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Table of Content

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Review

Shea butter extraction technologies: Current status and future perspective

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Shea butter is a high–value shea nut fat used as an edible oil, antimicrobial and moisturiser in the food, pharmaceutical and cosmetic industries, respectively. The annual worldwide export of shea nut from Africa is 350,000 MT of kernels with a market value of approximately $120 million to producing countries. The multifunctional properties of the shea butter depend strictly on its compositional properties: the peroxide value, moisture content, free fatty acid level and the insoluble impurities. Standard extraction technologies: the traditional, mechanized, enzymatic and chemical methods were used for shea butter extraction. Current extraction technologies which rely on different extraction parameters for shea butter extraction are yet to yield the desired qualities and efficiencies of butter. Application of hydrolysing enzymes during enzyme extraction however eliminates the laborious, tedious and labour–intensive extraction processes creating alternative, selective and mild extraction conditions. The current review gives an overview of shea butter extraction technologies, the efficiencies, qualities and a perspective into the shea butter industry.

Key words: Shea butter, mechanical, chemical, traditional, enzymatic, technologies.

INTRODUCTION

Shea butter is the oleaginous material obtained from the kernel of the shea nut tree *Vitellaria paradoxa*. Research has consistently described the shea butter as a vegetable fat extracted from the kernels of the fruit of *V. paradoxa*, Sapotaceae (Hall et al., 1996; Pontillon, 1996; Kengue and Ndo, 2003; Elias and Carney, 2004; Schreckenberg, 2004). Others described the shea butter as a yellowish–grey solid material (Abdul–Mumeen et al., 2013) or yellowish white in colour with a strong smell (Tessy, 1992) extracted as fat from the kernels of the shea nut fruit. Shea butter extracted from shea kernels is raw and can be refined.

Shea butter is as good as table oil because of its high nutritive value and low cholesterol levels; widely used locally for curing leprosy and other ailments and has various industrial uses that include soap making, cosmetics, lubricants and paints (Olaniyan and Oje, 2007b). Shea butter is ideal for use as raw materials for

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cooking oil, margarine, cosmetics, soap, detergents and candles due to the presence of solid fat (stearin) and liquid oil (olein) (Chevalier, 1943; Boffa et al., 1996; Russo and Ethrington, 2001). Shea butter is used extensively in the food, pharmaceutical, cosmetic industries and often as cocoa butter substitute by chocolate manufacturers and for margarine and baking purposes (Martin et al., 1987; Williams and Bolton, 1950; Hall et al., 1996).

The American Shea Butter Institute (ASBI, 2004) reports that 100% pure natural shea butter is an all–natural vitamin A cream which has shown to be a superb moisturizer, with exceptional skin healing properties. ASBI (2004) has also asserted that shea butter has proved to be effective against skin and other skin related conditions such as dry skin, skin rash, skin peeling after tanning, blemishes and wrinkles, itching skin, sunburn, shaving cream for a smooth silky shave, small skin wounds, skin cracks and tough or rough skin, cold weather, frost bites, stretch mark prevention during pregnancy, insect bites, health skin, muscle fatigue, aches and tension, skin allergies such as poison ivy or poison oaks, eczema, dermatitis and skin damage from heat.

Africa produces about 1,760,000 tons of raw shea nuts annually from its wild trees (Mohammed et al., 2013). In Ghana, there are estimated 94 million shea nut trees which were projected to produce at least 60,000 metric tonnes of shea nuts per annum for the production of all shea butter processed locally (Ofosu, 2009). This yields about 150 tonnes of shea butter, 60% of which is used locally with 25% exported. Over 80% of the woody vegetation in Northern Ghana is *Vitellaria* (Lovett and Haq, 2000).

In the new global economy, shea butter also known as ‘kpakahili’ (raw oil) has become a central commodity for most industries and thus, in addition to the kernels, plays a significant role in poverty alleviation in Northern Ghana (Moore, 2008; Abdul–Mumeen, 2013). The shea nut market in Ghana is well established, being sold both locally and internationally.

In 1996, Ghana exported 21,467 tons of shea nuts worth $4,484,600 (Chaffin, 2004). It is reported that shea butter export estimates from Ghana for 2002/2003 reached 2,000 mt, although demand existed for over 6,000 mt per annum (p.a.) (Lovette, 2004), while the export of 4,969 mt of shea nuts earned the country US$1,339,000.00 (FAO, 2002) the same year. Shea kernel exports reached the highest of close to 180,000 mt in 2007 (Figure 1) between the period of 1996 and 2013 (GEPA, 2014). That cannot be said of shea butter export which was only 40,000 mt in 2013, the highest ever within the same period (GEPA, 2014). Carette et al. (2009) observed that records on overland exports, records on export quantities of shea butter and finished shea products were not documented consistently until when Lovett (2005) made export estimations.

Shea is mainly exported in the form of shea kernels, rather than shea butter or finished shea products. Recent reports however show that annual exports of shea kernels are still high but shea butter exports have also increased from 12,561.37mt (US$19,010,304) in 2009 to 32,782.61mt (US$24, 764,995) in 2010 representing an increase of 61.7% (GEPA, 2014).

Raw shea butter is obtained primarily by the traditional method of extraction (Abdul–Mumeen et al., 2013), from the shea fruit kernel, but can also be obtained by mechanical (Olaniyan and Oje, 2007a), enzymatic (Didia et al., 2018) and chemical methods (Apea and Larbi, 2013). It can be consumed raw without any further physical or chemical treatments or refinement.

However, much uncertainty still exists about the standard method of extraction of shea butter which will meet the standards declared by the various certification and standard organizations for shea butter quality. Thus, there still remains huge information regarding the reasons for differing approach to the extraction of shea butter and their efficiencies that are yet to be collected. This sort of information would extremely benefit not only the communities and industries within Ghana but all the countries within the West African sub–region. Additionally, once the shea nuts have been harvested, a huge amount of time and effort is spent on the processing and extraction methods currently employed. To date, no extensive qualitative review of these methods has been carried out and as a result there is limited expert advice available to the local communities and women groups that would benefit most from it. This research aimed to fill some of these information gaps through carrying out a comprehensive review of the shea butter processing for the local women groups in particular, researchers, policy directors and other actors in Ghana who are committed to the development of the shea butter industry. Therefore, the current review gives an overview of shea butter extraction technologies, the efficiency and quality of the shea butter produced and the perspective into the shea butter industry.

General treatment of shea fruits prior to shea butter extraction

The processing of shea butter is seasonal. The fruiting and gathering of the nuts occur between the months of May to August every year (Moore, 2008) during which the shea nuts are processed into kernel (Owoo and Lambon–Quayefio, 2017). The raw and ripe fruits are green but the ripe fruit is occasionally yellowish (Moore, 2008) and soft when felt. The fruit is composed of four layers: the epicarp, mesocarp, shell and the kernel. The epicarp together with the mesocarp is called the pulp; the shell and the kernel compose the nut. The kernel is the oil–bearing material that can be obtained by processing the shea nut fruit. The fruit primarily undergoes several

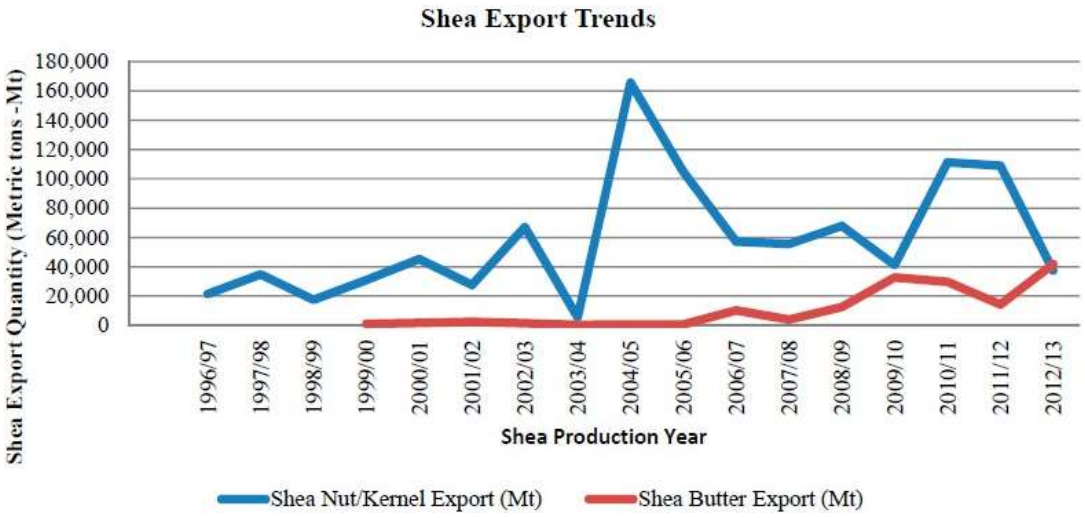

Figure 1. Shea production and export trends (Ghana Export Promotion Authority (GEPA) 2014.

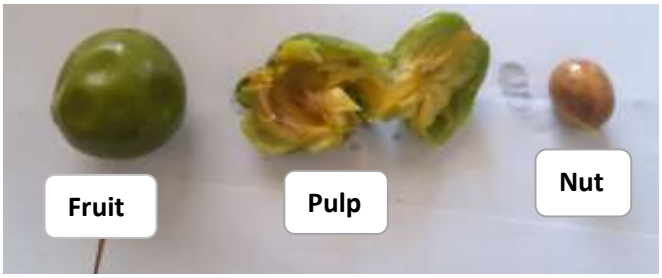

Figure 2. Shea nut fruit, pulp and nut.

processes for example, de-pulping, boiling, drying, de-shelling, winnowing and sorting to obtain the kernel from which the shea butter is extracted.

De–pulping

The fresh mature fruit of the shea tree is covered externally by the pulp (Figure 2) consisting of an epicarp (greenish) and a mesocarp (yellowish). De–pulping is the removal of the pulp (the epicarp and the mesocarp) when the shea fruit is ripe. The pulp which is mostly green becomes soft when the fruit ripens (Gyedu-Akoto et al., 2017). It has been well documented that the fruits are collected by African women from the ground and the pulp is removed by fermentation or manual peeling (Chaffin, 2004; Moharram et al., 2006). Fruit storage before de pulping, especially after three days, negatively affected the quality (Aculey et al, 2012) and quantity of the resulting butter because of the sugar rich pulp which assists fungal growth and thereby reduces oil content of the kernel (Carette et al., 2009). Ojo and Adebayo (2013) confirmed this when, during the bio–deterioration of the shea nut fruit pulp, they isolated eight fungi species (*Aspergillus flavus, Aspergillus niger, Botrydiplodia theombromae, Botryosphaeria* spp*., Colletotrichum gleosphoriedes, Lisidiplodia* spp*., Pseudofasicocum* spp*. and Trichoderma viridae*) from the fruit natural environment and from parboiled kernels (Aculey et al., 2012).

Boiling shea nuts for butter production

The shea nut (Figure 2), comprising the shell and the kernel, is obtained after the pulp has been removed. The shea kernel sticks to the shell wall and to separate them, the nuts are immersed in boiling water or on rare occasions smoked (Honfo et al., 2013) although Kpelly (2014, unpublished) hinted that smoking raised the FFAs and PAHs levels and could be carcinogenic. Smoking the nuts is specific for the Otamari socio–cultural group (Honfo et al., 2012). The nuts are usually boiled for about 30 – 45 min (Honfo et al., 2013) to temperatures ranging between 100 and 105ºC to deactivate all biological and enzymatic activities in the nut (Abdul–Mumeen, 2013). Boiling increases the fat output of the kernel and a possible explanation is that boiling softens the nuts leading to cell disruption and a better release of the oil (Honfo et al., 2013; Moore, 2008). Thus, to allow efficient extraction of the fat, research (Womeni et al., 2006; Lovette, 2004) stresses that boiling of shea nuts was necessary. Boiling also clean the surface of the nut of any remaining fruit pulp (Moore, 2008) that has the tendency to promote microbial growth.

Drying the shea nuts

After boiling, shea nuts are allowed to dry either via sunlight

for 5-10 days or by using oven for 2-3 days (Moore, 2008). Sun drying is a widespread practice to reduce the moisture content of the nuts and to facilitate the shelling operation (Honfo et al., 2013). The nuts sun–dried after boiling can lead to mold contamination during the rainy season and this affects the quality of the shea butter and shea butter products (Moharram et al., 2006; Senyo, 2014). On rare occasions the nuts are solar dried. The advantage of solar dryers is that it checks the activity of *Aspergillus fungi* and *Euphenestia caufella larvae*, even during long–term storage (CRIG, 2002) of the nuts. Both parboiling duration and drying method significantly affect shea butter yield and quality and the free fatty acids levels especially (Aculey et al., 2012).

De–shelling or de–husking

Removal of shells from the nut after cracking and winnowing is a process described as de–husking or de–shelling (AOS, 2011). During the drying period, the kernels become detached from the shell wall. De-shelling is carried out using stone, hammers and pistles (Alonge and Olaniyan, 2007). Winnowing is achieved by holding basket filled with a mixture of the shells and kernel at arm's length and allowing a gradual pour–out (Alonge and Olaniyan, 2007). If there is a strong wind, the pieces of shell will be blown away, if not, then the process is repeated many times (Fleury, 1981).

Sorting and further drying

Sorting is the removal of the remains of the shell pieces from the shea kernels after winnowing (Mohammed et al., 2013). At this stage, shea kernels that are broken, infected by mould or are black in colour are also removed to obtain clean unbroken shea kernels. The shea kernels can now be stored for several months without deterioration or processed into shea butter.

The pre–treatment and storage of the shea kernels before the butter extraction process is a critical stage that affect the quality of shea butter produced. The first adverse effects are seen in the decrease in oil phenols and in the reduction of volatile compounds responsible for the various properties of shea butter (Hee, 2011). Angerosa et al. (2004), notes that in several operative conditions involving long–term storage of seeds and high relative humidity, mould contamination increases the free acidity due to the production of fungal enzyme lipase, and simultaneously forms the characteristic sensory defect of "mould". This condition can affect the fatty acid (arachidic, linoleic oleic, palmitic, and stearic) composition and the free fatty acid content in particular thereby dictating the quality parameters of the butter and hence the international standards as set by the West African Regional Standards in 2006 (Table 1). There are several factors including the moisture content, pre–treatment of the shea kernel and the kneading session that affect the quality of shea butter (Abdulai et al., 2015). The research further reveals that the sandy soil, higher soil nitrogen levels, higher soil carbon levels and lower soil cation exchange capacity in general had significant positive impact on the quantity of fat produced in the kernels. Low levels of phosphorus in soils require higher levels of nitrogen for optimum shea seedling development and thus shea regeneration in shea parklands could still benefit from nitrogen supplementation Abubakari et al. (2012). Kapseu et al. (2001) and Womeni et al. (2006) showed that the drying time and roasting time of shea nut kernels affected the physico–chemical quality of shea butters. Overall, the target for the various pre–treatment processes is to extract all the 60% fat present in the kernel (Axtell et al., 1993). Figure 3 is a flow diagram of the various pre–treatment processes.

Shea butter extraction technologies

Addaquaye (2004) classified the processing technologies of shea butter into three methods: the traditional manual method, traditional semi–mechanized method and the fully mechanized method. However, recent studies have suggested that the traditional butter extraction encompass the traditional manual and the traditional semi–mechanized methods (Mohammed et al., 2013; Abdul–Mumeen, 2013) and that shea butter can be extracted by chemical and enzymatic processes (Didia et al., 2018; Otu et al., 2015; Apea and Larbie, 2013). Whether the various extraction technologies have answered the numerous challenges bedevilling the shea butter industry is discussed below.

The traditional extraction technology

In Africa and Ghana, shea butter is mainly produced by women by the traditional method (Abdul–Mumeen, 2013) also known as wet extraction process (Olaniyan and Oje, 2011) and this has become the most accessible income generating activity for most women up north of Ghana (Rammohan, 2010). This process is the main method of processing oils in most West African countries, including Ghana (Addaquaye, 2004). Indeed, about 80 per cent of Ghana's shea butter is produced through traditional processing techniques (Mensah, 2001). For production of substantial amounts of oil the process takes 20–30 h (Hall et al. 1996) since the total time needed to process the shea butter (for one cycle) is between 5–6 h (Boffa, 1999) and that kneading alone, for one session, takes about 30 min (Abdul–Mumeen, 2013).

Kernel size reduction and dehydration

The traditional extraction technology begins with kernel

Table 1. Characteristics of the shea nut kernel for enzyme–assisted butter extraction potential.

Characteristic	Percentage
Total lipids	59.04
Crude fat	54.85
Crude protein	7.81
Carbohydrate	34.77
Soluble sugars	5.45
Starch	7.59
Pectic substance	2.93
Hemicellulose	10.84
Cellulose	5.95
Total fibre	20.35

Source: Tano–Debrah and Ohta, 1994.

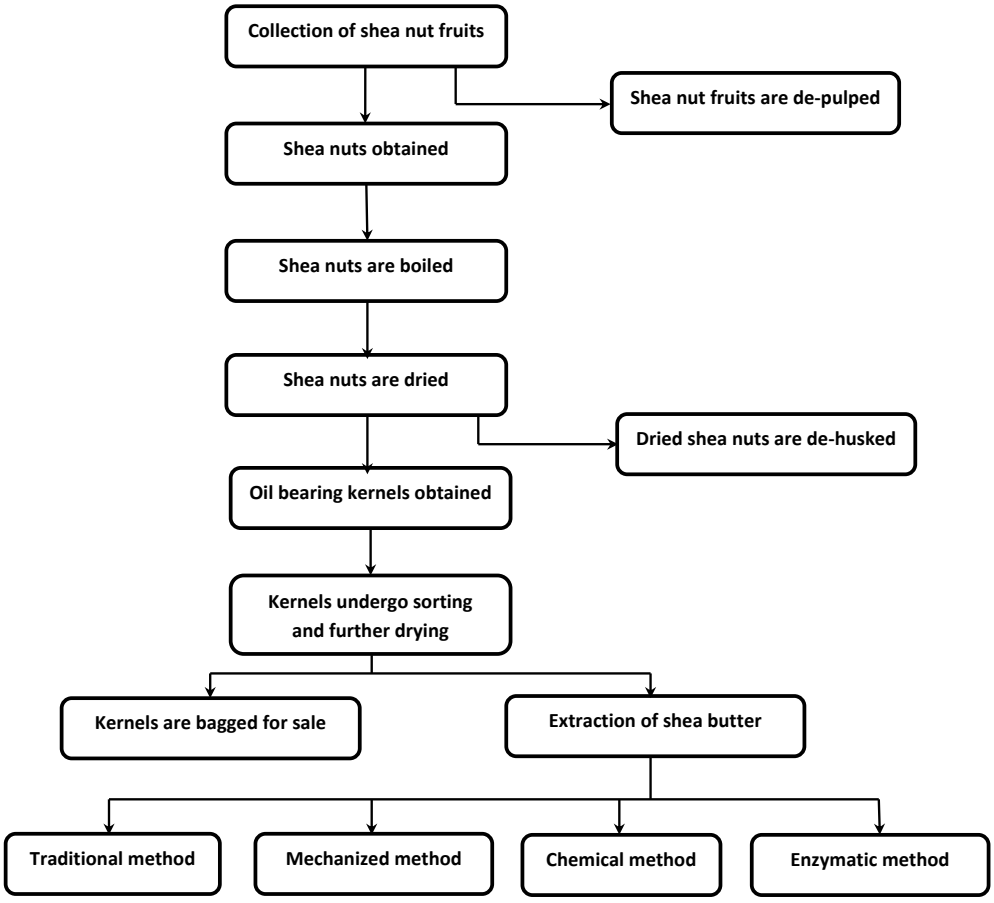

Figure 3. General pre–treatment of the shea nut fruit to obtain the kernel (Abdul–Mumeen, 2013; Moore, 2008 descriptions).

size reduction by pounding in the mortar using the pestle and further dehydration by roasting (Abdul–Mumeen, 2013; Moore, 2008; Olaniyan and Oje, 2007b) to aid oil extraction. The roasted grits of kernel are ground to paste by either the use of stones in the pure traditional extraction process or by the use of grinding mill in the semi–mechanized traditional method. The size reduction and further milling increases the surface area (Abdul–

Mumeen, 2013) for effective hydrolysis during kneading. Other studies determined the factors influencing the quality of fats during their preparation (Louppe et al., 1995; Hall et al., 1996; Kapseu et al., 2001; Womeni et al., 2006) and noted that blanching shea nuts improved shea butter quality. Hall et al. (1996), Semmelroch and Grosch (1998) and Sanz et al. (2001), underlined that the sensorial characteristics of shea and cocoa butters were linked to the kernel roasting time.

Kneading

A kneading process takes place to break up oil cells and ease oil extraction and women take an average time of 30 min to complete one kneading session. Abdul–Mumeen (2013) explains that a kneading session involves taking a reasonable quantity of shea paste, adding an initial amount of about 3 litres of cold water, stirring slowly and then vigorously later, with the hand until the butter begins to rise in crude milky–white form. Some researchers suggest that traditional extractors boil water and skim off the released oil from the kernel (Alander, 2004) or by kneading and hand beating (Moharram et al., 2006). At this stage 10– 20 kg of finely pulverized paste is mixed with three litres of water and kneaded until a white bloom appears which marks an important enzymatic step and followed addition of hot water (ASBI, 2004; Abdul–Mumeen, 2013; Mohammed et al., 2013). Kneading is successful depending on the individual's recognition of changes in temperature, consistency and appearance and this can only be assessed correctly with experience (CRIG, 2002).

Heating, oil separation and cooling

Once kneading is over the oily layer is harvested from the surface of the water layer leaving behind the water layer and particulate matter in the bottom of the pan (Tano–Debrah and Ohta, 1994). The oily layer or fat emulsion is washed with water, boiled to evaporate the water and the crude fat is obtained by decanting or gentle pouring. Finally, the decanted oil is allowed to cool to solidify taking 6–12 h and the product is Shea Butter (ASBI, 2004; Abdul–Mumeen, 2013).

Shea butter from traditional extraction technology is increasingly required abroad by cosmetic and pharmaceutical industries, to the detriment of solvent extracted shea butter (Elias and Carney, 2004). That not notwithstanding, the traditional extraction method is still considered low yielding and has several uncontrolled processes which account for the wide variability of shea butter quality in the market (Louppe et al., 1995; Hall et al., 1996; Kapseu et al., 2001; Womeni et al., 2006). About 23% of fat still remain in the shea nut cake after a successful extraction (Abdul–Mumeen, 2013) and it is considered grossly inefficient, yielding not more than 35% of the oil with the product quality often low (Ata, 1978; Olaniyan and Oje 2007b; Niess, 1983). But Ofosu (2009) asserts that shea butter extraction by the traditional method has reached 35–40% extraction efficiency.

Several studies have been undertaken in order to increase shea butter extraction rate but also to improve the shea butter quality using the traditional processing conditions (Louppe et al., 1995; Hall et al., 1996). Attempts were made to incorporate appropriate technology into a number of the processing stages, both to improve efficiency and to reduce the amount and drudgery of the labour, as well as impact on the environment (Hall et al., 1996; Elias and Carney, 2004; Schreckenberg, 2004).

Mensah (2010) discusses an attempt by GRATIS Foundation and Technology Consultancy Center (TCC) to remove the production bottlenecks in the traditional method of shea butter extraction. A successfully developed manually operated bridge press for the extraction of shea butter used the Intermediate Moisture Content (IMC) method to produce an average of 67 % extraction efficiency. The method was first tested in Gbimsi in the Northern Region and later used by some women processors in the region. The principle of operation involves grinding dry kernels of moisture content (4–6%) into paste using a local plate mill. The paste obtained at a temperature of 70°C is used in the extraction process without any heat treatment. The moisture content is raised to 12% by kneading with a predetermined amount of hot water. The moisturized paste at 60°C is put in small bags, and pressed in a "bridge press" to release the oil.

The IMC method comes with some advantages over the normal traditional processing such that it increases the extraction efficiency by 5% of the normal extraction rate; increases in daily production capacity by 200%; drastic decrease in firewood consumption: for every 85 kg of kernels processed about 8 kg of firewood is used instead of 72 kg; drastic decrease in water use, that is about 8 l of water used in place of 160 litres for 85 kg of kernels processed; reduction in the extraction operations stages from 7 to 5 by the removal of roasting and cream boiling; milder shea smell; environmentally friendly since few fuel wood and less waste water are involved.

With the pounding, roasting, milling, kneading, heating, decanting and cooling to solidify the shea butter, the process has been described as cumbersome, tedious, time–consuming and energy sapping (Olaniyan and Oje 2007b; Coulibaly et al., 2009). The returns do not commensurate the energy, material and financial input by women in shea extraction and besides their incomes are unstable due to low extraction efficiencies, inconsistent butter production, and supposedly low quality of the butter.

These challenges among others called for semi–mechanization technologies which later got developed

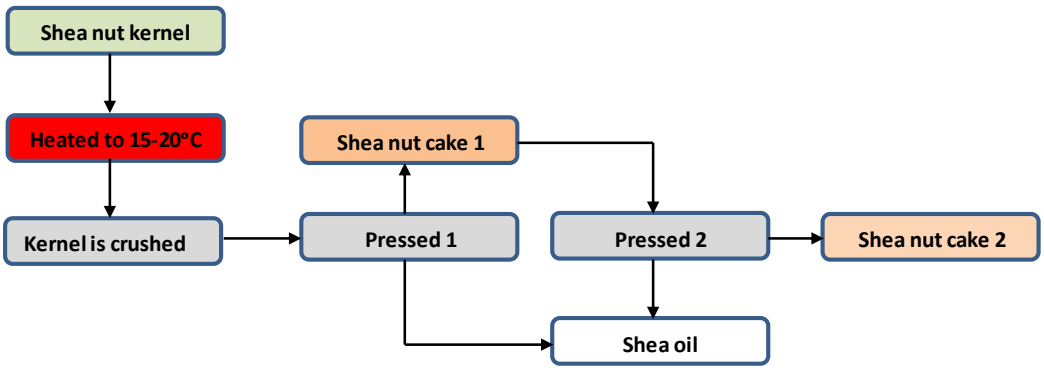

Figure 4. Flow chart of mechanical extraction of shea nut oil (Abdul–Mumeen, 2013).

further there was equipment designs designed to perform specific operations including oil digestion and oil pressing and eventually machines that combine several operations in the process (FAO, 2002).

The mechanical processing technology

The mechanical processing technology is usually referred to as the Cold Press Extraction method (Sekaf, 2008), so called because it does not involve the various different heating stages of the traditional procedure (Figure 4).

The mechanical press method of shea butter extraction has been reported by FAO and CFC (2002) but one of the earliest researches works on the use of the mechanical press was Marchand (1988). His research revealed that equipped with a jack that exerts 30 tonnes of force, a shea butter press could crush more than 3 kg of shea kernels within 20 min. The press could extract up to 85% of the fat contained in the kernel in a simplified process (Marchand, 1988) through a reduction in the various heating stages of the kernel and subsequently saves fuel wood.

The emergence and proliferation of processing shea butter by this method in the shea producing zones of Ghana was mainly due to the collaborative work between women groups and some development partners and Non–Governmental Organizations. The United Nations Fund for Women's Development, Technoserve Ghana and the Netherlands Development Organisation (SNV) introduced these innovations in the form of mechanized technologies such as hydraulics and mechanical presses, which were locally designed and manufactured. The CRIG (2002) however notes that the Dagomba women of Ghana were the first to initiate the mechanization of the butter extraction process. These have reduced processing times and enhance water use.

The processing of shea butter by this technique is carried out in a plant comprising of a boiler, mechanical press system and a filter press system. The mechanical press applies a great deal of pressure to the pulverized seed (Sekaf, 2008) to turn out more shea butter from the process (Yonas, 2014). Other inventions targeted single unit operations among which were a kneading machine, grinders, a hydraulic hand press, solar dryers, a heater and mixer. These inventions collectively achieved extraction efficiencies of 60 to 85% (CRIG, 2002; Marchand, 1988). Others have reported lower (35.9 to 45%) fat output at 82.28°C for the press (Alonge and Olaniyan 2007; Olaniyan and Oje, 2007b). About 30–33% of shea butter is extracted from the shea nuts with the mechanical expeller (Abdul–Mumeen, 2013) although combination of the mechanical with chemical methods has achieved 98% extraction efficiencies (Abdul–Mumeen, 2013).

In extraction, the dry kernels are fed into boiler or heating chamber where they are first heated to temperatures of between 15–20ºC and then directed into a crushing unit where they are reduced in size to increase the surface area for effective butter yield. The oil is pressed out from the pulverized nuts with some traces of the residue which are filtered out through the filter press to obtain clear oil. The cake remaining as a result of the first extraction is directed into another expeller where it is pressed the second time to produce more butter which is then allowed to cool and solidify (Abdul–Mumeen, 2013) or directed into another chamber for further refinement.

Unfortunately, the shea butter press still leaves huge problems for the village woman into shea butter production. The affordability and availability of the butter press in addition to its operation remains a problem for the local woman and for the local industries manned by these women.

It is a method recommended for the large production of commercial quantity of shea butter. The method was not only developed to increase productivity and save time, but to reduce stress on the processors since traditional boiling method was found to be labour intensive and time consuming (Masters and Puga, 1994).

The advantages of the mechanical press method notwithstanding, the equipment are expensive, scarce

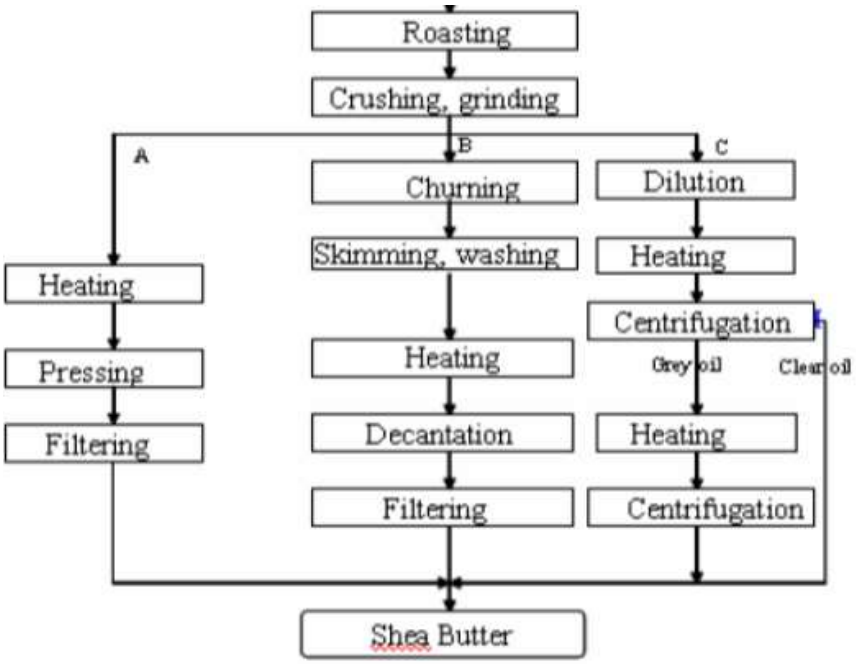

Figure 5. Flow diagram for traditional (**B**) and mechanical extraction [press (**A**) and centrifugal(**C**)] methods for shea butter. Source: Coulibaly et al. (2009).

and unaffordable by most local industries (Alonge and Olaniyan, 2007) which predominates developing countries including Ghana. Another shortcoming of the mechanical separation process using the press machine is that it does not completely remove all the oil from the mass of the paste (Apea and Larbi, 2013), that is about 19% fat remains in the cake (Abdul–Mumeen et al., 2013).

The centrifuge method

The centrifuge method is one of the mechanical extraction technologies and Coulibaly et al. (2009), focused on shea butter extraction with a centrifuge machine (Figure 5) but the extraction efficiency was found to be barely higher than 30% on average. This was similar to the mean efficiency values with traditional methods. The procedure adopted during the said research is outlined in the diagram below. The process was compared to traditional and the mechanical press.

The extraction principle involved separating the oil, water and shea nut cake from a pre–prepared water–paste emulsion. The extraction machine encompassed a movable unit driven by a motor/engine. A shaft driven by the motor at one end was equipped with a rotating drum at the other end.

The drum built with 10 kg kernel loading capacity had an angular frequency of 1,000 rpm. The separation entities (oil, water and cake) occurred in layers and according to their mass. The oil which was light and floating was discharged into 2 bailing devices fitted in the drum. The process was repeated until clear oil was obtained. However, the extraction efficiency was not different from the traditional and screw press methods.

The chemical extraction processes

With this method, the dried kernels are first crushed into paste and fed into the Soxhlet extractor. Afterward an organic solvent such as n–hexane or ether is added. The mixture is allowed to stand for some number of h for the oil to be separated which is decanted and allowed to solidify.

The types of the solvents used in the extraction have some influence on the quality characteristics of shea butter especially the peroxide value of the butter. In a study conducted by Kar et al. (1981), on the best solvent for shea butter extraction, petroleum ether, n–hexane, chloroform, benzene, and water were employed. Hexane extraction gave the highest amount of fat from the kernel.

The principle with hexane extraction is that, the pulverized kernel is mixed with hexane which then unlocks the polymeric mass allowing all the oily and fatty constituents of the kernel to dissolve in it. The resulting oil–hexane mixture is later separated from the seed residue by filtration. The oil–hexane mixture is then heated to 68ºC to vaporise and recover the hexane to obtain the crude Shea Butter (Abdul–Mumeen, 2013; ASBI, 2004). The choice of hexane over other solvents for shea butter extraction is also informed by several factors: the physical properties of the solvent, the

commercial economics of the butter and the edibility of shea oil from the extraction (Abdul–Mumeen, 2013).

Solvent extraction method has been reported to yield 47.5% of SB (Ikya et al., 2013), which is 32.9% greater than that of mechanical extraction (Olaniyan and Oje, 2007b) but have reached 98% when combined with the mechanical methods (Abdul–Mumeen, 2013). The use of organic solvent extraction has been acknowledged to showed low or no detectable level of peroxides.

Although the use of organic solvent for shea butter extraction gives a high yield, it is considered not wholesome for consumption due to some traces of the solvent that may remain in the butter (Apea and Larbi, 2013). According to FAO and CFC (2002), solvent extraction method is not usually used in domestic and commercial shea butter extraction in developing countries due to the high costs involved, environmental problems and the lack of technical skills associated with it. The natural integrity of unrefined shea is interrupted or changed during the chemical extraction process. For safety reasons, shea butter prepared by the chemical method, must be refined before it is permitted on the market. Additionally, hexane extraction removes most if not all of the healing properties from shea butter. Hexane is a liquid alkane whose chemical properties are similar to that of gasoline. Its use for shea butter extraction therefore requires a well–trained chemist and a well–equipped chemical laboratory (ASBI, 2004). Thus, the general view is that the use of chemicals in the extraction process take shea butter away from natural (Apea and Larbi, 2013).

Enzyme assisted extraction technology

Enzymatic extraction of vegetable oil with water–soluble enzymes involves the degradation of the cell wall and then the release of the oil (Perez et al., 2013). Aqueous enzymatic extractions are potentially used in the oil industries due to their high specificity and low operating temperatures (Ahmadi and Karimi, 2013). Several enzymes such as: amylase, glucanase, protease, pectinase, cellulase and hemicellulase have been used for the extraction of various vegetable oils from their kernels (Dominguez et al., 1995). Cellulase and hemicellulase have been reported to be the most suitable enzymes for cell wall degradation while pectinase has been identified as an effective enzyme for vegetable oils extraction (Dominguez et al., 1994; Perez et al., 2013).

Lipases can be produced by animals, plants, and microorganisms. Microbial lipases however primarily catalyze the hydrolysis of triacylglycerols and have been extensively studied due to their interesting characteristics of stability in organic solvents, action under mild conditions, and high substrate specificity (Gandhi, 1997; Sharma et al., 2001; Kempka et al., 2008).

Enzymatic extraction offers numerous advantages such as: increased yield, improved and high-quality vegetable oil, improved quality of the residual meal; reduced fibre content, preserved protein properties of defatted meal, low–peroxide and free fatty acid values (Soto et al., 2007; Perez et al., 2013). These are the reasons which make enzyme processes more economical for oil extraction processes (Rosenthal et al., 1996).

Potential of shea kernel for enzymatic extraction

The kernel, according to Axtell et al. (1993), contains about 60% edible fat and the residual product, from which the butter is extracted, is an excellent ingredient for livestock feed production. The enzymes break down the cell structure of plants and the cell wall of plants consists mainly of pectic substances. Hydrolytic enzymes like pectinase break down the cell wall of plants, while proteases permeabilize the liposome membrane and facilitate oil release from the oil body (Rosenthal et al., 1996; Fullbrook, 1983). Table 1 shows the characteristic potential of the shea nut kernel for enzymatic extraction of its oil.

Recent studies by Didia et al. (2018) suggest that enzymes technology is the way for shea butter extraction in Ghana. An aliquot equivalent to 50 g of shea nut biomass in 600 mL beaker was stirred with water in the ratio of 1:4 wt/vol and treated with commercial enzymes at optimized conditions. Three percentage (3%) enzyme–substrate concentrations for combined enzyme effect at 60°C for 2 h in a medium of pH 5 for three different substrates used: ‘raw kernels’, ‘roasted kernels’ and ‘roasted kernels ground to paste’ was subjected to the traditional procedure and this yielded 50, 54 and 70% butter respectively. The reaction was terminated after adding boiling water (100°C). The emulsion which formed the top layer was collected into another beaker and gently boiled until clear oil was obtained. It was then decanted into a weighed aluminium dish, cooled and weighed to estimate the percentage yield.

Otu et al. (2015), extracted shea butter using locally produced pectinases from *S. cerevisiae* ATCC 52712 via corn cobs substrate in a solid-state fermentation and compared its efficacy with commercial enzymes. The crude pectinase whose estimated optimal protein concentration was 7.00 mg/mL with 0.86 u/mg activity used against Viscozyme L (beta endo–1,3 (4)–glucanase (100 FBG/g) and Pectinex 5XL (4500 PECTU/mL). At 1.20% enzyme–substrate concentration the crude pectinase gave an optimum oil recovery of 44.00% while the commercial Pectinex and Viscozyme gave 58.60% and 72.00% at enzyme–substrate concentration of 0.80%.

Previously Tano–Debrah and Ohta (1994) carried out preliminary analysis of crude enzyme extraction of shea butter using: amylases, proteases, hemicellulases, cellulases and pectinases obtained from Shin Nihon Chemicals Co. (Anjoh, Japan). Although the traditional extraction method was adopted, it was done under

Table 2. Summary of the various shea butter extraction technologies.

Method	Pre–treatment required	Operating conditions	Advantages	Disadvantages
Traditional: Hand kneading	Roasting Size reduction Milled to paste	Knead with hand Energy input > 30 Kw per ton biomass Add cold water initially About 60°C of water added later	Simple for the local women Natural shea butter is produced Readily available source of shea butter for family use It's wholesome vegetable oil	Arduous, labour–intensive and time onsuming Requires large amounts of water and firewood Low recovery efficiency (25–30%) Product requires further refinement
Physical: Mechanical press Yonas et al. (2016)	Sorting and cleaning	Particle size (grits) Heating temperature (15–70°C) time (28–30 min) Moisture content (3–5%) Applied pressure (30 tonnes) Duration of pressing (10 – 20 min)	Volumes of water use is avoided Improves fat output over the traditional method Large scale production	Equipment are expensive Equipment are Scarce Physicality required in operation Capacity building training
Chemical: Soxhlet/hexane extraction	Sorting and cleaning	Kernel reduced to paste Mix 1 tonne with 5 L of hexane for 4 h Obtain hexane–oil mixture Retrieve the hexane at 68°C	Quiet efficient Fast results No drudgery No waste water	Technical knowhow Not friendly to women For laboratory scale production High Cost of chemicals Product may be unwholesome
Biological: Enzymatic	Reduce size Roast Mill to paste	Temperature (50 – 70)°C Hydrolysis time (2–4 h) Concentration (1–3%) pH (5–7)	Friendly Simple and safe Adoptable by women Efficient	Enzymes import are costly so local production required

strict laboratory principles. To deactivate the supposedly inherent lipases, shea kernel paste was weighed mixed with water in 1:4 ratio and autoclaved at 100~ for 5 min. The crude enzymes were added to the paste–water colloid singly and in combination each at the same level of activity (500 u/g). A uniform meal weight of 20 g was used in each treatment. The enzyme–paste uniform mixtures were incubated in a water–bath–shaker at 50°C and shaked at 100 revolutions per min for 4 h. The mixtures were autoclaved at 100°C for 3 min and centrifuged at 12,300 rpm for 20 min. The supernatants were extracted with petroleum ether in separatory funnels, and the ether phase was washed several times with warm water until the washings were clean. The ether phases were collected in weighed dishes, evaporated on a water–bath and dried in an air–oven at 100°C for 2 h. Weights of oil extracted were determined after cooling the dishes and were expressed as a percentage of the value obtained by the Soxhlet method. For all enzyme single dosage and combinations, the extraction efficiency ranged from 47–74%. Table 2 summarises the various methods for their extraction conditions, merits and demerits.

Quality and efficiency of shea butter generated

Quality of shea butter generated

The general quality of shea butter, according to most international standards, is pinned on four shea nut quality parameters: peroxide value, moisture content, free fatty acid levels, and the insoluble impurities.

Peroxide value (PV) is an indicator for stability and level of deterioration of shea butter and it measures the milli–equivalents of oxygen or hydro–peroxides in 1 g of fat or oil (Ikya et al.,

Table 3. Quality characteristics of shea butter from the various technologies.

Method	Peroxide value (meq/Kg)	Moisture content (%)	Free fatty acids (%)	Insoluble impurities (%)	Efficiency (%)	Reference
Traditional	8.06 – 14.2	5.39 – 13	5.01– 21.2	0.13 – 0.14	25 – 40	Asuquo et al. (2010); Hee (2011); Abdulai et al. (2015); Olaniyan and Oje (2007b); Coulibaly et al. (2009); Ata (1978); Ofosu (2009); Niess (1983); Adomako (1985); Nkouam et al. (2007); Agyente–Badu (2010)
Mechanical	3.55 – 44.9	0.1 – 0.56	0.6 – 15.5		30 – 45	Olaniyan and Oje (2007b) Gezahegn et al. (2016) Francis 2009.
Chemical	2.20–11.46				40 – 66	Akingbala et al. (2006); Francis (2009); Tano–Debrah and Ohta (1994); Okullo et al. (2010)
Enzymatic	3.67–11.18	0.17 – 0.18	1.60 – 2.71	0.02	47.0– 74.0	Didia et al. (2018) Otu et al. (2015) Tano–Debrah and Ohta (1995)
IUPAC 2501 AOAC 920.116 IUPAC 2. 201 ISO 663: 2000	1.0 – 10.0	0.05 – 3.0	1.0 – 3.0	0.09 – 0.2		FAO/WHO (2017)

2013). It is a valuable measure of oil quality as it serves as an indicator of degradation of the long fatty acid chains through auto–oxidation into peroxides that can later break down into other chemicals including foul–smelling ketones and aldehydes. The parameter deprives the butter of its stability and thus butter with high peroxide value (PV>10 meq kg^{-1}) is associated with the development of rancidity, which eventually limits their use in the food industry (Shahidi, 2005). Peroxide value is the most common determinant of lipid oxidation (Shahidi, 2005). Hydro–peroxides under normal condition is remarked to have no flavour or odour of their own, they are however unstable and usually break down rapidly to other products such as aldehydes and thereby developing strong, disagreeable flavour and scent.

Free Fatty Acids (FFA) by definition are the fatty acids present in oil or fat which has not been neutralized (Guy, 2009) or just unattached fatty acids present in a fat (Sapna and Nirmali, 2009). FFAs are related to their acid values. The acid value is a parameter expressed as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids contained in one gram of fat or oil (Kardash and Tur"yan, 2004) and it is twice the FFA of a fat and therefore acid value is directly proportional to free fatty acids (Roger et al*.,* 2010) and thus the lower the acid value of oil, the fewer FFA it contains.

Moisture is a chemical contaminant usually mixed with oil. Presence of moisture in oil affects the quality of the oil and significant amount of moisture in oil support microbial growth (Alirezalu et al., 2011; Hee, 2011) and lipid oxidation leading to rancidity (Hee, 2011) thereby reducing the shelf life of the fat and its corresponding products.

Conversely, low moisture content of shea butter is indicative of good quality (Olaniyan and Oje, 2007b). Difference in moisture content of shea butter can be attributed to shea vegetation (Quainoo et al*.,* 2013), although the minimum moisture content of shea butter is 0.05% but can go as high as 2.0% (West African Regional Standards, 2006).

Insoluble impurities refer to dirt and other foreign materials in shea butter (Hamilton et al*.,* 1986; Hee, 2011). It has been reported that some of these materials are bonded to the butter via the machinery employed in the extraction of the butter. Insoluble impurities may also make their way into the butter through physical contact of the butter with the soil, water, ground as well as packaging materials. The amount of insoluble impurities is an important quality parameter which determines shea butter deterioration since metals can catalyse the oxidation of shea butter and thus decreases its market value (Hee, 2011). Table 3 summarises the general range of values of the

different quality parameters by different researchers with their respective efficiencies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Association of metabolic syndrome with the risk of developing liver disease in chronic hepatitis B patients

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Metabolic syndrome is a constellation of abnormal glucose and lipid metabolic parameter that increases ones risk of developing cardiovascular diseases. Metabolic profiles have been linked to progression of varying stages of liver disease in chronic hepatitis B infection. The main objective of this prospective cross sectional study was to establish a link between metabolic syndrome indicators and markers of progression of liver disease in chronic hepatitis B infection. This could provide data leading to an alternative to managing the complications of chronic hepatitis B infection by possibly targeting metabolic precursors and their pathways which will be more targeting, sensitive and has minimal treatment complications than the conventional treatment regimes. In all, 200 chronic hepatitis B patients were sampled of which 100 met the United State National Cholesterol Education Program – Adult Treatment Panel III (US NCEP ATP III) 2005 criterion for metabolic syndrome. Anthropometric data and biochemistry analysis were performed. Obesity and dyslipidemia markers except HDL were higher in metabolic syndrome while haematological makers except WBC were lower in metabolic syndrome. Markers of liver carcinogenesis were generally higher in metabolic syndrome and strongly associated (p=0.01) with initial hepatocellular necrosis and cirrhosis stages of liver carcinogenesis than the intermediary fibrosis stages suggesting virologic mechanism may be responsible more for the fibrosis than metabolic factors. Metabolic syndrome was associated with the developing of various hepatitis B related liver complications. A long term study to elucidate viral genomic and dietary contributions to liver complications due to hepatitis B is necessary.

Key words: Metabolic syndrome, cardiovascular disease, carcinogenesis, anthropometry, chronic hepatitis, dyslipidemia, haematological, hepatocellular, fibrosis.

INTRODUCTION

The US NCEP-ATP III (2005) defines metabolic syndrome as the co-occurrence of any three of obesity, portal hypertension, atherogenic dyslipidemia, diabetes or impaired glucose utilization and microalbuminuria (Chackrewarthy et al., 2013; Pedroza-Tobias et al., 2014). Metabolic syndrome indicates one's risk of developing cardiovascular diseases and dysregulation in the body's energy metabolism (Cheng et al., 2016).

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In 850 hepatitis B cohort, metabolic syndrome was found to be 5%, of which elevated fasting blood glucose (≥ 100 mg/dL) was most prevalent. The degree of liver fibrosis was higher in metabolic syndrome group. Higher body mass index (BMI), high aspartate transaminase (AST)/Alanine transaminase (ALT) and metabolic syndrome were correlated with advanced fibrosis (p<0.001) (Aygun, 2015; Hsiang et al., 2014). In a long term population-based study, diabetes was correlated (p< 0.01) with advanced cirrhosis while extreme obesity (BMI ≥ 30 kg/m^2) was 4 fold associated with liver cirrhosis, and 100 fold increase in risk of advanced liver cirrhosis and hepatocellular carcinoma when both diabetes and extreme obesity were present (Hoebel, 2010, Pathik et al., 2017).

Stages of liver disease in chronic hepatitis B infection

The hepatocyte necrosis stage or cell injury stage is the first phase of carcinogenesis. The infected hepatocytes display the viral surface antigens (HBsAg) and the core antigens (HBcAg) leading to activation of immune-mediated target cell lysis or necrosis (Liaw and Chang, 2014).

Fibrosis or the wound healing stage is the development of tough, fibrous scar tissues due to excessive accumulation of extra matrix protein such as collagen, laminin, elastin and fibronectin (Papastergiou et al., 2012; Howell et al., 2015). Prompt detection of hepatic fibrosis is essential to making therapeutic decisions, predicting clinical prognosis and future complications (Papastergiou et al., 2012; Castera, 2011). Patients with mild or absent fibrosis have 25 to 30% risk of developing cirrhosis in the next 20 years whereas patients with septal fibrosis develop cirrhosis in 8 to 10 years (Lee et al., 2016, El-Serag and Rodulf, 2007).

Advanced cirrhosis or hepatocellular carcinoma is characterized by massive morphological disruption of the liver architecture and aberration in liver hepatocyte regeneration mechanisms resulting in changes in hepatocytes nodules and vascular formations (Gristina et al., 2015).

Mechanism of effect of metabolic syndrome on development of liver disease

Metabolic syndrome is linked to liver carcinogenesis in chronic hepatitis B through insulin resistance, molecular regulation, generation of oxidative stress and host immunity suppression (Brian and Ma, 2012; Katoonizadeh et al., 2016).

Insulin resistance leads to accumulation of visceral fat which disrupt cortisol homeostasis resulting in a surge in adiponectin synthesis, reduced sero-clearance of triglycerides and Very-low-density lipoprotein (VLDL) (Aygun, 2015; Mazzanti et al., 2016). These accumulatively generate insulin antagonist inflammatory cytokines such as tumor necrosis factor alpha, interleukin-6, insulin growth factor -1 (IGF-1) and resistin which initiate proliferation and antiapoptotic effect of hepatic stellate cells (Rahman et al., 2013; Jarcuska et al., 2016). Oncogenic mechanisms such as the C-Jun N-terminal Kinases (JNK) and nuclear factor Kb-1 (NF-kB-1) pathways that lead to hepatocellular carcinoma are activated by proinflammatory cytokines (Deng et al., 2015).

The NS3 and NS5 core proteins promote promitogenic in the hepatic stellate cells, stimulate the activation of NADPH oxidase and repress hemeoxygenase in hepatocytes further causing oxidative stress by accumulating lipid peroxides and free radicals (Svegliati-Baroni et al., 2014).

Immune response systems that produce multiple growth factors, inflammatory cytokines and chemostatins activate hepatic stellate cells, while those that generate kuffer cell-derived transforming growth factor and bile-induced activation of the epidermal growth factor promote hepatic stellate cells proliferation. Fibrogenic cytokine tumor growth factor B1 activates quiescent HSCs into contractile myoblast which eventually become a sustained source of extra metric proteins disrupting the extracellular matrix proteins turn over (Kuo et al., 2016; Rahman et al., 2013).

The surge in the prevalence of hepatitis B and metabolic syndrome, treatment cost and compliance challenges require early and comprehensive identification of persons at risk. The main objective of the study was to establish a link between metabolic syndrome indicators and markers of progression of liver complications in chronic hepatitis B infection. This could provide alternative to managing the disease by targeting metabolic precursors and their pathways.

MATERIALS AND METHODS

Ethical considerations and sample selection

Ethical approval was granted by the Committee on Human Research, Ethics, and Publication of the KATH and School of Medical Sciences, KNUST (CHRPE-SMS/KNUST-KATH). Subjects were selected from hepatitis B-infected patients attending clinical services at the Tamale Teaching Hospital. Inclusion into the study involved signing of an informed consent form by the volunteers and serological detection of HBsAg. Volunteers were recruited as they report to the Hospital.

Determination of sample size

In all, 200 chronic hepatitis B subjects were recruited using the population based formula by Krejcie and Morgan (1970). The formula takes into consideration the population of the study area, the prevalence of hepatitis B infection and 5% margin of error. Of this, 100 meet the US NCEP-ATP III (2005) criterion and were set as the test sample while 100 chronic hepatitis B patients which did not meet the US NCEP-ATP III (2005) criterion were set as control.

Recruits were excluded based on either one or more of evidence of smoking, use of drugs that affect the lipid profile, insulin administration, alcoholism, presence of liver complications and Hepatitis C virus (HCV) or have not met the US NCEP ATP III (2005) criteria.

Gathering of personal and anthropometry data

Data on age, gender, residency, marital status, level of education and presenting clinical symptoms were acquired using self-administered questionnaire. The body mass and height were measured with multipurpose stadiometer, Seca™ (Medical Measuring Systems and Scale, USA). The systolic and diastolic blood pressures were taken with sphygmomanometer, Baumanometer (Medical EXPO, UK). The waist circumferences were taken with a measuring tape KOMELON® (KOMELON Inc, USA). The BMI was calculated as body mass (kg) divided by the square of the height (m) (Svegliati-Baroni et al., 2014).

Biochemistry and haematological analysis

5.0 ml venous blood was drawn after 12-hour overnight fast using sterile syringes by aphlebotomist into sterile vacutainers. About 2 ml of blood samples were collected into EDTA tube for haematological estimation while 3.0 ml was collected into dry tube for biochemical estimation. Glucose was estimated at the point of collection using glucometer (GLUCOCARD Expression, USA).

The viral serological test was performed using commercial Hepatitis BVirus profile test kit, Diaspot (Fortress Diagnostics Ltd., Antrim BT14 1QS, UK). The biochemistry analysis were performed using NADH oxidation-dependent spectrophotometric technique with commercial chemistry analyser Horiba (HORIBA medicals, France), while full blood count (FBS) was estimated using automated hematology analyzer, Humacount 30^{TS} (Human Diagnostic Worldwide, Germany).

Calculation of biochemical indices

The fibrosis-4-index (FIB-4) index and the aspartate to platelet ratio index (APRI) were calculated according to the formula by Van Der Meer et al. (2012) and Castera (2011) as follows:

$$APRI = \left(\frac{AST}{40}\right)\left(\frac{1}{PLATELETS(10^9/\mathrm{L})}\right) \times 100$$

Fibrosis-4-index= (Age x AST) / (Platelets × (ALT)^2

$$\text{Females: VAI} = \left(\frac{\text{WC}}{36.58 + (1.89 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right)$$

$$\text{Males: VAI} = \left(\frac{\text{WC}}{39.68 + (1.88 \times \text{BMI})}\right) \times \left(\frac{\text{TG}}{1.03}\right) \times \left(\frac{1.31}{\text{HDL}}\right)$$

Where APRI is aspartate to platelet ratio index, AST is aspartate transaminase, HDL is High density lipoprotein, TG is triglycerides, and WC is waist circumference

Statistical analysis

Statistical analyses were performed with Minitab^{tm} Version 16 (Minitab Inc, Pennsylvania, USA). Discrete data were reported as percentages while continuous data were reported in mean and standard deviation. Correlation analyses were reported in p-values and correlation coefficient while regression analyses were reported in S-values, R-square and R-square adjusted.

RESULTS

Anthropometric, haematological and biochemical parameters of study population

Table 1 shows the anthropometric, haematological and biochemical parameters of the study population. Both groups had similar heights. Subjects with metabolic syndrome (MS) weighted 65.60±6.52 kg while the NMS group weighted 52.82±7.69 kg).

The BMI of MS category were in the obese category (>30 kg/m^2) while that of the without MS (NMS) group was in overweight category (> 25 kg/m^2). The waist circumference (WC) and visceral adiposity index (VAI) of MS group were 92.7±7.6 cm and 1.18±0.024, respectively whereas those in the NMS group were 85.11±8.13 cm and 0.42±0.25, respectively.

The lipid profiles also followed this trend, except for HDL cholesterol, where NMS group was 1.42±0.025 mmol/L) while subjects with the syndrome was 1.28±0.61 mmol/L. Fasting blood glucose (FBG), coronary ratio and blood pressure of MS category were 8.24±2.16 mmol/L, 11.5±3.15% and 152/98±8.28 mmHg whereas NMS group were 6.19±2.98 mmol/L, 8.29±1.98% and 128/18.58 mmHg, respectively.

All the liver disease markers were generally higher in the MS category than NMS category, except for AST/ALT ratio where the MS group was lower (0.28±0.07) than the NMS group (0.78±0.29). For the haematological properties, the Hb and platelets counts were lower (11.6±4.21 g/dL and 218.61±97.41×10^9/L, respectively) in the MS group compared to the NMS group (12.10±3.0g/dL and 258.63±108.19 g/dL, respectively). The WBC counts were slightly higher in the MS group than the NMS group (7.85±2.16×10^9 and 7.29±1.20×10^9/L, respectively). Regarding the number of subjects with results outside the reference limits, Body mass index (BMI), VAI, WC, VLDL, triglycerides, alanine aminotransferase (ALT), APRI and α-fetoprotein (AFP) of the MS group were generally higher than the NMS group except for fibrosis-4 index and haemoglobin counts, where the MS group were lower (14.7 and 28.14%, respectively) than the NMS group (32.16%).

Association of biochemical, viral serological and demographic parameters with metabolic syndrome

In MS group, AST/ALT was significantly correlated with BMI (p=0.12), FBG (p=0.024), Hepatitis B envelop antigen (HBeAg) seropositivity (p=0.01) and the total WBC count (p=0.027). The correlation with FBG and

Table 1. Anthropometric, haematological and biochemical features of study population stratified by the syndrome.

Marker	Metabolic syndrome (MS)		Without metabolic syndrome (NMS)		Total (Out of reference range)	*p*-Value
	Mean value	No. above reference limit n (%)	Mean value	No. above reference limit n(%)		
Anthropometries						
Height (m)	1.62±0.43		1.61±0.78		0.779	
Weight (kg)	65.6±6.52		52.8±7.69		0.769	
BMI (kg/m^2)	35.7±3.25	42 (21)	27.01±4.72	26 (13.0)	68 (34.0)	0.113
WC (cm)	92.7±7.62	9 (4.5)	85.11±8.13	5 (2.0)	14 (7.5)	0.000
VAI	1.18±0.02	48 (24)	0.42±0.25	29 (14.5)	77 (38.5)	0.012
Lipid profile/CVD						
HDL-CHOL	1.28±0.61	25 (12)	1.42±0.025	12 (6.0)	37 (16.5)	0.931
LDL (mmol/L)	4.93±0.78	32 (16)	4.31±0.98	23 (11.5)	55 (27.5)	0.031
TRI (mmol/L)	1.73±0.65	45 (22.5)	1.42±0.05	20 (10)	65 (32.5)	0.949
TCHOL(mmol/L)	7.93±2.98	20 (10.0)	5.92±0.97	11 (5.5)	33 (16.5)	0.736
VLDL (mmol/L)	1.91±0.30	35 (17.5)	0.96±0.19	32 (16.0)	67 (33.5)	0.000
FBG (mmol/L)	8.24±2.16	28 (14)	6.19±2.98	13 (6.5)	41 (20.1)	0.941
CR (%)	11.5±3.15	8 (4.0)	8.29±1.98	1 (0.05)	9 (4.5)	0.885
BP (mmHg)	152/98±8.28	35 (17)	128/70±8.5	32 (16.0	67 (33.5)	0.945
HBeAg RR	14(7.0)	2 (1.0)			8 (4.0)	0.021
Liver disease						
AST/ALT	0.28±0.07	31 (15.5)	0.78±0.29	30 (15%)	61 (30.5)	0.513
APRI	0.64±0.13	24 (12)	0.41±0.18	12 (6%)	36 (18.0)	0.000
AFP (µg/L)	3.73±2.12	12 (6.0)	2.67±1.20	10 (5%)	22 (11.0)	0.000
FIB-4	0.90±0.69	14 (7.0)	0.80±0.21	32 (16.0%)	46 (23.0)	0.000
Haematology parameters						
Hb (g/dL)	11.6±4.21	28 (14)	12.10±3.20	32 (16.0%)	60 (30.0)	0.718
T.WBC	7.85±2.16	32 (16)	7.29±1.20	20 (10.0%)	58 (29.0)	0.512
Platelets	218.61±9	23 (11)	258.63±108.1	18 (9.0)	41 (20.5)	0.012

APRI, Aspartate to platelet ratio index; T.WBC, Total white blood cells; FIB-4, Fibrosis-4 index; CR, Coronary ratio; AFP, Alpha feto-protein.

HBeAg seropositivity was stronger (co-efficient of 0.86 and 0.84, respectively) than BMI and WBC (co-efficient of 0.26 and 0.36, respectively). For the NMS group, AST/ALT ratio only correlated significantly with FBG and HBeAg seropositivity (*p*=0.02 and 0.024, respectively) (Table 2).

APRI also correlated significantly with BMI (*p* =0.021) only in the MS group and no parameter in NMS group. Similarly, FIB-4, correlated with only HBeAg seropositivity in both categories (*p* =0.032 and 0.024, respectively). AFP also correlated with VLDL, VAI and HBeAg seropositivity (*p*=0.040, 0.024 and 0.01, respectively) in the MS group and with only FBG (*p* =0.03) in the NMS category.

HBeAg seropositivity correlated with only VLDL in both categories (*p*=0.040 and 0.032, respectively) while CR correlated with VAI, VLDL and triglycerides (*p*=0.02, 0.032 and 0.03 respectively) in subjects with the MS.

Correlation of metabolic syndrome with hepatitis B related liver disease

Table 3 shows the correlation of MS and NMS groups with liver disease markers. MS category generally correlated with AFP, AST/ALT ratio, APRI and CR levels. The correlation varied with CR being the strongest, followed by AFP, AST/ALT ratio and APRI with p-values indicated in their category in Table 3. Subjects that did meet the criteria for metabolic syndrome correlated with FIB-4 (p=0.049) and CR (*p*=0.029).

CR was more associated with the MS markers (Table 4) having the lowest S-value but the highest R-square and R-square adjusted values as indicated in Table 4. Necrotic markers, AST/ALT and APRI, had similar strength of association of their respective related variables as shown. FIB-4 was weakly associated with MS markers having the highest S-value and lower R-

Table 2. Association of biochemical, viral serological and demographic parameters with metabolic syndrome.

Parameter	Metabolic syndrome	Without metabolic syndrome
AST/ALT	BMI-0.26 (0.012) FBG-0.86 (0.024) HBeAg-RR-0.84 (0.01) WBC-0.36 (0.027)	FBG-0.213 (0.02) HBeAg RR-0.18 (0.024)
APRI	BMI-0.26 (0.021)	None
FIB-4	HBeAg RR-0.86 (0.032)	HBeAg RR-0.86 (0.024)
AFP	VLDL-0.58 (0.040) VAI-0.68 (0.024) HBeAg RR-0.184 (0.01)	FBG-0.23 (0.03)
HBeAg seropositivity	VLDL-0.58 (0.040) VAI-0.92 (0.02) VLDL-0.58 (0.032) TRI-0.84 (0.03)	VLDL-0.58 (0.032) None

Values are represented as correlation coefficient in brackets the corresponding p-value. APRI, Aspartate to platelet ratio index; T.WBC, Total white blood cells; FIB-4, Fibrosis-4 index; CR, Coronary ratio; AFP, Alpha feto-protein.

Table 3. Correlation of metabolic syndrome with Hepatitis b related liver disease.

Category	Total n(%)	Female n (%)	Male n (%)	Liver disease markers	*p*-value
With metabolic syndrome	100 (50.0)	62 (31.1)	38 (18.0)	AFP AST/ALT APRI CR	0.01 0.01 0.017 0.003
Without metabolic syndrome	100 (50.0)	49 (24.5)	51 (25.5)	FIB-4 CR	0.049 0.029

Table 4. Extent of association of metabolic syndrome with liver diseases.

Liver disease marker	S-value	R^2-value (%)	R^2 (adjusted value) (%)
Metabolic syndrome group			
Hepatocellular necrosis			
AST/ALT ratio	0.61	10	5.8
APRI	0.62	11.9	6.8
Liver fibrosis			
FIB-4	2.11	6.1	0.6
Liver cirrhosis/HCC			
AFP	234.44	5	5
Cardiovascular disease risk			
CR	1.03	67.4	65.5
Non-metabolic syndrome group (Control)			
FIB-4	1.31	49.5	58.7
CR	3.3	63.7	52.7

square and R-square adjusted values as shown in Table 4. Generally the studied metabolic syndrome markers correlated with their liver disease markers in decrease strength from CR, APRI, AST/ALT ratio, AFP and FIB-4 using the indices shown in Table 4.

DISCUSSION

Subjects with abnormal levels of the studied liver and cardiovascular disease markers were higher in the metabolic syndrome category than NMS category except FIB-4. FIB-4 indicates the risk of hepatic fibrosis and poor extra-matrix protein turn over (Kasmari et al., 2017). FIB-4 correlated significantly with HBeAg seropositivity in both categories (p=0.032 and 0.024, respectively). FIB-4 was also strongly associated with the absence of metabolic syndrome than its presence (s=1.31, R=49.5%, R^2=58.7% and s=2.11, R=6.1%, R^2=0.6%) respectively. HBeAg seropositivty is an indication of high viremia, high rate of replication or infectivity and an immune escaping genotype (Kasmari et al., 2017). These suggest that, though metabolic syndrome poses risk to the development of liver and cardiovascular diseases, viral load and genotype contribute significantly to the development of hepatic fibrogenesis.

The necrosis stage of hepatic complication relies on host immune capacity to destroy HBsAg antigen displaying hepatocytes, minimize immune evasion and inhibit the c-Jun N-terminal kinase (JNK) pathway from initiating hepatic stellate cells synthesis. The fibrosis stage rather relies on microinflammation to initiate the JNK pathway and accumulate matrix protein (Dragut et al., 2016).

Alteration in AST/ALT ratio and APRI are markers of hepatocytes necrosis resulting from the viral infection, FIB-4 for the extent of fibrosis and AFP indicates the presence of substantial cirrhosis and hepatocellular carcinoma (Kaur, 2014, Boyd et al., 2017). AST/ALT ratio correlated significantly with BMI (p=0.012), fasting blood glucose (p=0.024) and HBeAg (p=0.01). APRI correlated significantly with BMI (p=0.021); AFP correlated significantly with FBG and VLDL (p=0.03 and 0.04) respectively while HBeAg significantly correlated with VLDL (p=0.040). Metabolic syndrome is a constellation of signs of both extra and intracellular energy metabolism disorder and the more the markers present, the higher the risk of development of the liver or cardiovascular diseases (Maud et al., 2017). These however suggest that all the risk factors do not contribute and if at all not equally to the development of the stages of liver disease. Further, high BMI, FBG and VLDL may contribute greatly to the development of liver disease than the other risk factors.

Metabolic syndrome influences the development of hepatic steatosis by fluxing the circulatory system with VLDL and small dense (sd)-LDL, generation of reactive oxygen species and induction of non-alcoholic fatty liver (Sugihara et al., 2016). VLDL and sd-LDL are precursors for the packaging of the viral outer protein coat, which is required for the virulence and replication of the virus (Li and Zhao, 2017). Fatty liver is also associated with lipotoxicity and generation of reactive oxygen species which is toxic to the membranes of the infected hepatocytes. Diabetes or impaired glucose utilization and visceral obesity are also associated with generation low grade inflammatory cytokines and tumor necrosis factor-1 which are essential initiators of the JNK pathway for the initiation of the hepatic stellate cells synthesis (Maud et al., 2017).

These outcomes agrees with Nau et al. (2014), where metabolic syndrome was significantly associated with the necrosis, fibrosis and cirrhosis stage of liver disease development (p=0.01). HBeAg seropositivity had positive significant correlation with AFP (p=0.01). HBeAg seropositivity indicates high infectivity or active replication suggesting the essence of virologic factors in the expansion of the hepatic stellate cells, extra matrix protein turn over disruption and subsequent morphological deformation of the liver. It further confirms the importance of metabolic precursors such as VLDL in the packaging of the outer protein coat of the virus which favor the development of hepatocellular carcinoma, cirrhosis and hepatoma (Changotra et al., 2008).

A study by Hsiang et al. (2014) confirmed that metabolic syndrome delays serum disappearance HBeAg positive cohorts by 18.9% (p=0.001). Metabolic syndrome was also used as baseline predictor of delayed HBeAg seroclearance when adjusted for viral load and antiretroviral therapy (Hsiang et al., 2014).

VLDL was associated with presence of HBeAg irrespective of the presence or absence of metabolic syndrome. VAI identified more obesed and overweight persons than the anthropometric obesity markers waist circumference and BMI. Metabolic syndrome is a factor of genetic, dietary and environmental disposition; it is therefore recommended that a follow up study on the role of genotypes, viral load and dietary roll call on the development of these liver conditions.

Metabolic syndrome was associated with the hepatocyte necrosis stage and development of hepatocellular carcinoma or cirrhosis stages, however viral load and genotype contribute significantly to the development of hepatic fibrosis.

Limitations of the study

The study did not take into consideration the role of the different genotypes of the hepatitis B virus as well as the viral loads which are also determinants of the clinical outcomes of the infection.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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