflour as a function of temperature (T) and time (t)

By the end of the twelfth week storage period the processed flour had not shown any decline in the P.V. indicating that a longer storage time was required for the P.V. to reach the maximum level. This also meant that the rate of production of the thiobarbituric reactive substances (TBARS) was still low since the rate had been found to increase sharply as soon as the P.V. began to decline.

In general the raw soyflour showed a much higher increase in both the P.V. and the TBA Number than the processed soyflours. These results suggest that both moist and dry heat processing of soybeans have the ability to increase lipid stability of soy products. The difference in the rate of lipid oxidation in raw and processed soyflour could be attributed to the fact that the heat treatment given to the processed flours might have inactivated the lipid oxidising enzyme, lipoxygenase (Baker and Mustakas, 1972). The oxidation of lipids in the processed samples might therefore be due to autoxidation, thermal oxidation and photo-oxidation whereas in the raw flour there was an additional mode of oxidation, that is, enzymatic (Hamilton and Berger, 1995). The decline in the P.V. after reaching a maximum value could be due to a decrease in the level of substrate (oxygen or unsaturated fatty acids) as peroxidation progressed (deMan 1990). The fact that the samples were packaged in high-density polythene might have reduced the transfer of oxygen into the package hence the oxygen inside could be used up almost completely (Hamilton and Berger, 1995).

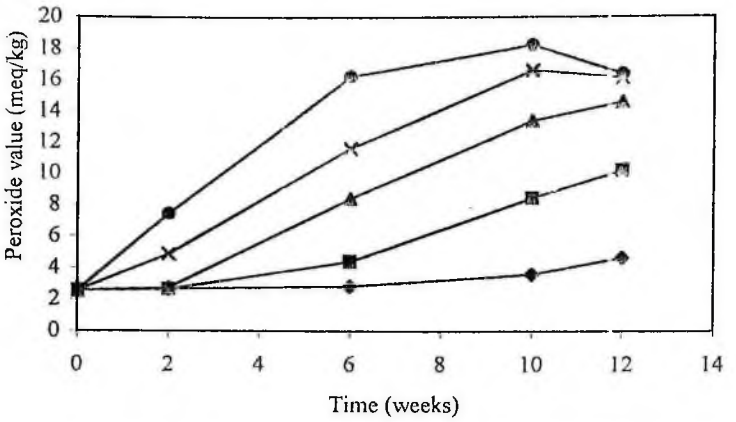


Fig 4.6 Effect of storage temperature and time on the **peroxide value** of **raw** soy flour.

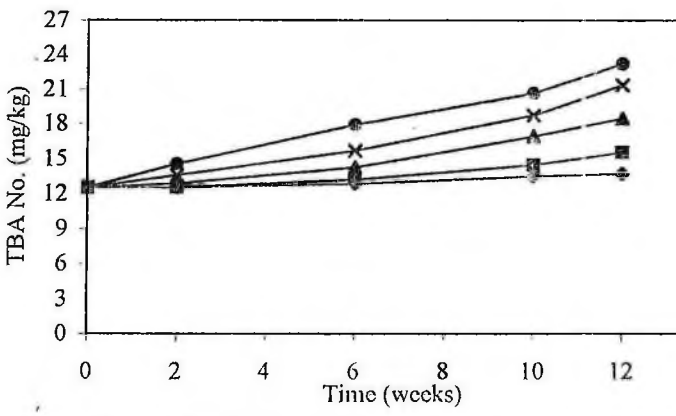


Fig 4.7 Effect of storage temperature and time on the **TBA Number** of **raw** soyflour.

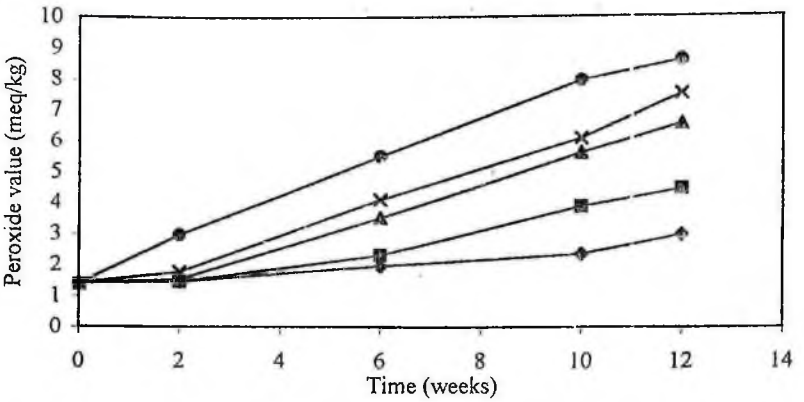


Fig 4.8 Effect of storage temperature and time on the **peroxide value** of **roasted** soyflour.

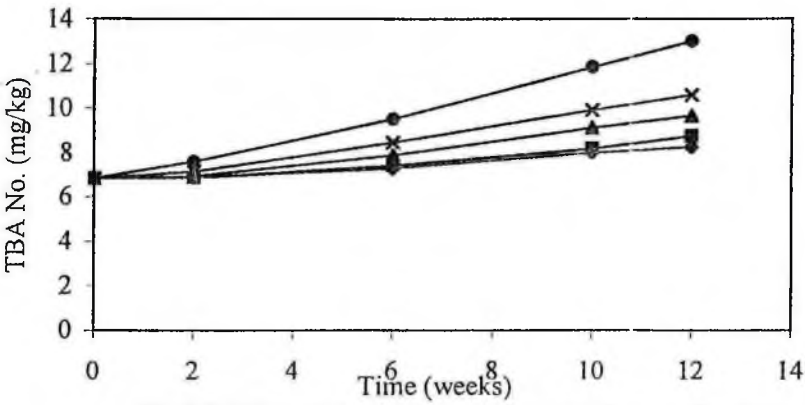


Fig 4.9 Effect of storage temperature and time on the **TBA Number** of **roasted** soyflour.

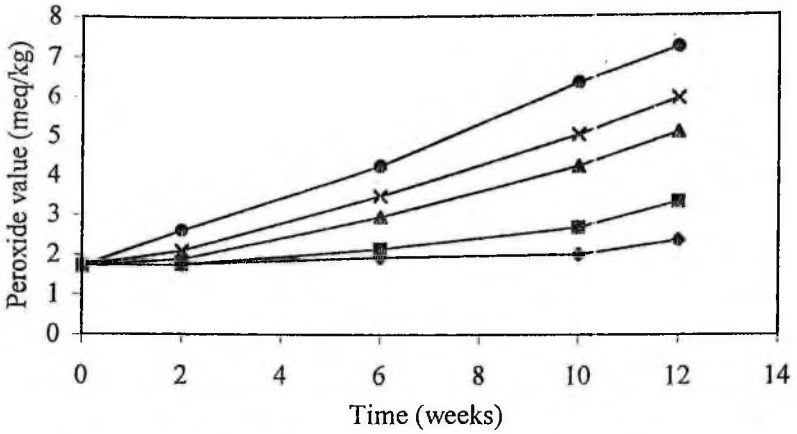
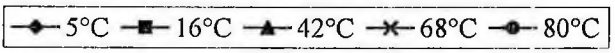


Fig 4.10 Effect of storage temperature and time on the **peroxide value** of **cooked-dried** soyflour.



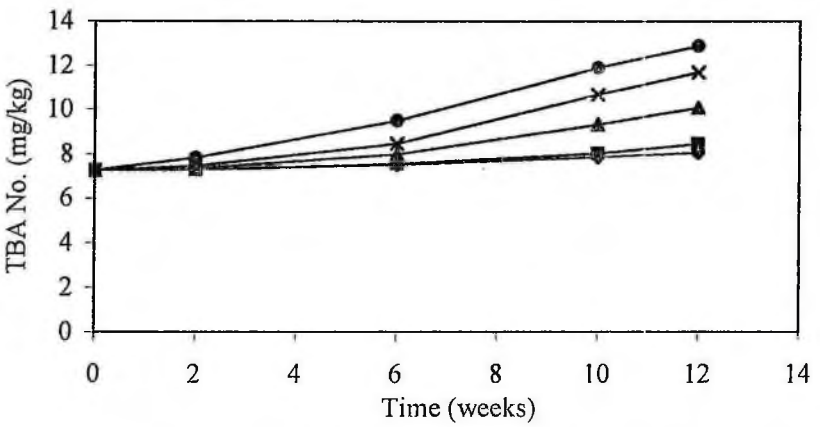
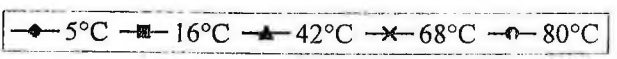


Fig 4.11 Effect of storage temperature and time on the **TBA Number** of **cooked-dried** soyflour.



The general increase in the P.V. and the TBA Number as temperature increased confirms the general rule that the oxidation of lipids approximately doubles for every 10° rise in temperature (Hamilton and Berger, 1995).

A multiple regression analysis gave a model with an R^2 of 96%, 98% and 92% for P.V. in raw, cooked-dried and roasted soyflours respectively. The regression models for TBA Number in soyflour gave R^2 of 99%, 98% and 97% for raw, cooked-dried and roasted soyflours respectively. None of the models had a significant "lack of fit" (Appendices 5,6, 7,8,9,and 10).

In all the three soyflours the linear terms of temperature and time had significant effects on the P.V. The quadratic term of temperature however affected only the P.V. in the roasted flour significantly. The quadratic term of time also affected only the P.V. in the cooked-dried soyflour significantly. The interaction between temperature and time had significant effect only on the raw and cooked-dried soyflours (Tables 4.5 and 4.6). The TBA Numbers of all the three soyflours were affected significantly by the linear terms of temperature and time and also by the interaction between the two factors. The quadratic terms of temperature and time affected significantly the TBA Number of raw and cooked-dried soyflours only.

3-D response surface plots for P.V. and TBA Number of soyflour as a function of temperature and time were generated from the regression equations and are shown in Figs 4.12-4.17.

Table 4.5 Further Anova for variation in the order fitted (showing only the P-values) for Peroxide value in soyflours stored at various temperatures over a twelve-week period.

Source of variation	P-values		
	Raw	Cooked	Roasted
Time	0.0001*	0.0001*	0.0001*
Temp	0.0001*	0.0001*	0.0004*
$(Time)^2$	0.7848	0.0150*	0.4548
$(Temp)^2$	0.4582	0.8491	0.0253*
Time *Temp	0.0337*	0.0001*	0.1195

* = Significant P-value

Table 4.6 Further Anova for variation in the order fitted (showing only the P-values) for TBA Number in soyflours stored at various temperatures over a twelve-week period.

Source of variation	P-values		
	Raw	Cooked	Roasted
Time	0.0001*	0.0001*	0.0001*
Temp	0.0001*	0.0001*	0.0001*
$(Time)^2$	0.0001*	0.0001*	0.1532
$(Temp)^2$	0.0258*	0.0012*	0.0562
Time *Temp	0.0001*	0.0001*	0.0082*

* = Significant P-value

Regression equation for Fig 4.12

$Z = 0.399762 + 0.534072t + 0.015502T - 0.006632t^2 + 0.000464T^2 + 0.014082Tt$

Z = P.V.

T = storage temperature

T = storage time

$R^2 = 96\%$

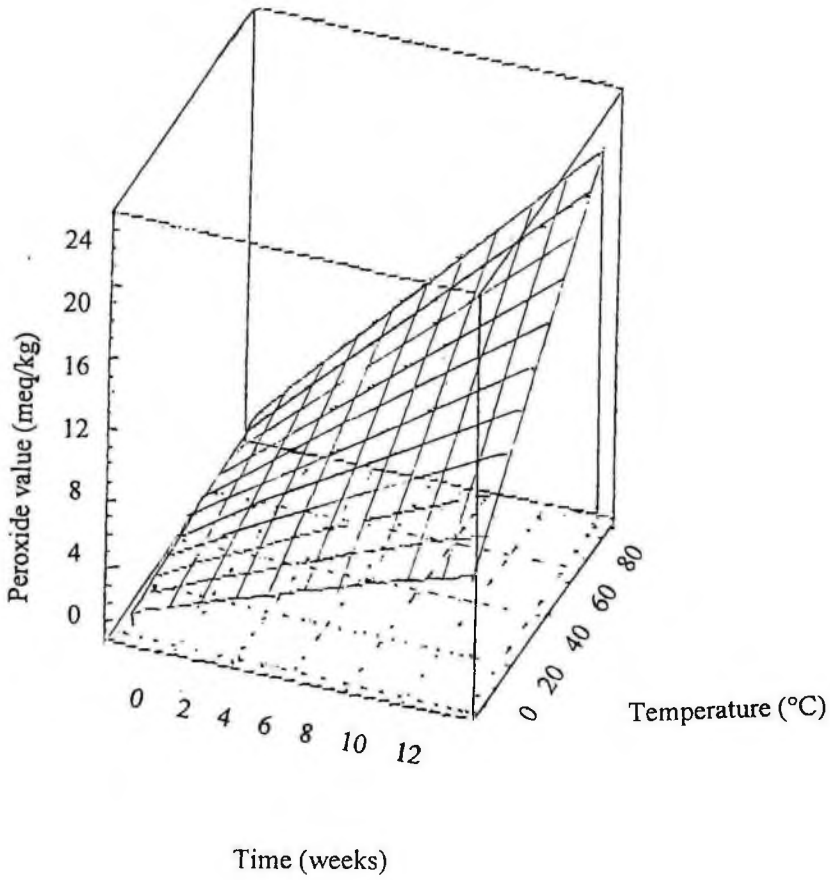


Fig 4.12 The 3-D response surface for the peroxide value (Z) in **raw** soy flour as a function of temperature (T) and time (t).

Regression equation for Fig 4.13

$Z = 1.787188 - 0.071507t - 0.003508T - 0.006632t^2 + 0.00001T^2 + 0.005366Tt$

Z = P.V.

T = storage temperature

t = storage time

$R^2 = 98\%$

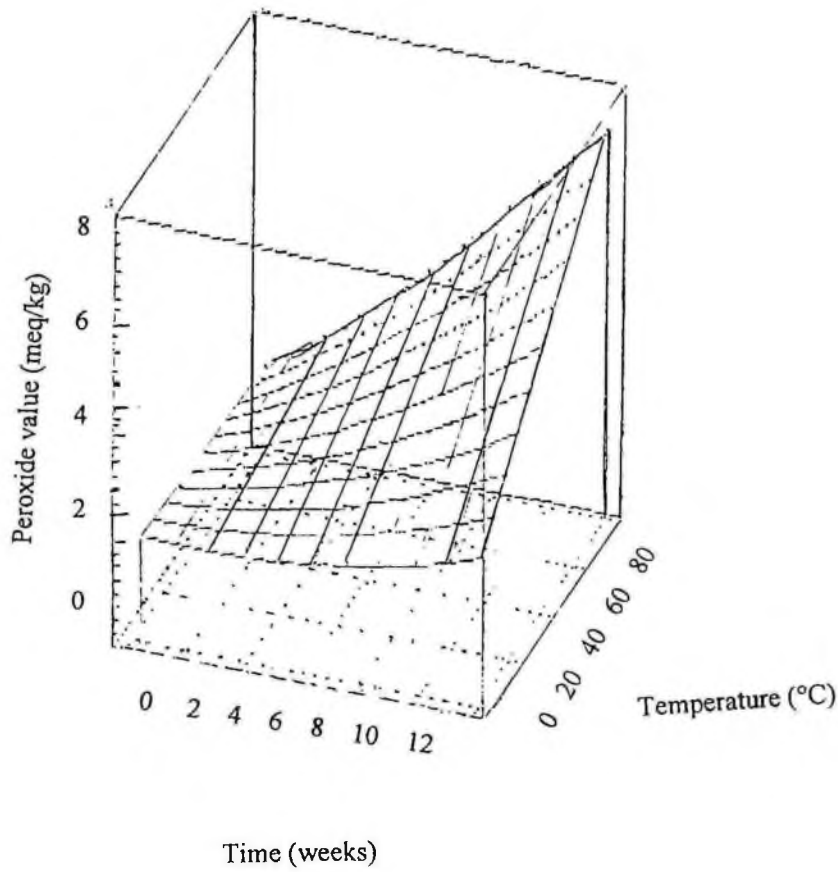


Fig 4.13 The 3-D response surface for the peroxide value (Z) in **cooked-dried** soyflour as a function of temperature (T) and time (t).

Regression equation for Fig 4.14

$Z = 1.75484 + 0.024894t - 0.048513T + 0.008391t2 + 0.00074T2 + 0.004132Tt$

Z = P.V.

T = storage temperature

t = storage time

$R^2 = 97\%$

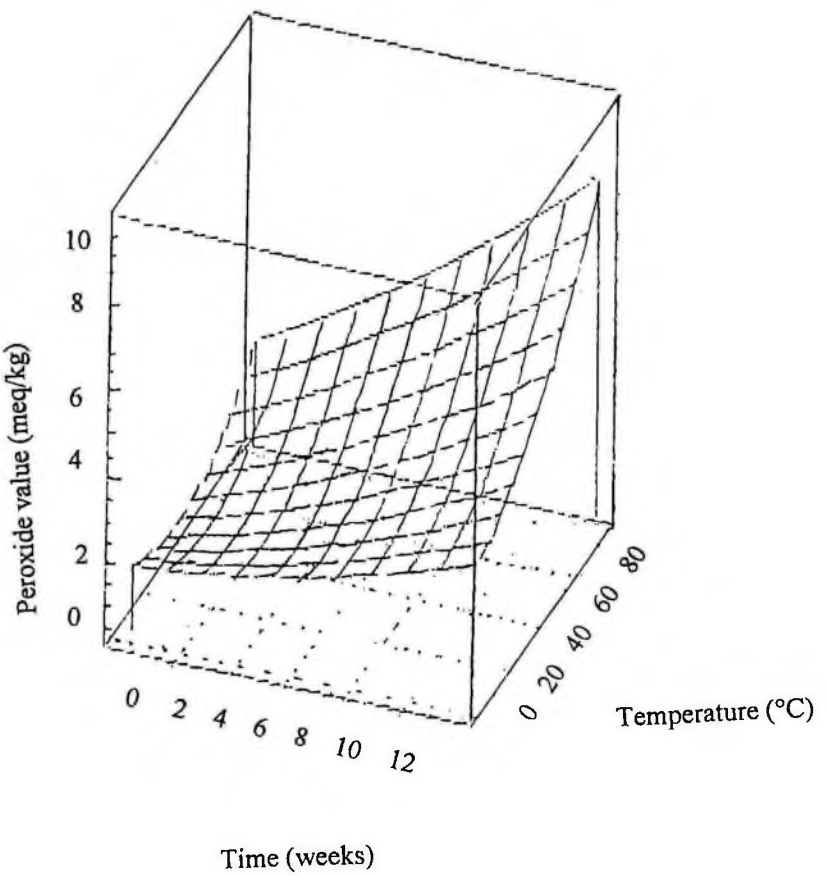


Fig 4.14 The 3-D response surface for the peroxide value (Z) in **roasted** soyflour as a function of temperature (T) and time (t)

Regression equation for Fig 4.15

$Z = 12.922889 - 0.232634t - 0.025856T + 0.030753t^2 + 0.000382T^2 + 0.007883Tt$

Z= TBA no.

T = temperature

t = storage time).

$R^2 = 99\%$

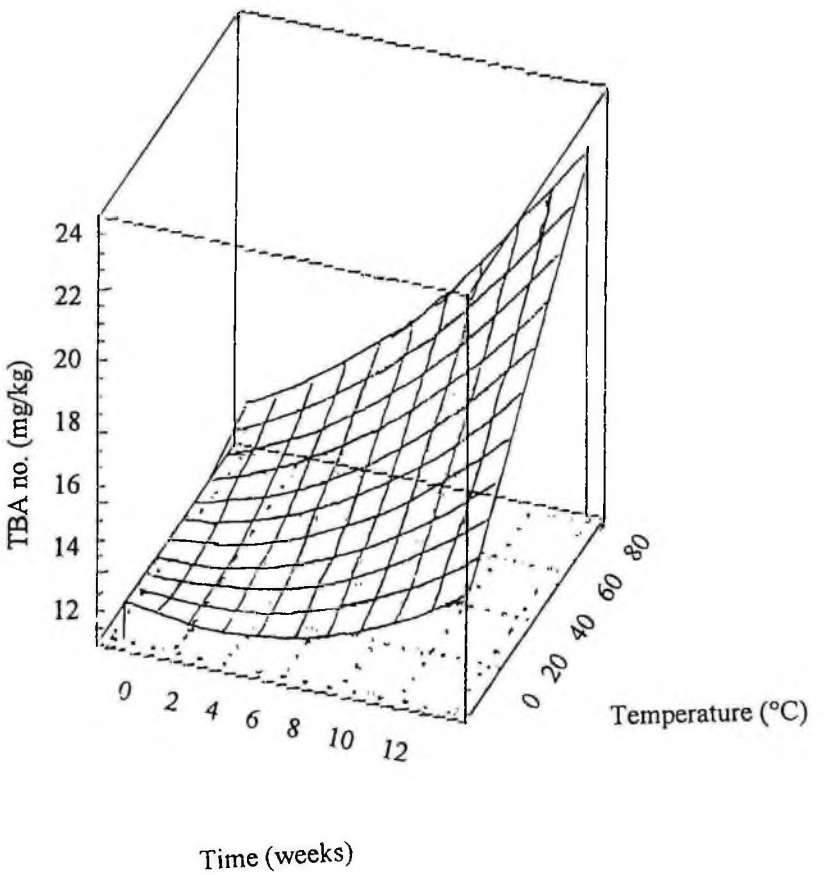


Fig 4.15 The 3-D response surface for the TBA no. (Z) in **raw** soyflour as a function of temperature (T) and time (t).

Regression equation for Fig 4.16

$Z = 8.007328 - 0.208362t - 0.033737T + 0.017071t^2 + 0.000304T^2 + 0.005794Tt$

Z =TBA no.

T = storage temperature

t = storage time

$R^2 = 98\%$

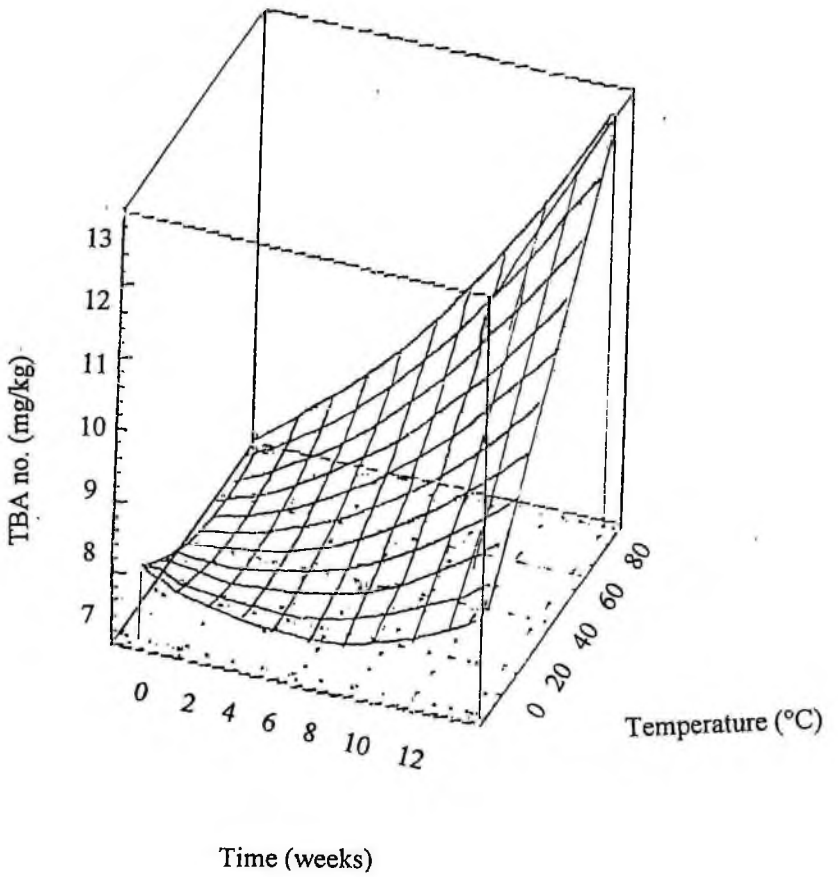


Fig 4.16 The 3-D response surface for the TBA no. (Z) in **cooked-dried** soyflour as a function of temperature (T) and time (t).

Regression equation for Fig 4.17

$Z = 6.932735 + 0.021054t - 0.01765T + 0.006741t^2 - +0.000256T^2 + 0.003372Tt$

Z = TBA no.

T = storage temperature

t = storage time

$R^2 = 94\%$

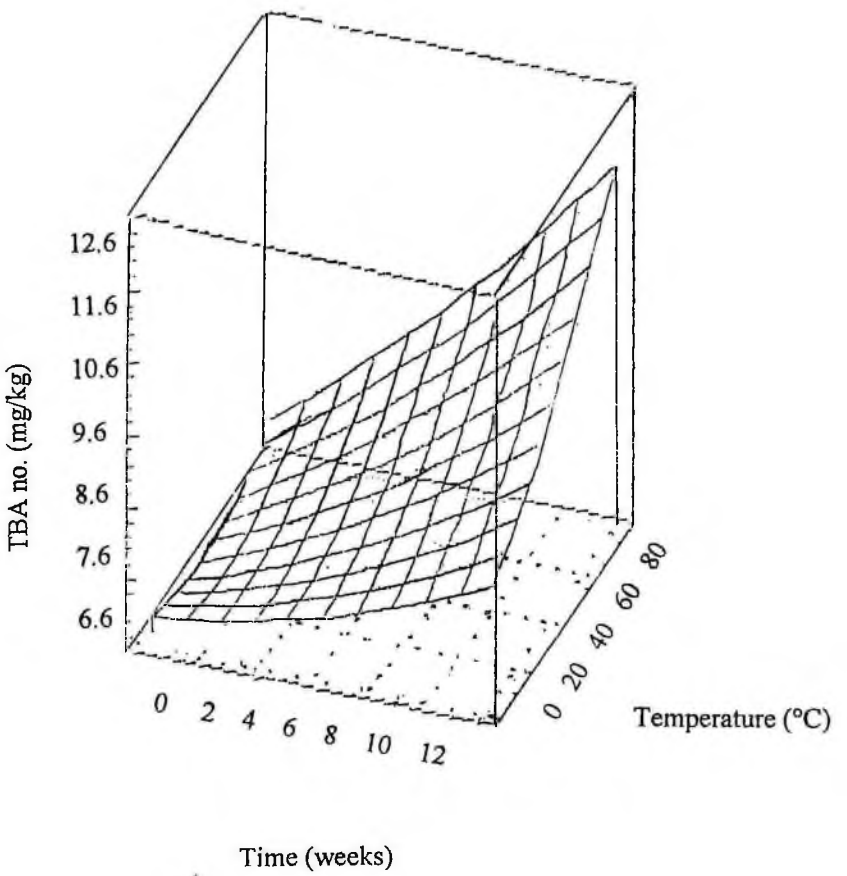


Fig 4.17 The 3-D response surface for the TBA no. (Z) in **roasted** soyflour as a function of temperature (T) and time (t).

4.5 Effect of water activity (a_w) and time on lipid stability in stored soyflour.

4.5.1 Moisture sorption isotherms and B.E.T. monolayer values

According to Labuza (1975), moisture content close to the monolayer coverage of food has a protective action against lipid oxidation and results in minimum lipid oxidation rate. Based on these findings, the BET monolayer values of all the soyflour samples were determined from the BET plots shown in Figs 4.33-4.35, which were plotted using the following BET equation:

$A/(1-A)M = 1/M_0C + (C-1/M_0C)A$, where A = water activity; M = equilibrium moisture content; M_0 = monolayer value; C = a constant related to heat of adsorption.

Moisture sorption isotherms for all the three soyflours are shown on Fig. 4.18.

The calculated B.E.T. monolayer values and the corresponding a_w are shown on Table 4.7. The corresponding a_ws were obtained from the sorption isotherms (Fig.4.18).

Table 4.7 B.E.T. monolayer values for different types of soyflour at 30°C.

Soyflour	B.E.T. monolayer value	Corresponding a_w
Raw	2.87	0.24
Roasted	2.96	0.23
Cooked-dried	2.93	0.22

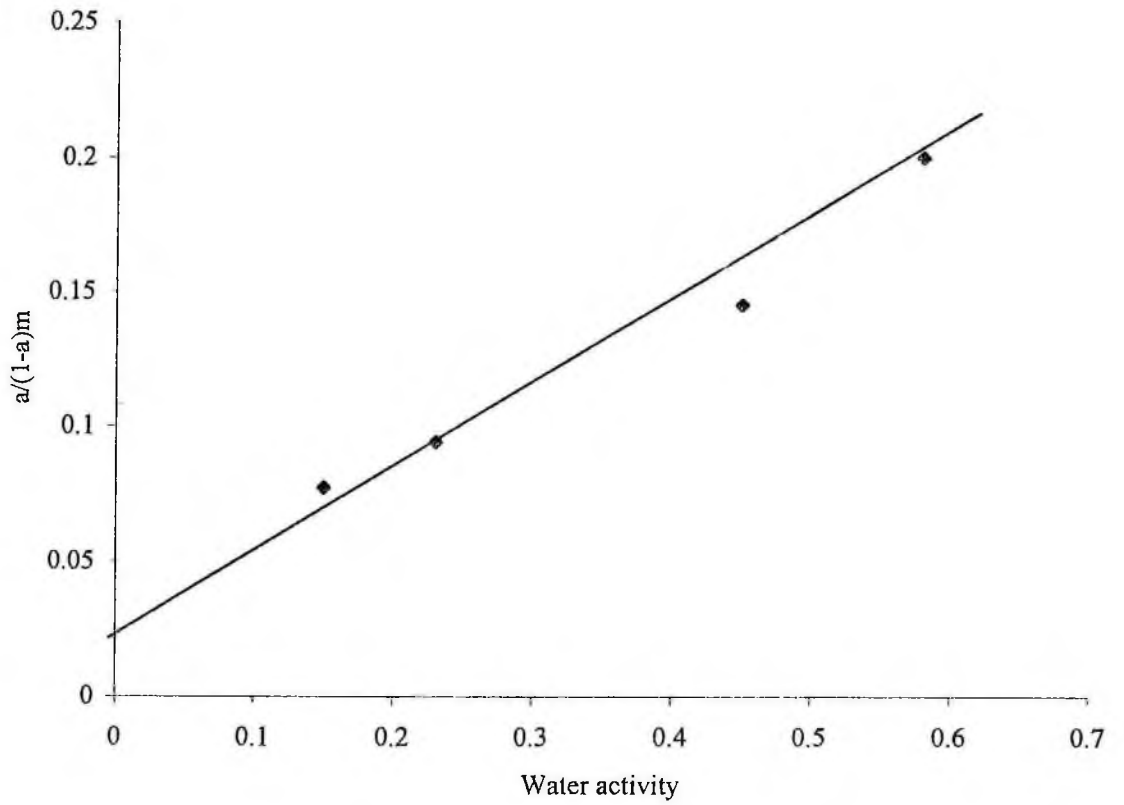


Fig 4.33 B.E.T plot for raw soyflour at 30°C

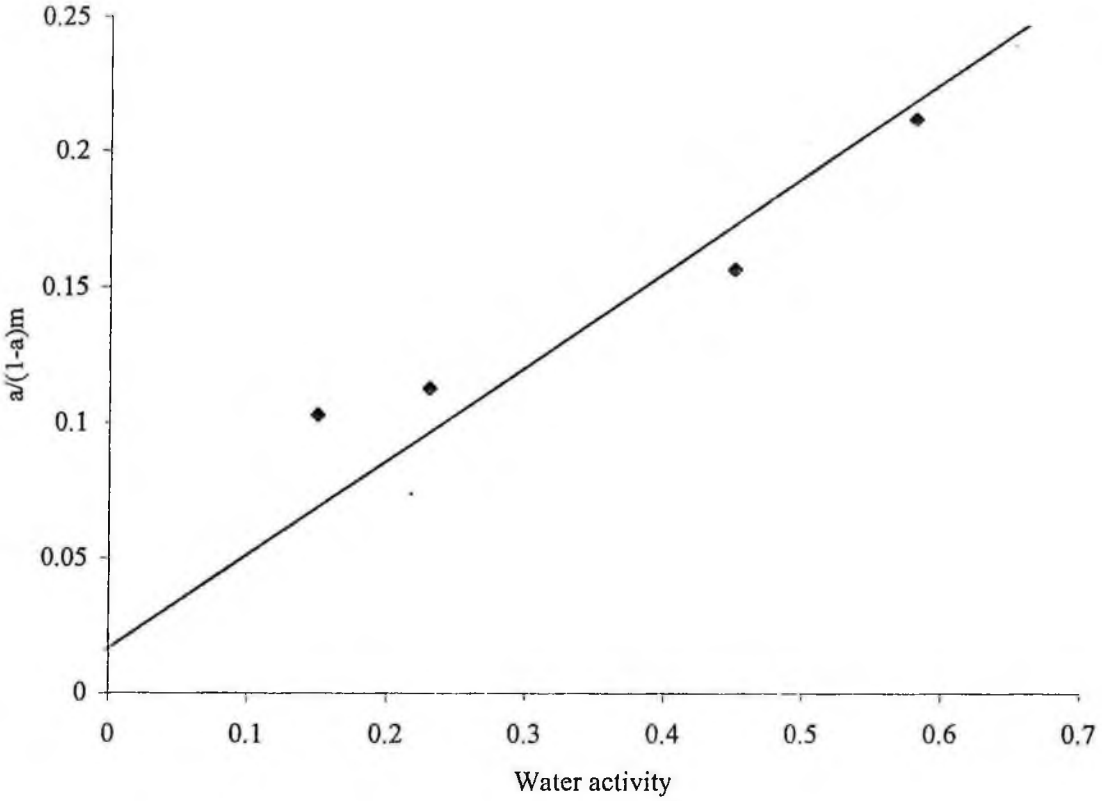


Fig4.34 B.E.T. plot for **cooked-dried** soyflour at 30°C.

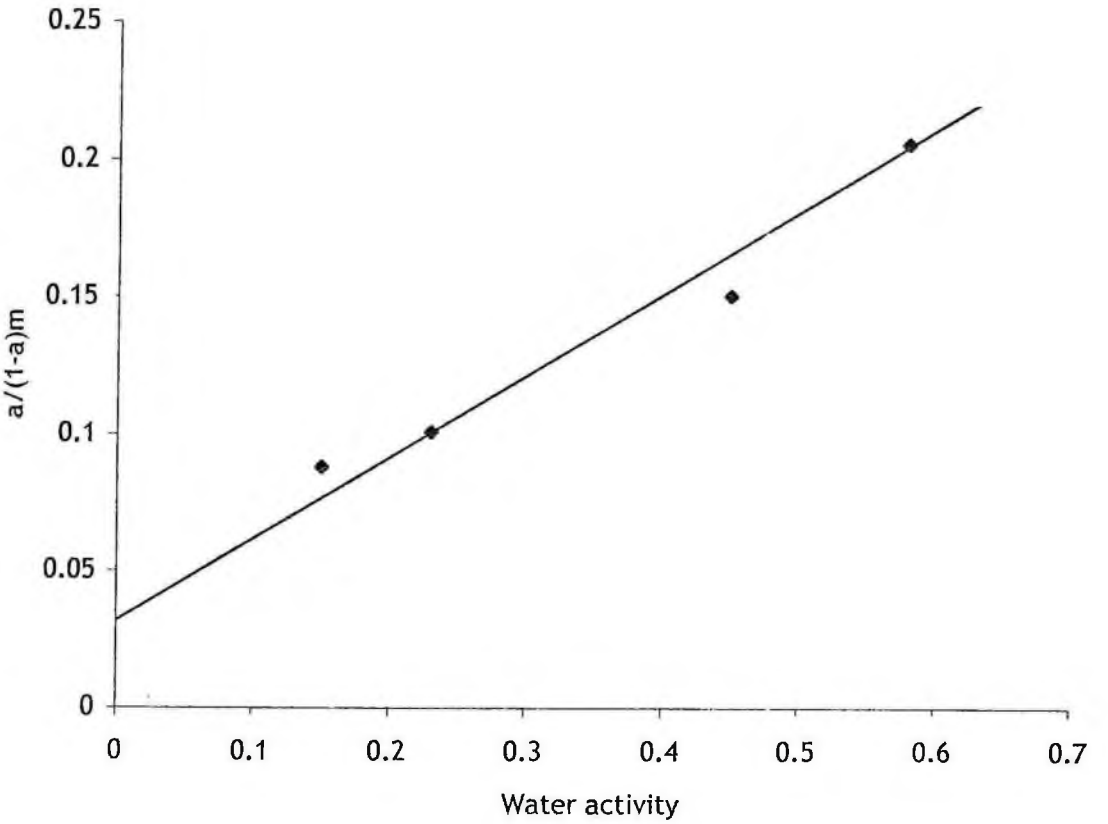


Fig4.35 B.E.T plot for **roasted** soyflour at 30°C

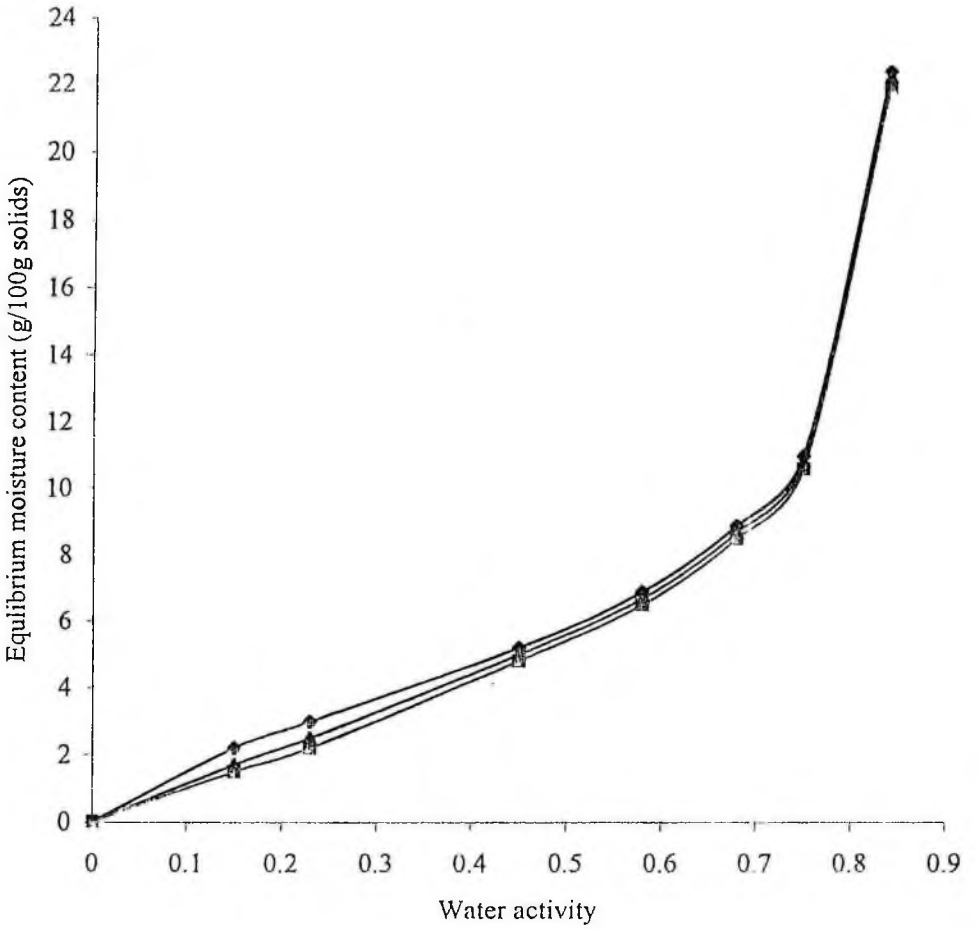


Fig 4.18 Moisture sorption isotherms for raw, cooked-dried and roasted soyflours at 30°C

The monolayer water is the amount of water in a system, which is unavailable as a solvent (Labuza, 1975). As indicated in Table 4.18, there was no significant difference in the monolayer values as well as the corresponding water activities in all the three soyflours.

4.5.2 Effect of water activity and time on peroxide value and TBA Number of stored soyflours

In all the three soyflours the P.V. and the TBA Number generally increased with time at all the selected water activities, that is, 0.15, 0.23 (average BET monolayer value), 0.45, 0.68 and 0.75. The P.V. was high at 0.15 a_w and then decreased to a minimum value at 0.23 a_w (that is, about 1.94 meq/kg in roasted soyflour, 2.21 meq/kg in cooked-dried soyflour and 3.89 meq/kg in raw soyflour) and thereafter increased gradually as a_w was increased (Fig.4.19).

A similar trend was observed for the TBA Number (Fig 4.20). The minimum value of P.V. and that of TBA Number obtained at 0.23 a_w (the approximate a_w corresponding to the monolayer value of all the three soyflours could be due to the water present in each sample, that is, 2.87% in raw, 2.96% in roasted and 2.93% in cooked-dried soyflours. This water might have formed a protective barrier, preventing the oxygen from reaching the underlying unsaturated fatty acids (Salwin, 1959). Halton and Fischer (1967) also proposed that the monolayer water retarded the diffusion of oxygen to the sites of the unsaturated double bonds.

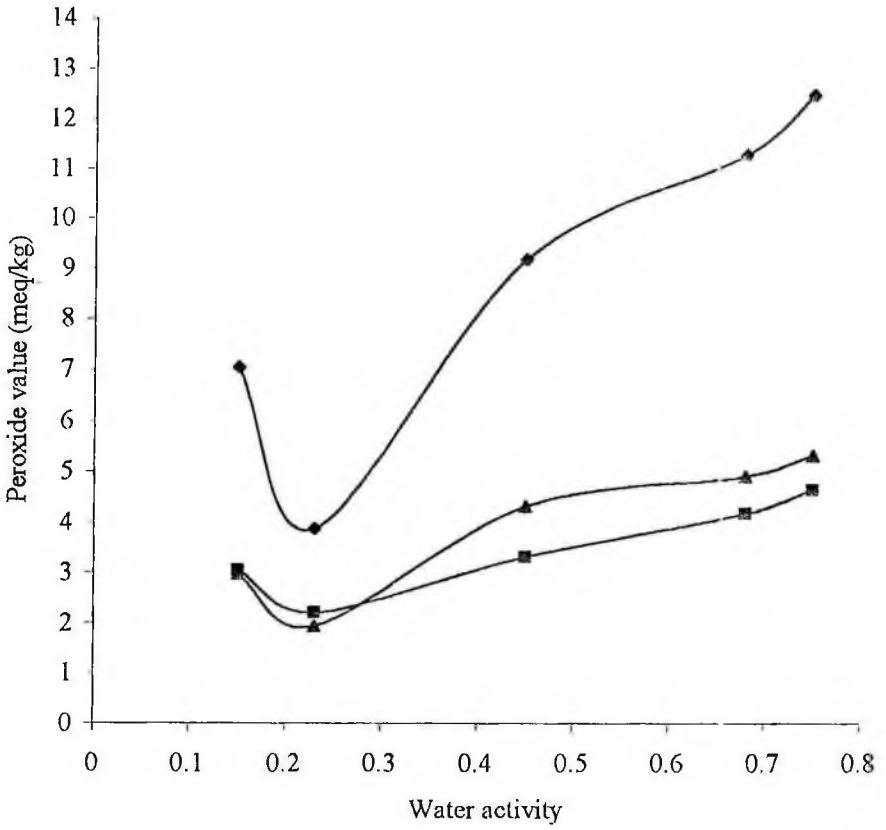


Fig 4.19 Peroxide values of raw, cooked-dried and roasted soyflours stored at various water activities, at 30°C, for a period of twelve (12) weeks.

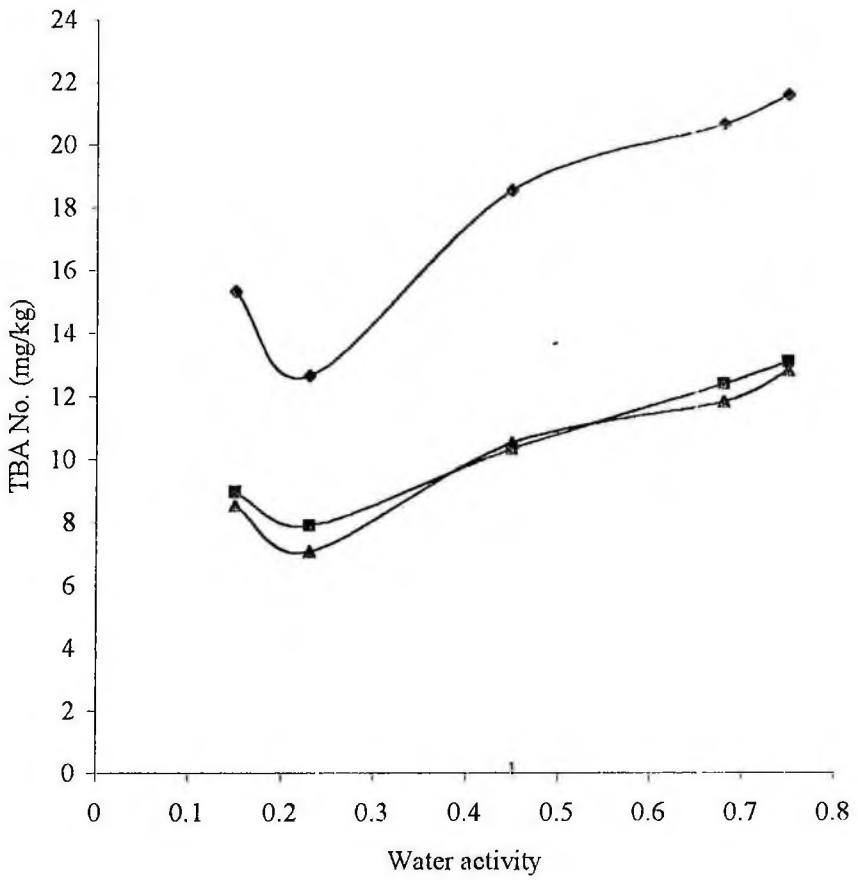


Fig 4.20 TBA Nos. of different types of soyflour stored at various water activities, at 30°C, over a period of twelve (12)weeks.

At a_w below the monolayer value the P.V. and the TBA Number decreased with increasing a_w and this could be explained from the fact that water was acting as an antioxidant at those water activities. This is attributed to water hydrogen- bonding to hydroperoxides and hydration of metal catalysts hence reducing the peroxides concentration and overall lipid oxidation rate (Heidelbaugh and Karel, 1970). The increase in the rate of oxidation at a_w above the monolayer value could be attributed to the increased water content, which reduces the viscosity of the solution hence promoting mobility of reactants (Heidelbaugh and Karel, 1970). Labuza (1975) also proposed that the increase in the rate of lipid oxidation as a_w increases might be due to dissolution of precipitated catalysts and swelling of solid matrices hence exposing new catalytic surfaces.

A multiple regression analysis for P.V in soyflour gave an R^2 of 94%, 92% and 95% for raw, cooked-dried and roasted soyflours respectively. None of the models showed a significant "lack of fit" (Appendices14, 15, and 16) implying that all the models are sufficient for predicting the P.V. in soyflour at any given value of water activity and time.

In all the soyflour samples both the linear terms of water activity and time, and the interaction between time and water activity affected the P.V. significantly. Storage time however had no significant second power effect on the P.V. in all the three soyflours (Table 4.8).

A 3-D response surface plot showing the P.V. as a function of water activity and time were generated from the regression equations and are shown in Figs 4.21 – 4.23.

Table 4.8 Further Anova for variation in the order fitted (showing only the P-values) for peroxide value in soyflours stored at various water activities (a_w) over a twelve-week period.

Source of variation	P-values		
	Raw	Cooked	Roasted
Time	0.0001*	0.0001*	0.0015*
a_w	0.0004*	0.0001*	0.0001*
$(Time)^2$	0.5142	0.6211	0.2993
$(aw)^2$	0.1518	0.0422*	0.3168
Time * a_w	0.0019*	0.0016*	0.0107*

* = Significant P-value

Table 4.9 Further Anova for variation in the order fitted (showing only the P-values) for TBA Number in soyflours stored at various water activities (a_w) over a twelve-week period.

Source of variation	P-values		
	Raw	Cooked	Roasted
Time	0.0001*	0.0001*	0.0001*
a_w	0.0006*	0.0001*	0.0001*
$(Time)^2$	0.5240	0.0339*	0.0275*
$(a_w)^2$	0.2344	0.0012*	0.2106
Time * a_w	0.0047*	0.0001*	0.0016*

* = Significant P-value

A multiple regression analysis for TBA Number in soyflour produced models with R^2 of 92%, 98% and 97% for raw, cooked-dried and roasted soyflours respectively. All the models had no significant "lack if fit" (Appendix17, 18, and19) hence the models could predict the TBA Number in soyflour for any given water activity and storage time.

The TBA Number in all the three soyflours were affected significantly by the linear terms of water activity and storage time as well as the interaction between the two factors (Table 4.9). Storage time had a second power effect on only the heat processed soyflours.

3-D response surface plots showing the TBA Number as a function of water activity and time were generated from the regression equations and are shown in Figures 4.24-4.26.

Regression equation for Fig 4.21

$Z = 0.972049 - 0.002047t + 7.825284w - 0.007642t^2 - 9.836994w^2 +$

$1.3338256wt$

Z = P.V.

w = water activity

t = storage time

R^2 =92%

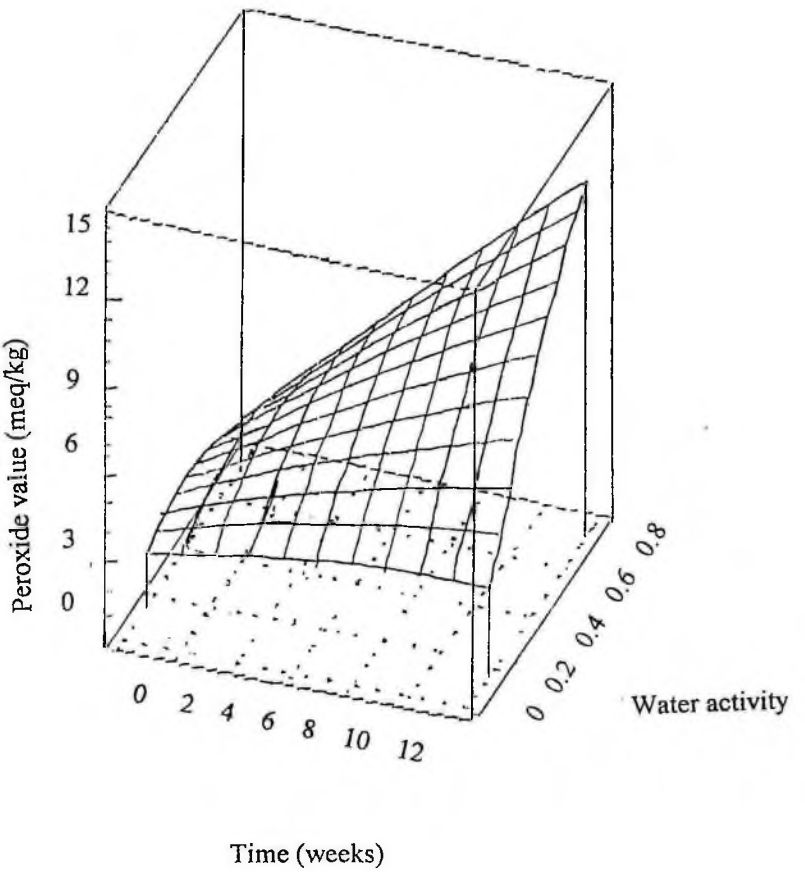


Fig 4.21 The 3-D response surface for the peroxide value (Z) in **raw** soyflour as a function of water activity (w) and time (t).

Regression equation for Fig 4.22

$Z = 1.33564 - 0.1213t + 1.266546w + 0.006453t^2 - 2.499652w^2 + 0.552486wt$

Z = P.V.

w = water activity

t = storage time

R^2 =95%

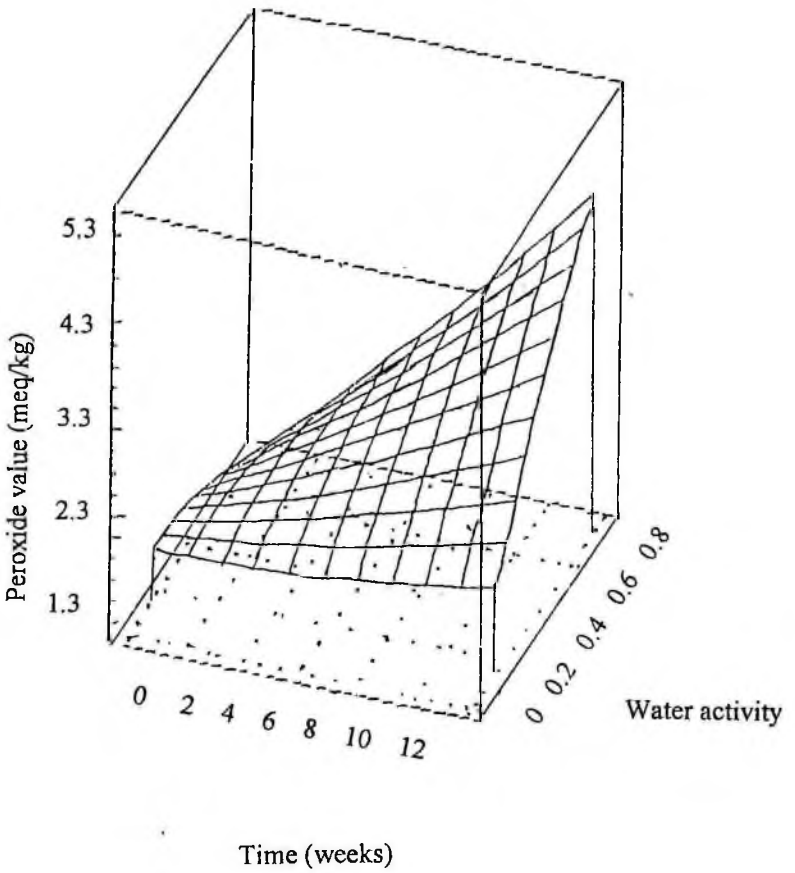


Fig 4.22 The 3-D response surface for the peroxide value (Z) in **cooked-dried** soyflour as a function of water activity (w) and time (t).

Regression equation for Fig 4.23

$Z = 1.38135 - 0.1122t + 1.256866w + 0.006293t^2 - 2.487654w^2 + 0.540447wt$

Z = P.V.

w = water activity

t= storage time

R2 = 95%

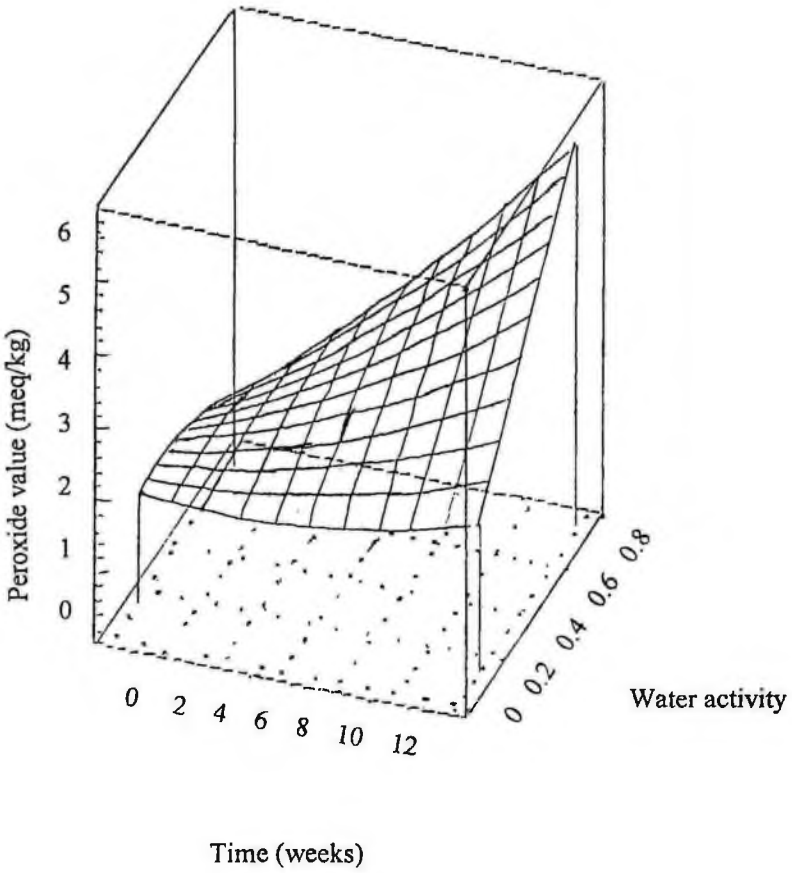


Fig 4.23 The 3-D response surface for the peroxide value (Z) in **roasted** soyflour as a function of water activity (w) and time (t)

Regression equation for Fig 4.24

$Z = 12.168641 - 0.125327t + 3.853998w - 0.011933t^2 - 7.480869w^2 + 1.581264wt$

Z = TBA no.

w = water activity

t = storage time

$R^2 = 92\%$

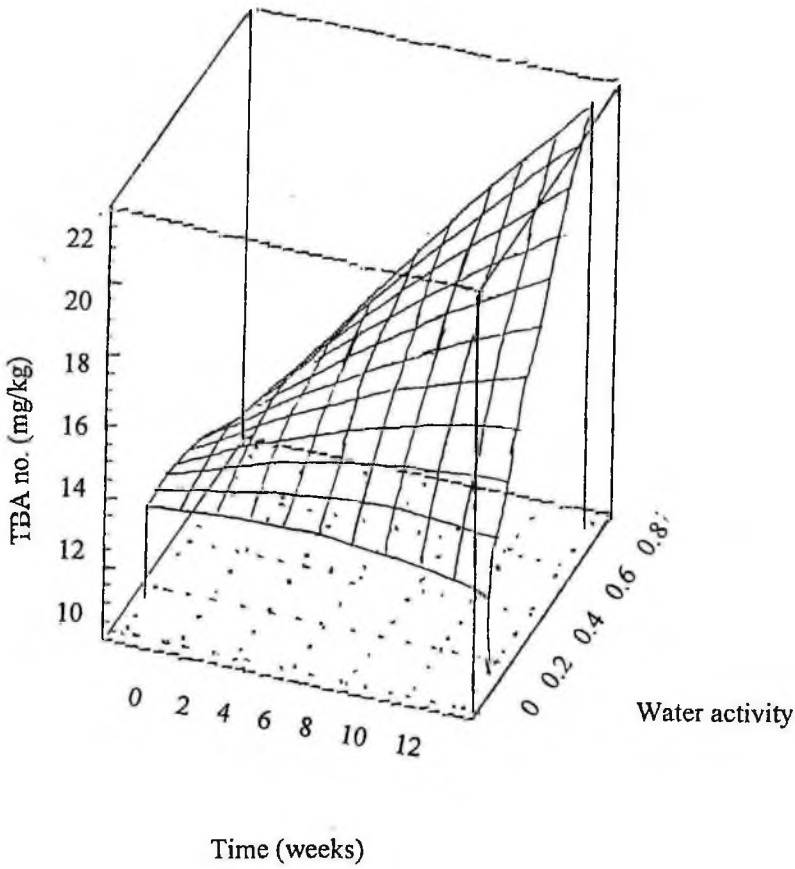


Fig 4.24 The 3-D response surface for the TBA no. (Z) in **raw** soyflour as a function of water activity (w) and time (t).

Regression equation for Fig 4.25

$Z = 8.125469 - 0.195971t - 4.025325w + 0.010167t^2 + 4.217257w^2 + 0.715521wt$

Z = TBA no.

w = water activity

t = storage time).

R^2 =98%

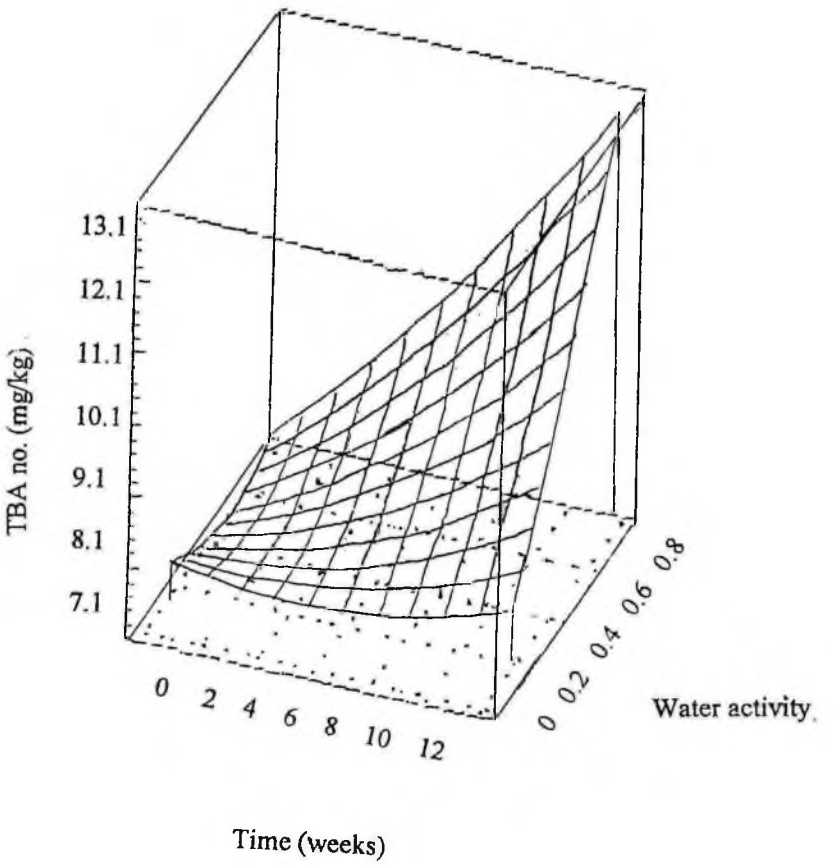


Fig 4.25 The 3-D response surface for the TBA no. (Z) in **cooked-dried** soyflour as a function of water activity (w) and time (t).

Regression equation for Fig 4.26

$Z = 7.346747 - 0.161642t - 2.648091w + 0.014505t^2 + 2.6804w^2 + 0.643557wt$

Z = TBA no.

w = water activity

t = storage time).

$R^2 = 97\%$

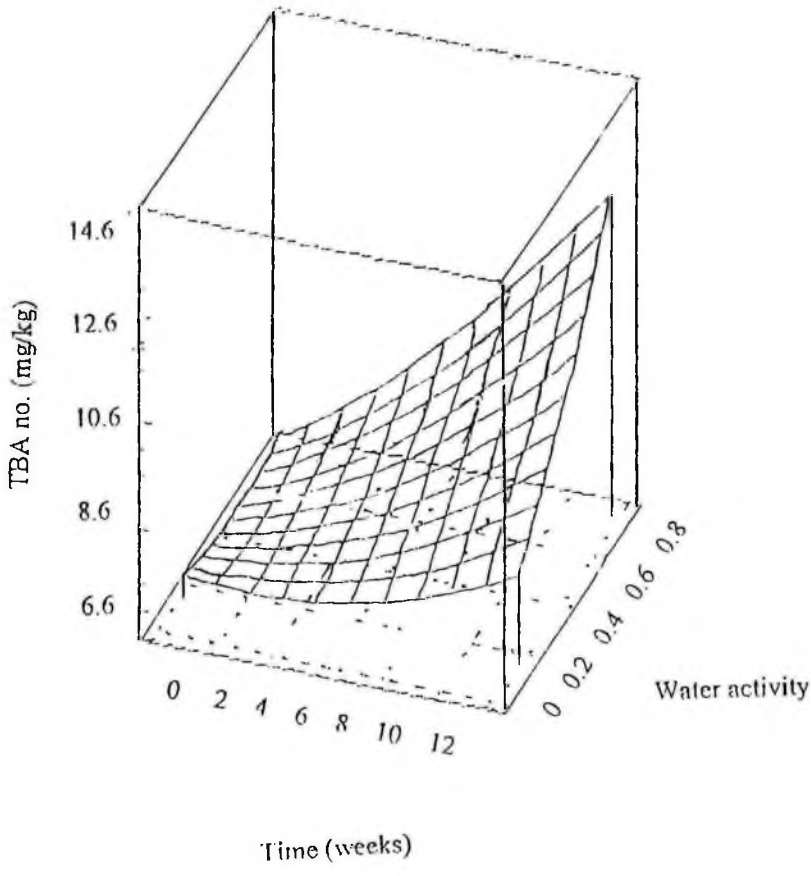


Fig 4.26 The 3-D response surface for the TBA no. (Z) in roasted soyflour as a function of water activity (w) and time (t).

4.5.3 Effect of water activity and time on free fatty acids in stored soyflours

The free fatty acid content of all the three soyflours increased with storage time and a_w as shown on Figures 4.27-4.29. These findings confirm the report by Acker and Wiese (1972) that lipid hydrolysis by the enzyme lipase increases with increase in a_w from the dry state.

Among the three soyflours raw soyflour recorded the highest rate of lipid hydrolysis and this might probably be due the fact that the sample was not given any heat treatment hence the lipase was not inactivated. It was also observed in all the three samples that the rate of FFA production increased at a_w 0.45, 0.68 and a_w 0.75 after about eight (8) weeks of storage. This might be explained from the fact that at these a_ws the samples might have absorbed more moisture hence growth of moulds and other microorganisms might have been encouraged. Some of these microorganisms might have produced lipases, and in the presence of adequate moisture rate of lipid hydrolysis might be increased (Whitaker, 1972).

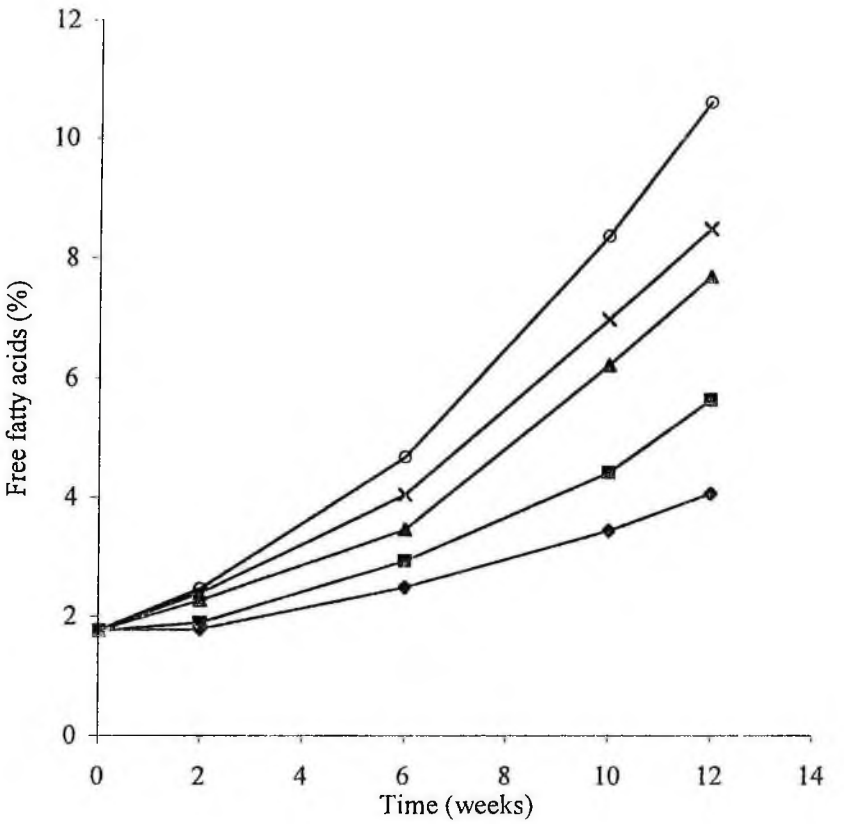


Fig 4.27 Effect of water activity and storage time on the **free fatty acids** in **raw** soyflour at 30°C.

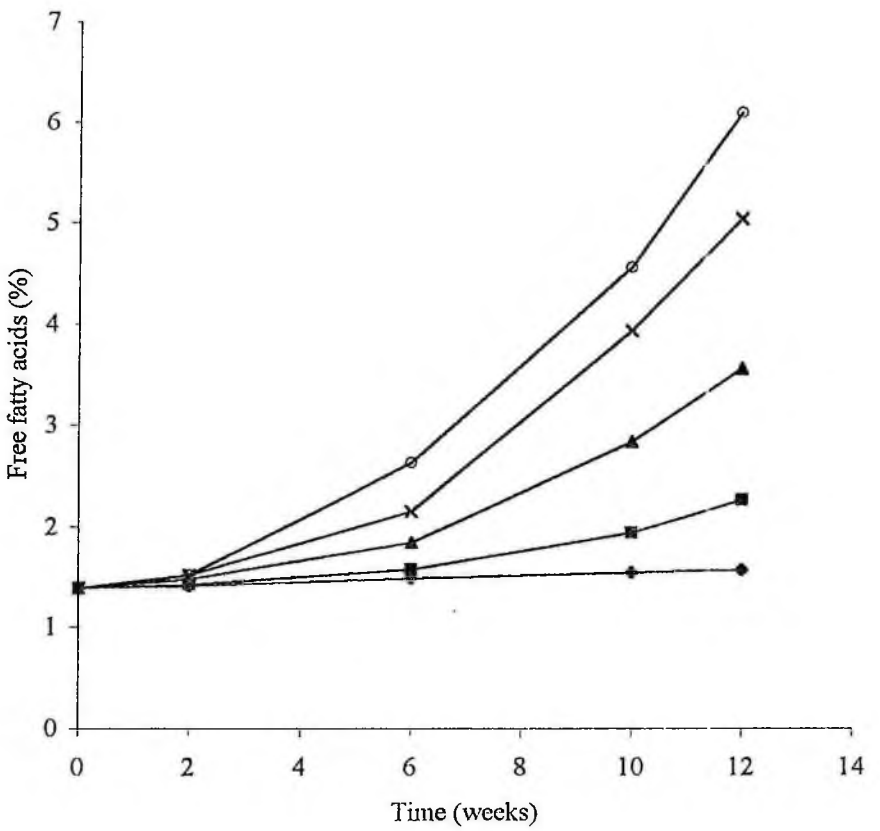


Fig 4.28 Effect of water activity and storage time on the **free fatty acids** in **cooked-dried** soyflour at 30°C.

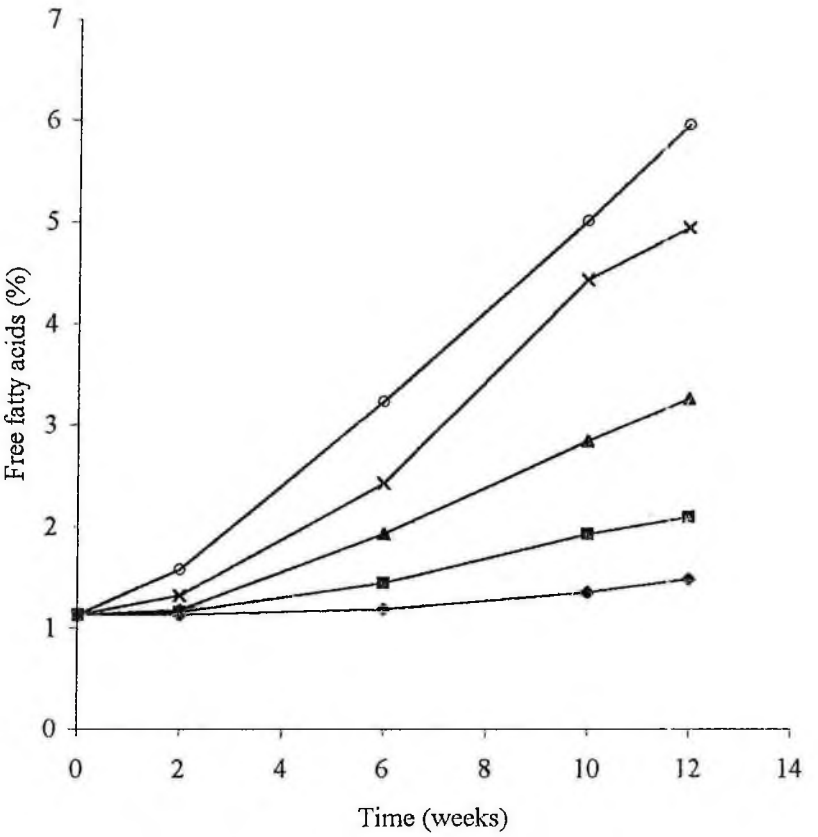


Fig 4.29 Effect of water activity and storage time on the **free fatty acids** in **roasted** soyflour at 30°C.

A multiple regression analysis for FFA in soyflour produced regression models with R^2 of 99%, 99% and 98% for raw, cooked-dried and roasted soyflours respectively. None of the models had a significant "lack of fit" (Appendix11, 12, and 13).

The linear terms of the two factors, water activity and time, had significant effect on the FFA in all the three soyflours (Table 4.10). Interaction between the two factors also affected the FFA significantly. The quadratic term of time however affected only the FFA in raw and cooked-dried soyflours significantly. Water activity also had a second power effect on the free fatty acids in cooked-dried and roasted soyflours.

3-D response surface plots showing the FFA in soyflour as a function of water activity and storage time, generated from the regression equations are shown in Figs 4.30 – 4.32.

Table 4.10 Further Anova for variation in the order fitted (showing only the P-values) for FFA content in soyflours stored at various water activities (a_w) over a twelve-week period.

Source of variation	P-values		
	Raw	Cooked	Roasted
Time	0.0001*	0.0001*	0.0001*
a_w	0.0001*	0.0001*	0.0001*
$(Time)^2$	0.0001*	0.0001*	0.0609
$(a_w)^2$	0.8391	0.0027*	0.0303*
Time $*a_w$	0.0001*	0.0001*	0.0001*

* = Significant P-value

4.5.4 Effect of lipid oxidation and hydrolysis on total lipids and crude fat content in soyflour

The total lipids in all the three soyflours were found to decrease at the end of the storage period as shown on Table 4.3. Raw soyflour had the highest total lipid loss of 6.50% followed by cooked-dried and roasted soyflours with losses of 1.64% and 1.58% respectively. Like the total lipids the crude fat content of the soyflours decreased with storage time. At the end of the twelve-week storage period raw soyflour lost 8.58% of the crude fat whilst cooked-dried and roasted flours lost 2.78% and 2.38% respectively.

Table 4.11 Total lipids in various forms of freshly prepared soyflour as well as those stored for twelve (12) weeks at 30°C.

Soyflour type	Moisturecontent (%)		Total lipids (%)		Storage loss
	Fresh	Stored	Fresh	Stored	Total lipids loss (%)
Raw	9.78	9.98	26.29 ± 0.09	24.58 ± 0.21	6.50
Roasted	5.51	5.71	26.56 ± 0.11	26.14 ± 0.15	1.58
Cooked-dried	4.94	5.02	26.82 ± 0.15	26.36 ± 0.24	1.64

Regression equation for Fig 4.30

$Z = 1.835393 - 0.183203t - 0.143718w + 0.032932t^2 + 0.247847w^2 + 0.572356wt$

Z = FFA

w = water activity

t = storage time).

R^2 =99%

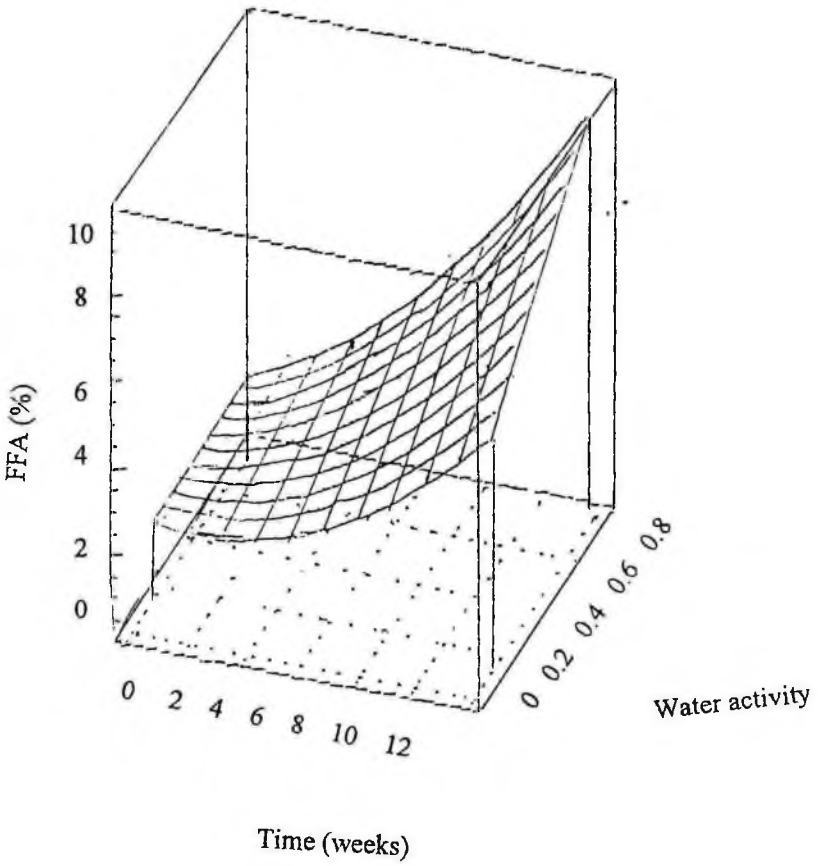


Fig 4.30 The 3-D response surface for the free fatty acids (Z) in **raw** soy flour as a function of water activity (w) and time (t)

Regression equation for Fig 4.31

$Z = 2.24105 - 0.256899t - 2.838473w + 0.016738t^2 + 1.984741w^2 + 0.53058wt$

Z = FFA

w = water activity

t = storage time

R^2 =99%

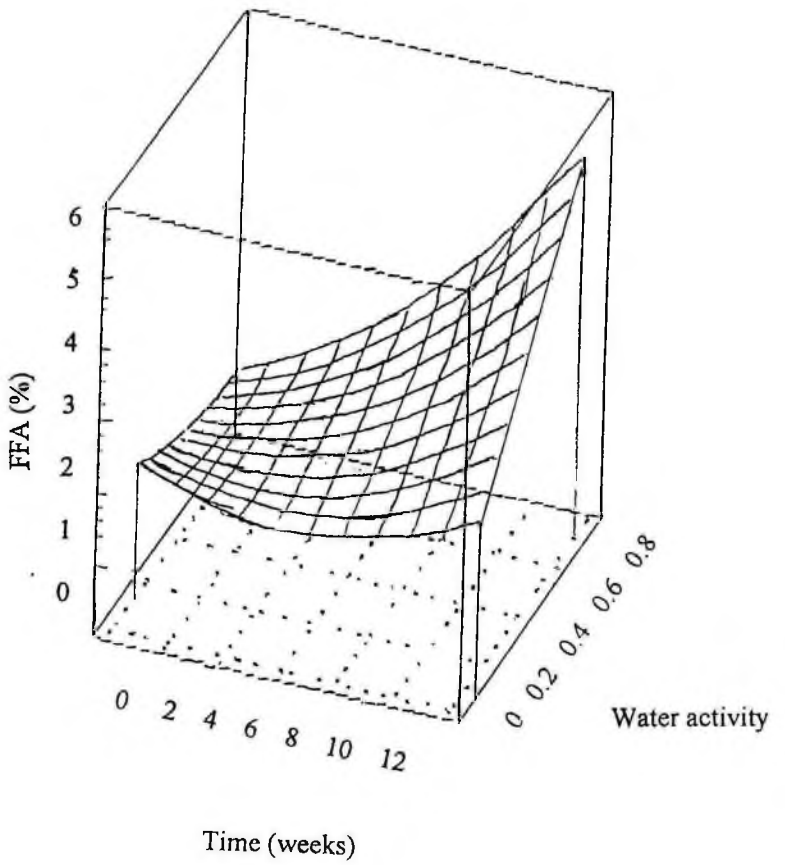


Fig 4.31 The 3-D response surface for the free fatty acids (Z) in **cooked-dried** soyflour as a function of water activity (w) and time (t).

Regression equation for Fig 4.32

$Z = 1.889946 - 0.171717t - 3.404782w + 0.006987t^2 + 2.920305w^2 + 0.658826wt$

Z = FFA

w = water activity

t = storage time

R^2 =98%

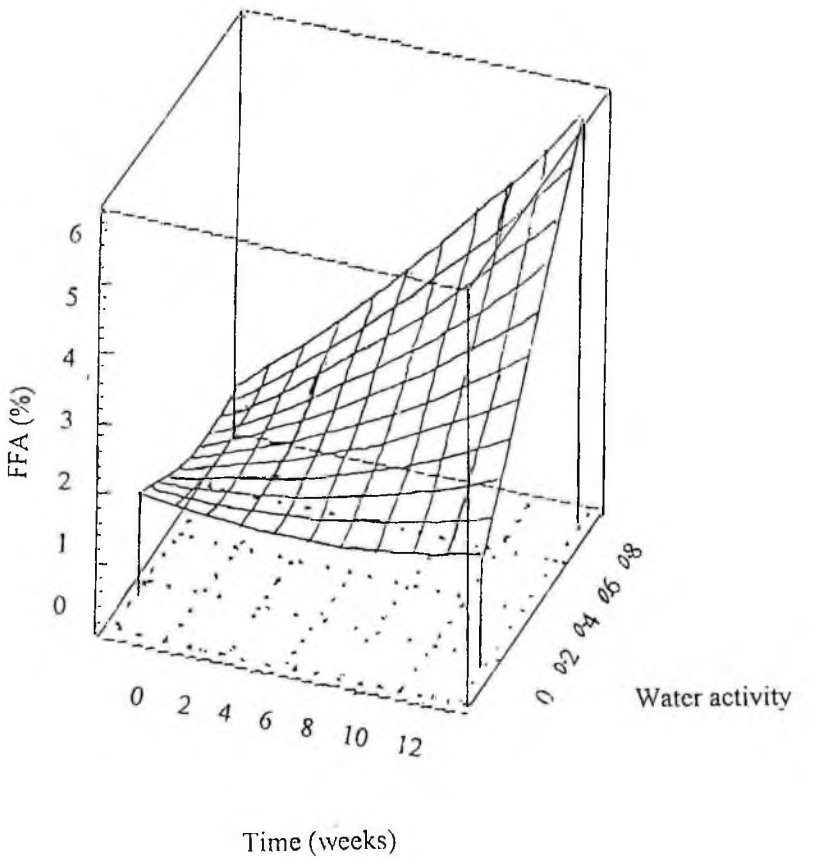


Fig 4.32 The 3-D response surface for the free fatty acids (Z) in roasted soyflour as a function of water activity (w) and time (t).

Table 4.12 Crude fat content in various forms of freshly prepared soyflours as well as that stored for twelve (12) weeks at 30°C.

Soyflour type	Moisture content (%)		Crude fat (%)		Storage loss
	Fresh	Stored	Fresh	Stored	Crude fat loss (%)
Raw	9.78	9.98	20.16 ± 0.09	18.43 ± 0.11	8.58
Roasted	5.51	5.71	20.56 ± 0.11	20.07 ± 0.15	2.38
Cooked-dried	4.94	5.02	20.48 ± 0.15	19.91 ± 0.12	2.78

4.5 Sensory evaluation of soyflour samples

Sensory evaluation was performed on the samples at four (4) different times during storage. A panel, made up of sixteen (16) judges, was used and each was given a fresh and a stored sample of each of the three soyflour types to compare in terms of flavour. The results are shown on Tables 4.13 and 4.14. Whenever at least 50% of panellists detected a difference in the flavour of the samples in each pair the results was considered significant.

According to the results shown on table 4.14, only 31.25% of the panellists were able to detect changes in the flavour of the raw soyflour stored at 30°C. All the sixteen panellists could not detect any change in the flavour of all the other samples. By the end of the eighth week however, all the panellists could detect a difference between the fresh raw soyflour and the stored one at 30°C.

At the end of the twelve weeks storage period, 50% and 43.75% of the panellists detected a change in the flavours of cooked-dried and roasted soyflours, stored at 30°C, respectively. This is an indication that, flavour changes in processed soyflour become detectable after storage for three months. This detection occurred at peroxide value and TBA Number of 4.21 meq/kg and 9.76 mg/kg respectively in cooked-dried soyflour and 4.01 meq/kg and 9.57 mg/kg respectively in roasted soyflour.

Zadernowski *et al.* (1983) reported that refined soybean oils in good condition have TBA Number in the range 0.02 to 0.08 mg/kg whereas crude oil or badly stored oils have TBA Number in the range 15 to 20 mg/kg. He also reported that the P.V. of fresh soybean oil is well below 10 meq/kg and that rancidity begins beyond 20 meq/kg. The disparity in this report and my findings could be explained as follows. The oil in the soybean flour is in the presence of non- lipidic substances such as proteins and polysaccharides. It was reported by Pokorny *et al.* (1983) that in the presence of protein there is rapid destruction of hydroperoxides as some amino acids react with the peroxides. As a result the peroxide value measured in the soyflour appears to be lower than in the extracted oil. Pokorny *et al.* (1983) also reported that the secondary oxidation products especially

malondialdehyde react with proteins to produce compounds including some volatile compounds which do contribute to flavour changes. As a result the TBA test does not measure all the TBA reactive substances produced from secondary oxidation hence the TBA Number measured appears to be lower than in the extracted oil. However since some of the volatile products produced from the reaction of the TBA reactive substances with proteins affect flavour, changes can thus occur in flavour of soyflour at a lower TBA Number than it will in the extracted oil.

Table 4.13 Sensory perceptions of flavour changes in various types of soyflour stored at 5°C.

Length of storage (weeks)	Judges' Response (%)					
	Raw		Cooked-dried		Roasted	
	Y	N	Y	N	Y	N
4	0	100	0	100	0	100
8	12.5	87.5	0	100	0	100
10	25	75	12.25	87.5	6.25	93.75
12	43.75	56.25	18.75	81.25	18 75	81.25

Y = percentage of judges who could detect a difference in the flavours of freshly prepared and stored soyflours.

N = percentage of judges who could **not** detect any difference in the flavours of freshly prepared and stored soyflours.

Table 4.14 Sensory perceptions of flavour changes in various types of soy flour stored at 30°C.

Length of storage (weeks)	Judges' Response (%)					
	Raw		Cooked-dried		Roasted	
	Y	N	Y	N	Y	N
4	31.25	68.75	0	100	0	100
8	100 **(s)**	0	25	75	18.75	81.25
10			31.25	68.75	25	75
12			50 **(s)**	50	43.75	56.25

Y = percentage of judges who could detect a difference in the flavours of freshly prepared and stored soyflours.

N = percentage of judges who could **not** detect any difference in the flavours of freshly prepared and stored soyflours.

(s) =Significant difference

Flavour changes in all the samples stored at 5°C occurred very slowly. The raw flour however showed the highest rate of flavour change. As shown on Table 4.13, 43.75% of the panellists detected flavour change in the raw soyflour stored at 5°C after twelve weeks while only 18.75% of the panellists detected flavour change in both the cooked-dried and the roasted flours. These results indicate that raw soyflour has the tendency to undergo flavour changes faster than processed soyflours. It also shows that both moist (cooked-drying) and dry (roasting) heat processing do not have any significant difference in terms of slowing down the rate of off flavour development in soyflour. Dry heat processing however appeared to have the most pronounced effect.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

1. Heat-treatment of soybeans prior to processing into flours yield flours with lower rates of chemical reactions, which result in minimal lipid oxidation and hydrolysis.
2. Cold storage of soyflour results in lower rates of chemical reactions, which cause increase in the stability of lipids in soyflour.
3. Maintaining the moisture content of soyflour at or close to the monolayer value of soyflour leads to lower rates of chemical reactions, which result in increased rate of lipid stability.
4. The shelf life of soyflour can be predicted when the peroxide value and the TBA Number of soyflour are known.
5. It is possible to extend the shelf life of soyflour by
    1. Heat-processing the beans prior to milling into flour
    2. Storing the soyflour under cold condition.

5.2 Recommendations

1. This studies has revealed that full-fat soyflour is liable to oxidative activities under tropical conditions therefore it is recommended that studies be conducted for the development of appropriate low-cost effective packaging for soyflour at ambient storage conditions.

2. This study has shown that soybean is used in the fortification of some cassava products and maize flour in Ghana. It is therefore recommended that more studies be conducted on soybean-fortified foods so as to determine the effect of other ingredients on lipid stability in soyflour.

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APPENDICES

APPENDIX 1: NUTRITION AND FOOD SCIENCE DEPARTMENT

UNIVERSITY OF GHANA, LEGON

MPHIL PROJECT.

SENSORY EVALUATION OF SOYFLOUR SAMPLES

Date..... Panelist number.....

You are provided with three (3) pairs of soyflour samples. Please taste and smell each pair (one at a time) and record whether the samples in each pair are different in flavour or not.

Note: where difference exists between a pair of samples indicate it by writing 'Y', where no difference is detected indicate it by writing 'N' at the appropriate place.

	Sample Pair	**Flavour**
1	---------	--------
2	---------	--------
3	---------	--------

Comment.....

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APPENDIX2: Analysis of variance for the full regression for FFA in **raw** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	7.7162	5	1.5433	16.9767	0.0004
Error	0.7272	8	0.0909		
Total (corr)	8.4435	13			
Lack of fit	0.4282	3	0.1427	2.3	
Pure error	0.2990	5	0.0598		

APPENDIX3: Analysis of variance for the full regression for FFA in **cooked-dried** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	1.2666	5	0.2533	48.0931	0.0001
Error	0.0421	8	0.0053		
Total (corr)	1.3087	13			
Lack of fit	0.0293	3	0.0098	3.78	
Pure error	0.0129	5	0.0026		

APPENDIX4: Analysis of variance for the full regression for FFA in **roasted** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	1.6231	5	0.3246	33.5679	0.0001
Error	0.0774	8	0.0097		
Total (corr)	1.7004	13			
Lack of fit	0.06	3	0.02	5.78	
Pure error	0.0173	5	0.0034		

APPENDIX5: Analysis of variance for the full regression for P.V in **raw** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	267.741	5	53.5482	39.9118	0.0001
Error	10.7333	8	1.3416		
Total (corr)	278.474	13			
Lack of fit	6.8333	3	2.2778	2.9	
Pure error	3.9	5	0.78		

APPENDIX6: Analysis of variance for the full regression for P.V in **cooked-dried** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	16.3158	5	3.2632	144.197	0.0001
Error	0.1810	8	0.0226		
Total (corr)	16.4969	13			
Lack of fit	0.1732	3	0.05776	37	
Pure error	0.0078	5	0.0016		

APPENDIX7: Analysis of variance for the full regression for P.V in **roasted** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	23.5544	5	4.7109	18.9013	0.0003
Error	1.9939	8	0.2492		
Total (corr)	25.5482	13			
Lack of fit	1.4439	3	0.4813	4.8	
Pure error	0.5	5	0.1		

APPENDIX8: Analysis of variance for the full regression for TBA in **raw** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	51.3228	5	10.2646	235.805	0.0001
Error	0.3082	8	0.0435		
Total (corr)	51.6711	13			
Lack of fit	0.3438	3	0.1146	127.33	
Pure error	0.0045	5	0.0009		

APPENDIX9: Analysis of variance for the full regression for TBA in **cooked-dried** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	14.2961	5	2.8592	280.274	0.0001
Error	0.08161	8	0.01020		
Total (corr)	14.3777	13			
Lack of fit	0.0691	3	0.0230	9.208	
Pure error	0.0125	5	0.0025		

APPENDIX10: Analysis of variance for the full regression for TBA in **roasted** soyflour as a function of temperature and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	12.2189	5	2.4438	59.0055	0.0001
Error	0.3310	8	0.0414		
Total (corr)	12.5499	13			
Lack of fit	0.2471	3	0.0824	4.9	
Pure error	0.084	5	0.0168		

APPENDIX11: Analysis of variance for the full regression for FFA in **raw** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	38.4550	5	7.6910	355.870	0.0001
Error	0.1729	8	0.0216		
Total (corr)	38.6279	13			
Lack of fit	1.629	3	0.0543	27	
Pure error	0.0099	5	0.0019		

APPENDIX12: Analysis of variance for the full regression for FFA in **cooked-dried** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	7.9576	5	1.5915	463.569	0.0001
Error	0.0275	8	0.0034		
Total (corr)	7.9851	13			
Lack of fit	0.0211	3	0.0070	5.48	
Pure error	0.0064	5	0.0013		

APPENDIX13: Analysis of variance for the full regression for FFA in **roasted** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	11.5539	5	2.3108	117.529	0.0001
Error	0.1573	8	0.0197		
Total (corr)	11.7112	13			
Lack of fit	0.1365	3	0.0455	10.9351	
Pure error	0.0208	5	0.0042		

APPENDIX14: Analysis of variance for the full regression for P.V in **raw** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	61.4134	5	12.2827	46.4994	0.0001
Error	2.1132	8	0.2641		
Total (corr)	63.5265	13			
Lack of fit	1.5032	3	0.5011	4.1	
Pure error	0.61	5	0.122		

APPENDIX15: Analysis of variance for the full regression for P.V in **cooked-dried** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	3.2732	5	0.6546	33.9167	0.0001
Error	0.1544	8	0.0193		
Total (corr)	3.4276	13			
Lack of fit	0.1271	3	0.0424	7.8	
Pure error	0.0273	5	0.0055		

APPENDIX16: Analysis of variance for the full regression for P.V in **roasted** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	8.9545	5	1.7909	20.7366	0.0002
Error	0.6909	8	0.0864		
Total (corr)	9.6454	13			
Lack of fit	0.5316	3	0.1772	5.56	
Pure error	0.1593	5	0.0318		

APPENDIX17: Analysis of variance for the full regression for TBA in **raw** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	52.8308	5	10.5662	19.6279	0.0003
Error	4.3066	8	0.5383		
Total (corr)	57.1374	13			
Lack of fit	2.6224	3	0.8741	2.59	
Pure error	1.6842	5	0.3368		

APPENDIX18: Analysis of variance for the full regression for TBA in **cooked-dried** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	17.0330	5	3.4066	112.259	0.0003
Error	0.2427	8	0.0303		
Total (corr)	17.2758	13			
Lack of fit	0.2374	3	0.0791	73.26	
Pure error	0.0054	5	0.0011		

APPENDIX19: Analysis of variance for the full regression for TBA in **roasted** soyflour as a function of water activity and time

Source of variation	Sum of squares	D.F	Mean square	F-ratio	P-value
Model	19.5235	5	3.9047	63.264	0.0003
Error	0.4938	8	0.0617		
Total (corr)	20.0173	13			
Lack of fit	0.4303	3	0.1434	11.3	
Pure error	0.0635	5	0.0127		