Functional characteristics of egusi seed (*Citrullus lanatus*) hydrocolloid and oil in instant egusi soup

by

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**Master of Science and Technology: Food Science and Technology**

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

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**Co-supervisor:** Mrs Joseline Felix-Minnaar

**Bellville**  
April 2018

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DECLARATION

I, Olakunbi Olubi, declare that the content of this thesis represent my own original work, and that the thesis has not previously been submitted for academic examination toward any qualification. Furthermore, it represents my own opinion and not necessarily those of the Cape Peninsula University of Technology.

2018/11/26

Olakunbi Olubi

Date
ABSTRACT

The use of egusi melon in soup has been domesticated and egusi has predominantly been regarded as a secondary crop. The aim of this study was to evaluate the functional characteristics of egusi seed (*Citrullus lanatus subsp mucosospermus*) hydrocolloid and oil in instant egusi soup. An instant production of egusi soup by adding boiling water to an instant soup mix will promote the availability of this nutritious seed as a healthy meal option. Egusi oil was successfully extracted from egusi seed using supercritical carbon dioxide method. The percentage oil yield which measures the amount of oil derivable from egusi seed ranged from 46-53% w/w. There was a significant difference in the oil yield as pressure and temperature are increased at a constant CO$_2$ flow rate of 30 g/h. The proximate composition of egusi oil was determined using standard AOAC method. The moisture contents were 1.3, 2.0 and 1.9% w/w, respectively for EO1 (60°C and 450 bar), EO2 (55°C and 600 bar) and EO3 (75°C and 600 bar). EO1 was significantly (p ≤ 0.05) lower in moisture content compared to EO2 and EO3. The fat content was 99.1% w/w (EO1), 98.3% w/w (EO2) and 98.9% w/w (EO3), with no significant difference in the three oil samples. The fatty acid composition was analyzed using gas chromatography. The fatty acid content of egusi oil was high in polyunsaturated and monounsaturated fatty acids, which was identified as linoleic (62%) and oleic (15%) acids. The saturated fatty acid (undecylic, myristic, palmitic and stearic) composition of egusi oil differed significantly (p ≤ 0.05) with EO1, having the lowest compared to EO2 and EO3. The index of atherogenicity (IA %) were significantly low 0.35, 0.38 and 0.38% w/w for EO1, EO2, and EO3, respectively. The thrombogenicity index (IT %) were 0.08, 0.09 and 0.09% w/w for EO1, EO2, and EO3, respectively with no significant difference. Peroxide Value (PV) measured using auto titrate Titrino plus, ranging from 11.60 for EO1 milliequivalents peroxide/kg to 12.60 for EO2 and 11.89 milliequivalents peroxide/kg for EO3. The oxidative stability index (OSI) was measured using Methrohm Rancimat at 120°C, expressed as the induction time of oxidation was 10.2, 11.5 and 5.3 h for EO1, EO2, and EO3, respectively, with EO3 significantly higher than EO1 and EO2 (p ≤ 0.05). The iodine number, determined by AOAC direct titration method was high and ranged from 95 g/100 g for EO1 to 129 g/100 g for EO3, with EO3 being significantly high (p ≤ 0.05). The nutritional and functional properties of defatted egusi flour and hydrocolloid extracted using supercritical carbon dioxide extraction was also successfully achieved. Proximate analysis of defatted egusi (DEF) flour after supercritical extraction was carried out according to standard AOAC procedures. The moisture content of DEF ranged from 5.3 to 10.1% w/w, crude protein 48.3 to 60.4% w/w, crude fibre 3.4 to 4.5% w/w) and ash 5.3 to 6.8 % w/w). The protein content of defatted egusi flour differed significantly (p ≤ 0.05) between samples. The amino acid compositions of DEF showed glutamic acid had the highest concentration of 12.9, 11.8 and 9.8
mg/100 g for DEF1, DEF2, and DEF3, respectively with a significant difference (p ≤ 0.05) across the samples. In functionality, the water absorption and solubility index at a low temperature of 50°C ranged between 52.5 to 57.6% w/w and 68.0 to 73.3% w/w respectively for DEF1 to DEF3, which significantly differed between samples. The final viscosity of defatted egusi flour ranged from 126.7 to 126.3 cP, which differed significantly (p ≤ 0.05) between samples. Egusi flour is high in protein 60% w/w and carbohydrate 25% w/w was treated with hot water to extract its hydrocolloid. Functional properties of egusi hydrocolloid for the three defatted flour shows a stable emulsifier as the breakdown viscosity remained constant at (8.00 cP). Breakdown viscosity of egusi hydrocolloid confirmed its stability. Based on the optimal mix, three soup samples were produced namely: instant egusi soup from boiled grits (IESBG), instant egusi soup from spherified grits (IESSG) and instant egusi soup from extruded grits (IESEG). Sixteen trace and five major mineral elements were found in egusi soup, with a high concentration of P (1220.4, 1326.2 and 1277.9 mg/100 g), K (1220.4, 1326.2 and 1277.9 mg/100 g), Mg (822.2, 905 3 and 863.70 mg/100 g), Ca (172.3, 190.9 and 183.4 mg/100 g) and Fe (53.7, 57.5 and 29.5 mg/100 g)), and for IESBG, IESSG and IESEG respectively. Instant egusi soup was also a source Zn (9.9, 12.3 and 11.8) respectively for IESBG, IESEG, and IESEG. IESSG and IESEG were significantly (p ≤ 0.05) higher in minerals when compared with IESBG. The effect of the ingredient on the final soup formulation was established, as the three component mix gave an optimum formulation. The optimal soup mix was 17.5% grit, 57.5% egusi flour and 10% hydrocolloid. The model fitness for appearance and taste has a significant F value of 5.14 and 8.95 respectively. The overall acceptance and desirability of the instant soup mix was (3.508 and 0.947 g/100g) respectively.
I wish to thank:

- Professor Victoria A. Jideani, my supervisor and mentor in the Department of Food Science and Technology at the Cape Peninsula University of Technology (CPUT), for her support and guidance. And for sharing her wealth of knowledge and expertise, challenging me to do my best.
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DEDICATION

This I dedicate to my heavenly Father, Lord Jesus Christ for blissful grace to finish this study. Jimi Shotunde for believing in me and his financial support: mere word cannot express my gratitude. To my siblings, Kikelomo, Olapeju, Oladimeji and Bisi Oluwabi Eri for her immense support and counsel, I appreciate you. To my daughter gracious Esther Derinmola Olubi and niece Mimiola Esther Olubi for your support and understanding.
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<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
</tr>
<tr>
<td>AAAC</td>
<td>Association</td>
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<tr>
<td>SFE</td>
<td>Supercritical fluid extraction</td>
</tr>
<tr>
<td>SCF</td>
<td>Supercritical fluid</td>
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<tr>
<td>EO</td>
<td>Egusi oil</td>
</tr>
<tr>
<td>PON</td>
<td>Peroxide number</td>
</tr>
<tr>
<td>OSI</td>
<td>Oxidative stability index</td>
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<tr>
<td>DEF</td>
<td>Defatted egusi flour</td>
</tr>
<tr>
<td>EH</td>
<td>Egusi hydrocolloid</td>
</tr>
<tr>
<td>TSM</td>
<td>Technicon Sequential Multisampling</td>
</tr>
<tr>
<td>AQC</td>
<td>Aminoquinolyl-N-hydroxysuccinimidyl carbamate</td>
</tr>
<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
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<tr>
<td>UPLC</td>
<td>Ultra Performance Liquid Chromatograph</td>
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<td>PDA</td>
<td>Photodiode array (PDA) detector</td>
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<td>NIST</td>
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<td>ICP-AES</td>
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<td>MSD</td>
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APPENDIX

Appendix A: Approved ethics clearance

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science*. This thesis represent a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has therefore, been unavoidable.
CHAPTER ONE
MOTIVATION AND DESIGN OF STUDY

1.1 Introduction

Plant protein is the most desired food in the world today due to its ability to replace the expensive meat and meat products. Egusi seed (*Citrullus lanatus* Thunb) from plant origin made available to consumers will serve the nutritional purpose of conventional foods from meat, fish and dairy products. Egusi seed a wild member of the gourd family (Abaelu *et al.*, 1990) has unlimited potentials for use in the food industries worldwide. *Citrillus* belongs to the family *Benincaseae* of the subfamily *Cucurbitoideae*. It comprises of four species, two of which (rehmii and ecrihosus) originated from Namibia, while the other two (*colocynthis* and *lanatus*) are endemic to West Africa and can be grown in any part of the world.

The *C. lanatus*, previously known as *Citrullus vulgariser* or *colocynthis*, comprises of three subspecies: subsp. *Vulgaris* Fursa, which is the most common edible cultivars of watermelon. The subsp. *mucosospermus* Fursa, growing in West Africa as a wild melon, occur in semi-cultivated and cultivated forms, and also has a similar species growing and under cultivation in southern Africa. The *caffer* (*tsamma*, *karkoer*, *bitterboela*), and the var. *citroides* (*Citron* melon, *makataan*), are under farming in South Africa (Department of Agriculture, 2013) and has been locally consumed by local cultivating these melons. Nyam *et al.* (2010) also reported the high tocopherol content in Kalahari melon seed (Bitterboela), defatted using supercritical method (Nyam *et al.*, 2010). The egusi plant *Citrullus lanatus* subsp *mucosospermus* species is widely cultivated in Nigeria, Ivory Coast, Central Africa and Cameroon, where it is commonly called gourd or egusi.

In South Africa, the fruit of egusi plant, known as bitter watermelon, is a source of water in dry seasons (Bankole *et al.*, 2005). Egusi is sensitive to cold temperatures and best suited to a temperature range from 18 to 35°C (Giwa *et al.*, 2010). Bitter watermelon is produced in the warmer parts of the entire country and in more or less sandy, drier areas of southern Africa, primarily in the Kgalagadi region of the Northern Cape (Compton & Gray, 1993). It is also found growing or under cultivation in the provinces in southern Africa (Modi & Zulu, 2013). No statistical data are available on the production levels of bitter watermelon in South Africa as the crop has not been commercialised (Modi & Zulu, 2013).

The nutrient density of egusi seed which comprises nearly 50% w/w edible oil and 30% w/w pure protein makes it a functional food (Iwuoha & Eke, 1996). Also, it is a potential
natural oil-seed to be evaluated as an emerging nutritionally acceptable food. The oil contains four essential fatty acids: linoleic acids oleic palmitic and stearic. Linoleic acid is the most abundant at 71.9 mg/100g of the oil. The fat of egusi is unsaturated, out of which 14.5% w/w are monounsaturated fatty acids and 57.4% w/w are polyunsaturated fatty acids (Jarret & Levy, 2012). However, despite these advantages, egusi seed is underutilised and primarily used in making egusi soup by indigenes of various communities that cultivate the egusi melon. Egusi oil which is high in omega 6 fatty acid has never been introduced into the food industry (Bankole et al., 2005).

The conventional oil extraction process is often laborious, and sometimes involves the use of harmful and toxic hydrocarbons for oilseeds. Thus, a more effective method of defatting using the supercritical fluid extraction (SFE) has been introduced into the food industry (Machida et al., 2011). Supercritical fluid extraction is the process of extracting the liquid substance from a solid matrix (seed) or any interphase using an extractant (Pourmortazavi & Hajimirasadeghi, 2007). SFE extracts chemical compounds using supercritical carbon dioxide instead of the usual organic solvent which can be toxic to human health (Reverchon & De Marco, 2006).

Moreover, after a defatting process, the meal of egusi which is high in protein (60% w/w), can be used as functional food ingredients such as hydrocolloid, protein isolate and flour (Trujillo, 2016). Hydrocolloids are a composition of different starch and proteins widely used in the food industries (Tsai et al., 2017). The use of a food hydrocolloid has seen a significant breakthrough in the food industries, to perform roles like thickening, gelling, emulsifying and water binding (Burey et al., 2008). Till date, the hydrocolloid properties of egusi seed have never been evaluated despite its use chiefly as an emulsifier and thickener in a soup known as egusi soup.

The traditional soup is known as "egusi soup" is common in Cameroon, Nigeria, and Benin, and referred to as "pistachio soup" in Côte d'Ivoire. This process of cooking egusi soup is laborious. Hence, more immediate processing of this soup into instant soup using boiled, spherified and extruded precooked egusi grits, will reduce the cooking time, enhance its flavours and increase its consumption. According to the recent reports on speciality food for "Food Consumer 2010" by the National Association for the Specialty Food Trade (NASFT), there was an increase in consumer preference to eat at home (Wang et al., 2010). Also, with improved economic conditions at the time, there was a surprising number of consumers who take a particular interest in speciality food, since young consumers have limited cooking skills and older consumers limited time to cook (Meixner & Krejc, 2007). Speciality meals, such as egusi instant soup, which comes with desirable characteristic and high nutritional composition, will meet the dietary needs of consumers and nutritious egusi will be readily available for consumption.
1.2 Problem Statement
The use of egusi seed (Citrullus lanatus subsp mucosospermus), with distinct nutritional benefits (Bankole et al., 2005) is limited to egusi soup by minor population in Africa. Preparation of the soup is laborious. This process cannot fit into the daily busy life of a modern man or woman. To date, the nutritional and functional properties of egusi seed have been well studied but not its hydrocolloid and oil. Furthermore, egusi has received little attention from scientists and decision makers. Efforts must be directed towards adding value to egusi melon to preserve the richness of this seed. Presently, defatted egusi meal and oil are not readily available in the market. Nothing is known about instant egusi grits which could improve the egusi soup process and thereby lead to commercial production of the soup. Hence, it is essential to investigate the functionality of egusi hydrocolloid, oil, and grits in instant egusi soup.

1.3 Broad Objective
The primary aim of this project was to determine the functional characteristics of Citrullus lanatus (egusi seed) hydrocolloid and oil for instant egusi soup.

1.3.1 Specific objectives
The specific objectives include:
   I. Characterize the fatty acid profile of the egusi oil extracted using the SFE and determine its functionality in the egusi seed.
   II. Establish the effect of processing on physicochemical, nutritional characteristic of defatted egusi meal.
   III. Establish the functional and pasting properties of hydrocolloids from defatted egusi meal.
   IV. Produce instant soup using egusi grit, hydrocolloid and defatted meal.
   V. Determine the shelf life and overall acceptance of instant egusi soup.

1.4 Hypotheses
It was hypothesised that:
   I. Egusi oil will be rich in polyunsaturated fatty acids.
   II. Defatted egusi flour will be high in protein.
   III. Egusi hydrocolloid extracted from defatted egusi flour will stabilise instant egusi soup.
   IV. Defatted grits from boiling, extrusion, and spherification will not differ in nutritional, physicochemical and nutritional properties.
V. Consumer acceptance of egusi soup from the different egusi grits will not differ significantly.

1.5 Delineation of Research
I. Egusi seed to be used in the thesis was the specie of *Citrullus lanatus subsp mucosospermus*, purchased from a seed store in Cape Town, South Africa.
II. Supercritical fluid extraction techniques will be used to defat egusi seed.

1.6 Importance of the Study
Food scientists have targeted the development of new oilseed crops which could be used for food, medicinal and industrial purposes (Al-Farga et al., 2016). Producing highly nutritious oil, hydrocolloid and food from egusi plant is a process that involves picking from lesser-known crops to contribute to the essential nutrient in the diet of the world at large (Ojieh et al., 2007). The hydrocolloid property of egusi flour can be applied in various foods and beverages, thereby creating more jobs and income for the men/women that cultivate egusi crop. Food industries will explore a new form of high protein flour and hydrocolloid from an underutilised source. Successful development of food made from egusi seed will promote its use, and it will help eradicate extreme poverty and hunger by providing a nutritious meal from a non-expensive plant source. Egusi seed farming will encourage sustainability and job creation as farmers will grow more of egusi melon seed which will be readily available throughout African region. Instant egusi grits which could improve the egusi soup process and thereby lead to commercial production of the soup will be made available to the food industries. This will eventually benefit the global world, as this new oilseed crop will offer medicinal and nutritional benefits.

1.7 Expected outcomes of the study
The expected outcome of the project includes:
I. An alternative highly nutritional plant food will be produced for the people of South Africa, Africa and the world at large.
II. One published article in an international accredited journal.
III. Conference presentation at national and international scientific meetings.
IV. M Tech Degree in Food Science and Technology

1.8 Thesis overview
This thesis was compiled in an article format and consists of six chapters. Chapter one gave the general design and motivation of the study, which includes the introduction, problem statement, objectives, significance of research and expected outcome. Chapter
two is the literature review, which explained the background of the study, with topics such as propagation and planting of egusi melon, the structure of egusi melon and the nutritional composition of egusi seed. Egusi seed is an underutilised oil seed in Africa. The functional and nutritional properties of egusi make it a plant food that should be thoroughly industrialised. Chapter three is the first research chapter and its focus on the extraction of egusi seed using the supercritical carbon dioxide extraction method. Egusi seed high in oil has been underutilised and has not seen much industrialisation. The process of defatting is often laborious and sometimes involves the use of solvent extraction which could be harmful to consumers. In this chapter, the fatty acid composition of egusi oil was analysed, and the two health index (index of atherogenesis and thrombogenetic) was evaluated. The physicochemical properties of egusi oil were also assessed for peroxide number, iodine number, pH and oxidative rancidity.

Chapter four is the second research chapter and its focus on the nutritional and functional properties of defatted egusi flour and hydrocolloid extracted using supercritical carbon dioxide extraction. The defatted residue after supercritical removal in chapter three was milled into flour and analysed for its macro and micronutrients. The following analytical procedures were conducted on of defatted egusi flour proximate composition (moisture, protein, carbohydrate, ash, and fibre), micronutrient (amino acids profiling, trace and significant mineral elements, sugars), and functional properties (water absorption and water solubility index and pasting properties). In chapter four, the hydrocolloid of egusi flour was extracted using hot water treatment and was subsequently freeze-dried at a desired temperature and pressure. Egusi hydrocolloid was tested for its functionality (water absorption index, water solubility index and pasting properties). Chapter five covers the final research chapter, which focuses on the nutritional and consumer acceptability of instant egusi soup. This chapter focus on the production of precooked egusi grits prepared using boiling, spherification and extrusion cooking. A quadratic mixture design was used to determine the best component mix of egusi grit, egusi flour, and egusi hydrocolloid. The optimum soup produced was analysed for traced and significant mineral composition, the colour of instant soup, microbiological and sensory properties. Chapter six is a summary of the conclusion and recommendation of the study, with a focus on the experimental findings.

Reference
Al-Farga, A., Zhang, H., Siddeeg, A., Shamoan, M., V.M. Chamba, M., Al-Hajj, N. & Zhang,


Department of Agriculture, F. and F. (2013). • PRODUCTION GUIDELINES • Citrullus lanatus (Thunberg) agriculture,


CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction
The egusi plant, *Citrullus lanatus* subsp *mucosospermus* belongs to the *Benincaseae* family and is a subfamily of *Cucurbitoideae* (Gusmini *et al.*, 2004) (Figure 2.1). The plant’s delicacy is in its seed. This indigenous plant species is mostly consumed as food in Asia, Central Africa, and West Africa, where it is commonly known as egusi melon or bitter melon seed. In some parts of South Africa, *Citrullus lanatus* (Thunberg), Matsum. & Nakai is known as bitter melon. *Citrillus* is a genus of seven species of a desert vines, among which *Citrullus lanatus* (Thunberg), Matsum. & Nakai is an important crop (Gusmini *et al.*, 2004). This main species was first described by Thunberg in 1794 as *Momordica lanata* and was later improved by Matsum and Nakai in 1916, and placed in the *Citrullus* family (Dane & Liu, 2007). The cucurbit family has more than 95 genera and 750 species (Mgidi *et al.*, 2007).

Melons are cultivated as a weak stem plant creeping along a surface and belong to the family *Cucurbitaceae* with eatable and sweet flesh as found in watermelon and cucumber. *Citrullus lanatus* comprises of four species which are (*colocynthis*, *lanatus, rehmii* and *ecrihosus*) (Levi *et al.*, 2001). In Namibia, *rehmii* and *ecrihosus* are widely grown, but, *colocynthis* and *lanatus* are more prominent in West and Central Africa. In Africa, specifically in Nigeria, Cameroon, and parts of Central Africa, *Citrullus lanatus* subsp *mucosospermus* can be differentiated by two seed types, distinguished by the presence or absence of a black edge as shown in Figure 2.2 (Guo *et al.*, 2013). These seed types can be identified as different cultivar, and are more prominent in Africa (Guo *et al.*, 2013).

In some parts of South Africa, Citron melon (Makaatan) is a source of water in dry seasons, while the leaves and unripe fruit of egusi melon are utilised as green vegetables (Dane & Liu, 2007). Roasted egusi seeds are edible with a nutty taste and aroma. Specie of *Citrillus* similar to egusi commonly known as *karkoer* or *bitterboela* can be found on the sandy, the drier soil of southern Africa, primarily in the Kgalagadi region of the Northern Cape (Henderson, 2007). It is under cultivation in the Western Cape, Northern Cape, North West and Eastern Cape provinces. No statistical data is available on the production levels of bitter watermelon in South Africa as it has not seen any industrialisation (Henderson, 2007).
Figure 2.1  Categories of melon  
[Adapted from Garcia-Mas et al.(2012)]
Figure 1.2  (a) Dehulled egusi: (b) Hard shelled egusi seed
[Adapted from Bankole et al. (2005)]
Egusi seed is mainly used as a thickener in a traditional soup known as "pistachio soup" in Côte d'Ivoire or as "egusi soup" in West Africa. Despite the social, nutritional and economic role played by this cucurbit, it is classified as a secondary crop (Koffi et al., 2008).

In Côte d'Ivoire, egusi seed is differentiated by its physical attributes (Loukou et al., 2007). Some Citrillus cultivar has a seed coat at the edge of connection and is locally called Bebu; some other seed types are smooth with no black borders at the point of attachment. Egusi gives high yield in various intercropping systems, which is typically characterised by minimal inputs to guarantee food security and sustainable incomes for farmers (Bankole et al., 2005).

### 2.1.1 Propagation and planting of egusi melon

Egusi melon can be grown in the tropical land with up to 1000 m altitudes. It gives a higher yield in the savannah region than in areas with constant heavy rainfall. The egusi seed needs an annual rainfall of 700–1000 mm and a temperature of 28–35°C (Compton & Gray, 1993). In southern Africa, bitter melon gets more than 400 mm of rain. High rainfall promotes the growth of egusi plant, giving high yield (Ikeorgu & Ezumah, 1991). Higher yields could be obtained by dry cropping, but lack of irrigation facilities in most African farms influences farmers to plant melon seeds during the raining season. But on the southern African soil, egusi melon can be planted during the spring, summer and autumn season. For a fruitful yield on a tropical land, a loamy soil with pH 6-7 can be used during plantation (Ogbonna, 2009).

Nigeria has the highest production/yield of egusi melon in Africa as shown in Figure 2.3. Egusi's full maturity is observed by the falling of its stalk and tendrils. The egusi fruit is ripened to the point at which it is cut in half with the aid of a sharp axe. The seeds are scooped out, covered with dry grass and allowed to ferment for about 14 days. After fermentation, the seeds were separated from the pulp, washed and sundried on dry mats. To ensure even drying, seeds are turned over several times.

Environmental temperature and hot wind conditions influence the drying process of egusi seed which dries within 5–7 days (Ijoyah et al., 2012). In West Africa, egusi seed yield is 225 kg/ha, Senegal having up to 1100 kg/ha and Nigeria with the highest yield of 6000 kg/ha (Ntui et al., 2009). Namibian seed yield for egusi is 550 to more than 3000 kg/ha, depending on the type of cultivar used. China has an average seed yield of *C. lanatus* 1500 kg/ha. In Nigeria, egusi seeds are removed and packed in baskets or perforated bowls (20–25 kg) to dry. After drying the seed are dehulled using a dehulling machine or manually hand peeled.
Figure 2.3   Top six melon producing countries (CAR = Central African Republic; DRC = Democratic Republic of Congo) [Adapted from Garcia-Mas et al. (2012)]

2.1.2 Structure of egusi melon
The *Citrullus lanatus* is the important melon species in Africa (Gusmini *et al.*, 2004). The egusi plant is a genus of a desert vine, having its stem climbing up its trail. Members bear small sepals, solitary staminate flowers, and a corolla that is 5-parted to the base. The leaves are not or rarely divided beyond the middle, the fruits are smooth, or at most green-lined or hairy and a ground trailer as seen in Figure 2.4 (Mao *et al.*, 2004). The leaves are soft to touch, with a broad sinus and distant lobes at the base. Egusi melon comprises of a fleshy mesocarp which occurs during the third week of cultivation.
Egusi seed is enclosed in the mesocarp and must be scooped out, dried and dehulled before utilisation (Ogbonna, 2009). The good seeded melon (*Citrullus lanatus*) which is small flat and oval (Figure 2.4) has a soft golden brown seed coat, which must be dehulled before the tinny white seed is obtained. The dehulling can be manually done by hand twisting its flexible shell, or mechanically using a dehulling machine (Ogbonna, 2009).

**Figure 2.4** (a) Egusi melon plant. (b) A dissection through egusi melon
[Source: Garcia-Mas et al. (2012)]
The egusi plant can withstand pests and diseases because it covers the ground as it grows and can help reduce the growth of unwanted plants (Gusmini et al., 2004). This attribute of egusi makes it the number one choice for farmers when it comes to intercropping with sorghum, cassava, coffee, cotton, maize or bananas. Egusi seed must be dehulled to obtain the inner white seed is obtained. Studies by researchers indicate that egusi melon pods have an almost spheroidal external shape and an ellipsoidal seed cavity (Figure 2.4). Mature egusi melons can also remain in the field for a long time without rotting, so crop loss and loss of plant nutrient due to leaching of soil and erosion is minimised while the waste of plant during harvest is rare. Once the seeds are harvested, rot and spoilage are rare (Bankole et al., 2010).

2.2 Nutritional Composition of Egusi Seed

In developing countries, *C.lanatus* is an important crop; the seeds of egusi are dehulled to obtain a white kernel, which contains approximately 50-60% w/w oil, 28-30% w/w protein, 20-10% w/w carbohydrate, 2-3% w/w ash and 3-4% w/w fibre. The milk extracted from melon seeds was reported to contain 3.6% w/w protein, 4.0% w/w fat and 2.5% w/w carbohydrates, values of which are comparable to soy milk (Akubor & Ogbadu 2003). Hence, egusi seeds can fortify modern and traditional food products.

In most Africa countries, the *C. lanatus* seed is consumed due to its nutritional benefits, with literature indicating the presence of 60% w/w fat (defatted flour) and 30% w/w protein as shown in Figure 2.5.

2.2.1 Egusi melon protein

The raw and defatted egusi seed shows a nutritional composition of approximately 26-28% w/w protein and 56-60% w/w in defatted flour [Figure 2.5], (El-Adawy & Taha, 2001). The protein in raw egusi is higher than protein in cowpea and slightly lower than that of soybeans (36%) (Ntui et al., 2010). The major protein in egusi is from storage salt-soluble globulins consisting of three components with molecular weight of 570,000-590,000, 310,000 and 160,000-200,000, large protein group of molecules that have a molecular weight of over 60 kDa and contain over 30% w/w hydrophobic amino acid. An example of this group is the haemoglobin and egg white albumin. Globular proteins of molecular weight lower than 23 kDa do not form gels under normal conditions. Protein from cucurbit species tends to possess nutraceutical properties, such as anti-fungi, anti-bacterial and anti-inflammatory properties (Ntui et al., 2010).

Egusi seed is high in essential amino acids and can contribute to the nutritional need of a child suffering from amino acid deficiency. Melon is high in glutamine as seen in Figure 2.6, offering a significant building block for protein in the body (Hlatky et al., 2002).
Glutamine is $\alpha$-amino acid that is used in the biosynthesis of proteins increases and glutamine must be obtained from the diet.

**Figure 2.5** Raw and defatted nutritional composition of egusi kernel
[Adapted from Igwenyi & Akubugwo (2010)]
Glutamine helps to improve the side effect of a drug and improve the effectiveness of medication on diarrhoea, pain and swelling inside the mouth (mucositis) (Calder & Yaqoob, 1999). Glutamine builds the immune system and helps patients undergoing chemotherapy to maintain a healthy lifestyle (Calder & Yaqoob, 1999). Glutamine (a building block for proteins), found naturally in the body but can be derived from food sources like egusi melon, soy meal most oilseeds (Akobundu, 1989). Glutamine counters some of the side effects of medical treatments. It is used to reduce side effects of chemotherapy which includes diarrhoea, pain and swelling inside the mouth (mucositis), nerve pain (neuropathy), and muscle and joint pains caused by the cancer drug. Glutamine is also used to protect the immune system and digestive system in people undergoing radiochemotherapy for cancer of the oesophagus.

Figure 2.6  Amino acids profile of egusi seed
[Adapted from Giwa et al. (2010)]
Considering the widely used oilseed in the world and their protein content (Table 2.1), soya bean contains 36.0%, rapeseed 22.0%, sunflower (19.8%), peanut (25.6%). While considering these varying percentages, egusi seed has a high protein content and amino acid store (Giwa et al. 2010). The high protein in egusi seed can help solve problems of malnutrition that is attributable to protein deficiencies in our societies. Protein-energy malnutrition (PEM) also known as Calorie Malnutrition occurs in children whose consumption of protein and energy is not sufficient to meet their dietary needs. In most cases deficiency will exist in both total calorie and protein intake (Garcia-Mas et al., 2012).

Table 2.1 Nutritional profile of some major oilseeds and egusi seed

<table>
<thead>
<tr>
<th>Contents % w/w</th>
<th>Major oilseeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cotton *seed</td>
</tr>
<tr>
<td>Protein</td>
<td>32.6</td>
</tr>
<tr>
<td>Fat</td>
<td>36.3</td>
</tr>
<tr>
<td>Saturated fatty acid</td>
<td>9.7</td>
</tr>
<tr>
<td>Monounsaturated fatty acid</td>
<td>6.7</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid</td>
<td>18.1</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>21.9</td>
</tr>
<tr>
<td>Fibre</td>
<td>5.5</td>
</tr>
</tbody>
</table>

[*Adapted from Standard Food Organization], [** Adapated from Giwa et al. (2010)]

However, PEM occurs with the diet that provides more energy but lacks an adequate amount of protein. It may also occur in children with an illness that leaves them unable to absorb vital nutrients or convert them to energy essential for healthy tissues (Calder & Yaqoob, 1999).

Egusi is a source of arginine, having between 12-13.3% w/w in composition. Arginine is mainly for the biosynthesis of protein, and it is classified as a semi-essential or conditionally essential amino acid. The use of arginine is dependent on the health or development stages of the individual in need of the amino acid. A premature infant cannot synthesise arginine by themselves, a food rich in arginine should be incorporated into their diet as this will help such children to synthesise protein naturally.
2.2.2 Egusi melon fat

Egusi oil contains essential fatty acids as seen in Table 2.2, with linoleic, palmitic, stearic and oleic acid being the highest. Linoleic is the most abundant in egusi seed; approximately 59% linoleic, 16-17.1% w/w oleic (Akobundu, 1989), palmitic 12.4% w/w and stearic acid 8.1% w/w (Akobundu, 1989) and relatively small amounts of linolenic acid (LA) (Bankole et al., 2005). Roasting melon seed at 133.1°C for 20.2 min was reported to produce an optimum yield of high-quality oil from melon seed (Ntui et al., 2009). A significant reduction in total cholesterol, as well as reduction in atherosclerosis, was reported in experimental rats fed with egusi oil suggesting that egusi melon can reduce the blood pressure (Ziyada et al., 2008).

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>% COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid</td>
<td>0.21</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>0.78</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>13.45</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>13.71</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>14.50</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>56.94</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>0.46</td>
</tr>
<tr>
<td>Saturated fatty acid</td>
<td>28.10</td>
</tr>
<tr>
<td>Monounsaturated fatty acid</td>
<td>14.50</td>
</tr>
<tr>
<td>Polyunsaturated fatty acid</td>
<td>57.40</td>
</tr>
<tr>
<td>Unsaturated fatty acid</td>
<td>71.90</td>
</tr>
</tbody>
</table>

Source: Jarret & Levy (2012)

Egusi melon has the possibility of preventing heart diseases and help with weight loss by burning adipose tissues in the body also known as brown fat due to the presence of linoleic acid. Linoleic acid is a polyunsaturated omega-6 fatty acid. It is a colourless liquid at room temperature. In physiological literature, it has a carboxylic acid with an 18-carbon chain and two cis double bonds; with the first double bond located at the sixth carbon from
Linoleic acid belongs to one of the two families of essential fatty acids, which means that the human body cannot synthesise it but can be found in food components (Kelly, 2001). A recent study by Harvard School of Public Health claims that conjugated linoleic acid (CLA) help reduces internal body fat (Khanal & Dhiman, 2004).

Conjugated linoleic acid raises the body metabolism, allowing the body burn visceral fat. In overweight people adipose tissue is inactive when food high in CLA is consumed, their fatty tissue is made active, and weight loss will be achieved (Kamphuis et al., 2003). Conjugated linoleic acid can also help to suppress appetite if it is incorporated into a weight loss diet (Dhiman et al., 2000). Furthermore, CLA has external benefit for the body. It produces prostaglandin which functions as anti-inflammatory and diuretic, helping the skin to maintain tone and moisturised after weight loss (Bassaganya-Riera et al., 2004). When the fat in the belly and around the heart is removed, coronary heart disease is prevented (Kamphuis et al., 2003). Egusi seed is high in polyunsaturated fatty acid 71.9 g/100 mg, making egusi a nutraceutical food option (Akobundu, 1989).

2.2.3 Egusi melon carbohydrate

Egusi carbohydrate at 10.6% w/w helps to produce functionally stable and nutritious food. The carbohydrate content of plant food mainly comprises of amylose and amyllopectin (Adelberg et al., 1994). The component of starch is in both its amylose and amyllopectin (Ponka et al., 2006). The difference between these two starches is in its absorption and solubility in water and its ability to swell in hot water.

Egusi carbohydrate is presumed to consist mostly of amyllopectin and less of amylose due to its ability to dissolve in water, swell and form a stable emulsion. The function of the amylose is to provide energy for plants (Blazek, 2008). In the creation of food products, amyllopectin is used more often as an emulsion stabiliser and as a way to thicken substance in industrial and food-based industries (Blazek, 2008).

Egusi which has been traditionally used as a thickener for ages proffers a hydrocolloid to the food industry in the area of emulsifying and stabilising food water balance (Wang et al., 2011). A hydrocolloid is defined as a colloid system wherein the colloid particles are hydrophilic polymers dispersed in water (Blazek, 2008). Egusi starch globule forms irreversible light gels because they easily interact with their hydrophobic fragments (Giwa et al., 2014).

2.2.4 Mineral composition of egusi seed

The minerals of egusi melon show it is high calcium and potassium source. Calcium is found in bones and teeth, helping in the formation of healthy bones and teeth (Gillooly et al., 2005). Phosphorus also helps the body define its usage of carbohydrate and protein
(Cahill et al., 2013). It’s needed for the maintenance of protein globules for the growth and repair of cells and tissues (Akobundu, 1989).

Egusi seed is an excellent source of magnesium. According to literature, the average body contains 25 g of magnesium (Saris et al., 2000). Magnesium performs more than 300 chemical reactions that help the body functions properly (Mordike & Ebert, 2001). Dietary source of magnesium include a diet rich in magnesium, but the supplement can be administered in extreme situation of deficiencies (Saris et al., 2000). Intake of magnesium is peculiarly low among women (Ford & Mokdad, 2003). Calcium is used for prevention of bone-related deficiencies leading to weak bones or low bone density, softening of bone, and painful softening of bone known as porous bone or osteoporosis (Saris et al., 2000). Egusi melon is also a good source of calcium (28.2% w/w) (Ijoyah et al., 2012).

2.3 Anti Nutrient in Egusi Seed

Anti-nutrient and toxins are present in a considerable amount of all raw food. The level of toxicity is affected by the quantity of these antinutrients in a fresh agricultural product (Enujiugh & Ayodele-Oni, 2003). Egusi has a trace amount of oxalate, phytate, tannins, and nitrate. Enujiugh & Ayodele-Oni [2003] reported the levels of antinutritional factors in egusi seed to be 1.8, 1.77, 0.50 and 0.11 g/kg dry matter respectively for oxalates, phytic acid, phytate-P and tannins (Enujiugh & Ayodele-Oni, 2003). These values are within limits, and it is expected that processing methods would considerably reduce the levels of these anti-nutrients further. These low anti-nutrients in egusi seed will more also increase the functionality of egusi melon in food.

2.4 Functional Properties of Egusi Seeds

The functional properties of food from a processing point of view indicate the desirable properties of food. At a proper concentration of the components and appropriate conditions, it provides for the beneficial rheological as well as sensory characteristics of the product as it relates to mouthfeel, aeration, juiciness and bulking properties (Kennedy & Mistry, 2003). Functional properties of a food can be classify into four major categories including: functional properties that relates to structure of protein and rheological features, which will results in the interaction of macromolecules and water; (2) properties related with addition of water leading to thickening, wettability and solubility: (3) properties related to the protein surface as it related to whippability and foaming properties: and (4) properties related to it sensory properties (Boye et al., 2010).
2.4.1 Functional properties related to hydration mechanisms

Water is the most abundant component in food systems. Thus, molecules in the food systems are generally in intimate contact with water. Functional properties as it relates to water in food, describes solubility, wettability, retention, and swelling (Raghavendra et al., 2004). The reaction of protein with water is related to the thermodynamic attribute of essential amino acids in the food. These properties affect the functions of proteins in the food system (Bande et al., 2013).

Protein isolate from egusi flour can be obtained using a one or 2 step sodium chloride extraction or by treating with hot water. Water absorption of melon flour was reported as 1.44 g/g (Giwa et al., 2010). Defatted egusi seed flour was reported by Giwa et al. (2010) to possess water holding and oil holding capacities of 0.7 ml/g and 2.6 ml/g, respectively. Defatting enhances the protein solubility, water and oil absorption of egusi flour (Eltayeb et al., 2011). Defatting increases the protein solubility, water and oil absorption capacities of the meals (Eltayeb et al., 2011). Melon seed meal incorporated into chicken sausage reduced the fat content, decreased refrigeration weight loss and improved the overall acceptability of the sausage (Akobundu, 1989). Hence, egusi seed could satisfy the growing demand for low fat and high protein food.

Proteins swell as they absorb water and it is an important functional property in foods like processed meat, dough, and custards where the proteins should imbibe and hold water without dissolving and concurrently impart body, thickening, and viscosity. Hence, egusi seed meal could satisfy the growing demand for low-fat sausages (Olivares et al., 2010) as well as in food systems requiring hydration.

2.4.2 Interactions of macromolecules-water-macromolecules interactions

In a food system, solubility, hydrodynamic properties, hydrophobicity and microstructure of proteins have been reported to play an important role in the rheological properties of proteins (Song & Zheng, 2007). The interactions of macromolecules-water-macromolecules relate to functional properties with the protein structure and rheological characteristics (viscosity, elasticity, adhesiveness, aggregation and gelling) due to gelation. Knowledge of the viscosity and flow properties of protein dispersions are of practical importance in product formulation, processing texture control, and mouthfeel properties as well as in clarifying protein-protein interactions and conditions affecting conformational and hydrodynamic properties (Blazek, 2008).

Gel formation by globular proteins involves several reactions such as denaturation, dissociation-association, and aggregation (Sorgentini & Wagner, 2002). Denaturation is the change of a native protein conformation to a more unfolded structure, which functional groups such as sulphydryl groups or hydrophobic groups become exposed allowing the
exposed groups to interact with each other to form aggregates (Henzler-Wildman & Kern, 2007). When the protein concentration is high enough, aggregation leads to the formation of a gel. Otherwise, collection leads to precipitation of the protein. Increase in viscosity of 10% w/v dispersion of melon seed flour in water from 81°C reaching a maximum of 80 Brabender units at 83°C was due to thermally induced aggregation and coagulation of the melon seed protein since there is little starch in a melon seed.

Aggregation of the water-soluble protein in distilled water at pH 6.4 starts at 60°C, coagulation and precipitation occurred at 85°C (Vignola et al., 2018). It is known that protein aggregation before emulsification is a requirement for the formation of gel-like protein stabilised emulsions (Berghout et al., 2014). The degree of molecular associations or aggregations affects not only the mechanical properties of gels but also the thermal properties [i.e. thermo-reversibility or –irreversibility of gel elasticity] (Bemiller, 2011). The mechanism of gel-like structure formation is due to colloidal destabilisation, especially droplet aggregation through strong attractive interactions.

The formation of a gel-like structure will improve the stability of emulsions against gravitational separation (creaming and sedimentation). When the egusi seed meal dispersion was heat-treated (at 0.15 M NaCl and pH 7.2), white slurry was obtained at 3% (w/v) egusi protein, whereas at higher protein concentrations (6–20%, w/v) an opaque white gel was formed. The rheological characteristics of whole egusi seed meal indicate increased crosslinking in egusi gels (very strong and elastic gel networks) as the protein concentration increased at 10% and 20% (w/v) egusi protein levels (Uruakpa, 2004). The formation of elastic networks may be due to the electrostatic interactions between NaCl and the macromolecules (protein, lipid, polysaccharides) present in whole egusi seed meal. Egusi protein (6%) was sufficient to produce properly-cross-linked networks (G' = 8724 Pa), with increased structural stability and more elastic systems as NaCl level increased. Elastic gels developed above the denaturation temperature (T_d = 93.7°C) in the heating phase and continued during cooling, indicating that egusi seeds can be used as a gelling food ingredient (Uruakpa, 2004). It appears the ability of egusi proteins to form gels in the presence of NaCl is related to the thermal denaturation temperature of the proteins when exposed to each salt concentration. As the T_d increases, structure development is enhanced, and the resulting gel networks were superior in overall strength (Uruakpa, 2004). Whole egusi seeds can be used as a texture modifier in food systems, and this functionality can be modified by interactions with ingredients such as NaCl. Texture modifiers are substances used to increase the viscosity or gel of aqueous solutions thereby retarding or preventing droplet movement (Ishihara et al., 2010).
2.4.3 Functional properties related to the protein surface

Protein surface of the food is affected by protein-lipid interaction which results in properties like whippability and emulsifying properties. Emulsified food which emanates from the use of liquid droplet in a continuous phase of another liquid is not miscible with the droplet material. Emulsified food includes mayonnaise, milk, cream, soybean milk, salad dressing and ice cream. An emulsified mixture is not stable naturally and can separate into two layers. To prevent this sort of separation, the protein with high gelling property must be used. Flocculation and coalescence are decreased when protein film is formed around the emulsion. Flocculation occurs when a cluster is created around fat droplets leading to creaming, caused by gravitational force (Piorkowski & McClements, 2013). The dissolved protein will add rheological properties of the dispersing phase, thus contributing to the decrease in the rate of creaming and coalescence. Creaming and flocculation can be accepted in some foods below the extent leading to separation of serum (Piorkowski & McClements, 2013). Defatted egusi flour forms a stable emulsion in an alkaline medium and a stable foam at pH 5 (Akobundu, 1989).

The capability of protein to form a stable film is an excellent functional characteristic in cake production, and general confectionaries (Linke & Berger, 2011). Egusi melon seed is nitrogen soluble at pH 10 (Akubor, 2004). Hence egusi proteins have potential to stabilise foams. Foams are gaseous droplets encapsulated by a liquid film containing soluble surfactant protein resulting in reduced interfacial tension between gas and water. Properties of good foaming proteins include solubility in the aqueous phase and rapid adsorption during shipping and bubbling; (Martin et al., 2002), concentrate at the air-water interface and unfolding to form cohesive layers of protein around air droplets with reduction of surface tension; and possess sufficient viscosity and mechanical strength to prevent rupture and coalescence (Martin et al., 2002).

2.4.4 Functional properties related to organoleptic/kinesthetic

Organoleptic attributes of food such as sound, appearance, temperature, flavour and texture determine food palatability while considering taste and feel like the major factors (Zushi & Matsuzoe, 2011). The texture is the perception of food in the mouth, combination of both mechanical and thermal properties exhibited by the mouth and oesophagus (Pascua et al., 2013). It is reported that more than 30% of the pleasant taste of food is determined by the texture. Consumer's acceptance of food is attributable to the texture of the food products, because people derive pleasure in meals, with a perceived different texture which contributes to a release profile of flavour in the retro nasal passage (Zushi & Matsuzoe, 2011).
Food hydrocolloids (polysaccharides and proteins) are used to manipulate food texture. Egusi protein has the potential to modify flavour by holding together flavour and non-flavour in cooking. This will enable the release of a complete flavour profile by reactants that may produce flavour, especially following the breakdown of protein into smaller amino acids and also the release of the water molecule in food substance (Uruakpa, 2004).

2.5 Prospects and Uses of Egusi Melon Seed

*Citrullus lanatus* seeds are increasingly used locally for their oil and meal for food in semi-arid regions by minor population (Achigan-Dako *et al.*, 2008b). But its oil can also be used in food, cosmetic and pharmaceutical industry. Traditionally, egusi seed is prepared in Western Africa through hand kneading of the meal to extract the oil from its seed. Egusi dough from the process is hand cut into bits and rolled into a small ball like shapes. This laborious method often discourages the use of this seed as a food and a thickener Egusi seed high in protein, and healthy fat has not seen any industrial utilisation. Introducing egusi plant into cropping system will assist in suppressing weed as egusi blanket itself on the ground allowing minimum possibilities of weed interruption in a plantation, reducing production/maintenance cost (Ikeorgu *et al.*, 1989). Further research must be made to improved agronomic practices, labour saving by upgrading the dehulling and defatting equipment and a more cost-effective method of defatting egusi seed (Giwa *et al.*, 2010).

2.5.1 Egusi melon as a functional food ingredient

Hydrocolloids which comprise of the protein and polysaccharides in food is the primary constituent that helps in the structure and texture of food products (Bemiller, 2011). Egusi starch and protein has the potential to form a thick gel, which can be used as a bulking agent. Egusi’s gelling property will encourage it is used in the production of pastries in composite flour. Factors that can affect egusi gelling and pasting properties include the pH, ionic content or strength, urea content, temperature and the physical force used during processing (Bemiller, 2011). The properties of a good gel are determined by its ability to react thoroughly with water, forming a coagulated gel of a transparent gel. Proteins containing non-polar residue forms a coagulated gel, while amino acids that melt in water creates a transparent gel (Rojas *et al.*, 1999).

Defatted egusi flour and concentrates are expected to form a firm, hard, resilient gels, whereas egusi protein isolates will form the semi-liquid gel. Acceptable texture and water-holding are a challenging aspect, particularly in low fat and low salt food protein systems. Hence, egusi meal/protein is a potential ingredient in these systems. In multiphase systems such as emulsions and foams where stability is not only controlled by
the interface between the immiscible components but also by the viscosity of the continuous phase, egusi hydrocolloid will serve as a useful foaming and emulsifying agent will enhance stabilisation of the constant phase but also strengthen the interface (Kundu, 2005). *Citrullus lanatus* has the potential to fortify cereals and staple food by serving as a rich protein source (Uruakpa, 2004).

However, before this could happen, future perspectives on the full utilisation of the potential of egusi seeds as food ingredients will be directed in the area of publicity and its potential in malnutrition programs as shown in Figure 2.7. Further research on its functional properties relating to thickening, stabilising and fortifying processed products is required. Hydrocolloid from plant source has more advantage than hydrocolloid from animal source and microorganisms (Burey *et al.*, 2008).

The water-holding and oil holding the capacity of defatted egusi flour have been reported to be 0.7 ml/g and 2.6 ml/g respectively (Akubor & Ogbadu, 2003). Hence egusi proffers a thickening power in its ability to form a gel. However there is no published report on the hydrocolloids properties of egusi seeds, despite its being locally used as a thickener in soup in some parts of Africa (Achigan-Dako *et al.*, 2008). Exploring the hydrocolloid properties of egusi in its ability to form gel will encourage its use in the food and non-food industries. Also, egusi nutritional composition will enhance its utilisation in a food system.

They exhibit more user-friendly potentials and offer more nutritional benefits that are health friendly. Reduced interaction with water increases the hydrophobic nature of a polysaccharide. The hydroxyl group in carbohydrate preferably reacts with two water molecule, leading to less interference or interaction with another hydroxyl group. Hydrocolloids are very important in food industries and can also be explored in other non-food sectors (Armstrong & Barringer, 2013). Naturally, hydrocolloid can be present in some food, while some food will require the use of hydrocolloid from a different source to control the functional behaviour of such food (Armstrong & Barringer, 2013).
2.5.2 Egusi melon as cooking oil

Oilseeds used in the preparation of diets abound in several parts of the world. Castor oil and flaxseed oil are used in the development of meals and can sometimes be used for its nutraceutical purposes. When cooking at high heat, stable oils are most preferred, oil that will not go rancid due to heat (Innawong et al., 2004). During oxidation of the oil, oxygen reacts with free profound compounds that lead to off flavour in food, which can be harmful to health when consumed. Important factors when considering oxidation and rancidity is the degree of saturation at both high and low heat processing of the free fatty acids present in the food component (Quintal, 2012). Saturated, monounsaturated and polyunsaturated
fatty acids (PUFAs) comprise of single bonds, one double bond, and two or more double bonds respectively (Smith et al., 2007). Saturated and monounsaturated fatty acids are sensitive to heat and should be avoided when cooking. This is due to the formation of radicals that will lead to toxin formation and off flavour in the food produced from such oil. Egusi melon is reported to be high polyunsaturated fatty acids and a low level of saturated fatty acids, this makes egusi oil heat stable.

![Scheme of conjugated linoleic acid](image)

**Figure 2.8** Scheme of conjugated linoleic acid  
Source: (Perakis et al., 2005)

The oil in egusi melon is cholesterol free, making it a heart-friendly oil for all age. Sunflower (*Helianthus annuus* L.), world production is estimated at $26.5 \times 10^6$ t with a harvested area of about $21.7 \times 10^6$ ha. The oil capacity of sunflower oil enables its huge production as cooking oil. It is light, tasteless and contains a relatively high concentration of the polyunsaturated fatty acids and a high linoleic acid shown in Figure 2.8 (Perakis et al., 2005).

The recent innovation on the use of a natural, non-toxic supercritical CO$_2$ method of extracting oil from seed enhances the quality of oil made from such processing (Perakis et al., 2005). The meal of egusi seed is extracted by solvent method becomes darker with temperature increase and at constant pressure. At the high temperature of 75°C, the meal located at the reactor inlet for most major seeds solvent extraction will change from off-white to light brown and finally to dark brown when the temperature reaches 95°C (Mbah et
al., 2014). Egusi meal will appear very red and less yellow after extraction; the meal becomes a waste while the oil will need purification before usage (Machida et al., 2011).

**Supercritical carbon dioxide oil extraction**
Vegetable oils are one of the most exciting products proposed for supercritical fluid extraction [SFE] (Aladedunye & Przybylski, 2013). Indeed, in these cases, supercritical fluid processing is very promising for improving the traditional industrial techniques that are based on hexane extraction for vegetable oils (Perakis et al., 2005). Supercritical fluid extraction is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent (Pourmortazavi & Hajimirsadeghi, 2007). Different oilseeds have been studied by several authors from the processing point of view, and a wide range of seed specie has been explored, which includes wheat germ, oats corn germ, cottonseed, soybean, rice brans, evening primrose jojoba, rapeseed, peanut and grape seed. The extraction of solids by liquid solvents is a well-known process which is used in various industries. Natural substances are conventionally extracted by liquid solutions at normal pressure; an example is the extraction of hops, spices or oilseeds.

General problems with solvent extraction methods are the presence of residues in products and the need for additional processing steps to remove the solvent from the mixture of solvent and extract which involves a further financial obligation and stress (Nwu, 2005). Alongside conventional solid/liquid extraction, the supercritical fluid extraction of natural substances has recently become established (Akanda et al., 2012). This process employs unproblematic non-insipid fluids as the solvent and yields practically solvent-free products in a thermally gentle manner. Simple fractionation of the products is possible by a variation of pressure and temperature (Gouda et al., 2017). The SFE process has become particularly familiar with the food, coffee and tobacco industries. The local conventional solid/liquid extraction plants are typically optimised energetically by squeezing and pressing using the hydraulic press which is not the case for all operated supercritical fluid extraction plants (Nyam et al., 2010).

Supercritical carbon dioxide extraction has attracted considerable attention in recent years as a promising alternative to conventional solvent extraction and mechanical pressing for extracting oils and other materials as it offers some advantages including non-solvent residues and better retention of aromatic compounds (Eckert et al., 2005).

**The principle of supercritical fluid extraction**
The advantages of using supercritical fluids (SCF) as solvents in chemical synthesis offer environmental, health safety and chemical benefits (Goto, 2009). The low energy consumption of the process is also an environmental benefit. Supercritical fluids also have
health and safety benefits because the most important SCFs which are CO₂ and H₂O are non-carcinogenic, non-toxic, non-mutagenic, non-flammable and thermodynamically stable. One of the major process benefits derived from these thermo physical properties of SCFs is its high diffusivity, low viscosity, density and dielectric constant of SCFs (Tenllado et al., 2011). These properties can be fine-tuned by changes in the input of the pressure/temperature during the process of extractions. The use of high pressure in a wide range of technologies and processes is based on chemical and physicochemical, effects it has on the oil or process obtained from such effects (Aladedunye & Przybylski, 2013).

The advantages of using supercritical fluids for the isolation of natural products have been well explored by food and non-food industries (Tenllado et al., 2011), which results in solvent-free products, low temperature, and zero by-products. The most important advantages of using supercritical fluids are the selective extraction of components or the fractionation of total extracts, which is made possible by using different gases for isolation or fractionation by changing the process parameters. The limitation of the process is the relatively high price of the equipment when compared with the material used in the conventional methods of extraction involving hydrocarbon or the use of hydraulic press method for extraction (Goto, 2009). More so, the meal obtained after a supercritical extraction process, maintain its nutrient density.

2.6 Conclusion and Future Recommendation

*Citrullus lanatus subsp mucosospermus* with its profound benefits could become a good source of protein in many foods and an excellent supplement to known cereal and root-based staple foods. Egusi high in linoleic acid and essential minerals is a potential food ingredient that has been underutilised for so long. However, before this could be achieved future perspectives on the full utilisation of the potential of egusi seeds as food ingredients will be directed in the area of publicity of its potential in malnutrition programs. As a hydrocolloid in the food industry, egusi has inherent functional characteristics as a stabiliser. Egusi seed should not be seen as a secondary food, as most big markets in West Africa and the most African nations sell its seed to make some necessary income. The use of egusi flour in the improvement of infant nutrition given its high protein and fat content must be explored.

Reference


Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., González, V.M.,


Ijoyah, M.O., Bwala, R.I. & lheadindueme, C.A. (2012). Response of cassava, maize and


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75.
CHAPTER THREE

PHYSICOCHEMICAL AND FATTY ACID PROFILE OF EGUSI OIL FROM SUPercritical Carbon Dioxide EXTRACTION

Abstract

Egusi is a high oil seed that could have potential benefits to human health but has remained unexploited. The only method of extraction is by solvent and mechanical means, a process which destroys the meal obtainable thereafter and discarded as waste. Thus is the emergence of egusi oil (EO) extracted using supercritical CO₂ extraction method. A series of operational parameters (temperature, flow rate, pressure) for supercritical fluid extraction of EO at 60°C, 30 g/h and 450 bar (EO1); 55°C, 30 g/h and 600 bar (EO2) and 75°C, 30 g/h and 600 bar (EO3) were investigated using a plant scale supercritical equipment. The oil yield which measures the amount of oil derivable from egusi seed ranged from 46-53% w/w. The moisture contents were 1.3, 2.0 and 1.9% w/w, respectively for EO1, EO2, and EO3. EO1 (60°C and 450 bar), was significantly (p ≤ 0.05) lower in moisture content compared to EO2 and EO3. The fat content was 99.1% w/w (EO1), 98.3% w/w (EO2) and 98.9% w/w (EO3), with no significant difference in the three oil samples. Egusi oil was predominantly high in polyunsaturated and monounsaturated fatty acids, which was identified as linoleic (62%) and oleic (15%) acids. The index of atherogenicity (IA%) were significantly low 0.35, 0.38 and 0.38% w/w for EO1, EO2, and EO3, respectively. The thrombogenicity index (IT %) were 0.08, 0.09 and 0.09% w/w for EO1, EO2 and EO3, respectively. The pH of egusi oil was 4.2, 5.4 and 4.9 for EO1, EO2, and EO3, respectively indicating slightly acidic oil. Peroxide Value (PV) measured using auto titrate Titrino plus, ranging from 11.60 milliequivalents peroxide/kg for EO1 to 12.60 for EO2 and 11.89 milliequivalents peroxide/kg for EO3. The oxidative stability index (OSI) was measured using Methrohm Rancimat at 120°C, expressed as the induction time of oxidation was 10.2, 11.5 and 5.3 h for EO1, EO2, and EO3, respectively, with E03 significantly higher than EO1 and EO2 (p ≤ 0.05). The iodine number ranged from 95 g/100 g for EO1 to 129 g/100 g for EO3. Hence, egusi oil is high in linoleic acid and iodine number but low in peroxide number being stable at high temperature, and not susceptible to microbial spoilage due to its level of acidity.
3.1 Introduction

Egusi seed (*Citrullus lanatus subsp Mucosospermus*), a family of *Benincaseae* of the subfamily *Cucurbitoideae*, can be grown in any part of the world with long summer of three months (Ntui *et al.*, 2009) and is more prominent in Africa. The flesh of egusi has a bitter taste and a spongy feel and is not considered edible in raw form in communities that consume egusi seed. Egusi is well adapted to extremely divergent agro-ecosystems and can survive extreme weather conditions (Lagoke *et al.*, 1983). The meal is high in protein and oil with literature indicating 28.4% w/w protein (60% in defatted flour), 52% w/w fat, 3.6% w/w ash, 2.7% w/w fibre and 8.2% w/w carbohydrate (Akobundu, 1989). Akubor & Ogbadu (2003) reported that the primary fatty acids in egusi oil are 59-63% w/w linoleic acid and 16% w/w oleic acid. Egusi oil, with no cholesterol and high omega-3 and 6 fatty acids have never been introduced into the food industry (Adegoke & Ndife, 1993).

The conventional oil extraction process is often laborious, and sometimes involves the use of harmful and toxic hydrocarbons for defatting. A more effective method of defatting using the supercritical fluid extraction method is necessary. Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids (Akanda *et al.*, 2012).

A supercritical fluid (SCF) is a substance prevailing at a temperature and pressure above the critical point. It is neither a gas nor a liquid and is best described as an intermediate to these two extremes. The supercritical phase has solvent strengths close to those of liquid properties. Carbon dioxide is the logical choice since it has properties most ideal for extraction with a relatively low critical temperature (31°C) and critical pressure (73 atm). Carbon dioxide is non-toxic, non-flammable, relatively cheap and commercially readily available.

Carbon dioxide is regarded as an environmentally friendly gas, replacing the hazardous organic solvents and results in extracts free from solvent residue. Chemical substances that have also been used successfully as supercritical fluids include ammonia, argon, propane, xenon, and water. Supercritical CO$_2$ fluid extraction (SCF-CO$_2$) of a wide range of oilseed species including wheat germ, oats, cottonseed, soybean, rice bran, evening primrose, jojoba, rapeseed; peanut and grape seed have been studied by several authors from the processing point of view (Akanda *et al.*, 2012).

SFE has attracted considerable attention in recent years as a promising alternative to conventional solvent extraction and mechanical pressing for extracting oils and other materials as it offers some advantages. These advantages include non-solvent residues and better retention of aromatic compounds (Balachandran *et al.*, 2006); chemical, environmental, health, and safety benefits (Fiori, 2009). The ecological benefits of most
SCF in industrial processes result from their replacement of the widely known environmentally damaging conventional organic solvents (Wang et al., 2011).

There is an increasing public health awareness of the environmental hazard associated with solvent contamination in the use of organic chemicals to separate components. Also, the high cost of organic solvents is also to be considered during extraction (Veli, 2012).

To date, the nutritional and functional profiles of egusi seed have been studied (Onuora & King, 1984; Kouebou et al., 2013), but not the nutritional and physical properties of its oil extracted using supercritical CO$_2$ extraction method. Hence, the objective of this study was to characterise the proximate, fatty acid profile and physicochemical properties of egusi seed oil extracted using SCF-CO$_2$ with a view to drawing the attention of the consumer and the food industries to the potential of egusi oil.

3.2 Materials and Methods

3.2.1 Source of egusi seed, reagents, and equipment

The overview of the methodology used in this chapter was shown in Figure 3.1. Dehulled egusi seeds (Citrullus lanatus sub-Muscoparmerus) were purchased from a local store in Capetown, South Africa. All chemical reagents were obtained from Merck Pty, South Africa. Deionized water was used all through the experiment. The supercritical fluid extraction equipment (Swiss Nova), a plant extractor, was operated manually at the Process Engineering Department of Northwest University, Potchefstroom Campus, South Africa. Egusi oil was tested for proximate, physicochemical and fatty acid profile (Figure 3.1).

3.2.2 Egusi seed preparation

Dehulled egusi seeds were sorted for chaff and damaged seeds as well as stones and pebbles, and all extraneous matter removed and discarded. The seeds were stored in a perforated sack bag inside a refrigerator before use.

3.2.3 Supercritical carbon dioxide extraction of egusi Oil

Supercritical CO$_2$ extraction of egusi oil was performed using a plant scale unit. An extraction run was initiated by weighing a thimble (sample holder) and inserting the weighed 2 kg egusi seeds into the thimble and weighing again. The mass of the sample of egusi seed in each thimble was obtained by subtracting the weight of thimble from the mass of egusi seed. A collection vial was weighed and positioned for oil collection. After each extraction run, the collection vial was weighed again.
Figure 3.1  Overview of chapter three.
The extraction run was initiated by clicking "analyse" from the "samples" menu after entering the extraction parameters required for the egusi seed. Firstly, the system was pressurised (at intervals of approximately 400-600 bars) until the desired pressure of 450 bar, and 600 bar was reached. The temperature at the extractor outlet was varied between 55, 60 and 75°C by a thermoregulatory device to allow the separation between the extract and the solvent during depressurisation. After column temperature and the pressure was stabilised, the system was kept in contact with the egusi seed for at least 15 minutes to allow system stabilisation. Then, the supercritical CO₂ was pumped into the bed of egusi seed at 30 g/hr. The volumetric flow rate of the solvent was regulated using the expansion valve at the outlet of the extractor. The pump pressure at 450 bar was regulated at the set value by high variable restrictors (HVRs), electronic flow meters and solenoid valves.

Solenoid valves lowered the thimbles into the chamber and automatically shut-off the sample compartment. A pump was turned on to compress the CO₂ to the set pressure. A pump cooler was also turned on, which cools the pump and CO₂ to approximately 0°C to enhance compression and increase the pump flow capacity. Pump pressure (CO₂) continues to increase until the default pressure of 600 bars (9000 psi) was reached. Three experimental runs were conducted using (1) a low temperature, low pressure (450 bar, 60°C), (2) high pressure, low temperature (600 bar, 55°C), and (3) high pressure, high temperature (600 bar, 75°C) at constant flow rate of 30 g/h.

As the run continues, oil was extracted from egusi seed and collected once ambient conditions were restored at the end of the extraction run (Tenllado et al., 2011). The extracted egusi oil (EO) was collected via an amber glass container and weighed every 10-20 minutes until constant weight.

### 3.3 Characterization of SCF-CO₂ Extracted Egusi Oil

#### 3.3.1 Determination of oil yield

The oil yield was calculated by taking into account the mass of the extracted oil and the mass of the seeds used for oil extraction by using equation 1 (Rebolleda et al., 2012).

\[
\text{Oil yield (\%)} = \left( \frac{\text{Mass of extracted oil}}{\text{Mass of seed}} \right) \times 100
\]  

#### 3.3.2 Total fat and fatty acid determination of egusi oil

The total fat and fatty acid of egusi oil were determined according to the standard AOAC official method 996.06 (AOAC, 2005). Samples were digested and analysed with Gas
chromatography Agilent technology (7890A) GC system as discussed in the following paragraph.

**Sample digestion**

Homogenized egusi oil (100 mg), was weighed accurately into 70 ml tube in triplicate. Pyrogallic acids (100 mg), internal standard (2 ml) and ethanol (2 ml) were added and mixed well using a vortex mixer until the product was dispersed. An aliquot (10 ml) of hydrochloric acid (32% concentration) was added to the mixture. The tubes were placed in the water bath at 70-80°C and shaken gently for 40 minutes. The content of the tubes was gently swirled at every 12 minutes. After digestion, the tubes were removed and cooled to room temperature. Extraction of the sample was carried out by adding 25 ml of diethyl ether, and the mixture gently swirled for 5 minutes. Petroleum ether was also added and swirled for 5 minutes. After the separation of two layers, the transparent upper layer was removed and placed in a 150 ml beaker. Ether fumes were evaporated in the fume hood till dryness.

**Gas chromatography analysis**

Derivatization/methylation of the sample was achieved by reconstituting the residue with 3 ml of chloroform and 3 ml diethyl ether. The solution was transferred into 10 ml tube to evaporate under nitrogen streams. Two millilitres of hydrogen sulphate (H$_2$SO$_4$) in methanol (1:2) and 1 ml toluene were added; the tube was closed and placed in the oven at 100°C to prevent dryness. After 45 minutes the tube was removed and cooled to room temperature. Five millilitres of water and 1 ml of hexane was added, capped and shook for 1 minute. After separation of layers, the transparent upper layer was carefully transferred to another tube. Anhydrous sodium sulphate (Na$_2$SO$_4$) was added to the mixture until the solution was clear. The clear sample was transferred into a vial and capped. The vial was placed in the auto sampler and the content analysed by Gas chromatography (GC) analysis. The load method for the gas chromatography (GC) was set using fatty acid methyl esters (FAME); suitable and individual fatty acids were run to confirm retention time (AOAC, 2005).

### 3.3.3 Determination of egusi oil quality

To establish the genetic effect of egusi oil on human health the index of atherogenicity (IA) and index of thrombogenicity (IT) (Siano & Straccia, 2015) was evaluated.

\[
IA = \frac{[4 \times C14:0) + C16:0 + C18:0]}{[\sum MUFA + \sum PUFA- n6 + \sum PUFA - n3]}
\]
where IA = Index of atherogenicity; C14:0 = Myristic, C16:0 = Palmitic, C18:0 Stearic; MUFA = Monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n6 = omega 6 fatty acid; n3 = omega 3 fatty acid.

The index of thrombogenicity (IT) was estimated using equation 3

\[
IT = \frac{\text{C14:0} + \text{C16:0} + \text{C18:0}}{0.5 \times \text{MUFA} + 0.5 \times \text{PUFA} - \text{n3} + \text{PUFA} - \text{n6}}
\]  

(3)

where IT = Index of Thrombogenicity; C14:0 = Myristic; C16:0 = Palmitic; C18:0 = Stearic; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; n6 = Omega 6 fatty acid and n3 = Omega 3 fatty acid

3.4 Physicochemical Properties of Egusi Oil

Standard AOAC methods were used to determine the physicochemical properties of egusi oil, peroxide value (AOAC method 965.33), iodine value (Horowitz 1975), pH and oxidative stability (AOCS) Method Cd 12b – 92 (1996).

3.4.1 The peroxide value

The peroxide value of egusi oil was carried out using auto titrate 848 Titrino plus with 801 magnetic stirrers (AOAC, 2005). The blank value of reaction was obtained by pipetting 10 ml of solvent (glacial acetic acid) containing 1-decanol in ratio 3 to 2. Iodine (10 mg) was dissolved in 200 ml glacial acetic acid (300 ml), and the solution was mixed. The solvent was pipetted into a titration beaker and treated with 200 mL potassium iodide, mixed and placed in a dark cupboard for one minute. The mixture was removed from the dark, and 50 ml water was added. The solution was titrated against sodium thiosulfate Na₂S₂O₃ = 0.001 mol/L, till auto titrate system reached the endpoint.

The peroxide number of egusi oil was determined by weighing 2 g of egusi oil into titration beaker, dissolved in 10 ml solvent (glacial acetic acid) containing 1-decanol in ratio 3:2 and treated with 200 µL potassium iodide. The solution was thoroughly mixed and placed in a dark place for one minute, thereafter 50 ml of distilled water was added to the solution and titrated against 0.01 mol/L sodium thiosulfate (Na₂S₂O₃) till auto titrate endpoint was reached, and a clear solution is observed at the endpoint. The peroxide number (PON) of egusi oil was measured in milli-equivalents of O₂ per kg sample as seen in equation 4.
where EP1 = Thiosulphate for sample (egusi oil); COO = Sample weight in g; CVO1 = 10: = Titer of thiosulphate solution

3.4.2 Iodine value of egusi oil
Egusi oil was weighed in triplicate into a 500 ml conical flask containing 20 ml chloroform. Iodine (25 ml) was pipetted into the flask, swirled and allowed to stand for 30 minutes. Potassium iodide (20 ml) with a concentration of 15% and 100 ml freshly boiled water were added to the sample. The mixture was titrated against sodium thiosulfate (Na$_2$S$_2$O$_3$) of 0.1 N until the yellow colour has almost disappeared using a magnetic stirrer. Few drops of starch indicator were added to the mixture and titrated until blue colour fades. The conical flask was stirred continuously to entirely remove iodine from the chloroform phase till a pale blue colour was observed, and final titer recorded William (Horowitz, 1975). The iodine number calculated using equation (5).

\[
\text{Iodine Number} = \frac{[B-S] \times N \times 12.69}{g \text{ sample}}
\]

where B = Blank titre value; S = Titre value; N = Number of mole of solvent; g = Weight of samples in gram

3.4.3 pH determination of egusi oil
A calibrated pH meter was used to determine the pH of egusi oil. Egusi oil (10 ml) was weighed into a beaker. An electrode was placed into the egusi oil to determine the pH of the oil. pH meter was standardised with buffer 4 and 7.

3.4.4 Oxidative stability index (OSI) of egusi oil
The oxidative stability of egusi oil was carried out using Methrohm 743 Rancimat American Oil Chemists’ Society America 1996 Method Cd 12b – 92. The Rancimat was assembled by placing the air tube below the connection of the reaction vessel. The O-ring was positioned at the end of each air tube. Thread adapter (M8/M6) was gently screwed into the link and the air tube was simultaneously pressed against the thread adapter. Polytetrafluoroethylene (PTFE) tube was inserted into the opening of the measuring vessel cover that has been filled with the double distilled water. The vessel was covered and
placed into the rancimat, and simultaneously the connection plugs were inserted into the electrode (AOCS, 1996).

To prepare the rancimat equipment, the heating block was heated up to the desired temperature; the reaction vessel was also prepared by filling the measuring vessel with 60 ml deionised water. Approximately 4 g of egusi oil was weighed in duplicate directly into the reaction vessel. The filled vessels were placed on the rancimat together, while the lids were placed tightly until the required temperature of 80°C was reached. Thereafter, the start button was initiated and left till the induction time was reached. Once the process stops, the peak curve on the induction graph is recorded as the maximum induction time (h).

3.5 Data Analyses

All data were collected in triplicate and results expressed as the mean ± standard deviation. The data were subjected to multivariate analysis of variance to establish mean differences between treatments. Duncan multiple range tests were used to separate means where differences existed. All data analyses were carried out using IBM SPSS software (2016).

3.6 Results and Discussion

3.6.1 Effect of temperature and pressure on the SC-CO$_2$ extraction of egusi oil

Egusi oil (Figure 3.2) was produced using three different extraction parameter (1) 60°C, 30 g/h, 450 bar (EO1), (2) 55°C, 30 g/h, 600 bar (EO2) and (3) 75°C 30, 600 bar (EO3). Increasing the temperature from 55 to 75°C increased the oil yield from 46 to 50 and 53% w/w, for EO1, EO2, and EO3 respectively. EO1 (low temperature and pressure) has the lowest yield compared to EO2 and EO3 which was extracted at high temperature and pressure. Özkal & Yener (2016) had a similar report for flaxseed particles, which contained 32.74% w/w oil. Also, a similar report was given by Opoku-Boahen et al. (2013) on Castor Ricinus communis L, a very ancient oilseed crop cultivated because of the high oil content of the seeds, which ranges between 42 and 58% extracted by cold pressing. An oilseed gives more than 50% oil yield during any method of extraction. The only disadvantage to cold press method is the discoloured meal obtained after extraction.

In supercritical extraction process, the effect of temperature on the extraction rate, at constant pressure, is due to two mechanisms (1) the increase in process temperature increases the solubility due to solute vapour pressure enhancement and (2) reduction in the solubility due to the decrease in solvent density. The solvent power is described regarding the supercritical carbon dioxide (SC-CO2) density under the operating conditions and is influenced by the temperature (Gouda et al., 2017).
Figure 3.2  Egusi oil from supercritical CO$_2$ extraction

The high pressure and temperature increased the solubility and diffusivity of the egusi oil so that the mass transfer resistance decreased (Martins et al., 2015).

A steady high carbon dioxide flow rate of 30 g/hr in this study increased convection, such that the mass transfer resistance decreased (Eckert et al., 2005; Huang et al., 2011).
This could also be due to the high solubility of the fluid phase which is due to a high driving force exerted on the solid material (Santos et al., 2017). The general rule in the SC-CO$_2$ extraction is that the higher the pressure, the larger the solvent power and the larger the extracted yield. At high temperature and pressure of 75°C and 600 bar (EO3), egusi oil yield increased significantly.

### 3.7 Effect of SC-CO$_2$ Extraction on Proximate Composition of Egusi Oil

Proximate compositions of egusi oil are shown in Table 3.1. The moisture content of the oil was 1.1% w/w for EO1, 2.0% w/w for EO2 and 1.9% w/w for EO3, which significantly (p ≤ 0.05) differed. The low moisture content could be due to the low moisture content (5-8% w/w) of raw egusi seed and also due to the use of high-grade supercritical CO$_2$. High-grade supercritical CO$_2$ is dry with no trace of the water molecule (Perakis et al., 2005).

<table>
<thead>
<tr>
<th>Proximate (%)</th>
<th>Egusi oil$^{1, 2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>EO1: $1.1 \pm 0.0^a$</td>
</tr>
<tr>
<td>Protein</td>
<td>EO1: $0.8 \pm 0.3^a$</td>
</tr>
<tr>
<td>Ash</td>
<td>EO1: $0.7 \pm 0.1^a$</td>
</tr>
<tr>
<td>Fat</td>
<td>EO1: $99.1 \pm 1.4^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation. Means with different superscript in each row differ significantly (p ≤ 0.05).

$^2$EO1 = oil extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EO2 = oil extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EO3 = at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

This finding is not similar to the 4.1% moisture content of egusi oil as reported by Onyeike and Acheru (2002) in melon seeds. Özkal and Yener (2016) also reported the moisture of flaxseed oil after supercritical extraction process to 3.4% w/w In vegetable oil, the high moisture of 10-14% increases the propensity of the hydrolytic breakdown of the oil, leading to a higher free fatty acid content and rancid flavour. Hence low moisture below 2% in egusi oil, will proffer oil that is less prone to rancidity.

Egusi oil protein was 0.8, 1.5 and 1.8% w/w for EO1, EO2, and EO3, respectively. EO1 was significantly (p ≤ 0.05) lower in protein than EO2 and EO3. The low protein was due to the non-leaching of the nutrient composition of the residue (defatted flour) into the
oil. High pressure and temperature led to the separation of the solid matrix, releasing more of the liquid component of the solid during extraction.

The total fat content ranged from 98.3-99.1%, EO1 (99.1% w/w), EO2 (98.3% w/w) and EO3 (98.9% w/w), with no significant difference. The high-fat contents could be attributed to the high pressure, temperature and high CO₂ flow rate used. An increase in the temperature increased the diffusivity of the oils in a solid matrix and hence, the mass transfer resistance decreased (Martins et al., 2015). Also at high pressure, there is a high yield of soluble nutrient in the liquid phase. This could be due to the high solubility of the fluid phase which indicates high driving force exerted of the solid material (Yener & Bayındırlı, 2005). El-Adawy & Taha (2001) reported that using solvent extraction, the fat content of 21.33% was observed in watermelon seeds, while the fat content in the fluted pumpkin was 50%.

The total fat content of 98.3 to 99.1% obtained in this study indicates that egusi oil has significantly high-fat content compared to watermelon and fluted pumpkin seeds. The proximate properties of egusi oil (EO) decreased with a rise in pressure, due to the destructive effect of high pressures on the solid structure of the seed. As pressure increased from 450 bar to 600 bar, a slight increase in the released oil from 46% to 53% w/w was observed. This result is similar to that obtained by Davarnejad et al. (2008) which shows that β-carotene solubility increased with increasing pressure at a constant temperature of 40°C.

The ash content of egusi oil was 0.7% w/w (EO1), 1.4% w/w (EO2) and 0.7% w/w (EO3), with a significant (p ≤ 0.05) difference, having the highest ash content. The ash content of egusi oil was relatively low, which could be due to less reaction time of the solute with the extraction solvent (Turner et al., 2001). Extraction was done at a pulse speed, allowing minimal contact of egusi seed with the supercritical CO₂. The separation only allows the release of the liquid component, leaving the solid matrix and its constituents intact. This is one of the benefits of the supercritical extraction procedure. The residue obtained after a supercritical carbon dioxide extraction procedure retained its nutritional composition and can be made into more functional food. The shortcoming of any other extraction methods, as the residue obtained is discarded as waste due to its off colour, flavour and low nutritional composition.

### 3.8 Fatty Acid Composition of Egusi Oil

The fatty acids compositions of egusi oil are shown in Table 3.2. The saturated fatty acids in egusi oil were undecanoic (C11), myristic (C14), palmitic (C16) and stearic (C18). Palmitic and stearic acid were (12, 13, and 13 mg/100 g), (13, 13, and 15 mg/100 g) for EO1, EO2, and EO3, respectively. Egusi oil is a source of undecanoic fatty acid 7.8, 8.0
and 8.3 mg/100 g for EO1, EO2 and EO3, respectively with no significant difference. Undecanoic fatty acid helps with wound healing and can be used as an antifungal agent in the treatment of ringworm (Özkal & Yener, 2016). The only monounsaturated fatty acid found in egusi oil was oleic acid (C18:1n9t) 17.3, 18.2 and 19.2 mg/100 g respectively, for EO1, EO2 and EO3, which differed significantly (p ≤ 0.05).

Monounsaturated fatty acid (MUFA) averagely contributed to 18.2% w/w of the total fatty acids, while polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) added to 53% w/w and 21.2% w/w, respectively. The primary fatty acid in egusi oil was C18:2n6t (linoleic acid), which was the only PUFA found in egusi seed oil (Table 3.2). Among the three oil samples, EO3 was significantly (p ≤ 0.05) different from EO1 and EO2, having the highest linoleic acid content. This could be due to a different temperature (55, 60 and 75°C) and pressure (450 and 600 bar) used for extraction.

![Chemical structure of conjugated linoleic acid](image)

**Figure 3.3** Chemical structure of conjugated linoleic acid
<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>EO1</th>
<th>EO2</th>
<th>EO3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undecanoic (C11)</td>
<td>7.81 ± 0.76a</td>
<td>8.01 ± 0.70a</td>
<td>8.27 ± 0.20b</td>
</tr>
<tr>
<td>Myristic (C14)</td>
<td>0.06 ± 0.00a</td>
<td>0.06 ± 0.00a</td>
<td>0.06 ± 0.00a</td>
</tr>
<tr>
<td>Palmitic (C16)</td>
<td>12.15 ± 0.75a</td>
<td>13.35 ± 0.18b</td>
<td>13.33 ± 0.42b</td>
</tr>
<tr>
<td>Stearic (C18)</td>
<td>12.62 ± 1.09a</td>
<td>14.12 ± 0.51b</td>
<td>14.99 ± 0.03b</td>
</tr>
<tr>
<td><strong>Mono</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic (C18:1n9c)</td>
<td>17.31 ± 0.54a</td>
<td>18.18 ± 0.21b</td>
<td>19.16 ± 0.16c</td>
</tr>
<tr>
<td><strong>Poly-n-3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-linolenic (C18:3n3)</td>
<td>0.32 ± 0.02a</td>
<td>0.32 ± 0.02a</td>
<td>0.38 ± 0.01b</td>
</tr>
<tr>
<td><strong>Poly-n-6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic (C18:2n6t)</td>
<td>53.30 ± 0.32a</td>
<td>54.13 ± 0.16b</td>
<td>56.21 ± 0.21c</td>
</tr>
<tr>
<td>ΣSFA (Saturated fatty acid)</td>
<td>20.02 ± 1.51a</td>
<td>21.41 ± 0.25</td>
<td>21.66 ± 0.61a</td>
</tr>
<tr>
<td>ΣPoly n-3 (omega 3)</td>
<td>0.32 ± 0.02a</td>
<td>0.32 ± 0.02a</td>
<td>0.38 ± 0.01b</td>
</tr>
<tr>
<td>ΣPoly n-6 (omega 6)</td>
<td>53.62 ± 0.34a</td>
<td>54.14 0.16b</td>
<td>56.22 ± 0.21c</td>
</tr>
<tr>
<td>IA (index atherogenicity)</td>
<td>0.35 ± 0.02a</td>
<td>0.38 ± 0.01b</td>
<td>0.38 ± 0.00b</td>
</tr>
<tr>
<td>IT (index thrombogenicity)</td>
<td>0.08 ± 0.01a</td>
<td>0.09 ± 0.01a</td>
<td>0.09 ± 0.05a</td>
</tr>
</tbody>
</table>

1Values are mean ± standard deviation. Means with different superscript in each row differ significantly (p ≤ 0.05).
2EO1 = oil extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EO2 = oil extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EO3 = at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar
The concentrations of linoleic, oleic, palmitic acid and stearic acids increased with increase in temperature and pressure. Giwa et al (2010) reported similar result for egusi oil, high in: C18:1 (15.8 mg/100 g), C18:2 (64.0 mg/100 g) and C16:0 (10.3 mg/100 g). Linoleic acid plays a significant role in nervous cell construction.

It is also fundamental to the prevention of cardiovascular diseases (Santos et al., 2017). Omega-6 fatty acid [conjugated linoleic acid] (Figure 3.3) was identified using gas chromatography-mass spectrophotometer (GCMS) and was high in the three egusi oil samples, making egusi oil a nutraceutical oil to be explored in the food industries.

The index of atherogenicity (IA) (Table 3.2) was low 0.35, 0.38 and 0.38 mg/100 g for EO1, EO2, and EO3, respectively, with a significant (p ≤ 0.05) difference, EO1, having the lowest IA value. While the thrombogenicity index (IT) was 0.08, 0.09 and 0.09 mg/100 g for EO1, EO2 and EO3, respectively with no significant differences. These lipid indices were reported and calculated according to Ghaeni et al. (2013).

All IT and IA values were lower than those reported for pomegranate and cherry, where IT of 0.30 and 0.75 mg/100 g, respectively and IA of 0.15 and 0.42 mg/100 g, respectively (Siano and Straccia, 2015) were reported. However, the values obtained were similar to that of pumpkin seed oil with IA (0.34 mg/100 g) and IT (0.65 mg/100 g) (Siano & Straccia, 2015). Omega-3 fatty acid present in egusi seed was 0.32, 0.32 and 0.38 mg/100 g for EO1, EO2, and EO3. EO3 was low compared to the samples.

Index of atherogenicity (IA) indicates the relationship between the sum of the principal saturated and unsaturated fatty acids, the primary saturated being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory system), and the anti-atherogenic inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro and macro coronary diseases (Ruiz-cara & Garcia, 2007; Akanda et al., 2012).

3.9 Physicochemical Characteristics of Egusi Seed Oil

The physicochemical properties of egusi oil are shown in Table 3.3. The pH of egusi oil for EO1, EO2, and EO3 was 4.20, 5.40 and 4.91, respectively, indicating slightly acidic oil. It is a desirable property for oils to be slightly acidic because acidity discourages early microbial spoilage (Raes et al., 2004; Jarret & Levy, 2012). Hence, egusi oil will be less susceptible to microbial spoilage.

Peroxide Value (PV) measures the primary oxidation products in an oil sample. The PV of egusi oil EO1, EO2, and EO3 was 11.6, 12.6 and 11.8 mequiv peroxide/sample, respectively, with EO3 significantly (p ≤ 0.05) higher compared to EO1 and EO2 (Table 3.3).
Nkafamiya *et al.* (2010) reported that the PV of oil varied depending on fatty acid composition and oxidation conditions of the sample.

The oxidation level of egusi oil was low as the PV was lower than the maximum limit (10-15 mequiv peroxide/sample) of peroxide value for edible oils, indicating a less oxidised oil (Adegoke & Ndife, 1993; Smith *et al.* 2007). The reason for low PV could be attributed to the presence of a large number of natural antioxidants (oleic acid) in the egusi oil. Egusi oil could be preserved for a long period without deterioration because of their low PV values as oils become rancid when their peroxide value reaches 20-30 mequiv peroxide/sample. The peroxide number of fresh oil is reported in the literature to be 10 mequiv peroxide/sample; this confirms the low level of rancidity of egusi oil after few weeks of extraction.

Oxidative stability index (OSI) is a measure of overall oxidation level of oil samples, which can give a better estimation of the progressive oxidative deterioration of oil (Adegoke & Ndife, 1993; Smith *et al.*, 2007). The oxidative stability of oil will be high when polyunsaturated fatty acid is low due to the presence of high degree of saturation in the bond of the oil. The stability of EO1, EO2, and EO3 at 120°C was expressed as induction time of oxidation, which ranged from 5.3 to 10.2 h. These values were higher than those reported for linseed oil (1.1 h) and olive oil (6.1 h) (Nwu, 2005). The high induction time of egusi oil (EO) could be attributed to the presence of a significant amount of natural antioxidants such as oleic acid.

### Table 3.3 Physicochemical properties of egusi oil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Egusi oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EO1</td>
</tr>
<tr>
<td>pH</td>
<td>4.20 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Peroxide number (meqO&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>11.60 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iodide value (g/l&lt;sub&gt;2&lt;/sub&gt;/100 g)</td>
<td>98.66 ± 5.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxidation stability (h)</td>
<td>10.17 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).

<sup>2</sup>EO1 = oil extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EO2 = oil extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EO3 = at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.
Oxidation stability of EO1, EO2, and EO3 were 10.2, 11.5 and 5.3 h, respectively (Table 3). EO1 and EO2 (10.2 and 11.5 h, respectively) had the highest oxidation induction time, which was significantly \((p \leq 0.05)\) higher from EO3 (5.26 h). The differences in the oil sample can be attributed to the different temperature and pressure used during extraction. This could also be due to experimental error of equipment failure.

The iodine number of egusi oil was relatively high 98.7, 95.3 and 129.3, respectively for EO1, EO2, and EO3. EO3 has a significantly \((p \leq 0.05)\) higher iodine value when compared to EO1 and EO2. This could be due to the presence of polyunsaturated fatty acids which was highest in sample EO3 in egusi oil. The iodine number equals the number of mg of iodine required to saturate the fatty acids present in 100 mg of the oil or fat.

Oil-rich in saturated fatty acids has low iodine numbers, while oils rich in unsaturated fatty acids have high iodine numbers (Abelu et al., 1990; Igwenyi & Akubugwo, 2010). Egusi oil extracted using high pressure (600 bar) and temperature (75°C), had the highest iodine number. Iodine numbers are often used to determine the amount of unsaturation in fatty acids (Igwenyi & Akubugwo, 2010). High temperature and pressure denature the nutrient density of the oil, especially its protein and mineral content, leading to less stable oil (Abelu et al., 1990; Igwenyi & Akubugwo, 2010). EO1 (60°C and 450 bar) shows a low peroxide number, high iodine number and high stability at high temperature.

### 3.10 Conclusion
Supercritical \(\text{CO}_2\) extraction of egusi seed oil was achieved by three experimental runs, which was divided into three. The amount of the oil recovered in the process was high due to the high pressure and temperature used. The oil obtained from the high pressure, the high-temperature process has the lowest nutritional composition. The oil with the best yield in term of nutritional composition was obtained from the low-temperature 60°C, low pressure 450 bar process, which was conclusively the best process parameter to be explored for future processing of egusi seed using supercritical fluid extraction. Nutritious and functional oil was extracted from *Citrullus lanatus* subsp *Mucospermus*. Egusi oil should be introduced into the food industry, due to its high omega-6 fatty acid content (conjugated linoleic acid) which will significantly reduce the risk of coronary heart disease and obesity, and serve as new functional oil for the food industry.

### Reference


AOAC 996.06 (2005) 18th edition, Chapter 41, page 20-24 (Oils and Fat)


Fiori, L. (2009). Supercritical extraction of sunflower seed oil: Experimental data and model validation. The Journal of Supercritical Fluids.


of temperature, pressure and flow rate, 77, 112–119.


of long-chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: A review. *Animal Feed Science and Technology*.


**CHAPTER FOUR**
Defatted egusi flour (DEF) was extracted using three runs. Firstly as 60°C, 30 g/h and 450 bar (DEF1), secondly at 55°C, 30 g/h and 600 bar (DEF2) and thirdly, extraction was done at 75°C, 30 g/h and 600 bar (DEF3). It was analysed for its nutritional properties as it relates to proximate, minerals, amino acid, sugars and physicochemical composition. The moisture content of DEF ranged from 5.3 to 10.1% w/w, crude protein 48.3 to 60.4% w/w, crude fibre 3.4 to 4.5% w/w) and ash 5.3 to 6.8 % w/w). The amino acid compositions of DEF were determined using the Technicon Sequential Multisampling Amino acid Analyser (TSM). Glutamic acid had the highest concentration of 12.9, 11.8 and 9.8 mg/100 g for DEF1, DEF2, and DEF3, respectively with a significant difference (p ≤ 0.05) across the samples. The essential amino acids were leucine, lysine, threonine, valine, and phenylalanine. The only sulphur containing amino acid in egusi flour methionine with values of 1.9, 1.7 and 1.5 mg/100 g for DEF1, DEF2, and DEF3, respectively differed significantly (p ≤ 0.05) between the samples. Arginine had the second highest concentration among the indispensable amino acids with the value of 9.8, 9.0 and 7.6, respectively for DEF1, DEF2, and DEF3. The concentrations of aspartic acid in defatted egusi flour were high at 5.7, 5.3 and 4.4 mg/100 g for DEF1, DEF2, and DEF3, respectively, which differed significantly (p ≤ 0.05). In functionality, defatted egusi flour water absorption and solubility index at a low temperature of 50°C ranged between 52.5 to 57.6% w/w and 68.0 to 73.3% w/w respectively for DEF1 to DEF3, which significantly differed between samples. The final viscosity of defatted egusi flour ranged from 126.7 to 126.3 cP, which differed significantly (p ≤ 0.05) between samples. Egusi flour is high in protein 60% w/w and carbohydrate 25% w/w was treated with hot water to extract its hydrocolloid. Functional properties of egusi hydrocolloid for the three defatted flour shows a stable emulsifier as the breakdown viscosity remained constant at (8.00 cP) confirming its stability as an emulsifier. Final viscosity also remained low across the samples, indicating a stable emulsion that will not be affected by an increase in temperature.
4.1 Introduction

Egusi melon (C. lanatus subsp muscospermus) prominent in Western Africa, has bitter pulp and is often cultivated on a large scale mainly for its seed. Egusi pulp can be eaten either raw or in soup, depending on the degree of sweetness or bitterness (King & Onuora, 1984; Onyeike & Acheru, 2002). Egusi seed is common across Africa and Asian countries; it is being sold in the markets as a dehulled seed, for food and as a soup thickener. The raw meal of egusi is dense in oil and often regarded as too oily by the consumers. The oil is usually obtained by cold pressing of egusi meal (Uruakpa, 2004). This process is time wasting and discourages the use of egusi melon.

Several methods of defatting egusi have been adopted by past findings, but these methods are often faced with the shortcomings of discoloured, off-flavoured and non-nutritious flour. Also was the use of toxic solvents, which are often detrimental to the health of consumers. This has led to the use of supercritical fluid extraction (SFE) method of defatting in this study. SFE proffers non-toxic flour that retains its nutritional composition (Özkal and Yener, 2016). Supercritical extraction is a process of separating the extractant from a solid medium with a supercritical carbon dioxide (SC-CO$_2$) operating in a fluid state of where it is held at or above its critical temperature and pressure (Goto, 2009).

Supercritical CO$_2$ extraction has been a success since the 1980’s in the defatting of grape seed, rapeseed, coriander and many more process such as decaffeination of green coffee beans and black tea leaves; production of hop extracts; extraction of essential oils (Turner et al., 2001). The defatted meal obtained after SFE extraction could be used as a functional ingredient. For years, egusi meal has been used as a thickener, and this suggests its possible hydrocolloid properties.

Hydrocolloids are a range of polysaccharides and proteins that are widely used in the food, cosmetic and pharmaceutical industries (Armstrong & Barringer, 2013). The food industry, in particular, has seen a significant increase in the use of hydrocolloids to perform some functions, such as thickening and gelling aqueous solutions, stabilising foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavours (Bemiller, 2011). The use of hydrocolloid in food as a functional ingredient is to improve the stability and texture as well as the nutritional quality of the product or for economic reasons (Hansen, 1993). Nevertheless, this application in food trade is often limited to some selected seed meal, whereas other vegetable proteins like egusi seed are underutilised (Berghout et al., 2014). Nothing is known of defatted egusi flour obtained using supercritical extraction method. This study aimed to (1) evaluate the nutritional and functional properties of the defatted egusi flour obtained by supercritical fluid extraction in Chapter 3 (2) extract hydrocolloid from the defatted flour and examine its functional properties.
4.2 Materials and Methods

4.2.1 Source of egusi seed, chemicals, and reagents
Defatted egusi flour was obtained as a residue after supercritical fluid extraction (Chapter 3). The overview of the methodology used in this chapter as shown in Figure 4.1. South Africa. All chemical reagents were obtained from Merck Pty. Deionized water was used all through the experiment. The supercritical fluid extraction equipment (Swiss Nova), a plant extractor, was operated manually at the Process Engineering Department of Northwest University, Potchefstroom Campus, South Africa. Defatted egusi flour was tested for proximate, physicochemical and functional properties. Egusi hydrocolloid was extracted at Research and Development (R&D) laboratory of CPUT and freeze-dried using pilot freeze drier (Virtin™ Sp Scientific).

4.2.2 Production of Defatted Egusi Flour Using Supercritical CO₂ extraction
Defatted egusi flour was obtained after supercritical fluid extraction in Chapter 3. Three experimental runs were conducted using low temperature, low pressure (60°C, 450 bar), Low temperature, high pressure (55°C, 600 bar) and high temperature, high pressure (75°C, 600 bar). In each experiment, the extractor was loaded with approximately 2000 g of raw dehulled egusi seed. Firstly, the system was pressurised (at intervals of approximately 400-600 bars) until the desired pressure of 450 bar and 600 bar. After column temperature and the pressure were stabilised, the system was kept in contact with the egusi seed for at least 15 minutes to allow system stabilisation. Then, the supercritical CO₂ was pumped into the bed of egusi seed at constant 30 g/hr. The volumetric flow rate of the solvent was regulated, using the expansion valve at the outlet of the extractor.

The temperature at the extractor outlet was varied between 55, 60 and 75°C by a thermoregulatory device to allow the separation between the extract and the solvent during depressurisation. The defatted egusi flour (DEF) was collected via a glass container and weighed every 10-20 minutes until constant weight. Defatted egusi flour was tested for its nutritional and functional properties. The flour with the best nutritional and functional composition was used in the production of precooked egusi grits.
4.3 Production of Egusi Hydrocolloid

Defatted egusi flour was suspended in distilled water (1:25 w/v flour: water ratio), and the suspension was heated on the electric stove to 100°C for one hour (PS & V, 2016). After
filtering through several layers of cheesecloth, the residue was suspended a second time in boiling water, and the process was repeated twice. The filtrate was combined and placed in a freezer (-70°C) overnight, to keep the sample frozen before freeze drying. The hydrocolloids were recovered after freeze-drying at a temperature of -53 to +55°C and pressure of ±150 Torr.

4.4 Macro and Micro Nutrients Composition of Defatted Egusi Flour

4.4.1 Proximate analysis of defatted egusi flour

The recommended methods of the Association of Official Analytical Chemists (AOAC, 2000) were adopted for the determination of moisture, crude protein, fibre, and ash. Crude protein \((N \times 6.25)\) was determined by the Nitrogen analyser. Ash was determined by the incineration of 1 g of the sample and placed in a muffle furnace maintained at 550°C for 6 h until ash is obtained. Carbohydrate content was obtained by the difference method.

4.4.2 Amino acids profiling of defatted egusi flour

Reagent preparation

Eluent A and B were used as received in the AccQ Tag Ultra Derivatization Kit – Water kit. Eluent A (50 ml) was prepared by mixing with 950 ml deionised water, while Eluent B was used as supplied. Weak wash solvent was made by adding 5% acetonitrile in water, while strong wash solvent was at the concentration of 95% acetonitrile in water. Preparation of derivatising agent (6-Aminoq., quinoline N-succinimidyl ester) (AQC) was done by drying acetonitrile of 1 ml, which was added to the reagent, contained in a vial containing 3 mg of AQC (Jama, 2013). This vial was then heated, vortexed and sonicated to ensure the reagent dissolves completely. The derivatising agent of 20 µl was used for each sample, allowing 1 ml sample for each reaction. The derivatising agent was prone to hydrolysis; and was stored in a desiccator to maintain its stability for approximately one week.

Preparation of Internal Standard (L-Norvaline) was done by weighing 10 mg of L-Norvaline into a 15 ml centrifuge tube and making up to 10 ml with MilliQ water. This gives a 1000 ppm solution which was diluted 5 times to produce a 200 ppm solution used during sample preparation. The derivatization reaction was also speeded up by heating the vials at 55°C for 10 minutes before analysis. The amino acids were measured in solution either as free amino acids or after hydrolysis of proteins using standard 6 M hydrochloric acid (HCl) acid digestion as 800 µl sample + 200 µl. Dilution of 10 µl was used during derivatization. The standards were prepared, using 80 µl std solution + 20 µl, with no dilution factor was involved.
**Derivatization procedure of amino acids**

Borate buffer of 70 µl was transferred into a 200 µl glass inserted in a 2 ml glass vial. Diluted egusi flour/standard solution of 10 µl was added. AQC reagent of 20 µl was also added, and the sample was capped and vortexed well to mix. The mixture was transferred into preheated vials previously heated in an oven/heating mantle at 55°C for 10 minutes. After 10 minutes, the vials were ready for analysis and can be loaded into the auto sampler tray (Jama, 2013).

**Chromatographic analysis of amino acids**

Amino acid separation and detection were performed using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector. Sample or standard solution of 1 µl was injected into the mobile phase which conveys the derivatized amino acids onto a Waters UltraTag C18 column (2.1 x 50 mm x 1.7 µm) held at 60°C. Elution of analytes of the column was performed by running a gradient (Jama, 2013).

Analytes eluting off the column were detected by the PDA detector, with each amino acid coming off the column at a unique retention time. Instrument control and data acquisition were performed by Mass Lynx software which integrates the peaks at the defined retention times and plots calibration curves for each amino acid based on the peak response (peak area/internal standard peak area) against concentration.

**4.4.3 Mineral content of defatted egusi flour**

**Sample digestion**

To solubilise the acid-extractable elemental content of the defatted egusi flour, digestion was performed on a MARS microwave digester, using ultra-pure HNO₃, or HNO₃ + HCl, at elevated temperature and pressure. After a cooling period, the extractant was made up to 50 ml volume with deionised water, then analysed by ICP-AES and ICP-MS for the selected analytes (Kan, 2015).

**Trace element analysis**

Trace elements were analysed on an Agilent 7700 quadruple ICP-MS. Samples were introduced via a 0.4 ml/min micro mist nebuliser into a Peltier-cooled spray chamber at a temperature of 2°C, with a carrier gas flow of 1.05 L/min. The elements V, Cr, Mn, Fe, Co, Ni, Cu, and Zn, were analysed under He-collision mode to remove polyatomic interferences. The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. A NIST-traceable quality control standard of a separate supplier than the main calibration standards was analysed to verify the accuracy of the calibration before sample analysis. Where
samples have undergone a digestion step, the results were corrected for the dilution factor resulting from the digestion procedure.

**Major element analysis**

Major elements (Na, K, Ca, Mg, P and Si) were analysed on a Thermo Cap 6200 ICP-AES. The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards purchased from Inorganic Ventures (Inorganic Venture 300 Technology Drive Christiansburg, VA 24073) to quantify selected elements. NIST-traceable quality control standards from De Bruyn Spectroscopic Solutions, Bryanston, South Africa, were analysed to verify the accuracy of the calibration before sample analysis, as well as throughout the analysis to monitor drift.

**4.4.4 Sugars in defatted egusi flour**

Approximately 10 mg of the egusi flour was extracted with 1000 μl of 70% methanol/water (v/v). The mixture was briefly vortexed and subsequently incubated for 180 min at 60°C. The sample was centrifuged, and the resultant supernatant was transferred into clean 100 μl centrifuge tubes. Ribitol was added as internal standard. The supernatant of 500 μl then dried in a speed vac. The dry samples were reconstituted in 100 μl methoxyamine hydrochloride (2.5%) in pyridine and incubated for 120 minutes in an oven maintained at a temperature of 40°C. Subsequently, samples were trimethylsilylated with 50 μl N-Bis (trimethylsilyl) trifluoroacetamide (BSTFA + TMCS, 99:1) and further incubated for 30 min at 60°C (Medeiros and Simoneit, 2007).

**Chromatographic separation of egusi sugars**

Separation was performed on a gas chromatograph (6890N, Agilent technologies network) coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent Technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL auto sampler. Separation of sugars was performed on a non-polar ZB-5MS Guardian (30 m, 0.25 mm ID, 0.25 μm film thickness) ZB 7HG-G010-11 capillary column.

Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector temperature was maintained at 250°C. Sample (1 μl) was injected in a split ratio and set at 5:1 split ratio. The oven temperature was programmed as follows: 80°C for 1 min; and finally ramped up to 300°C at a rate of 7°C/min and held for 2 min. The MSD was operated in a full scan mode, and the source and quad temperatures were maintained at 230°C and 150°C, respectively. The transfer line temperature was maintained at 280°C. The mass spectrometer was operated under electron impact mode at ionisation energy of 70 eV, scanning from 35 to 500 m/z.
4.5 Functional Properties of Defatted Egusi Flour

4.5.1 Water absorption and solubility index of defatted egusi flour

The water absorbance index (WAI) of defatted egusi flour was determined by the method of (Eltayeb et al., 2011) with slight modification. The WAI was determined using 2.5 g of extracted defatted egusi flour. The sample was suspended in 30 ml distilled water at 30°C in a previously weighed 50 ml centrifuge tube, stirred intermittently over a 30 min period and then centrifuged at 3500 rpm/10 min. After pouring the supernatant into a tarred evaporating dish, the gel was weighed, and WAI was defined as the gram of gel per gram solids using equation 1.

\[
\text{WAI (\% w/w)} = \frac{\text{Weight of sediment}}{\text{Weight of dry solid}} \times 100
\]  

(1)

Water solubility index (WSI) is defined as the water-soluble fraction in the sample extract (El-Adawy & Taha, 2001). This was determined from the number of dried solids recovered by evaporating the supernatant from the flour water absorption test. The water solubility index was measured using equation 2.

\[
\text{WSI (\% w/w)} = \frac{\text{Weight of dissolved supernatant}}{\text{Weight of dry sample}} \times 100
\]  

(2)

4.5.2 Pasting properties of defatted egusi flour

Fundamental rheological measurements were performed by using a controlled stress-strain rheometer (MCR 300, manufactured by Physical /Anton Paar Ostfildern, Germany) in a system of coaxial cylinders (CC27). The instrument measured the shear stress (Pa) and the apparent viscosity (Pas) and the shear rate (s1). Defatted egusi flour (3.5 g) was loaded into the sample cup with 25 g of water and allowed to equilibrate for 3 min to achieve temperature equilibrium (25°C) and stress relaxation. The samples were subjected to a programmed shear rate logarithmically increasing from 0 to 300 s in 5 min (Bolade, 2017).

4.6 Statistical Data Analysis

All data were collected in triplicate. The data were subjected to multivariate analysis of variance to establish mean differences between treatments. Duncan multiple range tests were used to separate means where gaps existed. All data analyses were carried out, using IBM SPSS, 2016.
4.7 Results and Discussion

4.7.1 Effect of processing on proximate composition of defatted Egusi flour

The proximate composition of defatted egusi flour in Table 4.1 showed moisture (10.1, 6.6 and 5.3% w/w), protein (60.4, 48.4 and 60.1% w/w), carbohydrate (19.5, 34.9 and 23.4% w/w), crude fibre (4.53, 3.4 and 4.6% w/w), and ash (5.3, 6.8 and 6.1% w/w). The moisture content of defatted egusi flour samples was low and differed significantly (p ≤ 0.05), with DEF3 (60°C and 600 bar) having the lowest moisture content. The protein content which ranges between 48.4 for DEF2 to 60.4% w/w for DEF1, differed significantly. This could be due to the effect of high pressure (600 bar) and low temperature used during extraction of DEF2.

At high pressure, protein is denatured, while low temperature reduces the extractible tendencies of a component in a defatted flour (Veli, 2012). The protein content of defatted egusi flour was higher than that of cereals (7.5–12 g/100 g) (Vignola et al., 2016) and eggs (12.8 g/100 g) (Lin et al., 2017). This result is similar to the result obtained by Lagoke et al. (1983), indicating a 60% w/w of protein in defatted egusi flour. Protein is an essential component of every cell in the body. Low protein in the body affects nails and hair; this is because protein makes up the significant part of our body (Lin et al., 2017).

<table>
<thead>
<tr>
<th>Table 4.1 Proximate composition of defatted egusi flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defatted egusi flour¹²</td>
</tr>
<tr>
<td>Proximate (%)</td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Crude protein</td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Fibre</td>
</tr>
<tr>
<td>Ash</td>
</tr>
</tbody>
</table>

¹Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).
²DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.
Figure 4.2 Defatted egusi flour
DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar).

Repair and building of muscle require protein in a significant amount. Egusi flour is high in protein (60% w/w) offers high protein flour to vegetarians and those that cannot afford the high protein meat and meat products. Egusi flour could be used as a major raw material for the ready-to-use-therapeutic food (RUTF).

The ash content was 5.3, 6.8 and 6.1% w/w for DEF1, DEF2, and DEF3, respectively with no significant difference observed. The defatted egusi flour was also a good source of carbohydrates 19.5, 34.9 and 23.4, respectively for DEF1, DEF2, and DEF3, with a significant (p ≤ 0.05) difference between the samples. DEF2 was significantly high in carbohydrate at low temperature (55°C), high pressure (600 bar). Starch granules are not
destroyed at low temperature during a supercritical extraction procedure (Gouda et al., 2017).

The dietary fibre was 4.5, 3.4 and 4.6% w/w for DEF1, DEF2, and DEF3, respectively with DEF2 significantly lower compared to DEF1 and DEF2. Fibre is crucial for digestibility enhancement, blood cholesterol reduction and reduces the risk of large bowel cancers (Al-Farga et al., 2016). The high dietary fibre content of egusi flour makes it a potential ingredient for functional food formulation. It is therefore suggested that the consumption of defatted egusi flour which could be made into precooked, processed food, may result in many positive health benefits such as protection against acute malnutrition and obesity.

4.7.2 Effect of process on the amino acid composition of defatted egusi flour
The amino acid composition indicated in Table 4.2 shows the presence of 14 amino acids among the 22 amino acids found in nature (Jama, 2013). Glutamine is the most common amino acid found in muscles, over 61% of skeletal muscle is glutamine. Glutamine consists of 19% nitrogen, making it the primary transporter of nitrogen into muscle cells; it has a flavour enhancing role in food. In defatted egusi flour, glutamine was (12.9, 11.8, 1.03 and 9.8 g/100 g), arginine (9.8, 9.0 and 7.64 g/100 g), aspartic (5.7, 5.3 and 4.4 g/100 g) and leucine (4.3, 4.2 and 4.2 g/100 g) and histamine (1.5, 1.4 and 1.10 g/100 g) for DEF1, DEF2 and DEF3, respectively.

Methionine, being the only sulphur-containing amino acid identified in the flour were 1.9, 1.8 and 1.5 g/100g for DEF1, DEF2 and DEF3, respectively and differed significantly (p ≤ 0.05), DEF3, having the lowest value. The concentration of the sulphur-containing amino acids (methionine) decreased with increase in pressure and temperature to 75°C and 600 bar. This is a reflection of the instability of amino acids when subjected to heat treatment and high pressure. Methionine contributes substantially to the maintenance and integrity of cellular systems by influencing cellular redox state and helps cell detoxification, free radicals and reactive oxygen species (Heimi et al., 2016).

According to Akande (2011), the amino acid composition of sunflower seeds is variable, with levels of lysine and methionine ranging from 0.6-0.7 g/100 mg and 0.3-0.5 g/100 mg, respectively. Lysine is an essential amino acid, It is not synthesised in animals or humans, and hence it must be ingested as lysine or lysine-containing proteins in food (King & Onuora, 1984). Defatted egusi flour was a source of lysine (2.6, 2.4 and 2.1 g/100 g) for DEF1, DEF2, and DEF3, respectively with no significant difference.

The high glutamic and aspartic acids contents in defatted egusi flour were similar to those found in seaweeds (Astorga-españa et al., 2016). Isoleucine, threonine, lysine, histidine, and methionine were found to be at a higher concentration, while the remaining
acids were at a lower level compared to that described in the FAO pattern. The recent FAO pattern for the amino acid, advised the consumption of 15-22 mg/kg/day of methionine and 30 mg/kg/day of lysine (John, 2008). These compositions are high in egusi flour as the essential amino acid present is similar to the recommended value. Arginine in egusi flour was 9.8, 9.0 and 7.6 g/100g for DEF1, DEF2 and DEF3, respectively with no significant difference between the three flour samples.

Arginine is classified as a conditionally essential amino acid, depending on the developmental stage and health status of the individual in need of this supplement. Infants are unable to synthesise or create arginine internally, making the amino acid nutritionally

Table 4.2  Amino acid content of defatted egusi flour

<table>
<thead>
<tr>
<th>Mineral (g/100g)</th>
<th>DEF1</th>
<th>DEF2</th>
<th>DEF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>1.5 ± 0.0a</td>
<td>1.4 ± 0.1b</td>
<td>1.1 ± 0.0c</td>
</tr>
<tr>
<td>Serine</td>
<td>3.0 ± 0.2a</td>
<td>2.8 ± 0.1b</td>
<td>2.3 ± 0.1c</td>
</tr>
<tr>
<td>Arginine</td>
<td>9.8 ± 1.1a</td>
<td>9.04 ± 0.5ab</td>
<td>7.64 ± 0.3b</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.8 ± 0.2a</td>
<td>3.8 ± 0.1b</td>
<td>2.8 ± 0.2c</td>
</tr>
<tr>
<td>Aspartic</td>
<td>5.7 ± 0.4a</td>
<td>5.3 ± 0.5b</td>
<td>4.4 ± 0.2c</td>
</tr>
<tr>
<td>Glutamic</td>
<td>12.9 ± 1.0a</td>
<td>11.8 ± 1.0c</td>
<td>9.8 ± 0.4b</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.5 ± 1.4a</td>
<td>2.0 ± 0.2a</td>
<td>1.1 ± 1.0a</td>
</tr>
<tr>
<td>Alanine</td>
<td>3.1 ± 0.1a</td>
<td>2.9 ± 0.2b</td>
<td>2.4 ± 0.1a</td>
</tr>
<tr>
<td>Proline</td>
<td>2.4 ± 0.2a</td>
<td>2.2 ± 0.1b</td>
<td>1.7 ± 0.1a</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.6 ± 0.4a</td>
<td>2.4 ± 0.4a</td>
<td>2.1 ± 0.2a</td>
</tr>
<tr>
<td>Thyrosine</td>
<td>2.0 ± 0.2a</td>
<td>1.9 ± 0.1b</td>
<td>1.4 ± 0.1a</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.9 ± 0.3a</td>
<td>1.8 ± 0.1ab</td>
<td>1.5 ± 0.2a</td>
</tr>
<tr>
<td>Valine</td>
<td>2.9 ± 0.1a</td>
<td>2.7 ± 0.1b</td>
<td>2.1 ± 0.1a</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.2 ± 0.2a</td>
<td>2.1 ± 0.1b</td>
<td>2.2 ± 0.3a</td>
</tr>
<tr>
<td>Leucine</td>
<td>4.31 ± 0.1a</td>
<td>4.21 ± 0.0b</td>
<td>4.21 ± 0.0a</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>3.09 ± 0.3a</td>
<td>2.95 ± 0.1b</td>
<td>2.24 ± 0.1c</td>
</tr>
</tbody>
</table>

1Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).
2DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.
essential for such class of individuals. More so, a healthy body synthesises arginine on its own, making it conditionally necessary for those with health-related issues. Egusi flour showed a significant difference in their amino acid content for the pressure and temperature of extraction. At low temperature and pressure, there was an increase in the nutritional properties of defatted egusi flour because there was minimal impact of pressure and temperature on the solid matrix.

4.7.3 Effect of process on the mineral composition of defatted egusi flour
Sixteen trace mineral element and five major elements were detected in defatted egusi flour obtained after supercritical extraction (Tables 4.3 and 4.4). The principal element, phosphorus 1698.9 1877.8 and 2046.4 mg/100 g for DEF1, DEF2, and DEF3, respectively was the most abundant element in the defatted egusi flour with a significant (p ≤ 0.05) difference in the samples followed by K, Mg, Ca, and Fe.

The concentrations of elements in the different flour were significantly influenced by temperature and pressure used during extraction. The mineral content was significantly increased with increase in temperature, and significantly (p ≤ 0.05) high in defatted flour DEF3 (75°C 600 bar) except for trace elements (Fe, Al, Ba, Hg, Pb and Si), where the highest concentration was found in DEF1 (60°C and 450 bar). This could be due to the affiliation of elements to low-temperature processing, causing minimal disruption in its constituents (Gouda et al., 2017). The effect of high temperature and pressure on the extracted flour during a supercritical extraction ensures minimum contact between solvent and solute, as solvent extraction CO2 was released at pulse speed. This led to complete separation of the liquid from its solid base, and thus more retention of nutrient was observed in the residue (Perakis et al., 2005).

Phosphorus, being the highest in egusi flour is an element considered necessary for human life. In medicine, phosphate deficiency syndrome may be caused by malnutrition, non-absorption of phosphate, metabolic problems which occur with reduced phosphate in the blood and recovery from illness. All causes of deficiency can be considered hypophosphatemia, which is a condition associated with low level of phosphate in the blood serum and its cells. Symptoms of hypophosphatemia include neurological dysfunction and disruption of muscle and blood cells due to lack of ATP. High phosphate intake can lead to diarrhoea and hardening of the organs and softening tissue, a condition known as calcification and can interfere with the body's ability to digest iron, calcium, magnesium, and zinc (Ford & Mokdad, 2003). In this view, egusi flour, being high in phosphorus proffers an additional functional food to be incorporated into the daily diet. Potassium plays a crucial
role in maintaining an electrical connection across cell membranes and in the conduction of nerve impulses alongside sodium (Enujiugha & Ayodele-Oni, 2003).

Calcium is an essential structural component of bone; it is vital in the formation and maintenance of a healthy body frame. Egusi contains a high concentration of calcium, making it an alternative calcium food source (Table 4.4).

Egusi is also high in magnesium 807.5, 880.1 and 957.8 mg/100g for DEF1, DEF2 and DEF3, respectively with a significant (p ≤ 0.05) difference between the three samples. Magnesium helps in maintaining healthy muscle and nerve functions that keeps the heart in a steady rhythm. More so, calcium, potassium, and magnesium are all needed in

<table>
<thead>
<tr>
<th>Mineral (mg/100 g)</th>
<th>DEF1</th>
<th>DEF2</th>
<th>DEF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2.7 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3 ± 0.045&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Al</td>
<td>17.1 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.6 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.0 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ti</td>
<td>0.7 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.6 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cr</td>
<td>0.3 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mn</td>
<td>7.7 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.5 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fe</td>
<td>44.4 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.7 ± 0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.0 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ni</td>
<td>0.5 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cu</td>
<td>3.7 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.1 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zn</td>
<td>9.6 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.0 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.8 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sr</td>
<td>0.9 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mo</td>
<td>0.2 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.4 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ba</td>
<td>0.8 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Si</td>
<td>14.0 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.7 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.7 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).

<sup>2</sup>DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2= flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.
Table 4.4  Major mineral composition of defatted egusi flour

<table>
<thead>
<tr>
<th>Mineral (mg/100 g)</th>
<th>DEF1</th>
<th>DEF2</th>
<th>DEF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>166.8 ± 0.79a</td>
<td>186.1 ± 0.96b</td>
<td>201.27 ± 1.26c</td>
</tr>
<tr>
<td>K</td>
<td>1208.1 ± 17.34a</td>
<td>1298.6 ± 15.34b</td>
<td>1413.3 ± 18.58c</td>
</tr>
<tr>
<td>Mg</td>
<td>807.5 ± 2.16a</td>
<td>880.1 ± 2.03b</td>
<td>957.8 ± 13.52c</td>
</tr>
<tr>
<td>Na</td>
<td>3.4 ±10.11a</td>
<td>8.5 ± 0.15b</td>
<td>9.4 ± 0.11c</td>
</tr>
<tr>
<td>P</td>
<td>1698.9 ± 8.52a</td>
<td>1877.8 ± 8.21b</td>
<td>2046.4 ± 5.95c</td>
</tr>
</tbody>
</table>

1Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).
2DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

Repairing worn-out body cells and making of red blood cells (Kamda et al., 2015). DEF1, DEF2, and DEF3 consist of trace elements such as iron, zinc, and manganese in an appreciable amount. These elements are essential for enzyme metabolism and proper functioning of individual cells in the body. The high phosphorus, magnesium, potassium and calcium content in egusi flour make it a good supplement for pregnant and lactating women, as well as for children and older adults. The mineral content was significantly high in the defatted flour extracted with high temperature and pressure which was similar to findings reported by Siano & Straccia (2015), who observed that at high pressure the separation of components is high, yielding a more nutrient dense extract.

4.7.4 Sugars of defatted egusi flour

There were nine sugars present in defatted egusi flours as seen in Table 4.5. Sucrose in defatted egusi flour (106 4, 109.4 and 86.5 mg/l) for DEF1, DEF2, and DEF3, respectively differed significantly (p ≤ 0.05), with the lowest sucrose found in DEF3. This could be due to the caramelization of sugars when exposed to high temperature. When sugar is exposed to heat, sugar will melt into thick syrup. As the temperature continues to rise, sugar syrup changes colour, from bright to light yellow to a progressively deepening brown (Ghiase et al., 1982). As the temperature increased, the sugar concentration of defatted egusi flour decreased significantly. This browning process called caramelization reduced the quality of sugar in flour and can lead to discolouration of the flour. This phenomenon was responsible for the pale white colour found in the defatted flour which slightly differed in the
three flour samples. Sucrose is a common carbohydrate found in many plants and plant parts. Sucrose is a disaccharide, a combination of two monosaccharaides glucose and fructose, with the formula C$_{12}$H$_{22}$O$_{11}$. In humans and other mammals, sucrose is broken down into its constituent monosaccharide, glucose, and fructose, by sucrase or isomaltase glycoside hydrolases.

Sucrose is an easily assimilated macronutrient that provides a quick source of energy. As a pure carbohydrate, sucrose has an energy content of 3.87 kilocalories per gram (or 16.2 kilojoules per gram) (UW Hospital & family Clinics, 2016).

<table>
<thead>
<tr>
<th>Table 4.5</th>
<th>Sugar metabolite in defatted egusi flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars (mg/l)</td>
<td>DEF1</td>
</tr>
<tr>
<td>D–fructose</td>
<td>2.2 ± 0.21$^a$</td>
</tr>
<tr>
<td>D–galactose</td>
<td>0.8 ± 0.00$^a$</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.1 ± 0.05$^a$</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.8 ± 0.14$^a$</td>
</tr>
<tr>
<td>Mannitol</td>
<td>8.0 ± 0.37$^a$</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>8.2 ± 0.41$^a$</td>
</tr>
<tr>
<td>Sucrose</td>
<td>106 4 ± 75.00$^a$</td>
</tr>
<tr>
<td>Alpha – lactose</td>
<td>7.7 ± 0.26$^a$</td>
</tr>
<tr>
<td>Trehalose</td>
<td>2.2 ± 0.75$^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).

$^2$DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2= flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

Sucrose is digested rapidly but has a relatively low glycaemic index due to its fructose content, which has a minimal effect on blood glucose (Al-Farga et al., 2016). In defatted egusi flour, sorbitol was 8.2, 9.2 and 4.0 mg/l for DEF1, DEF2 and DEF3, respectively with DEF3 significantly (p ≤ 0.05) lower in sorbitol. Sorbitol is a sugar alcohol with a sweet taste which the human body metabolises slowly. It can be obtained by reduction of glucose, transforming the aldehyde group to a hydroxyl group. Sorbitol is mostly made from corn syrup, but it is also found in apples, pears, peaches, and prunes (Wang et al., 2013). It is converted to fructose by sorbitol-6-phosphate 2-dehydrogenase.
Sorbitol is an isomer of mannitol, another sugar alcohol; the two differ only in the orientation of the hydroxyl group on carbons chain (Filip et al., 2016).

While similar, the two sugar alcohols have very different sources in nature, melting points, and uses. Alpha-lactose in defatted egusi flour was 7.7, 5.6 and 6.6 mg/l in DEF1, DEF2, and DEF3, respectively, with DEF2 significantly ($p \leq 0.05$) low in alpha lactose. Alpha-Lactose was present in defatted egusi flour at a considerably small amount, constituting to 5-6% of the sugar in the flour (Table 4.5). Lactose sugar which is a combination of galactose and glucose is a disaccharide which is only produced as part of the milk of mammals and can be found as storage carbohydrate in the seeds of a few plants (Kellam, 1996).

The purest form of α-lactose has been the second most widely used compound and used as a binder in tablets, capsules and other oral product forms. In the food industry, α-lactose helps the bulking of food and can enhance the hydrocolloid properties of food produced as found in mayonnaise production. This further explains the stability of egusi hydrocolloid as discussed in Chapter 3.

### 4.7.5 Effect of extraction condition on functional properties of defatted egusi flour.

The water absorption index (WAI) and water solubility index (WSI) of defatted egusi flour on Table 4.6 showed WAI for DEF1, DEF2, and DEF3 (56.3, 52.5 and 57.6% w/w), respectively. There was an increase in the water absorption index of defatted egusi flour with no significant difference among samples. Increase in pressure and temperature increased the rate of absorption of water significantly. This could be due to the effect of temperature on the starch structure in the defatted flour, expanding its crystalline structure for easy water absorption (Venn & Mann, 2004).

<table>
<thead>
<tr>
<th>Parameters (% w/w)</th>
<th>DEF1</th>
<th>DEF2</th>
<th>DEF3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WAI</strong></td>
<td>56.3 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.5 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.6 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>WSI</strong></td>
<td>68.0 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.3 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.3 ± 6.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are the mean ± standard deviation. Means with different superscript in each row differ significantly ($p \leq 0.05$).

<sup>2</sup>DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.
The WSI shows an increase in water solubility index which is not significantly different from the samples. The water solubility index of defatted egusi flour 68.0, 65.3 and 73.3% w/w for DEF1, DEF2, and DEF3, respectively was significantly (p ≤ 0.05) different as the pressure and temperature increased. This has proven that supercritical CO$_2$ method of extraction improved the functionality of the flour obtained from the extraction process (Fiori, 2009). The WAI and the water solubility index (WSI) of the three defatted flour were similar at temperatures below 50°C. WAI and WSI provide evidence of the magnitude of the interaction between starch chains within both the amorphous and crystalline domains (Oikonomou & Krokida, 2003).

4.7.6 Effect of extraction condition on pasting properties of defatted egusi flour

The pasting properties of DEF1, DEF2, and DEF3 in Table 4.7 showed a significant difference in the viscosity of defatted egusi flour. Peak viscosity of defatted egusi flours respectively 386.0, 512.0 and 398.3 j for DEF1, DEF2 and DEF3 significantly (p ≤ 0.05) differed across the three samples. DEF2 (512.0 cP) showed the highest peak viscosity and was the most viscous among the samples. This could be due to the use of low temperature during extraction (55°C, 600 bar). Low-temperature extraction prevents the breakdown of starch which encourages pregelatinization and enables a viscous substance (Spies & Hoseney, 1982; Bemiller, 2011). These results indicated that low-temperature extraction had a significant effect on the pasting viscosities under the experimental conditions and egusi starch would behave differently during cooking and processing. Meanwhile, the pasting temperature and final viscosity did not differ significantly.

The holding strength and breakdown viscosity were low in the sample extracted at high temperature and pressure (75°C, 600 bar) [DEF3] (49.0 cP). This could be due to the effect of high pressure and temperature on the starch structure, causing thinness in the starch, thereby reducing the rate of water absorption and retention (Rakszegi et al., 2014). During the hold period of the test, the samples were subjected to a period of constant high temperature (90-93°C) and mechanical shear stress which further disrupts the starch granules in the defatted flour, resulting in amylose leaching and realignment (Bemiller, 2011).

This holding period is commonly associated with a breakdown in viscosity. The breakdown viscosity of defatted egusi flour showed flour with stable thickness for DEF1 and DEF2, the low temperature of 55 and 60°C, respectively making low-temperature low pressure the best process parameter for optimal flour based on properties for egusi flour.
Table 4.7  Pasting properties of defatted egusi flours

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DEF1</th>
<th>DEF2</th>
<th>DEF3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak viscosity (cP)</td>
<td>386.0 ± 51.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>512.0 ± 13.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>398.3 ± 9.61&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Breakdown viscosity (cP)</td>
<td>306.0 ± 45.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>373.0 ± 36.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>191.3 ± 10.97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Holding strength (cP)</td>
<td>53.3 ± 4.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.7 ± 3.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.0 ± 2.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pasting temperature (°C)</td>
<td>92.7 ± 1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.0 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.8 ± 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final viscosity (cP)</td>
<td>126.7 ± 2.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.7 ± 2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>126.3 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).

<sup>2</sup>DEF1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; DEF2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; DEF3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

4.8 Functional Properties of Egusi Hydrocolloid

4.8.1 Effect of extraction condition on the water absorption and water solubility index of egusi hydrocolloid

The functional properties of egusi hydrocolloid (EH) in Figure 4.3, made from defatted egusi flour extracted with 60°C, 30 g/h and 450 bar (EH1), 55°C, 30 g/h and 600 bar (EH2) and 75°C, 30 g/h and 600 bar (EH3) is shown in Table 4.8. The water absorbance index (WAI) revealed sample EH2 (89.0% w/w) was significantly (p ≤ 0.05) lower compared to EH3 (98.0% w/w) and EH1 (95.3 % w/w). This could be due to the use of high pressure during extraction of the defatted flour. At high-temperature solutes dissolve more into solution, thereby leading to a less porous structure in the hydrocolloid (Hansen, 1993). Egusi hydrocolloid has good water absorbance and solubility property. This could be due to the use of temperature below boiling temperature. At a high temperature above 100°C starches are destroyed and are weak in texture (Patindol et al., 2015).

The water solubility index of egusi hydrocolloid considering the parameters in view showed samples had a similar water solubility index, with only sample EH3 significantly (p ≤ 0.05) low compared to EH1 and EH2. This indicated that low temperature has a significant effect on the solubility of the residue obtained from a supercritical extraction process. WAI and WSI provide evidence of the magnitude of the interaction between starch chains within both the amorphous and crystalline domains (Oikonomou & Krokida, 2003). Egusi hydrocolloid shows the functional property of an emulsifier due to its ability to dissolve completely in water to form a stable emulsion.
Figure 4.3  Images for egusi hydrocolloid

EH1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EH2= flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EH3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.
### Table 4.8 Water absorption and solubility indices of egusi hydrocolloid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EH1</th>
<th>EH2</th>
<th>EH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAI (% w/w)</td>
<td>95.33 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.00 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.00 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WSI (% w/w)</td>
<td>72.00 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.00 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.03 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are the mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).

<sup>2</sup>EH1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EH2 = flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EH3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

Stabilizers are used to provide long-term emulsion stability, some of them by absorbing into the interface while others were only modifying the viscosity of the continuous phase due to their non-adsorbing character. Usually, proteins, such as whey protein isolate are the main emulsifiers whereas polysaccharides contribute to the emulsion stability through their thickening and steric stabilising characteristics (Chung & Ferrier, 1992; Armstrong & Barringer, 2013). Egusi hydrocolloid is an emerging stabiliser with potential for the food and non-food industry.

### 4.8.2 Pasting properties of egusi hydrocolloid

Table 4.9 shows the pasting profiles of egusi hydrocolloid extracted from the defatted flour obtained after supercritical oil extraction process. A large peak viscosity was observed at the beginning of the line elated with the high-speed stirring of the paddle (960 rpm). This initial step allowed dispersing egusi particle as well as facilitated water absorption by starch granules preventing clump formation.

The peak viscosity of EH1 (17.7 Cp) and EH2 (18.7 Cp) were similar and significantly (p ≤ 0.05) different from EH3 (16.7 Cp). The differences in the starch and protein composition in the egusi hydrocolloid could affect pasting viscosity and properties (Uruakpa, 2004; Berghout et al., 2014). Egusi hydrocolloid has low peak viscosity, this could be due to the water binding capacity of the mixture, and this often correlates with final product qualities.

Breakdown viscosities (BD) were found to be constant 8.0 Cp for all samples. This may be due to restricted swelling of the starch granules, which increased the tendency of the hydrophilic chain of the fibre in the hydrocolloid to bind with hydrogen bonds of the
water, causing a decrease in available water for starch granules. Also, this could be due to the low carbohydrate content of egusi flour (Enujiugha & Ayodele-Oni, 2003; Ponka et al., 2006). The results of the pasting characteristics of egusi hydrocolloid showed that egusi hydrocolloid has a reduced the peak viscosity (PV), break down viscosity (BD), and setback viscosity (SB). This was due to the existence and interaction of component protein from the defatted egusi flour with the protein present in the hydrocolloid after extraction.

Table 4.9 Pasting properties of egusi hydrocolloid

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EH1</th>
<th>EH2</th>
<th>EH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak viscosity (cP)</td>
<td>17.67 ± 0.57\textsuperscript{ab}</td>
<td>18.67 ± 0.57\textsuperscript{b}</td>
<td>16.67 ± 0.58\textsuperscript{a}</td>
</tr>
<tr>
<td>Breakdown viscosity (cP)</td>
<td>8.00 ± 0.00\textsuperscript{a}</td>
<td>8.00 ± 0.00\textsuperscript{a}</td>
<td>8.00 ± 0.00\textsuperscript{a}</td>
</tr>
<tr>
<td>Holding strength (cP)</td>
<td>9.66 ± 0.57\textsuperscript{a}</td>
<td>9.00 ± 1.00\textsuperscript{a}</td>
<td>9.33 ± 0.57\textsuperscript{a}</td>
</tr>
<tr>
<td>Pasting temperature (°C)</td>
<td>76.73 ± 1.44\textsuperscript{a}</td>
<td>77.76 ± 0.55\textsuperscript{a}</td>
<td>88.30 ± 6.70\textsuperscript{b}</td>
</tr>
<tr>
<td>Final Viscosity (cP)</td>
<td>96.33\textsuperscript{y} ± 1.15\textsuperscript{a}</td>
<td>97.33 ± 0.57\textsuperscript{a}</td>
<td>96.67 ± 0.58\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are mean ± standard deviation. Means with different superscript in each column differ significantly (p ≤ 0.05).  
\textsuperscript{2}EH1 = flour extracted at 60°C, carbon dioxide flow rate 30 g/h, and pressure 450 bar; EH2= flour extract at 55°C, carbon dioxide flow rate 30 g/h and pressure 600 bar; EH3 = flour extracted at 75°C, carbon dioxide flow rate 30 g/h and pressure 600 bar.

The holding strength of egusi hydrocolloid did not significantly differ across the samples for EH1, EH2 and EH3 9.7, 9.0 and 9.3 Cp, respectively. The final viscosity was 96.3, 97.3 and 96.7 cP for EH1, EH2, and EH3, respectively with no significant difference. This could be due to stable emulsifier having a similar texture as were reported by other authors (Bos & van Vliet, 2001; Funami, 2011).

The final viscosity (FV) indicated the re-association of starch granules especially amyllose during the cooling time after gelatinisation and the formation of gel network (Fernández-muñoz \textit{et al.}, 2011). The final viscosity (FV) showed the ability of the hydrocolloid to form a viscous paste or gel after cooking and cooling as well as the resistance of the paste to shear stress during stirring (Bos & van Vliet, 2001).

The pasting temperature of egusi hydrocolloid for EH1, EH2 and EH3 were 76.7, 77.8 and 88.3 Cp respectively. EH3 was significantly higher in pasting temperature than EH1 and EH2, indicating a higher temperature was required for the starch granule in the sample to form a complete paste without further changes in its texture. This confirmed
egusi hydrocolloid dissolves entirely in solution at a high temperature above 70°C, offering a stable emulsion, thereby proffering a stable hydrocolloid to the food and food industries.

4.9 Conclusion
Defatted egusi flour produced from supercritical CO₂ extraction is creamy white flour, with 60% w/w of protein and approximately 30% w/w of carbohydrate. It can fit as a primary raw material for a nutrition program. The flour produced with low temperature, low pressure (60°C and 450 bar) offer the highest/best nutritional and physicochemical properties. Defatted egusi flour can be used to fortify flours with low dietary composition, as composite flour in the food industry. Defatted egusi flour obtained from the three processing parameters was confirmed as a potential thickener and a binder for food systems. The defatted flours were similar in water absorption, water solubility and pasting properties. The hydrocolloid obtained from defatted egusi shows it was more of an emulsifier than a thickener, maintaining a stable emulsion. Egusi hydrocolloid can be a beneficial functional ingredient for the food and non-food industry.

References


CHAPTER FIVE

NUTRITIONAL PROFILE AND CONSUMER ACCEPTABILITY OF INSTANT EGUSI SOUP

Abstract

The objective of this work was to study the nutritional profile and consumer acceptability of instant egusi soup (IES), produced using egusi grit, egusi flour, and egusi hydrocolloid. The d-optimal quadratic mixture model was used to study the effect of the independent variables (grit, flour, and hydrocolloid) and optimum levels were obtained using Numerical optimisation while maximising on the sensory quality of the soup. Appearance and taste of IES were used to obtain optimal soup mix. The quadratic model was adequate to navigate the design space for taste and appearance. The optimal soup mix was 17.5% grit, 57.5% egusi flour and 10% hydrocolloid with the desirability of 0.947. Based on the optimal, three soup samples were produced with boiled grit (IESBG), spherified grit (IESSG) and extruded grit (IESEG), while varying the other ingredients (spice, egusi oil, and water). Sixteen trace and five major mineral elements were found in egusi soup, with a high concentration of P (1220.4, 1326.2 and 1277.9 mg/100 g), K (1220.4, 1326.2 and 1277.9 mg/100 g), Mg (822.2, 905 3 and 863.70 mg/100 g), Ca (172.3, 190.9 and 183.4 mg/100 g) and Fe (53.7, 57.5 and 29.5 mg/100 g), and for IESBG, IESSG and IESEG respectively. Instant egusi soup is also a source of Zn (9.9, 12.3 and 11.8 mg/100 g) respectively for IESBG, IESSG, and IESEG. Phosphorus content was significantly (p ≤ 0.05) high across the three soup 1742.8, 1836.3 and 1838.2 mg/100 g for IESBG, IESSG, and IESEG, respectively. Instant egusi soup was also high in potassium (1220.4, 1326.2 and 1277.9 mg/100 g), and magnesium (822.2, 905 3 and 863.7 mg/100 g), respectively for IESBG, IESEG, and IESEG. IESSG and IESEG were significantly (p ≤ 0.05) higher in minerals when compared with IESBG. Instant egusi soup differed significantly (p ≤ 0.05) in lightness (L*) while the redness (a*) and yellowness (b*) did not vary significantly. The soup did not differ significantly in taste appearance, colour, texture and overall acceptability. The model fitness for appearance and taste has a significant F value of 5.14 and 8.95 respectively. The overall acceptance (3.508 g/100 g) and desirability (0.947 g/100 g) of the instant soup mix. The instant soup was moderately liked and has the potential to meet the dietary need of consumers.
5.1 Introduction
The seed of *Citrullus lanatus subsp muscospermus* is high in essential fatty acids, with a high amount of essential amino acid has long been domesticated (Kapseu *et al*., 1993). The method of processing which is laborious has for long negatively influenced its consumption. Food processing not only improves flavour and palatability of foods but also increases the bioavailability of nutrients, by inactivating anti-nutritional factors (Sodjinou, 2006). Traditional processing of the egusi meal involves laborious and prolonged hand rolling and kneading into boiling water. Precooking defatted egusi flour, using conventional boiling, chemical cooking (spherification) and extrusion cooking into grits will reduce the laborious cooking process of soup made from egusi meal, thereby offering an instant processed food to the consumers (Tsai *et al*., 2017b). Conventional boiling brings about some changes in physical characteristics and chemical compositions of egusi flour which is traditionally cooked by boiling. Boiling technology provides high-quality food products with enhanced flavour, colour, biological and active components (Iwuoha & Eke, 1996; Akubor & Ogbadu, 2003). Boiling egusi flour in water and subsequent drying to form grits will proffer a precooked grit with an improved functional property. More so, is the process of producing egusi grit with chemical cooking (spherification), which is an old technique in the world of modernist cuisine, and was pioneered at El Bulli in 2003, a cornerstone in experimental kitchens around the globe (Kurozawa & Hubinger, 2017).

Spherification is the culinary process of shaping a liquid into spheres which visually and texturally has a moist inner texture. In modern cuisine, this technique is central to the formation of faux caviar, eggs, gnocchi, and ravioli. These spherical elements of a dish range from having a thin membrane, which is filled with a non-gelled liquid to a gelled food (Lupo *et al*., 2015). Although alginate gels allow gel formation, alginates and a calcium source were introduced in the technology of spherification in 2003, and have been around for decades in the food industry. The first use of alginates was to restructure red peppers for manufacturing pimentos in olives. Unlike most edible gels, which are stable throughout, alginate spheres typically contain a physical outer gel membrane with a liquid core (Lee & Rogers, 2013). Spherification can occur using the basic or the reverse technique (Tsai *et al*., 2017). The reverse method utilises a calcium source added to the edible liquid, broth or slurry and is dispensed into a sodium alginate bath. The reverse spherification which was explored in this study involves calcium (Ca$^{2+}$) first cross-linking at the film surface, drawing the polymer chains closer together. This results in the formation of a less permeable surface slowing the diffusion of Ca$^{2+}$ (Ubbink, 2008). Another possible method for producing precooked grits is extrusion cooking.
Extrusion is the modern pre-heat cooking technique using a single screw and mechanical shearing at relatively low levels of moisture content (Moscicki, 2011). Extrusion allows starch gelatinisation, denaturation of protein, microbial reduction, enzyme inactivation and colour changes. The extent of which is dependent on the conditions of the extrusion. Those changes at the constituents' level modify the rheological behaviour of flour batters (Camire, 1991). Extrusion cooking is also responsible for changing the extent of molecular associations between components such as the amylose-lipid complex that can affect the in vitro starch digestibility of the flours (Li, 2001).

Egusi seed is locally defatted by cold pressing with hand and predominantly consumed as soup or soup thickener in Western, Central and Eastern Africa in a local relish called egusi/melon soup (Kamda et al., 2015). Egusi soup widely known for its characteristics taste and flavour has been consumed for decades without a formal introduction into the food industry. The laborious method of its preparation has been a significant deterrent in its consumption. The widely known instant soup is the traditional freeze-dried instant vegetable soups, which are in the global market, it is often prepared with various vegetables as primary raw materials, followed by seasoning, and freeze-drying (Wang et al. 2010).

Freeze drying produces the highest-quality of dried foods, but a significant problem with conventional freeze-drying is the long drying time needed, which in turn leads to high energy consumption and high capital costs (Wang et al., 2010). This is partly due to the poor heat transfer associated with the conventional electric heating method which transfers heat for drying by conduction (Song et al., 2012). A high cost of production restricts the popularity of freeze-dried instant soups. Thus, an instant cook-mix which involves less processing and low energy utilisation was explored in this study.

An instant cook-mix soup from egusi has never been industrialised. The ease of its preparation will fit into the busy life of an average soup eater, thereby proffering a nutritionally dense soup. The continuous yearn towards modern life, and the increase in the number of people that live alone have resulted in changes in food preparation and the habits of consumption. Less time is available for cooking a healthy meal (Meixner & Krejc, 2007), and according to the report “Specialty Food Consumer 2010” by the National Association for the Specialty Food Trade (NASFT), there is a growing consumers’ preference for eating at home (Duan, 2015). Along with the improving economic conditions, there were a surprising number of consumers who had re-engaged with speciality foods in 2010 (Wang et al. 2010). Speciality meal such as egusi instant soup is generally easier to handle over the time-consuming conventional cooking (Wang et al., 2010). The objectives of this study were to (1) produce precooked egusi grits (2) produce an optimal instant soup
mix from egusi grits; flour and hydrocolloid and (3) determine the physicochemical, microbiological and sensory properties of the instant egusi soup.

5.2 Materials and Methods

5.2.1 Source of egusi seed, chemical, and equipment
All laboratory chemicals and food grade reagents were obtained from Merck Pty South Africa. Egusi oil was obtained from oil extracted by supercritical CO\textsubscript{2} extraction in Chapter 3. Hydrocolloid and defatted egusi flour were obtained from processed defatted egusi flour and hydrocolloid in Chapter 4. The soup spice was obtained from GR Spice, Cape Town, South Africa. The overview of this chapter is shown in Figure 5.1.

5.2.2 Production of instant egusi grits from defatted egusi flour using different processing methods
Defatted egusi flour was subjected to various processing techniques to precook the flour into grits. These processing techniques include boiling, spherification, and extrusion.

Production of instant egusi grits using boiling method
Defatted egusi flour (800 g) was weighed into a bowl, mixed with 200 g of water to form a thick paste, which was cut into small balls (1 cm in diameter) into boiling water to boil for 5 minutes. The cooked balls were removed from boiling water and dried with electric cabinet drier at 50\textdegree C for 24 h. The dried egusi crumbs/balls were milled with a Corona hand mill (Landers, Columbia) and sieved to pass through a 355 \mu mesh. Boiled egusi grits obtained were then stored in air-tight plastic containers and held at 4\textdegree C until further use.

Production of instant egusi grits using spherification
Egusi slurry was prepared by mixing defatted egusi flour with water in ratio 1:1.5 g/mL to form thick slurry. To a 250 g of egusi slurry, 5 g of food grade calcium lactate was added to increase its calcium content. The sodium alginate bath was prepared by adding 5 g of sodium alginate in 1000 g of distilled water and blended using an electric blender for 5 min. The mixture was allowed to hydrate for 24 h in the refrigerator before cooking to remove the bubbles formed during blending. Egusi slurry enriched with calcium lactate was filled into a syringe and 0.1 ml dropped from the syringe into the alginate bath. The balls were taken out from the bath with a spoon, rinsed twice with distilled water and dried by electric cabinet drier at 50\textdegree C for 24 h. The dried flakes were milled with a Corona hand mill and sieved to pass through a 300 mic sieve. The spherified grits were then stored in air-tight plastic containers and held at 4\textdegree C until further use.
Figure 5.1  Overview of chapter five
**Production of instant egusi grits using extrusion**

Defatted egusi flour (DEF) was subjected to manual extrusion utilising a pasta extruder (Automatic pasta maker OTTIMO). Defatted egusi flour and water ratio 2.5:1 g/ml was used during the extrusion. Defatted egusi flour of 250 g and 100 ml of water was weighed into the mixing chamber of the extruder, the chamber was closed, and "mix" button was pressed to initiate the mixing process. After mixing for 2 min, the "extrude" button was pressed to extrude the mixed egusi. Extruded egusi was dried at 50°C for 24 h in a cabinet dryer and then milled with a Corona hand mill and sieved with a 355-micron sieve. Extruded grits were then stored in air-tight plastic containers and held at 4°C until further use.

### 5.2.3 Optimization of instant soup mix using mixture design

A D-optimal mixture design was used to determine the effect of boiled egusi grit ($X_1$), defatted egusi flour ($X_2$) egusi hydrocolloid ($X_3$), on the sensory quality of instant egusi soup.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Grit (%)</th>
<th>Flour (%)</th>
<th>Hydrocolloid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.2</td>
<td>56.7</td>
<td>9.2</td>
</tr>
<tr>
<td>2</td>
<td>15.0</td>
<td>60.0</td>
<td>10.0</td>
</tr>
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<td>3</td>
<td>20.0</td>
<td>55.0</td>
<td>10.0</td>
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<tr>
<td>4</td>
<td>20.0</td>
<td>60.0</td>
<td>5.0</td>
</tr>
<tr>
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<td>18.3</td>
<td>58.3</td>
<td>8.3</td>
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<tr>
<td>6</td>
<td>17.5</td>
<td>57.5</td>
<td>10.0</td>
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<td>7</td>
<td>17.5</td>
<td>60.0</td>
<td>7.5</td>
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<tr>
<td>8</td>
<td>20.0</td>
<td>55.0</td>
<td>10.0</td>
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<td>60.0</td>
<td>10.0</td>
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<td>19.2</td>
<td>59.2</td>
<td>6.7</td>
</tr>
<tr>
<td>11</td>
<td>20.0</td>
<td>57.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Each component was constrained by lower and upper limits. The three components in real scale amounted to 85% (5 to 10% for egusi hydrocolloid, 55 to 60% for egusi flour, and 15 to 20% for egusi grits), and the remaining ingredients, egusi oil, and spice were constrained to 10% of the soup composition. The design consisted of 11 formulations, 2 vertex points, 2 replicated vertices, 2 interior points, 2 centre edge and 3 edge points. Grits were mixed
with defatted egusi flour and hydrocolloid following the 11 formulations detailed in Table 5.1, with egusi oil (3%) and spice (7%), added to the mix. Instant preparation of the soup was obtained by adding boiling water (61.4 g/100 g). Benchtop sensory evaluation was conducted on the soups using five consumer panellists to determine some sensory qualities. Before the sensory assessment, ethics approval was given by the CPUT ethical committee.

5.2.4 Benchtop sensory evaluation of the soup mixes and data analysis

Five consumer panellists were chosen among the staff of the Cape Peninsula University of Technology to assess the products for appearance, colour, aroma, taste and overall acceptability. The 11 soup mixes were prepared into instant soup by adding boiling water (61.4 ml/100 g of soup mix) and mixed. The panellists received prior information about the procedure and were requested to sign an informed consent form before tasting the products. Three digital coded samples were presented to each panellist, with a cup of water to rinse their palate between tasting each sample. A five-point hedonic scale was used to rate each sample as (1) dislike extremely, (2) dislike moderately, (3) neither like nor dislike, (4) like moderately, (5) like extremely.

The sensory data was modelled using a quadratic model in equation (1) to establish the relationship between the sensory response variables and the three components of the instant soup. A mixture of three component forms a triangle with vertices corresponding to formulations that are a total composition a percentage of a single component (Jeirani et al., 2012).

\[
Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3
\]  

[1]

where \(Y\) represents the sensory attribute, while \(\beta\) represents the coefficients (\(\beta_1, \beta_2, \beta_3\) referring to the coefficient of egusi grit, egusi flour and egusi hydrocolloid) respectively and \(X\) the component proportion or independent variable (\(X_1, X_2\) and \(X_3\) referring to the egusi grit, flour and hydrocolloid).

5.2.5 Optimal soup mix from the quadratic mixture model

To determine the optimum instant soup formulation, numerical optimization was used to obtain an optimal mixture of egusi grits, egusi flour and egusi hydrocolloid based on having the components in range, while maximising the appearance and taste of the instant egusi soup. The optimum formulation was then used to produce three soups using the three grits (boiled, spherified and extruded grits), and was subjected to physicochemical, microbial and consumer acceptability tests.
5.2.6 Colour measurement of instant egusi soup

The colour of instant soup mix was determined using Minolta Chroma Meter (CR300) (Bolade, 2017). The instrument was calibrated using white calibration tile CR-A44. The L* measures the degree of whiteness/darkness and the higher the L* value, the higher the whiteness intensity. The a* indicates the balance between redness and greenness of the samples with a positive value corresponding to redness and negative value to greenness, while b* value shows the balance between yellowness (+) and blueness (-). For a* and b* readings, the values closer to zero indicate less intense colour whereas reading further from zero correspond to more intense chroma characteristics. The readings were collected in triplicate and mean values were calculated.

A CIE standard illuminates D65 was used to determine CIE colour space coordinates, L*a*b* values. The chroma and hue value of the lab was calculated using the method of Pathare et al. (2013) as shown in equation 2 and 3 below:

\[
C = \sqrt{a^*^2 + b^*^2}
\]  \[2\]

Where \( C \) = Chroma; \( a^* \) = redness; \( b^* \) = greenness

\[
H = \tan^{-1}\left(\frac{b^*}{a^*}\right)
\]  \[3\]

Where \( H \) = Hue angle; \( a^* \) = redness; \( b^* \) = greenness

5.2.7 Mineral content of instant egusi soup

The mineral content of egusi soup was carried out according to standard AOAC official method (AOAC, 2000). Samples were prepared by solubilising the acid-extractable elemental material for instance egusi soup (IES). Digestion was performed on a microwave digester, using ultra-pure HNO₃, at elevated temperature and pressure. After a cooling period, the extractant was made up to 50 ml volume with deionised water, then analysed by Inductive Coupled Plasma Mass Spectrophotometer (ICP-MS) or Inductive Coupled Plasma Atomic Emission Spectroscopy for the significant mineral analytes.

Trace elements were analysed on an Agilent 7700 quadrupole ICP-MS. Samples were introduced via a 0.4 ml/min micro mist nebuliser into a Peltier-cooled spray chamber at a temperature of 2°C, with a carrier gas flow of 1.05 L/min. The elements V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As and Se were analysed under He-collision mode to remove polyatomic interferences. The instrument was calibrated using NIST (National Institute of Standards
and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. Where the sample has undergone a digestion step, the results were corrected for the dilution factor resulting from the digestion procedure.

Major mineral elements (Na, K, Ca, Mg and P) were analysed on a Thermo ICap 6200 ICP-AES. The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards purchased from Inorganic Ventures (Inorganic Ventures 300 Technology Drive Christiansburg, VA 24073) to quantify selected elements. NIST-traceable quality control standards from De Bruyn Spectroscopic Solutions, Bryanston, South Africa, were analysed to verify the accuracy of the calibration before sample analysis, as well as throughout the analysis to monitor drift. The instant soup samples were analysed in triplicate.

5.2.8 Microbiological Evaluation of Instant Egusi Soup

Samples of instant egusi soup (10 g) were dispersed in 90 ml sterile Ringer’s solutions, using ¼ strength Ringer’s solution (Merck, Darmstadt, Germany) under the aseptic conditions, by using the sterile pipette. One tablet (BR001) in 500 mL of distilled or deionised water was stirred slowly until complete dissolution. The solution was dispensed in tubes (9 mL), and sterilise in an autoclave at 121°C for 15 minutes. The solution was cooled to 25°C after incubation.

A portion (10 g) of the egusi soup was aseptically placed into a sterilised bag (10-15 cm, Sunkyung Co., Seoul, ROK) with 90 mL of sterile peptone water (0.1%). The mixture was homogenised in a stomacher blender (Model 400, Teledyne Tekmar Co., Mason, OH USA) for 2 min. The following mediums were used for culturing: plate count agar (Difco Co., BD Diagnostic Systems, Sparks, MD USA) for total aerobic bacteria, 3M Petrifilm (3M Health Care, St. Paul, MN USA) coliform bacteria. A 1 mL aliquot was spread onto plates containing one of the above-mentioned media and incubated for bacterial growth at 35°C for 48 h under aerobic conditions (Song et al., 2012). Microbial populations from the sample cultured in triplicate on each medium were evaluated by manually counting the colonies on each plate (Bolade, 2017). Total count of mesophilic aerobic bacteria (TMAB) was enumerated in pour-plates of Plate Count Agar (Merck) after incubation at 30°C for 48 hours. Yeast and moulds were identified in agar plates of potato dextrose agar (Merck) after incubation at 30°C for 3 days (Song et al., 2012).

5.2.9 Sensory evaluation of instant egusi soup

A consumer tasting panel of 50 was invited among staffs and students of Cape Peninsula University of Technology to determine the quality attributes of instant egusi soup. Panellists received written and prior verbal instruction regarding the evaluation procedures. Panellists
were presented three coded digit samples in foam cups on a white tray of 9.6 x 6.6 cm; water was provided to the panellists to rinse their palate between tasting of each sample. Panellists were instructed to rate the samples for appearance, colour, aroma, taste, texture and overall acceptability using five-point hedonic scales. The five-point hedonic scale was used to rate each sample as (1) dislike extremely, (2) dislike moderately, (3) neither like nor dislike, (4) like moderately, (5) like extremely.

5.3. **Statistical Analysis**
All data were collected in triplicate. The data were subjected to a multivariate analysis of variance to establish mean differences between treatments. Duncan multiple range tests were used to separate means where differences existed. All data were analysed using IBM SPSS 2016 statistics version 24.

5.4 **Results and Discussion**
5.4.1 **Production of instant egusi grits**
Precooked grits were made from defatted egusi flour, using three different processing methods of boiling, spherification and extrusion cooking as shown in Figure 5.2. The boiled grits formed coarse crumbs after drying, and its colour becomes pale yellow. This was also observed for the grit made from extrusion, the extruded egusi pasta which formed the shape of the dye used was also pale yellow. Egusi grit made from reverse spherification formed a flaky irregular shape after drying. The flakiness of the spherified egusi grit could be due to the extra film formed by the alginate gum used during the spherification process.

5.4.2 **Optimization of instant egusi soup**
**Model fitting**
The sensory properties of instant egusi soup (IES) recorded in Table 5.2, showing the mean and standard deviation of the 11 instant egusi soup formulations. Using quadratic mixture design, the regression coefficient of the quadratic mixture model for instant egusi soup (IES) were shown in Table 5.3. Regression $R^2$ was significant ($p \leq 5$) for appearance, colour, taste, texture and overall acceptance of IES except for aroma ($0.406$), which was less than $0.5$. 

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Figure 5.2  (a) Spherified egusi grit unmilled: (b) Spherified egusi grit milled: (c) Extruded egusi grit unmilled: (d) Extruded egusi grit milled: (e) Boiled egusi grit unmilled: (f) Boiled egusi grit milled
<table>
<thead>
<tr>
<th>Formulation</th>
<th>( X_1 ) (Grit g)</th>
<th>( X_2 ) (Flour g)</th>
<th>( X_3 ) (Hydrocolloid g)</th>
<th>Appearance</th>
<th>Colour</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture</th>
<th>Overall acceptance</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>19.2</td>
<td>56.7</td>
<td>9.2</td>
<td>3.67 ± 1.03</td>
<td>3.50 ± 0.55</td>
<td>4.00 ± 1.10</td>
<td>3.67 ± 0.82</td>
<td>3.00 ± 1.10</td>
<td>3.50 ± 1.38</td>
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<td>3</td>
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<td>10.0</td>
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<tr>
<td>4</td>
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<td>5.0</td>
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<td>5</td>
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<td>6</td>
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<td>3.67 ± 1.52</td>
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<tr>
<td>7</td>
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<td>60.0</td>
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<td>57.5</td>
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<td>3.33 ± 1.51</td>
<td>3.50 ± 1.38</td>
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\(^1\text{Values are mean ± standard deviation}\)
### Table 5.3  Regression coefficients of quadratic mixture model for instant soup sensory quality

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Regression R²</th>
<th>Adjusted regression, R²</th>
<th>Adequate Precision</th>
<th>Lack of fit p-value</th>
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<tbody>
<tr>
<td>Appearance</td>
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<td>0.674</td>
<td>6.086</td>
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<td>Colour</td>
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<td>Aroma</td>
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<td>Overall acceptance</td>
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<td>0.442</td>
<td>5.670</td>
<td>0.876</td>
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### Table 5.4  F-ratio from quadratic mixture model for instant egusi soup

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<tr>
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<th>Sensory attributes</th>
<th>Appearance</th>
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<th>Aroma</th>
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<th>Texture</th>
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<tr>
<td>Model</td>
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<td>0.68</td>
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<td>0.71</td>
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<td>0.93</td>
</tr>
<tr>
<td>$X_1X_2$</td>
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<td>17.16*</td>
<td>8.35*</td>
<td>0.82</td>
<td>7.67*</td>
<td>4.58</td>
<td>0.98</td>
</tr>
<tr>
<td>$X_1X_3$</td>
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<td>7.41*</td>
<td>2.14</td>
<td>0.27</td>
<td>27.91*</td>
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<td>6.19*</td>
</tr>
<tr>
<td>$X_2X_3$</td>
<td></td>
<td>0.10</td>
<td>0.02</td>
<td>0.74</td>
<td>0.04</td>
<td>2.72</td>
<td>3.54</td>
</tr>
</tbody>
</table>

*p ≤ 0.05: where $X_1$ = egusi grit, $X_2$ = egusi flour; $X_3$ = egusi hydrocolloid
The adequate precision was greater than 4 in appearance, taste and overall acceptance except for colour, aroma, and texture which showed a model with the adequate goodness of fit. Adeq Precision 6.086 indicated the model has a desirable and adequate signal because adeq precision ratio greater than 4 shows the design can be used to navigate a model space (Table 5.3). This implies appearance, taste and overall acceptability of IES was fit into the design space with adequate signals.

A negative pred $R^2$ (-4.62) (Table 5.3), implies that the overall mean may be a better predictor of the response on aroma than the current model (Table 5.2). Adeq precision ratio of 5.670 for the overall acceptance of instant egusi soup indicates an adequate signal which can be used to navigate the design space (Table 5.3).

The lack of fit F-value of 0.42 implies the lack of fit was not significant relative to the pure error, and this proves the model can be used to navigate a design space. However, the adequacy of precision" which measures the signal to noise ratio, 3.03 indicates a low signal and this model should not be used to navigate the design space for the aroma of instant egusi soup. The quadratic model was fit for describing the effect of egusi grits, flour and hydrocolloid.

The F ratio from the quadratic mixture model for IES was obtained from the experiments and recorded in Table 5.4. There was a significant F-value for appearance (5.14) and taste (8.95) of the instance egusi soup (IES), indicating the model was adequate to describe the appearance and taste of IES using the quadratic model. The lack of fit p-value was not significant for all the quality attributes of IES measured indicating a model with the adequate goodness of fit. This implies the mixture component has a substantial effect on the overall acceptability of instant egusi soup.

The quadratic mixture model was found to be adequate to determine the effect of the three ingredients (grit, flour and hydrocolloid) on the instant soup mixture based on appearance, colour, taste, texture and overall acceptability of instant egusi soup.

**Effect of mixture component on the instant soup sensory parameters**

The linear effect of the component did not significantly affect the sensory attribute of the instant egusi soup as shown in Table 5.4. The interaction between $X_1X_2$ (egusi grit and egusi hydrocolloid) had a significant ($p \leq 0.05$) synergy effect on appearance, colour and taste. The relationships are shown in equation 4, 5 and 6 below:

\[
\text{Appearance} = 3.26X_1 + 3.34X_2 + 3.28X_3 + 2.78X_1X_2 + 1.94X_1X_3 - 0.22X_2X_3 \quad [4]
\]
\[
\text{Colour} = 3.26X_1 + 3.41X_2 + 3.45X_3 + 1.92X_1X_2 + 1.03X_1X_3 - 0.089X_2X_3 \quad [5]
\]
\[
\text{Taste} = 3.00X_1 + 3.50X_2 + 2.80X_3 + 1.86X_1X_2 + 3.76X_1X_3 + 0.13X_2X_3 \quad [6]
\]

where $X_1 = \text{egusi grit}; X_2 = \text{egusi flour}; X_3 = \text{egusi hydrocolloid}$
Figure 5.3  (a) Trace (Piepel) plot and (b) Response surface plot for the effect of three component (A: Egusi grit, B: flour and C: hydrocolloid) on instant egusi soup
Figure 5.4  (a) Trace (Piepel) plot and (b) Response surface plot for the effect of three component (A: Egusi grit, B: flour and C hydrocolloid) on instant egusi soup
Figure 5.5  (a) Trace (Piepel) plot and (b) Response surface plot for the effect of three components (A: Egusi grit, B: flour and C hydrocolloid) on instant egusi soup
Figure 5.6  (a) Trace (Piepel) plot and (b) Response surface plot for the effect of three components (A: Egusi grit, B: flour and C hydrocolloid) on instant egusi soup
Figure 5.7  (a) Trace (Piepel) plot and (b) Response surface plot for the effect of three components (A: Egusi grit, B: flour and C hydrocolloid) on instant egusi soup
Figure 5.8  (a) plot and (b) Trace (Piepel) response surface plot for the effect of three components (A: Egusi grit, B: flour and C hydrocolloid) on instant egusi soup
This implies that there was a maximum turning point beyond which appearance decreased. As egusi grit and flour was increased, the appearance of the instant soup was reduced. This observation was confirmed by the trace Piepel plot (Figure 5.3a) indicating that the appearance of instant egusi soup was more sensitive to the quantity of components $X_1X_2$. The 3D response surface plot (Figure 5.3b) showed the quadratic curve was exerting a positive influence on the curve resulting in a concave curve for appearance. Egusi grit and egusi flour ($X_1X_3$) also had a synergistic effect on the appearance of IES.

The colour of IES also had a synergistic effect as egusi grit, and egusi flour ($X_1X_2$) was increased in the instant soup, with the vertex edges indicating the impact of each ingredient (Rao & Baral, 2011). As the interaction between $X_1X_2$ (egusi grit and flour) was increased, a significant ($p \leq 0.05$) effect on the colour of the instant soup was observed. This observation was confirmed by the trace Piepel plot (Figure 5.4a) indicating that the colour of instant egusi soup was more sensitive to the quantity of components $X_1X_2$.

The 3D response surface plot (Figure 5.4b) showed the quadratic curve was exerting a positive influence on the curve resulting in a concave curve for appearance. Component $X_1X_3$ (egusi grit and egusi hydrocolloid) and $X_2X_3$ (egusi flour and egusi hydrocolloid) showed an antagonistic effect as an increase has no significant effect on the colour of the instant soup. A significant ($p \leq 0.05$) synergistic effect was observed for the taste of IES, as the component effect of $X_1X_2$ and $X_2X_3$ was increased. This was confirmed with the trace Piepel plot (Figure 5.5a) and 3D response surface plot (Figure 5.5b), with a concave curve displaying the effect of an increase in the component. An antagonistic effect was reflected in the component mix of $X_2X_3$ (egusi flour and hydrocolloid), as the increase in the component mix does not affect the taste of the instant soup. The component mix effect on aroma and texture of instant egusi soup showed an antagonistic effect, as there was no significant effect of the component mix on the two attributes.

This non-effect was confirmed by the trace Piepel plot (Figure 5.6a and 5.7a), showing the increase in $X_1X_2$, $X_1X_3$ and $X_2X_3$ had no significant effect on the aroma and texture of IES. The 3D plot also confirmed the non-significant effect of the increased addition of mixture component. A significant ($p \leq 0.05$) synergistic effect was observed with the component mix of $X_1X_3$ (egusi grit and hydrocolloid) for the overall acceptability IES. The interaction between $X_1X_2$ and $X_2X_3$ indicated an antagonistic effect of the component mix, as showed in the trace Piepel plot (Figure 5.7a). The concave curve displayed in the 3D response surface plot (Figure 5.8b) showed the quadratic curve was exerting a positive influence on the curve resulting in a concave curve for overall acceptance of IES.
**Optimal instant egusi soup formulation and model validation**

Based on high regression ($R^2$), the instant egusi soup mix was optimised by the components. The goal of optimising was to have component A (egusi grit) in range, component B (egusi flour) in range and component C (egusi hydrocolloid) in range while maximising the effect of appearance and taste. The best optimal solution with desirability of 0.947 was found as 57.2% defatted egusi flour, 17% egusi grit and 10% for egusi hydrocolloid. The accuracy of the model was confirmed by producing egusi soup based on 57.2% flour, 17% egusi grit and 10% egusi hydrocolloid. The result obtained were (4.00) for appearance and (3.67) for the taste of instant egusi soup, indicating the mean value of the mixture component. The relates to the predicted value for appearance and taste of instant egusi soup as the model was fit to describe the effect of the mixture on IES. The objective of production of instant soup from egusi grits was achieved.

5.4.3 **Colour of egusi soup**

Table 5.5 shows the CILAB colour parameters for instant egusi soup (IES) in the fresh form after processing. The values for the $L^*$, $a^*$ and $b^*$ coordinates of the instant soup made from boiled grit (IESBG) were 63.47, 5.77 and 18.15 respectively. The boiled egusi grit, cooked at boiling temperature, showed a pale colour which could be due to Maillard reaction. A Maillard reaction is a chemical reaction between amino acids and reducing sugars that gives browned food its distinctive flavour.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Instant egusi soup$^{1,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IESBG</td>
</tr>
<tr>
<td>$L^*$</td>
<td>$63.47 \pm 0.16^a$</td>
</tr>
<tr>
<td>$a^*$</td>
<td>$5.77 \pm 1.25^a$</td>
</tr>
<tr>
<td>$b^*$</td>
<td>$18.51 \pm 1.59^a$</td>
</tr>
</tbody>
</table>

$^1$Values are mean ± standard deviation. Means with different superscript in each row differ significantly (p ≤ 0.05).

$^2$IESBG = Instant egusi soup boil grit, IESSG = instant egusi soup spherified grit, IESEG = instant egusi soup extruded grit

This change in colour was observed after processing, resulting in low redness of instant egusi soup made from boiled grit. A similar report was given by Akobundu (1989), who reported that the colour of egusi meal changes to a pale brown colour when cooked, which invariably is responsible for the pale colour of sausages made using egusi meal. $L^*$, $a^*$ and
b* coordinate for instant egusi soup made with spherified grit (IESSG) were 63.32, 6.00, 17.75 in Table 5.5. While the L*, a* and b* coordinate of instant egusi soup made with extruded grit (IESEG) were 62.89, 6.34 and 17.56, which differed significantly (p ≤ 0.05) in lightness L, from IESBG and IESSG. This could be due to the breakdown of starch granules during extrusion, thereby causing more absorption of water. The results for chroma and hue angle showed colour stability for the instant soup samples shifting towards the slightly yellow region (Jarad et al., 2007). The hue angle of fresh egusi soup was about 72°, which represents a colour in the light/yellow region (hue angle between 0° and 90°). However, the effect of temperature was smaller in L and a* coordinate than b* in parameters, which turned the samples more yellowish as the temperature rises.

Comparing the three soup samples, it is possible to conclude that the soups were more of pale yellow and no significant redness and yellowness colour difference. The lightness L* significantly differ in the soup made with extruded grit (IESEG), which could be due to the effect of extrusion on the protein and starch structure of the extruded grit thereby causing more water absorption in the soup. The hue angle for IESBG, IESSG and IESEG was 72.0°, 71.3° and 70.1° respectively, indicating the soups were within the lightness/yellowness region.

5.4.4 Mineral content of instant egusi soup

Sixteen trace mineral elements and five significant elements were detected in instant egusi soup as shown in Table 5.6 and 5.7. The instant egusi soup (IES) produced with boiled (IESBG), grit, spherified grit (IESSG) and extruded grit IESEG, were high in major mineral elements (Table 5.7). Phosphorus was high in instant egusi soup 1742.8, 1836.3 and 1838.2 mg/100 g for IESBG, IESSG and IESEG, respectively, differed significantly (p ≤ 0.05) differed. This was not similar to report made by Enujiugha & Ayodele-Oni (2003) who reported that phosphorus (2.8 g/kg), magnesium (3.43 g/kg) and potassium (3.9 g/kg) is present in egusi melon which is low to the proportion found in instant egusi soup.

The mineral content was significantly high in the soup made with spherified and extruded grit. This could be due to the method of obtaining the precooked grit. The spherified grit was obtained using reverse spherification techniques (Ubbink, 2008). This involves the use of calcium chloride and a bath of alginate to form the spherified ball, which was subsequently dried and milled into a precooked grit (Lee & Rogers, 2012). IESSG was significantly (p ≤ 0.05) high in minerals except for Fe (53.7, 57.5 and 29.5 mg/100 g) and Al (23.0, 15.6 and 10.6 mg/100 g) which was high in the instant soup made from boiled grit.

Phosphorus, being the highest in IES is essential in the human diet as it helps to combat diseases. In medicine, hypophosphatemia, a phosphate deficiency syndrome may be caused by malnutrition, non-absorption of phosphate, also from metabolic problems
resulting in acute illnesses. All causes of deficiency are associated with low level of phosphate in the blood serum and its cells. Symptoms of hypophosphatemia include neurological dysfunction and disruption of muscle and blood cells due to lack of ATP (Vignola et al., 2016). High phosphate intake can lead to diarrhoea, hardening of the organs and soft tissue, a condition known as calcification. Calcification interferes with the body’s ability to digest iron, calcium, magnesium, and zinc (Gillooly et al., 2005). In this view, instant egusi soup, being high in phosphorus proffers an instant functional meal which should be explored by the food industry.

Calcium is an essential structural component of bone; it is crucial in the formation and maintenance of a healthy body frame (Varghese et al., 2013).

<table>
<thead>
<tr>
<th>Parameters (mg/100 g)</th>
<th>Instant egusi soup(^1,2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IESBG</td>
</tr>
<tr>
<td>Bo</td>
<td>2.8 ± 0.03(^a)</td>
</tr>
<tr>
<td>Al</td>
<td>23.0 ± 0.28(^a)</td>
</tr>
<tr>
<td>Ti</td>
<td>0.9 ± 0.02(^a)</td>
</tr>
<tr>
<td>V</td>
<td>0.1 ± 0.00(^a)</td>
</tr>
<tr>
<td>Cr</td>
<td>0.1 ± 0.00(^a)</td>
</tr>
<tr>
<td>Mn</td>
<td>7.8 ± 0.04(^a)</td>
</tr>
<tr>
<td>Fe</td>
<td>53.7 ± 0.29(^a)</td>
</tr>
<tr>
<td>Ni</td>
<td>0.4 ± 0.00(^a)</td>
</tr>
<tr>
<td>Cu</td>
<td>3.7 ± 0.03(^a)</td>
</tr>
<tr>
<td>Zn</td>
<td>9.9 ± 0.13(^a)</td>
</tr>
<tr>
<td>Sr</td>
<td>1.0 ± 0.00(^a)</td>
</tr>
<tr>
<td>Mo</td>
<td>0.2 ± 0.00(^a)</td>
</tr>
<tr>
<td>Ba</td>
<td>0.9 ± 0.07(^a)</td>
</tr>
<tr>
<td>Si</td>
<td>17.0 ± 0.05(^a)</td>
</tr>
</tbody>
</table>

\(^1\)Values are mean ± standard deviation. Means with different superscript in each row differ significantly (p ≤ 0.05).

\(^2\)IESBG = Instant egusi soup boil grit, IESSG = instant egusi soup spherified grit, IESEG = instant egusi soup extruded grit

The instant soup contains a high concentration of calcium 172.3, 190.9 and 183.4 mg/100 g for IESBG, IESSG and IESEG (Table 5.5) respectively, making it an alternative calcium
source from a plant. Egusi was also high in magnesium 807.5, 880.1 and 957.8 mg/100 g respectively for IESBG, IESSG and IESEG.

Table 5.7  Major mineral composition of instant egusi soup

| Parameters (mg/100 g) | Instant egusi soup  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IESBG</td>
</tr>
<tr>
<td>Ca</td>
<td>172.3 ± 0.1 a</td>
</tr>
<tr>
<td>K</td>
<td>1220.4 ± 26.67 a</td>
</tr>
<tr>
<td>Mg</td>
<td>822.2 ± 3.36 a</td>
</tr>
<tr>
<td>Na</td>
<td>3.9 ± 0.06 a</td>
</tr>
<tr>
<td>P</td>
<td>1742.8 ± 16.39 a</td>
</tr>
<tr>
<td></td>
<td>IESSG</td>
</tr>
<tr>
<td>Ca</td>
<td>190.9 ± 0.46 b</td>
</tr>
<tr>
<td>K</td>
<td>1326.2 ± 5.47 b</td>
</tr>
<tr>
<td>Mg</td>
<td>905 3 ± 0.83 b</td>
</tr>
<tr>
<td>Na</td>
<td>8.8 ± 0.07 b</td>
</tr>
<tr>
<td>P</td>
<td>1836.3 ± 12.40 b</td>
</tr>
<tr>
<td></td>
<td>IESEG</td>
</tr>
<tr>
<td>Ca</td>
<td>183.4 ± 8.13 c</td>
</tr>
<tr>
<td>K</td>
<td>1278.0 ± 48.79 a</td>
</tr>
<tr>
<td>Mg</td>
<td>863.7 ± 1.97 c</td>
</tr>
<tr>
<td>Na</td>
<td>8.9 ± 0.18 b</td>
</tr>
<tr>
<td>P</td>
<td>1838.2 ± 7.51 c</td>
</tr>
</tbody>
</table>

1Values are the mean ± standard deviation. Means with different superscript in each row differ significantly (p ≤ 0.05).

2IESBG = Instant egusi soup boil grit, IESSG = instant egusi soup spherified grit, IESEG = instant egusi soup extruded grit

Magnesium helps in maintaining healthy muscle and nerve functions that keeps the heart rhythm steady. More so, calcium, potassium and magnesium are all needed in repairing worn-out body cells and making of red blood cells (Kamda et al., 2015). The three instant soups were a source of trace elements such as iron, zinc and manganese. These elements are essential for enzyme metabolism and proper functioning of individual cells in the body. The high phosphorus, magnesium, potassium and calcium content in egusi soup make it an alternative healthy meal for pregnant and lactating women, as well as for children and older adults (Akobundu, 1989).

5.4.5 Microbiological characteristics of instant egusi soup

There were no aerobic bacteria detected and no cluster of colony growth seen after 48 h incubation. Coliform bacterial not detected in the three instant soups inoculated. The total coliform bacteria are an indicator of the presence of pathogenic intestinal bacteria. Yeast and moulds were not detected after 3 days incubation, making instant egusi soup microbiologically safe.

5.4.6 Sensory evaluation

The demography of the 50 consumer panellists, is detailed in Table 5.7, shows 86.0% female, 14.0% male, 94.0% students (52.0% local students, 48.0% international students), 6.0% staff, 90% <20-29 age group, 8.0% 30-39 age group and 2.0% 40 and above age
group. The black race was 92.0%, coloured race 6.0% and white race 2.0% of the panellist (Table 5.7). Panellists’ response significantly (p ≤ 0.05) differed in evaluation of the soup samples. However, there was no significant (p > 0.05) difference in the quality attributes of the soup samples.

The appearance of the soup was neither liked nor disliked by the panellists 3.0, 3.0 and 3.2, respectively for IESBG, IESSG and IESEG (Table 5.4). Based on various panellist comments, the soup appearance, taste and colour will improve if the soup had a smooth texture. The grittiness of the soup was not welcomed by most of the panellists, and this gave the soup less rating. Panellists gave the soups positive comment as it relates to the aroma which includes "nice aroma", "good aroma and good taste. But negative comments such as "bad appearance", "I love the aroma not sure about the texture and colour", were indicated.

<table>
<thead>
<tr>
<th>Item</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>45 (90.0)</td>
</tr>
<tr>
<td>30-39</td>
<td>4 (8.0)</td>
</tr>
<tr>
<td>40 &amp; above</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>46 (92.0)</td>
</tr>
<tr>
<td>White</td>
<td>1 (2.0)</td>
</tr>
<tr>
<td>Coloured</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
</tr>
<tr>
<td>Staff</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td>Student</td>
<td>47 (94.0)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (14.0)</td>
</tr>
<tr>
<td>Female</td>
<td>43 (86.0)</td>
</tr>
<tr>
<td><strong>International</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (48.0)</td>
</tr>
<tr>
<td>No</td>
<td>26 (52.0)</td>
</tr>
</tbody>
</table>

*Numbers are frequency and percentage in the bracket
Figure 5.9  Mean sensory scores of instant egusi soup (1 = Dislike very much, 2 = Dislike moderately, 3 = Neither like nor dislike, 4 = Like moderately, 5 = Like very much).  IESBG = Instant egusi soup with boiled grit: IESSG = Instant egusi soup with spherified grit: IESEG = Instant egusi soup with extruded grit
Figure 5.10  Instant egusi soup made from (a) boiled grits, (b) spherified grits and (c) extruded grits. Instant egusi soup made from
Figure 5.11  Sensory word cloud for instant egusi soup made with (a) boiled grit; (b) spherified grit (c) extruded grit.
The taste of instant egusi soup was moderately liked, with 3.6 average rating by 60% of the panellist due to low saltiness, with some comment like “not a good taste, not enough salt” “needs more salt”, on the soup samples could be due to the new salt regulation. The new salt regulation has reduced daily salt intake to 5 g person/day. Hence the spice used for the instant soup was formulated on a low salt scale, according to the new salt regulation.

The overall acceptability of instant egusi soup ranged from 3.4–3.6 (liked moderately) for IESBG, IESSG and IESEG with no significant difference among the three soup samples. Based on all sensory attributes, egusi instant soup with spherified grit and extruded grit had the best overall qualities. According to word cloud in Figure 5.8., boiled egusi soup has some negative comment like: “soup is too weak”, “soup is too gritty”.

Some positive comment like nice "soup", "good taste" and "good soup" This comment displayed the different option of each panellist and therefore, instant egusi soup made from boiled grit could be accepted if subsequently improved. Egusi soup made from spherified grit has mostly positive comment than negative. Few negative comments for IESSG were "bad appearance" and "watery soup". This response confirms the diversity of each panellist, making instant egusi soup made from spherified and extruded grit, the most preferred soup among the soup samples. Egusi soup made with spherified grit, has positive comments from word cloud, which includes "nice, good aroma, taste nice, good texture ", while few negative comments were "gritty soup and bad texture". These positive comments also revealed the soup made from spherified grit and extruded grit was moderately accepted by the panellists (Figure 5.11).

5.5 Conclusion

Instant egusi soup produced with boiled, spherified and extruded grit offers a pre-cooked meal that could fit into the daily busy meal schedule of an average soup consumer. The instant egusi soups were high in 21 essential and non-essential minerals with phosphorus (1838.2 mg/100 g), magnesium (905.3 mg/100 g) and potassium (1326.2 mg/100 g) being the highest mineral in instant egusi soup and is microbiologically safe. The three soups were free of a pathogen with no detection of *Escherichia coli*, and *Staphylococcus aureus* observed. Instant egusi soup was moderately liked by the panellists. Overall acceptance for the soups is in the order of spherified > extruded > boiled. The objective of producing instant egusi soup with egusi grits was achieved.

Reference

Kurozawa, L.E. & Hubinger, M.D. (2017). Hydrophilic food compounds encapsulation by


CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Egusi seed (*Citrullus lanatus* subsp *muscospermus*), has for a long time been locally consumed by the indigene of various communities cultivating egusi melon. Egusi seed high in its nutritional and functional composition has never been introduced into the food industry. Despite some previous research work indicating the potential of egusi oil, nothing has been known of its ability to fit into a weight loss program, and its nutraceutical functions. To highlight the importance of egusi seed, some objectives were worked on in this study:

1. Characterize the fatty acid profile of the egusi oil extracted using the supercritical fluid extraction and determine its functionality in the egusi seed.
2. Establish the effect of processing on physicochemical, nutritional characteristic of defatted egusi meal.
3. Establish the functional and pasting properties of hydrocolloids from defatted egusi meal.
4. Produce instant soup using egusi grit, hydrocolloid and defatted meal.
5. Determine the shelf life and overall acceptance of instant egusi soup.

The first objective was achieved by extracting oil from egusi seed using supercritical carbon dioxide extraction (SCF) and subsequently characterized for proximate, physicochemical and fatty acid profile to determine its functionality in egusi seed. The best oil yield as it related to nutritional and physicochemical attributes was the oil extracted using a high-temperature low pressure of (60°C and 450 bar). The high polyunsaturated fatty acid in egusi seed confirmed its potential as nutraceutical oil, which could be made into functional cooking oil that will help nourish the body. In recent years, the demand for plant-based polyunsaturated fatty acids (PUFAs) is increasing rapidly due to dietary and lifestyle requirements. Egusi oil being high in PUFA, with its two health's related indices, confirms its health benefits. The index of atherogenicity (IA%) were low, which implies that lipid composition of egusi oil is a source of fatty acid essential and beneficial to human health and thus can be incorporated into a good diet. The supercritical method of extraction has proven effective in extracting oil that retains its nutrient density.

The second objective was achieved by milling the defatted meal obtained after the supercritical extraction procedure and characterizing the flour for its functional properties, proximate and micronutrients. The defatted flour was high in amino acids, mineral and sugars. The protein content of defatted egusi flour 60% and carbohydrate of 30%, proffering
egusi flour as a major raw material in a nutrition program. Defatted egusi flour was high in 16 trace mineral and 5 major minerals (P, Ca, K, Na, Mg) and a source of essential and non-essential amino acids.

The third objective was achieved by extracting hydrocolloid from the defatted egusi flour, which was characterized by it pasting properties. Egusi hydrocolloid (EH) remained stable at a high temperature of 70-80°C. The hydrocolloid property of defatted egusi flour obtained from the three processing parameters used in the extraction process reveals egusi hydrocolloid as a stabilizer and emulsifier. Egusi hydrocolloid can be a very useful functional ingredient for the food and non-food industry. The flour produced with low temperature, low pressure (60°C and 450 bar) offer the highest/best nutritional and physicochemical properties. Defatted egusi flour can be used to fortify flours with low nutritional composition, serving as composite flour in the food industry. The defatted flour obtained from the three process displayed similar functional properties as it relates to water absorption, water solubility and rheological properties.

The fourth and the fifth objectives were also attained by producing an instant soup using egusi grit, hydrocolloid and defatted meal. The three ingredients were optimized using mixture design and the overall acceptance of instant egusi soup was achieved by sensory evaluation of the Instant egusi soup was high the mineral.

The following conclusion can be drawn from this study:
1. Egusi oil was extracted from egusi seed using supercritical carbon dioxide extraction.
2. Egusi oil was high in conjugated linoleic acid.
3. Defatted egusi flour was high in protein (60% w/w) and major minerals (P, Ca, K, Mg and Na).
4. Hydrocolloid was extracted from defatted egusi flour.
5. The extruded hydrocolloid stabilized instant egusi soup.
6. Consumer acceptance of egusi soup from the different egusi grits did not differ significantly.
7. An invention disclosure was submitted awaiting approval
8. Literature information on this study titled Citrillus lanatus Mucosospermus a novel; structural ingredient in the food system was submitted to Food review international.