CHAPTER 1

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Shea butter, extracted from the shea kernels, is the main source of edible oil for the people of Northern Ghana, and therefore the most important source of fatty acids and glycerol in their diet. The shea nut industry serves as the main source of livelihood for rural women and children who are engaged in its gathering. In addition to its use for food, shea butter is also used as an unguent for the skin and has anti-microbial and soothing properties, which give it a place in herbal medicine for the indigenes. It is also used in the pharmaceutical and cosmetic industries as an important raw material and/or a precursor for the manufacture of soaps, candles, and cosmetics (Adomako, 1985). Shea butter also finds use as a sedative or anodyne for the treatment of sprains and dislocations and also for the relief of minor aches and pains (*Personal Communication*, A. S. Sulemana, October 5, 2006).

Other important uses include its use as an anti-microbial agent for the promotion of rapid healing of wounds**,** as a material for greasing bread tins in baking and as a lubricant for donkey carts **(**Marchand, 1988**)**. Its by-products, the brown solid that is left after extracting the oil (shea nut cake) and the hard protective shell, are used as a water-proofing material on the walls of mud-buildings to protect them from wind and rain erosion. Poor quality butter is not only applied to earthen walls but also to doors, windows, and even beehives as a waterproofing agent (Marchand, 1988). In a traditional setting, shea butter of poor quality is used as an illuminant (or fuel, in lamps or as candles). The use of shea nut cake in animal feeding has been tried with some success (Okai and Bonsi, 1989). Although shea nut cake has been used as a substitute for maize in the diets of growing gilts, various anti-nutritional factors limit its use in large quantities (Okai and Bonsi, 1989).

Despite all these attributes, shea butter of commerce has a relatively short shelf – life owing to its high degree of unsaturation (Adomako, 1977).

1.1.1 Problem Statement

Shea butter has approximately 50% by weight of unsaturated fatty acids consisting of oleic (40.8%), linoleic acid (6.9%) and linolenic acid (1.6%), and a degree of unsaturation of 0.59% (Adomako, 1977, 1985). It is characterised by low palmitic and high stearic acid content as well as high iodine number (IV), acid number and free fatty acid (FFA) values (Adomako, 1977). The high unsaturated fatty acid content of shea butter accounts for its high iodine number, making it prone to oxidative rancidity. This rancidity results in the pungent odour or taste of shea butter within a short period during storage (Adomako, 1985). Although naturally occurring antioxidants in shea butter impart certain amount of protection against oxidation, these antioxidants are often lost during processing, necessitating the addition of exogenous antioxidants (Coppen, 1994).

Traditional processors have tried to solve this problem of oxidative rancidity during storage by using different quantities of extracts from the roots of the plant *Cochlospermum planchonii.*, resulting in wide variation in the colour of shea butter found on the Ghanaian market. There is also no reported characterisation of the *C. planchonii* root dye/extract in the literature to enable the determination of optimum levels of the extract required by processors.

The high concentration of *C. planchonii* root extract used during traditional processing may affect formulation of products in the pharmaceutical, confectionery and cosmetics industries. Therefore traditionally processed shea butter treated with varying concentration of *C. planchonii* root extract make them unsuitable for the International/export market. More importantly, although *C. planchonii* root extract has been in use for several decades in the shea butter industry, the Ghana Standards Board, the Food Research Institute of the Council for Scientific and Industrial Research and the Food and Drugs Board have very little information on its uses as well as its

effectiveness in retarding rancidity in shea butter. Furthermore, there is no documentary evidence of the type of antioxidants used to retard rancidity in shea butter.

1.1.2 General Objectives

The project seeks to characterize the root extract of *C. planchonii* and determine its effective concentration which can impart a definite shelf–life to shea butter under storage conditions similar to those practised by the traditional processors.

1.1.2.1 Specific Objectives

a). To chemically characterise the *C. planchonii* root dye/extract

b). To monitor stability of the dye during storage

c). To determine the effect of different concentrations of the root extract on free fatty acid, iodine value levels and peroxide values of shea butter stored under aerobic and anaerobic conditions.

1.2.0 LITERATURE REVIEW

1.2.1 Ecology of Shea Tree

The shea tree (**Plate 1)** also known as *Vitellaria paradoxa* (Maranz *et al.*, 2003) or *Butyrospermum parkii* belongs to the *Sapotaceae* family. It grows wildly in the dry savannah belt of West Africa, from Senegal, in the west, to Sudan in the east. It occurs on an estimated 1million

km^2, where annual rainfall ranges from 500 to 1200 mm (Salle *et al.*, 1991; Boffa, 1995). The species is found on various soil types on dry open slopes but avoids alluvial hollows or land subject to flooding. It is a fire-tolerant plant and therefore indigenous to the Guinea Savannah Woodland. It usually grows to an average height of about 15 metres. In Ghana, it virtually covers about two-thirds of the country, mostly in the wild state (Abbiw, 1990).

The tree grows slowly from seed, taking 12 to 15 years to bear fruit and about 30 years to mature (Adomako, 1985). It starts flowering in early November, with picking or gathering of fruits lasting from April to August every year. When the shea fruits (**Plate 2**) ripen, they fall under their own weight to the ground and are gathered by hand. The fruit (**Plate 3**) is green in colour, has a fleshy edible pulp which contains protein and carbohydrates, and is very sweet. Maranz *et al.*, 2003). The fruit pulp is a particularly rich source of ascorbic acid, iron and calcium (FAO, 1988). Within the fleshy pulp is the nut (**Plate 4**), which in turn, houses the kernel (**Plate 5**). Although shea nuts are a major commodity, it is not a plantation crop (Olaniyan *et al.*, 2007). The nuts sold on the International market are harvested from village tree populations in several West African countries. The fat content of the kernel and fatty acid profile are, however, extremely variable across this zone (Maranz *et al.*, 2004). The fat obtained from the shea kernel is referred to as shea butter and it is the most valued product from the shea tree (Hall *et al.*, 1996).

Plate 1. Shea Tree

Plate 2. Shea Tree with Fruits

PLATE 3. Ripe Shea fruits

PLATE 4. Shea nuts

PLATE 5. Dry Shea Kernels

1.2.1.1 Local Processing of Shea butter

The process involves gathering of the fruits from the wild, pre-treatment and then extraction to obtain the butter. The process of gathering fruits from the wild brings with it unwanted foreign materials. The first stage of pre-treatment, therefore, involves the removal of such unwanted materials. Shea fruits are then left in the open to ferment for between 3 – 5 days after which they are de-pulped to separate the fruit pulp (mesocarp and epicarp) from the nuts. The fermentation facilitates the removal of the fleshy pulp. The nuts are then sun-dried for 5 – 10 days to reduce their moisture content. The nut, which is made up of a hard outer shell with the kernel inside, is pounded in a mortar with a pestle, cracked between two stones or trampled upon with the feet to liberate the kernel (Salunkhe *et al.,* 1992). The nuts are then thoroughly dried for 10 - 20 days, depending on weather conditions (*Personal communication*, A. S. Sulemana, October, 2006), bagged and stored for sale or processed to obtain the butter.

The first stage of butter extraction involves dry-roasting of the kernel in a pan. The kernels are stirred from time to time to ensure even roasting. The roasted kernels are allowed to cool and then ground into a powder using a mortar and pestle or with an ordinary corn mill. The powder is then mixed with some cold water to form a paste which is then kneaded continuously with the fingers into a semi-solid mixture. Kneading continues until the original dirty brown colour of

the paste turns into dirty grey. More cold water is then added to the mass until slurry is formed. The slurry is allowed to stand for some time, followed by continuously working in with the fingers until there is flocculation of a grey matter. The suspended grey matter is scooped or skimmed into a basin and washed repeatedly with clean water to remove residual dirt. The grey fluffy matter is put into a cooking pot and heated. The grey matter, which is dirty or impure fat, melts and floats to the surface, leaving a brown solid residue to settle at the bottom of the pot. The oil is skimmed off into open calabashes and allowed to solidify. It is stirred continuously to facilitate solidification. It can also be made into smaller balls of between 100 – 200g, which are put in water to cool and solidify. The shea butter is now ready for marketing (*Personal communication*, F. Amantana, October 5, 2006). Figures 1 and 2 show the flow charts for the processing of shea nut and how shea butter is extracted locally.

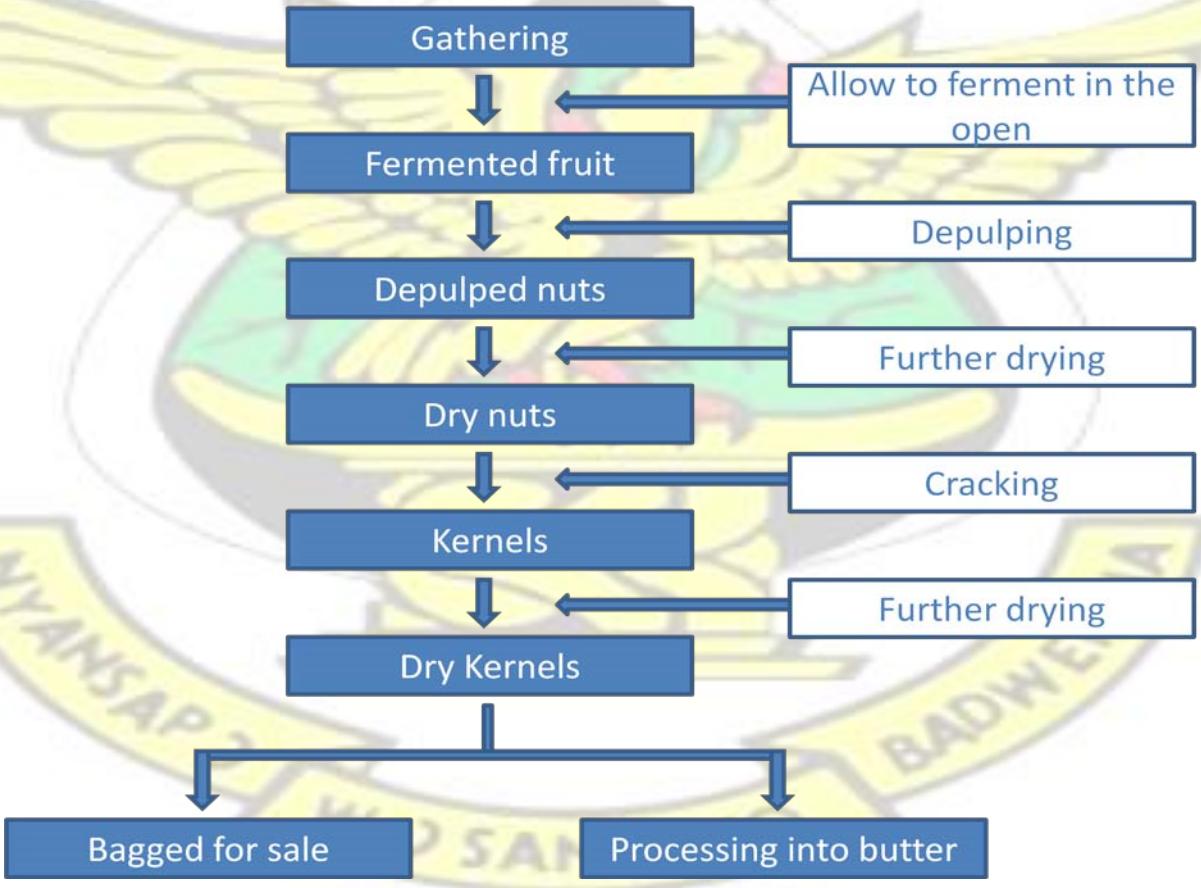


Fig 1. Flow Chart for Local Collection and Pre-treatment of Shea Nuts.

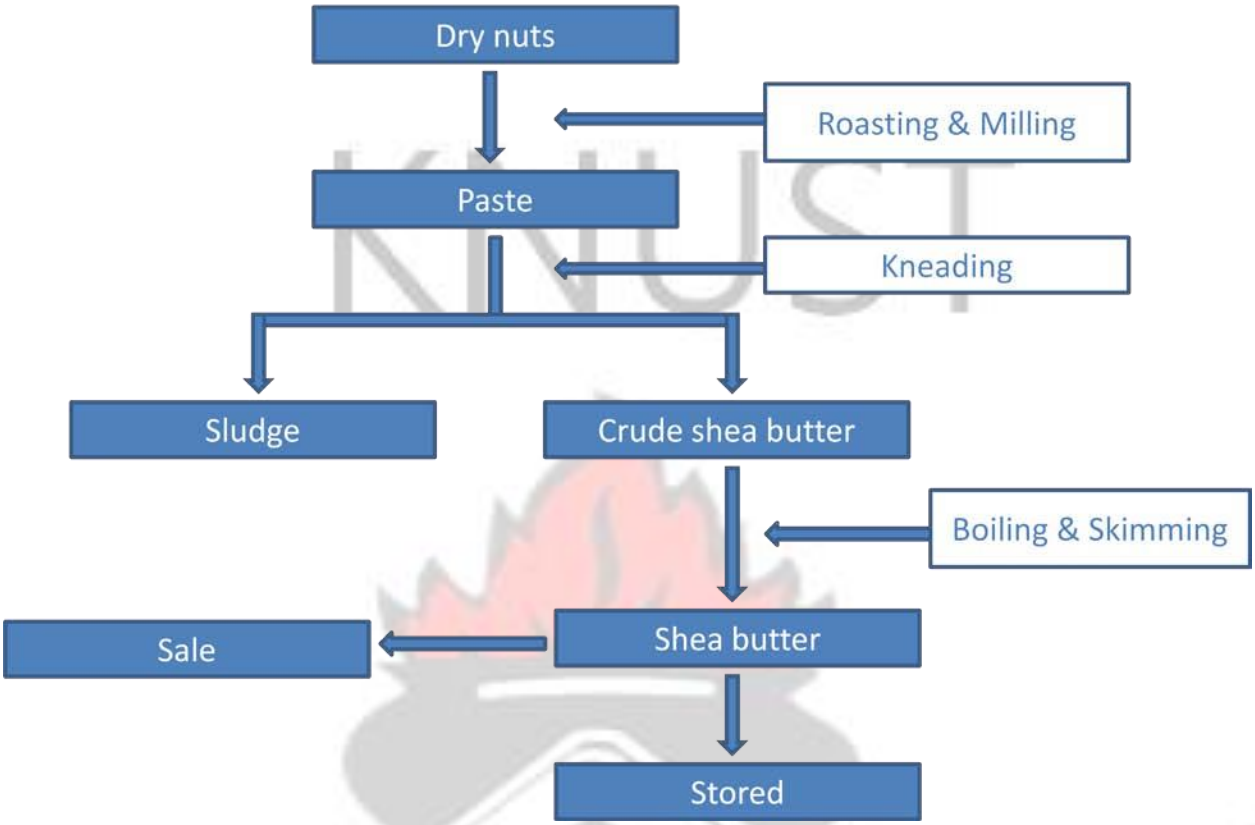


Fig. 2. Flow Chart for Local Processing of Shea Butter

1.2.1.2 Characteristics of Shea Butter.

The chemical and physical properties of shea butter have been documented by Adomako (1985) as shown in Table 1.

Table 1: Chemical and Physical Properties of Shea Butter and Cocoa Butter

	Shea butter	Cocoa butter
Fat content (%)	52.1	53.4
Ash content (%)	3.2	2.8
Melting point (°C)	38.0 – 39.5	34.3
Slip point (°C)	36.7 – 37.4	33.3 – 33.5
Iodine number	64.2	36.1
Acid number	13.4	1.8
Free fatty acids (as oleic) (%)	6.8	0.9
Saponification number	179.6 – 190.0	190.6 – 195.0
Unsaponifiable matter (%)	7.3 – 9.0	0.1 – 0.3
Solidification point (°C))	26.5 – 30.0	28.0 – 28.6
Linoleic acid (%)	6.9	3.2
Linolenic acid (%)	1.6	1.2
Degree of unsaturation	0.59	0.42

* Source: Adomako, 1985

The high iodine number, acid number and free fatty acids, as presented in Table 1, result in the pungent odour or taste characteristic of shea butter within a short period during storage (Adomako, 1985). Fatty acid composition of shea butter is shown in Table 2, an indication that palmitic, stearic, oleic, linoleic and linolenic acids are the main fatty acids in shea butter and that it has a relatively high degree of unsaturation.

Table 2: Fatty acid characteristics of tallow (*Pentadesma butyracea*) fat, shea butter and cocoa butter.

Fatty acids (%)	Tallow fat	Shea butter	Cocoa butter
Myristic	nil	nil	0.2
Palmitic	3.1	4.8	26.8
Palmitoleic	0.2	nil	0.3
Stearic	45.5	45.5	36.1
Oleic	50.5	40.8	31.9
Linoleic	0.4	6.9	3.2
Linolenic	nil	1.6	1.2
Degree of	0.51	0.59	0.42 *Unsaturation*

*Source: Adomako, 1977.

1.2.1.3 Uses of Shea Tree / Butter

Wood from the shea tree is used for tool making, and its roots and bark have medicinal applications. The shea tree has a great untapped potential for producing copious amounts of sap that can constitute an important source of raw material for the gum and rubber industry (Fobil, 2008). The bark and latex play a role in ethnomedicine (Neuwinger, 1996).

The shea tree is sacred to many ethnic groups and plays an important role in religious ceremonies (Millee, 1984). The butter from the shea tree is used locally by many as a culinary fat or oil, for soap manufacture, as an ointment, cosmetic, and illuminant, as well as for waterproofing houses or cracked walls (Marchand, 1988). Traditionally, shea butter is probably the primary cooking fat for a large proportion of the rural populations of Northern Ghana.

The butter is used in pastry for its high dough pliability and in confectionery as a cocoa butter substitute (Hall *et al.*, 1996). Because of its characteristics, shea butter is used as a base for cosmetic and pharmaceutical preparations for the treatment of dry hair, skin, burn and multiple skin ailments (Bonkoungou, 1987; Ezema and Ogujiofor, 1992) and also to massage pregnant women and little children (Marchand, 1988).

Whereas the composition of shea and cocoa butter are fairly similar, the unsaponifiable matter content is much higher in shea butter (8% on average). Currently, shea butter is mostly treated as a substitute for cocoa butter and priced like other vegetable oils. The future development of shea butter will depend on uses being found for its high UV absorption properties and the unsaponifiable matter which displays several interesting physical and biomedical properties that could have pharmacological and cosmetic applications. Examples of unsaponifiable matter in shea butter are the steroids. Other uses include the prevention of skin drying, the soothing of sore skin, protection and lubrication, fast release and long retention of active ingredients (Louppe, 1994). Shea butter contains tocopherols, collectively known as vitamin E and represents an important class of antioxidants (Maranz and Wiesman, 2004).

With all its good attributes, the high percentage of unsaturated fatty acids in shea butter (oleic - 40.8%, linoleic - 6.9% and linolenic - 1.6%) makes it prone to oxidative rancidity (Adomako, 1977; 1985). Shea butter quality is high when its free fatty acid content is low. Free fatty acid content is naturally low in fresh nuts, but increases rapidly, through hydrolysis, under poor storage conditions, leading to a relatively short shelf-life, low quality and subsequently, low economic value. Hydrolysis occurs through the lipolytic activity of the fruit lipase and micro-organisms; it is halted by heating and reducing the moisture content to lower than 8% (Louppe, 1994).

1.2.2.0 Basic Chemistry / Structure of Fat

Lipids form one of the major bulk constituents in foods and other biological systems. They occur in plants as storage lipids and contain highly unsaturated fatty acids (Jadhav *et al.,* 1995). Shea butter can be considered as having the basic typical fat / oil structure as shown in Fig. 3. Shea butter is, therefore, made up of glycerides or glycerol esters of long chain fatty acids. The fatty acids are either saturated or unsaturated. The unsaturated fatty acids of shea butter, oleic, linoleic and linolenic acids can undergo oxidation and deterioration during storage, to form other products.

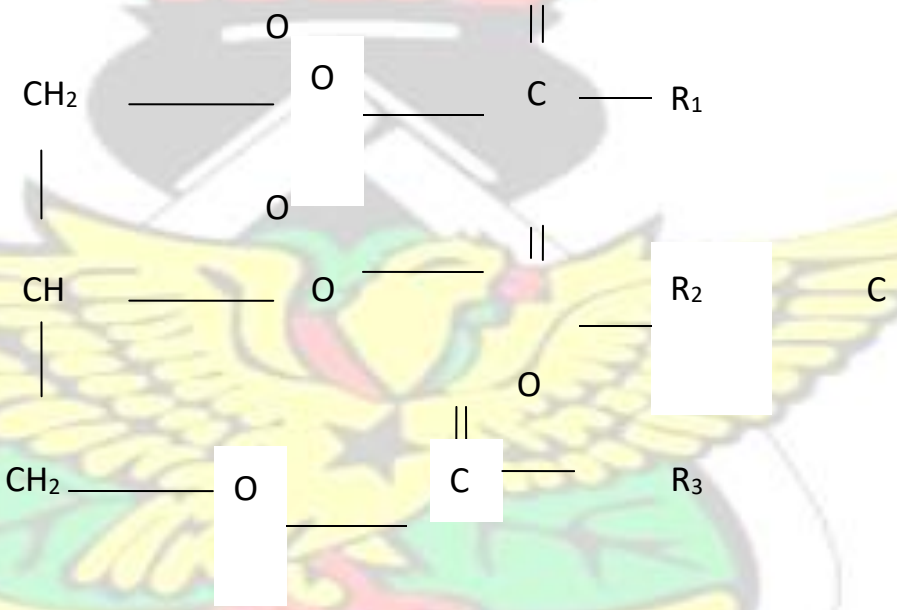


Fig. 3: Basic Structure of a Triacylglycerol

R_1, R_2 and R_3 represent individual alkyl groups. These groups are either saturated or unsaturated.

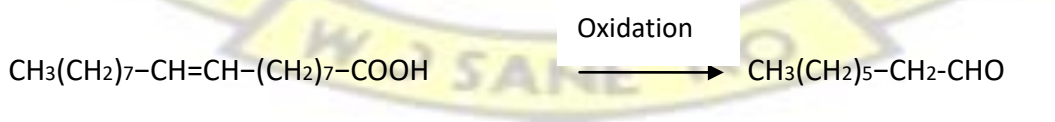
1.2.3.0 Rancidity (Lipid Oxidation)

Lipid oxidation has long been recognized as a major problem in the storage of fatty foods. Oxidative changes can result in repugnant flavours. This reaction occurs by several molecular mechanisms which all lead to the generation of oxygen–rich precursors of reactive, chainpropagating, free radicals (Kanner and Rosenthal, 1992). The chemistry of lipid oxidation

can be applied to shea butter since it is also a lipid. Like any other lipid, shea butter starts deteriorating from the moment it is isolated from its natural environment. It also undergoes deterioration in various ways during processing, handling and storage. The decomposition or degradation of lipids is referred to as rancidity.

Oxygen may attack the double bonds of the unsaturated fatty acids in shea butter when conditions become favourable. The unsaturation in the fatty acids of shea butter therefore makes it susceptible to oxygen attack leading to complex changes that eventually manifest as the development of off-flavours (Adomako, 1977). Practically, all quality attributes of a fat can be affected by this process. Thus, aroma changes result from new volatile, odorous compounds formed which make it unacceptable on the consumer market. Rancidity may be caused by light, moisture, heat, cations and oxygen. Light, moisture and heat are some of the factors which affect the structure of fat and greatly accelerate fat reaction with oxygen **(**Lipids Part 5, 2007). Rancidity in fats can be categorized into five main types; oxidative, hydrolytic, autoxidation, enzymatic and free radical.

(i) Oxidative rancidity (Fig. 4) is caused by oxygen attack on the fat, with the development of oxidized products and associated off flavours (Rossell, 1983). The initial step in fat deterioration is thought to involve the formation of free fatty acids (FFA), which are susceptible to oxygen attack in the presence of light. This is perhaps the most prevalent or important type of rancidity in fats. Oxygen normally attacks the double bond of the fats and this is catalysed by high temperatures. Peroxides are first formed which are then broken down to aldehydes (Sattar and Demen, 1973)**.**



2-undecenal

Oleic acid

+

$CH_3(CH_2)_6-CH=CH-CHO$

Nonanal Fig. 4: Unsaturated Fatty Acid undergoing Oxidative rancidity Reaction.

(ii) Hydrolytic rancidity is caused by hydrolysis of triacylgycerols, in the presence of moisture and heat. Here, the resulting products are glycerol and fatty acids, (C_4 to C_{20} chain length) which are either saturated or unsaturated. Hydrolysis of triglycerides in the presence of moisture is illustrated in Fig. 5a.

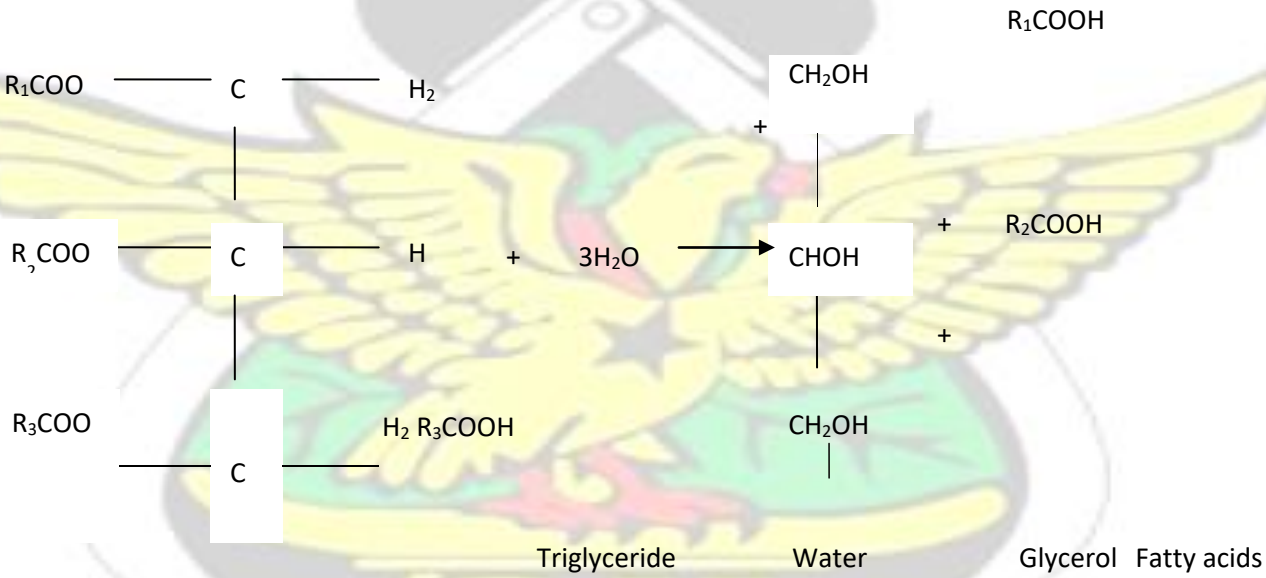


Fig. 5a. Hydrolysis of triacylglycerol in the presence of moisture

R_1, R_2 and R_3 represent individual alkyl groups. These groups are either saturated or unsaturated. Hydrolytic rancidity can also cause triglyceride molecules to break down to keto acids, which eventually lose carbon (IV) oxide readily as illustrated in Fig. 5b. These keto acids have much stronger off flavours and dominate the flavour of traditionally produced butter (Rossell, 1983)

$R'OCOCH_2$ O O OH

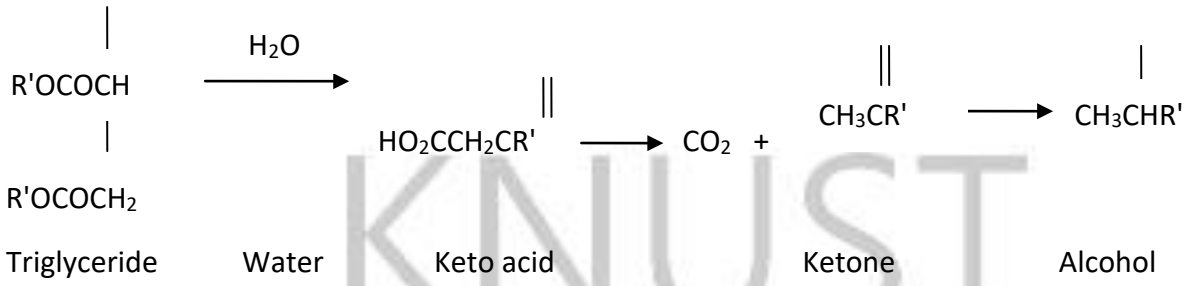


Fig. 5b: A triglyceride undergoing hydrolytic rancidity.

R_1, R_2 and R_3 represent individual alkyl groups. These groups are either saturated or unsaturated

(iii) Autoxidation occurs in lipids by way of free radical mechanism. After an induction period, hydroperoxides are formed. Ultimately these hydroperoxides break down; and secondary products, eg, aldehydes, ketones, organic acids and hydrocarbons are formed (Hamilton, 1994). Generally, the generation of free radicals is a natural phenomenon in food systems. Unless mediated by other oxidants or enzyme systems, autoxidation in lipids proceeds through a freeradical chain reaction mechanism (Pryor, 1976; Gordon, 1990; Pokorny, 1987; Kappus, 1991).

Autoxidation has been distinguished in three steps:

1. Initiation – Formation of free radicals
2. Propagation – Free radical chain reaction
3. Termination – Formation of nonradical products.

Initiation Step

Autoxidation is thought to be initiated with the formation of free radicals from unsaturated lipids. It takes place either by abstraction of a hydrogen atom at alpha- methlylene group of an unsaturated fatty acid or the addition of a radical to a double bond in the presence of light, heat, trace metals or an enzyme (Ohio State University, 2008). Also lipid hydroperoxides, which exist

prior to the oxidation reaction, break down to yield free radicals. The hydroperoxide can also undergo homolytic cleavage to form alkoxy radicals as well.

More free radicals are formed and a series of chain reactions are propagated. During the chain reaction, oxygen is consumed to yield new free radicals (peroxy radicals; $ROO^{\bullet}$) and also form peroxides as shown below:

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

$$ROOH \longrightarrow RO^{\bullet} + {}^{\bullet}OH$$

$$RO^{\bullet} + RH \longrightarrow ROH + R^{\bullet}$$

Thus, the radicals $R^{\bullet}$ and $ROO^{\bullet}$ can further propagate free radical reactions and initiate a chain reaction with other molecules. This reaction when repeated many times produces an accumulation of hydroperoxides and the propagation reaction becomes a continuous process as long as unsaturated lipid molecules are available.

Free radicals, with unpaired electrons are unstable and very reactive tend to react whenever possible, with each other to restore normal bonding. When there is a reduction in the amount of unsaturated lipids present, radicals bond to one another, forming stable non-radical compounds.

The termination step therefore leads to the interruption of the repeating sequence of propagating steps of a chain reaction as shown below:

$$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R$$

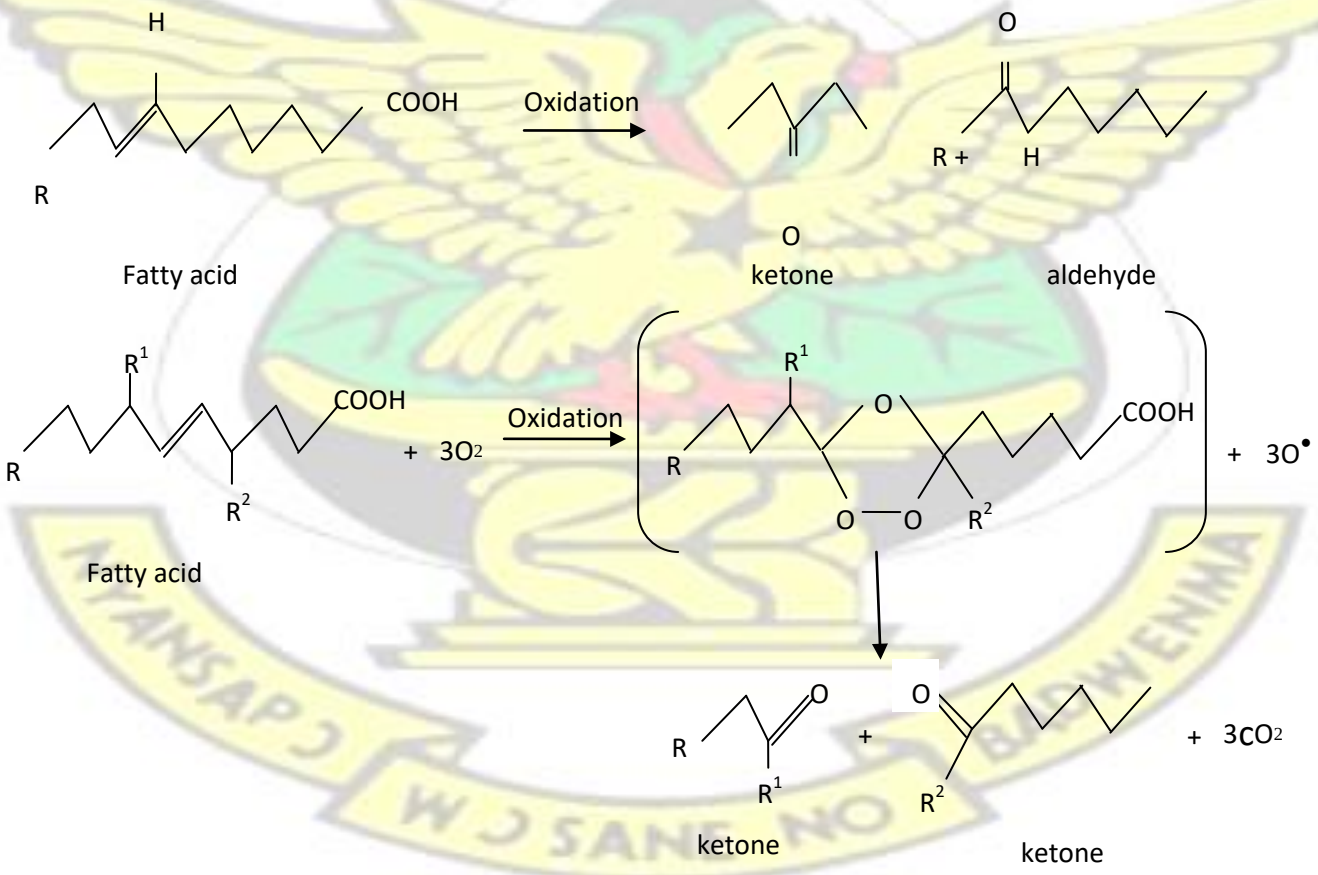
$$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$$

$$RO^{\bullet} + R^{\bullet} \longrightarrow ROR$$

$$2RO^{\bullet} \longrightarrow ROOR \quad ROO^{\bullet} + ROO^{\bullet}$$

$$ROOR + O_2$$

According to Coppen (1994), the breakdown products of autoxidation are usually ketones, aldehydes and low molecular weight fatty acids as shown in Fig. 6.



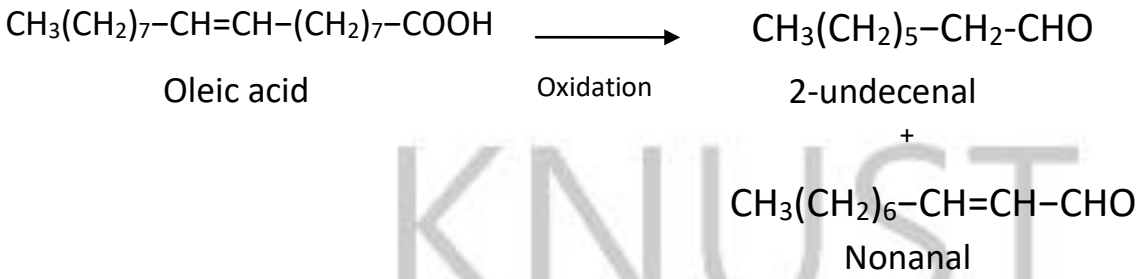


Fig. 6: Reaction and breakdown products of autoxidation

The production of free radicals can also occur during normal aerobic metabolism in microorganisms for the release of energy (Garrow and James, 1993), and oxygen is required (Frankel, 1985). During these processes oxygen acts as an electron acceptor. The reduction of oxygen is often incomplete under normal conditions, and a series of reactive intermediates are produced. These intermediates are referred to as free radicals. Free radicals are unstable and tend to seek electrons from surrounding molecules including unsaturated fatty acids in order to stabilize themselves and this could trigger auto-oxidation in any electron deficient environment (Wardlaw and Kessel, 2002). This is illustrated in Fig. 7.

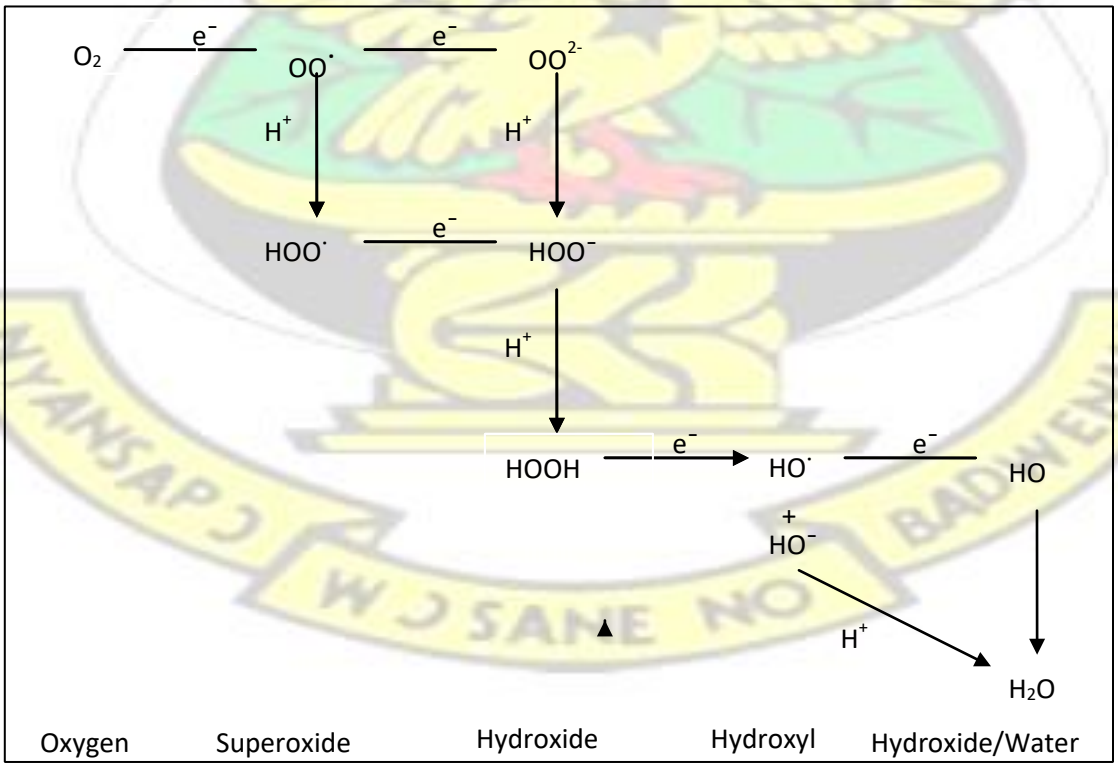


Fig. 7: Electron reduction of oxygen. Source: Sinclair *et al.*, 1990

(iv) Photo-oxidation has now been recognized as an alternative to the free radical mechanism, because it is believed that the different hydroperoxides are formed when light and certain photosensitive molecules are present (Hamilton, 1994).

Hydrolysis will split fatty acid chains away from the glycerol backbone in the glycerides and become free radicals at the alpha-methylenic group. The unsaturated acids are therefore susceptible to free radical attack due to their methylene - interrupted double bonds. According to this mechanism, oxidation proceeds as a chain reaction by a free radical extracting hydrogen from an unsaturated free fatty acid molecule leading to the formation of an unsaturated fatty acid radical. The double bond in the unsaturated fatty acid radical is rearranged to form a conjugated diene, which then combines with oxygen to produce a lipid peroxyl radical. The lipid peroxyl radical formed in turn, reacts with more unsaturated fatty acids to form lipid hydroperoxide that generates more radicals of the unsaturated fatty acids (Sinclair *et al.*, 1990; Garrow and James, 1993).

Unsaturated fatty acid

Hydrogen

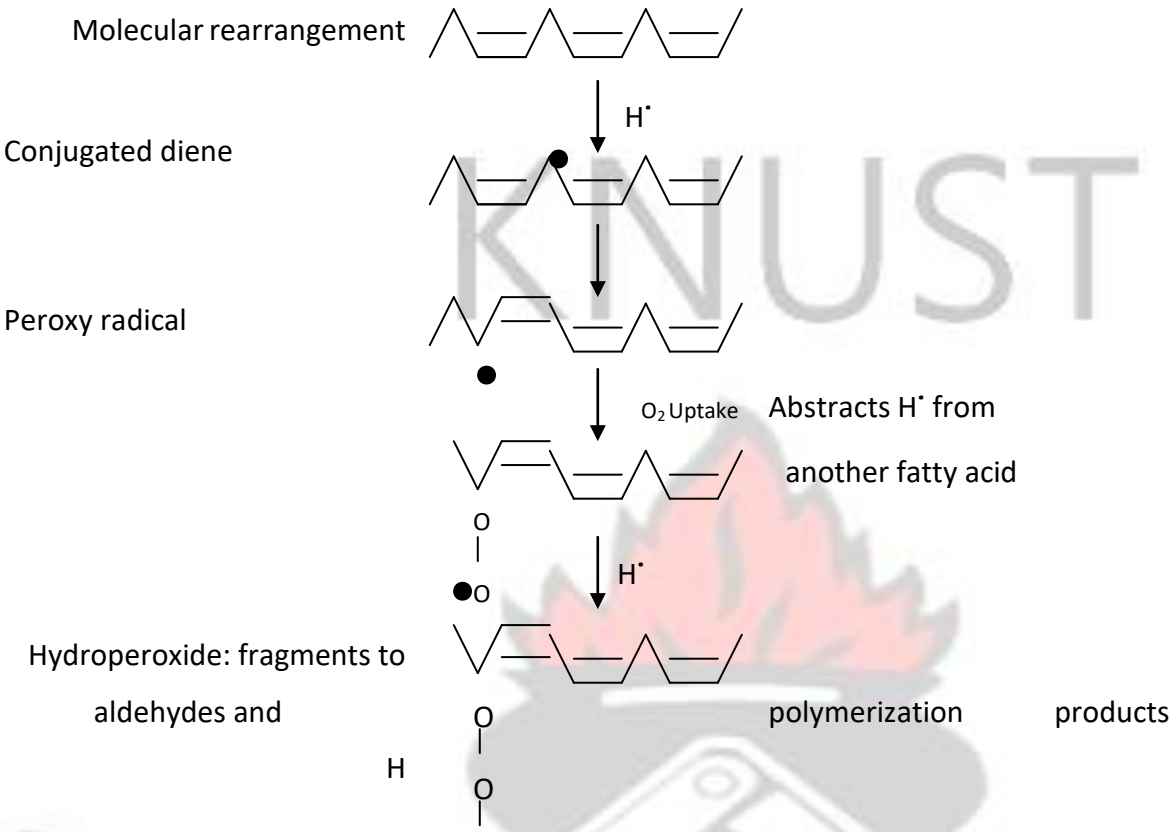


Fig. 8. Chain reaction of lipid peroxidation. Source: Sinclair *et al.,* 1990

These free radicals can then undergo further autoxidation. The free radical-mediated mechanism generates reactive molecules that are responsible for producing the unpleasant or obnoxious odours and flavours characteristic of rancid fats, oils or foods. Three groups of molecules – aldehydes, alcohols and hydrocarbons arise from hydroperoxides produced by photo-oxidation (Sinclair *et al.,* 1990).

(iv) Ezymatic cleavage is the simplest form of rancidity in glyceride esters (triglycerides) or fats/oils. This process is also referred to as de-esterification and is the liberation of short- chain or lower molecular weight fatty acids by lipase enzyme activity. The polyunsaturated free fatty acids liberated are prone to enzymatic oxidation by lipoxygenase (Leverentz *et al.*, 2002). Lipoxygenase is found in higher plants, in general, and specifically in most oil-containing seeds. It is believed that this oxidative breakdown involves the intermediate formation of peroxides

(Jadhav *et al.*, 1995). These peroxides could activate a secondary non-enzymatic oxidation (Kosary *et al.*, 2004).

1.2.3.1 Overall Mechanism of Lipid Oxidation

Labuza (1971) has summarized the mechanism of lipid oxidation as shown in fig. 9.

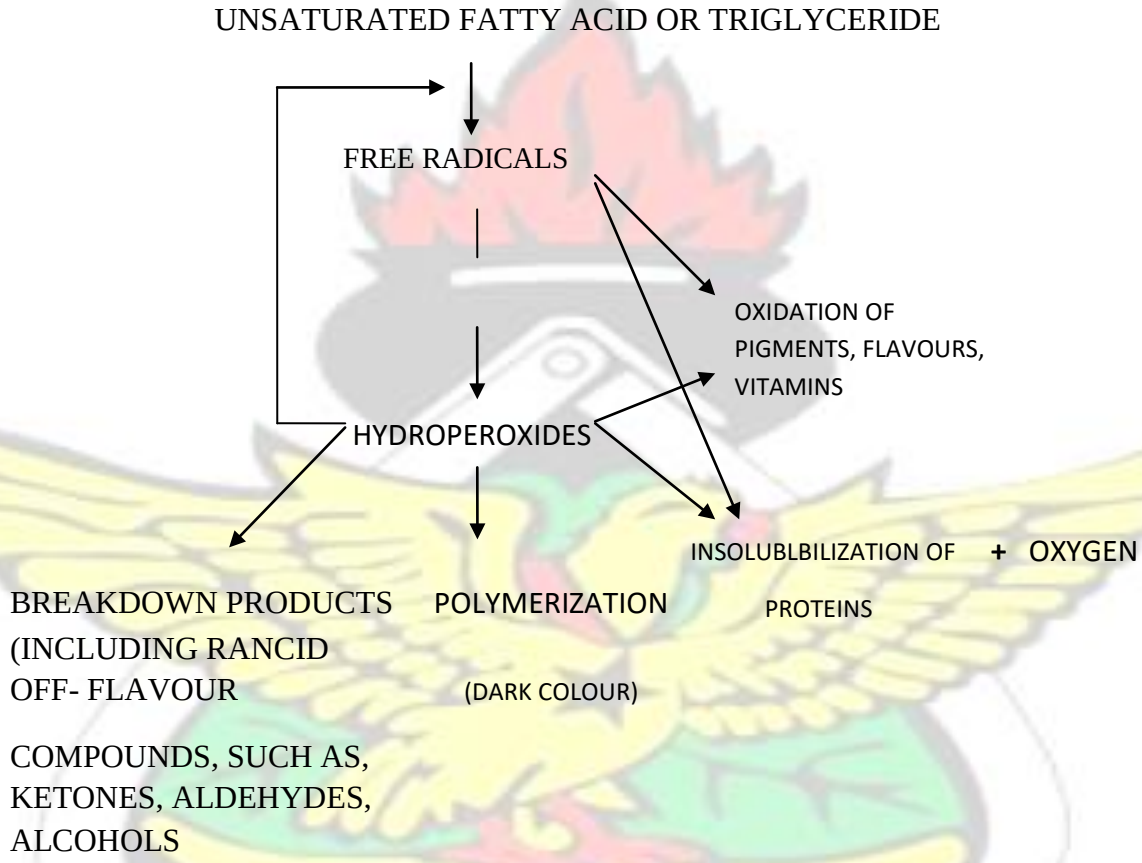


Fig. 9: Overall Mechanism of Lipid Oxidation. Source: Labuza, 1971

1.2.4.0 Retardation of Rancidity

The substitution of oxygen by an inert gas, or creating a vacuum in a particular environment may retard or prevent rancidity. However, the more practical method is the use of antioxidants (Hubbert *et al.*, 1996).

Antioxidants are chemical substances that are capable of donating hydrogen radicals while they become oxidized antioxidant radicals. Antioxidants therefore inhibit or cease free radical chain

reactions by stabilizing the free radicals through the donation of hydrogen or electrons, thus preventing further oxidation (Rajalakshmi and Narasimhan, 1995). Antioxidants, when incorporated in lipids, minimize rancidity, retard the formation of toxic products and increase the shelf life of the lipids. The discovery of the inhibition of lipid oxidation by phenolic compounds during the late 1940s has also contributed to the application of synthetic antioxidants in the food industry. Some commonly used synthetic anti-oxidants are *tert*-butyl hydroxyanisole (BHA), *tert* - butylhydroxytoluene (BHT), propyl gallate (PG), Nordihydroguaiaretic acid (NDGA), ethoxyquin and *tert*-butylhydroquinone (TBHQ) (Sherwin, 1990).

There are two types of antioxidants; primary and secondary antioxidants. Primary antioxidants, when present in small quantities, can react with peroxyl radicals before reacting further with unsaturated lipid molecules, converting them into stable products. Examples of primary antioxidants are BHA, BHT, TBHQ and polyhyrophenolic gallates. Secondary antioxidants on the other hand, are compounds that retard the rate of chain initiation reactions. They reduce the rate of autoxidation of lipids by binding metal ions, scavenging oxygen, decomposing hydroperoxides to nonradical products and deactivating singlet oxygen. Typical examples are sequestering agents, metal ions, reducing agents, and tocopherols or other phenolics (Gordon, 1990).

Plant phenolics are known to prevent lipid oxidation because of their redox properties which allow them to act as reducing agents (free radical terminators), hydrogen donors, singlet quenchers and chelators of pro-oxidant catalytic metals (Pascale *et al.*, 1999; Goupy *et al*., 1999; Kaur and Kapoor, 2002).

Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists (Larson, 1988).

A number of studies have shown that carotenoids also act as antioxidants by quenching singlet oxygen and free radicals (Palozza and Krinsky 1992; Tsuchiya *et al.*, 1992).

Naturally-occurring antioxidants impart a certain amount of protection against oxidation. However, these natural antioxidants are often lost during processing, necessitating the addition of exogenous antioxidants (Coppen, 1994). Shea butter has tocopherols but they are lost during processing.

1.2.4.1 *Cochlospermum planchonii* Plant

There are two species of *Cochlospermum* plant which belong to the *Cochlospermaceae* family. They are *Cochloserprmum planchonii (*Plate 6*)* and *Cochlospermum tinctorium* (Plate **7**). The *Gonja, Dagarti* and *Lobi* people of Northern Ghana refer to these plants as *kpokpolima, gbelemu* and *vilikyir* respectively (*Personal communication*, Sulemana, A. S., October 5, 2006).

According to Achenbach (1986), root extracts of the plant, *Cochlospermum planchonii*, contain polyphenols, and preliminary studies indicated the presence of gallic acid and its derivatives, especially glycosides and esters. An essential oil, Bisabolene, which has a conjugated double bond system, has been extracted from *C. planchonii* root by Benoit-Vical *et al.*, (2001).

Some novel long-chain compounds such as triacylbenzenes, 1,3,5-tri(dodecanoyl)benzene, 1,3di(dedocanoyl)-5-tetradecanoylbenzene, 1-dodecanoyl-3,5-di(tetradecanoyl)benzene and 1,3,5tri(tetradecanoyl) benzene, have been isolated from the same plant. A beta-keto alcohol has also been isolated from *C. planchonii* root by HPLC and identified by mass spetra, UV, and 1H NMR and 13C NMR (Addae-Mensah *et al.,* 1985).

These plants are abundant within the Guinea Savanna zone of West Africa. The *C. planchonii* plant possesses an extensive underground growth system with remarkable powers of regeneration after perennial bush fires (Lawson, 1986).

Plate 6: *C. planchonii* plant

Plate 7: *C. tinctorium* plant

Plate 8: *C. planchonii* plant with its underground xylopod

The plant has developed large underground xylopod as its normal mode of growth and this may be a modification for survival as a result of the perennial bush fires (Plate. 8)

The presence of polyphenols, gallic acid and its derivatives, 1, 3, 5 – substituted triacylbenzenes, bisabolene and carotenoids in *C. planchonii* root extract make the plant a potential antioxidant source even though studies conducted at the Cocoa Research Institute of Ghana by Ayeh (1993) on the use of *C. planchonii* root dye on rancid shea butter was not conclusive. .

Aqueous decoctions from the root have also been used by Benoit – Vical *et al.,* (2003) in a study that sought to enhance the traditional use of *C. planchonii* as an alternative therapy for treatment of non – severe malaria. Aqueous extracts of *C. planchonii* rhizomes have also been used by native medical practitioners in Northern Nigeria to treat jaundice (Aliyu *et al.,* 1995).

Pharmacological work indicates that aqueous extracts from *C. planchonii* root have been used as a hepatoprotective agent (Wolga, 1989) and that the active agent was cytochrome P-450 inhibitor which was classified as a zinc salt (Aliyu *et al.,* 1995). Jean-Paul, *et al.,* (2005), have suggested that plant essential oils and active components of *C. planchonii* can be used as alternatives or

adjuncts to current antiparasitic therapies. Antiplasmodial activity of the root extract and essential oils from *C. planchonii* have been reported by Vonthron-Senecheau *et al.*, (2003) and Benoit-Vical *et al.*, (2001)

Decoctions of the root of *C. planchonii* plant have also been used to flavour soups in Nigeria, while the dye from the root has been used for dying fabrics, leather and, mats and also to colour shea butter (Abbiw, 1990).

1.2.4.2 Mechanism of Antioxidant Activity

Antioxidants reduce the rate of autoxidation of lipids by binding metal ions, scavenging oxygen, decomposing hydroperoxides to nonradical products and deactivating singlet oxygen (Larson, 1988). The mechanism of antioxidant activity, as to how it stops the free radical-chain reaction, has been reviewed by a number of investigators (Gordon, 1990; Kappus, 1991; Pokorny, 1987) and is illustrated below:

$$AH + R^{\bullet} = RH + A^{\bullet}$$

$$AH + RO^{\bullet} = ROH + A^{\bullet}$$

$$AH + ROO^{\bullet} = ROOH + A^{\bullet}$$

Where AH represents the antioxidant, $R^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$, free radicals and $A^{\bullet}$, antioxidant free radical, while RH, ROH, and ROOH become reduced alkyl and alkoxy compounds.

The antioxidant free radicals also interfere with the chain-propagation step of lipid oxidation by reacting with more alkoxyl and hydroperoxides radicals, thereby becoming alkoxyl and peroxy antioxidant compounds as illustrated below.

$$R^{\bullet} + A^{\bullet} = RA$$

$$RO^{\bullet} + A^{\bullet} = ROA$$

$$ROO^{\bullet} + A^{\bullet} = ROOA.$$

These processes terminate the oxidation process. However, if the antioxidant in the lipid becomes exhausted, oxidation proceeds.

An effective antioxidant therefore is one which can extend the induction period of lipids before rapid deterioration sets in.

Rancidity in lipids cannot be completely eliminated but can be controlled by the use of the appropriate antioxidants. The antioxidant, when present in appreciable concentration would be oxidized in preference to the lipid molecules. Antioxidants therefore, effectively retard the process of oxidation, but cannot reverse it. They are however not effective in suppressing hydrolytic rancidity (Jadhav *et al.,* 1995).

CHAPTER 2

2 .0 MATERIALS AND METHODS

2.1 MATERIALS

C. planchonii roots were harvested at Bole, washed and cut into pieces to facilitate drying. Shea fruits were also gathered from Bole, in the Northern Region of Ghana.

96% ethanol (GPR grade) was purchased from Fisher Scientific Laboratories; diethyl ether (GPR grade) was from BDH Chemicals, Poole, England; n- hexane, HPLC grade, methanol and Acetonitrile, Bisabolene, Methylmyristate, 1,3,5,-Triacylbenzene, 2-Tridecanone, and nDodecyl-aldehyde and all chemical reagents used for the extraction or analysis were obtained from Acros Organics (Morris Plains, NJ, USA) except otherwise stated.

2.2 METHODS

2.2.1 Preparation of Dry *Cochlospermum planchonii* Root

Pieces of dry *C. planchonii* root were milled through a 1 mm mesh using a Thomas Willey Mill Model ED – 5 (Arthur H. Thomas Co. Philadelphia, PA, USA). The milled root was stored until ready to be used.

2.2.2 Scheme for Extraction of Dry Root of *C. planchonii*

Extracts from 50 g dry powdered root of *C. planchonii* were prepared by successive cold percolation with 250 ml quantities of n-hexane, diethyl ether and 96% ethanol for a period of 16 hours (Fig. 10).

Fifty gram dried and powdered roots were also extracted with successive 250 ml quantities of n-hexane, diethyl ether and 96% ethanol for a period of 16 hours using Soxhlet apparatus. Scheme of extraction procedure is shown in Fig. 10.

In another experiment 50 g of dried and powdered roots were extracted with Soxhlet apparatus, using n-hexane, diethyl ether and 96% ethanol (Fig. 11).

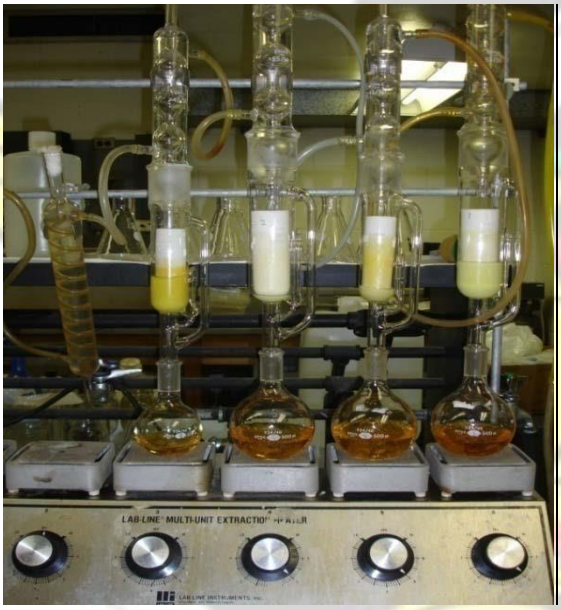


Plate 9: Set-up of Soxhlet extraction

Plate 10: Set-up, Cold Percolation

The Set-up of the extraction procedures are shown in Plates 9 and 10

The solvents were evaporated using a rotary evaporator (Brinkman Rotavapor RE 111, Switzerland) at 40^0C under vacuum. The residue was freeze dried to obtain powdered extracts (a), (b) and (c), which were determined gravimetrically and stored for subsequent use.

The yield of each fraction was determined and expressed as percentage of the dry root.

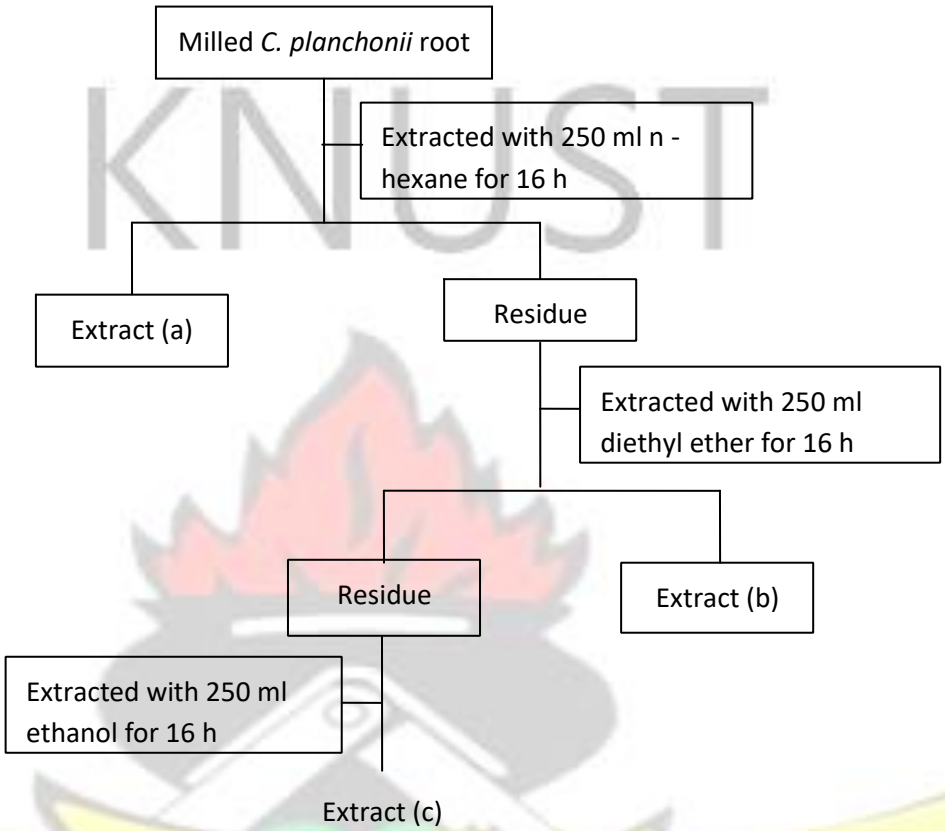


Fig. 10. Scheme for the extraction of dry *C. planchonii* root using Soxhlet / Cold percolation

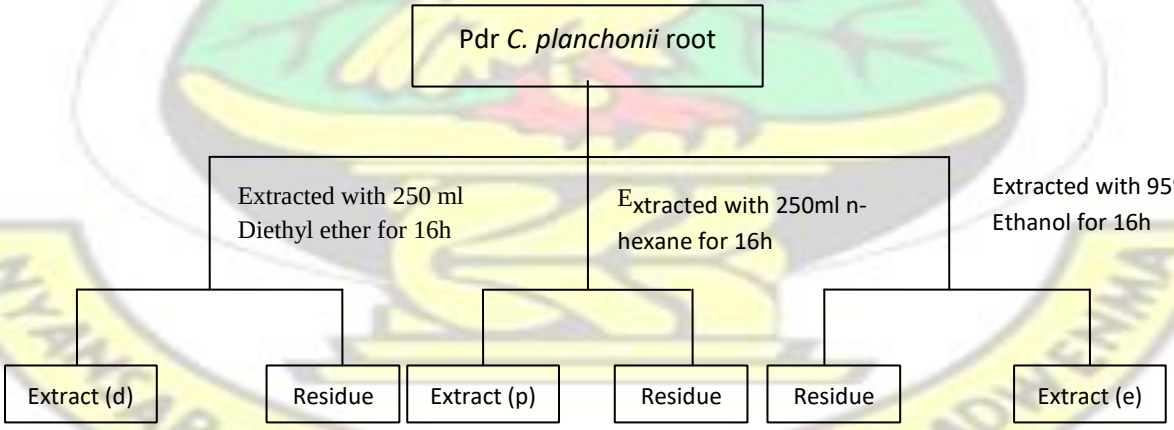


Fig. 11. Scheme for one-time Soxhlet extraction of dry *C. planchonii* root

2.2.3 Phytochemical Screening Tests of *Cochlospermum planchonii* Root Extract

Phytochemical screening tests were conducted on each extract for tannins, polyphenols, alkaloids, flavonoids, anthraquinones, terpenoids and saponins (Appendix I).

2.2.4 Analytical Methods

2.2.4.1 Absorbance Measurements

Absorbance measurements of the ethanol extract were used to determined the concentration as gallic acid equivalents at 760nm (Singleton *et al.*, 1999) using Ceci CE 700 Series Spectrophotometer for the stability studies.

2.2.4.2 HPLC Analysis

Samples were extracted as in Fig. 11 using n-hexane, diethyl ether and ethanol by soxhlet (Plate 9), concentrated at 40^{o}C under vacuum and freeze dried. Fifty parts per million (50 ppm) of each was dissolved in HPLC grade methanol and transferred to a 1.5ml vial before introduction into a Waters 2695 Separation Model HPLC, with symmetry C18 (3.9 x 150mm) column and Waters 996 Photodiode Array Detector as the detector.

The mobile phase consisted of a solution of 95% methanol and 5% water with a flow rate of 0.9 mL/min. The selected wavelength was 190 – 600nm, column temperature of 40^{0}C, sample temperature of 25^{0}C and elution time was 10 minutes.

Seven reference compounds, 2-Pentadecanone, Bisabolene, Methylmyristate, 1,3,5-Triacylbenzene, 2- Tridecanone, ß-Carotene and n-Dodecyl-Aldehyde were run as internal standards with the n-hexane, diethyl ether and ethanolic *C. planchonii* root extracts.

The mobile phases used were:

1. MeOH (98%) / Water (2%)

2. Acetonitrile (98%) / Water (2%)

3. Acetonitrile (98%) / Water (5%)

Waters 996 Photodiode Array Detector was later replaced with an Evaporative Light Scattering Detector (ELS) to run using the mobile phases listed above. Nebulising control temperature was 55%, drift tube temperature, 52^0C, Column temperature, 40^0C, flow rate, 1ml/minute, gain, 50, pressure, 45 psi and a run time 20 minutes.

2.2.4.3 Determination of Total Phenols

Total phenols in *C. planchonii* root extract were estimated as gallic acid equivalents according to the Folin- Ciocalteu method (Singleton *et al.,* 1999).

A 0.20 g powdered sample was extracted in 30 mL acidified methanol (80% MeOH containing 1% HCl). Three (3) millilitres aliquot was taken into a 25 mL volumetric flask and made up to the mark with the acidified MeOH. A 0.01 g gallic acid (Fluka Chemie, Buchs, Switzerland) was also treated as above.

Hundred microlitres (100 µL) of the extract was transferred to a 10- mL volumetric flask containing 6 mL of H_2O, to which was subsequently added 500 µL of undiluted Folin- Ciocalteu phenol reagent (Sigma-Aldrich Inc, St Louis, MO, USA). After 1 min, 1.5 mL of 20% Na_2CO_3 was added, and the volume was made up to 10 mL with H_2O. The control contained all the reaction reagents except the extract. After 2 h of incubation at 25^oC, the absorbance was measured at 760 nm using Cecil CE 700 Series spectrophotometer and compared to a gallic acid standard which was treated as the sample extract. Total phenol was determined as gallic acid equivalent and presented as the mean of 3 analyses using equation

(1).

$$\% \text{ Equivalent Gallic acid} = \frac{A_{sample}}{A_{standard}} \times \frac{Wt_{standard}}{Wt_{sample}} \times 100 \quad (1)$$

Where, A was the absorbance at 760nm and Wt, weight used for the assay.

2.2.5 Collection, Treatment and Extraction of Shea Butter from Fruits

2.2.5.1 Collection and Treatment of Shea Fruits

Shea fruits were collected from under the trees around the CRIG Sub- station, Bole, de-pulped, blanched or par-boiled immediately to stop any further enzymatic action within the kernels and sun-dried for five days. The dried nuts were de-husked using a pestle and a mortar and winnowed to obtain the kernels. The kernels were again dried further for 14 days until a constant weight was obtained with a moisture content of 6%. The kernels were then bagged and stored for subsequent use (Fig. 12).

2.2.5.2 Laboratory Extraction of Shea Butter

The kernels were roasted / baked at an oven temperature of 120^{o}C for 1h and pounded in a mortar. The pounded kernels were further milled into a fine paste /mass using the ordinary corn mill.

Ten (10) kg milled shea kernel was weighed into a bowl. Five litres (5L) of water was added to the contents of the bowl and kneaded for about thirty minutes. 10L water was again added to the kneaded mass and mixed thoroughly. The crude grey / off white coloured fat flocculated with some of the brown mass, while the aqueous phase with a lot of residue settled at the base of the bowl. The supernatant was skimmed into a cooking pot and heated until the oil layer became conspicuous and was skimmed off. The shea butter collected was washed with hot water and again skimmed into another cooking pot. The resultant shea butter was again washed with hot

0.00IM sodium hydroxide solution and a hot 0.001M NaCl solution to remove any free fatty acid and any residual dirt associated with the extraction process. The fat was further heated for 2h at 100^{o}C to expel any residual water (Figure 13). Shea butter yield was then determined and presented as a mean of three determinations.

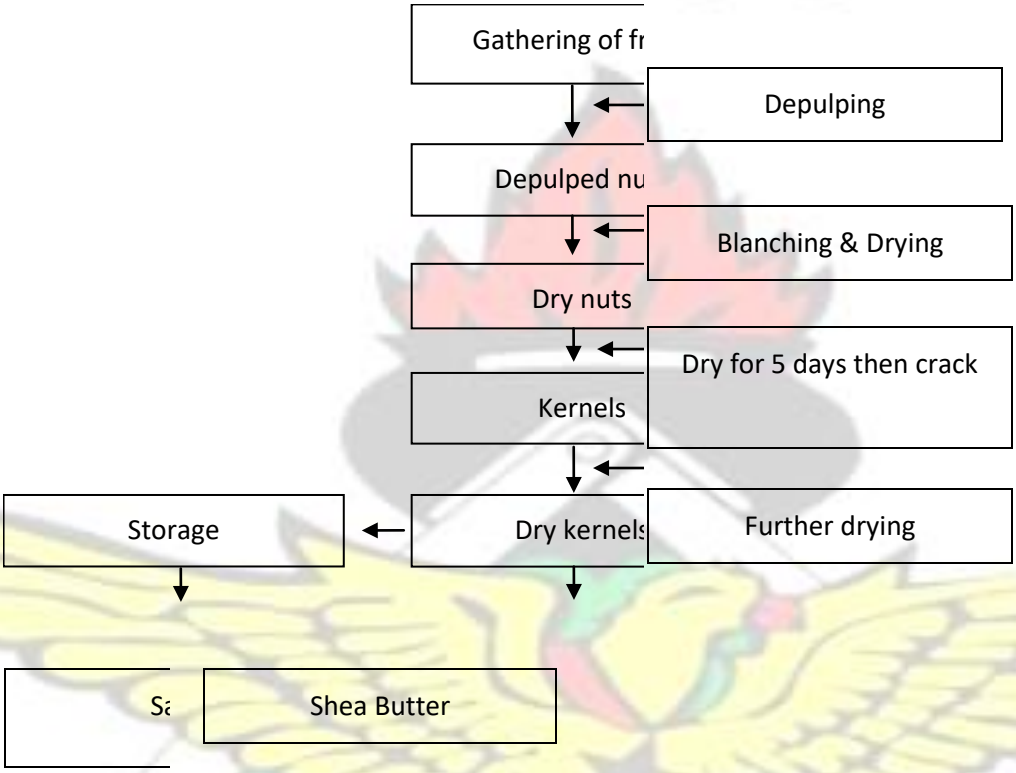


Fig. 12. Flow chart for collection and treatment of shea fruits.

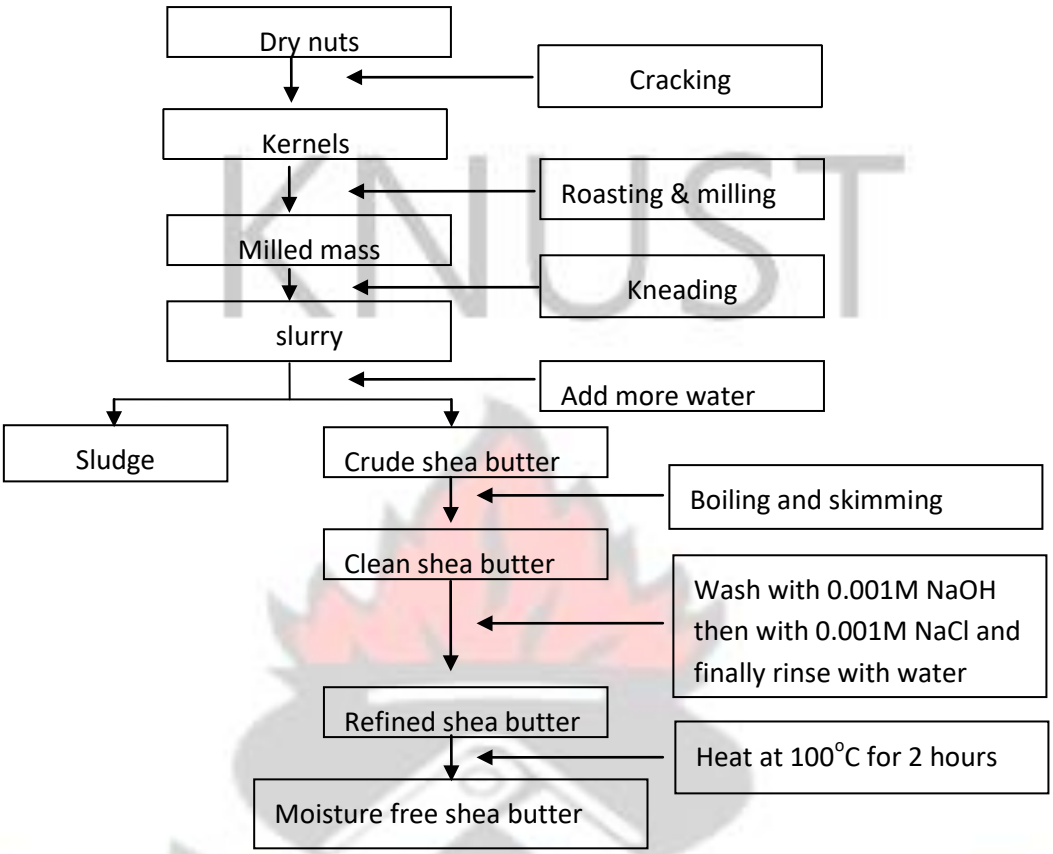


Fig. 13. Flow chart for laboratory extraction of shea butter

2.2.6 Experimental Design

2.2.6.1 Treatment of Shea Butter with *C. planchonii* Root Extract

Shea butter produced at the Cocoa Research Institute by the method described above as well as shea butter bought from the Bole market was used. The two types of shea butter were treated in exactly the same way in two sets of experiments which were carried out concurrently.

Ethanolic extract of *C. planchonii* root was used in the study since the yield was highest.

Different concentrations of *C. planchonii* root extract were incorporated into 100g of shea butter and put in plastic opaque jars.

Two factors were investigated in a factorial design.

Factor 1: Mode of storage (close, loose and open plastic jars).

Factor 2: Concentration of *C. planchonii* root extract.

Concentration of *C. planchonii* root extract was increased sequentially by 0.02% as follows:

1 Six (6) concentrations with three (3) replicates using *C. planchonii* root dye at 0 to 0.10% and stored in closed plastic jars.

2. Six (6) concentrations with three (3) replicates using *C. planchonii* root extract at 0 to 0.10% and stored in plastic jars with loose lids.

3. Six (6) concentrations with three (3) replicates using *C. planchonii* root extract at 0 to 0.10% and stored in open plastic jars.

The control in each case had no plant extract.

b) The mode of storage of the shea butter was as follows: when the plastic jar was not covered with its lid, it was described as "open". When the lid of the jar was on but not screwed tightly, allowing some air to enter the jar it was described as "loose" and when the lid of the jar was firmly screwed on, it was described as closed.

c) The samples were stored over a 12-month period and monitored monthly for free fatty acid (FFA) levels, peroxide value (PV) and iodine value (IV). The moisture content of the samples were also determined quarterly within 12-month period.

2.2.6.2 Determination of Moisture

Moisture in the shea butter samples was determined as weight loss on drying at 105ºC for 3 h.

2.2.6.3 Determination of Free Fatty Acid (FFA)

The free fatty acid formed during the storage of shea butter over the 12-month period was determined as oleic acid (BP, 1993) by titrating with standard 0.1M potassium hydroxide solution using phenolphthalein to a neutral point. The FFA was expressed as milligram of potassium hydroxide required to completely neutralise one gram shea butter, using the formula below:

$$\text{Free Fatty Acid Content} = \frac{5.610V}{W}$$

Where V is the volume of 0.1M KOH used and W the weight of fat used for the assay **(**Appendix II (1))

2.2.6.4 Determination of Peroxide Value (PV)

The hydroperoxide or peroxide formed during the storage of shea butter over a 12- month period was estimated iodometrically. The sample was reacted with a saturated aqueous solution of potassium iodide, and the iodine liberated by the peroxides was titrated with a standard solution of 0.1M sodium thiosulphate. The peroxide Value (PV) was expressed in units of milliequivalent of oxygen per kilogram of fat (BP, 1993),

$$\text{Peroxide Value (PV)} = \frac{10\ V}{W}$$

Where V is the titre and W, weight of sample used (Appendix II(3))

2.2.6.5 Determination of Iodine Value (IV)

Treated samples of stored shea butter were reacted with iodine bromide and the excess iodine liberated, titrated with standard 0.1M sodium thiosulphate. A blank titration was also performed. The iodine value was expressed as milligrams of iodine to completely saturate all the double bonds in the shea butter using the formula below:

$$\text{Iodine Value (IV)} = \frac{1.69V}{W}$$

Where V was the difference in volume between the blank and the sample titre, while W was the weight of the sample used (BP, 1993, Appendix II (2)).

2.2.6.6 Data Collation and Analysis

Changes in the values of FFA, IV, PV and moisture over the twelve month period were plotted and the data used to assess the point at which the extract could be said to have lost its power to act as an anti-oxidant. Analysis of variance (Steel *et al.,* 1997**,** GENSTAT, 2008 **)** was also carried out on data from locally produced shea butter at one (1) and three (3) months; and at six (6) and eight (8) months for laboratory-extracted shea butter. Initial values of FFA, IV and PV were used as covariates in the analysis. Significant means ($P < 0.05$) were separated by LSD.

The effect of extract concentration on the container type was also subjected to interaction analysis (Cochran and Cox, 1992**).** Interactions involve looking at two factors simultaneously and evaluate how they affect shea butter during storage. Although both factors may act independently, more commonly they interfere or interact with each other. The interaction is analyzed as a two-way analysis of variance (level of significant $p<0.05$) with degrees of freedom as the product of the degrees of freedom of the two factors involved. Interactions are most often depicted graphically. In this experiment, the influence of the type of container (first factor) and extract

concentration (second factor) on keeping quality of shea butter, measured as level of free fatty acid, peroxide value, iodine value and moisture were investigated simultaneously.

Stability of the extract during storage was determined by plotting changes in gallic acid concentration over a 12-month period.

CHAPTER 3

3.0 RESULTS AND DISCUSSION

3.1 Yield, Phytochemical Components, Stability and HPLC Analysis of *C. planchonii* Root Extract

Yield of extract and colour in the various solvents are reported in Tables 3a and 3b. Yields of extract obtained with Soxhlet apparatus were higher than the corresponding extract obtained with cold percolation and higher for the single Soxhlet extractions than the successive Soxhlet extractions. This implies that during the successive extractions, the solvents extracted only with the same polarity **(**Fig. 10). Colour of extract ranged from orange to dark brown as indicated in Tables 3a and 3b.

Table 3a. Yield, Colour and State of Extracts of *C. planchonii* Root in n-Hexane, Diethyl Ether and Ethanol (Successive Extraction /Cold Percolation)

	Colour/State of extract		Yield of extract g/100g dry root	
Method of extraction	**Cold percolation**	**Soxhlet**	**Cold percolation**	**Soxhlet**
n-Hexane	Yellowish/ Oily	Deep Orange/Oily	1.78 ± 0.16	1.95 ± 0.16
Diethyl ether	Deep orange/ Oily	Deep Orange/ Oily	0.68 ± 0.12	0.89 ± 0.09
Ethanol	Brown	Brown	9.12 ± 0.18	12.42 ± 0.16

(Results presented as a mean of three determinations ± SD)

Table 3b. Yield, Colour and State of Extracts of *C. planchonii* Root in n-Hexane, Diethyl Ether and Ethanol (Single Extraction, Soxhlet).

	Solvent	Colour of Extract/state	Yield g/100g dry root
	n-Hexane	Yellowish	1.99 ± 0.16
	Diethyl ether	Orange / Oily	2.29 ± 0.18

Ethanol	Dark brown / Oily	15.52 ± 0.13

(Results presented as a mean of three determinations ± SD)

It was difficult evaporating the diethyl ether and alcohol extracts completely using the rotary vapour and so dry samples were obtained by freeze drying.

Phytochemical screening results of the extracts are shown in Table 3c. The colour intensity of the extracts was so high that it was very difficult to observe colour reactions in the extracts with exact certainty. The n - hexane and diethyl ether extracts tested positive for terpenoids, while the diethylether and ethanol extracts tested positive for tannins, phenolics, terpenoids and saponins. The presence of saponins in the diethyl ether and ethanol extracts perhaps explains why it was difficult to evaporate the solvents after extraction, since saponins have distinctive detergent properties and in the presence of moisture make a foamy, sudsy soapy solution (Natural Herbalism, 2009).

Table 3c. Phytochemical Constituents of *C. planchonii* Root Extracted in n-Hexane, Diethyl Ether andEthanol.

Suspected group of Compounds	Extract in n-Hexane	Extract in diethyl ether	Extract in Ethanol
Tannins	-	+	+
Phenolics	-	+	+
Alkaloids	-	-	-

Flavonoids	-	-	-
Anthroquinones	-	-	-
Terpenoids	+	+	+
Saponins	-	+	+

+ = present, - = absent

3.2 Stability of *C. planchonii* Root Extract.

The stability of *C. planchonii* root extract in 96% ethanol over a twelve-month period is shown in Fig.14. The results ranged from an initial value of 12.85% ± 0.213 to 12.85 ± 0.489% by the 4th quarter with respect to its gallic acid equivalent (GAE) and showed no appreciable variation over the 4-quarter period. This implies that the extract was stable within the storage period of 12 months**.** Thus the incorporation of this extract in shea butter will not be affected by stability problems.

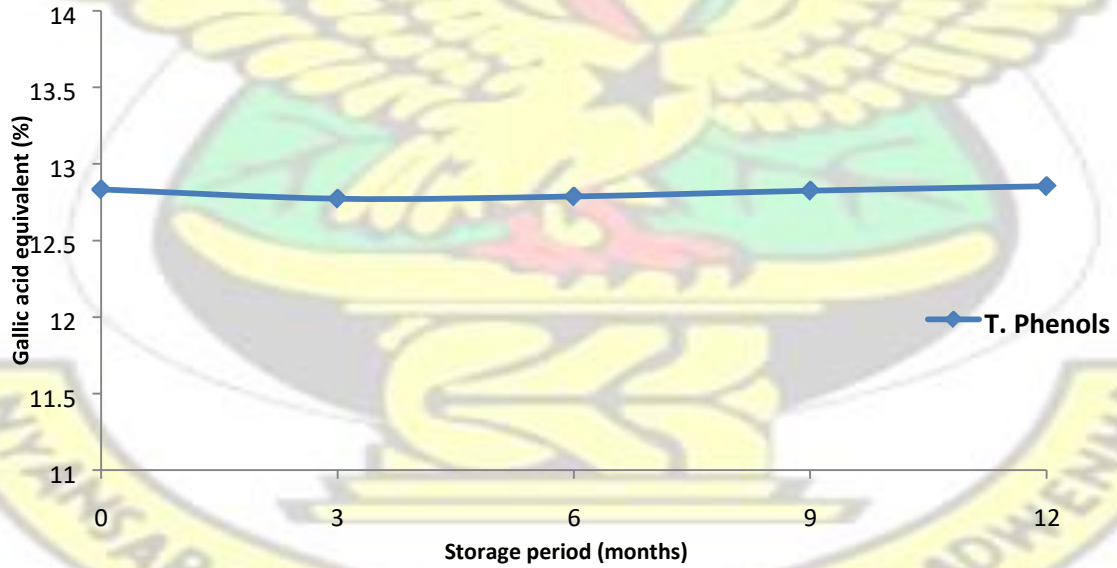


Fig. 14. Effect of storage on the stability (Total Phenols) of *C. planchonii*

3.3 HPLC Analysis of *C. planchonii* Root Extract

HPLC is a tool that could be used to determine components of extracts even when the constituents are present in very minute quantities. HPLC separates constituents of extracts according to their wavelengths or retention times. The retention times and wavelengths can be compared with that of known standards to establish the identity of the constituents.

The chromatogram of n – Hexane extract of *C. planchonii* root showed a peak at 260nm and a retention time of 2 minutes using a solvent system of 98% Acetonitrile and 2% Water (Fig. 15b). The peak matched the internal standard, 1,3,5 tri - acylbenzene isolated by Addae Mensah *et al.*,(1985) from *C. planchonii* root. A peak appeared after 1.5 minutes of elution; using a solvent system of 98% Acetonitrile and 2% Water which had a λ_{max} of 267nm (Fig. 15a). This matched the internal standard ß –Bisabolene using the same mobile phase. ß-Bisabolene has also been established to be one of the compounds present in *C. planchonii* root (Benoit- Vical *et al.*, 2001). Chromatogram of the ethanol extract is shown in Fig. 15c. Using a mobile phase of 98% MeOH and 2% Water, after an elution time of 1.5 minutes, a peak occurred at λmax of 450nm. This peak also matched the internal standard ß- carotene.

From the HPLC analysis, it can be concluded that *C. planchonii* root has gallic acid derivatives (1, 3, 5 tri- acylbenzene), ß - Bisabolene and ß – carotene as some of its constituents. The extract therefore could act as a source of antioxidants.

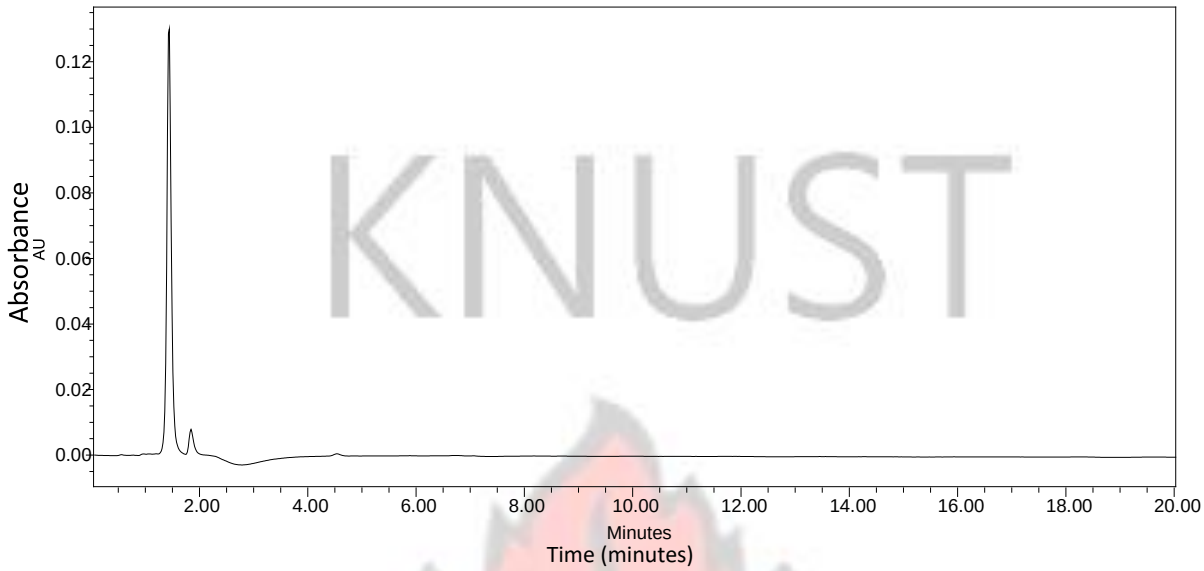


Fig. 15a. HPLC of n-Hexane extract of *C. planchonii* root at ʎmax 267nm.

(Mobile phase, Acetonitrile (98%) / Water (2%), retention time, 1.5minutes, Nebulising control temperature was 55%, drift tube temperature, 52^{0}C, Column temperature, 40^{0}C, flow rate, 1ml/minute, gain, 50, pressure, 45 psi and a run time 20 minutes)

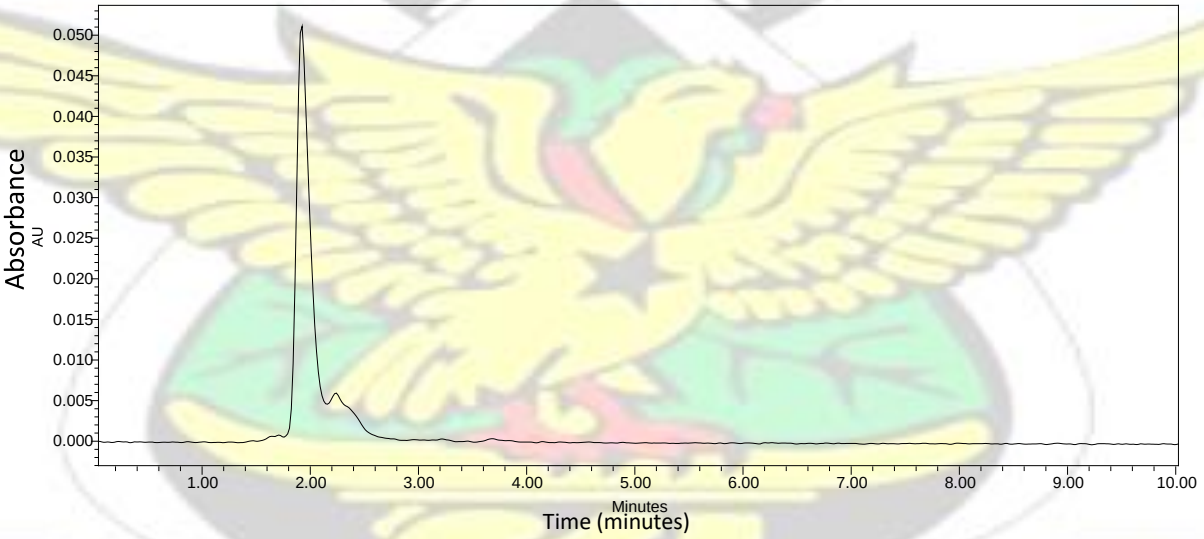


Fig. 15b. HPLC of n-Hexane extract of *C. planchonii* root at ʎmax 260nm.

(Mobile phase, Acetonitrile (98%) / Water (2%), retention time, 2 minutes, Nebulising control temperature was 55%, drift tube temperature, 52^{0}C, Column temperature, 40^{0}C, flow rate, 1ml/minute, gain, 50, pressure, 45 psi and a run time 20 minutes)

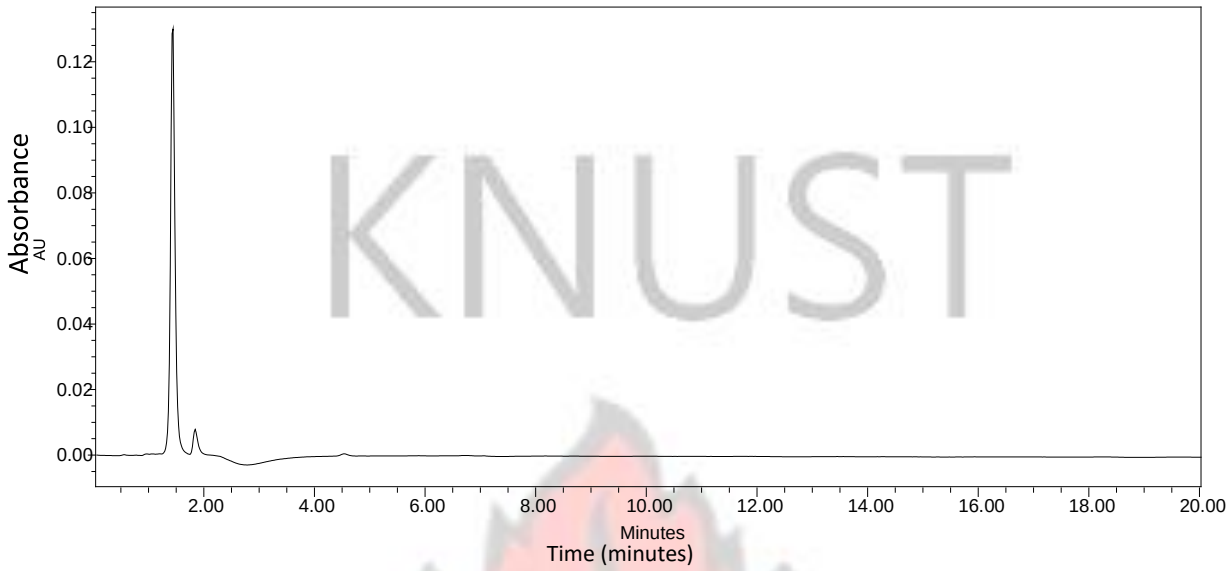


Fig. 15c. HPLC of ethanolic extract of *C. planchonii* root at λmax 450nm.

(Mobile phase MeOH (98%) / Water (2%), retention time, 1.5 minutes, Nebulising control temperature was 55%, drift tube temperature, 52^{0}C, Column temperature, 40^{0}C, flow rate, 1ml/minute, gain, 50, pressure, 45 psi and a run time 20 minutes)

3.4 Effect of *C. planchonii* Extract Concentration and Mode of Storage on Stability of Laboratory-Extracted Shea Butter.

To evaluate the effectiveness of the *C. planchonii* root extract in extending the shelf-life of shea butter, the butter produced under laboratory conditions and treated with the *C. planchonii* root extract was analysed over a 12-month storage period for changes in moisture, free fatty acid (FFA), peroxide values (PV), and iodine values (IV). The results were then subjected to analysis of variance and the interaction between extract concentration and container types evaluated.

3.4.1 Changes in Moisture Levels of Butter during Storage

Fig. 16 shows changes in moisture levels over a 12- month period for laboratory-extracted shea butter as affected by type of containers used during storage. The moisture levels remained constant for all three types of containers until the third month, when it begun to increase rapidly in the open container. The moisture levels in the loose and close containers were however the same until the 6th month when it also started to rise in the loose container. The moisture was however constant for the close container over the entire storage period (Fig. 16). Since the

samples in both open and loose containers were exposed to the atmosphere, moisture in the air was responsible for its rise in both containers. This implies that, shea butter when stored in open or loose containers is predisposed to rancidity (Lipids Part 5, 2007). Again, analysis of variance also showed significant differences ($P < 0.05$) for the different storage conditions as shown in Table 4. Thus from the analysis, shea butter treated with *C. planchonii* extract and stored in close containers can be protected best from absorbing moisture followed by the loose and the open containers respectively, thereby preserving its quality.

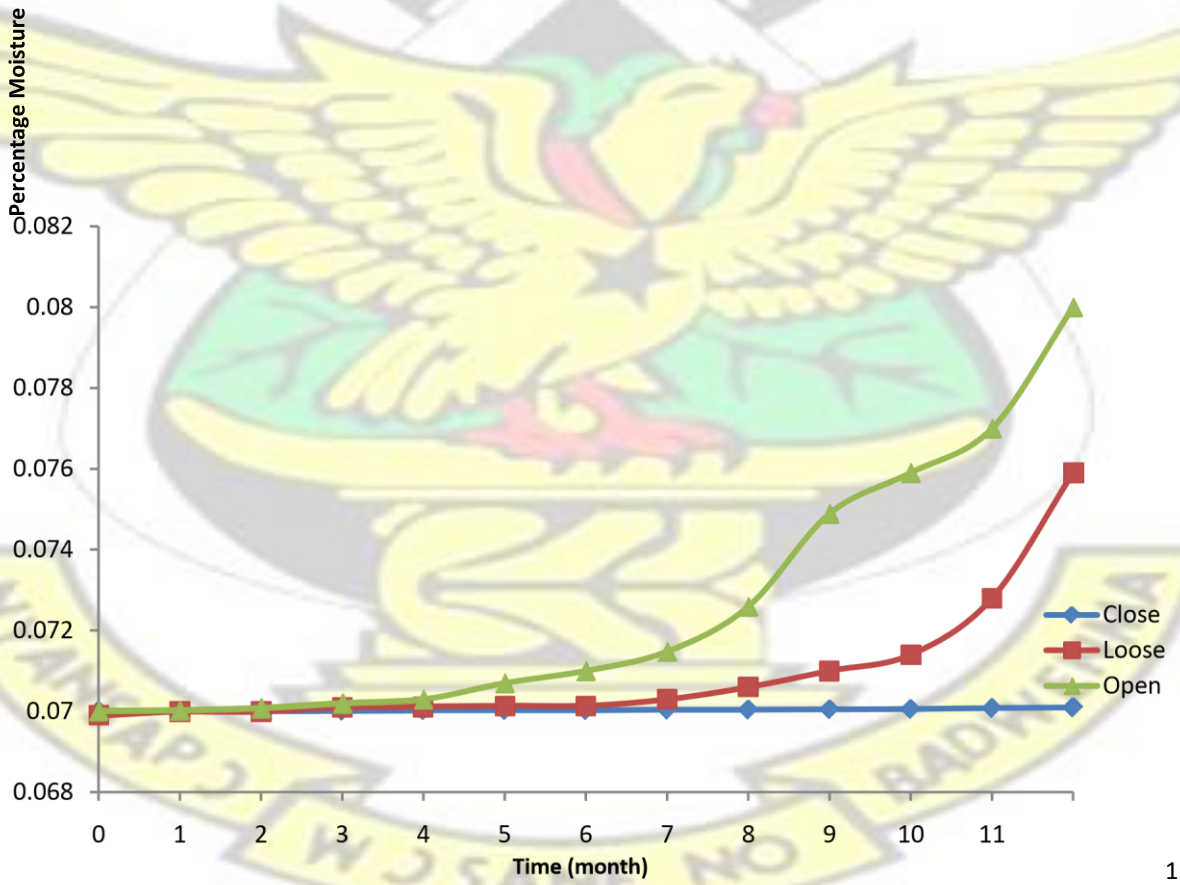


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Fig. 16. Changes in moisture levels over a 12-month storage period for laboratory-extracted shea butter treated with *C. planchonii* root extract in different modes of storage

Table 4. Mean moisture levels for laboratory-extracted shea butter over 12 months storage period in different modes of storage.

----------MODE OF STORAGE ----------			
CLOSE	LOOSE	OPEN	SED
0.000**a**	0.006**b**	0.010**c**	0.0001

N.B: Means with different postscripts (a, b, c) are significantly different (P < 0.05)

3.4.2 Changes in Free Fatty Acid (FFA) Levels in Shea Butter during Storage.

Fig. 17 and 18 show changes in the FFA levels over a 12 - month storage period, as affected by extract concentration and mode of storage used respectively. Both figures indicate that by the eighth month, the untreated shea butter (0.00%) as well as some of the treated samples (0.02% and 0.04%) had started to go rancid as indicated by sharp increase in fatty acid levels. The increase in FFA is indicative of the commencement of reactions which lead to formation of free fatty acids.

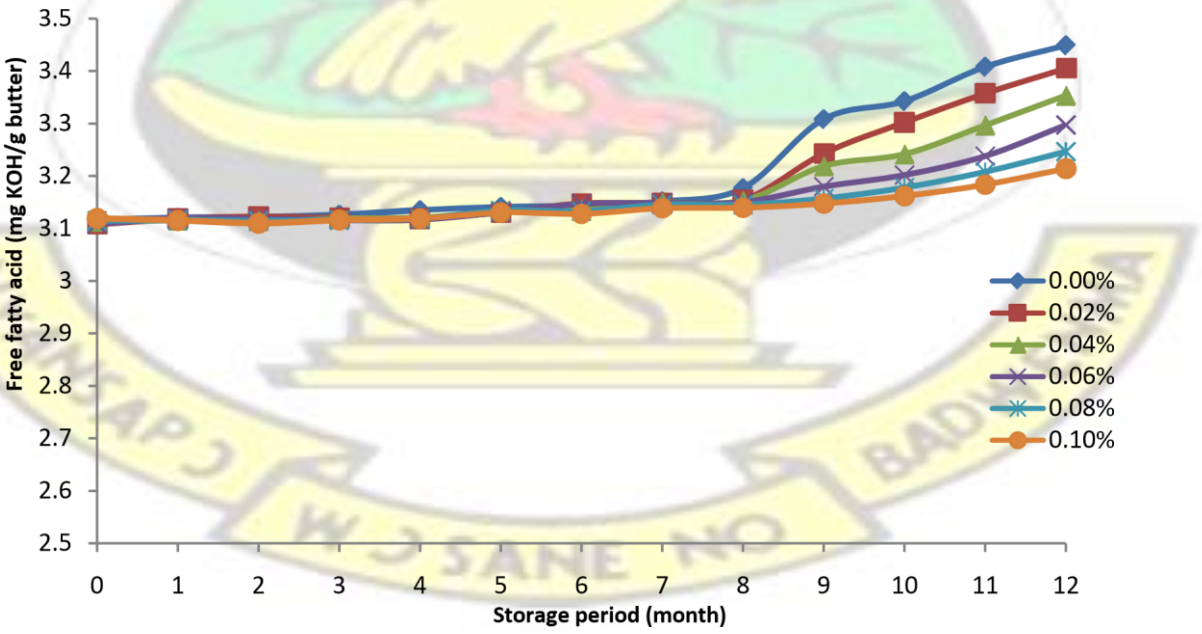

Fig. 17. Changes in free fatty acid levels over a 12-month storage period for laboratory-extracted shea butter treated with *C. planchonii* root extract of various concentrations.

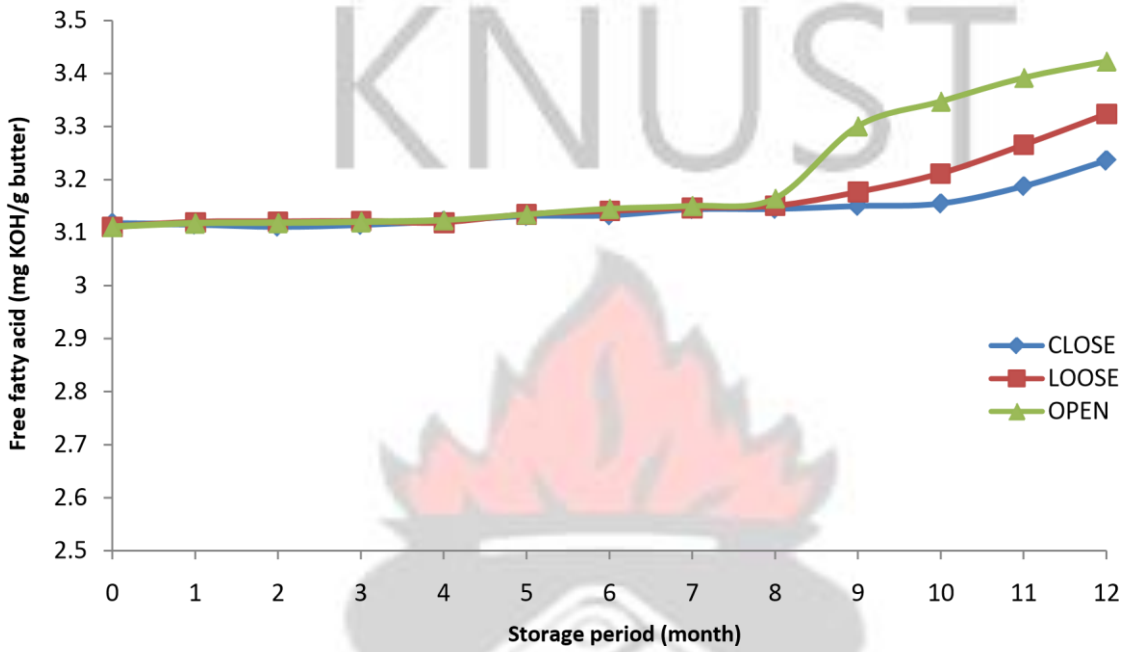

Fig. 18. Changes in free fatty acid levels over a 12-month storage period for laboratory extracted shea butter treated with *C. planchonii* root extract stored in different modes of storage

Treatments with 0.06%, 0.08% and 0.10% extract concentration however did not show sharp increases. It appears the formation of breakdown products was substantially suppressed by the higher extract concentrations. At higher extract concentrations therefore, and under particularly the closed storage conditions, where the moisture and air were virtually absent, the shea butter could not undergo rapid hydrolytic rancidity (Louppe, 1994) as compared to the aerobic environment within the open and loose containers. However, after the 8th month, when the butter in the loose and open containers might have absorbed substantial amount of moisture from the atmosphere (Fig. 16), de-esterification of the butter started at all extract concentrations, hence the rise in FFA for all the treatments for the de-esterification reaction. *C. planchonii* extract, even at higher concentrations could not completely suppress the hydrolysis in the shea butter. In contrast, the higher extract concentrations in the closed container reduced the rate of hydrolysis with lower FFA values up to the end of the 12-month storage period (Fig.

17 and18).

There was a significant ($P < 0.05$) effect of dye concentration (Table 5a) and type of container (Table 5b) on the rate of rancidity. Extract concentrations 0.02%, 0.04% and 0.06% were however not significantly ($P > 0.05$) different from each other but significantly ($P < 0.05$) different from 0.08% and 0.10%, which were also significantly ($P < 0.05$) different from each other (Table 5a). All mode of storage were significantly ($P < 0.05$) different from each other (Table 5b). These imply that butter quality is very dependent on both the extract concentration and the mode of storage.

Interactions between extract concentrations and mode of storage are shown in (Fig. 19). The graph shows that at extract 0 (untreated shea butter or control) concentration, the differences were very large with respect to the mode of storage. These were reduced at extract concentration 0.02%. For 0.04% extract concentration, the differences between open and close containers were smaller compared to that for the loose container. At 0.06%, the differences between the three modes of storage was pronounced but at 0.08% open and loose containers were virtually the same while there was a large difference between them and the closed container.

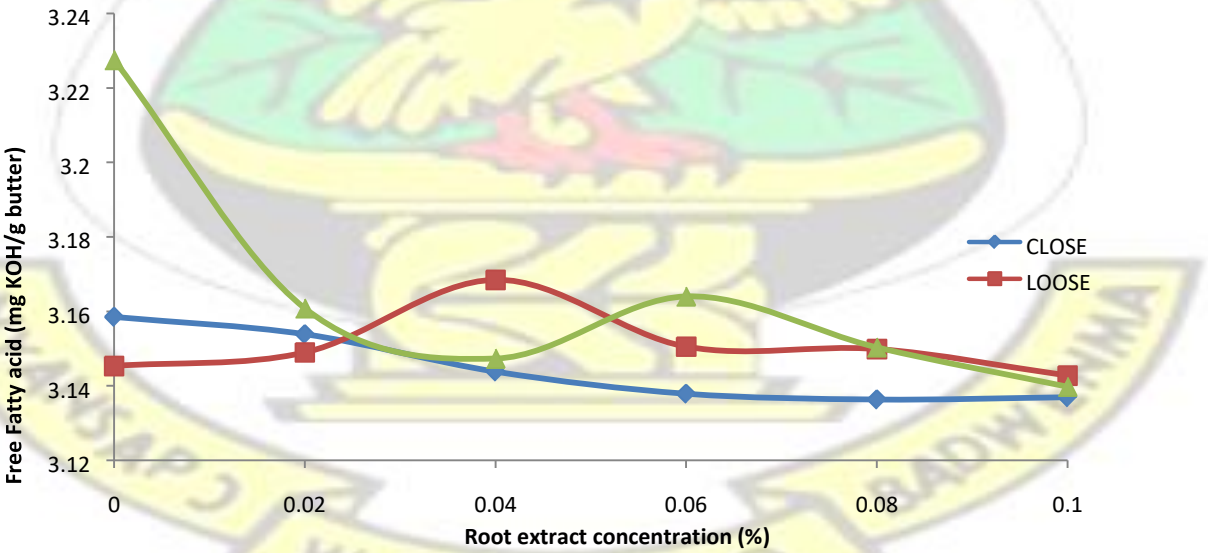

Fig. 19. Interaction between mode of storage and *C. planchonii* root extract concentration at eight (8) months for free fatty acid levels in laboratory-extracted shea butter.

At 0.10%, the differences between the storage conditions were very much reduced. The deduction from this analysis is that shea butter produced by the laboratory process, could be kept under any of the storage conditions and still maintain its butter quality in terms of FFA up to the 8th month of storage but the close container always had an edge over both the loose and open containers in terms of butter keeping quality.

Table 5a. Mean free fatty acid levels at 8 months for laboratory-extracted shea butter: Effect of *C. planchonii* root extract concentration

---------- C. *planchonii* ROOT EXTRACT CONCENTRATION ----------						
0.00%	0.02%	0.04%	0.06%	0.08%	0.10%	SED
3.177**a**	3.155**b**	3.153**b**	3.151**b**	3.145**c**	3.140**d**	0.0023

N.B: Means with different postscripts (a, b, c, d) are significantly different (P < 0.05)

Table 5b. Mean free fatty acid levels at 8 months for laboratory-extracted shea butter: Effect of mode of storage

----------MODE OF STORAGE----------			
CLOSE	LOOSE	OPEN	SED
3.145**a**	3.151**b**	3.165**c**	0.0016

N.B: Means with different postscripts (a, b, c) are significantly different (P < 0.05)

3.4.3 Changes in Levels of Peroxide Value (PV) during Storage

PV is one of the most common tests used to evaluate the extent of lipid oxidation, and is based on measuring peroxides produced during storage (Gan *et al.*, 2004; British Pharmacopeia, 1993; Rossell, 1983; Hudson and Gordon, 1994). High PVs indicate high degree of rancidity in a fat / oils and that more oxygen molecules are attached to the double bonds of the fatty acids of the fat / oils to produce peroxides or hydroperoxides.

Fig. 20 and 21 show changes in PV levels over a 12-month period, as affected by *C. planchonii* root extract concentration and type of container, respectively, for the laboratory extracted shea butter. Results in Fig. 20 show that PV steadily increased with storage time for all concentrations of root extract used. The results also show some degree of concentration dependency, as higher extract concentrations gave lower peroxide values over the entire storage period (Fig. 20). However, the rates at which PV changed differed for each type of container, as reflected in Fig. 21. The rate of production of peroxides or hydroperoxides was however slowest for the closed container over the 12-month period (Fig. 21). Under anaerobic conditions, where air supply is virtually not available, the rate of production of intermediate products would be low since PV is a measure of oxidative rancidity and requires oxygen to occur (Rossell, 1983). Oxygen attacks the double bonds to form hydroperoxides and peroxides. These peroxides subsequently break down to form oxidation products such as ketones and aldehydes.

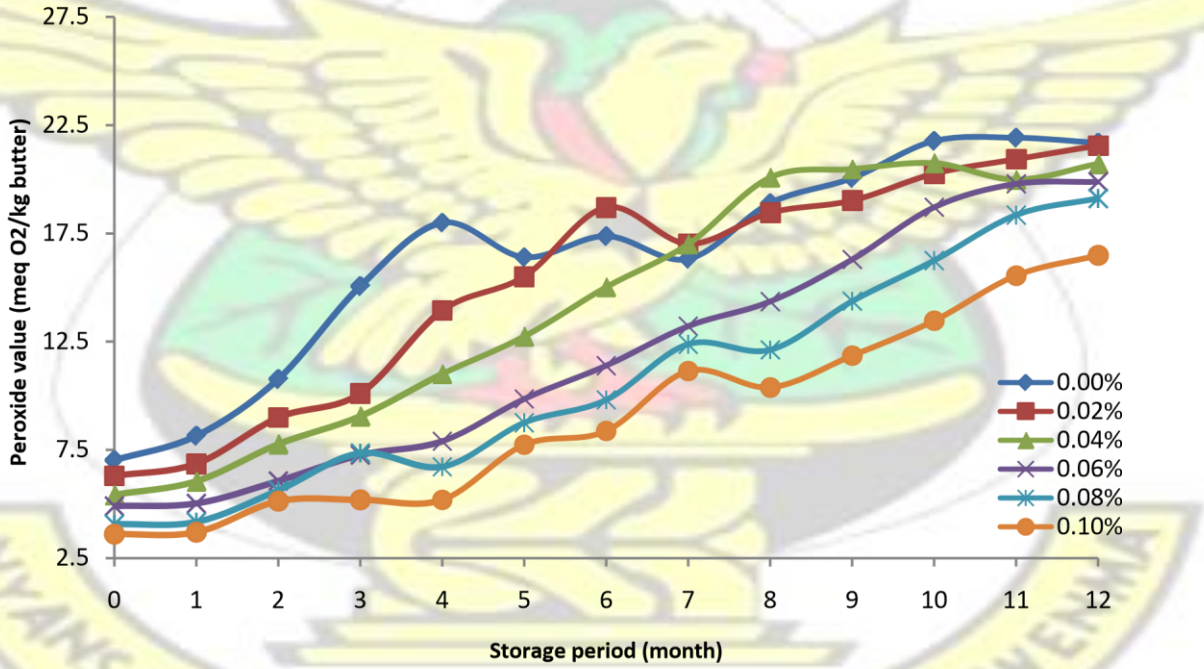

Fig. 20. Changes in peroxide values over a 12-month storage period for laboratory-extracted shea butter treated with *C. planchonii* root extract at various concentrations.

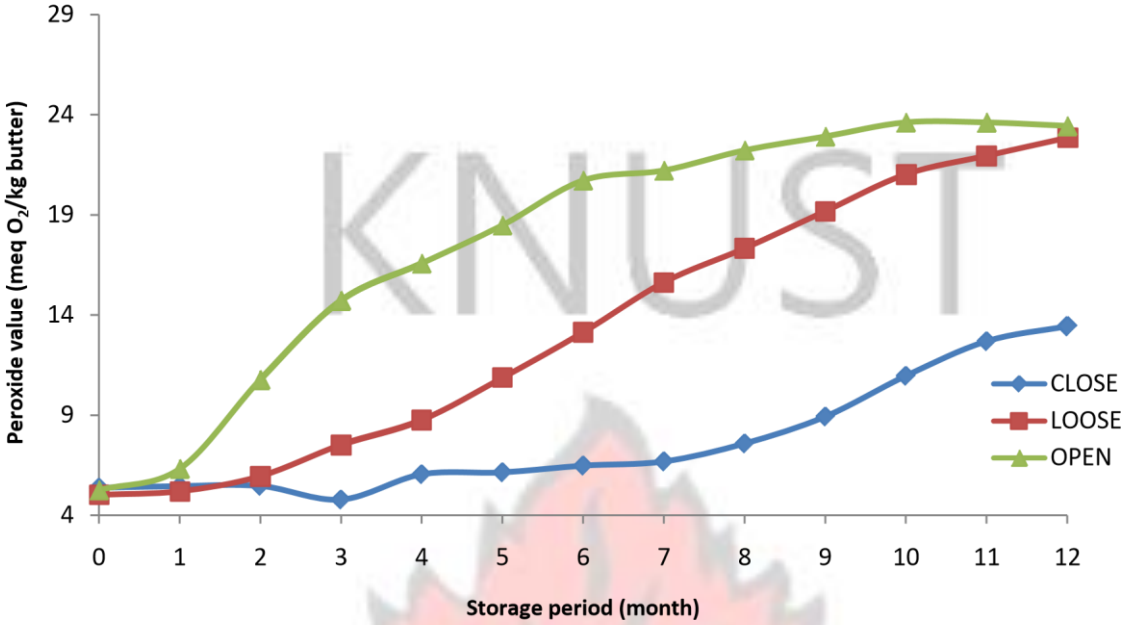

Fig. 21. Changes in peroxide value levels over a 12-month storage period for laboratory-extracted shea butter treated with *C. planchonii* root extract in different modes of storage

From Fig. 21, it can be seen that as far as the type of containers were concerned, a sharp rise in PV values occurred after the 1st month for the open and similarly after the second month for loose containers. The values for the loose and open containers were generally higher as compared to that for the close container. The above observation can be attributed to atmospheric oxygen and light being absorbed by the shea butter during storage, thereby producing intermediate oxidation products (Sinclair *et al*., 1990; Garrow and James, 1993).

After the 2nd month the high increase in PV values for the open and loose containers may also be due to the fact that the rate of production of hydro-peroxides or peroxides exceeded the rate of their decomposition. The presence of air and metallic ions like iron (during the milling of the kernels) may also have enhanced the oxidation of the shea butter, rapidly producing free radicals, peroxides, hydro-peroxides and other intermediate products as reported (Sattar and Demen, 1973; Pryor, 1976; Rossell, 1983; Frankel, 1984; Frankel, 1985).

C. planchonii root contains gallic acid, ß – bisabolene ß – carotene (Table 3c; Figures14; 15b and 15c) in its extract and therefore were preferentially oxidised or scavenged the free radicals.

These compounds were able to reduce the rate of chain initiation reactions or interfere with chain-propagation of free radical chain- reactions in the butter and probably terminated some of the free radical reactions (Larson, 1988; Kanner and Rosenthal, 1992; Palozza and Krinsky1992a; Pascale *et al.,* 1999; Goupy *et al.,* 1999; Kaur and Kapoor, 2002).

At higher extract concentrations therefore, the butter appeared to be protected from getting rancid quickly since plant–derived antioxidants have been shown to function as singlet or triplet oxygen quenchers, free radical scavengers and peroxide decomposers (Larson, 1988). The reduction of oxygen might not have been complete in the open and loose containers under normal conditions, thus probably more reactive intermediate products were produced in an electron deficient environment (Wardlaw and Kessel, 2002), and hence relatively higher PVs in the open and loose containers were observed. The extract was however able to slow down the rate of the production of the peroxides, hydroperoxides or free radicals (Gordon, 1990; Palozza and Krinsky, 1992a; Kanner and Rosenthal, 1992; Goupy *et al.,* 1999; Kaur and Kapoor, 2002) in all the treatments. It could however not stop completely the production of peroxides or hydro-peroxides but could only slow down the rate of their production (Jadhav *et al.,* 1995), hence the steady rise in PVs during the storage period in all treatments (Fig. 20).

Analysis of variance carried out on PV obtained after six (6) months indicated that there were significant differences ($P < 0.05$) in effects of root extract concentration (Table 6a), type of container (Table 6b) and the interaction between the two factors (Fig 22). Root extract concentrations of 0.00%, 0.02% and 0.04% were significantly different ($P < 0.05$) from the others. Root extract concentration 0.06% was significantly different ($P < 0.05$) from dye concentration of 0.10% whereas root extract concentration 0.08% was not significantly different ($P > 0.05$) from extract concentrations 0.06% or 0.10%. Close, open and loose containers were significantly different ($P < 0.05$) from each other. The interaction between *C. planchonii* root extract concentration and type of container is shown graphically in Fig. 22.

Table 6a. Mean Peroxide values at 6 months for laboratory-extracted shea butter: Effect of *C.planchonii* root extract concentration

----------C. *planchonii* ROOT EXTRACT CONCENTRATION----------						
0.00	0.02	0.04	0.06	0.08	0.10	SED
17.370**a**	18.700**a**	15.019**a**	11.389**b**	9.806**bc**	8.375**c**	1.2407

N.B: Means with common postscripts (a, b, c) are not significantly different (P > 0.05)

Table 6b. Mean Peroxide values at 6 months for laboratory-extracted shea butter: Effect of mode of storage

----------MODE OF STORAGE ----------			
CLOSE	LOOSE	OPEN	SED
6.474**a**	13.134**b**	20.721c	0.1717

Table 6c. Mean Peroxide values at 8 months for laboratory-extracted shea butter: Effect of *C.planchonii* root extract concentration

----------C. *planchonii* ROOT EXTRACT CONCENTRATION----------						
0.00	0.02	0.04	0.06	0.08	0.10	SED
18.903**a**	18.452**a**	20.089**a**	14.351**b**	12.122**c**	10.383**c**	0.8942

Table 6d. Mean Peroxide values at 8 months for laboratory-extracted shea butter: Effect of mode of storage

----------MODE OF STORAGE ----------			
CLOSE	LOOSE	OPEN	SED
7.583**a**	17.335**b**	22.233**c**	0.1242

N.B: For tables 6b, 6c and 6d, means with different postscripts (a, b, c) are significantly different (P < 0.05)

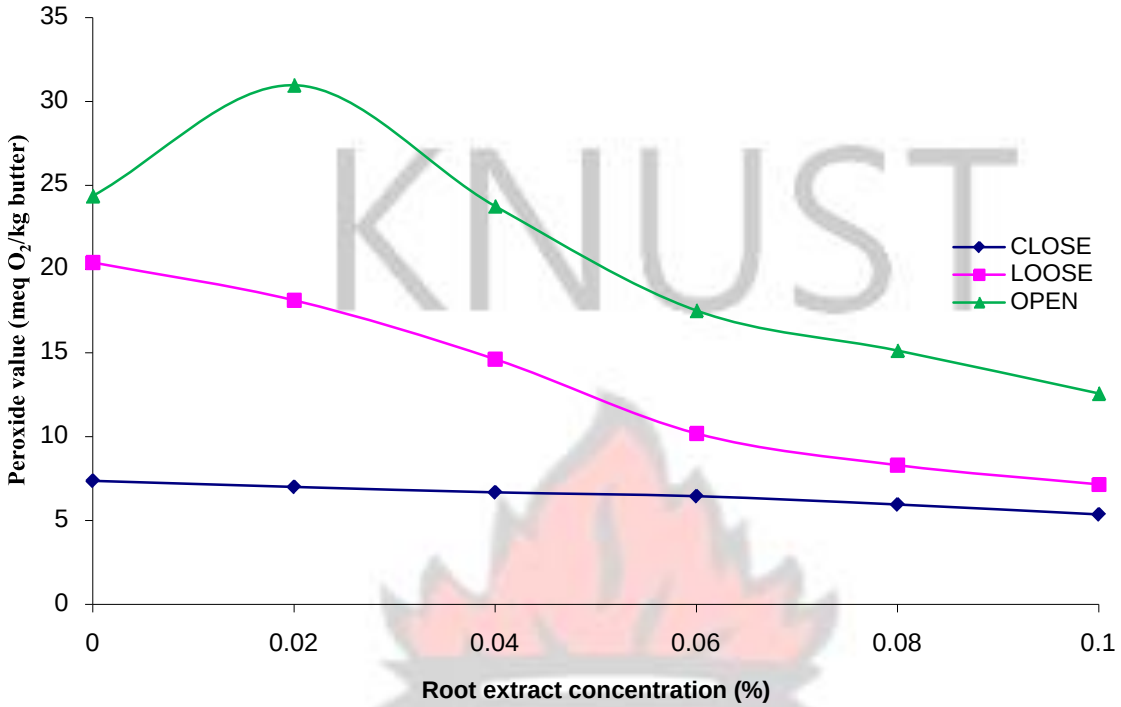

Fig. 22. Interaction between mode of storage and *C. planchonii* root extract concentration at 6 months for peroxide value in laboratory-extracted shea butter

Fig. 22 shows that there were large differences of PV in the untreated shea butter (control). These interactions with the mode of storage continued until root extract concentration 0.06%, with the close container showing the best results followed by the loose container and the open container in terms of keeping quality of the butter (lower peroxide values). At root extract concentrations 0.08% and 0.10%, however, the lines were nearly parallel, indicating that there were no interactions at these concentrations. It implies that at these high extract concentrations, the butter was being protected from rapid oxidation. Also, by the 6th month and at all extract concentrations the close container had an edge over the loose and open containers in terms of keeping quality of the butter. The closed container however maintained a about a constant PV, implying that, under anaerobic condition, air which is necessary for the break-down of butter to hydroproxides was not available.

Similarly, at eight (8) months, there was a significant difference ($P < 0.05$) in effect of *C. planchonii* root extract concentration (Table 6c) and mode of storage (Table 6d) and also of the interaction between the two factors (Fig. 23).

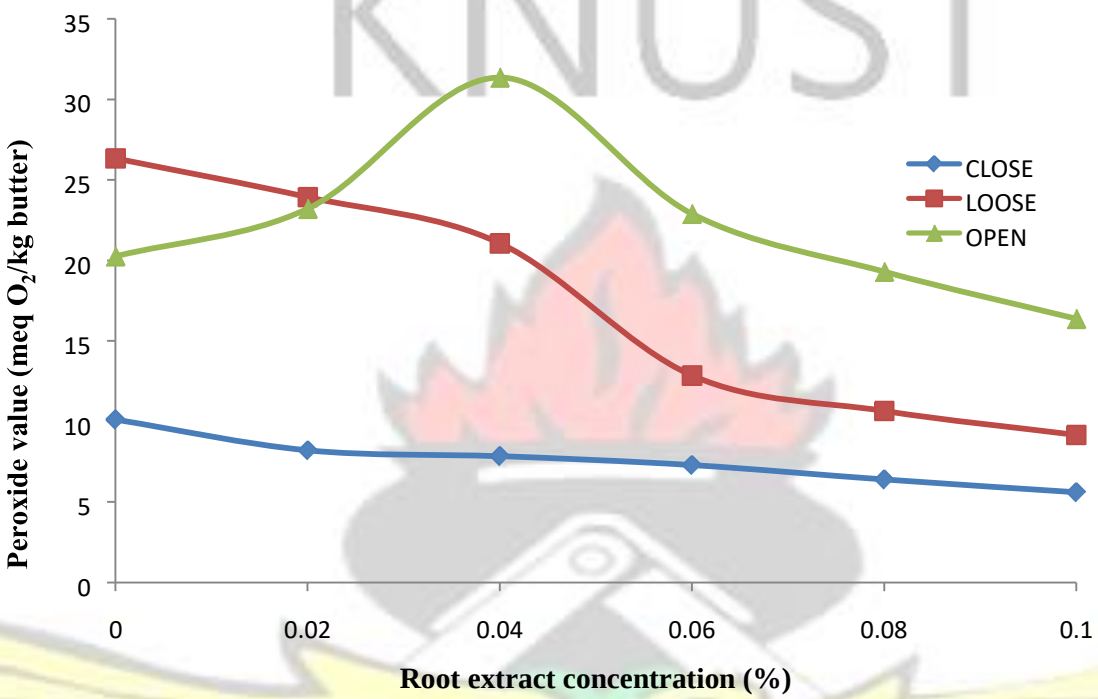

Fig. 23. Interaction between mode of storage and *C. planchonii* root extract concentration at 8 months for peroxide values in laboratory-extracted shea butter

The control and root extract concentrations of 0.02% and 0.04% were significantly ($P < 0.05$) different from the other root extract concentrations. Root extract concentration 0.06% was also significantly ($P < 0.05$) different from concentrations 0.08% and 0.10%, which were not significantly ($P > 0.05$) different from each other. All container types were significantly ($P < 0.05$) different from each other. Interactions are shown graphically (Fig. 23) after 8 months of storage. The graph shows that in the absence of extract (control), the differences were quite large. Some interaction is clearly observed at root extract concentration 0.04%, 0.06%, 0.08% and 0.10% with the closed container being better than the loose which is in turn better than the open container in terms of butter keeping quality. At root extract concentration 0.08% and 0.10%, even though these differences are maintained, the lines are basically parallel to each other indicating no interaction. The analysis showed that higher extract concentrations (0.06% and

0.10%) in the butter, produced lower PVs in loose than the open containers. PV however, did not change significantly ($P > 0.05$) in the close container during the period irrespective of extract concentration. It can be inferred generally that at higher extract concentrations, butter stored in close container had the lowest PVs and so the best in terms of quality.

3.4.4 Changes in Levels of Iodine Value (IV) during Storage.

Unsaturated fats/oils are very reactive toward iodine. The more iodine is absorbed, the higher is the iodine number or value, and the more reactive, less stable and more susceptible to oxidation or rancidity is the oil or fat. Unsaturated compounds which contain double or triple bonds are generally very reactive to iodine. The iodine value (IV) is therefore a measure of the degree of unsaturation in fatty acids (West *et al*., 1971). Iodine value can therefore be used to monitor the degree of rancidity. A reduction of IV in fat indicates that there is saturation or reduction of the number of double or triple bonds in a fat and signifies rancidity. The above principle was therefore used to monitor the effect of *C. planchonii* root extract on shea butter keeping quality during storage.

Fig. 24 and 25 show changes in IV over a 12-month period as affected by *C. planchonii* root extract concentration and mode of storage respectively, for the laboratory-extracted shea butter. Although clustered in the early stages, Fig. 24 shows clearly that by the twelfth month, higher IVs were produced by the higher concentrations of root extract. The control, with no root extract gave the lowest IVs. Similarly, Fig. 25 shows that by the twelfth month the close container had the best IV value followed by the loose container with the open container having the lowest value.

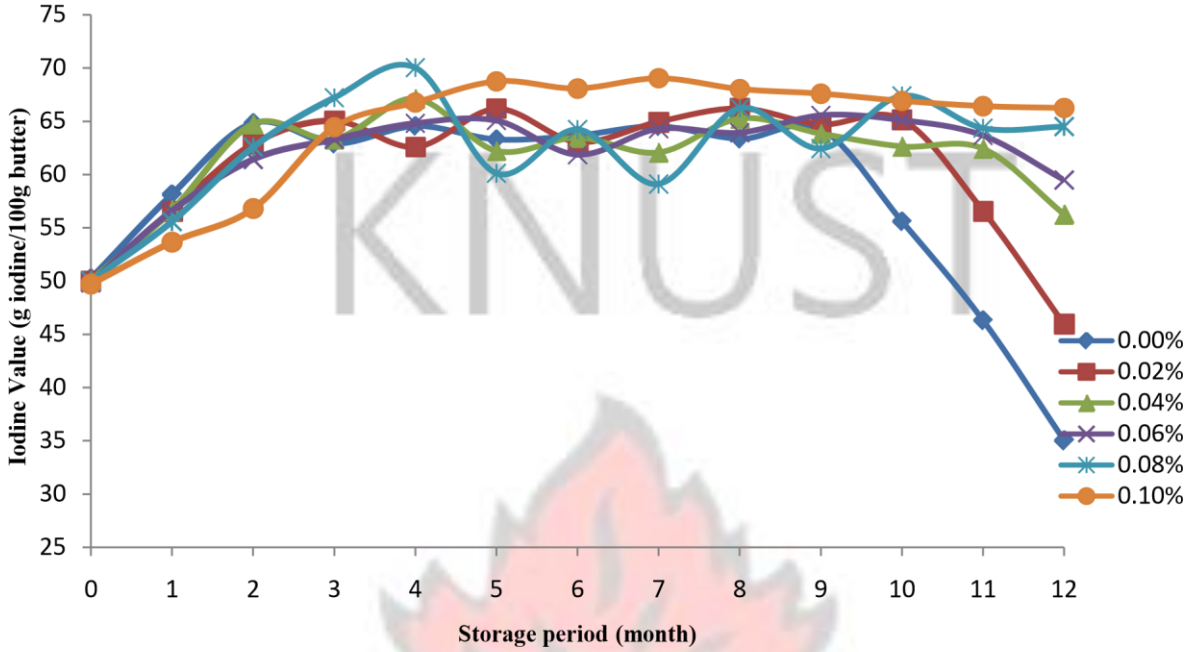

Fig. 24. Changes in iodine values over a 12-month storage period for laboratory-extracted shea butter treated with *C. planchonii* root extract at various concentrations.

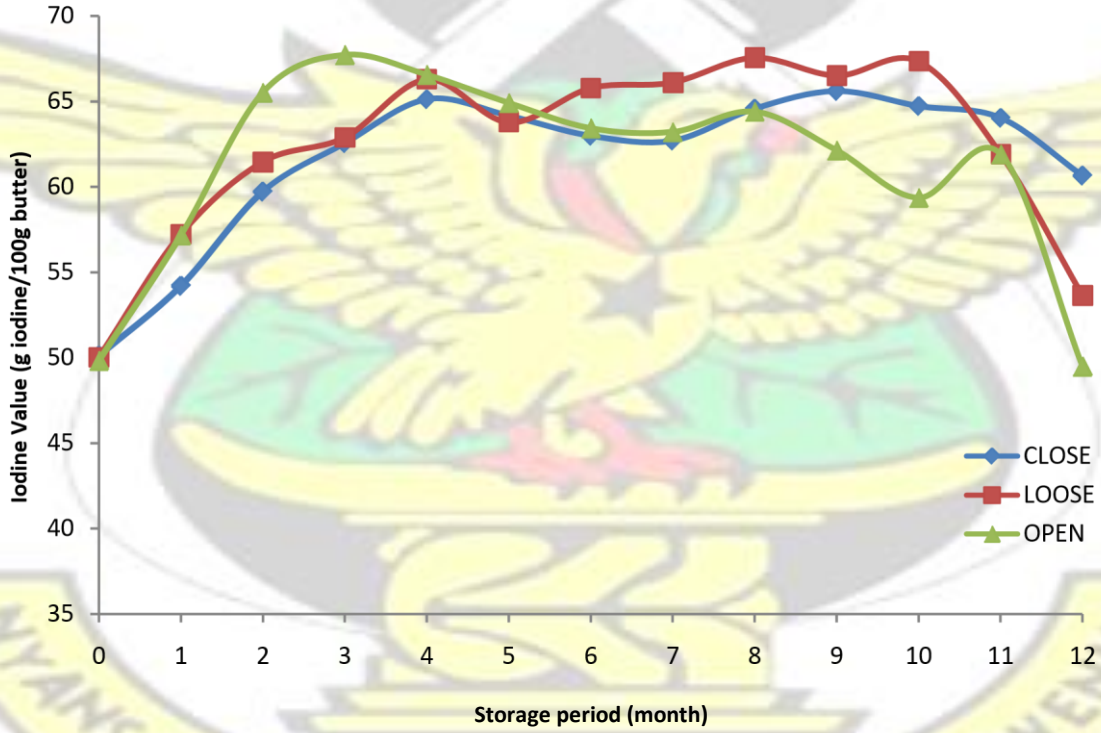

Fig. 25. Changes in Iodine valuesover a 12-month storage period for laboratory-extracted shea butter treated with *C. planchonii root* extract in different modes of storage

There were initial increases in the IVs for all treatments for the first 3 months. They all then fluctuated into a plateau-like form until the 10th month when the IV values begun to drop rapidly

for treatments 0.00 and 0.02% (Fig. 24). The decrease in iodine value is an indication of oxidation as a result of decreasing number of double bonds in the butter. Oxidative rancidity affects the unsaturated fatty acids; as it progresses, polyunsaturated fatty acids polymerize or break down to smaller molecules with fewer double bonds**.** The fluctuation in iodine values may be due to dehydrogenation or saturation of the fatty acid double bonds in the shea butter (Rossell, 1983; Nkafamiya *et al.,* 2007; Holiday and Pearson, 1979) working on the degradation properties of wild *Adansonia digitata* and *Prosopsis africana* and oil from soya beans respectively, reported similar observations.

The IVs were relatively higher in treatments with higher *C. planchonii* root extract concentrations. The above is an indication that the proportion of unsaturated fatty acids remained higher in the higher *C. planchonii* root extract concentration treatments during the 12month period. Thus the higher concentrations of the extract added to the butter protected it against development of rapid rancidity during the storage period. The gallic acid, ß – bisabolene ß – carotene (Fig.14, 15b, 15c) in the extract were preferentially oxidised or scavenged the free radicals, and so were able to reduce the rate of propagation of free radical chain reactions in the butter (Larson, 1988; Goupy *et al.,* 1999; Kaur and Kapoor, 2002).).

Results after six (6) months indicated that the main effects (*C. planchonii* root extract concentration (Table 7a) and mode of storage (Table 7b)) as well as the interaction between the main effects were significant ($P < 0.05$). The treatments with 0.00%, 0.02%, 0.04% and 0.08% root extract concentrations were not significantly different ($P > 0.05$) from each other. The highest concentration of 0.10% was however, significantly different ($P < 0.05$) from the other concentrations. The close and open containers were significantly ($P < 0.05$) different from the loose container. The interaction between the two factors at 8 months is presented in Tables 7c and 7d. With addition of 0.06% extract, shea butter quality in terms of its IV stored in close or open containers were almost the same. However at the highest concentration of 0.10%, shea

butter stored in close container had the highest IV, followed by the butter in the loose and open containers respectively. The analysis implies that the keeping quality of butter is greatly affected by the type of container used for storage. Also shea butter would retain most of its fatty acid characteristics when treated with 0.10% *C. planchonii* root extract and stored in a close container for up to 8 months storage period.

Table 7a. Mean Iodine values at 6 months for laboratory-extracted shea butter: Effect of *C. planchonii* root extract concentration

---------- C. *planchonii* ROOT EXTRACT CONCENTRATION----------						
0.00	0.02	0.04	0.06	0.08	0.10	SED
63.679**a**	63.059**ab**	63.446**a**	61.870**b**	64.225**a**	68.076**c**	0.7035

N.B: Means with common postscripts (a, b, c) are not significantly different (P > 0.05)

Table 7b. Mean Iodine values at 6 months for laboratory-extracted shea butter: Effect of mode of storage

----------MODE OF STORAGE----------			
CLOSE	LOOSE	OPEN	SED
62.988**a**	65.780**b**	63.420**a**	0.5540

Table 7c. Mean Iodine values at 8 months for laboratory-extracted shea butter: Effect of *C. planchonii* root extract concentration

----------C. *planchonii* ROOT EXTRACT CONCENTRATION----------						
0.00%	0.02%	0.04%	0.06%	0.08%	0.10%	SED
63.354**a**	66.230**b**	65.267**b**	63.932**a**	66.245**b**	68.033**c**	0.6683

N.B: For tables 7b and 7c means with different postscripts (a, b, c) are significantly different (P < 0.05)

Table 7d. Mean Iodine values levels at 8 months for laboratory-extracted shea butter: Effect of mode of storage

----------MODE OF STORAGE ----------

CLOSE	LOOSE	OPEN	SED
64.578**a**	67.551**b**	64.402**a**	0.5262

N.B: Means with different postscripts (a, b) are significantly different (P < 0.05)

3.5 Effect of *C. planchonii* Extract Concentration and Mode of Storage on Quality of Locally Extracted Shea Butter during Storage

The local method of shea butter production as shown in Fig. 2 is slightly different from the laboratory method shown in Fig. 13. There was therefore the need to subject the shea butter produced locally to the same treatment and analysis as the laboratory-extracted butter in order to compare the effects of *C. planchonii* extract treatment on the characteristics of the butter from both methods. Therefore, to evaluate the effectiveness of the *C. planchonii* extract in extending the shelf life of locally produced shea butter, butter was purchased from the open market and treated with the same concentrations of *C. planchonii* root extract and analysed over a 12-month storage period for changes in moisture, free fatty acid (FFA), peroxide values (PV), and iodine values (IV).

3.5.1 Changes in moisture levels during storage.

Fig. 26 shows changes in moisture levels over a 12-month period when the shea butter was treated with *C. planchonii* root extract and stored in different containers. There was a gradual increase in moisture level in the open container from the start (0 month) until about 6th month and then a rapid rise until the 12th month. Samples in the loose and close containers however maintained a constant moisture level from the start to 6 months of storage and then began to rise until the 12th month. The butter from the open market had higher initial moisture content compared to the laboratory-extracted one (Figs. 16, 26) and so rancidity must have occurred more readily in the butter from the open market compared with the laboratory-extracted one. Moisture has been established as one of the factors responsible for oxidation in fats and oils (Rossell, 1983) and so high initial moisture of the butter predisposes it to rancidity.

Moisture levels showed no significant differences (P > 0.05) for the various storage modes used as shown in Table 8. This means it does not matter which container type was used, changes in moisture levels would not be appreciable during the 12 – month storage period.

From the results, the degree of rancidity of the butter in the containers would be in the order open > loose >close. This means that wherever shea butter needs to be kept, it would be best to store in close containers so as to preserve its good characteristics with respect to moisture content and any oxidisable products.

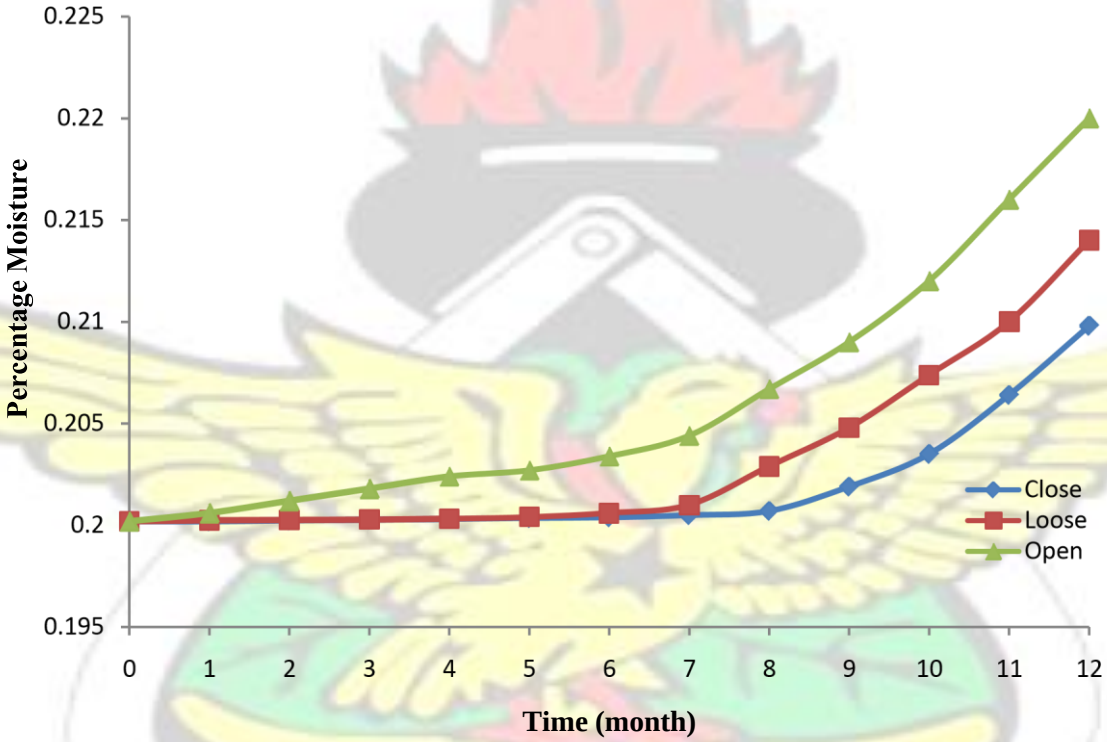

Fig. 26. Moisture levels over a 12-month period for locally extracted shea butter treated with *C. planchonii* root extract in different modes of storage

Table 8. Difference in moisture levels over 12-month period for locally extracted shea butter stored in different modes of storage

----------MODES OF STORAGE----------			
CLOSE	LOOSE	OPEN	SED
0.010a	0.014a	0.020a	0.0088

N.B: Means with common postscripts (a) are not significantly different (P > 0.05)

3.5.2 Changes in Levels of Free Fatty Acid (FFA) in Shea Butter during Storage Fig. 27 and 28 show changes in FFA levels over a 12-month period as affected by the extract concentration and the type of container used, respectively for locally produced shea butter. The point of inflexion occurred at 3 months after the start of monitoring as indicated by a sharp rise in FFA levels.

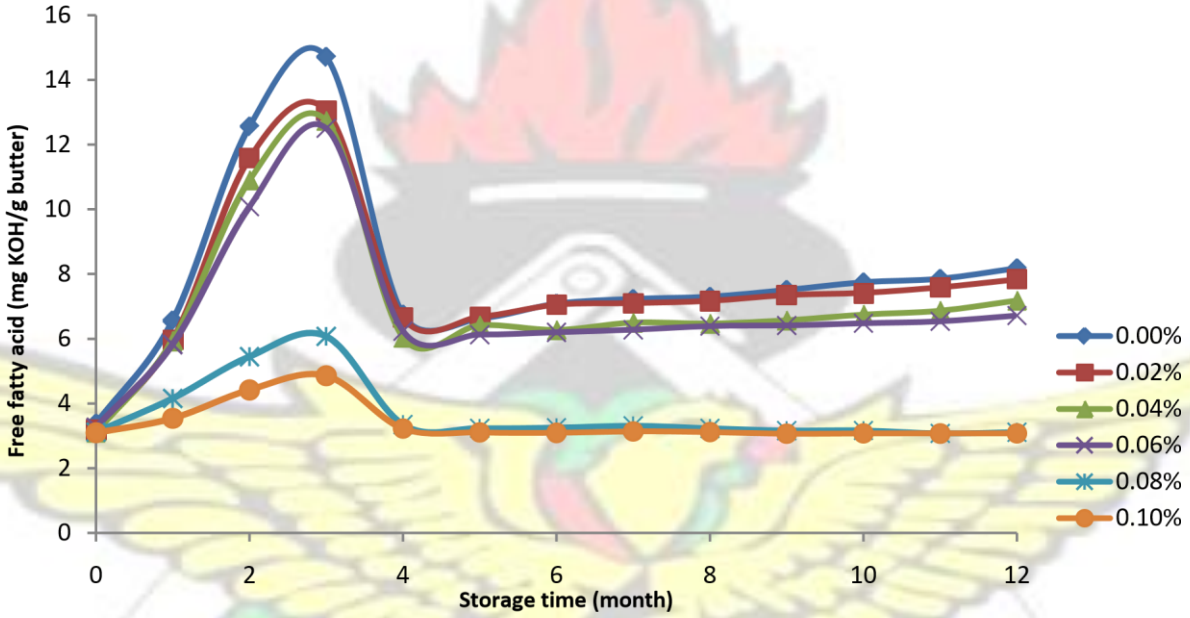

Fig. 27. Changes in fee fatty acid levels over a 12-month storage period for locally extracted shea butter treated with *C. planchonii* root extract at various concentrations.

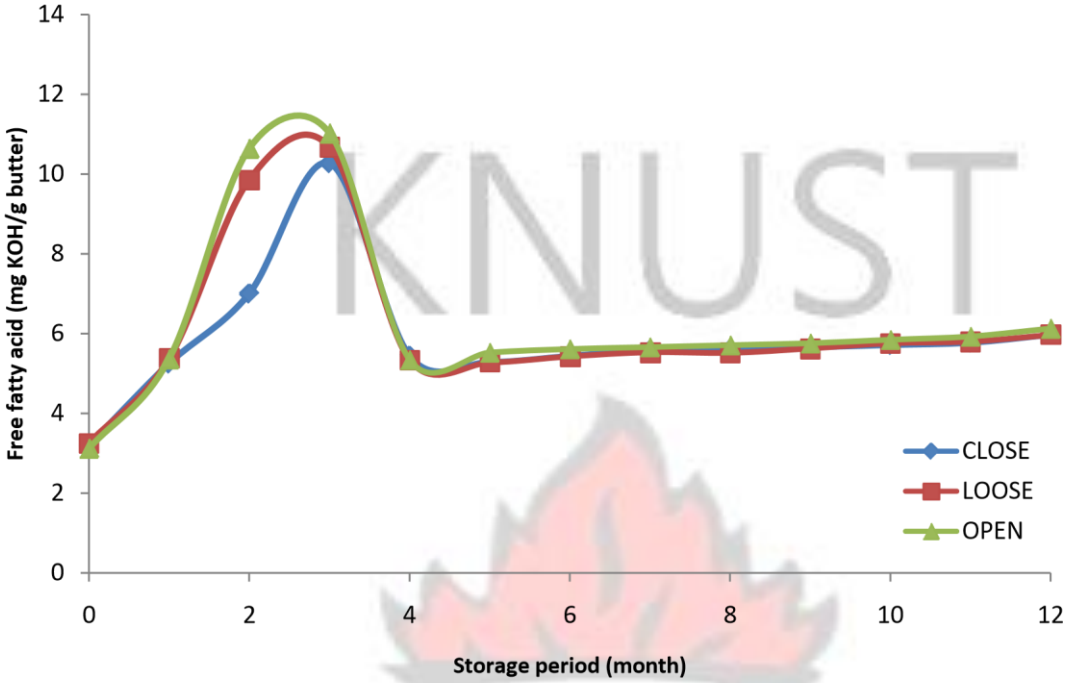

Fig. 28. Changes in free fatty acid levels over a 12-month storage period for locally extracted shea butter treated with *C. planchonii root extract* stored in different mode of storage

The *C. planchonii* root extract appears not to be able to control oxidation of the shea butter, hence the sharp increase in the FFA for all the treatments until the 3rd month (Fig. 27). This implies that the locally produced shea butter had no induction period at all. Rancidity had set in even before the commencement of the experiment due to the traditional method used for the production. The indigenes collect the ripe shea fruits and allow the pulp to ferment for between 3 - 5 days to facilitate depulping. During this period, enzymatic activity takes place, resulting in the hydrolysis of the triglycerides, thereby producing free fatty acids in the kernel prior to the extraction of the butter. Also, during the packaging of the butter, water is used to facilitate the solidification process (Section 1.2.1.1), hence the high initial FFAs in the locally produced butter. However after the point of inflection, the FFAs decreased rapidly until the 4th month when they levelled off for all extract concentrations used (Fig. 27). Treatments with higher extract concentrations however were able to lower the FFAs compared to that of the lower extract concentrations. This may imply that the components present in higher concentration of the *C. planchonii* root extract

(Table 3c; Achenbach, 1986; Addae- Mensah *et al.*, 1985) were available and became oxidized in preference to the shea butter and so any further release of FFA was suppressed (Larson, 1988). Even though shea butter contains tocopherols, these might have been lost during processing (Maranz *et al.*, 2004) and so could not protect the butter from becoming rancid. High initial FFAs, coupled with high initial moisture levels and presence of metallic ions are favourable conditions for oxidation of the shea butter (Lipids Part 5, 2007); so prior to the addition of the *C. planchonii* root extract, free radicals and FFA were already present. Any further release of FFAs, initiation of free radical reactions, interfering chainpropagating free radical- chain reactions appeared to have been suppressed by the C. *planchonii* root extract in the shea butter after the 4th month. The extract therefore appears to have played a dual role; it was able to suppress rancidity and also able to mop up existing free radicals in an already rancid shea butter (Larson, 1988). The rate of production of FFAs may then be assumed to be equal to the rate of its consumption from the 4th month. This may therefore explain the constant nature of the curves after the 4th month. Since the laboratory extracted butter was almost moisture-free, rancidity could not set in so fast (Fig. 17). It can also be inferred that, C. *planchonii* root extract at higher concentrations particularly above 0.08% can reduce the rate of rancidity of the shea butter but once oxidation has started, it cannot eliminate it completely (Jadhav *et al.*, 1995**)**.

Due to the seemingly peculiar nature of the curves (Fig. 27 and 28), ANOVA was done for the first and third months of locally produced shea butter storage. FFA values at the first month indicated that there were significant ($P < 0.05$) effects of extract concentration (Table 9a). This implies that changes in FFAs were dependent on the concentration of the extract. The interaction between the 2 factors was significant ($P < 0.05$). Type of container (Table 9b) however, did not affect the level of FFA ($P > 0.05$) as at the end of the first month. This also means that the mode of storage did not affect the keeping quality of the butter in terms of its FFA values within the first month and that it did not matter the container type used, butter quality would be the same.

At the third month, the amount FFAs present indicated significant ($P < 0.05$) effect of dye concentration (Table 9c) and mode of storage (Table 9d) and also of the interaction between the two factors (Fig. 29). The control (no dye) was significantly ($P < 0.05$) different from all those given various concentrations of dye treatment. Extract concentrations of 0.02%, 0.04% and 0.06% gave FFAs that were significantly ($P < 0.05$) different from those given by extract concentrations of 0.08% and 0.1%, which were not significantly ($P < 0.05$) different from each other. The closed container was significantly different ($P < 0.05$) from the loose and open containers.

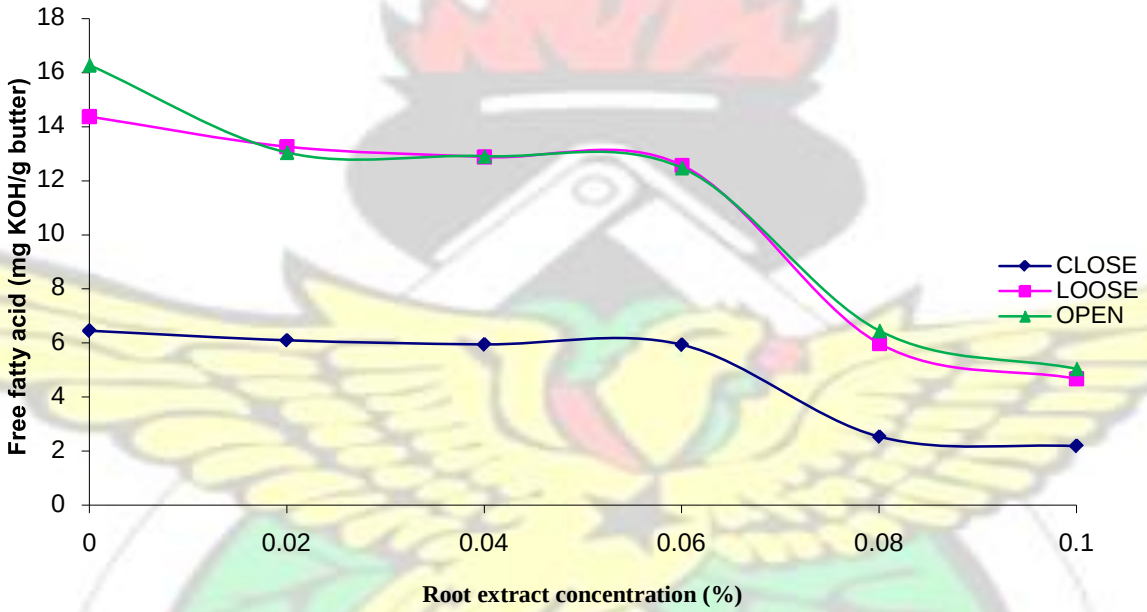

Fig. 29. Interaction between mode of storage and *C. planchonii* root extract concentration at 3 months for free fatty acid levels in locally extracted shea butter

The results indicate that the close container was most suitable for storing locally produced shea butter, compared to the loose and open containers three months after the start of monitoring shea butter quality. These differences were maintained at all levels of extract concentrations. The implication is that for locally processed shea butter treated with *C. planchonii* root extract, once rancidity has begun, the butter quality did not depend on which container type which was used for storage by the 3rd month. However, the butter in the closed container was superior in terms of its FFA values (lower) to the butter in both loose and open containers.

Table 9a. Mean free fatty acid levels at 1 month for locally produced shea butter: Effect of *C. planchonii* root extracts concentration.

---------- C. planchonii ROOT EXTRACT CONCENTRATION----------						
0.0%	0.02%	0.04%	0.06%	008%	0.10%	SED
6.591**a**	5.973**a**	5.944**a**	5.842**a**	3.108**b**	2.131**b**	0.7237

Table 9b. Mean free fatty acid levels at 1 month for locally produced shea butter: Effect of mode of storage

----------MODE OF STORAGE----------			
CLOSE	LOOSE	OPEN	SED
5.094**a**	4.883**a**	4.817**a**	0.3899

Table 9c. Means free fatty acid levels at 3 months for locally produced shea butter: Effect of *C. planchonii* root extract concentration

----------C. planchonii ROOT EXTRACT CONCENTRATION----------						
0.0%	0.02%	0.04%	0.06%	008%	0.10%	SED
12.369**a**	10.803**b**	10.579**b**	10.329**b**	4.989**c**	3.970**c**	0.7272

Table 9d. Means free fatty acid levels at 3 months for locally produced shea butter: Effect of mode of storage

----------MODE OF STORAGE ----------			
CLOSE	LOOSE	OPEN	SED
4.860**a**	10.625**b**	11.035**b**	0.3918

N.B: For tables 9a, 9b, 9c and 9d means with different postscripts (a, b, c) are significantly different (P < 0.05)

3.5.3 Changes in Levels of Peroxide Value (PV) during Storage

Fig. 30 and 31 show the changes in PVs over a 12-month period for effect of extract concentration and mode of storage, respectively. Fig. 30 shows a trend of steady increase in the values over

the period, with exception of the control and 0.02% concentration which showed a decline after 8 months. A similar trend was observed with respect to container type (Fig. 31).

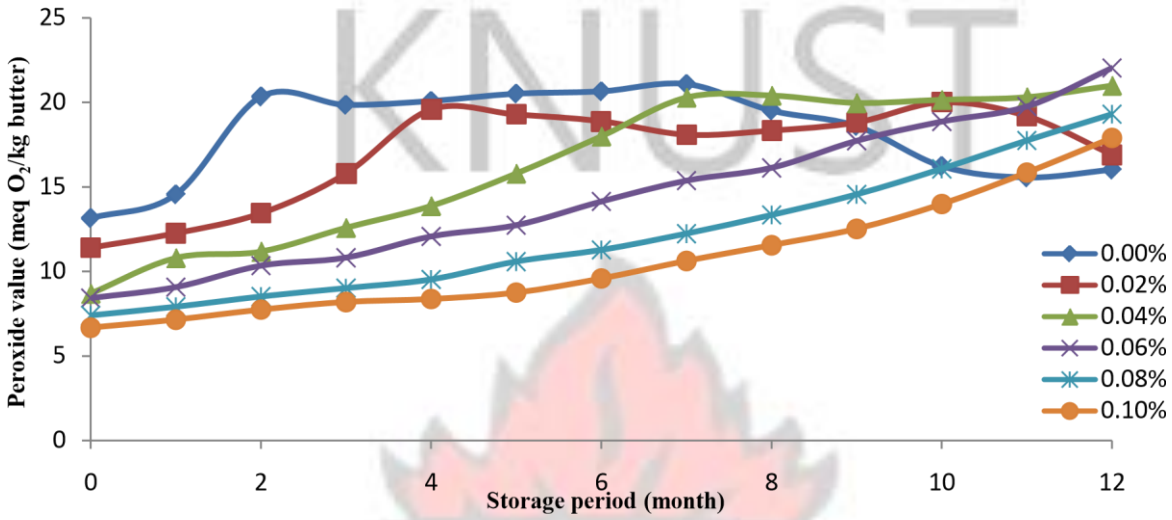

Fig. 30 Changes in Peroxide values over a 12-month storage period for locally extracted shea butter treated with *C. planchonii* root extract at various concentrations.

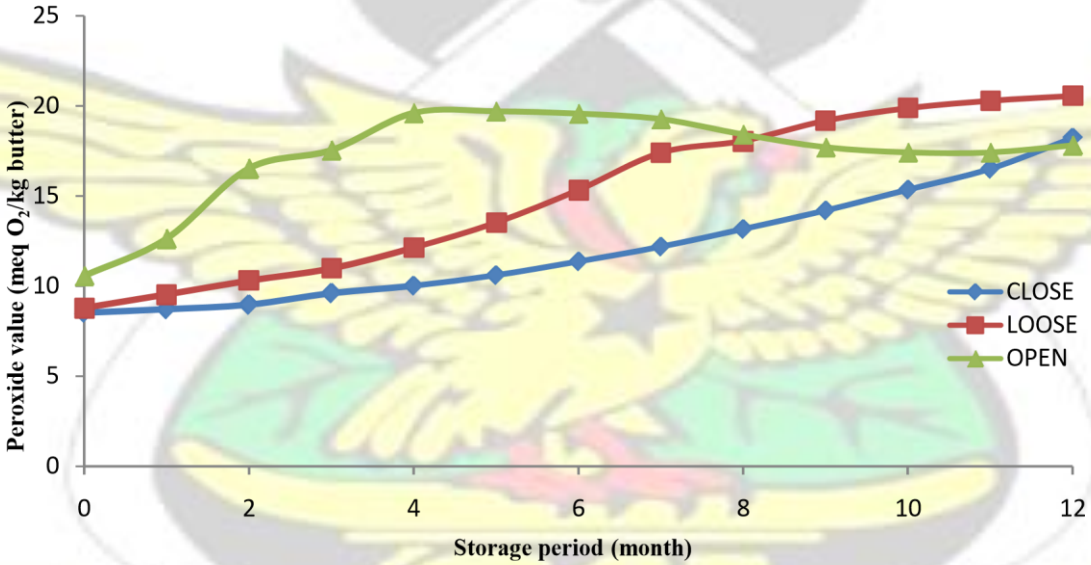

Fig. 31. Changes in Peroxide values over a 12-month storage period for locally extracted shea butter treated with *C. planchonii* root extract in different modes of storage.

The relatively high initial moisture content and atmospheric oxygen make locally extracted shea butter prone to hydrolytic and oxidative rancidity with the subsequent production of intermediate breakdown products (Rossell, 1983). The steady increase in the PV over the storage period may be due to the fact that the rate of production of hydroperoxides exceeded their rate of decomposition. Since changes in PV values were more rapid in the locally produced shea butter

than the laboratory-produced butter, it might be possible that low quality kernels were used in the production of the former. In addition, the presence of metallic ions like iron (during the milling of the kernels) may have enhanced the oxidation of the butter producing peroxides, hydroperoxides and other intermediate products (Rossell, 1983). Free peroxy or alkoxy radicals might be present, generating more free radicals of the fatty acids and thus propagating the chain reaction (Sinclair *et al*., 1990; Kanner and Rosenthal, 1992; Garrow and James, 1993) in a chain reaction in the butter before the addition of the *C. planchonii* root extract.

It appears however, that higher extract concentrations were able to interfere with chainpropagation reactions or terminate some of the free radical chain-reactions since the extract contains antioxidants. The gallic acid, ß – bisabolene ß – carotene in the extract may have been preferentially oxidised or may have scavenged the free radicals, and so were able to reduce the rate of propagation of free radical chain reactions in the butter (Gordon, 1990; Palozza and Krinsky, 1992; Kanner and Rosenthal, 1992; Goupy *et al.*, 1999, Kaur and Kapoor, 2002).

Plant–derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers and peroxide decomposers (Larson, 1988). The extract was able to slow down the propagation of the chain reaction by free radicals and thus reduced rate of the production of peroxides or hydroperoxides (Gordon, 1990, Goupy *et al*., 1999, Kaur and Kapoor, 2002). The extract could however not completely stop production of the intermediate products associated with rancidity but slowed the rate of their production (Jadhav *et al.*, 1995).

PVs after the first month indicated that there was a significant ($P < 0.05$) effect of *C. planchonii* root extract (Table 10a) and type of container (Table 10b). These imply that PV changes were both extract concentration and mode of storage dependent.

At three months of storage, the three modes of storage were significantly ($P < 0.05$) different from each other (Table 10d). This also implies that it did not matter which container type was

used during storage, the butter quality would not be affected by the storage mode with regard to PVs.

The interaction between the two main effects is shown in Fig. 32. A general decline in PVs was observed in all modes of storage. However, at higher extract concentrations (0.06%, 0.08% and 0.1%) the effects were almost the same for all modes of storage. The above observation implies that even though *C. planchonii* root extract at 0.06%, 0.08% and 0.10% inclusion levels could reduce the rate of peroxides or hydroperoxide formation, the mode of storage would not be important.

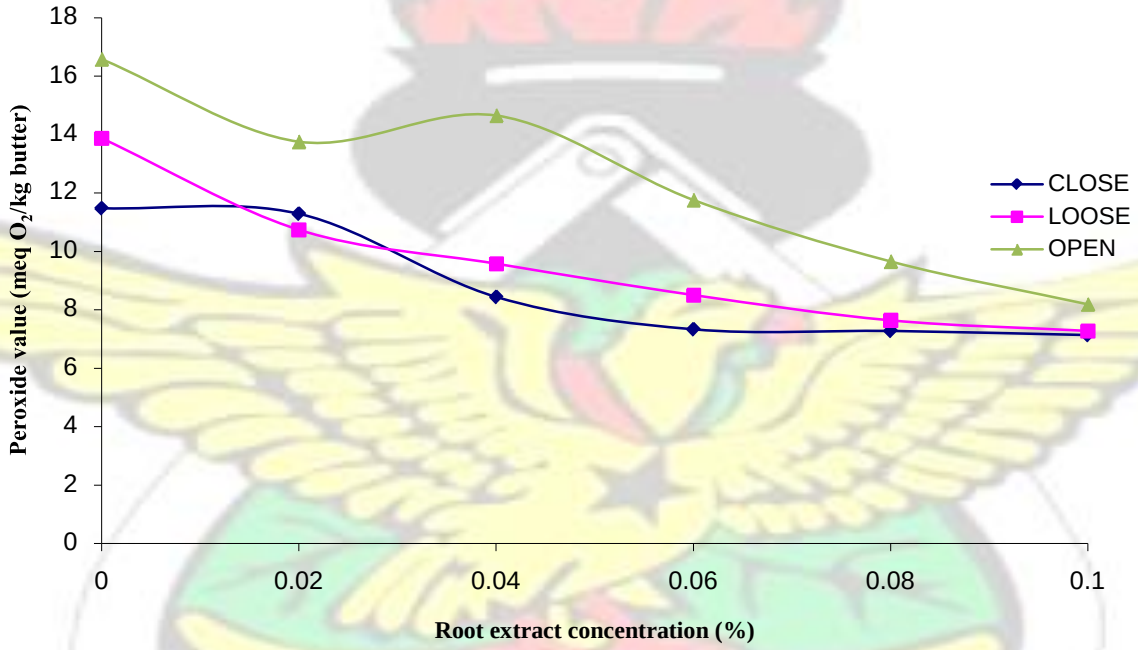

Fig. 32. Interaction between mode of storage and *C. planchonii* root extract concentration at 1 month for Peroxide values in locally extracted shea butter

Table 10a. Mean Peroxide values at 1 month for locally extracted shea butter: Effect of *C. planchonii* root extract concentration

----------C. *planchonii* ROOT EXTRACT CONCENTRATION----------						
0.0%	0.02%	0.04%	0.06%	008%	0.10%	SED
13.982**a**	11.931**b**	10.891**b**	9.199**c**	8.190**cd**	7.537**d**	0.7956

N.B: Means with common postscripts (a, b, c, d) are not significantly different (P > 0.05)

Table 10b. Mean Peroxide values at 1 month for locally extracted shea butter: Effect of mode storage

----------MODE OF STORAGE ----------			
CLOSE	LOOSE	OPEN	SED
8.825**a**	9.606**b**	12.434**c**	0.3550

Table 10c. Mean Peroxide values at 3 months for locally extracted shea butter: Effect of *C. planchonii* root extract concentration

----------C. *planchonii* ROOT EXTRACT CONCENTRATION----------						
0.0%	0.02%	0.04%	0.06%	008%	0.10%	SED
17.976**a**	14.764**b**	12.879**c**	11.228**d**	9.944**e**	9.468**e**	0.6210

Table 10d. Means Peroxide values at 3 months for locally extracted shea butter: Effect of mode of storage

----------MODE OF STORAGE----------			
CLOSE	LOOSE	OPEN	SED
9.970**a**	11.230**b**	16.929**c**	0.2771

N.B: For tables 10b, 10c and 10d means with different postscripts (a, b, c, d, e) are significantly different (P < 0.05)

Similarly, after the third month, root extract concentrations of 0.08% and 0.10% were not significantly (P > 0.05) different from each other but were significantly (P < 0.05) different from all the other concentrations (Table 10c). All the other concentrations (0.00%, 0.02%, 0.04% and 0.06%) were however, significantly (P < 0.05) different from each other indicating a concentration effect of the extract on the butter stability during the three months storage period. Similarly, all the modes of storage were also significantly (P < 0.05) different from each other which again implies that during the first three months of butter storage, the mode of storage used is very important. The interaction between the main effects is shown in Fig. 33. At each concentration, the order open > loose > close is maintained. Lower PV of butter is an indication

of higher quality. It implies that higher butter quality could be obtained if it is stored in close containers. It can therefore be concluded that it would be best to keep locally produced shea butter in close containers to obtain a fairly good product for a 12- month storage period.

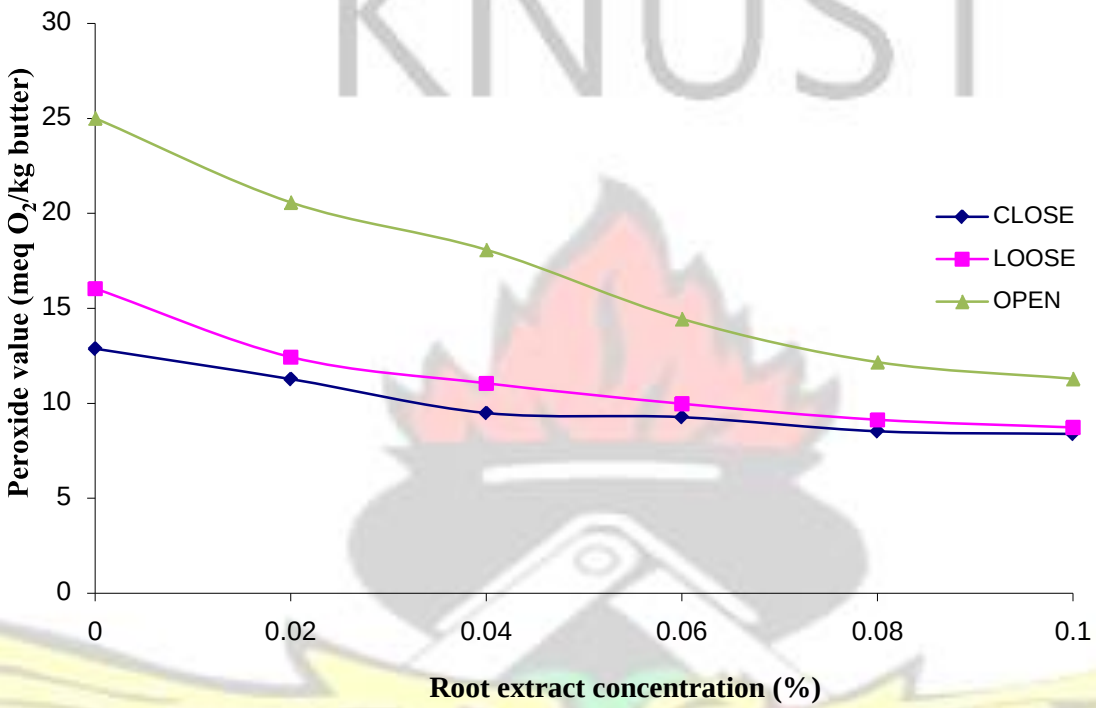

Fig. 33. Interaction between mode of storage and *C. planchonii* root extract at 3 months for Peroxide values in locally extracted shea butter

3.5.4 Changes in levels of Iodine Value (IV) during Storage.

Fig. 34 and 35 show changes in IV levels over a 12-month period for extract concentration and mode of storage used, respectively. Generally, iodine values for the butter treated with higher extract concentrations (0.06% – 0.10%) remained fairly constant over the 12-month storage period (with exception of a slight decline in the 0.06%), whereas the values for the three lower concentration treatments (0.0 – 0.02%) dropped within two months of storage (Fig. 34). Thus at higher extract concentrations, oxidation of the fatty acid double bonds was minimal, since the antioxidants in the extract were preferentially oxidized. With respect to mode of storage (Fig.35), clear differences were observed from 4 months up to the 12th month such that the closed container

provided the best stability while the open container was the worst in terms of the IV levels. Compared to the laboratory-extracted butter (Fig. 24), the decline in the IV levels with the control and lower extract concentrations were more rapid, starting from 2 or 3 months as opposed to 9 and 10 months respectively, for concentrations of 0.0% and 0.02% (Fig. 24 and 34).

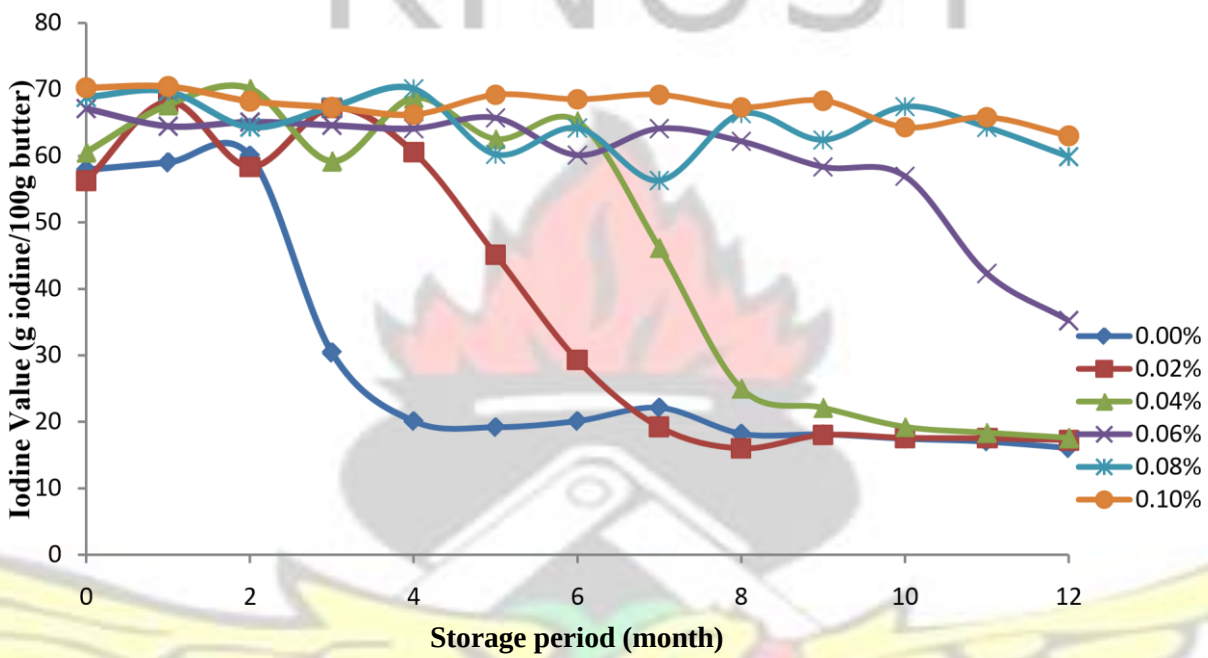

Fig. 34. Changes in Iodine values over a 12-month storage period for locally extracted shea butter treated with *C. planchonii* root extract at various concentrations.

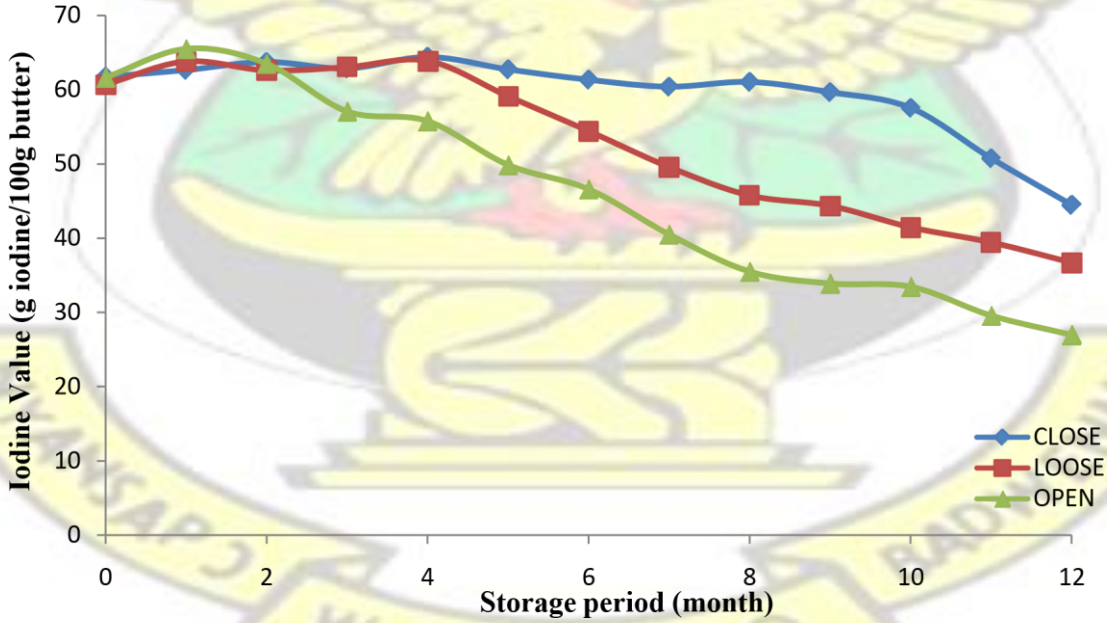

Fig. 35. Changes in Iodine values over a 12-month storage period for locally extracted shea butter treated with *C. planchonii* root extract in different modes of storage.

This supports the point that the process adopted for the shea butter extraction at the laboratory level will extend the keeping quality better than the traditionally processed on**e.**

The iodine value for the locally extracted shea butter also showed non–uniform decreases similar to the pattern observed in the laboratory-extracted shea butter. The decreases were however, more drastic with lower concentrations of the extract. This means that there were relatively small amounts of antioxidants in the lower extract concentrations which could not protect the double bonds in the butter from being oxidised.

Iodine values after the first month indicated that there was a significant ($P < 0.05$) effect of *C. planchonii* root extract concentration (Table 11a) and mode of storage (Table 11b) as well as the interaction between the two factors. It implies that changes in IVs at one month storage were extract concentration-dependent. The three modes of storage were however significantly ($P < 0.05$) different from each other. It also implies that after the first month there was no relationship between extract concentration and mode of storage regarding the keeping quality of butter in terms of IV.

However, IVs after the third month indicated that there was a significant ($P < 0.05$) effect of *C. planchonii* root extract (Table 11c) and mode of storage (Table 11d) as well as the interaction between the two factors.

The interaction between the two main effects is depicted graphically (Fig. 36). Generally the IVs for all the other treatments (0.02% to 0.10%) for 3 months storage period were the same with respect to all the modes of storage. This implies that container type did not influence keeping quality of locally produced shea butter with respect to IV.

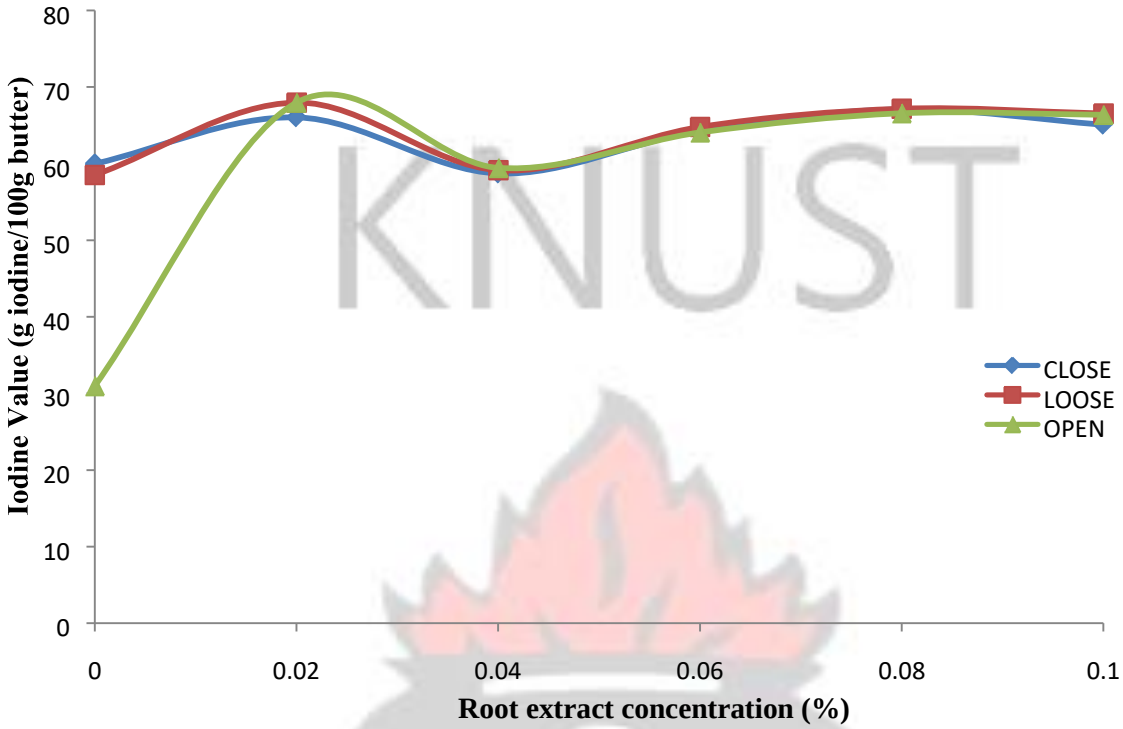

Fig. 36. Interaction between mode of storage and *C. planchonii* root extract at 3 months for Iodine values in locally extracted shea butter

Table 11a. Mean Iodine values at 1 month for locally extracted shea butter: Effect of *C. planchonii* root extract concentration

---------- C. *planchonii* ROOT EXTRACT CONCENTRATION----------

0.0%	0.02%	0.04%	0.06%	008%	0.10%	SED
56.889**a**	66.137**b**	67.081**c**	62.120**d**	69.664**e**	66.142**b**	0.1184

Table 11b. Mean Iodine values at 1 month for locally extracted shea butter: Effect of mode of storage.

----------MODE OF STORAGE----------

CLOSE	LOOSE	OPEN	SED
63.787**a**	63.674**b**	66.558**c**	0.0228

Table 11c. Mean Iodine values at 3 months for locally extracted shea butter: Effect of *C.planchonii* root extract concentration

----------C. *planchonii* ROOT EXTRACT CONCENTRATION----------

0.0%	0.02%	0.04%	0.06%	008%	0.10%	SED
49.806**a**	67.322**b**	59.094**c**	64.394**b**	66.945**b**	65.998**b**	1.8609

Table 11d. Mean Iodine values at 3 months for locally extracted shea butter: Effect of mode of storage

---------- MODE OF STORAGE ----------			
CLOSE	LOOSE	OPEN	SED
63.534**a**	64.014**a**	59.231**b**	0.3578

N.B: For tables 11a, 11b, 11c and 11d, means with different postscripts (a, b, c) are significantly different (P < 0.05)

The results from the experiment indicated that by the third month, locally produced shea butter had already shown signs of rancidity. The addition of the root extract was however effective in controlling rancidity even at low levels of *C. planchonii* extract treatment (Fig. 34). At higher extract treatment levels (0.08% and 0.10%) there was virtually no change in the iodine values up to the 12-month storage period (Fig. 34).

The IVs were relatively higher in treatments with higher *C. planchonii* root extract concentrations. This is an indication that the proportion of unsaturated fatty acids remained higher for the higher *C. planchonii* root extract concentration treatments during the 12-month period, an indication of good butter quality. The gallic acid, ß - bisabolene and ß - carotene which have antioxidant properties in the extract may have been preferentially oxidised or may have scavenged the free radicals, and so were able to reduce the rate of propagation of free radical chain- reactions in the butter during the 12-month storage period (Larson, 1988).

Even though the experiments with the laboratory-extracted shea butter and locally produced shea butter were run concurrently, the results were presented and discussed separately. Putting the

results from the two experiments together would have made the analysis very complicated due to some factors associated with their mode of processing (Fig. 2 and 13).

The general trend in this work was that, laboratory-extracted butter had lower moisture content, lower FFAs, lower PVs and higher IVs as compared to the locally processed butter. After the 12-month storage period the laboratory extracted butter was superior in terms of quality to the locally processed butter.

CHAPTER FOUR

4.0 CONCLUSIONS AND RECOMMENDATIONS.

4.1 CONCLUSIONS

Phytochemical analysis of ethanolic, diethylether and n-hexane extracts of *Cochlospermum planchonii* root contains tannins, phenolics, terpenoids and saponins; these have antioxidant properties.

HPLC analysis of the extracts showed the presence of bisabolene, carotene and gallic acid derivatives. These have antioxidants properties as well.

C. planchonii root extract at inclusion levels of 0.08% and 0.10% could suppress rancidity in shea butter (whether laboratory-extracted or locally extracted) when kept under different storage conditions for a 12- month period. However, the butter stores best in closed containers.

Locally produced shea butter showed more rancidity than the laboratory produced butter over the 12 month study period.

4.2 RECOMMENDATIONS

Local processors should be advised to par-boil the fruits immediately after collection. Water should not be used to facilitate solidification. The butter should not come in contact with water before storage and the use of mouldy kernels for butter production should be avoided as much as possible.

More data should be collected over a longer period to obtain a definite shelf -life for shea butter treated with *C. planchonii* root extract.

4.3 FURTHER WORK

Higher concentration of *C. planchonii* extract may be required to store shea butter longer than 12 months.

There is the need to use the treated shea butter in preparations to investigate whether the root extract will have any effect on formulation of products.

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