# EVALUATION OF PROCESS VARIABLES ON THE QUALITY OF EGUSI (*Citrullus vulgaris)* SOUP BALL USING RESP0NSE SURFACE METHODOLOGY

**BY**

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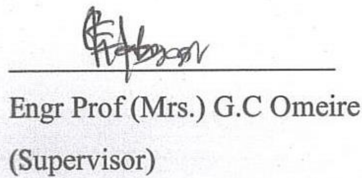
## CERTIFICATION

This is to certify that, this thesis "Evaluation of process variables on the quality of Egusi (*Citrullus vulgaris*) soup Ball using Response Surface Methodology was carried out by Onyejiaka, Ifeoma Philomena (Reg. No 20174080068) in partial fulfillment for the award of Master of Science (MSc) Food Science and Technology (Food Processing Technology option) Federal University of Technology Owerri.



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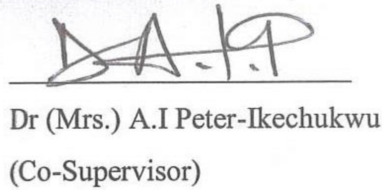

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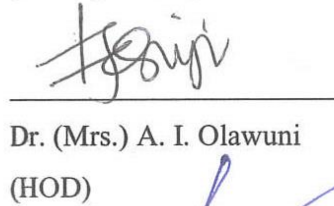

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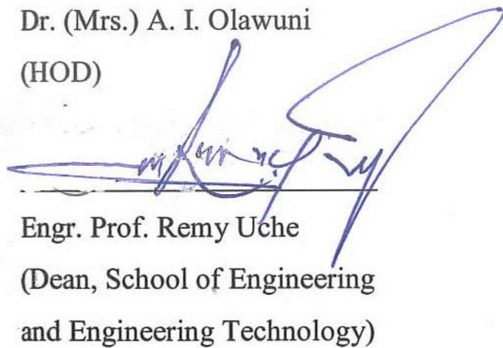

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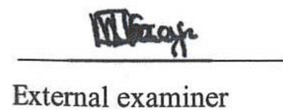

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## DEDICATION

I dedicate this work to God Almighty who made it possible for me to excel in life

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## ABSTRACT

This project was carried out to evaluate the effect of process variables on the quality of Egusi (*Citrillus vugaris*) soup balls from blends of defatted melon and "usu" *Pleurotus tuber*) flour. Egusi seeds were processed into flour and defatted while 'Usu' was processed into flour. The two flour samples were blended at different ratios of (90:10, 80:20, 70:30) egusi / usu. A three factor, three levels Response Surface Methodology (Box Behnken Design) was used to design the experiment. Seventeen (17) runs were designed for feed composition $X_1$(90:10, 80:20, 70:30) %, Cooking time$X_2$ (60, 90, 120) min and ball sizes $X_3$ (5, 10, 15) g. The runs were used to produce soup balls. Sensory attributes and proximate composition of the soup balls were determined. Shelf – life study on the soup balls for a period of four weeks were also carried out. Regression models describing the effect of variables on the product responses were obtained. Proximate composition showed that there were significant ($P \leq 0.05$) differences among the samples in terms of moisture content, crude protein, crude fibre, ash, fat, and carbohydrate samples. Responses were mostly affected to a large extent by feed composition, cooking time and to a lesser extent ball size. The moisture content of the soup ball samples was low, ranging from (5.75 -8.05%) which indicated that the samples would have longer shelf- life. The regression analysis results revealed that there were significant ($P<0.05$) differences in the interactions between feed composition and ball size and between ball size and cooking time. The coefficient of determination $R^2$ was 0.87, which signified that the model adequately represented the real relationship between the variables under consideration. All the $R^2$ were higher than 0.75. The lack of fits was not significant. The protein contents of the samples were high, ranging from (33.02 - 37.5%), which could be attributed to the protein content of the egusi. Fat content ranged from (7.67 – 10.23%). The fat content revealed that there was residual oil in the egusi flour after defatting. Screw oil expeller machine was used to defat the egusi which must have left residual oil in the flour. The response surface plot, fig. 4.5, revealed that increase in the percentage of egusi in the samples, resulted to increase in fat content of the samples. Increase of usu in the feed increased the crude fiber (2.95 – 6.63%) and ash content (4.01– 5.64%). Sensory scores showed that there were significant ($P< 0.5$) differences among the samples with respect to all the attributes. The scores were above 7.5 in a nine-point hedonic scale. The highest total viable count after one-month storage was $2.8 \times 10^4$cfu/ml from $MSB_{13}$.The highest fungal count was $2.0 \times 10^4$ cfu/ml from $MSB_1$. No coliform count was detected. Though the counts were within acceptable levels, the following microorganisms were isolated: *Bacillus subtilis, Pseudomonas aeruginosa (*disease–causing*); Aspergillus niger, Aspergillus flavus* which were spoilage organisms.



**Keywords**: Melon, Pleurotus tuber, meat substitute, response surface, process variables

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 BACKGROUND OF THE STUDY

Meat analogues also called a meat substitute, mock meat, faux meat or imitation meat are primarily vegetable based food products that contain proteins made from legumes (mainly soy), cereal protein or fungi which approximates certain aesthetic qualities and chemical characteristics of certain types of meat (Davis and Lightower, 1998, Saddler, 2004; Hoek *et al.,*2011), Meat analogue are primarily vegetable based food product that contain proteins made from pulses (mainly soy), cereal protein, or fungi (Jander and Trochia 2001;Alamu and Busie, 2019). Many meat analogue qualify as a complete protein and also contain substantial level of dietary fiber, natural antioxidants and phytochemicals, while possessing a low saturated fatty acid content and no cholesterol (Saddler, 2004). Traditionally, soy protein was used as a popular ingredient in the production of meat analogue such as tempeh and tofu (Hoek *et al*., 2011). These products have been consumed for centuries as the traditional dishes in Asian countries (Asgar *et al.,* 2010).

Recently several strategies have been developed to help consumers reduce their meat consumption. One of the strategies is based on the development of structured products. Here, vegetable protein-based food is texturized (using extrusion cooking to produce fiber-like structure) to imitate meat in chewiness and flavor (Asgar *et al*.,2010; Lin *et al.,* 2000; Yao *et al.,* 2004).

Another strategy is that meat in a meal could be replaced by the consumption of vegetables, beans, pulses, and/or nuts **(**Tan *et al.,* 2014). Melon soup ball "akpurukpu egusi" is a non-fermented, traditionally processed food product obtained from melon seeds *Citrullus vulgaris*), and *Pleurotus tuber* ("Usu") is consumed as a meat substitute mainly in the rural

areas of South Eastern Nigeria. (Nwokoma 2008; Chibundu *et al* 2018; Omeire *et al* 2019). The fibre- like structure of meat analogue or meat substitute depends on the material of its components, extrusion conditions and moisture content. (Lin et al., 2000; Yao et al.,2006). Addition of "Usu" increases the texture, fibrous characteristics, physical properties and functional properties of food (Sadler, 2004; Zhang et al., 2021). *Pleurotus tuber* is considered to be a good source of proteins, low in lipid content, possess "umami" flavor which is due to the sulphur containing amino acids and it imparts meaty taste to the products (Sadler, 2004). Melon is an oil and protein rich leguminous seeds which after being dried and ground are used as a major substrate in production of soup ball "akpuruakpu egusi" (Nwokolo, 1986). Standardization of food helps in maximizing compatibility, safety, repeatability and it ensures that best possible food items are produced every time. Careful cooking and storage would help retain the nutrients in the food (FAO/WHO, 1973). Response Surface Methodology (RSM) is a powerful mathematical and statistical technique for testing multiple process variables and their interactive and quadratic effects. It is useful in solving multivariable equations obtained from experiments simultaneously (Velioglu *et al.,* 2010). It is significant technique to determine interaction between responses (dependent variable) and factors (independent variables) and also to reduce number of experimental trials as compared to complete randomized design (Murphy *et al.,* 2004; Tiwari *et al.*2017; Ghodke *et al.,* 2009). It has been used for the simultaneous analysis of the effects of process parameters in fresh meat processing (Jakobsen and Bertelsen, 2000) and also in meat product (Desmond *et al*., 1998; Hsu and Chung 2000). In the present study, Box Behnken Design of response surface methodology was used to obtain the experimental runs for the factors feed composition$\mathbf{X_1}$ (70:30, 80:20, 90:10), cooking time$\mathbf{X_2}$ (60, 90, 120) min and Ball sizes $\mathbf{X_3}$ (5, 10, 15)g. Regression analysis was used to determine the models.

## 1.2 Statement of the problem

Egusi (melon) soup balls have not received adequate research attention, in the area of standardization of the processing methods thereby limiting its contribution to food availability and food security. Traditionally, it is consumed in different localities and is prepared in different ways, though many researchers have tried to standardize the recipe but standardized ball size, feed composition, and cooking time have not been established. Its preparation is laborious. The kneading and oil extraction from the melon are done manually, the cooking time is long because it is cooked overnight. This leads to loss of energy and time. It also has a shelf life of about 24 hours if not preserved. It is used in soup preparation though some researchers have tried to increase its shelf life through the use of preservatives. The use of faster and mechanized processing (use of screw press) method of oil extraction from the melon is yet to be explored.

## 1.2 Objectives of the study

### 1.3.1Main Objective

The main objective of this work was to evaluate process variables on the quality characteristics of egusi (Citrillus vulgaris) soup ball using Response Surface Methodology.

### 1.3.2 Specific Objectives

The specific objectives were to:

i. Produce defatted egusi and Usu flours
ii. Obtain different blends of egusi/usu flours
iii. Produce soup ball by varying the feed composition, ball size and cooking time
iv. Determine the proximate composition and sensory evaluation of formulated soup balls

v. Determine the microbial load on the soup balls at weekly interval for four weeks

vi. Use regression analysis to obtain models for the process variables

## 1.4 Justification of the study

Many researchers have produced soup balls using the traditional method with little modification in the processing techniques. Model that best represent how dependent variables are affected by independent variables are yet to be determined. This research addressed the problems of standardization of recipe, best cooking time, and acceptable ball size so that it can be reproducible. The Response Surface Methodology (RSM) would help in developing models for the processing conditions. Moreover, producing a plant-based meat substitute such as melon soup balls could substitute meat in the soup for the low income consumers, who cannot afford meat as part of their daily meal. It could also serve as a meat substitute for vegetarians. It would also reduce the problem of malnutrition since egusi is rich in protein. It would stimulate the establishment of food industry for the production of packaged melon soup ball and create employment opportunities.

## 1.5 SCOPE OF THE STUDY

The research covered the production of soup ball from blends of defatted melon and “usu” flours at different ratios of feed composition (90:10, 80:20, 70:30), different cooking time (60, 90, 120) min, and different soup ball sizes (15, 10,5) g. It also covers the determination of proximate compositions as affected by processing variables. It equally covers sensory evaluation, and microbial analysis of the soup balls.

# CHAPTER TWO

## 2.0 LITERATUREREVIEW

### 2.1 Melon Seed Soup Balls

Egusi soup ball 'akpuruakpu egusi' is a non- fermented, traditionally processed food product obtained from melon seeds (*Citrullus vulgaris*), and it is consumed as a meat substitute (Nwokoma 2008; Chibundu *et al.,* 2018; Omeire *et al.,* 2019). It is consumed mainly in the rural areas of South Eastern Nigeria as a substitute for meat. In umuahia (Abia state), it is called 'Ngbam', in Onitsha (Anambra State), and Imo state, it is called "Agbagheluatui', and 'agbarati (steamed snack)', respectively. In Igbo land, it is generally called 'akpuruakpu egusi'.(Nwokolo and Sim, 1987; Omeire *et al.,* 2019). Indigenous methods of soup ball production consist essentially of kneading and hand pressing of egusi meal to express the oil followed by addition of "Usu" flour, pepper, salt and other spices and shaping them into balls and cooking overnight. The shape of the balls varies (round, flat or cylindrical with the color of the balls ranging from dark brown to light grey (Nwokolo and Sim, 1987; Nwokoma, 2008).

The balls have short shelf life of 24h at ambient temperature and are usually used in preparation of egusi soup (Decker, 2004; Omeire *et al*., 2019). The soup containing the ball is commonly consumed with carbohydrate rich meal like garri and fufu (fermented and cooked cassava) according to (Nwokolo and Sim 1987). Apart from the regular melon seed (*Citrulus vulgaris*) used for preparation of Egusi soup balls, it can also be produced from 'egusikirikiri'(*Colocynthis citrullus*), sesame seeds(*Sesamum indicum*), and groundnut (*Arachis hypogaea*) as reported by Omeire *et al*., (2019). Quite a number of researchers have worked on melon Soup ball, for instance Chibundu *et al.,* (2018), worked on the fate of aflatoxin during traditional melon seed processing into melon cake and sauce. They found out

that the aflatoxin was significantly reduced in boiled melon cake and sauce when the product was cooked between 60-90 minutes. Another area of research is the preservation of “agbarati” using methods such as drying, packaging material and chemical preservatives to extend shelf life (Nwakudu *et al*.,2015).

### 2.2Description/Nutritional Composition of Melon seeds

Melon seed scientifically known as *Citrullus colocynthis* (under the family *Cucubitaceae*) is a cheap and readily available legume grows very well in warm and arid region of Africa and Asia. It is commonly known as “egusi” and is mostly cultivated in the southern part of Nigeria. It is a fat-and protein-rich leguminous seeds which after being dried and milled are used as a major ingredient in Western African cuisine (Nwokolo, 1986). Unextracted “full fat” melon seeds are rich sources of energy with oil content of 51- 55%, 32.5-38 crude protein, 2.7%, fiber, 3.6% ash, and 8.2% carbohydrate and some other important mineral nutrients (Abiodun and Adeleke, 2010; Oyenuga, 1968; Akobundu *et al*., 1982a; Ojeh *et al*., 2008). It contains alpha tocopherol (Vitamin E) that helps to maintain young and smooth skin and vitamin A (Shava, 2000; Abiodun and Adeleke, 2010). These are good sources of essential amino acids, especially arginine, tryptophan and methionine, vitamins B1, B2, and niacin, and sulphur, calcium, magnesium, manganese, potassium, phosphorous, iron and zinc. The oil contains mostly oleic acid (15.9%) and linoleic (62.8%) acid (Asgar, 2010). Melon is easily digestible and is reported to be as good as soybean in all the essential amino except lysine (Nwokolo and Sim 1987)

Melon seeds are rich in vitamin A, copper (3.37 ppm), Zinc (13.46 ppm). The oil expressed from the seeds is usually used for edible purposes (Ajibola *et al.,* 1993), while the residual cake is fried and consumed as a snack. The ground seeds are usually utilized as a thickener in the preparation of traditional soups. Melon seeds before consumption are usually processed by

commonly used traditional processing techniques which includes dehulling, sorting, and milling (Rachel, 2011). In tropical countries of Africa, melon seeds are milled and usually cold-pressed or solvent-extracted for the oil. Another form of local oil extraction involve crushing the seeds, boiling them in water until the oil floats to the surface and is separated (Nwokolo *et al.,* 1987). The oil extracted from the seeds is of high nutritional value as it is equally used for the production of pastries, margarines and soaps (Ajibola *et a*l.1993), and has found to have enormous potential in the biodiesel (Giwa *et al.,* 2010). The key problem regarding the nutritional exploitation of oil seed plant is the presence of anti-nutritional factors (Abiodun and Adeleke,2010), carried out a research on melon seed cake to ascertain the anti-nutritional factors present in the melon seed cake. Although egusi seed contains some amount of anti-nutritional factors such as oxalate, phytate, tannins and cyanogens, in relatively small amounts. Their presence do not interfere with the palatability of the seed as food. Processing techniques, such as cooking, roasting, washing with water (hard) and autoclaving, are some methods that have been previously shown to reduce the levels of anti-nutritional factors in seed (Ene-Obong, 1995).

**2.3 History of meat analogues**

Meat analogue, also referred to as meat substitute, mock meat, faux meat or imitation meat, approximates certain aesthetic qualities and chemical characteristics of certain types of meat (Saddler, 2004). Due to increasing health concern, global shortage of animal proteins, economic reasons and environmental issues, consumption of vegetable proteins in food products has increased (Craig *et al.,*2009). These vegetable protein products have been consumed for centuries as the traditional dishes in Asian countries (Asgar *et al*., 2010). Meat analogue are primarily vegetable based food product that contain proteins made from pulses mainly soybean, cereal protein, or fungi (Jander and Trochia 2001). Many meats analogue qualify as a complete protein and also contain substantial level of dietary fiber, natural

antioxidants and phytochemicals, while possessing a low saturated fatty acid content and no cholesterol. Many researchers have reported that fat plays a major role in meat or meat analogue quality such as juiciness, tenderness, mouth feel, texture, taste and flavor (Samard and Ryu, 2018) however, meat analogue are still known to be different from meat in terms of mouth feel,texture,taste and flavor release of the product. This is because vegetable oils differ considerably in their physicochemical properties from animal fat, and this could therefore negatively affect juiciness and texture parameters. Many modern commercial meat analogues are made from textured wheat protein from gluten and isolated soy protein. These products closely approximate the texture of real meat. Plant proteins, in general can be textured to mimic the properties of real meat (chicken, beef, pork, or seafood) using different factory processes such as spinning, jet-cooking, steam treatment and extrusion cooking. Among these processes, extrusion has the preferred technology. Developing a meat substitute that are attractive and acceptable to consumers is a challenge (Wansink *et al*.,2005). One strategy is encouragement of "meatless days' or smaller portion sizes (Laestadius *et al*., 2013). This approach recognizes that many people over consume proteins. This means that in processed meat products, it could be replaced by using plant-based meat extenders (Katayama *et al*., 2008). Meat extenders are plant based ingredients that act as fillers in processed meats. By doing so, the actual meat content in the product is lowered, leading to reduced meat intake. In second strategy, meat in a meal can be replaced by the consumption of vegetables, beans, pulses, and /or nuts (Tan *et al.,*2014). A third strategy is based on the development of structured products. Structured products are formed when vegetable proteins are combined with edible polymers.

### 2.4. 1 Description of *Pleurotus tuber-regium*(usu)

*Pleurotus tuber-regiun* is fungi and belongs to the group of fungi called Basidiomycetes and is the most developed fungi. It forms a large spherical to ovoid, subterranean sclerotium

composed of fungus tissue, sometimes up to 30 cm or more in diameter. The sclerotium is usually dark brown on the outside and white inside (Belowu and Belowu, 2005). Edible mushrooms are highly nutritious when compared with meat, eggs and milk (Okhuoya and Okogbo**,**1990). *Pleurotus tuber-regium* is known by many local names such as 'Ohu" (a growth), 'Otuako" (teeth cleaner), 'Ufetu' (swelling when ground in water) and 'Rumbagada' (having an underground sclerotum) as reported by Oso (1977**).**

### 2.4.2. Nutritional Compositions and Uses of *Pleurotus tuber-regium*

*Pleurotus tuber-regium* supplies the major food types such as protein (64.31% WW and 71.21% DW), carbohydrate (20.20 %WW and 22.15 %), crude fibre (2.89% WW and 3.20 DW), Sugar (18.6% having high concentration of galactose and low concentration of glucose and maltose), fats and oil as well as minerals (Oso, 1977; Okhuoya, and Okogbo, 1990; Zhang *et al.,* 2021). Chromatographic analysis of the sclerotum shows the presence of glucose, fructose, mannose, galactose, sucrose, inositol, maltose, cholesterol, palmitic acid, oleic and steric acid. Quantitative analysis shows 0.32% reducing sugar, 14.94% protein, 0.27% potassium and 0.39% sodium (Chang, 1996). According to Alofe *et al.,* (1991), the mushroom contains about 16.5% dry matter, 7.4% is crude fibre, 14.6% crude protein and 4.4% fat and oil. Protein level of usu compares to Shitake at 18%, it also compares with wheat at 13% and milk at 25% (all based upon dry weight). Fat levels are comparable to other mushroom species. It is a rich source of calcium for bones of aging men and women as well as treatment for boils, constipation and dental caries (Oso, 1977).

*Pleurotus tuber-regium* is useful for food (culinary) and for medicinal purposes such as alleviating pains and constipation (Oso, 1977). The tuberous sclerotum can be used as partial replacement for melon seed or groundnut cake in traditional preparation of sauces and soups. It is used as food stabilizer or thickening and binding agent. A local snack called "ogboloti" is

prepared from *Curcurbit apepo and Pleurotus tuber-regium.* It is normally seasoned and tied in a leaf of cola gigantean and boiled which yields a well-known health and energy sustaining snacks. It is cherished till today in the villages (Oso,1977).

**2.5 Response Surface Methodology**

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery, 2005). The parameters that affect the process are called independent variables, while the responses are called dependent variables for example, the hardness of a meat Y is affected by cooking time $X_1$ and cooking temperature $X_{2.}$ The meat hardness could be changed under any combination of treatment $X_{.1}$ and $X_{2.}$ Therefore, time and temperature could vary continuously. If treatment are from a continuous range of values, response surface methodology is useful for developing, improving, and optimizing the response variable. In this case the hardness of meat Y is the response variable and it is a function of time and temperature of cooking. It can be expressed as the dependent variable *Y* and is a function of $X_1$ and $X_2$

$$Y=f(X_1) + f(X_2) +e$$

Where Y is the response (dependent variable), $X_1$ and $X_2$ are independent variables and e is the experimental error. Response surface is a method based on surface placement. Therefore, the main goals of RSM study is to understand the topography of the response surface including the local, minimum and ridge lines and find the region where the most appropriate response occurs. Box-Behnken designs (BBD) and central composite design (CCD) are two main experimental designs used in response surface methodology. Central composite rotatable design (CCRD) has also been applied to optimization studies in recent years. The

experimental data are evaluated to fit a statistical model (Linear, Quadratic, Cubic or 2F1(two factor interaction). The coefficients of the model are represented by constant term, A, B, and C. The RSM is important in designing, formulating, developing and analyzing new scientific studying and products. RSM are commonly applied in industry, biological and clinical science, social science, food science, physical science and engineering.

### 2.5.1 Three –Level Factorial Design

The Factorial Designs are widely used in experiments when the curvature in the response surface is concerned. All treatment factors have 3-levels in the three level factorial designs. This design requires many runs; as a result, the factorial design can be an alternative approach when the number of factors gets cumbersome. The three-level factorial design is more efficient, it allows collecting information on the main effects and on the low-order interactions. The one problem with three-level factorial is that when number of factors is large, it becomes very complicated to separate the aliased effects and to interpret their significance. For this reason, when n is large, most of the time this kind of design is used for screening designs. After an appropriate design is conducted, the response surface analysis can be done by any statistical computer software and then statistical analyses could be applied to draw the appropriate conclusion ( Atashgah and Seifi, 2007).

This design was developed by Box and Behnken (1960). It provides three levels for each factor and consists of a particular subset of the factorial combinations from the 3n factorial design. The use of Box- Behnken design is popular in industrial research because it is an economical design and requires only three levels for each factor where the settings are -1, 0, and 1. Some Box-Behnken designs are rotatable, but, in general, this design is not always rotatable. Box and Behnken list a number of design arrangement for n=3, 4, 5, 6, 7, 9, 10, 11, 12, and 16 factors.

The Box- Benken Design (BBD) is a good design for response surface methodology because it permits

1. Estimation of the parametersof the quadratic model that is one containing squared terms, product of two factors, linear terms and an intercept
2. Building of sequential designs
3. Detection of lack of fit of the model
4. Use of blocks

A comparison between the Box- Benken Design and other response surface design (central composite, Doehlert Martrix and three level full factorial design) has demonstrated that the Box-Benken design and Doehlet Matrix are slightly more efficient than the three level full factorial designs, though the application of BBD for optimization of analytical methods is still limited (Ferrira *et al.,* 2007)

**2.6 OIL EXTRACTION FROM OIL SEEDS**

Extraction is the process of separation of a substance from a matrix and it could be done either by mechanical extraction {expression} or chemical extraction (solvent extraction) (Mohammed and Jorf-Thomas, 2003). Oil and fats are extracted from their original source (seeds, fruits or other oil bearing raw materials) using a variety of different methods. Currently worldwide, there are four basic methods for obtaining vegetable oil named chemical extraction, superficial fluid extraction, steam distillation and mechanical extraction as shown in Figure 2.1.

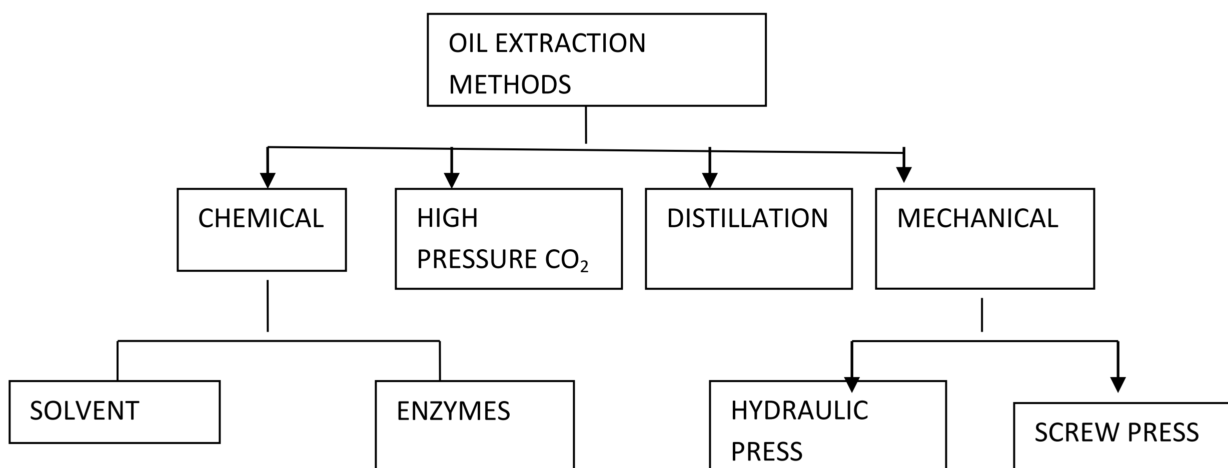


**Figure 2.1**: Basic methods for oil extraction (Sari, 2006)

### 2.6.1 Mechanical Oil Extraction

The most common method of extracting edible oil from oleaginous materials, which has been practiced for thousands of years, is mechanical pressing of oilseeds. Mechanical oil extraction (also known as pressing) is based on mechanical compression of oleaginous (plant that produces oil) materials. Through pressing, oil is separated from the oleaginous material (solid-liquid mixture) under the action of compressive external forces that arise in special machines called presses. This method ensures extraction of a non-contaminated, protein-rich low fat cake at relatively low-cost. The disadvantage of this method is that the mechanical presses do not have high extraction efficiencies, about 8-14% of the available oil remain in the press cake (Goering *et al.,* 1982).

### 2.6.2 Hydraulic press Technique

Currently, hydraulic presses are used in oil extraction which are driven by fluid pressure, or in screw presses, where the pressing force is created by a helical body (worm) which rotates in a closed space (press chamber). Hydraulic oil presses, so named because it works on the principle of the hydraulic ram, are originally from England and were first patented in 1795 by

Joseph Bramah. Hydraulic expression of oil involves application of pressure through a ram to digested oleaginous material mash in a cylindrical cage. The cylindrical cage is usually perforated laterally. This results in axial compaction and radial oil flow (Alonge, 2003).

In a typical hydraulic pressing of vegetable oil seeds three distinct stages could be identified The first cotton seed oil mill constructed in the United States (1920) utilized a hydraulic press. Seeds in filter bags were manually loaded into perforated, horizontal boxes between the head block and the ram. The cylindrical cage is usually perforated laterally. This results in axial compaction and radial oil flow (Williams, 2007). Hydraulic presses were in use until the 1950s. They are replaced with continuous screw presses and continuous solvent extraction plants, which are less labor intensive. The olive oil industry still utilizes hydraulic press today.

### 2.6.3 Screw press

The first screw oil press was developed in 1900 by V.D. Anderson in the United States. The mechanical screw press consists of a vertical feeder and a horizontal screw with increasing body diameter to exert pressure on the oilseeds as it advances along the length of the press. The barrel surrounding the screw has slots along its length, allowing the increasing internal pressure to first expel air and then drain the oil through the barrel. Oil is collected in a trough under the screw and the de-oiled cake is discharged at the end of the screw. The main advantage of the screw press is that large quantities of oilseeds can be processed with minimal labour, and it allows continuous oil extraction (Amalia *et al.,*2016). Lipid oxidation is one of the reasons that food deteriorates and is caused by the reaction of fats and oil with molecular oxygen leading to off- flavor that are generally called rancidity (Decker *et al.,* 2014). Rancidity is associated with characteristics off flavor and odour of the oil. In order to improve storage ability, the oil content in the melon seed is reduced by de-fatting using screw press (Fekria *et al*., 2012)

## CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 **Raw materials collection**

Raw materials that were used for this research includes; matured dehulled melon seeds, *Pleurotus tuber*s (Usu), pepper and salt. They were purchased from Relief Market in Owerri Imo State.

### 3.2 Raw Material Preparation

#### 3.2.1 Production of egusi (*Cittrullus colocynthis*) flour

The melon seed (egusi) flour was processed using the method of Omeire *et al.,* (2019) with modifications. One-kilogram (1 kg) melon seed were sorted to remove un-wanted materials, they were manually de-hulled, dried in a moisture extraction oven at 65$^{o}$C till a constant weight was attained and milled using a manual attrition mill (Corona hand grinder, model 685 China). The melon meal was defatted using screw oil expeller machine. (Aung *et al*., 2019) The defatted melon was packaged in air tight high-density polyethylene plastic container till analysis as shown in fig 3.1

#### 3.2.2 *Production of Usu (pleurotus) flour*

‘The 500g‘usu’ flour (*pleurotus tubers)* was dry-cleaned by scrapping off the outer layer with sharp, kitchen knive. They were cut into chunks of about 2cm thickness and milled using a manual attrition mill (Corona hand grinder model 685 China) Aung *et al*., (2019). The ‘Usu’ flour was sieved through 150µm sieve. The flour was packaged in high density air polyethylene bag prior to subsequent use. Fig 3.2 showed production of usu flour

### 3.2.3 Experimental Design

The defatted egusi flour and usu flour were blended at different ratios of egusi/usu (90:10, 80:20, 70:30) egusi/usu Table 3.1. Box Behnken Design response surface methodology was used to design the experiment. Three levels of each factor, which are feed composition ($X_1$), cooking time($X_2$) and ball size ($X_3$) were used (Table 3.1)

. The design was composed of three levels (low, medium and high) being coded as ( -1, 0 and +1) and a total of 17 runs were carried out. The range and levels used in the experiments are selected and listed in Table 3.1.and 3.2.

Table 3.1: Experimental Design of samples in their coded units and natural units.

| **Sample** | **Coded units$X_1$** | **$X_2$** | **$X_3$** | **Natural units $X_1$** | **$X_2$** | **$X_3$** |
|---|---|---|---|---|---|---|
| **1** | -1 | -1 | 0 | 70:30 | 60 | 10 |
| **2** | +1 | -1 | 0 | 90:10 | 60 | 10 |
| **3** | -1 | +1 | 0 | 70:30 | 120 | 10 |
| **4** | +1 | +1 | 0 | 90:10 | 120 | 10 |
| **5** | -1 | 0 | -1 | 70:30 | 90 | 5 |
| **6** | +1 | 0 | -1 | 90:10 | 90 | 5 |
| **7** | -1 | 0 | +1 | 70:30 | 90 | 15 |
| **8** | +1 | 0 | +1 | 90:10 | 90 | 15 |
| **9** | 0 | -1 | -1 | 80:20 | 60 | 5 |
| **10** | 0 | +1 | -1 | 80:20 | 120 | 5 |
| **11** | 0 | -1 | +1 | 80:20 | 60 | 15 |
| **12** | 0 | +1 | +1 | 80:20 | 120 | 15 |
| **13** | 0 | 0 | 0 | 80:20 | 90 | 10 |
| **14** | 0 | 0 | 0 | 80:20 | 90 | 10 |
| **15** | 0 | 0 | 0 | 80:20 | 90 | 10 |
| **16** | +1 | 0 | -1 | 90-10 | 90 | 5 |
| **17** | 0 | +1 | -1 | 80 -20 | 120 | 5 |

Samples (13, 14, 15, 17) are Centre points. $X_1$ -feed composition (%); $X_2$ – Cooking time(min) and $X_3$ – Ball size(g)

**Table 3.2: Independent Variable and Levels used for Box-Behnken Design ($x_1$) coded variable levels($X_1$)**

| **Variable** | **Symbol (xi)** | -1 | 0 | 1 |
|---|---|---|---|---|
| Feed Composition (%) | X1 | 90 -10 | 80 – 20 | 70 -30 |
| Cooking Time (min) | X2 | 60 | 90 | 120 |
| Ball size (g) | X3 | 5 | 10 | 15 |

Transformation of coded variables ($X_i$) levels to uncoded variables($X_1$) levels Where -1=minimum value, +1=maximum values, 0= Centre points

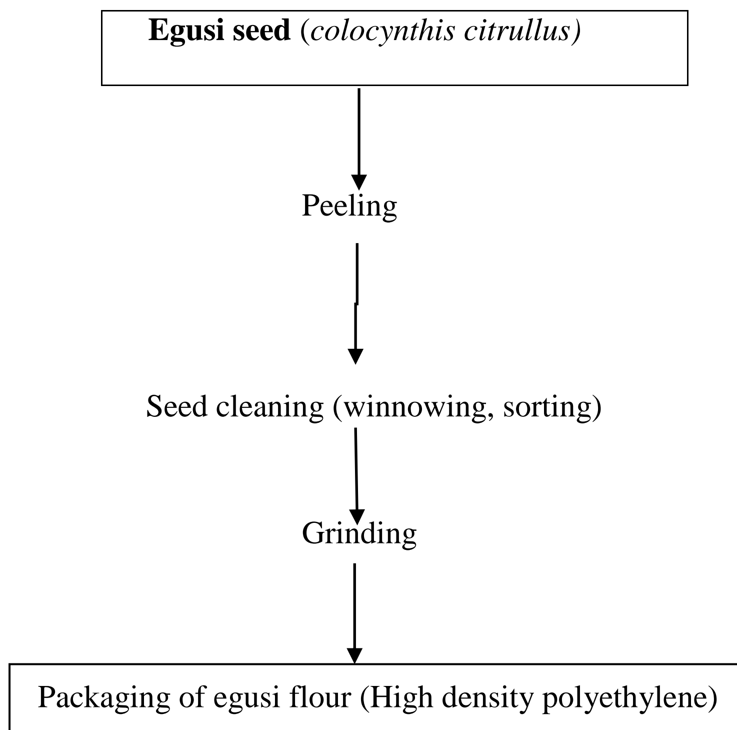


Fig 3.1 production of Egusi flour

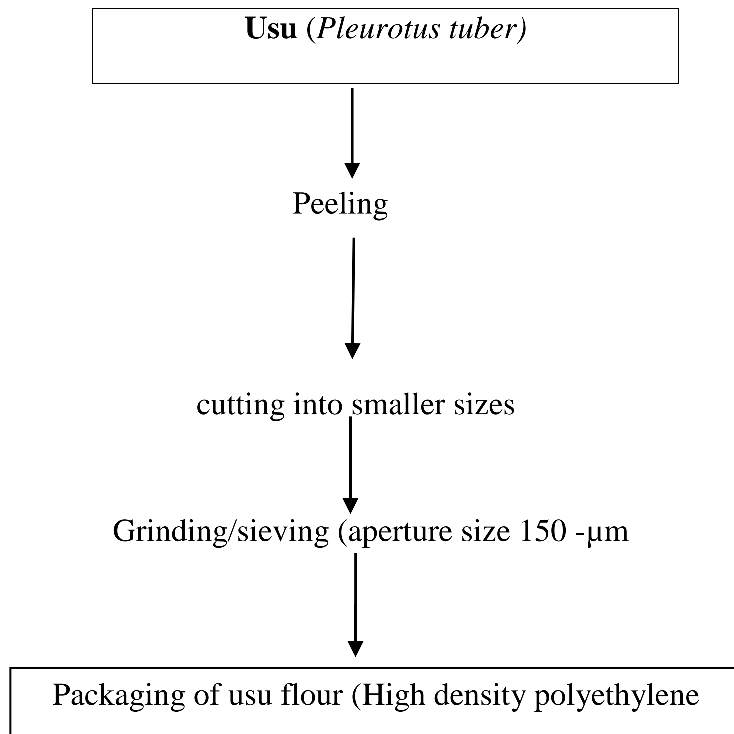


Fig 3.2 production of ‘Usu’ flour

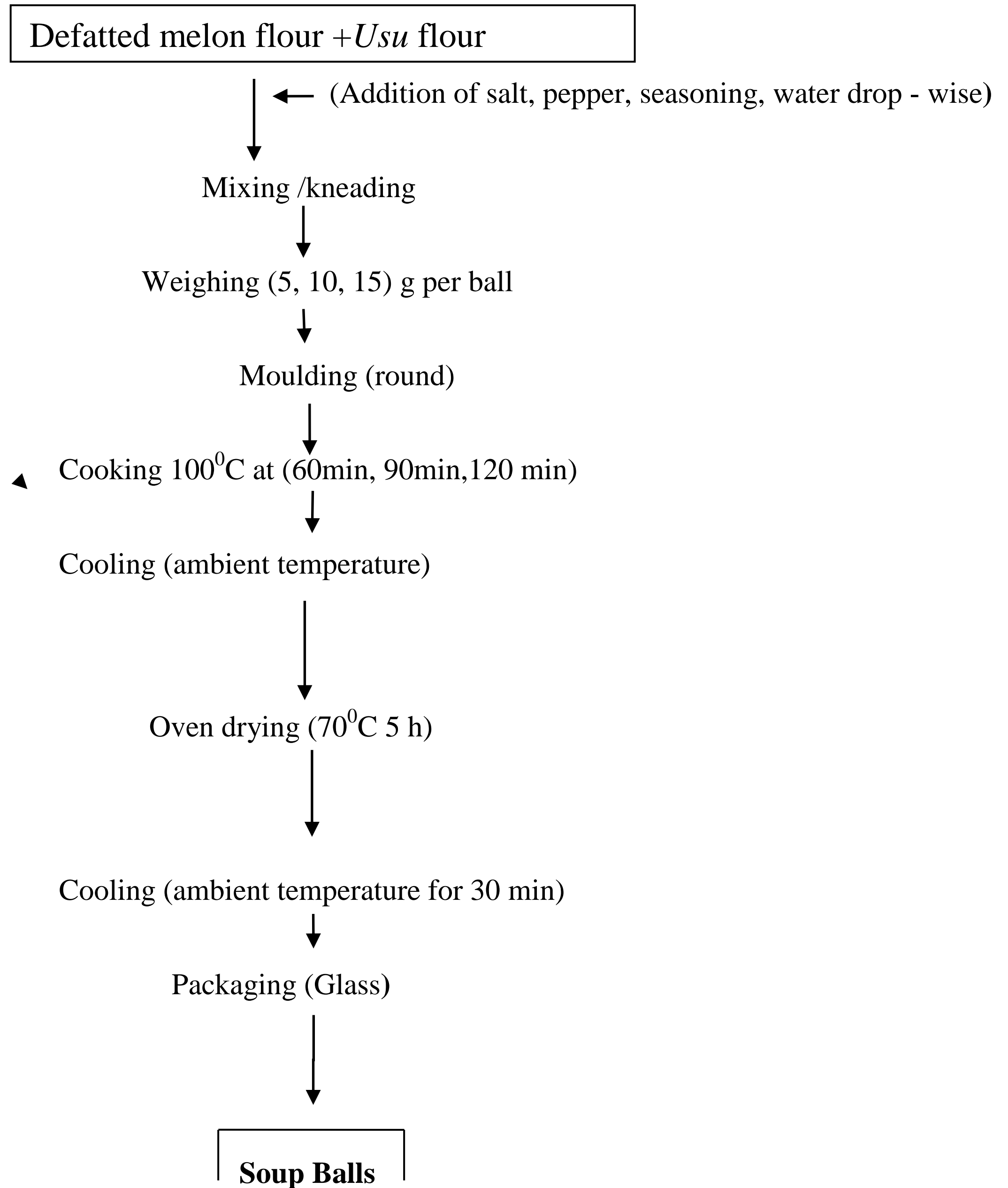


Figure 3.3: Production of soup balls

### 3.2.4 Storage of melon Soup Balls

The storage of the melon soup balls was done by the procedure of Nwakudu et al., (2015) The dried melon soup balls were aseptically picked with sterilized spatula into a sterilized glass bottle, covered, labeled and stored at ambient condition (34 $^{0}$C) for immediate and subsequent analysis. Microbial analysis was done weekly. The samples were pick from one bottle on 0, 7, 14, 21, 28 days

## 3.3 Analysis of Samples

### 3.3.1 Sensory Evaluation of soup ball

The sensory evaluation of the soup balls was conducted immediately after the production. Thirty (30) member semi-trained panelist drawn from regular consumers of the product were used for the sensory evaluation using a 9- point Hedonic scale (where 9 =like extremely and 1=dislike extremely. The panelists were trained to be objective, firm in their assessment and they were familiar with the product. The coded samples ($MS_1$ – $MS_{17}$) were served and assessed for the following attributes, colour, flavor, taste, texture and overall acceptability. Glasses of water at room temperature (34 $^{0}$C) were served to the judges to use in rinsing their mouths after each testing to avoid the taste of the preceding sample interfering with the taste of the next sample (Iwe, 2002)

### 3.3.2 Proximate Analysis

The methods of AOAC (2010) were used to determine the proximate composition of the soup balls. The parameters determined included moisture content, ash content, fat content, crude fiber content, protein content and carbohydrate content.

#### 3.3.2.1 **Moisture Content Determination**

Analytical method of Association of official Chemist moisture content of (AOAC, 2010) was used for moisture content determination by weighing into crucible 5g of sample and placed in an air drying oven at 105°C until a constant weight was obtained

$$\%moisture = \frac{ww1 - ww2}{ww2} \; x\ 100 \qquad (3.1)$$

$w_{w1}$= weight of product in wet form,

$w_{w2}$=weight of product in dry form

#### **3.3.2.2 Ash content**

Two gramme (2g) of test sample was put in a porcelain crucible and placed in temperature controlled furnace preheated to 600 $^0$C. The sample was allowed to stay in the furnace for 2 h, then transferred into desiccator, cooled, and reweighed. The percentage ash was recorded as:

$$\%(^{w}/_{w})\ ash = \frac{weight\ of\ test\ portion\ (g\ ) - weight\ loss}{weight\ of\ test\ portion\ ,(g)} x100 \qquad (3.2)$$

#### **3.3.2.3 Fat content**

To determine the fat content, Soxhlet method of oil extraction was used. Two hundred and fifty (250 ml) extraction flask was washed, dried in the oven for 30 minutes, cooled and weighed. Two grams (2g) of the sample was weighed and transferred into rolled filter paper and then placed inside the extraction thimble. The thimble was placed in the Soxhlet extractor and 300 ml of petroleum ether was poured into the weighed extraction flask. The Soxhlet extractor was connected to the flask and in turn to the condenser. The heater was switched on with the temperature not exceeding the boiling point of the petroleum ether (40 – 60°C). The

extraction process was allowed to proceed for 3 h. The ether was recovered and the thimble removed. The oil in the flask was dried at 100℃ for 1 h in the oven, cooled in the desiccator and weighed. The difference in the weight of empty flask and the flask with the oil gives the fat content of the sample. Solvent is separated from the extract(residue) by recycling

$$\% \text{ Fat} = \frac{C - A}{B} \text{ x } \frac{100}{1} \quad (3.3)$$

Where; A= weight of empty flask

B= weight of sample

C= weight of flask + oil after drying

#### 3.3.2.4 Crude fiber content

Two grams (2 g) of defatted sample was hydrolyzed in a beaker with 200mg of 1.25% $H_2SO4$ (tetraoxosulphate vi) for 30 minutes. The mixture was filtered under suction, washed with 200 ml of 1.25% NaOH. The digested sample was washed with 1%HCl to neutralize the Na0H and several times with hot distilled water. The residue collected was put into a weighed crucible and dried at $100^0$C for 2 h in an air oven. It was then cooled in a desiccator, weighed and ashed. The ash obtained was cooled and weighed. The percentage crude fiber was then calculated using the expression

$$\%\text{Crude fibre} = \frac{weight\ after\ drying - weight\ after\ ignition}{weight\ of\ sample} \text{x} \frac{100}{1} \quad (3.4)$$

#### 3.3.2.5 Crude Protein Content

The MicroKjeldahl method by Pearson (1976) was used in the determination of the crude protein content.

#### 3.3.2.6 Digestion/Distillation/Titration

**Digestion**:

Two grams 2 g of the sample was placed inside a 100 ml digestion flask and 2 g of anhydrous sodium sulphate, 1 g of hydrated cupric sulphate, a pinch of selenium powder and 10 ml of concentrated tetraoxosulphate vi were added to the flask. The moisture was placed on an electric coil heater and boiled gently at first until blacking occurred. The heating was then increased until the solution became clear and was continued for the 1 hr after the solution became cleared. The flask was then allowed to cool and the black specks rinsed down with distilled water. The contents were heated to further period until all specks disappeared. Distilled water was introduced into the flask up to the 100 ml mark and the flask was shaken thoroughly.

**Distillation:**

Steam was passed through MicroKjeldahl distillation apparatus for about 10 minutes. Five millilitres (5ml) of boric acid was placed into 250 ml conical flask and 2 drops of the indicator added. The conical flask was placed under the condenser such that the condenser tip is on the surface of the liquid. Also 5 ml of the diluted digest was placed in the distillation apparatus and rinsed down with distilled water. The cup was closed with the rod and 5 ml of 40% sodium hydroxide was added carefully to prevent ammonia from escaping. Steam was then let in for 5 min until the amount of liquid in the conical flask was about twice the volume. The boric acid indicator was titrated with 0.01M hydrochloric acid to a pinkish colour end point and the titre volume was recorded that is the volume of 0.01M hydrochloric acid that changes the indicator from green pinkish colour.

$$\% \text{ Crude protein} = \frac{Titre\ value\ x\ \ 0.14\ x\ 6.25}{weight\ of\ sample} \qquad (3.5)$$

#### 3.3.2.7 Carbohydrate Content

This was determined by difference that is, subtracting the sum of % ash, % fat, %crudefibre% moisture and % crude protein from 100%.

Carbohydrate = 100 - (%Ash + %Fat + %Crude fibre + %Moisture + %Crude protein) (3.6)

## 3.4 MICROBIAL ANALYSIS OF SAMPLES

Microbial analysis of the samples was determined using the method of (ICMSF, 2000) to determine total bacterial, fungal and coliform count in the samples. Also, biochemical test was used to characterize the isolated micro-organisms. This was done immediately after production and on weekly basis for a period of four weeks to ascertain wholesomeness of the soup balls.

### 3.4.1 Sterilization of Materials and Preparation of the Media

All materials to be used for this research were sterilized using standard techniques. Glass wares were washed with ethanol and water and sterilized in the autoclave at 120$^{o}$C for 15 min. The media (Nutrient agar and Potato Dextrose agar) that were used for this work was prepared according to manufacturer's instructions.

### 3.4.2 Preparation of Samples and Serial Dilution

Microbial isolation and identification were done by standard microbiological techniques using pour plate method. One (1 g) gram samples were homogenized in nine millilitres (9 ml) normal saline and serially diluted to $10^{-5}$.

An aliquot(0.1mL) of appropriate dilutions was inoculated in duplicate onto Nutrient agar, MacConkey and Potato Destrose Agar incubating (PDA). The inocula were spread with sterile spreader to ensure even distributions before incubating the plates. Nutrient agar and

Macconkey agar were incubated at 37±$^{0}$C for 24 – 48 h for the growth of heterotrophic bacteria and coliforms, while PDA plates were incubated at 28 ± 2$^{0}$ C for 5 days (Akinyele, and Oloruntoba, 2013). Colonies were enumerated at the end of incubation period using digital colony morphology, microscopic and biochemical characteristics to include indole production, methyl red, voges-proskauer, citrate utilization, motility, spore stain, urease production, catalase, oxidase, coagulase, starch hydrolysis, gelatin liquefaction, fermentation of glucose, lactose, sucrose, maltose, mannitol, xylose, raffinose, arabinose,temperature and salt tolerance test

#### 3.4.2.1Total Viable Count and Characterization

Pour plate method was used as described by Onyeagba (2004). The molten media was poured on the petri dishes and the samples pipetted aseptically on the agar. These were inverted and incubated at 29 $^{o}$C and 37 $^{o}$C for 96 h and 48 h for fungi and bacteria counts respectively. After solidification, colonies grow both inside and on the surface of the medium. The colonies growing inside the medium are confluent; those on the surface are used for viable counting. The colonies were counted with the use of a colony counter. The unit of measurement is cfu/ml. They were characterized after gram staining procedures using the methods of Ogbulie*et al*. (2005). Calculation of the colonies is done by multiplication of the counted number of colonies multiplied by the dilution used

#### 3.4.2.2 Coliform Test

**Presumptive test**: One milillitre (1ml) of each sample homogenate was transferred to sterile test tubes containing Lactose broth and inverted Durham tubes. Incubation was carried out for 48 h at 37$^{O}$C before tubes are checked for gas production.

**Confirmatory test**: A loop full of inocula from the gas positive tubes was streaked into Eosin Methylene Blue agar plates. Incubation was at 37 $^{o}$C for 24 h. After incubation, colonies

which showed bluish black colour with green metallic sheen and reddish/brown colonies was noted and isolated on agar slants.

**Completed test:** Colonies which formed green metallic sheen on Eosin Methylene Blue agar, was sub cultured into tubes containing lactose broth and incubated at 37°C for 24 h after which the tubes was observed for gas production.

#### 3.4.2.3 Identification of Isolates

The bacterial isolates were identified based on standard microbial methods of (ICMSF, 2000). Cultural characteristics such as shape, colour, size and consistency was carried out; Isolates was Gram stained and appropriate biochemical tests performed which include Catalase activity, Sugar utilization, Indole test, Methyl red and VogesProskauer test, Oxidase test, Motility test, Urease test and Coagulase activity.

#### 3.4.2.4 Gram Staining

A drop of distilled water was placed on a clean glass slid and suspension of the isolates was taken as especially and smeared over the slide. The smear was fixed to the slide by holding it over a Bunsen flame. Cystal violet stain was used to stain for a minute after which it was washed off by flooding with iodine for 1m washed in tap water and blotted dry. Fixed smear was further stained with safrinin (stain 2x) for 10 seconds, washed in tap water, dried and examined under oil immersion microscope to determine whether the organism is positive or negative (ICMSF, 2000).

#### 3.4.2.5 Indole Test

The isolated organism was incubated in tryptophan broth for 48 h at $37^0$C and5ml of the culture is added 0.5ml Kovac's indole reagent and then closed tube shaken gently after which

it was observed for colour formation and if any layer was formed at the surface which is an indication of indole production form tryptophan (Uzoegbu and Eke, 2001).

#### 3.4.2.6 Methyl Red Test

The isolated organism was inoculated into glucose phosphate broth and incubated for 120 h at $37^0$C. To 5 ml of culture, 5 drops of methyl red solution was added it was then observed for colour change and pH value taken to check for acidity.

#### 3.4.2.7 Voges – Proskauer (Vp) Test

The isolated organisms was inoculated into a phosphate buffered glucose peptone medium and incubate at $37^0$C for 48 h. Ethanoic solution (0.6 ml) was sequentially added to 1 ml of culture. Stopper tube was vigorously shaken while placed in slanting position. After about 40 min the formation of acetone was observed using colour change.

#### 3.4.2.8 Eijkman Test

A buffered tryptose lactose broth in tubes with inverted Durham's tube is inoculated with a culture of coliforms. It is then incubated in water jacketed incubator at $45^0$C for 48 hours. Gas production after incubation constitutes a positive test for fecal coliforms

#### 3.4.2.9 Citrate Test

A saline suspension of the growth was made from thus growth on a solid medium using a straight wire. Koser's medium was inoculated with the suspension and incubated at 37% for 48 h. The tube was examined therefore for turbidity/cloudy development.

### 3.5 Statistical Analysis

The data obtained from sensory evaluation, proximate analysis and microbial analysis of the samples were subjected to statistical analysis. Design Expert Software version 10.0.2 (Stat Soft. Inc.) was used to carry out the regression analysis. Response surface plots associated

with the results were also developed as well as response surface regressions of dependent data including ANOVA of the model. Model significance ($p<0.05$), lack of fit and adjusted regression coefficients ($R^2$ adj.) which indicated the model fitness were determined from the analysis. A quadratic polynomial regression model was assumed for predicting individual responses (Filli *et al.,* 2011). This is given by

$$Y= \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_3 x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + B_{23} x_2 x_3 \qquad (3.7)$$

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 RESULTS

The proximate composition of the soup ball samples was shown in Table 4.1. Plant protein of high-quality product was formulated as $MSB_8$ and it had the highest protein content of 37.5% which competes favourably with meat which has a protein content between (20 – 22) %. See formulation of samples in table 3.1

In order to improve the handling processes, the box Behnken design was adopted. There were 17 runs (samples), 4 center points (replicates). This design comprised of both the dependent and independent variables. The dependent variables (responses) comprises of moisture content (MC), protein (PRO), fat content (FAT), crude fiber (CF), ash content (ASH) carbohydrate (CHO) while the independent variables include feed composition, cooking time, and ball size. RSM design helped in the standardization of the processing method. This design helped to improve the processing time by giving a response of reduction in cooking time (from 24 hrs to 90 min) and it was able to give a standard recipe of feed composition of (80-20) %. Ball size of 10 g and cooking time of 90 min

Regression coefficient for significant and non-significant terms for proximate composition of the soup balls are shown in Table 4.2, it also showed the effect of the variables on the product samples. The polynomial equation comprises of the linear, quadratic and interactions. The $R^2$ of all the dependent variables, the moisture content, protein, fat, crude fibre, ash and carbohydrate are all satisfactory. $R^2$ for all the parameters are more than 75% (0.75), the lack of fit is not significant and models for each variable are significant.

Table 4.3 shows the suggested model summary statistics for the effects of variable on the proximate composition of soup balls as by the software design expert version 12 and suggestion was based on the model for prediction with their associated $R^2$, Adjusted $R^2$, standard deviation, Coefficient of variation (C.V), Adequate precision

A 3D surface plot is a three-dimensional graph that is useful for investigating desirable response values and operating condition. A surface plot contains the following elements: predictors on the x ad y-axis. A continuous surface that represent the response value on the z-axis

Table 4.4 shows sensory scores of the formulated soup balls based on these attributes (taste, aroma, appearance, texture, overall acceptability).

Table 4.5 shows the morphological and biological characteristics of isolates. The table explained the total viable counts from the samples. A total of four species of bacteria were isolated from the sample which are *Bacillus subtillis, Aspergilus niger, Aspergilus flavus, Pseudomonas aeruginosa*. Coliform was absent in all the soup ball samples. This could be attributed to the drying of the sample and the safe and hygienic condition observed in the preparation of the sample.

Table 4.6 shows the results of the microbial analysis of soup balls stored for 28 days. No coliform bacteria were found

Table 4.7 shows the bacteria count of the stored food ball in weeks

Table 4.8 shows the weekly fungal count

**Fig 4.1 -4.5** shows the 3D Response Graph

**Table 4.1: Proximate Composition(%)of the soup balls from Usu and Egusi**

| Runs | FC (g) | BS (g) | CT (min) | Moisture (%) | Protein (%) | Fat (%) | Crudefibre (%) | Ash (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 70:30 | 5 | 90 | 5.98$^{d}$ | 36.30$^{b}$ | 9.30$^{ab}$ | 4.59$^{b}$ | 4.26$^{b}$ | 39.40$^{e}$ |
| **2** | 90:10 | 5 | 90 | 7.01$^{b}$ | 34.58$^{c}$ | 8.48$^{b}$ | 3.30$^{c}$ | 4.98$^{b}$ | 41.65$^{c}$ |
| **3** | 70:30 | 15 | 90 | 7.00$^{b}$ | 34.58$^{c}$ | 8.49$^{b}$ | 3.31$^{c}$ | 4.99$^{b}$ | 41.63$^{c}$ |
| **4** | 90:10 | 15 | 90 | 8.05$^{a}$ | 34.87$^{c}$ | 8.01$^{bc}$ | 2.95$^{d}$ | 5.64$^{a}$ | 40.48$^{d}$ |
| **5** | 70:30 | 10 | 60 | 7.07$^{b}$ | 34.65$^{c}$ | 8.68$^{b}$ | 3.48$^{bc}$ | 5.00$^{ab}$ | 41.12$^{c}$ |
| **6** | 90:10 | 10 | 60 | 7.01$^{b}$ | 34.58$^{c}$ | 8.48$^{b}$ | 3.31$^{c}$ | 4.99$^{b}$ | 41.63$^{c}$ |
| **7** | 70:30 | 10 | 120 | 6.12$^{c}$ | 36.81$^{b}$ | 10.23$^{a}$ | 4.93$^{b}$ | 4.37$^{bc}$ | 37.54$^{f}$ |
| **8** | 90:10 | 10 | 120 | 5.90$^{f}$ | 37.50$^{a}$ | 10.23$^{a}$ | 6.63$^{a}$ | 4.32$^{c}$ | 35.42$^{g}$ |
| **9** | 80:20 | 5 | 60 | 7.00$^{a}$ | 34.58$^{c}$ | 8.48$^{b}$ | 3.30$^{c}$ | 4.98$^{b}$ | 41.66$^{c}$ |
| **10** | 80:20 | 15 | 60 | 5.75$^{d}$ | 36.18$^{b}$ | 9.92$^{ab}$ | 4.53$^{b}$ | 4.01$^{c}$ | 39.61$^{e}$ |
| **11** | 80:20 | 5 | 120 | 7.88$^{b}$ | 33.33$^{d}$ | 7.72$^{c}$ | 3.10$^{b}$ | 5.31$^{a}$ | 42.66$^{c}$ |
| **12** | 80:20 | 15 | 120 | 6.97$^{c}$ | 34.50$^{c}$ | 8.35$^{b}$ | 3.25$^{b}$ | 4.88$^{b}$ | 42.05$^{c}$ |
| **13** | 80:20 | 10 | 90 | 7.11$^{b}$ | 35.15$^{bc}$ | 8.79$^{bc}$ | 3.52$^{bc}$ | 5.10$^{a}$ | 40.33$^{d}$ |
| **14** | 80:20 | 10 | 90 | 7.01$^{b}$ | 34.58$^{c}$ | 8.48$^{b}$ | 3.31$^{b}$ | 4.98$^{b}$ | 41.63$^{c}$ |
| **15** | 80:20 | 10 | 90 | 7.27$^{b}$ | 33.02$^{d}$ | 7.67$^{c}$ | 3.01$^{b}$ | 5.21$^{a}$ | 43.79$^{b}$ |
| **16** | 90:10 | 10 | 60 | 7.01$^{b}$ | 34.58$^{c}$ | 8.48$^{b}$ | 3.31$^{c}$ | 4.99$^{b}$ | 41.63$^{c}$ |
| **17** | 80:20 | 10 | 90 | 7.27$^{b}$ | 33.02 | 8.48$^{b}$ | 3.01$^{b}$ | 5.21$^{a}$ | 41.63$^{c}$ |
| **LSD** | | | | 0.0027 | 0.3590 | 0.1909 | 0.0105 | 0.0836 | 0.0145 |

**Key:FC=feed composition; Bs=Ball size, CT=cooking time. Means with the same subscript in the same column are not significantly (P<0.05) different. Values are of triplicate determination**

**Table 4.2: Regression Equation Coefficient for Significant and Non-significant terms for Proximate Composition of soup balls**

| Coefficient | MC | PRO | FAT | CF | ASH | CHO |
|---|---|---|---|---|---|---|
| Linear | | | | | | |
| B0 | 6.31 | 21.29 | 10.39 | 13.72 | 2.95 | 53.9 |
| A | 3.58 | 17.61 | 9.33 | 8.97 | 2.69 | 45.08 |
| B | 0.2628 | 1.05 | 0.1800 | 0.0722 | 0.1326 | 6.73 |
| C | 0.5408 | 0.7503 | 0.7503 | 0.6105 | 0.0136 | 2.09 |
| Interaction | | | | | | |
| AB | 0.0420 | 0.3721 | 0.0002 | 0.0529 | 0.0004 | |
| AC | 0.9025 | 0.1722 | 0.1122 | 0.9900 | 0.0004 | |
| BC | 0.0025 | 0.0036 | 0.0030 | 0.0004 | 0.0002 | |
| Quadratic | | | | | | |
| $A^2$ | 0.6881 | 1.17 | 0.4918 | 2.44 | 0.1122 | |
| $B^2$ | 0.161 | 0.0545 | 0.0731 | 0.1888 | 0.0001 | |
| $C^2$ | 0.1468 | 0.0432 | 0.0014 | 0.3808 | 0.0001 | |
| $R^2$ | 0.8739 | 0.9578 | 0.9956 | 0.9478 | 0.9848 | 0.7563 |
| Adj $R^2$ | 0.7117 | 0.9578 | 0.9898 | 0.8806 | 0.9647 | 0.7000 |
| Lack of fit | < 0.0001* | <0.0001* | <0.0001* | <0.0001* | <0.0001* | |
| Model | 0.0185* | 0.0005 | <0.0001 | 0.0010 | 0.0001 | 0.0003 |

Key: A- feed composition; B-Ball size; C- cooking time; MC- moisture content; PRO-protein, Fat; CF; crude fibre; Ash; CHO * Significant at 5%level ($p<0.05$)

**Table 4.3: Model Summary Statistics for the Effects of Variable on the Proximate Composition of Soup Balls**

| Parameter | Source | $R^2$ | Adj $R^2$ | StdDev | C.V% | Adqprec |
|---|---|---|---|---|---|---|
| MC | Quadratic | 0.8739 | 0.7117 | 0.3607 | -1.0177 | 8.2678 |
| PRO | Quadratic | 0.9578 | 0.9578 | 0.3662 | 1.05 | 14.7281 |
| FAT | Quadratic | 0.9956 | 0.9898 | 0.0814 | 0.9362 | 39.9477 |
| CR | Quadratic | 0.9478 | 0.8806 | 0.3287 | 8.87 | 13.5823 |
| ASH | Quadratic | 0.9848 | 0.9606 | 0.0814 | 1.66 | 22.7141 |
| CHO | Quadratic | 0.7563 | 0.7000 | 1.16 | 2.82 | 11.7389 |

Keys: A- feed composition; B-Ball size; C- cooking time; MC- moisture content; PRO- protein, Fat; CF; crude fibre; Ash; CHO. - carbohydrate Adj$R^2$ = adjusted $R^2$; Std Dev =Standard deviation, C.V = Coefficient of variation, Adqprec =Adequate precision

**Table 4.4: Sensory Scores of Soup ball samples**

| Sample(Egusi:Usu) | Appearance | Taste | Aroma | Texture | Soup Ball size | Overall acceptability |
|---|---|---|---|---|---|---|
| MSB $_1$ (70:30) | $6.83^{b}$ | $7.23^{bcd}$ | $7.50^{ab}$ | $7.40^{abc}$ | $6.90^{b}$ | $7.56^{bcd}$ |
| MSB $_2$ ( 90:10) | $7.23^{b}$ | $6.73^{cd}$ | $6.80^{bc}$ | $7.20^{bc}$ | $6.80^{b}$ | $6.76^{cd}$ |
| MSB $_3$ (70:30) | $8.83^{a}$ | $8.56^{a}$ | $8.03^{a}$ | $7.83^{a}$ | $8.60^{a}$ | $8.93^{a}$ |
| MSB $_4$ (90:10) | $6.83^{b}$ | $6.60^{d}$ | $6.56^{c}$ | $7.20^{bc}$ | $7.13^{i}$ | $7.33^{bcd}$ |
| MSB $_5$ (70:30) | $6.77^{b}$ | $6.80^{bcd}$ | $7.66^{ab}$ | $7.63^{ab}$ | $7.03^{b}$ | $6.86^{cd}$ |
| MSB $_6$ (90:10) | $7.30^{a}$ | $6.90^{bcd}$ | $7.33^{abc}$ | $7.53^{abc}$ | $6.93^{b}$ | $7.33^{bcd}$ |
| MSB $_7$ (70:30) | $7.26^{b}$ | $7.56^{bcd}$ | $6.90^{bc}$ | $7.43^{ab}$ | $6.96^{b}$ | $7.90^{b}$ |
| MSB $_8$ (90:10) | $7.23^{b}$ | $7.20^{bcd}$ | $7.66^{ab}$ | $7.26^{abc}$ | $7.53^{b}$ | $7.16^{bcd}$ |
| MSB $_9$ (80:20) | $6.27^{b}$ | $7.33^{bcd}$ | $7.00^{bc}$ | $7.60^{ab}$ | $6.90^{b}$ | $7.56^{bcd}$ |
| MSB $_{10}$ (80:20) | $7.26^{b}$ | $7.26^{bcd}$ | $7.26^{abc}$ | $7.23^{e}$ | $7.26^{b}$ | $7.63^{bcd}$ |
| MSB $_{11}$ (80:20) | $7.20^{b}$ | $7.20^{bcd}$ | $6.83^{bc}$ | $6.90^{bc}$ | $7.16^{b}$ | $7.26^{bcd}$ |
| MSB $_{12}$ (80:20) | $6.70^{b}$ | $7.16^{bcd}$ | $7.00^{bc}$ | $6.66^{c}$ | $7.23^{b}$ | $7.73^{bcd}$ |
| MSB $_{13}$(80:20) | $7.20^{b}$ | $7.10^{bcd}$ | $7.03^{bc}$ | $6.76^{bc}$ | $7.30^{b}$ | $7.40^{bcd}$ |
| MSB14(80:20) | $7.26^{b}$ | $7.63^{b}$ | $7.10^{bc}$ | $6.86^{bc}$ | $6.70^{b}$ | $7.46^{bcd}$ |
| MSB $_{15}$(80:20) | $7.23^{b}$ | $7.16^{bcd}$ | $7.13^{bc}$ | $7.20^{bc}$ | $7.20^{b}$ | $7.80^{b}$ |
| **LSD** | **0.2622** | **0.25194** | **0.26061** | **0.2688** | **0.2599** | **0.2723** |

Mean values having different superscripts along the same column are significantly different ($P \leq 0.05$) of 30 panelists

**Key;**

Process variables codes were feed composition($X_1$) (Melon:Usu), Ballsize($X_2$), Cooking-time ($X_3$) in minutes of the melon soup ball samples produced **MSB$_1$**($X_1$)(70:30)($X_2$)(5g)($X_3$)(90mins) **MSB$_2$**($X_1$)(90:10)($X_2$)(5g)($X_3$)(90mins), **MSB$_3$**($X_1$)(70:30)($X_2$)(15g)($X_3$)(90mins), **MSB$_4$**($X_1$)(90:10)($X_2$)(15g)($X_3$)(90mins) **MSB$_5$**($X_1$)(70:30)($X_2$)(10g)($X_3$)(60mins) **MSB$_6$**($X_1$)(90:10)($X_2$)(10g)($X_3$)(60mins), **MSB$_7$**($X_1$)(70:30)($X_2$)(10g)($X_3$)(120mins) **MSB$_8$**($X_1$)(900:10)($X_2$)(10g)($X_3$)(120mins) **MSB$_9$**($X_1$)(80:20)($X_2$)(5g)($X_3$)(60mins), **MSB$_{10}$**($X_1$)(80:20)($X_2$)(15g)($X_3$)(60mins), **MSB$_{11}$**($X_1$)(80:20)($X_2$)(5g)($X_3$)(120mins), **MSB$_{12}$**($X_1$)(80:20)($X_2$)(15g)($X_3$)(120mins), **MSB$_{13}$**($X_1$)(80:20)($X_2$)(10g)($X_3$)(90mins), **MSB$_{14}$**($X_1$)(80:20)($X_2$)(10g)($X_3$)(90mins), **MSB$_{15}$**($X_1$)(80:20)($X_2$)(10g)($X_3$)(120mins)

**Table 4.5.: Morphological and Biochemical Characterization of Isolates**

| *Samples* | *Gram RXN* | *Morphological Appearance* | *Spore stain* | *Motitity* | *Catalase* | *Coagulase* | *Indole* | *Methyl Red* | *Voges Proskauer test* | *Citatrate* | *Oxidase* | *Sugar fermentation(fructose)* | *Probable microrganisms* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 2. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 3. | +Ve | Rod/Hyphae | + | - | - | - | - | - | - | - | - | - | *Bacillus subtillis, Aspergilus niger* |
| 4. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 5. | +Ve | Rod/Hyphae | + | - | - | - | + | - | + | | - | + | Bacillus *subtillis, Aspergillus niger, Aspergillusflavus, Pseudomonas* aeruginesa |
| 6. | +Ve | Rod/Hyphae | + | + | - | - | + | - | - | + | + | - | *Bacillus subtilis, Aspergillus niger, Aspergillusflavus* |
| 7. | +Ve | Rod/Hyphae | + | + | - | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 8. | +Ve | Rod/Hyphae /cocci | + | + | - | - | - | - | - | - | - | - | *Bacillus subtillis, Aspergilus niger, streptocococus sp.* |
| 9. | +Ve | Rod/Hyphae | + | + | - | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 10. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 11. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 12. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergillus niger Bacillus subtillis, Aspergilu sniger* |
| 13. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilu sniger* |
| 14. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Bacillus subtillis, Aspergilus niger* |
| 15. | +Ve | Rod/Hyphae | + | + | + | - | + | - | - | + | + | - | *Proteus, Bacillus subtillis, and Aspergillus niger* |

**Table 4.6: Microbial count of Soup Balls stored for 28days**

| Sample | Total viable ount (CFU/g) | | Fungalcount (CFU/g) | | Coliform (CFU/g) | |
|---|---|---|---|---|---|---|
| | **Zero Day** | **28 Days** | **Zero Day** | **28 Days** | **Zero Day** | **28 Days** |
| MSB $_1$ (70:30) | $3.0\times10^4$ | $2.5\times10^4$ | ND | $1.5\times10^4$ | ND | ND |
| MSB $_2$ (90:10) | $3.0\times10^4$ | $2.0\times10^4$ | $1.2\times10^4$ | $2.0\times10^4$ | ND | ND |
| MSB $_3$ (70:30) | $3.0\times10^4$ | $2.0\times10^4$ | ND | $1.3\times10^4$ | ND | ND |
| MSB $_4$ (90:10) | $3.0\times10^4$ | $2.3\times10^4$ | ND | $1.5\times10^4$ | ND | ND |
| MSB $_5$ (70:30) | $3.0\times10^4$ | $2.8\times10^4$ | $5.0\times10^4$ | $1.5\times10^4$ | ND | ND |
| MSB $_6$ (90:10) | $3.0\times10^4$ | $2.3\times10^4$ | $1.2\times10^4$ | $1.8\times10^4$ | ND | ND |
| MSB $_7$ (70:30) | $3.0\times10^4$ | $2.3\times10^4$ | ND | $1.5\times10^4$ | ND | ND |
| MSB $_8$ (90:10) | $3.0\times10^4$ | $2.5\times10^4$ | ND | $1.3\times10^4$ | ND | ND |
| MSB $_9$ (80:20) | $3.0\times10^4$ | $2.5\times10^4$ | ND | $1.3\times10^4$ | ND | ND |
| MSB $_{10}$ (80:20) | $3.0\times10^4$ | $2.3\times10^4$ | ND | $1.5\times10^4$ | ND | ND |
| MSB $_{11}$ (80:20) | $3.0\times10^4$ | $2.0\times10^4$ | ND | $1.3\times10^4$ | ND | ND |
| MSB $_{12}$ (80:20) | $1.5\times10^4$ | $2.5\times10^4$ | ND | $1.3\times10^4$ | ND | ND |
| MSB $_{13}$(80:20) | $3.0\times10^4$ | $2.8\times10^4$ | ND | $1.5\times10^4$ | ND | ND |
| MSB $_{14}$ (80:20) | $1.0\times10^4$ | $2.8\times10^4$ | $1.0\times10^4$ | $1.8\times10^4$ | ND | ND |
| MSB $_{15}$ (80:20) | $3.0\times10^4$ | $2.3\times10^4$ | $1.0\times10^4$ | $1.5\times10^4$ | ND | ND |

Process variables codes were feed composition($X_1$) = (Melon:Usu),Ball size($X_2$), Cooking time($X_3$) in minutes of the melon soup ball samples produced **$MSB_1$**($X_1$)(70:30)($X_2$)(5g)($X_3$)(90mins) **$MSB_2$**($X_1$)(90:10)($X_2$)(5g)($X_3$)(90mins)**,** **$MSB_3$**($X_1$)(70:30)($X_2$)(15g)($X_3$)(90mins),**$MSB_4$**($X_1$)(90:10)($X_2$)(15g)($X_3$)(90mins) **$MSB_5$**($X_1$)(70:30)($X_2$)(10g)($X_3$)(60mins) **$MSB_6$**($X_1$)(90:10)($X_2$)(10g)($X_3$)(60mins), **$MSB_7$**($X_1$)(70:30)($X_2$)(10g)($X_3$)(120mins) **$MSB_8$**($X_1$)(900:10)($X_2$)(10g)($X_3$)(120mins) **$MSB_9$**($X_1$)(80:20)($X_2$)(5g)($X_3$)(60mins), **$MSB_{10}$**($X_1$)(80:20)($X_2$)(15g)($X_3$)(60mins), **$MSB_{11}$**($X_1$)(80:20)($X_2$)(5g)($X_3$)(120mins), **$MSB_{12}$**($X_1$)(80:20)($X_2$)(15g)($X_3$)(120mins)**,** **$MSB_{13}$**($X_1$)(80:20)($X_2$)(10g)($X_3$)(90mins), **$MSB_{14}$**($X_1$)(80:20)($X_2$)(10g)($X_3$)(90mins), **$MSB_{15}$**($X_1$)(80:20)($X_2$)(10g)($X_3$)(120mins): ND = not detected

**Table 4.7 Bacterial weekly count (cfu/g)**

| *Samples* | *Period* | *No. of Isolates* | *Dilution Factor* | *THBC (cfu/mL)* |
|---|---|---|---|---|
| *Sample 1* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 2 | 3 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 3 | 7 | $10^4$ | 1.75x$10^4$cfu/mL |
| | Week 4 | 10 | $10^4$ | 2.5x$10^4$cfu/mL |
| *Sample 2* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 x$10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | 1.5x$10^4$cfu/mL |
| | Week 4 | 8 | $10^4$ | 2.0x$10^4$cfu/mL |
| *Sample 3* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 x$10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 3 | 5 | $10^4$ | 1.3x$10^4$cfu/mL |
| | Week 4 | 8 | $10^4$ | 2.0x$10^4$cfu/mL |
| *Sample 4* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 x$10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | 1.5x$10^4$cfu/mL |
| | Week 4 | 9 | $10^4$ | 2.3x$10^4$cfu/mL |
| *Sample 5* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | 2.0x$10^4$cfu/mL |
| | Week 4 | 11 | $10^4$ | 2.8x$10^4$cfu/mL |
| *Sample 6* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 x$10^4$cfu/mL |
| | Week 2 | 6 | $10^4$ | 1.5x$10^4$cfu/mL |
| | Week 3 | 8 | $10^4$ | 2.0x$10^4$cfu/mL |
| | Week 4 | 9 | $10^4$ | 2.3x$10^4$cfu/mL |
| *Sample 7* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 3 | $10^4$ | 5.0 x$10^4$cfu/mL |
| | Week 2 | 6 | $10^4$ | 1.5x$10^4$cfu/mL |
| | Week 3 | 8 | $10^4$ | 2.0x$10^4$cfu/mL |
| | Week 4 | 7 | $10^4$ | 2.0x$10^4$cfu/mL |
| *Sample 8* | Day zero | 1 | $10^4$ | 3.0 x$10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | 1.0x$10^4$cfu/mL |
| | Week 3 | 8 | $10^4$ | 2.0x$10^4$cfu/mL |
| | Week 4 | 10 | $10^4$ | 2.5x$10^4$cfu/mL |

| *Samples* | *Period* | *No. of Isolates* | *Dilution Factor* | *THBC (cfu/mL)* |
|---|---|---|---|---|
| *Sample 9* | Day zero | 1 | $10^4$ | $3.0x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 $x10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | $1.0x10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 4 | 10 | $10^4$ | $2.5x10^4$cfu/mL |
| *Sample 10* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 $x10^4$cfu/mL |
| | Week 2 | 5 | $10^4$ | $1.3x10^4$cfu/mL |
| | Week 3 | 8 | $10^4$ | $2.0x10^4$cfu/mL |
| | Week 4 | 9 | $10^4$ | $2.3x10^4$cfu/mL |
| *Sample 11* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 2 | 3 | $10^4$ | $1.0x10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 4 | 8 | $10^4$ | $2.0x10^4$cfu/mL |
| *Sample 12* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 $x10^4$cfu/mL |
| | Week 2 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 3 | 7 | $10^4$ | $1.8x10^4$cfu/mL |
| | Week 4 | 10 | $10^4$ | $2.5x10^4$cfu/mL |
| *Sample 13* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 3 | $10^4$ | $1.0x10^4$cfu/mL |
| | Week 2 | 5 | $10^4$ | $1.3x10^4$cfu/mL |
| | Week 3 | 9 | $10^4$ | $2.3x10^4$cfu/mL |
| | Week 4 | 11 | $10^4$ | $2.8x10^4$cfu/mL |
| *Sample 14* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 $x10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | $1.0x10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 4 | 11 | $10^4$ | $2.8x10^4$cfu/mL |
| *Sample 15* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 3 | $10^4$ | $1.0x10^4$cfu/mL |
| | Week 2 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 3 | 8 | $10^4$ | $2.0x10^4$cfu/mL |
| | Week 4 | 9 | $10^4$ | $2.3x10^4$cfu/mL |
| *Sample 16* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 $x10^4$cfu/mL |
| | Week 2 | 4 | $10^4$ | $1.0x10^4$cfu/mL |
| | Week 3 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 4 | 11 | $10^4$ | $2.8x10^4$cfu/mL |
| *Sample 17* | Day zero | 1 | $10^4$ | 3.0 $x10^4$cfu/mL |
| | Week 1 | 2 | $10^4$ | 5.0 $x10^4$cfu/mL |
| | Week 2 | 6 | $10^4$ | $1.5x10^4$cfu/mL |
| | Week 3 | 7 | $10^4$ | $1.8x10^4$cfu/mL |
| | Week 4 | 10 | $10^4$ | $2.5x10^4$cfu/mL |

**Table 4.8: Fungal Count weekly count**

| *Samples* | *Period of storage* | *Fungal count on (PDA) Isolate* | *Dilution Factor* | *TFC (cfu/g) Total fungi count* |
|---|---|---|---|---|
| *Sample 1* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | 3 colonies | $10^{4}$ | 1.75x$10^{4}$cfu/g |
| | Week 4 | 6 colonies | $10^{4}$ | 1.5x$10^{4}$cfu/g |
| *Sample 2* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | 3 | $10^{4}$ | 7'5x$10^{-4}$cfu/g |
| | Week 4 | 8 colonies | $10^{4}$ | 2.0x$10^{5}$cfu/g |
| *Sample 3* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | ND | $10^{4}$ | ND |
| | Week 4 | 5 colonies | $10^{4}$ | 1.3x$10^{4}$cfu/g |
| *Sample 4* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | ND | $10^{4}$ | 1.0x$10^{4}$cfu/g |
| | Week 4 | 6 colonies | $10^{4}$ | 1.5x$10^{4}$cfu/g |
| *Sample 5* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | 3 colonies | $10^{4}$ | 1.75x$10^{4}$cfu/g |
| | Week 4 | 6 colonies | $10^{4}$ | 1.5x$10^{4}$cfu/g |
| *Sample 6* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | 4 colonies | $10^{4}$ | 1.0x$10^{4}$cfu/g |
| | Week 4 | 7 colonies | $10^{4}$ | 1.8x$10^{4}$cfu/g |
| *Sample 7* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | ND | $10^{4}$ | ND |
| | Week 4 | 6 | $10^{4}$ | 1.5x$10^{4}$cfu/g |
| *Sample 8* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | 2 colonies | $10^{4}$ | 5.0 x$10^{4}$cfu/g |
| | Week 4 | 5 colonies | $10^{4}$ | 1.3x$10^{4}$cfu/g |
| *Sample 9* | Day zero | ND | $10^{4}$ | ND |
| | Week 1 | ND | $10^{4}$ | ND |
| | Week 2 | ND | $10^{4}$ | ND |
| | Week 3 | 3 colonies | $10^{4}$ | 7.5x$10^{4}$cfu/g |
| | Week 4 | 5 colonies | $10^{4}$ | 1.3x$10^{4}$cfu/g |
| ***Samples*** | **Period of** | **Fungal count on** | **Dilution Factor** | **TFC (cfu/g)** |

| | storage | (PDA) Isolate | | |
|---|---|---|---|---|
| *Sample 10* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | ND | $10^4$ | ND |
| | Week 4 | 6 | $10^4$ | $1.5x10^4$cfu/g |
| *Sample 11* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 2 colonies | $10^4$ | $5.0\ x10^4$cfu/g |
| | Week 4 | 5 colonies | $10^4$ | $1.3x10^{-5}$cfu/g |
| *Sample 12* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 2 | $10^4$ | $5.0\ x10^4$cfu/g |
| | Week 4 | 5 colonies | $10^4$ | $1.3x10^{-5}$cfu/g |
| *Sample 13* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 2 colonies | $10^4$ | $5.0\ x10^4$cfu/g |
| | Week 4 | 4 colonies | $10^4$ | $1.0x10^{-5}$cfu/g |
| *Sample 14* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 2 colonies | $10^4$ | $5.0\ x10^4$cfu/g |
| | Week 4 | 7 colonies | $10^4$ | $1.8x10^{-5}$cfu/g |
| *Sample 15* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 3 colonies | $10^4$ | $7.5x10^4$cfu/g |
| | Week 4 | 6 colonies | $10^4$ | $1.5x10^4$cfu/g |
| *Sample 16* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 3 colonies | $10^4$ | $7.5x10^4$cfu/g |
| | Week 4 | 6 colonies | $10^4$ | $1.5x10^4$cfu/g |
| *Sample 17* | Day zero | ND | $10^4$ | ND |
| | Week 1 | ND | $10^4$ | ND |
| | Week 2 | ND | $10^4$ | ND |
| | Week 3 | 2 colonies | $10^4$ | $5.0\ x10^4$cfu/g |
| | Week 4 | 7 colonies | $10^4$ | $1.8x10^4$cfu/g |

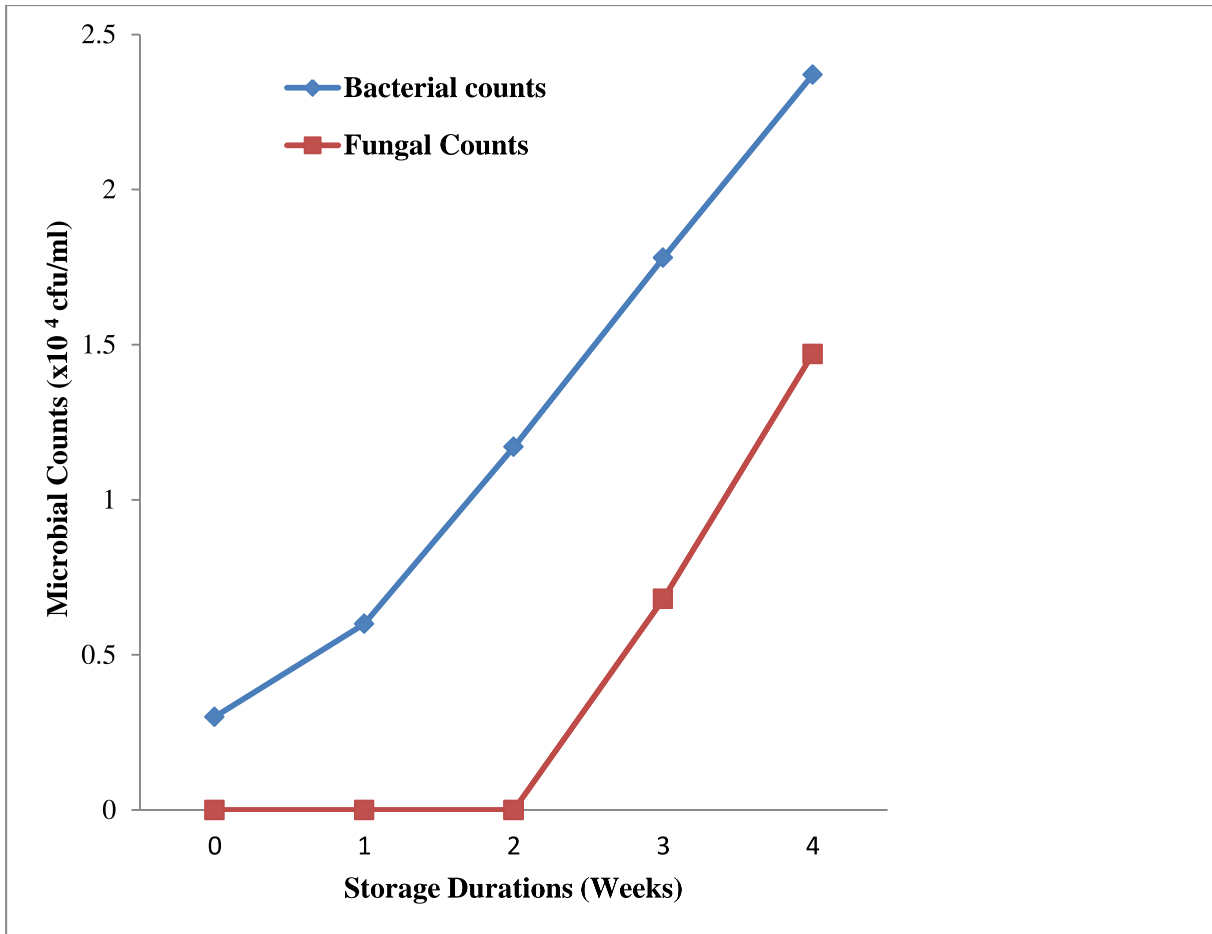


**Fig 4.1Microbial counts (x $10^5$ cfu/ml) of soup ball as affected by storage duration**

`+-

Factor Coding: Actual

**Moisture (%)**

Design Points:

Above Surface

Below Surface

5.75 8.05

X1 = A: FEED COMPOSITION

X2 = B: BALL SIZE

**Actual Factor**

C: COOKING TIME = 90

Factor Coding: Actual
Moisture (%)
Design Points:
Above Surface
Below Surface
5.75 8.05
X1 = A: FEED COMPOSITION
X2 = B: BALL SIZE
Actual Factor
C: COOKING TIME = 90

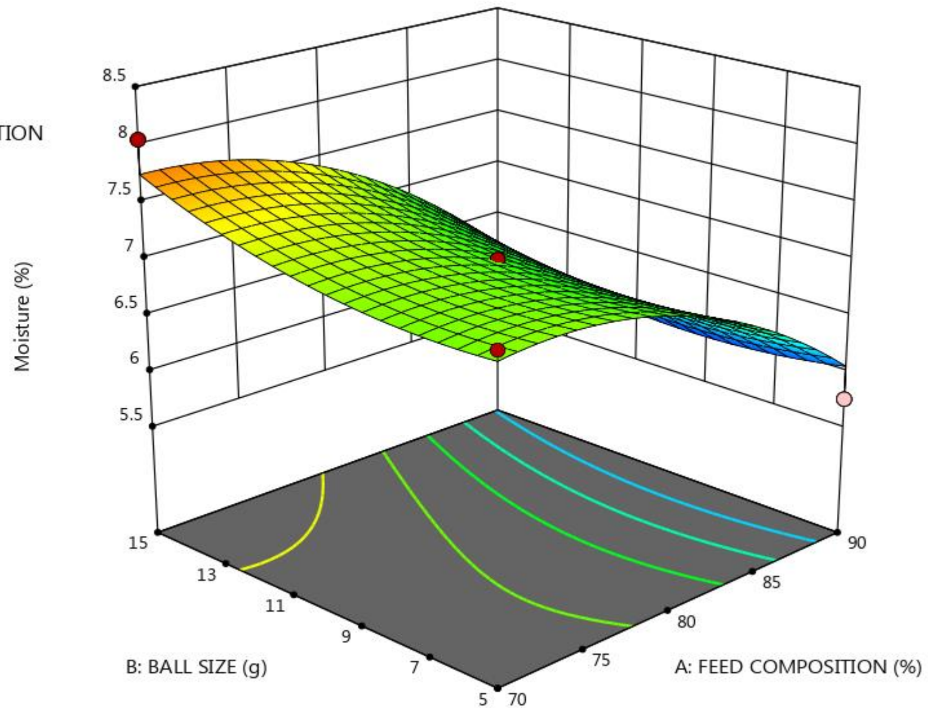


**Fig 4.2 Effect of feed composition and ball size on moisture content**

As feed composition increases, moisture content decreases. As ball size increases, there is no significant effect on moisture content

Design-Expert® Software

3D Surface

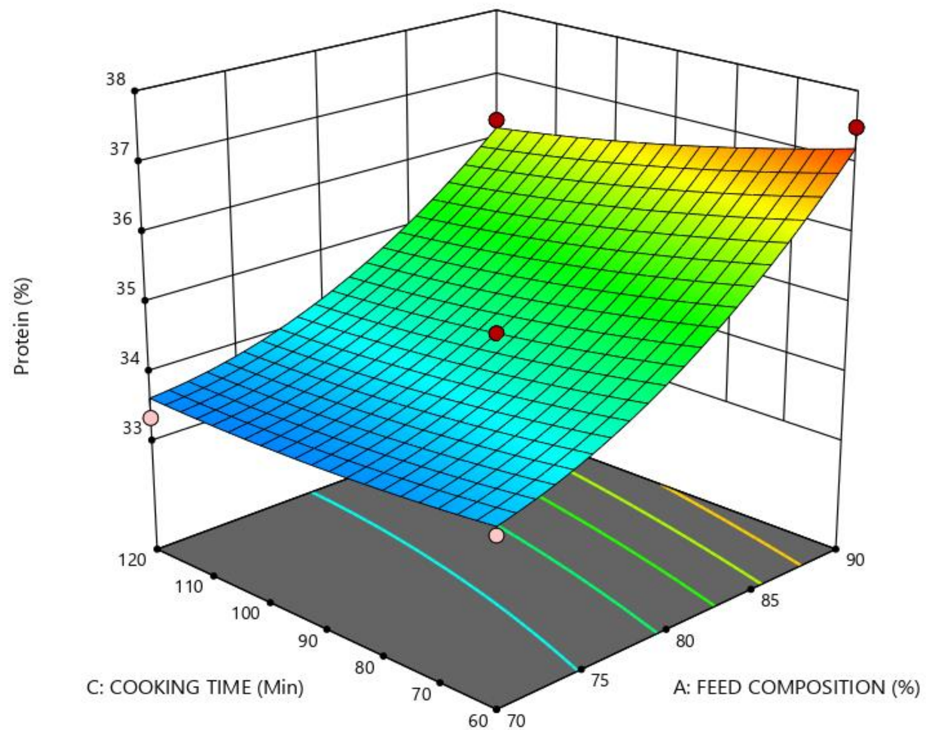


**Fig 4.3 Effect of feed composition and cooking time on protein content**

As feed composition (percentage of egusi in the sample) increased, protein content also increased

A 3D surface plot is a three-dimensional graph that is useful for investigating desirable response values and operating condition. A surface plot contains the following elements: predictors on the x ad y-axis. A continuous surface that represent the response value on the z-axis

Design-Expert® Software

3D Surface

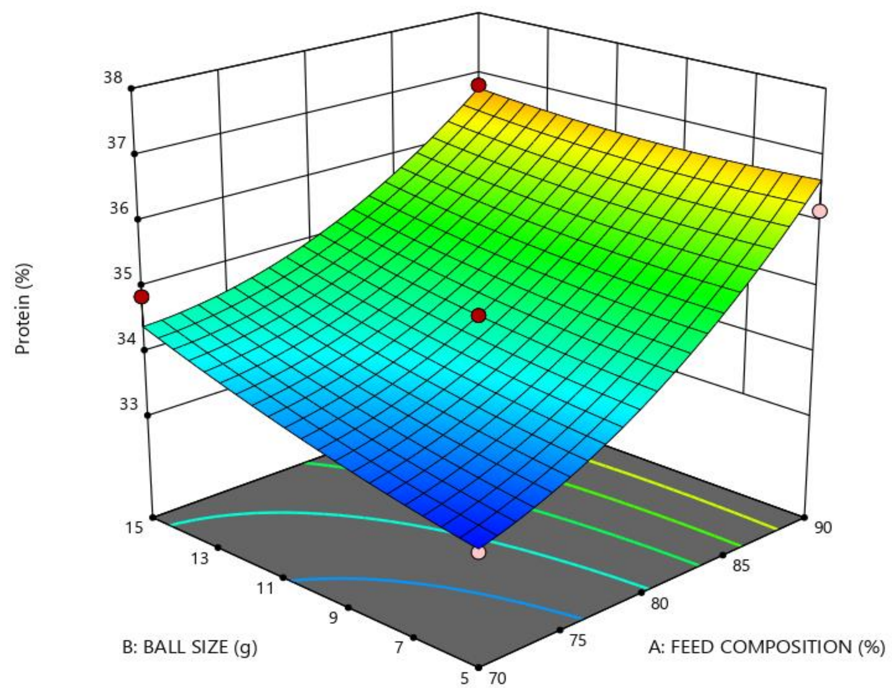


**Fig 4.4 Effect of feed composition and Ball size on the protein content of soup Ball**

As feed composition increased, protein content increased as well but increase in ball size had no significant increase on protein content

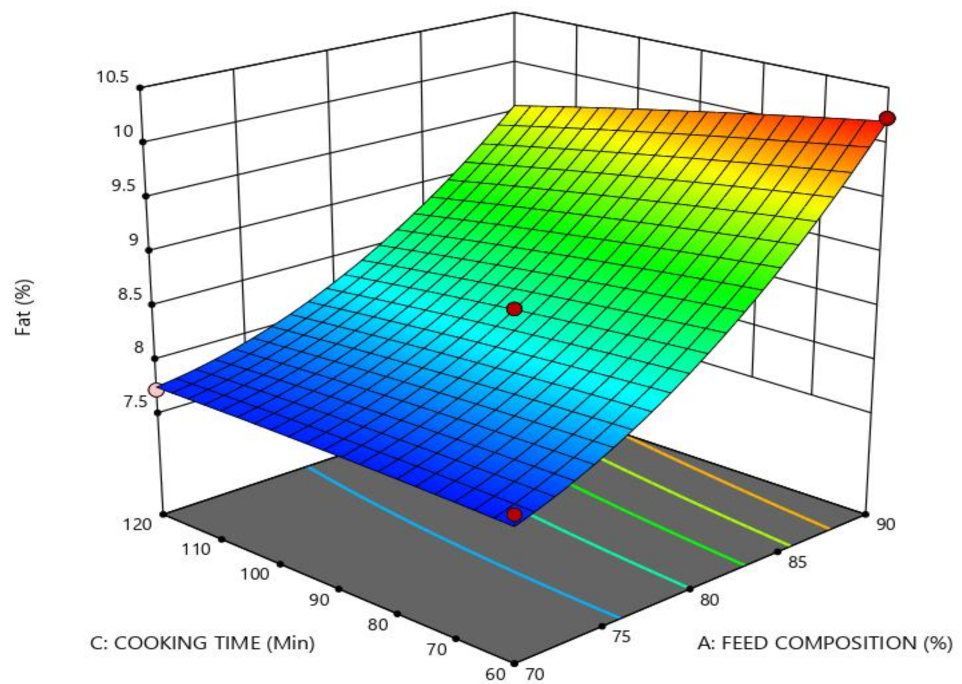


**Fig 4.5: Effect of feed Composition and cooking time on Fat content of soup Ball**

Increase in cooking time has slight decrease on fat content but fat content increased as the feed composition (melon) increased.

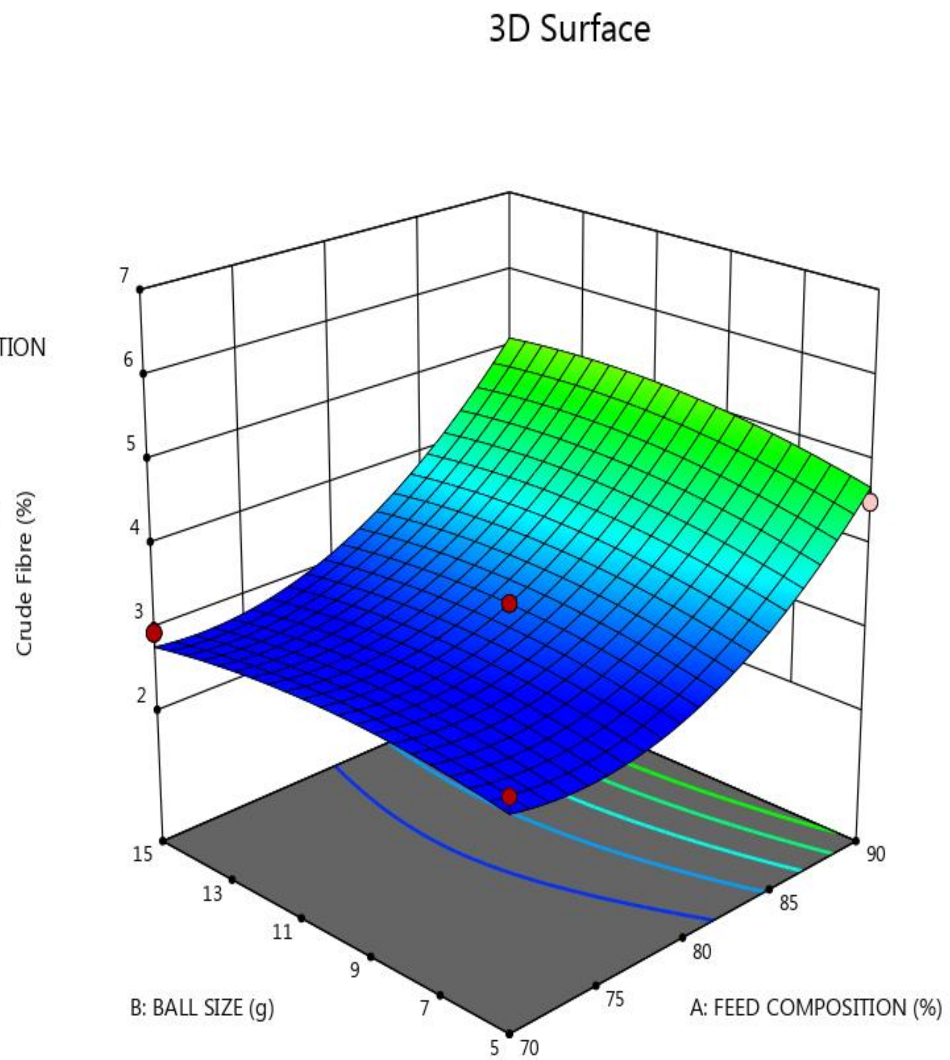


**Fig 4.6: Effects of Feed Composition and Ball size on the Crude Fibre**

As feed composition increased, crude fiber of the soup ball increased

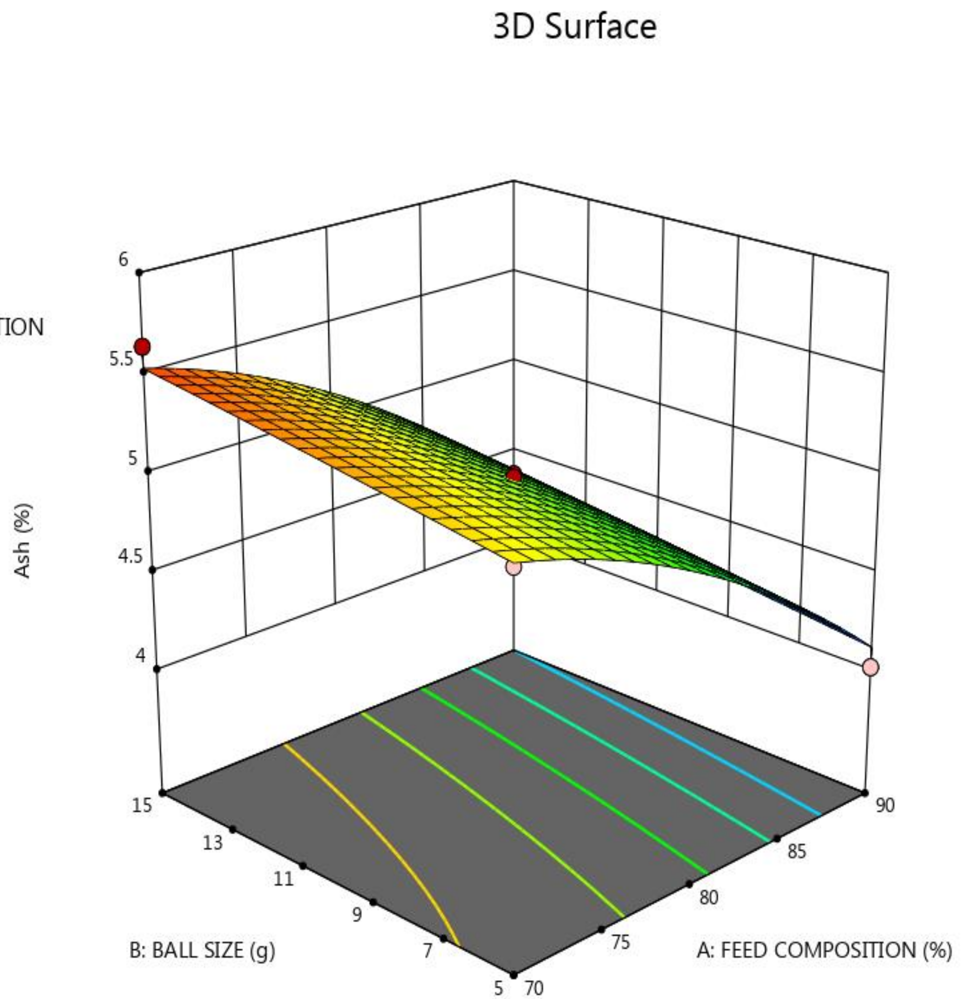


**Fig 4.7:Effect of feed Composition and ball size on Ash content of Soup Ball**

As the feed composition increased, the ash content decreased. High ash content indicates a substantial amount of inorganic minerals in a product

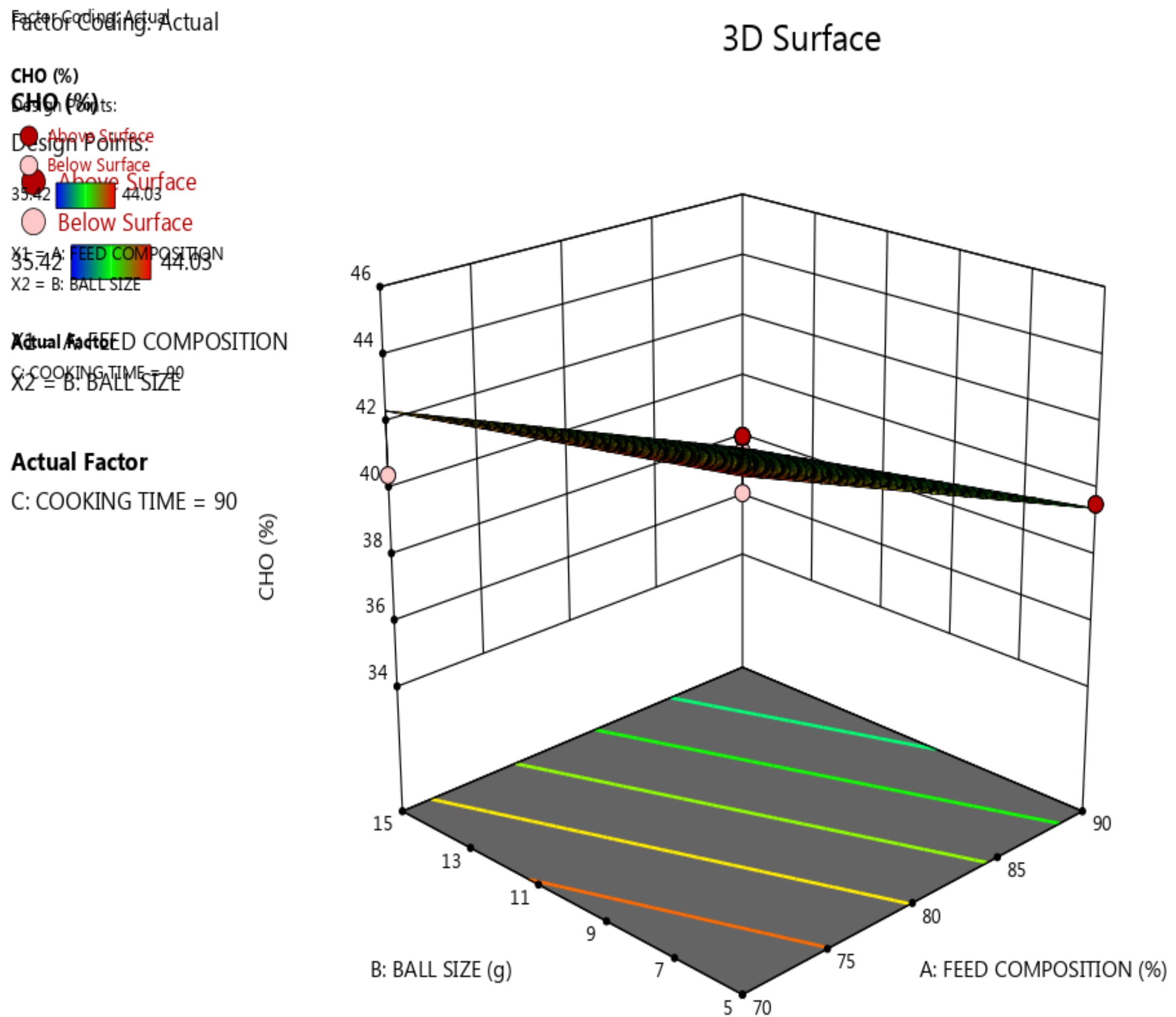


Fig 4.8: Effect of Feed Composition and Ball size on carbohydrate content of soup ball

There is no significant effect of feed compositions and ball size on carbohydrate content

## 4.2 DISCUSSION

### 4.2.1 PROXIMATE COMPOSITION

Table 4.1 represent the proximate composition of soup ball made from three levels each of feed composition, cooking time and ball size.

#### 4.2.1.1 Moisture content

The moisture content of the melon soup ball samples determined ranged from 5.75 to 8.05% as shown in Table 4.1. Heat treatment could lead to desirable or undesirable modification or changes in structure of food (Dominguez, *et al.,*2014). Dehydration or drying of food reduces the moisture content which supports microbial growth. Moisture content of food material is a key factor influencing the quality of storage thereby reducing post – harvest loss.

**Table 4.2** shows the estimated Surface regression coefficient for moisture of soup ball made from different process variables. Regression model analysis produces equation where the coefficients represent the relationship between each independent variable and the dependent variable. Regression equation is used to make predictions and model multiple independent variables. The values obtained showed that these responses were adequately described by the factors in the polynomial model. There were significant ($p<0.05$) differences in the linear effect (A), and the interaction between feed composition and cooking time (AC)

From Table 4.3, the model was significant ($p< 0.05$) and the result of the polynomial after removing non-significant terms in Table 4.2 gave

$$MC = 6.31 +0.042AB + 0.0025BC \quad (4.1)$$

The Response Surface plot in **Fig 4.1** showed that as cooking time increased, the moisture content decreased slightly, and as feed composition increased, there is a corresponding linear

decrease in moisture content. Fig 4.2 showed that as ball size increased, there was no significant effect on the moisture content.

Table 4.3 shows the estimated surface regression coefficient for moisture content. The coefficient of determination ($R^2$) of the model was 87.3% which indicated that the model adequately represented the real relationship between the variables under-review. The coefficient of variation (C.V) obtained was 5.29%. The $R^2$ indicated that 87.39% of the variability was explained by the model and 12.61% was as a result of chance or accounts for factors not included in the model. It was also observed that for every cooking time and feed composition the moisture content was affected by 0.042, while the effect on combination of ball size and cooking time was 0.0025. The low moisture content indicated low microbial activity hence long shelf life as this is in agreement with Omeire *et al,* (2019).

#### 4.2.1.2 Protein content

Protein content of the melon soup ball protein ranged from (33.02-37.5) %., as shown in Table 4.1. In Fig 4.3, the result of the protein content of the samples showed that protein content in the soup ball samples increased as feed composition (A) (egusi/usu) in the samples increased. The protein content of the soup balls decreased slightly with increased cooking time which showed that denaturation of protein might have occurred as reported by (Uzozie, 2018). Fig. 4.4 showed that ball size has no significant effect on the protein content. The high protein content in the sample 8 in Table 4.1(90:10 feed composition, 10g ball size, 120 min cooking time) with 37.5% protein could be attributed to higher quantity of melon meal toUsu feed composition as reported by Ifegbo, (2015). This is in tandem with the reports of other researchers that melon seed are rich in oil and protein.(Kpikpi et al.,2009). Protein is necessary for proper immune function, cell structure, growth and replenishment of lost body tissues (Obasi, 2000).

The values obtained (eqn.4,2) from protein content showed that these responses were adequately described by the factors in the polynomial model. The samples were significantly (($P<0.05$) different in the quadratic effect on Ball size (B) and cooking time (C), square of cooking time($C^2$). The coefficient of determination $R^2$0.9578 (95.78%) which measures how a model predicts or explains an outcome in the linear regression setting was high. The model from the table was significant ($P<0.05$) and the result of the polynomial after removing the non-significant terms gives.

**Protein content = 21.29 + 0.042AB + 0.04$C^2$ (4.2)**

The response surface plot in Figure (4.2) indicated a progressive linear increase in the protein content as the feed composition increases from 70 to 90 % for egusi. The coefficient of determination ($R^2$) of the model was 0.95% which indicates that the model adequately represented the real relationship between the variables under consideration. The $R^2$ value indicated that 95.78% of the variability was explained by the model and only 4.22% was as a result of chance or account for factors not included in the model.

The equation (4,2) in terms of coded factors could be used to make predictions about the responses for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The protein content which ranged from 33.02-37.5 % were within the Daily Recommended Intake (adult) which is 0.36grams of protein per pound or 0.8grams per kg of weight according to World Health Organization (2007).

### 4.2.1.3 Fat content

The fat content of the melon soup ball samples determined ranged from 7.67 to 10.23 % as shown in Table 4.1.In Fig 4.5 the result of the fat content of the samples showed that fat content in the soup ball samples increased as feed composition (A) increased, that is the

percentage of egusi in the samples, but decreased with increasing cooking time (C).The decrease in fat content of the samples could be attributed to prolonged cooking time of 120 minutes resulting in melting of the fat globules in the soup ball samples. According to Nzelu, (2010) increase in temperature of a food material during processing increases the yield of fats and oil, from oil bearing seeds such as melon during processing, thus resulting in decrease in fat/oil content in the soup ball samples. Fat content of any food sample aids in the improvement of mouth feel and flavor of food. It helped to improve the texture of the soup ball samples which increases consumers demand (Abiodun and Adeleke, 2010). Fig 4.5 showed that ball size has no significant effect on fat content. Screw oil expeller was used to defat the egusi flour which resulted to residual oil in the egusi flour.

Table 4.1, values obtained showed that the responses were adequately described by the factors in the polynomial model. There were significant ($P<0.05$) differences. In the quadratic and linear effect, feed composition (A) interaction between cooking time(C) on fat content were significant ($P<0.05$) on the result of the polynomial after removing non-significant terms, the model is as shown in eqn.(4.3)

$$\text{Fat content} = 10.39 + 0.0002AB + 0.003BC + 0.0014C^2 \qquad (4.3)$$

**Table 4.2** shows the estimated surface regression coefficient for the fat content. The coefficient of determination ($R^2$) of the model was 99.56% which indicated that the model adequately represented the real relationship between the variables under-review. The co-efficient of variation (C.V) obtained was 0.93%.

The graph (Figure 4.5) showed that as the percentage of feed composition of the melon soup balls increased the percentage fat content of the melon soup balls increases.

The correlation of the processing variables feed composition, ball size and cooking time showed that there was significant($P<0.05$) difference on the fat content of the samples at a

correlation value of 0.946. The correlation graph of fat content against feed composition showed that feed composition of the samples (70, 80, and 90%) increased fat content.

On the effect of cooking time on the fat content of the samples, the study revealed that as the cooking time (60, 90 and 120 min) increased, fat content of the soup balls decreased, because increase in cooking time could lead to leaching of oil. Also as the moisture, ash and carbohydrate decreased in the samples, fat content of the soup balls increased. This could be attributed to increase in the composition of melon quantity which is higher than that of usu portion as shown in the composition table 3.1

#### 4.2.1.4 Crude Fibre

Crude fibre content of the soup ball samples ranged from 2.95 to 6.63%. There were significant($p \leq 0.05$)differences in the crude fibre content of the samples, which could be attributed to the presence of "Usu". Presence of fibre in meal helps in the removal of waste products from the body, thereby preventing constipation and many health disorders (Obasi, 2000). Fibre in one's diet also plays a key role in preventing cancer of the large intestine commonly called colon cancer (Olusanya, 2008). Fibre aids in lowering blood cholesterol level and slows down the process of absorption of glucose; thereby helping in keeping blood glucose level in control (Anderson *et al.,* 2009). It also ensures smooth bowel movements and thus helps in easy flushing out of waste products from the body, increases satiety and hence imparts some degree of weight management (Omah and Okafor, 2015). As ball size and feed composition increased, crude fibre increased to an extent and decreased slightly at larger ball size. This could be attributed to the effect of heat on particle size of the product. The model from the Table 4.2 was significant ($P<0.05$) and the result of the polynomial after removing the non-significant terms gives.

**Crude fiber = 13.72 + 0.004BC** **(4.4)**

The model explained that interaction of ball size and cooking time has significant effect on the fibre content. As the feed composition increased, the fibre content increased, and as ball size increased, the fibre content increased slightly

The equations in terms of coded factors can be used to make predictions about the responses for given levels of each factor. The coded equation is for identifying the relative impact of the factors by comparing the factor coefficients.

#### 4.2.1.5Ash Content

Ash content of the soup balls ranged from 2.95% for to 6.63%. Fig 4.7 showed that as feed composition increased the ash content decreases. Ash content shows the level of minerals present in a particular food sample. Some of the minerals identified in melon include magnesium, calcium, potassium, zinc, manganese and phosphorous. The ash contents of the samples were significantly ($p \leq 0.05$) different). Also, the ash content values obtained in this study are higher when compared to the range 0.64% to 0.92% reported by (Adedoke, 2003) The difference might be due to the specie of egugi used and percentage of other proximate content of the defatted egusi. Ash content measures mineral composition of food samples and gives an indication of the mineral in a food sample (Nzelu, 2010). The model from the Table 4.2 ($p \leq 0.05$) was significant. In this case, A, B, $A^2$ are significant and the result of the polynomial after removing the non-significant terms gives

$$\textbf{Ash content} = 2.95 + 0.0136C + 0.0004AB + 0.0004AC + 0.0002BC + 0.0001B^2 + 0.0001C^2 \quad \textbf{(4.9)}$$

The graph (fig.4.7) showed that as the ball size of the melon soup balls increased in grams, there was significant ($P<0.05$) increase in ash content and as the feed composition increased, the ash content decreased.

There were significant lack of fit ($P<0.05$) and the coefficient of determination was 0.9848. The $R^2$ value indicated that 98.48% of the variability was explained by the model and only 1.52% was as a result of chance or account for factors not included in the model.

#### 4.2.1.6 Carbohydrate content

Carbohydrate content of a sample aids in determination of energy content of the food. The carbohydrate content of the soup ball samples ranged from 35.42 to 44.03%. Fig 4.8 showed that as feed composition increased, there is no significant increase in carbohydrate content but as ball size increases there is there is a corresponding increase in carbohydrate content. The proximate composition (%) in Table 4.1 showed significant ($p \leq 0.05$) differences in the carbohydrate content of the samples. The graph showed that as the ball size of the melon soup balls increases, there is significant increase in the carbohydrate content, but increase in feed composition has no significant effect on carbohydrate content

### 4.2.2 SENSORY SCORES

#### 4.2.2.1 Appearance

The appearance of the soup balls samples analyzed (Table 4.4) ranged from 6.27 for $MSB_9$ (80:20) to 88.3 for sample $MSB_3$ (70:30). Appearance is an important sensory attribute of any food because of its influence on acceptability among consumers (Falola, 2012).

#### 4.2.2.2 Taste and Aroma

The taste of the sample $MSB_3$ was significantly($P<0.05$).different. Taste of the sample which was perceived through the tongue ranged from 6.60 for sample $MSB_4$ to 8.56 for sample $MSB_3$ (70:30). The sample $MSB_3$ had the highest value in terms of the taste which could be attributed to the sample blends. According to Ayodele (2003), the feed composition and ingredients used during food preparation help in improving the taste of food samples.

Aroma of soup ball samples which was perceived through sense of smell ranged from 6.56 for sample $MSB_4$ to 8.03 for sample $MSB_4$. The aroma of the samples were significantly ($P<0.05$). different This might be attributed to the sample blend, cooking time and feed composition (Adeniyi, 2008).

#### 4.2.2.3 Texture

Texture assessment of these products was governed by surface response of touch in mouth during mastication a**n**d o**n** individual perception of huma**n** subject which vary among them

The texture of the samples were significantly ($P<0.05$) different. Texture of the samples ranged from 6.66 for sample $MSB_{12}$ to 7.80 for sample $MSB_3$and ranged from 6.70 for sample $MSB_{14}$ to 8.60 for sample $MSB_3$. The mean value of texture assessed by the panelists on sensory evaluation of the product was 7(seven)

#### 4.2.2.4 Overall acceptability

The overall acceptability ranged from 6.76 for sample $MSB_2$ to 8.93 for sample $MSB_3$. This could be attributed to the high value of sample $MSB_3$ in terms of appearance, taste, texture, soup ball size and overall acceptability. Also the sample $MSB_3$ was significantly ( $P \leq 0.05$) different compared to other samples analyzed.

### 4.2 Microbial Status of the Soup Balls stored for 28days

The microbial status of soup ball samples preserved for 28 days is presented in Table 4.6. From the result, the total bacterial count of the soup ball samples for day zero ranged from 3.0 $x10^4$Cfu/g($MSB_1$ etc) to $1.5x10^5$Cfu/g for $MSB_{12}$. The sample $MSB_{12}$ had the highest bacteria count. The melon soup balls ($MSB_5$, $MSB_{13}$ and $MSB_{14}$) had the highest total bacterial count at Day 28 with2.8 $x10^5$Cfu/g among the samples. The values were below the maximum number 8.0 $x10^5$Cfu/g of micro-organism affecting cooked food samples as reported by (Frazier and Westhoof, 2004). According to Henshaw (2002), bacteria are the most important and troublesome of all the microorganism for food processor. However, the result showed that the samples were prepared under hygienic condition with wholesome raw materials and therefore safe for consumption. The bacteria isolated were *Bacillus subtillis,, Pseudomonas aeruginosa* while *Aspergillus niger* and, *Aspergillus flavus* were the fungi found.It could be deduced from the result that these microrganisms were associated in traditionally processed foods(Frazier and Westhoof, 2004; (Obadoni, and Ochuko,2001). The fungal count recorded in the samples ranged from 5.0 $x10^4$Cfu/g to $1.2x10^5$Cfu/g for sample $MSB_2$ and $MSB_6$. The microbial status on total bacterial and fungal counts revealed that sample $MSB_3$ had the least microbial and fungal count

The storability of melon soup ball prepared is dependent on microbial quality of water, the packaging material and storage conditions such as humidity, temperature and levels of moisture in the soup balls (Frazier and Westhoff, 2004). Coliform was absent in all the soup ball samples thus making the product safe for human consumption (Gadada, 2004). Since coliforms are common inhabitants of the intestinal tract, their presence in food might indicate fecal contamination (Yusuf and Carlstrom, 2003). Hence their absence could mean hygienic processing of the soup balls.

The morphological characteristics of the isolates carried out showed that the bacteria isolated were gram positive, rod shaped while the fungi have hyphae. The bacterial isolates were Gram positive(Roberts and Greenwood, 2003) They retained the colour of the primary dye(crystal violet). This means that the thickness of their peptidoglycans rigidly adhered to their cell walls as such they were not decolorized by alcohol (acetone).

**Morphological and Biochemical characteristics of Isolate**

From the morphological and biochemical characterization, the isolates were majorly rod-like having hyphae. It is advised that the Food processors should reduce potential problems from microorganism by minimizing contamination from environment and equipment as well as proper sanitization (Falegan, 2011)

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

It has now been established that soup ball can be produced by varying its feed composition, with acceptable ball size and reduced cooking time without compromising its quality.Soup ball sample three (3) gave the highest sensory property with feed composition of (70:30) %, Ball size of 15g and cooking time of 90min.This could be attributed to portion of usu – melon ratio where the quantity of usu is higher in sample three(3) than the other ratios. Usu acts as binder and also as a bulk agent. It has 'umami' flavor that contributed to the good sensory property. The proximate composition of soup balls showed that they are rich source of nutrient. Moisture content of the product ranged from 5.57 to 8.05 %.Soup ball (sample ten ($MSB_{10}$ ) had the least moisture content of 5.75%. Drying the food reduced the amount of moisture available to support microbial growth, thereby increasing shelf life. The result showed that the moisture content was within acceptable limit. The fat content of soup ball ranged from (7.67-10.23) %. The fat content is low due to defatting of the melon seed ball before production. Seeds of melon contain up to 50% oil. Increase consumption of fatty food may promote hypertension; therefore, soup ball produced is healthy for consumption.

Protein content of the soup ball ranged from 33.02 – 37.5%. Soup ball sample eight ($MSB_8$) gave the highest protein content of 37.5%. However, it was discovered from Response Surface graph that protein content of soup ball increased with increasing feed composition (Egusi/Usu) but decreased lightly at elevated temperature. It is possible that at this elevated temperature, denaturation occurred. Denaturation leads to conformational change and loss of solubility due to exposure of hydrophobic groups. Proteins are building blocks and our body requires protein every day to repair worn out tissues. Plant proteins are healthier than animal

protein because meat contains saturated fat and cholesterol and this could cause heart disease while Plant proteins are typically low in saturated fat.

The variables that gave the best response as suggested by Response Surface methodology (RSM) were found to be 10g ball size, feed composition 80 – 20% and cooking time of 90 min. During production of soup ball, some ingredients(melon and Usu) were used to provide variety and improve cooking properties and sensory characteristics, increase stability and reduced production cost. Soup ball sample ($MSB_3$) gave the highest sensory property with feed composition of 70:30, ball size of 15g and cooking time of 90mins. The heat treatment during product preparation provides physical, chemical and structural changes to its components by the effect of heat.(Borba*et al.,*2013) and can change the quality and yield due to the composition of product

The soup ball samples were able to store for 28days. Factors such asmoisture content, packaging material and storage conditions (humidity, temperature and levels of moisture in the soup balls) contributed immensely to the storability.The microbial status carried out on soup ball showed that the product stored for 28 days and remained safe. Coliform was absent in all the soup ball samples thus making the product safe for human consumption. The absenceof coliform from the samples indicates good sanitary post processing condition and their absence contributes to delay in food spoilage.

The Response Surface Plots results showed that protein content increased with increasing feed composition(melon).due to the fact that, melon seeds are known for their richness in protein. Ball size generally has no significant effect on the overall quality.

## 5.2 Recommendation

All the egusi soup balls formulated had high protein content and could be used as meat substitute.

Sample eight ( $MSB_8$) hadthe highest protein content of 37. 50%.The relatively higher value in nutritional composition reported for melon soup ball sample ($MSB_8$)could be used to augment the nutritional need of man recommended Sample $MSB_3$ could also be recommended for good sensory property in terms of texture and overall acceptability. Feed composition of 80-20%, Ball size of 10g and cooking time of 90 min would give a standard recipe. Soup ball is a rich nutrient source that could be used to augment the nutritional need of man

Further work is recommended on the effect of other processing variables such as varying temperature, egusi particle size and use of different oil extraction methods to defat the egusi on quality of soup balls.

### 5.3 Recommendation to knowledge

Soup ball which was traditionally cooked overnight could now be produced within 90 min. The existing technique was laborious and was not cost effective. Response surface methodology (RSM) was effective for estimating the effect of three independence variables. The model equation developed could be used for predicting proximate composition and importance of process variable on soup ball could be ranked in the following order: feed composition ($X_1$) > Cooking time ($X_2$) >Ball size ($X_3$). This means the feed composition has the highest effect on the product followed by cooking time and lastly by ball size.

Regression models were established for the proximate composition results obtained which could be used to predict responses in further studies. Regression model was used to describe the relationship between a set of independent variable ad dependent variable. It was used to make prediction. Regression model was used to model multiple independent variable The coefficient of determination $R^2$ were all high above 85% in the parameters determined which indicated that the models were adequate.

Microbial count showed that soup ball could store for at least 28days without presence of diarrhea causative organism like Escherichia coli and it remained safe for consumption. Cooking method was used during processing and the product were dried at $70^0C$

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# APPENDIX

**SENSORY EVALUATION QUESTIONAIRE**
**FEDERAL UNIVERSITY OF TECHNOLOGY OWERRI**
**DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY**
Name of panelist (optional) ……………………………………….. Time: ………
Address of panelist: ………………………………………………………..……..

Product**: INSTANT SOUP BALL**
**Instruction(s)**
Dear panelists, you are provided with fifteen (15) instant soup ball samples. Please, you're required to indicate the degree of your likeness or dis-likeness on the under listed sensory attributes using assigned numerical values ranging from 9-points for extremely good to 1 for extremely poor.

| **Degree** | **Point** |
|---|---|
| Extremely good | 9 |
| Very good | 8 |
| Moderately good | 7 |
| Good | 6 |
| Neither good nor poor | 5 |
| Poor | 4 |
| Moderately poor | 3 |
| Very poor | 2 |
| Extremely poor | 1 |

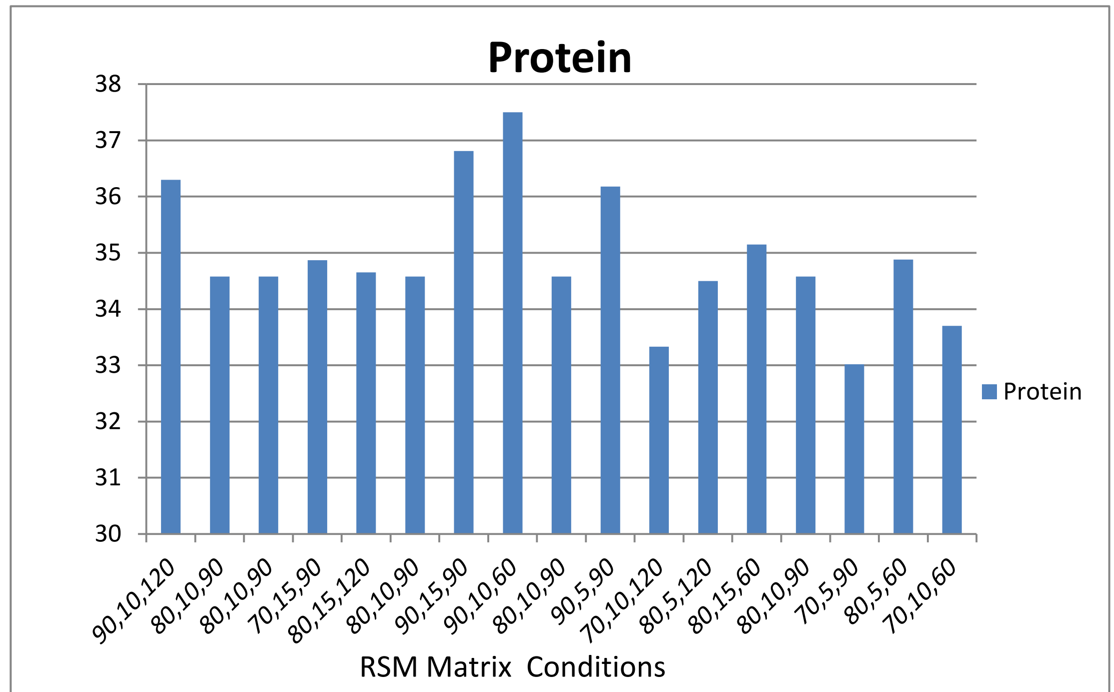
**Samples**

| **Attribute** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Colour | | | | | | | | | | | | | | | |
| Taste | | | | | | | | | | | | | | | |
| Aroma | | | | | | | | | | | | | | | |
| Texture | | | | | | | | | | | | | | | |
| Generall acceptability | | | | | | | | | | | | | | | |

Comment(s)
……………………………………………………..………………………….……..…
……………………………………………………………………………………..……

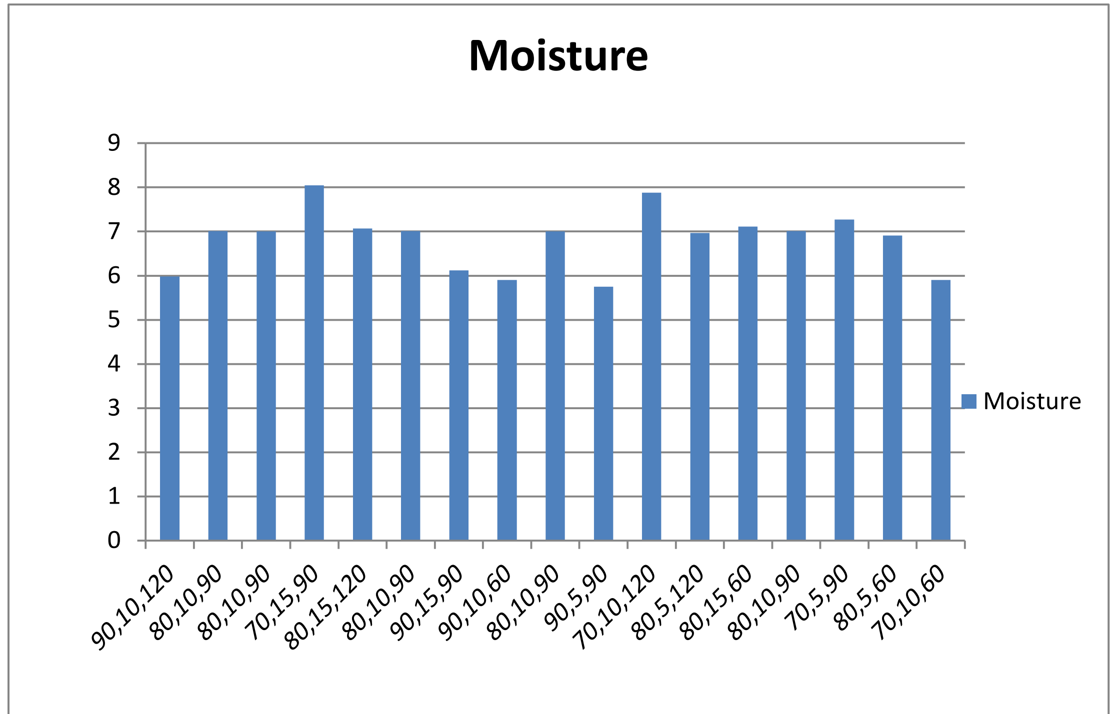
**BAR CHART FOR PROTEIN**

| Conditions | Protein |
|---|---|
| *90,10,120* | 36.3 |
| *80,10,90* | 34.58 |
| *80,10,90* | 34.58 |
| *70,15,90* | 34.87 |
| *80,15,120* | 34.65 |
| *80,10,90* | 34.58 |
| *90,15,90* | 36.81 |
| *90,10,60* | 37.5 |
| *80,10,90* | 34.58 |
| *90,5,90* | 36.18 |
| *70,10,120* | 33.33 |
| *80,5,120* | 34.5 |
| *80,15,60* | 35.15 |
| *80,10,90* | 34.58 |
| *70,5,90* | 33.02 |
| *80,5,60* | 34.88 |
| *70,10,60* | 33.7 |



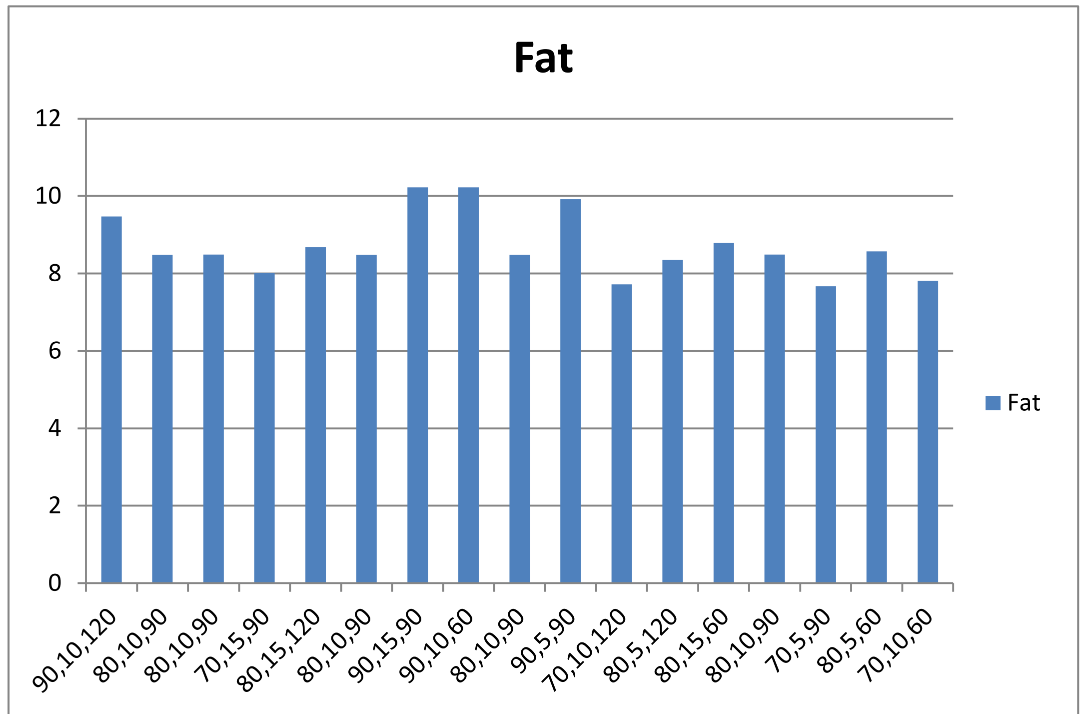
**BAR CHART FOR MOISTURE**

| Conditions | Moisture |
|---|---|
| *90,10,120* | 5.98 |
| *80,10,90* | 7.01 |
| *80,10,90* | 7 |
| *70,15,90* | 8.05 |
| *80,15,120* | 7.07 |
| *80,10,90* | 7.01 |
| *90,15,90* | 6.12 |
| *90,10,60* | 5.9 |
| *80,10,90* | 7 |
| *90,5,90* | 5.75 |
| *70,10,120* | 7.88 |
| *80,5,120* | 6.97 |
| *80,15,60* | 7.11 |
| *80,10,90* | 7.01 |
| *70,5,90* | 7.27 |
| *80,5,60* | 6.91 |
| *70,10,60* | 5.9 |



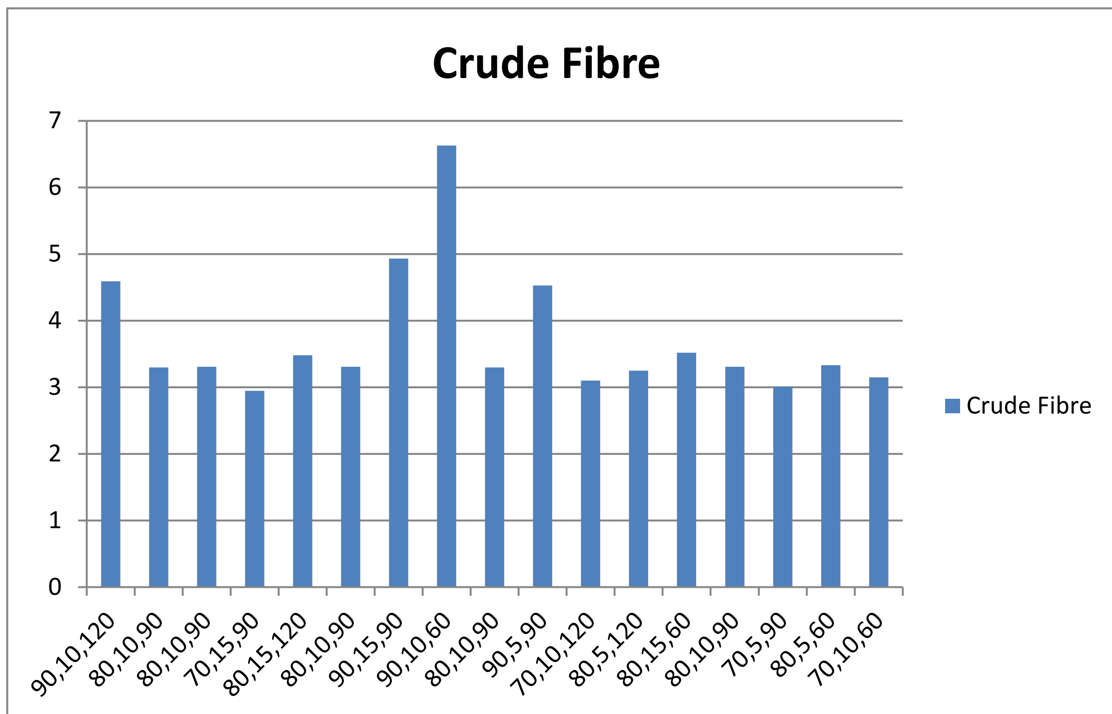
**BAR CHART FOR FAT**

| Conditions | Fat |
|---|---|
| *90,10,120* | 9.47 |
| *80,10,90* | 8.48 |
| *80,10,90* | 8.49 |
| *70,15,90* | 8.01 |
| *80,15,120* | 8.68 |
| *80,10,90* | 8.48 |
| *90,15,90* | 10.23 |
| *90,10,60* | 10.23 |
| *80,10,90* | 8.48 |
| *90,5,90* | 9.92 |
| *70,10,120* | 7.72 |
| *80,5,120* | 8.35 |
| *80,15,60* | 8.79 |
| *80,10,90* | 8.49 |
| *70,5,90* | 7.67 |
| *80,5,60* | 8.57 |
| *70,10,60* | 7.81 |



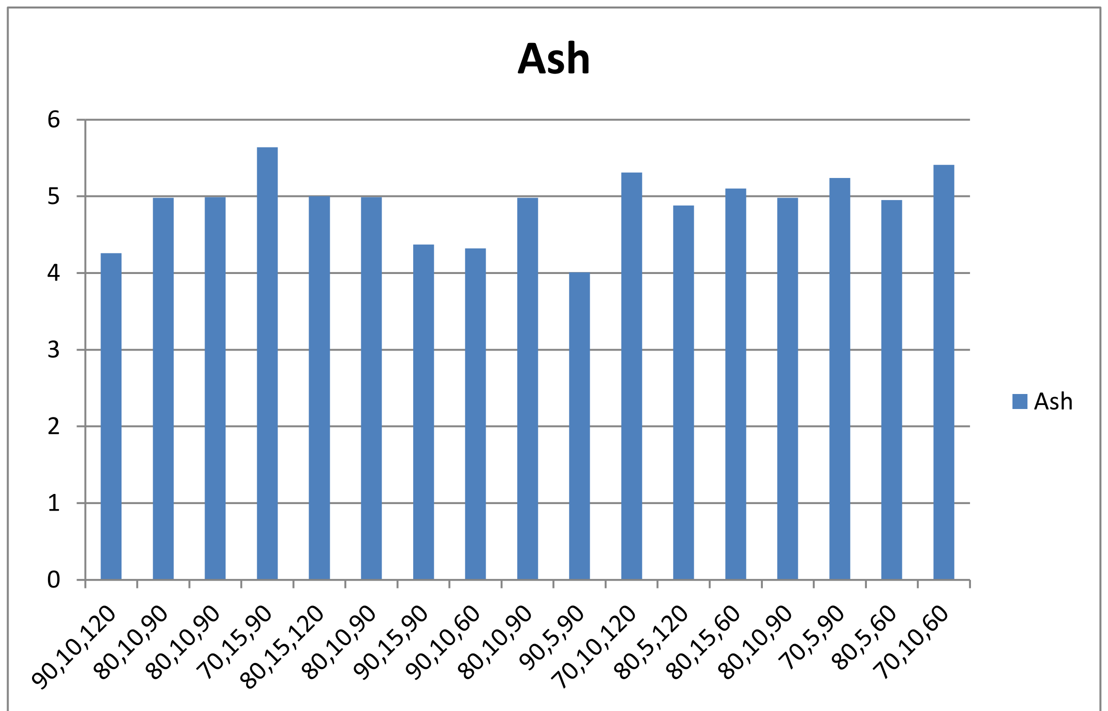
## BAR CHART FOR ASH CRUDE FIBRE

| Conditions | Crude Fibre |
|---|---|
| 90,10,120 | 4.59 |
| 80,10,90 | 3.3 |
| 80,10,90 | 3.31 |
| 70,15,90 | 2.95 |
| 80,15,120 | 3.48 |
| 80,10,90 | 3.31 |
| 90,15,90 | 4.93 |
| 90,10,60 | 6.63 |
| 80,10,90 | 3.3 |
| 90,5,90 | 4.53 |
| 70,10,120 | 3.1 |
| 80,5,120 | 3.25 |
| 80,15,60 | 3.52 |
| 80,10,90 | 3.31 |
| 70,5,90 | 3.01 |
| 80,5,60 | 3.33 |
| 70,10,60 | 3.15 |



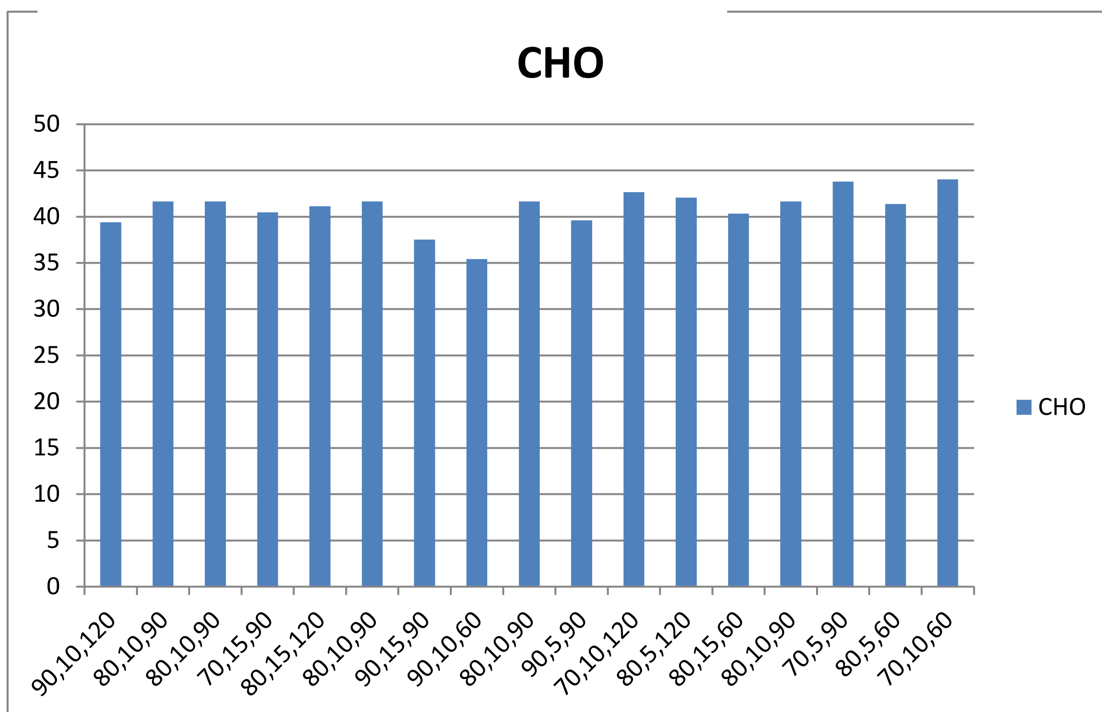
**BAR CHART FOR ASH CONTENT**

| Conditions | Ash |
|---|---|
| 90,10,120 | 4.26 |
| 80,10,90 | 4.98 |
| 80,10,90 | 4.99 |
| 70,15,90 | 5.64 |
| 80,15,120 | 5 |
| 80,10,90 | 4.99 |
| 90,15,90 | 4.37 |
| 90,10,60 | 4.32 |
| 80,10,90 | 4.98 |
| 90,5,90 | 4.01 |
| 70,10,120 | 5.31 |
| 80,5,120 | 4.88 |
| 80,15,60 | 5.1 |
| 80,10,90 | 4.98 |
| 70,5,90 | 5.24 |
| 80,5,60 | 4.95 |
| 70,10,60 | 5.41 |



**BAR CHART FOR CARBOHYDRATE**

| Conditions | CHO |
|---|---|
| 90,10,120 | 39.4 |
| 80,10,90 | 41.65 |
| 80,10,90 | 41.63 |
| 70,15,90 | 40.48 |
| 80,15,120 | 41.12 |
| 80,10,90 | 41.63 |
| 90,15,90 | 37.54 |
| 90,10,60 | 35.42 |
| 80,10,90 | 41.66 |
| 90,5,90 | 39.61 |
| 70,10,120 | 42.66 |
| 80,5,120 | 42.05 |
| 80,15,60 | 40.33 |
| 80,10,90 | 41.63 |
| 70,5,90 | 43.79 |
| 80,5,60 | 41.36 |
| 70,10,60 | 44.03 |



ANOVA TABLE FOR PROTEIN CONTENT

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 21.29 | 9 | 2.37 | 17.64 | 0.0005 | significant |
| A-FEED COMPOSITION | 17.61 | 1 | 17.61 | 131.35 | < 0.0001 | |
| B-BALL SIZE | 1.05 | 1 | 1.05 | 7.84 | 0.0265 | |
| C-COOKING TIME | 0.7503 | 1 | 0.7503 | 5.60 | 0.0499 | |
| AB | 0.3721 | 1 | 0.3721 | 2.78 | 0.1397 | |
| AC | 0.1722 | 1 | 0.1722 | 1.28 | 0.2944 | |
| BC | 0.0036 | 1 | 0.0036 | 0.0268 | 0.8745 | |
| $A^2$ | 1.17 | 1 | 1.17 | 8.70 | 0.0214 | |
| $B^2$ | 0.0545 | 1 | 0.0545 | 0.4063 | 0.5441 | |
| $C^2$ | 0.0432 | 1 | 0.0432 | 0.3219 | 0.5882 | |
| **Residual** | 0.9386 | 7 | 0.1341 | | | |
| Lack of Fit | 0.9386 | 3 | 0.3129 | | | |
| Pure Error | 0.0000 | 4 | 0.0000 | | | |
| **Cor Total** | 22.23 | 16 | | | | |

FIT STATISTICS TABLE FOR PROTEIN CONTENT

| | | | |
|---|---|---|---|
| **Std. Dev.** | 0.3662 | **$R^2$** | 0.9578 |
| **Mean** | 34.93 | **Adjusted $R^2$** | 0.9035 |
| **C.V. %** | 1.05 | **Predicted $R^2$** | 0.3245 |
| | | **Adeq Precision** | 14.7281 |

ANOVA TABLE FOR MOISTURE CONTENT

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 6.31 | 9 | 0.7013 | 5.39 | 0.0185 | significant |
| A-FEED COMPOSITION | 3.58 | 1 | 3.58 | 27.49 | 0.0012 | |
| B-BALL SIZE | 0.2628 | 1 | 0.2628 | 2.02 | 0.1983 | |
| C-COOKING TIME | 0.5408 | 1 | 0.5408 | 4.16 | 0.0809 | |
| AB | 0.0420 | 1 | 0.0420 | 0.3229 | 0.5876 | |
| AC | 0.9025 | 1 | 0.9025 | 6.94 | 0.0337 | |
| BC | 0.0025 | 1 | 0.0025 | 0.0192 | 0.8937 | |
| $A^2$ | 0.6881 | 1 | 0.6881 | 5.29 | 0.0550 | |
| $B^2$ | 0.1613 | 1 | 0.1613 | 1.24 | 0.3023 | |
| $C^2$ | 0.1468 | 1 | 0.1468 | 1.13 | 0.3234 | |
| **Residual** | 0.9109 | 7 | 0.1301 | | | |
| Lack of Fit | 0.9108 | 3 | 0.3036 | 10120.28 | < 0.0001 | significant |
| Pure Error | 0.0001 | 4 | 0.0000 | | | |
| **Cor Total** | 7.22 | 16 | | | | |

FIT STATISTICS TABLE FOR MOISTURE CONTENT

| | | | |
|---|---|---|---|
| **Std. Dev.** | 0.3607 | **$R^2$** | 0.8739 |
| **Mean** | 6.82 | **Adjusted $R^2$** | 0.7117 |
| **C.V. %** | 5.29 | **Predicted $R^2$** | -1.0177 |
| | | **Adeq Precision** | 8.2678 |

ANOVA TABLE FOR FAT CONTENT

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 10.39 | 9 | 1.15 | 174.04 | < 0.0001 | significant |
| A-FEED COMPOSITION | 9.33 | 1 | 9.33 | 1407.12 | < 0.0001 | |
| B-BALL SIZE | 0.1800 | 1 | 0.1800 | 27.14 | 0.0012 | |
| C-COOKING TIME | 0.1740 | 1 | 0.1740 | 26.25 | 0.0014 | |
| AB | 0.0002 | 1 | 0.0002 | 0.0339 | 0.8591 | |
| AC | 0.1122 | 1 | 0.1122 | 16.92 | 0.0045 | |
| BC | 0.0030 | 1 | 0.0030 | 0.4562 | 0.5211 | |
| $A^2$ | 0.4918 | 1 | 0.4918 | 74.16 | < 0.0001 | |
| $B^2$ | 0.0731 | 1 | 0.0731 | 11.02 | 0.0128 | |
| $C^2$ | 0.0014 | 1 | 0.0014 | 0.2115 | 0.6596 | |
| **Residual** | 0.0464 | 7 | 0.0066 | | | |
| Lack of Fit | 0.0463 | 3 | 0.0154 | 514.44 | < 0.0001 | significant |
| Pure Error | 0.0001 | 4 | 0.0000 | | | |
| **Cor Total** | 10.43 | 16 | | | | |

FIT STATISTICS TABLE FOR FAT CONTENT

| | | | |
|---|---|---|---|
| **Std. Dev.** | 0.0814 | **$R^2$** | 0.9956 |
| **Mean** | 8.70 | **Adjusted $R^2$** | 0.9898 |
| **C.V. %** | 0.9362 | **Predicted $R^2$** | 0.9290 |
| | | **Adeq Precision** | 39.9477 |

ANOVA TABLE FOR CRUDE FIBRE CONTENT

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 13.72 | 9 | 1.52 | 14.11 | 0.0010 | significant |
| A-FEED COMPOSITION | 8.97 | 1 | 8.97 | 83.02 | < 0.0001 | |
| B-BALL SIZE | 0.0722 | 1 | 0.0722 | 0.6684 | 0.4405 | |
| C-COOKING TIME | 0.6105 | 1 | 0.6105 | 5.65 | 0.0491 | |
| AB | 0.0529 | 1 | 0.0529 | 0.4897 | 0.5066 | |
| AC | 0.9900 | 1 | 0.9900 | 9.17 | 0.0192 | |
| BC | 0.0004 | 1 | 0.0004 | 0.0037 | 0.9532 | |
| $A^2$ | 2.44 | 1 | 2.44 | 22.56 | 0.0021 | |
| $B^2$ | 0.1888 | 1 | 0.1888 | 1.75 | 0.2277 | |
| $C^2$ | 0.3808 | 1 | 0.3808 | 3.53 | 0.1025 | |
| **Residual** | 0.7561 | 7 | 0.1080 | | | |
| Lack of Fit | 0.7560 | 3 | 0.2520 | 8400.28 | < 0.0001 | significant |
| Pure Error | 0.0001 | 4 | 0.0000 | | | |
| **Cor Total** | 14.48 | 16 | | | | |

FIT STATISTICS TABLE FOR CRUDE FIBRE CONTENT

| | | | |
|---|---|---|---|
| **Std. Dev.** | 0.3287 | **$R^2$** | 0.9478 |
| **Mean** | 3.71 | **Adjusted $R^2$** | 0.8806 |
| **C.V. %** | 8.87 | **Predicted $R^2$** | 0.1644 |
| | | **Adeq Precision** | 13.5823 |

ANOVA TABLE FOR ASH CONTENT

| | | | | | | |
|---|---|---|---|---|---|---|
| **Model** | 2.95 | 9 | 0.3280 | 49.54 | < 0.0001 | significant |
| A-FEED COMPOSITION | 2.69 | 1 | 2.69 | 406.48 | < 0.0001 | |
| B-BALL SIZE | 0.1326 | 1 | 0.1326 | 20.03 | 0.0029 | |
| C-COOKING TIME | 0.0136 | 1 | 0.0136 | 2.06 | 0.1947 | |
| AB | 0.0004 | 1 | 0.0004 | 0.0604 | 0.8129 | |
| AC | 0.0004 | 1 | 0.0004 | 0.0604 | 0.8129 | |
| BC | 0.0002 | 1 | 0.0002 | 0.0340 | 0.8590 | |
| $A^2$ | 0.1122 | 1 | 0.1122 | 16.95 | 0.0045 | |
| $B^2$ | 0.0001 | 1 | 0.0001 | 0.0210 | 0.8888 | |
| $C^2$ | 0.0001 | 1 | 0.0001 | 0.0115 | 0.9177 | |
| **Residual** | 0.0463 | 7 | 0.0066 | | | |
| Lack of Fit | 0.0462 | 3 | 0.0154 | 513.61 | < 0.0001 | significant |
| Pure Error | 0.0001 | 4 | 0.0000 | | | |
| **Cor Total** | 3.00 | 16 | | | | |

FIT STATISTICS TABLE FOR ASH CONTENT

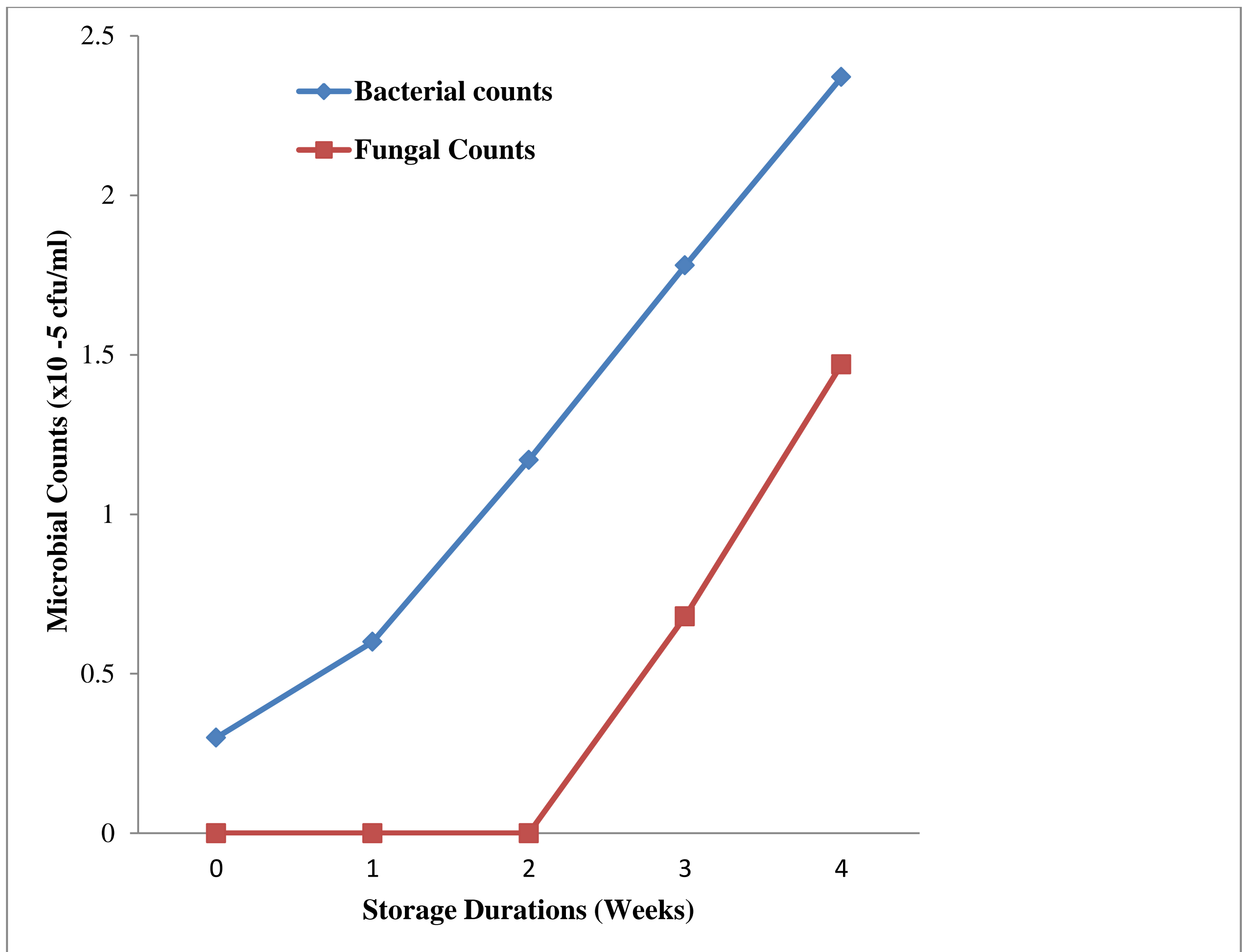
| | | | |
|---|---|---|---|
| **Std. Dev.** | 0.0814 | **$R^2$** | 0.9845 |
| **Mean** | 4.91 | **Adjusted $R^2$** | 0.9647 |
| **C.V. %** | 1.66 | **Predicted $R^2$** | 0.7532 |
| | | **Adeq Precision** | 22.7141 |

ANOVA TABLE FOR CARBOHYDRATE CONTENT

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 53.90 | 3 | 17.97 | 13.45 | 0.0003 | significant |
| A-FEED COMPOSITION | 45.08 | 1 | 45.08 | 33.73 | < 0.0001 | |
| B-BALL SIZE | 6.73 | 1 | 6.73 | 5.04 | 0.0428 | |
| C-COOKING TIME | 2.09 | 1 | 2.09 | 1.56 | 0.2330 | |
| **Residual** | 17.37 | 13 | 1.34 | | | |
| Lack of Fit | 17.37 | 9 | 1.93 | 9650.84 | < 0.0001 | significant |
| Pure Error | 0.0008 | 4 | 0.0002 | | | |
| **Cor Total** | 71.28 | 16 | | | | |

FIT STATISTICS TABLE FOR CARBOHYDRATE CONTENT

| | | | |
|---|---|---|---|
| **Std. Dev.** | 1.16 | **$R^2$** | 0.7563 |
| **Mean** | 40.94 | **Adjusted $R^2$** | 0.7000 |
| **C.V. %** | 2.82 | **Predicted $R^2$** | 0.5229 |
| | | **Adeq Precision** | 11.7389 |




**Microbial counts (x $10^5$cfu/ml) of soup balls as affected by storage duration**

15 g soup balls

10 g soup balls

5 g soup balls

**Samples of soup balls**