

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND  
TECHNOLOGY



COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

**PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF  
DIFFERENT ACKEE (*BLIGHIA SAPIDA*) ARIL FLOURS**

**A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND  
TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE  
AWARD OF MSc. FOOD SCIENCE AND TECHNOLOGY DEGREE**

BY

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## DECLARATION

I hereby declare that this submission is my own work towards the M.Sc. Food Science degree and that to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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## ABSTRACT

The ripe ackee (*Blighia sapida*) fruit arils are poorly exploited as food in Ghana and parts of West Africa. The goal of this research, therefore, was to investigate the potential of ackee aril flour for inclusion in the food basket by investigating the physicochemical and functional properties of different ackee aril flours and the effect the drying method or defatting had on these properties. Moisture, crude fat, crude protein, crude fibre, ash and carbohydrate content of the flours were in the range of 4.83 - 7.30 %, 21.42 - 59.54 %, 11.54 - 23.00 %, 3.83 - 4.08 %, 8.45 - 9.05 % and 15.13 - 42.48 % db, respectively. The flours also contained appreciable minerals (Ca, P, Mg, Na, K and Zn), with K (462.21 - 968.29 mg/100g) being the most abundant. The flour samples had functional properties ranging from 24.09 to 39.45 % solubility, 11.03 to 23.02 % swelling power, 111.75 to 139.57 % oil absorption capacity, 4.33 to 5.67 % foaming capacity, 76.34 to 84.35 % foam stability, 61.67 to 69.17 % emulsion capacity and 5.83 to 46.67 % emulsion stability. In general, defatted flours had higher proximate composition and functional properties than the full fat flours. Freeze-dried defatted flour (FDDF) had the highest proximate and mineral content whereas oven-dried defatted flour (ODDF) had highest functional properties investigated except for foam stability and swelling power. The results indicated that the drying method had significant effect ( $p < 0.05$ ) on mineral composition as well as fat and carbohydrate content but not on the ash, protein, fibre contents or oil absorption capacity and foam stability of the flour samples. Also, with the exception of ash and fibre, the defatting was significant for all physicochemical properties investigated but not for functional properties such as foam capacity, foam stability or emulsion capacity of the ackee aril flours. The present findings suggest oven dried ackee aril flours as potential ingredients for food systems such as emulsions, processed meats, bakery or fried products.



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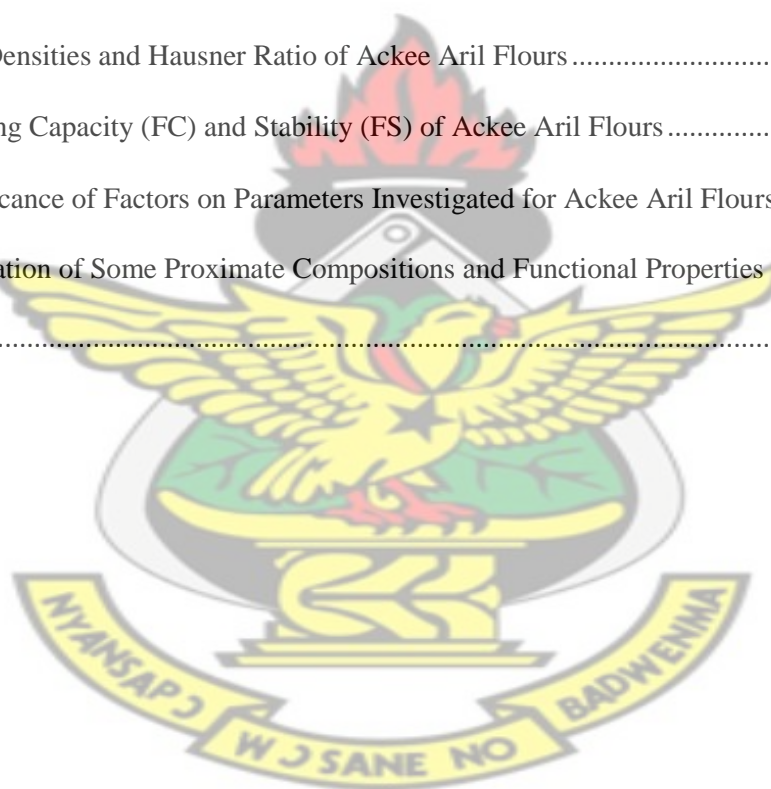
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## CHAPTER ONE

### 1.0 INTRODUCTION

### 1.1 BACKGROUND

The world has rich diversity of plant food sources but domestication and over reliance on only few plant species for food has made food security a challenge in Africa and a major concern in the world (Prescott-Allen and Prescott-Allen, 1990). Fruits and vegetables significantly contribute to improving world food security and nutrition. Being rich in vitamins, minerals and phyto-chemicals, they help to maintain good health and prevent diseases (Pamplona, 2008). Conservation, domestication and utilization of many indigenous wild fruit and vegetable species can contribute to hunger reduction and improved nutrition in Africa and the world (Prescott-Allen and Prescott-Allen, 1990; Ekué *et al.*, 2011).

Ackee (*Blighia sapida*) is a fruit tree which originates from the Guinean forests of West Africa. It is widely distributed across countries like Cote D'Ivoire, Ghana, Guinea, Liberia, Senegal, Togo, Benin, Cameroon and Nigeria (Ekué *et al.*, 2010, Agunbiade *et al.*, 2012) where it is noted particularly for its medicinal and aesthetic values. The tree is mostly admired as an ornamental and shade tree in Ghana where it is mainly grown along streets. The bark, roots and leaves of the tree are applied in traditional medicine to treat various ailments. Although the ripe fruit pulp (aril) is edible and nutritious, its utilization as food is limited in the West African sub-region. The ripe fruit arils can be eaten fresh, dried, fried, roasted or made into sauce or soup. In Benin, the dried ackee arils and ackee soap, made from the ash of fruit pods and seeds, are traded in local markets providing substantial revenue to local farmers and traders who are mostly women (Atolani *et al.*, 2009; Ekué *et al.*, 2010).



Introduced in Jamaica in the 16th century, mainly as a food for its residents, the ackee fruit has gained recognition as the 'National Fruit of Jamaica' with the fruit arils being the primary component of the national dish (ackee and salted codfish), where it is boiled in brine and prepared into a dish with onions, tomatoes and codfish (Moya, 2001; Rashford, 2001). Jamaica produces several tonnes of canned 'ackee in brine' for export to the United Kingdom and Canada generating more than 13 million U. S dollars per year (Blake *et al.*, 2006).

Drying is a common practice for preserving perishable food crops. It reduces the moisture content of the food and consequently the water activity, inhibiting enzymatic degradation of the food and limiting microbial growth (Ratti, 2001). Most local farmers in the tropical regions employ the sun or open-air and solar drying methods because of the low cost of operation and the availability of the sun for drying. However, the increased risk of contamination and several uncontrollable factors associated with open-air and solar drying methods calls for safer, more controllable methods for drying agricultural produce (Ratti, 2001).

Oven and freeze drying are employed in industrial food processes due to the fact that aside the high initial cost of ovens and freeze dryers, operation costs are relatively cheap and the quality and market value of the dehydrated product, which is not altered with changing weather conditions, is higher than in sun or solar drying. Oven or freeze drying conditions can be controlled to give the dehydrated product with most desired quality characteristics.

## **1.2 PROBLEM STATEMENT**

Ackee trees are widely distributed in the West African sub-region and mature ackee arils are edible and highly nutritious but the arils are poorly utilized as food and the commercial potential of ackee arils are also poorly exploited by the people of the sub-region.



Poor storage stability of dried ackee arils further limits its food use and commercialization. Inefficient drying and storage techniques as well as the high fat content of the arils affect the shelf stability of the dried arils. Dried ackee arils last up to two weeks on the shelf after which textural, colour and flavour changes make them unappealing to consumers. This problem was a major drawback for ackee traders in Benin because it limits the extended mass storage of dried arils for marketing (Ekué *et al.*, 2010).

Further processing of the ackee arils into secondary or tertiary products to increase its market value and food application prospects is limited in the West-African sub-region. Save for dried ackee arils and ackee soap, no other commercial ackee products have been reported in the West African sub region.

### 1.3 JUSTIFICATION

Food flours are one of the major ingredients in industrial food processes and provide increased food application prospects for many agricultural produce. They offer more convenience in use and storage than the raw products. For high oil seeds and fruits flours, defatting can contribute to improved flow properties and shelf stability as well as help rid the flours of certain undesirable flavours (Mandal *et al.*, 2013). A defatted ackee aril flour product could help increase the market value and the food application prospects of ackee arils.

Drying helps in food preservation by reducing the moisture available in foods for microbial growth. However, the drying method employed produces a unique effect on the quality properties of the dehydrated product which may or may not be desirable. It is therefore necessary to study the effect of different drying methods on dehydrated products to determine



which drying method will yield a dehydrated product with the most desired quality characteristics.

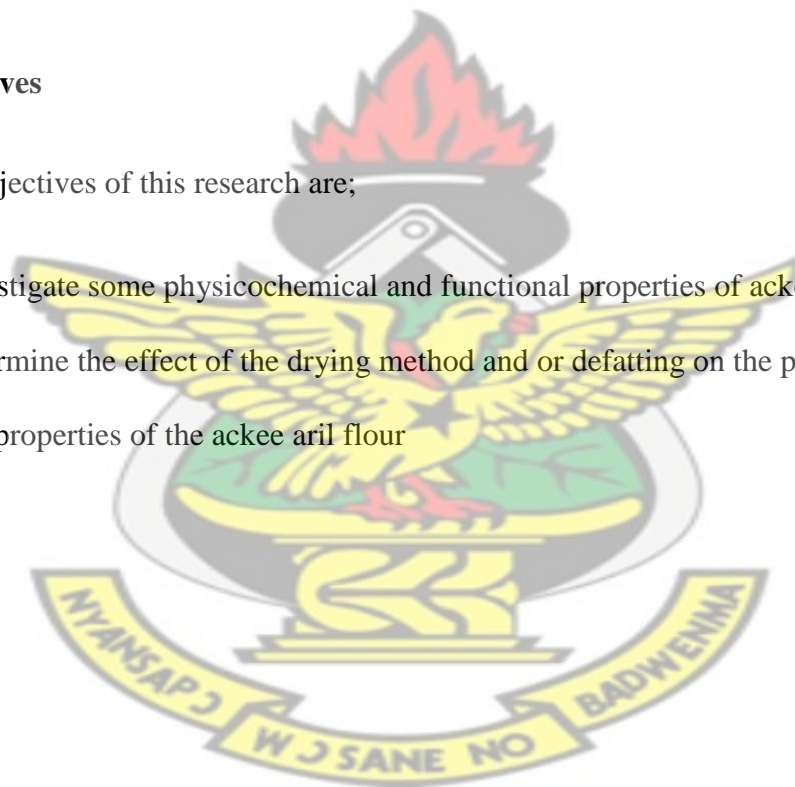
## 1.4 GOAL

In line with the Food and Agriculture Organization's goal to investigate alternative food sources to improve food security and widen the world food basket, the goal of this research is to investigate the potential of ackee arils as flour for inclusion into the food basket.

### 1.4.1 Objectives

The specific objectives of this research are;

- To investigate some physicochemical and functional properties of ackee aril flour
- To determine the effect of the drying method and or defatting on the physicochemical and functional properties of the ackee aril flour





## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 HISTORY AND DISTRIBUTION OF ACKEE

The ackee (*Blighia sapida*) tree, native to West Africa, is widely distributed across Cote D'Ivoire, Ghana, Guinea, Liberia, Senegal, Togo, Benin, Cameroon and Nigeria (see Figure 2. 1), where it has very little historical or cultural significance (Morton, 1987; Ekué, 2011). The Caribbean Islands especially Jamaica has become home to the ackee tree. It was initially introduced into Jamaica in the 18<sup>th</sup> century by slave ships from West Africa for propagation as food for the slaves on the Island.



**Figure 2. 1:** Geographical Distribution of Ackee (shaded) in Africa (Ekué, 2011)



Captain William Blighia drew the attention of the world to ackee when in 1793 he transported ackee from Jamaica to the Kew Gardens in England. To his credit, it was given the generic name *Blighia sapida* (Rashford, 2001). It is believed that the ackee was subsequently introduced to other islands by slaves from Jamaica from where it spread to different parts of the world (Morton, 1987; Kean and Hare, 1980, Rashford, 2001). The cultural and industrial significance of the ackee to Jamaica eventually earned it the name; *The Big Ackee* (Rashford, 2001).

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## 2.2 ACKEE BOTANY AND AGRICULTURE

The ackee tree is an evergreen tree with a smooth grey bark and densely branched symmetrical canopy, growing as tall as 35 m (Figure 2. 2). The ackee trees are propagated from seeds directly on the field or in a nursery for later transplanting. When vegetative propagation by stem cuttings or air-layering is employed, viability is low and the trees formed lack the tap root system of the seed-propagated trees (Stair and Sidrak, 1997; Mitchell *et al.*, 2008).

In Benin, sowing of seeds, transplanting of wild seedlings and saplings and assisted regeneration (staking and protection of wild seedlings from damage) are the main forms of propagating the ackee (Ekué *et al.*, 2010). The tree starts bearing fruit after 3 to 6 years when cultivated from the seed but when properly cared for, trees can bear fruit in 2 to 3 years. Trees from stem cuttings can flower and bear fruits in 18 to 24 months (Mitchell *et al.*, 2008).





**Figure 2. 2: Ackee tree bearing fruit**

The leaves are alternate compound leaves with 3 to 5 pairs of glossy leaflets. Ackee inflorescence is about 3 to 7 inches long with clusters of greenish-white fragrant flowers, each having 5 petals, in axillary racemes. The flowers are either male or hermaphroditic and are pollinated by insects. From the flowers arise green fruit capsules which turn bright yellow to red when matured (Figure 2. 3)



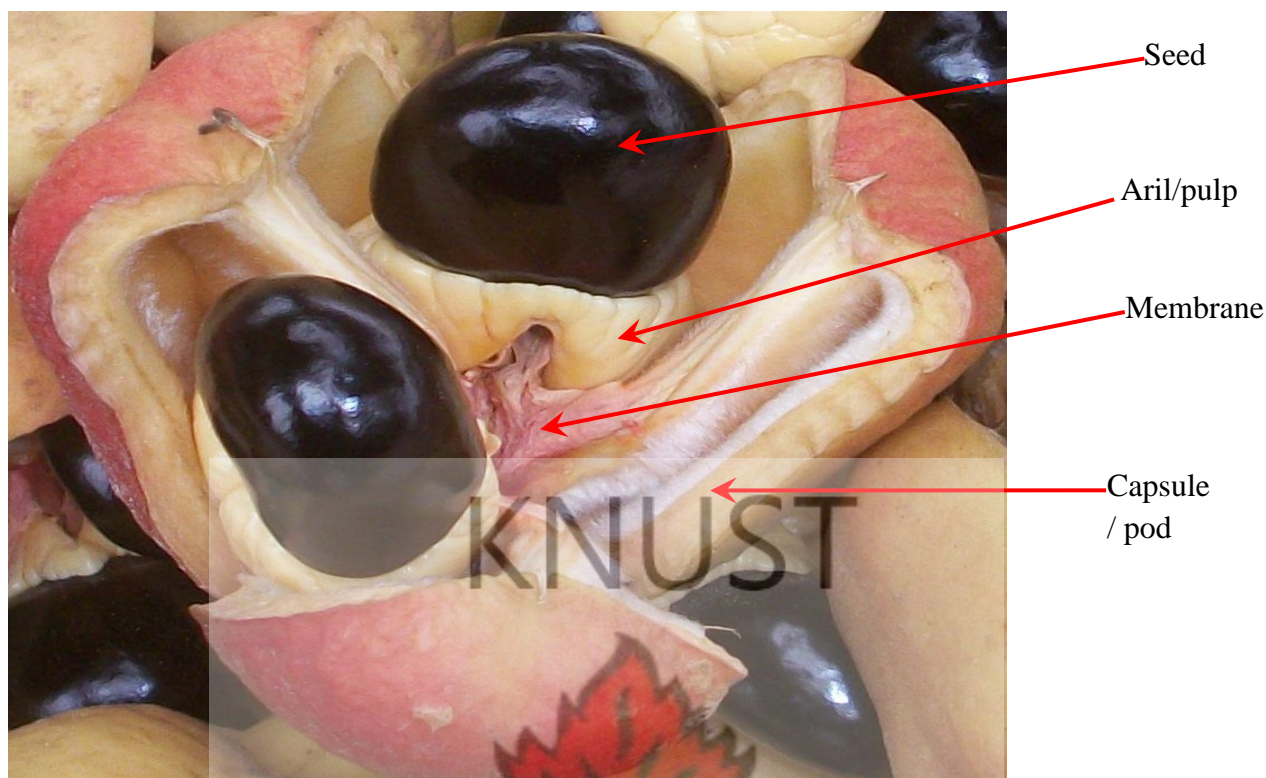


**Figure 2. 3:** Ackee leaves and Inflorescence (left), unripe ackee fruits (middle and right) (Mitchell *et al.*, 2008)

When ripe, the mature fruit capsules split open to reveal cream coloured arils attached to smooth, shiny black oblong seeds. The arils are attached to the capsule by pink to red fibrous membranes (Figure 2. 4) (Morton, 1987).

The ackee tree can grow on lands with elevations at sea level up to 1000 m above sea level. At higher altitudes, the wind prevents growth. Favourable temperatures for the cultivation of ackee range from 21 °C to 27 °C. The tree grows well in fertile soils that have good drainage with optimum pH between 5.5 and 7.5 although it can tolerate slightly alkaline soils. Black ants and scale aphids are the common pests of the ackee tree and the most common disease is fruit rot (Mitchell *et al.*, 2008). Ackee trees bear fruits year round but usually have two major fruiting seasons; from January to March (spring) and June to August (summer). In the Bahamas, harvesting of fruits from July through to October has been reported (Moya, 2001).





**Figure 2. 4:** Ripe Ackee fruit with the different parts labeled

Two main varieties of ackee are recognized in Jamaica from the colour and texture of the fruit arils; the cheese and butter varieties. The cheese ackee variety has cream coloured arils with firm texture while the butter ackee variety has yellow arils with soft texture. Ackee processors prefer the cheese variety for export because it retains its shape during cooking (Mitchell *et al.*, 2008). In West Africa, there are no documented varieties of the ackee but farmers in Benin identified the varieties mainly based on fruit size, seed size and aril taste (Ekué *et al.*, 2010).

*Blighia unijugata* and *Blighia welwitschii* are two species related to the ackee. The leaves of *Blighia unijugata* are edible but *Blighia welwitschii* is utilized mainly for medicinal purposes. These species are distributed in forest and savanna regions from Sierra-Leone to Cameroun (Kean and Hare, 1980; Ekué *et al.*, 2010).



## 2.3 USES OF ACKEE

Several uses of the different parts of the ackee tree have been reported in literature; food, medicine, fodder, furniture, charcoal, construction, cosmetics (Ekué *et al.*, 2010). Thus the ackee tree can serve various commercial purposes for farmers and industries alike.

### 2.3.1 Medicinal Uses

The medicinal use of the ackee tree is popular in West Africa. Ekué *et al.* (2010) identified 22 diseases which are treated with the use of different parts of the ackee tree in Benin; the roots, bark, leaves, capsules and seeds. Among the common diseases treated were fever, malaria, internal hemorrhage, dysentery, yellow fever and constipation. The bark and leaves of the ackee tree were most commonly used, being involved in the composition of drugs for the treatment of 13 and 8 different diseases respectively. However, only old people and traditional healers had such knowledge of the traditional medicinal use of the ackee tree parts and the knowledge varied among different ethnic groups in Benin.

The use of ackee for treating dysentery, yellow fever as well as epilepsy in parts of Africa was also reported by Kean and Hare in 1980. In Nigerian traditional medicine, ackee leaves and pulp are used to treat eye conjunctivitis and the roots are used to manage diabetes (Atolani *et al.*, 2009; Gbolade, 2009). Saidu *et al.* (2012) investigated the hypoglycemic effect of aqueous *ackee* root bark extract on normoglycemic albino rats. They observed that the consumption of ackee roots bark extract exerted significant hypoglycemic effect on the normoglycemic albino rats. This finding supported the traditional use of ackee roots in the treatment of diabetes in Nigeria. Although recorded among 160 medicinal plants in Jamaica, the ackee tree is not a popular medicinal plant in Jamaica (Rashford, 2001).



### 2.3.2 Food and Feed Uses

The aril or pulp is the edible portion of the ackee tree although young leaves are used as vegetables in some parts of West Africa. The ripe fruit arils are eaten fresh, dried, fried, roasted or made into sauce or soup in parts of West Africa (Ekué *et al.*, 2010). Ghanaians in the Upper-West region and parts of the Volta, Northern and Upper-East Regions exploit ackee arils as food; eaten fresh, dried or roasted and milled for use in soup (Personal communication).

In Jamaica, parboiled arils are made into various dishes. ‘Ackee and saltfish’ is the main ackee dish in Jamaica: The ackee arils are parboiled and prepared into a dish with onions, tomatoes and saltfish and eaten with bread or rice. It is a dish which holds cultural significance for all Jamaicans; found on the menu of most restaurants, hotels and cafeterias in Jamaica with chefs developing various alternatives like *ackee loaf* (ackee and saltfish filled bread-like pastry) and *ackee quiche* (Rashford, 2001). Fresh ackee arils have a nutty cheese-flavour and could be exploited in industrial food applications as a vegetable substitute for cheese. Aderinola *et al.* (2007) reported that ackee leaves are good fodder for West African dwarf goats especially during the harmattan season.

### 2.3.3 Other Uses

Immature ackee fruits are crushed and used directly as soap because they are rich in saponins and lather in water. This application is well known by people in parts of Kumasi in the Ashanti region of Ghana (Personal communication). Mature pods and seeds are burnt and the ash used in soap making. Ackee soap is common in Benin, traded in local markets and providing substantial revenue to local farmers and traders who are mostly women (Ekué *et al.*, 2010).



Ash of burnt capsules is applied to cowpea, corn and other food grains to repel insect pests during storage while the wood is burnt for charcoal (Ekué, 2011). Ackee wood, being termite resistant, is used for furniture, building construction, oars and canoes. The trees are used as live fence posts in Jamaica. The seeds and capsules are used as fish poison which is sprinkled on water surfaces to anesthetize fish and make them easy to catch (Rashford, 2001; Ekué *et al.*, 2010).

## 2.4 TOXICITY OF ACKEE FRUIT

The ackee fruit was implicated in several cases of food poisoning and fatalities in Jamaica and other countries. This had a negative effect on the ackee industry in Jamaica as the fruit was labeled as poisonous and banned in several countries like Trinidad and the United States of America (Rashford, 2001). Thus, research on ackee has focused mainly on causes of toxicity of the ackee fruit, the chemical compounds responsible and mechanisms and processes for reducing the toxins in the arils. The toxicity of the ackee arils has been found to be associated with the amino acids, hypoglycin A and B. This led to the development of several quantitative methods for hypoglycin A and B analysis as well as several ackee harvesting and processing protocols for reducing the toxins (Morton, 1987; Rashford, 2001; Mitchell *et al.*, 2008).

Unripe ackee fruit arils are poisonous, containing high amounts of hypoglycin A and B which when consumed cause hypoglycemia leading to death. Hypoglycin A intoxication, also known as Jamaican vomiting sickness (JVS) or toxic hypoglycemic syndrome is characterized by persistent vomiting, coma and death within 12 hours of ingestion in severe cases (Hill, 1952; Mitchell *et al.*, 2008). At full maturity, when fruit pods split open, the hypoglycin A content of the arils decreases to negligible amounts, which make the arils of



self opened fruits safe for consumption (Chase *et al.*, 1989; Brown *et al.*, 1991). In 2006, the Bureau of Standards, Jamaica developed the ‘Ackee Maturity Index Chart’ which serves to guide ackee manufacturers and consumers on the appropriate time to harvest the ackee for processing and/or consumption using visual indicators on the fruits (Mitchell *et al.*, 2008).

## 2.5 NUTRITIONAL COMPOSITION OF ACKEE ARILS

### 2.5.1 Proximate Composition

Available literature indicates that proximate composition of ackee arils vary depending on the origin, variety and or processing method (Table 2. 1). It has been reported that proximate composition of the ackee arils is comparable to many known legumes and oil seeds (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013).

**Table 2. 1:** Proximate composition of dried ackee arils

Components	Oven dried Arils	Sun dried Arils	Sun dried Arils
Moisture %	13.8	6.84 ± 1.13	3.95± 0.01
Crude fat %	45.5	45.32 ± 2.90	51.5 ± 0.04
Crude fibre %	4.23	3.21 ± 0.34	16.14 ± 0.01
Crude protein %	24.3	11.99 ± 1.12	15.27± 0.04
Ash %	5.6	4.90 ± 1.07	6.2 ± 0.03
Carbohydrate %	6.53	24.43 ± 2.24	6.86 ± 0.01
Adapted from:	(Akintayo <i>et al.</i> , 2002;	Howélé <i>et al.</i> ,2010;	Oyeleke <i>et al.</i> , 2013)



Oyeleke *et al.* (2013) reported that ackee arils have higher crude fibre content than most legumes and suggested their inclusion in the diet to help relieve constipation. Ackee arils have been reported to have high fat content, comparable to peanuts, rapeseed and sunflower seeds and higher than soybeans (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013). Ackee arils thus have potential for use in the food, and vegetable oil industry due to their high oil content.

### 2.5.2 Mineral Composition

Several researchers have reported varying results for the mineral composition of ackee arils (Table 2. 2). The arils are generally reported to be rich in potassium, magnesium, calcium and sodium but low in phosphorus and zinc with potassium being the most abundant mineral (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013).

Oyeleke *et al.* (2013) postulated that dried ackee arils can serve as a good source of minerals for bone formation. This has been attributed to its calcium/phosphorous ratio being greater than 2, a condition which facilitates the absorption of calcium in the small intestine (Nieman *et al.*, 1992). Also, inclusion of dried ackee arils in the diet will be good for the prevention of high blood pressure since its sodium/potassium ratio is less than 1. Howélé *et al.* (2010) concluded that with zinc content higher than in roasted peanuts, ackee arils are a good source of zinc which is an important mineral for pregnant women as zinc is vital for the normal growth and development of the fetus.



**Table 2. 2:** Mineral Composition of Dried Ackee Arils

	ODA Nigeria	SDA Cote d'Ivoire	SDA Nigeria
Mineral	(mg/100g)	(mg/100g)	(mg/100g)
Na	124	53.17±1.03	29.2
K	951	1503.3±1.89	29.52
Mg	271	215.33±1.03	21.12
Ca	32.6	139.67±0.85	25.07
Mn	NR	NR	0.7
Fe	17.5	17.33±0.24	1.95
Cu	8.1	NR	0.09
Pb	NR	NR	ND
Zn	6.5	4±0	0.2
P	240	NR	0.49

\*ND- Not Detected

\*NR-Not Reported

\*ODA- Oven dried Arils

\*SDA- Sun dried arils

Adaptedfrom: (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013)

### 2.5.3 Amino Acid Profile of Ackee Arils

Ackee arils were reported to contain all essential amino acid, with arginine being the predominant amino acid in the ripe arils. Unripe arils however had glutamic acid as the predominant amino acid. Aspartic acid was not detected in the ripe arils (Table 2. 3). While all other amino acids were higher, lysine and arginine contents were lower in the unripe arils than in the ripe arils (Golden *et al.*, 2002).



**Table 2. 3:** Amino Acid Profile of Ripe and Unripe Ackee Arils (fresh weight)

Amino Acid	Unripe Aril (mg/100g)	Ripe Aril (mg/100g)
Aspartic Acid	4.37± 0.24	ND
Glutamic Acid	80.80 ± 7.20	26.51 ± 0.35
Hydroxyproline	1.64 ± 0.15	1.40 ± 0.12
Serine	9.58 ± 1.50	3.06 ± 0.28
Glycine	11.34 ± 0.48	7.13 ± 0.64
Threonine	26.86 ± 4.09	5.73 ± 0.44
Alanine	34.46 ± 2.84	14.42 ± 2.90
Histidine	6.38 ± 1.10	2.84 ± 0.29
Proline	1.45 ± 0.16	0.45 ± 0.01
Arginine	53.54 ± 5.99	62.88 ± 5.87
Tyrosine	2.02 ± 0.40	0.58 ± 0.08
Valine	2.52 ± 0.15	0.80 ± 0.11
Methionine	2.32 ± 0.21	5.67 ± 0.45
Isoleucine	2.47 ± 0.55	0.18 ± 0.00
Leucine	5.92 ± 1.04	3.77 ± 0.38
Hyp-A	124.4 ± 6.70	6.40 ± 1.10
Phenylalanine	6.03 ± 1.56	2.65 ± 0.27
Tryptophan	1.46 ± 0.49	0.24 ± 0.00
Lysine	5.68 ± 0.75	10.56 ± 1.67

(Adapted from Golden *et al.*, 2002)



#### 2.5.4 Fatty Acid Profile of Ackee Arils

It has been reported that ackee aril oil is rich in linoleic, oleic palmitic and stearic fatty acids which are known to help reduce the risk of coronary heart diseases when included in the diet (Anderson-Foster *et al.*, 2012). According to Emanuel *et al.* (2013), oleic acid was the most abundant fatty acid in the mature arils (Table 2. 4). Unsaturated fatty acids were more abundant than saturated fatty acids, with the ratio of unsaturated: saturated fatty acids varying from 1.23 to 3.26 in the arils of both cheese and butter varieties.

**Table 2. 4:** Fatty Acid Profile of Mature Arils of Cheese and Butter Ackee Varieties

Fatty Acid	Cheese Variety (mg/g)	Butter Variety (mg/g)
Palmitic acid (16:0)	80.2 ± 15.6 <sup>a</sup>	42.2 ± 9.0 <sup>a</sup>
Stearic acid (18:0)	47.0 ± 9.7 <sup>a</sup>	36.7 ± 9.4 <sup>a</sup>
Oleic acid (18:1)	143.5 ± 41.3 <sup>a</sup>	146.2 ± 4.3 <sup>a</sup>
Linoleic acid (18:2)	11.4 ± 2.8 <sup>a</sup>	7.2 ± 1.7 <sup>a</sup>
Linolenic acid (18:3)	1.7 ± 0.4 <sup>a</sup>	9.9 ± 2.3 <sup>a</sup>

Values in the same row with different letter superscripts are significantly different ( $p < 0.05$ ) (Adapted from Emanuel *et al.*, 2013)

## 2.6 FUNCTIONAL PROPERTIES OF FOODS

Food constituents play some non-nutritive roles through physical and chemical interactions with other molecular constituents, which affect the overall behavior of food systems. These non-nutritive roles (functionality) are important in the processing, storage, sensory attributes and overall quality of foods. Functional properties of food systems include; solubility,



swelling, foaming properties, emulsification properties, water absorption properties, oil absorption properties, pasting properties and gelation properties (Fennema, 1996)..

Foams are mixtures of gases and liquids, in which the gas is dispersed in the liquid in the form of droplets. Proteins are important molecules in foam formation due to their hydrophobic and hydrophilic parts. The proteins migrate to the interface between air bubbles and the aqueous phase, then unfold and orient their hydrophilic regions towards the aqueous phase and their hydrophobic regions towards the air phase until they encapsulate the air bubbles and prevent the foam from collapsing (Kinsella, 1979; Fennema, 1996).

An emulsion is a mixture of two immiscible liquids, in which one is dispersed in the other in the form of droplets. Emulsification is a function of proteins in food systems because they contain both hydrophobic and hydrophilic groups. The proteins decrease the surface tension of the oil droplet by forming a film around the oil surface, interacting through their hydrophobic groups with the oil, while their hydrophilic groups interact with the aqueous phase. The concentration, solubility, structure and distribution of hydrophobic and hydrophilic groups of the protein contribute to emulsification properties of the protein (Fennema, 1996; Friberg *et al.*, 2003).

Emulsion capacity or activity measures the amount of emulsion formed by a food material under specified conditions while emulsion stability measures the amount of emulsified layer after a given period of time (Fennema, 1996).

The entrapment of water by macromolecules in such a way as to prevent it from leaching out of the matrix is termed water hydration. Water binding capacity, water absorption capacity, swelling and viscosity are properties related to water hydration. These properties are important in food formulations as they affect the yield (weight and volume), sensory and shelf quality of foods. Proteins and carbohydrates are the major macromolecules responsible



for water hydration because they contain polar groups (amino, carbonyl, hydroxyl, sulfhydryl etc), which through hydrogen bonding, covalent bonding or physical associations with water, help to trap water in the food matrix. Water absorption capacity measures the amount of water absorbed by the food material after forces of compression have been applied to it. Water absorption by proteins is affected by the conformation, temperature, pH, ionic strength and composition of proteins in the food matrix (Fennema, 1996).

Oil absorption is the physical entrapment of oil in foods, especially by proteins. Oil absorption plays a major role in flavor retention by interacting with hydrophobic groups of flavour compounds and trapping them in the food matrix. Also, oils play an important role in mouth feel of foods as they bind to the walls of the mouth during eating. Oil absorption capacity measures the amount of oil absorbed by the food material forces of compression have been applied to it (Kinsella, 1979; Fennema, 1996).

Starches absorb water, swell and lose their crystalline structure when heated in water, becoming more soluble. The amylose molecules of the starch leach out and form a matrix that traps water, and increase the viscosity of the system. Swelling power measures the amount of water taken up by starch granules when heated (Fennema, 1996).

Solubility measures the amount of solids that dissolve or solubilize in a given solvent under specified conditions. Heat facilitates the dissolution of solids by increasing the energy of the system and the easy breaking of the intermolecular bonds of the solid material (Fennema, 1996).



### 2.6.1 Functional Properties of Ackee Aril Flours

Functional properties of ackee aril flour were comparable to other legume flours currently employed in the food industry. Akintayo *et al.* (2002) found similarities in the protein solubility curve of the ackee arils with that of soybean, great northern bean and *Telfaira occidentalis* and concluded that ackee aril soluble proteins may thus be used in the formulation of acid foods, such as meat and milk-analogue products and protein-rich beverages. Emulsion capacity of ackee aril flour was greater than that of soy flour and wheat flour but lower than that of sunflower while foaming capacity was found to be higher than that of *A. breviflorus benth* seeds but lower than that of soy flour and sunflower seed flour. The foaming stability of ackee arils after 2 h was better than that of soy-protein concentrate. Water absorption capacity of ackee aril flour was lower than that of soy flour and sun flower seed flour. The oil absorption capacity was found to be higher than that of soy and wheat flours but lower than that of sunflower seed flour. Ackee aril flour has been found to have least gelation concentration of 8 % which is lower than that of pigeon pea, lupin seed and winged bean flours. These results were based on oven-dried non-defatted ackee aril flours (Akintayo *et al.*, 2002). Information on functional properties of defatted aril flours under different drying condition is, however, limited.

### 2.7 PHYSICOCHEMICAL PROPERTIES OF ACKEE ARIL OIL

Crude ackee aril oil is reported to have a reddish-brown colour which when purified turns to bright yellow. Like other oils, it is less dense than water with specific gravity less than 1. It is also reported to be less dense than canola, cottonseed, corn and sesame oils. It is a non drying oil with low iodine value (90 - 94.5). The iodine value of ackee aril oil although comparable to palm oil, is lower than that of groundnut oil, soybean oil and olive oil.



The iodine value gives an indication of the degree of unsaturation in the oil and the susceptibility of the oil to oxidation (Howélé *et al.*, 2010; Anderson-Foster *et al.*, 2012; Adepoju *et al.*, 2013; Oyeleke *et al.*, 2013).

**Table 2. 5:** Physicochemical Properties of Ackee Oil in Relation to Other Pharmaceutical Oils

Property	Ackee aril oil	Arachis (Peanut oil)	Arachis (Peanut oil) USP NF23/PhEur 2005	Corn Oil (Pharm. Excip.)
Acid value	1.8326 (0.006)	0.4488 (0.11)	0.5	2 - 6
Ester value	64.5200 (0.018)	33.6600 (0.05)	-	-
Hydroxyl value	28.0100 (0.037)	4.9952 (0.07)	2.5 - 9.5	8 - 12
Saponification value	198 (0.19)	190 (0.03)	185 - 195	187 - 196
Freezing point (°C)	9.0 (1)	5.5 (0.7)	-5	-
Melting point (°C)	25.7 (0.6)	18.5 (0.7)	-	-18 - 10
Specific gravity	0.9045 (0.008)	0.9191 (0.05)	0.912 - 0.920	0.915 - 0.918
Polarimetry (25°C)	1.4532	1.1011	1.462 - 1.464	1.474

(Adapted from: Anderson-Foster *et al.*, 2012)

Ackee aril oil is reported to have a low acid value of 8 % which translates into a low rate of deterioration and is thus safe for consumption (Howélé *et al.*, 2010). Corn oil and peanut oil are major oils for pharmaceutical applications. Corn oil, however, was found to have higher acid value than ackee aril oil as shown in

**Table 2. 5.** Ackee aril oil thus has potential for use in pharmaceutical applications similar to that of corn oil (Anderson-Foster *et al.*, 2012).



The saponification value of ackee aril oil is comparable to that of most plant oils used in the soap industry (Howélé *et al.*, 2010; Anderson-Foster *et al.*, 2012). Ackee oil is being employed successfully in soap making in Benin (Ekué *et al.*, 2010)

These research findings indicate the potential for development of an ackee oil industry from which low-fat ackee arils will be a by-product. Defatted ackee flours produced from this by-product for other food applications could generate more revenue for the ackee industry.

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## **2.8 EFFECT OF DRYING METHOD ON DRIED PRODUCT QUALITY**

Different drying methods have been employed in the dehydration of food. It has been reported that drying rate, physicochemical properties, sensory properties as well as microbiological quality of dried foods are influenced by the drying method employed (Abdelhaq and Labuza, 1987; Musa *et al.*, 2005). Sun drying method was reported to be slow, unreliable, posing a higher risk of microbiological contamination of food as well as being labour intensive (Abdelhaq and Labuza, 1987). Karanthos and Belessiotis (1995) recommended the use of hot-air ovens for drying because it is fast and yields hygienic, uniformly dried and attractive products. Most conventional drying techniques may impart undesirable colour, textural and flavor changes due to the high temperatures employed (Musa *et al.*, 2005). Freeze drying, which involves crystallization of water molecules at low temperature and direct sublimation from the solid state into vapour state by a change in pressure, preserves heat sensitive biological materials. Freeze drying is an expensive technology due to high cost, energy consumption and maintenance of freeze dryers. However, it offers some advantages over conventional drying methods which include; defined porous structure of dried product as well as retention of colour and heat sensitive compounds in the dried product (Ratti, 2001; Ciurzyńska and Lenart, 2011).



Sun and oven drying methods have been employed in the drying of ackee arils (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013). Although much information exists for other agricultural products, there is a dearth of information about the effect of different drying methods on the quality of dried ackee arils.

Bankole *et al.* (2005) dried melon seeds employing sun, oven, smoke and solar drying, studying the effect of each drying method on the physicochemical properties and storability of the melon seeds. It was observed that, although oven dried products had significantly lower moisture content and highest consumer acceptability, the drying method employed generally had no significant effect on proximate composition of the melon seeds. In a similar study on green banana, solar and biomass drying as well as combinations of freeze drying and conventional drying at different time intervals was employed to investigate the effect of the drying method on green banana flour quality. It was shown that, bananas that were freeze dried for two and four hours had higher scores for all sensory attributes as compared to the other drying treatments employed in the study (Kaddumukasa *et al.*, 2005).

Ogunlakin *et al.* (2012) also reported that the drying method (sun, oven and cabinet) had significant effects on the proximate and physicochemical compositions of cocoyam flour, with sun dried cocoyam flour having the highest values in all parameters tested except for foam stability and least gelation concentration.

## **2.9 EFFECT OF DEFATTING ON FLOUR QUALITY**

Available literature indicates that, defatting of flours generally improves most physicochemical and functional properties of the flour, with the effects being significant especially in high oil seeds (Jitngarmkusol *et al.*, 2008; Akinyede and Amoo, 2009; Ndie *et*



*al.*, 2010). Defatted African walnuts were found to have higher protein content, water absorption capacity solubility and set-back value but lower bulk density, pasting temperature and time than the undefatted flour, which makes the defatted African walnut flour better as composite flour for bread and confectionaries than the undefatted flour (Ndie *et al.*, 2010). It has been reported that fat, zinc and magnesium contents were lower whereas other proximate and mineral composition of defatted *Cassia fistula* flour were higher than that of the undefatted flour. Also, with the exception of emulsion and least gelation concentration capacities which were lower, defatted *Cassia fistula* flour had higher values for all other functional properties investigated than the undefatted flour (Akinyede and Amoo, 2009). Totally defatted flours of different macadamia cultivars had significantly higher protein and carbohydrate contents as well as higher water/oil absorption capacities and foaming capacities than their corresponding partially defatted flours, although emulsion activities and stabilities were not different. Foaming stabilities of the totally defatted flours, however, were lower than in the partially defatted flours (Jitngarmkusol *et al.*, 2008).

Defatting has also been asserted to have significant effects on the flavour, flow properties and shelf life extension of flours (Mandal *et al.*, 2013). Lipid oxidation or rancidification is a major factor for undesirable flavor changes in high oil foods. The effect of defatting on the flavour and shelf life extension of flours could be due to the reduction in available fat for lipid oxidation.

## **2.10 COMMERCIAL PROCESSING OF ACKEE ARILS**

Canned 'ackee in brine' is the major commercial ackee product. Ripe arils after harvesting are cleaned and boiled in brine and canned for export. Jamaica produces several tonnes of canned 'ackee in brine' for export to the United Kingdom and Canada generating more than



13 million U. S dollars per year (Blake *et al.*, 2006). Fresh arils are also sold on Jamaican markets, purchased mainly by tourists. Dried ackee arils are the main commercial ackee product in West African communities where ackee is exploited for food. In Benin, the dried ackee arils are sold mostly by women in market places. The dried ackee arils, however, have short shelf stability and cannot be stored for more than two weeks (Ekué *et al.*, 2010). This could be due to poor storage conditions, inefficient drying methods or the nature of the dried arils which have high fat content and thus are prone to oxidation. Other drying methods could be exploited to ascertain their effects on dried aril quality. Defatting could also be employed to reduce deterioration due to oxidation and prolong shelf life.

The need for development of ackee products for commercial applications is evident especially in West Africa where it is poorly exploited for food. Ackee aril flour could possibly become a major commercial ackee product for application in the food industry.





## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 SOURCE OF MATERIALS

The ripe ackee fruits (Figure 3. 1) were harvested from trees at the Crop Research Institute of Ghana- Fumesua and the Presbyterian Primary and Junior High School- Bantama in Kumasi- Ashanti Region. All chemicals used were analytical grade reagents.

#### 3.2 SAMPLE PREPARATION

##### 3.2.1 Harvesting and Cleaning of Samples

The ackee fruits (Figure 3. 1) were harvested from the trees using a sickle. The fruit arils were separated from the pink membranes and seeds. The separated arils were cleaned by washing under running tap water.



**Figure 3. 1:** Harvested ackee fruits



### 3.2.2 Drying and Milling of Samples

About 2 kg of cleaned arils was dried for 72 h using a vacuum freeze dryer (model: YK 115-50, True Ten Industrial Co. Ltd, Taiwan) and another 2 kg of arils was dried at 50 °C for 72 h in a Beveilinging Conventional Oven Dryer (model: DMV 1250, Holland). After drying, the arils were milled into flour using a Binatone kitchen grinder (model: BLG 402) and passed through a sieve of 1mm mesh size.

### 3.2.3 Defatting of Ackee Flours

The flour was defatted by soaking in petroleum ether in a ratio of 1:3 (w/v) for 120 h while shaking periodically (Figure 3. 2). After defatting, the mixture was filtered, using a cheese cloth, to remove the liquid filtrate and obtain the defatted flour. The defatted flour was dried for 2 h at ambient temperature in a fume hood, to remove the remaining solvent and packed in airtight polyethylene bags and stored in a freezer at -18 °C for further analysis.



**Figure 3. 2:** Set up for defatting of ackee flours



### **3.3 PROXIMATE ANALYSIS**

Proximate (moisture, ash, crude protein, crude fat, crude fibre and carbohydrate) analysis of the ackee aril flours was performed in triplicates as described in the following sections.

#### **3.3.1 Moisture Content Determination**

The standard method of the Association of Official Analytical Chemists (AOAC) (1997) was used to determine the moisture content. About 2 g of each sample was weighed into a previously dried petri-dish and dried in a thermostatically controlled electric oven (OSK 9500C, Ogawa Seiki Co. Ltd., Japan) at 105 °C for 8 h. The petri dishes were transferred into a desiccator for cooling after which they were weighed.

Moisture content was determined by difference and expressed as a percentage.

#### **3.3.2 Ash Content Determination**

About 2 g of sample was weighed into a pre-ignited and pre-weighed crucible. The crucible with the sample was placed in a muffle furnace and ignited at 600 °C for 2 h after which it was cooled to about 105 °C in a forced convection oven and further cooled in a dessicator to room temperature. The crucible and its contents were weighed and the ash content determined by difference and expressed as a percentage (AOAC, 1997).

#### **3.3.3 Crude Fat Content Determination**

The standard method of the AOAC (1997) was used for the crude fat determination. About 2 g of sample was transferred into a filter paper bag and placed in a thimble holder. About 200 mL of petroleum ether was measured into a pre-dried and reweighed round-bottom flask and assembled together with the thimble holder and its contents. The quick-fit condenser was



connected to the Soxhlet apparatus and refluxed for 16 h on a heating mantle at low heat. The flask was removed and the solvent evaporated after which it was heated at 105 °C for 30 min, cooled in a desiccators and the weight determined by difference and expressed as percentage crude fat.

#### **3.3.4 Crude Fibre Determination**

Approximately 2 g of defatted sample was transferred into a 250 mL Erlenmeyer flask and 0.5 g of asbestos added. About 200 mL of boiling 1.25 % sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was added and immediately transferred onto a heating mantle. A cold finger condenser was attached to it and the sample boiled for 30 min. The contents of the flask were filtered with linen cloth placed in a funnel. The residue was washed with boiling water until the washings were no longer acidic (determined using blue litmus paper). The washed sample with asbestos was then washed back into the flask (previously washed) with 200 mL of boiling 1.25 % sodium hydroxide (NaOH) solution. The flask was reconnected to the condenser and allowed to boil for 30 min. The mixture was filtered through a linen cloth and the residue washed with about 300 mL of boiling water and with 15 mL of alcohol. The residue was transferred into a previously dried and weighed crucible and dried at 100 °C for 1 h in an oven, cooled in a desiccator to room temperature and then ignited at 600 °C for 30 min after which it was cooled and reweighed. The loss in weight after ignition was determined and expressed percentage crude fibre.

#### **3.3.5 Crude Protein Content Determination**

The Macro-Kjeldahl method (AOAC, 1997) was adopted for the determination of crude protein content of ackee flours



### ***Digestion***

To about 35 mg of sample in a digestion tube, 1 g catalyst mixture, 2 mL of 30 % hydrogen peroxide and 2 mL concentrated sulphuric acid were added sequentially.

The sequence was followed to avoid spattering. The mixture was allowed to digest for 30 min and then cooled. About 200 mL of deionized water was added to dissolve the digest residue and the resultant solution, allowed to cool to room temperature.

### ***Distillation***

The digest solution was transferred into a cleaned Kjeldahl distillation apparatus. The digestion flask was rinsed twice with 2 mL of deionized water to ensure that no residue was left. About 6 mL of boric acid and 3 drops of indicator solution were put in a 250 mL Erlenmeyer flask and fitted to a condenser. The condenser tip was adjusted to extend well below the surface of the solution. About 8 mL of sodium hydroxide-sodium thiosulphate solution was added to the digest in the distillation apparatus and distillation allowed to continue until about 50 mL of distillate was collected.

### ***Titration***

The distillate was titrated against 0.2 M HCl solution until first appearance of violet colour. The same quantity of reagents and procedure of digestion, distillation and titration was followed for the blank. Percent nitrogen and crude protein was calculated using the formulae below;

*Crude nitrogen (%)*

$$= \frac{(\text{vol. of HCl in sample} - \text{vol. of blank}) \times \text{normality of HCl} \times 14.007}{\text{Wt. of Sample (mg)}} \times 100$$

*Crude Protein (%)* = % Nitrogen x 6.25



### 3.3.6 Carbohydrate Content Determination

The percentage carbohydrate content was estimated by difference using the formula below:

$$\begin{aligned} \text{Carbohydrate}(\%) \\ = 100 - \%(\text{Protein} + \text{Crude Fat} + \text{Ash} + \text{Crude Fibre} + \text{Moisture}) \end{aligned}$$

### 3.3.7 Calculated Metabolizable Energy Determination

The calculated metabolizable energy (CME) of the samples was estimated using the formula of FAO (2003) as below:

$$CME(KJ/100g) = (\text{Protein} \times 17 + \text{Crude Fat} \times 37 + \text{Carbohydrate} \times 17)$$

## 3.4 MINERAL ANALYSIS

Mineral contents of the ackee flour samples were determined by atomic absorption spectrometry, flame photometry and spectrophotometry according to the method of AOAC (2005), as described in the following sections.

### 3.4.1 Sample Preparation

One gram of each flour sample was placed in a porcelain crucible and ashed at 550 °C for 6 h. The ash was then dissolved in 2 ml concentrated HNO<sub>3</sub> and allowed to boil for 1 min.

The mixture was cooled, filtered through Whatman No. 42 filter paper into to a 100 mL volumetric flask and made to the mark with distilled water. The solution was well mixed and the minerals were determined from the resulting (ash) solution. A blank was also prepared using similar experimental procedure (AOAC, 2005).



### 3.4.2 Determination of Zinc (Zn), Calcium (Ca) and Magnesium (Mg) by Atomic Absorption Spectrometry

Atomic absorption spectrophotometer (model: 210VGP, Buck Scientific, USA) was used to analyze for calcium, zinc and magnesium contents. Different electrode lamps were used for each mineral. The equipment was run with standard solutions of each mineral before determination. The dilution factor for all minerals except Mg was 100. For determination of Mg, further dilution of the original solution was done by using 1 mL original solution and enough deionized water was added to it to make the volume up to 100 mL. Also for the determination of Ca, 1 mL lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentration of each mineral (ppm) was recorded and the total mineral concentration in mg/100 g was calculated using the following equation:

$$\begin{aligned} & \text{Total Mineral Concentration (mg/100g)} \\ &= \frac{\text{Concentration (ppm)} \times \text{Dilution factor}}{\text{Wt. of Sample} \times 1000} \times 100 \end{aligned}$$

### 3.4.3 Determination of Sodium (Na) and Potassium (K) by Flame Photometry

Na and K analysis of the sample were done using a flame photometer (model: Jenway PFP7). The ash solutions as used in AAS were used for the determination of Na and K. Standard curves were plotted using standard solutions of NaCl and KCl with concentrations of 20, 40, 60, 80 and 100 ppm. The concentration of Na or K in the ash solution was determined from the corresponding standard curve. The concentrations of minerals (ppm) were recorded and the mineral concentration in mg/100 g was calculated using the following equation:

$$\begin{aligned} & \text{Total Mineral Concentration (mg/100g)} \\ &= \frac{\text{Concentration (ppm)} \times \text{Dilution factor}}{\text{Wt. of Sample} \times 1000} \times 100 \end{aligned}$$



### 3.4.4 Determination of Phosphorus (P) by Spectrophotometry

Phosphorus content of each sample was determined using a spectrophotometer (model: Lemfield Spectrulab 23A). Approximately 22.5 g of aqueous ammonium heptamolybdate ( $(\text{NH}_4)_6\text{MO}_7\text{O}_2 \cdot 4\text{H}_2\text{O}$ ) was dissolved in 400 mL of deionized water to make solution A. About 1.25 g of ammonium vanadate was dissolved in 300 mL of boiling deionized water to make solution B. Solution B was added to solution A and allowed to cool to room temperature, after which 250 mL of concentrated  $\text{HNO}_3$  was added and the mixture was diluted to 1 L to obtain the colour reagent. One milliliter of each ash solution was taken and 4 mL deionized water added in a beaker. Approximately 5 mL of the colour reagent was added to this volume and the total volume of the final solution was made up to 25 mL. After some time, the colour of this final solution turned yellow. Sample from final yellow solution was taken in a cuvette and introduced into the spectrophotometer. The absorbance readings from the spectrophotometer were plotted on a standard curve to determine the phosphorus concentration (ppm). Total phosphorus concentration in mg/100 g was calculated using the following equation:

$$\text{Total P Concentration (mg/100g)} = \frac{\text{Concentration (ppm)} \times \text{Dilution factor}}{\text{Wt. of Sample} \times 1000} \times 100$$



### 3.5 BULK DENSITY AND HAUSNER RATIO DETERMINATION

Bulk Density was determined according to the method described by Maninder *et al.* (2007). Each flour sample was gently filled into a 10 mL graduated cylinder, previously tarred and the weight was recorded. The bottom of the measuring cylinder was gently tapped on a laboratory bench several times until there was no further decrease in the volume of the sample in the cylinder. Bulk (loose and tapped) densities were calculated as weight of sample per unit volume of sample (g/mL). Measurements were made in triplicate. Hausner ratio was determined as a ratio of the tapped bulk density to the loose bulk density of the flour (Ogunsina *et al.*, 2010).

### 3.6 FUNCTIONAL PROPERTIES DETERMINATION

The functional properties (water/oil absorption capacity, solubility, swelling power and foaming capacity and stability and emulsion activity and stability) analysis of the ackee aril flours was performed in triplicates as described in the following sections.

#### 3.6.1 Determination of Water and Oil Absorption Capacities

The modified method of Medcalf and Giles (1965) was used to determine water and oil absorption capacities of the flour samples. About 2 g of each flour sample was weighed into 50 mL pre-weighed centrifuge tubes and stirred into 40 mL distilled water or refined soybean oil for 1 h on a shaker (Edmund Buhler SM 30). The mixtures were placed in a centrifuge (Spectra Scientific Merlin) and centrifuged at 2200 rpm for 15 min. The water or oil released on centrifugation was drained and the wet flour weighed to determine by difference, the



weight of bound water or oil. The percentage water absorption capacity (%WAC) or oil absorption capacity (%OAC) was calculated using the following equation:

$$WAC \text{ or } OAC (\%) = \frac{\text{Weight of bound water or oil}}{\text{Weight of sample}} \times 100$$

### 3.6.2 Determination of Foaming Capacity and Stability

The method of Jitngarmkusol *et al.* (2008) was used for the determination of the foaming capacity and stability of ackee flours with some slight modifications. Two grams of each flour sample was mixed with 100 mL of distilled water and the suspension was whipped with a kitchen blender. The whipped suspension was transferred into a 250 mL graduated cylinder. Volumes of the whole mixture were recorded before and after whipping. The experiment was done in triplicate. The foaming capacity (FC) and stability (FS) were calculated using the following equations:

$$FC(\%) = \frac{(V_2 - V_1)}{V_1} \times 100$$

$$FS(\%) = \frac{V_3}{V_2} \times 100$$

Where  $V_1$  is the volume of initial mixture and  $V_2$  is the volume of the mixture after whipping and  $V_3$  is the volume of the foam after 5 h.

### 3.6.3 Determination of Emulsion Activity and stability

Emulsion activity and stability were measured according to the modified method described by Jitngarmkusol *et al.* (2008). About 1 g (db) of each flour sample was dispersed in 50 mL of distilled water. The mixture was homogenized for 30 s; 25 mL of soybean oil were added, and the mixture was homogenized again for 30 s. Then another 25 mL of soybean oil was



added and the mixture was homogenized for 90 s. Each emulsified sample was divided equally into two centrifuge tubes.

The first centrifuge tube, directly centrifuged at 1100 g for 5 min, was used to determine the emulsion activity. In order to determine the emulsion stability, the second centrifuge tube was heated in a water bath at 85 °C for 15 min, cooled down to room temperature, and centrifuged under the same conditions as the first tube. The experiment was conducted in triplicate.

The emulsion activity (EA) and emulsion stability (ES) were calculated according to the following equations:

$$EA(\%) = \frac{V_2}{V_1} \times 100$$

$$ES(\%) = \frac{V_3}{V_1} \times 100$$

Where  $V_1$  is the volume of emulsion before centrifugation,  $V_2$  is the volume of the emulsified layer and  $V_3$  is the volume of the remaining emulsified layer after heating.

#### 3.6.4 Solubility and Swelling Power Determination

The solubility and swelling power (SP) of ackee aril flour were determined using the method described by Leach *et al.* (1959), with some modifications. Approximately 1 g of flour sample was weighed into a 50 mL centrifuge tube containing 40 mL deionised water and vortexed for 30 min. The tubes were then heated in a thermostatically regulated water bath at 85 °C for 20 min and cooled to room temperature. The tube and its contents were centrifuged at 2200 rpm for 15 min and the clear supernatant carefully decanted into a pre-weighed petri dish. The weight of the residue/ sediment was then noted. The water in the supernatant was evaporated and the difference in weight of petri dish was recorded as the weight of soluble fraction. Solubility and swelling power (SP) were calculated using the following equations:

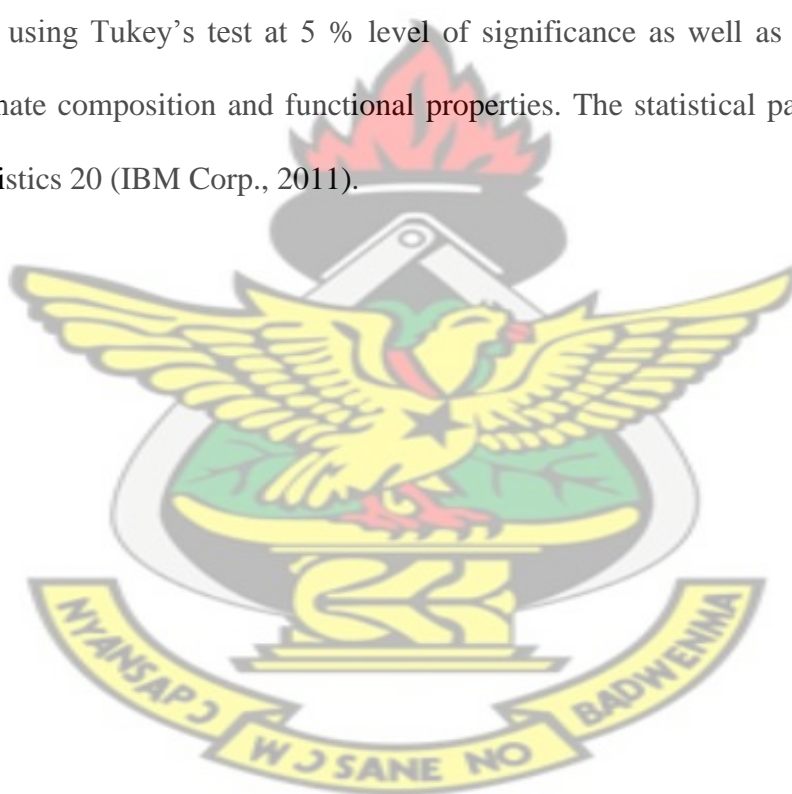


$$\text{Solubility}(\%) = \frac{\text{Weight of soluble fraction}}{\text{Weight of sample}} \times 100$$

$$SP(\%) = \frac{\text{Weight of sediment}}{\text{Weight of sample} \times (100 - \text{Solubility})} \times 100$$

### 3.7 STATISTICAL ANALYSIS

The data reported are averages of triplicate observations. Analysis of variance was performed and interpreted using Tukey's test at 5 % level of significance as well as correlation tests between proximate composition and functional properties. The statistical package used was IBM SPSS Statistics 20 (IBM Corp., 2011).





## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

From the oven-dried and freeze-dried arils (Figure 4. 2) four different ackee aril flours samples were obtained, namely; oven-dried full fat (ODFF), freeze-dried full fat (FDFF), oven-dried defatted (ODDF) and freeze-dried defatted (FDDF) (Figure 4. 3 and Figure 4. 4 respectively).

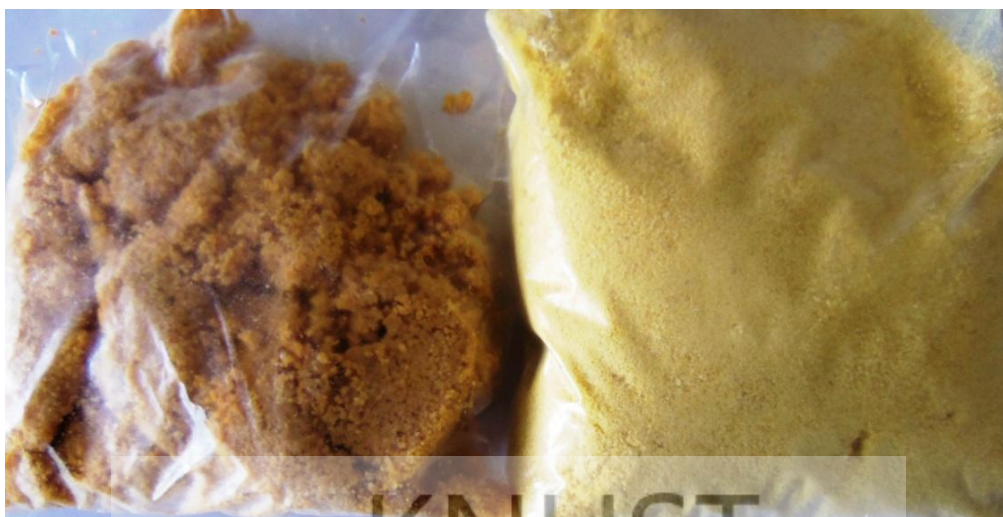


**Figure 4. 1:** Fresh ackee arils



**Figure 4. 2:** Oven dried (left) and freeze dried (right) arils





**Figure 4. 3:** Oven dried (left) and freeze dried (right) ackee flours prior to defatting



**Figure 4. 4:** Freeze dried (left) and oven dried (right) defatted flours

It was observed that the freeze-dried arils and full fat flours retained the original yellowish-cream colour of the fresh arils (Figure 4. 1) whereas the oven-dried arils and full fat flours had brownish colour. The browning of oven-dried arils could be attributed to Maillard reaction (a non-enzymatic browning reaction involving amino groups and reducing sugars, which yields brown polymeric pigments) which is facilitated by heat application (Friedman, 2005). However, after defatting, the freeze-dried defatted flour turned brown, which could be attributed to enzymatic browning (hydroxylation or oxidation of phenolic compounds,



catalyzed by enzymes) in the freeze-dried flour after defatting. Freeze drying, due to the low temperature it employs, preserves the biological activity of enzymes, which can be activated under favourable environmental conditions (Ciurzyńska and Lenart, 2011). The flours were dried under ambient conditions after defatting, which probably favoured the activation the enzymes responsible for browning. Oven drying, on the other hand, denatures the enzyme proteins and thus irreversibly destroys enzyme activity.

The results of the physicochemical and functional properties of the ackee were interpreted as discussed in the following sections.

#### **4.1. PHYSICOCHEMICAL PROPERTIES OF ACKEE ARIL FLOURS**

##### **4.1.1. Proximate Composition of Dried Aril Flours**

Results of proximate composition of the ackee flour samples, on dry weight basis, are presented in Table 4. 1. The moisture content of ODFF, FDFF, ODDF and FDDF were 4.83 %, 5.20 %, 5.12 % and 7.30 %, respectively. There was no significant difference between moisture contents of ODFF and FDFF ( $p > 0.05$ ). Moisture values of the full fat ackee aril flours were lower than previously reported for sun dried and oven-dried ackee arils (Akintayo *et al.*, 2002; Howélé *et al.*, 2010). Oyeleke *et al.* (2013) however reported lower moisture (3.95 %) for sun dried arils (Table 2. 1 on page 13).

Low moisture content of food contributes to its expected shelf extension by limiting the moisture available for microbial growth (Andrew and Harrison, 2006). Howélé *et al.* (2012) reported that ackee arils` with moisture content of 6.25 % can be stored for long without spoilage. Thus it can be inferred that the lower moisture content of the full fat ackee flours investigated will extend the expected shelf life of the flours. According to the Codex



Standard for wheat flour, moisture content of up to 15.5 % is recommended for wheat flour (CODEX STAN 152, 1985).

Moisture content increased in the defatted ackee flours; 5.12 % for ODDF and 7.30 % for FDDF, with differences being statistically significant ( $p < 0.05$ ). After defatting, the flours were exposed to ambient conditions in a fume hood to dry. Powdered food products are known to be hygroscopic, capable of absorbing moisture from the environment of high humidity (Jaya and Das, 2004). The exposure of the flours to atmospheric air after defatting could therefore be a contributing factor to the increased moisture content observed for the defatted flour samples. The absence of the liquid phase of the material during freeze drying makes the freeze-dried product more porous than air dried products (Karel, 1975; Krokida and Maroulis, 1997). The higher porosity of the freeze-dried flour could have accelerated the flow of the atmospheric air and consequently moisture through the flour matrix, resulting in more moisture being absorbed by the freeze-dried flour than the oven-dried flour. According to Meda and Ratti (2005), freeze-dried products rehydrate at a rate, 4 to 6 times higher than air-dried foods. This could have accounted for the higher moisture content of the freeze-dried defatted flour.

The crude fat content of full fat ackee aril flours ranged from 54.43 % (FDDF) to 59.54 % (ODFF), indicating that dried ackee arils are a better source of oil than many known oilseeds; cottonseed kernel (36.3 %), linseed/flaxseed (34.0 %), groundnut/peanut (46.0 %), safflower (38.5 %) and sunflower (47.5 %) (McKevith, 2005). The high crude fat contents reported for the full fat ackee aril flours are in agreement with values previously reported for dried ackee arils (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013).



**Table 4. 1: Proximate Composition of Ackee Aril Flours in % dry basis**

PARAMETER	ODFF	FDDF	ODDF	FDDF
Crude fat	59.54 (0.28) <sup>a</sup>	54.43 (2.35) <sup>b</sup>	25.82 (2.75) <sup>c</sup>	21.42 (0.9) <sup>c</sup>
Ash	8.99 (0.47) <sup>a</sup>	8.45 (1.19) <sup>a</sup>	8.68 (0.42) <sup>a</sup>	9.05 (0.01) <sup>a</sup>
Crude Fibre	4.08 (0.16) <sup>a</sup>	3.83 (0.01) <sup>a</sup>	3.88 (0.18) <sup>a</sup>	4.05 (0.36) <sup>a</sup>
Crude Protein	12.26 (0.38) <sup>a</sup>	11.54 (0.20) <sup>b</sup>	22.04 (0.16) <sup>c</sup>	23.00 (0.19) <sup>d</sup>
Carbohydrate	15.13 (0.96) <sup>a</sup>	21.75 (3.55) <sup>b</sup>	39.58 (3.11) <sup>c</sup>	42.48 (1.20) <sup>c</sup>
CME (kJ/100g)	2668.50 (7.17) <sup>a</sup>	2579.99 (32.15) <sup>b</sup>	2002.88 (49.46) <sup>c</sup>	1905.71 (13.37) <sup>d</sup>

Note: Values in a row with different letters in superscript are significantly different ( $p < 0.05$ ; Appendix 1)  
Values in brackets are standard deviations of triplicate results  
CME: Calculated Metabolizable Energy

There was significant difference ( $p < 0.05$ ) in the crude fat contents of the oven-dried and freeze-dried full fat flours. Enzymes after freeze drying still maintain their activities because the low temperature employed in the freeze-drying process does not denature the enzyme proteins. It is thus possible that some lipolytic enzymes, maintaining their activities after the freeze drying process, degraded triglycerides to form by products (e.g. lipid-starch complexes) which were unavailable for fat determination, thus affecting the crude fat composition of the freeze dried arils.

The defatting method employed significantly reduced the fat content of the ackee flours, yielding low-fat ackee flours with crude fat contents of 21.42 % (FDDF) and 25.8 %



(ODDF). This implies a 56.63 % and 60.66 % reduction in the fat contents of ODDF and FDDF respectively. Although FDDF had lower crude fat content than the oven-dried defatted flour, the difference was not statistically significant ( $p > 0.05$ ). This could be due to the freeze-dried flour having an initial lower crude fat content (54.43 %) than the oven dried flour (59.54 %) prior to the defatting. The crude fat contents of the defatted ackee flours are comparable to that of defatted peanut flour (21.90 %) as reported by the USDA National Nutrient Database for Standard Reference (2014). Foods with high fat content are susceptible to lipid oxidation during storage, which affects shelf stability. The reduced fat content of the defatted ackee aril flours imply their increased shelf stability as compared to the full fat ackee aril flours.

Ash contents of the ackee aril flour samples ranged from 8.45 to 9.05 % dry basis (% db) with no significant differences between oven and freeze-dried samples or defatted and full fat samples ( $p > 0.05$ ). The ash contents recorded were, however, higher than those previously reported in literature for ackee arils (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013). The ash content of a food sample generally reflects the total mineral content of the food. Thus it can be assumed that the ackee flour samples investigated are richer in minerals than jack bean, pigeon pea and cowpea flours, which have ash contents of 6.51, 4.58 and 4.73 % respectively (Arawande and Borokini, 2010).

Crude fibre content of the ackee arils and defatted flour ranged from 3.83 to 4.08 % db. There were no significant differences ( $p > 0.05$ ) between crude fibre contents of the ackee aril flour samples investigated. Results were in agreement with the work of Howélé *et al.* (2012) but lower than the values (4.23 and 16.14 %) reported by Akintayo *et al.* (2002) and Oyeleke *et al.* (2013) respectively. Defatted macadamia flours were reported to have lower crude fibre contents in the range of 2.65 to 3.77 % db (Jitngarmkusol *et al.*, 2008). Lower crude fibre



contents (2.36 – 3.67 %) have been reported for whole sesame seed and defatted sesame flours (Bukya and Vijayakumar, 2013).

High fibre foods contribute to sustaining the internal stretching of the intestinal tract for normal peristaltic movements during digestion and thus help promote free bowels. Fibre also helps in the reduction of total serum cholesterol and LDL-cholesterol levels as well as reduces the risk of cardiovascular diseases (IFT, 1990; Mehta and Kaur, 1992; Pereira, 2004). Due to their high fibre content, regular consumption of fruits and vegetables (at least 400g daily) has been recommended by the World Health Organization (WHO/FAO, 2003).

Protein content of the full fat ackee aril flours were 11.54 % for FDFF and 12.26 % for ODFD with differences being statistically significant ( $p < 0.05$ ). The values were comparable to that of sun dried arils reported by Howélé *et al.* (2012) but lower than values reported for sundried and oven dried arils by Oyeleke *et al.* (2013) and Akintayo *et al.* (2002), respectively. Abiodun and Adeleke (2010) reported higher protein contents for melon seed varieties (33.80 - 39.96 % d.b.). The protein contents of the arils significantly increased after defatting, yielding defatted ackee flours with protein contents of 22.04 % (ODDF) and 23.00 % (FDDF), which compared favourably with that of many known legume flours; cowpea (24.1 %), horse gram (22.5 %), chickpea (23.7 %), pigeon pea (19.9 - 24.0 %) and field pea (25.6 - 26.2 %) (Maninder *et al.*, 2007; Sreerama *et al.*, 2012).

The Food and Agriculture Organization recommends minimum protein content of 12 - 15 % in the diet (WHO, 2002). The defatted ackee aril flours meet the minimum recommended protein by the WHO and are thus a better source of protein than their corresponding full fat aril flours. The full fat ackee aril flours however will require complementing with other protein-rich food sources to meet the protein requirements in the diet.



The carbohydrate contents (determined by difference) of the full fat ackee aril flours were 15.13 % for ODFD and 21.75 % for FDFD, with the difference being statistically significant ( $p < 0.05$ ). Howélé *et al.* (2012) reported higher carbohydrate content for sun-dried arils while Akintayo *et al.* (2002) and Oyeleke *et al.* (2013) reported lower carbohydrate contents (6.53 % and 6.86 % respectively) for oven-dried and sun-dried arils (Table 2. 1 on page 13). Defatted ackee aril flours had significantly higher carbohydrate contents than their corresponding full fat ackee aril flours, with ODDF having carbohydrate content of 39.58 % and FDDF, 42.48 %. Carbohydrates and fats account for the greater energy fraction of the diet (Duncan *et al.*, 1983). Thus although the fat content is reduced in the defatted aril flours, the increased carbohydrate content ensures that the defatted aril flours contribute significant amount of energy in the diet.

The calculated metabolizable energy (CME) was 2668.50 kJ/100g for ODFD and 2579.99 kJ/100g for FDFD. The difference was statistically significant ( $p < 0.05$ ). The CME of the full fat ackee aril flours is higher than was reported for sun-dried ackee arils by Oyeleke *et al.* (2013). CME of the defatted ackee aril flour samples were 1226.10 kJ/100g for FDFD and 1369.61kJ/100g for ODFD, with differences being statistically significant ( $p < 0.05$ ). Fat provides more than double the amount of energy per gram compared to carbohydrates or proteins. Thus a reduction in the fat content of food will translate into reduced energy content of the food as seen with the CME values reported for the defatted ackee aril flours.

Low energy-dense foods are recommended for people who want to lose weight as they help lower caloric intake while ensuring prolonged feelings of satisfaction (Duncan *et al.*, 1983). Thus defatted ackee aril flours will serve as a better dietary option for people who want to watch their weights than the dried ackee arils. However, the energy contents of the defatted



flours are too high to be classified as low-energy dense foods since low energy dense foods must not provide energy exceeding 100 kcal/100 g (418 kJ/100 g) (WCRF UK, 2012).

#### 4.1.2 Mineral Composition of Ackee Arils Flours

Results of mineral composition of the ackee aril flours (Table 4. 2) showed potassium to be the predominant mineral, ranging from 425.10 to 981.78 mg/100 g while zinc was the least mineral, ranging from 1.95 to 3.58 mg/100 g. Similar trends have been reported for ackee arils (Akintayo *et al.*, 2002; Howélé *et al.*, 2010; Oyeleke *et al.*, 2013).

Arawande and Borokini (2010) reported lower Ca, K and Mg but higher Zn, Na and P contents for jack bean, pigeon pea and cowpea found in Nigeria. Also, the Ca and P contents of the ackee aril flours were lower than was reported for full fat and defatted cashew kernel flours. While K and Na contents were lower in the full fat ackee aril flours, defatted ackee aril flours, however, had higher K and Na contents than either full fat or defatted cashew kernel flours (Alobo *et al.*, 2009).

ODFF recorded higher mineral contents than FDFF except for Ca and Zn. However, for the defatted ackee aril flours, FDFF recorded higher mineral content than ODDF. This was probably due to the loss of the biological activity of the oven dried flour during heating, which allowed some of the minerals to wash off with the defatting solvent. For all minerals investigated in the full fat ackee flours, the drying method had no significant effect on the mineral composition ( $p < 0.05$ ). The defatted flours generally had significantly higher mineral contents than their corresponding full fat flours.



**Table 4. 2: Mineral Composition of Ackee Aril Flours**

Mineral (mg/100g)	ODFF	FDFE	ODDF	FDDF
<b>Ca</b>	160.00 (0.00) <sup>a</sup>	200.00 (0.00) <sup>b</sup>	240.00 (0.00) <sup>c</sup>	240.00 (0.00) <sup>c</sup>
<b>Mg</b>	240.00 (0.00) <sup>a</sup>	185.00 (7.07) <sup>b</sup>	390.00 (0.00) <sup>c</sup>	500.00 (14.14) <sup>d</sup>
<b>P</b>	152.43 (0.00) <sup>a</sup>	152.43 (4.15) <sup>a</sup>	222.78 (8.29) <sup>b</sup>	297.52 (2.07) <sup>c</sup>
<b>K</b>	475.71 (0.00) <sup>a</sup>	425.10 (14.31) <sup>a</sup>	804.66 (35.79) <sup>b</sup>	981.78 (0.00) <sup>c</sup>
<b>Zn</b>	1.95 (0.05) <sup>a</sup>	2.08 (0.02) <sup>b</sup>	2.90 (0.00) <sup>c</sup>	3.58 (0.02) <sup>d</sup>
<b>Na</b>	84.24 (0.00) <sup>a</sup>	73.37 (3.84) <sup>a</sup>	123.65 (1.92) <sup>b</sup>	171.20 (3.84) <sup>c</sup>

Note: Values in a row with different letters in superscript are significantly different ( $p < 0.05$ ; Appendix 2)

Values in brackets are standard deviations of duplicate results

#### 4.1.3 Bulk Densities and Hausner Ratio of Ackee Flours

Loose bulk density values (LBD) of ackee flours ranged between 0.31 g/mL and 0.50 g/mL while tapped bulk density (TBD) values ranged between 0.55 g/mL and 0.72 g/mL (Table 4. 3). FDFE had the lowest LBD and TBD while ODFE and ODDF had the highest TBD. LBD for the defatted samples were similar but was significantly different between ODFE and FDFE. There was significant difference in tapped bulk densities between FDFE and FDDF ( $p < 0.05$ ) but not between the oven dried flours, ODFE and ODDF ( $p > 0.05$ ).



Hausner ratios of the ackee aril flours ranged from 1.21 -1.78 and differences were significant for all flour samples, with defatted flours having significantly lower Hausner ratios than their corresponding full fat flours ( $p < 0.05$ ).

**Table 4. 3: Bulk Densities and Hausner Ratio of Ackee Aril Flours**

PARAMETER	ODFF	FDFD	ODDF	FDDF
<b>LBD (g/mL)</b>	0.44 (0.00) <sup>a</sup>	0.31 (0.00) <sup>b</sup>	0.50 (0.01) <sup>c</sup>	0.50 (0.00) <sup>c</sup>
<b>TBD (g/mL)</b>	0.72 (0.00) <sup>a</sup>	0.55 (0.01) <sup>b</sup>	0.72 (0.00) <sup>a</sup>	0.61 (0.02) <sup>c</sup>
<b>Hausner Ratio</b>	1.64 (0.08) <sup>a</sup>	1.78 (0.20) <sup>b</sup>	1.45 (0.12) <sup>c</sup>	1.21 (0.03) <sup>d</sup>

Note: Values in a row with different letters in superscript are significantly different ( $p < 0.05$ ; Appendix 1)

Values in brackets are standard deviations of triplicate results

LBD: Loose bulk density

TBD: Tapped bulk density

The TBD of the ackee aril flour samples was low as compared to that of soy ogi flours (0.7 - 0.84 g/cm<sup>3</sup>) as reported by Oluwamukomi *et al.* (2005). TBD of ODFF and ODDF were comparable to that of sesame cake and defatted sesame flour (0.67 g/mL and 0.76 g/mL respectively) and ogi flour (0.70 g/cm<sup>3</sup>) and higher than local Nigerian wheat flour (0.58 g/cm<sup>3</sup>), full fat and defatted moringa kernel flours (0.59 g/cm<sup>3</sup> and 0.38 g/cm<sup>3</sup> respectively) (Oluwamukomi *et al.*, 2005; Ogunsina *et al.*, 2010; Adejumo, 2013; Bukya and Vijayakumar, 2013). The LBD of the ackee flour samples were generally higher than for full fat and defatted moringa kernel flours (0.37 and 0.26 g/cm<sup>3</sup> respectively), comparable to that of ogi flour (0.42 g/cm<sup>3</sup>) but lower than that of soy ogi flour (0.47 - 0.68 g/cm<sup>3</sup>) (Oluwamukomi *et al.*, 2005; Ogunsina *et al.*, 2010). Hausner ratios of the defatted ackee aril flours were lower



than that of defatted moringa kernel flours while full fat ackee aril flours had higher Hausner ratios than that of full fat moringa kernel flours (Ogunsina *et al.*, 2010).

Porosity of flour affects its bulk density; the higher the porosity, the higher the volume occupied by a given mass of flour, which translates into a lower density of the flour. Freeze dried products are more porous than air-dried products, resulting in the lower density of the freeze-dried flours (Karel, 1975; Krokida and Maroulis, 1997). Loose bulk density determines the choice of container size as well as the strength of the food if prepared from a given volume while the tapped bulk density determines the packed structure or volume of the product after forces of compression and movement (e.g. during storage and distribution) have been applied to it. Larger storage spaces will be needed per weight for the freeze-dried ackee aril flours due to their lower bulk densities in comparison with the oven-dried ackee aril flours.

The Hausner ratio of flour determines its flowability, with flours of Hausner ratio up to 1.4 being able to flow easily. Flours/powders with Hausner ratios greater than 1.4 generally have poor flow properties (Barbosa-Canovas *et al.*, 2005; Ogunsina *et al.*, 2010). Food flours/powders with good flow properties facilitate their conveying, blending and packaging and thus encourage their application in industrial food manufacturing. The lower Hausner ratios of the defatted ackee aril flours indicate that the defatted flours have better flow properties which will facilitate their use in food industrial applications than the full fat ackee aril flours.



## 4.2 FUNCTIONAL PROPERTIES OF ACKEE ARIL FLOURS

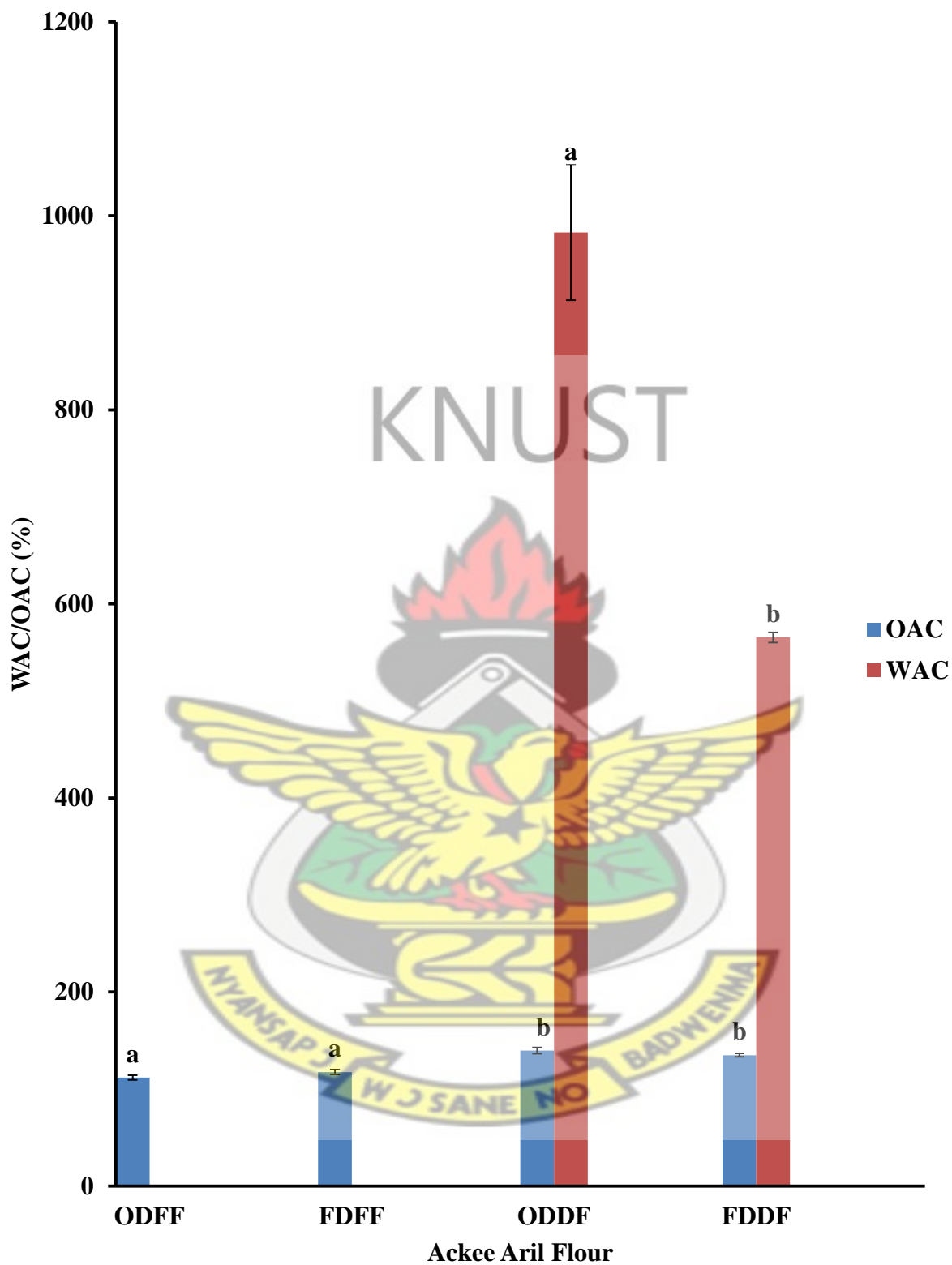
### 4.2.1 Water and Oil Absorption Capacities

Figure 4. 5 shows the results of water absorption capacities (WAC) and oil absorption capacities (OAC) of the ackee aril flour samples. The high fat contents of the full fat flours prevented the determination of their WAC as emulsion was formed, which floated on the surface and drained off along with the water. ODDF had a significantly higher WAC (982.88 %) than FDDF (565.53 %). A comparatively lower WAC was recorded for local Nigerian wheat flour (140 - 150 %), sorghum flour (219 - 235 %), irradiated and non irradiated cowpea flours (110 - 113 %), full fat and defatted mucuna flour (120 - 220 %) and different yam flours (194 - 207 %) (Iwuoha, 2004; Abu *et al.*, 2005; Elkhailifa *et al.*, 2005; Adejumo, 2013).

Carbohydrates and proteins have significant influence on the WAC of food due to the presence of hydrophilic components like polar or charged side chains. When the protein content, number of charged amino acid residues and hydrophilic groups are high, hydrogen bonding with water molecules increases as well as electrostatic repulsion between protein polymers, which facilitates binding and entrapment of water (Kinsella, 1979). Starches contribute to high WAC when the associative forces between starch granules are weak, allowing more starch surfaces to be available for binding water (Rickard *et al.*, 1991). Flours with the ability to absorb water and swell for improved consistency in food (high WAC) have beneficial applications in dough, processed meats and custards (Kinsella, 1979).

OAC of FDDF was higher (117.47 %) than ODDF (111.75 %). Results were however lower than previously reported by Akintayo *et al.* (2002) for full fat ackee pulp/aril flour (131.6 %). The OAC of defatted flour samples were significantly higher; 139.57 % for ODDF and 134.87 % FDDF.





**Figure 4. 5: Water and Oil Absorption Capacities (WAC and OAC) of Ackee Aril Flours**

\*Same coloured bars with different letters are significantly different ( $p < 0.05$ ; Appendix 3)



Although ODDF had higher OAC than FDDF, the difference was not statistically significant ( $p > 0.05$ ). OAC of the ackee aril flours were comparable to cowpea (83 – 113 %) but lower than for mucuna (200 – 260 %), sorghum (172- 185 %) and defatted macadamia flour (305 – 493 %) (Abu *et al.*, 2005; Elkhailifa *et al.*, 2005; Jitngarmkusol *et al.*, 2008).

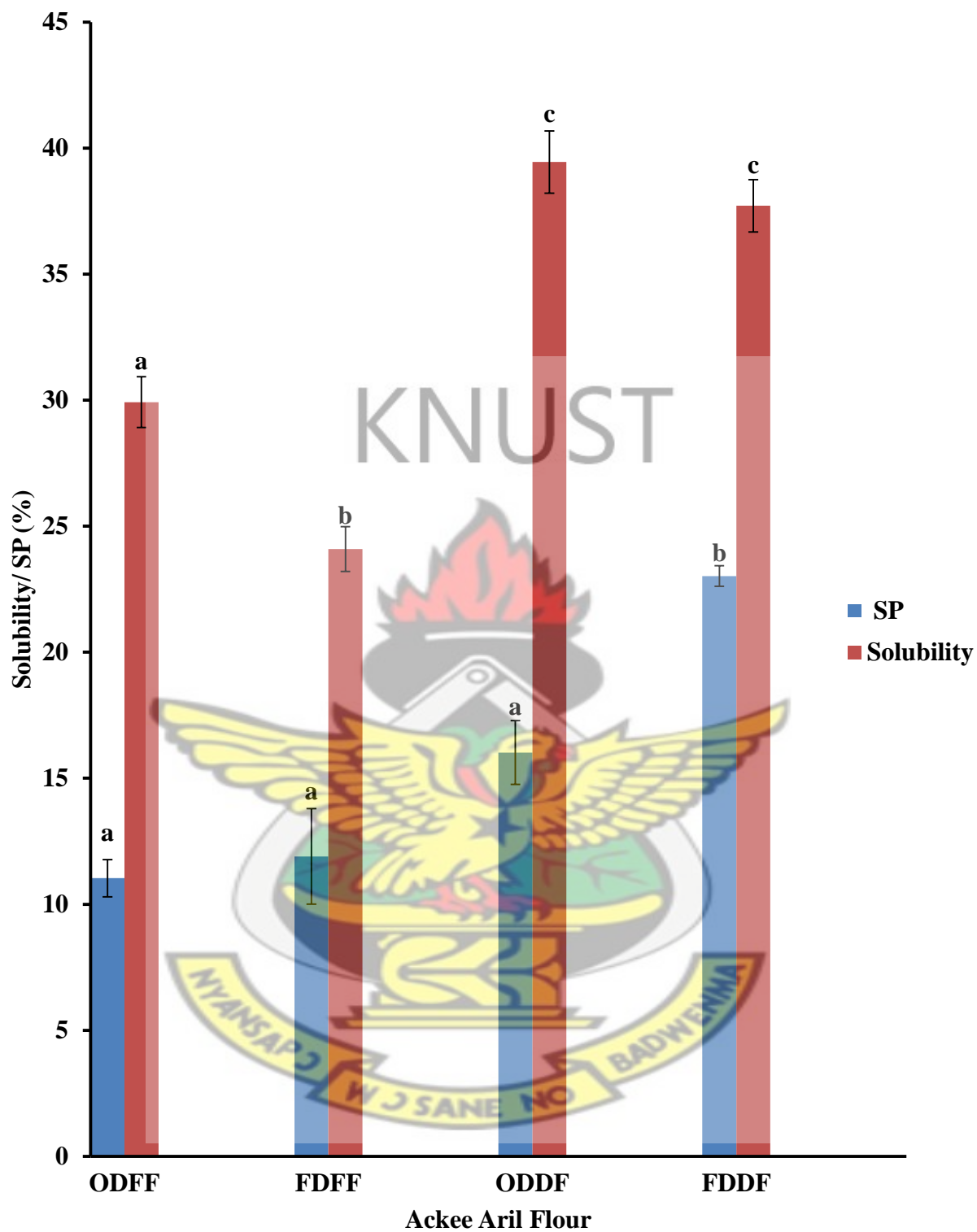
Proteins influence the OAC of food matrices, with increased non-polar amino acid residues enhancing hydrophobicity and fat binding through capillary action. The absorption of oil by protein surfaces increases the hydrophobic interaction of proteins with flavor compounds as well as the binding of food to the inner walls of the mouth during mastication. Thus, OAC of food determines the mouth-feel, flavour retention as well as shelf stability of baked or fried foods and meat products especially (Kinsella, 1976; Adebawale and Lawal, 2004).

#### 4.2.2 Solubility and Swelling Power

Solubility of ackee flours ranged from 24.09 to 39.45 % while swelling power ranged from 11.03 to 23.02 % as shown in Figure 4. 6. Oven-dried flours (ODFF and ODDF) had lower swelling power than the respective freeze-dried flours (FDFF and FDDF). The defatted flours had significantly higher solubility than their corresponding full fat flours. Swelling power increased for the defatted flours but only that of FDDF was significant ( $p < 0.05$ ).

Swelling power of the ackee aril flours were comparable to that of some cassava and sweet potato cultivars, which ranged from 10.59 to 27.53 %. However, the solubility of the ackee aril flours was higher than those reported for the cassava and sweet potato cultivars (12.06 - 24.44 %) (Baah *et al.*, 2005).





**Figure 4. 6: Solubility and Swelling Power (SP) of Ackee Aril Flours**

\*Same coloured bars with different letters are significantly different ( $p < 0.05$ ; Appendix 3)



Solubility and swelling power are influenced by the water binding capacity of the flour sample (which is a function of proteins and carbohydrates present in the flour). A low swelling power in association with high solubility is indicative of weak associative forces within the flour sample, which reduces its elastic and plastic properties. Viscosity is an important sensory attribute of dough, pasta and food gels. For a more elastic and viscous dough, flour with high swelling power in association with low solubility is recommended (Baah *et al.*, 2005). This implies that ackee aril flours may form less viscous gels or dough due their high solubility compared to cassava and sweet potato flours.

Pasta made from cassava or sweet potato composited wheat flour had less firmness in comparison with that of absolute wheat flour, although the cassava and sweet potato had low solubility (Baah *et al.*, 2005). Thus it can be inferred that the ackee aril flours having less potential to form viscous dough due to higher solubility may not be suitable for compositing with wheat flour in pasta formulation. This potential to form less viscous gels and dough could make them suitable as thickeners for sauces and soups and as binders for meat products.

#### **4.2.3 Foaming Capacity and Stability (%)**

Foaming capacity (FC) and foam stability (FS) after 5 h of the ackee aril flours ranged from 4.33 to 5.67 % and 74.36 to 84.35 % respectively (Table 4. 4). Oven-dried flours (ODFF and ODDF) had the highest FC while FDDF had the highest FS. The differences were, however, not statistically significant ( $p > 0.05$ ). The FC and FS values of the ackee aril flours were lower than that of flours of different macadamia cultivars as reported by Jitngarmkusol *et al.* (2008). Although foaming capacities of flours of six mucuna cultivars were higher, the



foaming stabilities after 4 h were lower than that of the ackee aril flour samples (Adebowale *et al.*, 2005).

**Table 4. 4: Foaming Capacity (FC) and Stability (FS) of Ackee Aril Flours**

Sample	ODFF	FDFD	ODDF	FDDF
FC (%)	5.67 (0.58) <sup>a</sup>	5.00 (1.00) <sup>a</sup>	5.67 (0.58) <sup>a</sup>	4.33 (0.58) <sup>a</sup>
FS (%) (after 5 h)	82.65 (1.38) <sup>a</sup>	82.88 (3.28) <sup>a</sup>	76.34 (0.95) <sup>a</sup>	84.35 (3.58) <sup>a</sup>

Note: Values in a row with different letters in superscript are significantly different ( $p < 0.05$ ; Appendix 3)

Values in brackets are standard deviations of triplicate results

Foaming capacity is influenced by the surface activity of proteins. The capacity of proteins to form stable foams with gas, by forming impervious protein films, is an important property in cakes (angel, sponge), soufflés, whipped toppings, fudges, ice cream and marshmallow. High protein content of a flour sample increases the foaming capacity of the flour. Denaturation of proteins increases their solubility and consequently foaming capacity due to the unfolding of the protein structure which allows more protein surface to be exposed and interact with the external environment (Kinsella, 1979). Heat application facilitates the denaturation of proteins. Oven drying, as opposed to freeze drying, employs heat application, which contributes to the denaturation of the proteins in the sample. Thus more proteins were probably denatured in the oven-dried samples allowing more protein surface to be exposed for foam formation, which may have accounted for the higher FC observed for oven-dried ackee aril flours than for freeze-dried ackee aril flours.



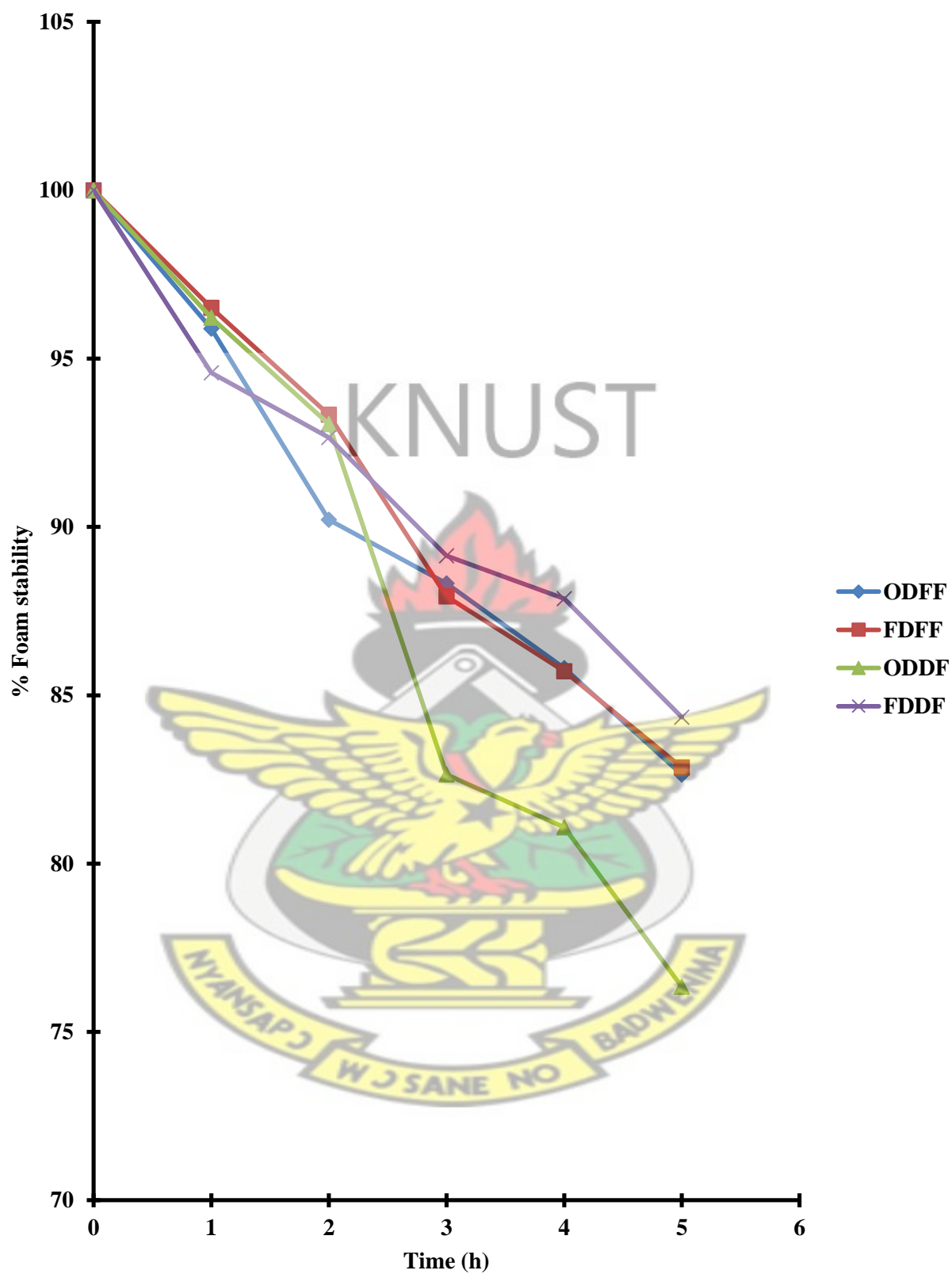


Figure 4. 7: Foam Stability (%) of Ackee Ari Flours at 0.02 g/mL concentration



Generally, a drop in foam stability was observed with time for all ackee aril flour samples (Figure 4. 7). Similar trends were reported for flours of different cultivars of mucuna and for different jackfruit-wheat flour blends (Adebowale *et al.*, 2005; Chowdhury *et al.* 2012). Although FDFF showed superior foam stability to ODFF in the first 2 hours, the foam stabilities of ODFF and FDFF were similar after 3 hours. ODDF showed superior foam stability to FDDF until after 2 h. when its foam stability decreased sharply. FDDF however maintained a slow reduction in foam stability, having markedly higher foam stability than ODDF after 5 hours.

Air-bubble size distribution, air/water surface tension and foam volume fraction all contribute to affect the stability of food foams (Müller-Fischer & Windhab, 2005). It is expected that ODDF, with higher protein content than full fat flours, would generally have better foam stability. ODDF probably had more foam destabilizing factors than the other flour samples.

#### **4.2.4 Emulsion Activity (EA) and Stability (ES) (%)**

Emulsion activity (EA) ranged from 61.67 to 69.17 % for the ackee aril flour samples while emulsion stability (ES) ranged from 5.83 to 46.67 % (Figure 4. 8). There were no significant differences between EA and ES of oven-dried ackee aril flours (ODFF and ODDF). For the freeze-dried ackee aril flours, while EA were not significantly different, FDDF had a significantly higher ES than FDFF. FDFF had the least EA (61.67 %) and ES (5.3 %) among the ackee aril flour samples investigated.

The EA of the ackee aril flours were higher, while ES were lower than those previously reported for partially defatted and totally defatted flours of different macadamia flours (EA from 49.05 to 56.21 % and ES from 50.44 to 54.26 %) (Jitngarmkusol *et al.*, 2008). Also, Plaher *et al.* (1977) reported comparable ES but lower EA for flours of different soybean

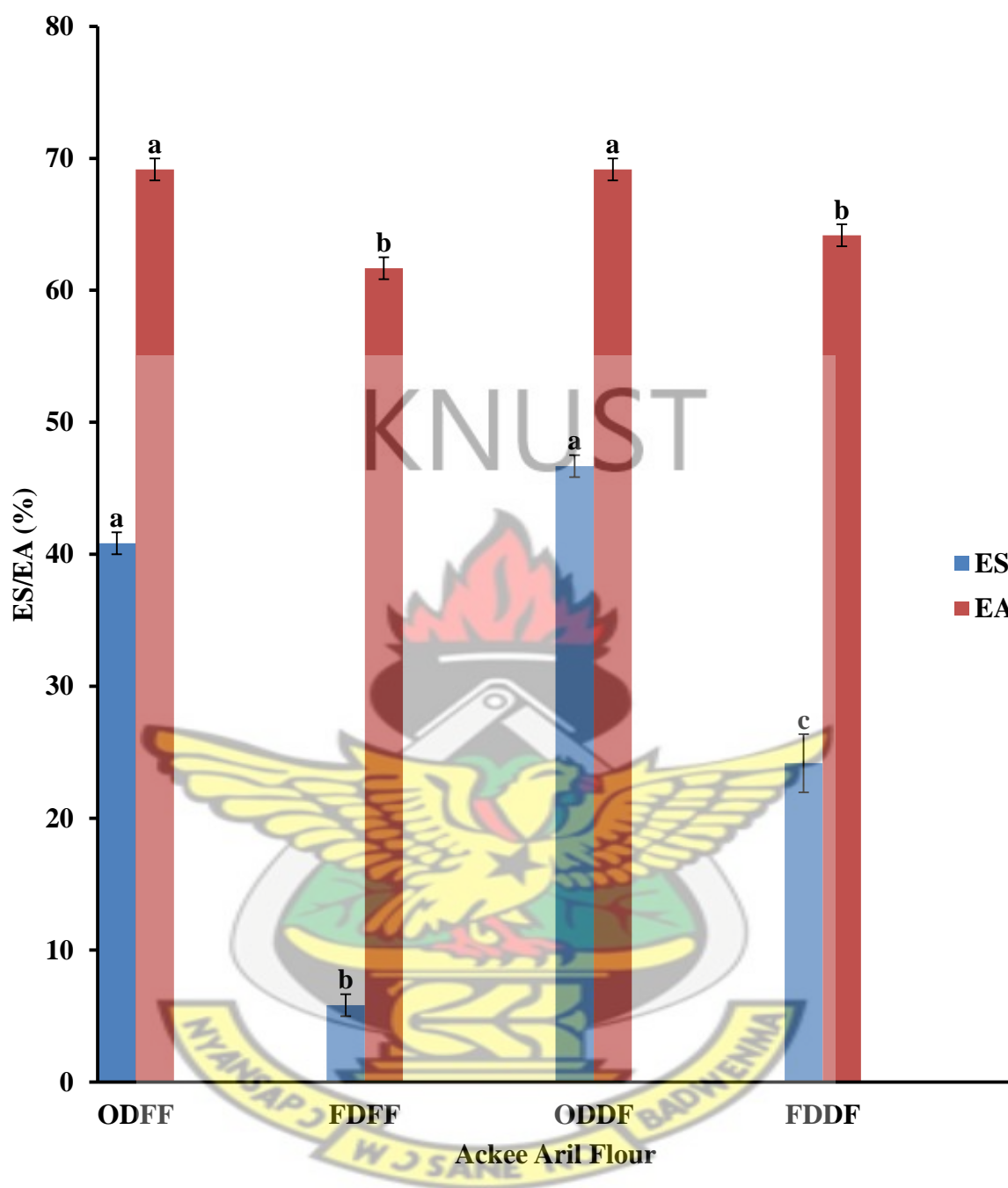


varieties grown in Ghana. The soy flours improved the EA and ES of wheat flour, meat or skimmed milk systems when added to those systems. Thus it can be inferred that the defatted ackee aril flours will have similar effects when complemented with wheat, skimmed milk or meat systems.

Emulsification is a function of proteins in food matrices. However, polysaccharides could contribute to emulsification properties. The proteins decrease the surface tension of the oil droplet by forming a film around the oil surface, interacting through their hydrophobic groups with the oil, while their hydrophilic groups interact with the aqueous phase. The concentration, solubility, structure and distribution of hydrophobic and hydrophilic groups of the protein contribute to emulsification properties of the protein (Friberg *et al.*, 2003). High emulsion activity and stability is important in food emulsions like mayonnaise, yogurts and ice-creams.

Some polysaccharides help increase the viscosity of the emulsion system and thereby stabilize the emulsion (Jitngarmkusol *et al.*, 2008). Thus an increase in protein and carbohydrate content of flour could have positive influence on its EA and ES. This trend was observed for the freeze-dried ackee aril flours (FDDF than FDFF), where EA and ES were higher, although the difference in EA of the two flours was not statistically significant. Oven dried flours (ODFF and ODDF) had significantly higher EA and ES than the freeze-dried flours (FDFF and FDDF). This could be due to thermal denaturation of proteins during oven drying which caused the proteins to unfold, exposing more hydrophobic groups to enhance emulsification.





**Figure 4. 8: Emulsion Activity (EA) and Stability (ES) of Ackee Aril Flours**

Note: Same coloured bars with different letters are significantly different ( $p < 0.05$ ; Appendix

3)



### 4.3 THE EFFECT OF DRYING METHOD AND/OR DEFATTING ON THE PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF ACKEE ARIL FLOURS

As summarized in Table 4. 6, investigations using the univariate ‘Test of Between-Subject Effects’ using SPSS software indicated that the drying method had significant effect on mineral composition as well as crude fat and carbohydrate content but not on the ash, protein and fibre contents as well as oil absorption capacity and foaming stability of the ackee aril flours. Defatting also had significant effects on all parameters investigated except foaming capacity, foaming stability and emulsion capacity values as well as ash and fibre contents of the ackee aril flours. The effect of defatting on the water absorption capacity could not be determined because of inadequate data available for the software to use for the computations

For proximate composition of the ackee aril flours, the interactions between the drying method and defatting generally had no significant effect except for protein composition. Interaction between drying method and defatting was, however, significant for mineral composition (except Ca) as well as swelling power, emulsion stability, tapped and loose bulk densities and Hausner ratios of the ackee aril flours. Full details of the analysis are given in Appendix 4. This implies that defatting of ackee aril flour is necessary in order to improve the proximate, mineral, density, flow and most functional properties of the ackee aril flour, for food and industrial applications, irrespective of the drying method employed.



**Table 4. 5: Significance of Factors on Parameters Investigated for Ackee Aril Flours**

Parameter	Significance of p-values		
	A: Drying Method	B: Defatting	Interaction: AB
Fat (%)	*	*	-
Protein (%)	-	*	*
Ash (%)	-	-	-
Fibre (%)	-	-	-
Carbohydrate (%)	*	*	-
Energy KJ/100g	*	*	-
Ca (mg/100g)	*	*	-
Mg (mg/100g)	-	*	*
P (mg/100g)	*	*	*
K (mg/100g)	*	*	*
Zn (mg/100g)	*	*	*
Na (mg/100g)	*	*	*
WAC (%)	*	X	X
Solubility (%)	*	*	-
Swelling Power (%)	*	*	*
OAC (%)	-	*	-
Foaming Capacity (%)	*	-	-
Foaming Stability (%)	-	-	-
Emulsion Capacity (%)	*	-	-
Emulsion Stability (%)	*	*	*
Tapped Bulk Density (g/mL)	*	*	*
Loose Bulk Density (g/mL)	*	*	*
Hausner Ratio	*	*	*
*: Significant ( $p < 0.05$ )      -: Not significant ( $p > 0.05$ )      x: Not reported			



#### 4.4 CORRELATION BETWEEN SOME PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES

Results of correlation tests (Table 4. 6) showed that the fat content had a negative correlation with all functional properties except foaming capacity and stability, with correlations between fat content and oil absorption capacity, solubility and swelling power being significant ( $p < 0.01$ ). Thus, a reduction in the fat content will yield an increase in the functional properties of the ackee aril flours. Correlations of protein and carbohydrate contents with functional properties were generally positive, with those of oil absorption capacity, solubility and swelling power being significant ( $p < 0.01$ ). This implies that an increase in protein and carbohydrate contents will increase the functional properties of the ackee aril flours.

**Table 4. 6: Correlation of Some Proximate Compositions and Functional Properties of Ackee Aril Flours**

Functional Properties		Fat	Protein	Carbohydrate
OAC	Pearson Correlation	-.904**	.913**	.883**
	Sig. (2-tailed)	.000	.000	.000
Solubility	Pearson Correlation	-.861**	.918**	.812**
	Sig. (2-tailed)	.000	.000	.001
SP	Pearson Correlation	-.838**	.824**	.829**
	Sig. (2-tailed)	.001	.001	.001
FC	Pearson Correlation	.285	-.252	-.274
	Sig. (2-tailed)	.369	.429	.389
FS	Pearson Correlation	.159	-.128	-.201
	Sig. (2-tailed)	.622	.691	.531
EC	Pearson Correlation	-.049	.183	-.016
	Sig. (2-tailed)	.880	.568	.960
ES	Pearson Correlation	-.243	.379	.164
	Sig. (2-tailed)	.446	.224	.610

\*\* - Correlation is significant at the 0.01 level (2-tailed)

N = 12

\* - Correlation is significant at the 0.05 level (2-tailed)



## CHAPTER FIVE

### 5.0 CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 CONCLUSIONS

The ackee aril flour samples investigated had variable physicochemical properties. The results of their proximate and mineral compositions suggest that inclusion of ackee aril flours in the diet could contribute appreciable amount of nutrients to help meet human nutritional needs. Although full fat ackee aril flours showed higher calculated metabolizable energies than the defatted ackee aril flours, the latter, however, could provide significantly higher amounts of proteins and minerals than the full fat ackee aril flours when included in the diet. The high fat content of the full fat ackee aril flours could be exploited for oil production for soap and/or vegetable oil industries.

The ackee aril flour samples showed variations in the bulk densities and Hausner ratios. Oven-dried ackee aril flours had higher bulk densities than freeze-dried ackee aril flours while defatted ackee aril flours had lower Hausner ratios than the full fat ackee aril flours. This implies that smaller storage space would be required for oven-dried ackee aril flours than for freeze dried ackee aril flours while defatted ackee aril flours will present ease of application in food industrial applications due to their high probability to have good flow properties.

The functional properties varied for the different ackee aril flours investigated. The results showed that defatted ackee aril flours had high water absorption capacities which could be beneficial in sausages, processed meats and dough, where water absorption or binding is an important quality attribute. Also, oven-dried ackee aril flours showed superior foaming capacity, emulsion activity and stability, which could prove useful in food applications



requiring high emulsion activity and stability such as sausages and processed meats, mayonnaise, yogurt and ice-cream.

In general, the drying method had significant effect on mineral composition as well as fat and carbohydrate content but not on the ash, protein, fibre contents or oil absorption capacity and foam stability of the flour samples. Also, defatting was significant for all physicochemical properties investigated, except ash and fibre contents. However, defatting had no significant effect on the foaming capacity, foaming stability or emulsion capacity of the flour samples. For proximate composition of the ackee aril flours, the interactions between the drying method and defatting generally had significant effect only on the protein composition of the flours. Interaction between drying method and defatting was shown to be significant for mineral composition (except Ca) as well as swelling power, emulsion stability, tapped and loose bulk densities and Hausner ratios of the ackee aril flours.

Oven drying generally improved the foaming capacity, emulsion activity and emulsion stability of the ackee aril flour, probably due to the denaturation of the proteins which made more protein surfaces available for interaction with the environment and with other protein surfaces.

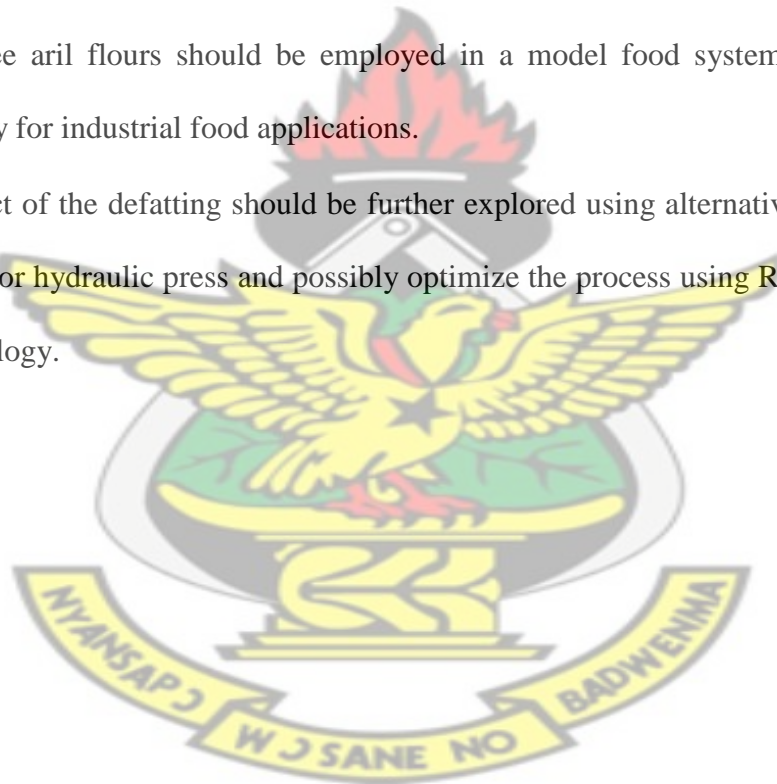
Defatting significantly reduced the fat content of the flours (56.63 % in oven dried flour and 60.66 % in freeze dried flour), which significantly improved the proximate, minerals, bulk density and hausner ratios as well as functional properties of the flour. This makes defatting a necessary step in improving ackee aril flour for food and industrial applications irrespective of the drying method employed



## 5.2 RECOMMENDATIONS

From the observations made and challenges encountered in this study, it is recommended that:

- The ackee aril flours should be screened for anti-nutritional components to ascertain their safety for human consumption
- Shelf life studies should be conducted on the ackee aril flours to establish how long they can be stored for and still be safe for consumption and/or without significant deterioration in nutritional and functional properties.
- The ackee aril flours should be employed in a model food system to assess their suitability for industrial food applications.
- The effect of the defatting should be further explored using alternative methods such as screw or hydraulic press and possibly optimize the process using Response Surface Methodology.





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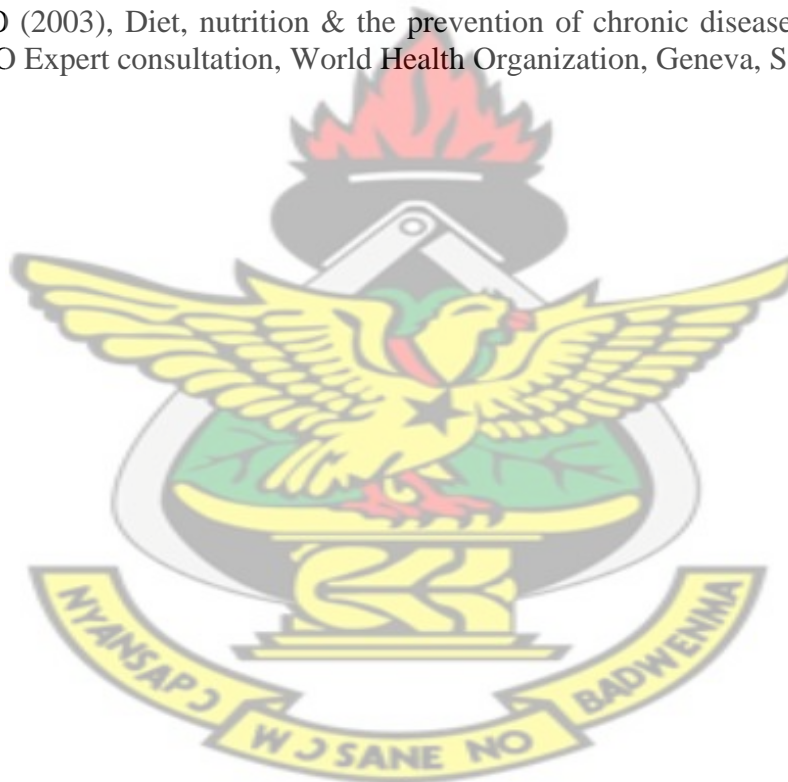
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## APPENDICES

### APPENDIX 1: ONE WAY ANOVA OF PROXIMATE ANALYSIS

#### Appendix 1a: ANOVA -Proximate Composition of Ackee Aril Flours

		Sum of Squares	df	Mean Square	F	Sig.
Moisture %	Between Groups	11.535	3	3.845	162.835	.000
	Within Groups	.189	8	.024		
	Total	11.724	11			
Fat %	Between Groups	3407.398	3	1135.799	323.028	.000
	Within Groups	28.129	8	3.516		
	Total	3435.527	11			
Protein %	Between Groups	340.282	3	113.427	1814.594	.000
	Within Groups	.500	8	.063		
	Total	340.782	11			
Ash %	Between Groups	.717	3	.239	.528	.675
	Within Groups	3.623	8	.453		
	Total	4.341	11			
Fibre %	Between Groups	.138	3	.046	.994	.443
	Within Groups	.369	8	.046		
	Total	.507	11			
Carbohydrate %	Between Groups	1608.474	3	536.158	86.866	.000
	Within Groups	49.378	8	6.172		
	Total	1657.852	11			

#### Appendix 1b: ANOVA of Results of Tapped and lose density and Energy

		Sum of Squares	df	Mean Square	F	Sig.
TBD	Between Groups	.062	3	.021	163.499	.000
	Within Groups	.001	8	.000		
	Total	.063	11			
LBD	Between Groups	.074	3	.025	980.000	.000
	Within Groups	.000	8	.000		
	Total	.074	11			
Energy	Between Groups	1372437.708	3	457479.236	493.215	.000
	Within Groups	7420.368	8	927.546		
	Total	1379858.076	11			



### Appendix 1c: Multiple Comparisons Moisture, Fat and Protein Composition

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Diff (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bnd.	Upper Bnd.
Moisture	Oven dried full fat	Freeze dried full fat	-.37670	.12547	.066	-.7785	.0251
		Oven dried defatted	-.29183	.12547	.171	-.6936	.1100
		Freeze dried defatted	-2.46397*	.12547	.000	-2.8658	-2.0622
	Freeze dried full fat	Oven dried full fat	.37670	.12547	.066	-.0251	.7785
		Oven dried defatted	.08487	.12547	.903	-.3169	.4867
		Freeze dried defatted	-2.08727*	.12547	.000	-2.4891	-1.6855
	Oven dried defatted	Oven dried full fat	.29183	.12547	.171	-.1100	.6936
		Freeze dried full fat	-.08487	.12547	.903	-.4867	.3169
		Freeze dried defatted	-2.17213*	.12547	.000	-2.5739	-1.7703
	Freeze dried defatted	Oven dried full fat	2.46397*	.12547	.000	2.0622	2.8658
		Freeze dried full fat	2.08727*	.12547	.000	1.6855	2.4891
		Oven dried defatted	2.17213*	.12547	.000	1.7703	2.5739
Fat	Oven dried full fat	Freeze dried full fat	5.10333*	1.53103	.042	.2004	10.0062
		Oven dried defatted	33.71667*	1.53103	.000	28.8138	38.6196
		Freeze dried defatted	38.11333*	1.53103	.000	33.2104	43.0162
	Freeze dried full fat	Oven dried full fat	-5.10333*	1.53103	.042	-10.0062	-.2004
		Oven dried defatted	28.61333*	1.53103	.000	23.7104	33.5162
		Freeze dried defatted	33.01000*	1.53103	.000	28.1071	37.9129
	Oven dried defatted	Oven dried full fat	-33.71667*	1.53103	.000	-38.6196	-28.8138
		Freeze dried full fat	-28.61333*	1.53103	.000	-33.5162	-23.7104
		Freeze dried defatted	4.39667	1.53103	.080	-.5062	9.2996
	Freeze dried defatted	Oven dried full fat	-38.11333*	1.53103	.000	-43.0162	-33.2104
		Freeze dried full fat	-33.01000*	1.53103	.000	-37.9129	-28.1071
		Oven dried defatted	-4.39667	1.53103	.080	-9.2996	.5062
Protein	Oven dried full fat	Freeze dried full fat	.72000*	.20414	.032	.0663	1.3737
		Oven dried defatted	-9.78000*	.20414	.000	-10.4337	-9.1263
		Freeze dried defatted	-10.73333*	.20414	.000	-11.3871	-10.0796
	Freeze dried full fat	Oven dried full fat	-.72000*	.20414	.032	-1.3737	-.0663
		Oven dried defatted	-10.50000*	.20414	.000	-11.1537	-9.8463
		Freeze dried defatted	-11.45333*	.20414	.000	-12.1071	-10.7996
	Oven dried defatted	Oven dried full fat	9.78000*	.20414	.000	9.1263	10.4337
		Freeze dried full fat	10.50000*	.20414	.000	9.8463	11.1537
		Freeze dried defatted	-.95333*	.20414	.007	-1.6071	-.2996
	Freeze dried defatted	Oven dried full fat	10.73333*	.20414	.000	10.0796	11.3871
		Freeze dried full fat	11.45333*	.20414	.000	10.7996	12.1071
		Oven dried defatted	.95333*	.20414	.007	.2996	1.6071
Ash	Oven dried full fat	Freeze dried full fat	.54333	.54950	.760	-1.2164	2.3030
		Oven dried defatted	.31667	.54950	.936	-1.4430	2.0764
		Freeze dried defatted	-.06000	.54950	.999	-1.8197	1.6997
	Freeze dried full fat	Oven dried full fat	-.54333	.54950	.760	-2.3030	1.2164
		Oven dried defatted	-.22667	.54950	.975	-1.9864	1.5330
		Freeze dried defatted	-.60333	.54950	.701	-2.3630	1.1564
	Oven dried defatted	Oven dried full fat	.31667	.54950	.936	-2.0764	1.4430
		Freeze dried full fat	.22667	.54950	.975	-1.5330	1.9864
		Freeze dried defatted	-.37667	.54950	.900	-2.1364	1.3830
	Freeze dried defatted	Oven dried full fat	.06000	.54950	.999	-1.6997	1.8197
		Freeze dried full fat	.60333	.54950	.701	-1.1564	2.3630
		Oven dried defatted	.37667	.54950	.900	-1.3830	2.1364

\*. The mean difference is significant at the 0.05 level.



## Appendix 1d: Multiple Comparisons Ash, Crude Fibre and Carbohydrate Composition

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bnd.	Upper Bnd.
Ash	Oven dried full fat	Freeze dried full fat	.54333	.54950	.760	-1.2164	2.3030
		Oven dried defatted	.31667	.54950	.936	-1.4430	2.0764
		Freeze dried defatted	-.06000	.54950	.999	-1.8197	1.6997
	Freeze dried full fat	Oven dried full fat	-.54333	.54950	.760	-2.3030	1.2164
		Oven dried defatted	-.22667	.54950	.975	-1.9864	1.5330
		Freeze dried defatted	-.60333	.54950	.701	-2.3630	1.1564
	Oven defatted	Oven dried full fat	-.31667	.54950	.936	-2.0764	1.4430
		Freeze dried full fat	.22667	.54950	.975	-1.5330	1.9864
		Freeze dried defatted	-.37667	.54950	.900	-2.1364	1.3830
	Freeze defatted	Oven dried full fat	.06000	.54950	.999	-1.6997	1.8197
		Freeze dried full fat	.60333	.54950	.701	-1.1564	2.3630
		Oven dried defatted	.37667	.54950	.900	-1.3830	2.1364
Fibre	Oven dried full fat	Freeze dried full fat	.25000	.17539	.519	-.3117	.8117
		Oven dried defatted	.19667	.17539	.688	-.3650	.7583
		Freeze dried defatted	.02667	.17539	.999	-.5350	.5883
	Freeze dried full fat	Oven dried full fat	-.25000	.17539	.519	-.8117	.3117
		Oven dried defatted	-.05333	.17539	.989	-.6150	.5083
		Freeze dried defatted	-.22333	.17539	.603	-.7850	.3383
	Oven defatted	Oven dried full fat	-.19667	.17539	.688	-.7583	.3650
		Freeze dried full fat	.05333	.17539	.989	-.5083	.6150
		Freeze dried defatted	-.17000	.17539	.770	-.7317	.3917
	Freeze defatted	Oven dried full fat	-.02667	.17539	.999	-.5883	.5350
		Freeze dried full fat	.22333	.17539	.603	-.3383	.7850
		Oven dried defatted	.17000	.17539	.770	-.3917	.7317
Carbohydrate	Oven dried full fat	Freeze dried full fat	-6.61667*	2.02850	.046	-13.1126	-1.1207
		Oven dried defatted	-24.44667*	2.02850	.000	-30.9426	-17.9507
		Freeze dried defatted	-27.34000*	2.02850	.000	-33.8360	-20.8440
	Freeze dried full fat	Oven dried full fat	6.61667*	2.02850	.046	.1207	13.1126
		Oven dried defatted	-17.83000*	2.02850	.000	-24.3260	-11.3340
		Freeze dried defatted	-20.72333*	2.02850	.000	-27.2193	-14.2274
	Oven defatted	Oven dried full fat	24.44667*	2.02850	.000	17.9507	30.9426
		Freeze dried full fat	17.83000*	2.02850	.000	11.3340	24.3260
		Freeze dried defatted	-2.89333	2.02850	.519	-9.3893	3.6026
	Freeze defatted	Oven dried full fat	27.34000*	2.02850	.000	20.8440	33.8360
		Freeze dried full fat	20.72333*	2.02850	.000	14.2274	27.2193
		Oven dried defatted	2.89333	2.02850	.519	-3.6026	9.3893

\*. The mean difference is significant at the 0.05 level.



## Appendix 1e: Multiple Comparisons of Results of Tapped and lose density and Energy

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Diff (I-J)	Std. Error	Sig.	95 % onfidence Interval	
						Lower Bnd	Upper Bnd
TBD	Oven dried full fat	Freeze dried full fat	.16433*	.00914	.000	.1351	.1936
		Oven dried defatted	-.00033	.00914	1.000	-.0296	.0289
		Freeze dried defatted	.11200*	.00914	.000	.0827	.1413
	Freeze dried full fat	Oven dried full fat	-.16433*	.00914	.000	-.1936	-.1351
		Oven dried defatted	-.16467*	.00914	.000	-.1939	-.1354
		Freeze dried defatted	-.05233*	.00914	.002	-.0816	-.0231
	Oven dried defatted	Oven dried full fat	.00033	.00914	1.000	-.0289	.0296
		Freeze dried full fat	.16467*	.00914	.000	.1354	.1939
		Freeze dried defatted	.11233*	.00914	.000	.0831	.1416
	Freeze dried defatted	Oven dried full fat	-.11200*	.00914	.000	-.1413	-.0827
		Freeze dried full fat	.05233*	.00914	.002	.0231	.0816
		Oven dried defatted	-.11233*	.00914	.000	-.1416	-.0831
LBD	Oven dried full fat	Freeze dried full fat	.13000*	.00408	.000	.1169	.1431
		Oven dried defatted	-.06000*	.00408	.000	-.0731	-.0469
		Freeze dried defatted	-.06333*	.00408	.000	-.0764	-.0503
	Freeze dried full fat	Oven dried full fat	-.13000*	.00408	.000	-.1431	-.1169
		Oven dried defatted	-.19000*	.00408	.000	-.2031	-.1769
		Freeze dried defatted	-.19333*	.00408	.000	-.2064	-.1803
	Oven dried defatted	Oven dried full fat	.06000*	.00408	.000	.0469	.0731
		Freeze dried full fat	.19000*	.00408	.000	.1769	.2031
		Freeze dried defatted	-.00333	.00408	.845	-.0164	.0097
	Freeze dried defatted	Oven dried full fat	.06333*	.00408	.000	.0503	.0764
		Freeze dried full fat	.19333*	.00408	.000	.1803	.2064
		Oven dried defatted	.00333	.00408	.845	-.0097	.0164
Energy	Oven dried full fat	Freeze dried full fat	88.51261*	24.86693	.030	8.8800	168.1453
		Oven dried defatted	665.62678*	24.86693	.000	585.9941	745.2594
		Freeze dried defatted	762.79763*	24.86693	.000	683.1650	842.4303
	Freeze dried full fat	Oven dried full fat	-88.51261*	24.86693	.030	-168.1453	-8.8800
		Oven dried defatted	577.11417*	24.86693	.000	497.4815	656.7468
		Freeze dried defatted	674.28502*	24.86693	.000	594.6524	753.9177
	Oven dried defatted	Oven dried full fat	-665.62678*	24.86693	.000	-745.2594	-585.9941
		Freeze dried full fat	-577.11417*	24.86693	.000	-656.7468	-497.4815
		Freeze dried defatted	97.17085*	24.86693	.019	17.5382	176.8035
	Freeze dried defatted	Oven dried full fat	-762.79763*	24.86693	.000	-842.4303	-683.1650
		Freeze dried full fat	-674.28502*	24.86693	.000	-753.9177	-594.6524
		Oven dried defatted	-97.17085*	24.86693	.019	-176.8035	-17.5382
Hausner ratio	Oven dried full fat	Freeze dried full fat	-.13794*	.01673	.000	-.1915	-.0844
		Oven dried defatted	.19643*	.01673	.000	.1428	.2500
		Freeze dried defatted	.42999*	.01673	.000	.3764	.4836
	Freeze dried full fat	Oven dried full fat	.13794*	.01673	.000	.0844	.1915
		Oven dried defatted	.33437*	.01673	.000	.2808	.3880
		Freeze dried defatted	.56793*	.01673	.000	.5143	.6215
	Oven dried defatted	Oven dried full fat	-.19643*	.01673	.000	-.2500	-.1428
		Freeze dried full fat	-.33437*	.01673	.000	-.3880	-.2808
		Freeze dried defatted	.23356*	.01673	.000	.1800	.2871
	Freeze dried defatted	Oven dried full fat	-.42999*	.01673	.000	-.4836	-.3764
		Freeze dried full fat	-.56793*	.01673	.000	-.6215	-.5143
		Oven dried defatted	-.23356*	.01673	.000	-.2871	-.1800

\*. The mean difference is significant at the 0.05 level.



## APPENDIX 2: ONE WAY ANOVA OF MINERAL ANALYSIS

### Appendix 2a: ANOVA for Results of Mineral Analysis

		Sum of Squares	df	Mean Square	F	Sig.
Ca	Between Groups	10800.000	3	3600.000	18.000	.001
	Within Groups	1600.000	8	200.000		
	Total	12400.000	11			
Mg	Between Groups	176266.667	3	58755.556	138.248	.000
	Within Groups	3400.000	8	425.000		
	Total	179666.667	11			
P	Between Groups	43481.735	3	14493.912	210.731	.000
	Within Groups	550.235	8	68.779		
	Total	44031.970	11			
K	Between Groups	578879.665	3	192959.888	142.164	.000
	Within Groups	10858.423	8	1357.303		
	Total	589738.088	11			
Zn	Between Groups	4.933	3	1.644	271.769	.000
	Within Groups	.048	8	.006		
	Total	4.981	11			
Na	Between Groups	13821.995	3	4607.332	55.042	.000
	Within Groups	669.646	8	83.706		
	Total	14491.641	11			



## Appendix 2b: Multiple Comparisons of Results of Minerals (Ca, Mg and P)

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Diff. (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bnd	Upper Bnd
Ca	Oven full fat	Freeze dried full fat	-33.33333	11.54701	.078	-70.3109	3.6442
		Oven dried defatted	-60.00000*	11.54701	.004	-96.9776	-23.0224
		Freeze dried defatted	-80.00000*	11.54701	.001	-116.9776	-43.0224
	Freeze full fat	Oven dried full fat	33.33333	11.54701	.078	-3.6442	70.3109
		Oven dried defatted	-26.66667	11.54701	.175	-63.6442	10.3109
		Freeze dried defatted	-46.66667*	11.54701	.016	-83.6442	-9.6891
	Oven defatted	Oven dried full fat	60.00000*	11.54701	.004	23.0224	96.9776
		Freeze dried full fat	26.66667	11.54701	.175	-10.3109	63.6442
		Freeze dried defatted	-20.00000	11.54701	.369	-56.9776	16.9776
	Freeze defatted	Oven dried full fat	80.00000*	11.54701	.001	43.0224	116.9776
		Freeze dried full fat	46.66667*	11.54701	.016	9.6891	83.6442
		Oven dried defatted	20.00000	11.54701	.369	-16.9776	56.9776
Mg	Oven full fat	Freeze dried full fat	33.33333	16.83251	.271	-20.5703	87.2369
		Oven dried defatted	-173.33333*	16.83251	.000	-227.2369	-119.4297
		Freeze dried defatted	-260.00000*	16.83251	.000	-313.9036	-206.0964
	Freeze full fat	Oven dried full fat	-33.33333	16.83251	.271	-87.2369	20.5703
		Oven dried defatted	-206.66667*	16.83251	.000	-260.5703	-152.7631
		Freeze dried defatted	-293.33333*	16.83251	.000	-347.2369	-239.4297
	Oven defatted	Oven dried full fat	173.33333*	16.83251	.000	119.4297	227.2369
		Freeze dried full fat	206.66667*	16.83251	.000	152.7631	260.5703
		Freeze dried defatted	-86.66667*	16.83251	.004	-140.5703	-32.7631
	Freeze defatted	Oven dried full fat	260.00000*	16.83251	.000	206.0964	313.9036
		Freeze dried full fat	293.33333*	16.83251	.000	239.4297	347.2369
		Oven dried defatted	86.66667*	16.83251	.004	32.7631	140.5703
P	Oven full fat	Freeze dried full fat	3.91333	6.77148	.936	-17.7713	25.5980
		Oven dried defatted	-77.18667*	6.77148	.000	-98.8713	-55.5020
		Freeze dried defatted	-141.67333*	6.77148	.000	-163.3580	-119.9887
	Freeze full fat	Oven dried full fat	-3.91333	6.77148	.936	-25.5980	17.7713
		Oven dried defatted	-81.10000*	6.77148	.000	-102.7847	-59.4153
		Freeze dried defatted	-145.58667*	6.77148	.000	-167.2713	-123.9020
	Oven defatted	Oven dried full fat	77.18667*	6.77148	.000	55.5020	98.8713
		Freeze dried full fat	81.10000*	6.77148	.000	59.4153	102.7847
		Freeze dried defatted	-64.48667*	6.77148	.000	-86.1713	-42.8020
	Freeze defatted	Oven dried full fat	141.67333*	6.77148	.000	119.9887	163.3580
		Freeze dried full fat	145.58667*	6.77148	.000	123.9020	167.2713
		Oven dried defatted	64.48667*	6.77148	.000	42.8020	86.1713

\*. The mean difference is significant at the 0.05 level.



## Appendix 2c: Multiple Comparisons of Results of Minerals (K, Zn and Na)

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Diff. (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bnd	Upper Bnd
K	Oven dried full fat	Freeze dried full fat	13.49667	30.08103	.968	-82.8334	109.8267
		Oven dried defatted	-317.14000*	30.08103	.000	-413.4701	-220.8099
		Freeze dried defatted	-506.07333*	30.08103	.000	-602.4034	-409.7433
	Freeze dried full fat	Oven dried full fat	-13.49667	30.08103	.968	-109.8267	82.8334
		Oven dried defatted	-330.63667*	30.08103	.000	-426.9667	-234.3066
		Freeze dried defatted	-519.57000*	30.08103	.000	-615.9001	-423.2399
	Oven dried defatted	Oven dried full fat	317.14000*	30.08103	.000	220.8099	413.4701
		Freeze dried full fat	330.63667*	30.08103	.000	234.3066	426.9667
		Freeze dried defatted	-188.93333*	30.08103	.001	-285.2634	-92.6033
	Freeze dried defatted	Oven dried full fat	506.07333*	30.08103	.000	409.7433	602.4034
		Freeze dried full fat	519.57000*	30.08103	.000	423.2399	615.9001
		Oven dried defatted	188.93333*	30.08103	.001	92.6033	285.2634
Zn	Oven dried full fat	Freeze dried full fat	-.11000	.06351	.369	-.3134	.0934
		Oven dried defatted	-.97000*	.06351	.000	-1.1734	-.7666
		Freeze dried defatted	-1.56000*	.06351	.000	-1.7634	-1.3566
	Freeze dried full fat	Oven dried full fat	.11000	.06351	.369	-.0934	.3134
		Oven dried defatted	-.86000*	.06351	.000	-1.0634	-.6566
		Freeze dried defatted	-1.45000*	.06351	.000	-1.6534	-1.2466
	Oven dried defatted	Oven dried full fat	.97000*	.06351	.000	.7666	1.1734
		Freeze dried full fat	.86000*	.06351	.000	.6566	1.0634
		Freeze dried defatted	-.59000*	.06351	.000	-.7934	-.3866
	Freeze dried defatted	Oven dried full fat	1.56000*	.06351	.000	1.3566	1.7634
		Freeze dried full fat	1.45000*	.06351	.000	1.2466	1.6534
		Oven dried defatted	.59000*	.06351	.000	.3866	.7934
Na	Oven dried full fat	Freeze dried full fat	9.05333	7.47020	.637	-14.8689	32.9755
		Oven dried defatted	-29.89667*	7.47020	.017	-53.8189	-5.9745
		Freeze dried defatted	-77.90667*	7.47020	.000	-101.8289	-53.9845
	Freeze dried full fat	Oven dried full fat	-9.05333	7.47020	.637	-32.9755	14.8689
		Oven dried defatted	-38.95000*	7.47020	.004	-62.8722	-15.0278
		Freeze dried defatted	-86.96000*	7.47020	.000	-110.8822	-63.0378
	Oven dried defatted	Oven dried full fat	29.89667*	7.47020	.017	5.9745	53.8189
		Freeze dried full fat	38.95000*	7.47020	.004	15.0278	62.8722
		Freeze dried defatted	-48.01000*	7.47020	.001	-71.9322	-24.0878
	Freeze dried defatted	Oven dried full fat	77.90667*	7.47020	.000	53.9845	101.8289
		Freeze dried full fat	86.96000*	7.47020	.000	63.0378	110.8822
		Oven dried defatted	48.01000*	7.47020	.001	24.0878	71.9322

\*. The mean difference is significant at the 0.05 level.



### APPENDIX 3: ONE WAY ANOVA OF FUNCTIONAL PROPERTIES

#### Appendix 3a: ANOVA of Results of Functional Properties

		Sum of Squares	df	Mean Square	F	Sig.
WAC	Between Groups	261266.567	1	261266.567	35.715	.004
	Within Groups	29261.106	4	7315.276		
	Total	290527.673	5			
OAC	Between Groups	1615.103	3	538.368	27.568	.000
	Within Groups	156.230	8	19.529		
	Total	1771.333	11			
Solubility	Between Groups	457.508	3	152.503	46.189	.000
	Within Groups	26.414	8	3.302		
	Total	483.921	11			
Swelling power	Between Groups	269.195	3	89.732	20.236	.000
	Within Groups	35.473	8	4.434		
	Total	304.668	11			
Foam capacity	Between Groups	3.667	3	1.222	2.444	.139
	Within Groups	4.000	8	.500		
	Total	7.667	11			
EC	Between Groups	126.563	3	42.188	20.250	.000
	Within Groups	16.667	8	2.083		
	Total	143.229	11			
ES	Between Groups	3034.896	3	1011.632	194.233	.000
	Within Groups	41.667	8	5.208		
	Total	3076.563	11			



### Appendix 3b: Multiple Comparisons of Results of Functional Properties

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bound	Upper Bound
OAC	Oven dried full fat	Freeze dried full fat	-5.71333	3.60820	.438	-17.2681	5.8414
		Oven dried defatted	-27.81333*	3.60820	.000	-39.3681	-16.2586
		Freeze dried defatted	-23.11000*	3.60820	.001	-34.6647	-11.5553
	Freeze dried full fat	Oven dried full fat	5.71333	3.60820	.438	-5.8414	17.2681
		Oven dried defatted	-22.10000*	3.60820	.001	-33.6547	-10.5453
		Freeze dried defatted	-17.39667*	3.60820	.006	-28.9514	-5.8419
	Oven dried defatted	Oven dried full fat	27.81333*	3.60820	.000	16.2586	39.3681
		Freeze dried full fat	22.10000*	3.60820	.001	10.5453	33.6547
		Freeze dried defatted	4.70333	3.60820	.586	-6.8514	16.2581
	Freeze dried defatted	Oven dried full fat	23.11000*	3.60820	.001	11.5553	34.6647
		Freeze dried full fat	17.39667*	3.60820	.006	5.8419	28.9514
		Oven dried defatted	-4.70333	3.60820	.586	-16.2581	6.8514
Solubility	Oven dried full fat	Freeze dried full fat	5.83333*	1.48363	.018	1.0822	10.5844
		Oven dried defatted	-9.52667*	1.48363	.001	-14.2778	-4.7756
		Freeze dried defatted	-7.79000*	1.48363	.003	-12.5411	-3.0389
	Freeze dried full fat	Oven dried full fat	-5.83333*	1.48363	.018	-10.5844	-1.0822
		Oven dried defatted	-15.36000*	1.48363	.000	-20.1111	-10.6089
		Freeze dried defatted	-13.62333*	1.48363	.000	-18.3744	-8.8722
	Oven dried defatted	Oven dried full fat	9.52667*	1.48363	.001	4.7756	14.2778
		Freeze dried full fat	15.36000*	1.48363	.000	10.6089	20.1111
		Freeze dried defatted	1.73667	1.48363	.660	-3.0144	6.4878
	Freeze dried defatted	Oven dried full fat	7.79000*	1.48363	.003	3.0389	12.5411
		Freeze dried full fat	13.62333*	1.48363	.000	8.8722	18.3744
		Oven dried defatted	-1.73667	1.48363	.660	-6.4878	3.0144
Swelling power	Oven dried full fat	Freeze dried full fat	-8.6667	1.71933	.956	-6.3726	4.6392
		Oven dried defatted	-4.98667	1.71933	.077	-10.4926	.5192
		Freeze dried defatted	-11.98667*	1.71933	.001	-17.4926	-6.4808
	Freeze dried full fat	Oven dried full fat	8.6667	1.71933	.956	-4.6392	6.3726
		Oven dried defatted	-4.12000	1.71933	.155	-9.6259	1.3859
		Freeze dried defatted	-11.12000*	1.71933	.001	-16.6259	-5.6141
	Oven dried defatted	Oven dried full fat	4.98667	1.71933	.077	.5192	10.4926
		Freeze dried full fat	4.12000	1.71933	.155	-1.3859	9.6259
		Freeze dried defatted	-7.00000*	1.71933	.015	-12.5059	-1.4941
	Freeze dried defatted	Oven dried full fat	11.98667*	1.71933	.001	6.4808	17.4926
		Freeze dried full fat	11.12000*	1.71933	.001	5.6141	16.6259
		Oven dried defatted	7.00000*	1.71933	.015	1.4941	12.5059

\*. The mean difference is significant at the 0.05 level.



### Appendix 3c: Multiple Comparisons of Results of Functional Properties

Tukey HSD							
Dependent Variable	(I) Sample	(J) Sample	Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
						Lower Bound	Upper Bound
Foam capacity %	Oven full fat	Freeze dried full fat	.66667	.57735	.669	-1.1822	2.5155
		Oven dried defatted	.00000	.57735	1.000	-1.8489	1.8489
		Freeze dried defatted	1.33333	.57735	.175	-.5155	3.1822
	Freeze full fat	Oven dried full fat	-.66667	.57735	.669	-2.5155	1.1822
		Oven dried defatted	-.66667	.57735	.669	-2.5155	1.1822
		Freeze dried defatted	.66667	.57735	.669	-1.1822	2.5155
	Oven defatted	Oven dried full fat	.00000	.57735	1.000	-1.8489	1.8489
		Freeze dried full fat	.66667	.57735	.669	-1.1822	2.5155
		Freeze dried defatted	1.33333	.57735	.175	-.5155	3.1822
	Freeze defatted	Oven dried full fat	-1.33333	.57735	.175	-3.1822	.5155
		Freeze dried full fat	-.66667	.57735	.669	-2.5155	1.1822
		Oven dried defatted	-1.33333	.57735	.175	-3.1822	.5155
EC %	Oven full fat	Freeze dried full fat	7.50000*	1.17851	.001	3.7260	11.2740
		Oven dried defatted	.00000	1.17851	1.000	-3.7740	3.7740
		Freeze dried defatted	5.00000*	1.17851	.012	1.2260	8.7740
	Freeze full fat	Oven dried full fat	-7.50000*	1.17851	.001	-11.2740	-3.7260
		Oven dried defatted	-7.50000*	1.17851	.001	-11.2740	-3.7260
		Freeze dried defatted	-2.50000	1.17851	.225	-6.2740	1.2740
	Oven defatted	Oven dried full fat	.00000	1.17851	1.000	-3.7740	3.7740
		Freeze dried full fat	7.50000*	1.17851	.001	3.7260	11.2740
		Freeze dried defatted	5.00000*	1.17851	.012	1.2260	8.7740
	Freeze defatted	Oven dried full fat	-5.00000*	1.17851	.012	-8.7740	-1.2260
		Freeze dried full fat	2.50000	1.17851	.225	-1.2740	6.2740
		Oven dried defatted	-5.00000*	1.17851	.012	-8.7740	-1.2260
ES %	Oven full fat	Freeze dried full fat	35.00000*	1.86339	.000	29.0328	40.9672
		Oven dried defatted	-5.83333	1.86339	.055	-11.8006	.1339
		Freeze dried defatted	16.66667*	1.86339	.000	10.6994	22.6339
	Freeze full fat	Oven dried full fat	-35.00000*	1.86339	.000	-40.9672	-29.0328
		Oven dried defatted	-40.83333*	1.86339	.000	-46.8006	-34.8661
		Freeze dried defatted	-18.33333*	1.86339	.000	-24.3006	-12.3661
	Oven defatted	Oven dried full fat	5.83333	1.86339	.055	-.1339	11.8006
		Freeze dried full fat	40.83333*	1.86339	.000	34.8661	46.8006
		Freeze dried defatted	22.50000*	1.86339	.000	16.5328	28.4672
	Freeze defatted	Oven dried full fat	-16.66667*	1.86339	.000	-22.6339	-10.6994
		Freeze dried full fat	18.33333*	1.86339	.000	12.3661	24.3006
		Oven dried defatted	-22.50000*	1.86339	.000	-28.4672	-16.5328

\*. The mean difference is significant at the 0.05 level.



## APPENDIX 4: TWO- WAY ANOVA OF PARAMETERS

### Appendix 4a: Tests of Between-Subjects Effects (Proximate Composition)

Dependent Variable: Moisture (%)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.535 <sup>a</sup>	3	3.845	162.835	0
Intercept	377.494	1	377.494	15986.513	0
Drying method	4.872	1	4.872	206.342	0
Defatting	4.245	1	4.245	179.776	0
Drying method * Defatting	2.418	1	2.418	102.387	0
Error	0.189	8	0.024		
Total	389.218	12			
Corrected Total	11.724	11			
a. R Squared = .984 (Adjusted R Squared = .978)					
Dependent Variable: Crude Fat (%)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3407.398 <sup>a</sup>	3	1135.799	323.028	0
Intercept	19492.304	1	19492.304	5543.729	0
Drying method	67.688	1	67.688	19.251	0.002
Defatting	3339.336	1	3339.336	949.727	0
Drying method * Defatting	0.375	1	0.375	0.107	0.753
Error	28.129	8	3.516		
Total	22927.831	12			
Corrected Total	3435.527	11			
a. R Squared = .992 (Adjusted R Squared = .989)					
Dependent Variable: Crude Protein (%)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	340.282 <sup>a</sup>	3	113.427	1814.594	0
Intercept	3554.898	1	3554.898	56870.779	0
Drying method	0.041	1	0.041	0.653	0.442
Defatting	338.141	1	338.141	5409.532	0
Drying method * Defatting	2.1	1	2.1	33.596	0
Error	0.5	8	0.063		
Total	3895.679	12			
Corrected Total	340.782	11			
a. R Squared = .999 (Adjusted R Squared = .998)					
Dependent Variable: Ash (%)					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.717 <sup>a</sup>	3	0.239	0.528	0.675
Intercept	927.873	1	927.873	2048.623	0
Drying method	0.021	1	0.021	0.046	0.836
Defatting	0.062	1	0.062	0.136	0.722
Drying method * Defatting	0.635	1	0.635	1.402	0.27
Error	3.623	8	0.453		
Total	932.213	12			
Corrected Total	4.341	11			
a. R Squared = .165 (Adjusted R Squared = -.148)					



#### Appendix 4b: Tests of Between-Subjects Effects (Proximate Composition)

<b>Dependent Variable: Crude Fibre (%)</b>							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.		
Corrected Model	.138 <sup>a</sup>	3	0.046	0.994	0.443		
Intercept	188.021	1	188.021	4074.86	0		
Drying method	0.005	1	0.005	0.104	0.755		
Defatting	0.001	1	0.001	0.012	0.917		
Drying method * Defatting	0.132	1	0.132	2.867	0.129		
Error	0.369	8	0.046				
Total	188.528	12					
Corrected Total	0.507	11					
a. R Squared = .272 (Adjusted R Squared = -.002)							
<b>Dependent Variable: Carbohydrate (%)</b>							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.		
Corrected Model	1608.474 <sup>a</sup>	3	536.158	86.866	0		
Intercept	10609.448	1	10609.448	1718.904	0		
Drying method	67.83	1	67.83	10.99	0.011		
Defatting	1530.247	1	1530.247	247.925	0		
Drying method * Defatting	10.397	1	10.397	1.685	0.23		
Error	49.378	8	6.172				
Total	12267.3	12					
Corrected Total	1657.852	11					
a. R Squared = .970 (Adjusted R Squared = .959)							
<b>Dependent Variable: Energy (KJ/100g)</b>							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.		
Corrected Model	1372437.708 <sup>a</sup>	3	457479.24	493.215	0		
Intercept	62889037.32	1	62889037	67801.528	0		
Drying method	25858.761	1	25858.761	27.879	0.001		
Defatting	1346522.723	1	1346522.7	1451.704	0		
Drying method * Defatting	56.224	1	56.224	0.061	0.812		
Error	7420.368	8	927.546				
Total	64268895.4	12					
Corrected Total	1379858.076	11					
a. R Squared = .995 (Adjusted R Squared = .993)							



#### Appendix 4c: Tests of Between-Subjects Effects (Mineral Composition)

Dependent Variable: Ca						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	10800.000 <sup>a</sup>	3	3600	18	0.001	
Intercept	529200	1	529200	2646	0	
Drying method	2133.333	1	2133.333	10.667	0.011	
Defatting	8533.333	1	8533.333	42.667	0	
Drying method * Defatting	133.333	1	133.333	0.667	0.438	
Error	1600	8	200			
Total	541600	12				
Corrected Total	12400	11				
a. R Squared = .871 (Adjusted R Squared = .823)						
Tests of Between-Subjects Effects						
Dependent Variable: Mg						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	176266.667 <sup>a</sup>	3	58755.556	138.248	0	
Intercept	1280533.333	1	1280533.3	3013.02	0	
Drying method	2133.333	1	2133.333	5.02	0.055	
Defatting	163333.333	1	163333.33	384.314	0	
Drying method * Defatting	10800	1	10800	25.412	0.001	
Error	3400	8	425			
Total	1460200	12				
Corrected Total	179666.667	11				
a. R Squared = .981 (Adjusted R Squared = .974)						
Tests of Between-Subjects Effects						
Dependent Variable: P						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	43481.735 <sup>a</sup>	3	14493.912	210.731	0	
Intercept	514900.327	1	514900.33	7486.263	0	
Drying method	2751.847	1	2751.847	40.01	0	
Defatting	37220.969	1	37220.969	541.165	0	
Drying method * Defatting	3508.92	1	3508.92	51.017	0	
Error	550.235	8	68.779			
Total	558932.296	12				
Corrected Total	44031.97	11				
a. R Squared = .988 (Adjusted R Squared = .983)						



#### Appendix 4d: Tests of Between-Subjects Effects (Mineral Composition)

Appendix 14: Table of Subject Effects (Continued)

Dependent Variable: K						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	578879.665 <sup>a</sup>	3	192959.89	142.164	0	
Intercept	5300995.834	1	5300995.8	3905.536	0	
Drying method	23083.518	1	23083.518	17.007	0.003	
Defatting	525062.718	1	525062.72	386.843	0	
Drying method * Defatting	30733.429	1	30733.429	22.643	0.001	
Error	10858.423	8	1357.303			
Total	5890733.921	12				
Corrected Total	589738.088	11				
a. R Squared = .982 (Adjusted R Squared = .975)						

Dependent Variable: Zn						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	4.933 <sup>a</sup>	3	1.644	271.769	0	
Intercept	84.27	1	84.27	13928.926	0	
Drying method	0.367	1	0.367	60.744	0	
Defatting	4.392	1	4.392	726	0	
Drying method * Defatting	0.173	1	0.173	28.562	0.001	
Error	0.048	8	0.006			
Total	89.251	12				
Corrected Total	4.981	11				
a. R Squared = .990 (Adjusted R Squared = .987)						

Dependent Variable: Na						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	13821.995 <sup>a</sup>	3	4607.332	55.042	0	
Intercept	154471.252	1	154471.25	1845.407	0	
Drying method	1138.216	1	1138.216	13.598	0.006	
Defatting	10241.61	1	10241.61	122.352	0	
Drying method * Defatting	2442.168	1	2442.168	29.176	0.001	
Error	669.646	8	83.706			
Total	168962.893	12				
Corrected Total	14491.641	11				
a. R Squared = .954 (Adjusted R Squared = .936)						



#### Appendix 4e: Tests of Between-Subjects Effects (Functional Properties)

Dependent Variable: WAC						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	261266.567 <sup>a</sup>	1	261266.57	35.715	0.004	
Intercept	3596347.44	1	3596347.4	491.622	0	
Drying method	261266.567	1	261266.57	35.715	0.004	
Defatting	0	0	.	.	.	
Drying method * Defatting	0	0	.	.	.	
Error	29261.106	4	7315.276			
Total	3886875.113	6				
Corrected Total	290527.673	5				
a. R Squared = .899 (Adjusted R Squared = .874)						
Dependent Variable: OAC						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	1615.103 <sup>a</sup>	3	538.368	27.568	0	
Intercept	190257.565	1	190257.57	9742.463	0	
Drying method	0.765	1	0.765	0.039	0.848	
Defatting	1532.958	1	1532.958	78.498	0	
Drying method * Defatting	81.38	1	81.38	4.167	0.076	
Error	156.23	8	19.529			
Total	192028.898	12				
Corrected Total	1771.333	11				
a. R Squared = .912 (Adjusted R Squared = .879)						
Dependent Variable: Solubility						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	457.508 <sup>a</sup>	3	152.503	46.189	0	
Intercept	12905.488	1	12905.488	3908.691	0	
Drying method	42.979	1	42.979	13.017	0.007	
Defatting	401.942	1	401.942	121.736	0	
Drying method * Defatting	12.587	1	12.587	3.812	0.087	
Error	26.414	8	3.302			
Total	13389.41	12				
Corrected Total	483.921	11				
a. R Squared = .945 (Adjusted R Squared = .925)						
Dependent Variable: SP						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	269.195 <sup>a</sup>	3	89.732	20.236	0	
Intercept	2880.521	1	2880.521	649.621	0	
Drying method	46.413	1	46.413	10.467	0.012	
Defatting	194.569	1	194.569	43.879	0	
Drying method * Defatting	28.213	1	28.213	6.363	0.036	
Error	35.473	8	4.434			
Total	3185.189	12				
Corrected Total	304.668	11				
a. R Squared = .884 (Adjusted R Squared = .840)						



#### Appendix 4f: Tests of Between-Subjects Effects (Functional Properties)

Dependent Variable: FC						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	3.667 <sup>a</sup>	3	1.222	2.444	0.139	
Intercept	320.333	1	320.333	640.667	0	
Drying method	3	1	3	6	0.04	
Defatting	0.333	1	0.333	0.667	0.438	
Drying method * Defatting	0.333	1	0.333	0.667	0.438	
Error	4	8	0.5			
Total	328	12				
Corrected Total	7.667	11				
a. R Squared = .478 (Adjusted R Squared = .283)						
Dependent Variable: FS						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	74.522 <sup>a</sup>	3	24.841	2.682	0.118	
Intercept	86948.178	1	86948.178	9385.857	0	
Drying method	34.007	1	34.007	3.671	0.092	
Defatting	5.153	1	5.153	0.556	0.477	
Drying method * Defatting	35.362	1	35.362	3.817	0.086	
Error	74.11	8	9.264			
Total	87096.811	12				
Corrected Total	148.632	11				
a. R Squared = .501 (Adjusted R Squared = .314)						
Dependent Variable: EC						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	126.562 <sup>a</sup>	3	42.187	20.25		
Intercept	52338.021	1	52338.021	25122.25		
Drying method	117.188	1	117.188	56.25		
Defatting	4.688	1	4.688	2.25		
Drying method * Defatting	4.688	1	4.688	2.25		
Error	16.667	8	2.083			
Total	52481.25	12				
Corrected Total	143.229	11				
a. R Squared = .884 (Adjusted R Squared = .840)						
Dependent Variable: ES						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	3034.896 <sup>a</sup>	3	1011.632	194.233	0	
Intercept	10354.688	1	10354.688	1988.1	0	
Drying method	2479.688	1	2479.688	476.1	0	
Defatting	438.021	1	438.021	84.1	0	
Drying method * Defatting	117.188	1	117.188	22.5	0.001	
Error	41.667	8	5.208			
Total	13431.25	12				
Corrected Total	3076.563	11				
a. R Squared = .986 (Adjusted R Squared = .981)						



#### Appendix 4g: Tests of Between-Subjects Effects (TBD, LBD and Hausner Ratio)

Dependent Variable: TBD					
Source	Type III Sum of Squares	df	Mean Square	F	
Corrected Model	.062 <sup>a</sup>	3	0.021	163.499	
Intercept	5.039	1	5.039	40176.861	
Drying method	0.057	1	0.057	457.741	
Defatting	0.002	1	0.002	16.587	
Drying method * Defatting	0.002	1	0.002	16.17	
Error	0.001	8	0		
Total	5.101	12			
Corrected Total	0.063	11			
a. R Squared = .984 (Adjusted R Squared = .978)					
Dependent Variable: LBD					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.074 <sup>a</sup>	3	0.024	980	0
Intercept	2.271	1	2.271	90828	0
Drying method	0.012	1	0.012	481.333	0
Defatting	0.048	1	0.048	1925.333	0
Drying method * Defatting	0.013	1	0.013	533.333	0
Error	0	8	2.50E-05		
Total	2.344	12			
Corrected Total	0.074	11			
a. R Squared = .997 (Adjusted R Squared = .996)					
Dependent Variable: Hausner ratio					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.549 <sup>a</sup>	3	0.183	435.371	0
Intercept	27.752	1	27.752	66077.204	0
Drying method	0.007	1	0.007	16.33	0.004
Defatting	0.438	1	0.438	1043.323	0
Drying method * Defatting	0.104	1	0.104	246.46	0
Error	0.003	8	0		
Total	28.304	12			
Corrected Total	0.552	11			
a. R Squared = .994 (Adjusted R Squared = .992)					