Int. J. Chem. Sci.: 6(2), 2008, 917-925

ANALYSIS OF FATTY ACID COMPOSITION OF KOMBO (*PYCNANTHUS ANGOLENSIS*) BUTTER

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ABSTRACT

Fatty acid esters, especially those essential to humans, are absolutely necessary for the maintenance of growth, reproduction and health. They are truly essential to normal cell structure and body function, which function as components of nerve cells and cell membranes. Lack of these in the body causes diseases such as arthritis. Hence, sources rich in cetyl myristoleate (CMO) are known to be a remedy for these diseases. One of such rich source is Kombo Butter, which is a fat obtained from *Pycnanthus angolensis*. This is a plant source, which is generally known to give a low level of saturated fat and relatively higher levels of essential fatty acids than animal sources. This research, therefore, aims to determine the constituent of the Kombo fat by finding out its fatty acid composition. This will contribute to scientific knowledge and also address the increasing demand for natural alternatives to synthetic medicines.

Key words: *Pycnanthus angolensis*, Myristicaceae, Ilomba, Lunaba, Pycnanthus, Calabo, Oje

INTRODUCTION

A lipid is defined as a naturally occurring organic compound that is insoluble in water but soluble in non-polar organic solvent such as a hydrocarbon or diethyl ether. The various classes of lipids are related to one another by the types of compounds in them but their chemical functional and structural relationships as well as their biological functions are diverse[1].

The lipid fraction of a fatty food therefore, contains a complex mixture of different types of molecules. Fats and oils are triglycerides or triacylglycerols (triesters of glycerol). At room temperature, a fat is a solid and an oil is liquid. The carboxylic acid obtained from

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the hydrolysis of fat or oil called fatty acid generally has a long unbranched hydrocarbon chain. Fats and oils are said to be complex lipids because they can be hydrolysed to smaller molecules. Lipids such as cholesterol that cannot be hydrolysed are called simple lipids[1].

Most naturally occurring fats and oils are mixed triglycerides. They form a major component of most foods. In the body, lipids provide energy and protection. They play a major role in determining the overall physical character such as flavour, texture, mouth feel and appearance in many foods[2].

Species identity

Taxonomy

Current name: *Pycnanthus angolensis*

Authority: (welw) Warb.

Family: Myristicaceae

Ecology and distribution

Natural habitat

Pycnanthus angolensis is found in the moist lowland rainforests from West Africa and Tanzania. The tree is mainly found in scattered fashion in the secondary formation of evergreen and semi – deciduous forests.

Uses of the plant

Pycnanthus angolensis has been found to be of various uses in some countries. For instance, the leaves are used locally with other herbs for the treatment of miscarriage in pregnant women. The bark is used by the Ibos in Nigeria to cure loss of appetite. It is also used as a toothache cure and a mouthwash. A decoction of the bark is used as an emetic and by mothers to purify their milk[3]. The Ewe tribe in Ghana uses the plant *Pycnanthus angolensis* in several ways. The bark exudates, twigs, leaf juice, and / or seed fat are used as mouthwash to treat oral thrush in children; the seed fat is applied topically to treat fungal skin infections[4].

In Ghana, a hot water extract of the root is taken orally as an antihelmintic[5]. The leaf and bark are used to treat toothache and the sap of the plant is applied topically to

arrest bleeding [6]. The Kombo tree is a commercially relevant timber source[7].

The fat, which is obtained from the kernels, has the following medicinal (therapeutically) and antioxidising applications; the seed fat is applied together with the reddish latex on skin diseases[8]**.** Arthritis is thought to affect more than 50 million Americans, and it is estimated that by the year 2020, about 18.2% of the American population will have arthritis. Kombo fat contains myristoleic acid, which is used for the production of cetyl myristoleate. Cetyl myristoleate is a relatively newly discovered natural substance, which is capable of treating arthritis[9].

Chemistry and functions of cetyl myristoleate oil (CMO)

Cetyl myristoleate oil is the hexadecyl ester of the unsaturated fatty acid cis-9-tetradecenoic acid. The common name for the acid is myristoleic acid. Myristoleic acid is found commonly in fish oils, whale oils, dairy butter, and Kombo butter. At the present time Kombo butter is the only known vegetable-derived source that contains commercially viable concentrations of myristoleate acid for the production of cetyl myristoleate[10].

The chemical formula for cetyl myristoleate is –

$$(Z)\text{-}ROCO\ (CH_2)_7CH{=}CH\ (CH_2)_3CH_3.$$

Chemistry and antioxidising properties of kombic acid

Kombo kernel fat has been reported to contain a compound known as kombic acid, which is a highly unsaturated, resin acid and has a molecular formula of $C_{28}H_{40}O_4$. The structure of the kombic acid was identified as 16(2', 5'-dihydroxy-3'-methylphenyl)-2, 6, 10, 14-tetramethyl-2, 6, 10, 14-hexadecatetraenoic acid. Kombic acid was found to belong to a class of natural terpenoids consisting of a polyisoprene system joined to a resorcinol, hydroquinone or quinine nucleus[11].

Kombic acid is suitable for preventing oxidation of various organic materials. It acts as an antioxidant in the stabilization of human and animal foods against rancidity, colour and odour development; for use in dietary supplements and other human and animal health formulations; as stabilizers in cosmetic and other personal care applications; and in the stabilization of plastics against oxidative degradation[11]. It is reported to be useful in prolonging the shelf life of edible oils that are susceptible to rancidity, the antioxidant being mixed with the edible oil, such oils include those obtained from soyabean, sunflower seed, canola, and cottonseed[12].

EXPERIMENTAL

Objectives

The objective of this study was to analyze and identify the type of fatty acids present in the lipid of Kombo butter.

Methodology

Extraction

Seeds collected were Sun dried for five days and then dehusked. They were then ground after the weight had been taken. The ground seeds were placed in the thimble of the sachets apparatus for extraction to be carried out. About 1 litre of methanol was poured in the thimble and placed in the apparatus. Extraction of the oil from the seeds was carried out for nineteen hours (19 hrs) for 97.9 g of the seeds. The methanol, in the extract, was then evaporated leaving the oil.

Colour of evaporated oil - Dark brown

Volume of oil – 6 mL

Saponification value

1.0 g of the fat was placed in a beaker and dissolved in 4 mL of solvent, in the ratio of 1 : 1 ethanol/ether. The dissolved triglycerides were transferred into a small distillation flask and the beaker was washed with 1 mL of 1 : 1 ethanol/ether in order to collect all residual material. It was added to the distillation flask. Also, 25 mL of 0.5M KOH/ethanol solutions was added.

The same process was followed for two other weighed fats (1 g each) into different flasks. A control was set up with 25 mL of the 0.5M KOH/ethanol solutions plus additional 1 : 1 ethanol/ether solvent. The hydrolysis was carried out by setting up a reflux condenser on each flask, which was placed in boiling water for 30 mins. The flasks were cooled and three drops of phenolphalein indicator was added. Each solution was titrated with 0.5 M HCl solutions.

After reflux: Volume of solution A = 29 mL

Volume of solution B = 21 mL

Volume of solution C = 29 mL

HPLC Spectroscopy

The lipid was converted into fatty acids by converting them into methyl esters, compounds that can easily be converted into gases. A sample of the fat was taken and 0.5M HCl was added. It was then heated on a water bath for 30 mins. 1 g of fat = 25 mL of 0.5M HCl. The different fatty acids were then separated and analysed using HPLC.

Type of HPLC - Thermo LC1

Detector used - UV detector at 242 nm

Solvent system - Acetonitrile/Water (50/50)

Flow rate – 1 mL/min

Column type - C18 column

Reagents

4-Bromophenacyl bromide, 50 mM potassium hydroxide (w/v) in methanol and acetonitrile.

Procedure

A sample of the free fatty acids was dissolved in methanol and neutralized with the KOH solution (with the help of phenolphthalein as an indicator) or triethylamine. The mixture was evaporated under nitrogen and 0.1 mL 4-bromophenacyl bromide was added to the residue. The mixture was heated at 80°C for 15 min mixing gently for several times. The vial was cooled and diluted to a larger volume with acetonitrile for the mixture to adapt the response to the fatty acid concentration.

The HPLC separation was made using a C18 column and a gradient of acetonitrile in acetonitrile/water (1 : 1, v/v) at 1 mL/min. The UV detection was set at 242 nm.

Refractive index

This is the ratio of the speed of light through vacuum to the speed of light through the oil. The refractive index of most organic compounds lies in the range between 1.35-1.70. They increase with increasing carbon chain.

The sample was injected and value recorded after the necessary calibration.

Infra red spectra

This spectrum gives a graph of absorption intensity (given as percent transmittance) as a function of radiation frequency (given as wave number in units of reciprocal centimetres). Frequency and wave number are directly proportional. Therefore, wave number is also directly proportional to energy; the higher is the wave numbers, the greater is the energy. The FTIR analysis was carried on the samples after the necessary calibration and sample preparation.

RESULTS AND DISCUSSION

Refractive index

The refractive index of the Kombo fat was found to be 1.4392. This indicates that the fat contains short fatty acid chains from lauric acid to stearic acid. This is because most organic compounds lie in the range between 1.35 – 1.70 and refractive index increases with increasing carbon chain. This trend can be seen in the table below –

Table 1. Refractive index of some fatty acids

Acid	N_D70	N_D50
Palmitic	1.4309	
Stearic	1.4337	
Oleic		1.4487
Linoleic		1.4588

The refractive index provides a very accurate physical constant for identification since the speed of light through a medium is directly related to the structure of the molecule and in particular to the type of functional groups present.

Infra red (IR) analysis

The infra red analysis gave the following results –

Table 2. The result of the infra red analysis

Absorption (cm^{-1})	Functional group
3400-3100	Stretching OH
2850	Aldehydic CHO
1730	Stretching C=O
1465	Scissoring CH_2
1375	Bending CH_2
710	Out of plane H-C=C

The above IR results show that the fat contains the following polar groups such as –OH and COOH. It also contains alkene groups, which forms the unsaturated part of the structure.

Saponification value

The saponification value analysis gave an average value of 319.2 mg. This is the mass of KOH required for the hydrolysis of the fat to obtain the fatty acyl esters. Since more milligrams of KOH were used, it implies that the fatty acid chains present in the Kombo fat are short in length.

HPLC analysis

The results obtained in the HPLC analysis are given in Table 3.

Table 3. The results of HPLC analysis

Acid	Major peak	Area covered	Area percentage
Extracted (Kombo fat) fatty acid	11.900	770161	20.704
Palmitic acid	11.870	2163387	49.282
Stearic acid	11.990	312641	17.040
Oleic acid	12.027	208321	8.646
Myristic acid	9.393	509827	22.645

Comparing the spectra, it could seen that the Kombo fat contains such fatty acids

as palmitic, stearic, oleic and myristic acids. This is because their retention times were almost the same even though that of myristic acid was quite smaller than the rest. Also, the largest area covered was of the palmitic acid with an area percentage of 49.282 and this was followed by myristic, stearic and oleic acids, respectively.

CONCLUSION

The study identified the presence of palmitic, stearic, oleic as well as myristic acids in the Kombo fat. The Kombo fat can be said to contain shorter fatty acid chains, which are saturated. Since this fatty acid is obtained from plant, they are much more preferred than those from animal sources. They tend to behave as essential fatty acids, which could be used as food and medicine supplement for the human body.

ACKNOWLEDGEMENT

The authors are thankful to the Kakum National Park (Cape Coast, Ghana) attendants where the samples were collected from their support. Also, we thank the staff of the Ghana Standard Board for the use of their HPLC.

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Accepted : 04.01.2008