UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY

THE CHEMISTRY OF THREE INDIGENOUS SEEDS, AND THE EXTRACTION, NUTRITIONAL AND INDUSTRIAL POTENTIALS OF THEIR OILS

A THESIS SUBMITTED TO THE BOARD OF POST GRADUATE STUDIES, UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI FOR THE AWARD OF MASTER OF PHILOSOPHY IN BIOCHEMISTRY

BY

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JUNE, 1993

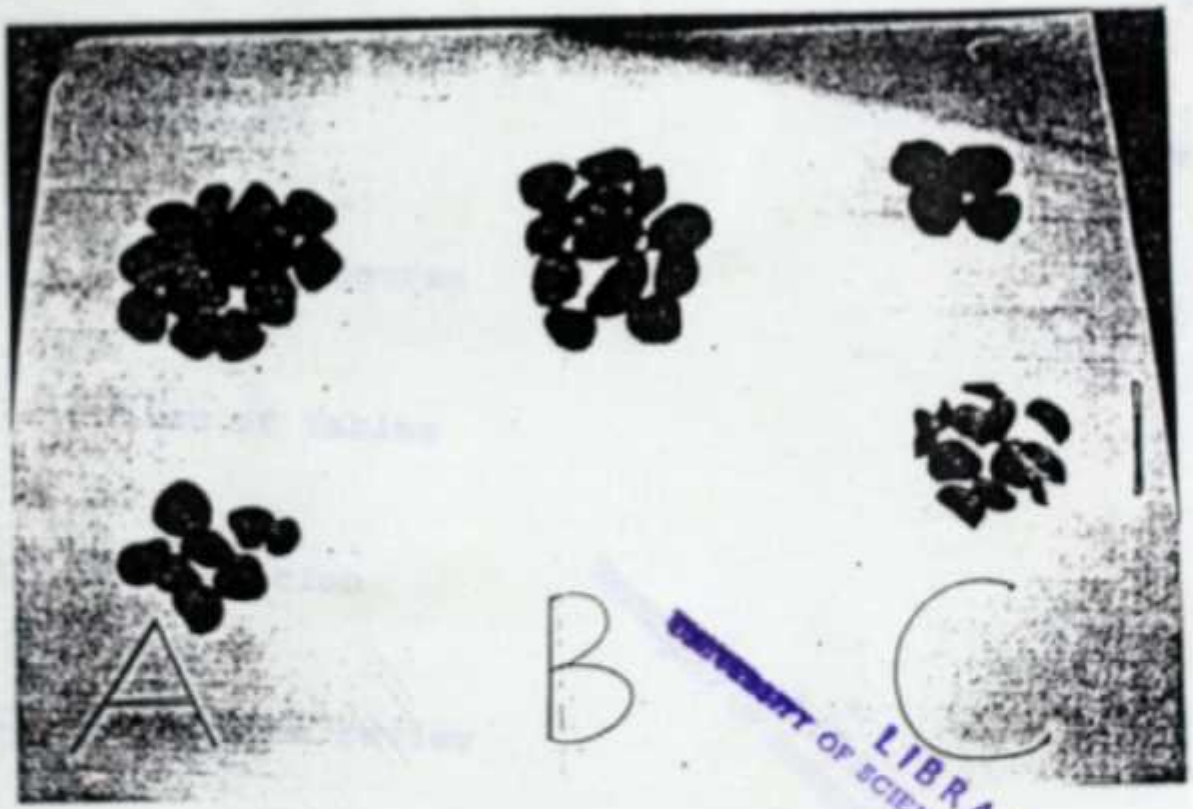

FIG 1 PROJECT MATERIALS

A CARAPA PROCERA SEEDS

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DEDICATION

This work has been dedicated to my daughter Emmanuella.

ACKNOWLEDGEMENT

My special thanks go to Dr. E. K. Marfo and Dr (Mrs.) V. P. Dzogbefia for suggesting and supervising this project work.

I am indebted to my friends especially Mr. and Mrs. Ahenkora and Mr and Mrs Osei for their moral support

I gratefully acknowledge the help of Mr. Michael Korsah-Brown, Mr Kofi Sarfo, Mr Alex Yeboah and Mr Kwame Manhyia for bringing this work into a printed form

Finally, my sincere thanks goes to my family for their financial support during this program.

DECLARATION

This thesis is my own work produced from research undertaken under supervision of Dr E. K. Marfo and Dr. (Mrs) V. P. Dzogbefia

DR. E. K. MARFO ..... DR. (MRS) V. P. DZOGBEFIA .....

ABSTRACT

This thesis describes the chemistry of the seeds of Carapa procera (Monkey cola), Pentadesma butyracea (Tallow tree) and Telfaira occidentalis (fluted pumpkin), the extraction, nutritional and industrial potentials of their seed oils. The oil content of the seeds ranged from 46.45% to 66.19% and protein, from 6.34% to 22.54%. Ash values ranged from 2.78% to 3.93% whilst seeds of P butyracea contained the highest fibre and carbohydrate values of 13.73% and 22.44% respectively. Chemical composition of defatted seeds showed protein values to be from 8.01% to 54.68%. Fibre contents ranged from 6.40% 15.22%. Ash and carbohydrate values ranged from 3.53% to 10.91% and from 24.69% to 64.56% respectively. Amino acid composition analysis revealed that the seeds were low in sulphur containing amino acids. The predominant fatty acids in all the seed oils were palmitic, stearic, oleic and linoleic acids. The iodine and saponification values ranges were 51.59% to 99.45% and 194.42% to 214.37% respectively. All the oils had low unsaponifiable matter and acid values.

Optimization of aqueous extraction parameters showed that more than 50% of the oils were extractable. Both C. procera and P. butyracea oils could be optimally extracted for 20 mins. using a meal : water ratio of 1:7

when the seeds were roasted at 100°C, for 30min and at 120°C for 45mins respectively. With T. occidentalis however, seed roasting at 100°C for 30 mins., using a meal : water ratio of 1:6 and an extraction time of 30 mins. were required to achieve optimum extraction.

Nutritionally, only P. butyracea oil was comparable to palm oil, however all the oils had a good potential for soap making.

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1.1 INTRODUCTION

Food supply has become an urgent priority as the world's population grows (Anon 1981). In reaction to this, several countries are advocating different food policies to augment available food sources. These include introduction of high yielding seeds, pest control, preservation and exploitation of new food sources (Josephine & Janardhanan, 1992). Among the new sources of food being considered are leafy vegetables, single cell proteins, agricultural and industrial waste and unconventional oilseeds.

Oilseeds are seeds which are exploited mainly for the use of their oil. Oilseed crops, especially those produced in developing countries have been used traditionally since the origin of humanity (Satin 1992). Besides food uses many also find application in soap manufacturing, flavouring and perfume. Since independence many indigenous plant species in Ghana which have little importance as timber trees, are being recognised as valuable sources of alkaloids, pulp and oil (Ambrevbille 1959). Already adequate information is available on several oil rich seeds including Adansonia digitata, Ceiba pentandra and Butyrospernum parkii (Watson 1971). However over-dependence of the nation on coconut oil, palm oil and palm kernel oil has at times led to severe

shortage of oil due to competition between food and industrial uses. After extracting the oil, the cake is also known to be rich in protein, carbohydrates and minerals. Although the quality of protein may be good, the, main set back against its exploitation is the presence of antinutritional factors (Huisman, 1989).

Carapa procera, Pentadesma butyracea and Telfaira occidentalis i.e,monkey cola,tallow tree and fluted pumpkin respectively are plants that grow wild in Ghana. They are common in Ashanti, Brong Ahafo, and Eastern regions. The seeds are known to contain oil that is extracted for soap and food. However little is known about their chemical composition, existing methods for the extraction of their oils and nutritional effects. Scientific literature is also inadequate on these information including its industrial potentials.

The purpose of this project is to research into the chemistry of the oilseeds,and the extraction, nutritional and industrial potential of the seed oils. This will help advise policy makers on their possible consideration for further studies.

1.2. OBJECTIVES OF THESIS

1. To determine the chemical composition of three defatted and undefatted oil bearing seeds.
2. To optimize parameters which affect the extraction of the oils by aqueous method and compare their characteristics with oils extracted by organic solvent and pressing methods.
3. To evaluate the nutritional and industrial potentials of the oils.

2 LITERATURE REVIEW

2.1 THE SEED

A seed is a reproductive unit formed from a fertilized ovule, and consisting of an embryo, food store and protective coat. They are produced by gymnosperms and angiosperms (Eleanor, 1989)

There has been 3 main classes of seed bearing plants of spermatophytes in the history of the earth's vegetation. These are:

1. The pteridospermae, or seed bearing ferns which had all the vegetative characteristics of pteridophytes but which bore seed-like reproductive structures. All are extinct but many fossilized remains give a good picture of their structure.
2. The gymnospermae, some of which still inhabit the earth. Most are of the tree habitat.
3. The angiospermae which have achieved the dominant position in the present era.

Seed Structure of angiosperms

Any angiosperm seed consists of the following essential parts: i. The embryo consisting of an axis differentiated into plumule or embryonic shoot at one end and radicle or embryonic root at the other, and bearing one or two cotyledons which may or may not contain food. ii. The testa or seed coat enclosing the embryo but perforated at one point by a pore or micropyle and carrying a scar or

hilum where it was attached to the ovary wall. The testa may carry a specialised fleshy out-growth or aril formed from placenta micropyle or funicle. In addition, within the testa there may be a mass of food-storing tissue, the endosperm (Vines & Rees, 1972).

2.1.1. OIL SEEDS

All seeds contain some amount of oil but to consider a seed as a commercially important source of oil or fat, the quantity and characteristics of the oil are important. The seed oils of many species have been investigated,(Mensier,1957), but comparatively few are of world wide importance (Godvin & Spensley, 1967). A considerable number of plant species, particularly in the tropics, provide oils and fats for domestic use, but these plants grow wild (Dalziel,1955).

The family of foods that have received the most attention as fortifiers or ingredients of formulated foods are oil seeds. In a 1971 survey for instance, 93% of new foods used oilseeds as the protein base (Berg, 1973). In general the oilseeds are both low in cost and high in nutritive value. Their most striking characteristic however is abundance. Approximately 110 million tons are harvested annually, mostly in countries with nutritional need (Berg, 1973). As the name implies, the oilseed is grown mostly for its oil which is primarily used for food and industrial purposes. The oil is extracted from the

seed either by screw pressing or by solvents or a combination of both. The residue, "oil cake" can be refined and used as the protein ingredient for human grade foods, animal feed or fertilizer. Ironically in some countries this by-product of vegetable poses a disposal problem (Berg, 1973).

2.1.2 CONVENTIONAL OILSEEDS

The use of oilseeds and their products are of nutritional significance in the country. Examples are soybean and sheanut. Soybean, a legume occupies a premier position as a world crop because of its high protein and oil content. Most of the soybean crop is processed by solvent extraction to yield edible oil with the meal primarily directed to animal feeding; only a minor percentage of the meal is processed directly for human consumption (Circle & Smith, 1975). The protein content of the whole seed, approximately 40% (%N x 6.25) of the seed weight is the highest of any commercially important crop with the non-protein nitrogen calculated as 2.9% to 7.9% (Smith & Circle, 1972). The soy contains several trypsin inhibitors, however 10 - 15 minutes of moist heat treatment at 100°C inactivates these inhibitors to render the defatted meal a useful source of protein.

Crude soybean oil contains small quantities of wax pigments and minerals including phosphorus, sodium, iron

and copper. Normally crude soybean oil undergoes refining before utilization

The shea tree grows naturally on the lateral slopes of the Savannah Zone of the hinterland of West Africa and throughout the equatorial region of Africa where rainfall is not too high (Irvine, 1961). Under protected conditions, for example, on cultivated land and near villages it grows up to 15m high and 175cm in girth (Coull, 1928). The tree grows slowly from seed, taking 12 to 15 years to bear fruit and about 30 years to mature (Hill, 1930; Dalziel, 1955).

The sheabutter obtained from the nut is extracted by the traditional method in northern Ghana. The method varies from town to town or village to village (Ata, 1982). The sheabutter serves as a frying fat, a pomade for the skin and hair, a muscle toning balm for aching muscle,and a base in traditional pharmaceutical preparation for curing boils and skin diseases. It also serves as an ingredient for soap manufacture and as fuel for locally manufactured lanterns (Ata, 1982). The fatty acid composition of 4.8% palmitic acid,45.9% stearic acid,40.8% Oleic acid,6.9% linoleic acid and 0.6% linolenic acid has been reported by Adomako, (1977). The cake is used for animal feed.

2.1.3 NON CONVENTIONAL OILSEEDS

2.1.3.1 PENTADESMA BUTYRACEA (TALLOW TREE)

Pentadesma butyracea is found in closed forest regions, especially in evergreen forest (Hall & Swine, 1981). It is also common in swampy areas and by stream sides. In Ghana it is mostly found in the Brong Ahafo region where there is sufficient rainfall. Its common name is the tallow tree and is known as "kisidwe" in the Akan language.

The tallow tree is a medium-sized buttressed evergreen tree. It grows to about 30m in height. It has a crown and the branches are conspicuously whorled and at right angles. The tree is easily reared from seed and matures fast. It regenerates freely from stump and from self sown seeds thus it is a suitable tree for reforestation purposes (Dalziel,1955, Manu-Boafo,1976). Flowering occurs in February to May and July to September. Fruiting occurs in February to June and July to October. Fruits are large, pointed and broadly ellipsoid.

Seeds of the tallow tree are brown with flattened sides resembling those of Carapa procera. It is red in section and very bitter when old, though it can be edible when young (Irvine, 1961).

Williams (1950) found considerable differences in the analytical values of fats extracted from tallow kernels from different areas. When air dry, the seeds yield a

fat content of about 32% - 42%. Traditionally, the fat is extracted from the seeds the same way as shea butter (Adomako, 1977). The fat has several unique characteristics that make it very suitable for edible and cosmetic purposes. Adomako (1977) again reported that tallow fat resembles shea butter in several characteristics but its relatively low unsaponifiable matter and higher saponification number makes it superior to shea butter. He reported the fatty acid composition to be 3.1% palmitic, 0.2% palmitoleic; 45.5% stearic; 50.5% oleic, and 0.4% linoleic. Natives use the fat from the seed as pomade for skin and hair, to kill lice, jiggers etc. It has been employed in the soap industry, candle making and sometimes for the production of margarine (Huthchinson & Dalziel, 1954).

The seed contains very little nitrogen and is only of value for fat. The residual meal, besides being almost free from carbohydrate, is high in tannin and other substrates rendering it unsuitable for feeding purposes and it is also of little value as manure.

2.1.3.2 **CARAPA PROCERA (MONKEY COLA)**

Carapa procera (D.C.) belongs to the family Meliaceae. It is spread throughout the western part of Africa from Senegal to Angola and occurs also in tropical America. It thrives best in evergreen, decidious and fringing forest. It is a quick growing tree which can reach

heights of 18m -20m and diameters up to 60cm (Irvine, 1961).

Carapa procera is generally a small tree with crooked bole which branches rapidly. It is often strangling and divides low into two stems like many other species in the family. It has compound leaves, the pinnately compound leaves consisting of 10 - 16 pairs of leaflets. The leaflets vary in size and shape from elliptic-oblong with entire margin. The young leaves are broad and reddish in colour (Irvine 1961, Hall & Swine,1981).

Flowering occurs in two main seasons. The major flowering period is in August - October, with the minor fruiting season occurring in May - July. The seeds resemble the common cola and is liked and eaten by monkeys. The Ashantis call it Kwakuo bese, the Twi's Sua bese, the Fantis and Wassa Krabese and the Nzema Asokoro (Irvine, 1961).

The kernels contain about 60% of a cream-coloured intensely bitter oil with a high acidity and unpleasant smell (Coursey, 1924). Natives extract the oil in the crude form like the extraction of shea butter. The Carapa family has stearic acid, palmitic acid and oleic acid as major fatty acid components but Carapa procera has palmitic acid as the most predominant whilst that of stearic acid is quite small. A composition of palmitic acid 21.0%, stearic acid 11.0% arachidic acid 0.2%, oleic

acid 47.6% and linoleic acid 18.1% have been reported by Hilditch & William (1964).

The dark yellow oil obtained from the seeds is used for the treatment of external sores, burns, rheumatic pains insect bites, jiggers, eruptions, ring worms, yaws and internally as vermifuge for both tapeworm and roundworm. It is also used as a purgative and also as an expectorant or taken in small doses for the treatment of syphilis and smallpox (Dalziel, 1955, Irvine, 1961). The oil is also used in some parts of West Africa with plantain ashes for making soap and pomade for skin and hair (Irvine, 1961).

2.1.3.3 **TELFAIRA OCCIDENTALIS** (FLUTED PUMPKIN)

Fluted pumpkin (Telfaira occidentalis, Hook f) belongs to the family Cucurbitaceae and is a native of tropical Africa (Dalziel, 1955). Fluted pumpkin is grown in Sierra Leone, Gold Coast (Ghana), Dahomey (Benin Republic), Southern Nigeria and Fernando Po (Equatorial Guinea) (Hutchinson & Dalziel, 1954).

Fluted pumpkin is a dioecious climber and has compound leaves with 3 - 5 shortly stalked leaflets and branched tendrils. It is fast growing; the vines may grow to 30.5m in length. Shoot elongation is seriously checked in the male plant by flowering (Esiaba,1982). The female plant continues to elongate while flowering. The Nigerian fluted pumpkin is grown as a minor crop at wide

spacing among field crops (Okigbo, 1975). It is planted among yams with which it shares common stakes,and also with maize and cassava plants. Pure stands of fluted pumpkin are also planted; seeds are sown at close spacing of 0.45m apart in rows down the centre of the bed or 0.76m between plant in rows 0.91m apart (Tindall,1968). In Ghana the plant grows wild and pods are usually collected during land preparation for farming. The whole fruit is not eaten, rather the seeds are boiled and the cotyledons eaten. The cotyledons which form the bulk of the seeds can also be roasted and made into paste and put in soup. The seed is also a good source of a non-drying oil suitable for cooking and soap (Dalziel,1955), however in Ghana the art of extracting the oil is not known. Work done by Lazos (1986) gave the following fatty acids composition;0.1% myristic,12.7% palmitic, 0.6% palmitoleic, 5.4% stearic, 37.8% oleic, 43.1% linoleic 0.3% linolenic and 0.3% arachidonic. The oil is pale yellow with faint odour and has an agreeably bland taste (Girgis & Turner, 1972).

The seed has a high nutritional and industrial value; it contains 4ml water; 2g fibre; 30g protein; 50g fat; 10g carbohydrate;40mg calcium; 10mg iron;30IU vitamin A potency; 0.2mg thiamine and ascorbic acid, nil (Tindall, 1968).

2.2 SEED LIPIDS

2.2.1 METHODS OF OIL EXTRACTION

The separation of oils and fats from oil-bearing animal and vegetable materials constitutes a distinct and specialized branch of fat technology (Bailey,1951).The widely differing characteristics of different fatty materials have given rise to such diverse extraction processes as rendering, pressing and solvent extraction. However, all extraction processes have certain objects in common. These are first, to obtain the oil as free as possible from undesirable impurities. Secondly, to obtain as high a yield as is consistent with the economy of the process and thirdly, to produce an oil cake or residue of greatest possible value.

2.2.1.1. RENDERING

The process consists of heating meat scraps so as to cause the fat to melt. The melted fat settles on top of the water whilst the remaining tissues settle below. The melted fat is often separated by skimming or centrifugation.

2.2.1.2 PRESSING OR EXPELLING

This extraction process is based on rupturing the cell walls by means of high pressure to force the oil out of the oil seed. Various types of mechanical presses and expellers are used to squeeze oil from the seeds. Examples include the screw and hydraulic presses.

Mechanical and thermal energies are usually used to pretreat the seeds before extraction for these reasons:

1. Breaking, grinding and rolling seeds.
2. To rupture or finish rupturing of oil cells.
3. To increase the fluidity of the oil by increase in temperature.
4. To coagulate or granulate the protein aleurone grain. This facilitates a separation of oil from the proteinaceous and other materials.
5. To "precipitate" phosphatidic materials in order to produce oil of lower refining loss.
6. To destroy mould and bacteria.
7. To destroy antinutritional factors e.g. gossypol in cotton seed.
8. To inactivate enzyme systems which have adverse effects on the quality of oil and meal (Fincher 1958, Davie & Vincent, 1980).

The heat for cooking or grinding seeds should not be excessive or it may result in darkening of the oil, reduction in oil extraction rate and low quality oil.

In general, the required cooking temperature is a function of the following factors:

i. cooking time.
ii. The type of oil extraction technology.
iii. The moisture content of the raw material.
iv. The type of seed being used.

All these variables must be considered simultaneously when considering cooking temperature (Anon,1987).

2.2.1.3 SOLVENT EXTRACTION

2.2.1.3.1 ORGANIC SOLVENT EXTRACTION

This is the extraction of oil from cracked seeds at low temperature with a non-toxic volatile fat-soluble solvent. Universally, hexane is largely used. Trichloroethylene, in small quantities is used to extract tallow from meat and bone (Davie & Vincent, 1980). Extraction is based on penetration and cross diffusion of the volatile solvent into the cells of the seed to release the oil. The mixture of oil and solvent is called miscella. The solvent is distilled from the oil and recovered for re-use.

Direct solvent extraction is generally used for low oil content (< 20% oil) seeds such as soya, rice bran and dry milled corn germ. Pressing followed by solvent extraction is used for high oil content (> 20% oil) seeds such as copra, cotton seed, palm kernel, groundnut, rapeseed, safflower and sunflower seeds, (Davie & Vincent, 1980).

Solvent extraction gives the maximum product of oil yield but the method has some disadvantages, which include:

i. Its restricted suitability for only large scale production.

ii. The high cost of investment.

iii. The need for highly skilled labour.

iv. Employment generation is low.

v. Danger of explosion because of the highly volatile solvents.

2.2.1.3.2. **AQUEOUS EXTRACTION**

Aqueous processing is the extraction of undefatted oil-seeds with water as the solvent. It is the most widely used method for the traditional extraction of oil from seeds. Presently it is commonly used to extract oil from coconut, sheanut, groundnut and palm kernel (Ata, 1982). During the last three decades, considerable progress has been made in perfecting aqueous processing as an alternative to conventional oilseed processing (Kim, 1989). It has successfully been applied to soybean, (Rhee et al, 1972), lupines (Aquilera et al, 1983) and palm kernel (Kim,1989). The extraction is based on the penetration of steam generated from the boiling water into the pulverised meal. The pulverised nature of the meal permits greater dispersion in the aqueous medium. An effective interaction between the granular particles and the heat generated from the boiling enhances release of oil. The heat absorbed by the cells causes an increase in pressure within the cells. The resulting expansion causes the cell wall to burst to release the oil. The heat also facilitates the release of oil from conjugated lipids. Fats which are semisolid at room

temperature melt to become oils in the presence of the heat. This enhances the efficiency of the extraction. The aqueous extraction method is inexpensive because the solvent for extraction is readily available.

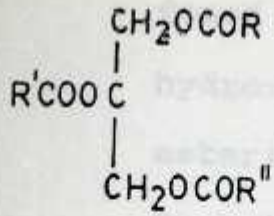
2.2.2. LIPID CHEMISTRY

The term lipid has traditionally been used to describe a wide variety of natural products which have in common a ready solubility in organic solvents (Harbone,1984). The principal lipid classes consist of fatty acid moieties linked by an ester bond to glycerol or by amide bond to long chain bases. Also they may contain phosphoric acid, organic bases, sugars and most complex components that can be liberated by various hydrolytic procedures.

2.2.2.1 GLYCERIDES

Glycerides are esters of the trihydric alcohol, glycerol, and fatty acids. Partial glycerides have only one or two position(s) esterified and are called mono and diglycerides respectively (Gurr & James, 1975). In the majority of mature seeds the quantitatively major class of lipid is triglycerides, see (fig 2). This may constitute between 10 - 70% of the tissue dry weight (Hitchcock & Nicholas,1972). The most abundant fatty acid in natural glycerides are palmitic, stearic, oleic and linoleic but generally plant glycerides have a relatively higher proportion of more unsaturated fatty acids (Gurr & James, 1975). Certain fungal and seed lipids have been

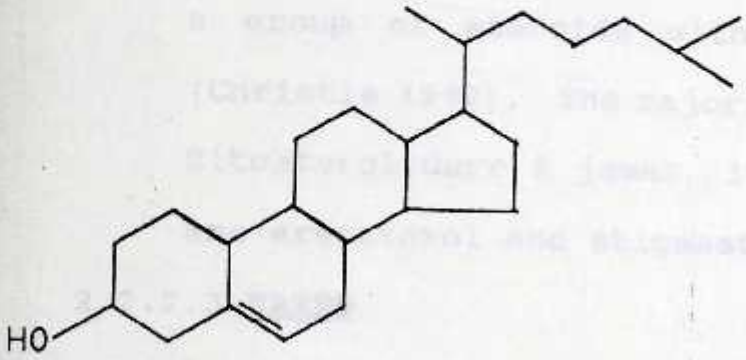
(i)



TRIACYLGLYCEROL

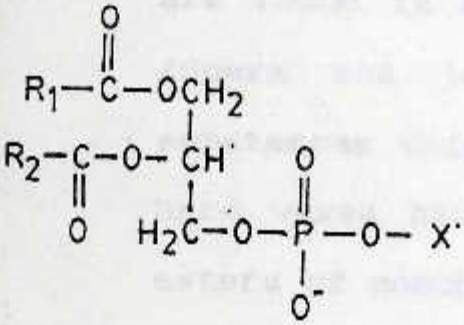
R R' R''-hydrocarbon chains of different fatty acids

(ii)



CHOLESTEROL

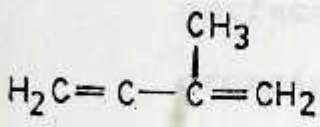
(iii)



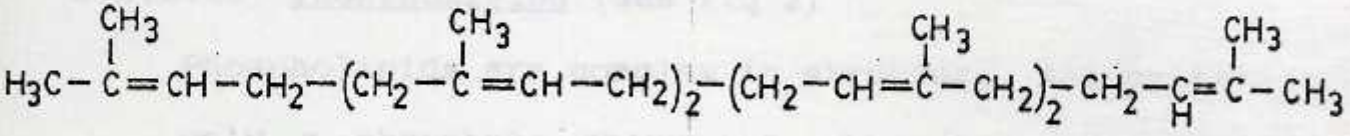
PHOSPHOLIPID

X-choline, ethanolamine, Inositol, Serine, glycerol

IV)



ISOPRENE



SQUALENE

FIG. 2 STRUCTURE OF LIPIDS

found with triglyceride components that contain hydroxyfatty acids. Additional fatty acids are esterified to these hydroxyl groups (Christie,1982). Such lipids are termed estolide.

2.2.2.2 **STEROL AND STEROL ESTERS**

Cholesterol see (fig 2) is by far the commonest member of a group of steroids with a tetracyclic ring system (Christie 1982). The major sterol in higher plants is B-Sitosterol(Gurr & james, 1975). Other related sterols are ergosterol and stigmasterol.

2.2.2.3 **WAXES**

Commonly, waxes are referred to as esters of long chain fatty acids with long chain primary alcohols. Wax esters are found in animals and insects. Also various oily (Sperm and jojoba oils) and greasy (wool grease) substances which in no wise resemble beeswax or other hard waxes have been found to consist essentially of esters of monohydric alcohols and monobasic fatty acids (Markley, 1960). The plant and animal waxes are responsible for the water repellant character of the surface and therefore important in conserving the organisms own water and providing a barrier against its environment (Gurr & James, 1975).

2.2.2.4. **PHOSPHOLIPIDS** (see fig 2)

Phospholipids are complex in structure. All contain not only a phosphate group but also at least one other

component usually a basic substituent (Harbone 1984). This basic substituent may be choline, ethanolamine or serine. Phospholipids are seldom major components of lipid extracts from plant tissues ; however, they are, like 1,2 diglycerides, an important intermediate in lipid biosynthesis (Hitchock & Nicholas, 1972).

2.2.2.5 **FATTY ACIDS**

In plants, fatty acids occur mainly in bound form esterified to glycerol as fats or lipids (Harbone,1984). Unlike fatty acids of animal origin, plant fatty acids are comparatively complex in structure. They can contain a variety of functional groups (Christie,1982). These include epoxy - hydroxy or keto groups and cycloprene. The common fatty acids of plant and animal origin contain even-numbered carbon atoms in straight chains with terminal carboxyl groups. They may be fully saturated or contain one, two or more (up to six) double bonds which generally but not always have a cis-cis configuration. Although numerous fatty acids are now known in plants most lipids have the same few fatty acid residues which make their identification that much easier. The common fatty acids are either saturated or unsaturated.

2.2.2.5.1 **SATURATED FATTY ACIDS**

Myristic acid is present in almost all vegetable and animal fats; usually in relatively small amount (1 - 5%), but in a number of fats it occurs to the extent of 40 -

70% or more (Markley, 1960). In palm oil it comprises about 15 - 30%,and in coconut oil, it is about 10% of the total fatty acid (Swern, 1964).

Palmitic and stearic acid are very common saturated fatty acids of C_{16} and C_{18} chain length. Palmitic acid is the major fatty acid in palm oil (Malaysian standard, 1983). It is also about 27% of cocoa butter fatty acids (Adomako, 1977). In the Chinese vegetable tallow (Jeffery & Padley, 1991) it comprises about 75% of the total fatty acids. Stearic acid is less prominent in leaf lipids but it is a major saturated acid in seed fats in a number of plant families (Shorland, 1963).

2.2.2.5.2 **MONOENOIC FATTY ACIDS**

Unsaturated acids are widespread in both seed and leaves. Palmitic $C_{16}H_{32}O_2$ and Oleic $C_{18}H_{34}O_2$ are by far the most commonly occurring and best known. Oleic acid forms 80% of the fatty acid content of Olive oil and 59% in peanut (Harbone, 1984). These oils are often accompanied by poly-unsaturated fatty acids.

2.2.2.5.3 **NON CONJUGATED POLY-UNSATURATED FATTY ACIDS**

Non conjugated polyunsaturated fatty acid (often abbreviated PUFA) of animal and plant origin can be sub-divided into several simple families according to the biosynthetic derivatives from simple fatty acid precursors. Linoleic acid is the commonest and most

TABLE 1

Carbon chain Length	Trivial name	Formula
	Saturated Acids	
C_{14}	Myristic	$CH_3(CH_2)_{12}CO_2H$
C_{16}	Palmitic	$CH_3(CH_2)_{14}CO_2H$
C_{18}	Stearic	$CH_3(CH_2)_{16}CO_2H$
C_{20}	Arachidic	$CH_3(CH_2)_{18}CO_2H$
	Unsaturated Acids	
C_{16}	Palmitoleic	$CH_3(CH_2)_5CH=CH_3(CH_2)_7CO_2H$
	Oleic	$CH_3(CH_2)_7CH=CH_3(CH_2)_7CO_2H$
C_{18}	Linoleic	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7CO_2H$
	Linolenic	$CH_3CH_2=CHCH_2CH_2=CHCH_2CH=CH(CH_2)_7CO_2H$
	Unusual Acids	
C_{16}	Petrosilinic acid	
	Erucic acid	
C_{18}	Ricinoleic acid	

simple fatty acid of this type. It occurs in sunflower about 80% (Pindry,1986) and 35.2%, 58.1% in rubber seed and corn oil respectively (Babatunde & Wilson, 1987). Linolenic acid is also a major component of plant lipids. It occurs in linseed oil to the extent of 51% of the total fatty acid (Harbone, 1984).

2.2.2.5.4 **UNUSUAL FATTY ACIDS**

In addition to the more common saturated monoenoic and C_{18} polyunsaturated fatty acids, plant lipids may contain a wide variety of unusual fatty acids. Petrosilinic acid, an isomer of the more common palmitoleic acid, occurs to the extent of 76% of the total acid in parsley seed, Petroselinium crispun (Harbone, 1984). It appears to be present in many other umbelliferal and also in the related Arialiaceae in Arabia. Erucic acid is another unusual acid (Appelquist & Ohlson, 1972). It is found especially in the crucifaerae and the tropaeoliaceae. It is present in high concentration in rape seed oil. Because of its toxic effects successful efforts have been made to breed rape varieties with low erucic content (Murthy et al,1983). Ricinoleic acid is similar to oleic acid except that it has a hydroxy group substituted for a hydrogen at the 12th carbon atom. It occurs to the extent of about 80 to 85% of the total fatty acids in castor oil and is responsible for the several unique properties of this oil (Bailey,1951). The commercial

value of cotton seed oil is also reduced as it contains cyclopropene fatty acids. This acid is small in concentration but it is sufficient to produce profound physiological effects in animals by inhibiting fatty acid desaturation, when the oil is incorporated into animal feedstuffs (Christie, 1982).

2.2.3. LIPID SOLUBLE PHYTOCHEMICAL COMPOUNDS

2.2.3.1 TRITERPENOIDS

They are compounds with a carbon skeleton based on six isoprene units and which are derived biosynthetically from the acyclic C_{30} hydrocarbon squalene.See (fig.2) Many triterpenes are known in plants and new ones are regularly being discovered and characterised (Das & Mahota, 1983). Some are known for their taste properties particularly their bitterness. Example is limonin the lipid-soluble bitter principle of citrus fruits. It belongs to a series of pentacyclic triterpenes which are bitter, known as limonoids and quassinoids. They occur principally in Rutaceae, Meliaceae and Simaroubaceae (Waterman & Grundon,1985). Another group of bitter triterpenes are the cucurbitacins, confined mainly to the seeds of various cucurbitaceae.

2.2.3.2 SAPONIN

Saponins are glycosides of both triterpenes and sterols and have been detected in over seventy families of plants (Tschesche & Wulff, 1973). They are surface active

agents with soap-like properties and can be detected by their ability to cause foaming and to haemolyse blood cells. The search in plants for saponins has been stimulated by the need for readily accessible source of sapogenins which can be converted to animal sterols of therapeutic importance, saponins are also of economic interest because of the occasional toxicity to cattle.

2.2.3.3 **CAROTENOIDS**

Carotenoids are C_{40} - hydrocarbons containing long systems of conjugated ethylenic linkage which are responsible for their colour and for their characteristic absorption in the 450 -500nm of the visible spectra range.In animals one particular carotenoid, B-carotene, is an essential dietary requirement since it provides Vit A, a C_{20} isoprenoid alcohol (Bauernfiend, 1981). The pigments are often highly oxidized with heat and acid. During Industrial oil processing they are removed together with the gums and during bleaching.

2.2.4. **QUALITATIVE TESTS FOR OILS**

Qualitative examination aims not only at the identification of a sample as a lipid but also at information about its type and origin. Moreover qualitative assessment of the actual condition of the lipid samples as well as the detection of impurities are important guides for the analytical methods to be applied.

2.2.4.1 **SOLUBILITY**

Besides smell and taste which are often characteristic, important clues to the identification of a sample as lipid are obtained from its superficial appearance and its solubility especially in organic solvents. All fats are easily soluble or miscible with organic solvents (Christie, 1982), but with the exception of castor oil, lipids are completely immiscible with alcohol (Bockenogen, 1964).

2.2.4.2. **INFRA RED** (IR) SPECTROSCOPY

Infra red spectroscopy is one of the spectroscopic methods applied to the analysis of lipids in general and fatty acid in particular (Davenport, 1971). Although there has been comparatively few new developments involving the technique it is still probably the spectroscopic method that would be chosen for the preliminary examination of an unknown lipid (Christie, 1982). Infra red spectra are obtained when energy of light in infra red region at a given frequency is absorbed by a molecule, thereby increasing the vibrations in the atoms in the molecule. By far the most common use of the infra red absorption spectroscopy is for the determination of isolated trans-double bonds in the fatty acids and lipids in general (Christie,1982).

2.2.5 **QUANTITATIVE TESTS**

2.2.5.1 **MOISTURE**

Moisture is one of the very important factors which affect the storage quality of oils and fats. It activates lipases which increase free fatty acids. Several methods are used to determine moisture content in lipids. These include Hot Oven (Cocks & Van Rede, 1966) and Karl Fishers (Van Der Vet,1968). The AOAC (1984) prescribes a drying method in which 5gm of oil is dried in a vacuum oven at uniform temperature of 20°C to 25°C above the boiling point of water at a working pressure which should not exceed 100mm of Hg. This method determines the water and the volatile matter together.

2.2.5.2 **REFRACTIVE INDEX (R.I)**

The index of refraction is the degree of diffraction of a beam of light that occurs when it passes from one transparent medium to another. The R.I. of fats and oils are often measured because they can be rapidly and accurately determined. It is a useful characteristic for quickly sorting out oils of unknown identity and for observing the progress of catalytic hydrogenation. This is because of the close relation of R.I. to the degree of unsaturation (Swern, 1964).

2.2.5.3 **SPECIFIC GRAVITY**

The specific gravity of oils is determined by the usual methods, (AOAC, 1984). The temperature is controlled since significant changes in these compounds occur in

short ranges of temperature. The specific gravity of a fat or oil is usually measured at 25°C but it may be necessary to use temperatures of 40°C or 60°C. Variation in the specific gravity from one oil to another is not great (Codex,1984). In general either unsaturation of the fatty acid chains or increase in chain length of the fatty acid residues tend to increase the specific gravity (Meyer,1964).

2.2.5.4 **SLIP AND MELTING POINTS**

Unlike inorganic compounds, fats and oils have a melting temperature range. The slip points, also a range, is the temperature at which the fat begins to "flow". This is related to the air or water beaten into the fat during its manufacture as well as its composition (Meyer, 1964). On the other hand the melting point refers to the temperature at which the fat completely melts.

2.2.5.5 **UNSAPONIFIABLE MATTER**

These include the sum total of all components which are stable towards bases, insoluble in water and soluble in fat solvents. They are not volatile at 100°C (Boekenogen, 1964). Cholesterol, its esters and certain vitamins that are associated with lipids in the natural sources contribute largely to the unsaponifiable matter.

2.2.5.6. **SAPONIFICATION VALUE**

The saponification number of a fat or oil is the number of milligrams of KOH needed to saponify completely 1g

glyceride (Martin,1979). Since the number varies inversely with the molecular weight of the glyceride, it serves as a measure of mean molecular weight of the constituent acids (Cocks & Van Rede, 1966).

2.2.5.7 **IODINE VALUE**

It is the number of grams of iodine or iodine compounds absorbed by 100gm of fat (Martin,1979). The double bonds present in the unsaturated fatty acids react readily with iodine to form an addition compound, even while the fatty acid is combined with the glycerol in the fat. The wigs method (AOAC,1984) uses an iodine solution made up in glacial acetic acid but contain iodine chloride as an accelerator. The excess iodine reacts with sodium thiosulphate according to the following equation

$$2Na_2S_2O_3 + I_2 \text{ ---------> } 2NaI + Na_2S_4O_6$$

The end point is determined by the disappearance of the blue starch - iodine colour.

2.2.5.8 **ACID VALUE**

The acid value is a measure of the free organic acid content (Boekenogen, 1964). It is calculated based on the molecular weight of a given fatty acid or fatty acid mixture. It gives the number of milligrams of KOH which are required for the neutrilization of the free organic acids contained in 1g of fat.

2.2.6 **CHROMATOGRAPHIC TECHNIQUES**

Lipid samples obtained are complex mixtures of individual

lipid classes. Often no simple procedure will achieve the desired separation. Thus, combination of techniques must be used until the required pure lipid classes are obtained. Adsorption Chromatography procedures are generally used to separate each of the simple lipid classes from the complex lipids. The latter may be further fractionated by adsorption chromatography or by ion-exchange chromatography or by the combination of both until the necessary separations are obtained.

2.2.6.1 **THIN LAYER CHROMATOGRAPHY**

Thin layer chromatography (TLC) has been extensively used for the separation of glycerides, fatty acids and methyl esters. TLC permits rapid separation of triglycerides and free fatty acids from complex lipid mixtures as in tissue lipids (Mahadevan, 1967).

Briefly, the method is as follows: the adsorbent is spread on a glass plate in even layers about 0.5mm thick, the sample to be examined (10 - 50mg) is applied in solution to one spot on the plate and developed during 20 - 60min with an ascending flow of a solvent such as petroleum ether or diethyl ether or mixtures of solvents. Lipid classes may be identified by the reaction with specific chemical reagents, by various spectroscopic techniques or by their chromatographic behaviour relative to that of authentic standards.

2.2.6.2 **GAS LIQUID CHROMATOGRAPHY**

This is the most important single method available to lipid chemists. It is an efficient and rapid method for the separation of the component from small batches of mixtures of volatile compounds (Crammer & McNair, 1983). Briefly the sample is introduced at one end of the tube. An inert carrier gas (usually nitrogen or helium) is made to sweep past them and to pass through a long and narrow tube, containing a sorbent and kept at appropriate temperatures. The effluent gas emerging at the other end passes through a detector (Ambrose, 1971).

Fatty acids are normally separated as their methyl esters on two main types of stationary phases, polar and non polar. The non polar include hydrocarbons (Apeizon greases) silicones, or fluorinated silicone preparations. The polar phases are polyesters eg diethylene glycolsuccinate and ethylene glycolsuccinate in butenediol adipate (Christie, 1982). The normal form in which the result from a gas chromatographic separation is obtained is a chromatogram, the trace representing the detector output. Peaks in the gas chromatogram are identified by comparison of retention times with standards.

2.2.7 FOOD LIPIDS

2.2.7.1 DIETARY ROLE

The part played by animal and vegetable oils and fats as human foods is discussed briefly (Fox & Cameron, 1983),

however, its metabolism in man has also been the subject of increasing interest and investigation (Stryer 1981). Fats are desirable dietary constituents for six main reasons (Guthrie, 1983).

1. Energy: The supply of energy in an average mixed diet is slightly less than 9 available calories per gram; whereas carbohydrates or proteins supply 4 calories.
2. Fat decreases hunger between meals. Fats tend to leave the stomach relatively slowly, being released approximately 3½ hours after ingestion. The delay in emptying helps to delay the onset of hunger pangs and contributes to the feeling of satiety.
3. Fats act as carriers of fat soluble vitamins.
4. Fats act as sources of essential fatty acids.
5. Fats provide precursors of prostalglandins.
6. Fats increase the palatability of foods, partly by their lubricating effect.

In man the only human obligatory reasons for including fat in the diet is to supply the essential fatty acids.

2.2.7.2. **FAT REQUIREMENTS**

The optimal level of fat in the diet is not known, and since fat may be formed from carbohydrate or protein, no definite requirement can be formulated (Goldsmith, 1953). For the estimation of optimum level, information is not obtained from dietary survey, rather, it is from surveys

of the types and quantities of retail food on the market (Lloyd & McDonald, 1978).

2.2.7.3. **DIGESTION AND ABSORPTION OF FATS**

Not until fat reaches the small intestine do the chemical changes necessary for fat digestion take place. Agents from two major sources accomplish this work. Bile salts from liver, gall bladder and lipase from pancreas (Williams, 1982). Pancreatic lipase acts on oil-water interphase after emulsification of triglycerides (this is aided by the detergent properties of bile salts) to hydrolyse it.

Fine particles, monoglycerides and fatty acids are formed from the triglycerides. They combine with bile salts to form water-soluble complexes known as micelle. These micelles enter the minute spaces between the small projections of the intestinal tract cell lining. The fatty acids and monoglycerides diffuse into the mucosal cells lining the intestines, the bile salts remain in the lumen of the intestines to be reused.

The lower chain fatty acids and monoglycerides recombine to form triglycerides. The reformed triglycerides combine with proteins to make them sufficiently water-soluble to be transported in the blood and other body fluids for storage (Guttrie, 1983).

2.2.7.4. **STORAGE OF LIPIDS**

After absorption, fat may be oxidized immediately in the

liver and muscles or used to synthesize other lipids, such as phospholipids or stored in the adipose tissue until required (Bell & Smith, 1976). Fat is also stored in other tissues.

Fatty change, ie, accumulation of fat in cells other than adipose cells is an indication of a diseased organ. It is due to imbalance between fat and fatty acids entering the cell and the rate of utilization or release of fat by the cell (Anderson, 1975). Because of its major role in fat metabolism, the liver requires special consideration in fatty change. However, fatty change does not only occur in the liver cells, which are usually most seriously affected but also in various other organs and tissues (Anderson, 1975).

2.2.7.5 **LIPID LEVELS IN BLOOD**

Extraction of plasma lipids with a suitable solvent and subsequent separation of the extract into various classes show the presence of triacylglycerides, phospholipids, cholesterol and cholesterol esters. In addition, the existence of much smaller fraction of unesterified long chain fatty acids account for 5 percent of the total fatty acids present in the plasma (Mayes, 1988). Usually after a fatty meal glyceride level in the blood increases from 300 to 500mg per 100ml. Maximum hyperlipemia is observed 2½ hours to 3 hours after the meal,and fasting level is attained by the fifth hour (Goldsmith, 1953).

2.2.8. INDUSTRIAL USES OF OILS AND FATS

2.2.8.1 FOOD INDUSTRY

To a large extent the consistency of a lipid affects its industrial use. Fats and oils are used in the food industry for manufacturing products of high fat content such as margarine. Lipids are also incorporated into food as salad oils. They contribute to mouthfeel and are used as a carrier for flavor. They can be emulsified with other ingredients to produce semisolid fatty foods known as mayonnaise or salad dressings. Fats and oils are also used for frying. Their high temperature achieved before the oils start to deteriorate, and the high thermal capacity makes the food cook quickly and develop a crisp texture. (Heid & Joslyn, 1981). Cooking fats are also used as shortening agents in a variety of foods. These include biscuit, short bread and pastry. They impart a tender quality to the baked goods through a combination of lubrication and ability to alter interaction among their constituents. (Brownsel et al, 1989).

2.2.8.2 SOAP INDUSTRY

There are a variety of oil and fat derivatives which owe their industrial importance to their ability to modify the surface behaviour of liquids in which they are dissolved. These products may be grouped under the broad designation of" surface active materials." The most

important surface active material is ordinary soap (Swern, 1964). Soap comprises the sodium or potassium salts of various fatty acids but chiefly oleic, stearic, palmitic and myristic (Austin, 1986). The raw materials for the making of soap cover a wide range of substances which may be classified under the following headings: fats and oils, alkalis, filling agents, water, salt, perfume, colour and dyes. However, fats and oils constitute about 90 percent of the soap maker's raw materials (Donkor, 1986). Traditionally, soap in Ghana and many other African countries involved the use of various types of oils (Huthchinson & Dalziel, 1954). However the rapid increase in prices due to shortages of edible oil has resulted in the search for alternative sources of raw materials. Donkor(1986), has reported the potentials of using neem seed oil, castor oil and physic oil. Besides the saponifiable materials the most important raw material used by soap makers is caustic soda. Caustic potash can also be used. The basic chemical reaction in soap making is the saponification reaction.

$$3NaOH + (C_{17}H_{35}COO)_3C_3H_5 \longrightarrow 3C_{17}H_{35}COONa + C_3H_5(OH)_3$$

Caustic soda glyceride Soap glycerol

Other materials derived from fats and oils include sulphonated oils to textiles and leather processing, and

a great variety of wetting agents (Bailey, 1951). These find special application in many different industries.

2.2.8.3 **SURFACE COATING INDUSTRY**

Paint, varnishes and other decorative and protective coatings including paint inks comprise one of the three major fields of oils and fats utilization. Basically, specialized oils for these products are the drying oils. These oils owe their value as materials for protective coating to their ability to polymerize or "dry" after they have been applied (Swern, 1964). They form tough adherent, impervious and abrasion resistant films (Swern,1964). Their film-forming properties are closely related to their degree of unsaturation since it is through the unsaturation centres or double bonds that polymerization takes place (Bailey, 1951).

2.3 OIL CAKE

In addition to lipid, oilseeds frequently contain high levels of nitrogen-containing constituents and important among these are proteins (Woodham, 1969). They also contain starch, essential growth factors and a moderate amount of minerals. After expressing or solvent extracting the greater part of oil the residue that remains is the oilseed cake or meal. Coconut oil meal is obtained after extraction of the oil from the kernels of nuts of the coconut palm. The meal, containing about 20 percent crude protein and a small amount of oil is ground

into a meal and used for animal feeding (Child, 1974). The meal find common use in cattle feed as protein supplement (Miller, 1979). Palm kernel meal is obtained after expressing the oil. The meal is regarded as a low protein meal but its content of essential amino acids together with a favorable calcium to phosphorus ratio enables it to make a valuable contribution to animal feed (Mcdonald et al, 1988). Peanut meal is a ground product of the shelled peanuts. Peanut meal as usually sold contains 45 percent protein (lassister & Edwards, 1982). Peanut meal is poor in lysine, threonine and methionine (Ihenkoronye & Ngoddy, 1985). The presence of aflatoxin in peanuts and peanut products is one of the biggest problems associated with the commodity (Hao & Brackett, 1987). Not all oil seed meals are edible. The principal inedible meals are castor and tung. Castor seed is an excellent source of oil (50%) and protein (20%) and about 0.91 million tons of castor beans are available per year (Dua et al,1986). The oil is obtained by pressing. Even though the cake contains a good quality of protein and mineral, the cake contains an extremely potent allergin and a highly toxic protein (Weiss, 1971). Thus castor meal finds its principal application as a fertilizer.

2.3.1 TOXICITY OF OILSEED CAKES

The toxicity associated with oilseed products is one of

their most serious drawbacks and constitutes a notable point of difference from other protein sources such as animal products and cereals.

3.3.2 **SAPONINS**

Saponins are sterols or triterpeneglycosides found in a variety of plant species, a few of which are used as foodstuffs by man. Examples are soy beans, chickpeas and spinach (Oakenfull,1984). Saponins have been associated with bitter taste, form stable foams in aqueous solutions and haemolyse red blood cells. In plants saponins may have an antimicrobial action, but they appear to be virtually non toxic when taken orally (Merck index, 1976). Under normal circumstances the saponins in food are not absorbed into the blood stream but remain within the gut. When the integrity of the intestinal wall is compromised the saponins do pass through and severe poisoning occurs due to hemolytic activity (Martindale, 1972).

2.3.3. **TANNINS**

Tannins are water soluble polyphenolic compounds of high molecular weight (500 - 3,000). Tannins occur widely in vascular plants, their occurrence in the angiosperms being particularly associated with woody plants (Harbone,1984). There are two main types namely non-hydrolysable or condensed tannins and hydrolysable tannins. The latter are readily cleaved by enzymes and

dilute acids into sugar and a phenol carboxylic acids. The former may however undergo slight acid decomposition to liberate small amounts of anthocyanidins or under progressive polymerization to yield tanin reds (Strumeyer & Malin, 1975). The antinutritional effect of tannin is a result of its ability to form complexes with proteins and vitamin B_{12} making them unavailable. Tannins have been found to cause decreased laying rate, decreased digestibility and decreased efficiency of food utilization in hens and chicks (Cramsfield et al, 1980, Martin-Tanquay et al,1977). The decreased protein digestibility caused by tannins is believed to be due to inhibition of the digestive enzymes (amylase, trypsin, and to a lesser extent lipase) or due to the binding of dietary proteins into an indigestible form. The net effect is reduction in amino acid availability and increased faecal nitrogen (Tamir & Alumont, 1969).

2.3.4 OXALATES

Oxalic acid occurs throughout the plant world mainly as oxalates. The oxalic acid in food complexes with the mineral ions especially Ca^{2+} making them unavailable to the body (Oke, 1966). The calcium-oxalate complex is excreted in the faeces. In the kidneys, the crystals of calcium oxalate may be deposited in the urineferous tubules as granules or stones leading to the incidence of oxaluria. In general, it has been found that oxalic acid

content depends on the species, the parts of plant analysed, the climate, soil condition and other factors (Oke, 1972).

2.4 ENZYMES

Clinical enzymology is the application of enzymes in the diagnosis and treatment of diseases (Donald et al,1987). Enzymes are protein with catalytic properties. They are found in cells and also in blood serum. For diagnosis, the serum is commonly used because the enzyme levels in the serum are very low and therefore the slightest increase in levels due to cell damage can be detected. Also it is easy to obtain the serum (Donald et al, 1987). Generally elevation in the level of transaminase, alkaline phosphatase and lactate dehydrogenase are used to monitor damaged liver, heart and diseased pancreases respectively (Varley et al, 1980).

3 MATERIALS AND METHODS

3.1 Preparation of sample

Mature seeds of Carapa procera and sun dried Pentadesma butyraceae seeds were collected from UST Campus whilst pods of fluted pumpkin were obtained from Birim north district. Fluted pumpkin pods were first split open and together with Carapa procera nuts were dried between 50°c to 60°c for 7 days in an oven. The dried nuts were then cracked to obtain the seeds. The seeds were crushed and pounded in a mortar to paste. They were kept in brown sample bottles and stored in a freezer till required.

3.2 Proximate analysis (AOAC 1984)

3.2.1 Determination of moisture content

Two grams of sample was weighed into a previously tarred dish and dried to a constant weight in a thermostatically controlled oven at 105°c. The dish was removed, cooled in a desiccator, and weighed. The average loss in weight was taken to be the moisture content and expressed as a percentage of the total weight of the sample used (see Appendix C.1).

3.2.2 Determination of crude fat (soxhlet method)

Two grams of dried sample was weighed into a cellulose thimble and plugged with glass wool. The fat was extracted with petroleum ether in a soxhlet apparatus for 16 hours. The flask was removed and the petroleum ether evaporated over a steam bath. The flask and

contents were dried in an oven at 100°c for 1 hour and the flask reweighed. The difference in the weight of the flask gave the weight of the crude fat contained in the sample used. The percentage fat was calculated (Appendix C.2).

3.2.3 Determination of crude fibre

Two grams defatted sample was transferred into a 500 ml round bottomed flask and half gram asbestos was added. 200 ml of boiling 1.25% H_2SO_4 was added and the flask was immediately set on a hot heating mantle. A cold finger condenser was connected and boiled for 30 minutes. The content of the flask was filtered and the residue washed with boiling water until free from acid. The residue and asbestos were washed back into the flask with some boiling 1.25% NaOH solution and boiled again, for 30 minutes after which it was filtered and washed thoroughly with boiling water. The residue was transferred to a gooch crucible and washed with 15 ml of alcohol and dried at 100°c until a constant weight was obtained. The crucible and its contents were cooled and weighed. After cooling the difference in the two weights were recorded as the crude fibre content (calculation of fibre, see Appendix C.3).

3.2.4 Determination of ash

Two grams of sample was weighed into a previously ignited and weighed crucible, and ignited in a furnace preheated

at 550°c for 2 hours. It was cooled in a desiccator and reweighed. The difference was recorded as the ash content (see appendix C4 for calculation).

3.2.5 Determination of Crude Protein

Crude protein content was determined by the kjeldahl method. One gram sample was digested with 25ml of concentrated H_2SO_4 and 2 tablets of kjeldahl catalyst ($CuSO_4$ +K_2SO_4) in a kjeldahl digestion flask until the mixture was clear. The digest was transferred into a 100 ml volumetric flask and diluted to the mark. 10ml aliquot of the digest was pipetted into the decomposition chamber of a Markham distillation apparatus. 15ml of 40% NaOH was added and the ammonia released trapped into 25ml of 2% Boric acid solution containing 2 drops of bromocreosol green mixed indicator. A colour change from green to blue was observed as the ammonia was being trapped. Distillation was continued for about 10 minutes. The boric acid was then titrated with 0.01N HCl and the percentage protein calculated. (Appendix C.5).

3.2.6 Determination of Carbohydrate

The carbohydrate content is the difference between 100 and the sum of the moisture, protein, fat, ash and fiber contents. (see Appendix C.6).

3.3 Toxicant analysis (Oke 1965)

3.3.1 Determination of Total oxalate content

2g of the dry sample was digested with a mixture of

190ml water and 10ml 6N HCl. The digest was filtered and diluted to 250ml with 6N HCL, and the brown precipitate was filtered and washed with hot water. The solution and wash were combined and washed with concentrated NH_3 until the salmon-pink colour of the methyl red indicator changed to faint yellow. The solution was then heated on a hot plate to about 90°c and the oxalate precipated with 10ml of 5% $CaCl_2$ solution. The solution was allowed to stand overnight, filtered, and the precipitate was then washed into a beaker with hot 25% v/v H_2SO_4 and diluted to 25ml. It was warmed to 90°C and titrated with 0.5N. $KMnO_4$ (see Appendix C7 for calculation).

3.3.2 **Determination of saponin** (Gestetner 1965)

2g of defatted sample was dispersed in a solution of IN H_2SO_4 in dioxane-water (1:3) and hydrolysed under reflux for 8 hours. The mixture was cooled, diluted with 200ml water and the resultant sapogenins were extracted with 100ml and then with three portions of 50ml ethylether. The combined ether extracts were washed with water, dried with Na_2SO_4 and evaporated to dryness in a previously tarred dish on a water bath. The saponin content was calculated from the difference in weight and expressed as a percentage (See Appendix C8).

3.3.3 **Determination of Tannins** (AOAC 1970)

2.5 grams of sample were boiled with 200mls of distilled water cooled and transferred to a 500ml. Volumetric

flask, and diluted to volume (ie the 500ml mark). To 5ml infusion, 12.5 ml of indigo carmine solution and 375 ml distilled water were added. Potassium permanganate solution was added little by little from a burette while stirring until the solution turned light green, and then the potassium permanganate was added dropwise until a bright yellow coloration was observed.

The volume of $KMnO_4$ solution used was designated as x. A clear infusion of sample was mixed with 25ml of gelatine solution, 50ml acid Nacl solution,and 5 grams powdered kaolin and shaken for 20 minutes in a stoppered flask. The mixture was allowed to settle and then it was decanted through a filter paper. 12.5ml of the filtrate obtained was mixed with 12.5ml of indigo carmine solution, 275ml distilled water and titrated against $KMnO_4$ solution as previously done. The volume of $KMnO_4$ solution used subtracted from that previously obtained (designated x) gave the volume of $KMnO_4$ solution which was required to oxidize the tannin obtained in the sample (See Appendix C.9).

3.4 **Analysis of Mineral composition**

sample preparation.

One gram sample was accurately weighed, into a glazed, high - form porcelain crucible. It was ashed for 2 hours at 500°c and cooled. The ash was wetted with 10 drops of distilled water and 3-4ml HNO_3 (1+1) was carefully added.

Excess HNO_3 was evaporated on hot plate set at 100°c - 120°c. The crucible was returned to the furnace and ashed for an additional 1 hour at 500°c. The crucible was cooled, the ash dissolved in 10ml HCl (1+1), and transferred quantitatively to a 100ml volumetric flask.

3.4.1 **Determination of Potassium and sodium**

The calibration curves were determined using the direct - intensity method. Distilled water and the strongest standards,that is 10ppm of KCl and NaCl solutions (Appendix 2B2), were used to adjust the reading scale to zero and 100 divisions (or full scale), on the flame photometer. The emission intensities from the ashed solutions were determined. The potassium and sodium contents were extrapolated from the standard curves (Appendix C 10).

3.4.2 **Determination of Ca, Zn, Mg, Fe, and Cu. (AOAC 1984)**

The atomic absorption spectrometer was used to determine these elemental concentrations. The calibration curves were determined using the emission, intensity of the various standards using their respective lamps. The emission intensities of the elements in solution were read directly as computed by the spectrometer. (See Appendix C10 for calculation)

3.4.3 **Determination of Phosphorus (Carwell, 1955)**

An aliquot (Xml), containing 50-500mg of phosphorus of the sample solution was transferred into a 50 ml

graduated flask. 5ml of 5M Hcl and 5ml ammonium molybdate-ammonium metavanadate (appendix 2B3) reagent was added. The solution was diluted to 50ml and allowed to stand for 30 minutes. The absorbence was read at 400nm. Concentration was extrapolated from the standard phosphorus curve (figure P) See Appendix C10 for calculation.)

3.5 **Extraction of total lipids.**

The procedure of Bligh & Dyer,(1959) was used. It is assumed that 100g of the wet tissue to be extracted contains 80g of water. 100g of the sample was homogenized for 4 minutes in a waring blender with a solvent mixture consisting of 100ml of chloroform and 200ml of methanol. If the mixture had two liquid phases, more chloroform - methanol was added until a single phase was achieved. The mixture was filtered through a sintered glass funnel and the tissue residue rehomogenized with 100ml of chloroform and filtered once more.

The two filtrates were combined, transferred to a 1 litre graduated measuring cylinder, 100ml of 0.88% potassium chloride in distilled water was added and the mixture shaken thoroughly before being allowed to settle. The mixture was now biphasic (further aqueous solution could be added to ensure this). The upper layer with any interfacial material was removed by aspiration. The lower phase contains the purified lipid and was filtered

before the solvent was removed on a rotary evaporator. The lipid was stored in a small volume of chloroform at -20°C.

3.5.1 Methylation of Lipids (AOAC, 1984)

250mg of sample was weighed into a flask and then 4ml of 0.5N methanolic NaOH added together with boiling chips. An air condenser was attached and contents refluxed until fat globules disappear (about 10 min.). 5 ml of BF_3 solution was added through the condenser using a pipette and boiling was allowed to continue for 2 min. 5ml Hexane was added through the condenser and boiling allowed for another 1 min. Heat was removed and several mls of saturated NaCl solution added till hexane floated into the neck of the flask. The hexane fraction was pipetted and dried using Na_2SO_4.

3.6 Fatty Acid Analysis

Fatty acid analysis was carried out by using a varian 3700 gas chromatograph equipped with a flame ionization detector, coupled to a varian intergrater and chart recorder. A 2m X 2m (i.d.) 15% OV-275m chromoscorb W 80/100 column was used. Temperature was programmed from 170°c to 230°c at 6°c/min. Carrier gas was nitrogen at a flow rate 4ml/min. The injection port and detector temperatures were 220°c and 240°c respectively. Volume injected was 1 μl.

Identification and quantification of fatty acids were

based mainly on direct comparison of the gas chromatogram response to available fatty acid methylester standards.

3.7 **Infra red spectroscopy (Harbone, 1984)**

Carl zeiss Jena specord M50 was used in this qualitative analysis. Clarified liquid oils were transferred into the absorption cells by means of a hypodermal needle. Excess lipid was wiped off. The cell was placed into the holder in the sample compartment and the lever closed. Absorption was read between the wavelengths 4000cm^{-1} and 200cm^{-1}. The results were printed on a chart, (fig 30.31.32.)

3.8 Analytical thin layer chromatography (Harbone, 1984)

Technique	:	One way ascending
Adsorbent	:	Silica gel G. (merck)
Plate Size	:	20 cm X 20 cm
Plate preparation	:	A slurry was formed from 30g of the silica gel and 60 ml of distilled water and spread over 5 plates.
Layer thickness	:	0.25 mm
Activation	:	Plates were activated at 100°C for 30min after air drying for 30min.
Application of Sample	:	was done as spots with the aid of capillary tubes and spotting templates.
Solvent System	:	Hexane : ether: Acetic acid: 80: 20:1 (v/v)
Running time	:	10 minutes
Detection reagent	:	H_2SO_4 : H_2O 9:1(v/v)

3.9 **Determination of food energy value (Lloyd & McDonald, 1978)**

Reasonable estimates of the available calorific value were obtained by multiplying the percentages of protein and carbohydrate by the factor 4 and the percentage of ether extract by the factor 9 and adding the resultant figures together.

3.10 **Determination of Calorific value**

Between 0.20 to 0.25g of defatted sample was weighed into the ignition crucible provided for the instrument. The bomb was then closed. This was after a loose thread extending from the loop had been placed in the pan. Oxygen was admitted to a pressure of 25 atmosphere in the bomb. The loop (electric heating element) ignites the sample, the heat of this ignition is then directed to the lid of the bomb, which had a thermocouple mounted on it, connected to a sensitive galvanometer. The zeroed galvanometer is allowed to reach maximum deflection and the reading recorded. The exact calorie content is obtained by extrapolating on a standard benzoic acid curve.

3.11 **Amino Acid analysis**

Known weights of each sample was placed in a test tube and 4 ml of 6M HCl added. The test tube and its contents were placed in a dry block of model troika dry Block (MG-2). The temperature was set at 110°c and the sample

hydrolysed for 22 -24 hours. The hydrolysate was then freeze-dried to dryness using freeze-dryer model ED-1 Eyela. The freeze-dried sample was washed off residual acid with 2.5ml deionised water and freeze-dried again. Afterwards, 4ml of citrate buffer of pH 2.2 was added and 80ml of the sample together with 5ml of norleucine (as internal standard) was injected into the analyser of model A - 3300 (RICA). Authentic amino acids were similarly treated and run (80 ml of these amino acids plus 5 ml of norleucine). Peaks of various amino acids appeared on the aminogram and the information obtained was fed into a computer which gave the respective concentrations of the amino acids detected in the sample with respect to the equivalent standards.

3.12 **Solubility test**

To one drop of oil in individual test tubes, 3ml of chloroform, ether, petroleum ether, carbon tetrachloride, acetone, (hot and cold) 95% ethanol were added. Test tubes were shaken and their solubility observed.

3.13 **Phytochemical tests (Harbone 1984)**

3.13.1 **Terpenoids**

Lieberman Burchard Test.

A small amount of the extract was put in a small test tube and then evaporated to dryness on a water bath. The dry mass was then dissolved in a small amount of chloroform. Few drops of acetic anhydride were added

followed by gentle addition of a small amount of concentrated sulphuric acid. Shaking was avoided after addition of concentrated sulphuric acid to prevent charring.

The chloroform solution changed from orange colour to red. This confirmed the presence of terpenes.

3.13.2 **Saponin**

A small portion of the extract was boiled with 10ml of distilled water for 3-5 minutes, filtered hot and allowed to cool. A 3ml portion of the filtrate was then shaken vigorously. Froth of about the same height as the aqueous layer was obtained. Saponin was present.

3.13.3 **Carotenoids**

A layer of conc. H_2SO_4 was carefully formed under an ethereal solution of the sample. The absence of an intense dark blue violet or greenish blue colour in the acid layer showed absence of carotenoid.

The methods of Cocks and Van Rede (1966) were used for the determinations of the oil characters.

3.14 **Determination of specific gravity**

The weight of clean dry empty specific gravity bottle was taken accurately. The bottle was then filled completely with distilled water in an ice bath and transferred into a water bath at 25°C. At the end of 20 minutes the bottle was removed, all excess water wiped off and the weight taken. There after, the same empty dried bottle

was filled with the oil in an ice bath and placed into the water bath for 20 minutes at 25°C. The weight was once again taken accurately and recorded (Appendix C.12).

3.15 **Determination of refractive index**

Optically clear oil and free from water was dropped to fill the space between the prisms of an Abbe refractometer, which had previously been freed from dirt and air bubbles. Ample time was allowed for the oil and prism of the instrument to attain a steady temperature before reading.

3.16 **Determination of water and volatile matter**

About 10g of the sample was weighed into a beaker and placed on an electric plate. Temperature was maintained at 125°c while stirring with the thermometer until no more vapour evolved. (Before the determinations the empty beaker and thermometer were weighed). A watch glass was used to ensure that no vapour ensued. This was done by covering the beaker with it and ensuring that the glass is not dimmed before taking final reading (Appendix C 13).

3.17 **Determination of Slip and Melting points**

A sample of fat was melted, mixed thoroughly and then filtered. Melting point tubes were particularly filled by dipping them into the fat so that the column of fat was 1cm long. The liquid was allowed to solidify by

placing them at once against ice for a few seconds until solidification had set in. Not more than four (4) filtered tubes were attached to the thermometer with a rubber ring so that the fat column was at the height of the mercury bulb of the thermometer. The thermometer and the tubes were placed into a mixture of ice and salt at -10°C and kept for 5 minutes. Water reservoir was filled with water at a temperature of about 10°c for melting points up to 40°C. The thermometer with the filled melting point tubes were hung exactly in the centre of the glass water reservoir so that the lower end of the melting point tube was 3 cm below the surface of the water.

The electric hot plate was switched on and the temperature at which the fat, after forming a meniscus, loosens itself completely from the lower edge of the melting point tube and rises was determined with an accuracy of ½°C. The temperature at which the oil completely melts is the melting point.

3.18 **Iodine value determination**

Calculated weight of oil was weighed into an iodine value flask. The oil was dissolved in 15ml of carbon tetrachloride and 25ml of wijs solution. The flask was closed mixed and allowed to stand in the dark at about 20°c for 1 hour. After standing, 20ml of the potassium iodide solution was added and approximately 150ml water

was added. This was titrated with sodium thiosulphate (0.1M) with shaking. Starch indicator was added towards the end of the titration.

A blank determination was made with the same quantities of reagents at the same time and under the same conditions (See appendix C14 for calculation).

3.19 **Determination of acid value**

Ten grams of the oil was dissolved in 50ml of solvent and titrated while swirling with O.1M KOH using phenolphthalein as indicator (Appendix C15).

3.20 **Determination of Unsaponifiable matter**

Between 2 to 2.5g of the oil was weighed into a 250ml round bottom flask and 50ml ethanolic KOH solution added. This was refluxed using air condenser for 1 hour on a steam bath with shaking occasionally. The contents were then transferred to a 500ml separating funnel and washed first with 100ml water and then with diethyl ether. The water and the diethyl ether were added to the contents in the separating funnel. The contents were shaken vigorously. After allowing the separating funnel to stand until the layers were separated, the ethanol-water layer was drawn into the flask in which the saponification had taken place and the diethyl ether layer was transferred to a second separating funnel containing 40ml water. The ethanol - water layer was shaken twice more as before, each time with 100ml diethyl

ether and the three quantities of diethyl ether was collected in the second separating funnel. The second separating funnel was rotated about its own axis without shaking.

When the layer had separated, the wash water was drawn off and diethyl ether solution was washed twice by shaking vigorously with 40ml water and successively with 40ml KOH, 40ml water and again 40ml KOH; this was then washed with water until the wash no longer became pink on addition of phenolphthalein.

The diethyl ether solution was transferred to a 500ml round bottom flask. Part of the solvent was evaporated off and the residue transferred to a weighed 200ml round bottom flask to evaporate most of the residual solvent. A few mls of acetone was added. This was evaporated quantitatively by submerging the flask almost wholly in a boiling water bath in a sloping position, rotating it and introducing a gentle current of clean air.

This was then dried at 100°c for 30 minutes to constant weight and reading recorded (Appendix C.16).

3.21 **Determination of saponification Value**

Four grams of filtered fat with an accuracy of 1mg was accurately measured and 50ml 0.5M ethanolic potassium hydroxide solution (Appendix 2B5) was added and refluxed with occasional shaking for half an hour. 1ml of phenolphthalein indicator was added and slowly titrated

with 0.5M HCl whilst the soap solution was still hot. A blank determination was carried out under the same conditions. (See Appendix C.17 for calculations).

3.22 **Optimization of condition for maximum extraction of oil**

Preparation of samples.

Roasted samples were milled in a plate mill. Sieve analysis showed that for all the samples, more than 50% of the meal was retained by 0.5mm mesh.

3.22.1 **Aqueous Extraction**

50 grams of sample was dispersed in 5 parts of water and boiled for 30 minutes, whilst stirring intermitently. In this study roasting temperature,roasting time,dilution ratio, extracting time and number of reextractions were studied. In detail, 2 parameters were studied at a time. One parameter was varied whilst the other was kept constant till maximum extraction was obtained.

Quantification of extract.

At the end of the boiling period, the dispersion was centrifuged at 6,000rpm for 30min to separate the solid, aqueous and oil phases. The volume of fat layer was noted and was converted to weight by using specific gravity factor of 0.90g/ml. (See Appendix C.18 for calculation of percentage extract).

3.22.2 **Pressing**

Measured amounts of milled sample were wrapped in a

cheese

cloth (to prevent fines and cake being expressed with the oil). The wrapped cake is put into the press cage and oil squeezed out. The dimensions of the press (Fig.3) are :

ITEM	DIMENSION (MM)
Handle bar length	525
Retch of thread	2.5
Diameter of screw	20
Plate diameter	104
Seating depth	20
Shaft rod length	16
Cylinder	
Diameter	105
Height	150

Maximum pressure was obtained in 2 minutes. In the trial process, temperature of roasting, time of roasting, and cake thickness were varied. The expressed oil was collected in a measuring cylinder and the weight obtained by multiplying by specific gravity 0.90g/ml. (See Appendix C.18 for calculation of percentage extract).

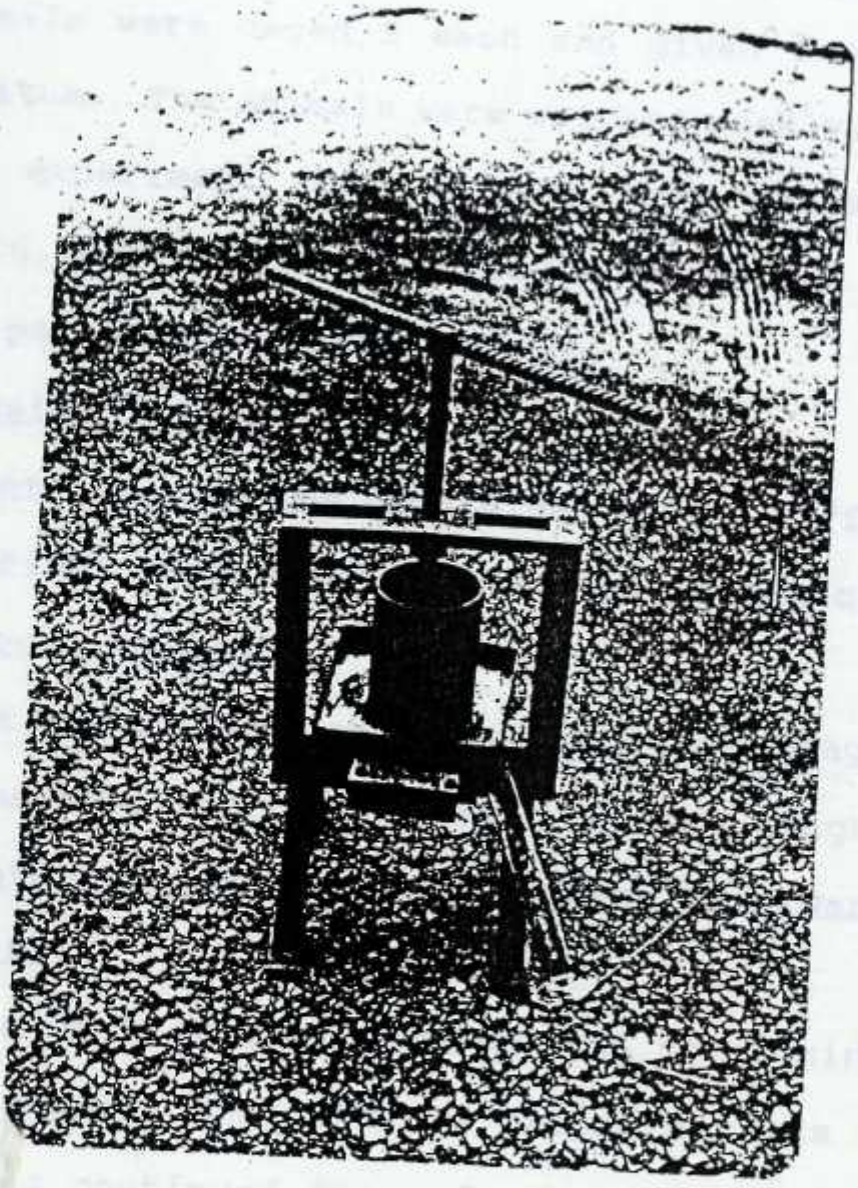


FIG 3. PRESS

3.23. Nutritional evaluation of oils

The nutritive value was determined by animal assay technique using weanling litter male rats from the department's animal house. The animals were weaned to the stock so that on commencement of the feeding trials, all the rats weighed between 50-60 grams. Six rats were put on the various diets (Table 2) for 28 days.The animals were caged 2 each and given food and water ad libitum. The animals were weighed weekly. At the end of the experiment, the animals were sacrificed and the blood, heart, liver, spleen and kidneys were collected for pathological examinations.

3.23.1 Weight increase

Weight increase was determined as the difference between the final weight and the initial weight of the rat.

3.23.2 Organ weights

Organ weights were expressed as a percentage of the ratio of the specific organ weight to body weight.

3.23.3 Determination of Lipid content of organ (Folch et al, 1957)

1 gram of tissue was homogenized for 1 minute with 10ml of methanol, then 20ml of chloroform was added and the process continued for a further 2 minutes. The mixture was filtered and the solid residue resuspended in chloroform - methanol (2:1 v/v 30ml) and homogenized for 3 minutes. After filtering, the solid was washed once

more with chloroform (20ml) and once with methanol (10ml). The combined filtrates were transferred into a measuring cylinder and one quarter of the total volume of the

Table 2 Composition of oil diets

CONSTITUENTS %	D I E T S											
	1	2	3	4	5	6	7	8	9	10	11	12
corn starch	75	70	65	76	70	65	75	70	65	75	70	65
casein	10	10	10	10	10	10	10	10	10	10	10	10
palm oil	5	10	15	-	-	-	-	-	-	-	-	-
P. butyracea oil	-	-	-	5	10	15	-	-	-	-	-	-
T. occidentalis oil	-	-	-	-	-	-	5	10	15	-	-	-
C. procera oil	-	-	-	-	-	-	-	-	-	5	10	15
fibre	8	8	8	8	8	8	8	8	8	8	8	8
vitamin premix	1	1	1	1	1	1	1	1	1	1	1	1
mineral premix	1	1	1	1	1	1	1	1	1	1	1	1

filtrate of 0.88% Kcl in water was added. The mixture was shaken thoroughly and allowed to settle The upper layer was removed by aspiration. One quarter of the vol of the lower layer of water - methanol (1:1) was added and the washing procedure repeated. The bottom layer containing the purified lipid was released into a weighed crucible. The solvent was evaporated and the weight is again taken. The difference in weight was the weight of lipid extracted. (See Appendix C19) for calculation).

3.23.4 **Determination of Total lipids in serum (Bloor 1925)**

30ml of Bloor's reagent (Appendix 2B7) was poured into a 50ml flask, 0.1 ml of serum was added with continuous stirring and heated to boiling. It was cooled to room temperature, diluted to 50ml with Bloor's reagent mixed, and filtered through a whatman number 1 paper (11cm). 40ml of the extract was poured into a 100ml beaker containing an amount of purified sea sand (for smooth boiling) and evaporated to dryness on a steam bath, in a fume hood.

A small amount of sand (0.01mg) was put into a weighed bottle and the exact weight of the bottle plus the sand determined (W1). 2ml of petroleum ether was poured into the 100ml beaker containing the residue and the residue filtered using whatman No.1 filter paper (7cm) fitted in a 35ml funnel. The filter paper was rinsed with 2ml ether and the ether portion evaporated. The bottle was

cooled to room temperature and weighed again (W2). (See Appendix C20 for calculation).

3.23.5 **Enzymatic Assay**

3.23.5.1 **Assay of serum aspartate transaminase (AST)** (Reitman & Frankel, 1957)

Test tubes labelled T,B,S and C for Test,Blank,Standard and control respectively were used. 0.5ml of SGOT substrate (See Appendix 2B8) was pipetted into all the tubes preincubated at 37°C in a water bath for 5 minutes. The tubes were removed and 0.1ml of distilled water pipetted into the blank tube B, 0.1ml of pyruvate standard (Appendix 2B8) solution pipetted into tube B and S respectively. 0.1ml of test serum was added to tube T and all the tubes incubated at 37°c for 60 minutes. 0.5mls Of 2, 4 dinitrophenyl hydrazine was added to each test tube after incubation and thoroughly mixed. 0.1ml of control serum was added to tube C. All the tubes were left to stand at room temperature for 20 minutes. 5mls of 400mmol/L sodium hydroxide was further added to all tubes, thoroughly mixed and left at room temperature for 5 minutes.

Absorbance was measured at 505nm using the reagent blank to set the instrument to zero. The control values were used as checks (See appendix C21 for calculation).

3.23.5.2 **Assay of serum alanine transaminase (ALT).**

(Reitman & Frankel, 1957)

The same labelled test tubes in the assay for SGOT was used. The substrate used in this assay was SGPT substrate (Appendix C 21) and incubation period used was 30 minutes. After incubation, 5ml of 400mmol/l sodium hydroxide solution was added to each test tube after 0.5ml of 2,4 DNPH had been added and thoroughly mixed. The tubes were allowed to remain at room temperature for 5 minutes, absorbances were also read at 505 nm on a spectrophotometer. The amount of pyruvate formed was calculated in mmol/mm/l and the table of standards (See Appendix C 21) was used to convert to SGPT activity in International units/litre (U/L). The control values were used as check.

3.23.5.3 **Assay for alkaline phosphatase (ALP)**

(King & Armstrong, 1934)

Test tubes with the same labelling as done for the transaminses were used. 1.0ml of the sodium carbonate,sodium bicarbonate alkaline buffer (Appendix 2B9) was pipetted into B and S test tubes. 1.1ml of the substrate followed by 0.8ml of 0.5M NaOH was then pipetted into tube C. 0.1ml of test and control sera were added to the respective test tubes and 1.0ml of distilled water also pipetted into tubes B and S. 1.0ml of warmed substrate and 0.1ml of the phenol working standard

solutions were then added to tubes T and S respectively and together, all the test tubes were incubated for 15 minutes at 37°C. 0.8mls of 0.5M NaOH was added to tubes T, B and S and 1.2 mls of 0.5M $NaHCO_3$ added to all the tubes. All tubes were thoroughly mixed after each addition. 1.0m 4-amino antipyrine and 1.0ml potassium ferricyanide were subsequently added and also thoroughly mixed. Absorbance was read at 510nm after setting the spectrophotometer to zero with the blank (B). 1.000 cm cuvette was used (See Appendix C 22).

3.24 **Industrial Application of Oils**

Shortening effect of Pentadesama butyracea fat

The shortening effect of the fat was evaluated in baked cake and bread. Margarine was used as the reference baking fat. Using the recipes below baking fat was substituted in the test products. The result was compared to the reference baking fat product. The difference was related to the shortening effect of the different fats.

3.24.1.1 **Bread rolls** (Cererani & Kinton, 1984)

1. sieve the flour into a bowl and warm in the oven or above the stove.
2. Cream the yeast and sugar in a small basin, add a quarter of the liquid.
3. Make a well in the centre of the flour, add the dissolved yeast.

4. Sprinkle over a little of the flour, cover with a cloth, leave in a warm place until yeast ferment (bubbles).
5. Add the remainder of the liquid (warm), the fat and salt.
6. Knead firmly until smooth and free from stickiness.
7. Return to the basin, cover with a cloth and leave in a warm place.
8. Knock back.
9. Divide into 8 equal pieces.
10. Mould into desired shapes.
11. Place on a floured baking sheet.
12. Brush carefully with egg wash.
13. Bake in a hot oven.

Table 3 Recipe for bread and cake production

Bread rolls	Cake
200g (hard) wheat flour 8oz	200g (soft) wheat flour 8oz
10g butter/magarine ½oz	200g sugar 8oz
salt	200g margarine 8oz
5g yeast ⅜oz	3 eggs 200g
12ml liquid (½ water, ½ milk)	½ teaspoons baking powder
¼ teaspoon sugar	

3.24.1.2 **Cake**

Sugar and fat were creamed together till fluffy (20min). The eggs were then beaten together and added. Flour and baking powder was added and together were put into "greased" cake tins, ready for baking. The oven temperature was 160°c and baking was timed for 50 minutes.

3.24.1.3 **Baked cookie Analysis**

Baked cookies were weighed and the weight and diameter measured with a calliper. Spread ratio was expressed as diameter/height.

3.24.1.4 **Sensory quality evaluation**

A ten member panel evaluated sensory qualities of the baked products. Whole cookies were arranged on a white sheet. Margarine cookies were labelled 1 and test fat cookies labelled 2 (See fig 28). Panellists were asked to use the reference cookie as a basis for determining acceptance by first assigning a score to it and then evaluating test cookie in comparison to it. Appearance, colour aroma, texture, taste and mouth feel were evaluated on a scale of 9 (excellent) to 1 (very poor). Paired comparison test and student T-test (Ihenkoronye & Ngoddy,1985) was statistically used to evaluate the scores.

3.24.2 Soap properties of oils

Procedure

100g of clarified oil was weighed in a 2 - litre beaker. The oil was heated and saponification started by adding 20ml of 23.5% NaOH. The remaining NaOH was added in bits until saponification was complete. In all about 60-65 grams of the NaOH solution was used for all the oils. 8g of salt was used to grain out the soap. The soap obtained was washed with water and filtered through a cheese cloth. After air drying, a small amount of water was added to soften it whilst heating. The molten soap was poured in casts and allowed to dry.

3.24.2.1 Analytical tests on soap

Physical tests.

Odour, lather stability, hardness and washing efficiency were determined. Odour was done by smelling whilst hardness was by pressing the casted soap in between the thumb and the third finger. Lather was created by vigrously shaking the casted soap in 200ml of water. The lather formed was observed for 1 hour and intentionally created oil spot was washed using the soaps to measure the washing efficiency.

3.24.2.2 Chemical tests

Total fatty matter (TFM)

5g of the finished soap was dissolved in 30ml distilled water. The fatty acid was precipitated from the salt

present in the solution with 0.05m Hcl. The solution was filtered and ether was added to the precipitated fatty acid to aid drying. The dried fatty acid obtained was then weighed and the percentage fatty acid determined.

Free caustic alkali (FCA)

5g of the sample soap was weighed and dissolved in 30ml of ethanol. Few drops of phenolphthalein indicator and 10ml of 20% Barium chloride was added. The resulting solution was then titrated against 0.05M H_2SO_4. The volume(v) of the acid obtained was multiplied by 0.0124 to get the percentage Na_2O present.

4. RESULTS AND DISCUSSION

The chemical composition of the whole and defatted seeds are presented in Tables 4 and 5. The most striking features of the tables are the high content of oil in the whole seeds and the high protein contents of defatted C. procera and T. occidentalis meals. The oil content of the seeds were C. Procera (66.19%), P. butyracea (6.45%) and T. occidentalis (55.49%). These are higher than the yield of common oil seeds such as sunflower (35%), safflower (30%), olive (25%) and soyabean (20%) (Osagie, 1988). The oil yield of P. butyracea (46.45%) compares very well with the oil content of shea (43.8%) palm, (46.8%), palm kennel (43.5%) and agushie (48.8%) (Eyeson & Ankrah, 1975). The oil yield obtained for P. butyracea also falls within the range (35%-54%) observed by Williams, (1950). However, that of T. occidentalis (55.49%) was higher than the (45.0%) obtained by Asiegbu, (1987). This may be due to the difference in the climate and soil properties (Jones et al, 1991). Ash values of the whole seed (Table 4) ranged from 2.78% to 3.43% whilst seeds of P. butyracea contain the highest fibre and carbohydrate value of (13.72%) and (23.44%) respectively. The quantity of protein in T. occidentalis seeds (22.54%) compares favourably with the protein content of cowpea, (Lasekan et al, 1987), which is being promoted as a high protein substitute in the Ghanaian

Table 4. Proximate composition of seeds

Constituent %	C.procera	P.butyracea	T.occidentalis
moisture	6.89 ± 0.58	7.52 ± 0.16	3.32 ± 0.53
fat	66.19 ± 1.87	46.45 ± 0.26	55.49 ± 0.96
crude protein	10.08 ± 1.26	6.34 ± 0.80	22.54 ± 1.11
ash	3.43 ± 0.54	2.87 ± 1.58	2.78 ± 0.52
fibre	10.67 ± 0.90	13.73 ± 0.20	7.38 ± 0.60
caborhydrate by difference	2.74	23.09	8.49
food energy value (kcal)	658.38	537.13	622.39

Values are the mean of triplicate determinations ± standard deviation of means.

Table 5. Chemical composition of defatted meal

constituent %	C. procera	P. butyracea	T.occidentalis
moisture	8.60 ± 0.90	7.81 ± 0.37	5.35 ± 0.05
crude protein	25.53 ± 0.52	8.01 ± 0.54	54.68 ± 0.42
crude fibre	10.54 ± 0.73	15.22 ± 0.15	6.40 ± 0.76
fat	0.94 ± 0.09	0.47 ± 0.52	1.46 ± 0.07
ash	10.91 ± 0.05	3.53 ± 0.63	7.42 ± 0.48
carborhydrate by difference	43.48	64.96	24.69
tannin	0.89 ± 0.01	7.51 ± 0.33	0.18 ± 0.01
saponins	4.58 ± 0.12	2.71 ± 0.57	3.89 ± 0.24
oxalate	9.08 ± 0.51	8.01 ± 0.23	0.78 ± 0.07
calorific value (kcal)	3.92	3.88	5.16

Values are the mean of triplicate determinations ± standard deviation of means.

Table 6. Mineral composiiton of defatted seed meals %

	C.procera	P.butyracea	T.occidentalis
Ca	0.250 ± 0.020	0.140 ± 0.000	0.020 ± 0.000
Fe	0.010 ± 0.000	0.040 ± 0.000	0.008 ± 0.000
K	4.58o ± 0.030	1.600 ± 0.000	2.310 ± 0.070
Na	0.080 ± 0.010	0.020 ± 0.050	0.001 ± 0.000
Zn	0.005 ± 0.000	0.002 ± 0.000	0.006 ± 0.000
Cu	0.003 ± 0.000	0.003 ± 0.000	0.001 ± 0.000
Mg	0.430 ± 0.230	0.290 ± 0.030	0.210 ± 0.010
P	1.670 ± 0.230	0.490 ± 0.110	1.950 ± 0.140

Values are the mean of triplicate determinations ± standard deviation of means.

diet. The fibre of contents of defatted samples were C. procera (10.54%), P. butyracea (15.22%) and T. occidentalis (6.40%). The protein content in defatted C. procera and T. occidentalis meals were (25.53%) and (54.68%) respectively. Even though the high value of T. occidentalis (54.68%) was lower than the defatted value of the melon seeds (66.02%) (Nwokolo et al,1987) it was significantly higher ($p < 0.05$) than linseed (35.0%) and sesame (50.0%) (Madhusudhan & Singh, 1983; Rivas et al 1981) which are promoted for animal feed. The very high content of oil in the whole seeds and of protein in the defatted meal of Telfaira occidentalis could make commercial cultivation of these seeds for edible oil and protein a lucrative business. Curently,it is cultivated in Nigeria and used a lot. Anti-nutritional factors were determined because they suppress the nutritional potentials of food materials. Data on antinutritional factors are presented in Table 5. They reveal that generally, there are significant differences between all the samples studied. Telfaira occidentalis was lowest in tannin (0.18%) and oxalate (0.78%) whilst Pentadesma butyracea was lowest in saponin (2.71%). Carapa procera was highest in oxalate with 9.08% followed by Pentadesma butyracea 8.01%. However Pentadesma butyracea was highest in tannin with 7.51%. The high tannin content of Pentadesma butyracea could be the cause of the reddish

colour of the seeds. The tannin of Telfaira occidentalis was lower than akashmoni and eucalyptus meals (Mandal et al 1985). It was also comparable to cowpea (0.144-1.015%) (Laurena et al,1984). Oxalates were higher in Pentadesma butyracea meal, (8.01%) and Carapa procera meal (9.08%) than Telfaira occidentalis meal (0.78%). Oke (1976) has noted that oxalate content depends on the species, the parts of plants analysed, the climate, soil conditions and other factors. Saponin content ranged from 2.72% to 4.25%. Toxicity of saponin is only effected if the intestinal walls are compromised. Oakenful (1984) also demonstrated that saponins have a cholesterol lowering effect.

Mineral composition of the defatted meals are shown in Table 6. Of the three seed meals Carapa procera meal contain the highest amount of Ca, 0.25%; mg, 0.43%;and K, 4.58% compared to Pentadesma butyracea or Telfaira occidentalis. Pentadesma butyracea on the other hand has significantly ($p < 0.05$) higher content of Cu 0.003% and Fe 0.04%. All the meals were generally low in the trace elements (Cu, Zn and Fe). However Telfaira occidentalis seems particularly low in most of the elements. In general the mineral compositions of Pentadesma butyracea and Telfaira occidentalis meals were lower, compared to other plant seeds(Ene-Obong & Cornovale,1992).

Amino acid composition

The amino acid composition of the various oil seeds are shown in Table 7. Tryptophan in all the meals could not be determined because of technical difficulties. Generally the essential amino acids composition compares favorably with FAO/WHO recommended requirement pattern (Anon 1973). However, like protein from legume sources they were relatively deficient in sulphur containing amino acids (Sosulski et al,1987,Enrigue et al,1986). The essential amino acids of Pentadesma butyracea meal compares better to soybeans (Asiegbe 1987) than Telfaira occidentalis; however, because of the low protein content of Pentadesma butyracea meal (8.01%) coupled with the high fiber content (13.73%), it will be more advantageous nutritionally to promote Telfaira occidentalis. Pentadesma butyracea and Telfaira occidentalis had better essential amino acid spectra than Carapa procera. Carapa procera was deficient in leucine and lysine. However all the meals were generally deficient in methionine and cystine.

Fatty acid composition

Table 8 shows the fatty acid composition of the oils. The major saturated fatty acids in all the oils were palmitic acid and stearic acid. The major mono unsaturated fatty acid in all the oils was oleic acid. These are in agreement with previous reports (Hilditch &

Table 7. Amino acid composition (g $100g^{-1}$) of seed

non essential	C.procera	P.butyrecea	T.occidentalis	FAO/WHO 1973 requirements
Asp	1.37	0.85	1.83	
Glu	2.69	1.49	6.14	
Ser	1.08	0.56	1.96	
His	0.59	0.42	1.25	
Gly	0.83	0.87	2.25	
Ala	0.73	0.56	2.49	
Arg	0.31	0.59	4.42	
essential				
Met	0.20 (0.79)	0.15 (2.00)	0.22 (0.39)	(3.5)
Cys	0.70 (2.77)	0.03 (0.40)	1.25 (2.20)	
Thr	0.78 (3.09)	0.50 (6.25)	2.31 (4.20)	(4.0)
Ileu	0.63 (2.70)	0.47 (5.87)	2.18 (3.96)	(4.0)
Val	1.17 (4.64)	0.68 (8.50)	2.21 (4.01)	(5.0)
Lys	0.71 (2.81)	0.54 (6.74)	1.71 (3.11)	(5.5)
Phe	0.86 (3.14)	0.57 (6.74)	2.61 (4.75)	(6.0)
Tyr	0.26 (1.03)	0.18 (2.25)	1.32 (2.40)	
Leu	1.15 (4.56)	0.80 (9.99)	4.31 (7.80)	(7.0)
Tryp	N.D.	N.D.	N.D.	(1.0)

N.D. - not determined
figures in brackets are represented in g $100g^{-1}$ seed protein.

Table 8. Fatty acid composition of lipids extracted (%)

FATTY ACIDS	O I L S		
	C. procera	P. butyracea	T. occidentalis
Myristic (14:0)	2.73	0.76	4.00
Palmitic (16:0)	25.53	4.42	12.50
Palmitoleic (16:1)	0.90	1.12	0.86
Stearic (18:0)	6.22	41.60	19.70
Oleic (18:1)	48.62	50.80	39.34
Linoleic (18:2)	13.05	0.87	33.14
Linolenic (18:3)	1.75	0.40	0.60
% Unsaturation	63.42	51.67	72.48

William, 1964, Adomako 1977, Osagie 1988). The results show Telfaira occidentalis oil has a higher degree of unsaturation than the other oils. This gives it a nutritional advantage over the others. This is because of the relationship which has been established between Omega 3 fatty acids and atheriosclerosis (Hornstra, 1980). The high degree of unsaturation makes Telfaira occidentalis oil more susceptible to rancidification. This will require an antioxidant to prevent deterioration. The percentage unsaturation of Telfaira occidentalis was less than the seed oils of Carica papaya (81.67%), corn oil (79.2%) and olive oil (84.3%) (Marfo et al,1986, Beyren et al,1987). However its degree of unsaturation was higher than palm oil (50.34%) and coconut oil (19.1%) (Arumugham et al, 1985, Beyren et al,1987) which are very common in the rural areas. Thus the promotion of Telfaira occidentalis oil could improve the nutritional status of the rural folk.The major poly-unsaturated fatty acid present was linoleic. The highest concentration of linolenic acid was observed in Telfaira occidentalis oil with traces in Carapa procera and Pentadesma butyracea. Carapa procera and Pentadesma butyracea also recorded traces of palmitoleic acid.

Physical and Chemical properties of the oils

Lipid class composition of seeds

The total lipids from the seeds were extracted by using

chloroform; methanol mixture (2:1 v/v) and non-lipid impurities removed using KCl solution (Folch et al 1957). The results of the lipid class separation are shown in Fig. 4. (Cholesterol was used in the absence of a plant sterol). The lipid class composition show that the seed lipids were predominantly triglyceride with traces of sterol and fatty acids, hence, their ready solubility in the various organic solvents. Hydrocarbons, diglycerides and monoglycerides were not detected by the method employed. Qualitatively the lipids showed the presence of terpenes, saponins and carotenoids.

Solubilty and organoleptic properties (Table 9)

Oils were extracted with petroleum ether. Telfaira occidentalis yielded a percentage extract of 55.49%, Carapa procera, 66.19% and Pentadesma butyracea 46.45%. The yellowish colouration of the oils was an indication of the presence of carotenoid pigment (which has qualitatively been confirmed). Carapa procera and Telfaira occidentalis oils remained liquid at room temperature but Pentadesma butyracea oil was solid. All the oils were soluble in all the organic solvents used, however they were partially soluble in hot ethanol and insoluble in water. With the exception of Carapa procera oil which had a bitter taste and a foul odour the other oils had a bland taste and pleasant aroma.

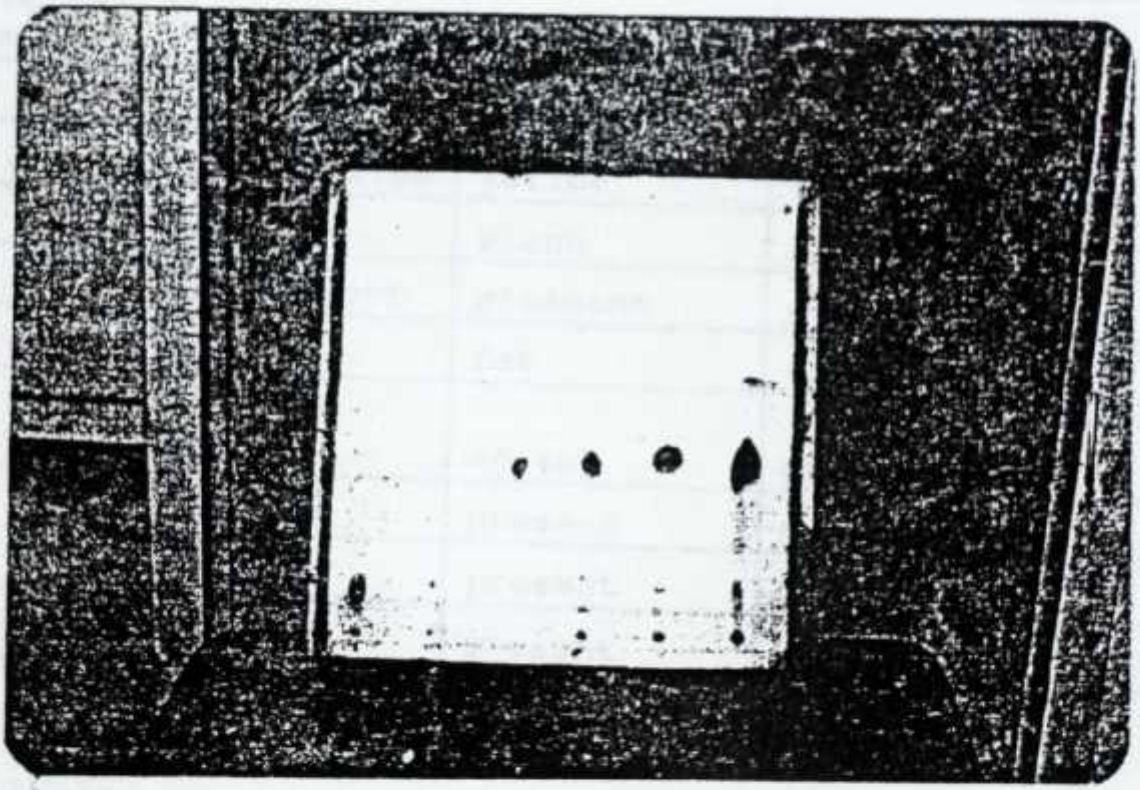


FIG 4 TLC SEPARATION OF SEED OILS

i. CHOLESTEROL

ii. FATTY ACIDS

iii. TRIACYLGLYCEROL

iv. CARAPA PROCERA OIL

v. PENTADESMA BUTYRACEA OIL

vi. TELFAIRA OCCIDENTALIS OIL

Table 9. Solubilty, organoleptic, spectroscopic & phyto chemical properties of oils.

		C.procera	P.butyracea	T.occidentalis
Pet Ether		+ + (T)	+ + + (C.L)	+ + + (C.L)
Chloroform		+ + + (C.L)	+ + + (C.L)	+ + + (C.L)
Ethanol	Hot	+ (T)	+ (T)	+ (T)
	Cold	(-)	(-)	(-)
Acetone		+ + + (C.L)	+ (Y)	+ + + (P.Y)
Ether		+ + + (C.L)	+ + + (C.L)	+ + + (C.L)
Carbon Tetra-chloride		+ + (C.L)	+ + + (C.L)	+ + (T)
Water		-	-	-
Colour		pale yellow	yellow	brownish yellow
taste		bitter	bland	bland
odour		unpleasant	pleasant	pleasant
form		oily	fat	oily
trans unsaturation		absent	absent	present
terpenes		present	present	present
saponins		present	present	present
carotenoids		present	present	present

Legend

+ :- soluble
++ :- very soluble
+++ :- very very soluble
T :- turbid
C.L :- colourless
Y :- yellow
P.Y :- pale yellow

The bitterness and odour of Carapa procera oil was comparable to other seed oils of plants in the same family Meliaceae (Watt & Breyer-Brandwick, 1962). Unlike some varieties of lupines in which bitter principles are only present in the cake (Kamel & Blackman, 1982), the bitter principle of Carapa procera oil is present in both cake and oil. The bitterness gives cause to suspect alkaloids, saponins and terpenes. Qualitatively, saponins and terpenes were confirmed in Carapa procera oil. This finding is in agreement with the observation of Bera (1963),who detected a lipid soluble triterpenoid of the limonine group in Carapa procera.

Physical properties

The physical properties of the oils are presented in Table 10. The specific gravity range of 0.9465 - 0.9592 was similar to most common oils eg. groundnut oil (Martin, 1976). The refractive index obtained for Telfaira occidentalis oil was 1.483. This was significantly different ($p < 0.05$) from the values obtained for Carapa procera (1.466) and Pentadesma butyracea (1.460) oils.

Table 10. Physical and chemical properties of organic solvent extracted oils

	C.procera	P.butyracea	T.occidentalis
Oil (% in seed)	66.19 ± 1.87	46.45 ± 0.26	55.49 ± 0.96
specific gravity 25°C	0.9503 ± 0.020	0.9592 ±0.018	0.9465 ± 0.015
moisture & volatile matter %	0.17 ± 0.01	0.31 ± 0.01	0.11 ± 0.02
iodine value	67.12 ± 1.33	51.59 ± 1.10	99.45 ± 2.00
saponification value %	214.37 ± 3.45	194.92 ± 2.46	199.67 ± 2.13
unsaponification matter %	1.41 ± 0.21	1.49 ± 0.03	1.67 ± 0.21
acid value %	2.33 ± 0.17	3.20 ± 0.11	0.26 ± 0.21
refractive index 28°C	1.466 ± 0.001	1.460 ± 0.003	1.483 ± 0.001
slip point °C	-	37.5 - 38.0	-
melting point °C	-	38.9 - 39.4	-

Values are the mean of triplicate determinations ± standard deviation of means.

With the exception of Telfaira occidentalis oil, the refractive indices of Carapa procera and Pentadesma butyracea oils compared favourably with soyabean (1.466 - 1.470) and arachis(1.460 - 1.465) oils (Martin, 1976). The determination of moisture and volatile matter gives an indication of the degree to which the oils can withstand deterioration. All the oils had low values. This makes them stable against deterioration by micro organisms and heat. This is because water acts as a medium for the growth of micro organisms which hydrolyse ester linkages of triglycerides thus, leading to high fatty acid content. Also, Ramanna & Sen, (1983) demonstrated that there was a positive corelation between high heat and the rate of deterioration of oils. The slip and melting points are particularly important in the selection of fats for catering and confectionery use (Brownsel, 1989). This is because of the role heat plays in these processes. Comparatively, Pentadesma butyracea values were higher than shea butter (36.7-37.4) and cocoa butter (33.3-33.5) (Adomako, 1977). This makes it harder and more brittle. Because of this more cost will have to go into heating and pumping of the oil if it is to be used for confectionery and commercial frying. The iodine value of an oil is a very important property which determines the degree of unsaturation. It also gives off-hand the application to which the oil can be put.

According to Pearson (1972) Carapa procera and Pentadesma butyracea are non-drying oils (because of low iodine value). This makes them comparable to palm oil, palm kernel oil and shea butter. However the higher iodine value (99.45) of Telfaira occidentalis oil suggests that it is of a semi drying type. This makes the oil economically important because it can be used together with others in the production of paints and varnishes. The saponification values were between 194 - 214. These were higher than values of shea butter (178-189) and castor oil (181) (Donkor 1986) and are comparable to palm oil (197) (Arumughan et al, 1985). However they are inferior to coconut oil (251-264) Codex (1984). Thus for better quality soap, blend together with other oils will be advantageous. The lower levels of acid value is indicative of low enzymatic hydrolysis. The low unsaponifiable matter is an indication that the steroids and related components are low in the oils.

Moisture and volatile matter were determined gravimetrically.

Optimisation of conditions for maximum extraction of oils

Three methods of extraction were used. They were organic solvent, pressing and aqueous methods. Organic solvent extraction gave the maximum extract. According to Christie (1982) organic solvent extraction represents about 95% of the total lipid present in the seed. All

other extracts by other methods were obtained as a percentage of the maximum extract by organic solvent. Particle size, a very important parameter in oil extraction, (Rhee et al, 1972) could not be varied due to lack of appropriate technology. However all the samples were milled in a plate mill. The milled Pentadesma butyracea seeds had a grainy nature unlike the Carapa procera and Telfaira occidentalis seeds that were paste like. However the particle size of the defatted meal showed that 50% of all the meals could not pass through 0.5mm mesh seive. The grainy nature of Pentadesma butyracea seeds could be attributed to its high fibre content, 13.73% compared to 10.67% and 7.38% for Carapa procera and Telfaira occidentalis seeds respectively.

Pressing

Variation of the parameters that affect pressing of oils are shown in Appendix D. Summary of the results of this investigation are shown in Table 11. Roasting temperature, roasting time and cake thickness were the prepressing parameters that were investigated. These parameters together with particle size and pressure were effectively combined by Ajibola (1987) to optimize conditions for extraction of palm kernel oil. Pentadesma butyracea meal was steamed to melt the oil thus higher than 200g of the milled seeds could not be investigated because steaming led to expansion (Press maximum was

200g). The draining time of Telfaira occidentalis oil (25 min) was far longer than the others (Table 11). This can be attributed to the more viscous nature of the oil. Weight of the meal was used as a parameter for thickness. Pentadesma butyracea meals showed significant increases in yield (57.9% to 88.8%) as thickness increased (fig.5) However negative effects were observed for Carapa procera and Telfaira occidentalis meals, (47.9% to 34.1%) and (70.4% to 50.9%) respectively (figs.6 & 7) This could be associated with their low fibre content which led to the "flowing" of the mass in the cage. This defect led to the clogging of air spaces and holes in the mass and cage. A similar observation is visible in the pressing of peanuts. However in the extraction of peanut oil, shells are introduced to minimize the clogging effect. This technique is learned through experience and perhaps could be applied to this also. The optimum conditions for the maximum extraction of the oils are shown in Table 11.

Aqueous extraction

In the aqueous extraction process, two significant observations were made. For all the 3 samples yield was affected by the temperature of roasting and boiling time (see Appendix D). This was because lipids are mostly attached to proteins and carbohydrates in lipid sacs which are released by among others thermal action (Gurr & James, 1975). Summary of the optimization study is

shown in Table 12. The higher roasting temperature and time (120°C, 45min) for Pentadesma butyracea (Table 12) compared to 100°C, 30min observed for Carapa procera and Telfaira occidentalis seed could be attributed to its hard stony nature. The meal to solvent ratios were Carapa procera, 1:7; Pentadesma butyracea, 1:6 and Telfaira occidentalis, 1:6. These were comparable to ratios reported for peanut (Rhee et al, 1972). In his study he observed that no significant yield was obtained after obtaining a ratio of 1:7. Kim (1989) however obtained a maximum meal solvent ratio of 1:4 for palm kernel when heating at 85°C. Lower dilutions could not be investigated. This was due to the thickening of the mass due to the evaporation and absorbtion of the water by the meal. The significantly ($P < 0.05$) lower extraction efficiency of Telfaira occidentalis oil compared to the others could be due to the sticky groundnut paste-like form of the mass. This made it difficult to disperse in water in effect making it difficult for hot water to penetrate into the mass. The sticky nature could also account for the longer extraction time (30min) compared to (20min) observed for Carapa procera and Pentadesma butyracea. A near complete extraction (figs 8,9,10) was achieved at double extraction of oil from the meals. The extraction efficiencies were respectively 76.1%,73.1% and 64.1% for Pentadesma butyracea oil, Carapa procera oil

Table 11. Summary optimum conditions for maximum extraction of oil by pressing (please refer to Appendix D)

	C.procera	P.butyracea	T.occidentalis
charging (min)	-	1	-
attainment of max. pressure (min)	2	2	2
draining time (min)	15	15	25
time of steaming (min)	-	15	-
weight of sample (g)	100	200	100
temp. of roasting (°C)	80	100	100
time of roasting (min)	30	45	60
% extract from seed	29.7 ± 0.38	39.1 ± 1.2	36.4 ± 1.0
% effeciency	47.9 ± 0.8	88.8 ± 1.2	71.4 ± 1.0

% efficiency was calculated as $\frac{\text{oil extracted (g)}}{\text{oil in seed (g)}} \times 100$

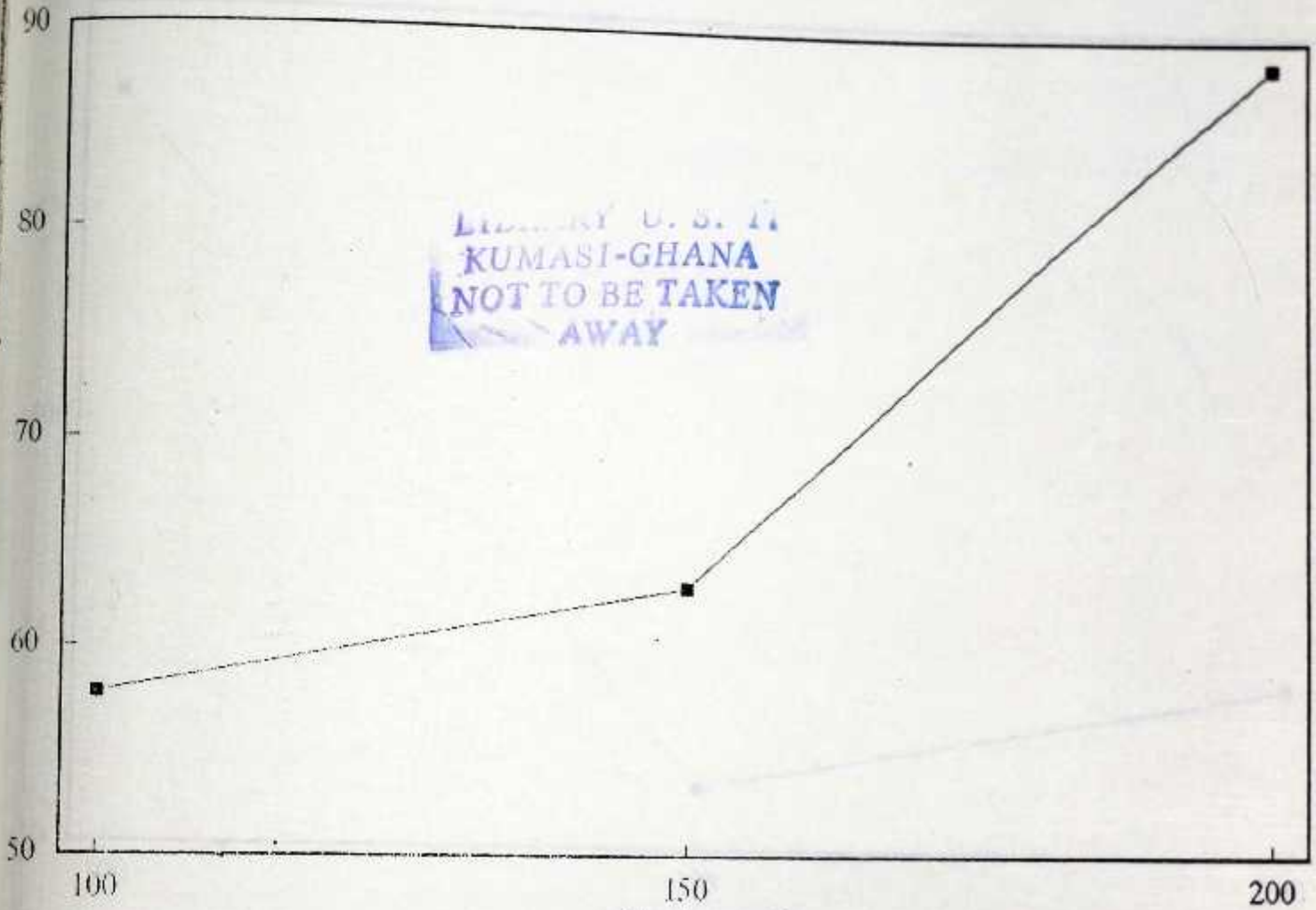


Fig.5: Effect of sample size on the extraction efficiency of of P. butyracea oil. (Seeds roasted at 100 degrees celcius for 45 mins)

(Maximum capacity of press was 200g)

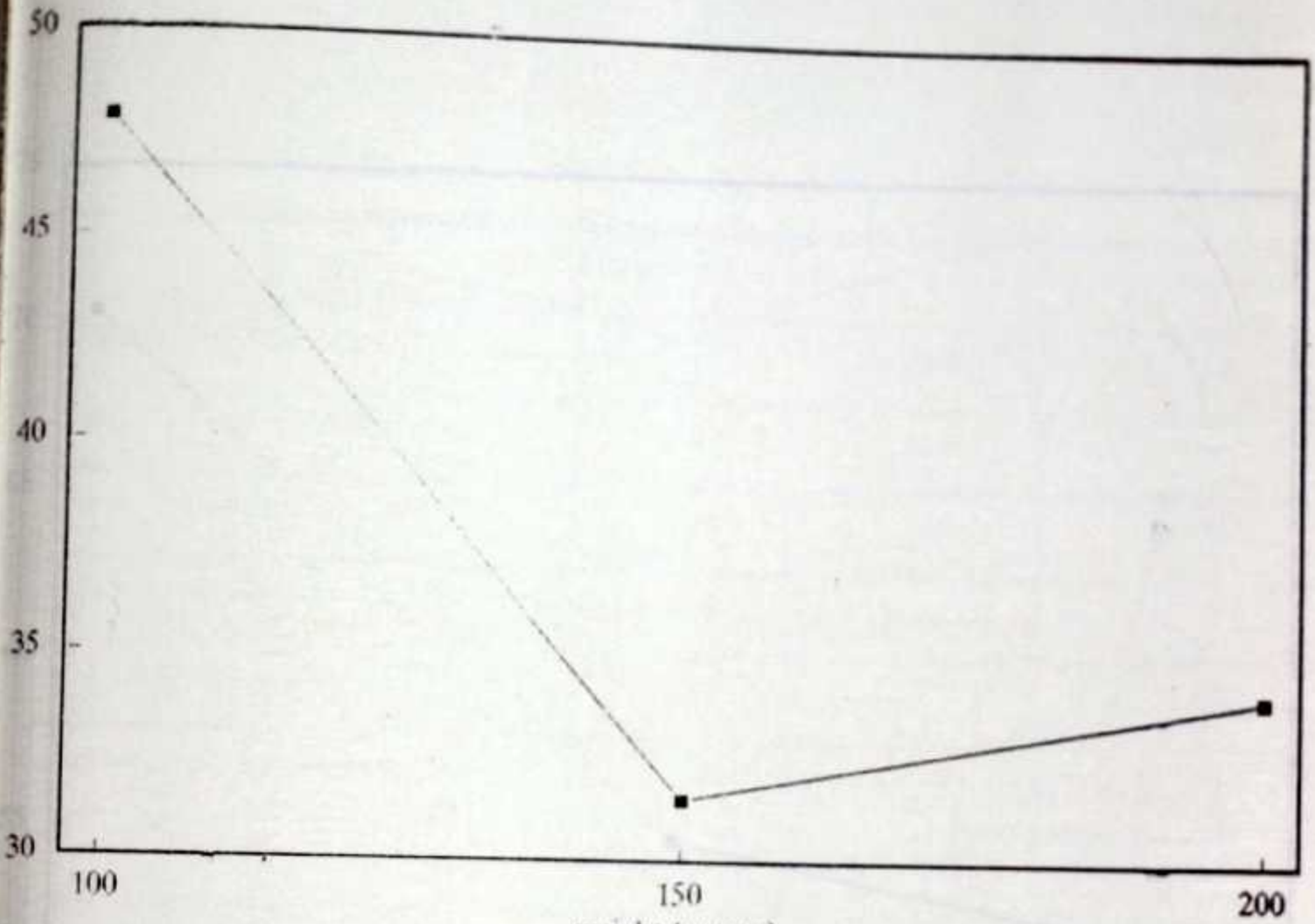


Fig.6: Effect of sample size on the extraction efficiency of C. procera seed oil (roasted at 80degrees, 30min)

(Maximum capacity of press was 200g)

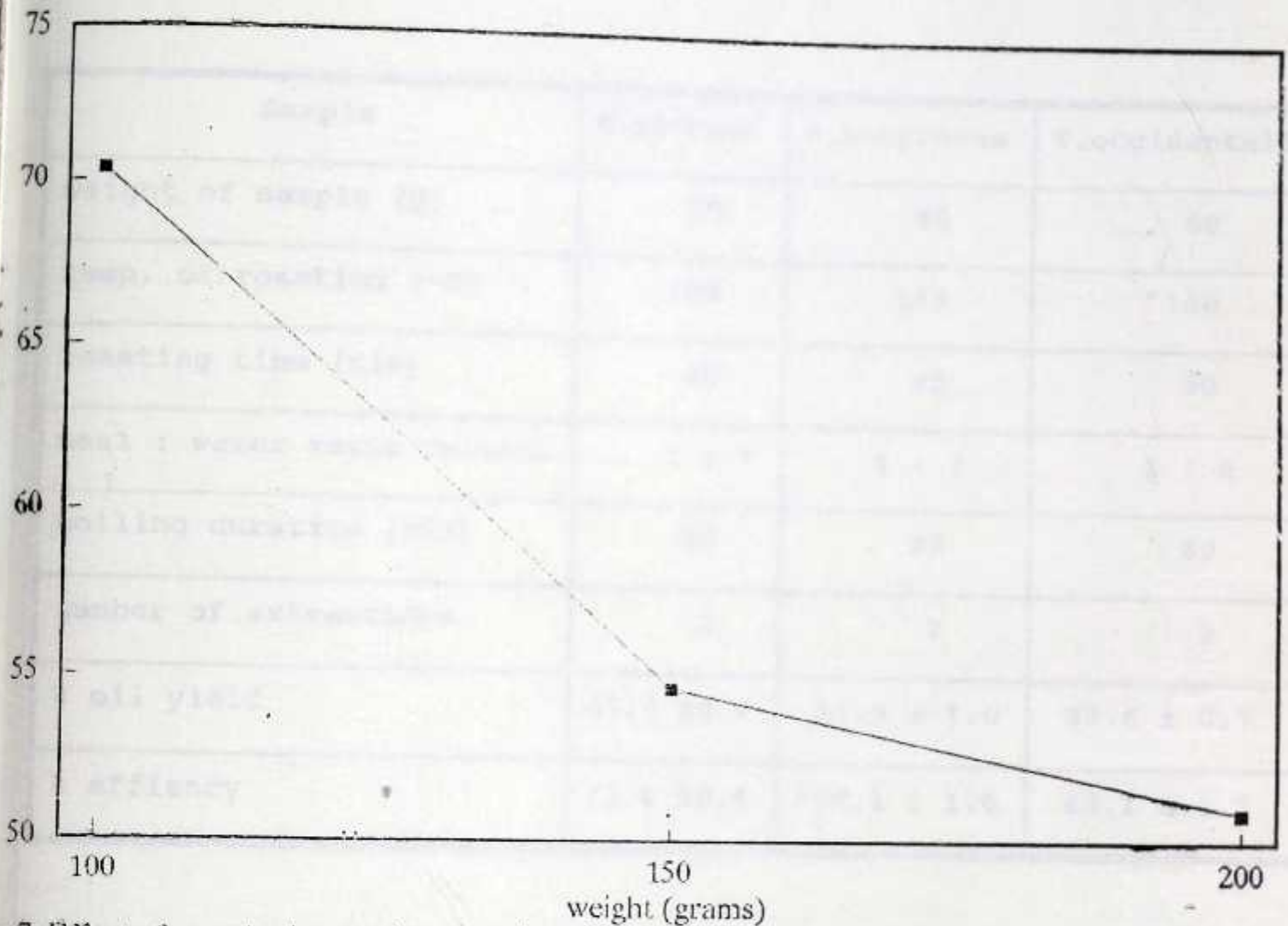


Fig.7: Effect of sample size on the extraction efficiency of T.occidentalis seed oil (seeds roasted at 120degrees for 30min)

(Maximum capacity of press was 200g)

Table 12. Summary of optimum conditions for maximum extraction of oil by aqueous method (please refer to Appendix D)

Sample	C.procera	P.butyracea	T.occidentalis
weight of sample (g)	50	50	50
temp. of roasting (°C)	100	120	100
roasting time (min)	30	45	30
meal : water ratio (m:vol)	1 : 7	1 : 7	1 : 6
boiling duration (min)	20	20	30
number of extractions	2	2	2
% oil yield	45.6 ±0.4	33.5 ± 1.0	32.6 ± 0.7
% effiency	73.6 ±0.4	76.1 ± 1.0	64.1 ± 0.7

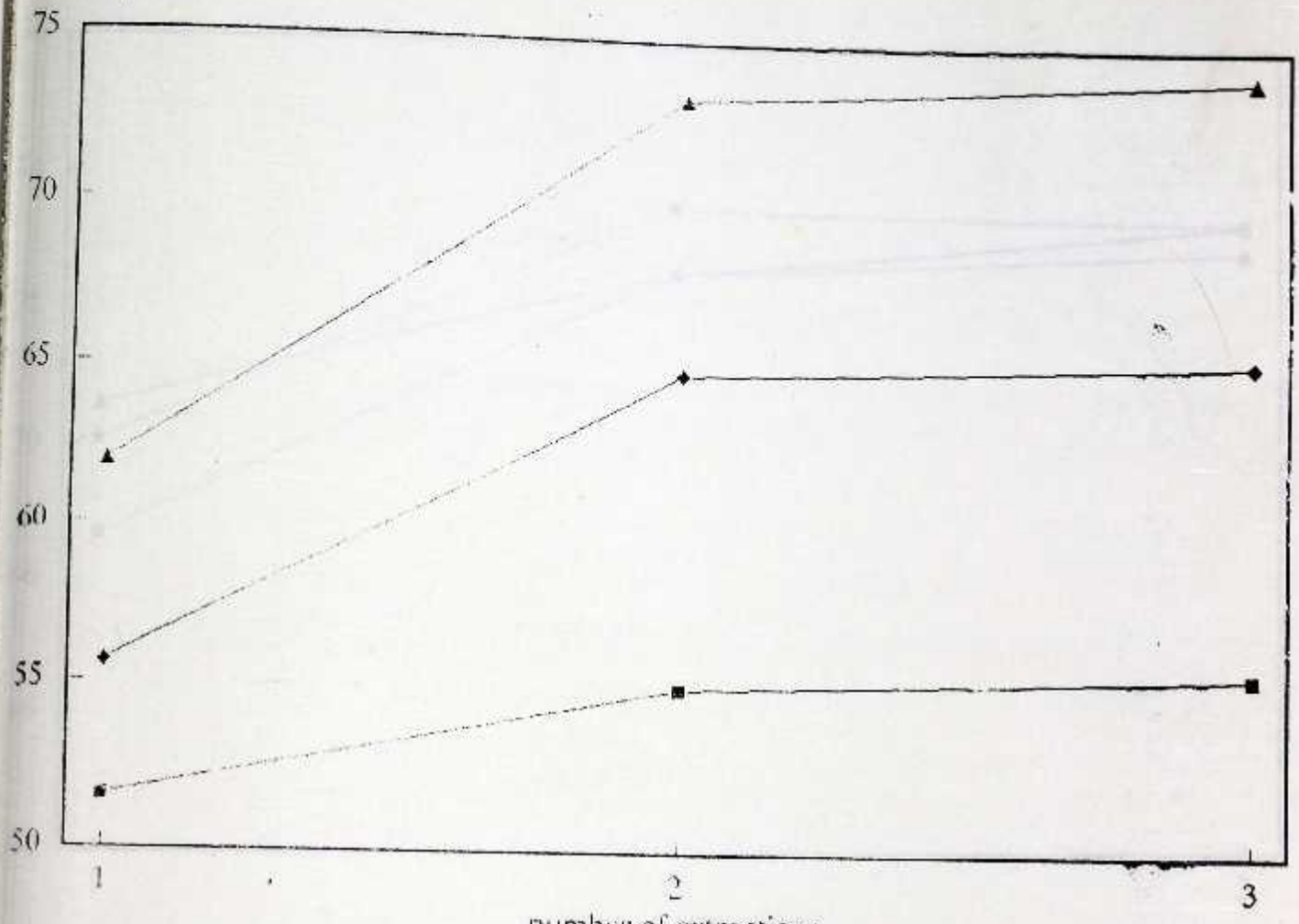


Fig.8: optimum extraction conditions for maximum extraction of P.butyracea seed oil

—■— seeds roasted at 80°c for 45 min, meal dilution ratio of 1:6 and extracting for 20 min.

—◆— Seeds roasted at 100°c for 45 min, meal dilution ratio of 1:6 and extracting for 20 min.

—▲— seeds roasted at 120°c for 45 min, meal dilution ratio of 1:7 and extracting for 20 min.

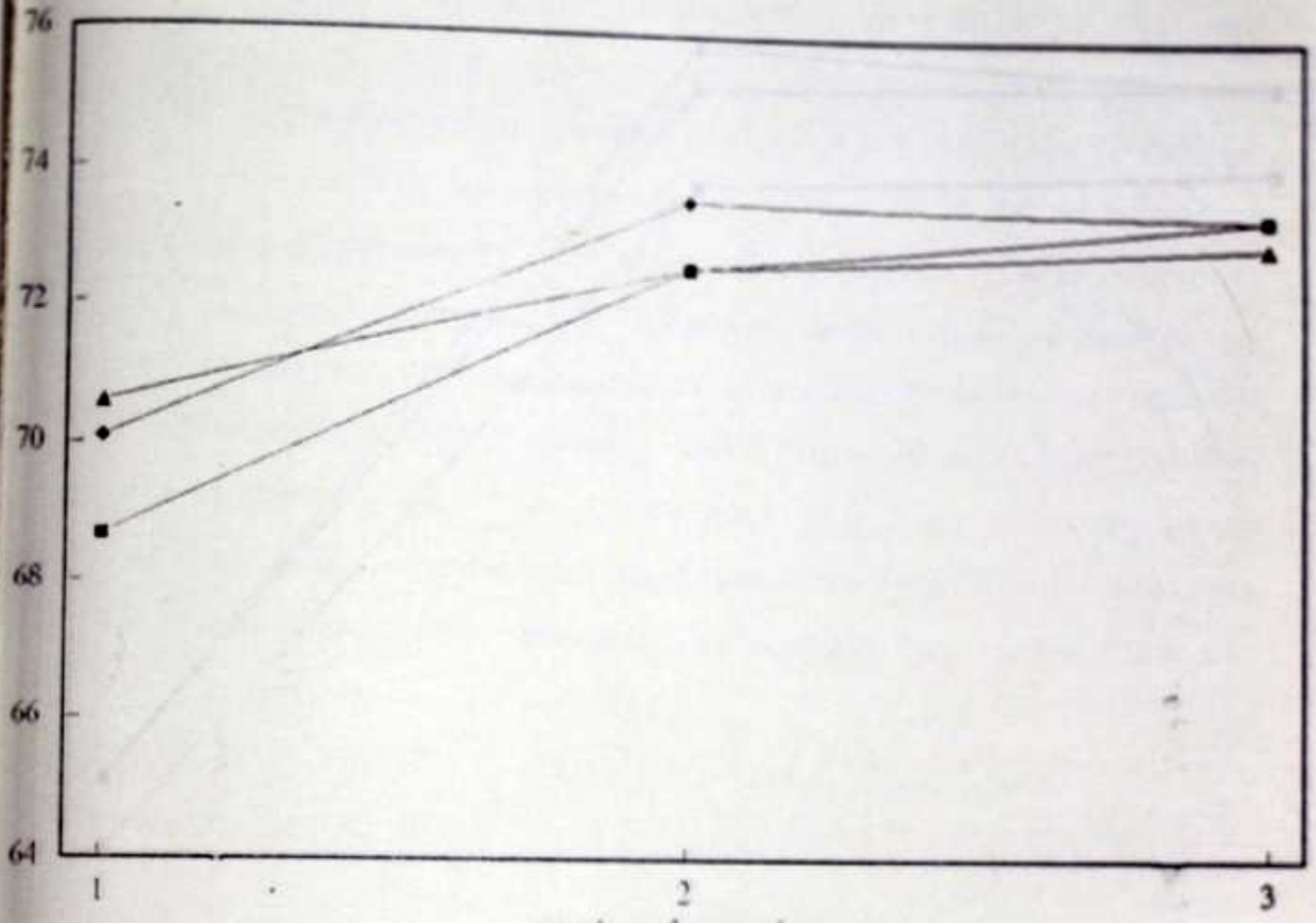


Fig.9: optimum extraction condition for maximum extraction of Carapa procera oil.

—■— seeds roasted at 80°c for 60 min, meal dilution ratio of 1:7 and boiling for 20 min

—◆— Seeds roasted at 100°c for 30 min, meal dilution ratio of 1:7 and boiling for 20 min

—▲— Seeds roasted at 120°c for 30 min meal dilution ratio of 1:7 and boiling for 20 min.

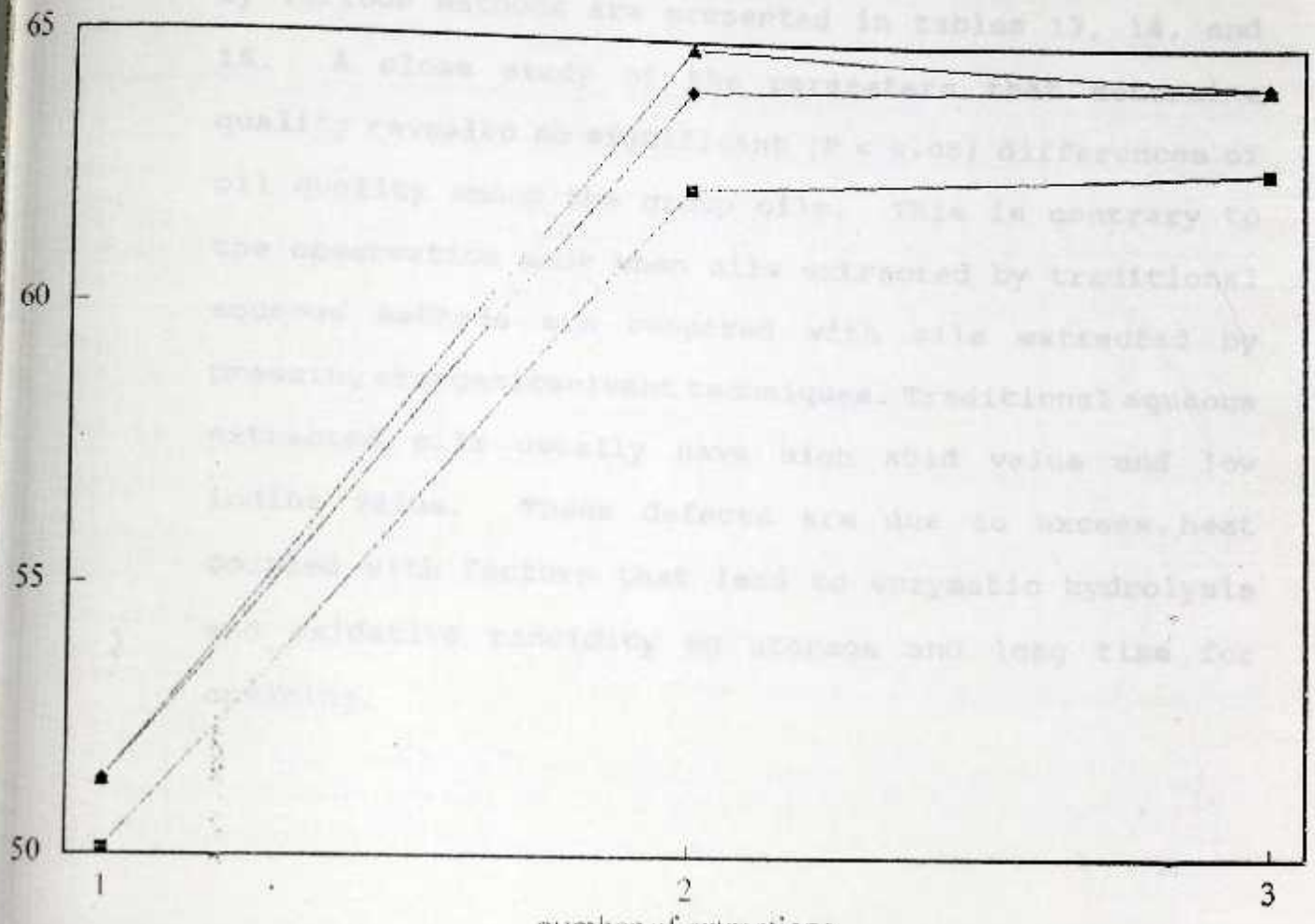


Fig.10: Optimum extraction conditions for maximum extraction of T. occidentalis

—■— seeds roasted at 80°c, 45 min; dilution of 1:6 and extracting for 30 min.

—◆— Seeds roasted at 100°c, 30 min; dilution of 1:6 and extracting for 30 min.

—▲— seeds roasted at 120°c, 30 min; dilution of 1:6 and extracting for 30 min.

and Telfaira occidentalis oil. Quality of oils extracted by various methods are presented in tables 13, 14, and 15. A close study of the parameters that determine quality revealed no significant ($P < 0.05$) differences of oil quality among the group oils. This is contrary to the observation made when oils extracted by traditional aqueous methods are compared with oils extracted by pressing ororganicsolvent techniques. Traditional aqueous extracted oils usually have high acid value and low iodine value. These defects are due to excess heat coupled with factors that lead to enzymatic hydrolysis and oxidative rancidity eg storage and long time for creaming.

Table 13. Characteristics of C.procera oil extracted by different methods

	Solvent	Aqueous	Pressing
specific gravity 25°C	0.9503 ± 0.020	0.9510 ± 0.020	0.9507 ± 0.023
refractive index 28°C	1.466 ± 0.001	1.466 ± 0.001	1.466 ± 0.001
moisture & volatile matter %	0.17 ± 0.01	0.18 ± 0.02	0.17 ± 0.01
iodine value	67.12 ± 1.33	67.12 ± 1.30	67.12 ± 1.07
unsaponifiable matter %	1.41 ± 0.21	1.41 ± 0.19	1.41 ± 0.30
saponification value %	214.37 ± 3.45	210.00 ± 2.40	213.83 ± 1.47
acid value %	2.33 ± 0.17	2.33 ± 0.15	2.33 ± 0.10

Values are mean of triplicate determinations ± standard deviations of means. Horizontal values are not significantly different at $P < 0.05$

Table 14. Characteristics of P. butyracea oil extracted by different methods.

	Solvent	Aqueous	Pressing
specific gravity 25°C	0.9592 ± 0.018	0.9592 ± 0.017	0.9592 ± 0.020
refractive index 28°C	1.460 ± 0.003	1.460 ± 0.003	1.460 ± 0.003
moisture & volatile matter %	0.31 ± 0.01	0.29 ± 0.01	0.29 ± 0.01
iodine value	51.59 ± 0.10	51.00 ± 0.25	51.00 ± 0.97
unsaponifiable matter %	1.46 ± 0.08	1.50 ± 0.05	1.47 ± 0.06
saponification value %	194.92 ± 2.46	197.01 ± 2.00	194.05 ± 2.87
acid value %	3.20 ± 0.11	3.19 ± 0.09	3.21 ± 0.05
slip point °C	37.5 - 38.0	37.5 - 38.0	37.5 - 38.0
melting point °C	38.9 - 39.4	38.9 - 39.5	38.9 - 39.5

Values are mean of triplicate determinations ± standard deviations of means. Horizontal values are not significantly different at $p < 0.05$

Table 15. Characteristics of T. occidentalis oil extracted by different methods.

	Solvent	Aqueous	Pressings
specific gravity 25°C	0.9465 ± 0.015	0.9465 ± 0.014	0.9465 ± 0.015
refractive index 28°C	1.483 ± 0.001	1.483 ± 0.001	1.483 ± 0.001
moisture & volatile matter %	0.11 ± 0.02	0.12 ± 0.01	0.12 ± 0.01
iodine value	99.45 ± 2.00	100.21 ± 1.87	99.7 ± 1.01
unsaponifiable matter %	1.67 ± 0.21	1.55 ± 0.36	1.60 ± 0.19
saponification value %	199.67 ± 2.1	1.99 ± 1.71	201.32 ± 2.56
acid value %	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.01

Values are mean of triplicate determinations ± standard deviations of means. Horizontal values are not significantly different at $p < 0.05$

Nutritional evaluation of oils.

The results of the nutritional evaluation of the three oils are presented in figures 11-15. Palm oil was used as the reference oil. Compared to palm oil, Pentadesma butyracea oil was comparable in the degree of unsaturation. However Carapa procera and Telfaira occidentalis oils were higher in unsaturation. This gave them nutritional advantage over palm and Pentadesma butyracea oils but the transaminase activities of the sera of rats on these oils diets did not confirm this. Figures 11 and 12 show the mean values of food eatwn and weight gain.Generally increase in oil concentration of diets led to a reduction of diet intake. This effect was similar to observation on farm animals. According to McDonald et al,(1988) farm animals reduce intake of diet as calorific values of diet increases. The high intake of Telfaira occidentalis oil diets could be attributed to the pleasant aroma the oil imparted to the diet, and the low intake of Carapa procera oil diets was principally due to its bitterness. Even though rats on Telfaira occidentalis oil diets ate more of the diet, rats on palm oil diets gained more weight. This could be attributed to negative growth factors present in the oil. Nwokolo & Sim, (1987) reported relatively poor growth in chicks when they were fed with Telfaira occidentalis cake. This suggests that the factors

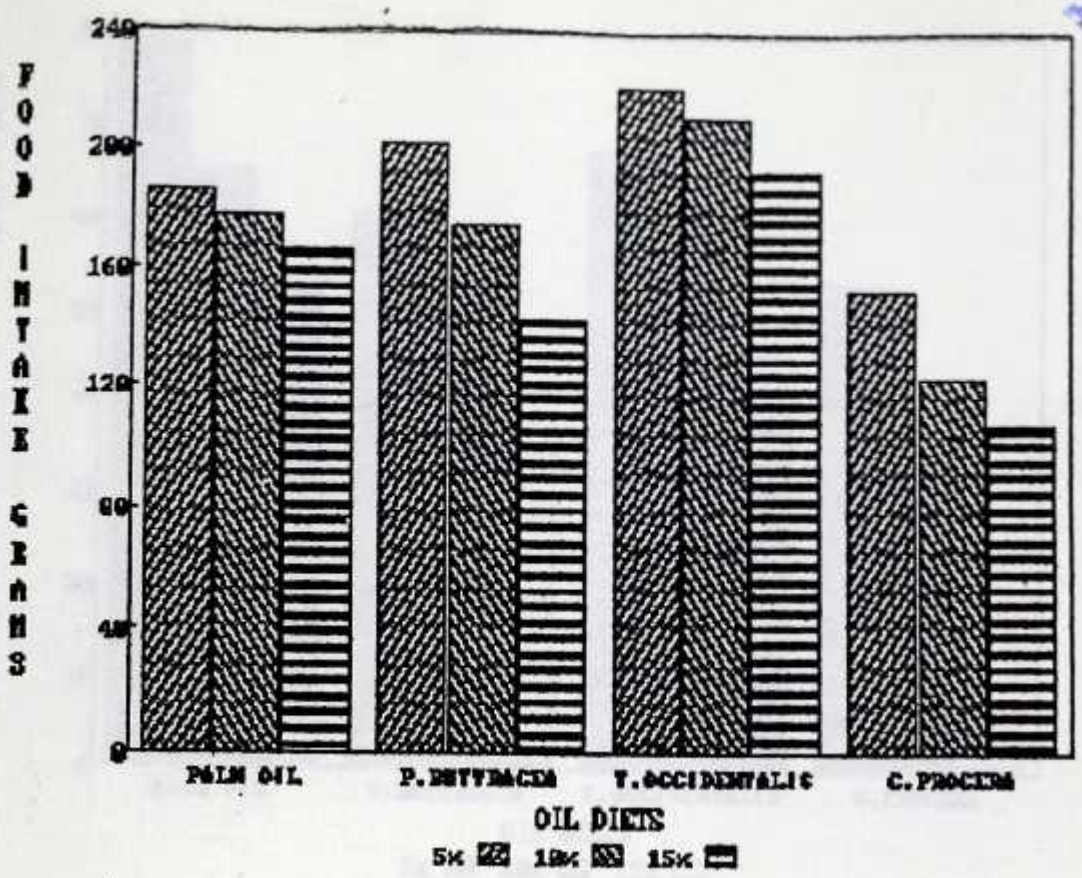


FIG II FOOD INTAKE OF RATS FED DIFFERENT OIL DIETS OF VARYING CONCENTRATION.

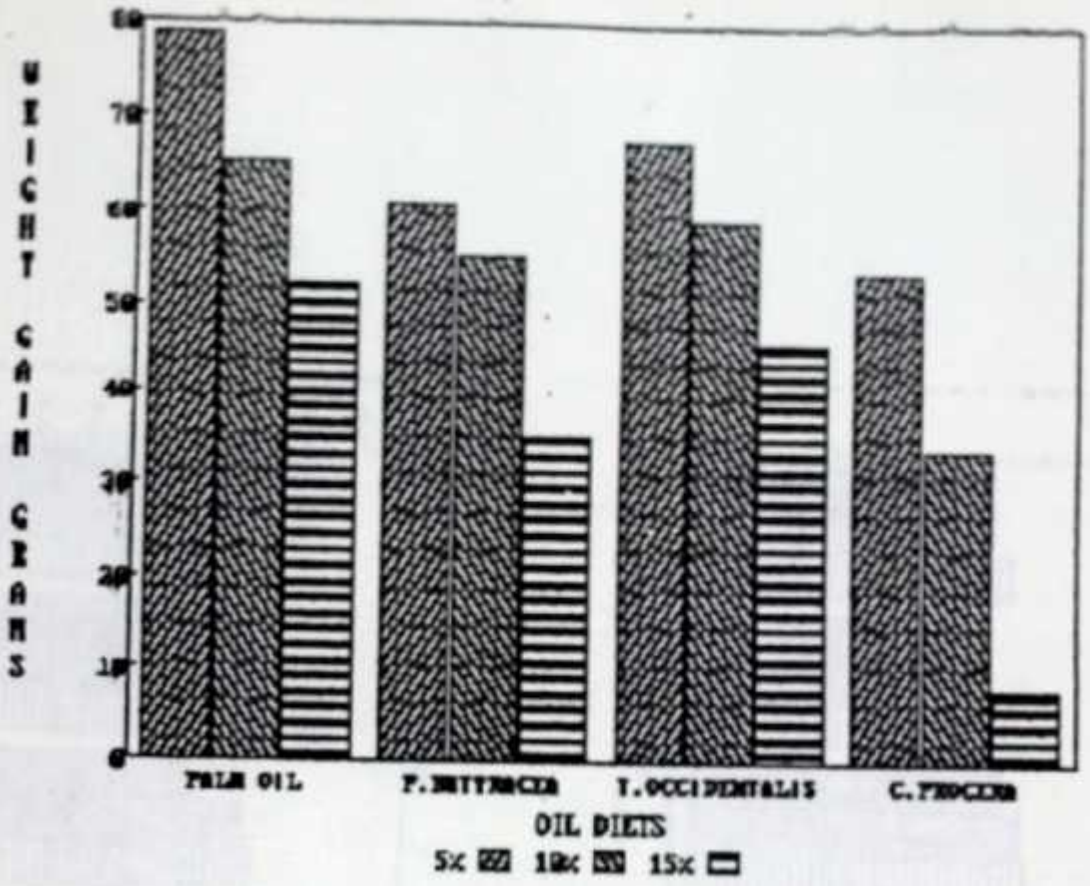
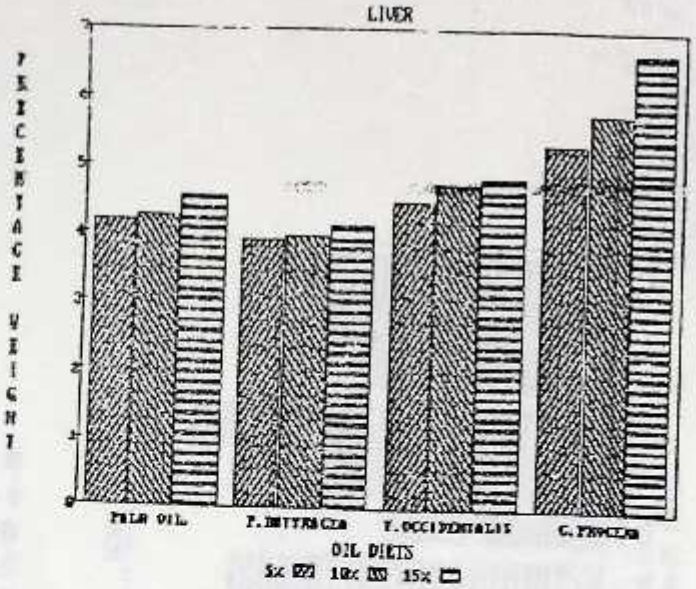
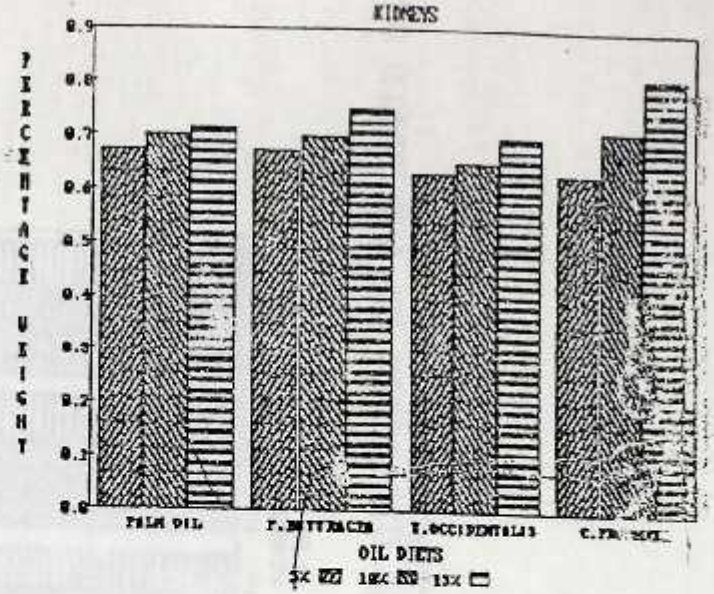
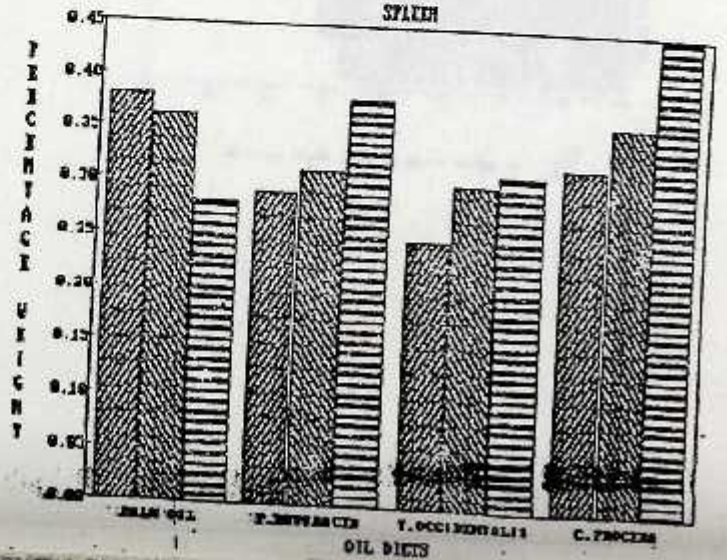
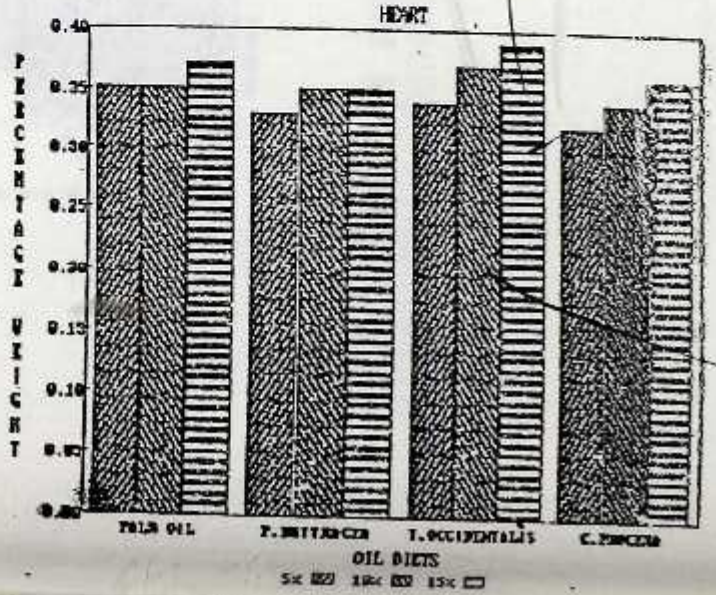


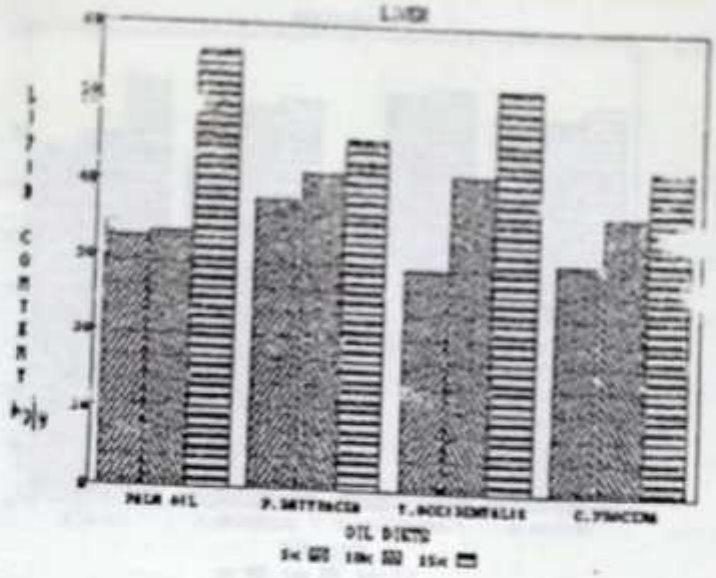
FIG 12 EFFECT OF OIL DIETS ON WEIGHT GAIN.

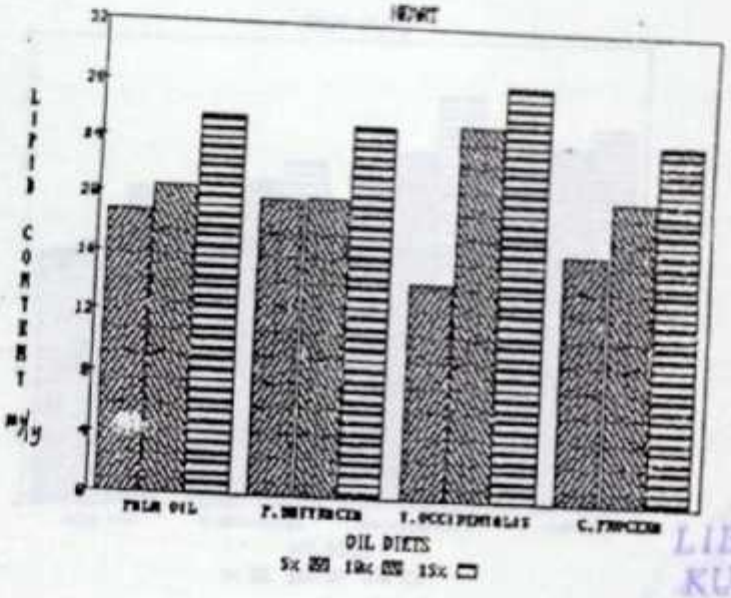












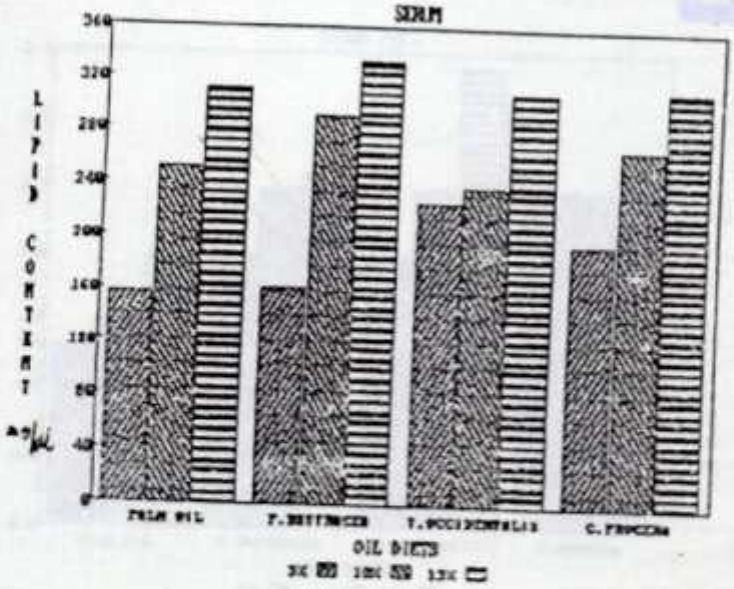
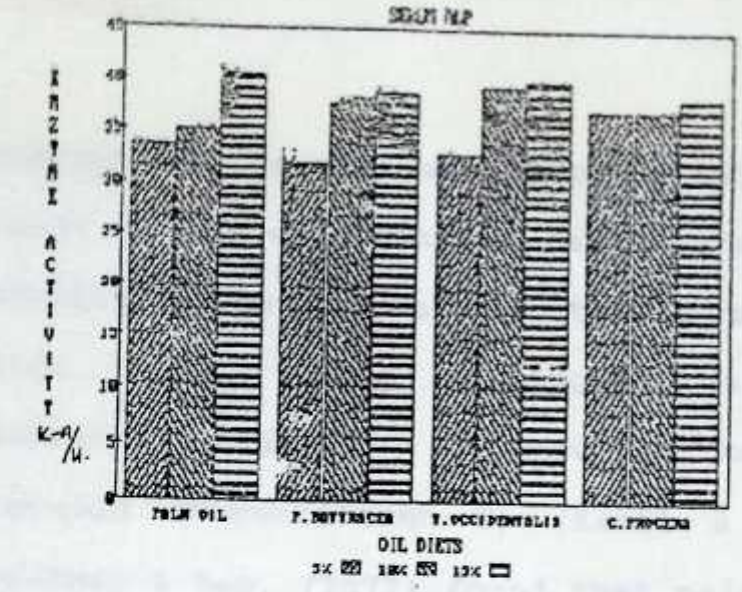
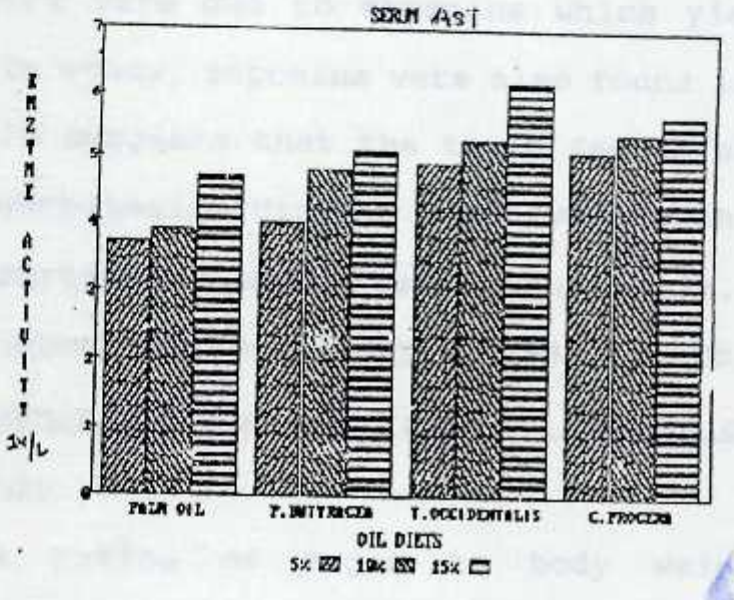


FIG 14 EFFECT OF OIL DIETS ON THE LIPID CONTENT OF ORGANS AND SERUM.





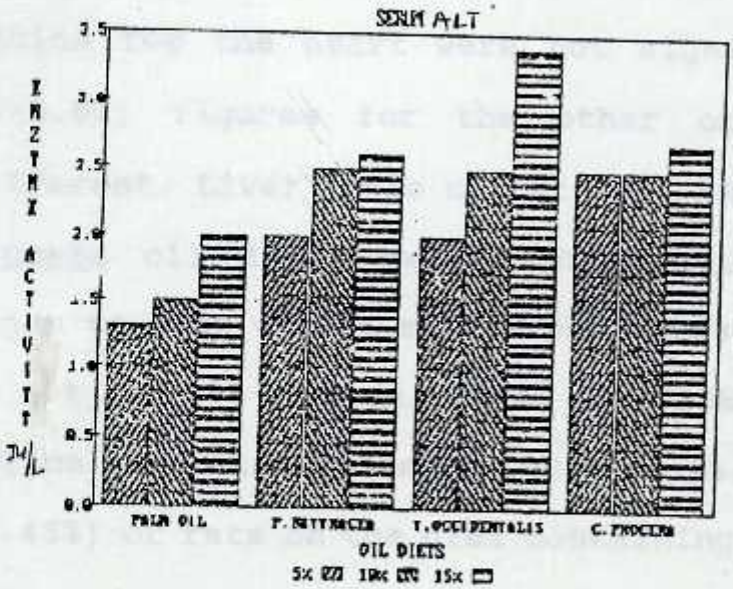


FIG 15 EFFECT OF OIL DIETS ON THE SERUM ENZYME ACTIVITIES.

inherent in the cake are probably oil soluble. Similar plants in the cucurbitacea family are known to contain negative growth factors collectively called cucurbitacins (Anon 1984). Luffa acutangula, a member of the cucurbitacea family is reported to contain bitter steroids (cucurbitacen B) (Kamel & Blackman, 1982). Varshney & Beg, (1977) found that poisnous materials in luffa were due to saponins which yield sapogenins. In this study, saponins were also found in the cake and oil. This suggests that the toxic factor could probably be a cucurbitacin. Similar negative growth effects have been reported for some lesser known oils, quar, pawpaw and flambouyant (Varade et al 1984, Marfo et al 1988).

Organ to body weight ratio of rats on graded level of oil diets

The ratios of organ to body weight, expressed in percentages are presented in figure 13. Even though ratios for the heart were not significantly different ($P<0.05$) figures for the other organs studied were different. Liver ratio of rats on 10% and 15% of Carapa procera oil diets gave significantly higher ($P<0.05$) organ to body weight ratios of, 5.78% and 6.66% compared to 10% and 15% palm oil diets of 4.25% and 4.57%. Higher ratios were also recorded for kidneys, (0.81%) and spleen (0.45%) of rats on the diet containing 15% Carapa procera oil.

Effects of diets on lipid content of organs.

Lipid content of heart and liver are shown in figure 14. A range of 18.6mg/g to 25.59mg/g was obtained for the heart of rats on palm oil diets. Comparable ranges of 19.74mg/g to 25.10mg/g and 16.36mg/g to 24.38mg/g were also obtained for rats on Pentadesma butyracea oil and Carapa procera oil diets respectively. The range for rats on Telfaira occidentalis oil diets, 14.36mg/g to 28.33mg/g however was significantly different. The range of 32.85mg/g to 52.0mg/g lipid in livers of rats on palm oil diets, compared favorably with the ranges 38.19mg/g to 43.95mg/g;28.72mg/g to 52.34mg/g and 30.20mg/g to 42.59mg/g lipid in the livers of rats on Pentadesma butyracea oil , Telfaira occidentalis oil and Carapa procera oil diets respectively. Basically, of interest nutritionally and toxicologically are the liver and kidneys. The liver plays a central role in regulating lipid metabolism whilst the kidney also plays a major role in detoxification in the body. From the results (fig 13) the liver weights of the rats on 10% and 15% Carapa procera oil diets showed significant ($p < 0.05$) differences from the range obtained for rats on 5%,10% and 15% palm oil diets ie 5.78 to 6.66% compared to 4.20 to 4.57%. This suggests that the diets led to metabolic impairment which is usually exhibited by an extremely large organ commonly caused by fatty change (Lombadi,

1966). However the lipid contents of the liver organs did not confirm this. This was because, due to the bitterness of the diets the rats ate little. This led to a low body weight hence even though their livers were normal in size a high ratio was obtained.

Serum parameters

The amount of lipid in the serum and transaminase and alkaline phosphatase enzyme activities are shown in figures 14 and 15. The general observation was that gradual increase in oil content of diets led to corresponding increases in serum lipid content and transaminase activities. Elevation of aminotransferases and alkaline phosphatase activities have been used as indicators of tissue damage particularly in the liver and kidneys (Ariens et al 1976, Mandal et at, 1985). In the study (ALT/AST) ratio was less than one for all the study rat groups. This is usually the ratio in normal and other related cases (Tietz 1986). Even though gradual elevation of aminotransferase activities were noted as oil content increased in the diets, significant increases were observed only in the sera of rats on 10%, 15% Telfaira occidentalis oil and 10%, 15% Carapa procera oil diets, compared to 15% of the reference oil. This suggests that these diets contained factors that damaged the cells of the liver and kidneys hence increasing the concentration of AST and ALT in the sera. Necrosis could

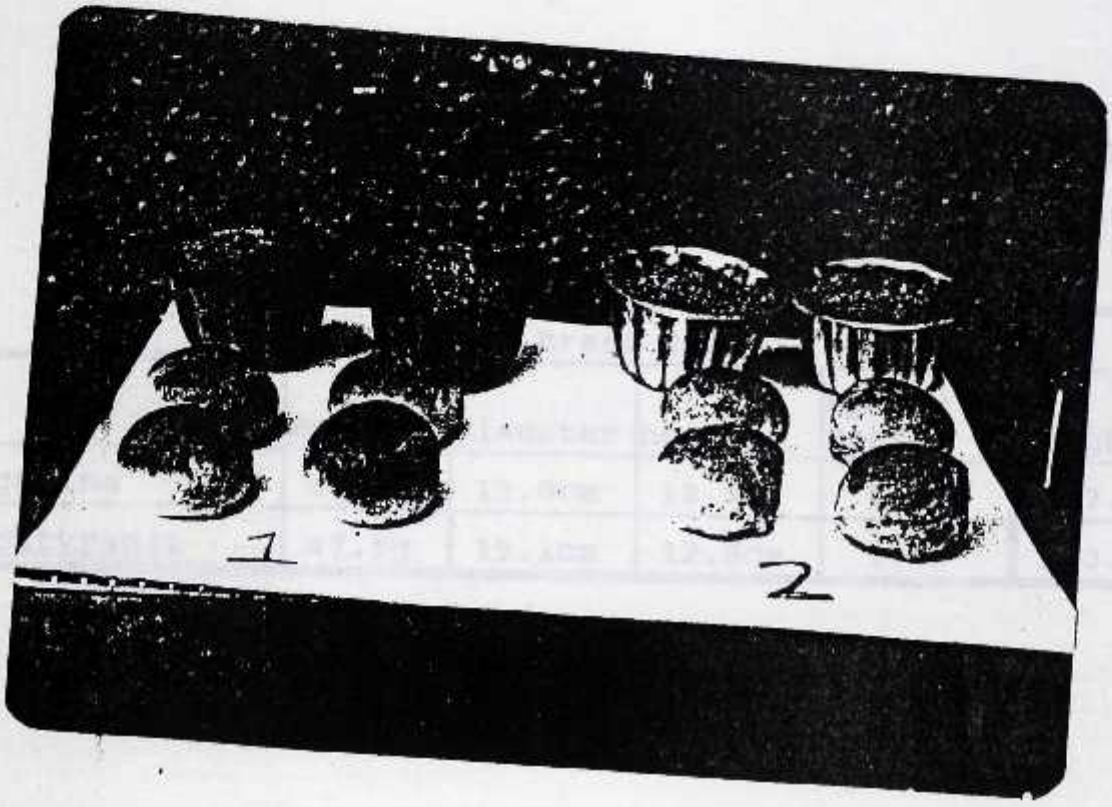
be a possible effect of the toxins.This is because in necrosis ✓ of the cell, considerable amounts of mitochondrial AST are released which depresses the ALT/AST ratio (Tietz 1986). The toxic effect of the oils could also be attributed to the suspected cucurbitacins present in Telfaira occidentalis oil,and a toxin present in Carapa procera oil (Nwokolo & Sim,1987). No significant differences were observed for alkaline phosphatase activities in the sera of rats on all the oil diets. This indicates the absence of a hepatobiliary disease.

Industrial Potentials of oils.

The shortening and soap potentials of the oils are shown in figure 16 and Tables 16, 17 and 18. Bread rolls prepared from margarine was lighter (46.2g) than Pentadesma butyracea fat (47.5g). Even though the ir diameters were not different, there were differences in height. Pentadesma butyracea bread was 12.8cm whilst margarine was 13.1cm. This led to differences in spread factors. Results obtained for the shortening effects of P. butyracea fat compared to margarine in cake production were, margarine gave lighter (197.6g) and bigger surface diameter (9.8cm) than Pentadesma butyracea fat (203.5g and 8.1cm). This could be due to poor evaporation of water be sidesthe poor loss of carbon dioxide due to the poor shortening effect of the fat,(Sweetman & Keller,

1963). The poor diameter of the Pentadesma butyraceae oil cake could also be attributed to the poor shortening effect of the fat. This is because fat is the only ingredient in shortened cakes which is active in entrapping air and has a considerable effect on volume (Charley 1952). During baking the fat melts releasing air cells to give expansion. The colour, odour, taste, mouth feel and flavour were the criteria used for acceptability of the cookies. Generally, margarine cookies fared significantly better ($P<0.5$) in this criteria than Pentadesma butyracea fat. This could also be due to the natural colour, flavour and the shortening effect of the Pentadesma butyraceae fat. This is because these are the factors that play significant roles in baking, and the acceptability of the end-product.

SHORTENING EFFECTS OF PENTADESMA BUTYRACEA FAT COMPARED TO MARGARINE



KEY

FIG. 16

1. Baked products using margarine
2. Baked products using Pentadesma butyracea fat

TABLE 16 Physical properties of baked products of margarine and P. butyracea fat.

	Bread Rolls				Cake	
	weight	diameter	height	spread factor	weight	diameter (surface)
Margarine	46.2g	19.0cm	13.1cm	1.45	197.6g	9.8cm
P. butyracea	47.5g	19.1cm	12.8cm	1.49	203.5g	8.1cm

TABLE 17 Organoleptic properties of baked products (taste panel) of margarine and *P. butyracea* fat.

	Appearance	Color	Aroma	Texture	Taste	Mouthfeel
Bread						
Margarine	6.6	6.6	5.5	6.7	6.2	6.0
P. butyracea fat	3.7	4.9	3.2	4.3	4.7	3.6
difference	s	s	s	s	s	s
Cake						
Margarine	7.6	7.6	7.35	7.3	7.7	6.6
P. butyracea fat	3.0	3.7	3.9	3.7	3.9	3.9
difference	s	s	s	s	s	s

s = significantly different at $p < 0.05$

TABLE 18

Physical and Chemical Properties of soap prepapared from C. procera, P. butyracea and T. occidentalis

Physical Properties					
Oil	Texture	Lathering stability	Washing efficiency	Odour	Colour
P. butyracea	very hard	very good	good	agreeable	cream
C. procera	hard	very good	good	agreeable	brown
T. occidentalis	hard	very good	good	agreeable	brown

Chemical Properties			
	Moisture %	Total fatty matter (TFM) %	Free caustic alkali (FCA) %
P. butyracea	10.21	63	0.18
C. procera	11.31	65	0.20
T. occidentalis	13.42	64.8	0.20
Ghana Standards	< 28%	> 59%	< 0.25%

The quality of soap is greatly determined by the fatty acid composition, iodine value and the saponification value of the oil used. The lathering stability and the washing efficiencies were similar. This is because of the similar fatty acids present in the individual oils. The softer nature of Telfaira occidentalis oil soap relative to the others could be attributed to the higher degree of unsaturation of the oil and the fairly higher moisture content. This will make the soap more susceptible to rancidification.

Soap produced from the different oils (Carapa procera, Pentadesma butyracea and Telfaira occidentalis) did not show any significant differences amongst them in terms of texture, lathering stability washing efficiency and odour. Pentadesma butyracea oil soap was cream in colour compared to the brown colour of Carapa procera and Telfaira occidentalis oils soaps. All the soaps satisfied Ghana standards specification for hard soaps.

CONCLUSION

The study has confirmed that Carapa procera, Pentadesma butyracea and Telfeira occidentalis are a rich source of oil, and aqueuos and pressing techniques can be used to extract good quantities and quality oils from the seeds. This study also suggests that Pentadesma butyracea oil can be used for food however the use of Telfeira occidentalis oil for food should be controlled.

Furthermore, all the oils have a good potential for soap making. The high level of protein coupled with the low level of toxic factors in Telfeira occidentalis cake makes it a good substitute for imported oil seed cakes for animal feed production. However, because its amino acid composition is not well balanced, its incorporation into animal feed should be carefully planned.

RECOMMENDATION

Further work should be done on Carapa procera to debitter the oil and cake to make it more economically useful. Investigation into good horticultural practices for the cultivation of T. occidentalis should be conducted so that it can be adapted as a cash crop.

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A P P E N D I X A

Equipment

Thermostically Controlled Oven

Chemical Balances

Heating Mantle

Steam Bath

Digestion Burner

Spectrometers

Abbé retractometer

TLC applicator and supporter

Amino acid analyser

Muffle furnace

Plate mill

Dessicator

Cotton wool

Soxhlet Extractor

Paper thimbles

Glass wool

Round bottom flasks

Thermometer

Condenser

Funnel

Elenmeyer flasks

Asbestos

Kjeldanl digestion flask

Markham distillation apparatus

Burettes

Volumetric flasks

Variable temperature hot plate

Pipettes

Measuring cylinder

Glass rods

Reflux apparatus

Filter paper

Specific gravity bottles

Glass plates

Disecting kit

Crucibles

TLC tank

Separating funnel

Centrifuge tubes

Centrifuge

Baking pans

Mini oven

PH meter

Capillary tubes

Watch glass

Flame photometer

Atomic absorption spectrometer

Infra red spectrometer

Ballistic bomb calorimeter

APPENDIX 1B

Reagents

Petroleum ether

Carbon tetrachloride

Potassium hydroxide

Hydrochloric acid

Phenolphthalein indicator

Wigs iodine

Sodium thiosulphate

Methanol

Starch

Acetone

Ethanol

Silica gel

Potassium dihydrogen phosphate

Potassium hydrogen phosphate

Hexane

Glacial acetic acid

Diethyl ether

Sodium hydroxide

Sulphuric acid

Boric acid

Kjedahl tablets

Concentrated ammonia

Calcium chloride

Potassium permanganate

Ammonia hydroxide

Potassium iodide

Dioxane

Ammonium molybdate

Ammonium metavanadate

Toluene

Bromocresol green

Methyl red

Indigo carmine

Gelatine

NaCl

Kaolin

Boron trifluoride

Potassium chloride

Fatty acid standard

Triacyl glyceride

Cholesterol

2, 4 Dinitrophenyl hydrazine

Sodium hydrogen carbonate

4-amino antipyrine

Potassium ferricynide

Benzoic acid

A P P E N D I X 2B

Reagent Details

2B1Determination of Tannin,

a) $KMnO_4$

Dissolve 1.33g in 1000ml of water

b) Indigo Carmine

6 grams of indigo carmine (free from indigo blue) is dissolved in 50ml H_2SO_4 in 1000ml water.

c) Gelatin

Soak 25grams gelatin 1 hour in saturated NaCl solution. Heat until gelatin dissolves. Cool and dilute with saturated NaCl solution to litre.

d) Acid Sodium Chloride

Acidify 975ml of Saturated NaCl solution with 25ml H_2SO_4.

2B2 Preparation of standard elemental solutions

a) Standard Potassium Curve

Stock solution containing 500ppm of K was prepared by dissolving 0.9335g of KCl in 300mls of distilled water followed by 10mls of conc. HCl, and then diluted to 1 litre. After the solution was complete and CO_2 evolution had ceased, reference solutions containing 2, 4, 6, 8 and 10ppm were prepared from stock.

b) Standard Sodium Curve

Stock solution containing 500ppm of Na was prepared by dissolving 1.2780g of NaCl in 300mls of distilled water followed by 10mls of conc. HCl, and then diluted to 1 litre. After the solution was complete and CO_2 evolution had ceased, reference solution containing 2, 4, 6, 8 and 10ppm were prepared from the stock.

2B3 Determination of phosphorus

Ammonium molybdate - ammonium metavanadate

Add 25g of ammonium molybdate and 1.25g of ammonium metavanadate to approximately 300ml of water. Warm to dissolve and cool. Dilute to 500ml and filter if necessary.

2B4 Methanolic Sodium Hydroxide (0.5 N)

Dissolvce 25g NaOH in 100ml methanol.

2B5 Methanolic potassium hydroxide (0.5)

Dissolve 35-40grams of KOH in 20ml distilled water, make it to 1 litre with ethanol. Leave overnight and filter.

2B6 TLC locating reagent

Mixture of conc. H_2SO_4: water (1:9)

2B7 Bloor's reagent

To 600ml ethanol, add 200ml ether and store in a glass stoppered bottle.

2B8 Transaminase reagents

a) Phosphate buffer, 0.1M pH 7.4

Dissolve 11.9g of disodium hydrogen phosphate (anhydrous) and 2.2g of potassium dihydrogen phosphate (anhydrous) in distilled water and make up to 1 litre. Check pH, adjust to 7.4 if necessary using small amounts of appropriate phospahte (eg if the pH more than 7.4 add KH_2PO_4). This solution is stable to about 2 months at 2-8°C.

b) Buffered substrate reagent (SGOT)

Place 50.0mg of aplha (α) ketoglutarate and 2.66g of Dl-aspartate into a small beaker. Dissolve 20ml of NaOH solution (1 mol/L) then adjust pH to 7.4 with more NaOH solution. Transfer to a 100ml volumetric flask with phosphate buffer and make up to 100ml with phosphate buffer. Add 1ml of chloroform as preservative and store at 4-8°C. This solution is normally stable for 2 weeks at 2-8°C but discard sooner if it becomes turbid.

c) Buffered substrate reagent (SGPT)

Place 300mg of aplha (α) ketoglutarate and 17.8g of DL-Alanine into a small beaker. Dissolve in 20ml of NaOH solution (1 mol/L) add more NaOH solution and adjust pH to 7.4 with more NaOH solution if necessary. Transfer to a 100ml volumetric flask with

phospahte buffer. Add 1ml of chloroform as preservative and store at 4-8°C. This solution is normally stable for 2 weeks at 2-8°C but discard sooner if it becomes turbid.

d) 2, 4 - Dinitrophenyl hydrazine (DNPH) 1 mol/L

Dissolve the equivalent of 23.8mg of 2,4 DNPH in 100ml 1 mol/L HCl. This solution is stable for 2 months at 2-8°C.

e) Triethanolamine - ethylenediamine tetra-acetate (EDTA) buffer.

Weigh out 3.8g triethanolamine and 1.0g EDTA disodium salt, dissolve in about 200ml of distilled water and add 15 ml 1 mol/L HCl. Make up to 500ml distilled water. Check that the pH is between 7.5 - 7.6. This buffer is stable indefinately at 2-8°C.

f) Pyruvate standard solution, 4m mol/L

Weigh out 44mg of sodium pyrurate and make up to 100ml in a volumetric flask with triethanol amine - EDTA buffer. Mix well, divide into small portions of 1 ml and store in the freezer compartment of a refrigerator. The standard solution is stable for about six months when frozen or 1 week at 2-8°C.

2B9 Alkaline phosphate reagents

a) Buffer, pH 10.14

6.36g of anhydrous sodium carbonate and 3.36g of sodium hydrogen carbonate dissolved in distilled water and made up to 1 litre. Keep at 4°C. The pH should lie within ±0.1 unit of its normal value.

b) Substrate, 0.01M disodium phenyl phosphate

Dissolve 2.18g of disodium phenyl phosphate in 1 litre of distilled water. Bring the solution quickly to boil to kill any organism. Cool immediately and preserve with a little chloroform (4m/L) keep at 4°C.

c) Stock phenol standard, 1mg/ml.

Dissolve 1g pure crystalline phenol per litre in 0.1N hydrochloric acid, keep at 4°C in a brown bottle.

d) Working phenol standard, 1mg/100ml.

Dilute 1ml of stock phenol standard to 100ml with distilled water. Preserve with a few drops of chloroform and keep at 4°C in a bottle.

e) 0.5N Sodium hydroxide solution

Dissolve 20g of NaOH in 1 litre of distilled water.

f) 0.5N Sodium hydrogen carbonate

g) 4-Amino anti pyrine

Dissolve 6g of aminoantipyrine in 1 litre of

distilled water. Store in a brown bottle.

h) Potassium ferricyanide

Dissolve 24g of potassium-ferricyanide in 1 litre of distilled water. Store in a brown bottle.

APPENDIX C

Calculations

APPENDIX C1

$$\text{Percentage moisture} = \frac{\text{Loss in weight of sample}}{\text{Original weight of sample used}} \times 100$$

APPENDIX C2

$$\text{Percentage fat} = \frac{\text{Weight of fat obtained}}{\text{Dry weight of sample used}} \times 100$$

APPENDIX C3

$$\text{Percentage crude fibre} = \frac{\text{Weight of fibre}}{\text{Dry weight of sample used}} \times 100$$

APPENDIX C4

$$\text{Percentage ash} = \frac{\text{Weight of ash}}{\text{Dry weight of sample used}} \times 100$$

APPENDIX C5

$$\text{Percentage crude protein} = \frac{V_A \times M_A \times 14 \times 6.25 \times V_T}{1000 \times W \times V_s} \times 100$$

where V_A = volume of acid used in titration
M_A = molarity of acid used in titration
14 = molecular weight of nitrogen
6.25 = protein factor
V_T = total volume of digest
V_s = volume of digest distilled
W = dry weight of sample used.

APPENDIX C6

% carbohydrate by difference = 100% - (%moisture + %ash + % Protein + % Fat + % Fibre).

APPENDIX C7

$$\% \text{ total oxalate} = \frac{a \times c \times Df \times V_T \times 100}{b \times W(mg) \times V_a}$$

where a = concentration (mg/25ml) of standard oxalate solution used
b = volume of 0.5N $KMnO_4$ required to neutralize the standard solution
c = volume of 0.5N $KMnO_4$ required to neutralize 25ml of sample extract
w = dry weight of sample used
V_T = total volume of extract
V_a = volume (aliqout) of extract which was concentrated before titration with 0.5N $KMnO_4$ solution
Df = dilution factor involved due to concentration of the aligout taken

APPENDIX C8

$$\% \text{ saponin} = \frac{\text{weight of saponin}}{\text{dry weight of sample used}} \times 100$$

APPENDIX C9

$$\% \text{ tanin} = \frac{(X-Y) \times 0.0525}{2 \times W}$$

where W = weight of sample
X = volume of $KMnO_4$ (designated X)
Y = titre value (light green)
Z = titre value using 12.5 ml filtrate
Actual volume of $KMnO_4$ used = (X-Z)
1 ml of 0.1 N oxalic acid ≡ 0.0042 tanin

$$\text{thus } 12.5 \text{ mls} = \frac{12.5 \times 0.0042g}{1}$$

APPENDIX C10

$$\% \text{ mineral (Ca, Fe, K, Na, Zn, Cn, Mn, P)} = \frac{V_B \times V_F \times V_C}{W \times V_A} \times 10^{-4}$$

where V_B = volume of ashed sample
V_F = final volume of diluted ashed sample
V_C = extrapolated concentration from standard curve
W = dry weight of sample
V_A = volume of aliqout used for reading

APPENDIX C11

$$\text{Amino acid (g } 100^{-1}\text{g) sample} = \frac{X \times 1000^{-1}}{100}$$

where X is the reading extrapolated on the standard chart recorded mg/g sample.

APPENDIX C12

$$\text{Specific gravity} = \frac{A - B}{C - B}$$

where A = weight of bottle + oil
B = weight of bottle
C = weight of bottle + water

APPENDIX C13

$$\% \text{ water and volatile matter} = \frac{(A - B)}{A} \times 100$$

where A = initial weight of oil
B = weight after heating

APPENDIX C14

$$\text{Iodine value} = \frac{(A - B) \times 12.69 \times N}{W}$$

where A = volume in ml of thiosulpahte solution used in the blank titration
B = volume in ml of thiosulpahte solution used in titration
W = weight in grams of oil taken

N = normality of the thiosulphate solution

APPENDIX C15

$$\text{Acid value} = \frac{V \times N \times 56.1}{W}$$

where V = volume in mls of KOH
N = normality of KOH
W = weight of oil

APPENDIX C16

$$\% \text{ Unsaponifiable matter} = \frac{P}{P_1} \times 100$$

where P = weight of residue
P_1 = weight of sample

APPENDIX C17

$$\text{Saponification value} = \frac{56.1N\ (X - V)}{W}$$

where N = normality of hydrochloric acid
X = volume in mls of hydrochloric acid used in blank titration
W = weight in gram of fat
V = volume in ml of hydrochloric acid used in test titration

APPENDIX C18

$$\% \text{ Extract} = \frac{X \times 0.9}{W} \times 100$$

where X = volume in mls of oil extracted
W = weight of oil in the cake

APPENDIX C19

$$\text{Total lipid} = \frac{X - Y}{W}$$

where X = weight of empty crucible + sample
Y = weight of empty crucible
W = weight of organ

APPENDIX C20

Serum lipid = $W_2 - W_1$

where W_2 = weight of bottle + sample
W_1 = weight of bottle
since 40ml of 0.1ml : 50 dilution of sample used

the aligout represents $\frac{40}{50} \times 0.1 = \frac{0.4}{5}$ ml of serum

to get per 100ml(mg/dl) multiply the mg of lipid in the aligout by 5/0.4 x 100.

APPENDIX C21

a) Calculation of SGOT and SGPT

$$\text{Pyruvate/min} = \frac{Ab_T - Ab_{Tc}}{Ab_s}$$

where Ab_T = absorbance of test
Ab_{Tc} = absorbance of test control
Ab_s = absorbance of standard

b) Chart for SGOT values

Pyruvate /min µmol/min /L	SGOT result IU / L	Pyrurate /min µmol/min /L	SGOT result IU / L
1	2.0	13	28.0
2	3.5	14	30.0
3	5.5	15	33.0
4	6.5	16	35.0
5	9.0	17	37.0
6	11.0	18	40.0
7	14.0	19	42.0
8	16.0	20	46.0
9	18.0	21	51.0
10	20.5	22	56.0
11	23.0	23	60.0
12	25.0		

c) Chart for SGPT values

Pyrurate /min µmol/min /L	SGOT result IU / L	Pyrurate /min µmol/min /L	SGOT result IU / L
1	2.5	12	24.5
2	3.5	13	26.5
3	5.0	14	30.0
4	7.0	15	33.5
5	8.0	16	36.0
6	10.0	17	38.0
7	12.5	18	41.0
8	15.0	19	46.0
9	17.0	20	51.0
10	19.5	21	56.0
11	22.0	22	61.0

APPENDIX C22

$$\text{Serum alkaline phosphatase (K-A untis/100ml)} = \frac{R_T - R_C}{R_S} \times \frac{10}{103} \times \frac{100}{0.1}$$

where R_T = reading test
R_C = reading of control
R_S = reading of standard

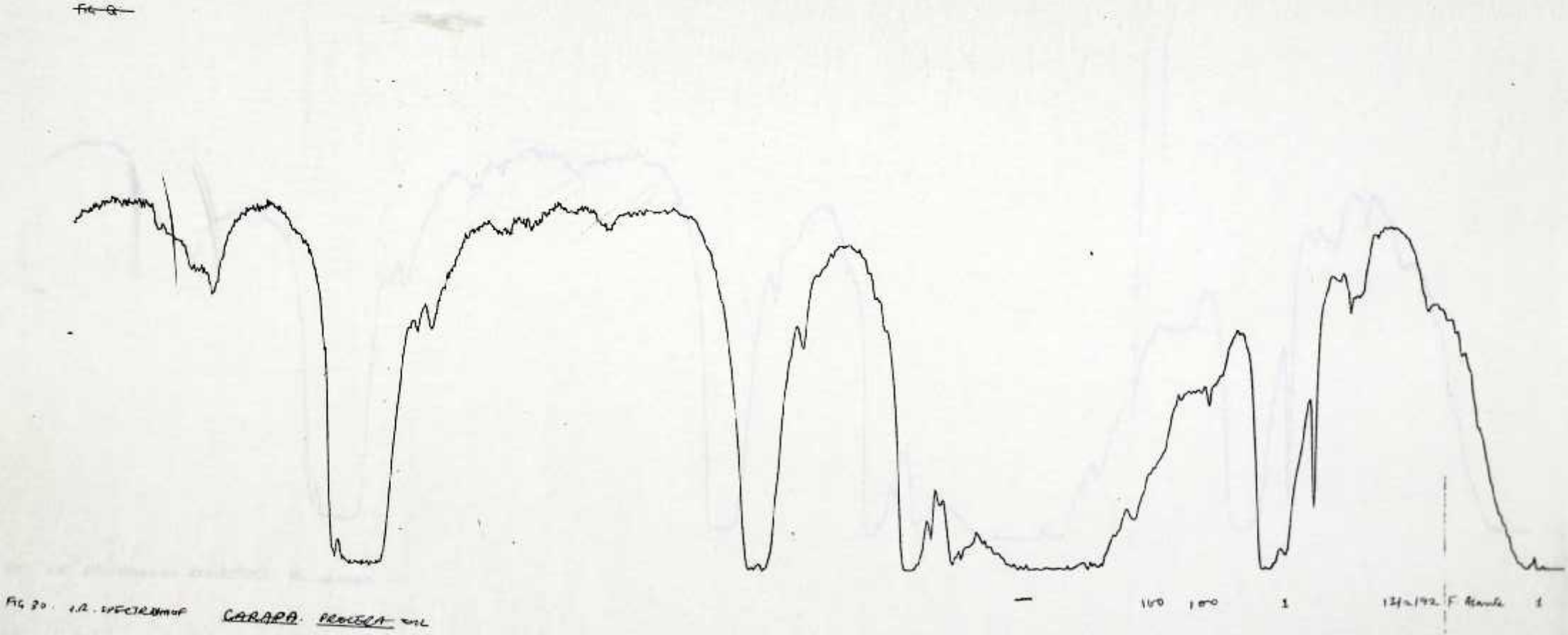

FIG. 17 i.e. SPECTRUM OF CARAPA PROCERA OIL

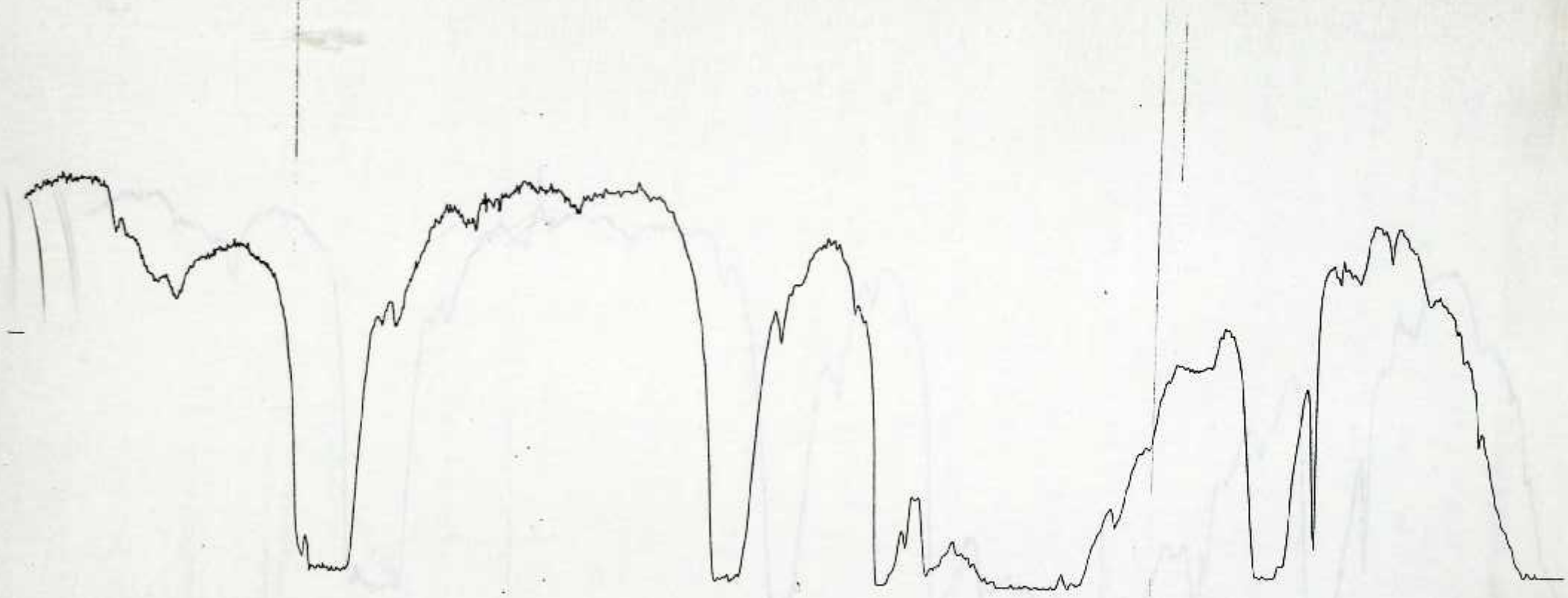

FIG 21 I.R. SPECTRUM OF PENTADESMA BUTYRACEA OIL

FIG. 18 I.R. SPECTRUM OF P. BUTYRACEA OIL

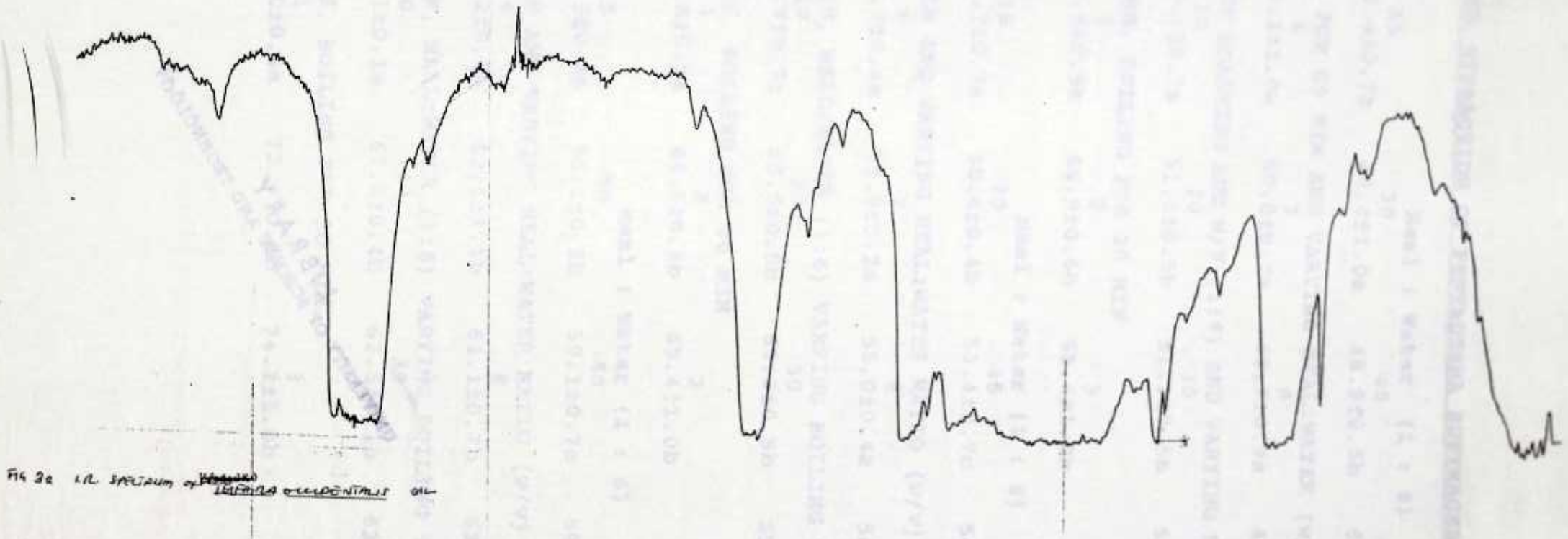

FIG. 19 I. R. SPECTRUM OF TELFAIRA OCCIDENTALIS OIL

APPENDIX D

AQUEOUS EXTRACTION OF PENTADESMA BUTYRACEA

Roasting at 80°C

Time (min)	Meal : Water (1 : 6)			
	15	30	45	60
% yield	46.4±0.7a	47.4±1.0a	48.9±0.5b	51.0±0.5c

ROASTING AT 80°C FOR 60 MIN AND VARYING MEAL:WATER (w/v)

Water (vol)	6	7	8	9
Yield (%)	51.1±1.0a	50.0±0.7a	49.7±0.9a	49.8±0.4a

AT CONSTANT TIME OF ROASTING AND W/V (1:6) AND VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	48.9±0.7a	51.6±0.5b	51.2±0.5b	51.3±0.4b

NO OF REEXTRACTIONS, BOILING FOR 20 MIN

Times	1	2	3
Yield	55.6±0.5a	64.9±0.8b	65.4±1.0b

Roasting at 100°C

Time (min)	Meal : Water (1 : 6)			
	15	30	45	60
Yield (%)	48.7±0.7a	50.4±0.4b	53.4±0.7c	54.5±1.0c

ROASTING FOR 60 MIN AND VARYING MEAL:WATER RATIO (w/v)

Water (vol)	6	7	8	9
Yield (%)	55.7±0.4a	55.9±0.2a	55.0±0.4a	56.0±0.3a

ROASTING FOR 60 MIN, MEAL:WATER (1:6) VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	50.9±0.7a	55.6±0.5b	55.2±0.5b	55.3±0.4b

NO OF REEXTRACTIONS, BOILING FOR 20 MIN

Times	1	2	3
Yield	55.6±0.5a	64.9±0.8b	65.4±1.0b

Roasting at 120°C

Time (min)	Meal : Water (1 : 6)			
	15	30	45	60
Yield (%)	54.5±0.2a	56.8±0.5b	59.1±0.7c	59.9±0.4c

ROASTING FOR 45 MIN AND VARYING MEAL:WATER RATIO (w/v)

Water (vol)	6	7	8	9
Yield (%)	60.2±0.8a	62.8±1.1b	63.1±0.7b	63.9±1.0b

ROASTING FOR 45 MIN, MEAL:WATER (1:6) VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	59.1±0.1a	62.0±0.4b	62.5±0.1b	62.4±0.1b

NO OF REEXTRACTIONS, BOILING FOR 20 MIN

Times	1	2	3
Yield	62.0±0.4a	73.[illegible]±0.2b	74.1±1.0b

AQUEOUS EXTRACTION OF CARAPA PROCERA

Roasting at 80°C

Meal : Water (1 : 6)

Time (min)	15	30	45	60
Yield (%)	48.4±0.5a	49.8±0.4b	50.0±1.0b	54.8±0.4c

ROASTING FOR 60 MIN AND VARYING MEAL:WATER (w/v)

Water (vol)	5	6	7	8
Yield (%)	50.5±0.2a	55.0±0.4a	68.5±0.2a	68.8±0.5a

ROASTING FOR 60 MIN, AT MEAL:WATER (1:6) AND VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	64.5±0.3a	68.[illegible]±0.2b	68.9±0.5b	68.1±0.5b

NO OF REEXTRACTIONS

Times	1	2	3
Yield	68.7±0.2a	72.[illegible]±0.5a	73.4±0.6b

Roasting at 100°C

Meal : Water (1 : 6)

Time (min)	15	30	45	60
Yield (%)	62.4±0.2a	64.8±0.1b	64.9±0.3b	64.5±0.7b

ROASTING FOR 30 MIN, MEAL:WATER (1:7), VARYING BOILING TIME

Water (vol)	5	6	7	8
Yield (%)	60.5±0.7a	64.7±0.7B	70.6±0.4c	69.1±0.1c

ROASTING FOR 30 MIN, MEAL:WATER (1:7), VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	65.5±0.5a	70.1±0.1b	70.6±0.3b	69.8±0.3b

NO OF REEXTRACTIONS

Times	1	2	3
Yield	70.1±0.1a	73.6 0.4b	73.4±0.9b

Roasting at 120°C

Meal : Water (1 : 6)

Time (min)	15	30	45	60
Yield (%)	63.8±0.3a	64.5±0.5b	64.3±0.4b	64.3±0.1b

ROASTING FOR 30 MIN AND VARYING MEAL:WATER RATIO (w/v)

Water (vol)	5	6	7	8
Yield (%)	64.0±0.4a	66.7±0.2a	71.6±0.8b	72.5±0.4b

ROASTING FOR 30 MIN, W/V (1:7) VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	66.5±0.7a	70.6±0.2b	71.0±0.6b	72.0±0.8b

NO OF REEXTRACTIONS

Times	1	2	3
Yield %	70.6±0.4a	72.6±0.2b	73.0±1.1b

AQUEOUS EXTRACTION OF TELFAIRA OCCIDENTALIS

Roasting at 80°C

Time (min)	15	30	45	60
Yield (%)	38.5±0.2a	43.3±0.5b	48.9±0.3b	50.0±0.1d

ROASTING FOR 60 MIN AND VARYING MEAL:WATER (w/v)

Water (vol)	5	6	7	8
Yield (%)	43.3±0.7a	50.1±0.1b	50.7±0.5b	50.5±0.9b

ROASTING FOR 60 MIN, AT MEAL:WATER (1:6) AND VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	22.7±1.0a	40.3±1.1b	50.1±0.1c	50.9±1.0c

NO OF REEXTRACTIONS, BOILING FOR 30 MIN

Times	1	2	3
Yield	50.1±0.1a	62.3±0.4b	62.7±1.0b

Roasting at 100°C

Time (min)	15	30	45	60
Yield (%)	48.1±0.7a	51.3±0.7b	51.4±0.5b	51.8±1.0b

ROASTING FOR 30 MIN AND VARYING MEAL:WATER (w/v)

Water (vol)	5	6	7	8
Yield (%)	45.26±1.2a	51.4±0.5b	51.6±0.1b	51.6±0.4b

ROASTING FOR 30 MIN, w/v AT 1:6 AND VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	27.9±0.4a	42.1±1.1b	51.4±0.5c	51.7±0.8c

NO OF REEXTRACTIONS, BOILING FOR 30 MIN

Times	1	2	3
Yield	51.4±0.5a	64.1±0.7b	64.3±1.0b

Roasting at 120°C

Time (min)	15	30	45	60
Yield (%)	49.0±0.1a	51.4±0.9b	51.5±0.7b	51.5±1.0b

ROASTING FOR 30 MIN AND VARYING MEAL:WATER RATIO (w/v)

Water (vol)	5	6	7	8
Yield (%)	45.3±0.8a	51.4±0.9b	51.0±1.0b	51.7±0.7b

ROASTING FOR 30 MIN AT W/V (1:6) AND VARYING BOILING TIME

Time (min)	10	20	30	40
Yield (%)	29.1±0.7a	43.1±0.8b	51.4±0.9c	51.7±0.7c

NO OF REEXTRACTIONS

Times	1	2	3
Yield %	51.4±0.9a	64.9±1.0b	64.3±1.1b

COMPARING AQUEOUS EXTRACTION RESULTS

SAMPLE	C. PROCERA	P. BUTYRACEA	F. PUMPKIN
Weight of sample (g)	50	50	50
Temp of roasting (°C)	100	120	100
Roasting time (min)	30	45	30
Meal:Water (w/v)	1:7	1:7	1:6
Boiling time (min)	20	20	30
Number of reextractions		2	22
Oil Yield (%)	45.6±0.4	33.5±1.0	32.6±0.7
Efficiency	73.6±0.4	76.1±1.0	64.1±0.7
% Extract of unroasted seeds	47.3±0.2	29.3±0.7	30.7±1.0

All figures are percentage extraction relative to solvent extraction

R E S U L T S

HYDRAULIC PRESSING OF *P. BUTYRACEA*

PRESS CYCLE

Charging (Filling after steaming) min	1
Time for attaining maximum pressure min	2
Draining time min	15
Total time (1 cycle) min	18
Weight of sample 150g	
Time for steaming min	15

TIME OF ROASTING (min)

	15	30	45	60
80°	44.3±1.1a	48.6±0.7b	51.4±1.0c	55.7±0.9d
100°	57.1±1.2a	58.6±1.0a	62.9±0.8b	63.0±1.5b
120°	62.5±1.0a	42.4±0.7a	62.0±0.9a	62.1±0.9a

VARYING THICKNESS (Roasting at 100°C for 45 min)

Weight of sample (g)	100	150	200
% Extract	47.9±1.1a	62.9±0.5b	88.8±1.2a

* All extracts are relative to the total extract by organic solvent

* Identical subscripts are not significantly different at ($P<0.05$)

HYDRAULIC PRESSING OF CARAPA PROCERA

PRESS CYCLE
Charging -

Time for attaining maximum pressure
min 2
Draining time
min 15

Total time (1 cycle)
min 17

Weight of sample
100g
Time for steaming -

TIME OF ROASTING (min)

	15	30	45	60
80°	45.0±0.7a	47.9±0.8a	46.3±1.0b	44.1±0.9ab
100°	42.5±0.8a	41.9±1.0a	42.9±1.2a	42.1±0.9a
120°	42.9±1.1a	42.0±1.1a	42.7±1.0a	41.5±1.1a

VARYING THICKNESS (Roasting at 80°C for 30 min)

Weight of sample (g)	100	150	200
% Extract	47.9±0.8a	31.5±0.9b	34.1±1.2b

* All extracts are relative to the total extract by organic solvent
* Identical subscripts are not significantly different at (P<0.05)

HYDRAULIC PRESSING OF TELFAIRA OCCIDENTALIS

PRESS CYCLE
Charging -

Time for attaining maximum pressure
min 2

Draining time
min 25

Total time (1 cycle)
min 27

Weight of sample
100g

Time for steaming -

TIME OF ROASTING (min)

	15	30	45	60
80°	51.7±0.7a	52.3±1.2a	55.7±1.0b	56.3±0.7b
100°	63.0±0.8a	68.7±0.8b	70.0±0.7bc	71.4±1.0c
120°	71.9±0.5a	72.3±1.1a	72.3±1.2a	72.2±1.0a

VARYING THICKNESS (Roasting at 100°C for 60 min)

Weight of sample (g)	100	150	200
% Extract	70.4±1.0a	54.7±1.2b	50.9±1.0c

COMPARISON OF RESULTS

SAMPLE	C. PROCERA	P. BUTYCACEA	F. PUMPKIN
Charging	-	1	-
Attainment of max press (min)	2	2	2
Draining time (min)	15	15	25
Time for steaming	-	15	-
Weight of sample	100	200	100
Temp of roasting (°C)	80	100	100
Time of roasting (min)	30	45	60
% Extract from whole seed	29.7±0.8	39.1±1.2	36.4±1.0
Extraction efficiency	47.9±0.8	88.8±1.2	71.4±1.0
% extract unroasted seeds	43.0±0.1	42.6±0.9	48.1±0.4

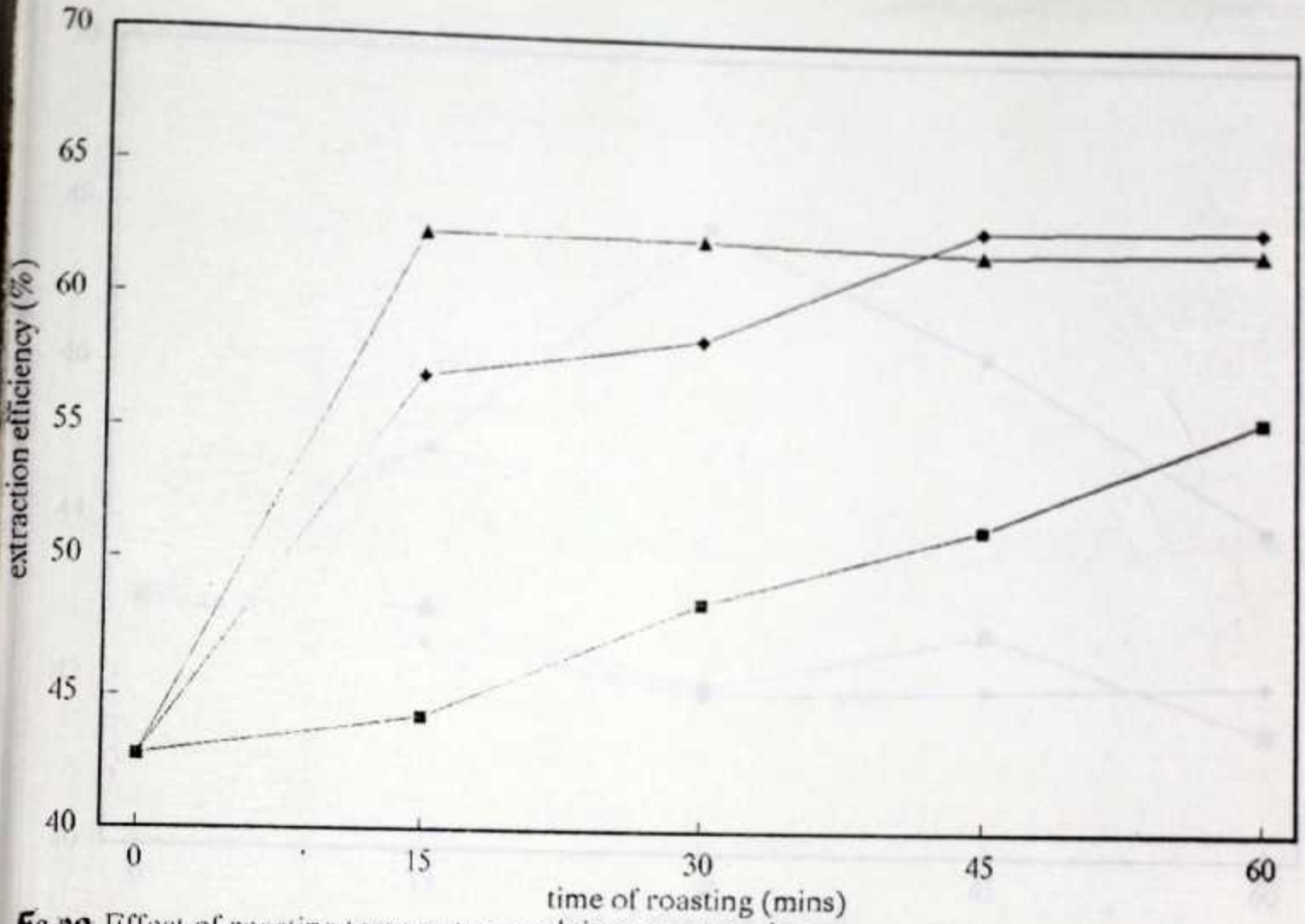

Fig. 20. Effect of roasting temperature and time on extraction efficiency of P. butyracea seed oil

seeds roasted 80 degrees
seeds roasted at 100 degrees
seeds roasted at 120 degrees

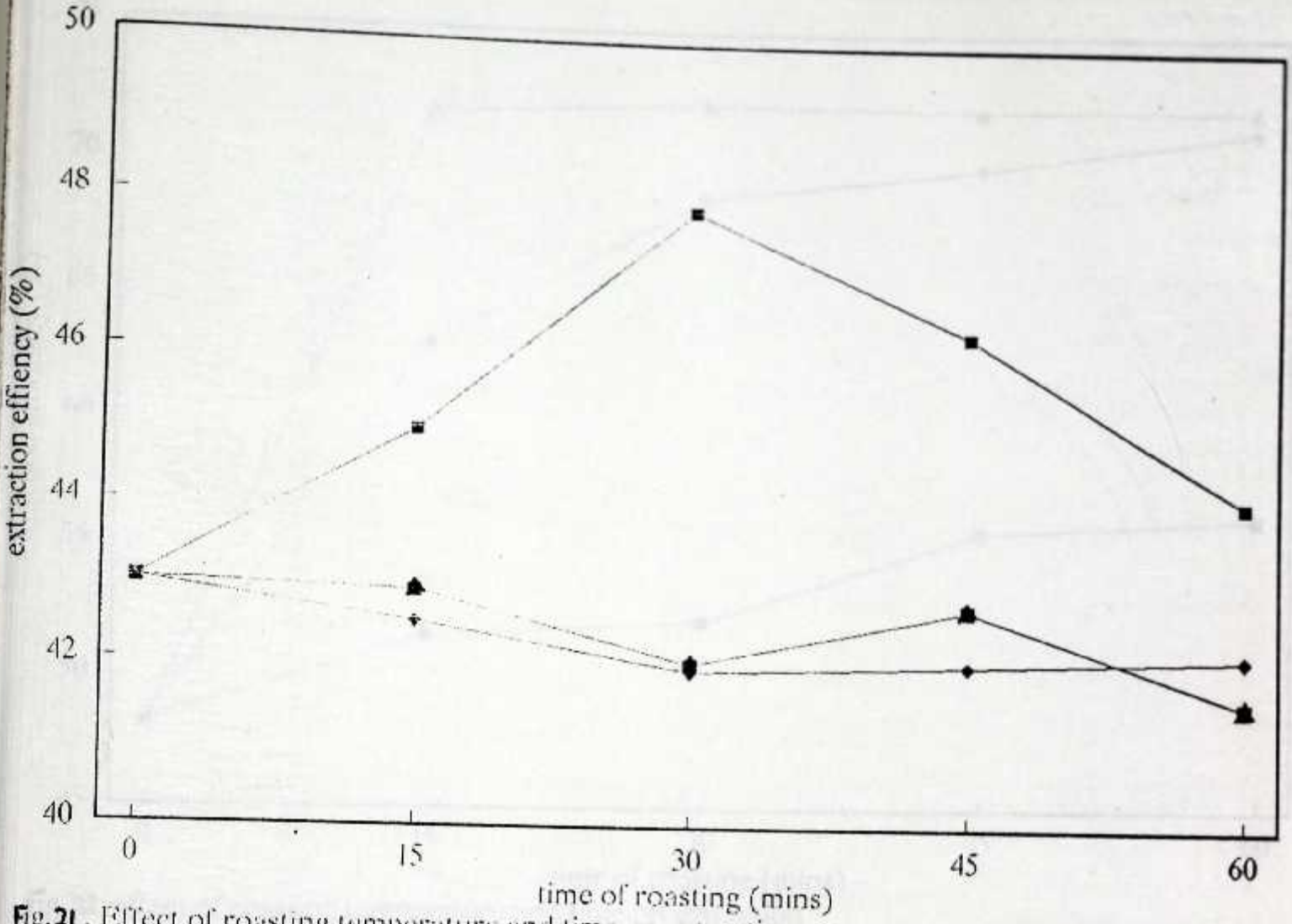

Fig.21. Effect of roasting temperature and time on extraction efficiency of C.procera seed oil

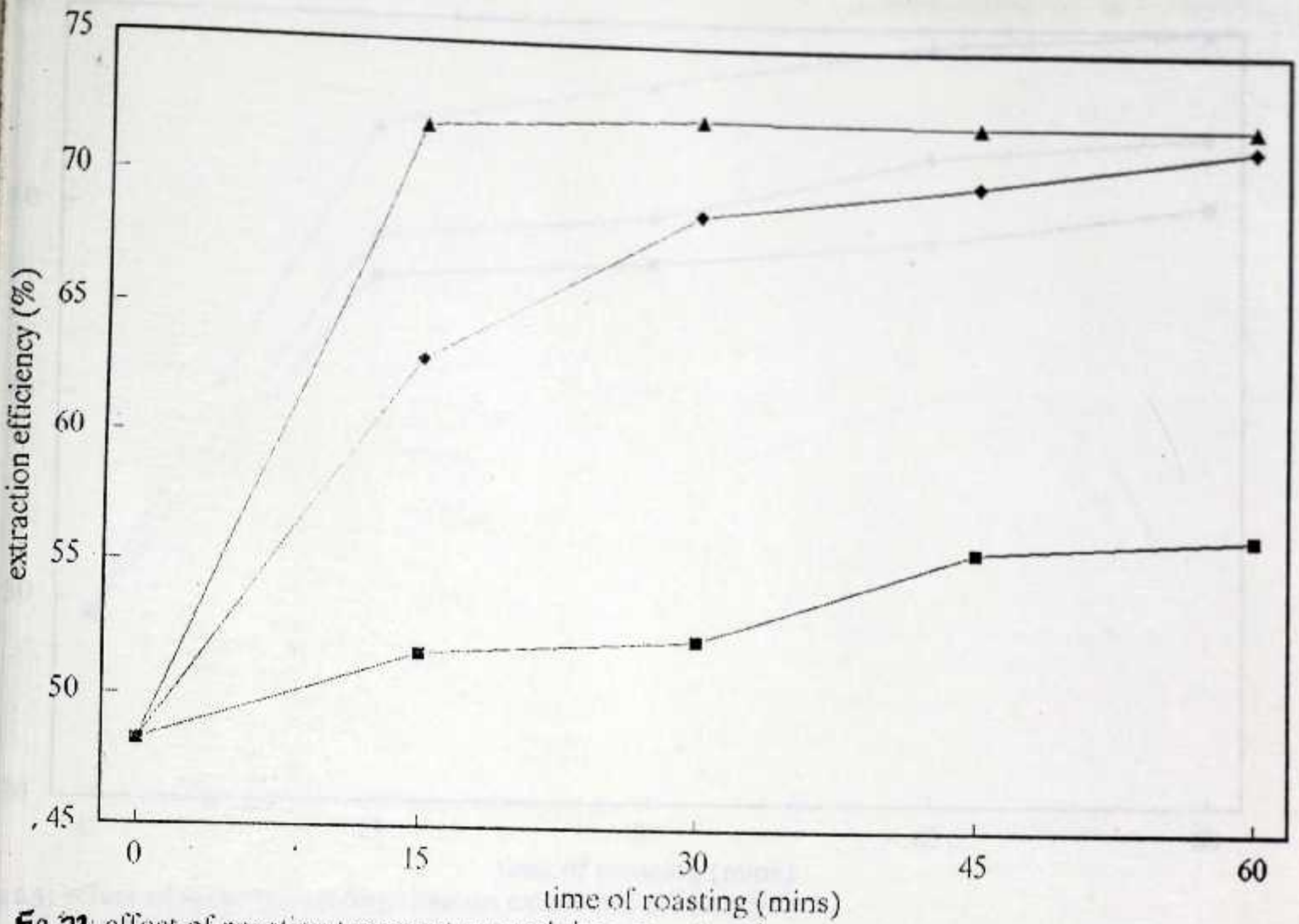

Fig. 22: effect of roasting temperature and time on extraction efficiency of T.occidentalis seed oil

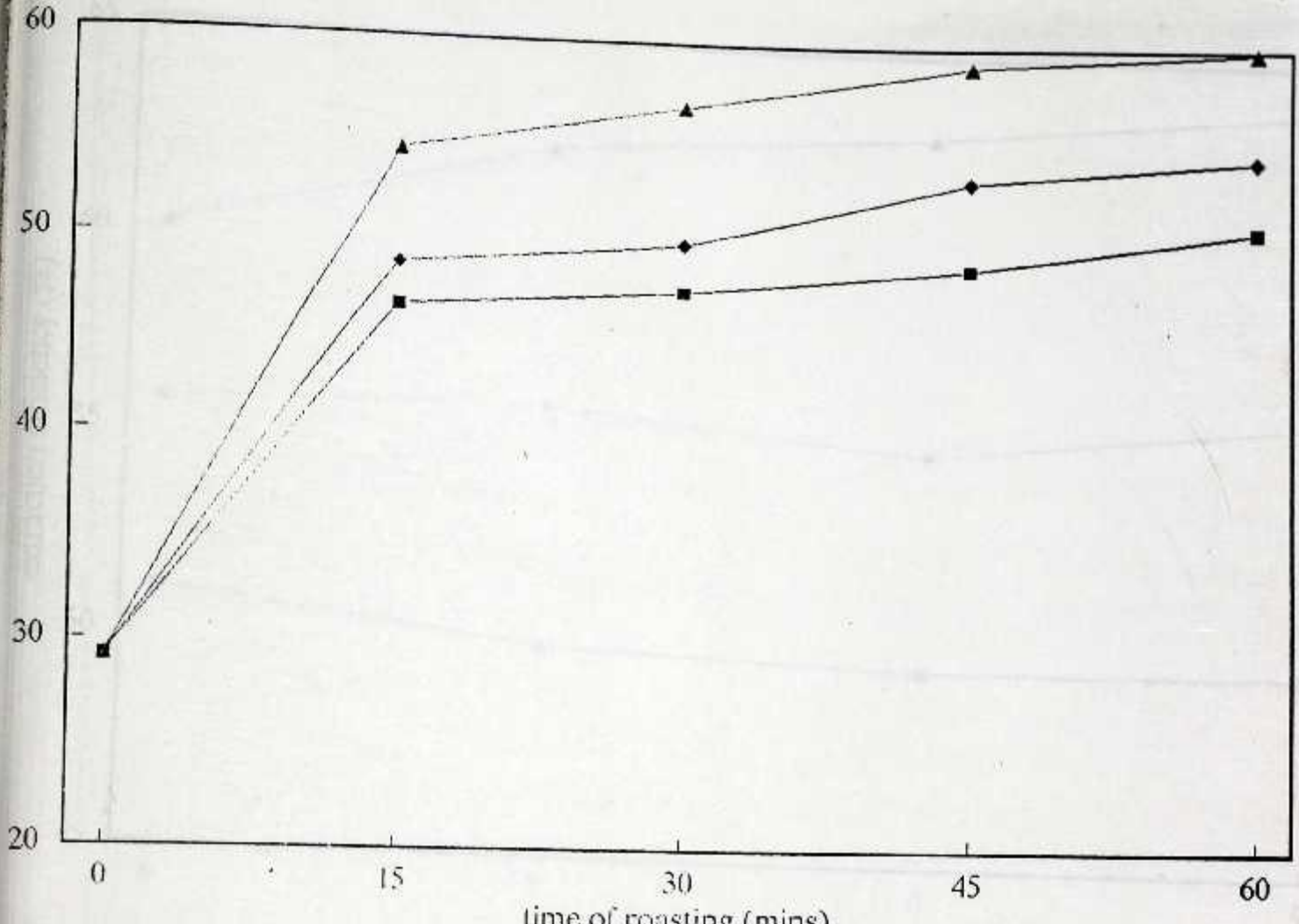

Fig. 23: effect of varying roasting time on extraction efficiency of P.butyracea oil (meal dilution ratio was 1:6)

seeds roasted at 80 degrees
seeds roasted at 100 degrees
seeds roasted at 120 degrees

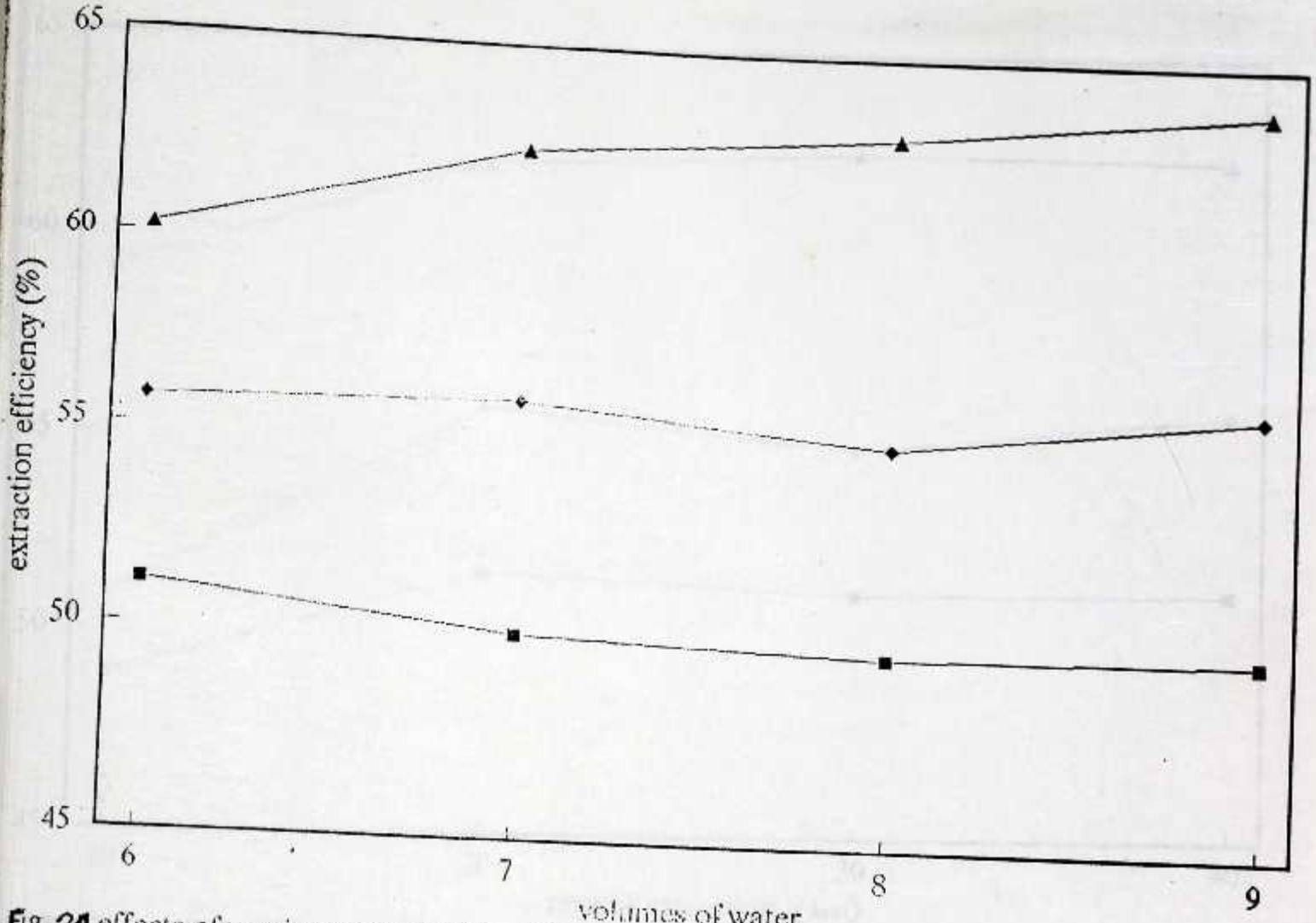

Fig. 24 effects of varying meal dilution ratio on extraction efficiency of P.butyracea oil

seeds roasted at 80 degrees, 45mins

seeds roasted at 100 degrees, 45 mins.

seeds roasted at 120 degrees, 45mins

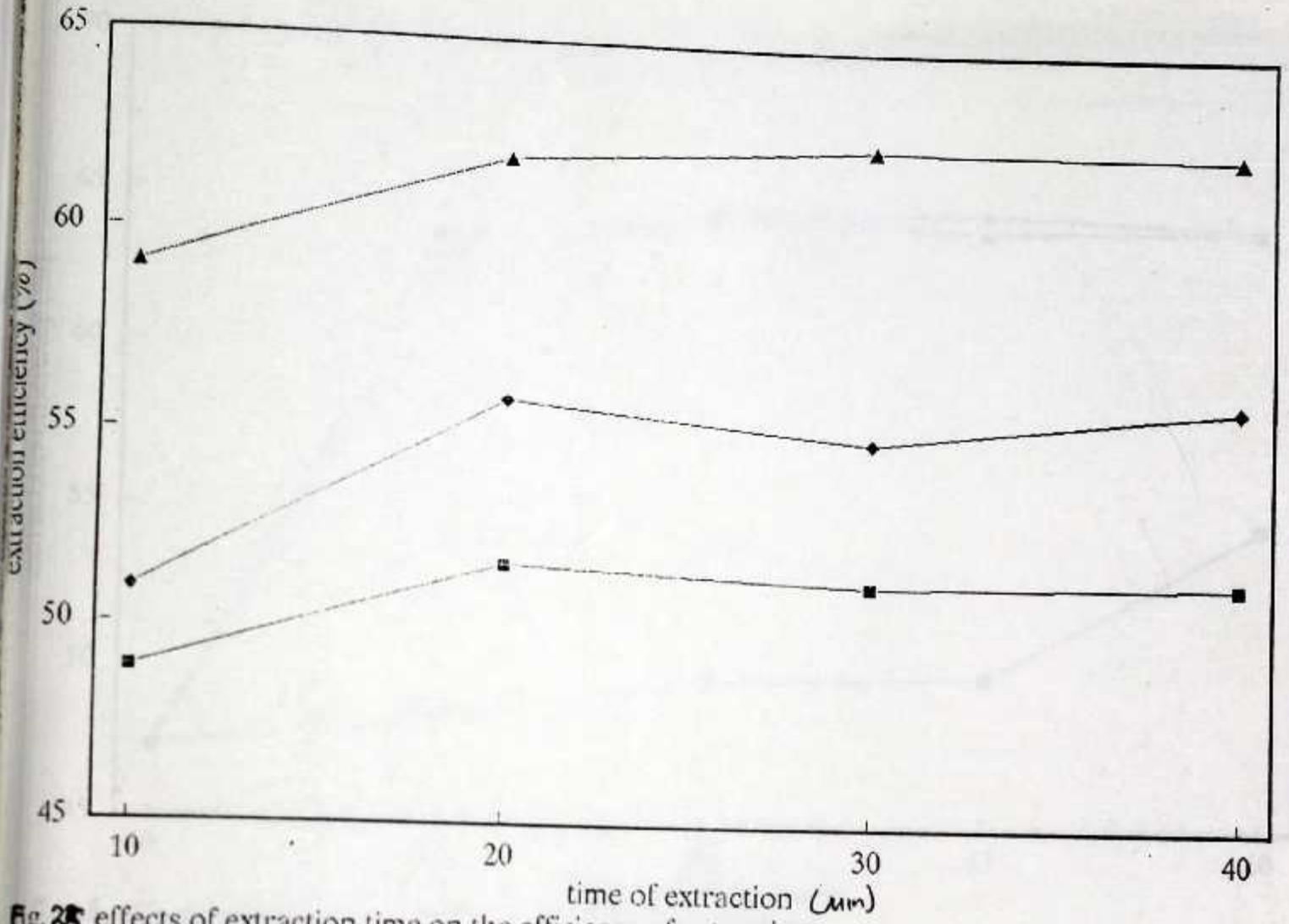

Fig. 25 effects of extraction time on the efficiency of extracting of P.butyracea oil

- seeds roasted at 80°c for 45 min and meal dilution ratio of 1:6
- seeds roasted at 100°c for 45 min and meal dilution ratio of 1:6
- seeds roasted at 120°c for 45 min and meal dilution ratio of 1:7

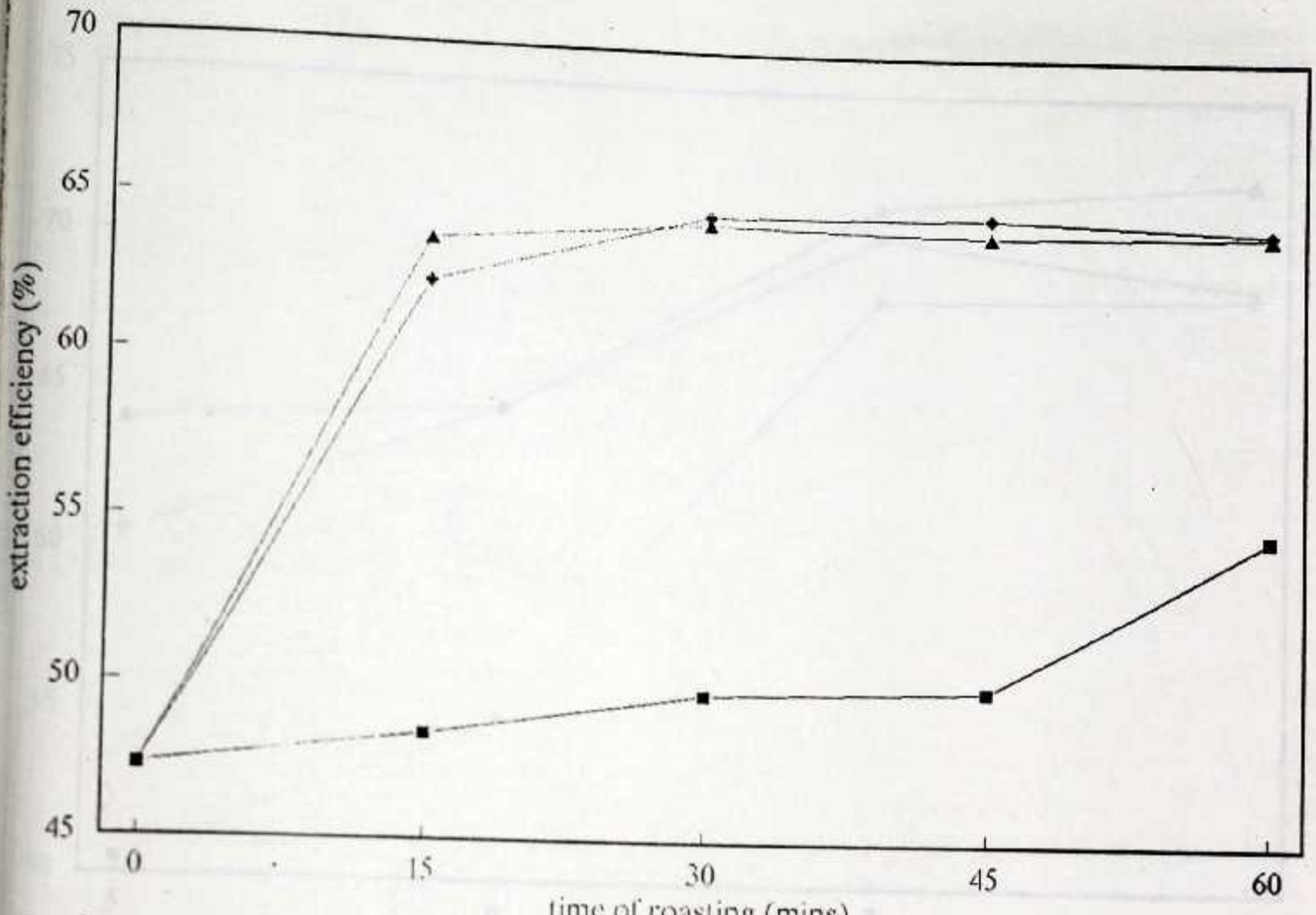

Fig.26 effects of varying roasting time on extraction efficiency of Carapa procera oil. meal dilution ratio was 1:6

seeds roasted at 80 degrees
seeds roasted at 100 degrees
seeds roasted at 120 degrees

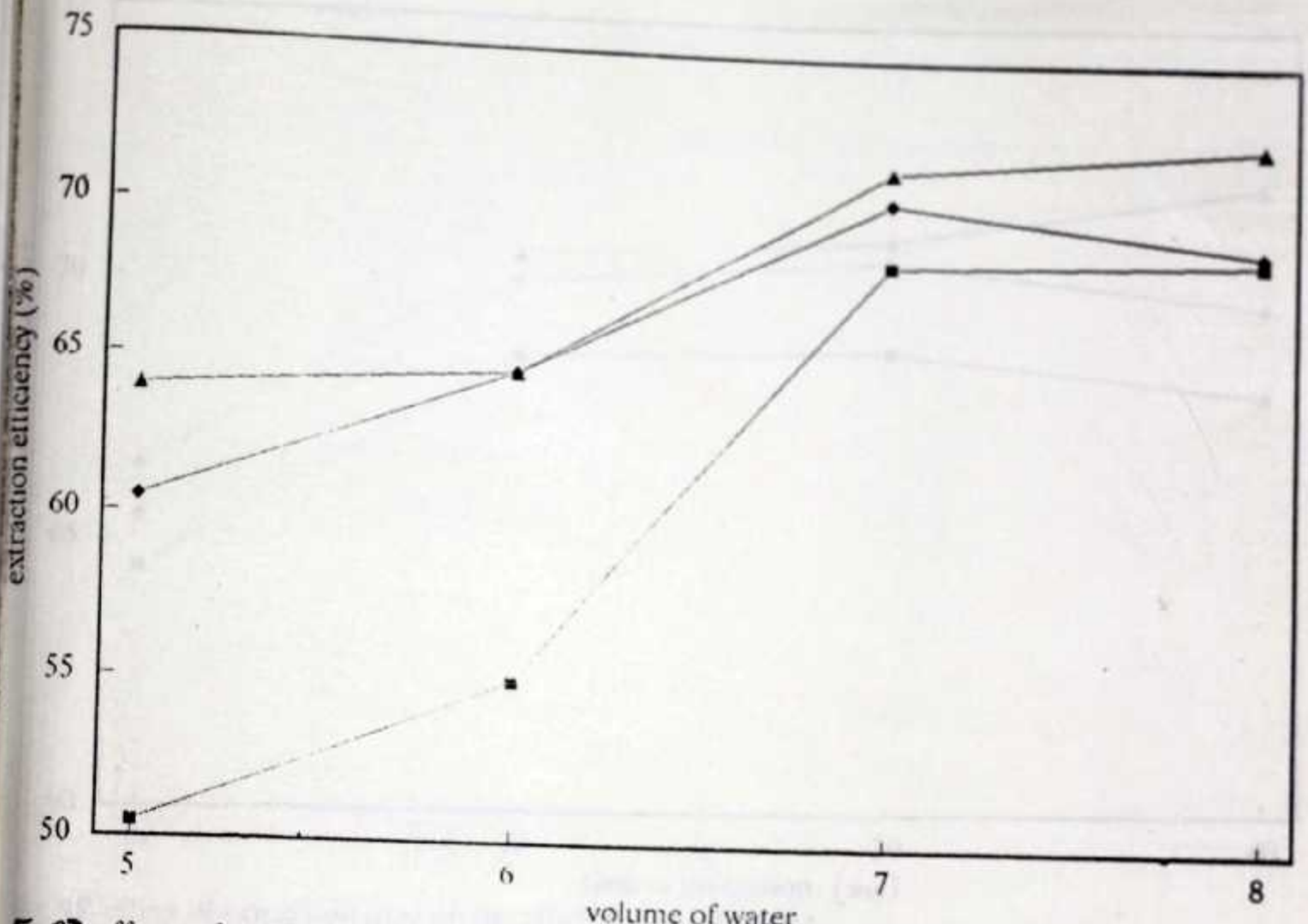

Fig. 27: effects of varying meal dilution ratio on extraction efficiency of Carapa procera oil.

seeds roasted at 80 degrees for 60 min

seeds roasted at 100 degrees for 30 min.

seeds roasted at 120 degrees for 30 min