KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCE

FORMULATION OF WEANING FOOD USING COMPOSITE OF MAIZE GROUNDNUT AND SOYBEAN AND ASSESSING ITS NUTRITIONAL EFFECT USING ANIMAL MODEL.

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMSITRY AND BIOTECHNOLOGY IN PARTIAL FUFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (FOOD SCIENCE AND TECHNOLOGY)

BY

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DECLARATION

It is hereby declared that this thesis is the outcome of the research work undertaken by the author. Any assistance obtained has been dully acknowledged. It is neither in part nor whole been presented for another degree elsewhere.

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DEDICATION

This Research work is dedicated to my parents **Mr. Anyan Asiedu** and the **late Madam Agnes Adwoa Sarfoa** for their love, support in diverse ways and always being there for me.

ACKNOWLEDGEMENT

To God be the Glory. I am very thankful to God for bringing me this far as well as His love, guidance, provision and protection. I would like to express my sincerest thanks and most profound gratitude to Mr. E.A. Amankwah, Mr. C.K. Frempong and Mr. J. Barimah, who supervised my work and whose valuable input, help and corrections led to the success of this project. I am very thankful to all the lecturers in Biochemistry and Biotechnology Department as well as the laboratory assistant in the Department. Mr. Thomas Ansah (Uncle Tom), a laboratory assistant in Faculty of Pharmacy, was very instrumental in helping carry out the Animal trial. I am indeed very grateful to him. My heartfelt thanks go to my parents, Mr. Anyan Asiedu and the late Madam Agnes Adwoa Sarfoa for their love and support, and to all my siblings. To my friends and course mates, I am indeed thankful to you all especially Miss Lucy Agyei- Berfi whose help has made this work a success.

ABSTRACT

In this study two composite weaning diets (Diet 1 and 2) made up of groundnut, soybean and maize were formulated. The diets were each in proportion of groundnut (1): soybean (8): maize (16). A control (Diet 3) made entirely of maize was also studied. Meanwhile, the difference between diet 1 and 2 were varietal. Diet 1 contained *Anidaso* variety while Diet 2 contained *Salintuya* 1 variety of soybeans. Material balance method was used to predetermine the nutrient to meet standards in the final product. The protein content of Diet 1 was 20.58%, with 10.65% fat and 64.39% carbohydrate. Diet 2 had 21.02% of protein, 10.71% fat and 64.06% carbohydrate. Diet 3 gave 9.54% protein, 3.92% fat and 80.36% carbohydrate. Iron and magnesium contents suited for infants between the ages of 0-5 months for diet 1, 2 and 3. Meanwhile calcium and phosphorus content for diet 1 and 2 were suitable both for children between ages of 0-5 and 5-12 months as compared to recommended dietary allowance for infants and children. Though the moisture increased marginally compared to changes in weeks, the microbial load count (Cfu/g) did not change significantly. Meanwhile, the level fell below allowable recommended range of 25- 250 Cfu/g. Sensory evaluation showed that sample C (Fermented Maize flour, Groundnut cake and blanch soybean(*Anidaso*) and cinnamon was the most preferred by both untrained and weaning babies with the help of their mothers. The animal study indicated growth and development in rat fed with diet 1 and 2, with no adverse biochemical and haematological effect with the exception of rats fed on Diet 3 which showed low growth and development with adverse biochemical and haematological disorders ($P<0.05$). A high and positive correlation existed (r-value) between anthropometric measurements (weight and length) and biochemical and haematological indices for rats fed on diet 1 and 2 with the exception of rats fed on diet 3 which showed weak correlation between anthropometric measurements and total protein, serum albumen and a negative correlation with haemoglobin. Though there was a high correlation between anthropometric measurement and WBC it was rather due to a disease condition as a result of the absence of soybean in diet 3. The formulation with soybean can therefore be used as a weaning food to improve upon the nutritional status of Ghanaian children and also help solve problems associated with protein energy malnutrition.

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LDL	Low density lipoproteins
CT SCAN	Computerized tomogram scan
PEM	Protein Energy Malnutrition
WBC	White Blood Cells
HDPE	High Density Polyethylene
PAG	Protein Advisory Group

CHAPTER ONE

1.0 Introduction

Malnutrition has become one of the major world health problems facing developing countries. Throughout the developing world, malnutrition affects almost 800 million people, or 20 percent of the world population (WHO, 2000; USAID, 2000). Clinically, malnutrition is characterized by inadequate or excess intake of protein, energy, and micronutrients such as vitamins, and the frequent infections and disorders that result (Dutra-de-Oliveira, 1991. Protein energy malnutrition (PEM) generally occurs during the crucial transitional phase when children are weaned from liquid to semi-solid or fully adult foods. During this period, because of their rapid growth, children need nutritionally balanced, calorie-dense supplementary foods in addition to mother's milk. In Sub-Saharan Africa, extreme poverty, inadequate caring practices for children, low levels of education and poor access to health services are among the major factors causing under nutrition (WHO, 2000; Fashakin and Ogunsola, 1982). Conflicts and natural disasters in many countries have further exacerbated the situation. Worldwide, the most common form of malnutrition is iron deficiency, affecting up to 80 percent of the population, as many as four or five billion people (WHO, 2000; Agyepong, 1991). According to the 1987 Ghanaian National Survey, 58% of the children were below 80% of the National Center for Health Statistics (NCHS) weight-for-age, 8% suffered from severe malnutrition, 40% were wasted, and 52% were stunted (Agyepong, 1991). Armar-Klemesu and Wheeler, (1991) observed that 30% of the infants who were fed on cereal porridge and adult foods as weaning foods were malnourished. They attributed this to inadequate complementation to the breast milk. However, high price of proprietary

weaning foods, vegetable, animal proteins and the non-availability of low-priced nutritious foods, combined with bad feeding practices and late introduction of supplementary foods, are mostly responsible for aggravating the disorder among children (Dutra-de-Oliveira, 1991). Good nutrition, particularly during infancy and childhood can promote adequate physical and mental development. Certain nutrients such as protein, fats and oils in food maintain life; thus used for cells growth, repair, and regulation of function (Berggren, 1998, Cameroon and Hofvander, 1971). According to researchers, West African mothers usually breastfeed for 12 months. Many urban poor and rural women breast feed for up to 18 and 24 months (Armar, 1989). However in many West African countries, exclusive breastfeeding is usually adequate up to three to four months of age, but after this period it may become increasingly inadequate to support the nutritional demands of the growing infant. Thus, in a weaning process there is always the need to introduce soft, easily swallowed foods to supplement the infant's feeding early in life.

The weaning process may be gradual, lasting for months until the infant is finally introduced to the family diet. On the other hand, in abrupt weaning, the infant is introduced straight into the family menu with various foods like rice, cassava, corn and groundnuts (Eschleman, 1991). This latter option creates a problem, as the child may not be able to eat enough of the adult diet to meet his or her nutritional needs (Kazimi and Kazimi, 1979). These supplements are carbohydrate rich foods, which are actual portions of the adult diet and hence are not suitable for infants especially babies, because of its components, their bulky nature and consistency as well as their inbalance in the nutrient

composition. This is the underlying cause of malnutrition in Ghana (Armar-Klemesu and Wheeler, 1991). Over 70% of dietary protein in the developing countries is supplied by cereals that are relatively poor sources of protein (Glover, 1976). Research showed that most of the weaning foods consumed in communities of developing nations are deficient in essential nutrients (FAO/WHO, 1998). Several strategies have been used to improve the nutritive value of weaning foods (Gopaldas *et al.,* 1988).

The traditional West - African weaning foods could be improved upon by combining locally available foods that complement each other in such a way that new pattern of amino - acids created by this combination is similar to that recommended for infants (Fashakin and Ogunsola, 1982). However, the high lysine content of legumes improves the nutritional quality of cereals by complementing their limiting amino acids sulphur containing amino acids are limiting in legumes and relatively high in cereals, whereas lysine is limiting in cereals and high in legumes (Bressani and Ellias, 1966; Ekpenyong *et al*., 1977). Soya beans have recently become popular in the West African sub-region due to their high protein content and quality, and is being cultivated at an increasing stead rate. It is a grain legume, which holds many advantages over animal products. Hence, there will be improvement of the nutritional value of the food as well as the nutritional status of the consumer (the infant) if both cereals and legumes are blended in the preparation of the food. Soybeans, groundnuts and maize are locally produced and this may make the soy weaning product very affordable. This study was undertaken to evaluate the nutritive value, biochemical and haematological effect, physiological characteristic and consumer acceptability of a locally produced cereal-based traditional

weaning using an animal model. It is in this light that this project seeks to formulate weaning food using soybean, groundnut and maize which would have a high protein content to reduce malnutrition among the children of the Ghanaian populace.

1.1 JUSTIFICATION

The animal study is very necessary because food nutrient present in a food may not necessary be available for absorption. However, there is much evidence from studies on experimental animals and human subjects that substituting soy-protein for animals in the diets improved upon their health by reducing the concentration of total and Low Density Lipoprotein cholesterol in the plasma or serum. From the above reason this project seeks to: Formulate weaning diet using composite flours of groundnut, maize and soybean and assessing its effect using animal model. The objectives therefore are as follows:

1.1.1 General Objective

Employing the material balance method to produce a weaning diet that meets the nutritional needs of growing infants and children.

1.1.2 Specific Objectives

- Assessing the nutritional composition value of the raw soybean (*Anidaso* and *Salintuya* 1), maize, groundnuts and the formulated weaning food.
- To evaluate the consumer acceptability of the formulated diet
- To assess the keeping quality of the formulated diet through microbial and moisture determinations and
- To determine the effect of the formulated diets on anthropometric measurements, biochemical indicators and haematological indices using ANOVA and correlation.

CHAPTER TWO

2.0 Literature Review

2.1 Malnutrition

Malnutrition is a state of impaired health resulting from under nutrition – a lack of nutrients or from over nutrition i.e. excessive intake of nutrients. Malnutrition also may result if the body cannot properly use the food it receives. For example, a child who has frequent bouts of diarrhoea may be poorly nourished, because the nutrients in the food he eats cannot be absorbed from the intestinal tract (Armar Klemesu and Wheeler 1991; Ojofeitimi, 1982). Under nutrition is widespread in the developing countries of the world. Through out the world it is estimated that four hundred (400) million people suffer from serious nutritional deficiencies (Ojofeitimi, 1982). Asia, Africa and Latin America have about twenty five percent to thirty percent (25%-30%) of the population suffering from semi starvation (Armar Klemesu and Wheeler 1991). Children are the hardest hit. The poorly nourished child is highly susceptible to infection, and infections are more severe and last longer in malnourished than in a well-nourished child. The child is then more vulnerable to next infectious disease to which he or she is exposed. The annual death toll due to common childhood diseases is extremely high in developing countries-fifteen (15) million children under the age of five (5) years (Ojofeitimi, 1982; Dutra-de-Oliveira, 1991). This represents one fourth of the death throughout the world. The growth patterns of a child are useful means for judging nutritional well-being. When a child is poorly nourished, the growth rate diminishes particularly because of delay in bone development (Ojofeitimi, 1982; Armar, 1989). Both the quality of the bone (the amount of calcium and phosphorus it contains) and its capability for growth is influenced by nutrition. Sexual

maturity appears to occur late in populations that are malnourished than in developed countries (Armar Klemusu and Wheeler 1991).

2.1.1 Nutritional concerns of Malnutrition

Every child admitted to the hospital for poor weight gain or malnutrition should be screened for the presence of illnesses and condition that could lead to protein-energy malnutrition. Children with higher-than average risk for malnutrition should be more closely assessed and evaluated often. Children who cannot or will not eat, or who are unable to absorb nutrients taken by mouth, may be fed by the use of tube feeding. Tube feeding is often used to provide nutrients to children who have burns, inflammatory bowel disease, or other long-term conditions, that case chronic malnutrition or malabsorption (e.g. cystic fibrosis or AIDS), and interfere with the ability to take in enough calories. This procedure involves inserting a thin tube through the nose and carefully guiding it along the throat until it reaches the stomach or small intestine (FAO/WHO, 1998; USAID, 2002). If long-term tube feeding is necessary, the tube may be placed directly into the stomach or small intestine through an incision in the abdomen (Bennett and Fred Plum, 1996). Tube feeding cannot always deliver adequate nutrients to children who:

- Are severely malnourished
- Require surgery
- Are undergoing chemotherapy or radiation treatments
- Have been seriously burned
- have persistent diarrhoea or vomiting
- Have a gastrointestinal tract that is not functional

Intravenous feeding can also supply some of the nutrients these children need.

Doctors or registered dieticians can help parents monitor overweight or obese children. These professionals may suggest a weight loss program if the child is more than 40 percent overweight. Keeping weight gain under control is accomplished by changing eating habits, lowering fat intake, and increasing physical activity.

2.1.2 Causes and Symptoms of Malnutrition

Individual nutritional status depends on the interaction between food that is eaten, the overall state of health and the physical environment. Malnutrition is both a medical and social disorder often rooted in poverty. Poor water and sanitation are important determinants in this conception, but sometimes improvement do not benefit the entire population. Malnutrition, mainly affecting children under 5 years of age, is a common problem in many developing countries. It is caused by different factors including

- Lack of food and low quality of the food;
- Lack of time to take care of children by parents and/or caretakers;
- Lack of knowledge;
- Lack of good quality health care resulting in the occurrence of many diseases;
- Poverty (USAID, 2002).

Worldwide, poverty and lack of food are the primary reasons why malnutrition occurs (FAO/WHO, 1998, USAID, 2002). Families of low-income households do not always have enough healthy food to eat. When there is a household food shortage, children are the most vulnerable to malnutrition because of their high energy needs. There is an increased risk of malnutrition associated with certain chronic diseases, such as the disease

of the intestinal tract, kidneys, and liver. Children with chronic diseases like cancer, cystic fibrosis, AIDS, celiac disease, and intestinal disorders may lose weight rapidly and become susceptible to malnutrition because they cannot absorb valuable vitamins, iron, and other necessary nutrients. Children who are lactose intolerant have difficulty digesting milk and milk products, and may be at risk for malnutrition, particularly a calcium deficiency (USAID, 2002).

Symptoms of malnutrition vary, depending on what nutrients are deficient in the body. Unintentionally losing weight may be a sign of malnutrition. Children who are malnourished may be skinny or bloated and may be short for their age (stunted). Their skin is pale, thick, dry, and easily bruised (USAID, 2002). Rashes and changes in pigmentation are common. Hair is thin, tightly curled, and easily pulled out. Joints ache and bones are soft and tender. The gums bleed. The tongue may be swollen, or shrived and cracked. Visual disturbances include night blindness and increased sensitivity to light and glare (FAO/WHO, 1998, USAID, 2002). Other symptoms of malnutrition include:

- Fatigue
- Dizziness
- Anaemia
- Disorientation
- Goiter (enlarged thyroid gland)
- lack of coordination
- Muscle twitches

- Decreased immune response
- Scaling and cracking of the lips and mouth

Children who are over-nourished are visibly overweight or obese and consume more food than their bodies need.

2.1.3 Diagnosis of Malnutrition

Overall appearance, behavior, body-fat distribution, and organ function can alert a family physician, internist, or nutrition specialist to the presence of malnutrition. Parents may be asked to record what a child eats during a specific period. X rays or CT scan can determine bone density and reveal gastrointestinal disturbances, as well as heart and lung damage. Blood and urine tests are used to measure levels of vitamins, minerals, waste products. Where as anthropometric measurements is use for nutritional assessments (FAO/WHO, 1998, USAID, 2002).

2.1.4 Prognosis of Malnutrition

Some children with protein-energy malnutrition recover completely. Others have many health problems throughout life, including mental disabilities and the inability to absorb nutrients through the intestinal tract. Prognosis is dependent on age and the length and severity of the malnutrition, with young children having the highest rate of long-term complications and death. Death usually results from heart failure, electrolyte imbalance, or low body temperature. Children with semi consciousness, persistent diarrhoea, jaundice, low blood and low sodium levels have a poorer prognosis. A good prognosis exists for overweight children who make lifestyle changes and adhere to a diet and

exercise program (Bennett and Plum, 1996).

2.1.5 Prevention of Malnutrition

Proper nutrition is required to ensure optimal health. Consumption of a wide variety of food, with adequate vitamin and mineral intake, is the basis of a healthy diet. Researchers have stated that no single nutrient is the key to good health, but optimum nutrition is derived from eating a diverse diet, including a variety of fruits and vegetables. Because foods such as fruits and vegetables provide more nutrients than vitamin supplements, food is the best source for acquiring needed vitamins and minerals (FAO/WHO, 1998; USAID, 2002). Breastfeeding a baby for at least six months is considered the best way to prevent early-childhood malnutrition. The United States Department of Agriculture, Health and human Service recommended that all Americans over the age of two should:

- Consume plenty of fruits, grains, and vegetables
- Eat a variety of foods that are low in fats and cholesterol, and contain only moderate amounts of salt, sugars, and sodium.
- Engage in moderate physical activity for at least 30 minutes, at least several times a week.
- Use alcohol sparingly or avoid it altogether
- Achieve or maintain their ideal weight

Iron deficiency can be prevented by consuming red meat, egg yolks, and fortified breads, flour, and cereals (Bennett and Fred Plum, 1996).

2.1.6 Treatment of Malnutrition

Normalizing nutritional status starts with a nutritional assessment. This process enables a

registered dietician or nutritionist to confirm the presence of malnutrition, assess the effects of the disorder, and formulate a diet that will restore adequate nutrition. For children suffering from malnutrition due to an illness or underlying disorder, the condition should be treated concurrently (Bennett and Plum, 1996).

2.2 Protein Energy Malnutrition (PEM)

PEM, "the silent emergency of the world" which may have hunted mankind since the dawn of history is by far the most lethal form of malnutrition. It is an imbalance between the supply of energy and protein, and the body's demand for them to ensure optimal growth and function. It is currently the most widespread and serious health problem of children in the world being the moderate or severe forms (FAO/WHO, 1998, USAID, 2002). This syndrome is one example of the various levels of inadequate protein and/or energy intake between starvation (no food intake) and adequate nourishment. Although infants and children of some developing nations dramatically exemplify this type of malnutrition, it can occur in persons of any age in any country. Inadequate intake of food essential nutrients leads to under nutrition, resulting in deterioration of physical growth and health. On the other hand, excess intake of high-energy food relative to the body's needs results in overweight and obesity.

Children under 5 years of age are the most visible victims of PEM and most susceptible to PEM's characteristic growth impairment because of their high energy and protein need and their vulnerability to infection. PEM is self-perpetuating within the poor population of backwards and economically struggling nations. It must be clearly demarcated from generalized famine following massive disaster such as warfare, droughts, floods or

earthquakes. Peak incidence is immediately after epidemics of infections, illness and diarrhea or in hungry months. In any country, the prevalence rates will be influenced by season, the availability if food, incidence of infection and the state of development of the health services (Armar, 1989).

2.2.1 Classification of PEM

PEM may be viewed as a consequence of chronic and cumulative failure to meet physiological and nutritional requirements (Barners 1989; Guiro *et al.*, 1987).

Clinically PEM has three forms:

- Dry (thin, desiccated)
- Wet (edematous, swollen)
- Combined form between the two extreme

The form depends on the balance of non-protein sources of energy. Each of the three forms can be graded as mild, moderate or severe. The dry form, Marasmus, result from near starvation with deficiency of protein and non-protein nutrients. The marasmic child consumes very little food-often because his mother is unable to breastfeed making him very thin from loss of muscle and body fat. The wet form is called 'Kwashiorkor,' an African word meaning "first child-second child". It refers to observation that the first child develops PEM when the second child replaces the first child at the breast. The weaned child is fed a thin gruel of poor nutritional quality (compared with the mother's milk) and fails to thrive. The protein deficiency is usually identified than the energy deficiency and oedema results. Children with kwashiorkor tend to be older than those with marasmus and tend to develop the disease after they are weaned. The combined

form of PEM is called Marasmus Kwashiorkor. Children with this form have some oedema and more body fat than those associated with marasmus. Marasmus is associated with the early abandonment or failure of breastfeeding and with consequent infections, most notably causing infantile gastroenteritis. These infections results from improper hygiene and inadequate knowledge of infant caring that are prevalent in the rapidly growing slums of developing countries. Kwashiorkor is less common and usually manifest as maramic- kwashiorkor. It tends to be confined to parts of the world (rural Africa, the Caribbean and Pacific islands) where staple and weaning foods such as yam, cassava, sweet potato and green banana are protein deficient and excessively starchy.

2.3 Nutrition in Infants

Nutrition plays an important role in life even before birth and an infant's nutrition during the first year of life. This is for the growth, development and maturation of body tissues which occur rapidly during the first year of life. A healthy infant's birth weight doubles by about five month of age and triples by one year and thus infants have a higher basal metabolic rate about twice that of adults, based on body weight (Whitney and Rolfes, 1999). According to Wardlaw and Insel (2000), an infant typically increase in length by 50% in the first year. Such rapid growth requires both nourishment and sleep in abundance. They also need concentrated source of nutrient and energy to support their tremendous growth and development. When an infant is inadequately fed there is the risk of stunted growth and a range of biochemical changes that can impair development to the extent of permanently damaging the infant health. Table 1 shows the recommended dietary allowance for infants and children (Minerals)/100g.

During the first four to six months of life, all nutrients required by an infant can be provided by breast milk and so there's no dietary need for the introduction of solid food before then (Trussel, 2003). By the age of 6 month, most infant need additional foods, the purpose of which is to complement the breast milk and make certain nutrient that the young child continues to have enough energy and nutrients to grow normally. This goal is only achieved when these foods are prepared and fed to the infants under hygienic conditions and given in adequate proportions (Akaninwor and Okechukwu, 2004). Cereals should be introduced differently a week at a time to identify allergies and for the infant to develop preference (Akaninwor and Okechukwu, 2004).

Table 1 Recommended Dietary allowance for infants and children (Minerals)/ 100g

Age	Ca (mg)	P (mg)	Mg (mg)	Fe (mg)	Zn (mg)
0-5 months	210	100	30	0.27	2.0
5-12 months	270	275	70	11	3.0

(Source: Nutritional Information Centre, 2007)

2.3.1 Weaning

Professionally literature on infant feeding is with references to "weaning period" – transition from breast-feeding to complete reliance on other foods (Eschleman, 1991). However, the word "wean" is to accustom (Eschleman, 1991). Other authors have used the word wean to mean a complete cessation of suckling. Others also indicate that weaning is the process of gradual introduction of food to an infant so he gets accustomed to food other than breast milk notwithstanding the fact that is normally referred to as a cessation of breast feeding (Kazimi and Kazimi, 1979; Armar, 1989). For the purpose of

this project, weaning food is thought to be complementing breast milk when it can no longer provide for the nutritional needs of the child as well as the period of feeding the child when he has completely and permanently stopped sucking until he is old enough to derive his required nutritional needs from the family meal (Armar, 1989).

During this time the feeding pattern of infants gradually changes from an exclusive milk diet to diets similar to that of the rest of the family. It is also the time when the baby's feeding behaviour will progress from sucking to biting and chewing. There is no precise age at which weaning should start, between four and six months of age. The low nutrient density and high bulk of the weaning foods, early introduction of solid foods, and unhygienic practices predispose infants to malnutrition, growth retardation, infection, and high mortality (Eschleman, 1991). Weaning foods with low fibre content is very important since it helps in the safety of children considering their stomach capacity since they have to consume more to get satisfied to meet their daily energy requirement (Eka and Edijala, 1972). Multi-approach strategies, involving the development of improved recipes and processing, nutrition education, access to safe water and promotion of breastfeeding, are recommended solution to the problems.

On other hand, in abrupt weaning the infant is introduced directly into the family meal. This latter option creates a problem, as the child may not be able to eat enough of the adult diet to meet his or her nutritional needs (Eschleman, 1991). West African mothers usually breastfeed for 12 months. Most urban and rural poor women, breastfeed from 18 to 24 months (Kazimi and Kazimi, 1979, Armar, 1989). Most Ghanaian mothers start

weaning by the third month of life (Armar, 1989). A few mothers, however, start after one month. Based on interviews with breast-feeding Ghanaian mothers, Armar-Kelmesu and Wheeler (1991) reported that the main weaning food for infants up to six month of age was a traditional fermented maize porridge *(koko.)*. From six months onwards, the infants are given the family diet with complementary breastfeeding. The family foods on which the infant are weaned include dishes made from cereal, starchy tubers, legumes, and vegetables. These indicate that there is early supplementation with solid food or early weaning. Although the majority of women start weaning their infants at the age of three to four months, a few being within the first two months of life. The first solid food and the most popular weaning food is a thin cereal gruel, which is called by different names depending on the type of cereal or the West African country. For example, *koko* in Ghana, Ogi, prepared form maize or sorghum (couscous ogi), is a popular weaning food in Sierra Leone (Jonsyn, 1985). Legumes are rarely used for weaning and are introduced much late (after six moths of age) because of the problems of indigestibility, flatulence, and diarrhoea associated with their use (King *et al.*, 1985, Uwaegbute and Nnanyelugo, 1989). However, Uweagbute and Nnayelug noted that 67% of their study population satisfactorily used cowpea products for weaning (Uwaegbute and Nnanyelugo, 1989).

2.3.2 Weaning Foods

The quality of weaning foods used to feed a baby has been found to be very crucial to mental development of that baby. The most rapid growth of the brain occurs from 5 months before birth to 10 months after birth. At the end of the first year of life, the brain, first organ to attain full development has achieved 70% of its adult weight (wardlaw and Insel, 2000). Poorly nourished babies have fewer and smaller brain cells than those well

nourished. Most authors have indicated that early and severe malnutrition is an important factor in deficiencies in late mental development apart from social and hereditary influences (Wardlaw and Insel, 2000). According to the Protein Advisory Group (PAG, 1971) guidelines for weaning foods, protein content should be 20%, fat levels up to 10%, moisture 5% to 10%, total ash not more than 5%. Soybean in the diet is found to improve nutrient density of food and improve nutrient intake, which results in the prevention of malnutrition problems (Badamosi *et al*., 1995; Temple *et al*., 1996).

Legumes are now largely replacing milk and other sources of animal protein, which are expensive and not readily available, as stable substitute for high-quality protein

This notwithstanding, however, soybean is used in weaning because the factors responsible for these undesirable factors are removed through roasting and soaking etc of the soybean. A large variety of oilseeds and pulses, including cowpeas, groundnuts, pigeon's peas and melon seeds, grow well in Ghana, forming part of the traditional diets of many people. Traditional food uses of soybean are very limited; however, efforts are being made to promote their incorporation in diets.

Extensive research into the process characteristics, nutritional quality and consumer acceptability of soybeans and soy-cereal blends is necessary if these items are to be used effectively to improve the nutrition status of the vulnerable group of the population. At the Food Research Institution flour produced on pilot scale is gaining popularity among middle-income mothers as a protein supplement of cereal-based weaning foods. Table 2 shows the traditional weaning foods in some West African countries and the ages at which the foods are introduced. First step in weaning involve the introduction of different

tastes and textures. First weaning foods are suitable cereals usually mixed with the baby's usual milk or purred fruits and vegetables. Initially only small amounts of food of a smooth consistency are required to teach the baby to take food from a spoon and gets used to the texture of solids in the mouth. Later on, more solid foods can be introduced. First weaning foods are generally blend so that the taste is not too far removed from milk with more sophisticated tastes and textures being introduced, as the baby grow older. Commercial weaning foods are designed to help to provide a healthy mixed diet for babies. Products are developed using expert medical nutritional advice and follow current UK legislation (SI No 29472 1997, SI No 1999) and Department of Health recommendations for daily amounts of the major nutrients (Barners, 1989). They must also comply with specific regulations on baby foods as well as general food legislation. In addition, commercial baby foods should;

- Contain products that are consistent in composition and quality;
- Ensure that nutritional content and eating quality is maintained throughout processing and packing (commercial baby foods undergo more rigorous cooking than home-made foods);
- Be capable of being stored safely and maintaining quality throughout their shelf-life (unlike home-made baby foods which are usually eaten on the day of preparation; and
- Be easy to prepare and appropriate for the recommended age group.

The weaning period represents a stage of rapid growth and development and the weaning diet must supply adequate energy in the form of carbohydrate and fat and also protein and essential vitamins and minerals. Thus when developing commercial weaning foods

manufacturers take into account the following criteria:

Energy (Calories) – Growth of infants depends on the provision of adequate calories for tissue building and energy expenditure (Barners, 1989). The total energy content of the infant's diet must be maintained with controlled limits. An insufficient energy intake could lead to failure to thrive, whereas an energy intake in excess of requirements may lead to obesity. The "energy density" (amount of energy in a given quantity of food) is, therefore important. For example high fibre foods have a low energy density, whilst sugar and fat have a high energy density.

Carbohydrate — is the source of energy and its intake must be controlled. If given in excess it may be converted into fat and stores as such in the body. The type of carbohydrate that is included in the food is also important, as babies need carbohydrate that is easily digestible.

Protein —an adequate amount of protein must be provided in the diet, but too much protein should be avoided. Protein is the principal material of tissues and so is found in the body fluids and secretions as well. The protein ingested must contain specific amount of each of the essential amino acids. Example lysine; 4.2 g /100g, Cystine 2.0 g /100g (Barners, 1989).

Fat — Infants must receive an adequate amount of fat, as it is the most important sources of energy and it represents a concentrated form of energy. Fat is also important to provide essential fatty acids and certain vitamins. Lack of these essential fatty acids causes the skin of the infants to be dry and thickened and become susceptible to inflammation in the folds of the groin or axilla (Barness, 1979). Example, Linoleic acid 0.71 µg/100g

Fibre — unlike adults, infants do not benefit from a high fibre intake and therefore fibre levels must be controlled. Fibre can interfere with the absorption of essential minerals and because babies have a small stomach capacity it is difficult to consume sufficient quantities of fibre-rich foods to meet energy requirements.

Vitamins and minerals— added vitamins and minerals may be needed to ensure an adequate intake or restore loses, which may occur during processing. Example Thiamine (vitamin B_1; 40µg/100g, Riboflavin (vitamin B_2); 60 µg/100g, Magnesium 6mg/100g, Calcium 50mg/100g are suppose to be added to compensate for the losses (Barners, 1989).

Salt – there is no added salt (sodium chloride) in commercial baby foods.

Additives – by law, artificial additives such as preservatives, colourings, antioxidants and artificial sweeteners are not permitted in commercial weaning foods. No artificial flavouring is used in commercial baby foods (Barners, 1989).

2.4 Nutritive value and nutritional problems of weaning foods in West Africa.

Traditional weaning foods in West Africa are known to be of low nutritive value (Guiro *et al.,*1987; Akinrele and Bassir, 1978) and are characterized by low protein, low energy density, and high bulk density. Maize pap or koko has been implicated in the aetiology of protein-energy malnutrition on children during the weaning period (Naismith, 1973; Fashakin and Ogunsola, 1982). Table 2 shows some traditional weaning foods fed by West African mothers. Cereal-based diets have lower nutritional value than animal-based ones. Cereals form the primary basis for most of the traditional weaning foods in West African. The protein content of maize and guinea corn is of poor quality, low in lysine and tryptophan. These two amino acids are indispensable to the growth of the young child

(Oyenugu, 1968).

Table 2 Summary of traditional weaning foods fed by West African Mothers

Country	Food	Age of introduction (months)	Description
Nigeria	Ogi, pap, akamu, koko	3-6	Fermented cereals from sorghum, or guinea corn
Ghana	Koko, kinky	3-6	Femented corn porridge
Sierra Leone	Ogi, couscous Ogi	4-6	Cereal gruel from femented maize or sorghum
Benin	Ogi	3-6	Cereal gruel from fermented maize, sorghum, or millet

(Source: Armar, 1989)

2.4.1 Strategies for solving weaning food problems in Africa

Ignorance and food taboos in West Africa can result in weaning foods of poor nutritional quality. Improving the nutritional value of the weaning foods by itself will not eliminate the problems. Training and nutritional education of the mothers is necessary to change feeding practices and provide correct information. Nutritional education can easily be incorporated into primary health care programmes. Health workers and nutritionists can educate rural mothers about the importance of adequate weaning foods and practices, infant health, host defense system, home-scale drying, processing, and so on. The importance of varying the baby's diet and practicing good hygiene when handling and storing the baby's food can be included as well. The teaching and training of rural

mothers can have a long-term impact on weaning practices and nutritional status of children. In the Philippines, a weaning education Programme led to a reduction in the prevalence of malnutrition from 64% to 42% (Nnanyelugo *et al.*, 1990). In Nigeria, the African Child Survival Programme yielded similar results. The governments of some West African nations have yet to realize the importance of training education.

2.4.2 Formulation and development of weaning foods of high nutritive value

Several strategies may be used to improve the nutritive value of weaning foods. The traditional West African weaning foods could be improved by combining locally available foods that complement each other in such a way that the new patters of amino acids created by this combination is similar to that recommended for infants (Uwaegbute and Nnanyelugo, 1989). Cereals are deficient in lysine but have sufficient sulphur-containing amino acids that are limiting in legumes. Therefore, the combination of cereals and legumes has been found to produce amino acid patterns that adequately promote growth.

2.4.3 Nigerian experience

Many researchers have worked extensively on cereal-legume combinations in Nigeria. For example, Fashakin and Ogunsola (1982) formulated nut-ogi (a mixture of corn gruel and peanut), Akinrele and Edwards (1971) formulated soy-ogi (corn gruel plus soybean), and the Collaborative Research Support Programme (CRSP) Cowpea Linkage Project at the University of Nigeria, Nsukka, Nsukka, formulated cerebabe (corn gruel plus cowpea). Other useful combinations include ogi and melon protein (corn gruel plus melon seed) and cowpea-ogi (Fashakin *et al.*, 1989). Some of these combinations have

been adopted by the food-processing industries and are available on the Nigerian market. However, Fashakin *et al*., (1989) observed that no single protein from the above sources was adequate to promote growth or enhance nitrogen retention as well as a milk-based diet Fashakin *et al*., (1989). To this end, a mixture of cowpea, melon, soy bean, and ogi was found to be superior to any single protein source in protein efficiency ratio, net protein retention, biological value, and net protein utilization (Fashakin *et al*., 1989).

2.4.4 Ghanaian experience

Low-cost, nutritious, well-balanced weaning foods rich in protein and energy have been developed from locally available foods in Ghana. One such foods weanimix, a blend of legume (groundnut and/or cowpea) and cereal (maize) in the ratio of 1:4 w/w. However, Takyi *et al.,* (1991) suggested that alfalfa could be incorporated into the weaning diet of infants (Takyi *et al.,* 1991). This legume was found to contain higher levels of protein, minerals, and carotene and could support child growth better than weanimix.

2.5 Fermentation and germination

Fermentation enhances the nutritive value of food by increasing thiamine, nicotinic acid, riboflavin, and perhaps protein content as a result of microbial activity (Steinkraus, 1985, Odunfa, 1985). Fetuga *et al*., (1973) observed that the digestibility, protein efficiency ratio, net protein utilization, and biological value were much higher in fermented beans than in uncooked beans. Lopez *et al***.,** (1983) also noted that minerals were made more available and phosphorus was released from phytate during fermentation of corn (*Zea mays*). Fermentation can also reduce the high bulk of the traditional West African weaning foods by reducing the viscosity of the cereal gruel. During cereal fermentation,

microbial activity hydrolyses starch granules, resulting in reduced viscosity of the porridge (Svanberg, 1987; Mosha and Lorri, 1987). In addition fermentation and germination can improve the nutritional value of weaning foods by reducing the water-binding capacity of cereal flour. This allows the porridge to have a free-flowing consistency even with a high proportion of flour (Svanberg, 1987, Mosha and Lorri, 1987). Germination also converts insoluble proteins to soluble components and increases the levels of lysine as well as of vitamins B and C (Brandtzaeg *et al.*, 1981).

2.6 Soybean

Soybean has become the 'gold nugget of Nutrition' because of the combination of nutrient it provides. Soybean (*Glycin max*.) is a widely used, inexpensive, and nutritional source of dietary protein (McArthur *et al.*, 1988). Its protein content (40%) is higher and more economical than that of beef (18%), chicken (20%), fish (18%) and groundnut (23%) (IITA, 1990). Soybean is also of particular interest as a vegetable protein source because of its cholesterol lowering abilities in patients with type II hyperlipoproteinaemia (Sirtori *et al.*, 1985, Lovati *et al*., 2000). Apart from proteins, Soybeans also contain carbohydrate (32%), fat (20%), minerals/vitamins (5%) and fiber (3%). A lot of work has been reported on the chemical composition, cultivation and processing of Soybean. However, more studies need to be carried out to elucidate its nutritional value. Soybean may hold many advantages over animal products above and beyond the fact that Soybean is low in saturated fatty acids and of course, cholesterol free. Soybean is a legume and cultivated in many areas of the world, from tropics to temperate regions. For centuries, Soybean has played a major role in the diet of the population of Eastern Asia particularly China where soybean was termed sacred "grain" The other four being rice, barley, wheat

and millet. Now most countries have recognized the importance of soybean as a potential source of high quality protein and oil. Its high fibre and low carbohydrate content in diet generally improves the control of diabetes. However, varieties of low fibre content of less than 5% have corresponding higher protein levels (Kakad *et al.*, 1972; Kolar *et al.*, 1983). Supplementation of soy fibre significantly enhanced return of serum glucose levels towards fasting levels during the later half of the test meal though soy fibre had no effect on plasma insulin levels (American Academy of Pediatrics, 1998). Lactose intolerance means the body cannot digest or absorb the sugar, lactose in milk, other dairy products, and foods to which milk has been added. Because of this, lactose free soy-protein based formula in the management of long-term lactose restriction, a number of studies have addressed the role of these formulas in the recovery of acute infantile diarrhoea. Thus, soybean is the best substitute to relieve the person from complains of indigestion after drinking milk (American Academy of Pediatrics, 1998). There is much evidence from studies on experimental animal and human subjects that substitute soy-protein for animal in the diets reduces the concentration of total and LDL cholesterol in the plasma or serum (American Academy of Pediatrics, 1998). The suggested importance for lowering cholesterol levels is as follows:

- It improves the catabolism of cholesterol rich in Low Density Lipoprotein fraction (LDL)
- Absorption of lipids from gastrointestinal (GI) tract may be slow with soy protein.
- It increases billiard cholesterol excretion
- It may increase faecal steroid excretion.

However, the report of Olaleye *et al.,* (1999) showed that raw soy bean reduced red cell osmotic fragility and could also reduce the hematocrit in the rat depending on the processing methods applied. The presence of trypsin inhibitor in soy protein was ascribed to be responsible for these effects.

More recently, it was also reported that soy bean has a buffering effect on gastric acidity and ameliorates induced ulcers (Alada *et al*., 2004). In the study, the high protein content of soy bean was implicated as being responsible for its effect. Although, several studies (Alada *et al.,* 2004; Bolarinwa *et al.,* 1991; McArthur *et al*., 1988) have shown the relative importance of some animal and vegetable protein diets in the formation and composition of blood, there is little information except the report of Olaleye *et al*., (1999) on the effect of soy bean on the haematological indices in the rat. In view of the increasing use of soy bean as a major source of protein in several communities, there is a need to revisit its effect on some haematological and biochemical parameters. Soy-protein has proved to be an excellent food source for people suffering from allergies. They clear up rashes, pimples, eczema, and other skin troubles (American Academy of Pediatrics, 1998). The reason why soy protein is recommended for use in case of eczema and other skin rashes is that they render unnecessary use of animal protein such as meat and eggs lessen the inflammatory activities of the skin.

Soymilk is of great value to people who are allergic to cow's milk. Soymilk and Soy flour promote growth; improve appetite, and mental alertness (American Academy of Pediatrics, 1998). It is also a good source of vegetable oil which is important for growth and development. This attribute tends to agree with the recommendations of (FAO/WHO,

1998) that vegetable oils be included in foods meant for infants and children, which will not only increase the energy density, but also be a transport vehicle for fat soluble vitamins. The fat can also provide essential fatty acids like that of n-3 and n-6 Poly unsaturated Fatty Acids (PUF's) needed to ensure proper neural development Soybean contains antioxidants, which have anti-aging benefits. The ageing process has been defined as the inevitable decrease in the amount of healthy tissue in the organ of the body, resulting in a loss of the organ. Soybean contain adequate amount of vitamin-A, which is present as antioxidants as well as vegetable oil (Alada *et al.*, 2004). Thus, Soybean plays a beneficial role in all conditions. The overall positive nutritional profile, plentiful supply, excellent functional property, puts Soybean into focus, as an attractive underutilized raw material for innovative development.

2.6.1 Uses of Soybeans

The uses of soybean are numerous. They are processed to give soy milk, soy sauce, tofu (soybean curd) yogurt, soybean sprout, Tempeh (soy steak), which is extensively used in the Far East as infant feeding. The seed yield edible, semi-drying oil which is extensively used in the Far East as food. The bulk of the oil is used for edible purposes particularly as a salad oil or in the manufacture of margarine, shortening and soy meal. The residues after the extraction of the oil is a very rich protein feeding stuff for livestock and for the manufacture of synthetic fibre, textile sizes and fire fighting foam. Soy flour prepared from whole soybeans is used in bakery and other food product extends to cereal flour, meat products and in health foods. Soybean flour is becoming increasingly important as an ingredient of food stuffs and bakery products such as bread, biscuits and cakes (IITA, 1990).

2.6.2 Biochemical Composition of Soybean

2.6.2.1 Nutritional Composition of Soybeans

Protein, starch and vitamin content of the seed vary considerably according to the cultivar, origin of seed, climate and soil. Variations of 29.6%-50.3% in the crude protein content have been reported. There is also a considerable variation in amino acid content according to the cultivars and origin of the seed, but soybeans are relative in low sulphur containing amino acids that is cysteine and methionine. (Asiedu, 1989), (Table 3).

Table 3 Chemical Composition of Soybean per 100g Edible Portions

Constituent	Composition /100g
Moisture	7g
Protein	38g
Fat/ Oil	17g
Ash	4.6
Fibre	4.8
Carbohydrate	26g
Mineral substances	7g
Phosphorus	700mg
Calcium	250mg
Iron	7mg
Potassium	155mg
Nicotinic acid	2.1µg
Thiamine (Vit B_1)	1000µg
Riboflavin (Vit B_2)	310µg
Pyridoxine (Vit B_6)	1180µg
Pantothenic acid	2150µg
Biotin	80µg
Vitamin E	140µg
Vitamin K	100µg

Source: (Asiedu, 1989)

The major constitution of interests – oil and protein make about 55% of the bean but

more than a quarter consist of carbohydrate including polysaccharides, stanchions (3.8%) raffinose (1.1%) (Asiedu, 1989). Soy oil belongs to the group of oil most valuable for human nutrition because of its high content of polyunsaturated fatty acids-linoleic acid and linolenic acid. The high content of linoleic acid makes it superior in nutritive value to other vegetable oil. Since this acid cannot be synthesized by the body and hence must be provided by the diet. Linolic acid also has the ability of reducing serum cholesterol level hence decreasing the risk of developing heart coronary vessel diseases (Asiedu, 1989). Soy proteins which are nutritionally comparable to animal protein also contain sufficient amount of fat-soluble vitamins but low in sulphur containing essential amino acid etc. Soybean has been a popular foodstuff in various forms in different countries. It occupies a dominant position in the world in terms of its high protein in comparison to both animal and vegetable proteins.

2.6.3 Anti-Nutritive Properties of Soybean.

Raw soybeans contain anti-nutritional compounds, which has to be removed. Such chemicals include trypsin inhibitors; which prevent the action of the breakdown of protein by trypsin; haemaglutinins, which cause agglutination of red blood cells, phosphatidylcholine, which produces an objectionable flavour and bitterness and raffinose which cause flatulence. Some preprocessing treatment such as soaking the bean, removes the phosphatidylcholine, raffinose as well as the bitterness. Heat treatment inactivates the trypsin inhibitors and haemaglutinins. Other compounds such as urea which are undesirable, are also removed during debittering (soaking) process (Ologhobo and Fetuga, 1984)

2.7 Groundnuts (*Arachis hypogaea Linnaecus*)

Groundnut is the 13th most important food crop of the world. It is the world 4th most important source of edible oil and 3rd most important source of vegetable protein (Matz, 1969). Groundnut seeds contain high quality edible oil (50%) of monounsaturated fat, easily digestible protein (25%) and carbohydrates (20g) per 100g in terms of nutritional value (Smart, 1994). The oil may be used for margarine, for pharmaceuticals and cosmetic products. Groundnut contains small portions of threonine and sometimes leucine and valine. Groundnuts are useful source of thiamine, niacin, vitamin E and folic acid. They are also rich source of palmitic, oleic and linoleic acids. Groundnuts however contains some anti nutritive factors including trypsin inhibitors, haemagglutinnis, goitrogens, saponins and phytic acid but all these are destroyed or reduce to minimum through traditional cooking and processing techniques such as soaking in water and roasting (Ologhobo and Fetuga, 1984). Table 4 shows the nutritional value of groundnut per 100g of edible portions.

Table 4 Nutritional value of Groundnut per 100g of Edible portions

Constituents	Composition
Moisture (g)	5
Protein (g)	23.2
Fat(g)	44.8
Fibre(g)	2.9
Ash(g)	2
Carbohydrate	23
Calcium (mg)	49
Phosphorous (mg)	409
Iron (mg)	3.8
Thiamine(mg)	0.79
B-carotene equivalent (µg)	15
Riboflavbin(mg)	0.14
Niacin(mg)	15.5
Ascorbic acid(mg)	1

Source: (Tindall, 1983)

2.7.1 Uses of Groundnuts

Globally, 50% of groundnuts produced is used for oil extraction, 37% for confectionery use and 12% for seed purpose (Smart, 1994). In India 80% of the total groundnut production is used for oil extraction, 11% as seed, and 8% for direct food uses and 1% is exported. Groundnuts haul (vegetative plant parts) provide excellent hay for feeding livestock. It is also a valuable source of E, K and B vitamins (it is the richest plant source of thiamine (B1), and is also rich in niacin, which is low in cereals) (Smart, 1994). Groundnut cakes, formed after the oil is extracted, are a high protein animal feed. With proper processing, people also use the cake to make products, such as biscuits and baby foods.

2.8 Maize *zea mays*

Maize is a gigantic domestic grass [*Zea mays*] of tropical Mexican origin. The plant is used to produce grain and fodder that are the basis of a number of food, feed, pharmaceutical and industrial products. Cultivation of maize and the elaboration of its food products are inextricably bound with the rise of pre-Colombian Mesoamerican civilizations (Matz, 1959). Maize is composed mainly of carbohydrates but it also has an appreciable amount of minerals, vitamins and amino acid especially methionine. The nutritional compositions as well as mineral and vitamins composition of maize are shown in Table 5 and 6 respectively.

Table 5 Proximate Composition of Maize

Constituent	Composition (%)
Moisture	6
Total Ash	1.2
Crude Fibre	2.2
Crude Fat	4.4
Crude Protein	10
Carbohydrate	65

Source: (Matz, 1959)

Table 6 Mineral and vitamin composition of maize

Minerals/Vitamins	Composition mg/100g
Calcium	6
Phosphorus	300
Iron	2.5
Carotene or vitamin A	0.015
Ascorbic acid	11.4
Thiamine	0.5
Riboflavin	0.08
Niacin	2

Source: (Asiedu, 1989)

2.8.1 Uses of maize

In Ghana maize is used in the preparation of corn dough, which is used for various foods such as porridge, kenkey, banku and akple. The grains are fed to livestock and the husk is used for wrapping kenkey and other local food. The straw is used for making door mats. In the United States and Canada, the primary use of maize is a feed for livestock, forage, silage or grain. Silage is made by fermentation of chopped green cornstalks. The grain also has many industrial uses, including transformation into plastics and fabrics. Some is hydrolyzed and enzymatically treated to produce syrup, particularly high fructose corn syrup, a sweetener, and some fermented and distilled to produce grain alcohol. Grain alcohol from maize is traditionally the source of bourbon whiskey (Watson and Ramstad, 1987). Increasingly, ethanol is being used at low concentrations (10% or less) as an additive in gasoline for motor fuels to increase the octane rating, lower pollutants and reduce petroleum use (Watson and Ramstad, 1987; Doebley, 1994).

Human consumption of corn and corn meal constitutes a staple food in many regions of the world. Corn meal is made into a thick porridge in many cultures; from the polenta of Italy and the mamaliga of Romania to mush in the U.S or sadza, nshima, ugali and mealie pap in Africa (Doebley, 1994).

Sweet corn is a genetic variation that is high in sugars and low in starch that is served like a vegetable. Popcorn is kernels of certain varieties that explode when heated forming fluffy pieces that are eaten as a snack. Another common food made from maize is corn flakes. The floury meal of maize (cornmeal or masa) is used to make cornbread and Mexican tortillas. Teosinte is used as fodder, and can also be popped as popcorn. Some

forms of the plant are occasionally grown for ornamental use in the garden. Corncobs can be hollowed out and treated to make inexpensive smoking pipes, first manufactured in the United States in 1969. Corn cobs are also used as a biomass fuel source. In Canada and the U.S these are called “corn mazes” and are popular in many farming communities. Maize is sometimes used as biomass fuel, such as ethanol. Maize is also used as fish bait. It is particularly popular in Europe for coarse fishing. Stigmas from female corn flowers, known popularly as corn silk, are sold as herbal supplements.

2.9 Shelf life

Shelf life and product dating relate to quality, not food safety (Rodriguez, 2002). According to the Food Safety and Inspection Service (FSIS), Federal regulations do not require product dating except for infant formula and some baby foods (Rodriguez, 2002). No uniform or universally accepted systems for dating exist in the United State. Open dating on a food product helps retailers determine how long to delay a product and lets consumers know the time limit to purchase (“sell by” date). The “best is used by” date tells the buyer how to maximize quality. Shelf life determination is provided at the manufactures discretion it is intimately related to packaging. In the case of food products with short life, shelf life can be determined by direct method i.e. by monitoring quality and microbiological indexes in conditions similar to those of the distribution chain and determining the time when any of these reaches an acceptable level. However, this method cannot be applied to products with a long shelf-life, as this would imply a significant delay in the introduction of new or improved products on the market. Accelerated shelf life studies and mathematical modeling are very useful in this context.

Determining shelf life of a product involves studying over varying documented time increments, the interactions between food products, its packaging and the storage environment. Food packaging is designed to promote, apportion and protect the food inside it (Walter, 2000). Shelf life testing is conducted to determine the length of time that a food product will maintain its safety and quality.

Shelf life varies according to packaging type, method, and storage conditions. Temperature, humidity, and exposure to light must be controlled. Component of shelf life testing includes assessing the physical, chemical, microbiological and sensory attributes of the product (Walter, 2000). Physical attributes include colour, moisture content, water activity, pH levels, brix value and viscosity. Chemical attributes include peroxide value (rancidity indicator) free fatty acid content (rancidity indicator), vitamin retention or loss. Microbiological analysis is also conducted to determine the microbial load of products Sensory evaluation by trained panelist (objective testing) and also by using consumer panelist for (subjective testing) is done on the product as time goes on to determine the stability of the product. Each category of food (confectionaries, dairy, meat etc) has unique properties that are affected by warehouse, distribution display and in-home storage conditions (Rodriguez, 2002).

2.10 Microbiology

A microorganism or microbe is an organism that is microscopic (too small to be seen by the human eye) except with the microscope (Schopf, 2006). The study of microorganisms is called microbiology. Microorganisms include bacteria, fungi, archaea or protists, but not viruses and prions, which are generally classified as non-living. Most microorganisms

are single-celled, or unicellular, but some are microscopic, and some unicellular protists are visible to the average human or eye.

Microorganisms live almost everywhere on earth where there is water, including hot springs, on the ocean floor, and deep inside rocks within the earth's crust. Microorganisms are critical to nutrient recycling in ecosystems as they act as decomposers. As some microorganisms can also fix nitrogen, they are an important part of the nitrogen cycle (Gold, 1992). However, pathogenic microbes can invade other organisms and cause diseases that kill millions of people every year (Altermann and Kazmierczak, 2003). Microorganisms are vital to humans and the environment, as they participate in the Earth's element cycles such as the carbon cycle. They also help in the medical, agricultural and food productions. They are use in the food industries for brewing, baking and other food-making processes (Stanley, 1973). The lactobacilli and yeasts in sour dough bread are especially useful.

Microorganisms are also used to control the fermentation process in the production of cultured dairy products such as yogurt and cheese. The cultures also provide flavour and aroma, and also inhibit undesirable organisms. They are also use in the biological treatment of sewage and industrial waste effluents**.** However they can also lead to diseases and food spoilage whenever they exceed their threshold as a result of increase in certain parameters such as moisture (DeLong, 2001). The most obvious way to determine microbial numbers is through direct counting by using a counting chamber. Using a counting chamber is easy, inexpensive, and relatively quick; it also gives information about the size and morphology of micro organisms. The number of micro organisms in a

sample can be calculated by taking into account the number of colonies formed and any sample dilutions required. There are disadvantages to this technique. It is difficult or impossible to distinguish between living and dead cells in counting chambers. Counting chambers and electronic counters yield counts of all cells, whether alive or dead. There are also several viable counting techniques, procedures specific for cells able to grow and reproduce. Usually the count is made more accurate by the use of a special colony counter. In this way the spread plate or pour-plate techniques may be used to find the number of micro organism in a sample (Prescott *et al.,* 1999). Plating techniques are simple, sensitive, and widely used to count bacteria and other micro organism in samples of food, water and soil. Low counts will results if clumps of cell are not broken up and the micro organism well dispersed. Because it is not possible to be absolutely certain that each colony arose from individual cells, the results are often expressed in terms of colony forming units (Cfu/g) rather than the number of micro organisms. The sample should yield between 25 to 250 colonies for best result (Prescott *et al.*, 1999). Of course the counts will also be low if the agar medium employed cannot support the growth of all the viable micro organisms present. The hot agar used in the pour- plate technique may injure or kill sensitive cells; thus spread plates sometimes give higher count than pour- plates.

2.11 Anthropometric Measurements

The term anthropometry refers to comparative measurements of the body. Anthropometric measurements are used in nutritional assessments. Dimensions that are used to assess growth and development in infants, children, and adolescents include length, height, weight, weight-for-length, and head circumference (length is used in

infants and toddlers, rather than height, because they are unable to stand). Individual measurements are usually compared to reference standards on a growth chart. For anthropometric measurements to be valid indices of growth status, they must be highly accurate, requiring precision in measuring technique. (Niedhammer *et al*., 2000).

2.12 Haemoglobin (Hb)

Haemoglobin, is the iron-containing oxygen-transport metalloprotein in the red blood cells of the blood in vertebrates and other animals. In mammals the protein makes up about 97% of the red cell's dry content, and around 35% of the total content (including water) (Eshaghian *et al*., 2006). Haemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases its load of oxygen. Haemoglobin also has a variety of other gas-transport and effect-modulation duties, which vary from species to species, and may be quite diverse in invertebrates.

The name haemoglobin is the concatenation of heme and globin, reflecting the fact that each subunit of haemoglobin is a globular protein with an embedded haem group; each heme group contains an iron atom, and this is responsible for the binding of oxygen. The most common type of haemoglobin in mammals contains four such subunits, each with one haem group (Campbell, 1999). In humans, each haem group is able to bind one oxygen molecule, and thus, one hemoglobin molecule can bind four oxygen molecules.

Haemoglobin is synthesized in a complex series of steps. The haem portion is synthesized in a series of steps which occur in the mitochondria and the cytosol of the immature red blood cell, while the globin protein portions of the molecule are synthesized by ribosomes in the cytosol (Ganong, 2003). Production of Hb continues in the cell

throughout its early development from the proerythroblast to the reticulocyte in the bone marrow (Hardison, 1996). At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this haemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

The chemical empirical formula of the most common human haemoglobin is $C_{738}H_{1166}N_{812}O_{203}S_2Fe$, but as noted above, haemoglobin vary widely across species, and even (through common mutations) slightly among subgroups of human **(**Hardison, 1996).

Decrease in haemoglobin, with or without an absolute decrease in red blood cells, leads to symptoms of anaemia (Hardison, 1996). Anaemia has many different causes, although iron deficiency and its resultant iron deficiency anaemia are the most common causes in the world. The absence of iron decreases haem synthesis and red blood cells production.The normal levels range starts from 11.0 -14.0 g/dl for infants, 12.0-15.0 g/dl for women and 13.0-18.0 g/dl in adult men (Hercberg, *et al*., 1991). Low haemoglobin values may indicate: anemia, erythropoietin deficiency, haemolysis, haemorrhage, lead poisoning, malnutrition, nutritional deficiencies of Iron, folate, Vitamin B_{12} and Vitamin B_6 and over hydration. High haemoglobin may include congenital heart disease, pulmonary fibrosis, polycythemia vera and increased RBC formation associated with excess erythropoietin.

2.13 White cell Count (WBC)

White blood cells or leukocytes are cells of the immune system which defend the body against both infectious disease and foreign materials. Several different and diverse types of leukocytes exist example neutrophil, small lymphocyte, large lymphocyte, monocyte, eosinophil, Basophils but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.

The number of leukocytes in the blood is often an indicator of disease. There are normally between 4×10^9/l and 10×10^9/l white blood cells in a liter of blood, making up approximately 1% of blood in healthy human beings (Skala *et al*., 1981; Sarchielli and Chandra, 1991).

Low WBC Count may indicate:

- Viral, bacterial, parasitic infections e.g. HIV/AIDS viral hepatic, measles, rubella, influenza, rickettsial infections, overwhelming bacterial infections such as military tuberculosis, relapsing fever, typhoid etc. Parasitic Infections including, leishmaniasis and malaria.
- Drugs (e.g. cytotoxic) and reaction to chemicals.
- Hypersplenism
- A plastic anaemia
- Foliate and Vitamin B_{12} deficiency (megaloblastic anaemia)
- Anaphylactic shock and
- Ionizing radiation.

High WBC counts may indicate the following:

- Acute infections e.g. pneumonia, meningitis, abseess, whooping cough, tonsillitis, acute rheumatic fever, cholera, etc.
- Inflammation and tissue necrosis of burn fractures arthritis etc.
- Metabolic disorders e.g. eclampsia, uremia, diabetic coma and acidosis
- Poisoning e.g. chemicals, dry, snake venoms.
- Acute haemorrhage
- Leukaemia and myeloproliferative disorders.
- Malnutrition (Skala *et al*., 1981).

2.14 Serum Albumen

Serum albumin is the most abundant plasma protein in humans and other mammals. Albumin is synthesized in the liver as preproalbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum (Rajbar, 1968). The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin. Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartment and body tissues. Bovine serum albumin (BSA) is commonly used in molecular biology laboratories. Albumin is negatively charged (Jakus *et al*., 1999). The main function of albumin is as follows: maintains osmotic pressures, transport thyroid hormones, transport other hormones particularly fat-soluble one, transport fatty acids, transports unconjugated bilirubin, transports many drugs and competitively binds calcium ion (ca^{2+}) and buffer's pH. Serum albumin levels have been used extensively to assess the

nutritional status of individuals with and without malnutrition and chronic renal failure (CRF) (Mohamadi-Nejad *et al*., 2002). It follows that nutritional interventions that maintain or increase serum albumin concentrations may be associated with improved long-term survival, although this has not been proven in randomized, prospective clinical trials. Serum albumin levels may fall modestly with a sustained decrease in dietary protein and energy intake and may rise with increased protein or energy intake (Kańska and Boratynski, 2002). Conversely, serum albumin levels may fall acutely with inflammation or acute or chronic stress and increase following resolution or recovery. Normal levels range from 38-51 g/l (Rodkey, 1964; Doumas, 1971).

Low albumin levels may be caused by a poor diet (malnutrition, severe burnt, kidney decease, liver disease, uncontrolled diabetes hyperthyroidism and heart failure). High value may indicate severe dehydration.

2.15 Total Protein

Total Protein is a rough measure of serum protein. Protein measurement can reflect nutritional state, kidney disease, liver disease and many other conditions. If total protein is abnormal, further tests must be performed to identify which protein fraction, and then which specific protein, is abnormal. Proteins are important constituents of all cells and tissues. There are many different kinds of proteins in the body with many different functions. Enzymes, some hormones, haemoglobin (oxygen transport), Low Density Lipo proteins (LDL), cholesterol transport fabric oxygen (blood clothing) collagen, (structure of bone and cartilage) and immuno globulin's (antibodies) are some examples. Proteins are separated into two groups: albumin and globulins. Total protein equals albumin plus

globulin. Globulins are roughly divided into alpha-1, alpha-2 beta and gamma globulins. Albumin is the protein of highest concentration in the serum (plasma is serum plus fibrinogen). Albumin is a carrier of many small molecules, but is also important in maintaining the osmotic pressure of the blood that is keeping the fluid from leaking out into the tissues. Normal values ranges between 46-70 g/l for normal babies, adults and children, above the ages of 3years have level between 66 and 87 g/l (Weichselbaum and Amer, 1946; Josephson *et al*., 1957).

Low total protein values may indicate Burns, extensive glomerelone purities, malabsorption (inadequate absorption of nutrients from the intestinal tract) malnutrition, and protein –loss enteropathy and light chain disease. High total protein value may include; chronic inflammation or infection, multiple myeloma and waldenstrom's disease.

CHAPTER THREE

3.0 Materials and Methodology

3.1 Source of Material

Two varieties of stored soybeans, *Glycine Max* (salintuya 1 and Anidaso), maize (*Obaatampa*) and groundnut (*Chinese*) were obtained from Grains and Legumes Development Board at Abuakwa, a suburb of Kumasi. Flours of the cereal were prepared according to the flow charts shown in Figures. 1, 2, and 3.

3.2 Statistical Analysis

Statistically Analysis were carried out using Complete Randomized Design (CRD) with SPSS VERSION 15, and determinations were done in duplicates. Duncan's multiple range tests was used to determine the least significant difference (LSD) of the results obtained.

3.3 Samples Preparations

3.3.1 Preparation of soybean flour

Defective grains (with holes), stones, dried pods and other debris were removed from the soybeans. The beans were then washed and soaked in water for a day. This was done to remove some of the anti- nutritive factors such as trypsin inhibitors and haemaglutinins present in the beans. The soaked beans were then placed in a nylon sieve and allowed to drain. It was then lowered into a container containing already boiled water for about 20 minutes. This step is called "blanching". This was done to make dehulling easier, and to inactivate enzymes activity. The water was drained off and discarded. The dehulled beans were then solar dried after which it was roasted using an electric oven (Crompton, UK) to

further reduce anti- nutritive factors and improve upon the flavour of the final product. The roasted soybeans were milled into flour to obtain smooth and consistent particle sizes. The procedure is as shown in Figure 1.

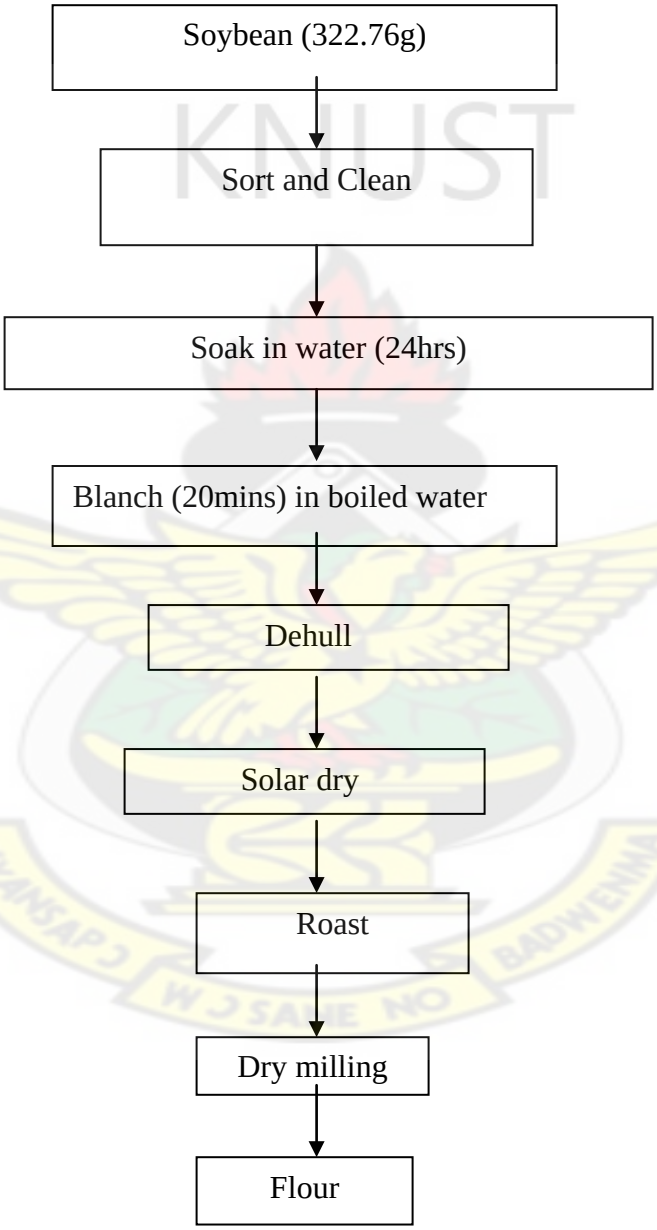

Fig. 1: Soybean flour preparation

3.3.2 Preparation of maize flour

Similar procedure was followed the maize was first sorted and washed. The grains were afterwards soaked for about a day to begin fermentation process. The soaked maize grains were washed and sieved to get rid of the water and foreign matter. It was wet-milled, made into dough and allowed to ferment for 48 hours. The fermented dough was then solar dried. The dried dough was milled into flour to obtain smooth and consistent particle sizes. The procedure is as shown in Figure 2.

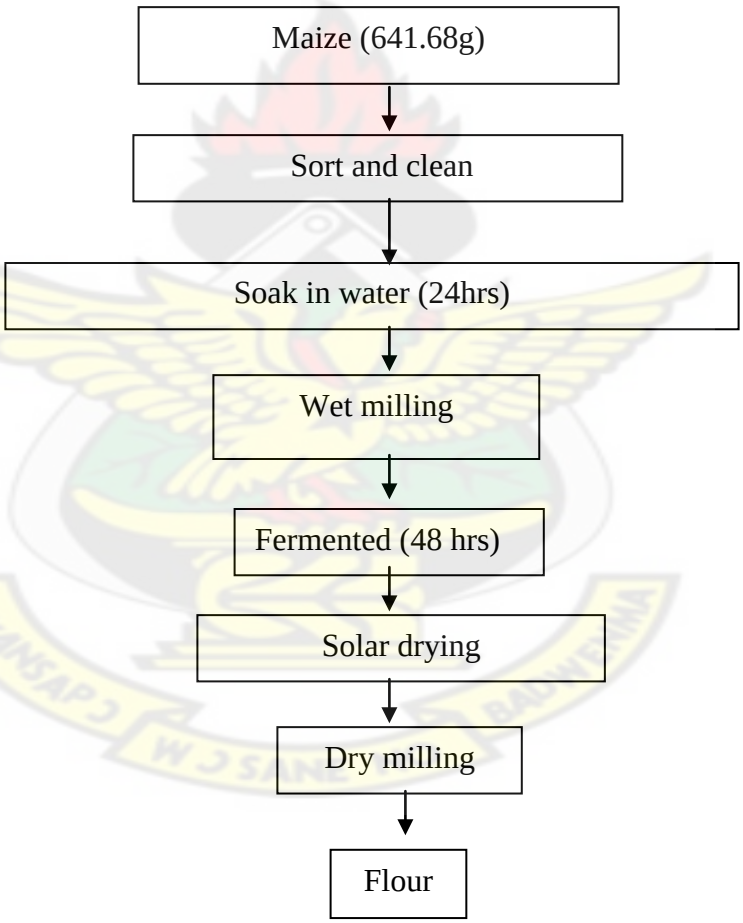

Fig. 2: Maize flour preparation

3.3.3 Preparation of groundnut flour

Defective grains (with holes), stones, dried pod and other debris were removed from the groundnut. The groundnuts were then roasted using an electric oven (Crompton, UK) for about 20 minutes. This was done for easy removal of groundnut haul and the anti-nutritive factors present in the groundnut. It was milled into paste and screw pressed using the manual screw plate press from Technology Consultancy Center, K.N.U.S.T TCC to reduce the oil content (detating) and also to obtain a groundnut cake. The groundnut cake was pulverized to smooth particle sizes, after which the groundnut flour was dried as shown in Figure 3.

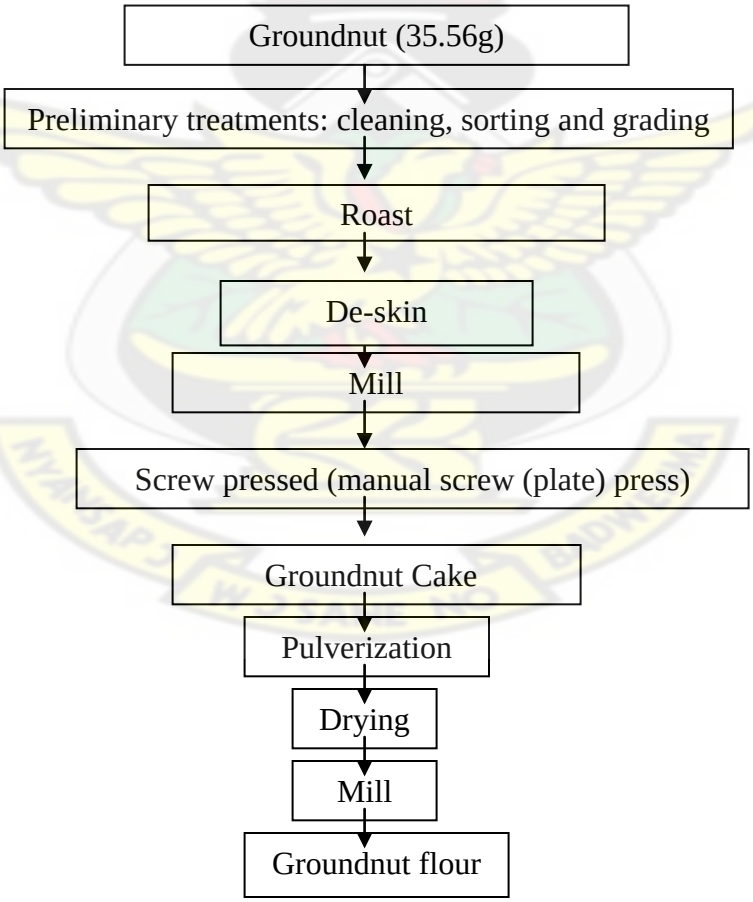

Fig. 3: Groundnut flour preparation.

The nutritional composition of the various flours were determined after which a composite flour was prepared by blending employing the material balance method (Appendix $3A_1$, $3A_2$) such that the blended flour will meet the standard set by the Protein Advisory Group (PAG, 1971) guideline for weaning food. This states that protein should be 20%, fat 10%, moisture 5% to 10%, ash not more than 5% and carbohydrate 65% in weaning foods (PAG, 1971).

3.4 Nutritional Analysis of samples

Nutritional composition on the raw, flour and formulated diets were determined. These were all done in duplicates.

3.4.1 Moisture Content

Five grams of sample was weighed into a previously dried and weighed glass crucible. The crucible and its content was placed in a thermostatically controlled oven at 105^0C for five hours. It was cooled in a desiccator and then weighed. The procedure was continued until a constant weight was obtained. The loss in weight was recorded as moisture content and it was expressed as a percentage of the total weight of sample used (AOAC, 1980).

3.4.2 Ash Content

Two grams of the dried samples from the moisture determination was weighed into a previously dried and weighed porcelain dish. It was then placed in a muffle furnace (Gallenkamp, UK) heated to 600^oC and kept constant at that temperature for six hours. The samples were removed and were then cooled in a desiccator and then weighed. The difference in weight of the residue and dish was recorded as the ash content and was expressed as a percentage of the total weight of the sample (AOAC, 1980).

3.4.3 Crude fat

Two grams of the dried sample (section 3.4.1) from the moisture determination was transferred into a 20 x 80mm paper thimble and plugged with cotton wool. It was then placed in a soxhlet extractor. Two hundred and fifty milliliters (250ml) of petroleum ether (60-80^{0}C) was measured into a previously dried and weighed round bottom flask. It was firmly attached to the soxhlet extractor, and extracted for sixteen hours on low heat. After the extraction, the flask was removed and the petroleum ether evaporated over steam bath. The flask was dried in an oven for an hour at 100^{0}C with the door of the oven not latched. It was then cooled in a desiccator and weighed. The difference in weight of the flask gave the weight of the crude fat present in the sample. This was expressed as a percentage of the total weight of sample (AOAC, 1980).

3.4.4 Crude Fibre

The defatted sample was transferred into a 750 ml Erlenmeyer flask and 0.5g asbestos added. Two hundred milliliters (200ml) of boiling 1.25% sulphuric acid was added to the flask and the flask connected to a cold finger condenser and boiled for thirty minutes. The contents of the flask was filtered and the residue washed with boiling water until the washings was no longer acidic (as tested with litmus paper). The charge and the asbestos were washed back into the flask with 200ml of boiling 1.25% NaOH solution and again attached to the cold finger condenser for thirty minutes, after which it was filtered and washed thoroughly with boiling water. The residue was transferred to a gooch crucible and washed with 15ml alcohol and dried for one hour at 100^{0}C. The crucible and contents was cooled in a desicator and then weighed. The crucible was then placed in a muffle furnace previously heated to 600^{0}C and kept constant for thirty minutes and then removed

and cooled again in a desiccator. The weight was taken and the difference in weight of the crucible and content gives the crude fibre content of the sample (AOAC, 1980).

3.4.5 Crude Protein

To the digestion tube, 2.0g of sample and a half of selenium based catalyst tablet and a few anti-bumping agents were added. Twenty five milliliters (25ml) of 5% concentrated H_2SO_4 was added and flask shaken so that the entire sample was thoroughly wet. The tube was then placed into the digestion burner and heated slowly until boiling ceased and the resulting solution was clear. It was cooled to room temperature. Digested sample was transferred into 100ml volumetric flask and made up to the mark (AOAC, 1980).

Distillation

To flush out the apparatus before use, distilled water was boiled in a steam generator of the distillation apparatus, with the connections arranged to circulate through the inner decomposition flask and out through the condenser, for 30 minutes.

Ten milliliters (10ml) of the digested sample solution was measured. The stop cock of the funnel on the steam jacket was removed and 10ml of the digested sample solution poured into it. Forty percent (40%) NaOH (excess) 15ml was added to the decomposition flask. The funnel stopcock was closed. To drive the liberated ammonia into the collection flask, steam was forced through the decomposition chamber by shutting the stopcock on the steam trap outlet. The boric acid changes to bluish-green as soon as it came into contact with ammonia and distillation was continued for 1 to 5 minutes. The receiving flask was then lowered so that the condenser tip was just above the liquid. The end of the condenser was washed with a little distilled water, and distilled for another 30 seconds. The burner was removed from the steam generator.

Titration

The distillate was titrated with 0.1M HCL solution. The acid was added until the solution was colorless. If additional acid is added, the solution becomes pink.

The same procedure was followed for the blank without the sample.

3.4.6 Carbohydrate

The carbohydrate content in the foods was obtained by calculating the difference between the sum of all the other food nutrients analyzed and subtracted from 100 (the total nutrient composition) (AOAC, 1980).

3.4.7 Mineral Determination

Two grams of the dried samples were used in the determination. The sample was ignited in a muffle furnace at a temperature of 600^0C. The ash was dissolved in 10ml of 5M HCl. Acid digestion of the ash was then carried out on a steam plate and the digested sample was carefully washed with distilled water and filtered using Whatman's filter paper into a 50ml volumetric flask and diluted to volume. The samples and blanks were then directly analyzed for the following minerals (Iron, magnesium, calcium and phosphorus) at wavelengths of 248.3, 285.2, 422 and 640 nm respectively (James, 1995) using the Atomic Absorption Spectrophotometer (SOLAR 929 Unicam A.A. Spectrophotometer, UK) at the Chemistry Department, K.N.U.S.T.

3.5 Microbial analysis of samples.

3.5.1 Preparation of samples for microbial studies

Ten (10) samples of 20g each of diet 1, 2 and 3 were packaged in a high density polyethylene (HDPE) and stored on the shelf at room temperature for microbial analysis.

3.5.2 Preparation of culture media

The culture media was prepared by suspending 23g nutrient agar into one litre of distilled water. It was then boiled to dissolve completely and sterilized by auto claving (Midas 36 Prior clave, U.K) at 121ºC for 15 minutes. It was allowed to cool to a temperature of 45ºC.

3.5.3 Microbial load count

The pour plate method was used to determine the load count. One gram of each of the three weaning food formulations was suspended into 9ml of sterile distilled water in a Mac Cartney bottles to give 10^{-1} dilution. Serial dilutions were made up to 10^{-3}. Each diluent of the samples was plated out in duplicate using the pour plating technique by transferring 1ml from each Mac Cartney bottle into 2 different Petri dish and pouring 15ml of the nutrient agar media on each sample as described by Harrigan and McCance, (1987); Nkama *et al.*, (1994); Badau *et al*., (1999) and Badau *et al*., (2001). Incubation of microorganism was done in an aerobic incubator for 48 h at 37°C. After incubation period the colonies appearing on the agar plates were counted using a colony counter (Gallenkamp colony counter, U.K). The average colony obtained from the countable duplicate plates, were expressed as colony forming unit per gram (Cfu/g).

3.6 Sensory Evaluation of formulated Diets

3.6.1 Sensory evaluation of formulated diets using untrained panelist

Ten (10) table spoonful of each sample was made into paste and poured into 1000 ml of boiling water. This was allowed to cook for 20 minutes. However, for samples C and D one table spoonful each of cinnamon was added to improve the flavour. The cooked samples A-E was presented for sensory evaluation by fifty untrained panelists. The panelists were students and workers from the Department of Biochemistry and Biotechnology, K.N.U.S.T. The panelists were people familiar with weaning diets. The attributes that were looked out for were appearance, aroma, taste, mouth feel, after taste, colour and overall acceptability. The panelists were to assign score to their preference for the various attributes using a seven (7) point hedonic scale with 1 being like extremely and 7 dislike extremely. Order of serving was critically considered and adhered to.

The samples presented to the panelists were:

A= Fermented maize flour, groundnut cake and blanch soybean (*Anidaso*)

B= Fermented maize flour, groundnut cake and blanch soybean (*Salintuya* 1)

C =Fermented maize flour, groundnut cake and blanch soybean (*Anidaso*) and cinnamon

D=Fermented maize flour, groundnut cake and blanch soybean (*Salintuya* 1) and cinnamon

E= Fermented maize flour.

The responses were presented on a bar graph and analyzed statistically.

3.6.2 Sensory evaluation of formulated diets using weaning babies

Samples A-D were presented for sensory evaluation by twenty weaning babies between the ages of 6-9 months aided by their mothers from the K.N.U.S.T hospital (Anti natal section). The panelists were babies familiar with weaning diets. The attributes that were looked out for were appearance, aroma, taste, mouth feel, after taste, colour and overall acceptability. The panelists were to assign score to their preference for the various attributes using a seven (7) point hedonic scale with 1 being like extremely and 7 dislike extremely. Mothers were to score based on the reaction, facial expression and general response by children.

The samples presented to the panelists were:

A = Fermented maize flour, groundnut cake and blanch soybean (*Anidaso*)

B =Fermented maize flour, groundnut cake and blanch soybean (*Salintuya* 1)

C = Fermented maize flour, groundnut cake and blanch soybean (*Anidaso*) and cinnamon

D =Fermented maize flour, groundnut cake and blanch soybean (*Salintuya* 1) and cinnamon

3.7 Animal Study

Ninety (90) healthy albino rats of 4 weeks maturity were obtained from the Animal House, Faculty of Pharmacy, Kwame Nkrumah University of Science and Technology, Kumasi. These rats were divided into three (3) groups labeled A, B and C. Each group contained six (6) cages of five animals each. The cages were numbered 1^{st} to 6^{th} in each group. Groups A, B and C were fed with diets 1, 2 and 3 respectively. The rats were acclimatized on 75g of the animals own commercial diet (concentrate) and 500 ml water

for one month followed by starving them on 25 g of this same diet as well as 500 ml of water for two weeks. The rats in the first cage in each group were sacrificed for various determinations such as Anthropometric (length and weight), biochemical (total protein and serum albumin), haematological (white cell count and haemoglobin) measurements after the fasting or starving. The rest of the rats in each cage were fed on 75 grams of the test diets as well as 500 ml of water each day. The animals in the 2nd to 6th cages in each group in that order were respectively sacrificed after two weeks intervals for the various determinations as mentioned above.

3.7.1 Anthropometric Measurement

3.7.1.1 Weighing of Albino Rats

The weights of the animals were determined in a weighing balance (SALTER, SL20348, UK) as shown in Figure 4. The animals were centered in the weighing tray and the weight recorded in grams. The weighing balance was checked and frequently adjusted to zero weight before each measurement was taken.

Figure 4: Picture of albino rats being weighed.

3.7.1.2 Measurement of length of Albino Rats

The animals were placed on fixed hardboard with a length measuring device (Rotring meter rule, Germany) that is marked in centimeters segments, with the zero ends at the edge of the head of the animal on the hard board. The animal was stretched by holding the back whiles pressing it firmly against the board to allow the animal to stretch appropriately. The length was read from the meter rule in centimeters as shown in Figure 5.

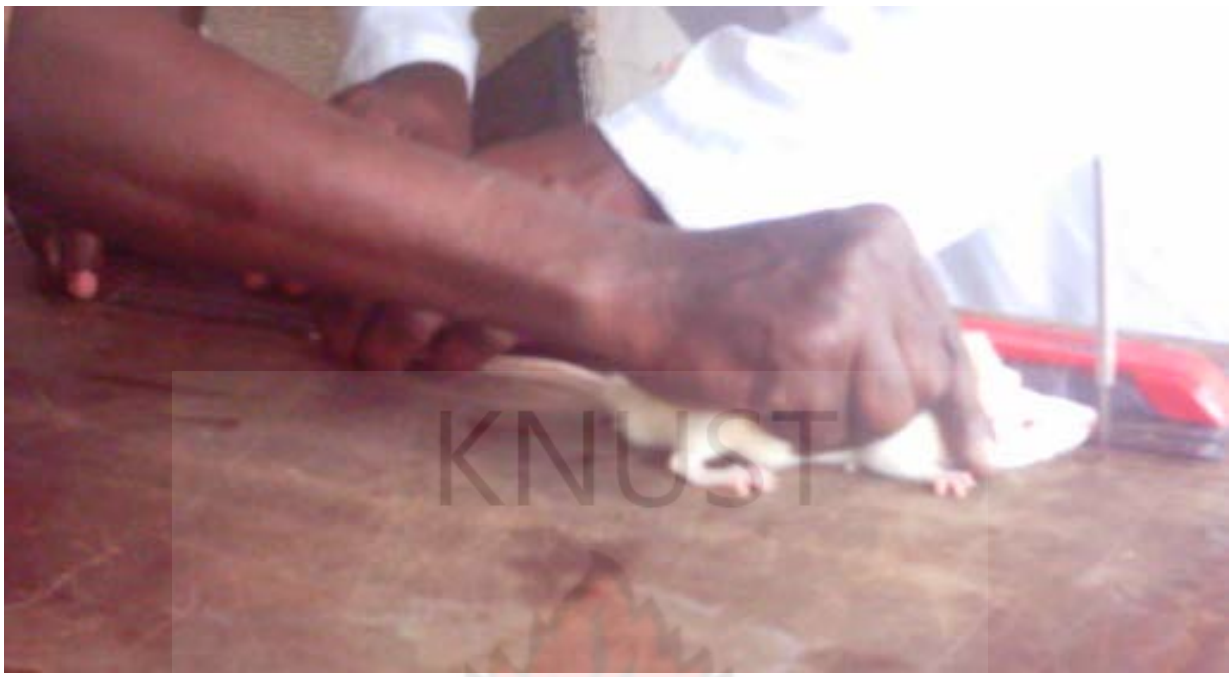

Figure 5: Picture of measuring the length of the Albino Rats

3.8 Biochemical and Haematological Analysis

Blood for haematological analysis was drawn by jugular incision and put into tubes containing ethylenediaminetetraacetate (EDTA) solution. Blood for serum protein and serum albumin determination was also drawn by jugular incision Fig. 6 into test tubes Fig. 7 and centrifuged for 30 minutes at 12000 x g*. The serum was stored in a refrigerator at a temperature of 2^{o}C for subsequent analysis (Monica, 2000).

NB: * = Acceleration due to gravity

Figure 6: Picture for taking blood sample of Albino Rat by jugular incision


Figure 7: Blood samples in storage tubes to be stored in a refrigerator for subsequent analysis.

3.8.1 Determination of Haemoglobin of Albino Rats using Colourimetric Method

A 0.2ml of whole blood was pipetted with RAININ Pipet (plus L60926, U.S.A) into a test tube containing 5ml Drabkin's reagent. The pipette was rinsed 3 to 4 times with the content in the test tube. The content in the test tube was mixed thoroughly by swirling and allowed to stand for 15 minutes at room temperature (25°). The absorbance of the content was measured using an auto analyzer (humalyzer junior, U.K) against the reagent blank at a wavelength of 540nm (Monica, 2000). The value of the haemoglobin level was determined based on the calculation method shown in appendix 16.

3.8.2 Determination of WBC count of Albino Rats using an improved Neubauer ruled Chamber

A 0.38ml of WBC diluting fluid was measured and dispensed into small containers of twenty micro litres (20µl) of well mixed EDTA and anti coagulated venous blood was added and mixed. The counting chamber was then assembled making sure that the central grid areas and the special haemolytometer cover glass were completely clean and dry. The cover glass is slide in a position over the grid areas and pressed down on each side until the rainbow color (Newton's ring) was seen. Prior to moistening of the chamber surface of each grid areas the cover glass was strongly adhered to the chamber. A capillary, Pasteur pipette held at angle of about 45^{Θ} was used to remix the diluted blood. One of the grids chamber was filled with the sample making sure that it does not over fill the area. The chamber was then left undisturbed for about 2 minutes to allow the white cells to settle. The underside of the chamber was dried and placed on the microscope stage. The white cells were counted in the four large corner squares of the chamber (Monica, 2000). The number of white cells per liter of blood was reported using the

following sample calculation:

(a) The total number of cells counted was divided by 20 and expressed as 10^9/l of white cells.

3.8.3 Determination of Serum Albumin of Albino Rats Bromo cresol Green Method (BCG)

Ten micro liters (10µl) of serum was pipetted into 1000µl of Bromo Cresol Green reagent. It was then mixed well and incubated (Griffin cool incubator UK) for 5 minutes at 20ºc. The absorbance of the sample and the standard against the reagent blank was measured within 30minutes using the auto analyzer (humalyzer junior, U.K) at a wave length of 578nm and this represent the change in absorbance (Rodkey, 1964, Doumas, 1971). The albumin levels were calculated using the formula shown in appendix 17.

3.8.4 Determination of total protein of Albino Rats using (Biuret Method)

Twenty micro liters (20µl) of serum was pipetted into 1000µl of the reagent. It was then mixed well and incubated (Griffin cool incubator, UK) for 10 minutes at 20ºc. The absorbance of the sample and the standard against the reagent blank was measured within 30 minutes using the auto analyzer (humalyzer junior, U.K) at a wavelength of 580nm and this represent the change in absorbance (Weichselbaum and Amer, 1946; Josephson *et al*., 1957). The total protein levels were calculated using the formula shown in appendix 17.

CHAPTER FOUR

4.0 Results and Discussion

4.1 Composition of Raw and Flour Sample

The proximate and mineral compositions of the raw and flour samples were as shown in Table 7.

Table 7 Proximate and mineral compositions of the raw and flour samples

Components	Contents of Samples							
	Soybean				Maize		Groundnuts	
	Anidaso		*Salintuya 1*		(*Obaatampa*)		(*Chineses*)	
	Raw 1	**Flour 1**	**Raw 2**	**Flour 2**	**Raw**	**Flour**	**Raw**	**Flour**
Moisture (%)	2.09^{d} (±0.18)	1.50^{b} (±0.04)	3.15^{e} (±0.04)	1.08^{a} (±0.25)	3.48^{f} (±0.05)	1.90^{c} (±0.05)	4.23^{g} (±0.09)	3.20^{e} (±0.09)
Crude Protein (%)	40.93^{f} (±0.25)	41.43^{g} (±0.5)	39.43^{e} (±0.13)	41.83^{g} (±0.45)	8.10^{a} (±0.25)	9.54^{b} (±0.6)	23.99^{c} (±0.5)	28.08^{d} (±0.5)
Crude Fat (%)	19.05^{d} (±0.74)	19.30^{d} (±0.5)	18.05^{c} (±0.55)	19.25^{d} (±0.1)	3.08^{a} (±0.48)	3.92^{b} (±0.4)	43.17^{f} (±0.13)	39.25^{e} (±0.13)
Ash (%)	4.02^{g} (±0.01)	1.53^{a} (±0.05)	4.49^{h} (±0.05)	1.67^{c} (±0.05)	1.76^{d} (±0.06)	1.41^{b} (±0.2)	2.98^{f} (±0.12)	1.43^{e} (±0.12)
Crude fibre (%)	4.61^{d} (±0.49)	2.42^{a} (±0.05)	4.63^{d} (±0.58)	2.61^{b} (±0.02)	3.66^{d} (±0.35)	2.62^{b} (±0.06)	4.78^{f} (±0.73)	3.60^{e} (±0.73)
Carbohydrate (%)	29.30	33.82	30.25	33.56	79.92	80.61	20.85	24.44
Iron (mg)/100g	7.19^{e} (±0.00)	8.28^{f} (±0.23)	8.26^{f} (±0.13)	9.30^{g} (±0.03)	1.24^{a} (±0.05)	2.04^{b} (±0.01)	3.47^{c} (±0.25)	4.16^{d} (±0.25)
Magnesium (mg)/100g	8.52^{e} (±0.45)	9.64^{g} (±0.17)	8.63^{f} (±0.18)	10.09^{h} (±0.25)	3.70^{a} (±0.06)	5.70^{b} (±2.56)	7.46^{c} (±0.45)	8.10^{d} (±0.45)
Calcium (mg)/100g	203.63^{d} (±0.25)	215.34^{e} (±0.44)	217.38^{e} (±0.45)	220.0^{f} (±2.34)	5.90^{a} (±0.71)	7.34^{b} (±9.86)	48.52^{c} (±0.19)	49.30^{c} (±0.23)
Phosphorus (mg)/100g	578^{e} (±18.60)	662^{g} (±20.57)	604^{f} (±24.7)	689^{h} (±24.9)	298^{a} (±9.64)	305^{b} (±9.87)	400^{c} (±17.90)	410^{d} (±19.9)

All values in the same row with different superscripts are significantly different at ($P<0.05$)

NB: Raw 1= *Anidaso***;** Raw 2= *Salintuya* 1**;** Flour 1= *Anidaso***;** Flour 2= *Salintuya* 1

It is observed that groundnut gave the highest moisture content of 4.23 % and 3.20 % for both raw and flour samples respectively. This was followed by maize with 3.48 % and 1.90 % for raw and flour samples with soybean (*Salintuya* 1 and *Anidaso*) recording 3.15 % and 2.09 % for raw samples and 1.08 % and 1.50 % for flour respectively. Meanwhile, the moisture content of all the samples were below 5%, 6 % and 7 % of literature for groundnut, maize and soybeans respectively as reported by Tindall, (1983); Matz, (1959) and Asiedu, (1989). Statistical analysis shows that significant difference ($p< 0.05$) exist between these values (Appendix 1, Table 1). The crude protein content for raw samples ranged from 8.10% to 40.94% with maize recording the least and soybean (*Anidaso*) recording the highest. With the flour the protein content was between 9.54% and 41.83% with maize recording the least and soybean *Salintuya* 1 recording the highest. The changes in crude protein of the two varieties of soybeans were as a result of changes in levels of moisture content.

However, research have shown that *Anidaso* has a higher protein content compared to that of *Salintuya* 1 and this may be attributed to the milling process that might have caused a decrease in moisture content of *Salintuya* 1 there by leading to an increase in the protein content. The protein content of maize observed in this study was low compared to the literature value of 10 % (Matz, 1959). However, that of soybeans (*Salintuya* 1 and *Anidaso*) and groundnut was significantly higher compared with literature value of 38 % and 23.2 % (Tindall, 1983; Asiedu, 1989). Also the slight increase in protein content from the raw sample to flour could be ascribed to the processing techniques such as roasting etc which resulted in the breakdown of lipocytes in soybean to release protein

and fat. Not only does the roasting increase these parameters but also helps remove the anti nutritional factors in the haul of these crops (Ologhobo and Fetuga, 1984).

Though statistical analysis showed significant difference ($p< 0.05$) between these crude protein values, the duncan's test showed no differences between the flours of the two varieties of soybeans (Appendix 1,Table 2). The fat content of raw samples was between 3.08% and 43.17%, and from 3.92% to 39.25% for flour with maize and groundnut recording the least and highest values respectively as compared to 4.4 % and 44.8 % of literature as reported by Matz, (1959) and Tindall, (1983) with the two varieties of soybean recording higher value of fat/oil content as compared with literature value of 17 % (Asiedu, 1989). The decrease in fat content of raw groundnut to flour could be attributed to screw pressing the oil out to obtain the cake (Table 7). Significant difference ($p< 0.05$) was observed between these values while no difference ($P>0.05$) existed between the flour of the two varieties of soybeans (Table 7). The crude fibre content of the raw samples were between 3.66% and 8.78% with maize and groundnut recording the least and highest values respectively. Meanwhile, that of flour was between 2.42% and 6.60% with soybean and groundnut recording the least and the highest values respectively. It could however be observed that with *Anidaso* and *Salintuya* 1, *Anidaso* had significantly low amount of fibre content compared to literature value of 5 % (Kakad *et al.,* 1972). This confirms the fact that varieties high in protein turns to have corresponding low fibre content (Kolar *et al.,* 1983). Maize and groundnut gave higher fibre content compared to literature value of 2.2 % and 2.9 % respectively (Matz, 1959; Tindall, 1983). The two varieties of soybean gave relatively low values of fibre compared

with literature value of 4.8% (Asiedu, 1989). There was no significant difference between the raw varieties of soybean and maize (Table 7) (P>0.05). The low value of fibre is important for infants and children considering their stomach capacity since they have to consume more to get satisfied to meet their daily energy requirement (Eka and Edijala, 1972).

Values obtained for raw samples showed that *Salintuya* 1 had the highest ash content of 4.49% followed by *Anidaso* with 4.02% while groundnut and maize gave 2.98% and 1.76% respectively (Table 7). Thus, the two varieties of soybean recorded slightly lower values of ash compared to literature value of 4.60 % (Asiedu, 1989) with groundnut and maize recording significantly higher value of 2.98 % and 1.76 % compared to 2 % and 1.2 % respectively reported by Tindall (1983) and Matz (1959). For flour it was between 1.41% and 1.67% with maize recording the least and soybean *Salintuya* 1 recording the highest. Significant difference ($p< 0.05$) was observed between these values (Appendix 1, Table 5). The sharp decrease in ash content from raw to flour is attributable to the dehulling process and this is very important since some of the anti nutritive factors are deposited in the haul of these crops. Ash content of food samples is an indication of the mineral content in a particular sample. Of the mineral that were analyzed, calcium and phosphorus were observed to be high in value for the two varieties of soybean compared to maize and groundnut. Among them, phosphorus seems to have the highest value ranging from 298 to 604 mg for the raw samples and from 305 to 689 mg/100g for flour with maize recording the least and soybean (*Salintuya* 1) recording the highest. It was observed that phosphorus content of all varieties studied compared well with literature

values of 300 mg/100g, 409 mg/100g and 700 mg/100g for maize, groundnut and soybean respectively (Tindall, 1983; Asiedu, 1989) though maize gave slightly low values. On the other hand the flours from these varieties gave higher values and this could be attributed to the processing technique such as fermentation which increased vitamins content and drying increasing the mineral content in these varieties as reported by Lopez *et al.*, (1983), Steinkraus, (1985), and Odunfa, (1985) ($P<0.05$) (Appendix 1, Table 9). Calcium content followed after phosphorus content with values ranging from 5.90 to 217.38 mg/100g for raw samples and between 7.34 and 220 mg for flour with maize and soybean (*Salintuya* 1) recording the least and the highest values respectively. Comparatively maize, groundnut and soybean experienced slightly low values compared to literature values of 6 mg/100g, 49 mg/100g and 250 mg/100g respectively (Tindall, 1983; Asiedu, 1989). Meanwhile, the flours of each corresponding raw sample gave higher values in calcium content ($P<0.05$) (Appendix 1, Table 8). However, no difference ($P>0.05$) was observed between *Anidaso* (flour) and the *Salintuya* 1 (raw) samples of the two varieties of soybean. All the raw samples gave relatively lower magnesium values as compared to flour samples. These increases can be attributed to drying effect during the milling process. Raw and flour samples of soybean and groundnut compared favourably with literature values (Asiedu, 1989; Tindall, 1983) with exception of maize which fell below literature values (Asiedu, 1989) ($P<0.05$) (Appendix 1, Table 7). However, iron content gave quite low values (2.24 – 8.63 mg/100g) for raw samples and between 3.52 to 9.30 mg/100g for flour with maize recording the least and soybean (*Salintuya* 1) recording the highest value ($P<0.05$) (Appendix 1, Table 6).

4.2 Formulation of Supplemented Diets

Figure 8 shows packaged composite flours of Diet 1 and Diet 2 as compared to Diet 3.

Diet 1: Fermented maize, groundnut and Soya bean flour composite (Anidaso)

Diet 2: Fermented maize, groundnut and Soya bean flour composite (Salintuya 1)

Diet 3: Fermented maize

Fig 8: Packaged samples of formulated products.

4.3 Nutritional Composition of the Supplementary Diets

The proximate and mineral composition of the formulated product was determined to ascertain its overall nutritional content. This is shown in Table 8.

Table 8 Proximate and mineral composition of Supplementary Diets

Nutrients	Diet1	Diet2	Diet3
Moisture (%)	1.82[a] (±0.5)	1.71[a] (±0.4)	1.90[b] (±0.05)
Crude protein (%)	20.58[b] (±0.2)	21.02[b] (±0.4)	9.54[a] (±0.6)
Crude fat (%)	10.65[b] (±0.1)	10.71[b] (±0.1)	3.92[a] (±0.4)
Crude fibre (%)	1.12[a] (±0.04)	1.14[a] (±0.03)	2.63[b] (±0.06)
Total ash (%)	1.44[a] (±0.2)	1.36[a] (±0.3)	1.65[b] (±0.2)
Carbohydrate (%)	64.39	64.06	80.36
Energy (kcal)	435.73 (±79.39)	436.71 (±79.59)	394.88 (±89.42)
Iron (mg)/100g	2.52[b] (±0.007)	2.85[b] (±0.005)	2.04[a] (±0.01)
Magnesium (mg)/100g	38.50[b] (±0.5)	40.25[b] (±0.005)	5.70[a] (±2.56)
Calcium (mg)/100g	98.90[b] (±10.5)	107.15[c] (±17.16)	7.34[a] (±9.86)
Phosphorus (mg)/100g	197.84[a] (±15.75)	203.33[b] (±20.5)	305[c] (±9.87)

All values in the same row with different superscripts are significantly different at ($P<0.05$)

Diet 1: Fermented maize, groundnut and blanch soy bean flour composite (Anidaso)
Diet 2: Fermented maize, groundnut and blanch soy bean flour composite (Salintuya 1)
Diet 3: Fermented maize (control).

Soybean, maize and groundnuts flours produced were used in the formulation of the supplementary weaning diets. The proportion of Groundnuts, soy bean and maize were in the ratio of 1: 8: 16 respectively (Appendix 3) employing the material balance method.

Protein and fat contributions to the blend were mainly provided by soybean while groundnut and the main source of carbohydrate is maize.

The results of this project has shown that soybean in diets is a good nutritional supplement because of its high protein and fat content for infant. In the processing of the soy flour, the beans were not defatted and hence the flour produce was full fat soy flour. The protein and fat content of Diet 1 and 2 almost doubled that of the Diet 3 with values ranging from 9.54% to 21.02% and 3.92% to 10.71% respectively. This could be attributed to the processing technique such as roasting which helped in the break down of lipocytes to release fat and protein. Not only does roasting improve the fat and protein content of soy bean but also removes the anti nutritive factors in soybean as reported by (Ologhobo and Fetuga, 1984). It can also be inferred that there is very little fat and protein in the testa of the soybean since dehulling during processing did not affect these values. Though Significant difference existed between these values ($P<0.05$) (Appendix 2, Table 11 and 12) there was no significant difference ($P>0.05$) in protein and fat content of diets 1 and 2 (Table 8).

The higher percentage of the fat in the beans confirms the fact that soybeans itself is a good source of edible oil production (FAO/WHO, 1998). This tends to agree with the recommendations of (FAO/WHO, 1998) that vegetable oils be included in foods meant for infants and children, which will not only increase the energy density, but also be a transport vehicle for fat soluble vitamins. The fat can also provide essential fatty acids

like that of n-3 and n-6 Polyunsaturated Fatty Acids (PUF's) needed to ensure proper neural development.

Soybean is also of particular interest as a vegetable protein source because of its cholesterol lowering abilities in patients with type II hyperlipoproteinamia (Sirtori *et al.*, 1985, Lovati *et al.*, 2000). The moisture contents of the test diets had values ranging from 1.71% to 1.90% with Diet 2 recording the least and the Diet 3 the highest. The decrease in moisture content of Diet 2 formulated from *Salintuya* 1 could be attributed to the milling process leading to an increase in both fat and protein content. Significant difference ($P<0.05$) existed between Diets 1, 2 and 3 (Appendix 2, Table 10). Meanwhile, there was no significant difference ($P>0.05$) in moisture content between diets 1 and 2 (Table 8).

Crude fibre and total ash decreased with values ranging between 1.12% and 2.63% for crude fibre, and between 1.36% and 1.65% for total ash for diets 1, 2 and 3 respectively though there was no significant difference ($P>0.05$) between Diets 1 and 2 (Table 8) ($P<0.05$) (Appendices 2, Table 13 and 14). These values were still less than 5% which is the maximum value for ash as recommended by (PAG, 1971) since this has nutritional implications. Dehulling of soybean may have contributed to the decrease in total ash content, but this was necessary since the anti-nutritive factors are concentrated in the haul of soybeans as reported by (Ologhobo and Fetuga, 1984). According to the Protein Advisory Group (PAG, 1971) guidelines for weaning foods, protein content should be at least 20%, fat levels up to 10%, moisture between 5% to10%, total ash not more than 5% and carbohydrate 65%. The result for the composition of the formulated diets falls within

the acceptable range of the recommendations given by PAG, 1971. Diet 3 was well below standard as a result of its low level in nutritional values. In terms of caloric worth, the protein content in Diet 1 gave 20.58 g which is equivalent to 82.32 calories (18.89 %) while that of carbohydrate gave 64.39 g which is equivalent to 257.56 calories (59.11 %). Fat gave 10.65 g which was also equivalent to 95.85 calories (21.99 %) (Appendix 4). Diet 2 had a protein content of 21.02 g which is equivalent to 84.08 calories (19.25 %) while that of carbohydrate gave 64.06 g which is equivalent to 256.24 calories (58.68 %). Fat gave 10.71 g which was also equivalent to 96.39 calories and energy 22.07 % (Appendix 4).

However, the protein content for Diet 3 was 5.54 g which is equivalent to 22.16 calories (5.62 %) while that of carbohydrate was 84.16 g which is equivalent to 336.64 calories (85.42 %) and fat was 3.92 g which is equivalent to 35.28 calories (8.95 %) (Appendix 4). Literature requires that protein should be between 10-20% of the total calories, carbohydrate between 50-60%, while fat should be less than or equal to 30% (Wardlaw, 2000). This implies that Diet 1 and 2 are excellent products in terms of caloric worth and will supply the needed energy to infants with the exception of Diet 3.

The mineral analysis conducted showed that calcium and phosphorus were observed to be higher in value (Table 8). Among them, phosphorus had higher values ranging from 197.84 to 203.33 mg/100g with Diet 1 recording the least and Diet 2 the highest value respectively ($P<0.05$) (Appendix 2, Table 18). Meanwhile diet 1 and 2 had low phosphorus levels compared to literature value of 275mg/100g (Nutritional information

centre, 2007). This was followed by calcium with values between 98.90 and 107.15 mg/100g with Diet 1 and 2 recording the least and the highest values respectively. Calcium content of diet 1 and 2 observed in this study was low compared with literature value of 270mg/100g. Significant difference ($P<0.05$) was observed between these values (Appendix 2, Table 17). The value for magnesium was between 38.50 to 40.25 mg/100g with Diet 1 and 2 having the least and the highest values respectively. Magnesium content for diet 1 and 2 were low compared with 70mg/100g of literature (Nutritional information centre, 2007). Even though significant difference ($P<0.05$) existed between these values there was no difference ($P>0.05$) between Diet 1 and 2 (Table 8). However, iron recorded the lowest mineral value (2.52 – 2.85mg/100g) with Diet 1 and 2 recording the least and the highest values respectively as compared with literature value of 11mg/100g. ($P<0.05$) but no differences existed between Diet 1 and 2 (Table 8). Generally both Diet 1, 2 and 3 had low concentration of minerals with none of them falling within the range of literature (Nutritional information centre, 2007). Therefore fortification with appropriate micronutrients or micronutrient-dense foodstuffs will be necessary.

4.4 Moisture Monitoring and Determinations

The Diets were packaged using high density polyethylene (HDPE). After two months the moisture content of the diets were determined to find out if the packaging material could serve as a good barrier between diets and environment. This was done at two (2) weeks interval. The moisture content increased gradually throughout the analysis with values ranging from 2.92- 6.20 %, 2.81- 6.10 % and 2.90-6.13 % for Diet 1, 2 and 3 respectively from week zero to eighteen. These values obtained could be attributed to perhaps the

packaging material (HDPE) which could not efficiently serve as a good barrier to the environment (Walter, 2000). However, since the range of moisture content determined for the three samples were below 10 % as recommended by (PAG, 1971) it is envisaged that the Diets could stay for a longer period if a better and hematically sealed packaging material is used. The result is shown graphically in Figure 9.

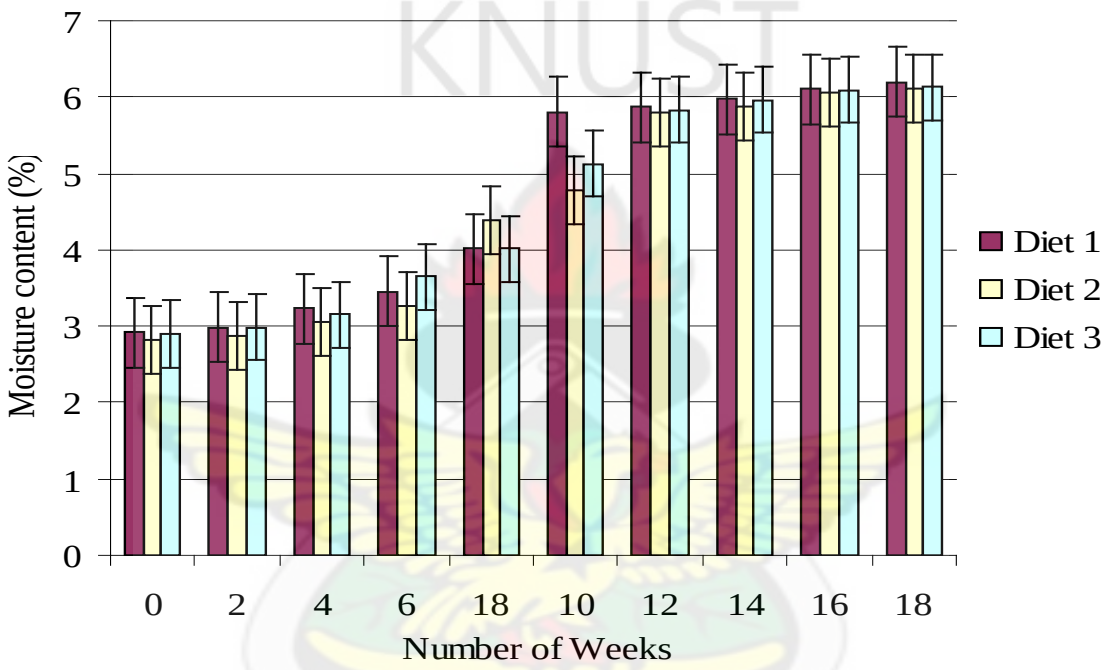

Figure 9: Moisture content against Number of Weeks

4.5 Microbial Analysis

Microbial analysis was conducted on the 12^{th} week whiles moisture determination was on going on the packaged products at 2 weeks interval. It was observed that there was general decrease in the microbial load counts with changes in dilution (10^{-1}-10^{-3}). On the 12^{th} week the microbial load count at 10^{-2} dilution ranged between 1.42 and 1.82 Cfu/g with Diet 3 having a moisture content of 5.83% recording the least and Diet 2 with moisture contents of 5.79% recording the highest. After 14^{th} week at 10^{-2}dilution the load count was between 1.62 and 1.82 Cfu/g with Diet 2 with moisture content of 5.87% recording the least and both Diet 1 and 3 with moisture content of 5.97% and 5.96% recording the highest respectively.

However, on the 16^{th} and 18^{th} week the microbial load count at 10^{-2}dilution for Diets, 1, 2 and 3 ranged from 1.62 to 2.01 Cfu/g with all the Diets recording different moisture contents (Table 9). Preliminary processes such as roasting at high temperature eliminated a large number of micro-organisms. However, the population of micro-organisms in relation to moisture content was not high enough to produce any harmful effective as at the 18^{th} week. The results obtained fell below the recommended range of 25-250 Cfu/g **(**Prescott *et al.,* 1999). This makes the product acceptable for consumption.

Table 9 Microbial load counts of Diet 1, 2 and 3 with respect to increase in moisture content.

Packaging Material (HDPE)	Moisture Content (%)	10^{-1} Cfu/g	10^{-2} Cfu/g	10^{-3} Cfu/g
Week 12				
(Diet 1)	5.87(±0.03)	24.1	1.61	0.042
(Diet 2)	5.79(±0.01)	24.1	1.82	0.091
(Diet 3)	5.83(±0.04)	22.2	1.42	0.082
Week 14				
(Diet 1)	5.97(±0.03)	24.2	1.82	0.092
(Diet 2)	5.87(±0.01)	22.2	1.62	0.022
(Diet 3)	5.96(±0.01)	24.2	1.82	0.091
Week 16				
(Diet 1)	6.10(±0.03)	24.2	2.01	0.041
(Diet 2)	6.06(±0.01)	24.1	1.62	0.092
(Diet 3)	6.09(±0.06)	24.2	1.81	0.081
Week 18				
(Diet 1)	6.20(±0.03)	24.1	1.62	0.042
(Diet 2)	6.10(±0.01)	24.1	1.81	0.091
(Diet 3)	6.13(±0.05)	24.2	2.01	0.041

Diet 1= fermented maize flour, groundnut and soybean (*Anidaso*)

Diet 2= fermented maize flour, groundnut and soybean (*Salintuya* 1)

Diet 3= fermented maize flour, (Control)

4.6 Sensory Evaluation of formulated diets

4.6.1 Sensory Evaluation using untrained panelist

Samples A-D were formulated from fermented maize flour, groundnut cake and blanch soybean with samples C and D containing cinnamon spices and E being fermented maize flour alone. Cooked samples A-E were presented for sensory evaluation by fifty untrained panelists. The attributes that were looked out for were appearance, flavour, taste, mouth feel, after taste, colour and overall acceptability. The panelists were to assign scores to their preference for the various attributes using a seven (7) point hedonic scale with 1 being like extremely and 7 dislike extremely. The results of the sensory evaluation obtained are presented in graphical form (Figure 10).

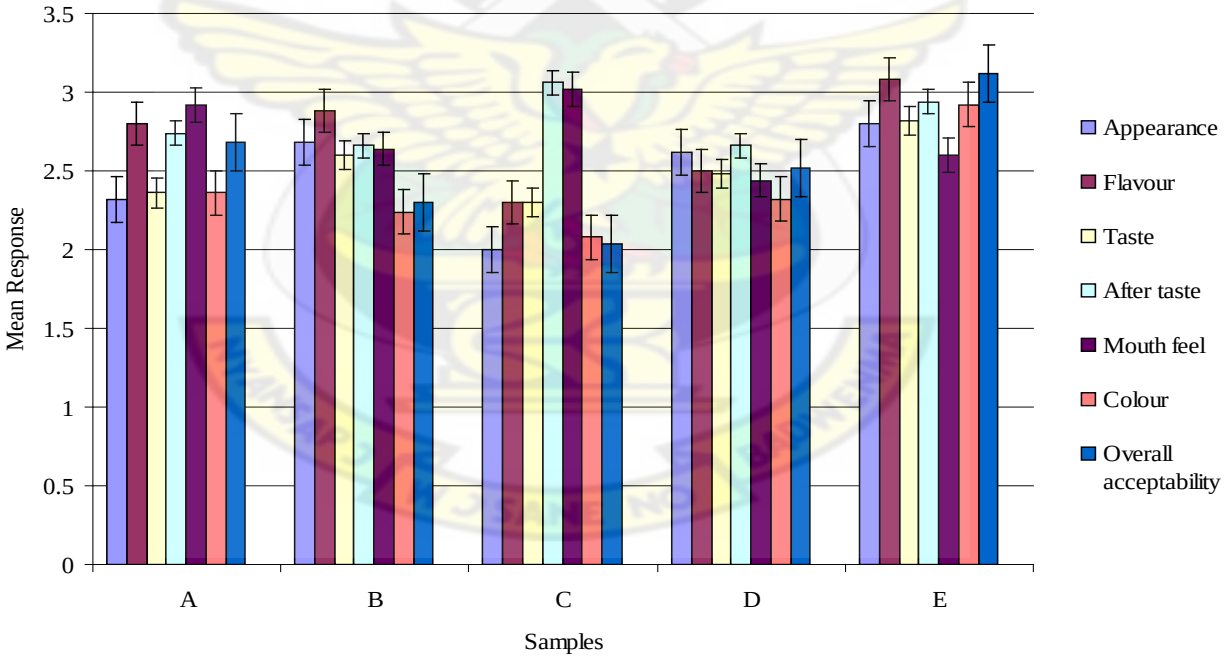

Fig. 10 Mean Response of Sensory attribute of cooked samples by untrained panelist

Appearance, flavour and taste of cooked samples ranged from 2.0 to 2.8, 2.3 to 3.08 and 2.3 to 2.82 for sample C and E respectively (fig. 14) Sample C was the most preferred and E the least preferred. There was significant difference ($P<0.05$) between both the samples and the respondent (Appendix 8, Table 27, 28 and 29). This could be ascribed to the fact that sample C was a blend of cinnamon spices, fermented maize, groundnut and soybean flour (*Anidaso*) which had good organolytic properties such as appearance, flavour etc and these are easily perceived by the senses compared to sample E which was fermented maize flour alone.

Mouth feel of cooked samples ranged from 2.44 to 3.02 for sample D and C. Samples B and D were preferred over the rest in terms of after taste with values ranging from 2.66 to 3.06. Significant difference ($P<0.05$) was observed between the respondent however, no difference ($P>0.05$) was observed between the samples (Appendices 8, Table 30 and 31).

Colour and overall acceptability of cooked samples had values ranging from 2.08 to 2.92 and 2.04 to 3.12 for sample C and E respectively. Sample C was the most preferred and E the least preferred. Significant difference ($P<0.05$) was observed between both the samples and the respondents (Appendices 8, Table 32 and 33). This could be assigned to the fact that sample E was purely fermented maize flour compared to sample C which is blend of cinnamon spices, fermented maize, groundnut and soybean flour (*Aniadso*) which has good organolytic properties which were easily perceived by the senses.

4.6.2 Sensory Evaluation using weaning Babies

Samples A-D were formulated from fermented maize flour, groundnut and soybean with samples C and D containing cinnamon spices. These cooked samples were presented for

sensory evaluation by twenty (20) weaning babies between the ages of 6-9 months from the K.N.U.S.T hospital. The attributes that were looked out for were appearance, flavour, taste, mouth feel, after taste, colour and overall acceptability. The panelists (mothers) were to assign score to their preference (using the facial expression of their babies as well as their general reaction) for the various attributes using a seven (7) point hedonic scale with 1 being like extremely and 7 dislike extremely. The results of this study are presented in graphical form (figure 11).

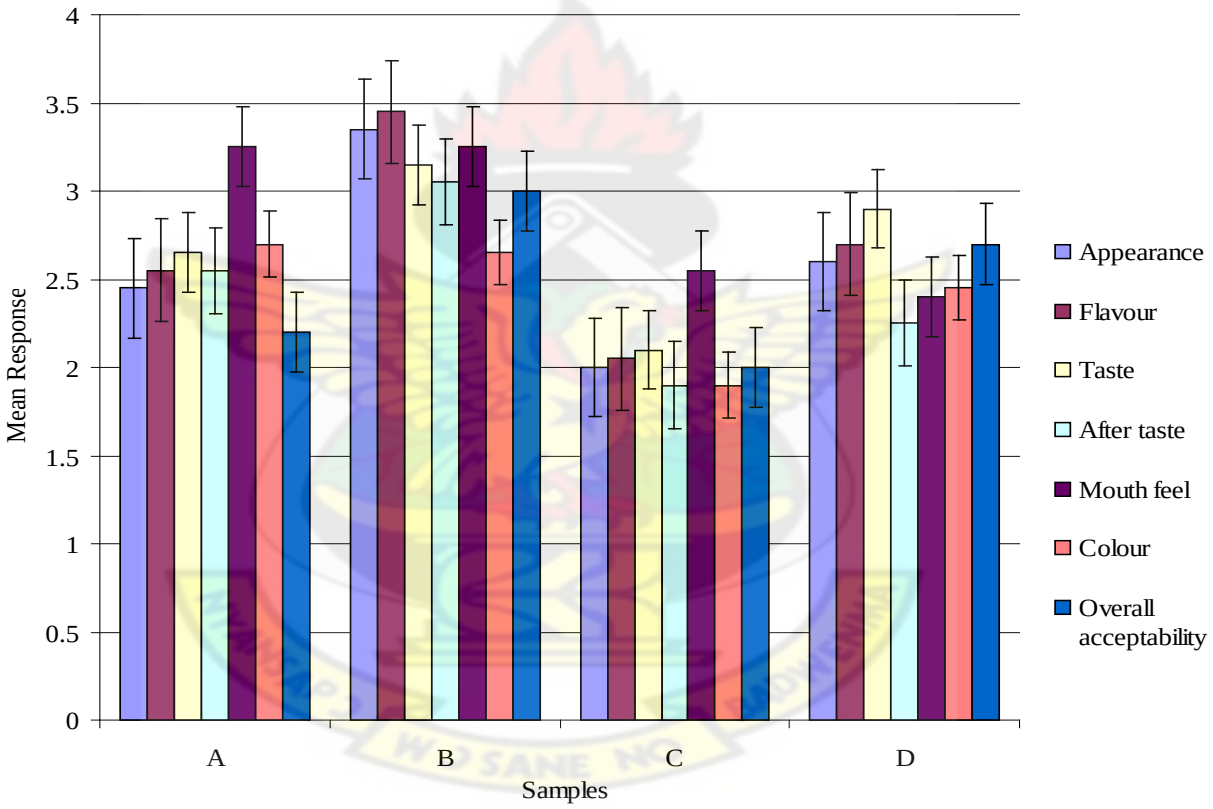

Fig. 11 Mean Response of Sensory attribute of cooked samples by Weaning Babies.

Appearance, flavour and taste of the samples range from 2.0 to 3.35, 2.05 to 3.45 and 2.1 to 3.15 for sample C and B respectively. Sample C was the most preferred and B the

least preferred. There was significant difference ($P<0.05$) between both the samples and the respondent (Appendix 11, Table 41, 42 and 43). This could be attributed to the fact that sample C was a blend of cinnamon spices, fermented maize, groundnut and soybean flour (*Anidaso*) which had good organolytic properties such as appearance, flavour etc and these are easily perceived by the senses.

Mouth feel of cooked samples had values ranging from 2.4 to 3.25. Sample D was most preferred. Significant difference ($P<0.05$) was observed between the respondent however, no difference ($P>0.05$) was observed between the samples (Appendix 11, Table 45).

After taste, colour and overall acceptability of cooked samples had values ranging from 1.9 to 3.05, 1.9 to 2.65 and 2.0 to 3.0 for sample C and B respectively. Sample C was the most preferred and B the least preferred. Significant difference ($P<0.05$) was observed between both the samples and the respondents (Appendix 11, Table 44, 46 and 47).

4.7 Anthropometric, Biochemical and Haematological Studies on Albino Rats

To determine the actual effect of formulated diets on rats an average value of weight, length, total protein, serum albumin, WBC and haemoglobin were calculated from the 2nd to the 10th week. This value was compared with the starvation value on the 0 week. A decrease or increase was expressed in terms of percentage with respect to the starvation value. According to their physical appearance, the rats in this study experienced growth and development with the formulated diets. Rats fed with Diet 3 had spiky and rough tail where as those on formulated diets 1 and 2 had smooth fur and fine tails.

The efficacy of fat and protein contents of the soy supplemented diets on growth was demonstrated in the animal model. Soy supplementary diets 1 and 2 with high fat and protein content had a remarkable effect on improving the growth of the rats compared to those on diet 3 (control) which had irregular growth. The growth measurements are as shown below:

Table 10: Length and Weight variation of Animals fed on Diets 1, 2 and 3 for the Trail period

weeks	Diet 1		Diet 2		Diet 3	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
0	178 (±29.59)	34.30 (±1.64)	170 (±25.49)	34.14 (±1.02)	169 (±34.99)	33.50 (±1.56)
2nd	196 (±20.59)	35.68 (±0.85)	189 (±17.15)	36.36 (±1.55)	179 (±24.08)	35.16 (±0.69)
4th	198 (±30.98)	35.86 (±1.04)	205 (±18.44)	36.40 (±0.77)	177 (±26.03)	35.40 (±0.64)
6th	199 (±13.89)	36.46 (±1.23)	223 (±18.28)	38.32 (±0.79)	190 (±5.47)	34.58 (±1.96)
8th	202 (±20.15)	37.04 (±1.06)	225 (±5.48)	38.52 (±0.74)	190 (±7.75)	36.46 (±0.32)
10th	207 (±19.93)	37.38 (±1.29)	227 (±9.27)	38.76 (±0.61)	183 (±5.09)	36.40 (±0.77)
Percentage (%) gain	**12.58**	**6.37**	**25.76**	**10.35**	**8.78**	**6.27**

Diet 1= fermented maize flour, groundnut and soybean (*Anidaso*)
Diet 2= fermented maize flour, groundnut and soybean (*Salintuya* 1)
Diet 3= fermented maize flour, (Control)

The percentage increase in weight by rats fed on test diets 1 and 2 were higher with values of 12.58 % and 25.76 % respectively compared to those fed on Diet 3 which had a value of 8.76 % (Appendix 16). There were significant differences in weight of the rats fed on diets 1, 2 and 3 ($p<0.05$) (Appendix 13, Table 49). The steady increase in weight could be attributed to the high protein content of the soy bean as well as the high fat content of the full fat soy flour used in formulating the diets relative to the low protein

and fat content of Diet 3. This confirms the earlier research by Badamosi *et al*., (1995) where soybean in the diet was found to improve the nutrient density of food and which resulted in the prevention of malnutrition problems. Diet 1 and 2 met the balanced nutritional needs of the rats as compared to diet 3 which was made entirely of maize. An earlier report by Fashakin and Ogunsola, (1982) suggested that maize flour alone could not meet nutritional needs for tissue formation.

A high and positive correlation was observed between body weight and length of albino rats fed on diets 1, 2 and 3 though diet 3 gave the least. There was also a positive correlation between body weight and total protein level; serum albumin; white blood cells count and haemoglobin for Diet 1 and 2 with correlation ranging from 0.8663 to 0.9611 (Table 11). Rats on Diet 3 showed low correlation between body weight and total protein; serum albumin with values ranging from 0.0735 to 0.4501. The reason being that rats suffered protein-energy malnutrition. The same reason was given by Hegsted (1968) and Laditan (1976). Though there was a positive correlation between body weight and white cell count with correlation value of 0.8525 for diet 3 there was an inverse correlation between body weight and haemoglobin of albino rats with a correlation of -0.8943 indicating symptoms of anemia confirming report by Hardison (1996). The inadequate protein level as a result of the absence of soybean in diet 3 could constitute the low level of haemoglobin.

The percentage increase in length by rat fed on test Diets 1 and 2 were 6.37 % and 10.35 % respectively, whereas rats fed on Diet 3 had 6.27 % (Appendix 18). This could be

inferred that supplementing weaning diets with plant protein can maintain adequate growth. There was a positive correlation between the length of albino rats and total protein; serum albumin; white blood cells count and haemoglobin for Diets 1 and 2. Correlation ranged from 0.86203 to 0.9860 as shown in (Table 11) with the exception of Diet 3 which showed low correlation between the length, total protein and serum albumin with values ranging from 0.0502 to 0.0791. Positive correlation (0.8566) was also observed between the length and white cell count for albino rats on Diet 3 with an inverse correlation (-0.8288) between length and haemoglobin it was due to anaemic condition. The lack of protein in Diet 3 became obvious throughout the trial period where animals fed on Diet 1 and 2 had better growth pattern. It could therefore be inferred that the amino acids in Diets 1 and 2 were available for utilization by tissues in its desired quantity. Therefore supplementing soybean in the diets of children could improve their physical growth during weaning. These dietary intervention results have implication for both human and animal nutrition.

Table 11: CORRELATION ANALYSIS ON ANTHROPOMETRIC, BIOCHEMICAL AND HAEMATOLOGICAL INDICES OF ALBINO RATS

		weight			length			Total protein			Serum albumin			WBC			haemoglobin		
		D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3
weight	D1	-			0.9808			0.9122			0.9028			0.8284			0.9611		
	D2		-			0.7682			0.8846			0.8663			0.8565			0.9409	
	D3			-			0.6000			0.0735			0.4501			0.8525			-0.8943
length	D1	-			-			0.9226			0.9145			0.8620			0.9876		
	D2		-			-			0.9063			0.8898			0.8670			0.9435	
	D3			-			-			0.0791			0.0502			0.8566			-0.8288
Total protein	D1	-			-			1			0.994			0.9777			0.8599		
	D2		-			-			1			0.9992			0.7856			0.9480	
	D3			-			-			1			0.3865			0.2239			-0.0020
Serum albumin	D1	-			-			-			1			0.9836			0.8434		
	D2		-			-			-			1			0.7765			0.8703	
	D3			-			-			-			1			0.4761			-0.4882
WBC	D1	-			-			-			-			1			0.7884		
	D2		-			-			-			-			1			0.6688	
	D3			-			-			-			-			1			-0.9699
haemoglobin	D1	-			-			-			-			-			1		
	D2		-			-			-			-			-			1	
	D3			-			-			-			-			-			1

NB; D1= DIET 1= fermented maize flour groundnut and soybean (*Anidaso*)
D2= DIET 2 = fermented maize flour groundnut and soybean (*Salintuya* 1)
D3= DIET 3= fermented maize flour, (Control)

The mean total protein content in rats fed on diet 1 and 2 increased steadily with percentage values of 17.8 and 23 respectively as compared to those fed on diet 3 which experienced a decreasing trend with percentage value of 4.6 (Figure 12).

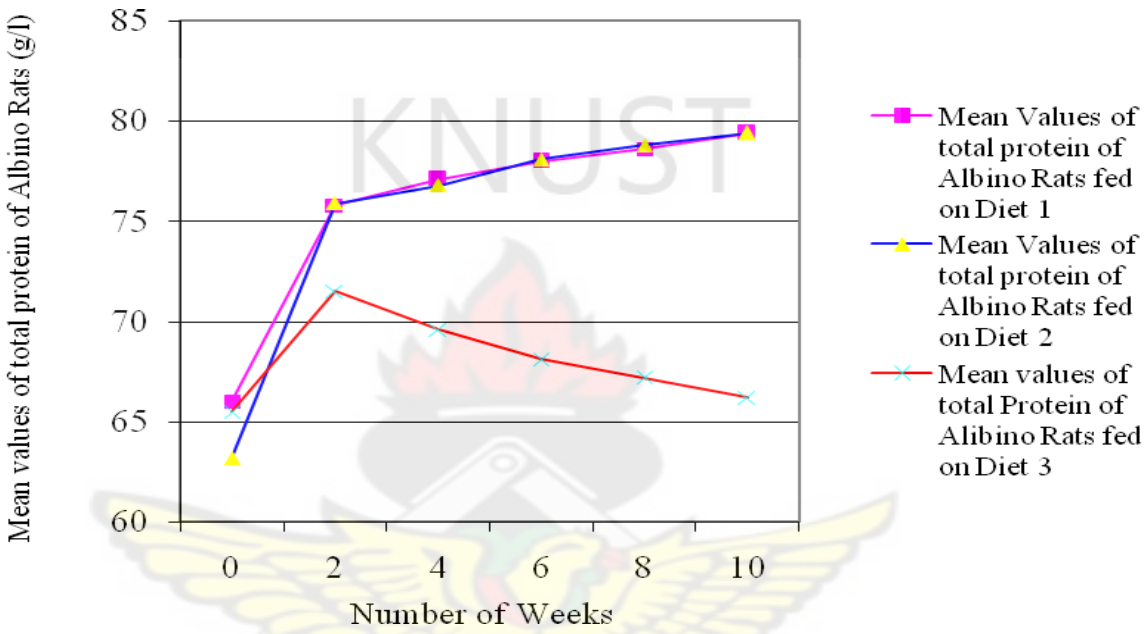

Figure 12: Total protein of Albino Rats against Number of Weeks

This could be attributed to the fact that after starvation the protein levels required to sustain the animals on diet 3 might have reduced and therefore the body system tried to synthesize amino acids through amination. However, since diets 1 and 2 are sufficient in essential amino acids it was able to support the production of complete protein to sustain growth and development. Though the results obtained fell within the recommended range of 66-87g/l as reported by Weichselbum and Amer (1946) and Bender (1965) it is envisaged that beyond the study period the protein content of rats fed on diet 3 could go below the recommended range. This is consistent with the report of (Bolarinwa *et al.*,

1991), in which there was a significant reduction in the levels of total protein in protein-calorie malnourished rats.

Hegsted (1968) and Laditan (1976) had earlier drawn a correlation between total protein levels and severity of protein energy malnutrition. The protein content of soy bean is quite high ranging from 40% in full fat soybean to 90% in isolate (IITA, 1990) and could be responsible for the high levels of the total protein. Indeed, the protein content of soybean is considerably higher than that of meat, fish, egg and other diary products on the same weight basis (McArthur *et al*., 1988). Though significant difference existed in the total protein levels between rats fed on Diets 1, 2 and 3 ($p < 0.05$) (Appendix 15, Table 57) there was no difference ($P>0.05$) in protein content between rats fed on diets 1 and 2 from the duncan's test.

Consumption of food product containing low protein levels may result in nutritional disorders particularly protein energy malnutrition. Normal protein levels in humans range from 46-70 g/l for normal babies. Adults and children, above the ages of 3years have levels of 66-87 g/l (Weichselbum and Amer, 1946) **(**Bender, 1965). All test diets which produces protein levels with values below standard are inadequate to support normal growth as shown in Figure 12.

Serum albumin levels in rats given test diets 1 and 2 increased steadily with percentage values of 46.5 and 57.3 respectively compared to those on diet 3 which experienced a decreasing trend in albumin levels with percentage value of 43 (Appendix 19) (Equation 13, 14 and 15).

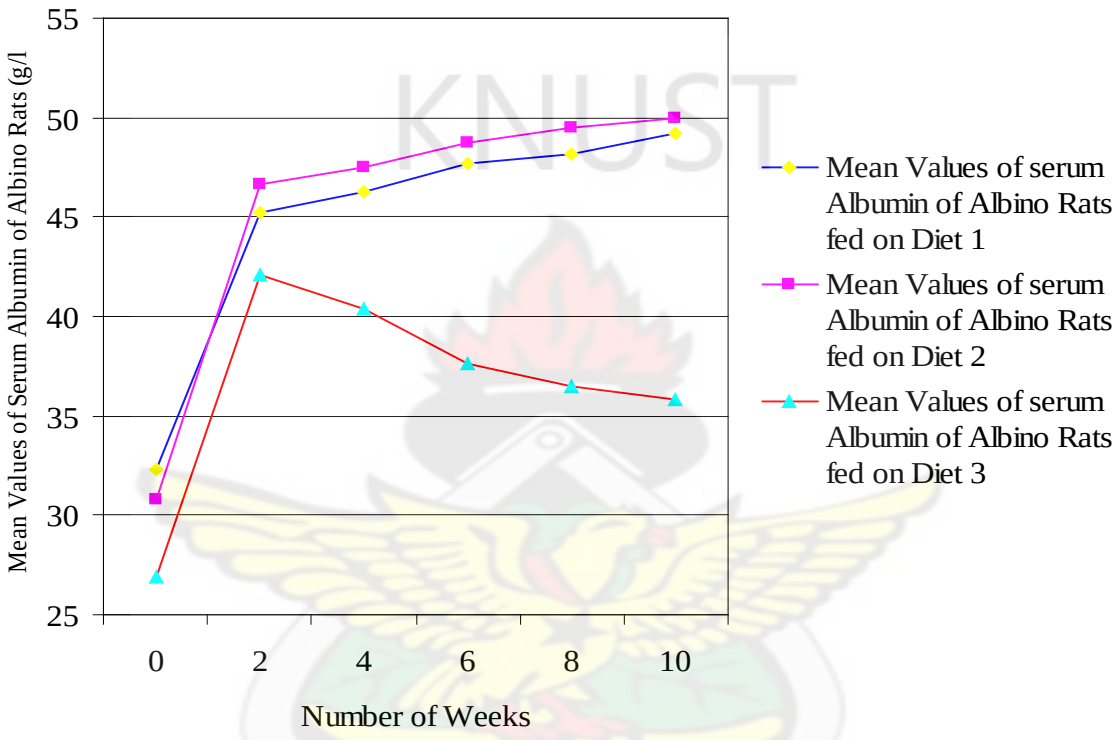

Figure 13: Serum Albumin of Albino Rats against Number of Weeks

This is because after starvation the protein levels required to maintain normal circulation of albumin in the animals might have reduced and therefore the body system tried to synthesize amino acid through amination however since diet 3 was deficient in protein it could not support the process resulting in the trend experienced.

Although significant difference was observed in the albumin levels of rats fed on Diet 1, 2 and 3 ($p < 0.05$) (Appendix 15, Table 58) yet there was no difference ($P > 0.05$) in albumin levels in rats fed on diet 1 and 2 from the duncan's test. Normal levels in humans ranged from 38-51g/l (Rodkey, 1964; Doumas, 1971). Therefore all test diets, with the exception of Diet 3 which produced albumin levels slightly below normal values were nutritionally adequate to maintain normal circulating levels of albumin. This is shown in Figure 13.

White blood cell count (WBC) showed similar effects of formulated diets on test animals. Normal white cell counts range from 4-10 $X10^9$/l (Skala *et al*., 1981).

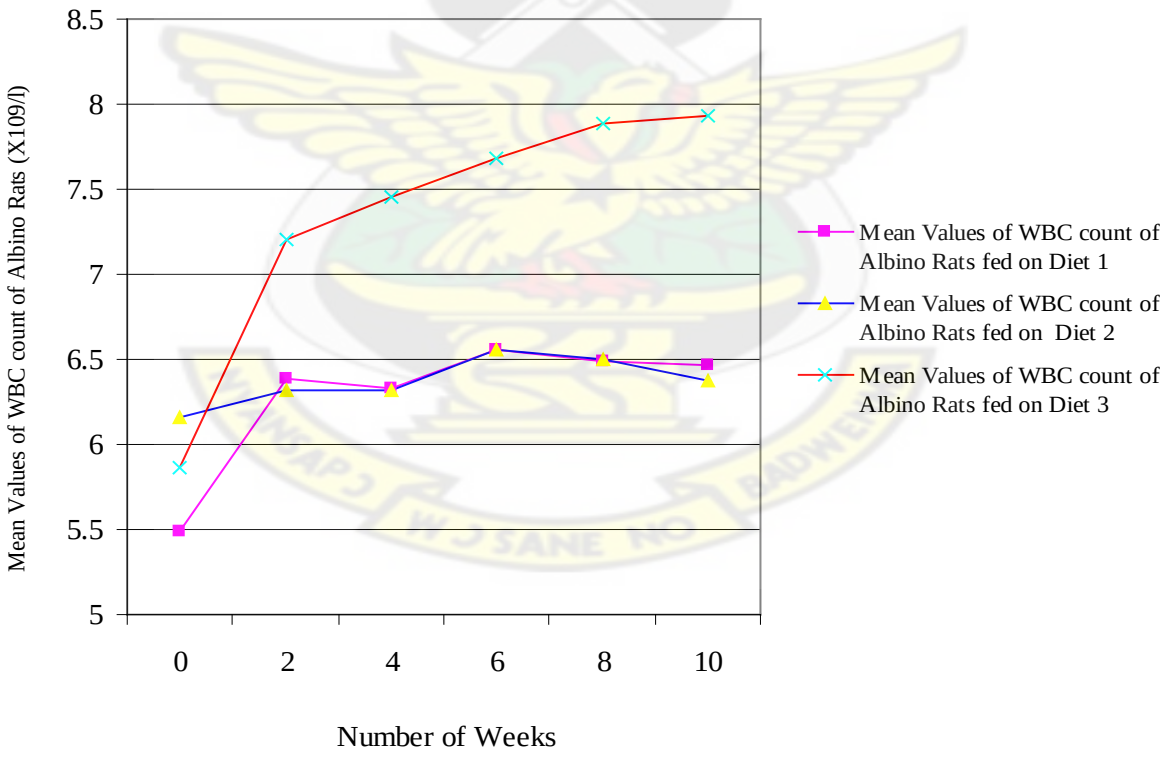

Figure 14: WBC count of Albino Rats against Number of Weeks

All the weaning foods studied produced white blood cell counts within the normal range through out the trial period with diet 1 and 2 having values of 16.6 and 3.9 % respectively compared to diet 3 which experienced a gradual increased with percentage gain of 30 (Appendix 19). Even though the results obtained fell within the range of literature it is observed that beyond the study period the WBC count of rats fed on diet 3 may exceed the recommended range. And this trend could be attributed to the absence of protein containing food (soybean) in the diet, which is required to contain all the nutrients and immunological factors which is required to maintain optimal health and growth and also help protect against the two leading cause of mortality, upper respiratory infection and diarrhea (WHO/OMS, 2000) ($P< 0.05$) (Appendix 15, Table 59). However, there was no difference between the WBC count of rats fed on diet 1 and 2 from the duncan's test.

Secondly starvation and debilitating conditions, such as nutritional disorders, particularly protein energy malnutrition can cause an increase in WBC count (Skala *et al*., 1981, Sarchielli and Chandra, 1991). This confirms that Diet 3 was inadequate for maintaining healthy nutritional status in albino rats for that matter human as reported by (Badamosi *et al*., 1995; Temple *et al*., 1996). This trend is shown in Figure 14.

The test diets 1 and 2 showed increased haemoglobin levels in rats over the period of study, with mean percentage increase value of 5.2 and 6.7 respectively with the exception

of Diet 3 which decreases through out the trial period with mean percentage decrease value of 12 (Appendix 19).

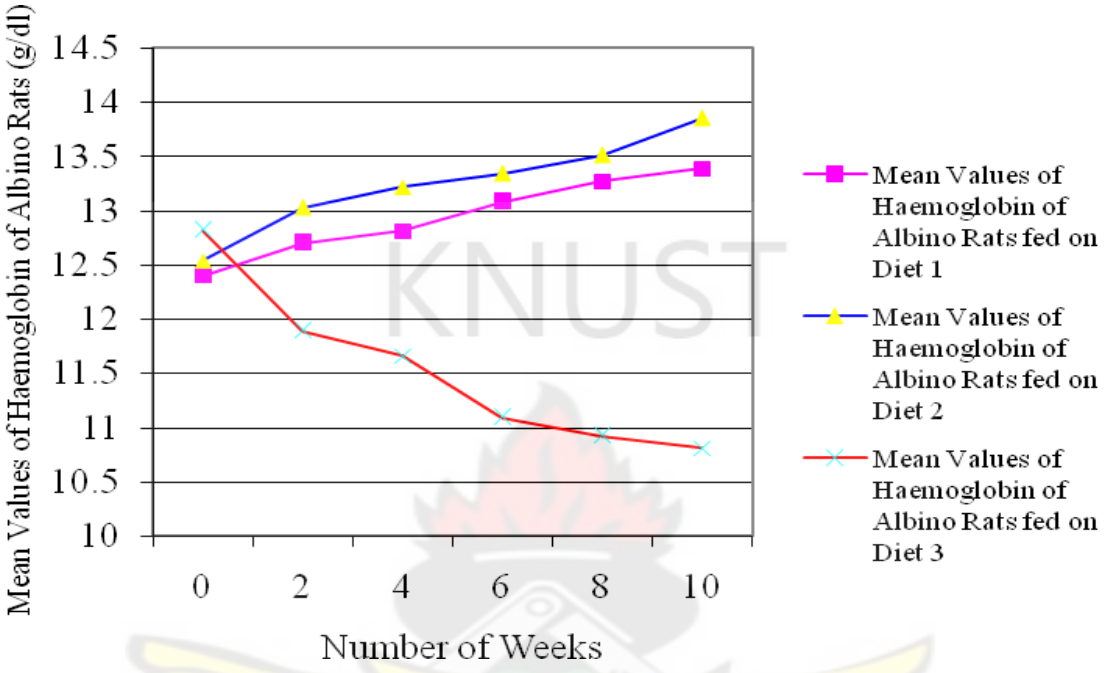

Figure 15: Haemoglobin of Albino Rats against Number of Weeks

This could be attributed to deficiency of protein in the diet. This indicates that supplementing weaning diets with plant protein can maintain adequate levels of haemoglobin in rats and for that matter humans. Significant difference in haemoglobin levels existed between rats fed on Diet 1, 2 and 3 ($p < 0.05$) (Appendix 15, Table 60). Several protein-rich diets have been shown to increase haemoglobin concentrations in human and animal studies (Bolarinwa *et al*., 1991; Mitchell, 1966). Indeed, animals fed on protein calorie malnourished diets have been reported to have significant reduction in haemoglobin concentrations (Bolarinwa *et al*., 1991). Again, it is well-documented that

kwashiorkor and marasmus patients have low levels of haematological indices (Mitchell, 1966; Adesola, 1968; and Coward and Whitehead, 1972).

Apart from the high level of protein in soybean, the protein is also of high quality consisting of most of the essential amino acids (IITA, 1990). Soybean also contains minerals and vitamins such as iron, zinc, copper, thiamine, riboflavin, niacin and patholenic acid (McArthur *et al*., 988). Most of these minerals and vitamins are well-known haematinics and are essential in the formation of red blood cells (Ganong, 1993, Mitchell *et al.,* 1976).

An advanced stage of iron deficiency is also associated with a significant decrease in circulating of haemoglobin. Subjects with levels below normal range according to sex, age, or other physiological circumstance are considered anaemic. The reference levels range from 11.0 -14.0 g/dl for infants, 12.0-15.0 g/dl for women and 13.0-18.0 g/dl in adult men. (Hercberg *et al*., 1991). The effect of this is shown in Figure 15.

CHAPTER FIVE

5.0 CONCLUSION

Soybeans can effectively be used in traditional cereal based weaning foods as an acceptable protein supplements. The process parameters and formulation developed through this study successfully produced a high protein energy weaning food with acceptable functional and sensory characteristic as well as excellent nutritional quality. Diet 2 containing *Salintuya* 1 was relatively high in protein, fat and minerals such iron, calcium, magnesium and phosphorus compared with Diet 1 containing *Anidaso*. However, sensory evaluation performed showed that sample C (fermented maize flour, groundnut cake, blanch soybean and cinnamon) was the most preferred by both untrained and weaning babies with the help of their mothers.

Though moisture content of sample increased with weeks since the packaging material could not serve as an effective barrier between food and environment, the levels of microbial load did change from the 12^{th} to 18^{th} week. The results obtained for microbial count fell below the recommended range of 25-250 Cfu/g. Animal studies indicated good growth and development in rat fed with diets 1 and 2, with no adverse biochemical or haematological effect with the exception of Diet 3 (control) which experienced irregular growth as well as biochemical and haematological defect. The animal study gave a strong correlation within and between anthropometric measurements (weight and length) and biochemical and haematological indices for rats fed on diets 1 and 2 with the exception of rats fed on diet 3 which showed weak correlation between anthropometric measurements and total protein, serum albumen and a negative correlation with haemoglobin. Though

there was a high correlation between anthropometric measurement and WBC it was due to an anaemic conditions.

5.1 RECOMMENDATIONS

During post-natal sessions, mothers should be encouraged to supplement the diet of their children with soybean/flour as the main protein source in weaning food preparation for most part of the weaning period. Fortification with appropriate micronutrient and macronutrient-dense food stuff will be necessary since the diets were found to be low in mineral content such as iron and magnesium for infants between the ages of 0-5 month. Human dietary interventions should be carried out with weaning children to assess its effect on growth in general since nutrients present in food may not give the expected effect it known to have. Further studies should also be done to determine the amino acid profile, the anti nutritive factors (if present) in supplemented foods, the essential elements profile as well as shelf life studies since all these play a crucial role in good nutrition. The department should also have its own milling machine so as to obtain products with good texture, consistency and hygiene. Animal farmers could also supplement crude protein with soy meal, instead of fish to reduce cost of production as well as maximizing profit since the animals would grow better and faster.

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APPENDICES

APPENDIX 1

Table 1: Analysis of variance (ANOVA) for % moisture of raw and flour samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Between Groups	16.52388	7	2.360555	2484.794	2.43E-11	3.787044
Within Groups	0.00665	7	0.00095			
Total	16.53053	14				

Table 2: Analysis of variance (ANOVA) for % crude protein of raw and flour samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Between Groups	2872.14	7	410.3058	580963.9	3.51E-22	3.500464
Within Groups	0.00565	8	0.000706			
Total	2872.146	15				

Table 3: Analysis of variance (ANOVA) for % crude fat of raw and flour samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Between Groups	2909.963	7	415.709	22888.31	1.46E-16	3.500464
Within Groups	0.1453	8	0.018163			
Total	2910.108	15				

Table 4: Analysis of variance (ANOVA) for % ash of raw and flour samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Between Groups	19.23778	7	2.748254	54965.07	4.38E-18	3.500464
Within Groups	0.0004	8	5E-05			
Total	19.23818	15				

Table 5: Analysis of variance (ANOVA) for % crude fibre of raw and flour samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Between Groups	69.65549	7	9.950785	3799.822	1.92E-13	3.500464
Within Groups	0.02095	8	0.002619			
Total	69.67644	15				

Table 6: Analysis of variance (ANOVA) for % iron (mg) composition of raw and flour samples

Source of Variation	*SS*	D. f	MS	F	P-value	F crit
Between Groups	136.6729	7	19.5247	29195.81	5.51E-17	3.500464
Within Groups	0.00535	8	0.000669			
Total	136.6782	15				

Table 7: Analysis of variance (ANOVA) for % magnesium (mg) composition of raw and flour samples

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	142048.5	7	20292.65	79569.25	9.98E-19	3.500464
Within Groups	2.04025	8	0.255031			
Total	142050.6	15				

Table 8: Analysis of variance (ANOVA) for % calcium (mg) composition of raw and flour samples

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	2287406	7	326772.3	1.308396	3.59E-19	3.500464
Within Groups	1998002	8	249750.3			
Total	4285408	15				

Table 9: Analysis of variance (ANOVA) for % phosphorus (mg) composition of raw and flour samples

Source of Variation	SS	D. f	MS	F	P-value	F crit
Between Groups	350096	7	50013.71	40010.97	1.56E-17	3.500464
Within Groups	10	8	1.25			
Total	350106	15				

APPENDIX 2

Table 10: Analysis of variance (ANOVA) for % moisture of formulated diets

Source of Variation	*SS*	D f	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0364	2	0.0182	364	0.000263	9.552094
Within Groups	0.00015	3	5E-05			
Total	0.03655	5				

Table 11: Analysis of variance (ANOVA) for % crude protein of formulated diets

Source of Variation	*SS*	D f	*MS*	*F*	*P-value*	*F crit*
Between Groups	200.6037	2	100.3019	2006037	6.47E-10	9.552094
Within Groups	0.00015	3	5E-05			
Total	200.6039	5				

Table 12: Analysis of variance (ANOVA) for % crude fat of formulated diets

Source of Variation	*SS*	D f	*MS*	*F*	*P-value*	*F crit*
Between Groups	60.93373	2	30.46687	609337.3	3.86E-09	9.552094
Within Groups	0.00015	3	5E-05			
Total	60.93388	5				

Table 13: Analysis of variance (ANOVA) for % ash of formulated diets

Source of Variation	*SS*	D f	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.089733	2	0.044867	897.3333	6.82E-05	9.552094
Within Groups	0.00015	3	5E-05			
Total	0.089883	5				

Table 14: Analysis of variance (ANOVA) for % crude fibre of formulated diets

Source of Variation	*SS*	D f	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.0004	2	1.5002	30004	3.53E-07	9.552094
Within Groups	0.00015	3	5E-05			
Total	3.00055	5				

Table 15: Analysis of variance (ANOVA) for iron (mg) composition of formulated diets

Source of Variation	SS	D. f	MS	F	P-value	F crit
Between Groups	0.672233	2	0.336117	3361.167	9.42E-06	9.552094
Within Groups	0.0003	3	0.0001			
Total	0.672533	5				

Table 16: Analysis of variance (ANOVA) for magnesium (mg) composition of formulated diets

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	1521.504	2	760.7521	90565.72	6.74E-08	9.552094
Within Groups	0.0252	3	0.0084			
Total	1521.529	5				

Table 17: Analysis of variance (ANOVA) for calcium (mg) composition of formulated diets

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	12265.57	2	6132.784	901880	2.14E-09	9.552094
Within Groups	0.0204	3	0.0068			
Total	12265.59	5				

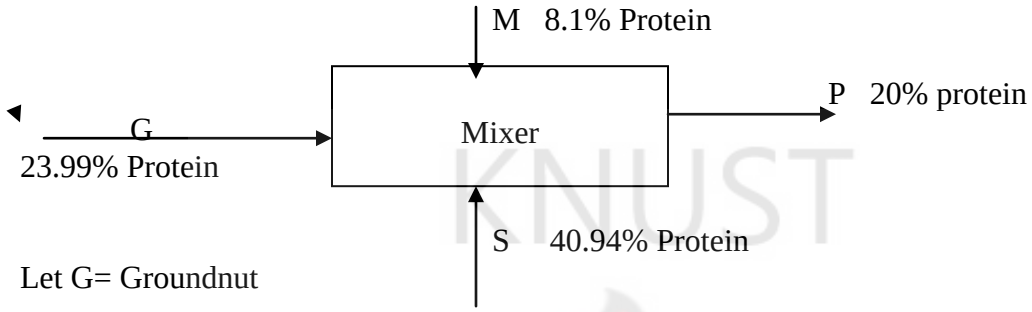
Table 18: Analysis of variance (ANOVA) for phosphorus (mg) composition of formulated diets

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	14430.68	2	7215.34	43257.44	2.04E-07	9.552094
Within Groups	0.5004	3	0.1668			
Total	14431.18	5				

APPENDIX 3

CALCULATION OF % COMPOSITIONS USING MATERIAL BALANCE METHOD FOR DIET 1

Assumption: the flow process is under steady state condition



Let G= Groundnut

M= Maize

S= Soy bean

P= Product

G + M + S = P

Total Balance

G + M + S= 100 g ……. (1)

Component Balance on Protein

0.2399G + 0.081M +0.0.4094S = 20 …… (2)

Component Balance on Fat

0.4313G + 0.0308M + 0.1905S =10…… (3)

From (1) G = 100-M-S …… (1)*

Substitute (1)* into (2)

0.2399 (100-M-S) + 0.081M + 0.4094S = 20

0.1589M – 0.1695S = 3.99…… (4)

Substitute (1)* into equation (3)

0.4313 (100-M-S) + 0.0308 M + 0.1905S = 10

0.4005M + 0.2408S = 33.13……. (5)

From (4)

M = 25.11 -1.07S…….. (6)

Substitute (6) into (5)

0.4005 (25.11 -1.07S) + 0.2408 S = 33.13

S = 32.28 g

Substitute S into (5)

0.4005M + 0.2408 (34.43) = 33.13

M = 64.17 g

Substitute M and S into (1)*

G = 100 – (62.02 + 34.43)

G = 3.55 g

=> G = 3.55 g
S = 32.28 g
M = 64.17 g

Ratio = ¼: 2:4 multiply through by 4
Ratio = 1:8:16
Mass of final product =1kg = 1000g

Mass of G to be weighed = (3.55/100) x 1000
= 35.5 g

Mass of M to be weighed = (64.168/100) x 1000
= 641.68 g

Mass of S to be weighed = (32.276/100) x 1000
= 322.76 g

CALCULATION OF % COMPOSITIONS USING MATERIAL BALANCE METHOD FOR DIET 2

Let G= Groundnut

M= Maize

S= Soy bean

P= Product

G + M + S = P

Total Balance

G + M + S= 100 g ……. (1)

Component Balance on Protein

0.2399G + 0.081M +0.3943S = 20 …… (2)

Component Balance on Fat

0.4313G + 0.0308M + 0.1805S =10…… (3)

From (1) G = 100-M-S …… (1)*

Substitute (1)* into (2)

0.2399 (100-M-S) + 0.081M + 0.3943S = 20

0.1589M – 0.1544S = 3.99…… (4)

Substitute (1)* into equation (3)

0.4313 (100-M-S) + 0.0308 M + 0.1805S = 10

0.4005M + 0.2508S = 33.13……. (5)

From (4)

M = 25.11 -0.9716S…….. (6)

Substitute (6) into (5)

0.4005 (25.11 -0.9716S) + 0.2508 S = 33.13

S = 32.43 g

Substitute S into (5)

0.4005M + 0.2508 (32.432) = 33.13

M = 62.07 g

Substitute M and S into (1)*

G = 100 – (62.71 + 32.43)

G = 3.86g

=> G = 3.86 g
S = 32.43 g
M = 62.71 g

Ratio= ¼: 2:4 multiply through by 4
Ratio 1:8:16

Mass of final product =1kg = 1000g

Mass of G to be weighed = (3.55/100) x 1000
= 35.5 g

Mass of M to be weighed = (64.168/100) x 1000
= 641.68 g

Mass of S to be weighed = (32.276/100) x1000
= 322.76 g

APPENDIX 4

CALORIC ENERGY CALCULATION FOR DIET 1, 2 AND 3

DIET 1

1g Fat = 9 calories energy
1g Carbohydrate = 4 calories energy
1g Protein = 4 calories energy

Protein = 20.58 g
Carbohydrate = 64.39 g
Fat = 10.65 g

1g Fat = 9 calories energy

=> 10.65 g Fat = 10.65 x 9

= 95.85 calories

1 g carbohydrate = 4 calories energy
=> 64.39g carbohydrate = 64.23 x 4
= 257.56 calories

1g protein = 4 calories energy
=> 20.80 g protein = 20.58x 4
=82.32 calories
Total calories = 435.73
% protein = 18.89

% carbohydrate = 59.11

% fat = 21.99

DIET 2

Protein =21.02 g

Carbohydrate = 64.06 g

Fat = 10.71 g

1g Fat = 9 calories energy

=> 10.71 g Fat = 10.71x 9

= 96.39 calories

1 g carbohydrate = 4 calories energy
=> 64.06g carbohydrate = 64.06 x 4
= 256.24 calories

1g protein = 4 calories energy
=> 21.02 g protein = 21.02x 4
=84.08 calories
Total calories = 436.71
% protein = 19.25

% carbohydrate = 58.68

% fat = 22.07

DIET 3

Protein = 8.54 g

Carbohydrate = 81.56 g

Fat = 3.92 g

1g Fat = 9 calories energy

=> 1g Fat = 3.92 x 9

= 35.28 calories

1 g carbohydrate = 4 calories energy
=> 81.56 g carbohydrate = 81.56 x 4

= 326.24 calories
1g protein = 4 calories energy

=> 8.54 g protein = 8.54x 4
= 34.16 calories

Total number of calories = 395.68

% protein = 8.63
% carbohydrate = 82.45
% Fate =8.92

APPENDIX 5

Table 19: Moisture determinations of Diets 1, 2 and 3

Number of weeks	Moisture content		
	Diet 1	Diet 2	Diet 3
0	2.92(±0.05)	2.81(±0.05)	2.90(±0.03)
2	2.98(±0.07)	2.88(±0.07)	2.99(±0.05)
4	3.23(±0.05)	3.05(±0.03)	3.15(±0.04)
6	3.45(±0.05)	3.27(±0.02)	3.65(±0.06)
8	4.01(±0.04)	4.40(±0.01)	4.01(±0.07)
10	5.81(±0.02)	4.79(±0.04)	5.13(±0.02)
12	5.87(±0.03)	5.79(±0.01)	5.83(±0.04)
14	5.97(±0.05)	5.87(±0.04)	5.96(±0.01)
16	6.10(±0.06)	6.06(±0.02)	6.09(±0.06)
18	6.20(±0.05)	6.10(±0.05)	6.13(±0.05)

APPENDIX 6

Questionnaire for sensory evaluation by untrained panelist

Msc Food Science and Technology Research project, Depart of Biochemistry and Biotechnology, K.N.U.S.T

SENSORY EVALUATION FORM: ACCEPTABILITY TEST USING UNTRAINED PANALIST

NAME……

DATE……

PRODUCT……

INSTRUCTION

You are provided with five cooked samples A-E. They are samples of a weaning food formulated from groundnuts, soybeans and fermented maize flour. Evaluate them for the following characteristics: appearance, flavour/aroma, taste, mouth feel, after taste, colour and overall acceptability.

	A	B	C	D	E
Appearance					
Flavour/Aroma					
Taste					
Mouth feel					
Colour					
Overall acceptability					

Score the samples based on the ff.

1= Like extremely
2= Like very much
3= Like slightly
4= Neither like nor dislike
5= Dislike slightly
6= Dislike very much
7= Dislike extremely

Comment ……..

……

……

APPENDIX7

Table 20: Score of panelist's response on appearance of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	2	2	2	2	3
2	5	5	1	2	3
3	3	5	2	1	5
4	5	2	3	6	2
5	3	3	3	2	5
6	2	2	1	2	2
7	3	1	1	3	1
8	2	2	1	1	2
9	1	1	3	4	1
10	2	2	2	2	3
11	1	6	7	7	2
12	1	1	1	1	1
13	1	2	2	3	2
14	2	2	1	1	1
15	2	2	2	2	3
16	3	3	3	2	5
17	1	3	3	3	2
18	4	2	2	3	2
19	2	2	3	3	2
20	3	3	3	2	3
21	3	5	1	3	1
22	3	2	3	3	3
23	3	4	3	2	1
24	3	3	3	3	4
25	3	5	3	4	2
26	2	3	3	3	2
27	3	3	3	3	2
28	3	2	4	2	3
29	3	3	3	3	4
30	1	1	1	2	5
31	1	2	1	3	5
32	3	3	2	2	3
33	3	3	2	3	2
34	1	2	2	3	2
35	3	2	2	2	4
36	3	3	1	2	4
37	1	3	1	3	2
38	3	2	2	3	2
39	1	2	1	2	2
40	1	3	2	2	1
41	1	3	1	3	5
42	1	2	1	3	4
43	3	3	1	2	5
44	3	2	1	3	3
45	1	3	1	2	5
46	3	2	1	3	3
47	3	2	1	2	5
48	1	3	1	2	3
49	3	3	1	3	2
50	2	4	2	3	1
Mean	**2.32**	**2.68**	**2**	**2.62**	**2.8**

Table 21: Score of panelist's response on flavour/aroma of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	2	2	2	2	3
2	5	5	1	2	3
3	3	5	2	1	5
4	5	2	3	6	2
5	3	3	3	2	5
6	2	2	1	2	2
7	3	1	1	3	1
8	2	2	1	1	2
9	1	1	3	4	1
10	2	2	2	2	3
11	1	6	7	7	2
12	1	1	1	1	1
13	1	2	2	3	2
14	2	2	1	1	1
15	2	2	2	2	3
16	3	3	3	2	5
17	1	3	3	3	2
18	4	2	2	3	2
19	2	2	3	3	2
20	3	3	3	2	3
21	3	5	1	3	1
22	3	2	3	3	3
23	3	4	3	2	1
24	3	3	3	3	4
25	3	5	3	4	2
26	2	3	3	3	2
27	3	3	3	3	2
28	3	2	4	2	3
29	3	3	3	3	4
30	1	1	1	2	5
31	1	2	1	3	5
32	3	3	2	2	3
33	3	3	2	3	2
34	1	2	2	3	2
35	3	2	2	2	4
36	3	3	1	2	4
37	1	3	1	3	2
38	3	2	2	3	2
39	1	2	1	2	2
40	1	3	2	2	1
41	1	3	1	3	5
42	1	2	1	3	4
43	3	3	1	2	5
44	3	2	1	3	3
45	1	3	1	2	5
46	3	2	1	3	3
47	3	2	1	2	5
48	1	3	1	2	3
49	3	3	1	3	2
50	2	4	2	3	1
Mean	**2.8**	**2.88**	**2.3**	**2.5**	**3.08**

Table 22: Score of panelist's response on taste of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	3	2	3	3	5
2	3	3	3	3	5
3	3	3	3	4	3
4	3	2	4	2	3
5	2	3	4	2	3
6	3	3	3	3	2
7	5	4	4	2	2
8	4	3	4	2	3
9	4	3	2	1	4
10	3	2	4	3	3
11	2	3	2	1	2
12	3	3	2	5	2
13	3	3	3	3	2
14	1	3	5	4	3
15	3	3	2	3	5
16	3	3	3	2	3
17	4	4	4	4	3
18	2	1	5	1	1
19	2	2	2	2	1
20	2	1	5	2	1
21	1	6	1	4	2
22	2	1	4	1	3
23	3	1	1	2	3
24	1	1	2	1	2
25	3	2	1	2	2
26	3	2	1	2	1
27	3	3	2	2	5
28	5	4	5	3	2
29	1	1	2	2	5
30	3	2	2	2	4
31	3	4	1	2	4
32	3	4	2	3	4
33	3	5	2	3	5
34	3	2	2	2	2
35	4	1	2	2	1
36	3	5	1	3	5
37	3	5	1	3	5
38	3	3	2	2	3
39	3	2	1	2	2
40	3	4	2	3	4
41	3	4	1	3	4
42	2	2	1	2	2
43	3	2	1	3	2
44	2	4	1	2	4
45	3	4	1	3	4
46	3	4	1	2	4
47	2	5	1	2	5
48	3	3	1	3	3
49	3	3	1	3	3
50	2	1	2	4	3
Mean	**2.36**	**2.6**	**2.3**	**2.48**	**2.82**

Table 23: Score of panelist's response on after taste of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	4	4	1	1	1
2	2	1	1	2	2
3	2	2	2	3	3
4	4	6	5	6	7
5	1	1	7	4	5
6	2	2	2	1	3
7	2	5	3	1	1
8	2	2	2	2	2
9	3	3	3	2	5
10	4	2	1	2	2
11	2	1	1	2	2
12	4	3	2	2	3
13	2	1	1	2	5
14	3	3	5	2	3
15	2	2	2	2	2
16	2	2	2	2	4
17	1	3	3	3	2
18	3	3	3	3	3
19	5	3	2	2	2
20	1	2	2	1	1
21	2	1	5	3	2
22	3	4	2	1	2
23	3	4	4	3	3
24	3	4	4	3	3
25	3	3	3	3	4
26	3	4	3	4	4
27	3	2	4	3	3
28	3	4	5	4	4
29	4	3	2	2	4
30	4	3	5	5	5
31	2	2	4	2	6
32	2	2	2	3	2
33	2	2	2	2	2
34	3	3	4	4	4
35	4	3	4	3	2
36	3	2	5	3	2
37	2	3	1	4	2
38	3	2	4	2	1
39	3	3	2	3	2
40	2	2	3	2	2
41	6	3	3	3	6
42	3	2	4	3	2
43	3	5	5	4	4
44	2	2	4	3	2
45	2	2	4	2	6
46	2	2	3	3	2
47	3	2	5	3	2
48	2	3	1	4	2
49	3	2	4	3	1
50	3	3	2	2	3
Mean	**2.74**	**2.66**	**3.06**	**2.66**	**2.94**

Table 24: Score of panelist's response on mouth feel of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	4	5	3	2	2
2	4	3	5	3	3
3	2	2	3	2	4
4	4	3	5	2	3
5	3	3	4	3	1
6	3	4	4	2	3
7	3	4	5	3	4
8	4	5	1	2	3
9	2	1	4	3	3
10	1	3	2	1	1
11	5	3	2	2	2
12	5	3	3	3	3
13	2	3	2	3	2
14	2	2	2	2	1
15	3	5	3	2	3
16	3	3	3	3	3
17	2	3	2	1	1
18	1	1	2	2	2
19	2	2	7	4	4
20	5	6	2	3	2
21	2	2	1	2	4
22	2	2	2	2	3
23	4	4	1	1	1
24	4	2	1	3	1
25	1	1	1	1	2
26	2	1	1	2	3
27	3	3	4	3	2
28	2	1	1	2	5
29	4	3	2	2	4
30	4	2	1	3	1
31	3	2	2	4	2
32	2	1	2	3	3
33	3	3	4	5	4
34	3	3	3	1	2
35	3	2	6	3	2
36	3	2	2	2	1
37	2	2	4	3	2
38	1	1	1	1	1
39	4	3	4	2	4
40	6	4	4	3	2
41	2	2	4	4	3
42	2	1	2	2	2
43	3	3	5	3	3
44	3	3	3	3	6
45	2	2	6	3	3
46	4	3	4	3	2
47	3	3	3	1	2
48	3	2	6	3	2
49	3	3	2	2	3
50	3	2	5	2	5
Mean	**2.92**	**2.64**	**3.02**	**2.44**	**2.6**

Table 25: Score of panelist's response on colour of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	5	5	3	2	3
2	4	3	2	2	4
3	4	3	3	3	4
4	2	2	4	3	3
5	3	3	3	3	4
6	3	3	3	3	3
7	2	3	3	4	2
8	2	2	2	2	2
9	3	4	2	3	1
10	3	1	2	2	2
11	2	3	1	1	1
12	2	2	2	2	2
13	2	2	3	3	2
14	2	2	1	2	2
15	1	3	3	3	2
16	2	1	2	3	2
17	1	1	1	1	1
18	3	2	2	4	5
19	3	2	2	2	3
20	5	3	3	1	3
21	3	2	2	4	3
22	3	2	3	3	5
23	2	2	2	2	3
24	2	1	1	1	1
25	1	1	2	2	3
26	1	1	1	1	2
27	1	1	2	1	5
28	2	2	2	3	2
29	1	2	2	3	2
30	1	1	1	2	3
31	2	2	2	2	2
32	3	2	3	2	2
33	1	1	1	1	2
34	4	4	2	2	4
35	2	4	1	4	4
36	3	3	2	3	5
37	3	2	2	3	4
38	2	2	2	2	3
39	1	1	1	1	1
40	2	2	2	2	3
41	1	1	1	2	3
42	2	2	1	1	5
43	4	4	4	4	4
44	2	2	2	2	5
45	4	3	2	2	4
46	2	2	2	2	3
47	4	3	3	3	2
48	1	3	3	4	3
49	2	2	1	1	5
50	2	2	2	2	2
Mean	**2.36**	**2.24**	**2.08**	**2.32**	**2.92**

Table 26: Score of panelist's response on over all acceptability of cooked samples A, B, C, D and E

JUDGE	A	B	C	D	E
1	4	3	1	1	1
2	2	2	2	3	2
3	2	2	2	3	3
4	1	1	1	3	5
5	3	3	5	5	5
6	1	2	2	2	3
7	1	1	3	3	4
8	3	3	3	3	3
9	3	2	3	2	3
10	4	1	1	3	3
11	3	2	2	2	4
12	1	1	1	1	5
13	3	3	3	2	5
14	1	1	1	2	2
15	3	3	1	3	5
16	2	2	5	4	3
17	2	3	3	3	3
18	2	3	2	3	3
19	1	1	2	3	2
20	2	1	4	3	5
21	1	2	3	4	4
22	3	3	4	3	4
23	3	3	4	4	4
24	2	3	3	3	3
25	2	3	2	3	3
26	2	3	2	3	4
27	3	3	2	3	4
28	4	4	1	3	2
29	3	2	3	5	5
30	3	2	1	1	3
31	2	1	2	2	3
32	5	3	2	3	2
33	3	3	1	2	3
34	2	4	2	2	3
35	2	1	2	3	2
36	4	3	2	1	2
37	4	1	3	4	3
38	2	1	1	1	3
39	2	3	2	2	2
40	2	3	1	1	2
41	1	2	1	2	3
42	5	1	1	2	3
43	4	3	1	3	2
44	5	3	1	1	3
45	3	4	2	2	2
46	5	1	1	1	3
47	3	3	1	3	2
48	5	3	2	2	2
49	3	1	1	1	3
50	2	3	1	2	3
Mean	**2.68**	**2.3**	**2.04**	**2.52**	**3.12**

APPENDIX 8

Table 27: Analysis of variance (ANOVA) on appearance of cooked samples

Source of Variation	*SS*	*D* f	*MS*	*F*	*P-value*	*F crit*
Row	0.255102	49	0.255102	0.217786	2.265E-10	3.940163
Columns	112.449	4	1.171344	4.12675	0.003117	2.417725
Error	95.184	196	0.485633			
Total	112.7041	249				

Table 28: Analysis of variance (ANOVA) on flavour/aroma of cooked samples

Source of Variation	*SS*	*D* f	*MS*	*F*	*P-value*	*F crit*
Row	102.896	49	1.44924	1.243129	0.00267596	3.938111
Columns	36.816	4	1.158367	5.93447	0.00158	2.429625
Error	303.984	196	1.550939			
Total	114.96	249				

Table 29: Analysis of variance (ANOVA) on taste of cooked samples

source of variation	*ss*	*d* f	*ms*	*f*	*p-value*	*f crit*
row	11.56	49	11.56	9.219401	0.003068	3.938111
columns	122.88	4	1.253878	4.12567	0.003117	2.417725
error	208.096	196	1.061714			
total	134.44	249				

Table 30: Analysis of variance (ANOVA) on after taste of cooked samples

Source of Variation	*SS*	*D* f	*MS*	*F*	*P-value*	*F crit*
Row	103.395	39	1.96	1.510063	5.81 E-14	3.938111
Columns	127.2	4	1.297959	2.363636	0.222072	2.467211
Error	124.08156	156	0.795385			
Total	129.16	199				

Table 31: Analysis of variance (ANOVA) on mouth feel of cooked samples

Source of Variation	*SS*	*D* f	*MS*	*F*	*P-value*	*F crit*
Row	2430.16	49	2.42016	0.149561	0.009793	2.578423
Columns	104.84	4	1.069796	1.563201	0.828625	2.417725
Error	117.548	196	0.879796	0.599918		
Total	277.476	249				

Table 32: Analysis of variance (ANOVA) on colour of cooked samples

Source of Variation	*SS*	*D* f	*MS*	*F*	*P-value*	*F crit*
Row	148.276	49	2.3064	0.816667	1.93 E-14	3.938111
Columns	102.64	4	0.783673	4.840659	0.00096	2.41775
Error	117.584	196	1.047347			
Total	213.65	249				

Table 33: Analysis of variance (ANOVA) on over all acceptability of cooked samples

Source of Variation	*SS*	*D* f	*MS*	*F*	*P-value*	*F crit*
Row	99.6	49	123.61	3.120303	2.74 E-18	3.938111
Columns	113.386	4	1.156939	1.579912	0.00436	2.417643
Error	172.44	196	0.879769			
Total	116.99	249				

APPENDIX 9 QUESTIONAIRE

Msc Food Science and Technology Research project, Department of Biochemistry and Biotechnology, K.N.U.S.T

SENSORY EVALUATION FORM: ACCEPTABILITY TEST USING WEANING BABIES

NAME OF CHILD……

AGE OF CHILD…….

DATE……

PRODUCT……

INSTRUCTION

You are provided with five cooked samples A-D. They are samples of a weaning food formulated from groundnuts, soybeans and fermented maize flour. Evaluate them for the following characteristics: appearance, flavour/aroma, taste, mouth feel, after taste, colour and overall acceptability. Mothers should assist babies based on their reactions to the products.

	A	B	C	D
Appearance				
Flavour/Aroma				
Taste				
Mouth feel				
Colour				
Overall acceptability				

Score the samples based on the ff.

1= Like extremely 5= Dislike slightly

2= Like very much 6= Dislike very much

3= Like slightly 7= Dislike extremely

4= Neither like nor dislike

Comment ……..

……

……

APPENDIX 10

Table 34: Score of panelist's response on appearance of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	2	3	2	2
2	5	5	1	2
3	3	5	2	1
4	5	2	3	6
5	3	3	3	2
6	2	2	1	2
7	3	4	1	3
8	2	2	1	1
9	1	2	3	4
10	2	2	2	2
11	1	6	1	7
12	1	5	1	1
13	1	2	2	3
14	2	7	1	1
15	2	2	2	2
16	3	3	3	2
17	2	3	3	3
18	4	4	2	3
19	2	2	3	3
20	3	3	3	2
Mean	**2.45**	**3.35**	**2**	**2.6**

Table 35: Score of panelist's response on Flavour of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	2	2	2	2
2	5	5	1	2
3	3	5	2	1
4	5	2	3	6
5	3	3	3	2
6	2	2	1	2
7	3	7	1	3
8	2	2	2	1
9	1	6	3	4
10	2	2	2	2
11	2	6	1	7
12	1	4	1	3
13	2	2	2	3
14	2	6	1	1
15	2	2	2	2
16	3	3	3	2
17	2	3	3	3
18	4	2	2	3
19	2	2	3	3
20	3	3	3	2
Mean	**2.55**	**3.45**	**2.05**	**2.7**

Table 36: Score of panelist's response on Taste of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	3	2	3	3
2	3	3	2	3
3	3	3	1	4
4	3	2	2	2
5	2	3	1	2
6	3	3	1	3
7	2	4	2	2
8	4	3	4	2
9	4	3	2	5
10	3	6	4	3
11	2	3	2	3
12	3	3	1	5
13	3	3	3	3
14	2	3	5	4
15	1	3	2	3
16	3	3	1	2
17	4	4	1	4
18	2	4	1	1
19	2	2	2	2
20	1	3	2	2
Mean	**2.65**	**3.15**	**2.1**	**2.9**

Table 37: Score of panelist's response on aftertaste of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	4	4	1	1
2	2	1	1	2
3	2	2	2	3
4	4	6	5	6
5	1	4	2	4
6	2	2	2	1
7	2	5	3	1
8	2	2	2	2
9	3	3	3	2
10	4	2	1	2
11	2	7	1	2
12	4	3	2	2
13	2	2	1	2
14	3	3	2	2
15	2	2	2	2
16	2	2	2	2
17	1	3	1	3
18	3	3	1	3
19	5	3	2	2
20	1	2	2	1
Mean	**2.55**	**3.05**	**1.9**	**2.25**

Table 38: Score of panelist's response on Mouth feel of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	3	5	4	2
2	5	3	4	3
3	3	2	2	2
4	5	3	4	2
5	4	3	3	3
6	4	4	3	2
7	5	4	3	3
8	2	5	4	2
9	4	2	2	3
10	2	3	1	1
11	2	3	2	2
12	3	3	5	3
13	2	3	2	3
14	2	2	2	2
15	3	5	3	2
16	3	3	1	3
17	2	3	2	1
18	2	1	1	2
19	7	2	2	4
20	2	6	1	3
Mean	**3.25**	**3.25**	**2.55**	**2.4**

Table 39: Score of panelist's response on Colour of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	5	5	3	2
2	4	3	2	2
3	4	3	1	3
4	2	2	1	3
5	3	3	3	3
6	3	3	3	3
7	2	3	3	4
8	2	2	2	2
9	3	4	2	3
10	3	1	2	2
11	2	3	1	1
12	2	2	2	2
13	2	2	3	3
14	2	2	1	2
15	1	3	1	3
16	2	1	2	3
17	1	4	1	1
18	3	2	2	4
19	3	2	2	2
20	5	3	1	1
Mean	**2.7**	**2.65**	**1.9**	**2.45**

Table 40: Score of panelist's response on Overall acceptability of cooked samples A, B, C and D

JUDGE	A	B	C	D
1	4	3	1	1
2	2	2	2	3
3	2	2	2	3
4	1	1	1	3
5	3	3	5	5
6	1	2	2	2
7	1	7	3	3
8	3	3	3	3
9	3	2	3	2
10	4	1	1	3
11	3	2	2	2
12	1	7	1	1
13	3	3	3	2
14	1	1	1	2
15	3	3	1	3
16	2	2	1	4
17	2	3	3	3
18	2	3	2	3
19	1	6	2	3
20	2	4	1	3
Mean	**2.2**	**3**	**2**	**2.7**

APPENDIX 11

Table 41: Analysis of variance (ANOVA) on appearance of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	18.9	3	6.3	3.619048	0.016832	2.724944
Columns	132.3	76	1.740789	2.17654	0.002563	3.942173
Error	197.096	124	1.06124			
Total	151.2	79				

Table 42: Analysis of variance (ANOVA) on flavour of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	20.1375	3	6.7125	3.722364	0.014853	2.724944
Columns	137.05	76	1.803289	1.253901	0.026843	1.347533
Error	203.145	119	1.24695			
Total	157.1875	79				

Table 43: Analysis of variance (ANOVA) on taste of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	12.1	3	4.033333	3.996523	0.010668	2.724944
Columns	76.7	76	1.009211	2.657431	1.23E-10	3.320252
Error	160.34	201.906	3.24597			
Total	88.8	79				

Table 44: Analysis of variance (ANOVA) on after taste of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	14.2375	3	4.745833	3.236279	0.026794	2.724944
Columns	111.45	76	1.466447	1.903687	1.265E-14	3.24190
Error	97.456	109.457				
Total	125.6875	79				

Table 45: Analysis of variance (ANOVA) on mouth feel of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	12.2375	3	4.079167	2.945527	0.038193	2.724944
Columns	105.25	76	1.384868	1.349856	0.789190	2.317903
Error	209.1478	125.48	3.369012			
Total	117.4875	79				

Table4 6: Analysis of variance (ANOVA) on colour of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	8.05	3	2.683333	2.934293	0.03872	2.724944
Columns	69.5	76	0.914474	3.483578	1.265E-14	1.24190
Error	214.901	195.170	1.239012			
Total	77.55	79				

Table 47: Analysis of variance (ANOVA) on overall acceptability of cooked samples

Source of Variation	SS	D f	MS	F	P-value	F crit
Row	13.1775	3	4.3925	2.993186	0.035705	2.718785
Columns	117.4	80	1.4675	1.780367	0.001458	1.780356
Error	226.091	201.56902	3.68484			
Total	130.5775	83				

APPENDIX 12

Table 48: Raw data on anthropometric measurements of albino rats at two weeks intervals

week 0						
Group/ Rats	**A_o**		**B_o**		**C_o**	
	weight	**length**	**weight**	**length**	**weight**	**length**
1	195	35	175	33.7	140	32.5
2	200	35.6	185	34.5	170	32.8
3	185	32.5	180	34.4	160	33.5
4	135	32.2	190	35.6	235	36.5
5	225	36.2	120	32.5	140	32.2
week 2						
Group/ Rats	**A_2**		**B_2**		**C_2**	
1	205	35.2	195	38.1	225	36.9
2	155	34.3	160	34.4	180	36.4
3	205	36.1	205	37.1	175	36.3
4	205	36.8	205	37.6	170	33.6
5	210	36	180	34.6	145	35.6
Week 4						
Group/ Rats	**A_3**		**B_3**		**C_3**	
1	225	36.3	190	37.1	175	34.9
2	220	36.7	200	35.5	220	36.4
3	220	36.8	240	37.4	160	34.6
4	180	35.5	205	36.4	150	35.8
5	145	34	190	35.6	180	35.3
Week 6						
Group/ Rats	**A_4**		**B_4**		**C_4**	
1	180	36.8	245	39.5	190	36.4
2	195	35.8	220	37.7	195	36.8
3	245	38.6	210	39	190	37.1
4	180	34.9	220	37.9	180	37.4
5	195	36.2	220	37.5	195	36.2
Week 8						
Group/ Rats	**A_5**		**B_5**		**C_5**	
1	195	37.8	225	39.6	185	36.2
2	240	35.9	235	38	195	36.6
3	180	38.6	225	39.2	195	37
4	200	35.9	220	38.1	180	36.4
5	195	37	220	37.7	195	36.1
Week 10						
Group/ Rats	**A_6**		**B_6**		**C_6**	
1	200	38.2	240	39.6	180	36.3
2	245	36.2	215	38.2	185	37.4
3	185	39.4	235	39.4	190	37.3
4	210	35.9	225	38.4	175	36.2
5	195	37.2	220	38.2	185	35.5

APPENDIX 13

Table 49: Analysis of variance (ANOVA) of mean weight again of albino rats for the trail periods (p< 0.05)

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	1978.111	2	989.0556	4.627281	0.02721	3.68232
Within Groups	3206.167	15	213.7444			
Total	5184.278	17				

Table 50: Analysis of variance (ANOVA) of mean length again of albino rats for the trail periods (p< 0.05)

Source of Variation	SS	D.f	MS	F	P-value	F crit
Between Groups	9.912933	2	4.956467	2.586157	0.008397	3.68232
Within Groups	28.74807	15	1.916538			
Total	38.661	17				

APPENDIX 14

Table 51: Raw data on biochemical and haematological analysis of albino rats for

Number of Albino Rats	Total Protein (g/1)	Serum Albumin (g/1)	Haemoglobin count (g/dl)	WBC count ($X10^9$/L)
A_1	67 (±1)	32 (±2)	12.2 (±0.07)	6.15 (±0.05)
A_2	68 (±1)	34.5 (±1.5)	12.4 (±0.1)	5.35 0.05)
A_3	65 (±1)	34 (±0.0)	11.5 (±0.07)	4.75 (±0.05)

week zero

A_4	64 (±1)	31 (±3)	13.1 (±0.1)	5.15 (±0.05)
A_5	66 (±1)	30 (±1)	12.8 (±0.07)	6.05 (±0.05)
B_1	60.5 (±1.5)	32.5 (±0.5)	12.8 (±0.1)	5.45 (±0.05)
B_2	64.5 (±1.5)	28 (±1)	11.7 (±0.07)	6.15 (±0.05)
B_3	64.5 (±0.5)	30.5 (±0.5)	12.5 (±0.1)	7.1 (±0.1)
B_4	61.5 (±1.5)	26 (±1)	11.9 (±0.1)	6.75 (±0.05)
B_5	65.0 (±1)	37 (±1)	13.8 (±0.07)	5.35 (±0.05)
C_1	67 (±1)	25 (±1)	13.2 (±0.07)	7.1 (±0.1)
C_2	65.5 (±1.5)	31.5 (±1)	13.5 (±0.07)	5.45 (±0.05)
C_3	65 (±1)	24 (±0.0)	12.8 (±0.07)	6.1 (±0.1)
C_4	63.5 (±1.5)	28.5 (±1.5)	11.4 0.07)	5.75 (±0.05)
C_5	66.5 (±1.5)	25.5 (±0.5)	13.2 (±0.07)	4.9 (±0.07)

Table 52: Raw data on biochemical and haematological analysis of albino rats for week two

Number of Albino Rats	Total Protein (g/1)	Serum Albumin (g/1)	Haemoglobin count (g/dl)	WBC count ($X10^9$/L)
A_1	75 (±1)	45.5 (±0.5)	12.55 (±0.05)	6.75 (±0.05)
A_2	76 (±0.0)	45.5 (±0.5)	12.40 (±0.0)	6.20 (±0.1)
A_3	76 (±1)	43.5 (±0.5)	11.75 (±0.25)	6.20 (±0.0)

A_4	75 (±1)	45.5 (±0.5)	12.75 (±0.05)	6.45 (±0.05)

Number of Albino Rats	Total Protein (g/1)	Serum Albumin (g/1)	Haemoglobin count (g/dl)	WBC counts ($X10^9$/L)
A_5	67 (±0.0)	47 (±0.0)	11.35 (±0.05)	6.35 (±0.55)
B_1	75.5 (±0.5)	47.5 (±0.5)	13.55 (±0.05)	6.15 (±0.05)
B_2	77.5 (±0.5)	48.5 (±0.5)	12.85 (±0.05)	6.35 (±0.55)
B_3	74 (±0.0)	46.5 (±0.5)	12.85 (±0.-05)	6.25 (±0.05)
B_4	74 (±0.0)	45.5 (±0.5)	12.05 (±0.05)	6.60 (±0.2)
B_5	77.5 (±0.5)	45 (±0.0)	13.85 (±0.05)	6.25 (±0.05)
C_1	72.5 (±0.5)	42.5 (±0.5)	11.55 (±0.05)	7.1 (±0.1)
C_2	71 (±1)	41.5 (±0.5)	12.75 (±0.05)	7.05 (±0.05)
C_3	72.5 (±0.5)	42 (±0.0)	11.75 (±0.05)	7.2 (±0.0)
C_4	71.5 (±0.5)	43.5 (±0.5)	11.35 (±0.5)	7.2 (±0.1)
C_5	70 (±0.0)	41 (±0.0)	12.05 (±0.5)	7.45 (±0.05)

A_1	77 (±1)	46.5 (±0.5)	12.8 (±0.2)	6.70 (±0.0)
A_2	76 (±1)	45 (±0.0)	12.75 (±0.05)	6.25 (±0.5)
A_3	77.5 (±1.7)	46 (±0.0)	12.45 (±0.05)	6.15 (±0.05)
A_4	78 (±00)	46 (±1)	13.25 (±0.45)	6.50 (±0.0)
A_5	77 (±1)	48 (±0.0)	12.8 (±0.1)	6.35 (±0.05)
B_1	76.5 (±0.5)	48.5 (±0.5)	13.7 (±0.1)	6.25 (±0.05)
B_2	78.5 (±-0.5)	49.5 (±0.5)	12.85 (±0.05)	6.35 (±0.05)
B_3	75. 5 (±0.5)	47.0 (±1)	12.85 (±0.15)	6.30 (±0.1)
B_4	76 (±0.0)	47.0 (±0.0)	12.8 (±0.1)	6.45 (±0.05)
B_5	77.5 (±0.5)	45.5 (±0.5)	13. 9 (±0.1)	6.25 (±0.05)
C_1	70.0 (±0.5)	39.5 (±0.5)	11.05 (±0.05)	7.8 (±1)
C_2	69.5 (±0.5)	40.5 (±0.5)	12.55 (±0.5)	7.20 (±0.1)
C_3	69.5 (±1.5)	41.5 (±0.5)	11.6 (±0.0)	7.40 (±0.0)
C_4	70.0 (±0.0)	42.0 (±0.0)	11.15 (±0.05)	7.65 (±0.15)
C_5	68.5 (±05)	38.5 (±0.5)	11.95 (±0.05)	7.25 (±0.05)

Table 53: Raw data on biochemical and haematological analysis of albino rats for week four

Table 54: Raw data on biochemical and haematological analysis of albino rats for week six

Number of Albino Rats	Total Protein (g/1)	Serum Albumin (g/1)	Haemoglobin count (g/dl)	WBC count ($X10^9$/L)
A_1	78.5 (±0.5)	48.5 (±0.5)	13.05 (±0.5)	6.80 (±0.0)
A_2	77.5 (±0.5)	46.5 (±0.5)	12.85 (±0.0)	6.60 (±0.1)
A_3	78.0 (±0.0)	47.5 (±0.5)	12.80 (±0.1)	6.45 (±0.05)
A_4	78.5 (±0.5)	47.5 (±0.5)	13.60 (±0.4)	6.40 (±0.5)
A_5	77.5 (±0.5)	48.5 (±0.5)	13.05 (±0.15)	6.55 (±0.05)
B_1	77.5 (±0.5)	49.0 (±0.0)	13.8 (±0.2)	6.65 (±0.05)
B_2	79.0 (±1)	50.5 (±0.5)	12.95 (±0.05)	6.55 (±0.15)
B^3	78.5 (±0.5)	48.5 (±0.5)	13.10 (±0.2)	6.50 (±0.0)
B_4	77.0 (±0.0)	47.5 (±0.5)	13.0 (±0.1)	6.70 (±0.0)
B_5	78.5 (±0.5)	48.0 (±0.0)	14.05 (±0.15)	6.40 (±0.0)
C_1	68.5 (±0.5)	38.5 (±0.5)	10.6 (±0.5)	7.95 (±0.5)
C_2	69.0 (±0.0)	38.5 (±0.5)	11.1 (±0.1)	7.45 (±0.05)
C_3	68.0 (±0.3)	36.5 (±0.5)	11.4 (±0.1)	7.60 (±0.1)
C_4	67.5 (±0.5)	38 (±1)	10.95 (±0.05)	7.90 (±0.0)
C_5	67.5 (±0.5)	37.5 (±0.5)	11.40 (±0.5)	7.50 (±0.0)

Table 55: Raw data on biochemical and haematological analysis of albino rats for week eight

Number of Albino Rats	Total Protein (g/1)	Serum Albumin (g/1)	Haemoglobin count (g/dl)	WBC count ($X10^9$/L)
A_1	79 .0 (±0.0)	49.0 (±0.0)	13.20 (±0.1)	6.80 (±0.0)
A_2	78.5 (±0.5)	47.5 (±0.5)	12.95 (±0.05)	6.55 (±0.05)
A_3	78.5 (±0.5)	48.0 (±0.0)	13.05 (±0.05)	6.45 (±0.0)
A_4	79.0 (±0.0)	47.5 (±0.5)	13.95 (±0.4)	6.35 (±0.05)
A_5	78.0 (±0.0)	49.0 (±0.0)	13.20 (±0.1)	6.35 (±0.05)
B_1	78.0 (±0.0)	50.0 (±0.0)	13.95 (±0.15)	6.55 (±0.05)
B_2	80.0 (±0.0)	51.0 (±1)	13.0 (±0.0)	6.45 (±0.05)
B_3	79.0 (±0.0)	49.0 (±0.0)	13.10 (±0.25)	6.50 (±0.0)
B_4	78.0 (±0.0)	48.5 (±0.5)	13.20 (±0.1)	6.65 (±0.05)
B_5	79.0 (±0.0)	49.0 (±0.0)	14.15 (±0.25)	6.35 (±0.05)
C_1	67.5 (±0.5)	36.5 (±0.5)	10.85 (±0.05)	8.45 (±0.45)
C_2	68.0 (±0.0)	37.0 (±0.0)	10.90 (±0.1)	7.70 (±0.1)
C_3	67.0 (±00)	36.0 (±0.0)	11.0 (±0.1)	7.75 (±0.05)
C_4	66.5 (±0.5)	37.0 (±0.0)	10.90 (±0.0)	7.85 (±0.05)
C_5	67.0 (±0.0)	36.5 (±0.5)	10.95 (±0.05)	7.55 (±0.05)

Table 56: Raw data on biochemical and haematological analysis of albino rats for week ten

Number of Albino Rats	Total Protein (g/1)	Serum Albumin (g/1)	Haemoglobin count (g/dl)	WBC count ($X10^9$/L)
A_1	79 .5	49.5	13.25	6.75
	(±0.5)	(±0.5)	(±0.0-5)	(±0.05)
A_2	79.0	48.5	13.10	6.50
	(±0.0)	(±0.5)	(±0.5)	(±0.0)
A_3	79.5	49.0	13.15	6.35
	(±0.5)	(±0.0)	(±0.05)	(±0.05)
A_4	79.0	48.5	14.1	6.35
	(±1)	(±0.5)	(±0.1)	(±0.05)
A_5	79.0	50.5	13.35	6.40
	(±0.0)	(±0.5)	(±0.05)	(±0.0)
B_1	78.5	50.5	14.10	6.50
	(±0.5)	(±0.5)	(±0.1)	(±0.0)
B_2	80.5	51.5	13.15	6.50
	(±0.5)	(±0.5)	(±0.05)	(±0.0)
B_3	79.5	49.5	13.45	6.45
	(±0.5)	(±0.5)	(±0.15)	(±0.05)
B_4	79.0	49.0	14.30	6.60
	(±0.0)	(±0.0)	(±0.2)	(±0.0)
B_5	79.5	49.5	14.25	6.40
	(±0.5)	(±0.5)	(±0.25)	(±0.0)
C_1	66.0	35.5	10.75	8.05
	(±1)	(±0.5)	(±0.05)	(±0.05)
C_2	67.5	36.0	10.85	7.95
	(±0.5)	(±0.0)	(±0.05)	(±0.05)
C_3	66.0	35.0	10.90	7.95
	(±00)	(±0.0)	(±0.0)	(±0.05)
C_4	66.0	36.5	10.80	7.95
	(±1)	(±0.5)	(±0.0)	(±0.05)
C_5	65.5	36.0	10.85	7.75
	(±0.5)	(±0.0)	(±0.05)	(±0.05)

APPENDIX 15

Table 57: Analysis of variance (ANOVA) for total protein of albino rats at two weeks intervals (p<0.05)

Source of variation	Sum of Squares	D f	Mean Square	F	Sig.
Between Groups	14414.405	2	7207.203	4.726	.0492
Within Groups	327742.189	33	9931.581		
Total	342156.594	35			

Table 58: Analysis of variance (ANOVA) for serum albumin of albino rats at two weeks intervals (p<0.05)

Source of variation	Sum of Squares	D f	Mean Square	F	Sig.
Between Groups	599.502	2	299.751	8.137	.001
Within Groups	1215.637	33	36.837		
Total	1815.139	35			

Table 59: Analysis of variance (ANOVA) for WBC count of albino rats at two weeks intervals (p<0.05)

Source of variation	Sum of Squares	D f	Mean Square	F	Sig.
Between Groups	8.286	2	4.143	17.683	.000
Within Groups	7.732	33	.234		
Total	16.018	35			

Table 60: Analysis of variance (ANOVA) for haemoglobin (hb) of albino rats at two weeks intervals (p<0.05)

Source of variation	Sum of Squares	D f	Mean Square	F	Sig.
Between Groups	62760.465	2	31380.233	4.968	.0390
Within Groups	1070027.118	33	32425.064		
Total	1132787.583	35			

APPENDIX 16

Equation 1: calculation used in the determination of haemoglobin levels of albino rats using colourimetric method

$$HB = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \text{ X Standard concentration (g/dl)}$$

APPENDIX 17

Equation 2: calculation used in the determination of concentration of serum albumin levels of albino rats using the Bromo cresol green method

$$C = 40 \text{ x} \frac{\text{Change in Absorbance of sample}}{\text{Change in Absorbance of standard}} (\text{g/l})$$

Equation 3: calculation used in the determination of total protein content of albino rats using the Biuret method

$$C = 80 \text{ x} \frac{\text{Change in Absorbance of sample}}{\text{Change in Absorbance of standard}} (\text{g/l})$$

APPENDIX 18

NB; Average value= Represent values from the 2^{nd} to 10^{th} week

Equation 4:% increase in weight gain for albino rats on diet 1

$$= \frac{\text{average weight gain- starvation value}}{\text{Starvation value}} \times 100$$

$$\text{Average weight gain} = \frac{196+198+199+202+207}{5} = 200.4$$

$$= \frac{200.4-178}{178} \times 100$$

$$= 12.58\%$$

Equation 5**:** % increase in weight gain for albino rats on diet 2

$$= \frac{\text{average weight gain- starvation value}}{\text{Starvation value}} \times 100$$

$$\text{Average weight gain} = \frac{189+205+225+223+227}{5} = 213.8$$

$$= \frac{213.8-170}{170} \times 100$$

$$= 25.76\%$$

Equation 6**:** % increase in weight gain for albino rats on diet 3

$$= \frac{\text{average weight gain- starvation value}}{\text{Starvation value}} \times 100$$

$$\text{Average weight gain} = \frac{179+177+190+190+183}{5} = 183.8$$

$$= \frac{183.8-169}{169} \times 100$$

$$= 8.76\%$$

Equation 7**:** % increase in length for albino rats on diet 1

$$= \frac{\text{average length gain- starvation value}}{\text{Starvation value}} \times 100$$

$$\text{Average length gain} = \frac{35.68+35.86+36.46+37.04+37.38}{5} = 36.48$$

$$= \frac{36.48-34.30}{34.30} \times 100$$

$$= 6.37\%$$

Equation 8**:** % increase in length for albino rats on diet 2

$$= \frac{\text{average length gain- starvation value}}{\text{Starvation value}} \times 100$$

$$\text{Average length gain} = \frac{36.36+36.40+38.32+38.52+38.76}{5} = 37.67$$

$$= \frac{37.67-34.14}{34.14} \times 100$$

$$= 10.35\%$$

Equation 9**:** % increase in length for albino rats on diet 3

$$= \frac{\text{average length gain- starvation value}}{\text{Starvation value}} \times 100$$

$$\text{Average length gain} = \frac{35.16+35.40+34.58+36.46+36.40}{5} = 35.60$$

$$= \frac{35.60-33.50}{33.50} \times 100$$

$$= 6.27\%$$

APPENDIX 19

NB; Average value= Represent values from the 2nd to 10th week

Equation 10: % Increase in total protein for diet 1

$$= \frac{average\ protein\ value - starvaton\ value}{Starvation\ value} x100$$

$$= \frac{75.8 + 77.1 + 78 + 78.6 + 79.4}{5} = 77.8$$

$$= \frac{77.8 - 66}{66} x100 = 17.8\%$$

Equation 11: % Increase in total protein for diet 2

$$= \frac{average\ protein\ value - starvaton\ value}{Starvation\ value} x100$$

$$= \frac{75.9 + 76.8 + 78.1 + 78.8 + 79.4}{5} = 77.8$$

$$= \frac{77.8 - 63.2}{63.2} x100 = 23\%$$

Equation 12: % Decrease in total protein for diet 3

$$= \frac{average\ \ protein\ \ value - starvaton\ value}{Starvation\ value} x100$$

$$= \frac{71.5 + 69.6 + 68.1 + 67.2 + 66.2}{5} = 68.5$$

$$= \frac{68.5 - 65.5}{65.5} x100 = 4.6\%$$

Equation 13: % Increase in serum albumin for diet 1

$$= \frac{average\ \text{serum albumin}\ value - starvaton\ value}{Starvation\ value} x100$$

$$= \frac{45.2 + 46.3 + 47.7 + 48.2 + 49.2}{5} = 47.3$$

$$= \frac{47.3 - 32.3}{32.3} x100 = 46.5\%$$

Equation 14: % Increase in serum albumin for diet 2

$$= \frac{average\ \text{serum albumin}\ \ value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{46.6 + 47.5 + 48.7 + 49.5 + 50.0 =}{5} 48.5$$

$$= \frac{48.5 - 30.8}{30.8} x100 = 57.3\%$$

Equation 15: % Decrease in serum albumin for diet 3

$$= \frac{average\ \text{serum albumin}\quad value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{42.1 + 40.4 + 37.6 + 36.5 + 35.8}{5} = 38.5$$

$$= \frac{38.5 - 26.9}{26.9} x100 = 43\%$$

Equation 16: % White cell count for diet 1

$$= \frac{average\ \text{white cell count}\ \ value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{6.39 + 6.33 + 6.56 + 6.49 + 6.49}{5} = 6.4$$

$$= \frac{6.4 - 5.49}{5.49} x100 = 16.6\%$$

Equation 17: % White cell count for diet 2

$$= \frac{average\ \text{white cell count}\ \ value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{6.32 + 6.32 + 6.56 + 6.50 + 6.37}{5} = 6.4$$

$$= \frac{6.4 - 6.16}{6.16} x100 = 3.9\%$$

Equation 18: % Increase in white cell count for diet 3

$$= \frac{average\ \text{white cell count}\ \ value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{7.20 + 7.46 + 7.68 + 7.89 + 7.93}{5} = 7.6$$

$$= \frac{7.6 - 5.86}{5.86} x100 = 30\%$$

Equation 19: % Increase in haemoglobin for diet 1

$$= \frac{average\ haemoglobin\ value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{12.70 + 12.81 + 13.08 + 13.27 + 13.39}{5} = 13.05$$

$$= \frac{13.05 - 12.40}{12.40} x100 = 5.2\%$$

Equation 20: % Increase in haemoglobin for diet 2

$$= \frac{average\ \text{haemog}lobin\ value - starvaton\ value}{Starvation\ value}\ x100$$

$$= \frac{13.03 + 13.22 + 13.34 + 13.51 + 13.85}{5} = 13.4$$

$$= \frac{13.4 - 12.54}{12.54} x100 = 6.7\%$$

Equation 21: % Decrease in haemoglobin for diet 3

$$= \frac{average\ haemoglobin\ value - starvation\ value}{Starvation\ value}\ x100$$

$$= \frac{11.89 + 11.66 + 11.09 + 10.92 + 10.81}{5} = 11.3$$

$$= \frac{11.3 - 12.82}{12.82} x100 = 12\%$$

APPENDIX 20

Table 61: Total protein (g/l) of Albino Rats fed on different weaning Diets

Number of weeks	Diet 1	Diet 2	Diet 3
0	66^{b} (±1.4)	63.2^{a} (±1.8)	65.5^{b} (±1.2)
2nd	75.8^{b} (±0.49)	75.9^{b} (±1.34)	71.5^{a} (±0.94)
4th	77.1^{b} (±0.66)	76.8^{b} (±1.07)	69.6^{a} (±0.54)
6th	78^{b} (±0.44)	78.1^{b} (±0.73)	68.1^{a} (±0.58)
8th	78.6^{b} (±0.37)	78.8^{b} (±0.74)	67.2^{a} (±0.50)
10th	79.4^{b} (±0.31)	79.4^{b} (±0.54)	66.2^{a} (±0.83)
Percentage (%) gain	**17.8**	**23.0**	**4.6**

All values in the same row with different superscripts are significantly different at ($P<0.05$)

Table 62: Serum Albumin (g/l) levels of Albino Rats fed on different weaning Diets

Number of weeks	Diet 1	Diet 2	Diet 3
0	32.3^{b} (±1.72)	30.8^{b} (±3.80)	26.9^{a} (±2.72)
2nd	45.2^{b} (±1.13)	46.6^{b} (±1.28)	42.1^{a} (±0.86)
4th	46.3^{b} (±0.97)	47.5^{b} (±1.37)	40.4^{a} (±1.78)
6th	47.7^{b} (±0.74)	48.7^{b} (±1.02)	37.6^{a} (±0.66)
8th	48.2^{b} (±0.67)	49.5^{b} (±0.89)	36.5^{a} (±0.38)
10th	49.2^{b} (±0.74)	50.0^{b} (±0.89)	35.8^{a} (±0.50)
Percentage (%) gain	**46.5**	**57.3**	**43**

All values in the same row with different superscripts are significantly different at ($P<0.05$)

Table 63: White cell count ($X10^9$/l) of Albino Rats fed on different weaning Diets

Number of weeks	Diet 1	Diet 2	Diet 3
0	5.49^a (±0.53)	6.16^b (±0.69)	5.86^a (±0.73)
2nd	6.39^a (±0.22)	6.32^a (±0.15)	7.20^b (±0.13)
4th	6.33^a (±0.20)	6.32^a (±0.62)	7.46^b (±0.23)
6th	6.56^a (±0.13)	6.56^a (±0.10)	7.68^b (±0.20)
8th	6.49^a (±0.17)	6.50^a (±0.30)	7.89^b (±0.41)
10th	6.47^a (±0.15)	6.37^a (±0.83)	7.93^b (±0.09)
Percentage (%) gain	**17.4**	**25.4**	**30**

All values in the same row with different superscripts are significantly different at ($P<0.05$)

Table 64: Haemoglobin (g/dl) levels of Albino Rats fed on different weaning Diets

Number of weeks	Diet 1	Diet 2	Diet 3
0	12.40^a (±0.54)	12.54^a (±0.74)	12.82^b (±0.74)
2nd	12.70^a (±0.53)	13.03^b (±0.62)	11.89^a (±0.48)
4th	12.81^a (±0.25)	13.22^b (±0.47)	11.66^a (±0.54)
6th	13.08^b (±0.28)	13.34^b (±0.45)	11.09^a (±0.29)
8th	13.27^b (±0.35)	13.51^b (±0.56)	10.92^a (±0.05)
10th	13.39^b (±0.39)	13.85^b (±0.46)	10.81^a (±0.05)
Percentage (%) gain	**5.2**	**6.7**	**12**

All values in the same row with different superscripts are significantly different at ($P<0.05$)