

**CHEMICAL, FUNCTIONAL AND PROCESSING CHARACTERISTICS OF  
VARIETIES OF MELONSEEDS: AGUSHIE (CUCUMEROPSIS EDULIS  
HOOK.F) AND NERI (CITRULLUS LANATUS Var. NERI. THUNB)**

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By

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for the Degree of Master of Philosophy  
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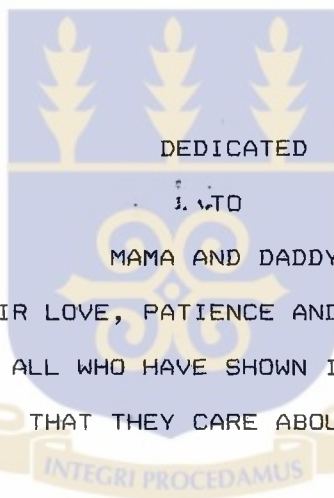
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DEDICATED  
TO  
MAMA AND DADDY  
FOR THEIR LOVE, PATIENCE AND UNDERSTANDING  
AND TO ALL WHO HAVE SHOWN IN VARIOUS WAYS  
THAT THEY CARE ABOUT ME.

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### ABSTRACT

Melonseeds namely Agushie variety 1 and variety 2 (Cucumeropsis edulis) and Neri (Citrullus lanatus) were studied. These were distinguished from each other by the colour of their seedcoat and their size.

Neri was found to have the lowest protein content (19.62%) compared to approximately 28% for the agushie. All three melonseeds contained approximately 47% oil. There was very little carbohydrate in all the varieties and starch was absent.

The oils extracted from the three varieties of melonseeds were pale yellow in colour. They had similar physicochemical characteristics. Their high iodine and saponification values suggest the presence of polyunsaturated fatty acids.

Distilled water, 5% sodium chloride and 0.4% sodium hydroxide solutions were used as protein extracting solvents. All three varieties showed very low protein solubility in water. Solubility in 5% sodium chloride was high and nearly all the protein was extracted by 0.4% sodium chloride in all the varieties. On the basis of pH, agushie variety 1 and neri had their lowest solubility at pH 3.0, and variety 2 had its lowest protein solubility at pH 4.0. The maximum protein solubility in sodium

chloride solutions occurred at ionic strength 0.6 for all the varieties.

Roasting at 140°C did not reduce protein solubility significantly but roasting at 160°C reduced protein solubility considerably. In water, solubility did not decrease when roasting temperature was increased from 160°C to 180°C. In sodium chloride solution however, solubility was further decreased when roasting temperature increased to 180°C.

Water absorption capacities of the melonseed flours were low. Agushie variety 2 had the highest water absorption capacity (WAC) at all ionic strengths. Increasing the incubation temperature above 50°C increased the WAC for all the agushie varieties considerably. The increase for neri was not great. Roasting at 140°C decreased WAC of the agushie but this increased on roasting at 160°C. It remained the same when roasting was at 180°C.

The fat absorption capacities of the three varieties were higher than their water absorption capacities. Roasting caused a decrease in fat absorption capacity. This was for all three varieties.

The melonseed flours showed good foaming properties. Foams of good structure and stability were obtained at pH 3.0. Foaming in sodium chloride solutions was better than in distilled water. Roasted samples showed very poor foaming properties in water though foams formed at pH 11.0 were very good. The temperature of whipping did not have a marked effect on the initial foam

volumes of neri and agushie variety 2. Whipping temperatures above 50°C however increased the foam volume of agushie variety 1 considerably. Stability at 5°C and 80°C was poor for all samples.

Emulsifying capacities of the three varieties were also good. When pH was varied the lowest emulsifying capacities were obtained at pH 4.0 for agushie varieties 1 and 2 and at pH 10.0 for neri. Emulsifying capacities of the three varieties were not markedly different from each other. Roasting above 140°C, and boiling drastically reduced emulsifying capacities of all the varieties. All the varieties showed good emulsifying stabilities.

Coagulation studies indicated that all varieties showed lowest coagulation temperature in sodium chloride solutions of ionic strength 0.2. On the basis of pH, the best coagulation temperature and biggest clots were obtained in slurries at the natural pH (6.2 to 6.8). No coagulation occurred at very acid and alkaline pH's. Slurries of roasted samples (160°C and 180°C) prepared with distilled water did not coagulate when heated. Coagulation however occurred on addition of sodium chloride.

An emulsion product was formulated, using agushie as emulsifier. The product did not have good shelf stability. Addition of cooked rice flour helped to reduce the separation of water from the emulsion. Stability was better when stored in the cold. A baby food product was also formulated. Agushie and rice flour on the proportion 3:1 was found to give the best product. Storage

under cold temperature improved shelf stability. Freezing and thawing gave the product an undesirable change of consistency and texture. More work needs to be done to improve both products to make them more acceptable and shelf-stable.

Examination of the microstructure of the cotyledons of the agushie varieties and neri, showed that their microstructure was very similar. Oil and protein bodies occurred in all the cells except the inner and outer epidermal cells.



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## 1. INTRODUCTION

### 1:1 World Food Supply.

There is a need to supply more food to the growing populations of the world. The rate of population growth increases each year, though food supply does not increase at the same rate for most countries. At the current population growth rate, the increase in food supplies should be over 50% if there is to be any meaningful improvement in the nutritional status of the undernourished populations of the world.

In most developing countries, the inadequate food supplies coupled with high prices of food especially animal products has created an increasing problem of protein-calorie malnutrition. The sectors of the population most affected being infants, pregnant and lactating mothers.

The emphasis on cash crops for export in many developing countries has led to the neglect of the production of a variety of highly nutritious foods. This means many crops of potential value in improving the nutritional status of the diet are underexploited. They are not cultivated in large enough quantities to form a significant part of the diet. Their scarcity leads to their being expensive and this is an additional

factor for their limited use. Most vegetable protein foods like legumes and oilseeds fall into this category

Table 1 shows the production of food crops in developing countries for a ten-year period. It can be seen from the annual change(%)<sup>a</sup> that there is no consistent increase in food production. In some years there is actually a reduction in food production compared to the previous year's production. This would mean an increase in malnutrition of large segments of the populations in these countries. The Table gives a general overview of food production in developing countries as a group. Individual countries may however show actual food production figures higher or lower than the average values given. The actual supply of food to different segments of the population would also vary from country to country. This means the proportion of undernourished persons differs in individual developing countries.

The problem of hunger and malnutrition in any country can only be dealt with successfully by increasing the supply of the right kind of food, improving the stability of food supplies and reducing poverty. The population growth rate would also have to be decreased.

### 1:2 Protein Demand and Supply

According to Hoshai (1984), the total protein supply needed for the future is estimated to increase from 108.2 million tonnes in

Table 1

Production of major crops in developing countries  
1971 to 1982

<u>Year</u>	<u>*Production (Tonnesx10<sup>6</sup>)</u>	<u>Annual change %</u>
1971	417.4	-
1972	407.1	-0.5
1973	423.2	4.0
1974	424.1	0.2
1975	461.8	8.9
1976	475.5	3.0
1977	478.7	0.7
1978	500.6	4.6
1979	487.5	-2.6
1980	505.8	3.7
1981	547.1	8.2
1982	549.2	-1.1

\* Production includes cereals, roots and tubers, pulses, groundnuts, bananas and plantains. Rice in milled form and the non-cereals are converted to cereal equivalents.

Source: Paulino and Mellor, 1984.

1978-80, to about 180 million tonnes by the year 2000. Vegetable protein is expected to increase from 71 million tonnes to 100 million tonnes or more whilst animal protein will increase from 37 million tonnes to about 70 million tonnes and over. If this trend is maintained, nutritional improvement at the world average might be slightly better, provided population growth rate is checked. In Africa and the Far East where nutritional improvement is most needed, the increase would have to be above the world average for a significant impact to be felt.

For a significant improvement in the quality of the diet to be seen, there is a need to direct agricultural policies towards utilisation of all available sources of protein, i.e. both animal and plant. For most people however, animal products are either too expensive, scarce or under-utilised because of ignorance and taboos. The main source of protein for developing countries is therefore from plants. Plant sources supply about 70% of the world's edible protein. Fifty percent of this is from cereals and the rest from legumes and oilseeds. Roots and tubers contribute very little protein. It is obvious that in places where roots and tubers are the staple food, protein deficiency would be high.

Nutritionally, animal protein is of better quality than plant protein. The amino acid composition of animal protein is such that its amino acid content is in the right proportions needed by

the body. Plant protein on the other hand does not contain all the essential amino acids in the right proportions. All cereal grains for example are deficient in lysine. Corn protein is deficient in tryptophan as well and wheat protein in threonine and methionine. Leguminous seeds whilst being rich in lysine are deficient in the sulfur amino acids. It is therefore important to encourage diversification in the cultivation of plant protein sources. This would ensure the utilisation of different kinds of plant protein to supplement each other. In this way the imbalance of plant protein essential amino acid in the diet would be eliminated. Increase in the direct use of legumes and oilseeds to supplement cereals would go a long way in alleviating protein malnutrition.

There is a large variety of plant protein foods in Africa which if produced in larger quantities and utilised effectively would significantly improve the nutritional quality of the diet. Melonseeds, from the family curcubitaceae, are one such group. They are still under-exploited despite their potential value as suppliers of much needed protein.

### 1:3 Status and Potential of Melonseeds

Melonseeds (Family-Curcubitaceae) whose utilisation is confined mainly to Africa have received hardly any attention in terms of research, compared to other oilseeds such as soybeans and groundnuts. As early as 1946, 1948, Curtis suggested the use of

curcubituos plants as a source of oil and protein for human use. After 40 years comparatively little use is made of them. Research studies by Food Scientists and Agronomists both in Africa and in the developed countries are minimal.

In Ghana, melons are grown as a minor crop. Few farmers grow them because of the intensive care they need and the laborious post-harvest treatment. Melonseeds are therefore not abundant on the market, consequently they are comparatively expensive. They are therefore not utilised on a large scale. If cost is brought down (through increase in cultivation) they would become an important part of the diet as they are desirable to most people in all parts of the country.

This is an area where Agronomists and Food Scientists can co-operate in research work to develop varieties which are easy to grow, have high yields and still possess desirable functional and processing characteristics.

If melonseeds are to be utilised in other ways apart from direct incorporation into soups and stews they must be investigated fully. Some of the research areas are:

- (1) Fundamental physical and chemical characteristics of melonseeds as related to their functional properties.
- (2) Determination of their functional properties with respect to their use in food.



(3) Modification of melonseed protein by chemical or enzymatic use.

To ensure correct processing methods and effective utilisation of melonseeds, an understanding of the molecular structure and physico-chemical interaction and inter-relationship of the various components of melonseeds may be necessary.

Clearly a lot of work needs to be done on melonseeds if they are to play an important role in solving the protein problem of Africa and even the rest of the world.

#### 1.4 Objectives

Agushie, (Cucumeropsis edulis) and neri, (Citrullis lanatus var. neri) have not been studied in any detail inspite of the important role they play in the West African diet. The two are of the same family but are grown under different climatic conditions. A comparision of the chemical, functional and processing characteristics would show whether they can be substituted for each other. If so, one can suggest the wider cultivation and utilisation of neri, since it requires less water for cultivation and does not demand as much attention as agushie. An evaluation of the functional characteristics of agushie and neri would show the potential they have for formulation and utilisation in protein foods. Funtional properties would be related to processing.

### 1:5 Specific Aims

- (1) To study the chemical characteristics of oil, carbohydrate and proteins of agushie and neri.
- (2) To study the functional characteristics of
  - (a) Undefined meal: (raw and roasted)
  - (b) Defatted meal: (raw and roasted)
- (3) To study the microstructure of the seeds using light microscopy.
- (4) To formulate an emulsion-type product and baby-food product from agushie.

## 2. REVIEW OF LITERATURE

### 2:1 Plant Protein Sources

Cereals, legumes and oilseeds are the most important sources of plant protein. About 70% of the world's protein supply is from these sources. Kohler and Lyon (1977) categorised plant protein into three groups. (Table 2). The nonendospermous seeds include the oilseeds in which the cotyledons make up the bulk of the seed. In starchy seeds, the endosperm makes up the major part of the seed. The difference between the oilseeds and legumes is that legumes have a low fat content (2-5%) and a high carbohydrate content (55-60%). Oilseeds have a high fat content (20-70%) and low carbohydrate with little or no starch. Oilseeds therefore serve the dual function of supplying protein as well as valuable vegetable oil.

### 2:2 Vegetable Oils and their Sources

#### 2:2:1 General

Vegetable oil-bearing materials can be divided into two groups

- (1) Fruits and nuts from trees: e.g. coconut, palm fruit, olive, palmkernel and babassu.
- (2) Seeds and nuts: e.g. Soybeans, sunflower seed, peanuts, cottonseed, safflower seed, rapeseed and melonseed. (Langstraat, 1976).

More than a hundred (100) varieties of plants are known to have oil-bearing seeds, but only a few are of commercial value. Fewer yet are used for human consumption.

Table 2. Plant Protein Sources

## A. \_ Nonendospermous "seeds" - usually achenes

soybean sunflowerseed sesameseed peanuts

cottonseed castorseed linseed Melonseed

rapeseed

## B Endospermous "seeds" usually caryopses

Wheat Triticale Oats

Rice Maize Coconut

## C Leafy plants

Alfalfa Clover Vegetable wastes

Aquatic plants Grasses Miscellaneous

Source: Kohler and Lyon, 1977.

The oil obtained from the different sources have different properties. Chemically, oils are triglycerides i.e. esters of glycerol and fatty acids. Since they all share the glycerol part, the differences in their properties are determined largely by their fatty acid content. For practical purposes it can be said that for vegetable oils, the carbon chain lengths of the fatty acids vary from 12 to 22 carbon atoms, with up to three double bonds. (Langstraat, 1976). Vegetable oils can therefore be classified according to their predominant fatty acids (Table 3).

In recent years special attention has been paid to the polyunsaturated fatty acids. The most important one being linoleic acid. The essential fatty acids cannot be synthesised by the human body in sufficient quantities and must therefore be supplied in the diet. Polyunsaturated essential fatty acids are thought to lower blood cholesterol levels and so help to reduce one of the risk factors involved in atherosclerosis. Vegetable oils which supply linoleic acid in large amounts are therefore nutritionally desirable for food use. Melonseed oil falls into this category and is therefore a nutritionally valuable oil whose use must be encouraged.

#### **2:2:2      Melonseed Oil**

The fatty acid composition of Colocynthis citrullus (egusi) melonseeds is shown in Table 4.



Linoleic acid is clearly the most abundant fatty acid in the melonseed oil. Its total saturated fatty acid content is low. The highly unsaturated fatty acid, linolenic acid is present in miniscule amounts. This means the oil would be stable to deterioration caused by oxidative rancidity. Apart from being nutritionally valuable, the oil is also more shelf-stable than other oils which have a higher linolenic acid content e.g. soybean oil. Its total saturated fatty acid content is also low.

Sawaya et. al. (1983) extracted edible oil from the Citrullus colocynthis melons grown in the desert regions of Africa and Asia. This oil also had a high linoleic acid content, and was relatively shelf-stable due to absence of linolenic acid.

## 2:3 Nomenclature, General Morphology and Varietal Differences of Agushie and Neri.

### 2:3:1 Nomenclature.

The family Curcubitaceae includes some important crops such as melons. Some melons have a sweet edible pulp. Other melons have

an inedible bitter fruit pulp. These are grown for their valuable seeds. Agushie (Cucumeropsis edulis) and neri (Citrullus lanatus var. neri) belong to this latter group.

There is some confusion in the literature as to nomenclature of melons grown solely for their seeds. They are variously referred to as Citrullus vulgaris Schrad; Citrullus lanatus Thunb; Colocynthis edulis hook.f.syn.; Cucumeropsis manii Naud. and Colocynthis citrullus. The last name is even sometimes mistaken for Citrullus colocynthis Schrad. This is another type of melon which grows in the warm arid and sandy areas of Morocco, Algeria, Sahara Desert, Saudi Arabia and India. Generally, it seems Cucumeropsis refers to the climbing melons and Citrullus are creeping melons.

In Ghana and Nigeria, the melonseed (agushie) is called by different names in the different languages. Some of the Ghanaian names are: Akyekyee, Akatewa, Akatoa and Akatsewa (Akan); Agushie (Ga and Anlo-Ewe) Agbesi, Samase Atsetsea (Ho-Ewe). The Nigerian names are Egusi (Yoruba) Ogili (Ibo) and Guna agushi (Hausa).

## 2:3:2 General Morphology

### A. Cucumeropsis edulis.

This is an annual herbaceous climber with angular-pubescent stem. the leaves are five-lobed, cordate at the base and alternate. male and female flowers are separate. Male flowers are small and

Table 3Main Vegetable Oil categories

<u>Principal Fatty Acid</u>	<u>Oil</u>
Lauric ( $C_{12:0}$ )	Coconut, Palmkernel, Babassu
Palmitic ( $C_{16:0}$ )	Palmoil
Oleic ( $C_{18:1}$ )	Olive, Peanut Winged bean
Linoleic ( $C_{18:2}$ )	Soybean, cottonseed, maize, sesame
Linoleic (high)	Sunflowerseed, safflowerseed melonseed
Erucic ( $C_{22:1}$ )	rapeseed

Source: Langstraat, 1976



Table 4Fatty acid composition of aquashie seeds

<u>Fatty acid</u>	<u>% of Oil</u>
Myristic (C <sub>14:0</sub> )	0.02
Palmitic (C <sub>16:0</sub> )	10.43
Palmitoleic (C <sub>16:1</sub> )	0.14
Stearic (C <sub>18:0</sub> )	9.84
Oleic (C <sub>18:1</sub> )	15.90
Linoleic (C <sub>18:2</sub> )	62.81
Linolenic (C <sub>18:3</sub> )	0.41

Source: Akobondou et. al. 1982.

Table 5

Characteristics of Melonseed Oil (Citrullus lanatus)  
syn. Colocynthis citrullus, Linn. from Ibadan.

	<u>Solvent extracted</u>	<u>Locally produced</u>
% oil in seed	5.1	-
Acid value	13-17.9	1.1-2.9
Saponification value	191.7	189.5-190.5
Hydroxyl value	1.7	2.6
Iodine value	112.9	114.7-121.1
Unsaponifiable matter%	0.98	1.04
Refractive Index $n_D^{20}$	1.4722	1.4725

Source: Girgis and Said, 1968

numerous whilst female flowers are solitary. The fruits are elliptical and green, turning whitish when ripe. The fruit pulp is whitish with numerous seeds embedded in the white jelly-like substance.

B Citrullus lanatus:

This is a creeping pubescent monoecious annual. The stems are angular with numerous hairs. Dark green leaves which are highly dissected are carried on fairly long petioles. The flowers are pale yellow and male and female flowers appear separately. The fruits which may be mottled green to whitish green in colour are of different sizes. They all have a hard smooth rind. The variety neri has the smallest fruit and seed size. The fruit pulp is white with numerous seeds embedded in it (Omidiji, 1977)

C. Varietal Differences:

The varieties are easily distinguished from each other by their seed types and to some extent by their fruits. Cucumeropsis melons differ from Citrullus melons in fruit colour, seed type and size.

Omidiji (1977) distinguished three varieties of Citrullus melons by their seed types. Those referred to "Bara" seeds in Nigeria have black thick edges more thickened at the apices. "Sofin" seeds have thick white edges more thickened at the base, whilst "Serewe" seeds are small with marginal thickening.

Oyolu (1977) reported five seed phenotypes for Colocynthis citrullus. These he referred to as 1, 2, 3, 4 and 5. The seed types did not have any correlation to with fruit colour.

Type 1 according to his description has miniature seeds with thin seed coat and flat edges. The size of these seeds makes them difficult to dehull. This description corresponds to that of neri used in this study. Type 2 seeds are small with thin seed coat and flat edges. Small seeds with an encrusted thick seed coat and flat edges are type 3. Type 4 seeds are large with thick seed coat and moulded edges. The last type (5) are large with thin seed coat and flat edges. They are easy to dehull by hand. His study however did not indicate whether the plants from which the seeds were obtained were climbers or creepers.

#### **2:4 Cultivation**

Cucumeropsis edulis and Citrullus lanatus are grown from Sierra Leone down to Cameroon, Gabon, Congo and Angola. In Ghana, agushie is cultivated on farms or found climbing other plants on abandoned farms in the closed forest areas. Neri on the other hand is extensively grown in the Guinea savanna-forest transistion zone (i.e.in the Northern regions). The ecological zone in which neri is grown shows that it is quite moderate in its water requirements as compared to agushie and some other oilseeds. Both agushie and neri are grown during the rainy season and they mature in 3-6 months depending on the variety.

## **2:5 Post-Harvest Treatment**

The steps involved in separating the seeds from the fruit pulp are outlined in the flow diagram (Figure 1).

The aim of the decomposition of the pulp is not fermentation of the seeds, but rather softening of the pulp to allow easy seed removal. During washing, immature and incompletely filled seeds are removed. The seeds are dried to a fairly low moisture content which allows prolonged storage. Both dehulled and unde-hulled seeds are available on the market.

## **2:6 Traditional uses of melon seeds**

The undefatted meal (ground seeds) is used in the preparation soups and stews. In Ghana, neri is roasted, ground into a paste and used in the preparation of soup in the same way that roasted groundnut paste is used. In this way it forms an important protein constituent of the diet. In Nigeria the oil is extracted and used in cooking. This oil is obtained by boiling the ground meal in water until the oil floats on top. This is then scooped off. The residual protein-rich meal is fried into patties known as igbalo. This serves as a meat substitute in the diet (Oyenuga and Fetuga, 1975). The seeds can also be fermented. This is achieved by wrapping the boiled seeds in leaves and keeping for 5 days in covered baskets. This fermented product is used as a soup condiment in Nigeria (Odunfa, 1982). The seeds are also roasted and eaten as a snack.

## 2:7 Nutritive value of melonseeds

Most of the research work done on melonseeds including the nutritive aspects have been conducted mainly by Nigerian workers (Oyolu, 1975; Omidiji, 1977; Oyenuga and Fetuga, 1975; Oke, 1978; Abaelu et. al. 1979; Akpapuman and Markarkis, 1981; Akobundu et. al. 1982; Achinewhu, 1983; Onuora and King, 1983;). No work has as yet been published about studies done in Ghana. Studies on melonseeds have generally not been as extensive as other oilseeds.

Melonseeds have a high oil content 45-52% hence their classification as oilseeds. The protein content is approximately 20-30%. When defatted, this increases to between 40-70%.

Orraca-Tetteh (1964) reported the Net protein utilisation values of Neri and Agushie to be 50 and 51 respectively, in contrast to cowpeas which have NPU value of 35. The amino acid composition has been studied by a number of workers.

Oyenuga and Fetuga (1973), Abaelu et. al. (1979), had an amino acid pattern showing lysine and methionine to be 1st and 2nd limiting aminoacids respectively (Table 6). In a later study, Akobundu et. al. (1982), had lysine and threonine as the limiting amino acids. Their findings are not surprising since lysine,





FIGURE 1

Flowchart showing Post-harvest Treatment of Melonseeds



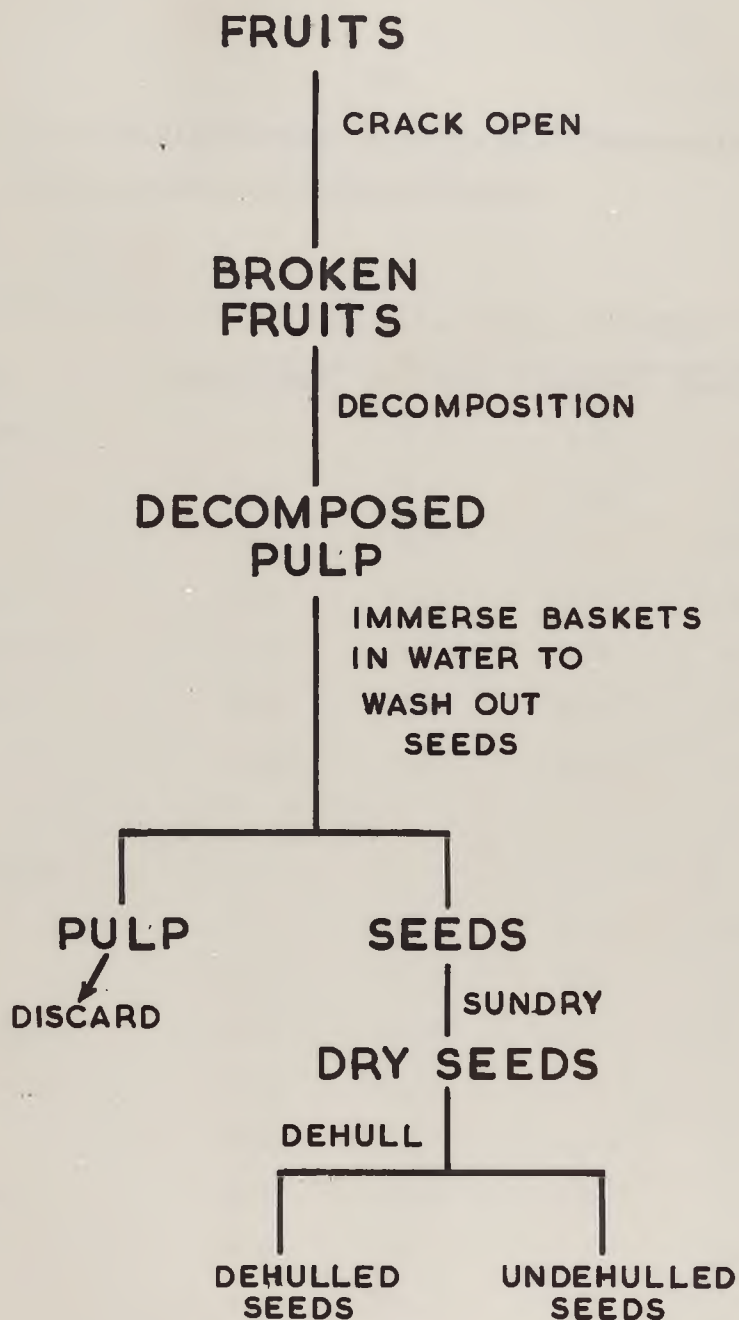


Table 6 Species Differences in Amino acid Composition  
of Melonseed and Three Oilseeds.

	g aminoacid/16g nitrogen			
<u>Essential</u>	<u>Melonseed</u>	<u>Soybean</u>	<u>Rapeseed</u>	<u>Sunflower</u>
Isoleucine	4.0	5.0	4.2	4.9
Leucine	6.1	7.0	7.6	5.8
Lysine	3.6	6.7	3.1	2.9
Methionine	2.0	1.3	2.1	1.7
Phenylalanine	4.7	4.8	3.9	4.3
Threonine	2.8	3.6	3.5	2.9
Valine	4.6	5.1	5.4	4.5
<u>Non-essential</u>				
Alanine	4.6	4.4	4.0	3.8
Arginine	13.4	7.0	5.6	8.7
Aspartic acid	6.3	11.5	6.8	9.6
Glutamic acid	17.5	18.7	21.9	22.2
Glycine	5.3	4.2	4.8	5.4
Histidine	3.1	2.3	2.8	2.3
Proline	5.0	5.4	6.7	4.5
Serine	3.8	4.5	3.0	3.1
Tyrosine	2.2	3.4	2.6	2.3
Total	92.0	93.0	88.0	89.0

Source: Abaelu *et. al.*, 1979.

threonine and methionine are the limiting amino acids in most oilseeds.

Though the biological indices of protein quality are lower than values obtained for soybeans, the biological value, net protein utilisation and protein efficiency ratio are comparable to or higher than most oilseeds (Oyenuga and Fetuga, 1975). Achinewhu (1983) fed two varieties of melonseeds to rats. He reported an apparent digestibility of 0.83 for climbing melon (Colocynthis vulgaris) and 0.82 for creeping melon (Citrullus vulgaris) compared to 0.93 for casein. Food conversion efficiency was 0.144 (climbing melon) and 0.145 (creeping melon). This is comparable to other oilseeds. Supplementing melonseeds with cowpeas increased protein efficiency ratio (PER) from 1.70 for melonseeds alone to 2.20. Casein had a PER of 2.50 (Akpapunum and Markakis, 1981).

Melonseeds contain several micronutrients that contribute significantly to the diet. Essential nutrients such as phosphorus, potassium, magnesium, calcium, zinc and iron are present. Melonseed flours also contain significant amounts of the vitamins thiamine and niacin. The potential of melonseed as a source of calcium and niacin is encouraging for low milk consuming regions of West Africa (Akobundu et. al. 1982).

Nutritional studies were conducted by the Nutrition Centre at Bawku in the Upper Region of Ghana on the use of neri as a source

of protein and fat. This proved satisfactory for malnourished children (Sinnadurai, 1984).

So far, toxic factors which are a major source of concern in the utilisation of oilseeds have not been reported in agushie. Since optimal heat processing eliminates most of these antinutritional and toxic factors it is likely that if any toxic constituents are present in agushie they would be eliminated during cooking.

## 2.8 Functional properties of oilseed proteins

The kind of protein in seeds determines the chemical and physical properties of the seed protein products and its amino acid composition. The proteins present in a seed are of two broad categories. (1) Structural proteins of the embryo--these are of predetermined and invariable composition; (2) storage proteins of endosperm and cotyledons --the amino acid composition of these can vary considerably (Hulse, 1981).

McWatters and Cherry (1977) stated that for plant proteins to be incorporated successfully as ingredients in food formulations, they must impart certain desirable qualities to the food. The functionality of plant proteins seems to be dependent on chemical characteristics inherent in the seed proteins.

Functional properties according to Fan and Sousolski (1974) are those characteristics which make isolated proteins useful as food

ingredients. These characteristics are emulsion stability, foaming, gel formation, solubility, wettability. fat and water binding.

Kinsella, (1976) gave a broad definition of functionality. He stated that physico-chemical properties of proteins which affect their processing behaviour in food systems as judged by quality attributes of the food product are functional properties. These reflect complex interactions between the composition, structure and conformation of the proteins and other food components as well as the nature of the environment in which these are associated.

The functional properties of proteins are affected by many factors. These include the source of the protein, method of isolation, precipitation and drying, protein concentration, modification (enzymatic or chemical). Temperature, pH, ionic strength, additives, amount, sequence and rate of addition of ingredients as well as mechanical processing also affect functionality (McWatters and Cherry, 1975).

The protein in a food commodity is made up of several discrete protein molecules each with different properties. The functionality associated with a protein preparation may therefore be that of one of the protein components instead of the total protein.



## 2:8:1 Protein solubility

Most industrial applications of proteins depend upon bringing the material into solution. A knowledge of the solubility properties is therefore an important factor in selecting vegetable proteins for possible industrial use.

The solubilisation of a protein molecule is a process which involves simultaneously wetting, swelling, solvation and dissolution. It is dependent on protein conformation, pH, ionic strength, temperature, protein concentration and other experimental conditions (Chou and Morr, 1979; Shen, 1976).

The dependence of solubility on pH is relatively easy to understand. At the isoelectric point (IEP) solubility is minimal because the net charge on the protein is zero, consequently electrostatic repulsive forces are almost non-existent whilst interaction between the molecules is maximal. On the alkaline side of the IEP the proteins have a negative charge and as these charges increase with increase in pH, repulsive forces also increase. This causes solubility to increase. At the acid side the net positive charge also causes increase in solubility through high repulsive forces (Ledward, 1979). Solubility over a range of pH values and other environmental conditions can be used as a guide to protein function. The pH solubility profile of a new protein is therefore usually the first functional property measured (Kinsella, 1976).

Akobundu et. al. (1982) found that melonseed protein had lowest solubility between pH 5.0-6.5. The highest solubilities occurred at the alkaline pH range. Gel electrophoresis showed that there was a variation in the types of protein extracted at the different pH's. At the alkaline pH's more types were extracted. Onoura and King (1983) also working with melonseeds had minimum protein solubility at pH 4.0. In salt solutions the pH-nitrogen solubility profile was different. Solubility was low at pH values less than 5.0 and there was a sharp rise in solubility at pH 7.0. The presence of the salt caused the protein molecules to behave differently at the different pH's from their behaviour in solvents of zero ionic strength. Minimum solubility occurred at pH 3.0 and 5.5 for two varieties of melonseeds (Ige et. al. 1984). Slight differences in the IEP in literature may be attributed to varietal differences.

Ledward (1979) stated that the relationship between salt concentration and solubility is complex. Globulins which are soluble in 5-10% salt solutions are insoluble in water at the IEP. Albumins on the other hand are readily soluble in both water and dilute salt solutions. Protein solubility however decreases in concentrated salt solutions ("salting out"). In dilute salt solutions, the ions of the neutral salt interact with the protein molecules decreasing protein-protein interactions. This leads to an increase in solubility ("salting in"). "Salting-out" can be explained in terms of dehydration of the

protein molecules by the salts. Protein solubility depends on water "clustering" around the hydrophilic groups. If this is prevented (by for example interaction with numerous salt ions) the protein is dehydrated and will not solubilise i.e. low solubility results. Onoura and King (1983) found that more than 67% of the melonseed protein was made up of globulins.

Curcubit seed proteins as a family are known to be made up of up to 80% or more globulins. The number and relative concentration of proteins that comprise the globulins of any given curcubit are not known with certainty. The major non-globular protein is water soluble albumin (Jacks et. al., 1972).

Heat treatment leading to denaturation also decreases protein solubility. Solubility is therefore used as an index of protein denaturation (Kinsella, 1976; Shen, 1976; McWatters and Cherry, 1975). King and Onuora, (1984) reported that nitrogen solubility of melonseed flour was reduced by heating. Moist heat had a greater effect. A large drop in solubility occurred after 30 minutes of heating. The combination of heat and moisture can be expected to cause denaturation of the protein. The pH-nitrogen solubility profile for melonseed flour differs from that of soyflour and peanut flour.

Although there is considerable diversity in solubility characteristics of seeds, there are correlations among the



solubilities within families. Legumes, cucurbits and mallow families are notable for general high solubilities of their nitrogenous constituents in NaOH,  $\text{Na}_2\text{PO}_4$ , and NaCl solutions. (Smith *et. al.*, 1959).

### 2:8:2 Water Absorption

Properties relating to the interaction of proteins with water are extremely important. The critical step in imparting the desired functional property to a food system is the interaction of proteins with water to rehydrate, swell or solubilise them. The ability of the proteins to bind, and immobilise water is itself one of its important functional characteristics in most food applications. Wettability and dispersability are mainly determined by hydrophilic and hydrophobic properties of the protein surface. Surface tension, the relative rates of water absorption and protein swelling also play a part. Swelling is caused by spontaneous uptake of water by the protein matrix. It therefore has a direct effect on the amount of water that a food system can absorb and upon the ultimate body and texture of a food product (Chou and Morr, 1979).

The water absorption capacity varies with protein source, composition, presence of carbohydrates, lipids and salts, and pH. It may also be influenced by previous processing such as heating and any other modification. (Hagenmaier, 1972; Lin *et. al.* 1974; Fleming *et. al.*, 1974). The water absorption capacity of

different proteins must therefore be determined to facilitate adjustments in food formulations when interchanging protein sources.

Ige et. al. (1984) measured the water holding capacity of three varieties of melonseed protein flours and isolates. The isolates had higher water holding capacities than the flours, though this was low in comparison to soy protein isolate. Akobundu et. al. (1982), in an earlier study also reported a low water absorption of melonseed flour.

### 2:8:3 Oil/Fat Absorption

The binding of fat by proteins is important for such applications as meat extenders and replacers and in some composite flour products. This is because it enhances flavour retention and improves mouthfeel. Oil absorption or oil-holding capacity is usually measured by adding excess oil to the protein sample, mixing thoroughly and centrifuging. The amount of bound oil is determined and can be expressed as oil absorbed per gram or 100 grams protein. The mechanism of absorption as assessed by the method is mostly due to physical entrapment of the oil. This can be explained by the fact that chemical modification of protein which increased bulk density also enhanced fat absorption (Kinsella, 1976).

Akobundu et. al. (1982) reported a higher oil holding capacity

for melonseed than water holding capacity. Ige et. al. (1984) found that fat absorption was higher for the melonseed flours and isolates than for soyflour and isolates. They were comparable to that of sunflower seed flours and isolates. Fat absorption of winged bean flour was also found to be higher than its water absorption capacity (Sathe, et. al. 1982b).

#### **2:8:4      Foaming Properties**

Foaming is the capacity of proteins to incorporate air to form stable foams. It is important in products such as sponge cakes, candy, whipped toppings and icings. Whippability and foamability are foaming property terms which are used interchangeably. They refer to the maximum volume increase of a protein dispersion after air is incorporated through whipping, agitation or aeration. The increase in volume is the foaming capacity whilst the ability of a foam to maintain its volume over a given time is the stability (Kinsella, 1976).

Food foams usually consist of air droplets dispersed in and enveloped by a liquid containing a surfactant. The surfactant lowers the surface tension of the liquid facilitating its deformation and a marked expansion in its total surface area against its own surface tension (Holm and Breedon, 1983) Cherry and McWatters, (1981) stated that for good foaming, proteins in a the liquid should

- (1) be soluble in the aqueous phase

- (2) readily concentrate at the liquid-air interface,
- (3) denature to form a cohesive layer possessing sufficient viscosity and mechanical strength to prevent rupture and coalescence of air cells.

Ideal foaming proteins therefore have the ability to form extended membranes around air droplets and to undergo a certain degree of denaturation which stabilises the foam and forms the structural network of the foam. An example is a protein like egg albumin. Oilseed flours and protein isolates can be whipped to produce good volume stable foams which can be used in a variety of foods.

Many factors affect the foaming properties of a protein. These include protein source, pH, temperature, protein solubility and presence of additives (salt, sugar, lipids) Townsend and Nakai (1983) investigated the relationships between hydrophobicity of a protein and foaming. Because proteins are extensively uncoiled at the air-water interface they found that a measure of total hydrophobicity rather than surface hydrophobicity had a significant correlation with foaming capacity. For effective stabilisation of air bubbles there should be a balance between hydrophilic and hydrophobic groups. Proteins with low hydrophobicity show poor foaming capacity. Molecular rigidity, solubility, hydrophobicity and viscosity are all important in the foaming mechanism of proteins in solution.

McWatters and Cherry (1977) reported that protein solubility was more related to the type of foam formed than to increase in volume. Maximum foaming of undenatured protein has been observed at acidic values above but close to the isoelectric point. The effect of pH can be over-shadowed in the presence of relatively large amounts of denatured or insoluble proteins. The true effects of pH are observed when a high percentage of surfactant protein is soluble. The effects of 'salting-out' at high concentration would reduce foaming.

In the work of Lawhon et. al. (1972) heat treatments during flour preparation from cottonseed reduced solubility and foaming. The presence of oil also depressed foaming, whilst addition of sucrose improved volume and strength of foams.

Akobundu et. al. (1982) found that melonseed flour gave very thin foams between pH 5 to 7. Foam viscosity varied from thin between pH 2 and 5 to thick at pH 5 to 6.7. and increased as more protein become soluble in the alkaline and acid regions. Foam at pH 5.0 had the highest stability though foam volume increase was lowest. In the alkaline pH range, when more storage protein became soluble, both foam capacity and stability were enhanced. Ige et. al. (1984) also reported that melonseed flours and protein isolates showed very good foam stability comparable to soyflour and isolate after 2 hours. The initial foam volume was however not as high as that of soy products.



## 2:8:5 Emulsifying Properties

Emulsification and foaming are surfactant properties related to the capacity of proteins to lower the interfacial tensions between the hydrophilic and hydrophobic components in foods (Kinsella, 1976).

Emulsions are systems composed of two or more immiscible phases in which one phase (discontinuous) is finely dispersed in the other phase (continuous). Typical emulsions are oil in water types. Because of their potential use in food emulsions, the emulsifying properties of protein are usually studied. Three methods have been used in studying the emulsifying ability of proteins.

- (1) Emulsifying capacity i.e. how much oil can be emulsified
- (2) Emulsion stability i.e. how stable the emulsion is to phase inversion.
- (3) Emulsifying activity.

The first two are the most commonly used methods and for full evaluation of emulsifying properties both must be considered.

According to Puski (1976) there are three general types of food emulsions:

- (a) comminuted meat emulsions
- (b) low viscosity emulsions e.g. milk, coffee whiteners
- (c) high viscosity emulsions e.g. mayonnaise.

A protein suited for one type of emulsion may not necessarily be suitable for another type. Model systems for studying the

emulsification properties of a protein for a particular emulsion should therefore simulate the emulsion type.

Most conditions used in determining the emulsifying properties of various proteins have been chosen arbitrarily. It is therefore difficult to compare the emulsifying capacity of different proteins since the methods are not standardised. Conditions such as equipment design, speed of blending, rate of oil addition, protein concentration, temperature, pH, protein solubility affect the emulsification. Additives such as salt and sugar also have an effect.

Although a positive correlation between emulsifying properties and solubility has been reported in some studies (Crenwelge et. al., 1974; Yatsumatsu et. al. 1972; Huffman et. al. 1975) other studies show a poor correlation between emulsifying properties and solubility (Smith et. al. 1973; McWatters and Cherry, 1975; McWatters and Holmes, 1979). Solubility by itself has been found not to be a very good predictor of emulsifying and other properties. The crucial role protein hydrophobicity plays is being increasingly understood (Li-Chan et.al. 1984). A closer correlation exists between hydrophobicity of proteins and emulsifying ability than between protein solubility and emulsifying ability.

Voutsinas et. al. (1983) noted that with proteins which have a



high surface hydrophobicity, solubility does not play an important role in emulsifying capacity (EC) and emulsifying stability (ES). At low and medium surface hydrophobicity, increasing solubility increases EC and ES. This explains their finding that with some proteins as heat denaturation increased (up to a certain point) with consequent decrease in protein solubility emulsifying properties were enhanced. In such proteins, the denaturation resulted in an exposure of hydrophobic amino acid residues which in the native protein are usually buried in the interior of the molecules. The increase in hydrophobicity therefore enhanced emulsification. The more hydrophobic the protein the greater the decrease in interfacial tension with consequent improvement of emulsifying properties. For proteins whose surface hydrophobicity is not increased on denaturation, heat treatment depresses emulsifying properties. Although the emulsifying properties of proteins ultimately depend on a balance between the hydrophobic and lipophilic groups factors such as molecular size, flexibility and charge also play a role.

Akobundu et. al. (1982) reported that agushie flour formed two types of emulsions within the pH range 2.5 -10.5. Thin emulsions were formed between pH 2.5 and 5.0. As pH increases from 6.5-10.5 progressively thicker emulsions were formed. This phenomenon could be related to the type of soluble protein present at the different pHs. This property of agushie flour

suggests that it can function as an excellent emulsifier in various foods. King and Onoura, (1984) observed that emulsifying activity of agushie flour increased with increasing solubility (based on pH change). The increase in emulsifying activity above pH 5.0 and below pH 3.0 was greater than the increase in solubility. This suggests the influence of other factors such as surface charge. The same pH and concentration dependence was observed for winged bean (Sathe et. al. 1982b). Sathe et. al. (1982a) explained the dependence of emulsifying capacity on the basis of adsorption kinetics. At low protein concentration, the rate of adsorption is diffusion controlled. At high concentrations, there is an activation barrier to absorption. Under such conditions, the ability of the protein molecules to create space in the existing film and to penetrate and rearrange on the surface is rate limiting. This depresses emulsifying capacity. There is therefore a critical concentration above which EC decreases. At lower protein concentrations, there is a greater degree of unfolding of the polypeptide chains during the shearing involved in emulsion formation. This is aided by hydrophobic association of peptide chains with the lipid droplets. The net result is that a greater volume to surface area of protein is made available and emulsification is enhanced.

#### **2:8:6      Gelation and Coagulation**

These are functional properties of proteins associated with

heating. Some protein molecules have the ability to link together and form a continuous three-dimensional network. Such a network with elastic properties is termed a gel (Powrie and Nakai, 1981). Gelation of proteins may result in the formation of a thermally reversible gel. Coagulation is however an irreversible random protein-protein aggregation reaction. It normally involves limited protein swelling due partly to the formation of covalent bonds such as disulphide bonds (Chou and Morr, 1979). Gels are characterised by a relatively high viscosity, plasticity and elasticity. Examples of protein gels and coagulates are gelatin, jelly, coagulated egg white and soybean tofu.

Variations in gelling and coagulating properties may be ascribed to the relative ratios of different constituents i.e. proteins, lipids, and carbohydrates. This suggests that interaction between such components may also have an effect on gelling and other functions (Sathe *et. al.* 1982b).

The functions of gelation and coagulation cannot be explained on the basis of hydrophobicity alone, but rather by a combination of the hydrophobicity of the unfolded protein and its sulphhydryl group content (Li-Chan *et.al.* 1984).

An interesting characteristic of melonseed meal is the ability of its suspensions to coagulate when heated. This imparts a desirable scrambled egg look to stews. It is this characteristic

which accounts for its desirability by housewives. The literature does not contain information on studies of the coagulation properties of melonseeds.

### 2:7 Protein-Rich Products from Oilseeds

Different products are made from oilseeds flours, protein concentrates and isolates. Soybean is the oilseed with the greatest usage in food formulations. It is being used in a variety of ways in various products, such as fortifiers in composite flour blends, and in textured forms. Soybean milk is a popular milk substitute for babies and even adults who are lactose intolerant.

Foods such as breads, snack type products and other cereal based foods are fortified by addition of oilseed flours, protein concentrates and isolates. Other products are imitation dairy products such coffee whiteners, simulated milks and cheese.

Pintauro, (1975) lists a patent for the preparation of soy-sesame-coconut simulated milk formula. The three constituents are ground to a fine powder in the ratio 4:3:2 or 3:5:2. In the plant manufacture, the finely ground powder is mixed with water to make a milk. This milk is then spray-dried to obtain a dry product. The product is made into liquid form by the consumer. The product was found to have a protein content of 1.5% which approximates that of human milk (1.4%).



Other beverages have also been made from oilseeds. 'Saci' a caramel-flavoured soybean beverage has been marketed by the Coca-cola company in Rio-de Janeiro. Vitasoy is another protein beverage marketed in Hong-Kong. A problem with the use of vegetable protein to produce beverages is the need to improve mouthfeel so that higher protein concentrations can be used. The micelles of casein unique to milk offer it a texture advantage which cannot be easily duplicated by other protein sources. The presence of a strong flavour is also a limiting factor in the use of oilseeds to produce beverages (Altschul, 1970).

In Ghana, the Food Research Institute developed a precooked high protein flour from agushie. The protein content of the defatted flour was approximately 70%. Various mixtures based on this flour were tested on rats. One such mixture was agushie flour, guineacorn and a little milk powder to provide an additional 4% protein. The growth attained by the animals on this mixture was about 92% of the growth of the control group fed on 16% milk and guinea corn mixture (Kordylas, 1974). No further product developments have been carried out by the Institute.

### 3. MATERIALS AND METHODS

#### 3:1 Materials

- (a) Two varieties of dehulled agushie (Cucumeropsis edulis) designated variety 1 ( $V_1$ ), and variety 2 ( $V_2$ ).
- (b) Undehulled neri (Citrullus vulgaris var. neri).
- (c) Refined soybean oil (B.V. Vereenigde,  
Oliefabrieken Holland)
- (d) Long grain rice
- (e) Granulated sugar
- (f) Heinz distilled vinegar

All raw materials were obtained from the local markets.

#### 3:2 Experimental Methods

##### 3:2:1 Sample Preparation and Storage

###### 3:2:1:1 Milling

The samples were sorted by hand to remove debris, spoilt seeds, and any undehulled seeds. The clean seeds were milled with a laboratory disc attrition mill (Straub Model 4E Grinding Mill) into a meal. The neri was ground whole due to difficulty in dehulling. A portion of each sample was roasted before milling. Roasting temperatures of 140°C, 160°C, and 180°C were used. Roasting time was 45 minutes at each temperature. The milled samples were kept in sealed polythene bags.

###### 3:2:1:2 Defatting

The Soxhlet extractor was used to defat samples using

petroleum ether (40-60°C b.p.). The defatted meals were sundried, and ground into flour using a laboratory mill (Casella and Co Ltd., London) with sieve opening of 0,063mm.

Solvent was evaporated from the oil extracted from raw meal with a rotary evaporator. The three (3) lots of desolventised oil were kept in bottles at 2-5°C.

### **3:2:1:3 Sample Storage**

All the samples i.e. whole seeds, undefatted meal, defatted flour and oil were kept under cold storage until used (2-5°C)

### **3:2:2 Proximate Composition**

The American Association of Cereal Chemists (AACC) Approved Methods (1969) were used. Each determination was carried out in triplicate.

Ash	AACC method	28 -16
Crude fat	" "	30 -25
Moisture	" "	44 -15A
Crude Fat	" "	46 -10

### **3:2:3 Available Carbohydrate**

Total available carbohydrate was determined by the Anthrone method. The method of Clegg as given by Osborne and Voogt (1978) was used.



### 3:2:4 Amylose and Amylopectin

#### A. Reagents

- i. Stock Iodine solution: 20g KI and 2.0g resublimed iodine were dissolved in a minimum of water the solution was transferred to a 100ml volumetric flask and made up to the mark.
- ii. Iodine Reagent: 10ml of the stock iodine solution was pipetted into a 100ml volumetric flask and made up to the mark.
- iii. 0.5M KOH
- IV. 0.1M HCl

#### B. Procedure

- i. Twenty (20)mg of defatted sample was weighed into a beaker- 10ml of 0.5M KOH was added and sample dispersed with a stirrer.
- ii. The dispersed sample was transferred to a 100ml volumetric flask and made up to the mark with distilled water.
- iii. Ten (10)ml of the sample solution was pipetted into a 50ml volumetric flask and 5ml of 0.1M HCl added followed by 0.5ml of iodine reagent.
- iv. The solution was diluted to the 50ml mark and left to stand for 5 minutes. The absorbance of the solution was read at 625nm on a Spectronic 20 Spectrophotometer (Bausch and Lomb).

- v. A blank solution was prepared using distilled water in place of sample solution.
- vi. A calibration curve for pure amylose was prepared using known concentrations of amylose solutions.

### 3:2:5 Physicochemical Characteristics of Oil

#### 3:2:5:1 Refractive Index and Saponification Value

The AACC approved methods (1969) were used.

- i. Refractive Index:           AACC method       58 -20
- ii. Saponification Value   AACC method       58 -50

#### 3:2:5:2 Acid Value

##### A. Reagents

- i. Solvent:     Equal amounts of 90% ethanol and diethyl ether were mixed. The solvent was neutralised just before use with 0.1M NaOH using phenolphthalein as indicator.
- ii. 0.1M NaOH

##### B Procedure

About 10g of the oil sample was dissolved in 50ml solvent. The solution was titrated against 0.1M NaOH using phenolphthalein as indicator. The acid value was calculated using the following formula:

$$\text{Acid Value} = \frac{56.1 \times 0.1 \times \text{titre value}}{\text{weight of sample}}$$

- v. A blank solution was prepared using distilled water in place of sample solution.
- vi. A calibration curve for pure amylose was prepared using known concentrations of amylose solutions.

### 3:2:5 Physicochemical Characteristics of Oil

#### 3:2:5:1 Refractive Index and Saponification Value

The AACC approved methods (1969) were used.

- i. Refractive Index: AACC method 58 -20
- ii. Saponification Value AACC method 58 -50

#### 3:2:5:2 Acid Value

##### A. Reagents

- i Solvent: Equal amounts of 90% ethanol and diethyl ether were mixed. The solvent was neutralised just before use with 0.1M NaOH using phenolphthalein as indicator.
- ii. 0.1M NaOH

##### B Procedure

About 10g of the oil sample was dissolved in 50ml solvent. The solution was titrated against 0.1M NaOH using phenolphthalein as indicator. The acid value was calculated using the following formula:

$$\text{Acid Value} = \frac{56.1 \times 0.1 \times \text{titre value}}{\text{weight of sample}}$$

### 3:2:5:3 Iodine Value

#### A. Principle

The oil is treated with an iodine monochloride solution. The excess of iodine monochloride after addition of the halogen is determined by titration with thiosulfate solution.

#### B. Reagents

- i. Carbon tetrachloride ( $\text{CCl}_4$ )
- ii. Potassium iodide:- 10% aqueous solution
- iii. Starch Indicator:- 1% solution
- iv. Sodium thiosulfate:- 0.1M aqueous solution
- v. Wijs iodine solution (BDH)

#### C. Procedure

About 0.15g of oil was weighed into a conical flask and dissolved in 15ml  $\text{CCl}_4$ . 25ml of Wijs iodine solution was added from a burette and mixed thoroughly. After standing for exactly one hour in the dark, 20ml of KI solution was added. The solution was titrated with thiosulfate solution with thorough shaking. The starch indicator was added towards the end of the titration. Blank determinations were carried out with the same quantities of reagents and reaction time.

**D. Calculation**

Weight of sample	W g
Volume of titre	$V_1$ ml
Blank titre volume	$V_2$ ml
Molarity of thiosulfate	M
Iodine Value	$= \frac{12.69M (V_2 - V_1)}{W}$

**3:2:5:4 Peroxide Value****A. Principle**

The peroxide value is determined by subjecting potassium iodide at room temperature to the oxidant effect of peroxides. The iodine thus liberated is titrated with sodium thiosulfate.

**B. Reagents**

- Solvent: glacial acetic acid : chloroform (2:1V/V)
- Potassium iodide solution (KI): 5% aqueous solution
- Freshly powdered potassium iodide.
- Sodium thiosulfate: 0.002M (freshly prepared)
- Starch indicator: 1% solution

**C. Procedure**

One (1)g of sample was accurately weighed into a 200ml conical flask. 25ml of solvent was added followed by 1g of powdered KI and 20ml KI solution. The mixture was shaken and left to stand in the dark for 1 minute. 35ml of water was added and titrated



with 0.002M sodium thiosulfate using starch as indicator. A blank determination was run at the same time.

#### D Calculation

$$\text{Peroxide Value} = \frac{1000(\text{Titre value} - \text{blank})0.002}{\text{weight of sample}}$$

### 3:6 Protein Solubility In Different Solvents

Distilled water, 5% NaCl and 0.4% NaOH were used to study the protein solubility of the samples.

3g of defatted raw sample was weighed into a conical flask. 100ml of extracting solvent was added and the suspension shaken for 45min. in a mechanical shaker. The extract was decanted and fresh solvent added to repeat the extraction for 30min. After a third extraction for 30min., the three extracts were added.

The protein content of the total extract was determined using the method of Lowry et. al. (1951).

### 3:2:7 Protein determination (Lowry et al. 1951)

#### A. Principle:

This determination depends on the colour developed when protein

reacts with copper in an alkaline medium and the resulting copper-treated protein reduces phosphomolybdic-phosphotungstic reagent.

B. Reagents:

1. 2%  $\text{Na}_2\text{CO}_3$  in 0.1M NaOH
2. 0.5%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 1% sodium potassium tartarate ( $\text{NaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ).
3. Alkaline copper solution: 50ml of (1) was mixed with 1ml of (2) just before use.
4. Diluted Folin-Ciocalteu reagent (BDH):- One volume of reagent diluted with 2 volumes distilled water before use.

C. Procedure:

- i. Five (5)ml of alkaline copper solution was added to 1ml of protein solution and mixed. This was left to stand for 10min. at room temperature.
- ii. 0.5ml diluted Folin reagent was added and mixed immediately.
- iii. After 30min. the absorbance of the solution was read at 750nm on the Spectronic 20 spectrophotometer.
- iv. A blank was prepared using distilled water in place of protein solution.
- v. A calibration curve was prepared using known concentrations of bovine serum albumin.





### 3:2:8 Functional Characteristics

The effects of pH, ionic strength, roasting of seeds, defatting and temperature on functional characteristics were studied.

The pH of distilled water was adjusted by the addition of HCl (0.1 or 1.0M) or NaOH (0.1 or 1.0M). The pH range was from 2 to 12. Sodium chloride solutions with ionic strengths ranging from 0.1M to 1.0M were used for studying the effect of ionic strength.

Roasted samples were used to study the effect of roasting. Roasting temperatures were 140°C, 160°C and 180°C for 45min.

Undefatted meal was used for the effect of defatting.

Apart from carrying out the experiments at room temperature (27°C), temperatures of 5°C, 50°C, 60°C and 80°C were used for some of the functional characteristics.

#### 3:2:8:1 Protein Solubility

One (1)g of defatted flour was weighed into a 250ml conical flask and 100ml distilled water was added. The dispersion was shaken with a mechanical shaker at a given temperature for 45min. The suspension was then centrifuged for 30min. at full speed (speed 10, MSE bench centrifuge)

The supernatant was decanted carefully and protein content determined using the method of Lowry *et. al.* (1951)

The effects of (i) changing pH, (ii) ionic strength, (iii)

roasting temperature and (iv) extraction temperature were studied.

### **3:2:8:2 Water Absorption**

Defatted flour (2g) was weighed into a 15ml centrifuge tube and 10ml distilled water added. After mixing thoroughly the suspension was left to stand for 30min. at a given temperature, then centrifuged for 20min. (speed 10, MSE bench centrifuge).

The volume of the supernatant was then measured. The effects of (i) ionic strength, (ii) roasting temperature and (iii) temperature of incubation were studied. The temperatures for incubation were 5°C, 27°C, 50°C, 60°C and 80°C.

### **Calculation**

$$\text{Water absorbed/g sample} = \frac{X - Y \text{ (ml)}}{\text{weight of sample(g)}}$$

X = initial volume of water

Y = volume of supernatant

### **3:2:8:3 Fat Absorption**

The procedure was the same as that for water absorption. 10ml of refined soybean oil was used instead of water. Defatted, raw and roasted flours were used.

**3:2:8:4 Foaming Properties**

100ml of distilled water was added to 1g of defatted flour. The dispersion was whipped for 1min in a Top-drive homogeniser (MSE). The whipped mixture was quickly transferred into a 250ml graduated measuring cylinder and foam volume quickly read. This was taken as foam volume at zero time. Foam volume was thereafter read at intervals of 5, 10, 15, 30, 60, 90 and 120 minutes.

The effects of pH, ionic strength and whipping temperature were studied. Comparisons were also made using undefatted meal and defatted roasted flour.

**3:2:8:5 Emulsification**

A modified version of the method of Yatsumatsu et. al. (1972) was used. The effect of sample concentration on emulsion capacity was first studied to determine the amount of sample to use. Defatted agushie  $V_{20}$  flour was used for this.

<u>Weight of sample (g)</u>	<u>Concentration %</u>
0.25	1
1.00	4
2.00	8
2.50	10

2g of sample was mixed with 25ml of water. 25ml of refined soybean oil was then added. The mixture was emulsified in a Top-drive homogeniser (speed 3) for 1 min. The emulsion was divided equally into 15ml centrifuge tubes and centrifuged at speed 8 (MSE bench centrifuge) for 5minutes. The volume of the emulsified layer was measured. To determine emulsion stability, the emulsion was prepared as described above, then heated for 30min. at 80°C before centrifuging.

The effects of pH, ionic strength, and roasting temperature were studied. Some seeds were boiled for 30min. before using. In this case sample concentration of 20% was used before any measurable reading could be obtained.

The viscosities of the emulsions prepared at the different pH's were measured, after centrifuging. The Brookfield Viscometer Model RVT was used with Spindle T-F rotating at 5 r.p.m.

#### Calculation

$$\text{Emulsion capacity/stability} = \frac{\text{Volume of emulsified layer} \times 100}{\text{Total volume}}$$

### 3:2:8:5 Coagulation Studies

For these studies only undefatted raw and roasted samples were used. Fifty (50)ml of water was added to 5.0g of whole seeds and blended into a slurry for 1.5min. with a Top-drive homogeniser. The slurry was poured into a beaker and heated to boiling point whilst stirring continuously with a glass rod. The temperature was also monitored continuously and the temperature at which agglomeration started was noted. The effects of pH, ionic strength and roasting temperature were studied.

### 3:2:9 Microscopy

#### Fixing Fluid

70% Ethanol	90ml
Glacial acetic acid	5ml
Formaldehyde	5ml

#### Stains

- i. Sudan IV to detect fat cells.
- ii. Millon's reagent to detect protein.

#### Procedure

##### i. Fixing and Dehydration

The dehulled seeds (cotyledons) were placed in fixing fluid for 20 hrs. They were then washed in 50% ethanol for 1hr,



70% ethanol for 30min, and 95% ethanol for 30min. Tissue sections of 50 $\mu$  to 75 $\mu$  thick were cut from the dehydrated cotyledons using a sledge microtome.

## ii. Staining

(1) Fat Stain: The sections were placed in the Sudan IV stain for 3-5 min. They were then put on microscope slides and mounted in glycerine.

(2) Protein Stain: The sections were put in the Millon's reagent and heated gently for 2min. The sections were removed, placed on microscope slides and mounted in glycerine.

## iii. Photomicrographs

After examining the slides under the microscope, photomicrographs of the sections were taken with a Nikkon EMF photomicroscope using a blue filter.

## 3:3 Product Development

### 3:3:1 Emulsion-type product

The agushie was roasted at 140°C for 60min before use.

Two batches of product were prepared. One batch had cooked rice paste added to it. The other batch was prepared without the



addition of rice.

The products were put into jars whilst still hot. The steps for the preparation of the product are shown in Figure 2.

Samples from each batch were kept at room temperature (25-27°C), others at 5°C (cold storage), and the rest were frozen. Changes in the products were observed up to 7 days. The frozen products were thawed after 3 days and examined. They were refrozen for a further 4 days, thawed and examined.

The following ingredients were used in the processing of the two batches.

<u>Ingredients</u>	<u>Batch 1</u>	<u>Batch 2</u>
Agushie	25g	25g
Rice paste	30g	-
Sugar	20g	20g
Salt	2g	2g
Oil	150ml	150ml
Water	150ml	150ml
Vinegar	10ml	10ml

### 3:3:2 Baby Food

The agushie was roasted at 140°C for 60min before blending to form a slurry. The steps for the preparation of the product are shown in Figure 3. Four batches of product were prepared. Three batches had rice flour added and one batch had no rice flour.

The amount of agushie used varied for the various batches.

Storage:- Some of each batch was kept at room temperature, some at 5°C, and the rest were frozen in a household freezer.

The products were observed up to 7 days noting changes in consistency and appearance. The frozen products were thawed after three days and examined. They were refrozen and rethawed for examination after four days.

The following ingredients were used for processing the different batches of products.

<u>Ingredients</u>	<u>Batch 1</u>	<u>Batch 2</u>	<u>Batch 3</u>	<u>Batch 4</u>
Agushie	150g	150g	100g	100g
Rice flour	50g	-	100g	50g
Sugar	70g	70g	70g	70g
Salt	2.5g	2.5g	2.5g	2.5g
Water	800ml	800ml	1000ml	800ml

batches had rice flour added and one batch had no rice flour.

The amount of agushie used varied for the various batches.

Storage:- Some of each batch was kept at room temperature, some at 5°C, and the rest were frozen in a household freezer.

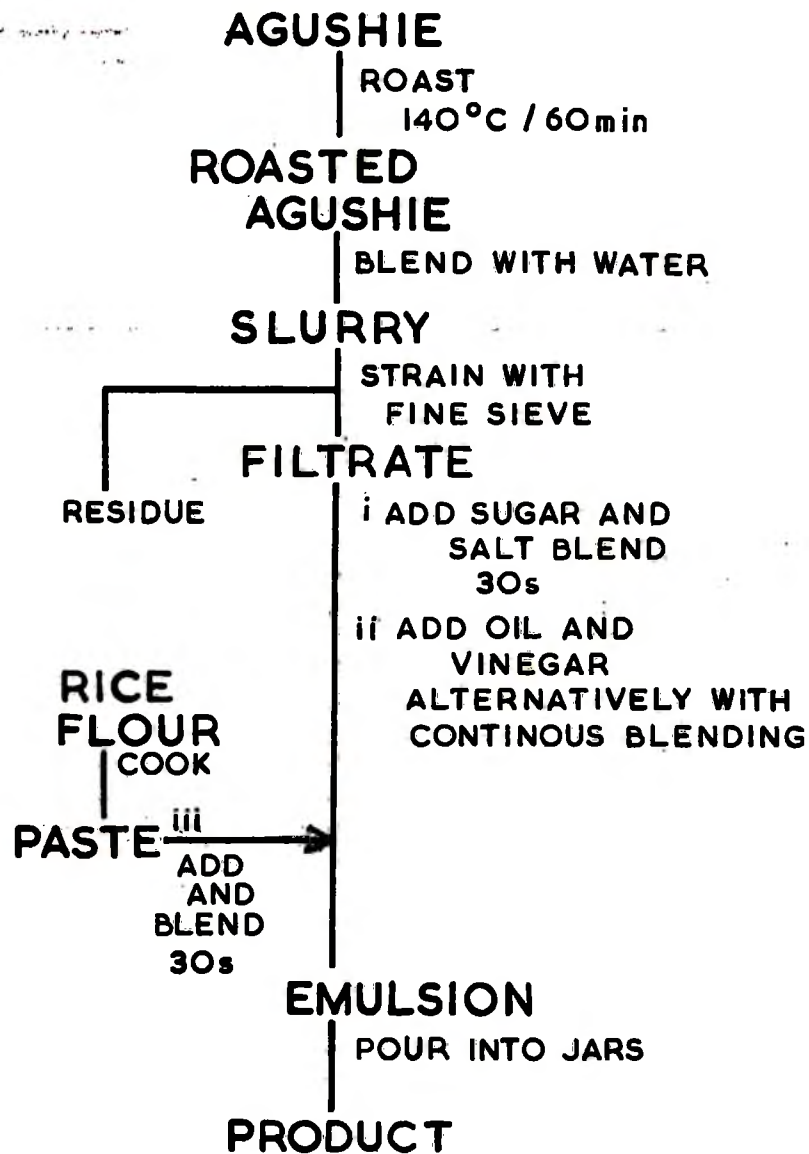
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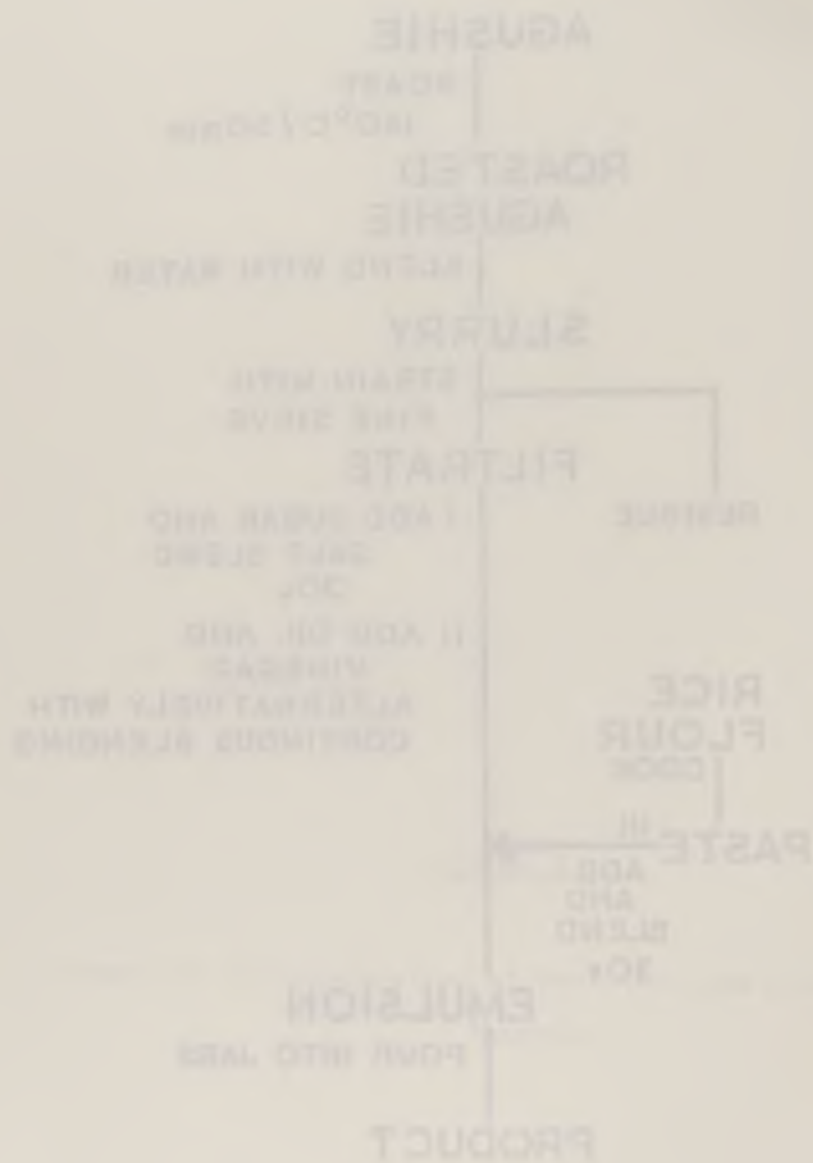
The following ingredients were used for processing the different batches of products.

<u>Ingredients</u>	<u>Batch 1</u>	<u>Batch 2</u>	<u>Batch 3</u>	<u>Batch 4</u>
Agushie	150g	150g	100g	100g
Rice flour	50g	-	100g	50g
Sugar	70g	70g	70g	70g
Salt	2.5g	2.5g	2.5g	2.5g
Water	800ml	800ml	1000ml	800ml

FIGURE 2

Steps for the preparation of Emulsion type product from  
Agushie





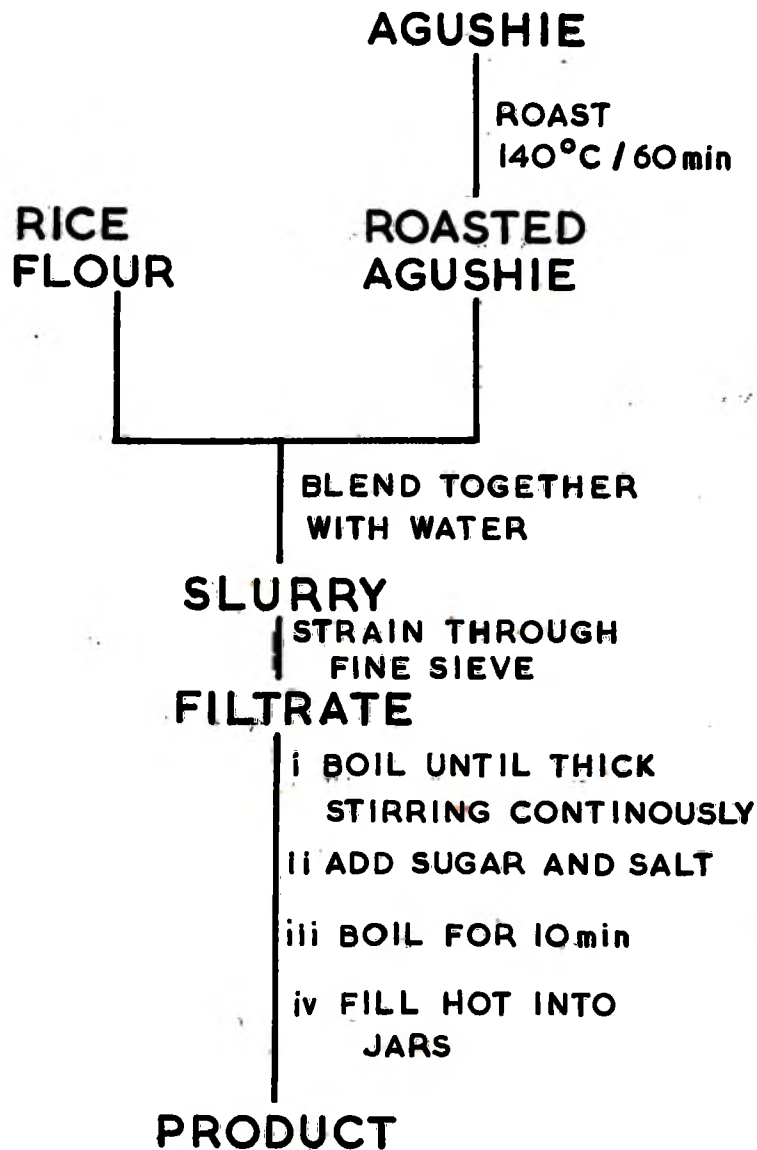


hence it is feasible to

to the

FIGURE 3

Steps for the preparation of Baby-food type product from  
Agushie





#### 4. RESULTS AND DISCUSSION

##### 4:1 Seed Description and Composition

##### 4:1:1 Seed Description

The three types of melonseeds used can easily be distinguished by their size and seedcoat (Figure 4). Their dimensions are given in Table 6.

Agushie variety 1 had a white seed coat which is thin and loose, making it easy to dehull. It is the longest of the varieties used (length, 1.66cm; width, 0.70cm; thickness, 0.05cm). Agushie variety 2 is flatter and slightly shorter than variety 1 though it is wider (length, 1.40cm; width, 0.72cm; thickness, 0.04cm). The seedcoat is yellow with moulded edges of a lighter colour. It is not as loose as that of variety 1 and it is thicker. Dehulling is not as easy as for variety 1.

The neri seeds are very small (length, 0.84cm; width 0.36cm; thickness, 0.05cm) and are about half the size of agushie variety 1. The seedcoat is thin and yellowish in colour. The size of the seeds makes dehulling very difficult.

##### 4:1:2 Composition

All the samples showed relatively low moisture levels.

During the post-harvest treatment, the seeds are sun-dried to a

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SCHOOL OF DISTANCE EDUCATION

B.A. POLITICAL SCIENCE

SEMESTER I EXAMINATIONS, 2011

POLITICAL SCIENCE (HONOURS) - 1

1. (20 marks)

a) Define the term 'politics'.

b) Discuss the role of the state in politics.

c) Explain the concept of 'good governance'.

d) Discuss the role of the media in politics.

e) Explain the concept of 'democracy'.

f) Discuss the role of the judiciary in politics.

g) Explain the concept of 'rule of law'.

h) Discuss the role of the executive in politics.

i) Explain the concept of 'development'.

j) Discuss the role of the legislature in politics.

k) Explain the concept of 'social justice'.

l) Discuss the role of the civil service in politics.

2. (20 marks)

a) Discuss the role of the state in development.

b) Explain the concept of 'economic growth'.



low moisture content. It appears that the size of the seeds may affect the moisture content. Neri, the smallest of the seeds had the lowest moisture content, whilst agushie variety 1 had the highest. This observation is not surprising, for when the same drying conditions are used, the smallest seeds are likely to dry faster and more thoroughly than larger seeds.

A low carbohydrate content was found for all the samples (3.18 to 4.97) (Table 6). The amylose and amylopectin determination did not register any values. To confirm the absence of starch, the defatted flours were examined under the microscope. Starch granules were absent from all three melonseed flours.

Jacks et. al. (1972) reported that starch was apparently absent from curcubit seeds. The carbohydrate content may be made up of sugar and other carbohydrate compounds. The review of Jacks et al (1972) indicated the presence of phytic acid and the terpenoid glycosides as part of the carbohydrate content of curcubits. The method used to determine the carbohydrate in this study will not account for these, so the actual carbohydrate content may be slightly higher than reported. Carbohydrate content of melonseeds reported in literature have varied from as high as 10 - 11% (Oyolu, 1977, Akpapunam and Markakis, 1981) to as low as 3% (Oyenuga and Fetuga, 1975). Variations in methods used to determine the carbohydrate may account for the differences observed, apart from varietal differences.





FIGURE 4

Varieties of melonseeds used









FIGURE 5

Diagram showing seed dimensions

L =length

W =width

T =thickness

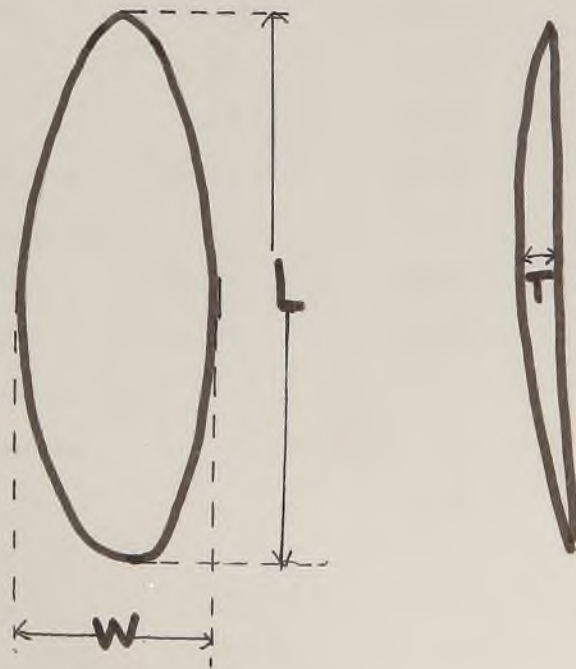




Table 6

Seed Dimensions and Composition of Agushie (Cucumeropsis edulis) and neri (Citrullus lanatus)

<u>Description</u>	<u>Agushie</u>		<u>Neri</u>
	Variety 1	Variety 2	
*Length cm.	1.66±0.14	1.40±0.11	0.84±0.15
*Width cm.	0.70±0.09	0.72±0.07	0.36±0.10
*Thickness cm.	0.05±0.02	0.04±0.02	0.05±0.03
Moisture%	6.27	4.58	3.75
Ash %	3.77	3.26	2.13
Protein(Nx5.7)%	28.91	27.48	19.62
Protein- defatted			
meal(Nx5.7)%	57.18	55.86	32.44
Crude fat %	46.71	48.79	46.93
Total available			
carbohydrate as			
% glucose	4.7	3.65	3.18
Amylose and			
Amylopectin	-	-	-

\* Mean±SD of 20 determinations



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3	ABU, A. A.	12345678	B
4	ABU, A. A.	12345678	B
5	ABU, A. A.	12345678	B
6	ABU, A. A.	12345678	B
7	ABU, A. A.	12345678	B
8	ABU, A. A.	12345678	B
9	ABU, A. A.	12345678	B
10	ABU, A. A.	12345678	B
11	ABU, A. A.	12345678	B
12	ABU, A. A.	12345678	B
13	ABU, A. A.	12345678	B
14	ABU, A. A.	12345678	B
15	ABU, A. A.	12345678	B
16	ABU, A. A.	12345678	B
17	ABU, A. A.	12345678	B
18	ABU, A. A.	12345678	B
19	ABU, A. A.	12345678	B
20	ABU, A. A.	12345678	B

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High crude oil content (46.71% to 48.79%) was obtained for all the samples. Agushie variety 2 had the highest. Neri and agushie variety 1 had similar values. The crude fat content compares well with other oilseeds. Since the yield of oil is so high, the use of melonseeds as a source of vegetable oil should be encouraged. The oil has been found to be nutritionally valuable because of its high linoleic acid content, and it is relatively shelf-stable due to the very low level of linolenic acid. Literature values have varied from 43-56%. Varietal differences may account for the differences observed. Kinsella, (1975) reported that the species and variety of oilseeds have a marked influence on the lipid content and composition. This being due to variation in genotype.

The protein content of neri (19.62%) was lower than that of the agushie varieties which were similar (28.91% and 27.42% for agushie variety 1 and variety 2 respectively). When compared with other legumes and oilseeds commonly used in Ghana, the protein content of agushie is higher than groundnut (26.75%) and cowpeas (21.32%). It is however lower than that of soybeans (37%). Defatting the melonseeds, leaves a protein-rich meal as shown by the protein content of the defatted meals. This defatted meal is used traditionally in Nigeria. It is fried as patties which can serve as a meat substitute. Because of the high protein content of the defatted meal, the use of agushie and neri in this way will greatly improve the protein quality of the



diet. The advantages of extracting the valuable oil from agushie and neri and using the protein-rich residue should therefore not be overlooked.

On the basis of the composition alone, agushie and neri can be recommended as very valuable additions to the diet.

#### 4:2 Physico-chemical Characteristics of Melonseed Oil

The values obtained for the characteristics measured are shown in Table 7. For each characteristic, the values obtained for the three oils do not differ significantly from each other.

The refractive index for all the oils was approximately 1.47. This indicates the similarity of the oils which were all yellow in colour (Figure 6).

The oil from agushie variety 1 had higher acid value, saponification and peroxide values (3.19, 185.52 and 10.52 respectively), than variety 2 and neri. The acid value for all the oils was lower than the codex standard for virgin oils which is 4. The low acid values indicate that the free fatty acid level (FFA) in the oils is low. A high FFA level is a result of deterioration of the oil. Evidently this had not occurred in the oils. This is confirmed by the low peroxide values obtained. A rancid oil has a high peroxide value. The low level of linolenic acid in the samples coupled with the storage conditions used (in the dark at 5°C) may account for the stability of the oil. The codex standard for peroxide value is 10. Agushie variety 2 and

FIGURE 6 Melonseed Oil

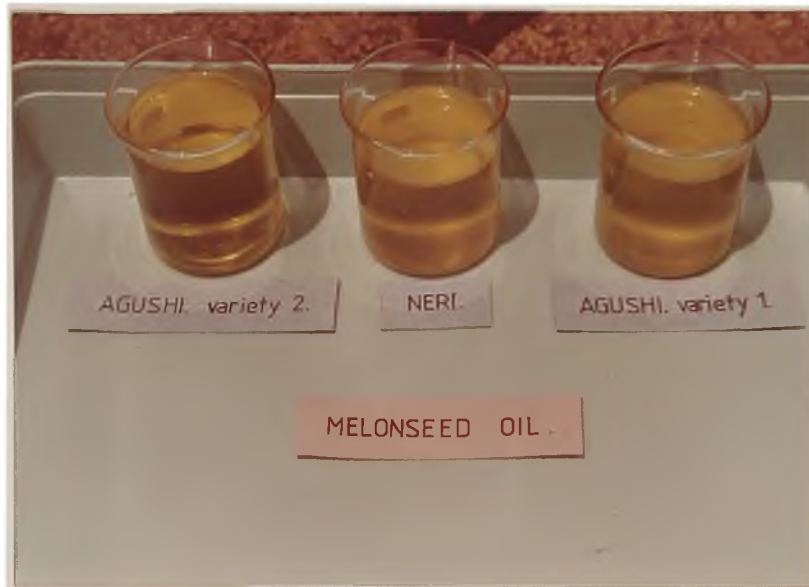


Table 7

Characteristics of Agushie (Cucumeropsis edulis) and  
Neri (Citrullus lanatus) oil.

	<u>AGUSHIE</u>		<u>NERI</u>
	Variety 1	Variety 2	
Refractive Index	1.4719	1.4728	1.4699
Acid value	3.19	2.83	2.77
Saponification			
Value	185.52	177.46	176.76
Peroxide value	10.52	6.77	6.64
Iodine value	106.013	106.79	109.32



neri oils had values much lower than 10 (6.77 and 6.64 respectively). The oil from variety 1 had a peroxide value almost equal to the standard.

The fairly high iodine and saponification values confirm the unsaturated nature of the oils. Both the iodine and saponification values are related to the fatty acids present in an oil, though they do not indicate the specific fatty acids present. The main fatty acid present in melonseed oil has been reported in literature to be linoleic acid (Girgis and Said, 1968, Akobundu *et. al.* 1982). The high iodine and saponification values obtained in this study could therefore suggest that the melonseeds used contain high amounts of linoleic acid. Use of agushie and neri oils in cooking would supply much needed linoleic acid which is also a precursor of other essential fatty acids like arachidonic acid.

On storing the oils under cold temperature (5°C) there was deposition of crystal (stearine). It was observed that crystals started forming at approximately 10°C. The oils solidified at 5°C. At room temperature the oils were completely liquid. This means that industrial processing of these oils should include the step of winterisation.

#### **4:3 Protein Solubility**

##### **4:3:1 Protein Solubility in different solvents**

The solubility of melonseed protein in three solvents was studied. The results are given in Table 8. The percentage of total protein extracted by each solvent was calculated based on the total protein of the defatted flours. These are presented in Table 9.

For all the melonseeds, the water extractable protein fraction was the lowest (less than 30%). This represents the albumin fraction. There was a marked increase in the protein extracted by sodium chloride (NaCl) and sodium hydroxide (NaOH) solutions. This suggests that the bulk of the proteins in melonseeds are not water extractable. Melonseed proteins differ in this way from soybean and cowpea proteins, the bulk of which are more soluble in water than sodium chloride solutions.

Neri seems to have a higher proportion of water extractable protein (20%) than agushie. Agushie variety 2 had the least amount of water extractable proteins (23%).

More than 50% of the total protein of all the melonseeds is extractable in 5% sodium chloride solution. Agushie variety 1 and the highest amount (76%) followed by variety 2 (60%) than neri (57%). The high solubility in 5% sodium chloride is to be expected, since the bulk of melonseed proteins have been found to be storage globulins. In this respect the agushie and neri proteins are like sunflower seeds proteins (Sosulski and Fleming,

Table 8Solubility of Melonseed protein in different Solvents

Solvent	Protein mg/ml		
	Agushie		Neri
	<u>Variety 1</u>	<u>Variety 2</u>	
Distilled water	1.56	1.28	0.94
5% NaCl	4.35	3.35	1.83
0.4% NaOH	5.70	4.35	2.87

Table 9

Percentage of total protein present in Agushie and neri  
extracted by different solvents.

	Agushie		Neri
	<u>Variety 1</u>	<u>Variety 2</u>	
Solvent	% of total protein		
Distilled water	27	23	29
5% NaCl	76	60	57
0.4% NaOH	98	78	88

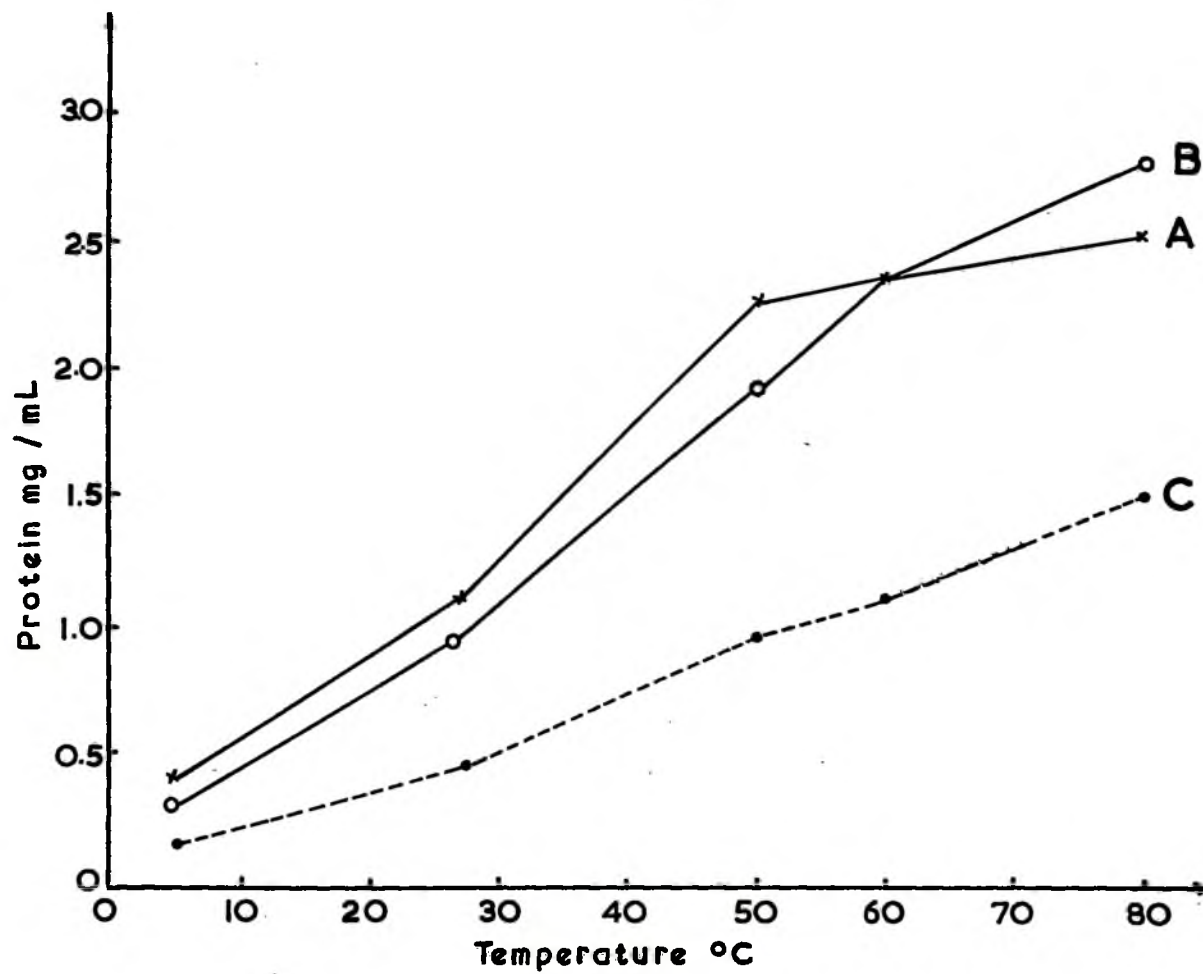
FIGURE 7

Effect of Extraction Temperature on Protein Solubility  
of Melonseeds (Cucumeropsis edulis) and (Citrullus  
lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri





1977, and safflower seed proteins (Betschart, 1975) The differences in the actual amounts extracted by the 5% sodium chloride solution may be due to certain differences in the specific proteins present in these three varieties of melonseeds. Solubility in the 0.4% sodium hydroxide (NaOH) was highest for all the samples. Almost all the protein (98%) in the agushie variety 1 was extracted. 88% of the neri and 78% of agushie variety 2 were extracted. This very high solubility may be explained by the fact that the high pH of the sodium hydroxide solution gave the proteins a net negative charge, leading to strong repulsive forces between the molecules. This results in the protein molecules going into solution. Another factor may be that there was dissociation of the large protein molecules into smaller fragments and this facilitated solubilisation of the proteins. Akobundu et. al., (1982) also explained the high solubility in NaOH as due to the easier rupturing of the membrane surrounding the protein bodies by the alkaline medium, thereby releasing the protein into solution. In water the membranes of the melonseed protein bodies are not ruptured easily therefore solubility is low.

#### 4:3:2 Effect of Extraction Temperature on Protein Solubility of melonseeds

Protein was extracted from the defatted melonseed flours with distilled water at different temperatures. It was observed that the temperature of extraction affected the protein solubility.

(Figure 7) As the temperature of extraction is increased from 5°C to 80°C, the protein solubility also increased. The percentage of protein extracted at each temperature based on total protein contents of the defatted agushie and neri flours was as follows:- Agushie variety 1; 6 to 44%; variety 2; 5 to 19% and neri: 5 to 46% (5°C to 80°C). At room temperature, 19%, 17% and 14% protein was extracted from agushie variety 1, variety 2 and neri respectively. At 5°C solubility was minimal.

The increase in solubility as temperature increases could be attributed to gain in energy with increase in temperature. This energy gain may be used to cause dissociation of the protein molecules making it easier for them to go into solution. High temperatures may also aid in rupturing of membranes surrounding the protein bodies thereby releasing more protein into solution. The increase in solubility from 5°C to 50°C was greater than from 50°C to 80°C, for all the samples. Since the amount of protein solubilised did not increase markedly from 50°C to 80°C, 60°C could be used as an optimum temperature for extracting water-soluble proteins. Temperatures up to 80°C clearly do not cause denaturation of the water-soluble proteins. This may hold for only the water extractable proteins, since the solvent used was distilled water

Solubility characteristics under various conditions are very useful in selecting optimum conditions for extracting protein from oilseeds.

#### 4:3:3 Effect of pH on Protein Solubility of Melonseeds

Figure 8 shows the effect of pH adjustment on the protein solubility of agushie and neri. The pH-solubility profiles of the three varieties follow a similar trend. The profiles are also similar to that of other oilseeds. Protein solubility is highest at the alkaline pH's (9 - 11) for all the samples. The point of minimum solubility is pH 4.0 for agushie variety 1 and neri. Agushie variety 2 has a minimum solubility at pH 3.0. These would be the isoelectric points for the agushie varieties and neri. It has been explained that at the isoelectric point, the net charge on protein molecules is zero, therefore there are strong attractive forces between the molecules. This reduces the solubility of the protein molecules markedly. On either side of the isoelectric point, as the net positive (acid) or negative (alkaline) charges increase, the repulsive forces become stronger and more protein goes into solution. This explains the shape of the pH-solubility curve. At very high pH's, apart from the strong repulsive forces, dissociation and/or fragmentation of the protein molecules occur, further enhancing protein solubility.

On the acid side of the pH range the highest solubility was at pH 2.0 for all the samples. The amount of protein soluble at pH 2.0 was however less than that at pH 9.0 and above. The melonseed protein is therefore more soluble in the alkaline medium than in acid. The low solubility of melonseed protein in the acid range would not favour its use in acid foods except in a function where

protein solubility is of minimum importance. The proportion of protein extracted at the various pH's shows that at pH 2.0 only about 35% was extracted for all samples. At pH 9.0 about 50% was extracted and at pH 11.0 more than 70%, for all samples. At the isoelectric point only about 16% of the total protein present in the melonseeds was extracted.

Other workers have found melonseed-proteins to have minimum solubilities between pH 3.0 and pH 5.5 (King and Onoura, 1984; Ige et al, 1984; Akobundu et al, 1982) Preparation of protein isolates from melonseed flours would involve the standard method of solubilising the protein at the pH of maximum solubility (above pH 9.0 in this case) and then precipitating the protein at the isoelectric point (pH 3 to 4).

#### 4:3:4 Effect of Ionic Strength on Protein Solubility of Melonseed

Protein was extracted from the melonseed flours at various ionic strengths. The changes occurring in protein solubility with changing ionic strength are shown in Figure 9.

For all the samples protein solubility increased as ionic strength increased from zero (0) to 0.6. After 0.6 the solubility started decreasing. The increase in solubility from ionic strength zero (0) to 0.6 may be attributed to the

FIGURE 8

Effect of pH on Protein Solubility of Melonseeds  
(Cucumeropsis edulis) and (Citrullus lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri

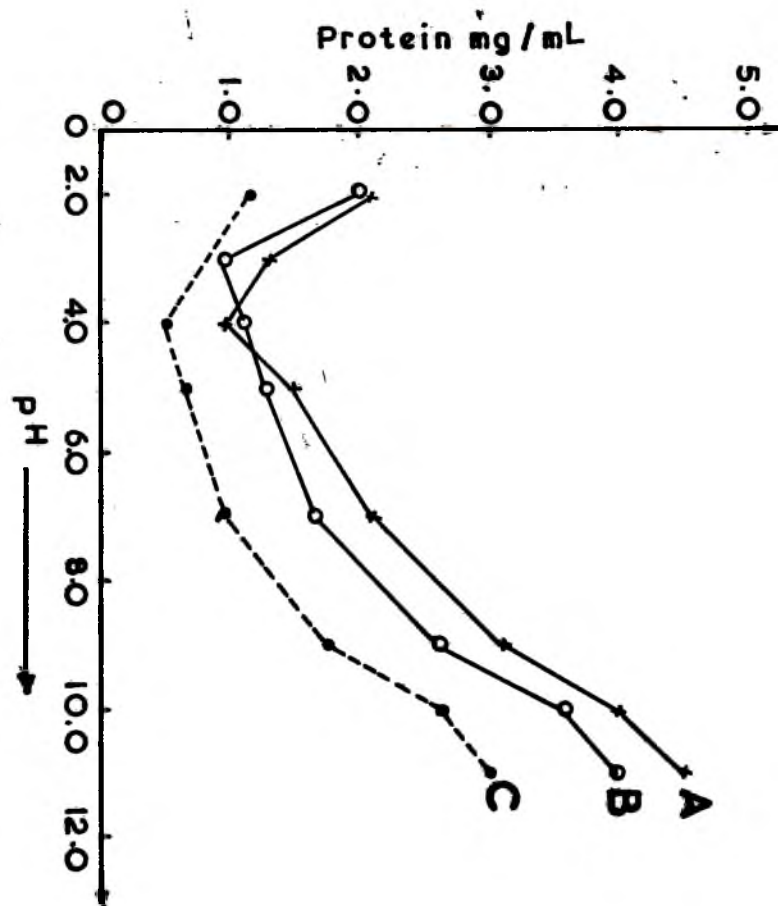




FIGURE 9

Effect of Ionic Strength on Protein Solubility of  
Melonseeds (Cucmeropsis edulis and Citrullus  
lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri

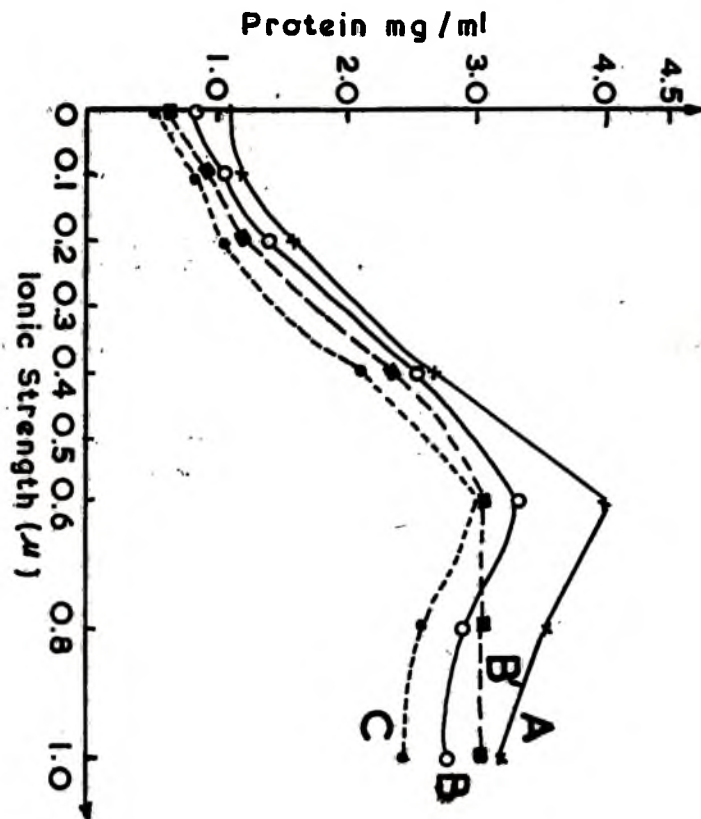




Table 10

Effect of Ionic Strength on Protein Solubility of Agushie (Cucumeropsis edulis) and Neri (Citrullus lanatus).

ANOVA SUMMARY TABLE

<u>Source of variation</u>	<u>df</u>	<u>Sum of</u> <u>squares</u>	<u>Mean square</u>	<u>F</u>
Variety	2	1.5622	0.7811	38.4778**
Ionic Strength	6	19.6847	3.2808	161.6158**
Error	12	0.2432	0.0203	
Total	20	21.4901		

\*\*Significant  $P < 0.01$

interaction of the protein molecules with the salt ions. This reduces protein-protein interaction and the protein goes into solution more easily. As the concentration of the sodium chloride solution increases (i.e. more than 0.6) the hydrophilic groups are surrounded by more salt ions producing interaction with water molecules. This leads to a kind of dehydration of the protein molecules making less of them go into solution (i.e. solubility is reduced).

The percentage of protein extracted at each ionic strength based on the total protein of the defatted flours was calculated. This showed that for agushie variety 1, the amount extracted ranged from 19% at ionic strength zero to 70% at the ionic strength of maximum solubility (0.6). For agushie variety 2 the range was from 15% to 60% and for neri it was 17% to 93%. The low solubility at ionic strength zero is not surprising since the bulk of melonseed proteins are not water-extractable.

Statistical analysis of the data using analysis of variance (Table 10) confirms that the changes in protein solubility as ionic strength was changed are significant and not due to chance. Apart from the 'salting-in' and 'salting-out' effects, association-dissociation reactions may also account for the observed changes in protein solubility. Dissociation of the protein molecules may occur in the sodium chloride solutions as ionic strength increases up to 0.6. After this there may be

reassociation of some of the fractions leading to reduction of the solubility. The analysis of variance also indicates that the differences in the solubility of the three varieties of melonseed at each ionic strength are significant. This may mean that the salt-soluble proteins of the agushie varieties 1 and 2 and neri react slightly differently from each other and this affects their solubility. There may be some differences in their conformation. The agushie variety 2 was roasted at 140°C for 45 min. and the defatted flour was evaluated. The effect of ionic strength on its protein solubility is also shown in Figure 9. From ionic strength zero to 0.6, there was increase in protein solubility as with the raw defatted flour. The amount of protein extracted did not change from ionic strength 0.6 to 1.0. This was 55% of the total protein. For the raw agushie variety 2 flour, amount of protein extracted was 60%. More protein was extracted from the raw flour than from the roasted flour at ionic strengths zero to 0.6. Protein denaturation during roasting may account for the decrease in solubility. The heating may have caused conformational changes in the protein molecules such that no further change occurred in solubility when maximum solubility was reached, even though the ionic strength was increased.

A t-test between the solubilities of the unroasted and roasted samples showed no significant difference between the two at different ionic strengths. This may mean that the degree of denaturation at 140°C was not significant, accounting for the

fact that the differences between the solubilities of the two samples were not great.

#### 4:3:5 Effect of Roasting Temperature on Protein

##### Solubility of Melonseed

The agushie and neri were roasted at 140°C, 160°C and 180°C for 45 min. defatted and evaluated. The results are shown in Table 11. Two solvents, distilled water and sodium chloride solutions of ionic strength 0.6 were used. For all the melonseed samples protein solubility decreased with increase in roasting temperature. This was more marked in the sodium chloride solution. In the distilled water protein solubility did not decrease when roasting temperature was increased from 160°C to 180°C. In sodium chloride solution there was a further decrease in protein solubility. It is evident from this results that solubility was higher in sodium chloride solution than in the distilled for all the samples. This is to be expected since most of the melonseed proteins are more soluble in sodium chloride solution than in water

The percentage reduction in solubility was calculated based on the solubility of the raw samples. Solubility at each roasting temperature was compared to the solubility of unroasted sample. Figure 10 shows the percentage reduction in solubility with roasting. It appears that roasting at 160°C caused all the



water extractable proteins to be denatured. Roasting at 180°C therefore did not cause further changes in these proteins and so there was no further reduction in the solubility (Figure 10I). This observation applies to all three melonseed varieties studied; suggesting similarities between their proteins which are water-extractable.

The salt-soluble proteins behaved differently. Protein solubility decreased with increasing roasting temperature (Figure 10II). At 140°C and 160°C the reduction in solubility of the salt-soluble proteins was lower than the reduction for the water extractable proteins. This was observed for both the agushie varieties and the neri. This could mean that the heating did not affect the salt-soluble proteins as much as the water-soluble proteins at these temperatures. This may be because the salt-soluble proteins which are the storage globulins are surrounded by membranes. At 180°C a marked reduction in protein solubility was observed, (86% for neri; 71% for agushie variety 1 and 62% for variety 2). This may indicate extensive denaturation of these proteins at 180°C.

Analysis of variance of the data indicates that the variation in protein solubility observed with change in extracting solvent and roasting temperature were significant. However the variation due to variety of melonseed used was not significant (Table 12). This suggests that when the melonseeds are roasted, changes occurring in their conformation may cause them to react similarly.

Table 11

Effect of Roasting Temperature on Protein Solubility of  
 Agushie (Cucumeropsis edulis) and Neri (Citrullus lanatus)  
 in Distilled Water and 0.6M NaCl solution.

Roasting Temp.	<u>Distilled</u> Water			<u>0.6M NaOH</u>		
	*AV <sub>1</sub>	AV <sub>2</sub>	Neri	AV <sup>1</sup>	AV <sub>2</sub>	Neri
Raw	1.1	0.825	0.55	4.00	3.30	3.03
140	0.55	0.625	0.35	3.45	3.05	2.55
160	0.40	0.40	0.30	2.175	2.25	1.45
180	0.40	0.40	0.30	1.15	1.25	0.50

\* AV<sub>1</sub> = Agushie variety 1

AV<sub>2</sub> = Agushie variety 2

Table 12

Effect of roasting temperature on Protein solubility of Agushie (Cucumeropsis edulis) and Neri (Citrullus lanatus) in Distilled Water and 0.6M NaOH solution.

ANOVA SUMMARY TABLE:

Source of Variation	df	Sum of squares	Mean square	F
Solvent	1	20.0842	20.0842	73.6879**
Roasting Temperature	3	7.5637	2.5212	9.2501**
Variety	2	1.1786	0.5893	2.1621ns
Error	17	4.6335	0.2726	
Total	23	33.4600		

\*\* Significant at  $P < 0.01$

ns not significant

FIGURE 10

Percentage (%) Reduction in Protein Solubility with  
change in Roasting temperature.

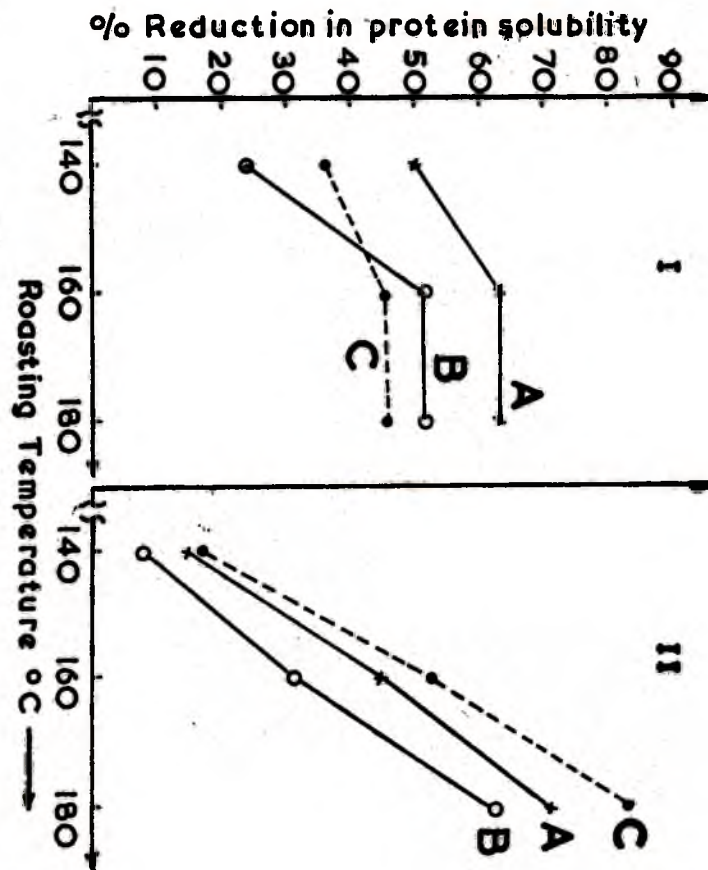
I Solvent:- Distilled water

II Solvent:- 0.6M NaCl solution

A = Agushie variety 1

B = Agushie variety 2

C = Neri



The fact that the roasting temperature had a significant effect on protein solubility suggests that protein solubility can be used as an index of protein denaturation. In most cases, the higher the degree of denaturation, the lower the protein solubility.

#### 4:4 Water Absorption of Melonseed Flour

The binding and immobilisation of water by proteins is an important functional characteristic. The extent to which this occurs is determined largely by the hydrophilic and hydrophobic groups on the protein molecules. Water absorption can lead to swelling of the protein matrix in some cases, but in some food products, the ability of a protein to absorb water without swelling is desired.

##### 4:4:1 Effect of Ionic Strength on Water Absorption Capacity of Melonseed flours

The water absorption capacity of the raw defatted melonseed flours as affected by changing ionic strength is presented in Figure 11.

Agushie variety 2 had the highest water absorption capacity (WAC) at all ionic strengths. From ionic strength zero to 0.4, neri had a higher WAC than agushie variety 1. From ionic strength 0.6 to 1.0 the WAC of agushie variety 1 was higher than that of neri.



For all the samples, there was a drop in water absorption capacity (WAC) from ionic strength zero to 0.2. WAC increased thereafter. The addition of small amounts of sodium chloride seem to depress WAC but higher quantities enhance the WAC of the melonseed flours.

The water absorbed depends on the hydrophilic groups present on the protein molecules. It may therefore mean that more hydrophilic groups are present on the protein molecules of agushie variety 2, hence its higher WAC. On the basis of the fact that water is absorbed by the proteins which are not in solution, the less protein that is solubilised the higher should be the WAC. The data on percentage of total protein solubilised at various ionic strengths indicate that less protein is solubilised at all ionic strengths by agushie variety 2 (15-59%) compared to agushie variety 1 and neri. At ionic strengths 0.6 to 1.00 much more protein is solubilised by neri (93% to 75%) than variety 1 (70% to 56%). This may explain why agushie variety 1 has a higher water absorption capacity at these ionic strengths than neri.

The interaction of hydrophilic/hydrophobic groups present on the protein molecules and the amount of protein solubilised contribute to determining the water absorption capacity at a particular ionic strength.

To test whether there was any correlation between the protein

solubility and water absorption at various ionic strengths, the correlation coefficient ( $r$ ) was calculated. The calculated  $r$  for each sample was found to be less than the critical values of  $r$  at 3 degrees of freedom. (Agushie variety 1;  $r = 0.5338$ ; variety 2;  $r = 0.8081$ ; neri:  $r = 0.3280$ ) This means there is no significant correlation between protein solubility at any ionic strength and the water absorption capacity. Protein solubility alone can therefore not be used to explain the water absorption capacities of the melonseed flours. Since flours and not protein isolates were used, other food components such as the sugars present, may have an effect on the water absorption capacity. Fleming et al (1974) noted that the addition of 5% NaCl increased the water absorption of soybean and sunflower seed flours, but decreased that of their protein concentrates. This suggests interference from other components such as carbohydrates present in the flours.

#### 4:4:2 Effect of Roasting Temperature on Water Absorption Capacity of Melonseed Flours

Samples which had been defatted after roasting at 140°C, 160°C and 180°C for 45 min. were evaluated for their water absorption capacities in distilled water. The results are graphically presented in Figure 12.

The agushie varieties 1 and 2 behaved similarly but the neri was different. For all the melonseeds water absorption capacities

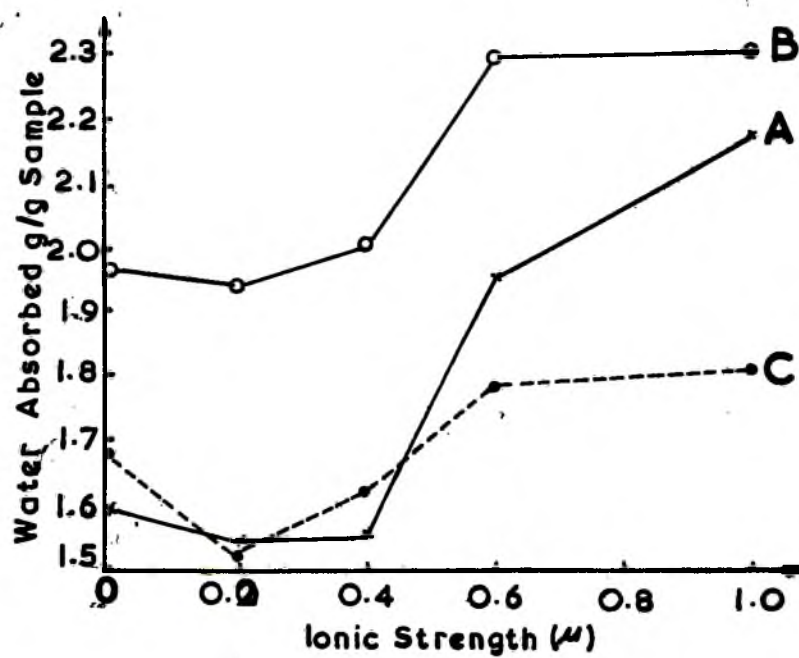
FIGURE 11

Effect of Ionic Strength on Water absorption capacity  
Melonseeds (Cucumeropsis edulis and Citrullus lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri



of the unroasted samples were highest. The water absorption capacities (WAC) of the agushie varieties reduced drastically when roasted at 140°C. The WAC rose slightly for samples roasted at 150°C. This did not change for samples roasted at 180°C. For the neri, the WAC of the sample roasted at 140°C was the same as that of the unroasted sample. The WAC decreased at 160°C but did not change when roasting was done at 180°C. Conformational changes of the proteins on heating may account for the water absorption capacities observed. Since water was used, it is likely that it was changes occurring in the water-extractable proteins which affected the water absorption capacities. From Fig. 10-I the solubility of the roasted melonseed samples in water decreased from 140°C to 160°C, but did not change on roasting at 180°C. This may mean that the same proteins are involved in the water absorption of the roasted samples when just water is used. The denaturation of the agushie water-soluble protein at 160°C may completely expose the groups which favour water absorption, so that a higher temperature has no further effect. The effect of roasting the neri at 150°C may also be such that the higher temperature caused no further changes in its water extractable proteins, hence no change in its water absorption capacity.

For all the roasted samples, a high significant negative correlation ( $r$ ) was found between water absorption capacity and protein solubility. (Agushie variety 1:  $r = 1.000$ ; variety 2: =

$r = -0.9444$ ;  $\text{neri} = r = -1.000$ ). This may mean that changes which occur in the protein molecules on roasting at  $140^{\circ}\text{C}$  and  $160^{\circ}\text{C}$  enhance their water absorption capacities even though these changes produce their solubility. This suggests that the less soluble the water extractable proteins are, the higher their water-binding ability. This is in contrast to the salt soluble proteins which showed no significant correlation between their solubility at different ionic strengths and water absorption capacity.

#### 4:4:3 Effect of Incubation Temperature on Water Absorption capacity of melonseed flours

The water absorption capacity of the defatted flours was measured at different temperatures. This was done using water at a given temperature and keeping the dispersion at that temperature for 30 min. The results are shown in Figure 13. Increasing temperature from  $5^{\circ}\text{C}$  to  $80^{\circ}\text{C}$  brought about an increase in water absorption capacity (WAC) for all samples. The increase from  $5^{\circ}\text{C}$  to  $50^{\circ}\text{C}$  was minimal, but from  $50^{\circ}\text{C}$  to  $80^{\circ}\text{C}$  a marked increase was observed. This marked increase in WAC may be due to gain in energy which may cause dissociation of the molecules and other conformational changes exposing more hydrophilic groups to bind the water molecules.

FIGURE 12

Effect of Roasting Temperature on Water absorption capacity of Melonseeds (Cucumeropsis edulis and Citrullus lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri



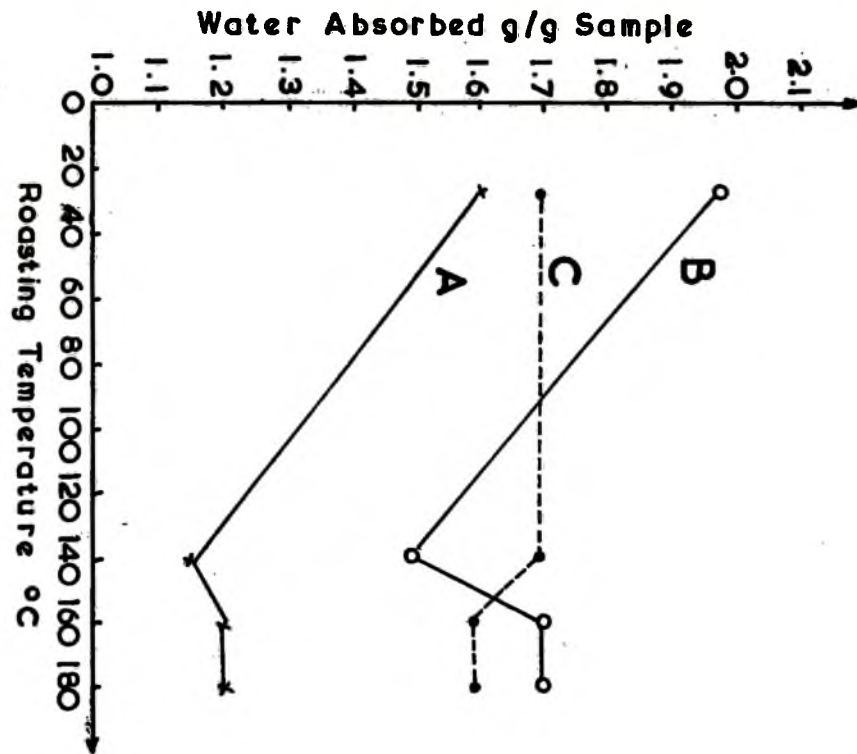


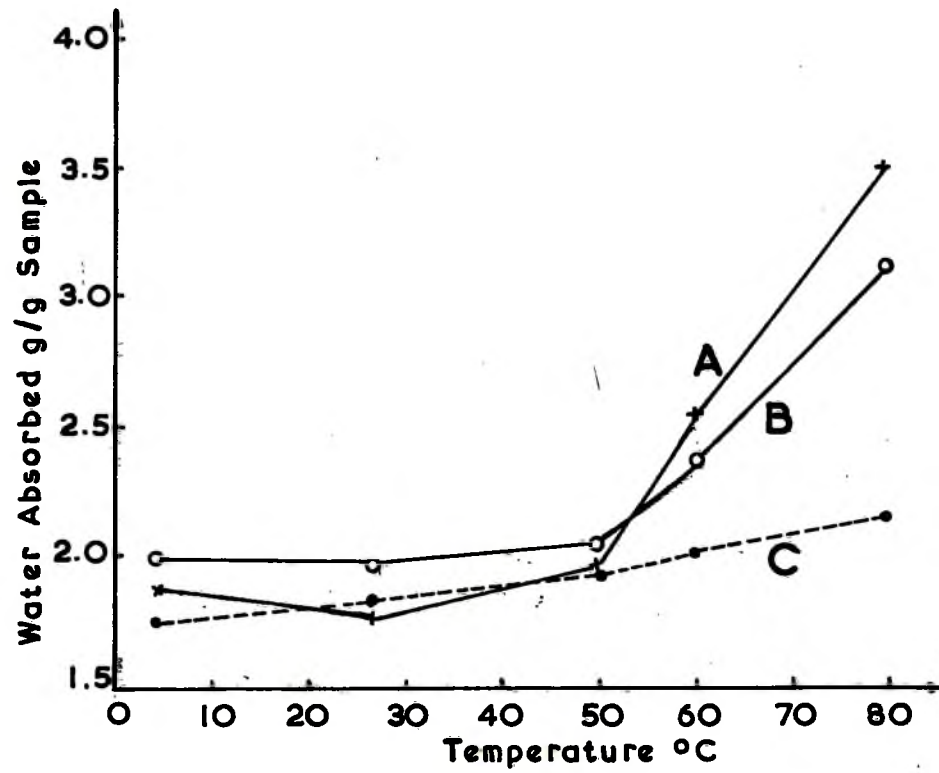
FIGURE 13

Effect of Incubation Temperature on Water absorption  
Capacity of Melonseeds (Cucumeropsis edulis and  
Citrullus lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri



Comparing Figure 7 (effect of dispersion temperature on protein solubility) and Figure 13, shows that when there was a marked increase in protein solubility from 5°C to 50°C water absorption capacity was not greatly increased. From 50°C to 80°C there was minimal increase in protein solubility but water absorption capacities increased markedly. It was observed that after standing for 30 min. at 80°C some coagulation of the melonseed flours had occurred. Agushie variety 1 had coagulated more than variety 2 whilst very little coagulation had occurred in neri. The very high water absorption capacities at 80°C could be partly due to more water being taken up by the flours on coagulating. This may be especially so for agushie variety 1 which coagulated most.

#### 4:5 Fat Absorption Of Melonseed Flours

Fat absorption is important in proteins to be used as meat extenders and replacers and other food products in which fat plays an important role.

##### 4:5:1 Effect of Roasting Temperature on Fat Absorption Capacity of Melonseed Flours

Raw and roasted defatted flours were the samples evaluated. The results are presented in Figure 14.

The trend shown by the flours in their fat absorption is clearly

different from that of the water absorption capacity (Figure 12) Fat absorption capacities (FAC) of the raw flours were highest and decreased as roasting temperature increased. For the raw samples, fat absorption was 2.96; 3.02; and 2.86g oil/g sample for agushie variety 1, variety 2 and neri respectively. These decreased to 1.56, 1.76 and 1.73 at roasting temperature 180°C for agushie variety 1, variety 2 and neri respectively. Agushie variety 2 had higher fat absorption capacities than variety 1 at all roasting temperatures. Neri's fat absorption capacity was in between the two. The change in fat absorption of neri roasted at 140°C and that roasted at 160°C was minimal. For the agushie varieties 1 and 2 however, each change in roasting temperature brought about a marked reduction in fat absorption.

Fat absorption is supposed to involve the lipophilic groups on the protein molecules. These are quite different from the groups involved in water absorption. It is therefore not surprising that the two characteristics showed different trends when heat denaturation caused changes in the lipophilic groups, making them less able to bind fat. This loss in fat binding ability continued when roasting temperature was increased to 180°C in contrast to water absorption which did not change. Physical entrapment of oil is also thought to play a part in fat absorption. Kinsella (1976) stated that chemical modification of protein, which increased its bulk density also increased fat absorption. Apart from the effects of heat denaturation the bulk density of the roasted samples may be lower than that of raw

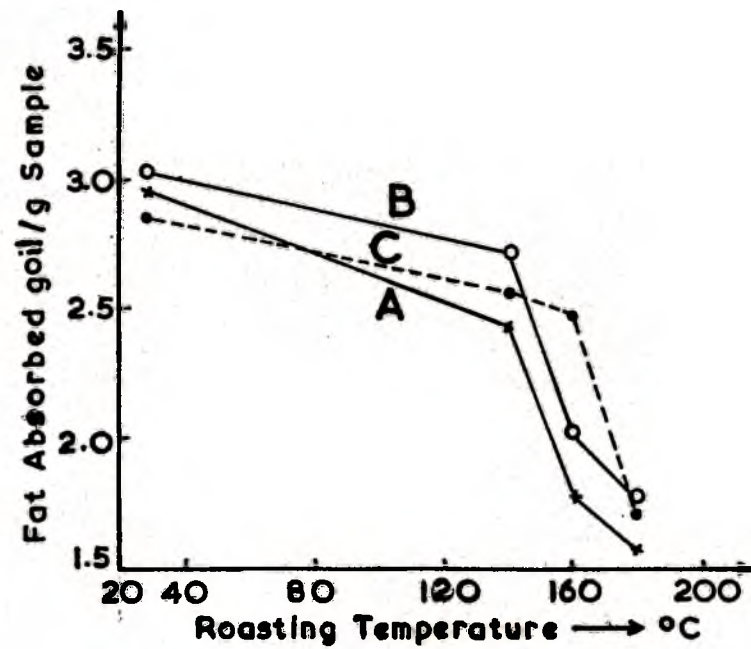
FIGURE 14

Effect of Roasting Temperature on Fat absorption Capacity  
of melonseed (Cucumeropsis edulis and Citrullus lanatus)  
flours

A = Agushie variety 1

B = Agushie variety 2

C = Neri





flours hence the marked reduction in fat absorption on roasting. Generally it seems proteins with high fat absorption capacities have lower water absorption capacities and vice versa because different groups are involved. (Lin *et. al.*, 1974; Sathe *et. al.*, 1982b; Akobundu *et. al.*, 1982).

The relatively high fat absorption capacity of the melonseed flours suggests that they may be useful in products where fat binding is of importance. Agushie variety 2 would fulfill this role better than variety 1 and neri based on the results obtained.

#### 4:6 Foaming Properties

##### 4:6:1 Effect of pH on foaming properties



Raw defatted flours were evaluated for the effect of changing pH on their foaming properties. Figure 15 shows the effect of pH adjustment on the initial foam volume of the three melonseed flours.

Agushie variety 2 had a higher initial foam volume than variety 1, whilst neri had the lowest at all pH's. The highest foam volume for all three flours was at pH 11.5, followed by pH 2.0. The difference in the foam volumes at these two pH's is however very great. At pH 11.5 foam volumes were 220ml, 225ml and 210ml

for agushie variety 1, variety 2 and neri respectively. At pH 2.0, the initial foam volumes were 47ml, 35ml, and 24ml for the three melonseeds respectively. Minimum foam volumes of 9ml, 13ml and 7ml were obtained at pH 3.0 for agushie variety 1, variety 2 and neri respectively. Between pH 3.0 and pH 8.0 the initial foam volumes of all the samples did not change much.

At pH 3.0 very little protein is soluble as seen from Figure 8 (pH-solubility curve). This may explain why very little foaming occurred, since for foaming to occur, the proteins must be solubilised. The initial foam volumes obtained at pH 11.5 and pH 2.0 are much more than can be explained solely on the basis of the amount of protein which is in solution. The state of the protein in solution at these pH's may play an important role. Dissociation of the large protein molecules into smaller polypeptide fragments occur under extreme alkaline and acid conditions. These small molecules apart from being readily soluble can readily migrate to the liquid-air interface lowering the surface tension of the liquid sufficiently to encapsulate air. This enhances foaming. The small molecules are also better able to form a cohesive layer around the air cells and this stabilises the foams. According to Townsend and Nakai (1983) for good stabilisation of air cells, there is a need for a good balance between the hydrophilic and hydrophobic groups of the protein. The fragmentation of the protein molecules at pH 2.0 and 11.5 may help bring about this balance. At pH 11.5, the

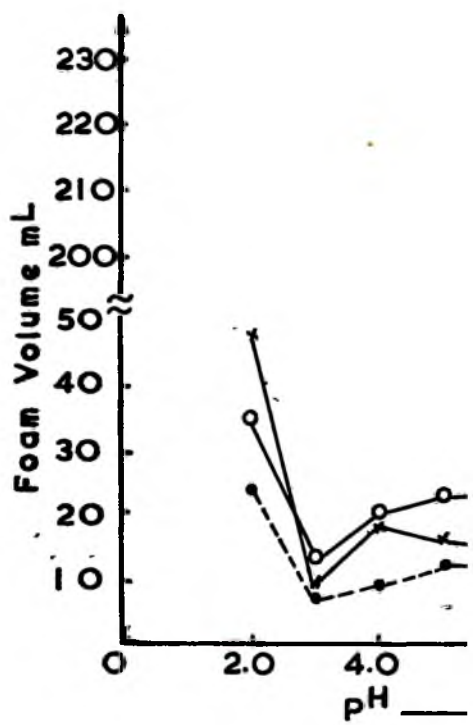
FIGURE 15

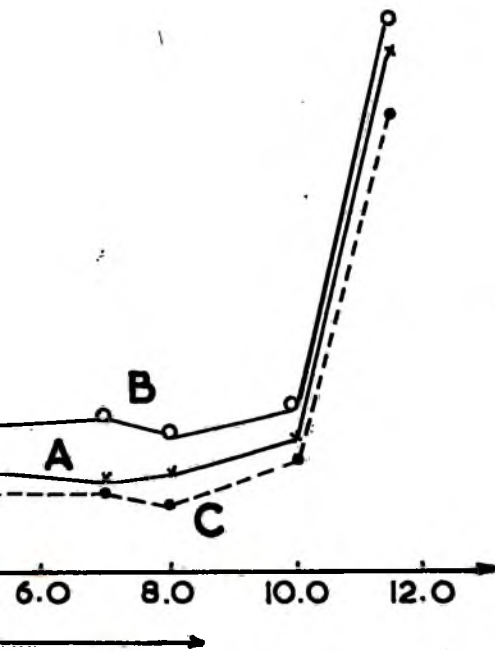
Effect of pH on Initial Foam volume of Melonseed flours  
(Cucumeropsis edulis and Citrullus lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri





differences in the initial foam volumes of the agushie varieties and neri are not wide. Complete dissociation of their protein molecules may have occurred and this counteracted any differences in protein solubility which may have brought about wider differences in their initial foam volumes.

The type of foam formed for all three samples at the different pH's were similar. At pH 2.0, 10.0 and 11.5, very thick stiff and compact foams were obtained. The air cells in these foams were very tiny. These foams looked like whipped egg white, and their stability was also good. Foam stability at the different pH's are presented in Figures 16a (pH 2 to 5) and 16b (pH 7 to 11.5). At pH 3.0 and 4.0, the foams were very thin with large air cells giving them an open look. Stability of these foams was also poor. From pH 5.0 to pH 9.0, the foams were less open, but not as thick as the foam at pH 2.0 and 11.5. On the whole the foam stability of the melonseed flours was quite good, considering that 1% (W/V) dispersions were used. Also at the end of 120 min. some foam remained at all the different pH's, and the foams formed at pH 2.0 and pH 11.5 had not showed much change in structure.

Melonseed proteins may therefore be quite useful in the preparation of whipped products due to their good foaming properties.

**4:6:2      Effect of Ionic Strength on Foaming Properties**

Raw defatted flours were made into 1% dispersions using sodium chloride solutions of different ionic strengths. These were whipped and their foaming properties evaluated.

The effect of ionic strength on initial foam volume of the agushie and neri is shown in Figure 17. Agushie varieties 1 and 2 had similar initial foam volumes. From ionic strength zero (0) to 0.2, variety 2 had higher volumes than variety 1. From ionic strength 0.40 to 0.80 variety 1 had higher volumes, and at ionic strength 1.00 the volumes were equal.

Neri had lower volumes at all ionic strengths. At ionic strength 0.40, the highest initial foam volumes for neri and agushie variety 2 were obtained. Agushie variety 2 had its highest initial foam volume at ionic strength 0.20.

Foam stability of the three melonseed varieties is shown in Figures 18a (ionic strength zero to 0.20) and 18b (ionic strength 0.40 to 1.00).

Initial foam volumes of all the samples were lowest in distilled water (i.e. ionic strength zero). The stability of these foams was also poor. It appears that sodium chloride enhances the foam volume and stability of all the samples. At the end of 120 min. more than 5ml of foam remained when sodium chloride was added.



FIGURE 16a

Foam stability of Melonseeds (Cucumeropsis edulis  
Citrullus lanatus) at different pH's (3-5)

A = Agushie variety 1

B = Agushie variety 2

C = Neri

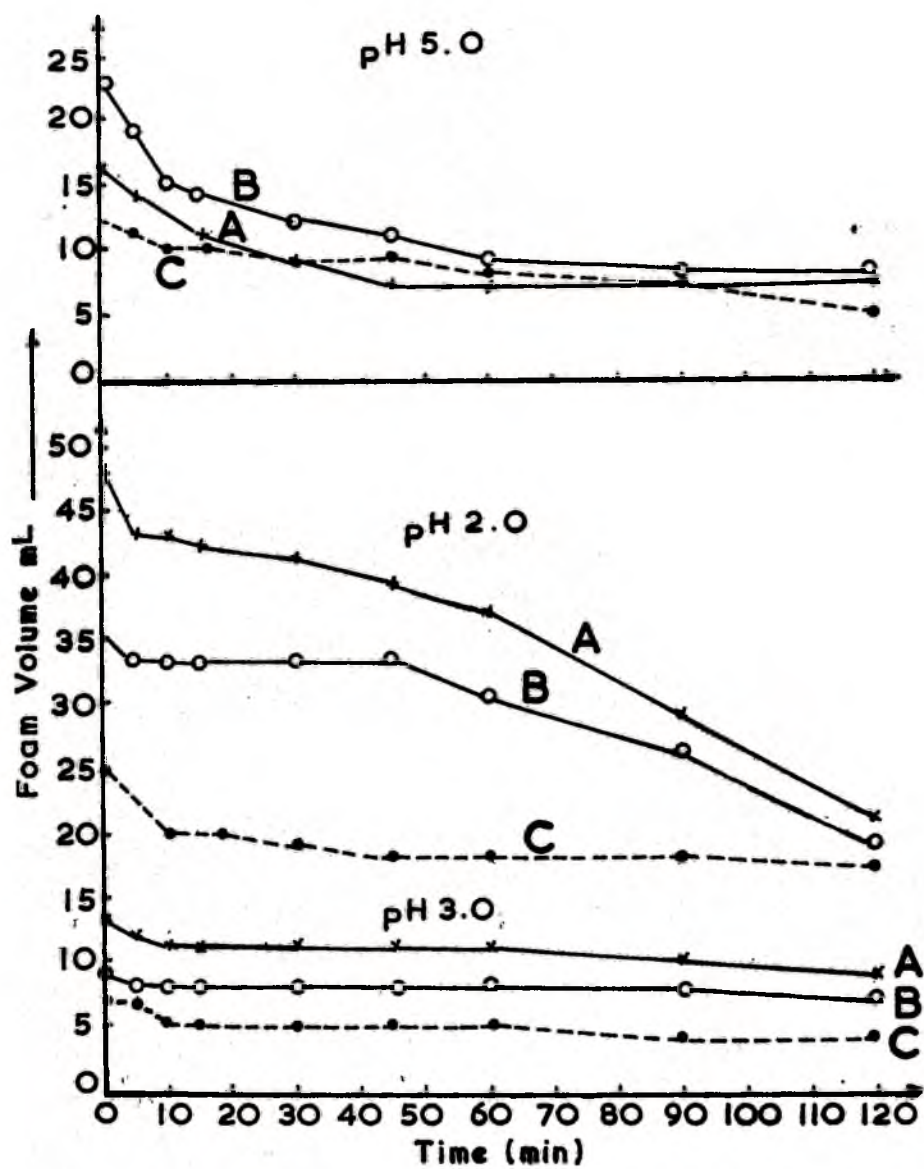


FIGURE 16b

Foam stability of Melonseeds (Cucumeropsis edulis and Citrullus lanatus) at different pH's (7 -11)

A = Agushie variety 1

B = Agushie variety 2

C = Neri

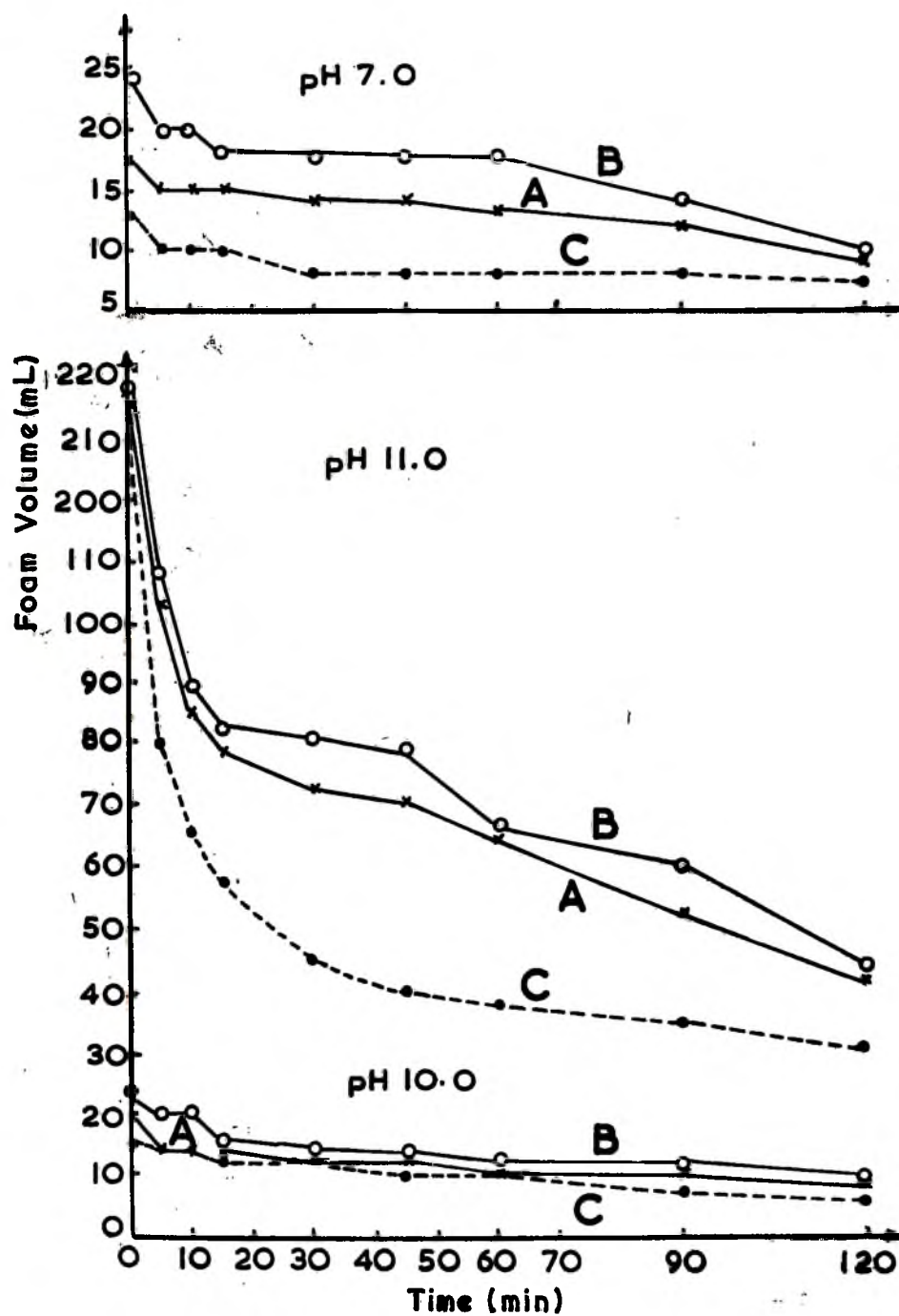


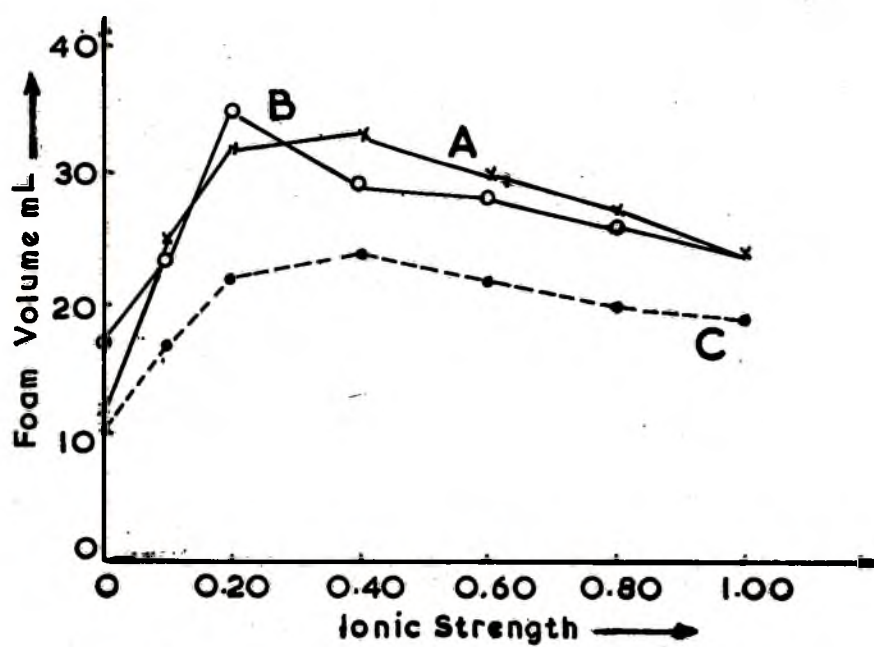
Figure 17

Effect of Ionic strength on initial foam volume of  
melonseeds (Cucumeropsis edulis) and (Citrullus lanatus)

A = Agushie variety 1

B = Agushie variety 2

C = Neri



In distilled water 4ml of foam remained for agushie variety 2, whilst variety 1 and neri had no foam. The highest foam volume and stabilities were obtained at ionic strengths 0.20 and 0.40 for all samples. The increase in foam volume and stability on addition of sodium chloride may be due to the type of protein solubilised apart from the fact that more protein comes into solution on addition of sodium chloride. Calculation of correlation coefficient between protein solubility and initial foam volume at the ionic strengths used, indicated that there was no significant correlation between the two characteristics (Agushie variety 1,  $r = 0.4251$ ; variety 2  $r = 0.3320$ ; neri  $r = 0.6717$ ). This suggests that on addition of sodium chloride, foaming does not depend so much on the amount of protein in solution, but rather on the properties and state of the protein in solution. The salt-soluble proteins may be more ideal for foaming than the water-soluble proteins. Their conformation and the amino acid groups present on them, being different from those of the water soluble proteins may make them ideal as surfactant proteins. The decrease in foam volume and stability at higher ionic strengths may be due to association of the protein molecules and other conformational changes which may reduce their foam forming ability. The fact that protein solubility reduces at these ionic strengths may also be a minor contributing factor to reduction in foam volume.

Very thick, compact foams were obtained with sodium chloride



solutions of ionic strengths 0.20 to 1.0. The foams obtained using distilled water were not strong. Their air cells were quite large, and their poor stability may be due to the easier collapse of these air cells. Sathe et al (1982b) found that the foaming capacity of winged bean protein concentrates which was only moderate, could be enhanced by addition of sodium chloride. Ionic strengths 0.2 to 0.4 are about the concentration of sodium chloride in most foods. This means use of melonseed proteins- especially agushie variety 2 - in whipped products may be feasible.

#### **4:6:3      Effect of Roasting Temperature on Foaming Properties**

For each defatted roasted sample, two determinations were carried out. Distilled water and sodium hydroxide solution of pH 11.0 were used to prepare the 1% (W/V) dispersions which were whipped. For each roasted flour, very different foam volumes were obtained in distilled water and at pH 11.0. The effect of roasting temperature on initial foam volume for the two determinations is shown in Figure 19.

Very weak foams of low volume were obtained when the roasted samples were whipped in distilled water. This was in contrast to foams obtained at pH 11.0 which were very thick and compact and of high volume.

FIGURE 18a

Foam stability of Melonseeds (Cucumeropsis edulis  
and Citrullus lanatus) at different ionic strengths  
( zero to 0.2)

A = Agushie variety 1

B = Agushie variety 2

C = Neri

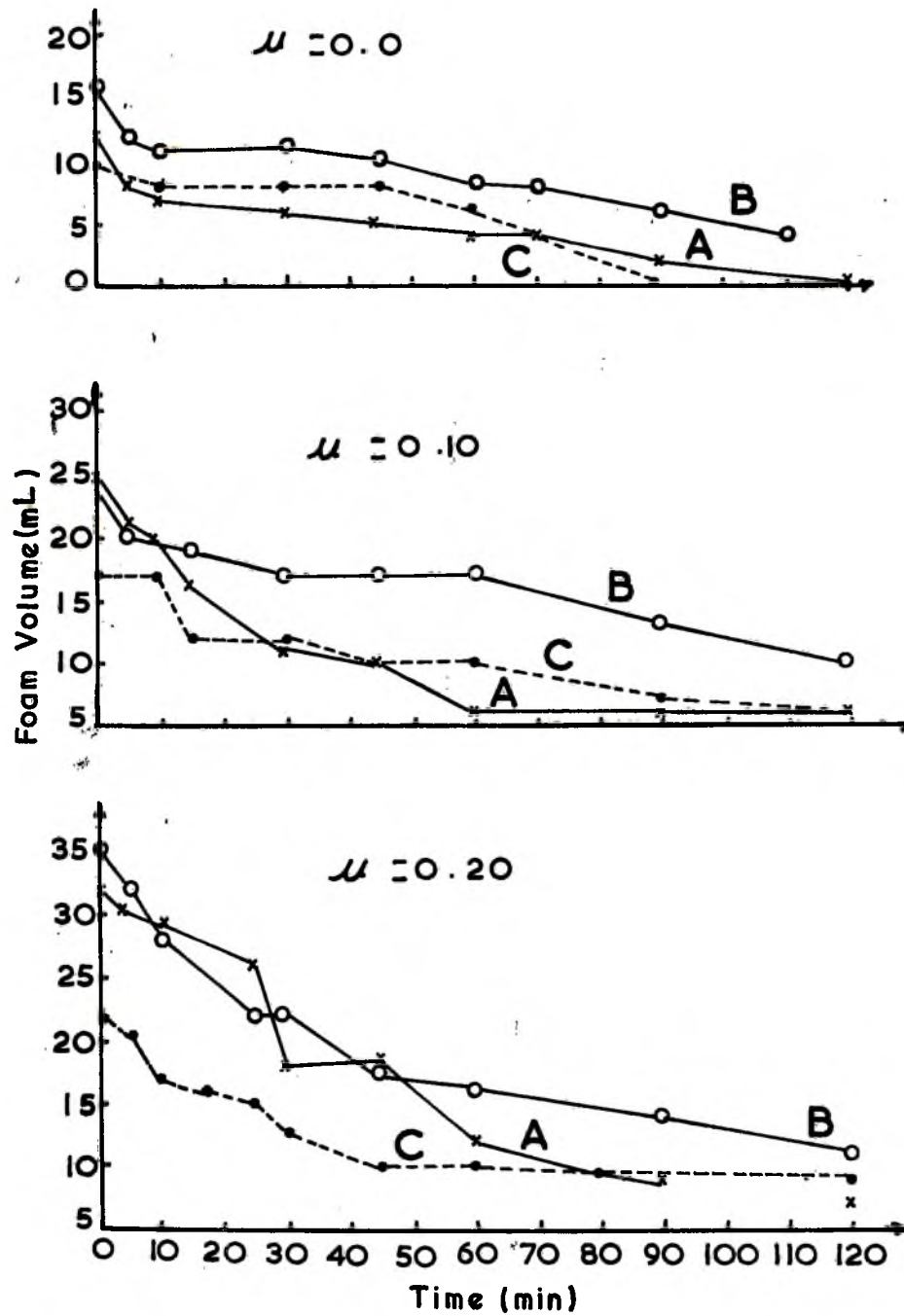


FIGURE 18b

Foam stability of Melonseeds (Cucumeropsis edulis and  
Citrullus lanatus) at different ionic strengths  
(0.4 - 1.0)

A = Agushie variety 1

B = Agushie variety 2

C = Neri

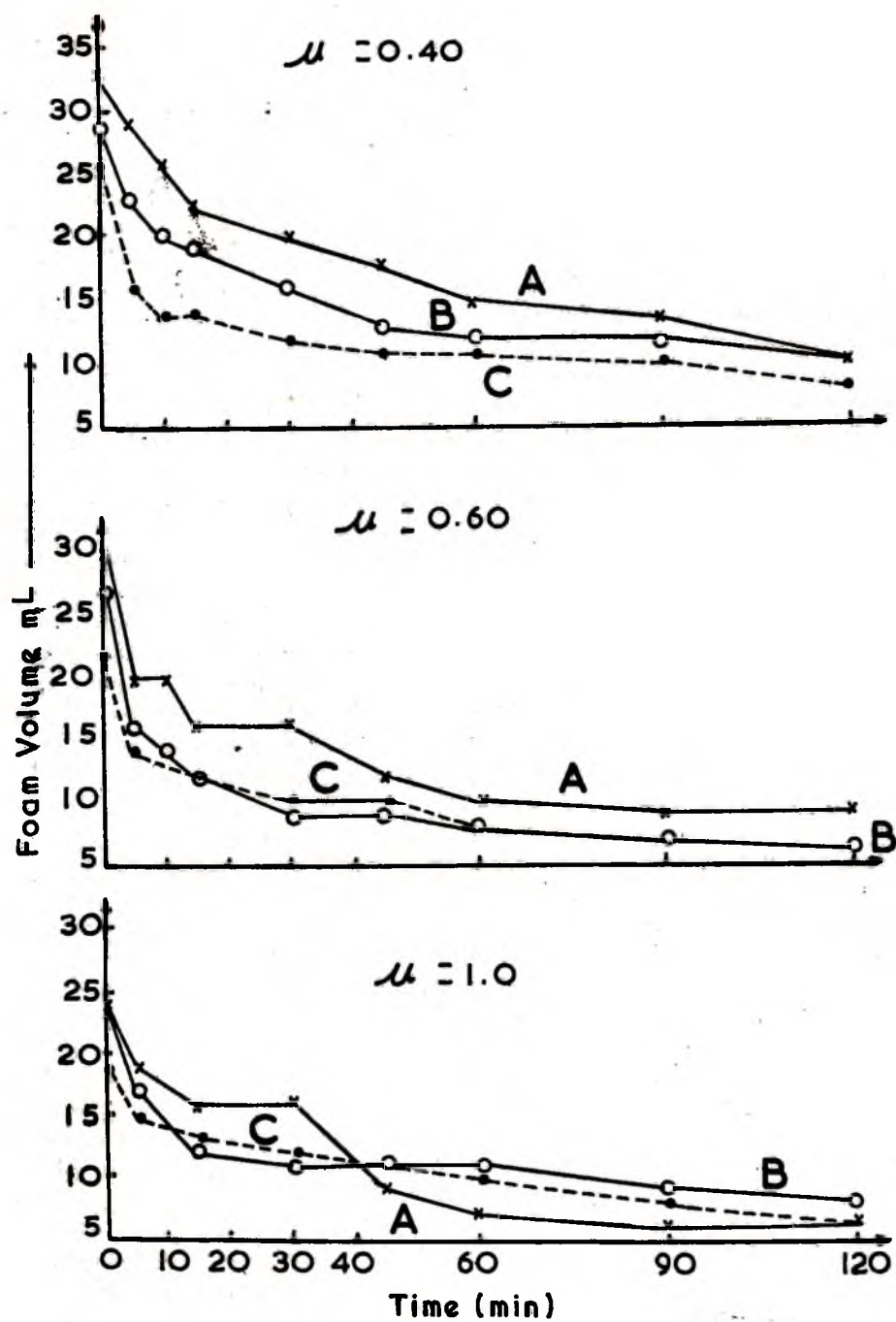


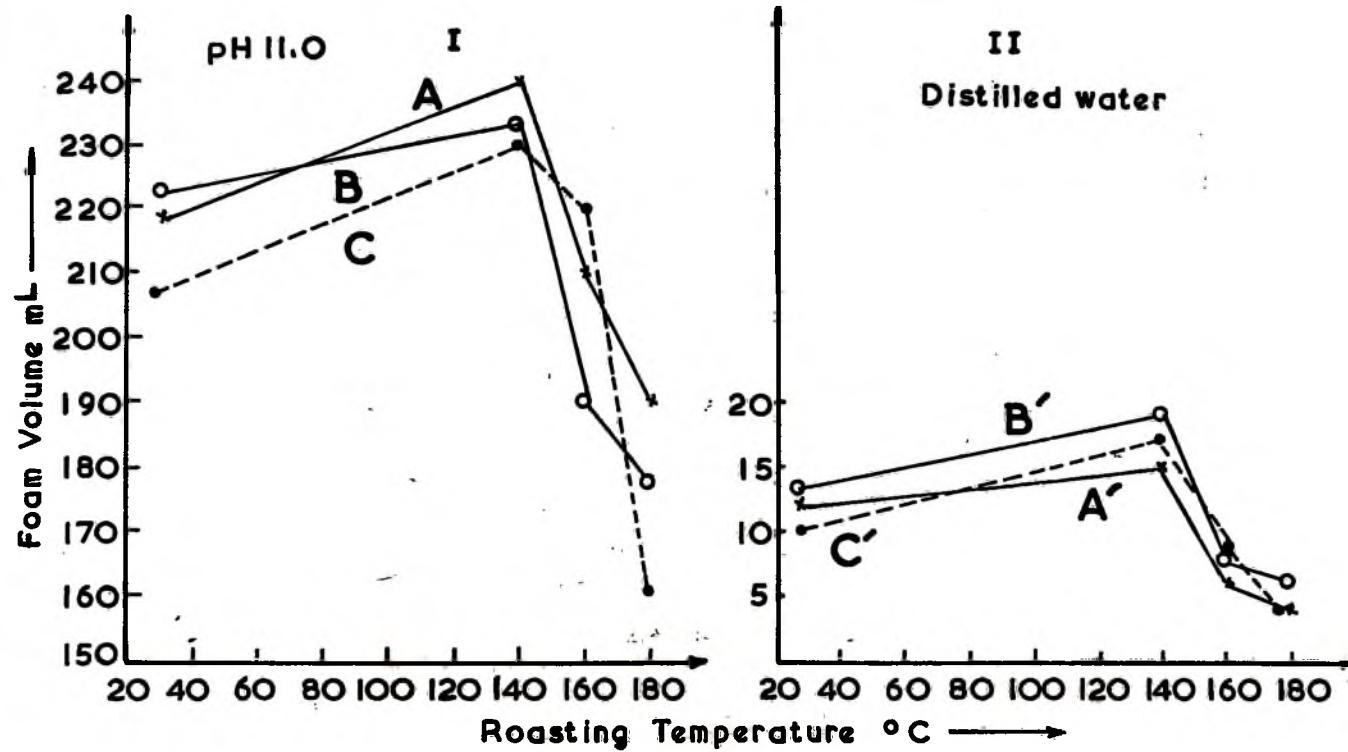
Figure 19

Effect of Roasting Temperature of Melonseeds (Cucumeropsis edulis and Citrullus lanatus) on their initial Foam volume at pH 11.5 and in Distilled water

A; A' = Agushie variety 1

B; B' = Agushie variety 2

C; C' = Neri





Samples roasted at 140°C had higher foam volumes than the raw samples both in distilled water and at pH 11.0. Foam volumes of samples roasted at 160°C and 180°C were however lower than foam volumes of the raw samples. The increase in foam volume may be due to unfolding of the protein molecules to expose groups which favour foam formation. At the higher temperature, further denaturation causes conformational changes in the molecules which reduce their foaming ability. Loss in protein solubility may also be a contributing factor.

At pH 11.0, even though the protein may have been heat denatured, the dissociation of the protein molecules into the smaller fragments suitable for foam formation counteracts the effect of heat denaturation. Foam volume and stability are therefore greatly enhanced. This observation further suggests that the state of the protein in solution plays a dominant role in determining foaming properties. At all roasting temperatures all the melonseed flours had foams of the same structure at pH 11.0 meaning their proteins may have been reduced to the same state by the alkaline medium.

Melonseed proteins may therefore be modified to obtain optimum foam volume and stability for use in whipped products.

#### 4:6:4 Effect of Whipping Temperature on Foaming Properties

One percent (1%) aqueous dispersions of raw defatted flours were whipped at temperatures of 5°C, 27°C, 50°C and 60°C. Figure 20 shows the effect of whipping temperature on initial foam volume, whilst Figure 21 shows the foam stability at the various temperatures.

The whipping temperature had different effects on the three melonseed flours. For agushie variety 1, there was a consistent increase in initial foam volume as whipping temperature was increased. For agushie variety there was no further increase in foam volume after 50°C. Initial foam volume for neri did not increase till after 60°C. At each temperature all the foams of the three melonseeds had the same structure though their initial volumes were different. Thick compact foams were formed at 50°C and 60°C, though the foams at 50°C had better stability. Foams formed at 80°C had the highest volumes, but they were more open than those at 50°C, and their stability was poor. At 5°C, very weak foams were formed.

Since distilled water was used for whipping, it is not likely that the water-soluble proteins were involved in the foaming. These seem to have poor foaming ability compared to the salt-soluble proteins. The increase in temperature may cause some dissociation of protein molecules especially at 50°C and 60°C,

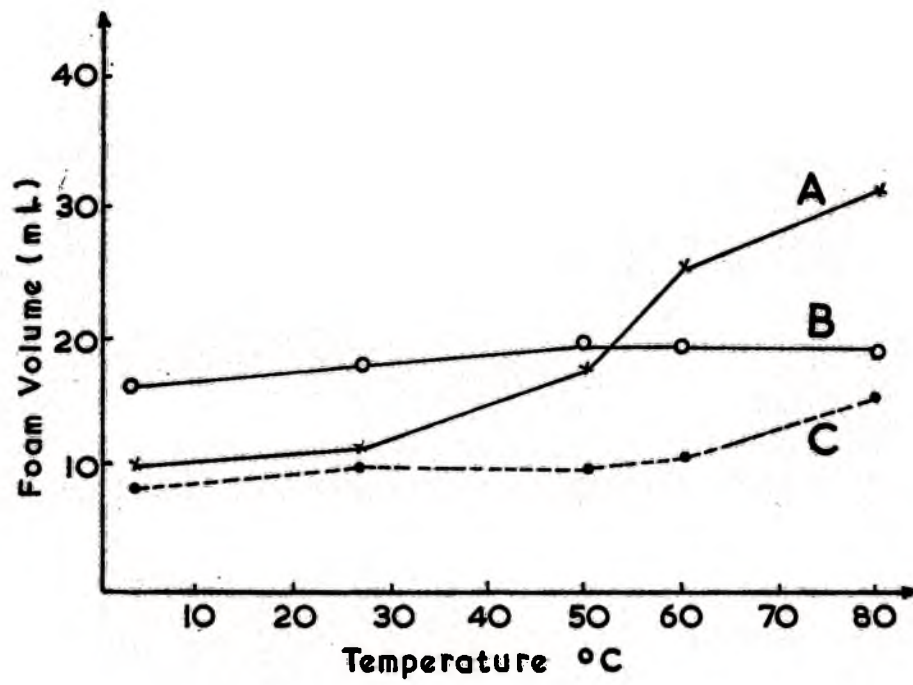


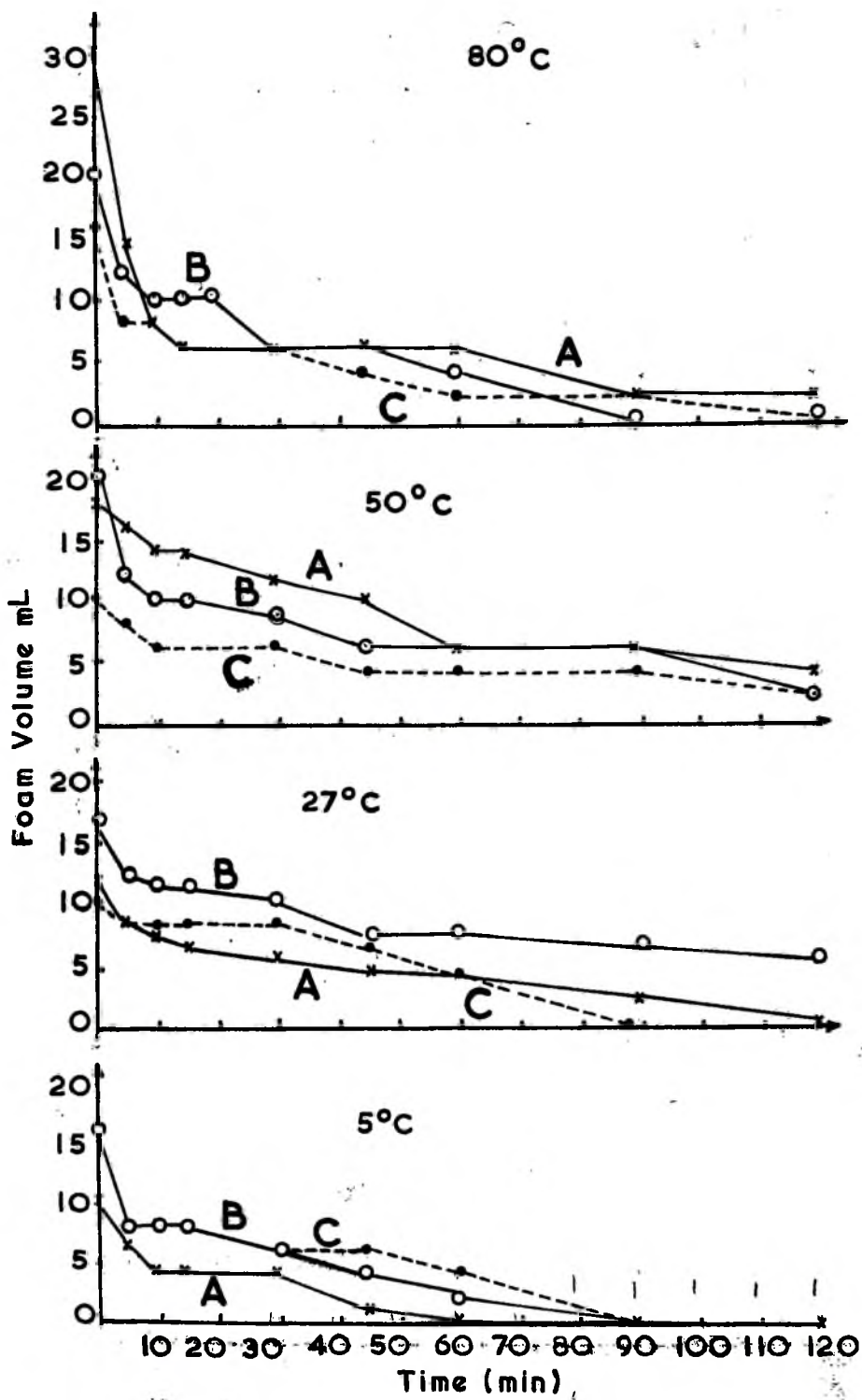
FIGURE 21

Foam stability of Melonseeds (Cucumeropsis edulis and Citrullus lanatus) at different whipping temperatures

A = Agushie variety 1

B = Agushie variety 2

C = Neri



resulting in better foaming than at the low temperatures. Increase in protein solubility with increase in temperature may also play a part. To get good foaming with water, it may be best to whip the melonseed flour at 50°C, since 60°C may be a bit too hot.

#### **4:6:5**      Effect of Defatting on Foaming Properties

Raw undefatted meals were used to study the effect of defatting. At 1% there was no foam formation for any of the samples. When the dispersions were made at 10% (W/V) and whipped, there was some foaming. The initial foam volume was less than 15ml for both agushie varieties and neri. Stability was also very poor. All the foam had broken down by the end of 30 min. The structure of the foam was open with very large air cells which were highly unstable.

The presence of oil in the meal clearly depressed foaming of the melonseed meal. This could be due to the reduction of the surfactant action of the protein molecules by the oil. The protein instead of forming a cohesive layer around the air cells, may be engaged in emulsification of the oil and water. Foam formation is therefore depressed. High concentration of protein in the presence of oil may result in some protein being available for foam formation. This may explain why some foaming occurred at 10% concentration.

This depression of foaming by lipid has been observed for other proteins as well. Lawhon et. al. (1972) reported that the presence of oil depressed foaming of cottonseed flour. The presence of small quantities of oil have also been observed to prevent foaming of egg white on whipping.

#### 4:7 EMULSIFICATION

##### 4:7:1 Effect of Concentration on Emulsification

This was a preliminary study conducted to find out the concentration of melonseed meal which would give optimum emulsifying capacity. This is because protein concentration has been reported in literature to affect emulsifying capacity.

Defatted agushie variety 2 flour was used for this experiment. The results are given in Table 13. Four per cent concentration gave the highest emulsifying capacity. This emulsion was however thinner than that obtained using 8% concentration. Emulsifying capacity increased with flour concentration from 1% to 4%. Then decreased as concentration was increased further

At 1% concentration, the amount of protein present was not enough for emulsification. At 4% there was an optimum amount of protein present. The proteins had enough room to unfold during the shearing to expose hydrophobic groups which would associate with



the lipid droplets and hydrophilic groups to associate with water molecules and bring about emulsification. Emulsifying capacity was therefore optimum. At the higher concentration (8% and 10%) the abundance of protein molecules may prevent full unfolding of the molecules to expose the groups involved in emulsification. Because of overcrowding, the molecules may not be able to align themselves at the oil-water interface in an orderly way. The net result would therefore be that even though a lot of protein molecules are in solution, not all of them would be available for emulsification. Emulsifying capacity would therefore be reduced.

Other workers have also observed this phenomenon in their work with various oilseeds (Crenwelge et al) 1974; Narayana and Narasinga, 1982; Sathe et. al.1982b).

#### 4:7:2 Effect of pH on Emulsification

The results of the study to find out the emulsifying capacity of three defatted melonseed flours are presented in Figure 22.

For all the samples, the highest emulsifying capacity was at pH 12, (62%, 60% and 57% for agushie variety 1, variety 2 and neri). At pH 2.0 high emulsifying capacity was also observed (58%, 57% and 55% for agushie variety 1, variety 2 and neri respectively). The lowest emulsifying capacity for the agushie varieties was at

Table 13

Effect of concentration on Emulsifying Capacity of Agushie  
Variety 2

Concentration%	Emulsifying Capacity%
1	48.05
4	51.12
8	50.82
10	50.62

pH 4.0 which is around their isoelectric point.

These were 48% and 47% for variety 1 and 2 respectively. The lowest emulsifying capacity for neri which was 50% was at pH 10.0.

For the agushie varieties the emulsifying capacities did not change much between pH 4.0 and pH 10.0. The emulsifying capacities of these varieties were also very close to each other. Neri had a markedly high emulsifying capacity than the agushie at pH 9.0 and between pH 3.0 and pH 5.0. At the isoelectric point of neri (pH 4.0) the emulsifying capacity was higher than expected compared to those of the agushie varieties.

Under extreme alkaline and acid conditions, the protein solubilised is dissociated into smaller fragments. These fragments may have their hydrophobic and hydrophilic groups exposed such that they can associate fully with the lipid water molecules, thereby increasing the emulsion formation, and raising the emulsifying capacity. This may explain why all three varieties have high emulsifying capacities at pH 2.0 and pH 12.0. At the pH where very little protein is in solution, emulsifying capacity would be expected to be low as observed at pH 4.0 for the agushie varieties. This may be because proteins need to be in solution in order to migrate to the oil-water interface to bring about a lowering of interfacial tension. If hardly any protein is in solution there would be very little emulsion used

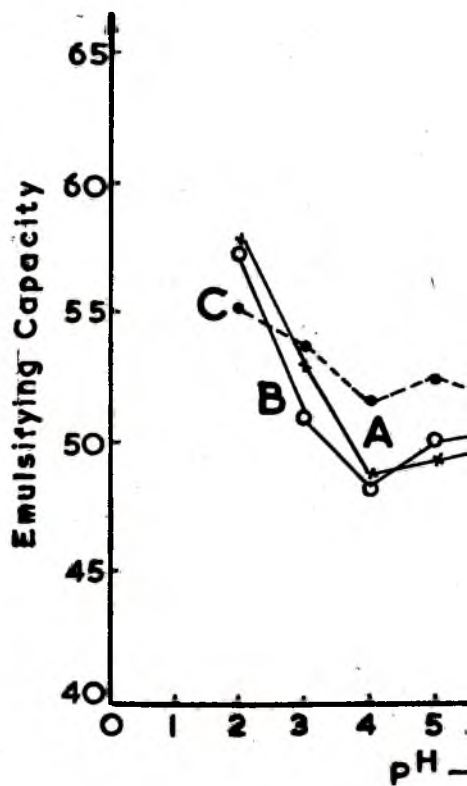
FIGURE 22

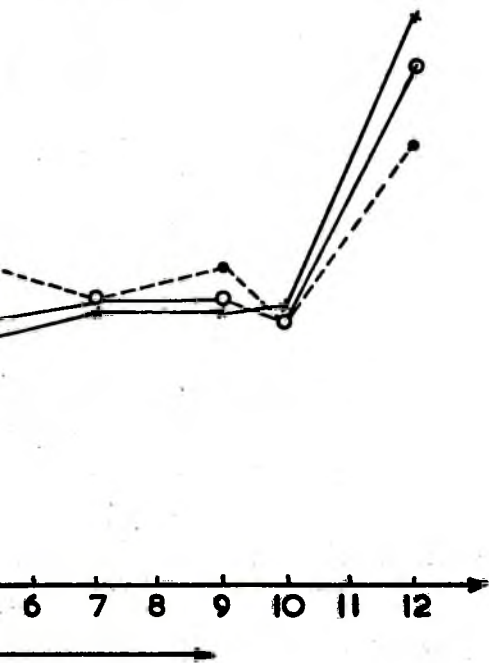
Effect of pH on Emulsifying capacity of Melonseed  
(Cucumeropsis edulis and Citrullus lanatus) flours

A = Agushie variety 1

B = Agushie variety 2

C = Neri





formation.

Calculation of the correlation coefficient ( $r$ ) between protein solubility at various pH's and emulsifying capacity showed that there was no significant correlation between the two (Agushie variety 1  $r = 0.5661$ , agushie variety 2,  $r = 0.5644$ ; neri,  $r = 0.1603$ ). This may mean that the amount of protein in solution is not the most important factor in determining the degree of emulsification. As seen from Figure 22, for the agushie varieties, emulsifying capacity does not change much between pH3 and 10 even though protein solubility increases. The neri also did not show emulsifying capacities which could be expected, if protein solubility was the main determining factor. Li-Chan et al (1983) stated that the emulsifying properties of a protein could not be predicted solely on the basis of its solubility. Rather the surface hydrophobicity of the protein molecule should be considered as well.

Between pH 3 and 10 the high emulsifying capacity of the neri may be because the neri protein has groups on its surface conducive to emulsion formation (i.e the state of the protein at these pH's favour emulsion formation more than the state of the agushie protein).

The viscosities of the emulsions formed at the various pH's were measured after centrifugation. The Brookfield Viscometer was

used with Spindle T-F rotating at 5.0 rpm. It was observed that viscosity was lowest at pH 4.0 for all the samples. The range was 35,000 to 45,000cp at the acid pH's and 50,000 to 65,000cp at the alkaline pH's. The higher the emulsifying capacity, the higher the viscosity was. The state of the protein involved in the emulsion formation may determine the viscosity of the emulsion formed. Akobundu et. al. (1982) also found that thin emulsions were formed from agushie flour at the acid pH's whilst thicker emulsions were formed at the alkaline pH's. The emulsifying capacities of more than 50% obtained for all the samples indicate that melonseed protein may act as good emulsifiers. Good emulsifying properties for melonseeds have been reported by other workers (Ige et. al. 1984; King and Onuora, 1984; Akobundu et. al., 1982)

#### **4:7:3      Effect of Ionic Strength on Emulsification**

The emulsifying capacities of defatted melonseed flours were measured in distilled water and sodium chloride solutions of increasing ionic strength. The results are shown in Figure 23.

For all the samples emulsifying capacity increased from ionic strength zero (0) to 0.6 after which it decreased. At the low ionic strengths neri had higher emulsifying capacities than agushie varieties 1 and 2. The emulsifying capacities of the and agushie did not differ markedly from each other at each ionic



strength. The highest emulsifying capacity for each melonseed flour was obtained at ionic strength 0.6 (57.4%, 56.18%, 57% for agushie variety 1, variety 2 and neri, respectively).

It seems the salt-soluble proteins have better emulsifying properties than the water-extractable proteins. It may be because the water-extractable proteins may not have a good balance of hydrophilic and hydrophobic groups on their surface compared to the salt-soluble proteins hence their lower emulsion forming ability. The fact that more protein is made soluble in the sodium chloride solution may partly account for the higher emulsifying capacities. This is because after ionic strength 0.6 when protein solubility decreases, the emulsifying capacity also decreases slightly.

A significant correlation was found between protein solubility and emulsifying capacity at the various ionic strengths for agushie variety 1 and neri. For agushie variety 2 there was no significant correlation. This may be because the emulsifying capacities were lower than was expected considering the amount of protein solubilised. The correlation coefficients were 0.9859, and 0.9357 for agushie variety 1 and neri respectively. For agushie variety 2 it was 0.9106.

This may mean that for proteins whose conformation remain the same and are ideal for emulsification the amount of protein in

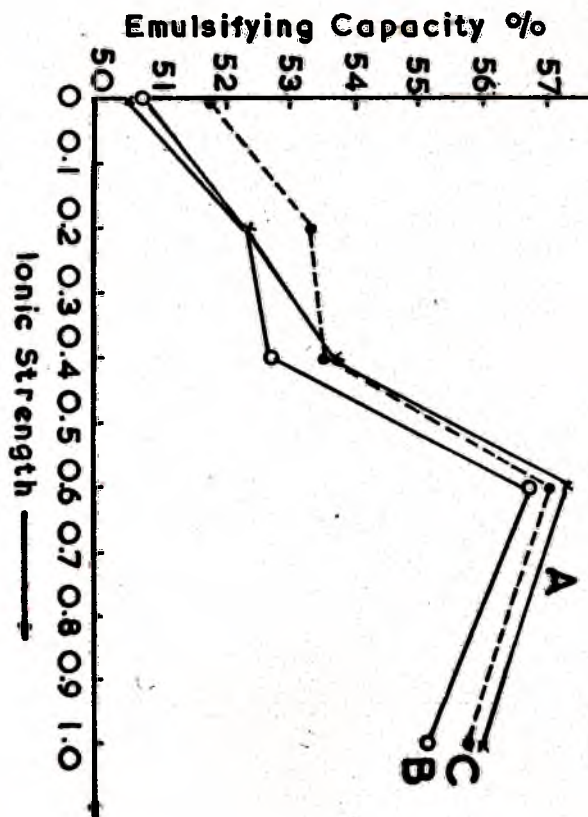
FIGURE 23

Effect of Ionic Strength on Emulsifying capacity of  
Melonseed (Cucumeropsis edulis and Citrullus lanatus)  
Flours

A = Agushie variety 1

B = Agushie variety 2

C = Neri





solution may play a dominant role in determining the degree of emulsification. At high ionic strengths the association of the protein molecules with the salt ions, may prevent them from associating with the water molecules. This, apart from reducing the solubility of the protein may prevent it from acting as a good emulsifying agent between the water and oil, since its charged groups are occupied by salt ions. This leads to a reduction of emulsifying capacity.

Work with other oilseeds have also shown that addition of NaCl up to certain levels have an incremental effect on emulsifying capacity (Ramanathan et al, 1978; Narayana and Narasinga, 1982). In these experiments, emulsifying capacities of more than 50% were obtained. This may mean that agushie and neri proteins are good emulsifying agents. Their emulsifying properties can also be enhanced by addition of salt at a concentration acceptable in most foods, making them useful as emulsifiers in food products.

#### 4:7:4 Effect of Roasting Temperature and Boiling on Emulsification

Defatted flours prepared from agushie and neri roasted at 140°C, 160°C and 180°C for 45 min. were evaluated for their emulsifying capacities. Distilled water was used in this experiment. The effect of roasting temperature on emulsifying capacity is shown in Figure 24.

From the graph there is a clear indication that roasting depresses the emulsifying capacity of the melonseeds. Roasting at 140°C did not affect the emulsifying capacities of agushie varieties 1 and 2, though that of neri was considerably lowered. Roasting temperatures of 160°C and 180°C lowered the emulsifying capacities of all the samples appreciably. At 180°C, roasting temperature, the emulsifying capacities were 12%, 6% and 3% for agushie varieties 1, 2 and neri respectively, compared to 50%, 51%, 52% for raw agushie varieties 1, 2 and neri respectively.

In addition to the reduction in protein solubility caused by heat denaturation, other factors may play a part in the severe depression of emulsifying capacity observed in samples roasted at 160°C and 180°C. The solubility of water-soluble proteins did not change when roasting temperature was increased from 160°C to 180°C (Figure 10) but the emulsifying capacity was depressed further. The denaturation may have reduced the surface charge and hydrophobicity of the protein molecules. The combined effect of reduced solubility and surface hydrophobicity of the protein molecules may have contributed to the very drastic reduction of emulsifying capacity of samples roasted at 160°C and 180°C. Roasting at 140°C may not have drastically affected the protein molecules of the agushie, that is why emulsifying capacity was not reduced, though the emulsions were a bit thinner than those formed from raw samples.

FIGURE 24

Effect of Roasting Temperature on Emulsifying capacity of  
Melonseed (Eucumeropsis edulis and Citrullus lanatus)  
flours

A = Agushie variety 1

B = Agushie variety 2

C = Neri

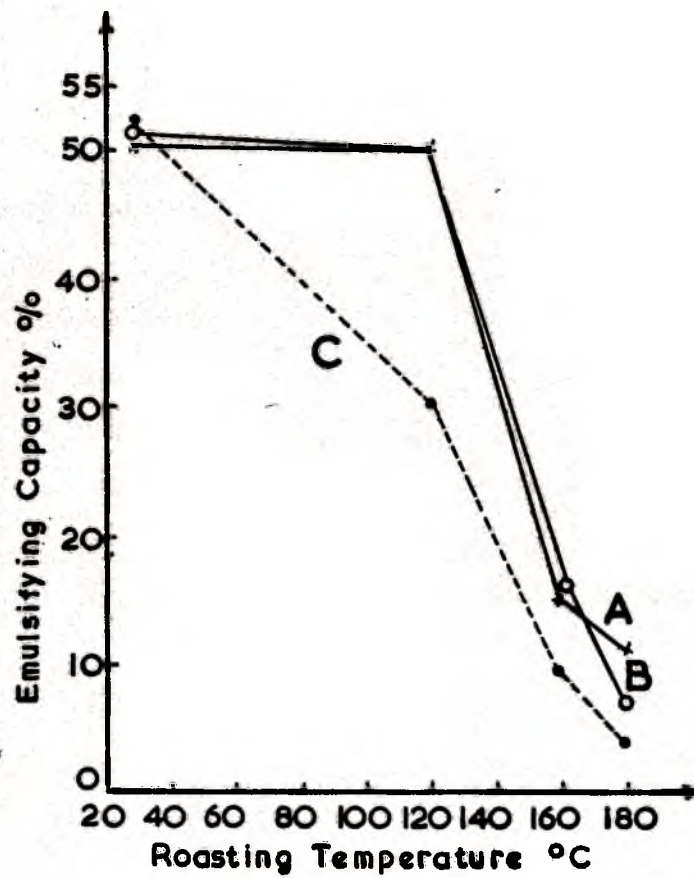




Table 14

Effect of boiling (moist heat) on Emulsification

Sample	Emulsifying capacity %	Remarks
Agushie V <sub>1</sub>	46.18	Very thin emulsions were formed. After centrifugation some oil not emulsified.
" V <sub>2</sub>	46.15	
Neri	30.32	A lot of oil remained unemulsified. Emulsion was very thin.

A weighed amount of melonseeds was boiled for 30 min. This was blended into a slurry, before oil was added for emulsification. When 2g of seeds ~~were~~ used to give 8% concentration, no emulsion was formed. Five grammes of melonseeds ~~were~~ therefore used to give 20% concentration. The results are presented in Table 14.

The effect of heat and moisture may have destroyed the groups on the protein surface which are necessary for emulsion formation. This may account for the need for a higher concentration to supply protein to bring about formation of stable emulsion. Even the emulsions formed with 20% concentration were thin and weak. Moist heat evidently has a detrimental effect on the emulsifying capacity of melonseed flour. King and Onuora (1984) reported that heating of melonseed flour depressed emulsifying ability. Moist heat had a greater effect than dry heat.

#### 4:7:5 Emulsion Stability

Emulsion stability was studied by heating the emulsion formed at 80°C for 30 min., before centrifuging.

It was observed that the emulsion did not break, on under-going this heating process. Some coagulation of the emulsion however occurred. This suggests that heat coagulable proteins are involved in the emulsification process. The stability of the emulsions may be partly due to this coagulation. The results

suggest melonseed protein can form emulsions with good stability which would not break under moderate heating conditions. However if the emulsions have to be heated above 80°C, the coagulation which occurs would be undesirable. This is because the viscosity of the emulsions would change. For such emulsions, the use of melonseed protein as emulsifier would be unsuitable.

#### 4:8 COAGULATION STUDIES

##### 4:8:1 General

Coagulation is one of the thermal functional properties of proteins. The others are thickening and gelation. These properties according to Li-Chan et al (1983) can be explained on the basis of a combination of the total hydrophobicity of unfolded protein molecules and their sulfhydryl group content.

Melonseed protein has the ability of coagulate when heated to certain temperatures. This property is exploited traditionally in the preparation of certain types of stews. The coagulated agushie in the stew gives it a desirable appearance. The agglomerates formed when the agushie coagulates look like scrambled egg. It is therefore useful in the absence of eggs when the housewife wants to give her stew a 'scrambled egg' appearance.

Preliminary investigations showed that as the concentration of the slurry increased, the size of the agglomerates formed

decreased. This could be because there was not enough room for the agglomerates to grow in size. At 20% concentration, the agglomerates were very tiny and very little liquid separated out. The separation of liquid from the agglomerates gives a 'curds and whey' appearance. The whey differed in appearance from clear to cloudy. The greater the degree of coagulation, the clearer the whey was. When there was definite formation of agglomerates of large size, the whey was very clear and droplets of oil floated on it. This may be because most of the protein had separated out. The emulsion formed from the oil in the meal and the water when the slurry was made therefore broke.

It is this breaking of the emulsion on prolonged heating at boiling point that allows oil to be separated out in the traditional preparation of melonseed oil.

#### **4:8:2      Effect of pH on Coagulation Temperature**

The raw melonseeds were blended into 10% slurries with distilled water. These slurries had pH's of 6.2 to 6.8, which were the natural pH's of agushie variety 1, variety 2 and neri. Other slurries were made at pH's on either side of the natural pH of slurries by adjusting the pH with either hydrochloric acid (HCl) or sodium hydroxide (NaOH). These slurries were evaluated for coagulation temperature and degree of coagulation. The behaviour of the three varieties of melonseeds was similar in degree of coagulation, though their coagulation temperatures

varied slightly from each other.

There was a marked difference in the degree of coagulation at the different pH's for all the varieties. At the natural pH of each variety, there was good coagulation on heating. The temperature of coagulation was also lowest at the natural pH of agushie varieties 1, 2 and neri. In cooking, the pH of the stew is likely to be between pH 6 and pH 7, that is why good coagulation is obtained.

It was observed that no coagulation occurred in slurries with very acid and alkaline pH when heated. Some coagulation occurred in slurries whose pH was near the natural pH, but the temperature at which coagulation occurred was higher. The full results are presented in Table 15.

At the natural pH, the proteins are likely to be in their native state. On heating, energy may be supplied to the protein molecules for them to associate through covalent bond formation. The heating may also cause the protein molecules to unfold and expose groups on their surfaces which would allow intermolecular bond formation. This causes the proteins to come out of solution as agglomerates. As the pH of the slurry moves away from natural pH, the state of the protein changes and higher temperatures are needed to cause the protein to coagulate. At the highly acid and alkaline pH's, the repulsive forces between the proteins may be

so great that heat does not have the effect of bringing them together so coagulation is inhibited.

At these pH's too, dissociation of the large protein molecules into smaller polypeptide chains may occur. The groups involved in intermolecular bond formation of these molecules may be too far apart, so that heating cannot bring about coagulation since no bond formation occurs. It was observed that when the pH of the slurry was changed from pH 12.0 to pH 7.0 whilst the slurry was still hot, agglomerates were formed. This may be due to the fact that the repulsive forces keeping the molecules apart were eliminated and association with subsequent intermolecular bond formation could then occur.

The effect of pH on the coagulation of the melonseed meals indicate that pH modification of melonseed proteins could be used to prevent coagulation in products which need heating but in which coagulation is undesirable.

#### 4.8.3 Effect of Ionic strength and Roasting Temperature on Coagulation Temperature

Raw and roasted (140°C, 160°C, 180°C) agushie and neri were blended in 10% slurries. Distilled water and sodium chloride solutions of ionic strengths 0.1, 0.2, 0.4 and 1.00 were used.

The results are presented in Figure 25.

Table 15

Effect of pH on Coagulation Temperature of Agushie (Cucumeropsis edulis) and Neri (Citrullus lanatus) slurries.

<u>Temperature of</u> <u>coagulation °C</u>				
<u>pH</u>	<u>AV<sub>1</sub></u>	<u>AV<sub>2</sub></u>	<u>Neri</u>	<u>Remarks</u>
1.20	-	-	-	No coagulation occurred on heating. However on standing, a precipitate was formed with clear liquid on top.
3.80	-	-	-	No coagulation occurred. A precipitate was formed on standing.
4.20	83	85	85	Small agglomerates formed at the temperatures given.
6.20- 6.80	79	82	82	Obvious coagulation started at the temperatures given. Big agglomerates were suspended in clear liquid.
7.30	90	92	93	Small clots were formed on heating. "Whey" was not clear.
12.0	-	-	-	No coagulation on heating. No precipitate formed on standing.

AV<sub>1</sub> = Agushie variety 1

AV<sub>2</sub> = Agushie variety 2

FIGURE 25

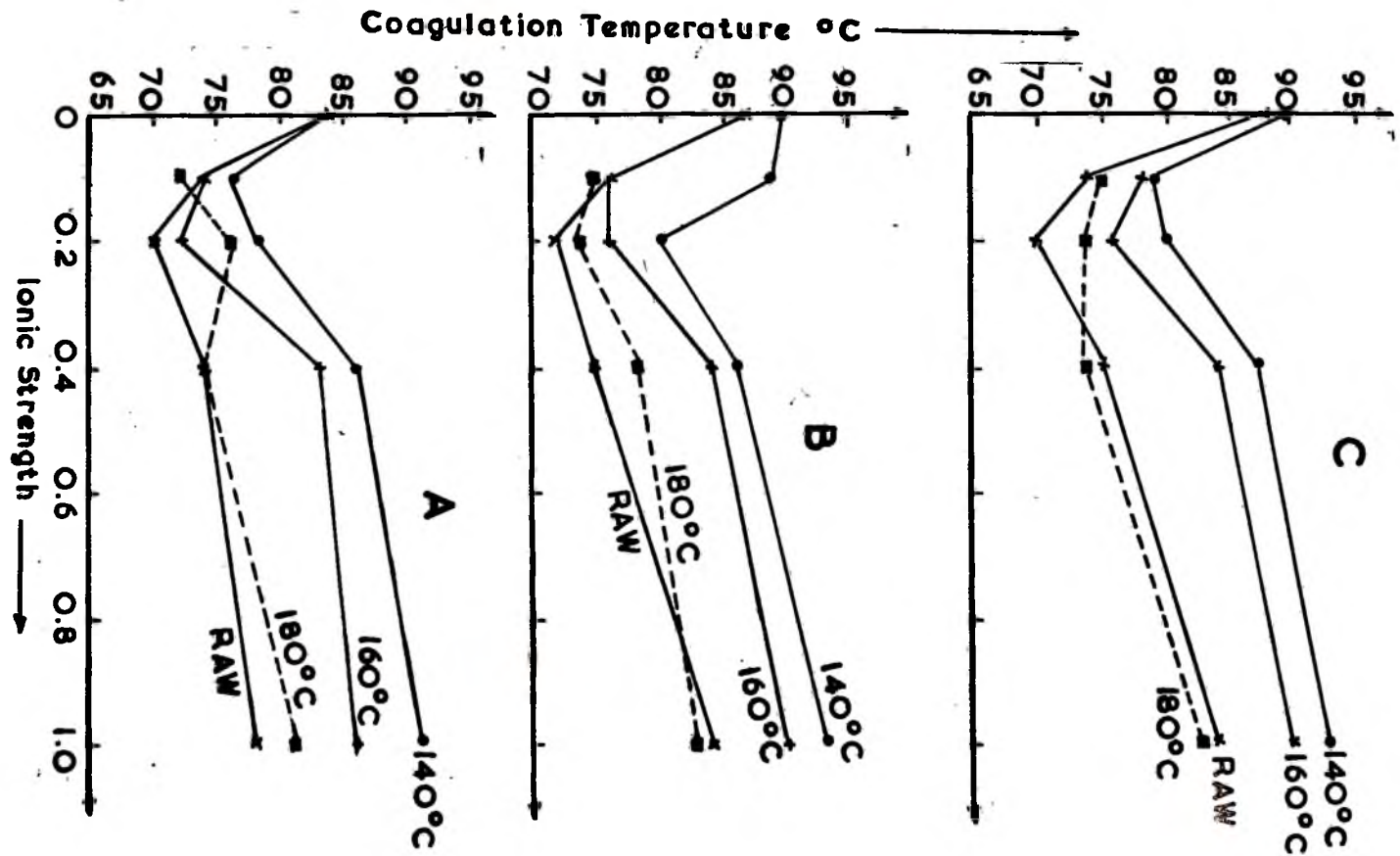
Effect of Ionic Strength on Coagulation Temperature of  
Raw and Roasted Melonseed (Cucumeropsis edulis and  
Citrullus lanatus) meals

A = Agushie variety 1

B = Agushie variety 2

C = Neri





For both raw and roasted melonseeds, the lowest temperature of coagulation was at ionic strength 0.20 and the highest was at ionic strength 1.0. The distilled water slurries prepared from melonseeds roasted at 160°C and 180°C did not coagulate at all on heating. Coagulation however occurred when the slurries were prepared with sodium chloride solutions.

Slurries made from unroasted melonseeds had the lowest coagulation temperatures at all ionic strengths. For the roasted samples the temperature of coagulation decreased as roasting temperature increased at each ionic strength. For example, in the case of neri, the temperatures of coagulation of raw sample and those roasted at 140°C, 160°C and 180°C (in sodium chloride solution of ionic strength 0.1) were 74°C, 79°C, 78°C and 74°C respectively.

The low coagulation temperatures observed at ionic strengths 0.1 and 0.2 suggest that the state of the heat coagulable proteins is such that less energy is required to bring about intermolecular bond formation. As the ionic strength increases, there may be changes in the structural conformation of the protein molecules. These changes may involve the groups which are necessary for the intermolecular bond formation. More energy may therefore be needed to bring these groups near each other for bond formation which leads to agglomerates forming. Bigger agglomerates were formed at ionic strengths 0.1 and 0.2, than at

the other ionic strengths. The trend was that the lower the temperature of coagulation, the bigger the agglomerates formed. The coagulation is a form of irreversible denaturation. At the high ionic strengths, the sodium chloride ions may form a protective barrier around these molecules, thereby requiring higher temperatures to cause this denaturation.

The denaturation caused when the seeds are roasted at 160°C and 180°C, may prevent coagulation in distilled water. When sodium chloride is added, other proteins, (i.e. salt-soluble proteins) are solubilised. They may still possess the ability to form the covalent bonds which would bring them out of solution as agglomerates, therefore coagulation occurs.

The reduction in coagulation temperature as the temperature of roasting increases may be due to changes in the native state of the protein molecules which favour association between the molecules. Less energy would therefore be required to bring the proteins out of solution as agglomerates.

Statistical analysis of the results using analysis of variance (Table 16) showed that the variety of melonseed did not have a significant effect on the temperature of coagulation. The differences observed could therefore be due more to chance than to significant differences in the protein structure of the three varieties of melonseeds used. The differences in the coagulation

temperatures may also not have been wide enough to cause a statistically significant effect. The ionic strength and roasting temperature however had a significant effect on the coagulation temperature. This suggests that these factors caused significant changes in the protein structure which affected the bond formation needed for coagulation. Hence the differences in coagulation temperatures observed.

In the use of agushie and neri to prepare soups, coagulation is undesired. The neri and agushie are therefore roasted before grinding into a paste to prepare the soup. During the soup preparation, salt is added when the soup is well-cooked and prevents coagulation. The neri and agushie are used as soup thickeners in this case. This study has confirmed the practice.

#### 4:8:4 'Whey' Protein

Daniellson (1949) reported that seed globulins of leguminosae had different components which behaved differently when heated. One component which was lighter coagulated on boiling. The heavier component did not coagulate on boiling. This shows that not all the protein components are heat coagulable.

Protein was therefore determined by the method of Lowry et. al. (1951) in the liquid that separated out ('whey') in a few of the coagulated samples. This was just to have an idea of how much

Table 16

Effect of Roasting Temperature and Ionic Strength on  
Coagulation Temperature.

ANOVA SUMMARY TABLE.

Source of variation	df	sum of squares	mean squares	F
Ionic strength	4	1322.9166	330.7292	10.48**
Temperature	3	566.6833	188.8944	5.99**
Variety	2	70.3334	35.1667	1.11ns
Error	44	1388.4001	31.5545	
Total	53			

\*\* Significant at  $P < 0.01$

ns not significant



protein was not heat coagulable. The results are given in Table 17.

The results show that with the melonseed proteins too, not all the components are heat coagulable. This suggests that the proteins in the melonseeds are not homogenous, but are made up of heterogeneous components which behave differently.

For the roasted samples, there was less 'Whey' protein as the roasting temperature increased. This may seem that more protein was coagulated from slurries made from seeds roasted at higher temperatures, because the denaturation on roasting enhanced covalent bond formation.

The general trend was that the higher the coagulation temperature, the higher the 'whey' protein content. This suggests that difficulty in bringing together the groups necessary for bond formation resulted in less protein molecules being coagulated.

There is a need to study the phenomenon of coagulation further to know the actual mechanism and the specific protein components involved. This could be done by characterising both the coagulated protein and the 'whey' proteins.

Table 17

"Whey" Protein of some Coagulated Samples.

Treatment	Ionic Strength	Protein (mg/ml) in "Whey" (10% slurry)		
		AV <sub>1</sub>	AV <sub>2</sub>	Neri
Raw	0.20	4.1 (70)*	4.2 (72)	3.25 (70)
Raw	0.40	5.3 (74)	4.55 (75)	3.50 (73)
140°C	0.20	4.7 (78)	4.15 (80)	3.35 (80)
160°C	0.20	4.5 (72)	4.0 (76)	3.70 (76)
180°C	0.20	4.1 (76)	3.3 (74)	2.50 (76)

\* Coagulation Temperature °C

AV<sub>1</sub> = Agushie variety 1AV<sub>2</sub> = Agushie variety 2

#### 4:9 PRODUCT DEVELOPMENT

##### 4:9:1 Rationale

This product development was an initial step in developing a fully acceptable product. The aim was to find out whether agushie can be used as an emulsifier, since the melonseed flours used showed good emulsifying properties.

The studies also showed that clotting of agushie meal could be prevented by roasting. Could a baby food (weaning food) therefore be made from agushie? This was of particular interest considering the nutritional value of agushie. Apart from the protein which would be supplied, the oil would supply energy and essential fatty acids of unsaturated nature. That is the advantage of using the full-fat meal. There is a practice of adding groundnuts to corn to prepare weaning foods for babies. If agushie can be used instead, it has the advantage of supplying high amounts of linoleic acid. The protein content is also higher than that of groundnuts and cowpeas commonly used in traditional weaning foods in Ghana. The supply of vitamins and minerals such as calcium is also good. To successfully develop weaning food from agushie would be nutritionally advantageous in the feeding of babies and infants.



#### 4:9:2 Emulsion-type product

##### 4:9:2:1 General

From the emulsification studies, it was found that roasting the sample at 140°C, did not markedly reduce the emulsifying capacity of the melonseed meal. The agushie was therefore roasted at 140°C for 60 min. This imparted a desirable flavour and colour to the product. The slurry prepared with the roasted seeds had a biege colour and the product had a desirable cream colour like mayonnaise. In the preparation of the slurry, foaming was reduced due to the roasting of the agushie.

Two batches of the product were prepared. They had the same composition except that Batch I which had the rice paste was thicker than Batch II. Both products were however pourable.

##### 4:9:2:2 Storage Studies

For the storage studies, some of each batch was kept at room temperature 27°C at 5°C and in a household freezer (below 0°C).

##### A. Batch I

Some water separated out from the emulsion of samples kept at room temperature, though no oil separated out after three days. From 50ml of emulsion about 10ml of water separated out and settled at the bottom of the jar. The emulsion which remained was still pourable. After 5 days, the emulsion became thin and

there was a change in odour. This could be attributed to microbial spoilage.

For the samples stored at 5°C, there was no separation of water till the 7th day. The emulsion remaining became very thick and was pourable. On shaking vigorously, there was a resuspension and the product was again pourable. The setting of the emulsion in the cold could be due to the cold temperature causing the rice starch to gel. Shaking breaks up the gel structure causing the emulsion to flow. After 7 days there was no noticeable change in colour or odour of the product. They were presumed to be still wholesome.

On thawing the frozen samples water separated out, though oil did not. The consistency of the emulsion did not change. Refreezing and thawing did not cause any further change.

#### **B. Batch II**

This batch did not contain any rice paste. It was made up of just the agushie slurry, oil, vinegar, salt and sugar.

With the samples stored at room temperature, water separated out after 24 hours. The emulsion however remained pourable. About 20ml of water separated out from 50ml of product. Thinning of the emulsion was not noticed till after 5 days. By the 7th day, very tiny droplets of oil could be seen on top of the emulsion, and the odour was becoming foul. Presumably microbial spoilage

was taking place.

The samples stored at 5°C had water separating out after 2 days. The emulsion remained pourable and did not set like the batch containing rice paste. Less water separated out than at room temperature. The frozen samples on thawing had water separating out, but oil did not separate out. The emulsion remained pourable and did not change in consistency on rethawing after refreezing.

To prevent the loss of water from the emulsion it may be necessary to incorporate stabilisers. Since the product with cooked rice paste added lost less water, this could be attributed to the rice binding up some of the water. Further work would have to be done to develop a more shelf-stable product.

#### **4:9:3      Baby Food**

##### **4:9:3:1    General**

Four batches of the product were prepared. Their composition is showed in Table 18.

Roasting the agushie prevented coagulation when the slurry was boiled. Adding salt after boiling did not cause any coagulation. In terms of consistency Batch I was the best. Batch II which had no rice flour, had liquid separating out of

it. The agushie settled and water floated on top. It was also the thinnest of the products. The absence of starch from agushie may account for the lack of water in the product. When rice flour was added this separation was eliminated. Batch III which had agushie and rice flour in the ratio 1:1 was too thick and the flavour of roasted agushie somewhat masked. More water had to be used in the preparation of this batch. Batch IV (agushie: rice, 2:1) was thinner than Batch I and the rice flavour came out too strongly compared to Batch I (agushie: rice 3:1).

#### 4:9:3:2 Storage Studies

After six (6) days all the products stored at room temperature had spoilt. Their odour had become foul and their consistency had changed to become watery. This may be due to improper sealing.

Batch III which contained agushie and rice flour in equal proportion set into a gel as soon as it cooled to room temperature. It was not pourable. Batches I and IV did not become like a gel though their viscosity increased on cooling to room temperature. They poured very slowly. Batch II with no rice flour did not set at all. There was rather a settling of the agushie.

Under cold storage (5°C) Batch II which had no rice flour, still had water separating out. All the other batches set into a gel after 24 hours. Batch III was the hardest of them. Batch I was

soft and easily spoonable. At the end of 7 days some water separated out of the gel of Batches I and IV. No water separated out of Batch III (agushie and rice, 1:1). The rice starch must have bound up all the water so none was free to separate. No microbial spoilage occurred.

The texture of the frozen samples changed on thawing. The texture had become rubbery and dry. All the batches acquired this change on freezing and thawing. Freezing the product would therefore not be recommended.

The product can either be used hot when it is like a thick porridge or after it has cooled and set into a spoonable gel. Using agushie and rice in proportion 3:1 would give the best product.

Further work needs to be done with regard to flavouring, use of stabilisers and other gelling agents like gelatin. Storage stability at room temperature would also have to be improved.

#### **4:10 Microstructure of Melonseed Cotyledons**

Transverse tissue sections of the cotyledons were prepared and stained with Sudan IV to show oil droplets and with Million's Reagent to show protein granules.

It was observed that the types of cells and their arrangement in

the cotyledon were similar in all three varieties. The shape of the cells changed from the outermost cells through to the innermost cells. The outermost and innermost cells were very small and regular in shape. These could be the epidermal cells (Figures 26, 27 and 28). The major part of the cotyledon showed typical parenchymatous cells interspersed with vascular bundles. These cells form about 12 - 14 layers and their arrangement was irregular. The shape of the cells about two (2) layers before the inner epidermal cells was considerably different from the parenchymatous cells which were polygonal. They were longitudinally elongated and arranged regularly. There were no intercellular spaces between any of the cells of the cotyledon.

Samples stained with Sudan IV showed the presence of oil droplets, whilst those stained with Millon's reagent showed protein-body like structures. The oil droplets and protein bodies occurred in all the cells except the epidermal cells. This may explain the high fat and protein contents of the melonseeds. Seeds which have these bodies localised in only certain cells tend to have a low content of fat or protein. For example wheat has a low protein content since the protein bodies are localised in mainly the outer aleurone layer.

FIGURE 26

Microstructure of the Cotyledons of Agushie Variety 1

(Cucumeropsis edulis)





FIGURE 27

Microstructure of the cotyledons of Agushie Variety 2  
(Cucumeropsis edulis)



Figure 28

Microstructure of the cotyledons of Neri  
(Citrullus vulgaris)



## 5. CONCLUSIONS

From the studies the following conclusions can be drawn:-

- (a) The melonseeds have relatively high protein (19-29%) and fat content (47-50%). The seeds will therefore be nutritionally valuable.
- (b) Protein extractability was found to be dependent on the type of solvent. Distilled water extracted the least amount of protein in all three varieties. The bulk of melonseed proteins are globulins which are extracted in sodium chloride solution. In sodium hydroxide nearly all the proteins of the melonseeds were extracted. This was confirmed by the very high solubility observed above pH 10.0.
- (c) In general, the melonseed proteins showed low solubility in acid medium, with the lowest solubility occurring at pH 3.0 to pH 4.0 which was the isoelectric point. In sodium chloride solutions of varying ionic strengths the highest solubility was obtained at ionic strength 0.6 for all the varieties.

- (d) For all the roasted samples, protein solubility was reduced. This may be attributed to protein denaturation. The water-extractable proteins were completely denatured at roasting temperature 160°C. The salt-soluble protein however seemed to be completely denatured at roasting temperature 180°C. This was shown by the reduction in protein solubility which occurred at these roasting temperatures.
- (e) It may be concluded that the proteins of the three varieties of melonseeds are similar since the effects of pH, ionic strength, roasting and extraction temperature on the protein extractability were similar.
- (f) The water absorption capacities of the three raw melonseed flours were not high. When the ionic strength of the water was changed by adding sodium chloride, the water absorption was increased. Agushie variety 2 had the highest water absorption capacity at all ionic strengths. Increasing incubation temperature increased the water absorption capacity of all the varieties. Roasting seemed to enhance the water absorption of the agushie but not neri.
- (g) Fat absorption was higher than water absorption for all three raw melonseed flours. Roasting however decreased the fat absorption capacities. Agushie variety 2 had the

highest fat absorption capacities for both raw and roasted flours. The agushie and neri flours may therefore be useful in products which need good fat binding agents.

- (h) The three melonseed flours showed good foaming properties. At pH's where the proteins had been dissociated into fragments, thick compact foams of high volume and stability were obtained. Foams of low volume and poor stabilities were obtained at pH 3.0 and pH 4.0 (isoelectric pH's). Foams in sodium chloride solution were of higher volume and better stability than those in water. The highest foam volumes and stabilities were obtained at ionic strengths 0.20 and 0.40 for all three raw melonseed flours. This indicates a potential use of these flours in whipped products if sodium chloride is added.

Roasting depressed foaming in water but not in sodium hydroxide solution of pH 11.0. Whipping the flours in water at 50°C and 60°C produced thick foams of good stability. Cold whipping (5°C) depressed foaming. Like all proteins, the presence of oil in the melonseed meals prevented foaming. For some foaming to occur, the concentration of the slurry had to be increased considerably.

- (i) The emulsifying properties of the agushie and neri flours were good. High acid (pH 2.0) and alkaline (pH 12.0) media, resulted in very high emulsifying capacities for all the raw melonseed flours. In between these pH's the emulsifying capacities did not change much. Emulsifying capacities were 50% and above. Alkaline pH's gave emulsions of higher viscosities than acid pH's.

The state in which the proteins are at these pH's therefore determines the thickness of the emulsion formed. Melonseed proteins may therefore be used in the formulation of emulsion products of varying thickness depending on the pH. Emulsifying capacity was better in sodium chloride solution than in distilled water.

Roasting temperatures of 160°C and above reduced the emulsifying capacities of both the agushie and neri drastically. Boiling had an even more detrimental effect on the emulsifying capacities. Raw melonseed flours therefore had the best emulsifying capacities.

Coagulation of the melonseed proteins above 80°C may prove undesirable in emulsions which need heating due to the change in viscosity which would occur. Heating however does not break the emulsion.



- (j) The raw melonseed meal coagulated when heated in water and in sodium chloride solutions. Sodium chloride solutions of ionic strengths 0.1 and 0.2, seem to favour the formation of big agglomerates at lower temperatures (below 80°C). Samples roasted at 160°C and 180°C coagulated only when sodium chloride was present in the slurry. The sodium chloride may therefore bring about changes in the conformation of the proteins which cause them to coagulate when heated.

The effect of pH on coagulation suggests that changes in protein structure at high acid and alkaline pH's prevent coagulation. At the natural pH (6.2 - 6.8) the protein structure is conducive to coagulation.

Not all the proteins of the melonseed were heat coagulable because some protein remained in the liquid that separated from the agglomerates.

- (k) The good emulsifying properties of agushie could be exploited in formulating an emulsion type product from agushie variety 2. More work however needs to be done to improve the storage stability of the products.

Agushie or neri (since they have similar properties) can be used to formulate a baby-food of good nutritional value.

The coagulation which occurs on heating, giving a 'curds and whey' appearance was prevented by roasting the agushie.

Rice flour or some form of starch could serve as a thickener and gelling agent if a product which would gel is desired. Further work needs to be done to develop a more shelf-stable product which would be fully acceptable.

- (1) Microscopic examination of sections of the cotyledons of all three melonseed varieties, showed the presence of oil droplets and protein bodies in all the cells except the inner and outer epidermal cells. This could account for their high protein and oil contents. Differentiation of the varieties on the basis of microstructure would be difficult since the types of cells and their arrangement in the cotyledons are the same.

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