QUALITY CHARACTERISTICS OF WEST AFRICAN SHEA BUTTER (*VITELLARIA PARADOXA*) AND APPROACHES TO EXTEND SHELF-LIFE

by

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ABSTRACT OF THE THESIS

Quality Characteristics of West African Shea Butter (*Vitellaria paradoxa*) and Approaches to Extend Shelf-life

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Shea butter is a versatile plant fat extracted from kernels of shea nuts, seeds of shea trees (*Vitellaria paradoxa*). Shea butter has long been used in sub-Saharan Africa for medicinal, culinary, and other applications and serves as a cocoa butter equivalent in the manufacture of chocolate as well as an ingredient in cosmetics. Shea butter, rich in unsaturated fatty acids undergoes hydrolytic and oxidative degradation during post-harvest processing and storage, resulting in inconsistent and degraded quality and limited shelf-life.

The objective of this study was to assess important quality characteristics of shea butter. Seven West African shea butters were analyzed to measure physicochemical parameters by wet chemical tests and to measure chemical composition by gas chromatographic analysis. Physical properties were consistent among samples and within the range of

typical shea butter. The samples also shared similar chemical compositions, showing palmitic (3.36-4.44 % of total fatty acids), stearic (39.74-44.62 %), oleic (40.71-44.48 %), and linoleic acids (5.73-6.41 %) as the major fatty acids and α-amyrin having anti-inflammatory property (57.26-64.37 % of total sterols and triterpenes) as the major unsaponifiable matter. Moisture, insoluble impurities, free fatty acids, and peroxide values were needed to be controlled. Free fatty acid level was the most variable parameter, ranged from 1.07 to 8.56 %. Peroxide value was low enough except the one which was as high as 15.32 mEq/kg. Total unsaponifiable matters were measured lower (2.21-4.18 %) compared to the previous studies (4-11 %) but still higher than many other plant oils and fats (~2 %).

This study also aimed at identifying the protective effect of selected synthetic (BHT) and natural (rosmarinic and gallic acids) antioxidants on shea butter from oxidation. Peroxide value, conjugated dienes, thiobarbituric acid reactive substances, and the amount of major fatty acids were measured as oxidative parameters at 0, 72 and 144 hours while the control and samples with 0.02 % of antioxidants were stored at 90 ℃ with air flow. The antioxidants were significantly effective in protecting shea butter from oxidation and no significant difference in the effect of synthetic and natural antioxidants was found.

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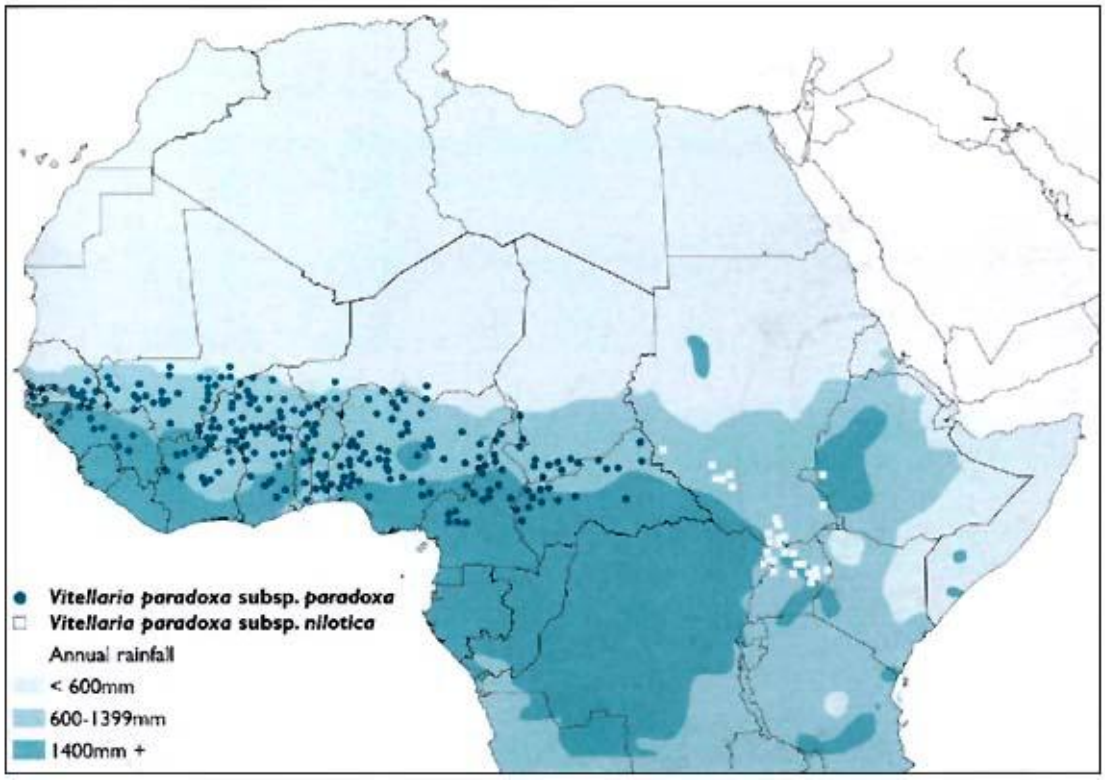
CHAPTER 1. INTRODUCTION

1.1. Overview of shea butter

1.1.1. The Botany of shea trees

Shea trees belong to Sapotaceae family and were first named by the German botanist Carl Gaertner (Gaertner, 1807) as *Vitellaria paradoxa* C.F. Gaertn. In 1961, the species already described by Gaertner was renamed as *Butyrospermum parkii* (Kotschy) (Maranz *et al.,* 2003a), with the epithet "*parkii*" referring to Mungo Park (1771-1805) who was the great Scots explorer introducing shea butter to Europe (Goreja, 2004). Many publications have considered *Butyrospermum parkii* as the synonym of *V. paradoxa*; the currently accepted name for the African shea trees is *V. paradoxa,* with the West African trees classified as the subspecies *"paradoxa"* and the East African one as *"nilotica"* (Ferris *et al.,* 2001; Di Vincenzo *et al.,* 2005; Mbaiguinam *et al*., 2007).

Shea trees grow wild across a 5000 km wide belt of savanna (**Figure 1-1**) (Maranz *et al.,* 2003a; Masters *et al*., 2004) including West African countries of Senegal, Mali, Côte d'Ivoire, Burkina Faso, Togo, Ghana, Benin, Nigeria, Niger, Cameroon, and further east in Uganda, Sudan and Ethiopia (Chalfin, 2004; Goreja, 2004). This savanna belt is also called 'shea belt' among traders (Ferris *et al.,* 2001). Among these countries, Ghana and Burkina Faso are the main shea nut exporters (Walter *et al.,* 2003).



Source: Hall *et al*., 1996

Figure 1-1. Distribution of shea trees which is called shea belt among traders.

In the wild, shea trees grow up to 9-12 m (30-40 feet) in height and begin to bear commercial quantities of fruit after approximately 20 to 50 years (**Figure 1-2**) (Tella, 1979; RAISE, 2002). Shea trees do not reach maturity until 45 years and after getting mature, they can continuously produce shea nuts for up to 200 years (RAISE, 2002; Alander, 2004). Compared to other trees grown as plantation crops, shea trees take much longer time to reach maturity, which have discouraged the commercial plantation. It is easy to understand how longer it takes for shea trees to reach maturation by simply comparing with coffee trees (*Coffea Arabica*). *Coffea Arabica* is commercially important coffee species belonging to Rubiaceae family and originated in South Western Ethiopia (Steiger *et al.,* 2002). Coffee plants in Salvador are reported to begin to bear after 2 to 3 years and reach full maturity after 7 to 8 years and continue to yield coffee beans up to nearly 30 years (Ukers, 1922). For this reason, the entire shea industry is based upon shea butter collected in many countries from naturally growing stands of shea trees.

Shea trees blossom during February to March, and the fruit become matured in June to July (**Figure 1-2**) (Alander, 2004). The fruit are harvested during June to September once they fall to the ground from the trees (RAISE, 2002; Alander, 2004). Shea fruit is light green colored with a diameter of 2-3 inches or 5-8 cm similar to a fig (Chalfin, 2004). Shea fruit consist of a green epicarp (the outer part), a fleshy mesocarp (pulp), and a relatively hard endocarp (shell) containing embryo (shea kernel) (Olaniyan *et al.*, 2007) (**Figure 1-2**). Mostly, shea fruit contain one or two kernels but occasionally have two to three from which shea butter is extracted (Alander, 2004).

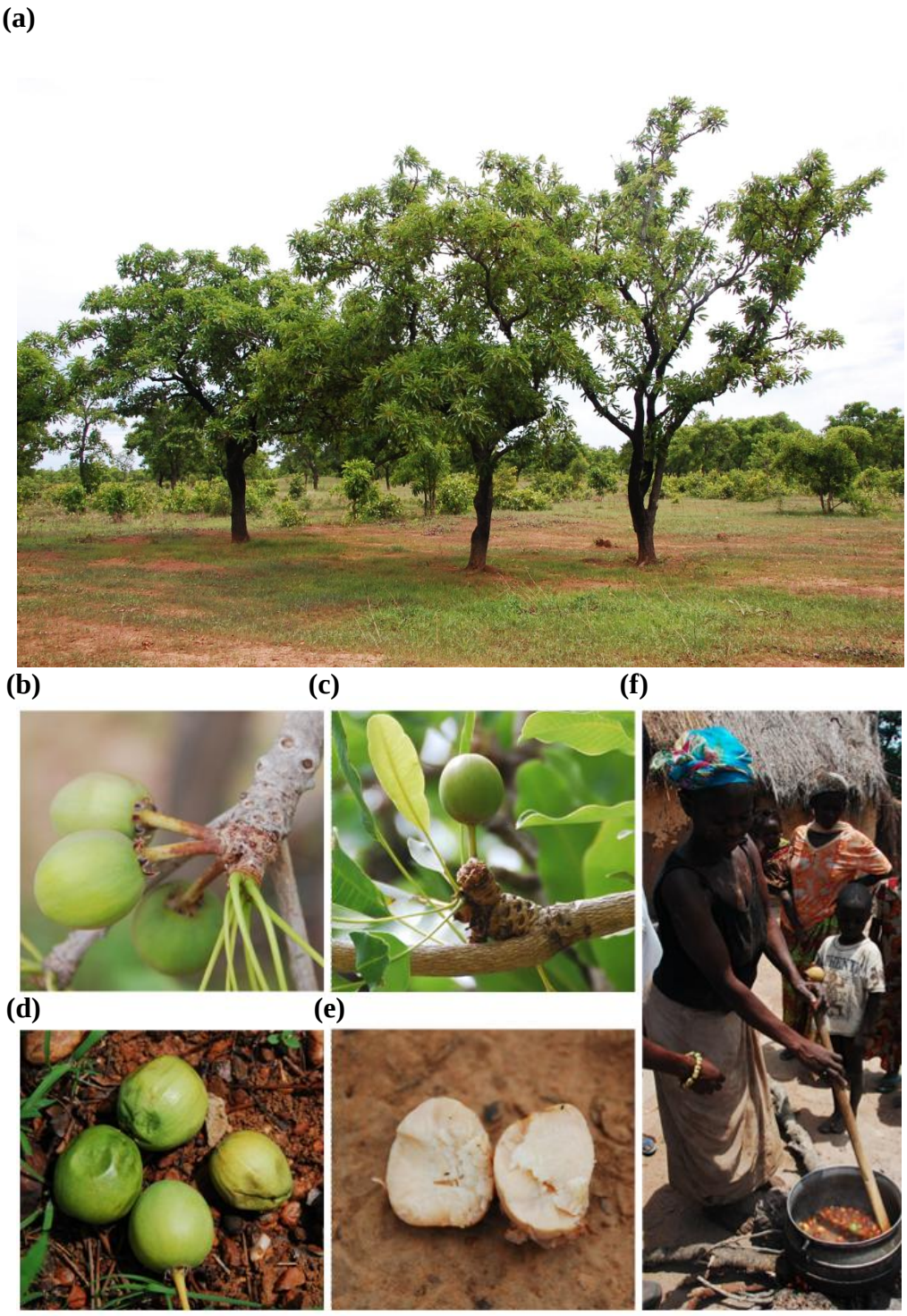


Figure 1-2. Wild growing shea trees (a), shea fruit on the trees (b-c) and fallen to the ground (d), shea kernels (e), and an African woman boiling shea nuts to kill embryo (f) as a part of shea butter making. Pictures were taken and provided by The New Use Agriculture and Natural Plant Products (NUANPP) in Rutgers University.

1.1.2. Extraction of shea butter

Traditionally, the extraction of shea butter has been done at the village level, where shea butter is sold in local markets. In recent years, the dried kernels have been exported to processing countries in Europe, Japan, and India where shea butter is extracted in large-scale industrial plants (Lovett, 2004). Traditional extraction has been usually done by boiling water and skimming off the released oil while commercial one is conducted by pressing or solvent extraction with further refining and deodorizing of shea butter (Alander, 2004). However, with the increased interest in naturally derived products, organic shea butter production is preferred and thus efforts have been made to industrially produce shea butter by following the traditional extraction methods. The shea butter obtained from the traditional extraction procedure not including a refining stage is called 'unrefined shea butter'.

Either at the village or industrial level, shea butter is extracted from dried shea kernels. Once shea fruit fall from the trees, the fruit are collected by African women from the ground and the pulp is removed by fermentation or manual peeling (Chalfin B. 2004; Moharram *et al.,* 2006). Then, the nuts can be processed according to one of the three distinct traditional procedures (Lovett, 2004) (**Figure 1-3**). In West Africa, the nuts can be boiled or roasted, while in East Africa the nuts are simply sun-dried.

In the West African boiling method, the nuts are boiled to kill the embryo and thus prevent germination of the seeds. This method has the additional advantage of inactivating the lipases that are responsible for hydrolytic degradation of shea butter.

However, boiling can also cause high peroxide values since the high temperature and water can accelerate oxidation (Lovett, 2004; Masters *et al.*, 2004; Bail *et al.*, 2009). After the boiling, the nuts are dried in the sun, though sun-drying of shea nuts during rainy season can lead to mold contamination and thus affect the quality of the final products (Moharram *et al.*, 2006). After then, the nuts are cracked to remove shells from the dried nuts and then kernels are further dried by roasting or sun-drying (Lovett, 2004; Moharram *et al.*, 2006)

In the West African oven method, the nuts are roasted or smoked on ovens and the dried nuts that still have husks are stored (Lovett, 2004). This procedure has the disadvantage that roasting or smoking in the oven can cause high amounts of polycyclic aromatic hydrocarbons (PAHs) known to be carcinogenic (Lovett, 2004).

While the West African methods include a heating stage, either boiling or roasting before or while drying, the East African method involves no heating step. Instead, the nuts are directly sun-dried, de-husked, and sun-dried again (Lovett, 2004). The dried kernels are stored with occasional re-drying. Since the nuts are not subjected to high temperatures, in this method, there is less chance of deactivating lipases, which is usually linked with high levels of free fatty acids (FFAs) (Lovett, 2004). In this case, sun-drying should be avoided during rains to prevent microbial deterioration of the nuts and kernels.

The dried kernels are then subjected to pounding or wet milling to make a paste which is then emulsified by kneading and hand beating (Moharram *et al.*, 2006). The paste is

then boiled to separate the fat from the shea nut cake and the resultant butter is scooped up, filtered through a filter cloth and placed in a cool place to solidify.

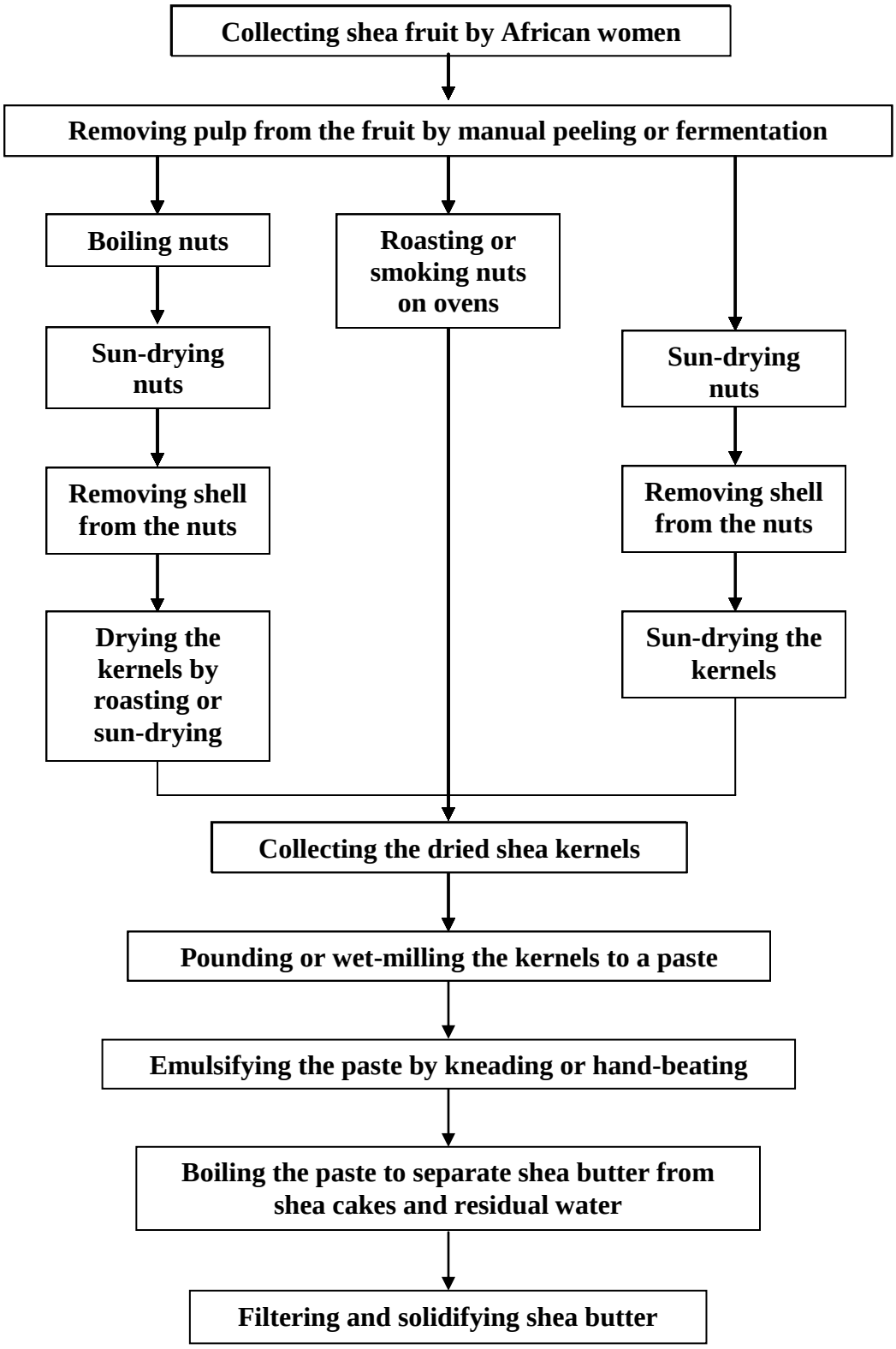


Figure 1-3. Diagram of extraction procedure for shea butter

1.1.3. Uses of shea butter

1.1.3.1. Traditional use of shea butter in Africa

Shea butter has long been used in the West African countries, dating back to ancient Egypt based on the record that during the Cleopatra's Egypt, caravans carried clay jars of valuable shea butter for cosmetic uses (Goreja, 2004). Many records on traditional uses of shea butter have focused on its ethnopharmacological uses. Shea butter was used by local healers as a treatment for rheumatism, inflammation of the nostrils, nasal congestion, leprosy, cough, and minor bone dislocation (Tella, 1979; Badifu, 1989; Goreja, 2004; Olaniyan, 2007). Shea butter has also been used for soothing and accelerating healing after circumcision, and for preventing stretch marks in African pregnant women (Goreja, 2004), which is frequently mentioned on the advertisement of shea butter products. Shea butter has also been used to massage newly born babies. In addition, shea butter has been used as an insect repellent, providing protection against *Simulium* infection (Goreja, 2004).

In addition to the ethnopharmacological uses, shea butter has been used in West African cuisine as edible oil due to its high nutritional value and affordable price (Kar *et al*., 1981; Njoku *et al*., 2000; Chalfin B. 2004; Olaniyan, 2007). Shea butter is used as the base of many soups and condiments (Goreja, 2004). For example, when shea butter is mixed with onion and pepper, it becomes a popular condiment (Chalfin B. 2004). Beverages made with shea butter combined with millet flour, water, and savory spices have been served during weddings, funerals, and work parties (Chalfin B. 2004).

African local communities have also found uses and applications of shea butter for lamp and heating oils, lubricants, weather-proofing roofs and soap manufacturing (Goreja, 2004; Lovett, 2004; Olaniyan, 2007). Shea butter, in addition, has been beneficial for domestic animals as moisturizer which is applied to dogs to protect their skin and paws against harsh sand and salt (Goreja, 2004).

1.1.3.2. Use of shea butter in the international market

Large quantities of shea butter are used in manufacturing chocolate and margarine in Europe. In the chocolate industry, shea butter is refined and deodorized to be used usually as cocoa butter equivalents (CBEs) since 1960s with its similarity in physical properties to a more expensive cocoa butter (Peers, 1977; Alander, 2004). CBEs are plant fats containing no lauric acid which have similar physical and chemical properties to cocoa butter, and are mixable with cocoa butter in every amount without altering the properties of cocoa butter (Lipp *et al.*, 1998). The European Union allows using a non-cocoa fat in the manufacture of chocolate up to 5 % under the current European Union Chocolate Directive, while, in the US, if the product contains any cocoa butter alternatives including CBEs, it is not allowed to be labeled as 'chocolate' (Alander, 2004; Lovett, 2004). Shea butter is less expensive than cocoa butter, and therefore it can reduce the cost of the chocolate production (Chalfin B. 2004). Besides the price competitiveness, shea butter has lower melting point compared to cocoa butter and thus can be easier to make hard candy coatings (Chalfin, 2004).

Even though the major market of shea butter has been found in the chocolate and confectionary industries, there is a fast-growing, popular market in cosmetics and personal care product industry. Due to highly moisturizing and emollient properties, shea butter can be often found in the products for improving drying skin and hair such as lip balms, hand creams, facial moisturizers, shampoos, conditioners, among others. The main marketing claims for shea butter products usually emphasize their highly moisturizing properties due to shea butter's semi-solid characteristics, providing a buttery consistency and ease of spreading on the skin (Alander, 2004). Shea butter, in addition, has been reported to have high levels of unsaponifiable matters compared to other vegetable fats and oils (Hamilton *et al.,* 1986; Alander, 2004; Rogers *et al*., 2009), and thus more potential exists to develop shea butter products stressing shea butter's medicinal properties including antioxidant, anti-inflammatory and other purported activities (Alander *et al.,* 2002; Maranz *et al.,* 2004; Rogers *et al*., 2009). The unsaponifiable fraction of shea butter is used as an ingredient in the treatment of inflammatory diseases due to its anti-inflammatory action (Masters *et al.,* 2004). It has been used to develop treatment for arthritis, eczema, and herpes lesions and to lower cholesterol levels by a pharmaceutical company, BSP Pharma (Masters *et al.,* 2004).

1.1.4. Benefit of using shea butter

1.1.4.1. Skin, scalp, and hair emollient and moisturizing activity

It is easy to find shea butter as an active ingredient in moisturizers (Kraft *et al*., 2005). Due to the semi-solid characteristics and buttery consistency, shea butter itself can be used as great emollient and moisturizer without further processing. In addition,

fractionated shea butter especially olein fraction is easily formulated in creams or surfactant based products such as bath products and shampoo to provide the skin, scalp, and hair with well-maintained or increased moisture and at the same time to deliver antioxidants in unasponifiable fraction to the skin and hair (Alander, 2004; Rogers *et al*., 2007).

1.1.4.2. Anti-aging of skin due to protease-inhibiting activity

Skin-rejuvenating effect of shea butter is usually stated on the shea butter products or their advertisement and this property of shea is likely to be attributed to the inhibition of structural protein-degrading enzymes. Collagen and elastin are the major structural proteins providing skin with toughness and plumpness and α-amyrin and lupeol, the triterpenes also found in the unsaponifiable fraction of shea butter, were found to contribute to the inactivation of proteases such as metalloprotease (e.g., collagenase) as well as serine protease (e.g., elastase) (Alander, 2004). However, further studies are needed to assess the effects of those triterpenes extracted from shea butter on the inhibition of proteases.

1.1.4.3. Sun-screening function

Sun-screens decrease the amount of ultraviolet (UV) radiation reaching the skin, preventing erythema or reducing further risk of sun-induced skin-cancer. Within the UV spectrum, UVB radiation (290-320 nm) is the major cause of photocarcinogenesis since UVB directly interacts with cellular DNA and subsequent formation of cyclobutane pyrimidine dimers and thymine glycols (Velasco *et al.,* 2008). Cinnamate esters of

triterpene alcohol which are the main constituent of shea butter's unsaponifiable fraction are known to have strong absorbance of UV radiation in the wavelength range at 250-300 nm, which make the addition of shea butter's unsaponifiables into sunscreens provide synergistic sun-protection by increasing absorption of UVB radiation. However, the effectiveness of the triterpenes is somewhat doubted since studies using double-fractionated shea butter with 20 % of triterpene esters found that this triterpenic fraction only provided the sun protection factor (SPF) of 3-4 (Alander *et al.*, 2002; Alander, 2004).

1.1.4.4. Anti-inflammatory effect

Traditional uses of shea butter in African folk medicine have been greatly attributed to the anti-inflammatory properties of shea butter, which may be related to the unsaponifiable fraction, especially triterpene alcohols and phytosterols. Although, there is a lack of studies using unsaponifiable components (lupeol, α-amyrin, and β-amyrin) specifically isolated from shea butter, several studies have focused on the same components found in other species. The triterpenes, α-, and β-amyrin extracted from *Protium heptaphyllum* were effective in reducing the production of proinflammatory cytokine TNF- α related to acute periodontal inflammation (Holanda Pinto *et al*., 2008). Lupeol from the latex of *Leptadenia hastata* showed slightly higher anti-inflammatory effect when compared to the well-known agent indometachin in edema models (Nikiéma *et al.*, 2001). In addition, *Acacia visco* extracts where the lupeol, α-amyrin, and β-amyrin are the main triterpenes, showed significant edema inhibition (Pedernera *et al.*, 2010).

1.1.4.5. Hypersensitivity-alleviating effect

Wiedner (2008) found that pharmaceutical composition containing at least 5 % of shea butter's triterpenes such as butyrospermol, lupeol, parkeol, germanicol, dammaradienol, 24-methylene-dammarenol, and α, and β-amyrins effectively suppresses hypersensitivity reaction in a mammal such as Immunoglobulin E (IgE)-mediated allergic reactions and autoimmune reactions in a mammal.

1.2. Chemical composition of shea butter

As a plant fat, shea butter consists of approximately 90% or more of triglycerides and a minor unsaponifiable fraction. Triglycerides are responsible for shea butter's emollient properties, while the unsaponifiable fraction contains the bioactive substances that include hydrocarbons, tocopherols, sterols, and alcohols and thus responsible for shea butter's medicinal properties (Esuoso *et al.*, 2000).

1.2.1. Triglyceride fraction

Shea butter fatty acids are composed dominantly of palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. Stearic and oleic acids dominate almost 40-45 % of total fatty acids respectively and linoleic acid generally ranges from 5-10 %, followed by palmitic acid at 4 % (Alander, 2004) with lower amounts of arachidic acid and others. Compared to grape seed oil (total saturated fatty acids: 10.4-14.3 % of total fatty acids), olive oil (12.7-16.2 %), and canola oil (5.5-7.7 %) which are liquid at room temperature and have saturated fatty acids less than 20 % of total fatty acids (Baydar *et al*., 2007; Damodaran *et al*., 2008; Samman *et al*., 2008), shea butter contains relatively high

amount of saturated fatty acids, which is responsible for solid to semi-solid state of shea butter at room temperature. Shea butter fatty acids were found to vary across the African countries (Di Vincenzo *et al.*, 2005).

The triacylglyceride fraction consists of fatty acids (acyl chains) attached to a glycerol backbone. Since different fatty acids are present in shea butter, different combinations of fatty acids attached to the glycerol are possible. In shea butter, the most common combination is SOS (S-Stearic, O-oleic) making up to 40% of the total triacylglycerol molecules, followed by SOO (27 %), POS (P-palmitic, 6%) and POP (1%) (Alander, 2004). The study conducted by Di Vincenzo *et al* (2005) found that SOO, OOO, and SOS were the major triglycerides in shea butter with regional variation. The shea butter's distinctive triglyceride composition dominated by SOS and SOO makes it easy to separate high-melting stearin fraction from the low melting olein fraction by fractionation and the resultant stearin fraction is further used in the mixture of cocoa butter equivalents (Alander, 2004).

Cocoa butter's triacylglycerides are composed of POP, PLP (L-linoleic), POS, POO, PLS, SOS, SOO, SLS, and OOO among which POP (18.3 %), POS (42.1 %), and SOS (26.4 %) are the major triglycerides (Lipp *et al.*, 2001). The composition of triglycerides in cocoa butter found in this study was quite similar to that of shea butter stated in Alander (2004). The similarity was also found in the fatty acid composition (Lipp *et al*., 2001). Of 42 samples of cocoa butter, they were found to contain palmitic (26.2 % of total fatty acids), stearic (35.8 %), oleic (33.6 %), linoleic (2.7 %), and arachidic acid (0.9 %).

Although the major fatty acids showed some degree of variation, the profile of the both cocoa and shea butters were characterized by the high levels of stearic and oleic acids.

1.2.2. Unsaponifiable fraction

Unsaponifiables are substances dissolved in the fat which are insoluble in aqueous solution but soluble in organic solvent after saponification (Hamilton *et al*., 1986). Unsaponifiable fraction comprises only a small part of shea butter compared to triglyceride fraction; however, shea butter has been characterized to have unusually high amounts of unsaponifiables compared to other vegetable oils and fats. The amount of unsaponifiables in shea butter ranged from 4 to 11 % by weight (Itoh *et al.*, 1974; Peers, 1977; Hamilton *et al*., 1986; Lipp *et al.*, 1998; Alander, 2004), while a typical vegetable oils and fats contained lower levels (0-2%) (Gutfinger *et al*., 1974; Itoh *et al*., 1974; Peers, 1977; Itoh *et al*., 1980; Hamilton *et al*., 1986). In the last decades, the unsaponifiables of plant oils and fats has been the subjects of intense research due to their various bioactivities including antioxidant, antimicrobial and anti-inflammatory.

Several studies have found that the unsaponifiable fraction of shea butter is dominated mostly by triterpene alcohols, followed by hydrocarbons, sterols, and other minor components such as vitamin E (Itoh *et al*., 1974; Lipp *et al.*, 1998; Alander, 2004), with minor differences within each group. According to Itoh *et al.* (1974), shea butter's unsaponifiable fraction was found to be composed of 75 % of triterpene alcohols which are usually esterified with cinnamic acid or fatty acids, 18 % of less polar compounds such as hydrocarbons, 5 % of sterols and 2 % of methylsterols. Lipp *et al.* (1998)

observed that the unsaponifiable fraction consisted of 65 % of triterpene alcohols, 27 % of hydrocarbons and 8 % of sterols. Alander (2004) reviewed that hydrocarbons (karitenes) made up 2-5 % of total unsaponifiables with lower amounts of tocopherols. Tocopherols in shea butter were extensively studied by Maranz *et al* (2004), revealing that the total tocopherol content ranged from 0.0029-0.0805 % of the total shea butter. Four isomers of tocopherols (α, β, γ, and δ) were detected among which α-tocopherol was found to be the major tochopherol (64% of the total tocopherols).

Triterpene alcohols and sterols were mostly esterified to cinnamic and fatty acids (Peers, 1977). The composition of triterpene alcohols in shea butter was studied by Itoh *et al.* (1974, 1980) and Peers (1977). Itoh *et al*. (1974, 1980) found α-amyrin as the major triterpenes (46-54.6 % of total triterpene alcohols) followed by butyrospermol (12.3-26 %), lupeol (16-17 %), β-amyrin (7.1-8 %) with the minor amounts of Ψ-taraxasterol, taraxasterol, parkeol, and cycloartenol. Peers (1977) found the major triterpene alcohols were α-amyrin (27.6 % of total triterpene alcohols), butyrospermol (26.0 %), lupeol (22.6 %), β-amyrin (10.6 %), and germanicol (9.4 %) with the minor levels of 24-methylene-lanost-9(11)-en-3-ol, parkeol, dammaradienol, and 24-methylene-dammarenol (**Table 1-1**).

The composition of phytosterols in shea butter was studied by Itoh *et al*. (1974), Peers (1977), and Badifu (1989). Itoh *et al.* (1974) found the major sterols were α-spinasterol (43 % of total sterols), Δ-7-stigmasterol (37 %), Δ-7-avenasterol (11 %), and 24-methyl-choest-7-enol (6 %). Peers (1977) observed that α-spinasterol (50 % of total sterol) and

Δ-7-stigmasterol (50 %) were the major sterols. Badifu (1989) found that β-sitosterol (68 % of total sterols), stigmasterol (20%), and campesterol (11 %) were the major phytosterols (**Table 1-1**).

Shea butter also contains other minor bioactives, phenols, within the unsaponifiable fraction. In the study conducted by Maranz *et al*. (2003b), the average value of total phenols of 101 samples was 97 ppm (**Table 1-1**), while the values in the kernel were more than 4000 ppm, showing that around 97 % of total phenols were lost during hexane extraction of shea butter from the kernel. This result suggested that the phenolic fraction is highly polar and thus far less soluble in fats.

Table 1-1. Composition of unsaponifiable fraction of shea butter as reported in the literature

Chemical constituents	**Itoh *et al.*, 1974**	**Peer, 1977**	**Itoh *et al.*, 1980**	**Badifu, 1989**	**Alander, 2004**	**Maranz *et al.*, 2003b**	**Maranz *et al.*, 2004**
Triterpene alcohols	75 % of UNS						
α-amyrin	46 % of TA	27.6 % of TA	54.6 % of TA		○	○	
β-amyrin	8 % of TA	10.6 % of TA	7.1 % of TA		○	○	
Parkeol		1.04 % of TA	1.5 % of TA			○	
Lupeol	16 % of TA	22.6 % of TA	17 % of TA		○	○	
Butyrospermol	26 % of TA	26.0 % of TA	12.3 % of TA		○	○	
Germanicol		9.4 % of TA					
Cycloartenol	○						
Ψ-taraxasterol			2.6 % of TA				
Taraxasterol			1 % of TA				
24-methylene-lanost-9(11)-en-3-ol)		1.3 % of TA					
Dammaradienol		0.7 % of TA					
24-methylene-dammarenol		0.7 % of TA					

Table 1-1. Continued.

Chemical constituents	**Itoh *et al.*, 1974**	**Peer, 1977**	**Itoh *et al.*, 1980**	**Badifu, 1989**	**Alander, 2004**	**Maranz *et al.*, 2003b**	**Maranz *et al.*, 2004**
Karitine hydrocarbons	18 % of UNS				2-5 % of UNS	○	
Sterols	5 % of UNS						
Campesterol				11 % of TS		○	
α-spinasterol	43 % of ST	50 % of ST			○	○	
Stigmasterol				20 % of ST			
Δ-7-stigmasterol	37 % of ST	50 % of ST			○	○	
β-sitosterol				68 % of ST	○	○	
24-methyl-cholest-7-enol	6 % of ST						
Δ-7-avenasterol	11 % of ST						
Tocopherols					○	○	0.021% of SB
A							64 % of TC
B							7 % of TC
Γ							38 % of TC
Δ							34 % of TC
Phenolics						97 ppm	

○: constituent reviewed to exist; SB: shea butter; UNS: total unsaponifiables; TA: total triterpene alcohols; ST: total sterols; TC: total tocopherols

1.2.3. Fractionation and chemical composition

The chemical composition of shea butter can be modified by fractionation that produces hard stearin (the fraction rich in stearic and thus solid at room temperature) and liquid olein (the fraction rich in oleic acid and thus in liquid state). To achieve higher content of unsaponifiable fraction, shea butter can be further concentrated. The chemical composition of unrefined, liquid (shea butter fractionated to be rich in liquid olein), and concentrated shea butter was recently compared by Alander *et al.* (2002) (**Table 1-2**).

As the level of fractionation increased from unrefined, liquid to concentrated, the levels of unsaponifiables increased from 8, 10 to 25% (**Table 1-2**). As the total unsaponifiables increased, total tocopherols were also increased from 100-150 ppm, 150-200, to 250-300 ppm, respectively (Alander *et al*., 2002). In addition, it was also found that the composition of fatty acids changed with the decrease in the ratio of stearic acid to oleic acid as fractionation furthered. As a result, the percentage of stearic acid decreased from 42 %, 27 % to 9 % while that of oleic acid increased from 45 %, 57 % to 68 %, respectively.

Table 1-2. Chemical composition of shea butter and the fractionated products

Component		**Unrefined shea butter**	**Liquid shea butter**	**Shea butter concentrate**
Glycerides (%)		92	90	75
Unsaponifiables (%)		8	10	25
Tocopherols (ppm)		100-150	150-200	250-300
Fatty acids (% of glyceride fraction)	**Palmitic**	4	5	5
	Stearic	42	27	9
	Oleic	45	57	68
	Linoleic	6	9	14

Source: Alander *et al.* (2002)

1.3. Factors causing inconsistency of the quality of shea butter

1.3.1. Regional variability of shea butter's characteristics

The fatty acid composition of the West African *V. paradoxa* subspecies *paradoxa* and the East African *V. paradoxa* subspecies *nilotica* shows considerable subspecific variation. The subspecies *paradoxa* exhibited the typical fatty acid profile of commercially available shea butter where stearic and oleic acids are almost in similar amounts, while the subspecies *nilotica* is characterized by higher levels of oleic acid, thus providing a better product for the cosmetic industry. However, the butter from subspecies *nilotica* is hardly found on the market due to political instability of northern Uganda and southern Sudan where this subspecies is the major source of shea butter and thus the inconsistent supply (Ferris *et al.,* 2001).

1.3.1.1. Regional variability of fatty acid composition

Di Vincenzo *et al*. (2005) found that the Ugandan shea populations were characterized by high levels of oleic acid (57.8 %) while the West African populations from Mali, Burkina Faso, and Nigeria showed lower percentages of oleic acids ranging from 42.0 to 46.2 %. The regional variation of the four major fatty acids, palmitic, stearic, oleic, and linoleic acids, has been reviewed by several studies (**Table 1-3**). Geographical variation appears to influence fatty acid composition. Shea butters from Chad and Uganda showed the relatively higher amounts of oleic acids (57.8-68.0 %) and lower amounts of stearic acid (22.5-28.9 %) than the shea butters from West Africa such as Benin, Burkina Faso, Côte d'Ivoire, Mali, and Nigeria with similar levels of stearic acid (31.1-46.8 %) and oleic acid (39.3-47.5 %) (Badifu, 1989; Lipp *et al*., 1998; Ferris *et al*., 2001; Di Vincenzo *et al*.,

2005). Shea butter samples coming from the same country can also show varied fatty acid composition (**Table 1-3**), which may be due to the genetics and specific population or the processing involved from harvest to storage. For example, the samples from Mali (Di Vincenzo *et al*., 2005) contained 3.3 % of palmitic acid and 43.3 % of stearic acid while the sample from the same country (Ferris *et al*., 2001) was also reviewed to have 19 % of palmitic acid and 31.1 % of stearic acid.

Table 1-3. Regional variability of fatty acid composition (% total fatty acids) of shea butter

Origin	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	References
Benin	3.8	44.1	43.8	6.65	Lipp *et al.,* 1998 [b)]
Burkina Faso	12.1	42.5	39.3	4.5	Ferris *et al.,* 2001[a)]
Burkina Faso	3.3	43.5	44.5	5.9	Di Vincenzo *et al.,* 2005
Côte d'Ivoire	6.6	46.8	51.4	8.4	Lipp *et al.,* 1998[b)]
Mali	3.3	43.3	44.6	6.0	Di Vincenzo *et al.,* 2005
Mali	19	31.1	42.6	5.7	Ferris *et al.,* 2001[a)]
Nigeria	4	46	41	7	Badifu, 1989
Nigeria	3.4	43.8	44.3	5.8	Di Vincenzo *et al.,* 2005
Nigeria	3.2	38.9	47.5	6.5	Ferris *et al.,* 2001[a)]
Chad	4.2	22.5	68.0	4.9	Lipp *et al.,* 1998 [b)]
Uganda	4.2	28.9	57.8	6.3	Di Vincenzo *et al.,* 2005
Uganda	6.5	26.4	59.3	6.2	Ferris *et al.,* 2001[a)]

a) reviewed the study conducted by the Ben Gurion Univ, Israel
b) reviewed the study conducted by Jacobsbers *et al.,* 1977 (Title: Causes de I'adification du beurre du karité au cours de la préparation et du stockage des amandes. Oléagineux 32:529-533).

1.3.1.2. Regional variability of triglyceride composition

Regional variation of triglyceride composition was also reviewed by Di Vincenzo *et al.* (2005), where Ugandan samples had major triglycerides as SOS (19.87 %), SOO (33.39 %), OOO (19.07 %), while the same triacylglycerides in samples from Mali, Burkina Faso, and Nigeria were 40 %, 26 %, and 10 %, respectively.

1.3.1.3. Regional variability of unsaponifiable fraction

Regional variability of unsaponifiable fraction of shea butter also appears to be affected by the climate of the origin. Maranz *et al.* (2003b) found a relationship between the phenolic compounds especially catechins in shea kernels and the climate. The concentration of phenolics was highly linked to the level of environmental stress, and thus the shea kernels from the hottest and driest areas (e.g. the lake Chad basin area) and those from much cooler and wetter area (Guinea and west Cameroon) showed the highest amount of total catechins compared to the shea kernels from regions with moderate rainfall and temperature (e.g. Burkina Faso). However, since gallic acid was mostly lost during hexane extraction, the shea kernel with large amounts of gallic acid yielded a shea butter with only small amounts of total phenolic compounds, which resulted in a weak regional variation in unsaponifiables of shea butter compared to that of shea kernels.

Tocopherol content, especially α-tocopherol, which is a dominant tocopherol of shea butter was directly correlated with temperature. The concentration of α-tocopherol in shea butter from hot, dry areas (N'Djamena in Chad) was higher (414 μg/g) than the butters originated from cooler areas of northern Uganda (29 μg/g) (Maranz *et al.,* 2004).

A study conducted in Mali, Burkina Faso, Nigeria, and Uganda by Di Vincenzo *et al.* (2005) also confirmed variation in the triterpene alcohols of the unsaponifiable fraction. The study found the West African shea butters from Nigeria (12.6 %), Mali (9.6 %) and Burkina Faso (7.1 %) contained more triterpene alcohols than the East African butters, with the Ugandan butters containing only 3.7%.

1.3.2. Effect of post-harvest processing on shea butter's characteristics

1.3.2.1. Effect of extraction method on shea butter

As reviewed in section 1.1.2., shea nuts can be processed to extract shea butter using several methods that can either stimulate or prevent the degradation of shea butter. The different methods in gathering, processing and initially treating the shea nuts are presumed to be a major underlying cause in the difference in the quality characteristics among the shea butter extracted from different places. Studies by Mbaiguinam *et al.* (2007) also found that hexane extraction or the extraction method including the parboiling of the seeds yielded shea butter with almost half level of free fatty acids compared to shea butter extracted without seed-boiling procedure. While the shea butters from hexane extraction and the procedure involving parboiling of seeds showed acidity of 5.1-5.5, the butters extracted from sun dried nuts, with no boiling, showed much higher levels of acidity (10.3-10.6).

1.3.2.2. Effect of solvent used for extraction on shea butter

The type of the solvents used in the extraction of shea butter from the kernel can influence several quality characteristics including total vitamin E content and peroxide value. In a study conducted by Kar *et al* (1981), petroleum ether, n-hexane, chloroform, benzene, and water were used in extraction of shea butter. This study found that water extraction yielded almost the half or less amount of total fat from the kernel. They also reported that the shea butters extracted with organic solvents contained nearly 0.01 % of vitamin E and showed no detectable level of peroxides while the butters extracted with

water contained no detectable levels of vitamin E and higher values of peroxides (5.0-8.3 mEq/kg), showing that water extraction can promote the oxidation of shea butter.

1.3.2.3. Effect of extraction temperature on shea butter

Temperature involved in the extraction of shea butter is also an important factor related to shea butter's quality. In a study conducted by Olaniyan *et al.* (2007), four different temperatures were used in dry extraction of shea butter from the kernels which were heated to 50, 70, 90, and 110 ℃ respectively. The researchers found that the moisture content decreased as the temperature increased. Though, temperature above 90 ℃ resulted in deleterious effect of other quality characteristics due to excessive burning of the shea butter. Increased temperature was linked to lower specific gravity due to expansion of the oil and thus the volume. Increased extraction temperature also led to the shea butter with high levels of free fatty acids due to thermal destruction of oil cells and increase in lipase activity, higher peroxide and rancidity values, as well as decreased palatability due to decreased ester value.

1.4. Quality control of shea butter

1.4.1. Currently available standards for shea butter

Quality standard for unrefined shea butter has been developed by ProKarité, a project managed by the World Agroforestry Centre and funded by CFC/FAO (Common Fund for Commodities/Food and Agriculture Organization) and approved by UEMOA (Union Economique Monétaire Ouest Africaine) (Lovett *et al.,* 2005) (**Table 1-4**). This regional standard board has proposed sensory, physical and chemical characters that

define the quality of shea butter, including color, odor, taste, free fatty acids, peroxide value, insoluble impurities, moisture, volatile matters, soap content, relative density, refractive index, saponification value, unsaponifiable matters, iodine value, and melting point. This organization has developed a grading system using the most important quality characters for shea butter, including moisture content, free fatty acid, peroxide value, and insoluble impurities (**Table 1-4**).

Table 1-4. Quality characteristics and grades of unrefined shea butter.

Parameters	**Unrefind shea butter**		
	Grade 1	**Grade 2**	**Grade 3**
	Min. Max	**Min. Max**	**Min.Max**
Moisture content (%)	- 0.05	> 0.05 – 0.2	> 0.2 – 2.0
Free fatty acid (%)	- 1.0	> 1.0 – 3.0	> 3.0 – 8.0
Peroxide value (meq/kg)	- 10.0	>10.0 – 15.0	>15.0 – 50.0
Insoluble impurities (%)	- 0.09	>0.09 – 0.2	> 0.2 – 2.0

Source: Regional Technical Committee Comments on Draft Africa Regional Standard for Unrefined Shea Butter (2006).

According to this standard, the best quality unrefined shea butter of grade 1 can be used by the cosmetic and pharmaceutical industries, and for direct consumption. The shea butter of grade 2 can serve the needs of food industry for manufacturing confectionary, chocolate, edible oil, and a basis for margarines. The shea butter of grade 3 is recommended to be used in soap-making or further refined for direct consumption.

The American Shea Butter Institute (ASBI) is a US based organization which also serves grading of shea butter with its own standards, where shea butter is graded from Grade A to Grade D, and even to Grade F. ASBI (2011) conducts routine laboratory analysis of

physical and chemical properties as well as other procedures (e.g., nuclear magnetic resonance (NMR) melting profile) and also studies on the potential shelf life of products.

1.4.2. Efforts made on locally produced shea butter to improve the quality

During the last decade, efforts have been made to enhance the quality of in-country processed African shea butter. Partnership between 'universities, non-government organizations (NGOs), and/or companies' and 'African private business sectors or unions of individual shea butter producers' have established quality control (QC) systems and trainings to improve the collection and processing practices of shea nuts and thus enhance the quality of shea butter. These efforts have been made ultimately to increase the market value of locally-processed African shea butter for the benefits of local producers.

Some of these partnerships include the Northern Uganda Shea Processors Association (NUSPA) which has been established with funding from the United States Agency for International Development (USAID). NUSPA has been producing pharmaceutical-grade shea butter for exports to the United States, Europe, and Japan. In addition, NUSPA assisted the neighboring country, Sudan, by providing training and by helping to establish its processing centers involving rural women (Harsch, 2001).

Agribusiness in Sustainable Natural African Plant Products (ASNAPP), a non-profit organization formed with funding from USAID has been working with a Ghanaian private sector and woman collectors and assisting the private sector to assess the quality of shea butter. A Ghanaian private sector, The Pure Company, has just started to

industrially produce shea butter with the collaboration of ASNAPP and Rutgers University. The New Use Agriculture and Natural Plant Products (NUANPP) Program in Rutgers University has provided technical support to develop QC protocols and trainings to lab technicians, and also cross-validated the results from the QC tests on shea butter by doing the same tests in labs in Rutgers University and The Pure Company at the same time.

L'Occitane, a France-based cosmetics company which has a series of shea butter products, buys shea butter directly from Burkina Faso through the "Union des groupements Kiswendsida (UGK) ", a network of shea producers, which have brought better returns to the producers since there is no middleman (Harsch, 2001). In addition, the company has contributed to the livelihood and the development of African women as well as shea butter production by providing training in quality control and by paying for the shea butter in advance (Harsch, 2001).

Currently, there is another movement to promote in-country processed shea butter. On October 11, 2010, an international shea alliance was launched (the alliance will be formally launched at Shea 2011: Sustainable Solutions, the international shea industry conference, April 4-7, 2011, in Accra, Ghana) with more than 50 major shea industry stakeholders including traders, exporters, service providers, and NGOs (Lamport, 2010; Global Shea, 2011). This alliance is aimed to promote shea butter worldwide, establish industry standards for quality and sustainable sourcing, and exchange information (Global Shea, 2011).

1.5. Effect of antioxidants on oxidative stability of plant-derived oils and fats

1.5.1. Oxidation that occurs in plant oils and fats

Most of the degradation of shea butter during processing is due to lipid oxidation, a major problem in edible lipids that leads to chemical, sensory, and nutritional deterioration (Velasco *et al*., 2002). Lipid deterioration is characterized by two distinct stages. The induction period is an early stage of oxidation (a lag phase), where the oxidative change occurs relatively slow. After the induction period ends, oxidative deterioration proceeds rapidly (i.e., an exponential phase) (Pokorny *et al*., 2001; Damodaran *et al*., 2008). The induction period can be extended with decrease in temperature, oxygen concentrations, fatty acid unsaturation, and activity of pro-oxidants as well as increase in concentrations of antioxidants (Damodaran *et al*., 2008).

1.5.2. Mechanism of autoxidation in lipids

Basically, oxidative rancidity is caused by the reaction of oxygen with the double bonds of unsaturated fatty acids, and thus oils and fats containing higher levels of unsaturated fatty acids are more susceptible to oxidation (McWilliams, 2001; Pokorny *et al*., 2001; Damodaran *et al*., 2008). In the initiation step of lipid oxidation, a free radical, a molecule or an atom that has unpaired electron, is formed (an alkyl radical: R·) with the presence of initiator such as heat, light, metal ions, among others (Shahidi *et al*., 2010).

Once the free radical is formed, the propagation stage begins with the addition of oxygen atoms to the free radical to form a peroxide (a peroxyl radical: ROO·**)**. Then the hydrogen removed from another unsaturated fatty acid attaches to the peroxide to form a

hydroperoxide (ROOH). While the hydrogen is removed from the other unsaturated fatty acid, the unsaturated fatty acid forms a new free radical and the same procedure occurred in the first free radical is auto-catalytically repeated and thus this whole process is called autoxidation. Hydroperoxides can be also formed by the action of naturally occurring lipoxygenases on the polyunsaturated fatty acids before or during the extraction of oils (Pokorny *et al*., 2001).

In the termination stage of oxidation, the autoxidation is ceased by the reaction of two radicals to form stable non-radical products such as ROOR (from peroxyl and alkyl radicals), RR (from two alkyl radicals), and ROOR + O_2 (from two peroxyl radicals) (**Figure 1-4**).

With the presence of oxygen, lipid oxidation will be accelerated or delayed depending on the presence of pro- or anti-oxidants respectively. Lipid oxidation is facilitated under the high temperature and/or high water activity, and the presence of metals such as iron and copper, light, among others. (McWilliams, 2001; Damodaran *et al*., 2008).

Initiation	**$X\cdot + RH \rightarrow R\cdot + XH$**
Propagation	**$R\cdot + O_2 \rightarrow ROO\cdot$**
	$ROO\cdot + R'H \rightarrow ROOH + R\cdot$
Termination	**$ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$**
	$ROO\cdot + R\cdot \rightarrow ROOR$
	$R\cdot + R\cdot \rightarrow RR$

Figure 1-4. Mechanism of lipid autoxidation (Pokorny *et al*., 2001)

1.5.3. Decomposition of hydroperoxides and rancidity

Hydroperoxides formed during lipid oxidation are not directly responsible for off-aroma and thus rancidity (Damodaran *et al*., 2008). However, their further decomposition into alkoxyl radicals (RO·) which have high energy causes β-scission, the cleavage of aliphatic chain of fatty acids into low molecular weight compounds that are responsible for perceived rancidity (Damodaran *et al*., 2008). The low molecular weight compounds include volatile hydrocarbons, alcohols, and aldehydes and non-volatile alcohols and ketones. Volatile aldehydes are the most important contributor to off-aroma. Hexanal, heptanal, octanal, nonanal, decanal, *trans*-2-heptanal, *trans*-2-nonenal, *cis*-2-decenal, *trans,trans*-2,4-nonadienal, *trans-cis*-2,4-decadenal are some examples of volatile aldehydes that can be found in oxidized lipids including shea butter (Pokorny *et al*., 2001; Bail *et al*., 2009).

1.5.4. Use of natural antioxidants to protect plant oils and fats

The protective effects of various antioxidants on the oxidative stability of plant-derived oils and fats have been the subject of intense research, showing great potential to protect oils and fats against oxidation. During the last decade, the use of natural antioxidants and natural products (e.g. plant extracts) have been widely studied by several researchers, in view of the concerns generated by the use of synthetic antioxidants (Abramovic *et al*., 2006; Kowalski, 2007; Azizkhani *et al*., 2009). However, up to now, there are no research reports on the effects of natural and synthetic antioxidants on shea butter oxidation.

Protective effects of antioxidants on oils and fats were assessed by measuring several oxidative parameters as well as induction period either under the normal storage condition or oxidation-accelerating condition (e.g., high temperature of 40, 60, 90, 110 ℃, air flow) (Judde *et al*., 2003; Kowalski, 2007; Jennings *et al*., 2009). The oxidative parameters include sensory characteristics such as off-flavor or odor, headspace volatile oxidation products, peroxide value, conjugated dienes and trienes, p-anisidine value, thiobarbituric acid reactive substances (TBARS), fatty acid composition, among others (Pokorny *et al*., 2001; Judde *et al*., 2003; Abramovic *et al*., 2006; Kowalski, 2007; Bouaziz *et al*., 2008; Azizkhani *et al*., 2009; Jennings *et al*., 2009; Konsoula *et al*., 2010). Induction period was measured using Rancimat test (Abramovic *et al*., 2006; Bouaziz *et al*., 2008) and oxidative stability index was also determined as the means to assess oxidative stability of oils with antioxidants (Jennings *et al*., 2009).

Judde *et al*.(2003) found that the addition of 1 % (w/w) soy lecithin was effective in delaying the oxidation in rapeseed, soy, walnut, and palm oils by increasing the induction time by 1.7-1.8 times when measured at 110 ℃ and decreasing the peroxide values by 2.2- 4.6 folds when heated at 40 ℃ for 35 days.

Abramovic *et al*. (2006) found the addition of 0.2 % rosemary extracts containing 0.024 % of carnosic acid to *Cemelina sativa* oil showed the reduced formation of hydroperoxides by more than 40 % and the extended induction period by 60 % compared to control when stored in room temperature (20-30 ℃) for 330 days.

Kowalski (2007) found that the addition of 0.02, 0.04, 0.06 % of natural antioxidants including quercetin, caffeic, and protocatechuic acids were effective in preventing the levels of fatty acids in olive oil and sunflower oil while heating at 90 ℃ for 120 hours. The natural antioxidants were no less effective than the same concentration of BHT, a synthetic antioxidant.

Bouaziz *et al*. (2008) found that the addition of olive leave extracts and hydrolysate (400 ppm) were effective in protecting refined olive oils and refined olive husk oils. The leaf extracts and hydrolysate decreased peroxide values and conjugated dienes and trienes of the oils compared to controls. The induction period was more than doubled with the presence of the leave extracts and hydrolysate.

In a study conducted by Azizkhani *et al*. (2009), the mixture of rosemary extracts (200 ppm) and ascorbyl palmitate (200 ppm), and the mixture of rosemary extracts (200 ppm), α,β, and γ tocopherols (200 ppm), and lecithin (1000 ppm) each showed antioxidant effect similar to that of tert-butylhydroquinone (TBHQ) (120 ppm) which is the most commonly used synthetic antioxidants when added to margarine made of sunflower oil and palm stearine. The two mixtures were effective in lowering p-anisidine of the margarine stored at 60 ℃ for 25 days and also effective in extending the induction period of the margarine while stored at 4 ℃ for 14 weeks.

Konsoula *et al*. (2010) found that olive, sunflower, soybean, and corn oils heated at 100 ℃ for 200 hours were protected against oxidation with the addition of sesame

extracts (unsaponifiable matter, sesame oil extracts, and sesame seed extracts) containing 50 to 1200 μL of sesamol, corresponding to a sesamol content of 0.5-12 mg/kg oil. Peroxide values of olive, sunflower, soybean, and corn oils were reduced by 17.9-81.4 %, 15.1-70.7 %, 14.3-67.6 %, and 13.2-61.8 %, respectively. The protected effect was superior when the sesame extracts were prepared from coated-unroasted sesame seeds rather than from dehulled-roasted sesame seeds.

1.6. Objectives

This study was started to aid in the improvement of shea butter produced from West Africa. Using shea butter from Ghana as the case study, and in concert with a Ghanaian private sector, particularly The Pure Company, located in Buipe, northern Ghana, where the industrial production of shea butter has just started, collaboration began with Rutgers University and Agribusiness in Sustainable African Natural Plant Products (ASNAPP), Acrra, Ghana, to support the natural products industry. The motivation for this study was to provide the Ghanaian private sector with well established quality control procedures for shea butter and to assess the quality of shea butter produced. However, for this master's thesis, this research was purposefully not limited to the shea butter from The Pure Company and Ghana; but rather included several other West African unrefined shea butters that were available in the market.

1.6.1. Problem statement

Traditionally, in West Africa, processing of shea nuts and production of shea butter were practiced by the collectors of the shea nuts who are, in most cases, women, who pass the

knowledge of shea butter handling to their daughters, each using their own approach and methods. Local production of shea butter revealed problems which include the inconsistent product and the difficulty to control or procure consistent product due to the lack of quality control and the varied and degraded quality shea butter. Shea butter, especially, undergoes hydrolytic and oxidative degradations during the post-harvest processing and storage, which results in the shea butter characterized by high values of free fatty acids and peroxide values. All these factors lead to inconsistency of quality and limited shelf-life of shea butter.

1.6.2. Objectives and hypothesis

This study was initiated to support shea butter industry in Ghana by setting up quality control procedures to assess the quality of shea butter from the Ghanaian private sector (The Pure Company, Buipe, Bron Ahafo region, Northern Ghana) as a case study.

The first objective of this work was aimed at assessing and comparing sensory and physicochemical characteristics, and chemical composition of West African shea butter samples from different origins. The hypothesis underlying this objective was that West African shea butters will share similar chemical properties particularly the composition of fatty acids regardless of the origin, production condition, or storage condition while some properties related to quality degradation will be significantly different among the shea butters from different origins.

The second objective of this study was to identify ways to extend its shelf life using selected synthetic and natural antioxidants, considering shea butter is fat comprised almost half of the unsaturated fatty acids which are susceptible to oxidative degradation. The hypothesis underlying this objective was that selected antioxidants will prevent the oxidative degradation of shea butter and thus result in extended shelf-life.

CHAPTER 2. MATERIALS AND METHODS

2.1. Characterization of shea butter

2.1.1. Plant materials

Seven West African shea butter samples were used in this study for the characterization of shea butter (**Table 2-1)**. The samples TPC 01-03 were graciously provided by the The Pure Company (Buipe, Northern Ghana) and were obtained on January, 2009 for TPC 01, May, 2009 for TPC 02, and September, 2009 for TPC 03. GHA 01 was purchased at a local store in New Brunswick, NJ in August, 2009 and GHA 02 was provided from Eden shea naturals on October, 2007. WAF 01 was purchased online (Sheabutterhut.com) and was informed as 'made in West Africa' on the package. The sample from Benin (BEN 01) was provided by Magatte Wade-Marchand on April 2010.

Table 2-1. Source, origin, year of production and acquisition date of the unrefined shea butter samples used in this study.

Sample ID	Source	Origin	Year of production	Date of acquisition
TPC 01	The Pure Company	Ghana	December, 2008	January, 2009
TPC 02	The Pure Company	Ghana	January, 2009	May, 2009
GHA 01	Africansheabutter.org	Ghana	N/A	August, 2009
GHA 02	Eden shea naturals	Ghana	September, 2007	October, 2007
WAF 01	Sheabutterhut.com	West Africa	N/A	August, 2009
TPC 03	The Pure Company	Ghana	August, 2009	September, 2009
BEN 01	Magatte Wade-Marchand	Benin	June/July 2009	April, 2010

2.1.2. Sample preparation

Most of the shea butter samples were filtered through the coarse filter paper (P8, Fisher Scientific, Pittsburgh, PA) at 45-50 ℃ in an oven. The samples were stored at 4 ℃ in a refrigerator and, just before the use, they were melted at 45-50 ℃ until all the crystals were dissolved and thus shea butter became clear. Specific gravity, melting point, moisture, insoluble impurities and sterols and triterpenoids were measured with unfiltered sample. The refractive index was tested on both unfiltered and filtered samples.

2.1.3. Chemicals and reagents

Methyl red, potassium dichromate, potassium hydroxide, potassium iodide, sodium sulfate, starch, and tert-butyl methyl ether, and ACS grade acetone and sodium carbonate were purchased from Acros (Morris Plains, NJ). Diethyl ether, Karl Fischer reagent, sulfuric acid, and toluene, HPLC grade chloroform, hexane, and methanol, and ACS grade glacial acetic acid, hydrochloric acid, and phenolphthalein were purchased from Fisher Scientific (Fairlawn, NJ). Iodine was purchased from Mallinckrodt (Paris, KY). ACS/USP grade ethyl alcohol 95 % was provided by Pharmco-Aaaper (Brookfield, CT). Ammonium chloride and sodium thiosulfate were purchased from Sigma Chemical (St. Louis, MO).

2.1.4. Measurement of sensory characteristics

Color, aroma, and texture were assessed upon arrival of the samples. Color of the samples was visually assessed. Aroma or note was assessed by smelling each sample.

Texture was assessed by applying the shea butter on the back of the hand. This study was done as routine sensory quality assessment without the involvement of trained panels.

2.1.5. Measurement of physicochemical characteristics

2.1.5.1. Specific gravity (Relative density)

Specific gravity of shea butter was measured with a 2mL capacity Gay-Lussac bottle which was calibrated with water. To calculate the specific gravity, the weights of (a) a completely dried Gay-Lussac bottle, (b) the completely dried Gay-Lussac bottle with distilled water which was heated to 20 ℃ in a water bath, and (c) the completely dried Gay-Lussac bottle with shea butter heated to 40 ℃ in the water bath were measured. The specific gravity was calculated by using the following formula:

$$\text{Specific gravity} = \frac{\text{density of shea butter}}{\text{density of water}} = \frac{c - a}{b - a}$$

2.1.5.2. Refractive Index

Refractive index was measured using a refractometer (Fisher Scientific, model 334620). The temperature of the refractometer was set up and maintained to 40 ℃ while used.

2.1.5.3. Melting Point

For this study, three different melting points, the clear, liquefying and dropping points were determined to identify which of the three methods will be the best method to determine the melting point, an important physical property of shea butter.

Clear Point

Melted shea butter samples were transferred to Pasteur pipettes of which the tips were sealed by melting the glass (**Figure 2-1**), which was prepared by the modification of Harris (2004). The pipettes containing shea butter samples were placed at 45 ℃ in an oven to prevent shea butter from sticking to the walls of the pipettes. The pipettes were then placed at 4 ℃ in the refrigerator to solidify the butter.

When the samples were entirely solidified, the pipettes were dipped into the water in a 2000 mL capacity beaker. The temperature of the water was gradually increased by placing it on the hot plate and by continuously stirring it with a magnetic stirrer (**Figure 2-1)**. The clear point was measured when the solidified butter is completely melted and turn into a clear liquid (Nielsen, 2010).

Figure 2-1. Apparatus for measuring clear point. Shea butter was expected to be filled in the apparatus up to the line on the pipett.

Liquefying Point and dropping point

For this procedure, a device made with a glass tube containing a 3mm hole in the bottom was used that was sealed with a parafilm, and then melted shea butter was transferred to the tube up to 1 cm from the bottom (**Figure 2-2**) (Lunge *et al*., 1908). Then the sample was placed at 4 ℃ in a refrigerator to solidify.

After the sample was solidified, a thermometer was placed through the top of the device, the parafilm was then removed, and the device was then placed in a bigger test tube. The test tube containing the device with thermometer was then submerged in a 2000 mL capacity beaker (**Figure 2-2**). The temperature of the water was gradually increased by placing the beaker on a hot plate and by stirring it with a magnetic stirrer. The liquefying point was measured as the temperature at which the shea butter samples started to melt and appeared from the bottom of the 3mm hole, while the dropping point was measured at the temperature at which the first drop of the melted butter went through the hole (Lunge *et al*., 1908).

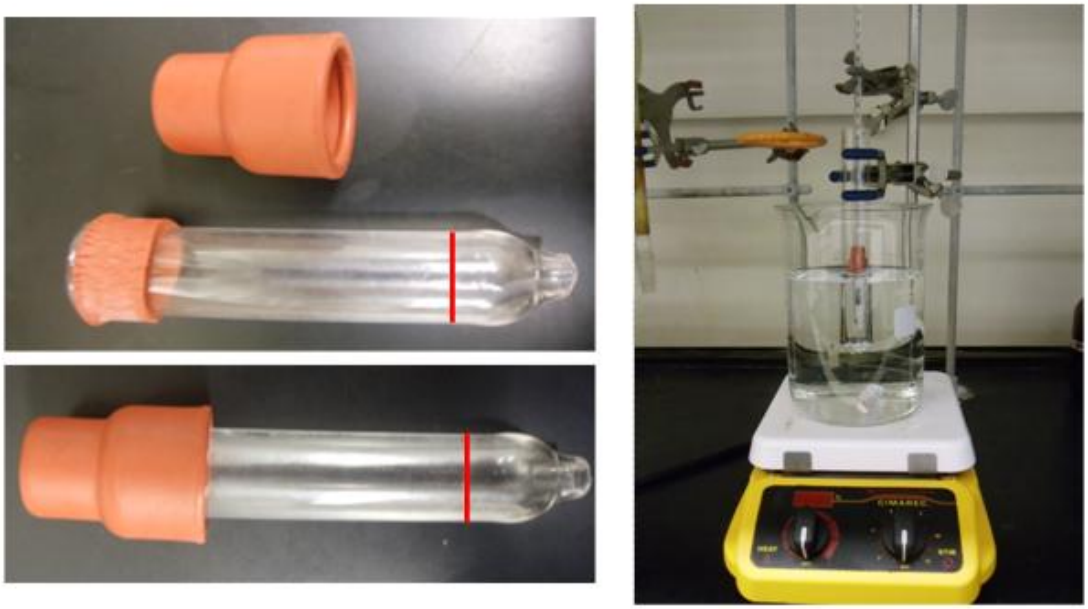


Figure 2-2. Glass device with a 3 mm hole (left) for measuring liquefying and dropping points (Shea butter was expected to be filled in the apparatus up to the line.). The glass device attached with a thermometer was then inserted in a bigger test tube and submerged in a 2000mL beaker.

2.1.5.4. Moisture content

Moisture content was measured with two methods: distillation method and modified Karl Fischer method. Since the distillation method requires a large amount of sample, this method was only used for the sample WAF 01 and TPC 03, the samples provided in large amounts.

Distillation method for the sample TPC03 and WAF01

Water content was determined following A.O.C.S. Official method Ca 2a-45 (Firestone, 1990). In this study 400 g of unfiltered shea butter and 200 mL of dry toluene were transferred to a 2000 mL capacity round bottom flask which was then attached with a water trap and placed on a heating mantle. Since shea butter was expected to yield very low amount of water, the water trap was filled with 1 mL of water to read the exact amount of water and thus facilitate recording of the moisture contents. Condenser was then mounted on the top of the water trap and the sample was boiled with toluene on the heating mantle at the temperature of 100 ℃. The mixture of toluene and shea butter was then allowed to boil for an hour, and the water trap was then cooled for another hour. After then, the water content was monitored and the amount of water in the sample (%) was calculated by the following formula:

$$\text{Moisture content (\%)} = \frac{\text{volume of water (mL)}}{\text{weight of shea butter test portion (g)}} \times 100$$

Modified Karl Fischer Reagent method for all samples

Moisture content was measured following IUPAC 2.603 modified Karl Fischer method which is appropriate for analyzing fats containing small amount of water (Paquot *et al,* 1987).

Calibration of Karl Fischer reagent

Karl Fischer reagent was calibrated by the following procedure. 25 mL of 3:1 (v:v) solvent mixture of chloroform and methanol was transferred into a 250 mL capacity Erlenmeyer flask and a magnetic stirrer was also placed in the flask. The flask was then masked with a rubber stopper with two holes one of which could be fitted with the delivery tip of a burette filled with Karl Fischer reagent. Once the burette was filled with Karl Fischer reagent, the solvent was titrated with the Karl Fischer reagent until the color of solvent turned from light yellow to orange red. To prevent the absorbance of moisture from air to the solvent, the burette was closed with a rubber stopper connected with a funnel which was filled with cotton ball and desiccant, cobalt chloride ($CoCl_2$).

Another set of experiment was followed with the same procedure mentioned above but the 250 mL capacity Erlenmeyer was filled with 25 mL of 3:1 (v:v) solvent mixture of chloroform and methanol and a drop of water which was precisely weighed to within 0.0001 g.

Determination of the water content of shea butter

Melted unfiltered shea butter sample (10 g) was weighed in a 250 mL capacity Erlenmeyer flask to within 0.0001 g and then 25 mL of 3:1 (v:v) solvent mixture of chloroform and methanol was added into the flask. Titration with Karl Fischer reagent was then performed in the same manner used in the calibration of the reagent. To calculate the moisture content of the shea butter samples, the following formulas were used:

$$A = \frac{m0}{V1 - V0}$$

where, A is the value obtained after the calibration of Karl Fischer reagent, V0 is the number mL of Karl Fischer reagent used with 3:1 (v:v) solvent mixture of chloroform and methanol alone, V1 is the number of mL of the reagent used with the solvent and the water added, and m0 is the mass of the water added in g.

$$\text{Moisture content (\%)} = \frac{100 \times (\mathrm{V} - \mathrm{V0}) \times \mathrm{A}}{\mathrm{m}}$$

where, V is the number of mL of Karl Fischer reagent used in the determination of moisture content of the sample, and m is the mass, in g, of the shea butter samples used.

2.1.5.5. Insoluble impurities

Pretests for the measurement of insoluble impurities using IUPAC 2.604 resulted in a considerable amount of residual fat on the edge of the filter paper with the diameter of 12 cm and thus % insoluble impurities were overestimated. Therefore, to minimize the fat

residue on the filter paper, the filter paper with the smaller diameter was used and the filter paper with shea butter was washed using syringe filtration.

A 10 mL capacity gastight syringe (Hamilton Company, NV) was filled with 5 mL of melted shea butter sample and the syringe was then weighed to 0.0001 g accuracy. A filter and the filter paper (G6, Fisher Scientific, Pittsburgh, PA) were previously weighed to 0.0001 g and then they were placed in a filter holder and attached to the tip of the syringe (**Figure 2-3**). Shea butter sample passed through the filter by applying pressure in the syringe, and then the filter and the filter paper in the holder were washed with 50 mL of hexane to remove the shea butter from the device. After the washing, the filter paper was then dried in an oven at the temperature of 100 ℃ for 10 minutes to evaporate all solvent and then weighed. The amount of insoluble impurities that remained in the filter paper (without any shea butter) was then calculated by the following formula:

$$\text{Insoluble impurities } (\%) = \frac{\mathrm{m2} - \mathrm{m1}}{\mathrm{m}} \times 100$$

where m is the mass, in g, of the test portion, m1 is the mass, in g, of the filter paper and the filter, m2 is the mass, in g, of the filter paper with insoluble impurities and the filter. Since the glass fiber filter circles used were too fragile to pick with tongs, the filter papers were weighed when they were placed on the filter.

A blank test using cooking oil known to have almost no insoluble impurities was used to make sure all the oil/fat was properly dried by the amount of hexane.

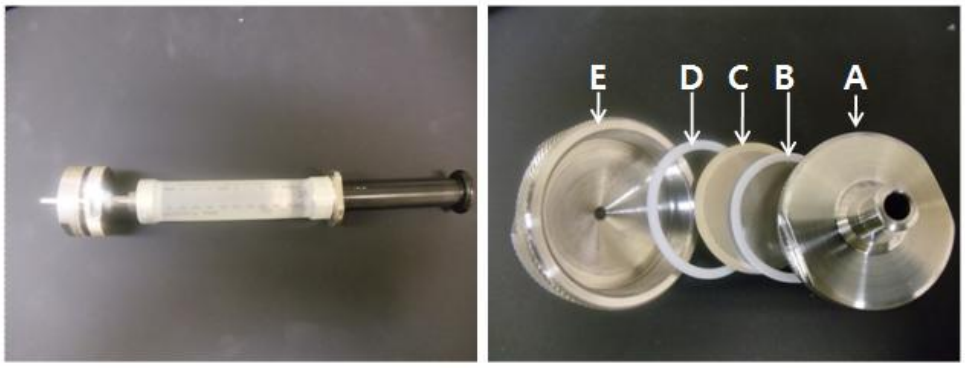


Figure 2-3. Syringe and filter holder used in the measurement of insoluble impurities. The figure in right side represents the constituents of syringe filter holder. Filter paper was supposed to be placed between the white ring (B) and filter (C).

2.1.5.6. Free fatty acids

Free fatty acids were measured according to IUPAC 2.201 indicator method (Paquot *et al*, 1987). 2.5 g of melted filtered shea butter sample was placed in a 250 mL Erlenmeyer flask. 150 mL of neutralized 1:1 (v:v) ethanol and diethyl ether solution was added to the flask and the mixture was titrated with potassium hydroxide 0.1 N ethanolic solution until the pink color appeared and lasted for at least 10 seconds. Acid value and free fatty acid value are calculated by the following formulas:

$$\text{Acid value} = \frac{56.1 \times \text{T} \times \text{V}}{\text{m}}$$

where, T is exact normality of standardized potassium hydroxide solution, m is the mass (g) of the test portion;

$$\text{Free fatty acid (\%)} = \frac{\text{acid value}}{1.99}$$

2.1.5.7. Peroxide value

Peroxide value was measured following IUPAC 2.501 (Paquot *et al*, 1987). 3g (weighed to 0.001 g accuracy) of melted filtered shea butter sample was placed in 250 mL capacity Erlenmeyer flask and the following reagents were added to it: 10 mL of chloroform, 15 mL of glacial acetic acid, 1 mL of potassium iodide saturated solution, 2 mL of starch solution, and 75 mL of distilled water. The resultant mixture showing dark purple to dark brown was titrated with standardized 0.01 N sodium thiosulfate solution

until the color of the mixture turned to ivory to white color. Peroxide value (PV) was calculated by the following formula:

$$\text{Peroxide value (mEq/kg)} = \frac{V \times T}{m} \times 1000$$

where V is the number of mL of standardized sodium thiosulfate solution used for the test corrected to take into account the blank test, T is the exact normality of the sodium thiosulfate solution used, and m is the mass in grams of the test portion.

2.1.5.8. Total unsaponifiable fraction

Total amount of unsaponifiable fraction was measured according to IUPAC 2.401 diethyl ether method (Paquot *et al*, 1987). 5 g of melted filtered shea butter sample was refluxed gently for 1 hour with 50 mL of potassium hydroxide ethanolic solution (6%, w/v). The sample was diluted with 100 mL of distilled water and unsaponifiable matters were extracted with 100 mL of diethyl ether for 3 times. The recovered ethereal fraction was then washed with 40 mL of distilled water for 3 times, 40 mL of potassium hydroxide 0.5 N aqueous solution, 40 mL of distilled water, and 40 mL of potassium hydroxide 0.5 N aqueous solution and then washed with 40 mL of water until the collected water from washing gave no pink color upon the addition of a drop of phenolphthalein solution.

The ethereal solution recovered was then evaporated in a round bottom flask with ground joint which was previously dried and weighed to the nearest 0.0001 g by distillation. After the distillation, 5 mL of acetone was added to the flask and the volatile solvent was

evaporated through a gentle current of air, resulting in creamy substances on the bottom of the flask. The flask was then dried in the oven at 101 ℃ and the residue was weighed based on the weight of the flask with the residue and the weight of the flask.

After weighing the residue, 4 mL of diethyl ether was added in the flask to dissolve the residue, followed by the addition of 20 mL of neutralized ethanol. The resultant solution was stirred for 10 minutes and additional 2 drops of phenolphthalein solution was added to it. The solution was then titrated with 0.1 N ethanolic potassium hydroxide solution until the color turned to pink. The amount of unsaponifiables (%) was calculated by the following formula:

$$\text{Unsaponifiables (\%)} = \frac{100 \times (\text{m1} - 0.28\text{VT})}{\text{m}}$$

where, m is the mass, in g, of the test portion, m1 is the mass, in g, of the residue, V is the number of ml of the standardized potassium hydroxide solution used, and T is the exact normality of the potassium hydroxide solution used.

2.1.6. Measurement of chemical compositions

Gas chromatography (GC) was used to analyze the chemical composition of shea butter: fatty acid composition of the triglyceride fraction and sterols and triterpenes in unsaponifiable fraction.

2.1.6.1. GC analysis of fatty acid composition

Transesterification of fatty acids to fatty acid methyl esters (FAMEs)

0.5 g of homogenized shea butter was refluxed with 5 mL of 0.5 N potassium hydroxide methanolic solution for 5 minutes. After the reflux, 15 mL of ammonium chloride and sulfuric acid in methanol solution was added and heated for 3 minutes and after the mixture cooled down, 10mL of hexane was added and a solvent fraction was recovered using separating funnel. Then 1.5 mL of the solvent fraction containing fatty acid methyl esters (FAMEs) was dried over sodium sulfate, and centrifuged at 13000 rpm for 5 minutes. After the centrifugation, the resultant solution was subjected to GC analysis.

Identification of fatty acid methyl esters (FAMEs) using gas chromatography with flame ionization detector (GC-FID)

FAMEs were analyzed on an Econo-Cap™ EC™-WAX Capillary Column (length 30m, internal diameter 0.25mm, phase Polyethyleneglycol, film 0.25µm, Alltech, Deerfield, IL) in an HP 6890 series gas chromatograph equipped with a flame ionization detector and an automated injector (Agilent, Wilmington, DE). Samples were injected at an initial oven temperature of 60 ℃ held for 1 minute. Then the column temperature was increased at a rate of 10℃ / min to 200 ℃. The injector and the flame ionization detector (FID) temperatures were set to 220 ℃. Helium was used as the carrier gas.

Peak identification was performed by comparison of retention times of standard solutions to that of individual fatty acid standards. Fatty acids were expressed as % of total fatty acids.

2.1.6.2. GC analysis of the composition of sterols and triterpenes

Analysis of the composition of sterols and triterpenes was performed following the procedure according to Ramadan *et al* (2007) with modifications.

Isolation of the unsaponifiables

Unsaponifiable fraction of shea butter was extracted as described in prior section 2.1.5.8 (total unsaponiable fraction). 1.2 g of melted unfiltered sample placed in a 125 mL capacity Erlenmeyer with ground joint was refluxed for 1 hour with 15 mL of potassium hydroxide ethanolic solution (6%, w/v). After 1 hour refluxing, the unsaponifiable fraction was washed with 25 mL of diethyl ether for 3 times, 10 mL of distilled water for 3 times, 10 mL of potassium hydroxide 0.5 N aqueous solution, 10 mL of distilled water, and 10 mL of potassium hydroxide 0.5 N aqueous solution. The recovered solvent layer was then washed with 10 mL of water until the collected water from washing gave no pink color upon the addition of a drop of phenolphthalein solution. The extract was then mixed with 7 mL of tert-butyl methyl ether and then transferred to a 125 mL capacity separating funnel, swirled and then drew the bottom layer to remove water. The extract was then dried over sodium sulfate while centrifuged at 13000 ppm for 5 minutes. The mixture was then transferred for glass vial for GC analysis.

Separation and identification of unsaponifiable fraction using gas chromatography with mass spectrometer (GC-MS)

Sterols and triterpenes in the unsaponifiable fraction were analyzed on an Econo-Cap EC-5 Capillary Column (length 30m, internal diameter 0.25 mm, phase (5% Phenyl)-95%

methylpolysiloxane, film 0.25 µm, Alltech, Deerfield, IL) in an HP 6890 series gas chromatograph equipped with a flame ionization detector and a mass spectrometer (Agilent, Wilmington, DE). Samples were injected at an initial oven temperature of 60 ℃ held for 1 minute. Then the column temperature was increased at a rate of 7℃/ min to 250 ℃ which was then held for 22 min. Helium was used as the carrier gas.

Peak identification was performed by comparing the mass spectra of each peak to the mass spectra available in Wiley Mass Spectrometer Library 2000 (Wilmington, DE).

2.1.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed on all physico-chemical parameters by using GraphPad Priam 5 (GraphPad Software Inc. La Jolla, CA) to see the difference among the seven West African shea butter samples. When the significant difference was observed as a result of ANOVA at $P<0.05$, further multiple comparisons were conducted with the Tukey's multiple comparison test at $P<0.05$. Results are presented as means ± standard error (SE).

2.2. Identification of the effect of antioxidants on shea butter's oxidative stability

2.2.1. Sample preparation

For the oxidative stability tests the sample TPC03 was used. The sample was filtered as described in section 2.1.2.

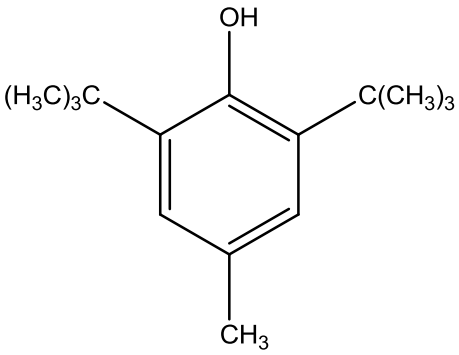
2.2.2. Chemicals and reagents

The chemicals 1-butanol, 95 % heptadecanoic acid, potassium dichromate, potassium hydroxide, potassium iodide, sodium sulfate, starch and ACS grade sodium carbonate were purchased from Acros (Morris Plains, NJ). Sulfuric acid, HPLC grade chloroform, hexane, iso-octane, and methanol, and ACS grade glacial acetic acid and hydrochloric acid were purchased from Fisher Scientific (Fairlawn, NJ). Iodine was purchased from Mallinckrodt (Paris, KY) and 95 % ethyl alcohol was provided by Pharmco-Aaaper (Brookfield, CT). 2-thiobarbituric acid, ammonium chloride, sodium thiosulfate, butylated hydroxyltoluene (BHT), gallic acid, rosmarinic acid were purchased from Sigma Chemical (St. Louis, MO).

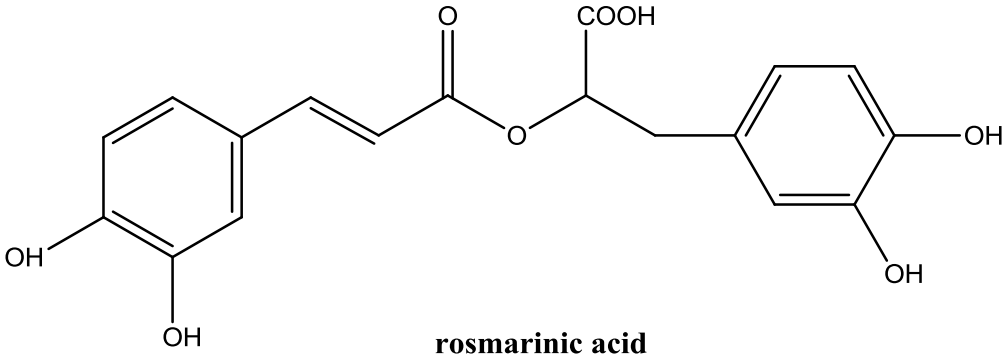
2.2.3. Addition of antioxidant into shea butter

BHT, rosmarinic acid, and gallic acid (0.02 % each) (**Figure 2-4**) were added to the shea butter. The concentration of antioxidant was selected based on the previous study of Kowalski (2007) as well as the maximum level to be used in food that is generally 0.02 % of the fat or oil content of food (Pokorny *et al.*, 2001). To achieve 0.02 %, 0.02g of antioxidant dissolved in 10 mL of ethanol was added to 100 g of shea butter sample and well stirred according to Kowalski (2007). Kowalski (2007) placed sunflower and olive oil samples with antioxidant dissolved in ethanol at room temperature for 24 hours for the evaporation of alcohol. However, considering that shea butter is characterized as solid in room temperature and evaporating alcohol in higher temperature for extended period can result in over-oxidation of shea butter, the procedure for the evaporation of alcohol

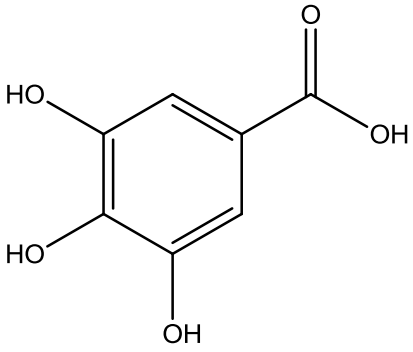
was limited. The sample was placed in an oven at 45-50 ℃ for 15 minutes to evaporate ethanol used as well as to homogenize the sample.



butylated hydroxytoluene (BHT)



rosmarinic acid



gallic acid

Figure 2-4. Chemical structures of the antioxidants used in the oxidative stability studies of shea butter (butylated hydroxytoluene (BHT), rosmarinic acid, and gallic acid).

2.2.4. Acceleration of oxidation of shea butter

The control (samples without antioxidants), and the samples with antioxidants were placed in an oven at 90 ℃ with a stream of air. The samples were then taken from the oven 0, 72, and 144 hours after they were placed in the oven and the samples were stored in a refrigerator at the temperature of 4 ℃ until used in experiments. The temperature and duration for accelerating the oxidation was based on a previous study conducted on sunflower and olive oils by Kowalski (2007).

Shea butter stored at 90 ℃ may not reflect the true lipid oxidation at the normal storage condition since that high temperature can lead to side effects such as polymerization or cyclization and also result in overestimated decomposition of the primary oxidation products (Frankel, 1993). However, 90 ℃ was used since the objective of this study was to assess the protective effect of selected antioxidants on shea butter against oxidation using a fast method and thus identify the effectiveness of antioxidant on the oxidative stability of shea butter. Initial experiments found that the antioxidants were effective in protecting shea butter from oxidation at 90 ℃, which means the same antioxidants are likely to be effective under the storage conditions of shea butter where the temperature is much lower.

2.2.5. Measurement of oxidative indicators

To assess the effect of antioxidants during the accelerated oxidation of shea butter, peroxide value, conjugated dienes, thiobarbituric acid reactive substances (TBARS), and change in the amount of major fatty acids were measured as indicators. Along with

peroxides, conjugated dienes are another indicator of oxidation as the primary lipid oxidation products. Conjugated double bonds are formed upon the abstraction of hydrogen from the polyunsaturated fatty acids in the initiation stage of lipid oxidation (Damodaran *et al*.. 2008). TBARS (e.g., aldehydes) as the secondary oxidation products are the substances that react with thiobarbituric acid (TBA) when heated under acidic condition, which produce pink chromophores (Seljeskog *et al*., 2006).

2.2.5.1. Peroxide value

Peroxide value was analyzed by following the same procedure stated in section 2.1.5.7.

2.2.5.2. Conjugated dienes (CD)

For the analysis of conjugated dienes (Abuzaytoun *et al*., 2006), 0.02-0.04 g of melted filtered shea butter sample (weighed to 0.001 g accuracy) was placed in a 25-mL volumetric flask and the flask was filled with iso-octane up to the mark. The flask was then sealed with a stopper and sonicated for 5 minutes for shea butter to be dissolved in iso-octane uniformly and completely. The absorbance was read at 234 nm in a 10 mm Hellma quartz cell using a Hewlett-Packard 8453 diode array spectrophotometer (Agilent, Wilmington, DE). Pure iso-octane was used as the blank and CD content was calculated by the following formula:

$$\text{Conjugated dienes} = A/(C \times d)$$

where, A is absorbance of the solution at 234 nm, C is concentration of the solution in g/100 mL solution, and d is the length of the cell in cm.

2.2.5.3. Thiobarbituric aicd-reactive substances (TBARS)

Thiobarbituric acid reactive substances were determined according to Abuzaytoun *et al.* (2006). 0.05-0.20 g of melted filtered shea butter sample (weighed to 0.001 g accuracy) was placed in a 25-mL volumetric flask and 1-butanol was transferred to the flask up to the mark. The mixture was placed at 45 ℃ in an oven for a while to avoid the solidification of shea butter, and then sonicated for 5 minutes. 5 mL of the mixture was transferred to a dry test tube and 5 mL of 2-TBA reagent (0.2 g of 2-TBA was dissolved in 100 mL of 1-butanol) was added to the same test tube. The mixture was well mixed and heated at 100 ℃ for 2 hours in a water bath and the intensity of the resultant colored mixture was measured at 532 nm with a Hewlett-Packard 8453 diode array spectrophotometer (Agilent, Wilmington, DE). Pure 1-butanol was used as the blank and the TBARS value was calculated by the following formula:

$$\text{TBARS value } \left(\frac{\mu\text{mol}}{\text{g}}\right) = \text{B} \times 0.415$$

where, B is the absorbance reading at 532 nm and 0.415 is a constant determined from a standard line prepared using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde acquired by Abuzaytoun *et al.* (2006).

2.2.5.4. GC analysis of the amount of major fatty acids

The content of fatty acids was determined by GC analysis as discussed in section 2.1.6.1. However, before the sample was refluxed with 5 mL of 0.5 N potassium hydroxide methanolic solution, 1 mL of heptadecanoic acid (1 % w/v in hexane) was added to the 5 g of the sample as an internal standard. After the GC analysis, the amount of the major

fatty acids, palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids were calculated by comparing the peak areas of the fatty acids with the peak area of known amount of heptadecanoic acid (internal standard).

2.2.6. Statistical analysis

Statistical analysis was conducted by using GraphPad Priam 5 (GraphPad Software Inc. La Jolla, CA). One-way ANOVA was performed on peroxide value, conjugated dienes, and thiobarbituric acid reactive substances (TBARS), and fatty acid contents to see whether those values were significantly increased or decreased over time within each sample, control and samples with antioxidants. For the samples where the significant increase in the oxidative indicators was observed at $P<0.05$, post-tests for multiple comparisons were conducted with the Tukey's multiple comparison test also at $P<0.05$.

In addition, two-way ANOVA was conducted on peroxide value, conjugated dienes, TBARS, and the amount of each major fatty acid of all samples with two variables, treatment and time. This analysis was conducted to see how protective the antioxidants were on shea butter against the oxidation and to see which antioxidant was the most effective one. When the significant difference was observed by ANOVA at $P<0.05$, the further multiple comparisons were performed on all pairs of sample with Bonferroni post test.

Results are presented as means ± SE.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Quality characteristics of West African shea butter

In this study, sensory, physical and chemical parameters of shea butter were assessed in all the shea butter samples. Especially, moisture content, insoluble impurities, free fatty acid, and peroxide value were measured to assess the quality of the shea butters as these parameters have been considered as the key parameters to determine the potential for degradation and thus to define the grades of shea (Regional Technical Committee Comments on Draft Africa Regional Standard for Unrefined Shea Butter, 2006). While these parameters will provide information on the degradation and potential rancidity of the shea butter, the active principle contents such as total unsaponifiables are characters that defines the bioactivities of shea butter and, therefore, the maximum amounts are usually preferred in the cosmetics, personal care product, or pharmaceutical industries, while for the food industry minimum amounts are usually preferred (Lovett, 2004).

3.1.1. Sensory characteristics

3.1.1.1. Color

Color of the shea butter samples ranged from whitish yellow to yellow, which is consistent with the typical shea butter color (Goreja, 2004; Moharram *et al*., 2006) (**Figure 3-1**). Since all the samples used in this study were 'unrefined' shea butter, yellow color was observed as expected possibly due to the retention of β-carotene, in that the butters did not undergo refining procedure which leads to the removal of carotenoids and thus the loss of characteristic color (Hamilton et al., 1986; Rossi *et al*., 2001).

Sensory analysis conducted by Akingbala *et al*. (2006) found that unrefined shea butter gained lower scores than refined butters regarding to color. However, refining procedure also cause the loss of minor but valuable components such as unsaponifiable fraction with medicinal properties (Tasan *et al*., 2005; Moharram *et al*., 2006; Van Hoed *et al*., 2006).

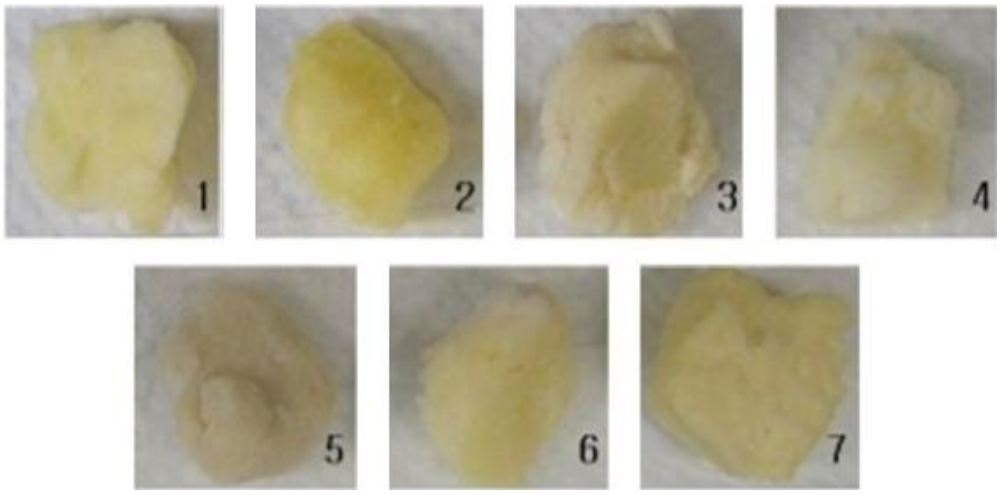


Figure 3-1. Color of seven West African shea butter samples. 1.TPC 01; 2.TPC 02; 3.GHA 01; 4. GHA 02; 5. WAF 01; 6. TPC 03; 7. BEN 01.

3.1.1.2. Aroma

All seven shea butter samples revealed characteristic oily and nutty aroma notes. However, the samples TPC 01, TPC 02, and GHA 03 also contained a hint of pleasant chocolate-like aroma. GHA 03 showed somewhat unpleasant smoky aroma. No rancid notes were observed.

3.1.1.3. Texture

Texture of shea butter was similar to those of commercially available body butter or Vaseline. Since the shea butter samples were all solid at room temperature, the first feeling to take the small portion from the bulk shea butter using finger tip was not as smooth as creams or lotion. However, once applied on the skin, all the samples were melted and showed nice creamy texture when spread over the back of the hand.

3.1.2. Physicochemical characteristics

3.1.2.1. Specific gravity

Specific gravity also referred to as relative density is an important physical character that can give information on the identity of the sample as well as detect adulteration of shea butter of which density may increase or decrease. It can also provide information for the shippers on the weight of the shea butter from the given volume while exporting it in large volumes (Hamilton *et al*, 1986).

In all seven West African shea butter samples, specific gravity was found to be consistent, showing values of 0.91 (**Table 3-1**). The specific gravity of 0.91 was in accordance

with the specific gravity of 0.87-0.9 obtained by Akingbala *et al*. (2006) and Olaniyan *et al*. (2007) but lower than the value (0.97) obtained by Njoku *et al*. (2000).

Table 3-1. Specific gravity of shea butter samples

Property \ Sample	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Specific gravity	0.91 ± 0.00	0.91 ± 0.00	0.91 ± 0.00	0.91 ± 0.00	0.91 ± 0.00	0.91 ± 0.00	0.91 ± 0.00

Values are expressed as average ± SE

3.1.2.2. Refractive Index

Refractive index is the ratio of the speed of light in a vacuum to that in the oil under examination which is related to the degree of saturation and the ratio of *cis/trans* double bonds, and can also provide hints on the oxidative damage (Hamilton *et al.*, 1986). Refractive index can be used for rapid sorting of fats and oils which are suspected to be adulterated (Olaniyan *et al*, 2007) as well as one of the important physical characteristics for identification of oils and fats. In this study, refractive index was measured on both unfiltered and filtered shea butter samples (**Table 3-2**).

Table 3-2. Refractive index of shea butter samples

Property \ Sample		TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Refractive index	Unfiltered	1.466 ± 0.00	1.464 ± 0.00	1.463 ± 0.00	1.465 ± 0.00	1.464 ± 0.00	1.464 ± 0.00	1.465 ± 0.00
	Filtered	1.466 ± 0.00	1.464 ± 0.00	1.463 ± 0.00	1.464 ± 0.00	1.464 ± 0.00	1.464 ± 0.00	1.464 ± 0.00

Values are expressed as average ± SE

Refractive index of the seven samples ranged from 1.463 to 1.466 in unfiltered samples and from 1.463 to 1.466 in filtered samples, which falls within the range of a typical refractive index of shea nut oil, 1.463-1.467 (Hamilton *et al*, 1986). There was almost no significant difference in the refractive indices between the unfiltered and the filtered samples (**Table 3-2**).

Refractive index was relatively consistent among almost all samples except for the sample TPC 01 which showed a significantly higher refractive index compared TPC 02, GHA 02, and BEN 01 ($P<0.01$) and compared to GHA 01, WAF 01, and TPC 03 ($P<0.001$) and GHA 01 showed significantly lower refractive index ($P<0.05$) compared to TPC 01, GHA 02, and BEN 01 in the both unfiltered and filtered samples. However, these differences were not noticeable since the refractive indexes were within the normal ranges of refractive index for shea butter.

3.1.2.3. Melting points

Since fats melt away over a range, there are various concepts of melting points depending on the amount of residual solid fat (Hamilton *et al.*, 1986). Since melting points of fats are related to triglyceride composition, it can be useful to identify the fats. In addition, the melting point of shea butter is closely related to the establishment of storage temperature.

In this study, three different melting points were measured: liquefying, dropping and clear points. The results indicated that higher temperatures were needed to reach the

clear point in relation to the liquefying and dropping point (clear point > dropping point > liquefying point) (**Table 3-3**). Liquefying point of seven shea butter samples ranged from 29 to 32 ℃ and dropping point from 31 to 34 ℃. While these two melting points were relatively consistent among different shea butter samples, clear point showed a higher variation (46 to 59 ℃) with significant difference among the samples (P<0.05) (**Table 3-4**).

Table 3-3. Liquefying, dropping, and clear points of shea butter samples

Property \ Sample	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Liquefying point (℃)	32 ± 0.17	29 ± 0.58	30 ± 1.26	30 ± 0.33	30 ± 1.04	31 ± 0.44	31 ± 0.33
Dropping point (℃)	34 ± 0.29	31 ± 0.76	31 ± 1.53	32 ± 0.50	31 ± 1.17	33 ± 0.58	32 ± 0.33
Clear point (℃)	54 ± 0.33	52 ± 0.33	55 ± 0.44	47 ± 0.67	52 ± 0.58	46 ± 1.67	59 ± 0.67

Values are expressed as average ± SE

Table 3-4. Tukey's multiple comparisons of the seven West African shea butter samples on clear point

	TPC 01	TPC 02	GHA01	GHA02	WAF01	TPC03
BEN01	**	***	*	***	***	***
TPC03	***	**	***	N/S	**	
WAF01	N/S	N/S	**	**		
GHA02	***	**	***			
GHA01	N/S	N/S				
TPC02	N/S					

N/S: not significant (P>0.05); *: significant (0.01<P<0.05); **: very significant (0.001<P<0.01); ***: extremely significant (P<0.001).

The dropping point obtained in this study was within the range of melting point on shea butter, 34.5 to 38 ℃, obtained by Olaniyan *et al*.(2007) on shea butter from Nigeria and Alander *et al*. (2002) that described shea butter's melting point ranged from 30 to 35 ℃. Hamilton *et al*. (1986) presented the slip melting point with values ranging from 37 to 42 ℃. The process for measuring slip melting point is quite similar to those for dropping point, and measured as the temperature when the stabilized melted fat becomes softened enough to rise or slip suddenly in a capillary tube with certain diameter while the tube is immersed in water with the increasing temperature at a rate of 1℃ /min (Hamilton *et al*., 1986). These melting points make shea butter solid or semi-solid at room temperature, but liquefied when applied on skin (37 ℃), thus making shea butter itself as a good body moisturizer with great spreadability.

All samples showed only 1-2 ℃ difference between liquefying and dropping points; however, the temperature difference between dropping and clear point of each sample was varying among the shea butter samples ranged from 13 to 27 ℃. This implies that the clear point is not a good bulk property of shea butter. Instead, this may provide information of how small difference in the amount of very high melting triglyceride can influence the clear point (Hamilton *et al.*, 1986).

In this study, we confirm that the dropping point was the best method to measure the melting point of shea butter due to 1) little variance between samples compared to the clear point which is more related to the final liquefaction of very high melting triglyceride rather than the bulk property and 2) easiness to read the temperature at the

precise time since the formation of drop is continuous process while the dropping of the drop occurs in a moment.

3.1.2.4. Moisture content

High moisture content in plant fats and oils usually leads to increase in microbial load as well as lipid oxidation resulting in rancidity. In this study, moisture content of all samples was planned to be measured by distillation method with toluene as this method can detect the right amount of water rather than the amount of all volatile components (i.e. the value obtained from the method using drying procedure). However, due to the requirement of having a larger sample size (400g), only samples WAF 01 and TPC 03 were available in sufficiently large amounts and as such only these were analyzed with distillation method. At the same time, all seven shea butter samples were analyzed to measure moisture content by modified Karl Fischer Reagent method which requires much less amount of sample and is appropriate for samples containing very low amounts of water.

Moisture contents of shea butter were quite low, ranging from 0.01 to 0.20 % in all the samples (**Table 3-5**). There were no significant differences in moisture content among the most pairs of samples when measured by modified Karl Fischer reagent method, even though significant difference was found between the moisture contents of GHA 01 (0.20 %) and TPC 03 (0.01 %) ($P<0.05$).

We also found that both distillation and modified Karl Fischer reagent methods can detect reproducible and reliable moisture content of shea butter. In addition, when comparing the moisture contents of WAF 01 and TPC 03 measured by distillation methods to those measured by modified Karl Fischer reagent method, no significant differences were found. This result also bolstered that both methods were equally reliable to measure the moisture content of shea butter. However, if the sample is available in large amounts, the distillation method would be more appropriate since it consists of much simpler procedures, requiring only one reagent (toluene), while the modified Karl Fischer reagent method requires more complex procedure which needs more care to prevent the absorbance of moisture into reagent or the sample from the environment, even though it is the standard method for samples containing low amounts of moisture.

Table 3-5. Moisture content of shea butter samples measured with distillation method and modified Karl Fischer reagent method

Sample / Property	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Distillation (%)	N/A	N/A	N/A	N/A	0.08 ± 0.00	0.03 ± 0.01	N/A
Modified Karl Fischer reagent (%)	0.06 ± 0.01	0.09 ± 0.04	0.20 ± 0.06	0.06 ± 0.02	0.12 ± 0.02	0.01 ± 0.06	0.06 ± 0.02

Values are expressed as average ± SE

3.1.2.5. Insoluble impurities

Insoluble impurities include dirt and other foreign materials (Hamilton *et al*., 1986). Some of these impurities can arise from machinery involved in the processing of shea butter as well as from physical contact with the soil, ground and packaging materials.

Along with moisture content, the amount of insoluble impurities is another important quality parameter which determines deterioration of shea butter since metals (particularly iron) can accelerate the oxidation of shea butter and thus decrease its market value.

In this study, insoluble impurities of shea butter ranged from 0.12 to 0.15 % (**Table 3-6**). Likewise moisture content, insoluble impurities are quite consistent among samples. No significant difference in insoluble impurities among the samples was observed at $P<0.05$.

Table 3-6. Insoluble impurities of shea butter samples

Property \ Sample	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Insoluble impurities (%)	0.14 ± 0.03	0.12 ± 0.03	0.15 ± 0.01	0.12 ± 0.02	0.12 ± 0.04	0.12 ± 0.01	0.12 ± 0.04

Values are expressed as average ± SE

3.1.2.6. Acid value and free fatty acids

The amount of free fatty acids were found to be variable among the samples, ranging from 1.07 to 8.56 % (**Table 3-7**), as the free fatty acids are heavily dependent on the processing practices. All pairs of shea butter samples, except between TPC 01 and TPC 03, showed significantly different level of free fatty acids at $P<0.01$ (**Table 3-8**). The free fatty acids found in this study was similar to the acidity values divided by 1.99 obtained by Mbaiguinam *et al*. (2007) on shea butter from Southern Chad, 2.1-6.2 %. The values were relatively higher than the free fatty acid commonly reported in literature, <0.05 % (Moharram *et al*., 2006) and also the value of 0.77 % from Njoku *et al*. (2000).

Several studies conducted free fatty acids analysis on commercial vegetable oil samples and the values were in a range of 0.03-0.45 % (Gan *et al.*, 2005) and 0.02-1.38 % (Osawa *et al.*, 2007), which were much lower than the crude shea butter samples used in this study.

Table 3-7. Free fatty acids of shea butter samples

Sample / Property	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Free fatty acids (%)	1.94 ± 0.00	3.92 ± 0.04	8.56 ± 0.00	1.46 ± 0.04	7.91 ± 0.07	2.11 ± 0.04	1.07 ± 0.12

Values are expressed as average ± SE

Table 3-8. Tukey's multiple comparisons of the seven West African shea butter samples on free fatty acids

	TPC 01	TPC 02	GHA01	GHA02	WAF01	TPC03
BEN01	***	***	***	**	***	***
TPC03	N/S	***	***	***	***	
WAF01	***	***	***	***		
GHA02	***	***	***			
GHA01	***	***				
TPC02	***					

N/S: not significant (P>0.05); *: significant (0.01<P<0.05); **: very significant (0.001<P<0.01); ***: extremely significant (P<0.001).

Free fatty acid levels of the shea butter samples used in this study were found to be higher. Free fatty acid levels of final shea butter can be reduced by neutralization or deodorization but the procedures also reduce several bioactive compounds naturally in

shea butter. Therefore, for unrefined shea butter, free fatty acids levels should be kept low by proper processing practices, considering that free fatty acids were observed to act as pro-oxidants due to the presence of unesterified carboxylic acid groups (Waraho *et al*, 2009).

3.1.2.7. Peroxide value

Peroxide values showed wide range from 2.15 to 15.32 mEq/kg (**Table 3-9**). The difference may be due to the condition where the shea fruits and nuts were collected, processed into the shea butter, and stored. While the range of peroxide values was wide, except the GHA 02, other samples showed no significantly different levels of peroxides. GHA 02 showed a significantly higher level of peroxides (15.32 mEq/kg) than that of TPC 01 (2.15 mEq/kg) and that of TPC 03(4.46 mEq/kg) at $P<0.05$.

Table 3-9. Peroxide values of shea butter samples

Property \ Sample	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Peroxide value (mEq/kg)	2.15 ± 0.43	6.01 ± 0.43	8.73 ± 0.56	15.32 ± 5.71	8.94 ± 0.64	4.46 ± 0.00	6.79 ± 0.00

Values are expressed as average ± SE

It was interesting to note that TPC01 showed the lowest peroxide value among the samples TPC01, TPC02, and TPC03 which were all from The Pure Company (Ghana) with the same production method and different production date of late 2008, early 2009, and mid 2009, respectively. Even though all the three samples showed relatively low amount of peroxide value which could be within the range of a grade 1 shea butter, the

extent of oxidation was relatively higher in TPC 02 (6.01 mEq/kg) compared to TPC 03 (4.46 mEq/kg) and TPC 01 (2.15 mEq/kg). This result suggests that slight differences in processing or storage practices can impact the oxidation of shea butter.

In addition, when the free fatty acids (**Table 3-7**) and peroxide values (**Table 3-9**) were compared, somewhat similar trends were found except the sample GHA 02, which means the hydrolytically degraded shea butter had more chance to get oxidative degradation. The reason for this is not clear but the oxidation of fatty acids might be linked with the hydrolytic breakdown of the fatty acids and the the glycerol molecule. Waraho *et al.* (2009) reported that the presence of unesterified carboxylic acid groups in free fatty acids endows the surface activity of free fatty acid and results in the increase of pro-oxidant-metal-lipid interaction. However, to confirm the correlation between hydrolytic and oxidative degradation, more samples are needed to be tested since, in this study, correlation coefficient (R) was 0.13, which means very weak correlation existed between free fatty acids and peroxide values.

No clear relations between moisture content or insoluble impurities and peroxide value were found since the levels of moisture and insoluble impurities were very similar among the samples.

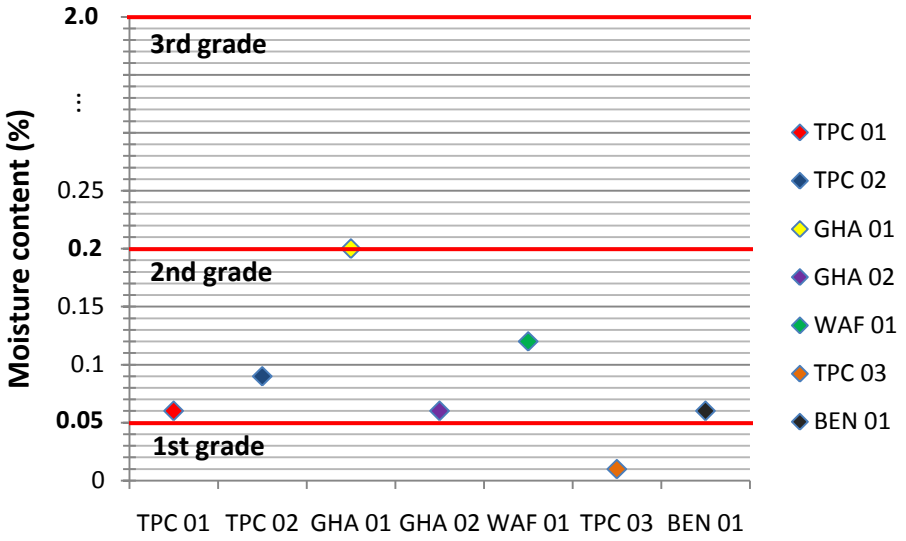
3.1.2.8. Grading of the seven West African shea butter samples

The shea butter samples used in this study were graded according to the West African standards developed by ProKarité and approved by UEMOA (Union Economique

Monétaire Ouest Africaine), where moisture content, free fatty acid, peroxide value, and insoluble impurities are mentioned as key parameters in grading of shea butter.

The grid lines represents the upper limit of the 1st, 2nd, and 3rd grade of shea butter, and how the quality characters fit in relation to the measured quality characters (**Figure 3-2**). Except the sample GHA 02, the levels of peroxide value of all samples were low enough to be the 1st grade while moisture, insoluble impurities, and free fatty acids were relatively high and thus most samples could not be the 1st grade. Sample GHA 01 showed the highest level of free fatty acids (>8 %) and Sample GHA02 showed the highest level of peroxide value (>15mEq/kg), which were the beyond the upper limit to be within a 3rd grade.

(a) Moisture content



(b) Insoluble impurities

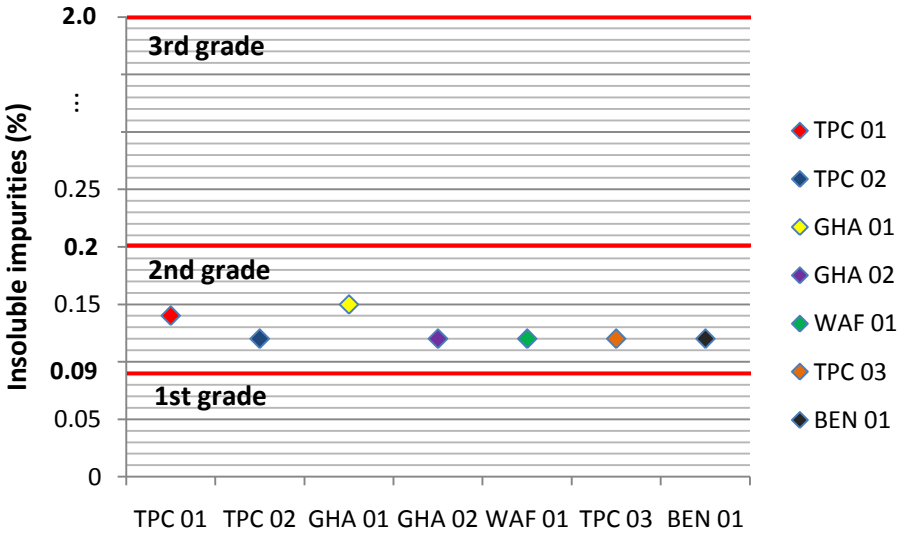
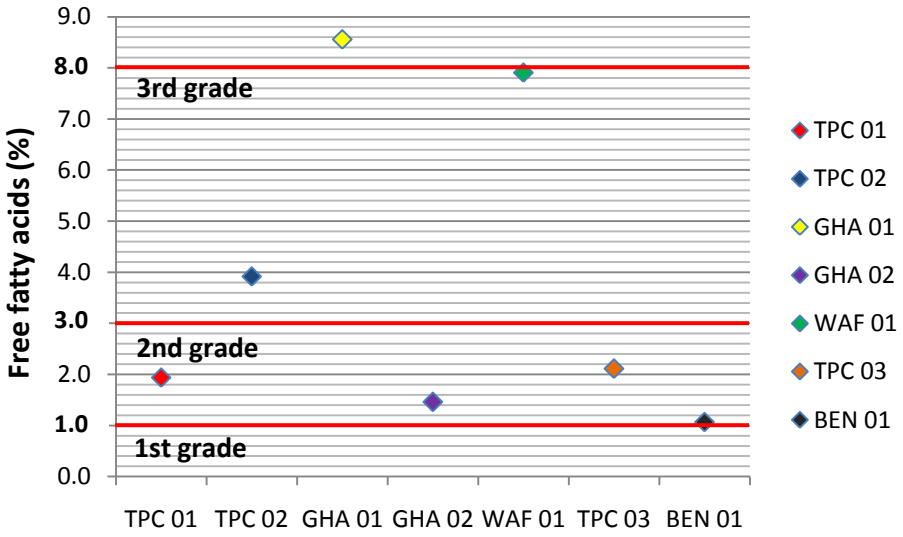


Figure 3-2. (a) Moisture, (b) insoluble impurities, (c) free fatty acids, and (d) peroxide value of the shea butter samples for grading. Red grid lines on the graphs represent the upper limit for the 1st, 2nd, and 3rd grade of shea butter as for each parameter. The best quality shea butter is the 1st grade.

(c) Free fatty acids



(d) Peroxide value

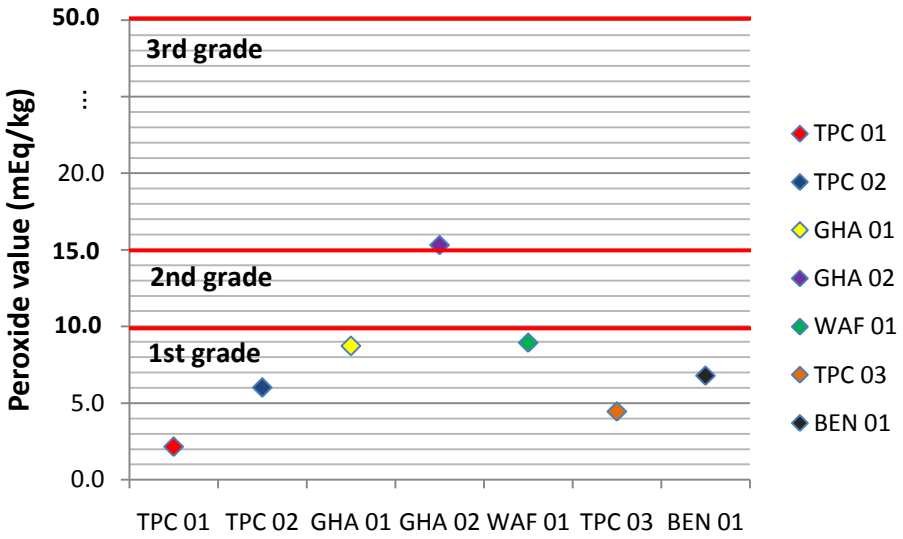


Figure 3-2. Continued.

Based on the value of each parameter of the samples, TPC 03 was the highest quality shea butter, belonging to grade 2. The next high quality samples were TPC01 (grade 2) and BEN01 (grade 2) followed by TPC 02 (grade 3) and WAF01 (grade 3), and GHA01 (grade 3) while the sample GHA02 showed the lowest quality (grade 3) (**Table 3-10**).

It is noticeable that all the samples revealed the 2nd grade level of insoluble impurities and thus, for ranking, other three parameters were used. TPC 03 was the only sample in which two parameters -moisture and peroxide values- were scored as 1st grade and free fatty acid was in 2nd grade level and thus evaluated as the best quality among the samples. This result showed that the recently produced shea butter sample (TPC 03) from The Pure Company was well processed as well as stored to keep the high quality, although more effort is needed to reduce insoluble impurities and free fatty acids.

The samples TPC 01, TPC 02, GHA 01, WAF 01 and BEN 01 all had 1st grade level peroxide value and 2nd grade level moisture content but the free fatty acid level was within the 2nd grade level in TPC 01 and BEN 01, 3rd grade level in TPC 02 and WAF 01, and beyond the 3rd grade level in GHA 01. The sample GHA 02 was found to have no parameters within the 1st grade level and instead, moisture content and free fatty acids were in 2nd grade level, and peroxide value, which is an important oxidative indicator, was in the 3rd grade level and thus the ranked as the worst quality sample.

The long period of storage (approx. 2 years) would have likely resulted in the increased peroxide value of GHA 02, even though the storage conditions were good to keep the

relatively low level of free fatty acids (no light and low temperature). The sample GHA 01 was scored as the second lowest quality that was possibly due to poor storing conditions. The sample GHA 01 was purchased in local market in New Brunswick, NJ in August, 2009 where the sample was placed in the shelf without air-conditioning in hot, humid summer and thus exposed to high temperature which might be perfect for lipase activity.

Table 3-10. Grading of the seven West African shea butter samples based on moisture content, insoluble impurities, free fatty acid, and peroxide value.

Grade \ Sample		TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
1st Grade	MC							
	II							
	FFA							
	PV							
2nd Grade	MC							
	II							
	FFA							
	PV							
3rd Grade	MC							
	II							
	FFA							
	PV							
3rd Grade ~	MC							
	II							
	FFA							
	PV							
Rank (Grade)		2 (2)	4 (3)	6 (3)	7 (3)	4 (3)	1 (2)	2 (2)

MC: moisture content; II: insoluble impurities; FFA: free fatty acids; PV: peroxide value

3.1.2.9. Total amount of unsaponifiable fraction

Total amount of unsaponifiable substances in the seven West African shea butter samples ranged from 2.21 to 4.18 % with no significant difference among the samples at $P<0.05$ (**Table 3-11**). Although several studies and commercial advertisements of shea butter have emphasized the unusual high amount of unsaponifiables as shea butter's uniqueness, the results from this study suggest all the samples contained lower levels. All the samples except TPC 02 and TPC 03 were found to contain lower amounts of unsaponifiables compared to previously reported values, 4-11 % (Itoh *et al*, 1974; Itoh *et al*, 1980; Hamilton *et al*, 1986; Lipp *et al*, 1998; Alander, 2004).

Table 3-11. Total unsaponifiables of shea butter samples

Property \ Sample	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Unsaponifiables (%)	3.99 ± 0.25	4.18 ± 0.43	2.69 ± 0.44	2.21 ± 0.30	2.77 ± 0.54	4.17 ± 0.67	2.56 ± 0.54

Values are expressed as average ± SE

However, compared to many other vegetable oils and fats, the range of unsaponifiables found in this study was still higher. Except few plant oils (e.g., olive and avocado), most plant derived oils revealed low amounts of unsaponifiables generally less than 2 % (**Table 3-12**).

Table 3-12. The amount of unsaponifiable matters in selected plant derived oils and fats.

Oil or fat	Unsaponifiable content, %	Family
Olive extracted	~2.5 [a)]	Oleaceae
Coconut	0-0.5 [a),b)]	Arecaceae
Cocoa butter	0.1-1.2 [a)]	Sterculiaceae
Linseed	0.1-1.7 [a)]	Linaceae
Groundnut	0.2-0.8 [a)]	Fabaceae
Palm kernel	0.2-0.8 [a)]	Arecaceae
Babassu kernel	0.2-0.9 [a)]	Arecaceae
Cotton seed	0.2-1.5 [a),b)]	Malvaceae
Rapeseed	0.2-2.0 [a)]	Brassicaceae
Palm	0.3-1.2 [a)]	Arecaceae
Safflower seed	0.3-1.3 [a)]	Asteraceae
Sunflower seed	0.3-1.3 [a)]	Asteraceae
Camellia seed	0.33-0.4 [c),d)]	Theaceae
Kapok seed	0.5-1.0 [a)]	Malvaceae
Walnut	0.5-1.0 [a)]	Juglandaceae
Soy bean	0.5-1.7 [a),b)]	Fabaceae
Corn (maize)	0.5-2.8 [a)]	Poaceae
Tea seed	0.6-0.82 % [c),d)]	Theaceae
Olive pressed	0.7-1.1 [a)]	Oleaceae
Mustard seed	0.7-1.5 [a)]	Brassicaceae
Illepe	0.7-2.0 [a)]	Dipterocarpaceae
Olive flesh	0.8-1.5 [b)]	Oleaceae
Pentadesma	0.8-1.8 [e)]	Clusiaceae
Sesame seed	0.9-2.0 [a)]	Pedaliaceae
Olive pit's kernel	1.5 [b)]	Oleaceae
Date palm seed	1.79 [f)]	Arecaceae
Wheat germ	2-5 [a)]	Poaceae

Table 3-12. Continued.

Oil or fat	Unsaponifiable content, %	Family
Mango kernel	2.01 [g)]	Anacardiaceae
Sal	2.02 [g)]	Dipterocarpaceae
Pokeweed seed	2.07 [d)]	Phytolaccaceae
Kokum	2.3 [a)]	Clusioideae
Mahua	2.41 [g)]	Sapotaceae
Spinach	2.9 [c)]	Amaranthaceae
Rice bran	3-7 [a)]	Poaceae
Alfalfa	3.3 [c)]	Fabaceae
Shea nut	1.2 [i)]; 4-11 [a),c),d),h),j),k)]	Sapotaceae
Avocado flesh	4.8-12.2 [b)]	Lauraceae
Olive pit's shell	4.9 [b)]	Oleaceae
Garden balsam	5.6 [c)]	Balsaminaceae
Avocado kernel	55.5 [b)]	Lauraceae

Sources: a) Hamilton *et al*, 1986; b) Gutfinger *et al*, 1974; c) Itoh *et al*, 1974; d) Itoh *et al*, 1980; e) Tchobo *et al*, 2007; f) Nehdi *et al*, 2010; g) Dhara *et al*, 2010; h) Lipp *et al*, 1998; i) Njoku *et al*, 2000; j) Alander, 2004; k) Moharram *et al*, 2006

With the relatively high amount unsaponifiables, future research is needed to focus on shea butter's bioactivities which most presume to be found in its unsaponifiable fraction, including antioxidant, anti-inflammatory, and insect-repellent activities based on the previous studies. Several studies showed the presence of tocopherols, which have antioxidant activities, in shea butter (Maranz *et al*, 2004) and provided examples of traditional use of shea butter for the treatment for rheumatism, and nostril infection (Tella, 1979; Goreja, 2004). Several studies can be performed *in vivo* and *in vitro*. For example, the effect of shea butter on cell regeneration, skin cancer and skin inflammation can be tested with topical application on skin on animal models which can bolster more

scientific evidence of the use of shea butter as ingredient for the cosmetics formula or ointments.

Unsaponifiable matters are known to be potential source of nutraceuticals as well as a good stabilizer in lipids (Shahidi *et al*., 2010). Therefore, the relatively large amount of unsaponifiables in shea butter compared to many other plant-derived oils suggest that shea is a promising source of natural ingredients for many applications in cosmetics, foods, and pharmaceutical industries.

3.1.3. Chemical composition of shea butter

3.1.3.1. Fatty acid composition

GC-FID analysis of fatty acid methyl esters (FAMEs) found triacylglycerides (TAGs) in shea butter were comprised of four major fatty acids, palmitic (3.36-4.44 %), stearic (39.74–44.62 %), oleic (40.71-44.48 %), and linoleic (5.73-6.41 %) acids, among which stearic and oleic acids were dominant (**Figure 3-3 and Figure 3-4**). The sum of these four major fatty acids ranged from 93.73 to 97.11 % of total fatty acids (**Table 3-13**). In addition, except the sample GHA 01, the relative amount of stearic acid was slightly higher than that of oleic acid and the percentage of stearic and that of oleic acid were quite similar among samples. The ratio of stearic to oleic acid ranged from 1.02 to 1.08 while the ratio of GHA 01 was as low as 0.89.

With exception of few pairs of samples, the relative amount of the most dominant fatty acids, stearic and oleic acids, were not significantly different. Relative amounts of

stearic acid in the sample GHA 01 was significantly lower ($P<0.05$) than that of the sample BEN 01 and relative amount of oleic acid of GHA 01 was significantly higher ($P<0.05$) than that of TPC 02. GHA 01 showed also significantly higher levels of relative amount of palmitic and linoleic acids than most of the samples (**Table 3-14** and **Table 3-15**). From this result, it is found that the fatty acid composition of the sample GHA 01 is distinct from other samples while the other samples shared almost same fatty acid composition.

Table 3-13. Fatty acid composition (% total fatty acids) of the shea butter samples

Sample / Fatty acid	TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Palmitic	3.69 ± 0.07	3.95 ± 0.26	4.44 ± 0.17	3.62 ± 0.10	3.63 ± 0.13	3.58 ± 0.10	3.36 ± 0.02
Stearic	42.67 ± 1.00	43.82 ± 1.00	39.74 ± 1.48	43.49 ± 0.68	43.62 ± 0.58	43.97 ± 0.60	44.62 ± 0.37
Oleic	41.53 ± 1.00	40.71 ± 0.86	44.48 ± 0.59	41.91 ± 0.57	42.61 ± 0.74	42.46 ± 0.43	43.40 ± 0.37
Linoleic	5.84 ± 0.15	5.77 ± 0.17	6.41 ± 0.09	5.86 ± 0.03	6.02 ± 0.04	6.06 ± 0.04	5.73 ± 0.05
Total percent	93.73	94.25	95.07	94.87	95.89	96.07	97.11
Stearic : oleic	1.03	1.08	0.89	1.04	1.02	1.04	1.03

Values are expressed as average ± SE (except, the sum of the four major fatty acids and the ratio between stearic and oleic acids)

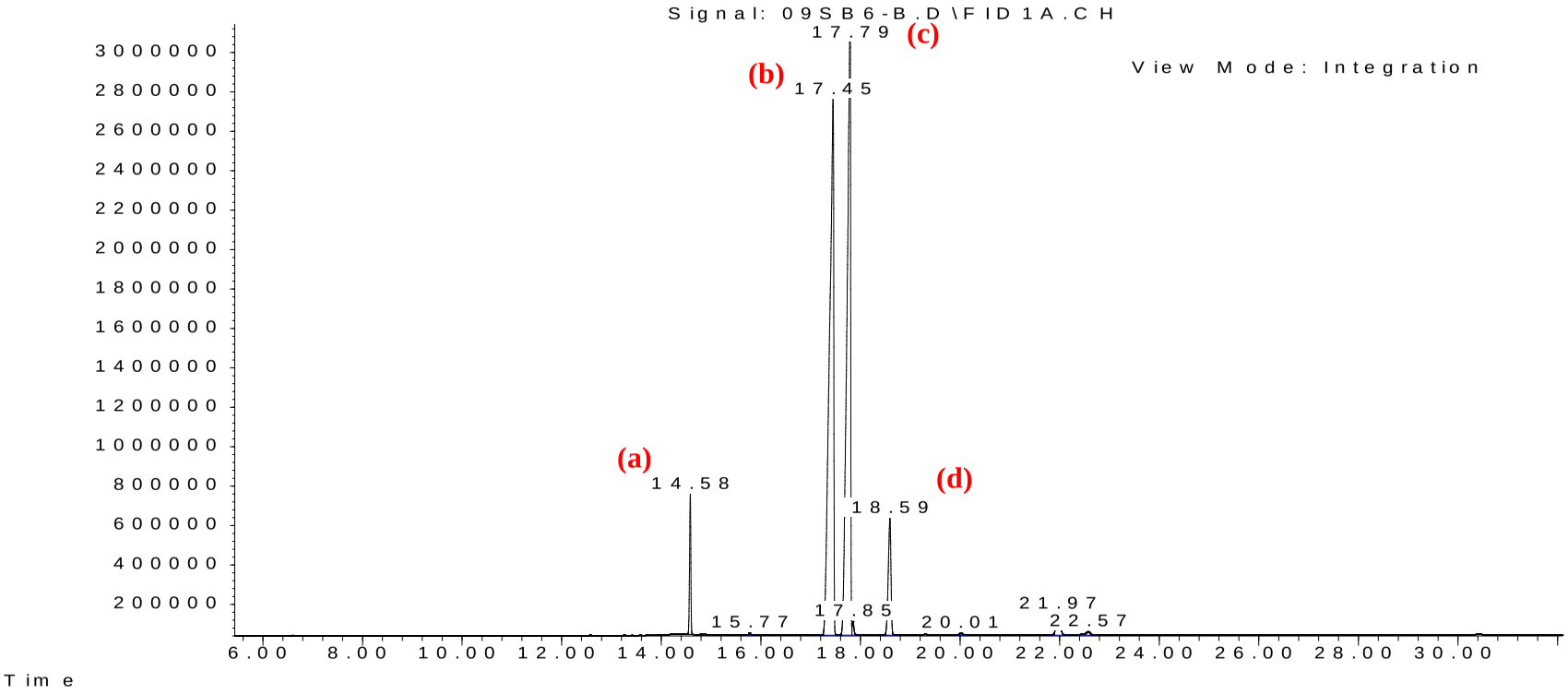


Figure 3-3. Chromatogram from GC-FID analysis of fatty acid composition. Peak (a), (b), (c), and (d) represent palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids, respectively. This chromatogram represents fatty acid composition of TPC 03 and all other six samples showed almost the same profile.

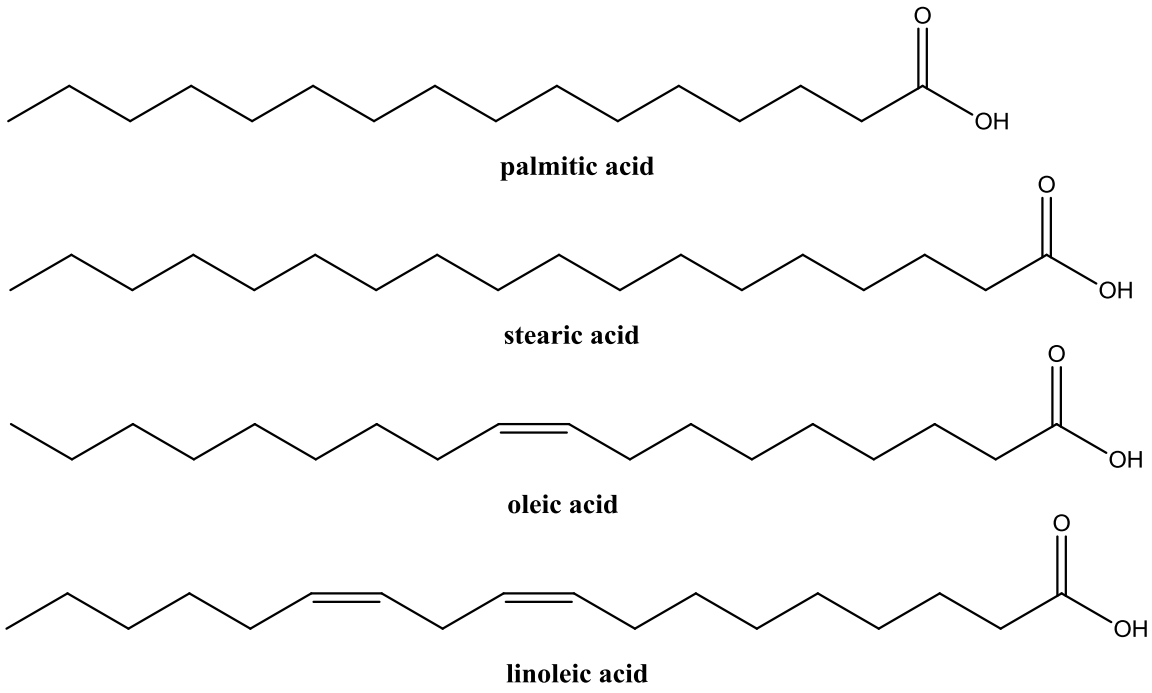


Figure 3-4. Chemical structures of major fatty acids in shea butter

Table 3-14. Tukey's multiple comparisons of the seven West African shea butter samples on relative amount of palmitic acid.

	TPC 01	TPC 02	GHA01	GHA02	WAF01	TPC03
BEN01	N/S	N/S	**	N/S	N/S	N/S
TPC03	N/S	N/S	*	N/S	N/S	
WAF01	N/S	N/S	*	N/S		
GHA02	N/S	N/S	*			
GHA01	*	N/S				
TPC02	N/S					

N/S: not significant (P>0.05); *: significant (0.01<P<0.05); **: very significant (0.001<P<0.01); ***: extremely significant (P<0.001).

Table 3-15. Tukey's multiple comparisons of the seven West African shea butter samples on relative amount of linoleic acid.

	TPC 01	TPC 02	GHA01	GHA02	WAF01	TPC03
BEN01	N/S	N/S	**	N/S	N/S	N/S
TPC03	N/S	N/S	N/S	N/S	N/S	
WAF01	N/S	N/S	N/S	N/S		
GHA02	N/S	N/S	*			
GHA01	*	**				
TPC02	N/S					

N/S: not significant (P>0.05); *: significant (0.01<P<0.05); **: very significant (0.001<P<0.01); ***: extremely significant (P<0.001).

East African shea butter is reported to have higher amounts of unsaturated fatty acids compared to West African variety. According to Di Vincenzo *et al* (2005) (**Table 3-16**), the average oleic acid in East African samples from Uganda was 57.8 % and that of stearic acid was 28.9 % while West African samples from Burkina Faso, Mali, and Nigeria contained the oleic acid and stearic acid on average at around 43 % and 44 %, respectively.

Our results conducted with these West African samples were in agreement with the results reported by Di Vincenzo *et al* (2005), showing similar levels of the main fatty acids.

Table 3-16. Fatty acid composition of shea butter from Burkina Faso, Mali, Nigeria, and Uganda

Origin / Fatty acid		Burkina Faso	Mali	Nigeria	Uganda
Fatty acid composition (%)	Palmitic	3.27	3.34	3.35	4.16
	Stearic	43.52	43.31	43.80	28.90
	Oleic	44.48	44.56	44.34	57.76
	Linoleic	5.90	5.97	5.80	6.31
	Sum	97.17	97.18	97.29	97.13

Source: Di Vincenzo *et al* (2005)

3.1.3.2. Sterol and triterpene composition in unsaponifiable fraction

GC-MS analysis of the unsaponifiable fraction of seven West African shea butter samples showed a similar profile in the unsaponifiables in all the samples, showing two major peaks (**Figure 3-5**).

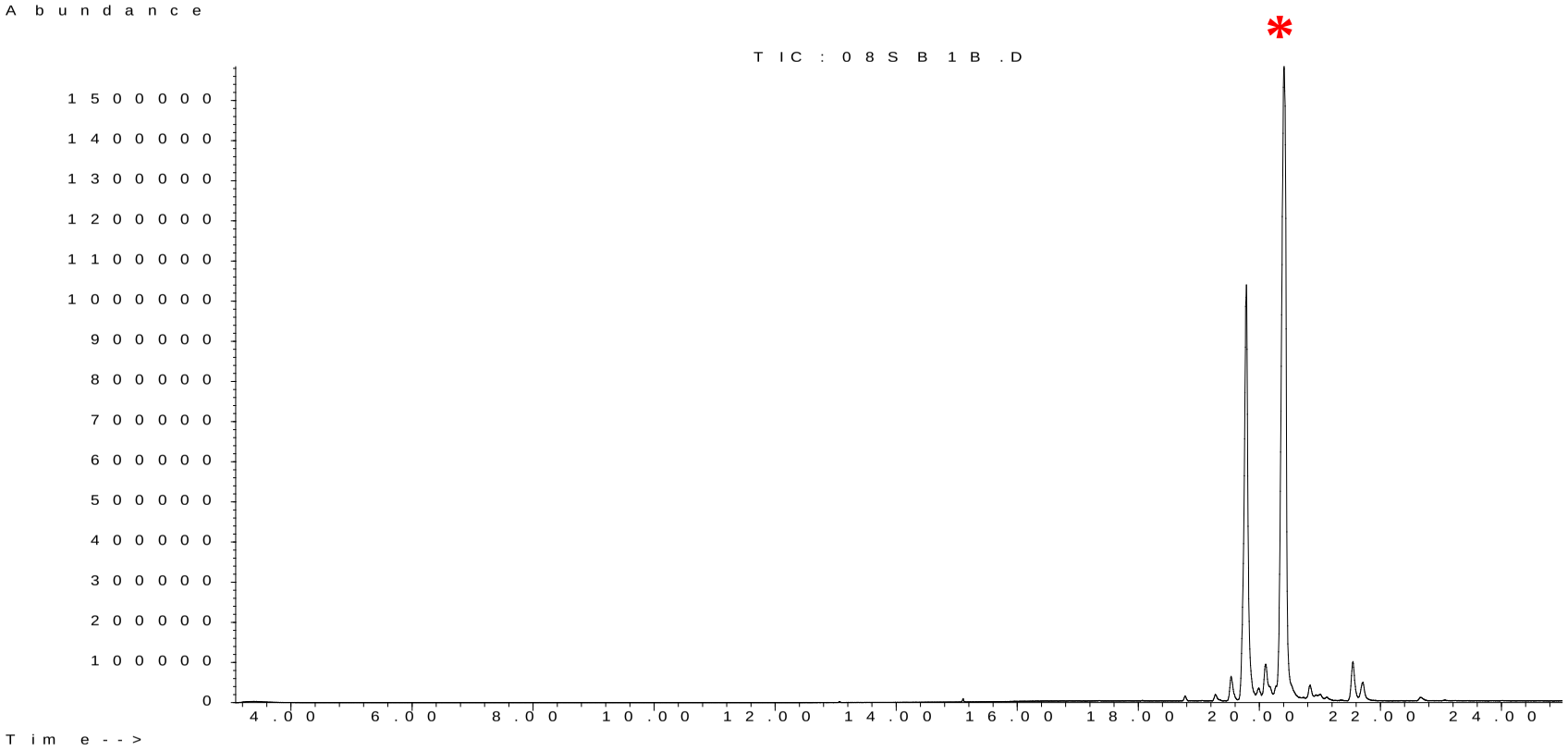


Figure 3-5. Chromatogram from GC-MS analysis of unsaponifiable fraction to identify major composition of sterols and triterpenes. This chromatogram represents unsaponifiable fraction of TPC 01 and all other six samples showed almost the same profile. The main peak (*) represents α-amyrin as the main unsaponifiable matter identified by GC/MS.

The retention time and mass spectra of each peak in all samples were compared and the shea butter samples were found to share 8 common constituents (**Table 3-17**). The relative amounts of sterols and triterpenes were found to be quite consistent among the samples. The mass spectra of the major peaks were then compared to the mass spectra in Wiley Library and only one peak was identified as α-amyrin, a pentacyclic triterpene, which dominated the unsaponifiable fraction in the range of 57.26 - 64.37 %. The mass spectra for α-amyrin which was acquired in this study is shown in **Figure 3-6**. There was significant difference in the relative amount of α-amyrin. Among the samples, especially the samples GHA 01 (64.37 %) and GHA 02 (63.53 %) contained significantly higher amounts of α-amyrin ($P<0.05$) compared to the samples TPC 01, WAF 01, TPC 03, and BEN 01 (57.26-58.53 %) (**Table 3-18**) and also TPC 02 (62.72 %) showed significantly higher amounts of α-amyrin than the sample TPC 01(57.26 %) ($P<0.05$).

The second dominant sterol was Peak-B ranging from 26.92 to 30.39 %. While the sample TPC 01 showed the lowest relative amount of α-amyrin, the sample showed significantly high amount of Peak-B (30.39 %) compared to the sample GHA 01 (26.92 %) and GHA 02 (27.50%) ($P<0.05$) which had the highest amounts of α-amyrin.

Significant difference in Peak-A and Peak-D which took relatively small portion of unsaponifiable fraction was also found in few pairs of samples at $P< 0.05$. The sample GHA 01 had significantly low amount of Peak-A (0.70 %) compared to TPC 01 (1.44 %), WAF 01 (1.44 %), and TPC 03 (1.54 %). The sample GHA 01 (1.95 %) and GHA 02

(1.89 %) showed significantly low amounts of Peak-D compared to the samples TPC 01(3.17 %), TPC 03(3.10 %), and BEN 01(3.14 %).

Table 3-17. Triterpenes and sterols in unsaponifiable fraction of shea butter determined by GC-MS analysis.

Component	R.T.[a)]	Percentage (% total sterols and triterpenes)						
		TPC 01	TPC 02	GHA 01	GHA 02	WAF 01	TPC 03	BEN 01
Peak[b)]-A	19.53	1.44 ± 0.09	0.92 ± 0.13	0.70 ± 0.10	0.87 ± 0.22	1.44 ± 0.12	1.54 ± 0.11	1.37 ± 0.08
Peak-B	19.78	30.39 ± 0.25	28.15 ± 0.45	26.92 ± 0.00	27.50 ± 0.80	29.47 ± 0.04	29.21 ± 0.25	29.30 ± 0.80
Peak-C	19.99	0.83 ± 0.04	0.69 ± 0.16	0.61 ± 0.06	0.57 ± 0.09	0.83 ± 0.05	0.96 ± 0.02	0.98 ± 0.04
Peak-D	20.10	3.17 ± 0.09	2.08 ± 0.02	1.95 ± 0.14	1.89 ± 0.13	2.82 ± 0.39	3.10 ± 0.33	3.14 ± 0.02
α-amyrin	20.41	57.26 ± 0.39	62.72 ± 0.11	64.37 ± 0.56	63.53 ± 1.70	58.27 ± 0.91	58.02 ± 0.73	58.53 ± 0.89
Peak-E	20.84	0.99 ± 0.11	0.65 ± 0.10	0.68 ± 0.03	0.72 ± 0.06	1.11 ± 0.18	0.93 ± 0.06	0.93 ± 0.13
Peak-F	21.55	2.53 ± 0.03	2.53 ± 0.02	2.61 ± 0.15	2.56 ± 0.10	2.75 ± 0.04	2.70 ± 0.09	2.64 ± 0.00
Peak-G	21.71	1.46 ± 0.04	1.42 ± 0.08	1.57 ± 0.20	1.45 ± 0.05	1.44 ± 0.01	1.47 ± 0.03	1.44 ± 0.04
Sum		98.07	99.16	99.41	99.09	98.13	97.93	98.33

a) retention time in min
b) Peak-A, B, C, D, E, F, and G were not identified.

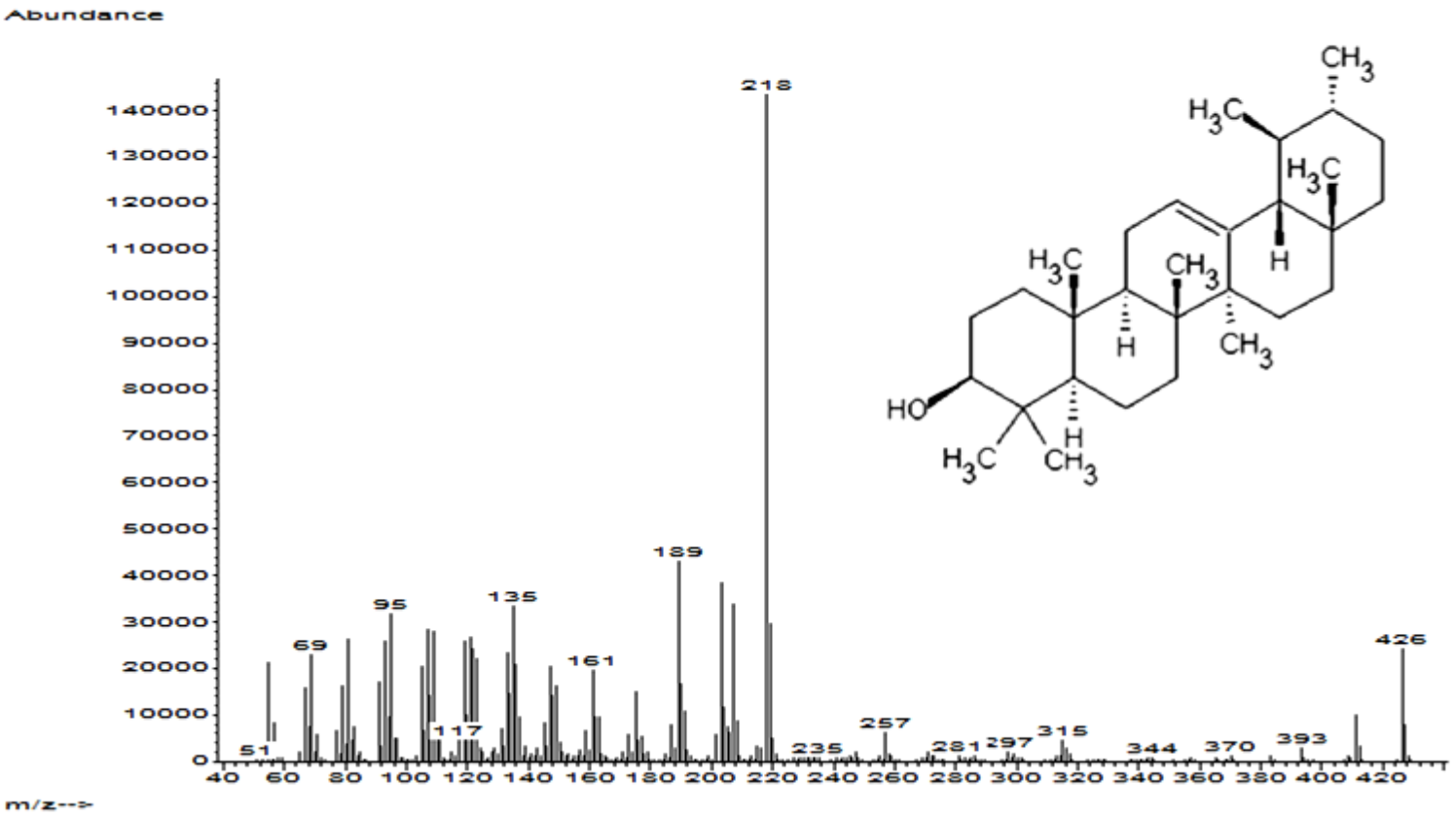


Figure 3-6. Mass spectra and chemical structure of α-amyrin, a major triterpene alcohol in shea butter

Table 3-18. Tukey's multiple comparisons of the seven West African shea butter samples on relative amount of α-amyrin.

	TPC 01	TPC 02	GHA01	GHA02	WAF01	TPC03
BEN01	N/S	N/S	*	*	N/S	N/S
TPC03	N/S	N/S	*	*	N/S	
WAF01	N/S	N/S	*	*		
GHA02	*	N/S	N/S			
GHA01	**	N/S				
TPC02	*					

N/S: not significant (P>0.05); *: significant (0.01<P<0.05); **: very significant (0.001<P<0.01); ***: extremely significant (P<0.001).

Among the triterpenes, α-amyrin was found to be the most dominant triterpene in shea butter's unsaponifiable fraction in this study. Bioactivities of α-amyrin have been studied especially with α-amyrin extracted from *Protium kleinii*, a plant used in Brazillian folk medicine belonging to Burseraceae family. When administered, α-amyrin was reported to show dose-related antinociceptive effect against the visceral pain when mixed with β-amyrin *in vivo* test on mice (Otuki *et al*, 2005a). The topical application of α-amyrin showed anti-inflammatory effects, inhibiting skin inflammatory responses such as edema formation, migration of polymorphonuclear leukocyte, and increase in tissue IL-1β levels (Otuki *et al*, 2005b). Another study on the anti-inflammatory effect of α-amyrin and β-amyrin of *Protium heptaphyllum* and the result showed they retarded acute inflammation in rat model of periodontitis (Holanda Pinto *et al*, 2008).

Beyond the current use of shea butter as an ingredient for pharmaceuticals and nutraceuticals, the findings implies the possible antinociceptive, analgesic, and inflammatory effect of α-amyrin in the shea butter's unsaponifiable fraction and potential use in pharmacological and personal care industries for example as an active ingredient for pain releaser, and toothpaste.

3.2. Effect of antioxidants on shea butter's oxidative stability

Shea butter has been reported to contain natural antioxidants such as tocopherols (0.003-0.080 % of shea butter) (Maranz *et al*., 2004) and, along with previous studies (4-11 %) (Itoh *et al*, 1974; Itoh *et al*, 1980; Hamilton *el al*, 1986; Lipp *et al*, 1998; Alander, 2004), this study also found relatively high amounts of unsaponifiable constituents (2.21-4.18 %). These minor components are known to provide better stability to oils and fats against oxidation (Shahidi *et al*., 2010). However, as a plant fat containing large amounts of unsaturated fatty acids, shea butter can undergo oxidative degradation when processed or stored improperly (Moharram *et al*., 2006), resulting in inconsistent quality as well as limited shelf-life. Therefore, in this study, butylated hydroxytoluene (BHT), rosmarinic acid, and gallic acid were used to identify their effects on shea butter against oxidation triggered by high temperature and air.

For identifying the effectiveness of the selected antioxidants on shea butter's stability against oxidation, the primary oxidation products (peroxide value and conjugated dienes), and the secondary oxidation products (thiobarbituric acid reactive substances, TBARS) were measured as oxidative parameters. In addition to these oxidation products, fatty

acid methyl esters (FAMEs) were measured as an indirect way to identify the effect of antioxidants in inhibiting oxidative degradation within the fat (Kowalski, 2007). The results are summarized in **Table 3-19**.

Table 3-19. Oxidative parameters of shea butter without antioxidant and with BHT, rosmarinic acid, and gallic acid

Parameter	Storage period (h)	Control	BHT	Rosmarinic acid	Gallic acid
Peroxide value (mEq/kg)	0	10.89 ± 0.61	10.22 ± 0.62	10.55 ± 0.62	8.76 ± 0.19
	72	25.22 ± 0.71	17.34 ± 0.20	14.10 ± 0.23	5.04 ± 0.34
	144	354.30 ± 3.19	18.46 ± 0.48	15.99 ± 0.84	4.37 ± 0.20
Conjugated dienes	0	2.72 ± 0.14	2.61 ± 0.43	2.72 ± 0.21	3.16 ± 0.03
	72	4.52 ± 0.04	3.01 ± 0.46	3.06 ± 0.23	2.95 ± 0.02
	144	13.08 ± 0.02	3.50 ± 0.56	3.12 ± 0.50	2.98 ± 0.09
TBARS (μmol/g)	0	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.01	0.05 ± 0.00
	72	0.05 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.00
	144	0.13 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	0.04 ± 0.00
Palmitic acid (g/100g sample)	0	2.84 ± 0.04	2.56 ± 0.11	2.85 ± 0.08	2.91 ± 0.08
	72	2.71 ± 0.21	3.05 ± 0.11	2.97	2.55 ± 0.08
	144	2.72 ± 0.05	2.83 ± 0.07	2.77 ± 0.14	2.42 ± 0.01
Stearic acid (g/100g sample)	0	34.76 ± 0.59	30.80 ± 1.07	33.87 ± 0.75	35.33 ± 1.55
	72	33.04 ± 2.37	37.20 ± 1.54	35.48	31.58 ± 1.01
	144	33.34 ± 0.59	34.26 ± 0.96	33.03 ± 1.97	30.45 ± 0.20
Oleic acid (g/100g sample)	0	34.33 ± 0.47	30.99 ± 1.47	34.29 ± 0.88	35.12 ± 1.55
	72	32.34 ± 2.39	37.44 ± 1.34	35.53	31.27 ± 1.00
	144	26.98 ± 0.45	34.35 ± 0.93	33.50 ± 2.01	29.92 ± 0.14
Linoleic acid (g/100g sample)	0	4.79 ± 0.05	4.47 ± 0.22	4.96 ± 0.13	4.97 ± 0.22
	72	4.09 ± 0.29	5.29 ± 0.16	4.96	4.40 ± 0.15
	144	0.78 ± 0.02	4.83 ± 0.15	4.75 ± 0.26	4.13 ± 0.02

Values are expressed as average ± SE

3.2.1. Effect of antioxidant on the primary oxidation products

3.2.1.1. Peroxide value

In the accelerated oxidative studies, the peroxide values were shown to be increased over time in all samples including control and samples with antioxidants, BHT, rosmarinic acid, and gallic acid, respectively. Especially, the peroxide value was increased to a greater extent in control between 72 and 144 hours compared to other samples with antioxidants (**Figure 3-7**). In the control, peroxide value significantly increased between 0 and 72 hours ($P<0.01$) from 10.89 to 25.22 mEq/kg and between 72 and 144 hours from 25.22 to 354.30 mEq/kg ($P<0.001$). Peroxide value was also significantly increased between 0 and 72 hours in the samples with BHT (from 10.22 to 17.34 mEq/kg) ($P< 0.001$) and rosmarinic acid (from 10.55 to 14.10 mEq/kg) ($P< 0.05$) each, while no significant increase was found between 72 and 144 hours in the same samples. It was interesting to see that the peroxide value was decreased significantly between 0 and 72 hours from 8.76 to 5.04 mEq/kg ($P<0.001$) in the sample with gallic acid while peroxide value was not significantly changed between 72 and 144 hours. From the result it was found that BHT, rosmarinic acid, and gallic acids remarkably inhibited the oxidation in the shea butter samples during the oxidation-accelerating process (**Table 3-19** and **Figure 3-7**).

At 0 hour, there was no significant difference in the amount of peroxides in all samples including control, while the peroxide values of control at 72 hour (25.22 mEq/kg) and 144 hour (354.30 mEq/kg) were significantly high, compared to the samples with BHT (17.34 mEq/kg at 72 hour and 18.46 kg at 144 hour), rosmarinic acid (14.10 mEq/kg at

72 hour and 15.99 mEq/kg at 144 hour), and gallic acid (5.04 mEq/kg at 72 hour and 4.37 mEq/kg at 144 hour) ($P<0.001$). There was no significant difference in the peroxide values between the sample with BHT and the sample with rosmarinic acid all the time, while the sample with gallic acid showed significantly low peroxide values, compared to the sample with BHT and the sample with rosmarinic acid at 72 hour and 144 hour ($P<0.0001$) (**Table 3-19** and **Figure 3-7**). From these results, all the antioxidants used were found to be effective in protecting shea butter from oxidation but when compared the effectiveness of the antioxidants, it is noticeable that the effectiveness of natural antioxidant was the same (rosmarinic acid) or better (gallic acid), compared to synthetic one (BHT). These results are promising considering that the consumer prefers more natural and organic products which contain no synthetic matters.

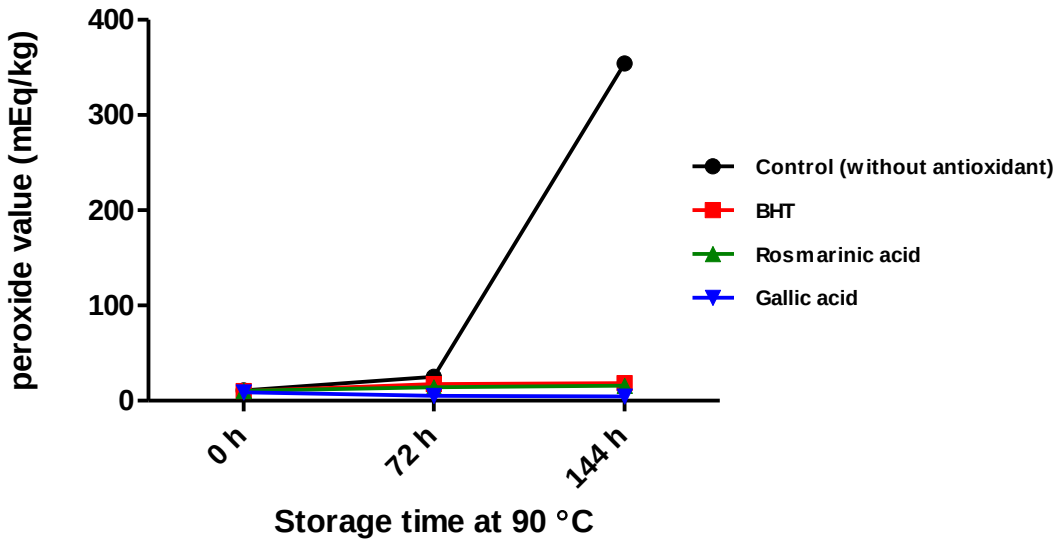


Figure 3-7. Change in peroxide value over storage time at 90 ℃ in control and shea butter samples with 0.02 % of BHT, rosmarinic acid, and gallic acid

3.2.1.2. Conjugated dienes

In this study, conjugated dienes were almost at similar level over time in the shea butter samples with antioxidants while there was huge increase in conjugated dienes in control especially between 72 and 144 hours (**Figure 3-8**). Conjugated dienes were significantly increased in control between 0 and 72 hours from 2.72 to 4.52 and between 72 and 144 hours from 4.52 to 13.08 ($P<0.001$). There was no significant increase in conjugated dienes in the samples with BHT and rosmarinic acid, respectively over all storage period. In the sample with gallic acid, significant decrease in the conjugated dienes were found between 0 and 72 hours from 3.16 to 2.95 ($P<0.01$) and no change was found between 72 and 144 hours (**Table 3-19** and **Figure 3-8**).

As observed in the peroxide value measurements, it was also found that all the antioxidants used in this study were significantly effective in protecting shea butter from oxidation. There were no significant differences in the amount of conjugated dienes at 0 hour among all the samples including the control. However, at 72 hour, conjugated dienes were significantly in low level in the samples with BHT (3.01), rosmarinic acid (3.06), and gallic acid (2.95) compared to the control (4.52) ($P<0.05$) and at 144 hour, the difference was even more distinct with significantly high amounts of conjugated dienes in control (13.08) compared to the samples with BHT (3.50), rosmarinic acid (3.12), and gallic acid (2.98) ($P<0.0001$) (**Table 3-19** and **Figure 3-8**). From this result protective effect of antioxidants was found to be more remarkable at 144 hour. The type of antioxidants had no significant effect on the level of conjugated dienes and thus all the antioxidants being equally effective.

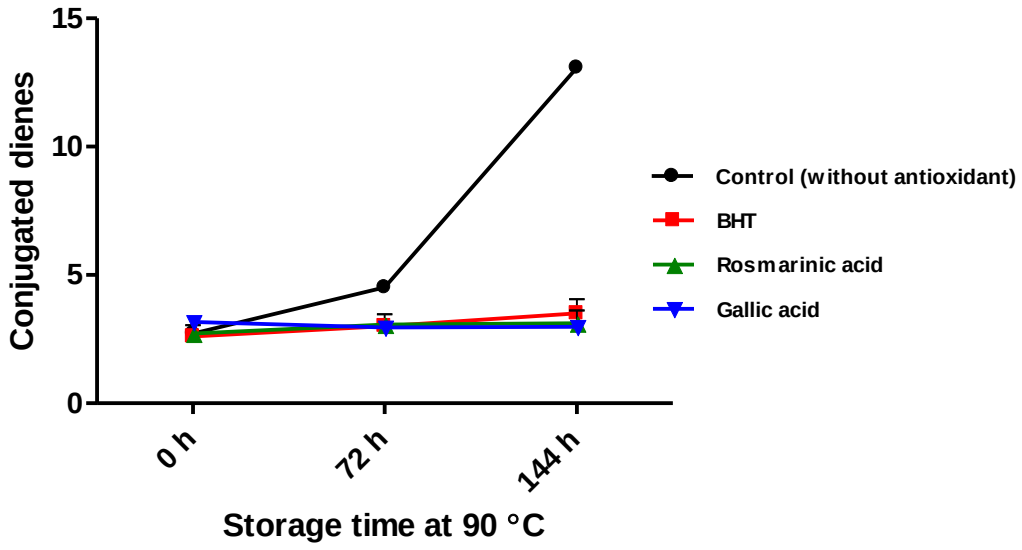


Figure 3-8. Change in conjugated dienes over storage time at 90 ℃ in control and shea butter samples with 0.02 % of BHT, rosmarinic acid, and gallic acid

3.2.2. Effect of antioxidant on the secondary oxidation products

3.2.2.1. Thiobarbituric acid reactive substances (TBARS)

In this study, TBARS values were quite low in both shea butter samples without and with antioxidants (**Figure 3-9**). No significant differences in the amount of TBARS were found in the samples with antioxidants for all the treatments including antioxidants. In the control, the TBARS values were significantly increased only after 72 hour and thus the values were increased from 0.05 μmol/g at 72 hour to 0.13 μmol/g at 144 hour ($P<0.001$) but the TBARS value at 144 hour was as not as high as peroxide value or conjugated dienes (**Table 3-19** and **Figure 3-9**). Results show that the rate of production of primary oxidation products was much higher than that of the decomposition of the primary oxidation products and the antioxidants were still effective in protecting shea butter against oxidative rancidity. The control at 144 hour showed bleached color possibly due to the degradation of β-carotene with rancid odor while the other shea butter samples with antioxidant kept almost the same color and aroma as the original ones.

TBARS values in the control were similar to those in the treatment containing the antioxidants almost as same as the samples with antioxidants at 0 hour and 72 hour, while at 144 hour, TBARS was significantly high in the control (0.13 μmol/g) compared to the samples with BHT (0.04 μmol/g), rosmarinic acid (0.05 μmol/g), and gallic acid (0.04 μmol/g) ($P<0.0001$) (**Table 3-19** and **Figure 3-9**), which showed BHT, rosmarinic acid, and gallic acid was all effective in protecting shea butter. However, the protective effect of each antioxidant was not significantly different.

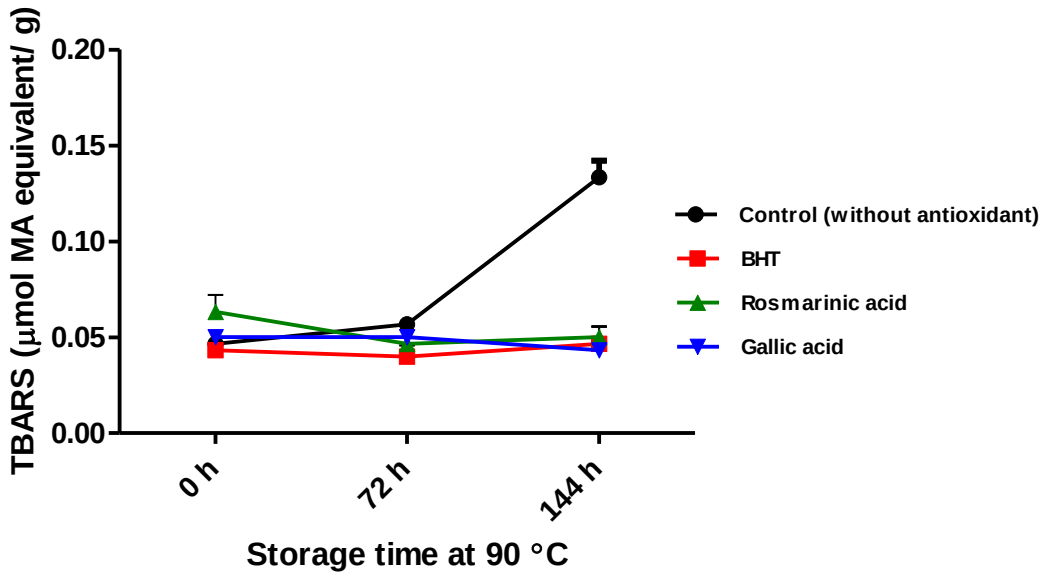


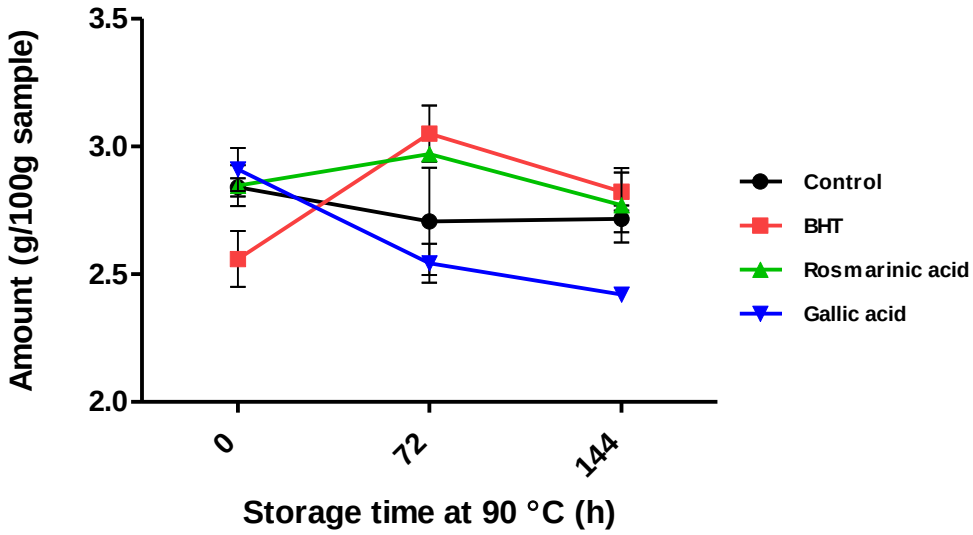
Figure 3-9. Change in TBARS (thiobarbituric acid reactive substances) over storage time at 90 ℃ in control and shea butter samples with 0.02 % of BHT, rosmarinic acid, and gallic acid

3.2.3. Effect of antioxidant on the amount of major fatty acids

3.2.3.1. Palmitic and stearic acids

There was no significant decrease in palmitic and stearic acids in all the samples including control except the sample with gallic acid in which 16.8 % of palmitic acids ($P<0.01$) and 13.8 % of stearic acids ($P<0.05$) were decreased from the initial value. Palmitic and stearic acids were slightly increased between 0 and 72 hours and decreased between 72 and 144 hours in the samples with BHT and rosmarinic acid respectively (**Figure 3-10**). The results showed that palmitic and stearic acids as saturated fatty acids were quite stable during the oxidation process even without the presence of antioxidants.

(a) Palmitic acid



(b) Stearic acid

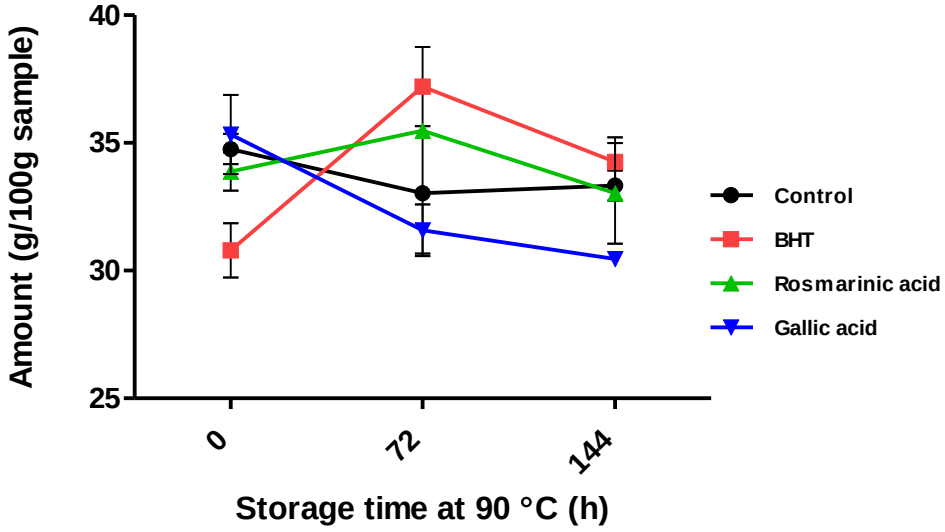


Figure 3-10. Change in the amount of (a) palmitic acid and (b) stearic acid in shea butter with and without antioxidant at 90 ℃ over the storage period

3.2.3.2. Oleic and linoleic acids

The addition of antioxidants was significantly effective on the unsaturated fatty acids, oleic and linoleic acids, in shea butter samples from accelerated oxidation (**Figure 3-11**). In the control, the amount of oleic acid was significantly decreased with the increase in storage period from 0 to 144 hours ($P<0.05$). Between 0 and 72 hours, and between 72 and 144 hours, there was no significant decrease in oleic acids, but compared to the initial value (34.33 g/100 g sample), the amount of oleic acid was significantly decreased by 21.4 % and thus the final amount of oleic acid was 26.98 g/100g sample ($P<0.05$). On the other hand, in the samples with BHT and rosmarinic acid, the amounts of oleic acid were maintained at the similar levels with the initial value during all period, which indicated that the addition of these antioxidants were significantly inhibited the oxidative degradation of oleic acids in shea butter. However, in the sample with gallic acid, the amount of oleic acid was significantly decreased with the increased storage period, although the extent to which oleic acid decreased was less than the control ($P<0.05$). Compared to the initial level (35.12 g/100g sample), 14.8 % of oleic acid was decreased and thus the final amount at 144 h was 29.92 g/100 g sample in the shea butter sample with gallic acid (**Table 3-19** and **Figure 3-11 (a)**). From these results, it was found that the addition of three antioxidants each significantly inhibited the oxidation of oleic acid. However, the results suggest that BHT and rosmarinic acid were more effective in protecting oleic acid than gallic acids, even though the differences were not significant.

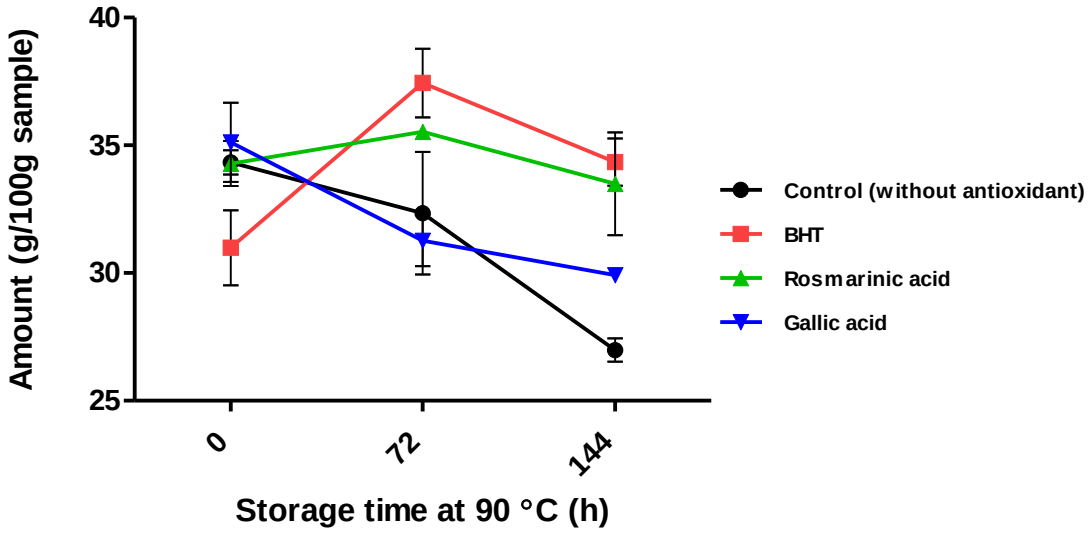
As linoleic acid contains two double bonds (18:2) the accelerated oxidation in the control was remarkably increased at 144 hour. In control, there was significant decrease in the

amount of linoleic acid especially between 72 (4.09 g/100g sample) and 144 hours (0.78 g/100g sample) ($P<0.001$). When compared to the initial amount of linoleic acid (4.79 g/100g sample), the result showed the 83.7 % of linoleic acid was degraded by oxidation in the control. Meanwhile, there was no significant decrease in linoleic acid in the samples with BHT and rosmarinic acid, which suggests the addition of BHT and rosmarinic acid were significantly effective in protecting linoleic acid against oxidation. However, the addition of gallic acid was less effective than BHT and rosmarinic acid and thus linoleic acid was significantly decreased by 16.9 % from 0 hour (4.97 g/100g sample) to 144 hour (4.13 g/100g sample) ($P<0.05$) (**Table 3-19** and **Figure 3-11 (b)**). These data shows the addition of BHT, rosmarinic acid, and gallic acid significantly inhibited the oxidative degradation of linoleic acid especially when the amounts of linoleic acid at 144 hour were compared ($P<0.0001$). The effectiveness of the three antioxidants was not significantly different but BHT and rosmarinic acid were slightly more effective than gallic acid.

Compared to the saturated fatty acids, palmitic (16:0) and stearic (18:0) acids, decrease in the unsaturated fatty acids, oleic (18:1) and linoleic (18:2) was more significant during oxidation. This result is consistent with the fact that degree of unsaturation is proportionally related to the oxidation rate (Shahidi *et al*., 2010). It was shown that the ratio of oxidation rate of stearic, oleic, and linoleic acids is 1:100:1200 (deMan, 1999) and the similar relation was also found in this study. For example, in control, the degrees by which stearic, oleic, and linoleic acids decreased during 144 hour-oxidation process were 4.08 %, 21.4 %, and 83.7 % respectively. In addition, this study found

BHT and rosmarinic acid were slightly more effective in protecting the fatty acid composition.

(a) Oleic acid



(b) Linoleic acid

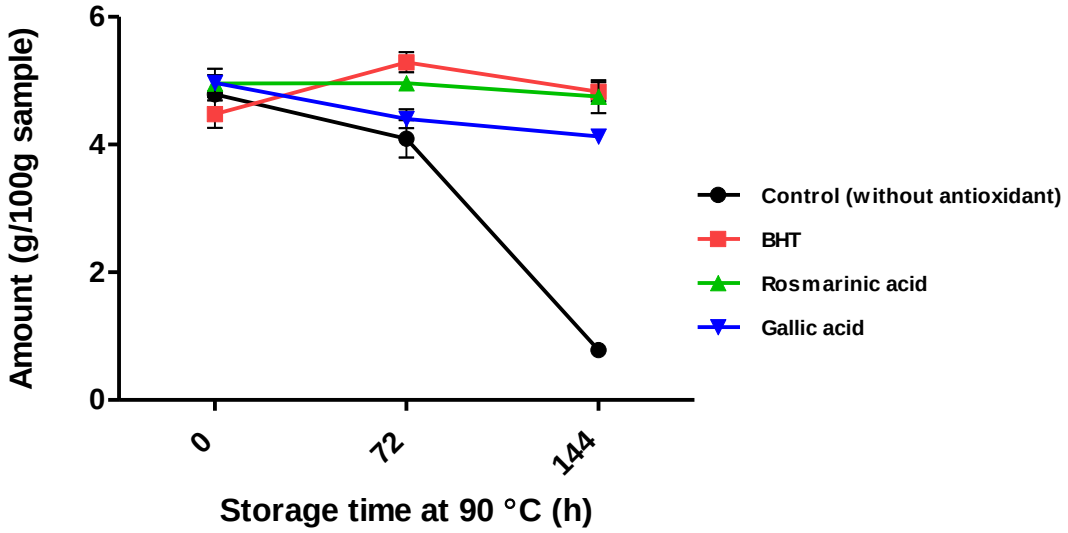


Figure 3-11. Change in the amount of (a) oleic acid and (b) linoleic acid in shea butter with and without antioxidant at 90 ℃ over the storage period

CHAPTER 4. CONCLUSION

This study showed variation in the quality characteristics of shea butter samples from the marketplace. Some samples showed very high levels of degradation, while the industrially extracted and processed samples (e.g. the samples from The Pure Company) showed low levels of degradation, although improvements are still needed to reduce the level of free fatty acids to produce grade 1 products. These results suggest that proper and controlled processing practices can produce a high quality butter with decreased degradation.

All the samples showed a consistent composition in the fatty acid profile thus showing almost equal physical properties (density and refractive index), which suggests that the West African trees from which these shea butter samples came were genetically homogeneous.

Relatively weak relationship between moisture, free fatty acid, and peroxide value were found, which implies all the parameters should be considered collectively in quality control of shea butter. This study has confirmed the use of the dropping point as a reliable method which is easy to conduct for the determination of melting point, important physical parameter of shea butter.

Shea butter showed relatively high amount of unsaponifiables compared to other plant derived oils and fats and the major constituent was found to be α-amyrin which was reported to exhibit anti-inflamatory properties. This characteristic can be used to

develop new products using shea butter or shea butter's unsaponifiable fraction, even though more research is needed on the bioactivity of shea butter and its isolated triterpenoids and phytosterols.

While it was observed that processing practices can affect oxidation parameters of shea butter, the addition of natural and synthetic antioxidants can delay the oxidation of shea butter and thus extend its shelf life. Future research should be focused on the synergistic effect of the mixture of various natural antioxidants on shea butter that may provide better ways to improve the shea butter's quality and thus the extended shelf-life. The result showing the effect of rosmarinic acid and gallic acid also suggests the natural plant extracts such as rosemary extract or white tea extract may be great to be added to or mixed with shea butter in the development of shea butter products which may have better bioactivities or better stability.

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