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RESEARCH ARTICLE

Food Technology

Comparison of physicochemical and sensory properties of African butter seed (*Pentadesma butyracea*) and cocoa fats for potential use in future food applications

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Abstract: African butter (*Pentadesma butyracea*) seeds are rich in edible fat, while cocoa butter is an expensive product obtained from fermented and dried cocoa beans. The aim of our study was to analyze the physicochemical and sensory properties of African butter seed fat and cocoa fat while determining their potential food applications. Chemically extracted fat was utilized for determination of physicochemical properties according to AOCS guidelines. Physically extracted fat was utilized for preparation of cookies and determination of sensory properties. Acid value, free fatty acid value and iodine value of African butter seed fat and cocoa fat were 1.05 ± 0.17 vs. 2.06 ± 0.14 mg KOH/g, 0.53 ± 0.09% vs. 1.14 ± 0.07% and 48.65 ± 3.03 vs. 34.31 ± 0.97 g I_2/100g respectively. Saponification values of African butter seed fat and cocoa fat were 177.0 ± 0.6 mg KOH/g and 194.2 ± 1.1 mg KOH/g respectively. Between 25 and 30 °C, the solid fat content ranged from 31.8 ± 0.05% to 6.85 ± 0.07% for African butter seed fat and 36.14 ± 0.87% to 11.15 ± 0.11% for cocoa fat. The contents of stearic and oleic acids which are abundant in African butter seed fat were 39.05 ± 0.16% and 56.97 ± 0.27% respectively while those of in cocoa fat were 37.75 ± 0.06% and 34.12 ± 0.14%. Results of hedonic test performed for cookies prepared by incorporating the two kinds of fats highlighted that there was a significant difference relative to the preference ($p < 0.05$) for colour, while there was no significant difference with respect to the preference for flavour, texture and overall acceptability ($p > 0.05$). There is a high potential to develop African butter seed fat as a fat spread, a cocoa butter alternative and in preparation of cookies.

Keywords: African butter seed fat, cocoa fat, cookies, fatty acid profile, physicochemical properties, sensory properties.

INTRODUCTION

African butter trees (*Pentadesma butyracea*) are naturally found in Africa from Guinea Bissau to the western regions of the Republic of Congo. In Sri Lanka, several plants are grown at the Royal Botanical Garden, Peradeniya and also, a small plantation could be seen in Hunuwala estate, Kahawatta. 'Tallow tree' and 'butter tree' are commonly used English names for *Pentadesma butyracea* plants (Ayegnon *et al.*, 2015). These plants belong to the family of *Clusiaceae* and they can grow up to a height of about 20 m (Tchobo *et al*., 2007). Butter tree kernels are found to be rich in edible fat and fresh kernels are consumed like kola in African regions (Ayegnon *et al.*, 2015). Different communities of the northern regions of Benin use tallow seed fat for edible purposes, mainly as a cooking oil rather than therapeutic and cosmetic applications. They employ various traditional processing techniques in preparation of butter from tallow seeds. Various pretreatment steps like boiling of fresh kernels followed by different drying techniques such as solar drying, direct sun drying, and smoking are employed, prior to the extraction which is associated with steps such as heating, crushing, milling, and churning (Badoussi, 2014).

Cocoa butter is a valuable product obtained from the cocoa bean processing industry. It is manufactured by pressing cocoa liquor obtained from grinding cocoa nibs separated from mature, fermented, and dried cocoa

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beans (Beckett, 2009). Blending of cocoa butter in chocolates and confectionery products yields numerous unique physicochemical and sensory properties, such as melting characteristics in mouth with typical mouth feel. Because of the prevailing gap between the supply and demand with respect to cocoa butter, the industry is switching to focus on cocoa butter alternatives at present. Shea butter is used as a cocoa butter alternative in the manufacturing of chocolate and confectionery items in Europe (Naik & Kumar, 2014). Palmitic, stearic, and oleic are the three major fatty acids common to African butter seed fat and shea butter (Adomako, 1977). Therefore, there is a potential to develop African butter seed fat as a cocoa butter alternative.

In the manufacture of a fat spread, different vegetable oils including palm, corn, cottonseed, soy, safflower, and sunflower oils are used. In order to obtain the semi solid nature of the fat spread either partial hydrogenation or blending of oils having different solid fat contents is required. Artificial colourants are also added to obtain a desirable yellow colour in some types of fat spread (Marcus, 2013). Because of the semi solid nature at room temperature and the unique bright yellow colour of African butter seed fat, hydrogenation and addition of colourants are not required. Therefore, the objectives of this study were to determine the applicability of African butter seed fat as a cocoa butter alternative, a type of fat spread, and its suitability in the preparation of cookies via determination of physicochemical properties of African butter seed fat and cocoa fat. Furthermore, a cookie product has been developed by incorporating African butter seed fat and cocoa fat separately and their sensory properties have been compared in order to evaluate their applicability in bakery products such as cookies.

MATERIALS AND METHODS

Materials and chemicals

Seeds of the African butter tree (*Pentadesma butyracea*) were obtained from the Royal Botanical Garden, Peradeniya, Sri Lanka. Ripened cocoa pods were obtained from the Export Agriculture Department, Matale, Sri Lanka.

Absolute ehanol, hydrochloric acid, potassium hydroxide, phenolphthalein, glacial acetic acid, potassium iodide solution, sodium thiosulphate, starch, Wijs solution, cyclohexane, n-hexane, sodium hydroxide, methanol, and concentrated sulphuric acid were purchased from Sigma Aldrich, USA. In addition, authentic standard for gas chromatography (SUPELCO 37 component FAME Mix, 1 x1 mL varied conc, in dichloromethane) and single standards were used for fatty acid profile analysis, with a column capillary gas chromatograph (Agilent HP-88; 100m length, 0.25mm ID).

Pretreatments and pre-preparation techniques

African butter seeds were obtained by removing the mesocarp of fallen fruits and subjected to washing and cleaning, followed by sun drying for 3 ds with 6 h of exposure to sunlight on each day. Then the seeds were cracked by using a hammer (4340 alloy steel) and the cracked pieces were sun dried on raised white polythene mats for 5 ds with 3 h of exposure to the sunlight per day. Cocoa beans were separated from pods and were fermented for 5 ds. Fermented seeds were sun dried on raised white polythene mats for 5 ds with 6 h of exposure to the sunlight. At the end of each hour, African butter seed pieces and cocoa beans were turned and mixed with hands covered with gloves.

A portion of each dried source (African butter seeds and cocoa beans) was ground into a fine powder by using a grinder (Jaipan, Model No.1000). Seed powder was stored in air tight plastic containers to be used in solvent extraction. The remainder of each type, were kept in non-ground form, to be used in physical extraction. The moisture content of the ground seed samples of African butter seeds and cocoa beans was determined (AACC 44-15A) prior to the chemical extraction of fat.

Extraction of fat

Fat was extracted by physical and chemical methods (Physical extraction was employed to avoid the contamination of fat samples with non food grade chemicals, since the cookies are evaluated by the panelists). Physical extraction was conducted using an industrial mini expeller (screw press, single barrel, alloy: steel and

cast iron). Extraction of oil was performed by feeding cracked seed parts, 2-3 cm in average size, to the expeller in small batches. A single extraction was performed for 5 min and the expeller was kept switched off for 10 min under ventilation via an electric fan. This process of extraction was continued with intervals of 10 min. Initially, fat was collected to aluminium vessels and such collected fats were filtered via a muslin cloth into glass containers and stored in freezing conditions (minimum level -2 ^{o}C) in a refrigerator until they were used for preparation of cookies.

Chemical extraction was performed using hexane in a Soxhlet apparatus. The extraction was carried out for 6 h. After the extraction procedure hexane was evaporated off using a rotary evaporator (Heidolph 2000) at 65 ^{o}C. Extracted fats were stored in labelled air tight glass containers under refrigerated conditions (4-6 ^{o}C) until they were taken for physicochemical tests. Oil yield from chemical extraction was determined before subjecting the extracted fats to physicochemical tests.

Determination of physicochemical properties

Slip melting point (Cc 3-25, AOCS 1998), smoke point (Cc 9a -48, AOCS 1998) and chemical properties such as acid value and free fatty acid content (Cc 5a-40, AOCS 1998) and iodine value (Cd Id-92 AOCS 1998), saponification value (Cd 3-25 AOCS 1998) were determined. In addition, colour was determined by using colorimeter (CHN Spec, CS-10) and expressed as L*, a*, b* values. All the measurements were taken for three replicates and the values were expressed as mean ± sd.

Solid fat content was measured at 10 different selected temperatures from 0-45 ^{o}C by using a Bruker NMR analyzer (ISO, 2008). Fat sample was initially melted at 80-100 ^{o}C for 15 min. A residue free well mixed sample was selected and filled in to 10 mm diameter sample tubes to a height of 4 cm. The sample tubes were maintained at 60 ^{o}C for 5 min and then at 0 ^{o}C for 60 min. Then the sample was transferred to the temperature at which the particular measurement is needed and maintained at that temperature for 30 min. The samples were measured in triplicates at each selected temperature, 0 ^{o}C, 5 ^{o}C, 10 ^{o}C, 15 ^{o}C, 20 ^{o}C, 25 ^{o}C, 30 ^{o}C, 35 ^{o}C, 40 ^{o}C, and 45 ^{o}C. Each sample was inserted into the pulsed NMR spectrometer for a 6 s analysis and a curve was created representing free induction decay (FID) vs. time while displaying the reading of SFC percentage on the monitor at the particular temperature concerned was read off the display on the monitor.

The fatty acid composition of each type of fat was analysed according to the method described by O'Fallon *et al.* (2007) with some modifications. The internal standard C17:0 (10.3 mg dissolved in 1 mL of dichloromethane) was added into a screw capped pyrex culture tube. This was dried under a stream of N_2 gas. Then, 40 µL of the oil sample was added to the culture tube and weighed. Then 0.7 mL of 10 N KOH and 5.3 mL of methanol were added to culture tube and vortexed for 30 seconds. The tubes were incubated in a water bath set at 55 ^{o}C for 1.5 h with vigorous shaking for every 20 min. The tubes were allowed to cool in cold tap water to bring to room temperature. Then, 0.58 mL of 24 N H_2SO_4 was added to each tube, the mixture was vortexed well and incubated at 55 ^{o}C for 1.5 h with vigorous shaking every 20 min. After completion of the incubation, each tube was cooled in tap water. Following that, about 3 mL of n-hexane was added, and the tube vortexed for 3 min and centrifuged for 5 min at 2000 rpm. The hexane layer was collected into a 5 mL test tube containing 0.1 g of Na_2SO_4. Then it was decanted, vortexed for 10 s, allowed to settle, dried under stream of N_2 and 1.5 mL of dichloromethane added. The tube was vortexed for 10 s and kept for 5 min. After that, the dichloromethane layer was transferred into a GC autosampler vial, and it was dried under N_2 gas. Finally, the volume was made up with 0.5 mL Suprasolv dichloromethane and the sample analyzed using GC-FID.

The weight (in g) of each individual fatty acid (W_X) in 100 g of test sample is expressed as

$$W_X = \frac{A_X \times W_{IS} \times 1.0047 \times R_X \times F_{FAX} \times 100}{A_{IS} \times W_{TS}} \quad \ldots(1)$$

where,

A_X - Peak area counts for $FAME_X$ in test sample
W_{IS} - Weight of C17:0 TAG internal standard (in g) added to the test sample

1.0047 - Conversion of C17:0 TAG internal standard from TAG to FAME
R_X - Theoretical flame ionization detector response factor (TCF) for FAMEs relative to 17:0 FAME internal standard
F_{FAX} - Conversion factor for conversation of $FAME_X$ in the test sample to its corresponding fatty acid
A_{IS} - Peak area counts of the C17:0 FAME internal standard added to the test sample as 17:0 TAG
W_{TS} - Weight of test sample in g

GC-parameters:
Injection port temperature: 250 ^oC; detector temperature: 250 ^oC; oven temperature: isothermal at 180 ^oC (32 min), ramped at 20 ^oC/min to 215 ^oC (hold 31.25 min); carrier gas: Helium; column head pressure 286 kPa, flow rate 1.0 mL/min, linear velocity 19 cm/s, split ratio 100:1. As the capillary column, Agilent HP-88 (100m length, 0.25mm ID, stationary phase of 88%-Cyanopropyl 12% -aryl-polysiloxane) was used.

Preparation of cookies

This procedure was followed for both African butter seed fat and cocoa fat. Initially, 40 g of fat was allowed to melt. Then 35 g of finely ground and sieved sugar was added in to the melted butter and it was followed by the addition of 80 g of wheat flour. After that, half of an egg yolk and 1 mL of vanilla essence were added and mixed. Then round shapes were made using a mold and baking was done using an oven at 150 ^oC for 15 min.

Determination of sensory properties of cookies prepared by incorporating the two fats separately

Similar to the acceptance test as described by Choi (2013), a set of 30 subjects were selected randomly for the study as panelists. Those subjects were within the age range of 20-60 years, belonging to both genders, and residing in the Polgahawela city area, North Western province, Sri Lanka. Subjects met following criteria; freedom from any oral disease, freedom from systemic diseases, non smoking, non-alcoholic, not chewing betel, and not under medication as described by Mabbithasri *et al*. (2020). All the panelists were informed in detail about the study after obtaining their consent to participate. A single cookie of weight 5-6 g made by incorporating each type of fat was offered on a white-coloured ceramic dish to every randomly selected panelist to get sensory properties (colour, flavour, texture and overall acceptability) evaluated based on their preference. Fifteen randomly selected subjects were presented with African butter seed fat incorporated cookies initially and then cookies incorporated with cocoa fat while it was done vice versa for the other 15 subjects in order ensure the randomness of presenting the samples. They were advised to cleanse their mouth with warm water (60 ^oC) before and after evaluating flavour of each sample. The main tool used in collecting data was a ballot paper comprised of 05-point hedonic scale based on consumer preference from like very much to dislike very much as described by Choi (2013). The collected responses were analyzed via Wilcoxon signed rank test by using SPSS (version 25).

RESULTS AND DISCUSSION

The moisture contents of the ground seed powder of African butter seeds and cocoa beans were determined to be 5.82% and 6.24% respectively. Employing a proper drying technique is essential to remove moisture, to facilitate the removal of oil from tissues during extraction and to inactivate some enzymes such as lipase. Oil yields of African butter seeds and cocoa beans via chemical extraction were 38.94% and 44.23% respectively. Tchobo *et al.* (2007) have shown that the oil yield of African butter seeds obtained from 10 different regions of Benin varies from 39.1 to 47.3%. The oil yield obtained for cocoa beans in the present study is lower than the value revealed by Adomako (1976), which was determined to be 53.4%. The oil yield might have been influenced by some factors such as geographical location, climate, and variety.

Physicochemical properties of African butter seed fat and cocoa fat

Table 1 shows physicochemical properties of African butter seed fat and cocoa fat. Slip melting point of African butter seed fat is closer to the value of 37.5 - 38.2 ^{o}C as reported by Adomako (1976). The slip melting point of cocoa butter also falls in the melting point range of 29 - 40 ^{o}C as reported by Naik and Kumar (2014). As shown by Tchobo *et al.* (2013), the average L* (lightness), a* (redness) and b* (yellowness) values obtained for African butter seed fat that was extracted using the traditional method (grinding and then churning with hot water until fat is separated) are L = 63 ± 20, a = -2.06 ± 1.64 and b = 24.73 ± 1.11. L* a* b* values of African butter seed fat that was extracted mechanically by using a screw expeller in the current study, also show closer values to the fat extracted by traditional means. Colour gets affected mainly due to different postharvest handling practices of the seeds such as drying. A dark-coloured fat is obtained when the seeds are not dried properly and during rainy seasons, as described by Ayegnon et al (2015). The temperature during the expulsion of oil also might influence the colour of the extracted fat. Since the colour values determined in the present study are closer to the previous study, it can be assumed that approximately similar conditions had been employed during postharvest handling of seeds and extraction of fat. Higher L* and b* values correspond to the unique bright yellow colour inherited by African butter seed fat. Because of this natural yellow colour, it is not required to add artificial colourants as in conventional margarine or fat spread production.

Table 1: Physicochemical properties of African butter seed fat and cocoa fat

Property	African butter seed fat	Cocoa butter
Slip melting point	37 ^{o}C	35.5 ^{o}C
Smoke point	225 ^{o}C	238 ^{o}C
Colour	L*: 60.47 ± 0.37, a*: -2.84 ± 0.6, b*: 24.14 ± 0.16	L*: 47.05 ± 0.68, a*: 2.74 ± 0.55 b*: 12.36 ± 0.16
Acid value	1.05 ± 0.17 mg KOH/g	2.06 ± 0.21 mg KOH/g
Free fatty acids value	0.53 ± 0.09%	1.04 ± 0 .06%
Iodine value	48.65 ± 3.03 g I_2/100g	34.31±0.97 g I_2/100g
Saponification value	177.0 ± 0.6 mg KOH/g	194.2 ± 1.1 mg KOH/g

Smoke point of African butter seed fat is slightly lower than that of in cocoa fat. In cocoa fat, because of the higher content of saturated fatty acids such as palmitic (C16:0) and stearic (C18:0) acid, the smoke point has a slightly higher value when compared with African butter seed fat. But both values are above 200 ^{o}C which is a basic characteristic of oils that are suitable for frying. Smoke point, flash point and fire point are the three specific temperatures that are determined, in order to investigate the thermal stability of fats. Smoke point is the temperature at which a continuous smoke begins to evolve from the sample. With the degradation of sample above the smoke point, volatiles are produced and the rate of evolving those volatiles determines the flash point and fire point. Flash point is the temperature at which the evolved volatiles produce a temporary ignition when a flame is applied, while the fire point is defined as the temperature at which a continuous combustion can be sustained with the application of a flame. Therefore, in this study the smoke point has been determined, since it can be considered that temperatures below that point are in the safe zone for application of fats in food products that are subjected to baking and frying. Further research is required to determine the frying stability of African butter seed fat.

The acid value of African butter seed fat is 1.05 ± 0.17 mg KOH/g while its free fatty acid content (expressed as oleic %) is in compliance with the range of 0.5 ± 0.1% to 2.36 ± 0.1% shown by Tchobo e*t al.* (2007) from analyzing fats extracted from *Pentadesma butyracea* kernels collected from 10 different areas in Northern Benin. The acid value of cocoa fat is 2.06 ± 0.21 mg KOH/g and its fatty acid content (expressed as stearic %) is 1.04 ± 0.06%. The acid value obtained for cocoa butter is slightly above the corresponding value reported by Adomako (1976) which was reported as 1.8 mg KOH/g. With that, the free fatty acids content (expressed as stearic %) also has a slight increase when compared with the value of 0.9%, reported by Adomako (1976). But still the free fatty acid content value obtained in the present study is lower than the critical value of 1.75% for cocoa fat as shown by Guehi *et al.* (2010). As revealed by Essien and Tettey (2016), the free fatty acid content is directly proportional to the storage time of cocoa beans and there is an interaction of the

fermentation period with the storage time finally affecting the FFA %. In the present study, the mean acid value of African butter seed fat is lower when compared with the mean acid value of cocoa fat, highlighting the fact that the quality of African butter seed fat is higher and refining steps can be applied effectively.

The iodine value of African butter seed fat is closer to the value of 47.3 g I_2/100g reported by Adomako (1976). In cocoa fat, the iodine value is within the range of 32 - 35 g I_2/100g reported by Naik and Kumar (2014). The iodine value is an index of the degree of unsaturation of fatty acids in a particular oil or a fat. When comparing the iodine values of the two types of fats, African butter seed fat has shown a higher iodine value than that of cocoa fat, indicating a higher level of unsaturated fatty acids in African butter seed fat in comparison to cocoa fat. The saponification value of African butter seed fat is lower when compared with cocoa fat. Saponification value gives an idea about mean molecular weight of the triacylglycerols in the fat sample. Therefore, the relatively lower saponification value of African butter seed fat can be related to its fatty acid composition and their high mean molecular weight.

Solid fat content

Table 2 shows solid fat content values obtained for African butter seed fat and cocoa fat separately at selected temperatures from 0 to 45 ^oC. SFC curve derived for African butter seed fat (Figure 1), depicts its hardness at temperatures below 20 ^oC, since the slope of the curve is very low. As depicted in the table 2, SFC percentages obtained for African butter seed fat are higher than 60%, which indicate its characteristic hardness. Within the temperature range 20-25 ^oC, the initiation of a slope in the curve shows the resistance of African butter seed fat to heat supplied or the resistance to melting. In the range 25-35 ^oC the curve depicts intensive melting of the African butter seed fat sample. Within this range, the solid fat content has been drastically reduced from 31.80 ± 0.05% to 2.11 ± 0.03%.

The SFC content varies from 31.80 ± 0.05% to 6.85 ± 0.07% within the temperature range of 25 - 30 ^oC which can be defined as working or utilization temperature range. Devi and Khatkar (2016) reveal that an SFC range of 15-20% at utilization temperature is appropriate for cookie preparation. The SFC content of African butter seed fat from 25 ^oC to 30 ^oC approximately fits the SFC range of 15-20%. According to Devi and Khatkar (2016), the liquid fraction of fat at this temperature is responsible for the lubricating effect which can improve the mixing process, whereas the solid fraction is involved in incorporation of air during mixing. The combination of the functionality of these solid and liquid fractions of fat at the utilization temperature results in improving the ultimate functional performance and textural nature of raw fat, as well as foods prepared by incorporating such fats. Since there is a relationship between SFC and characteristics of baked goods, as revealed by Lai and Lin (2006), African butter seed fat can be suggested as a favourable type of fat in preparing bakery items such as cookies.

In addition to that, the SFC profile of African butter seed fat can be compared with SFC curves of different margarine types such as soft margarine, brick margarine, and shelf stable and pastry margarines described by Sahri and Dian (2011), in order to determine the potential in developing it as a type of a margarine. The SFC value of African butter seed fat which is 60.84% at 20 ^oC is much closer to the SFC range of 65-70% at 20 ^oC which is the characteristic SFC range of pastry margarine type at that temperature. In addition to that, at 30 ^oC, African butter seed fat has exhibited a SFC value of 6.85% which is much closer to the SFC range of 8-10% in shelf stable margarine type at the same temperature. Therefore, it can be claimed that African butter seed fat shows an intermediate SFC profile, showing characteristics of both pastry margarine and shelf stable margarine within that particular temperature range of 20-30 ^oC. Similar to shelf stable margarine, African butter seed fat also exhibits nearly a complete melt down at temperatures above 35 ^oC, highlighting its convenience in spreading, working at room temperature, and favourable melting behaviour during consumption.

When focusing on cocoa butter (Table 2), higher SFC values at temperatures below 15 ^oC highlight its hard nature, while the temperature range from 15-25 ^oC shows its resistance to heating, with the initiation of the slope in the curve as shown in Figure 1. Within the temperature range of 25-35 ^oC, an intensive melting behaviour is depicted in the SFC curve of cocoa fat. The SFC content at 20 ^oC and 25 ^oC are 54.97 ± 0.24% and 36.18 ± 0.87% respectively and those values are lower when compared with the SFC values of cocoa butter in

some previous studies. Ribeiro *et al.* (2012) have depicted the SFC ranges at 20 °C and 25 °C as 60 - 70% and 50 - 60%, respectively. In addition to that, Shukla (2006) has indicated the SFC value of cocoa butter at 25°C as 53.3% for Brazilian cocoa butter. According to *Ribeiro et al.* (2012), there is a correlation between the solid fat content value and the triacyl glycerol (TAG) profile of cocoa butter. Disaturated-monounsaturated triacyl glycerols (TAGs composed of two saturated fatty acids and one monounsaturated fatty acid) are the predominant type of TAG class present in cocoa butter and SFC values vary depending on the content. When the disaturated-monounsaturated TAG level gets reduced in the cocoa butter sample, its solid fat content value decreases more than the expected level. Geographical factors and cocoa variety might be having an influence on this and it has been revealed by Shukla (2006) that SFC values range from 74.8 - 83.7% at 25 °C in cocoa butter available in countries like Ghana, Nigeria, and Malaysia. When considering the application of cocoa fat, its utilization in the preparation of cookies is minimal mainly because of its high cost and lesser availability in the market to purchase in retail amounts. But there is a potential to apply cocoa butter in manufacturing cookies, since there is an extensive melting shown from 25 - 35 °C, which highlights the cooling and creaminess sensation within the mouth during consumption. In addition, the presence of SFC values from 36.14 ± 0.87% to 11.15 ± 0.11% in cocoa fat within the utilization temperature range (25-30 °C) is an indication of its suitability to be used in cookie preparation since the ideal SFC in a fat that can be used for cookie preparation is 15 - 20% according to the study conducted by Devi and Khatkar (2016).

Table 2: Solid fat contents of African butter seed fat and cocoa fat measured by NMR (direct)

Temperature (°C)	Solid fat content (%)	
	African butter seed fat	Cocoa fat
0	81.57 ± 0.03	93.15 ± 0.84
5	82.17 ± 0.08	93.31 ± 0.68
10	79.36 ± 0.02	91.27 ± 0.69
15	72.91 ± 0.04	81.06 ± 0.86
20	60.84 ± 0.03	54.97 ± 0.24
25	31.80 ± 0.05	36.14 ± 0.87
30	6.85 ± 0.07	11.15 ± 0.11
35	2.11 ± 0.03	1.64 ± 0.03
40	1.75 ± 0.03	1.93 ± 0.08
45	1.83 ± 0.04	1.97 ± 0.05

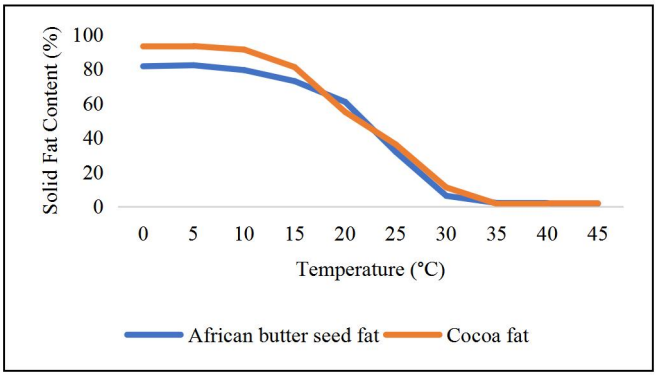

Figure 1: SFC curves for African butter seed fat and cocoa fat

When comparing the two SFC curves of African butter seed fat and cocoa fat, it is clear that there is a slight gap in the curves drawn relative to the SFC values at the same temperature range (Figure 1). A noticeable difference is shown in SFC values within 0 -15 °C while there is a smaller difference within 25 -35 °C among these two types of fats. These contrasts between the two curves can be merged by altering triacyl glycerol composition via

enzymatic transesterification to produce cocoa butter equivalents as highlighted by Naik and Kumar (2014). In addition to that blending of different compatible oils can also be done in order to alter the SFC values at selected temperatures as revealed by Sahri and Dian (2011).

Fatty acid composition of African butter seed fat and cocoa fat

GC chromatograms obtained for the fatty acid composition of African butter seed fat and cocoa fat are shown in the supplementary figures 1 and 2 respectively. Table 3 depicts percentages (by weight) of major fatty acids present in African butter seed fat and cocoa fat. In African butter seed fat, stearic and oleic are the two fatty acids present in abundance with respect to saturated and monounsaturated fatty acid types, respectively. The percentage of stearic acid is in the range 38.4 - 47% in African butter seed fat collected from 10 different regions of Benin, as reported by Tchobo *et al.* (2007). The fatty acid available in the highest percentage is oleic acid, and this value is close to 53.72% with respect to oleic acid in African butter seed fat, as revealed by Ayegnon *et al.* (2014). Linoleic and linolenic acids are the two major poly unsaturated fatty acids present in African butter seed fat, with percentage values of 0.82 ± 0.00% and 0.20 ± 0.00% respectively. In addition to that, there are no *trans* fatty acids detected in the analyzed sample of African butter seed fat.

When concerned about the fatty acid composition of cocoa fat, there are three fatty acids present in higher percentages, namely palmitic, stearic and oleic acids. Palmitic and stearic are the two major saturated fatty acid types and their percentage weights were found to be in the ranges of 24.5 - 33.7%, 33.7 - 40.2% respectively, as reviewed by Naik and Kumar (2014). Oleic acid is the major monounsaturated fatty acid present in cocoa fat which amounts to 34.12 ± 0.14%, and that value is close to 36.1% as reported by Adomako (1976). Also, that value fits in to the range of 26.3 - 35% as revealed by Naik and Kumar (2014). With regard to the major types of poly unsaturated fatty acids, linoleic acid content is 2.53 ± 0.00% while linolenic acid content is 1.23 ± 0.00% in cocoa fat. There are no trans fats detected in the analyzed sample of cocoa fat.

Table 3: Fatty acid composition of African butter seed fat and cocoa fat

Fatty acid	African butter seed fat weight (%)	Cocoa fat weight (%)
Palmitic (C16:0)	2.78 ± 0.11	24.14 ± 0.07
Stearic (C18:0)	39.05 ± 0.16	37.75 ± 0.06
Oleic (C18:1)	56.97 ± 0.27	34.12 ± 0.14
Linoleic (C18:2)	0.82 ± 0.00	2.53 ± 0.00
Linolenic (C18:3)	0.20 ± 0.00	1.23 ± 0.00

When comparing the two fatty acid profiles of African butter seed fat and cocoa fat, they can be considered as approximately similar. However, the content of palmitic acid is significantly higher in cocoa fat than in African butter seed fat. This has resulted in an increase of total saturated fatty acid content in cocoa fat compared to African butter seed fat. The fatty acid profile is directly linked with the triacyl-glycerol (TAG) composition. Most abundant TAGs present in the African butter seed fat are 1(3)-stearoyl-2-oleoyl-3(1)-stearoyl-sn-glycerol (SOS) and 1(3)-stearoyl-2 oleoyl-3(1)-oleoyl-sn-glycerol (SOO) as revealed by Aissi *et al.* (2018). This has led to the presence of a higher content of stearic and oleic acids in African butter seed fat. According to the findings of Adomako (1976), major triacyl glycerols present in cocoa fat are 1(3)-palmitoyl-2-oleoyl-3(1)-stearoyl-sn-glycerol (POS), 1(3)-stearoyl-2-oleoyl-3(1)-stearoyl-sn-glycerol (SOS), and 1(3)-palmitoyl-2-oleoyl-3(1)-palmitoyl-sn-glycerol (POP). This has resulted in increasing the content of the fatty acids, stearic, oleic, and palmitic acids, in cocoa fat. There is a potential to convert selected abundant TAGs of African butter seed fat, namely SOO and SOS, to POS which is the most abundant TAG type in cocoa fat in order to develop African butter seed fat as a cocoa butter alternative. Transesterification, which is performed by using either chemicals or enzymes, can be cited as a method to equalize the TAG composition of African butter seed fat to cocoa fat.

According to Tchobo *et al.* (2013), the major antinutritional constituents found in African butter seeds are phytates and oxalate, which have been quantified as 0.42% and 1.04% respectively. Phytate is present in many food items such as nuts, legumes, cereal germ or bran, beans, carrots, broccoli, potatoes, and wheat. Based on

the findings of Schiemmer *et al.* (2009), the phytic acid contents of oil seeds such as soybeans, linseed, sesame seed, and rape seed are 1.0 - 2.22%, 2.15 - 3.69%, 1.44 - 5.36% and 2.50% respectively. Therefore, the phytate content in African butter seeds can be considered as much lower when compared with these oil seeds. As described by Schiemmer *et al.* (2009), phytates are known to be partially degraded in the human stomach and intestine. And also, those can be degraded by employing different processing conditions at temperatures above 110 ^{o}C, which requires further research with respect to African butter seeds. Oxalates are also present in many foods such as spinach, almonds, cashews, beet, and potatoes. Since oxalates and phytates are water soluble, boiling of African butter seeds in water is recommended as a possible pretreatment method, which is already followed in traditional processing of African butter seed fat.

Apart from naturally occurring antinutritional compounds, there is a tendency towards forming some process induced toxins such as polycyclic aromatic hydrocarbons (PAH), due to exposure of oils to a high temperature above 400 ^{o}C. Pyrolysis of oil starts at temperatures above 200 ^{o}C according to Shahidi *et al.* (1997). Even though such very high temperatures are not achieved during the present study, necessary precautions were implemented to avoid generation of high temperature during the drying process and extraction of fat using an oil screw expeller. Use of non-heating surfaces when drying, limiting the time of exposure to sun light, performing oil extraction in a discontinuous manner allowing to cool down the interior of the machine and collecting oils initially into the aluminium containers are some of the precautionary measures taken.

Sensory evaluation of cookies prepared by incorporating the two fats

Cookies prepared by incorporating the two types of fats separately were presented to the selected panelists. Preference levels for sensory attributes of colour, flavour, texture and overall acceptability for cookies prepared by incorporating the two fats are shown in Supplementary Figure 3. Phrases with scores from 5 to 1, which correspond to different degrees of preference, from 'like very much' (corresponding score = 5) to 'dislike very much' (corresponding score = 1) have been used. In addition, Table 4 shows a summary of Wilcoxson signed rank test results which were obtained by analyzing sensory data collected for the two cookie types. There is a significant statistical difference between the preferences for colour of cookies prepared by incorporating the two kinds of fats ($p < 0.05$). The preference of the panelists for colour of cookies prepared from African butter seed fat is higher than that for cookies prepared from cocoa butter. There is no significant statistical difference with respect to the preference shown towards flavour of the two types of cookies ($p > 0.05$). Where the subjects' preference for texture is concerned, there is no significant statistical difference with respect to the two cookie types ($p > 0.05$). With regard to the preference for overall acceptability, there is no significant statistical difference in relation to two types of cookies ($p > 0.05$).

Table 4: Wilcoxson Signed ranks for the preference for cookies made by incorporating African Butter fat and cocoa fat

Sensory attributes of cookie samples	Ranks	N	Significance (p)
Colour, $AFBF_1$- Colour, CF_1	Negative ranks	4	0.018
	Positive ranks	14	
	Ties	12	
	Total	30	
Flavour, $AFBF_1$- Flavour, CF_1	Negative ranks	6	0.193
	Positive ranks	11	
	Ties	13	
	Total	30	
Texture, $AFBF_1$- Texture, CF_1	Negative ranks	9	0.617
	Positive ranks	7	
	Ties	14	
	Total	30	
Overall acceptability, $AFBF_1$- Overall acceptability, CF_1	Negative ranks	2	0.132
	Positive ranks	6	
	Ties	22	
	Total	30	

$AFBF_1$: Cookies prepared by incorporating African butter seed fat, CF_1: Cookies prepared by incorporating cocoa fat.

CONCLUSION

The physicochemical properties of African butter seed fat determined in this study reflect the potential of using this fat as an edible fat that can be utilized even under different processing conditions. Fatty acid profiles of the two fat types are approximately similar and there is a good potential to develop African butter seed fat as a cocoa butter alternative. The SFC profile of African butter seed fat, specially within the temperature range of 25-30 ^{o}C indicates the applicability of this fat in bakery items such as cookies. There is no significant difference with respect to the preference for flavour, texture, and overall acceptability among panelists for the two cookie types developed by incorporating African butter fat and cocoa fat ($p > 0.05$), while there is a significant difference for colour ($p < 0.05$).

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