Biochemical Effects of Shea Butter and Groundnut Oils on White Albino Rats

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ABSTRACT

The physical and chemical properties of edible oils influence their suitability for use in food and other process industries. The aim of this study was to determine the physico-chemical properties, the components of shea butter, also the effect of shea butter on enzymatic activity in the liver, kidney and serum. Results obtained showed that Shea-butter has the following chemical properties: acid value (3.825), iodine number (43.27), peroxide value (12.85), saponification value (196.90), Total Cholesterol, Alanine aminotransferase and Aspartate aminotransferase activity found to be higher in the liver as a result of the intake of shea butter. Shea butter was found to contain triacylglycerol and free fatty acids using the thin layer chromatographic technique. Other physico-chemical properties quantified were moisture content (1.37%), ash content (1.26%), and melting point (27°C). These results showed that the physico-chemical properties of Shea butter are comparable with the properties of groundnut oil which is widely used for cooking and industrial food processes, and also reveals the richness of shea butter in nourishing the liver by increasing enzymatic activity.

INTRODUCTION

Worldwide, natural vegetable oils and fats are increasingly becoming important in nutrition and commerce because they are sources of dietary energy, antioxidants, bio fuels and raw materials for the manufacture of industrial products. They are used in food, cosmetics, pharmaceuticals and chemical industries. Vegetable oils account for 80% of the world's natural oils and fat supply (FAO, 2000). With increasing awareness of the importance of vegetable oils in the food, pharmaceutical and cosmetic industries, there is need to search for indigenous plant species that can provide such oils and characterize them. *Vitellaria* paradoxa (the Shea tree), an indigenous wild tree is one such plant of African savannah parkland (Hall *et al*. 2006). *Vitellaria* paradoxa tree has been included in the priority list of African Genetic Resources by the FAO (2002).

FIGURE 1; REFINED SHEA BUTTER.

Shea butter is a slightly yellowish or ivory-colored natural fat extracted from the nut of the African Shea tree (Vitellaria paradoxa) by crushing, boiling and stirring. It is widely used in cosmetics as a moisturizer, salve or lotion. Shea butter is edible and may be used in food preparation. Occasionally the chocolate industry uses Shea butter as a substitute for cocoa butter, although the taste is different. (Davrieux et. al; (2010). The English word "Shea" comes from s'í, the tree's name in the Bamana language of Mali. The French name karité comes from ghariti, its equivalent in the Wolof language of Senegal

Shea butter oil botanically called *Butyrospermum parkii* is a soft paste of melted fat with a milky colour in solid form and brownish when melted. It has a characteristic odour. It contains fatty acid triglyceride and a high amount of unsaponifiable matter, which ranges from 2.5% to 15% (Eka, 2001). This exceptionally rich vegetable extract contains fatty acids, phytosterol and unsaponifiable matter which stimulate the skin's natural renewal process. The composition of the product depends on several criteria particularly the geographical occurence, its botanical origin, handling of the seeds and processing e.g. drying time, ripening (Asintoke, 2000). According to (Tella 2003), Shea butter oil contains cinnamic acid, a substance that helps protect the skin from harmful ultra-violet rays. Equally, unrefined Shea butter oil is superior in that it retains all its natural vitamins, especially vitamins A and E. Crude Shea butter has natural anti-oxidant properties due to its tocopherol content (Asintoke, 2000). Shea butter oil has the following fatty acid composition-palmitic acid (C16) 8.5%, stearic acid (C18)35.9%, oleic acid (C18) 49.9% and linoleic acid (C18) 5.3% (Tooley, 2002).
The V. paradoxa nuts/seeds are usually processed into Shea butter oil that constitutes an important source of fat (Okullo *et al., 2004). Th*e Shea butter fat can also be used in soap making, cosmetics and traditional medicine in many rural areas, (Maranz *et al.*, 2004),
(Aulander, 2004). Due to its richness in food nutrients, the shea butter oil has found market as baking fat, like other fatty spreads in Europe and Asia (Akhter *et al* 2008) . The use of shea butter oil alone for cosmetics in U.S.A. has been growing at an annual rate of 25%.

Shea butter extract is a complex fat that contains many nonsaponifiable components (substances that cannot be fully converted into soap by treatment with alkali): oleic acid (40-60%), stearic acid (20-50%), linoleic acid (3-11%), palmitic acid (2-9%), linolenic acid(<1%) and arachidonic acid(<1%) (Manosroi, (2010).

Shea butter melts at body temperature and absorbs rapidly into the skin without leaving a greasy feeling (Tella, 2010).

Vegetable fats and oils are lipid materials derived from plants. Physically, oils are liquid at room temperature, and fats are solid Piombo(2009). Chemically, both fats and oils are composed of triglycerides, as contrasted with waxes which lack glycerine in their structure (Diallo2002). Although many plant parts may yield oil, in commercial practice, oil is extracted primarily from seeds.

Unsaturated vegetable fats and oils can be transformed through partial or complete hydrogenation into fats and oils of higher melting point. The hydrogenation process involves "sparging" the oil at high temperature and pressure with hydrogen in the presence of a catalyst, typically a powdered nickel compound N (Kalu 2006). As each carbon-carbon double-bond is chemically reduced to a single bond, two hydrogen atoms each form single bonds with the two carbon atoms. The elimination of double bonds by adding hydrogen atoms is called saturation; as the degree of saturation increases, the oil progresses toward being fully hydrogenated. An oil may be hydrogenated to increase resistance to rancidity (oxidation) or to change its physical characteristics. As the degree of saturation increases, the oil's viscosity and melting point increase (Eisenmenger et.al (2006).

The use of hydrogenated oils in foods has never been completely satisfactory. Because the centre arm of the triglyceride is shielded somewhat by the end fatty acids, most of the hydrogenation occurs on the end fatty acids, thus making the resulting fat more brittle. A margarine made from naturally more saturated oils will be more plastic (more "spreadable") than a margarine made from hydrogenated soy oil (Kikuchi 2008).While full hydrogenation produces largely saturated fatty acids, partial hydrogenation results in the transformation of unsaturated cis fatty acids to trans fatty acids in the oil mixture due to the heat used in hydrogenation. Since the 1970s, partially hydrogenated oils and their trans fats have increasingly been viewed as unhealthy. Ghani". Banglapedia.). the Standard of Identity for a product labelled as "vegetable oil margarine" specifies only canola, safflower, sunflower, corn, soybean, or peanut oil may be used. Products not labelled "vegetable oil margarine" do not have that restriction.

INDUSTRIAL USES: Vegetable oils are used as an ingredient or component in many manufactured products (Beare-Rogers 2003)., in production of some pet foods, also used to make biodiesel, which can be used like conventional diesel (Vance2001).

EXTRACTION: The "modern" way of processing vegetable oil is by chemical extraction, using solvent extracts, which produces higher yields and is quicker and less expensive (Warner 2002). The most common solvent is petroleum-derived hexane. This technique is used for most of the "newer" industrial oils such as soybean and corn oils (Hornstra 2002).

Another way is physical extraction, called "crushing", which does not use solvent extracts. It is made the "traditional" way using several different types of mechanical extraction. This method is typically used to produce the more traditional oils (e.g., olive, coconut etc.), and it

is preferred by most "health-food" customers in the USA and in Europe (Gurr 2003) .Expeller-pressed extraction is one type, and there are two other types that are both oil presses: the screw press and the ram press. Oil seed presses are commonly used in developing countries, among people for whom other extraction methods would be prohibitively expensive. The amount of oil extracted using these methods varies widely, as shown in the following table for extracting mowrah butter in India (Lerman, 2003).

Crude oil, straight from the crushing operation, is not considered edible in the case of most oilseeds. The same is true for the remaining meal. For instance, animals fed raw soy meal will waste away, even though soy meal is high in protein. Researchers at Central Soya discovered that a trypsin inhibitor in soybeans could be deactivated by toasting the meal, and both licensed their invention, and sold soy meal augmented with vitamins and minerals as MasterMix, a product for farmers to mix with their own grain to produce a high quality feed. (Troisi, 2002).

The processing of soy oil is typical of that used with most vegetable oils. Crude soy oil is first mixed with caustic soda. Saponification turns triglycerides into soap. The soap is removed with a centrifuge. Neutralized dry soap stock (NDSS) is typically used in animal feed, more to get rid of it than because it is particularly nourishing. The remaining oil is deodorized by heating under a near-perfect vacuum and sparged with water. The condensate is further processed to become vitamin E food supplement, while the oil can be sold to manufacturers and consumers at this point. (Hennekens,2007).

Some of the oil is further processed. By carefully filtering the oil at near-freezing temperatures, "winter oil" is produced. This oil is sold to manufacturers of salad dressings, so that the dressings do not turn cloudy when refrigerated (Ascherio,2004).

The oil may be partially hydrogenated to produce various ingredient oils. Lightly hydrogenated oils have very similar physical characteristics to regular soy oil, but are more resistant to becoming rancid.

Margarine oils need to be mostly solid at 32 °C (90 °F) so that the margarine does not melt in warm rooms, yet it needs to be completely liquid at 37 °C (98 °F), so that it doesn't leave a "lardy" taste in the mouth.(Sampugna,2000).

Another major use of soy oil is for fry oils. These oils require substantial hydrogenation to keep the polyunsaturates of soy oil from becoming rancid. Hardening vegetable oil is done by raising a blend of vegetable oil and a catalyst in near-vacuum to very high temperatures, and introducing hydrogen. This causes the carbon atoms of the oil to break double-bonds with other carbons, each carbon forming a new single-bond with a hydrogen atom (Monoj,2007).

Hydrogenated vegetable oils differ in two major ways from other oils which are equally saturated. During hydrogenation, it is easier for hydrogen to come into contact with the fatty acids on the end of the triglyceride, and less easy for them to come into contact with the centre fatty acid (Michael, 2002).This makes the resulting fat more brittle than a tropical oil; soy margarines are less "spreadable". The other difference is that trans fatty acids (often

called trans-fat) are formed in the hydrogenation reactor, and may amount to as much as 40 percent by weight of a partially hydrogenated oil. Trans acids are increasingly thought to be unhealthy. (Kadam,2002)

In the processing of edible oils, the oil is heated under vacuum to near the smoke point, and water is introduced at the bottom of the oil. The water immediately is converted to steam, which bubbles through the oil, carrying with it any chemicals which are water-soluble. The steam sparging removes impurities that can impart unwanted flavours and odors to the oil.(Emken,2004).

MATERIALS

Shea butter seeds were obtained from Saaki in Oyo state, while processed shea butter was obtained from Akure, White albino rats were obtained at Ayoola farm in Ibadan.

2.2 REAGENTS

Hexane and all other reagents were purchased from Sigma Aldrich Chemicals Pvt , Ltd, Nigeria.

METHODS;

ETHER EXTRACT DETERMINATION: The ether extracts or oil content is the amount of the lipid present in the sample. The lipid content of the shea butter seed was determined by the AOAC method of analysis using the soxhlet extractor.

PURIFICATION OF EXTRACTED LIPID: The method described by fold et al (2007) was employed to purify the lipid extracted from the sample.

PHYSICOCHEMICAL PROPERTIES OF SHEA BUTTER

IODINE VALUE: Iodine value measures the degree of unstauration in a fat or vegetable oil (i.e. The number of double bonds) (Daintith, 2008). It determines according to the method of A.O.A.C (2001).

SAPONIFICATION VALUE: The saponification value helps determine the quantity of potassium (in mg) needed to neutralize the acids and saponify the esters contained in 1g of lipid (Roger *et al.*,2010) and it was carried out according to standard method described by A.O.A.C (2001).

DETERMINATION OF TOTAL ASH CONTENT The standard method described by A.O.A.C (2001) was used.

MOISTURE CONTENT: This is carried out in order to determine the moisture content of the shea butter sample.

ESTER VALUE

Ester value represents the number of milligrams of potassium hydroxide required to saponify the esters present in 1g of the oil. It is obtained as the difference between the saponification value and the acid value.

Calculation;
Ester value=saponification value-acid value

ACID VALUE: Acid value corresponds to the amount of potassium hydroxide needed to neutralize free fatty acid. The lower the acid value of an oil, the fewer free fatty acids it contains which makes it less exposed to the phenomenon of rancidification (Roger *et al.*,2010)

CHROMATOGRAPHIC SEPERATION OF LIPIDS
PRINCIPLE
The principle is based on differential affinities of the separating solutes in a given mixture of solvents for the absorbent. The affinity results due to Vander Waal forces. In the inorganic mobile phase, the solutes are eluted and consequently separated into different degrees depending on their affinities for the adsorbent. Silica gel is most often used as the absorbent in this study.

FEEDING FORMULAR
The feed was formulated by using already percolated proportion by weight of corn starch, soya bean meal, sucrose, vitamins-minerals(pre-mixed)the feed was compounded with shea butter oil and groundnut oil while the control contained neither of the oils in appropriate amounts as shown below;

FEED COMPOSITION	SHEA BUTTER BASED DIET(g)	GROUNDNUT OIL BASED DIET(g)
Corn starch	516	516
Soya bean meal	250	250
Cellulose	40	40
Sucrose	60	60
Shea butter oil	250	--
Groundnut oil	--	250
Minerals and vitamin mixture	50	50
D-L mixture	4	4

TABLE 1; FEEDING FORMULA.
Values are given as grams per 1170g of diet.
Mineral and vitamins premix contain: vitamin A 10,000,000iu, vitamin D,2,000,000iu, vitamin E 12,000iu, vitamin k 20000iu, vitamin B12 10,000mg, thiamine 1,500mg, riboflavin-B2 5,000mg,pyridoxine-B 500mg, biotin 20mg, niacin 15mg, pantothenic acid 5,000mg, folic acid 500mg, manganese 750mg, zinc 45,00mg, iron 20,000mg, copper 5000mg, iodine 1000mg, selenium 100mg, cobalt 200mg, choline chloride 100,000mg, B.H.T 125,000mg.
Rate of inclusion =2.5kg per 1 tonne of animal feeds

THE EXPERIMENTAL ANIMAL
9 male, and 16 female rats (Rattus novergious) of three weeks were obtained and grouped into four groups,
group A contained 3 female rats
group B contained 3 male rats,
group C contained 3 female rats,
group D contained 3 male rats

group E contained 3 male rats.
group A and B were fed with the shea butter feed,
group C and D were fed the groundnut oil feed,
group E was fed the feed that contained none of the oil, this group served as the control.

ANIMAL MAINTENANCE

The rats were kept in wooden cages barred with steel nets, and were constantly fed and supplied with water. Their surroundings were also cleaned and disinfected to maintain proper hygiene.

SACRIFICE OF ANIMALS

After being fed for two weeks and closely monitored the animals were sacrificed placing them in a jar containing cotton wool soaked in chloroform.

BIOCHEMICAL ASSAYS

Serum, liver and kidney enzymatic analysis were carried out for Alkaline Phosphatase(ALP), Aspartate aminotransferase(AST), Alanine aminotransferase(ALP), Total cholesterol, Total Billirubin were determined using commercially available enzymatic test kits (Randox Laboratories Ltd, San Francisco, USA) method following the manufacturer's instructions.

RESULTS

PHYSICOCHEMICAL PROPERTIES OF SHEA BUTTER

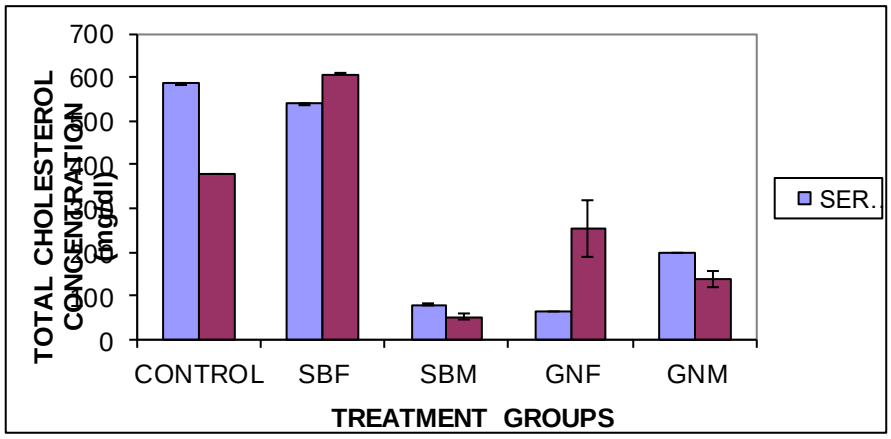
1.	Colour	Milky -cream
2.	Clarity	Fat (solid
3.	Odour	Milky odour
4.	Taste	Tasteless
5.	melting point	51-56°C
6.	Peroxide value	14.2
7.	Moisture content	10%
8.	Iodine value	63.45
9.	Saponification value	185.20
10.	Ester value	183.4
11.	Acid value	1.79
12.	solubility's	
(i)	Chloroform	soluble
(ii)	Petroleum ether	Partially soluble
(iii)	Carbon tetrachloride	Partially soluble
(iv)	Ethanol	soluble
(v)	Water	insoluble
(vi)	Hexane	Soluble
13.	Ash content	1.26%

TABLE 2; PHYSICOCHEMICAL ANALYSIS OF SHEA BUTTER.

SPOTS	OBTAINED Rf	STANDARD Rf	CORRESPONDING

	VALUE	**VALUES**	**NEUTRAL LIPIDS**
1	0.7391	0.670-0.740	Triacylglycerol
2	0.4637	0.325-0.440	Free fatty acid

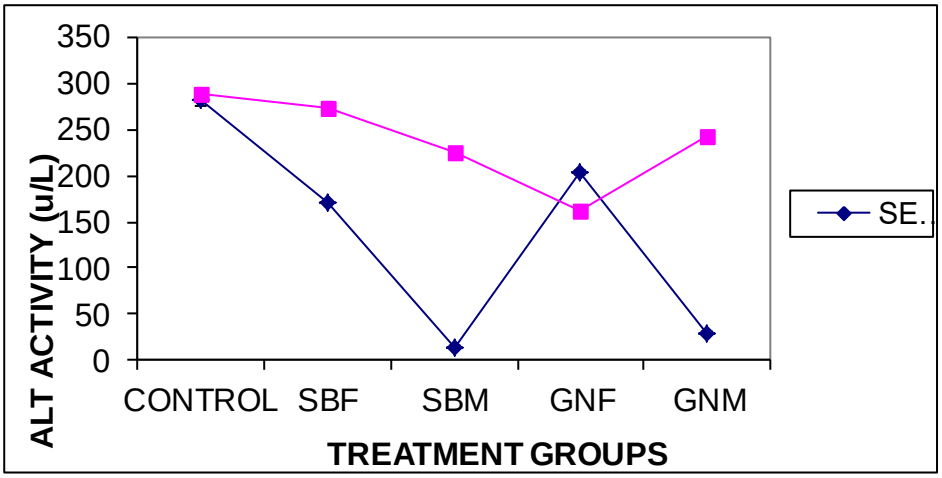
TABLE 3;THIN LAYER CHROMATOGRAPHIC VALUE



DETERMINANT OF 3 RATS ± 5.O

FIGURE 5: Effect of shea butter and groundnut oils on serum and liver cholesterol concentration in female and male Albino rats

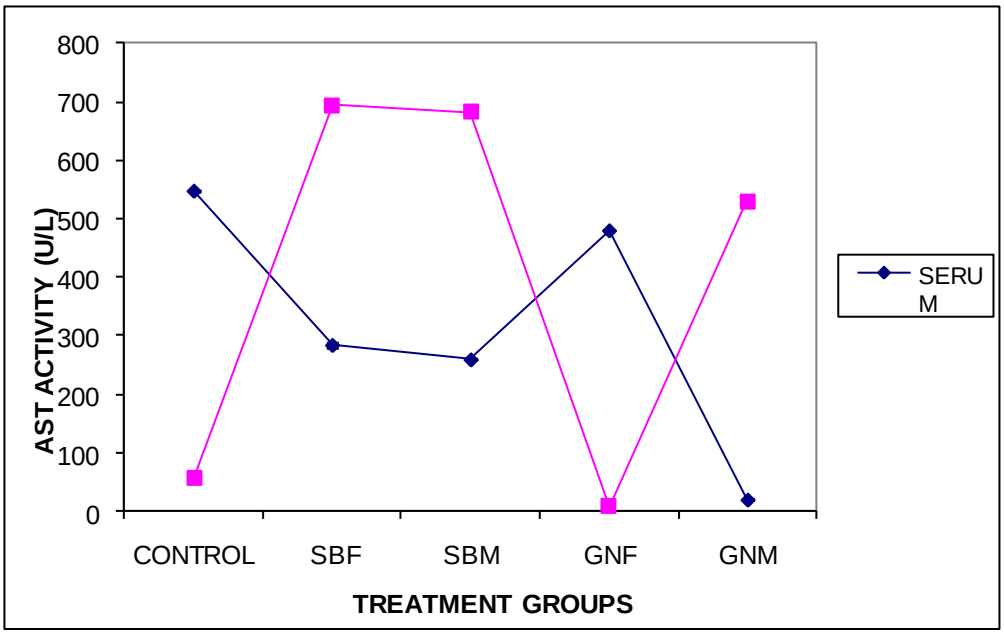
NOTE: SBF-Shea butter female
SBM-Shea butter male
GNF-Groundnut oil female
GNM-Groundnut oil male



EACH GROUP IS A DETERMINANT OF 3 RATS ± S.D

FIGURE 6: Effect of shea butter and groundnut oils on serum and liver ALT activity in female and male Albino rats

NOTE: SBF-Shea butter female
SBM-Shea butter male
GNF-Groundnut oil female
GNM-Groundnut oil male



EACH GROUP IS A DETERMINANT OF 3 RATS ± 5.0

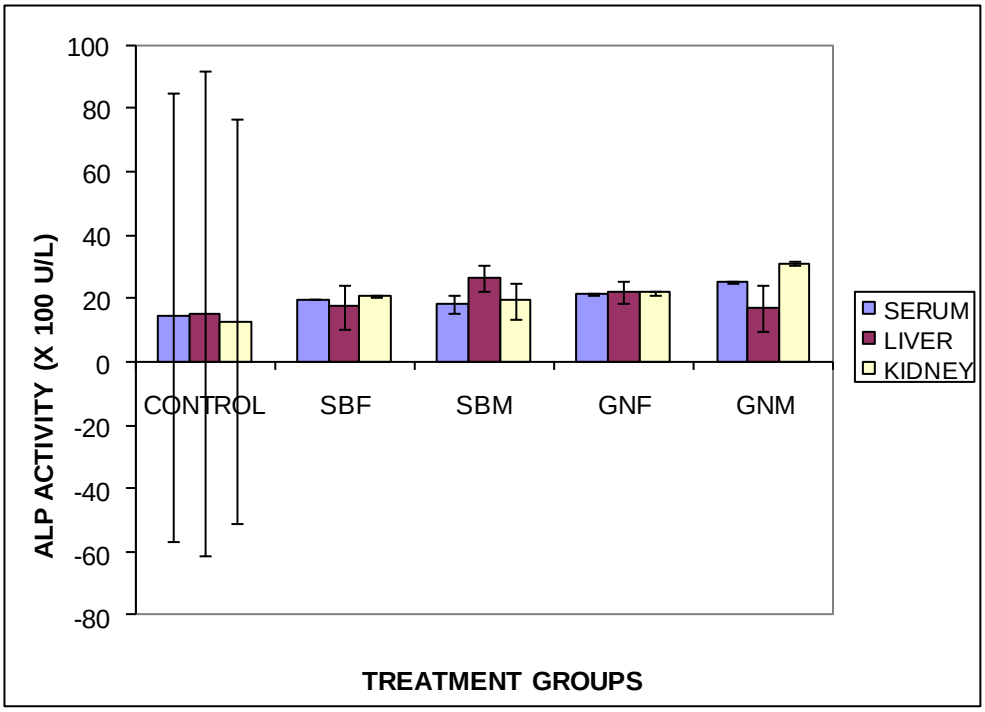
FIGURE 7: Effect of shea butter and groundnut oils on serum and liver AST activity in female and male Albino rats

NOTE: SBF-Shea butter female

SBM-Shea butter male

GNF-Groundnut oil female

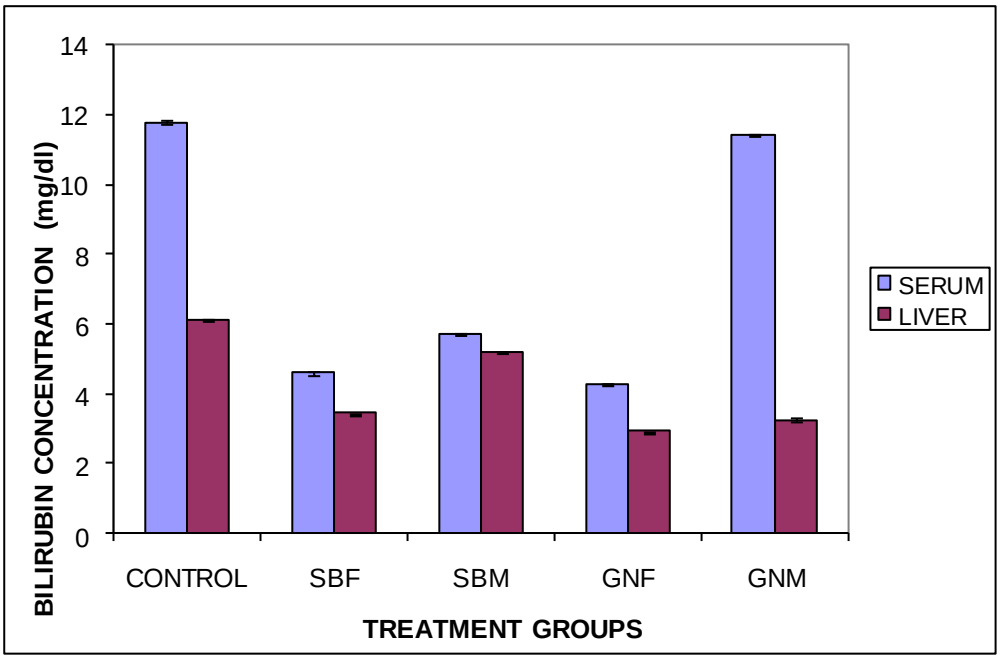
GNM-Groundnut oil male



EACH GROUP IS A DETERMINANT OF 3 RATS ± 5.0

FIGURE 8: Effect of shea butter and groundnut oils on serum and liver ALP activity in female and male Albino rats

NOTE: SBF-Shea butter female
SBM-Shea butter male
GNF-Groundnut oil female
GNM-Groundnut oil male



EACH GROUP IS A DETERMINANT OF 3 RATS ± 5.0

FIGURE 9: Effect of shea butter and groundnut oils on serum and liver bilirubin concentration in female and male Albino rats

NOTE: SBF-Shea butter female
SBM-Shea butter male
GNF-Groundnut oil female
GNM-Groundnut oil male

DISCUSSION

The physicochemical composition of shea butter oil obtained in this study also conforms to the regional standards for shea butter oils (Regional Technical Committee, 2007). The thin layer chromatography carried out indicated via the Rf which was 0.739 and 0.4360 value obtained and compare to the standard value reflects the presence of triacyglycerol and free fatty acids are present in shea butter.

Cholesterol level in the control was found to be higher in the serum than in the liver, while the cholesterol level in the shea butter female is higher in the liver than in the serum. in the groundnut oil female the cholesterol level was high in the liver but very low in the serum As shown in (Figure 8). The cholesterol level in the shea butter male was found to be low in both liver and serum, while in groundnut oil male, the cholesterol level was higher in the serum than in the liver. There is a significant difference in the cholesterol level of all groups. The shea butter female have the highest level of cholesterol than all other groups due to the intake of shea butter which contains triacylglycerol and fatty acids being deposited (Asuquo, 2008). It was found to be low in the shea butter male because the male rats did not eat as much as the female rats during feeding. The groundnut oil female and male have a high cholesterol level in their serum than in

the livers because of the monounsaturated, polyunsaturated,saturated and trans fat which are not metabolised and deposited in the serum.(Abayeh *et al.*,2000)

The activity of ALT in the liver which was 270u/l and serum which was 260u/l of the control was found to be higher in the control compared to the other groups. The activity of the enzyme was also high in the liver of shea butter female with a value of 262u/l almost as high as that of the control, but lower in the serum which is 155u/l, in groundnut oil female. The activity of ALT is higher in the serum with a value of 202u/l than in the liver which is 153u/l.In shea butter male it is higher in the liver 220u/l but very low in the serum 155u/l, compared with groundnut oil male which is higher in the liver 239u/l than in the serum 35u/l. There is a significant level of difference in the activity of the enzyme in all groups as shown in (Figure 6).

The activity of AST in the serum of the control was found to be higher compared to the liver. But in shea butter female the activity is the highest in the liver and lower in the serum. However, the activity of the enzyme in the liver 20u/l was lowest in the liver of groundnut oil female and higher in the serum 479u/l.the activity of the enzyme is also equally high in the liver 778u/l of shea butter male and lower in the serum 270u/l.As shown in(Figure 7)while in groundnut oil male the activity is higher in the liver 530u/l than in the serum 40u/l. Sex difference causes a significant difference in the activity of AST in all groups(Dörmann P,(2007).

The activity of ALP in the liver19u/l of the control was slightly higher than in the serum18u/l,and very low in the kidney 16u/l,in shea butter female the activity of the enzyme is lower in the liver 18u/l and high in the kidney 19u/l and in the serum19u/l as shown in(Figure 8), in groundnut oil female it is high in both the kidney and liver but slightly lower in the serum18u/l.but in shea butter male the activity of the enzyme is higher in the liver22u/l and equally lower in the kidney16u/l and serum17u/l ,in groundnut oil male it is higher in the kidney24u/l and slightly high in the serum 20u/l but it is lower in the liver 5u/l. In summary, from the biochemical assay of the enzyme activity it can be deduced that shea butter oil improves enzymatic activities in the liver, compared to that of groundnut oil which was used as the control oil. This compares favourably to the findings of (Tella, 2009), which shows that shea butter competes favourably with other conventional oils.

The bilirubin level in the serum is 11.7u/l for the control which is the highest compared to other groups, and lower in the liver 6u/l. In shea butter female it is higher in the serum than in the liver 3.6u/l, in groundnut oil female it is also higher in the serum 4.2u/l than in the liver. In shea butter male it is higher in the serum 3.8u/l than in the liver 3.4u/l. However in the serum11.6u/l of groundnut oil male it is almost as high as that of the control but considerably lower in the liver3.1u/l as shown in (Figure 9). This indicates that shea butter and groundnut oil have no effect in the total bilirubin concentration. (Honke et. al 2004).

CONCLUSION

This study was undertaken to determine the physico-chemical characteristics of the oil obtained from shea butter seeds from Saaki, Oyo state, Nigeria. The properties are in agreement with that of shea butter oil in the West African sub-region. The study has helped in filling the literature gap in this area of oil studies for Nigeria and globally, as the information from literature review is scanty. It also places Nigerian shea butter oil in the world map for various uses including domestic and industrial applications. It has also helped to broaden and widen the scope on the biochemical effect of shea butter and groundnut oil in the system.

RECOMMENDATION

Since shea butter as discovered from this research is a good source of oil that nourishes the liver with fat and promotes enzymatic activity, it is therefore recommended that further research should be carried out on it to determine its enormous potentials.

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