NUTRITIONAL ASSESSMENT OF CEREAL AND PULSE BASED WEANING FOOD SUPLEMENTED WITH EGG AND MILK POWDER

A THESIS
BY

Diti Das

Examination Roll No: 1605224

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Session: 2016-17

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MASTER OF SCIENCE (MS)
IN
FOOD SCIENCE AND NUTRITION

Department of Food Science and Nutrition

Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh

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The Author

Abstract

The aim of this study was to develop a weaning food for the children by using available resources, for this purpose three weaning food formulations based on wheat, rice, maize, bengal gram, egg and milk powder was processed and evaluated. The prepared formulations were investigated for proximate composition, vitamin and mineral content. The developed food was also evaluated for their sensory characteristics and microbiological quality. Analysis of variance (ANOVA) was used to establish any significant difference in the analytical data for formulated and control weaning foods. The proximate composition results showed that moisture content ranged between (6.81 to 7.70%), protein (17.90 to 19.20%), ash (1.62 to 1.89%) crude fiber (2.50 to 2.83%) and carbohydrate (61.64 to 62.10%). Even though the individual constitute of prepared weaning foods was found to be non significant yet the value was within standard and recommended values of weaning foods. Fat content was 7.67 to 8.10%. Calculated values for total energy provided by the blends ranged from 390.36 to 394.20 kcal/100g dry matter. A simple comparison on sensory evaluation revealed that overall acceptability of S_1 (25% Wheat flour+10% Maize flour+22.5% Rice flour +22.5% Bengal gram flour+10% Egg powder+10% Milk powder) was comparable with commercial weaning food. Therefore weaning food prepared with 25% Wheat flour+10% Maize flour+22.5% Rice flour +22.5% Bengal gram flour+10% Egg powder+10% Milk powder formulation can be commercialized in the countries like Bangladesh.

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Chapter 1

INTRODUCTION

Weaning includes the long critical period when the child is slowly used to other adult's food while continuing breast feeding. Hence it is vulnerable part of life and specially so when food resources are limited. Weaning food plays a vital role in the all-round growth, development and mental health of children (Amankwah *et al.*, 2000). Infants need nutritionally balanced, calorie-dense supplementary foods in addition to mother's milk because of the increasing nutritional demands of the growing body (Sajilata *et al.*, 2002: Umeta *et al.*, 2003).

The weaning period is a crucial period in an infant's life. At the age of 5 to 6 months, most infants begin to eat supplementary semisolid foods. At this stage, homogenized infant foods play a major role in their nutrition (FAO, 2004).

Infants and young children suffer from malnutrition in most developing countries. The Food and Agriculture Organization (FAO) reported that about 2.0 billion people globally are suffering from chronic malnutrition, which is manifested in diseases like kwashiorkor, marasmus and other related malnutritional problems. Of this population, about 1.2 billion are from the developing countries of Asia, Africa and Latin America, where the most prevalent cause of death in infants after weaning is protein energy malnutrition (Mahmoud and Ayman, 2014).

Protein energy malnutrition continues to be a major public health problem among children throughout the developing world (Brabin BJ and Coulter JBS, 2003; Schofield C and Ashworth A, 1996). Poverty and poor feeding practices are considered the major factors responsible for this nutritional problem (Sachs JD and McArthur JW, 2005). Childhood malnutrition caused by the consumption of weaning foods of low nutrient density is common in developing countries (Black RE *et al.*, 2008). The growth of infant in the first and second year of life is very rapid and breast milk alone cannot meet the child's nutritional requirements. The infant needs supplementary feeding starting from 4-6 months (Achinewhu, 1987; Ijarotimi and Famurewa, 2006).

In developing countries, traditional weaning foods are low in protein, vital nutrients and calories, required for normal child growth and development (FAO, 2004). Low-cost, high protein food supplement development for weaning infants is a constant challenge (Schmidt, 1983). This is particularly important in developing countries where malnutrition problems are still common particularly during weaning.

Many brands of preparatory foods have been developed and marketed; however these brands are too expensive and therefore are beyond the economics of low income families. The high price of weaning food and animal proteins combined with faulty feeding practices are mostly responsible for aggravating malnutrition among children (Dutra de Olivera, 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. Children therefore require nutritionally balanced calorie-dense supplementary foods in addition to mother's milk (Berggren, 1982; Cameroon and Hafvander, 1971).

Therefore, there is a real need to study means and ways of developing inexpensive, but equally nutritious weaning mixes from available food materials using simple technology as an intervention to childhood malnutrition, taking into account that this should be within the reach of the wider sectors of the population.

The formulation and development of nutritional weaning food from local and readily available raw material has received a lot of attention in many developing countries (Ijarotimi & Aroge, 2005). In developing countries, 70% of weaning foods are supplied by cereals which are relatively poor source of protein (D.V. Glover, 1976). Legumes and cereals are the main source of nutrients for traditional complementary foods in developing countries. Cereals are adequate in methionine and cysteine and are a good source of B-complex vitamins but limiting in lysine. Most legumes are rich in lysine but low in sulfur containing amino acids. It is evident that when cereals and legumes are judiciously selected and combined, a desirable pattern of essential amino acids of high biological value is obtained (Bekele M, 2011). Therefore, the combination of cereals and legumes has been found to produce amino acid patterns that adequately promote growth.

Cereal-based diets have lower nutritional value than animal-based one. Whole egg can make up for the deficits in energy and protein intake since it is a reference protein. Adding even small quantities of egg can increase protein content and quality of traditional complementary foods. Improvement of the nutritional quality of traditional complementary foods with egg is convenient, cheap and highly effective to promote quality of traditional complementary foods.

Hence in this research work, efforts were made to develop a weaning food from wheat, rice, maize, bengal gram flour, egg powder (source of energy & protein) and milk powder blended together in the different ratios to maintaining the nutritional requirements for babies of 6-9 months of ages.

Objectives:

- To develop weaning food using the available food sources.
- To evaluate macro and micro nutrients of the prepared formulations.
- To evaluate sensory attributes.
- To determine the microbial load (yeasts and molds count, salmonella count and Total Viable Count) of prepared weaning foods.
- To determine shelf life of the formulated weaning foods.

Chapter 2

REVIEW OF LITERATURE

2. 1 Weaning food

Weaning is a period of transition for the infant, during which the diet changes in terms of consistency and source. From a liquid milk-based diet, the child is gradually introduced to semi-solid foods (Draper, 1994). By the end of the weaning process, the infant no longer receives breast milk (or milk from a bottle), in the process of finally eating the family's table food .Thus, weaning is the substitution of solid foods or special childhood foods for breast or bottled milk. Gradual transition from single food milk to mixed diet. The American Academy of Pediatrics and the World Health Organization recommended waiting until 6 months to introduce baby food.

Weaning foods need to be soft, so that they are easy for baby to chew, swallow and digest. They should not be irritating for baby's digestive tracts and they should be free from contamination. Weaning food should be cheap, culturally acceptable, made of locally available foods and easily or quickly cooked or prepared. Solid weaning food should be concentrated in energy and good quality protein and should contain adequate iron and essential nutrients (Mitzner *et al.*, 1984).

Guidelines for weaning food indicate that weaning can occur between the ages of 6-12 months the food given should have characteristics according nutritional need, appropriate texture and viscosity and appropriate form to support mental and physical development. Special attention should be paid to microbial safety as the immune system is still maturing and protection provided by mothers milk may not be present (WISH, 2006).

2.1.1 Importance of weaning food

Even though breast milk is the ideal food for infants during the first six months of life, it cannot provide all of the nutrients and calories that allow infants to thrive after the first six months of life. From the age of six months to two years, infants and young children should gradually be introduced to provide more energy and vitamin for growth. In order to avoid malnutrition among children well balanced weaning food is needed. It is important to develop well balance nourish weaning food.

2.1.2 Weaning food around the world

In every culture, weaning aims to accustom babies to the staple diet, while relying on milk as the main source of nutrition. In countries where food is in short supply, mother may breast-feed for up to two years. Weaning foods are universally bland and of high carbohydrate composition. In many cases, recipes for traditional weaning foods have been passed down through generations. In Asian countries, rice is the basis of kedgeree or kongi, which is fed to babies from five months of age. Initially, boiled, sieved rice is added to cooked lentil juice.
(http://www.babyorganix.co.uk)

As babies mature, pureed lentil is added to rice; followed by peas, carrots, tomato pulp, coriander and sometimes yoghurt. In the Caribean, first foods are often steamed. For example, steamed white fish and pumpkin are pureed with cho-cho, a local vegetable. Parents also offer their babies' pureed chicken casserole or fish.

In Africa, maize porridge or rice forms the first food, to which vegetable and protein-rich foods are gradually added. In Mexico and much of South America, first foods are based on potatoes, corn, peppers, beans and tortillas (http://www.goodies.uk.com).

2.2 Weaning condition in developing countries like Bangladesh

Inappropriate feeding practices of infant and young children are the most serious obstacles to maintain adequate nutritional status and contribute to levels of malnutrition in Bangladesh that are amongst the highest in the world. Poverty, malnutrition and disease are inter-linked with each other. Malnutrition in children is the consequence of a range of factors, which are often related to poor food quality, insufficient food intake, severe and repeated infectious diseases; or frequently it involves some combination of the three (de Onis *et al.*, 1993).

A survey carried out in Bangladesh by WFP, UNICEF and Institute of Public Health Nutrition (IPHN) showed that two million children aged six months to five years affected by acute malnutrition (wasting 13.55%). Out of those two million malnourished children, half a million are suffering from acute malnutrition (severe wasting 3.4%), a highly vulnerable condition where the child need appropriate management and treatment. Almost half of the surveyed children aged six months to five years are stunted (48.6%) too short for their age, which depicts a very high prevalence of chronic malnutrition one of the highest in south

Asia. In addition 37.4% of the same children are under weight. Among the causes of malnutrition, the survey identified lack of dietary diversity as a key problem. Almost half of the children between 6-24 months did not receive the minimum meal frequency, while two third of the same age group did not meet the minimum dietary diversity of four groups per day (UNICEF, Bangladesh 2009).

According to Institute of Public Health and Nutrition (IPHN), Bangladesh (2007), almost one-half of children under five years are underweight and 42% are stunted. Only 42% of infants aged less than six months are exclusively breastfed and almost one-third (29%) of children aged 6 to 9 months do not receive any solid or semi-solid foods.

Recently World Health Organization opined that malnutrition is responsible for about 60% of all deaths, occurring among children less than five years in developing countries. In the world the number of malnourished children under five is about 60.5 million, 90% of which from developing countries (Faruque *et al.,* 2008).

Weaning foods used by many developing countries are low in energy density and nutrient level. Due to this, the energy and nutrient intake of children undergoing weaning is low, often less than the protein intake, relative to their recommended levels. The bio-availability of nutrients in cereals (which are abundantly used) is low. Coupled with infrequent food intake, the nutritional inadequacy of the child is further aggravated. The introduction of supplementation in terms of weaning foods prepared from easily available and low cost ingredients is of vital importance to meet the requirements of the growing children (Saeeda *et al.,* 2009).

2.3 Nutritional problems of traditional complementary foods

Traditionally, the diets of most societies consist of a starchy plant staple, such as cereal, root or tuber, combined with herbs, vegetables and fruits (mainly gathered or self-grown). Sometimes legumes serve as additional staple food. Only people with a good income or those who hunt, fish or keep livestock themselves consume significant amounts of animal protein.

A common feature of plant foods is their high content of water, fiber, low energy and micronutrient densities (Solomon, 2000). This characteristic becomes particularly worrisome during the complementary feeding period in infants and children. Hence, traditional weaning foods from plant staples often fail to meet the nutritional needs of the infants, due to stiff consistency and high volume which combine to offer a low-cost filling meal that often lacks adequate nutrients (Fernandez *et al.,* 2002). They are therefore known to poorly support growth and development.

2.4 Formulation of local complementary foods of high nutritive values

The use of high nutrient dense foodstuffs such as cereals, legumes, vegetables and animal food products to prepare complementary foods for infants and children has been suggested by a number of researchers (Temple *et al.,* 1996; Onofiok and Nnanyelugo, 1998; Nnam, 2002). Cereals generally are known to be relatively low in lysine and tryptophan, but fair in sulphur-containing amino acids i.e., methionine and cysteine (FAO, 1997; Okoh, 1998). On the other hand, legumes are relatively rich in proteins (19-26%) and fat (40–46%), and contain moderate quantities of tryptophan and threonine. This class of foodstuff can therefore form a good supplement to cereals. It is however evident that cereals and legumes are low in trace minerals and vitamins (Osagesand Eka, 1998). Fruits and vegetables are valuable sources of these micronutrients. They could therefore provide significant quantities of the nutrients if properly processed and blended with the staple foods. Animal foods like crayfish, egg and milk have further been suggested as source of enrichment (Badamosi *et al.,* 1995; Temple *et al.,* 1996; Ladeji *et al.,* 2000).

2.5 Previous Studies on Weaning Food Formulation

Ismail *et al.,* 2000, developed a supplementary food using 72% extraction of wheat flour, corn flour, mung bean flour and dried skimmed milk. The weaning food was found to have 15.1-22% protein, 1.30-16.5% fat, 1.4-2.90% ash, 63.70-77.8% carbohydrate and 0.90-1.90% fiber. Some essential fatty acids were found in some blends. Some antinutritional factor such as phytic acid, tannins and trypsin were removed from raw materials by standard method to make it acceptable and easily digestible by children.

In the study on the formulation of weaning foods from millet, cowpea and groundnut mixtures (70:20:10) and millet, cowpea, groundnut and millet malt mixtures (65:20:10:5), it was found that the addition of legumes improved the nutrient content of the weaning food, while addition of malt reduced the hot paste viscosity, making the weaning foods nutrients dense (Nkama *et al.,* 2001).

Nnam (2001) found that blends from sprouted sorghum, bambara groundnuts and fermented sweet potatoes (52:46:2) ratio had better nutritional attributes than their unprocessed counterparts and the traditional sorghum complementary food.

Ahmed (2005) formulated a weaning food using different proportion of wheat, soybean, supplemented with whole milk powder and sugar. Six samples of weaning food were prepared using 5% and 10% soya flour. The protein, ash and fat content of the sample with soya flour were higher and carbohydrate content was lower than that of control sample. There were no remarkable changes observed in moisture content, peroxide value, fatty acid and flavor during fourth month storage at ambient temperature.

Amankwah *et al.,* (2009) developed weaning foods from fermented maize, rice, soybean and fishmeal. Two blends were formulated with or without fishmeal respectively. The high protein content of the formulations was contributed by soybean and fishmeal. The blends had low level of minerals due to removal of fish bones and grain pomace during formulation. The microbial count of the formulations was within acceptable limit.

The nutritional and sensory properties of weaning food developed by Adengua (2010) from sweet potato, cowpea and peanut flour were investigated. The flours were combined in specific ratios (sweet potato: 60,65,70%, cowpea: 25,15,15% and peanut: 15,20,15%) to produce three weaning foods which were compared with commercial weaning food. The result of the nutritional properties revealed a significant increase of crude protein ranging from 18.9-38.5%, crude fiber 2.8-4.8%, fat 2.4-7.8, ash 2.4-3.6 and carbohydrate 42.3-62.5%. Fortification with 25% cowpea and 15% peanut flour was acceptable. It was observed that sensory characteristics of the sweet potato based products were comparable to the commercial baby food.

Weaning food based on millet-egg-soybean hull composite flour was developed by Ayo *et al.,* (2011). The preliminary investigation showed the 85:15 ratio of the millet: egg composite powder to be the best using sensory evaluation and was therefore used as the base for formulation to which soybean-hull was added. The sensory, chemical, energy value and microbial qualities were determined. The moisture, protein, fat, carbohydrate and energy content decreased from 13.23-10.44, 15.63-12.32, 10.57-6.31% and 386.99-376.15 Cal/100g, respectively, but the ash (mineral) and carbohydrate increased from 3.36-6.02 and 57.11-64.93% with increase in the added soybean-hull powder (0-25%).

Weaning foods from germinated lentil and germinated wheat flour was prepared by Habiba *et al.,* (2013). It was found that protein and ash content were higher than commercial brand weaning food. The developed weaning blends were also rich in essential amino acids especially leucine and lysine content. Prepared formulations had suitable functional and sensory properties.

Weaning foods were formulated using locally available cereals such as maize, millet, sorghum, wheat and Legumes and Soybean by V.I.E. Ajiwe *et al.,* (2014). Twenty composite blends were formulated in different ratios (cereal: legume); 60:30:10, 70:20:10, 50:40:10, 40:50:10 in that order. The weaning food formulations had reasonable percentage of the recommended daily allowance for protein, carbohydrate, energy, and some micro/macronutrient. The results of anti-nutritional levels showed that the tannin, trypsin inhibitor, saponin, phytate, alkaloid and HCN were significantly different at $p<0.05$ when compared with the commercial products (Cerelac and Nutrend).

2.6 Nutritional needs of infants

Good nutrition is essential for the growth and development that occurs during an infant's first years of life. When developing infants are fed the appropriate types and amounts of foods, their health is promoted. Positive and supportive feeding attitudes and techniques demonstrated by the caregiver help infants develop healthy attitudes toward foods, themselves, and others.

Energy needs

Infants need energy from food for activity, growth and normal development. Energy comes from foods containing carbohydrate, protein and fat. The number of kilocalories (often termed "calories") needed per unit of a person's bodyweight expresses energy needs. A kilocalorie is a measure of how much energy a food supplies to the body and is technically defined as the quantity of heat required to raise the temperature of 1 kilogram of water 1 degree Celsius. An infant's energy or caloric requirement depends on many factors, including body size and composition, metabolic rate (the energy the body expends at rest), physical activity, size at birth, age, sex, genetic factors, energy intake, medical conditions, ambient temperature, and growth rate. Infants are capable of regulating their intake of food to consume the amount of kilocalories they need.

Table 2.1 Estimated Energy Requirements (EER) for Infants (Boys)

Source: Butte NF *et al.*, 1997

Age (months)	Reference Weight(kg)	Reference Weight(lb)	Reference Length(cm)	Reference Length(in)	Estimated Energy Requirements (kcal/day)
1	4.4	9.7	54.7	21.5	472
2	5.3	11.7	58.1	22.9	567
3	6.0	13.2	60.8	23.9	572
4	6.7	14.8	63.1	24.8	548
5	7.3	16.1	65.2	25.7	596
6	7.9	17.4	67.0	26.4	645
7	8.4	18.5	68.7	27	668
8	8.9	19.6	70.2	27.6	710
9	9.3	20.5	71.6	28.2	746
10	9.7	21.4	73	28.7	793
11	10	22	74.3	29.3	817
12	10.3	22.7	75.5	29.7	844

Table 2.2 Estimated Energy Requirements (EER) for Infants (Girls)

Source: Butte NF *et al.*, 1997

Age (months)	Reference Weight(kg)	Reference Weight(lb)	Reference Length(cm)	Reference Length(in)	Estimated Energy Requirements (kcal/day)
1	4.2	9.3	53.5	21.1	438
2	4.9	10.8	56.7	22.3	500
3	5.5	12.1	59.3	23.2	521
4	6.1	13.4	61.5	24.2	508
5	6.7	14.8	63.5	25	553
6	7.2	15.9	65.3	25.7	593
7	7.7	17	66.9	26.3	608
8	8.1	17.8	68.4	26.3	643
9	8.5	18.7	69.9	27.5	678
10	8.9	19.6	71.3	28.1	717
11	9.2	20.3	72.6	28.6	742
12	9.5	20.9	73.8	29.1	768

Carbohydrates

Carbohydrates serve as primary sources of energy. Carbohydrates are necessary in the infant's diet because they supply food energy for growth, body functions, and activity.

Adequate intake (AI) for Infants

0–6 months 60 g/day of carbohydrate

7–12 months 95 g/day of carbohydrate

Protein

Infants require high quality protein from breast milk, infant formula or complementary foods. Protein perform very specialized functions in regulating body processes like building and repairing new tissues, manufacturing important enzymes, hormones, antibodies and other components. Protein also serves as a potential source of energy if the diet does not furnish sufficient kilocalories from carbohydrate or fat. As with energy needs, protein needs for growth per unit of body weight are initially high and then decrease with age as growth rate decreases.

Adequate intake (AI) for Infants

0–6 months 9.1 g/day of protein

Recommended Dietary Allowance (RDA) for older infants

7–12 months 11 g/day of protein

Protein deficiency

In developing countries, infants who are deprived of adequate types and amounts of food for long periods of time may develop kwashiorkor, resulting principally from a protein deficiency; marasmus, resulting from a deficiency of kilocalories; or marasmus-kwashiorkor, resulting from a deficiency of kilocalories and protein.

Lipids

Infants require lipids in their diets because they supply a major source of energy – fat supplies approximately 50 percent of the energy consumed in breast milk and infant formula, allow for the absorption of the fat-soluble vitamins A, D, E, and K and provide essential fatty acids that are required for normal brain development, healthy skin and hair, normal eye development, and resistance to infection and disease.

AI for Infants

0–6 months 31 g/day of fat

7–12 months 30 g/day of fat

Vitamin A

Vitamin A, a fat-soluble vitamin, refers to a group of compounds including preformed types of the vitamin found in animal products and carotenes, precursors of vitamin A, found in plants. Vitamin A is essential for formation and maintenance of healthy skin, hair, and mucous membranes, proper vision, growth and development and healthy immune and reproductive systems.

AI for Infants

0–6 months 400 μg Retinol Active Equivalent/day of vitamin A

7–12 months 500 μg Retinol Active Equivalent/day of vitamin A

Iron

This mineral is a vital component of hemoglobin, the part of red blood cells that carries oxygen throughout the body. Most infants are born with adequate iron stores that are not depleted until about 4 to 6 months of age. After 6 months this store starts to run low because breast milk doesn't provide enough to meet baby's growing needs, iron-rich foods are an essential part of a healthy weaning diet, even from the very early stages. Too little iron can lead to iron deficiency anemia – a condition that can affect a baby's development.

AI for Infants

0 - 6 months 0.27 mg/day of iron

RDA for Infants

7 - 12 months 11 mg/day of iron

Zinc

Zinc plays an important role in a baby's normal growth and development. Zinc, a mineral that is a component of many enzymes in the body, is involved in most metabolic processes. As with iron, the zinc content of breast milk is low compared to baby's increased needs, so it is important to include good sources from the start of weaning.

AI for Infants

0–6 months 2 mg/day

RDA for Infants

7–12 months 3 mg/day

Calcium

Calcium is needed for baby's bones and teeth development. This important nutrient helps to build baby's skeleton as they grow, and will make up around 2% of their body weight by the time they are an adult. Most of baby's calcium intake comes from milk, whether they're breastfeeding or formula feeding. Cows' milk also contains calcium but isn't suitable for babies under 12 months old, due to its lower levels of iron.

AI for Infants: 0–6 months 210 mg/day

2.7 Nutritive value of ingredients used in weaning food preparation

2.7.1 Cereals

Mature cereal grains consist mainly of carbohydrates, proteins, lipids, mineral salts and water. The minerals in the edible kernels of cereals include potassium, magnesium and calcium, mainly in the form of phosphates and sulphates (FAO, 1989). Vitamins are present in small quantities (Kent, 1975). Cereals provide energy from carbohydrates and fats together with a variable proportion of plant protein, depending on the species, variety and conditions of cultivation (FAO, 1989). Except for two amino acids, lysine and tryptophan, most cereals contain essential amino acids required by man as well as vitamins and minerals. In many developing countries, cereal is the main source of dietary protein where animal protein is scarce and expensive. The chemical composition of cereals used in formulation of weaning foods is shown in table:

Table 2.3 Chemical composition of wheat, rice and maize (100g edible portion)

	Wheat	**Maize**	**Rice**
Crude fiber(g)	2.0	2.8	1.0
Calories(Kcal)	348	355	362
Carbohydrates(g)	71	73	76
Proteins (g)	11.6	9.2	7.9
Fat (g)	2.0	4.6	2.7
Ash (g)	1.6	1.2	1.3
Calcium (mg)	30	26	33
lron (mg)	3.5	2.7	5.4
Riboflavin (mg)	0.10	0.20	0.15
Thiamine (mg)	0.41	0.38	0.38
Niacin (mg)	5.1	3.6	4.3

Source: FAO, 1995.

2.7.2 Bengal gram

Bengal gram is also called Chickpea or Gram (*Cicer arietinum* L.) in South Asia and Garbanzo bean in most of the developed world. It belongs to Family *Leguminosae* and is widely grown for centuries and accounts for nearly 40 percent of the total pulse production. It is one of the most important crops in the world because of their good balance of amino acid, high protein bio-availability, high levels of complex carbohydrates (low glycemic index) and rich in vitamins, minerals; and have relatively low levels of anti-nutritional factors (Wood and Grusak, 2007).

Chickpea is a good source of carbohydrates and proteins, which together constitute about 80% of the total dry seed mass. The starch content of chickpea cultivars have been reported to vary from 41% to 50%. The crude protein content of chickpea varies from 12.4 to 31.5%. Chickpeas contain about 6% fat that is important in the vegetarian diets of resource-poor consumers.

The protein quality is considered to be better than other pulses. Chickpea has significant amounts of all the essential amino acids except sulphur-containing amino acids, which can be complemented by adding cereals to the daily diet. Although lipids are present in low amounts, chickpea is rich in nutritionally important unsaturated fatty acids such as linoleic and oleic acids. Chickpea contains nutritionally important minerals, notably calcium and iron, and the availability of iron is reported to be good (Murty CM *et al.,* 2010).

Mg, P, K are also present in chickpea seeds. Chickpea is a good source of important vitamins such as riboflavin, niacin, thiamine, folate and the vitamin A precursor β-carotene. As with other pulses, chickpea seeds also contain anti-nutritional factors which can be reduced or eliminated by different cooking techniques. Overall, chickpea is an important pulse crop with a diverse array of potential nutritional and health benefits (Segev *et al.*, 2010).

Table 2.4 Chemical Composition of Bengal gram (100g edible portion)

Component	Value per 100 gram
Moisture	7.68g
Energy	378kcal
Protein	20.47g
Total lipid	6.04g
Carbohydrate by difference	62.95g
Fiber, total dietary	9g
Sugar, total	10.70g
Zn	2.76mg
Ca	57mg
Fe	4.31mg
Mg	79mg
P	252mg
K	718mg
Na	24mg
Vit C	4 mg
Thiamin	0.477 mg
Riboflavin	0.212 mg
Folate	557 mg
Vit B_6	0.535 mg
Vit A, RAE	3 µg
VitA, IU	67 IU

Source: USDA Nutrient Database, 2013

2.7.3 The importance of fortification with egg

The egg is important as a food and food ingredient. It is one of the most nutritious foods we consume. Eggs have the highest quality protein and most of the essential vitamins (Bergquist, 1974). The lipids are easily digested and there are more unsaturated fats than in any other animal product. Apart from this, eggs have negligible fiber, therefore, its proportion cannot increase the dietary fiber content of the final weaning food. The Biological Value (BV) of eggs is rated between 88 and 100 (NC egg Association, 2016). Research has shown that egg powder contains about 49% protein, 43% fat and a useful source of riboflavin, vitamin A, iron and phosphorus. (Okaka, 2002; Okaka and Okaka, 2001; Ndukwe, 2006).

Chicken eggs are used for the fortification of cereal flour baked goods such as cakes. Whereas cereal based weaning foods have not received much attention. In food processing, eggs have desirable attributes such as foaming capacity, gel formation, water-holding capacity and emulsifying activity. These are likely to improve the functional properties of the weaning food.

Improvement of the nutritional quality of traditional complementary foods with egg is convenient, cheap and highly effective to promote quality of traditional complementary foods. Adding even small quantities of egg can very much increase protein content and quality of traditional complementary foods.

Chapter 3

MATERIALS AND METHODS

The study was conducted in the Food Science and Nutrition laboratory in collaboration with Agricultural Chemistry and Microbiology laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.1 Materials

3.1.1 Food materials

The materials used in this study were locally available wheat, rice, maize, bengal gram, egg, milk powder and commercial weaning food were collected from local market.

3.1.2 Chemicals and reagents

Chemicals and reagents used in the study were of analytical grade.

3.2 The preparation process for cereal grains and legumes

The cereal grains (maize, wheat and rice) were processed according to the method followed by Ajiwe and Nwaigbo (2014) for the formulation of cereal and legume based weaning food using locally available cereals such as maize, millet, sorghum, wheat, legumes and soybean.

3.2.1 Processing of cereals (rice, wheat and maize)

Cereals were sorted and cleaned manually to remove broken seeds, dust and other extraneous materials

↓

The cereals were thoroughly washed and soaked for 24 hours in water separately

↓

Sun dried for 5 days and further oven dried at 60^{o}c for 1 hour

↓

Milled and sieved to get flours of fine texture

↓

The flours were separately packaged in thick polyethylene bags, sealed and stored until use

3.2.2 Processing of legume (bengal gram)

Pulses were sorted and cleaned manually to remove broken seeds, dust and other extraneous materials

↓

The legume was thoroughly washed and boiled with water for 45 minutes

↓

Sun dried

↓

Dehulled

↓

Roasted

↓

The legume was milled and sieved

↓

The flour was packed in thick polyethylene bags, sealed and stored until use

3.2.3 Preparation of egg powder:

The raw eggs (4 eggs) were beaten and poured into a nonstick frying pan and cooked for (5-7) minutes, during cooking the eggs were broken up with spatula because smaller pieces dehydrate quickly and more evenly. The eggs were spread out into baking sheet evenly and baked in oven at 50^0C for several hours until it had a crispy texture (Elizabeth Mnyandu, 2014). Then the dried eggs were grinded into powder using blender. Finally egg powder was packed in thick polyethylene bags, then sealed and stored in cool dark place.

Fig 3.1: Egg powder

3.3 Weaning food formulation: Weaning food formulation from rice, wheat, maize and bengal gram flour, egg and milk powder.

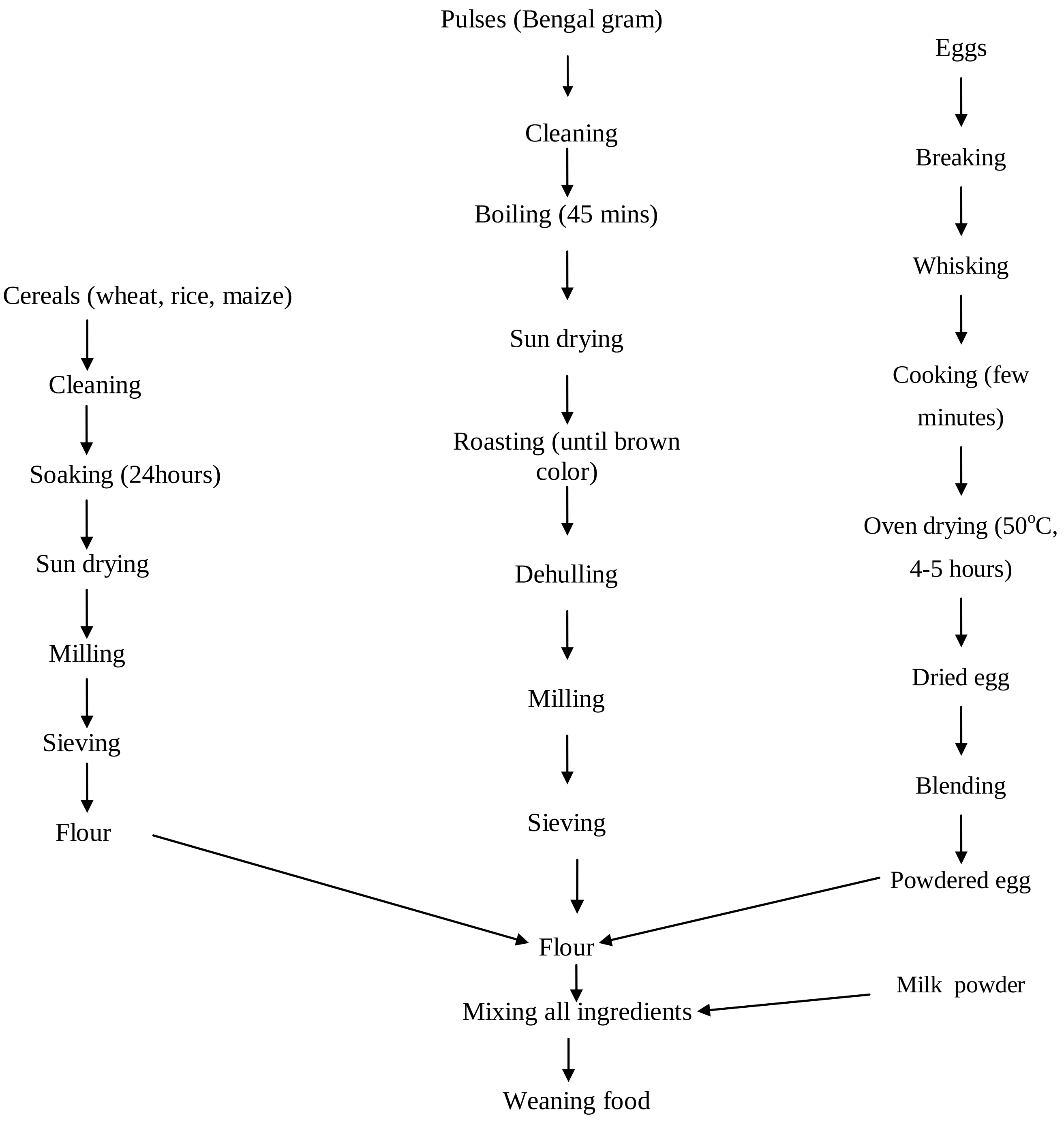

Figure 3.2: Flow diagram of weaning food preparation.

3.4 Formulation of weaning food by mixing the ingredients

The weaning mix was prepared in the laboratory by combining cereal and pulse in the ratio of 3: 1. The three samples (S_1, S_2, S_2) were prepared by addition of 10% egg powder and 10% milk powder. The formulation of the complementary food mix is shown in Table 3.1.

Table 3.1: Formulation of weaning food samples

Sample	Ingredients (%)					
	Cereals			**Pulse**		
	Wheat	Rice	Maize	Bengal gram	Egg	Milk powder
F_1	25	22.5	10	22.5	10	10
F_2	30	20	10	20	10	10
F_3	35	17.5	10	17.5	10	10

3.5 Proximate analysis of prepared weaning foods

3.5.1 Determination of moisture content

Moisture content was determined by (AOAC, 2005) method as described below.

Procedure

Five gram of sample was taken in previously weighed dried empty crucible in triplicate. Then the crucibles with sample were dried in a hot air oven at 100-105°C for overnight or more till constant weight. After drying the crucibles were removed from the oven and cooled in desiccators. The crucibles were removed from desiccators and weighed soon after reaching room temperature. The losses in weight were taken as the moisture loss of the samples. Each trial was given in triplicate. From these weights the percent of moisture in the samples were calculated as follows:

$$\text{Percent of moisture} = \frac{\text{Initial weight (g) - final weight (g)}}{\text{Weight of the sample (g)}} \times 100$$

3.5.2 Determination of ash

AOAC method (2005) was used to determine the total ash content as described below:

Procedure

Five gram of each sample was weighed and taken in dry, clean porcelain dishes. Hot air oven method was applied to remove the moisture. Then the samples were transferred into the muffle furnace and burnt at 550°C temperature for 4-6 hours and ignited until light gray ash resulted (or to constant weight). The samples were then cooled in desiccators and weighed. The ash content was expressed as:

$$\% \text{ ash} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.5.3 Determination of fat

AOAC (2005) method was used to determine crude fat content of the sample as described below.

Procedure

The dried sample remaining after moisture determination was transferred to a thimble and plugged the top of the thimble with a wad of fat free cotton. The thimble was dropped into the extraction tube attached to a Soxhlet flask. Approximately 75 ml or more of anhydrous ether was poured into the flask. The top of the fat extraction tube was attached to the condenser. The sample was extracted for 16 hr or longer on water bath at 70-80^0 C. At the end of the extraction period, the thimble was removed from the apparatus and distilled off most of the ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether reached a small volume, it was poured into a small, dry (previously weighed) beaker through a small funnel containing a plug of cotton.

The flask was rinsed and filtered thoroughly, using ether. The ether was evaporated on a steam bath at low heat, it was then dried at 100^0 C for 1hour, cooled and weighed. The difference in the weights gave the ether soluble material present in the sample.

Calculation

Fat content was calculated by the following formula:

$$\text{Percent of Fat} = \frac{\text{Weight of ether extracts}}{\text{Sample weight}} \times 100$$

3.5.4 Protein content

AOAC (2005) method was used to determine protein content of the sample as described below.

Principle

The estimation of nitrogen was made by modified Kjeldahl method, which depends on the fact that organic nitrogen, when digested with concentrated sulphuric acid (H_2SO_4) in the presence of a catalyst, is converted into ammonium sulphate $(NH_4)_2SO_4$. Alkali is added to the sample to convert ammonium (NH_4^+) to ammonia (NH_3). The ammonia is steam distilled into a receiver flask containing boric acid and titrated with a standard acid solution. This determines percent of nitrogen that is multiplied by 6.25 to give the value of crude protein.

Digestion mixture

Potassium sulphate (K_2SO_4) and dehydrated copper sulphate ($CuSO_4.5H_2O$) in a ratio of 5g: 1g were powdered with mortar and pestle and mixed well. Concentrated HCl was used for titration.

Sodium hydroxide (40%)

Sodium hydroxide (NaOH) 40 gm was dissolved in distilled water and the volume was made up to 100 ml.

Receiver solution

10g of boric acid was added in 500 ml deionized water in a one liter volumetric flask, heated it gently until the boric acid was dissolved. An amount of 0.02 g bromo cresol green was dissolved with 4ml ethanol (C_2H_5OH) in a separate beaker. An amount of 0.014g methyl red was dissolved with 4 ml ethanol (C_2H_5OH) in another beaker. Some bromo cresol green and methyl red solution mixture was then transferred into that volumetric flask and 0.5 ml 1N NaOH was added when the total volume was made 1000 ml with deionized water.

Procedure: The Kjeldahl method consists of the following steps:

- Digestion of the sample
- Distillation
- Titration

Digestion of the sample

Two grams of sample was measured accurately. This sample was poured into a 100 ml clean and dry Kjeldahl flask, to which 10 gm of digestion mixture and 25 ml of concentrated H_2SO_4 were added. To avoid frothing and bumping 2-5 glass beads was placed inside the flask. A blank sample was carried with all reagents except sample material for the comparison. The flask was then heated in a Fume hood Digestion chamber at 400^{0}C until the solution became colorless. At the end of digestion period, the flasks were cooled and diluted with 100 ml distilled water. A small piece of litmus paper was placed in the solution and the reaction was found to be acidic.

Distillation

The distilling set of Kjeldahl apparatus was thoroughly washed with distilled water before starting the distillation. In a measuring cylinder 60 ml of 40% NaOH was taken and it was carefully poured down the side of the Kjeldahl flask. The mouth of the flask was closed with a stopper containing connective tube, which was ultimately connected to the ammonia-receiving flask containing 25 ml receiver solution.

The mixture was boiled at such a rate that water and ammonia distilled over at a steady moderate rate. The heating was not too slow so that the receiver solution might be sucked into the Kjeldahl flask and not to fast so that the distilling ammonia did not escape the receiver solution without absorption.

Titration

The ammonia absorbed in the receiving flask containing receiver solution was titrated with 0.1 N HCl. Similarly a reagent blank was distilled and titrated.

Calculation

Crude protein content of the sample on the percentage basis was calculated by using the following formula:

$$\text{Percentage of nitrogen} = \frac{\{c - b \times p \times 0.014 \times d\}}{\text{weight of sample}} \times 100$$

Where, c = reading of the sample

b = blank reading

p = strength of the HCl solution

d = protein factor

The conversion factor of nitrogen to protein is 6.25 and atomic weight of nitrogen is 14.

3.5.5 Determination of crude fiber

Crude fiber of the sample was determined sequentially by acid and alkali hydrolysis followed by ignition of the residue as described the method AOAC (2005).

Procedure:

One gram of ground sample (oven dried) was taken in a one litre beaker covered with round bottom flask containing cold water. The system maintained constant volume during boiling. The content of beaker was refluxed to boiling point with 200 ml of 1.25% sulphuric acid exactly for 30min. Then the content was filtered quickly in a Buchner funnel through whatman no 42filter paper and washed to make it acid free. The acid free residue was refluxed with 1.25% alkali solution at boiling point for exactly 30minutes maintaining the constant volume as before. Then the mixture was filtered as before and washed water to make the residue alkali free. The residue was transferred to a crucible and in an oven at 100 ± 5^{0}Cuntil constant weight gained. It was then cooled in a desiccator and weighed (Residue contain ash and crude fiber).Then the sample was ignited in a muffle furnace at 600^{0}C for 4 hrs. It was cooled in a desiccator again. The weight of the ignited sample was recorded after subsequent cooling. The loss of weight after ignition gave the amount of crude fiber.

Calculation:

$$\%\text{crudefiber} = \frac{\text{loss of weight}}{\text{sample weight}} \times 100$$

3.5.6 Determination of total carbohydrate

Total percentage carbohydrate was determined by the difference method as reported by Osborne and Voogt (1978). This method involves adding the total values of crude protein, crude fat, crude fiber, moisture and ash constituents of the sample and subtracting it from 100.

Total %C= 100 - [%M+ %P+ %F+ %Fb+ %A]

Where:

C - Carbohydrate content, M - Moisture content, P - Protein content, F - Fat content,

Fb - Fiber content and A - Ash content.

3.5.7 Calorific value

Energy value (calorific value) is quantified using an indirect calculation method. The three groups of nutrients, which provide the body with energy, are carbohydrates, fats and proteins. One gram of carbohydrate was assumed to give 4 kcal energy; one gram of fat 9 kcalenergy and one gram of protein 4 kcal. Therefore, calorific value (Kcal/100g) was determined by following formula:

Energy value = (P* 4) + (F* 9) + (C* 4) in kcal/100g of the sample

Where:

- P = Protein content (%).
- F = Fat content (%).
- C = Available total carbohydrate (%).

3.6 Determination of mineral content of weaning food

3.6.1 Estimation of calcium and zinc

Adequate intake of micronutrients such as iron, zinc, and calcium are important for ensuring optimal health, growth, and development of infants and children (Huffman, 1994).

Ca and Zn were determined by the method of Pearson (1976).

Sample digestion:

1 ml sample with 15 ml of diacid mixture (2:1) (HNO_3:$HCLO_4$) was taken in a beaker and boiled until the solution become clear. The sample was then cooled and made the volume in 100 ml.

The solution was then ready for the estimation of minerals (Ca and Zn).

3.6.1.1 Estimation of Calcium:

Five ml solution mixed with 20-25 ml hot distilled water. Masking Reagent (10drops of each potassium pherocyanite,10 drops hydroxylamine hydrochloride,10 drops triethanolamine) and 5 ml of NaOH buffer(10%) was added to the flask. Six drops of calcon indicator was added. The flask was shaken vigorously and titrated against Na_{2-} EDTA(0.001M) solution from a burette until pink color completely turned topure blue color.

A blank was run following the same procedure as describe above. The data were recorded and the amount of calcium present in the sample was calculated. The percent of calcium was calculated according to the formula:

Calculation:

1ml 1M Na_{2-} EDTA=40.08 mg Ca^{++}

$$\text{mg of calcium} = \frac{\text{mg of calcium obtained}}{\text{weight of sample}} \times 100$$

3.6.1.2 Estimation of Zinc:

Five ml solution mixed with 20-25 ml hot distilled water. Masking Reagent (10drops of each potassium pherocyanite,10 drops hydroxylamine hydrochloride,10 drops triethanolamine) and 5 ml of NaOH buffer(10%) was added to the flask. Six drops of calcon indicator was added. The flask was shaken vigorously and titrated against Na_{2-} EDTA (0.001M) solution from a burette until pink color completely turned to pure blue color.

A blank was run following the same procedure as describe above. The data were recorded and the amount of calcium present in the sample was calculated. The percent of calcium was calculated according to the formula:

Calculation:

1ml 1M Na_{2-} EDTA=65.38 mg Zn^{++}

$$\text{mg of Zn} = \frac{\text{mg of Zn obtained}}{\text{weight of sample}} \times 100$$

3.6.1.3 Determination of iron

The iron content of the samples was determined by redox titration method described by Dr. Miertschin (2010). At first 100 or 150 ml beaker were cleaned with soap and water. Then the beaker rinsed with distilled water. The iron compounds to be used for the titration with potassium permaganate solution contain the salt ferrous ammonium sulfate hexahydrate

(Mohr's salt), $FeSO_4 (NH_4)_2SO_4.6H_2O$ which is abbreviated as FAS. Then directly into each beaker carefully weighted 0.5 gm of unknown sample, 15 ml distilled water was added to each sample and stirred to dissolved the solid. 8ml of 3M sulfuric acid was added to each sample which was required for the reduction of the permanganate ion. 55 ml of the standardized $KMnO_4$ solution was obtained for the titration. Then prepared the burette for titrating by clearing, rinsing and filling with $KMnO_4$ solution. Then titrated each sample to the end point. The end point was the first appearance of a permanent, pale pink color.

Calculation:

$$1 \text{ ml } 1N\ KMnO4 \text{ solution} = 0.05585 \text{ gm } Fe^{++}$$

3.7 Determination of β carotene content:

β carotene content of weaning food was determined by a slightly modified method described by Nagata and Yamashita (1992). At first prepared a mixture of acetone-hexane with a proportion of 4:6. Then 1 gm of sample was homogenized with 10ml of acetone hexane mixture. After that centrifuged the solution at 3600rpm for 10 minutes and collected the supernatant. A little amount of supernatant was taken in a cuvette of spectrophotometer and absorbance of the mixture was measured at 453,505and 663 nm. β carotene content was calculated using the following equation:

β carotene (mg/100g)= $0.216A_{663} - 0.304A_{505} + 0.452A_{453}$

ß carotene (mg/100gm) = Vitamin A×0.6

3.8 Microbial analysis of prepared weaning food

3.8.1 Determination of total viable microorganism

For total viable count of microorganism present in the samples standard plate count method was followed according to the method described by Rangana (2004).

Preparation of media

23.5 g of Plate Count Agar (PCA) powder was dissolved in 1000ml of cold distilled water and heated to boiling the dissolve ingredients completely. Then media was filled into different screw cap bottles and sterilized at 121ºC for 15 min in an autoclave. After sterilization the media was kept in water bath at 45ºC until used.

Sterilization of glassware

Glass wares (petridishes, pipette, test-tubes, etc) were sterilized in an autoclave at 15 psi for 20 min.

Preparation of dilution blank

In order to dilute the sample consecutively 1ml of the original sample was diluted stepwise through a series of tubes containing 9ml of salt solution. Salt solution was made by adding 0.85g of NaCl with 100ml of distilled water. At first 9ml of the salt solution was taken in a sterile test tubes and then 1ml of the original sample was taken to the first test-tube with a sterile pipette. Salt solution to the sample was vigorously shaken for homogenous distribution of the bacterial population in the solution. This tube was denoted as 1. From the tube 1 another 1ml aliquot was transferred to the second tube and this tube was denoted as 2. In this way 3, 4, 5, 6, 7, 8, 9 and 10 was prepared until the desired dilution is achieved. Now the tubes have got the dilution 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, $10^{-9,}$ and 10^{-10} respectively. The dilutions were as follows:

Tube No.	Dilution	Volume of original fluid per ml
1	1/10	0.1 or 10^{-1}
2	1/100	0.01 or 10^{-2}
3	1/1,000	0.001 or 10^{-3}
4	1/10,000	0.0001 or 10^{-4}
5	1/100,000	0.00001 or 10^{-5}
6	1/1,000,000	0.000001 or 10^{-6}
7	1/1,000,0000	0.0000001 or 10^{-7}
8	1/1,000,00000	0.00000001 or 10^{-8}
9	1/1,000,000000	0.000000001 or 10^{-9}
10	1/1,000,0000000	0.0000000001or 10^{-10}

Procedure of plating

Now from the test-tube 1, one ml of the sample solution was taken in a sterile petridish containing 9ml of melted PCA agar medium. The agar with bacterial sample was mixed by rotating the petridish. This petridish was marked as A. In this way B, C, D, E, F, G, H, I and J marked petridishes were prepared from the tubes 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Then these petridishes were placed on a level surface for a few minutes for solidifying the agar medium.

Incubation and colony count

After solidification petridishes were placed in the incubator at 37^{o} C for 24 hours, the petridishes over loaded with bacterial colonies were avoided and the petridishes containing countable colony were selected. Colonies in each plate were counted having 30-300 colonies using a magnifier and finally the total number of bacteria per ml of sample was calculated by the following equation:

$$\text{Total viable count (CFU/ml)} = \frac{\text{No. ofcolony} \times \text{Reciprocal of dilution}}{\text{sample taken(ml)}}$$

3.8.2 Determination of yeast and mold count:

Same procedure was followed for yeast and mold count. In this test, Potato Dextrose Agar (PDA) was used as a media. 17.5 g of PDA powder was dissolved in 1000ml of cold distilled water and heated to boiling till the ingredients dissolved completely.

3.8.3 Determination of salmonella:

Same procedure was followed for Salmonella count. In this test, Salmonella Shigella (SS) agar was used as a media. 39g of SS agar powder was dissolved in 1000ml of cold distilled water and heated to boiling till the ingredients dissolve completely.

3.9 Preparation of gruels:

Gruels were prepared from both controls and formulated food samples by mixing 20g of each sample dissolved in 400ml water and boiled at 100^{o}C for 15min. The boiled gruels were allowed to cool to about 45^{o}C.

3.10 Sensory evaluation

The sensory evaluations of all formulated weaning food samples was evaluated for color, flavor, taste and overall acceptability parameters by 10 panelists using 9 point hedonic scale. The panelists were selected from students and employees of the Department of Food Science and Nutrition, HSTU.

The hedonic scale arranged was such that; 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like or dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely.

3.11 Statistical analysis

Each experiment (proximate, mineral, vitamin, moisture, shelf life study) was repeated in triplicate. The obtained data found from these experiments were analyzed by SPSS (version 22.0). The results were expressed as mean ± standard deviation. One-way analysis of variance was performed using ANOVA procedures. Significant differences among the means were determined by Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

Chapter4

RESULTS AND DISCUSSION

4.1Formulation of supplemented diets

Figure 4.1 shows packaged composite flours of S_1, S_2 and S_3

S_1= 25% Wheat flour+10% Maize flour+22.5% Rice flour +22.5% Bengal gram flour +10% Egg powder+10% Milk powder

S_2=30% Wheat flour+10% Maize flour+20% Rice flour+20% Bengal gram flour+10% Egg powder+10% Milk powder

S_3=35% Wheat flour+10% Maize flour+17.5% Rice flour+17.5% Bengal gram+10% Egg powder+10% Milk powder

Fig 4.1: Packaged samples of formulated products

4.2 Nutritional comparison among the formulated blends and commercial weaning food

The proximate composition of the formulated blends and commercial brand is shown in table 4.1:

Table 4.1
Proximate composition of weaning food formulations and commercial weaning food:

Components (%)	Weaning food				Recommended	
	S_1	S_2	S_3	A(control)	Codex standard	PAG
Moisture	7.7±0.43 ^{a}	6.81±0.56 ^{b}	7.52±0.40 ^{ab}	4.5±0.14 ^{c}	<5	5-10
Ash	1.89±0.24 ^{b}	1.75±0.35 ^{b}	1.62±0.23 ^{b}	2.6±0.28 ^{a}	<3	≤5
Protein	17.9±0.16 ^{c}	19.2±0.14 ^{a}	18.6±0.14 ^{b}	15.2±0.30 ^{d}	15	20
Fat	8.1±0.22 ^{b}	7.8±0.29 ^{c}	7.67±0.26 ^{c}	9.0±0.50 ^{a}	10-25	≤10
Fiber	2.83±0.14 ^{a}	2.64±0.23 ^{b}	2.5±0.18 ^{b}	1.49±0.03 ^{c}	<5	≤5
Total Carbohydrate (By difference)	61.64±0.40 ^{b}	61.8±0.85 ^{b}	62.1±0.76 ^{b}	67±0.32 ^{a}	60-75	-

Values are mean ± SD. Means followed by the same letter are not significantly different ($p < .05$)

S_1=25%WF+10%MF+22.5%RF +22.5%BF+10%Egg powder+10% Milk powder

S_2=30%WF+10%MF+20%RF+20%BF+10%Egg powder+10%Milk powder

S_3=35%WF+10%MF+17.5%RF+17.5%BF+10%Egg powder+10% Milk powder

[WF=Wheat flour; MF= Maize flour; RF=Rice flour; BF=Bengal gram flour]
A=Commercial weaning food (control)
PAG=Protein Advisory Group requirements for weaning food formulation
(WHO/FAO/UNICEF 2007)

Moisture content

Moisture content and water activity are key factors affecting the storage, shelf life and safety of foods (Fontana, 2008). From table no 6 it is seen that, S_1 have highest moisture content (7.7%) as followed by S_3 and S_2 having (7.52%) and (6.81%) respectively. The moisture content of the whole blends was higher compared to the commercial product (4.5%). Monitoring the moisture content in foods and food products is crucial, because high moisture contents can reduce shelf life by increasing microbial degradation activity, resulting in bad odor and unacceptable taste of the product. Moisture contents are within the PAG recommended value [5-10%] except control (low moisture content 4.5%) probably due to the worry of prolonging or increasing the shelf life of that commercial product. According to Egyptian Standard No. 3284 the recommended moisture content of infant formula is not more than 7%. However, the moisture content of the formulations was higher than the maximum level (5%) recommended by the Codex standard for infant formula.

The moisture content of the study are lower to that of reported by Haque (2010) for sweet potato and soybean flour based weaning food (8.05-8.16%) but higher than fermented pearl millet and roasted cowpea based diet (3.37-4.5%) reported by Ijarotimi *et al.,* (2006).

Ash content:

Ash content is an important nutritional indicator of mineral content and an important quality parameter for contamination, particularly with foreign matter (for example, pebbles) (Fennema, 1996). The ash content of the prepared weaning food samples ranged from 1.62% to 1.89%. Formulated blend S_1 gave maximum ash content (1.89%). The increased ash content of S_1 could be due to the high ratio of bengal gram flour which is good sources of minerals. All formulated blends have ash contents significantly ($P<0.05$) lower than control. All the formulated blends meet the recommended codex standard and also fulfill the requirement reported by PAG for weaning food. Slightly similar result for ash has been found in wheat flour, corn flour, mug bean flour based weaning food (1.4-2.9% ash) reported by Ismail *et al., (*2000). The ash content of formulated blends is lower than that of reported by Adengua (2010) for sweet potato, cowpea and peanut flour based weaning food (2.8-3.6%).

Protein content

Protein is one of the most important nutrients required in weaning foods. The three blends of weaning food formulations were high in crude protein. The mean value ranged from17.9% to 19.2%. Weaning food blend S_2 contain highest protein content (19.2%) due to highest proportion of bengal gram flour and wheat flour ratio. All the formulated blends of this study are higher in protein content than commercial weaning food (15.2%). All the formulations meet the recommended codex standard and also fulfill the requirement reported by PAG for weaning food. The formulated complementary food complies with the permitted levels (15%) of Food and Nutrition Board 1989 and Egyptian Standard No. 3284. Diets with these high content of protein can be useful not only for weaning children but also for children suffering from PEM.

The protein content of formulated blends is similar to that reported by E.A. Amankwah *et al.*,(2009),for fishmeal, maize, soybean based weaning blends(17.18-19.13%). Higher protein content (31.1-38.5%) was found in weaning food formulated from sweet potato, cowpea, peanut by W. Adenuga (2010).

Fat content

The fat content of the prepared weaning foods ranged from (7.67% -8.1%). Among the prepared weaning foods S_1 have highest fat content (8.1%) as followed by S_2 and S_3 having (7.8%) and (7.67%) respectively. The control sample gave higher fat content (9%) than that of all formulated blends. All the formulated foods fulfill the requirement reported by PAG for weaning food while none of this could meet the recommended codex standard (10-25).

The fat content of formulated blends was higher than that reported by Suresha *et al.*, (2016), for milled based weaning blends (2.34-2.96%). The fat content of formulated weaning foods were lower than that reported by Ismail *et al.*, (2000), from wheat flour, corn flour, mung bean flour based weaning blends (13-16.5%).

Fiber content

The low crude fiber content is nutritionally appreciated because it traps less protein and carbohydrates. The crude fiber content of infant foods is expected to be low, as food with high fiber content tends to cause indigestion in infants. Hence, samples with low fiber content were rated good as potential complementary foods.

The percent crude fiber of the prepared weaning foods were 2.5% -2.83%. The fiber content of formulated samples is higher than commercial weaning foods (1.5%). It is seen that fiber content of all the blends decreased with the decrease in the ratio of bengal gram flour, because bengal gram flour contain highest amount of fiber (9%). Fiber is an important dietary component in preventing overweight, constipation, cardiovascular disease, and diabetes and colon cancer (Mosha *et al.*, 2000) but high dietary fiber content has been reported to impair protein and mineral digestion and absorption in human subjects (Whitney *et al.*, 1990). Some fiber related fractions such as polyphenols and non-starch polysaccharides, bind minerals such as Ca, Zn and Fe, making them unavailable for human nutrition (Fairweather-Tait and Hurrell, 1996). All the formulated blends meet the recommended codex standard and also fulfill the requirement reported by PAG for weaning food.

The fiber content of formulated weaning foods is lower than that reported by E.A. Amankwah *et al.*, (2009) for fishmeal, maize, soybean based weaning blends (8.75-9.38%). The fiber content of formulated diet is higher than that reported by Suliaman *et al.*, (2004), for sorghum based weaning blends (0.91-1.2%).

Carbohydrate content

The carbohydrate content in the prepared weaning foods ranged from (61.64-62.1%). The maximum carbohydrate content was calculated to be 62.1% in S_3 which is lower than the control sample (67%) but higher than other two prepared samples. All the samples meet the Recommended Dietary Allowances (≥60) for infant and babies. The carbohydrate content of current formulations are higher than that of soyabean and tigernut based weaning food (50.33-52.13%) reported by C. A. Emmanuel *et al.*, (2012).

The carbohydrate content of formulated blends are more or less similar to the results found by Tehseen *et al.*, (2014), from germinated wheat flour, rice flour, mug bean flour based weaning blends(57.85-62.57 %).

4.3 Calculated energy value

The gross energy value of percent protein, percent fat and percent carbohydrate is shown in table below:

Table 4.2:

Calculated energy	Weaning food			
	S_1	S_2	S_3	Control
Carbohydrate Energy(Kcal/100g	246.56	247.2	248.4	268
Protein Energy(Kcal/100g)	71.6	76.8	74.4	62
Fat Energy(Kcal/100g)	72.2	70.2	69.03	81
Gross energy (Kcal/100g)	390.36	394.2	391.83	411

The gross energy values were calculated, by multiplying the protein, fat and carbohydrate content by 4, 9 and 4 respectively.

The standard energy need from complementary foods is approximately 200 kcal/day for infants aged 6-8months, 300 kcal/day for infant's aged 9-11 months, and 550 kcal/day for children aged 12-23 months (FAO, 2001).

The energy content of the formulations fall within the recommended daily dietary allowance (RDA) for infants. The high energy requirement for the infant is due to the fact that growth is rapid at this stage (Onimawo, 2001).

4.4 The micronutrient content of weaning foods

To meet the mineral requirements of infant a variety of mineral rich complementary foods should be offered, since the consumption of these foods is relatively small among infants/children aged between 6 and 24 months [WHO/UNICEF, 1998].

Table 4.3: Mineral composition of prepared weaning blends and control

Sample	Calcium(mg/100g)	Zinc(mg/100g)	Iron(mg/100g)	VitA(µg/100g)
1	451±0.38 [a]	3.26±0.05 [c]	11.37±0.12 [b]	346±0.94 [a]
2	446±0.89 [b]	4.6±0.08 [b]	12.09±0.24 [a]	332.54±0.85 [b]
3	435.29±0.58 [c]	5.3±0.22 [a]	10.41±0.32 [c]	329.71±0.33 [d]
Control	420±0.05 [d]	2.5±0.10 [d]	10±0.27 [c]	330±0.56 [d]

Values are mean ±SD. Means followed by the same letter are not significantly different ($p < .05$).

Calcium

The mineral composition of control and formulated food samples presented in table 7 shows that calcium content is highest in S_1 (451mg/100g) followed by S_2 (446 mg/100g) while the low value obtained from S_3(435.29mg/100g). The control sample gave lower calcium content (420mg/100g) than that of all formulated diet. S_1 contain higher amount of bengal gram as compare to other samples and bengal gram contain higher amount of calcium (58mg/100g). According to FAO/WHO (2004), recommended level of calcium in weaning foods should be 400 mg/100g. On the basis of this standard, the prepared weaning foods had the calcium concentrations above the standard set by FAO/WHO (2004).

Zinc

S_3 contain highest zinc content (3.26mg/100g) due to highest proportion of wheat flour (35%). Wheat flour contains about 3 mg Zn per 100 g. On the other hand the S_1 had the least zinc value (3.26mg/100g). All the formulated diets are higher in zinc content than commercial brand (2.5%). According to FAO/WHO (2002), recommended level of zinc in weaning foods should be 4.6 mg/100g. S_2 and S_3 meet the minimum zinc requirement while S_1 and commercial brand were less. All the prepared weaning foods had the zinc concentrations above the minimum amount (3.2 mg/100 g) specified in the Codex Alimentarius Standards (FAO/WHO, 1991).

Iron

Iron concentrations in prepared weaning blends were 10.41 mg/100 g to 12.09mg/100g. High iron content is observed in S_2 (12.09mg/100g) and lower value obtained from S_3 was 10.41mg/100g. All the prepared food has higher iron than commercial weaning food (10%).

The prepared weaning food and the imported products had the iron concentrations above the minimum amount (9.3 mg/100 g) specified by (FAO/WHO, 2004).

In comparison, the minerals content of formulated food samples were higher than the commercial complementary food i.e., control sample. This observation indicates that formulated food sample would serve as a good source of minerals particularly calcium which is essential for bone and teeth formation and development in infant.

Vitamin A

According to Codex Alimentarius Standards (FAO/WHO, 1994), the recommended levels (µg/100 kcal) of vitamin A for processed cereal based foods for infants and young children ranged from 60 to 180 µg/100 kcal. Comparing with this standard, the prepared weaning food had vitamin A content of 88.64µg/100 kcal, 84.36µg/100 kcal and 84.14 µg/100 kcal. The imported commercial weaning food had vitamin A content of 80.29µg/100 kcal, respectively which were above the minimum limit (60 µg/100 kcal) specified in the Codex Alimentarius standards. The vitamin A content of the prepared weaning food was significantly higher ($p<0.05$) than the commercial weaning food.

4.5 Sensory evaluation:

The mean sensory scores of the weaning food formulations and commercial food are shown in table 4.4

Table 4.4: Sensory quality of the weaning foods

Sensory attributes	S_1	S_2	S_3	control
Color	7.4^b	7.2^b	7.1^b	8.4^a
Flavor	7.2^b	6.90^b	6.80^b	8.6^a
Taste	7.0^b	6.8^b	6.30^c	8.3^a
Consistency	7.2^b	6.9^b	6.7^b	8.1^a
Overall acceptability	7.1^b	6.8^c	6.5^c	8.00^a

Where, superscript a, b, c, indicates the rank on level of significant difference among samples. Sample means having the same letter meaning that do not differ at 5% level of significance.

Color:

Color is an important quality indicator of a food system that could affect consumer acceptance. Color scores ranged from 7.1 to 7.4. The commercial weaning formula had color score of 8.4 which was significantly (P>0.05) higher than those of the formulated weaning foods. Furthermore, among the formulated blends, there was no significant (P>0.05) difference in terms of color score. However, panelist ratings for all the samples were above the mid-point and therefore were acceptable. There were significant differences between the commercial and the prepared weaning foods in color.

Flavor:

Flavor is a very important attribute in case of weaning food as infants and babies would refuse to consume food of odd flavor. Flavor scores ranged from 6.8 to 8.6. The commercial weaning food had the highest flavor score of (8.6) followed by formulated sample S_1, while S_3 receive the least flavor ratings. There was no significant difference (P>0.05) in flavor scores among the formulated baby foods but there was a significant difference (P<0.05) between the control sample and formulated samples.

Consistency:

Consistency ratings ranged from 6.7 to 8.1. The commercial weaning food had significantly (P<0.05) higher consistency score compared to the formulated blends. The commercial blends had the highest consistency score of (8.1) followed by formulated sample S_1, while S_3 receive the least consistency ratings. There was significant (P<0.05) difference in consistency score of the commercial blends and formulated blends. On the contrary, there was no significant (P>0.05) difference in the overall acceptability score between the S_2(6.8) and S_3 (6.5).

Taste:

Taste is an important sensory attribute which determine products acceptability especially by an infant. The taste score ranged from 6.3 to 8.3. Commercial blends had the highest taste score of 8.3 which was significantly (P>0.05) higher than those of the formulated blends. The best taste ratings of the reference sample would be as a result of flavoring addition. There was no significant (P>0.05) difference in the taste score between the S_1 (7.1) and S_2 (6.8). However, the ratings of the samples were within acceptable range.

Overall acceptability:

The overall acceptability of the blends were influenced by the organoleptic attributes of color, flavour, taste, mouthfeel etc. The overall acceptability score ranged from 6.5 to 8.0. Commercial blends had the highest overall acceptability score of 8.0. There was significant ($P<0.05$) difference in the overall acceptability score of the commercial diet and formulated diets. On the contrary, there was no significant ($P>0.05$) difference in the overall acceptability score between the S_2 (6.8) and S_3 (6.5). The overall acceptability of the commercial product was greater than that of the weaning food. This could be because consumers are used to the commercial product and also because flavoring, sweetening, and other sensory enhancing agents are incorporated into the commercial product during its formulation. The current study recommends adding natural flavors or sweeteners during the preparation of the complementary food to increase its palatability and acceptability. Some of the organoleptic properties of the infant food formulation can be improved by addition of edible flavouring and colouring materials (Nwanekezi, 2001).

Formulated weaning food S_1, therefore, can be suggested as more acceptable and will be potentially suitable for use as weaning food, both at the home and commercial levels.

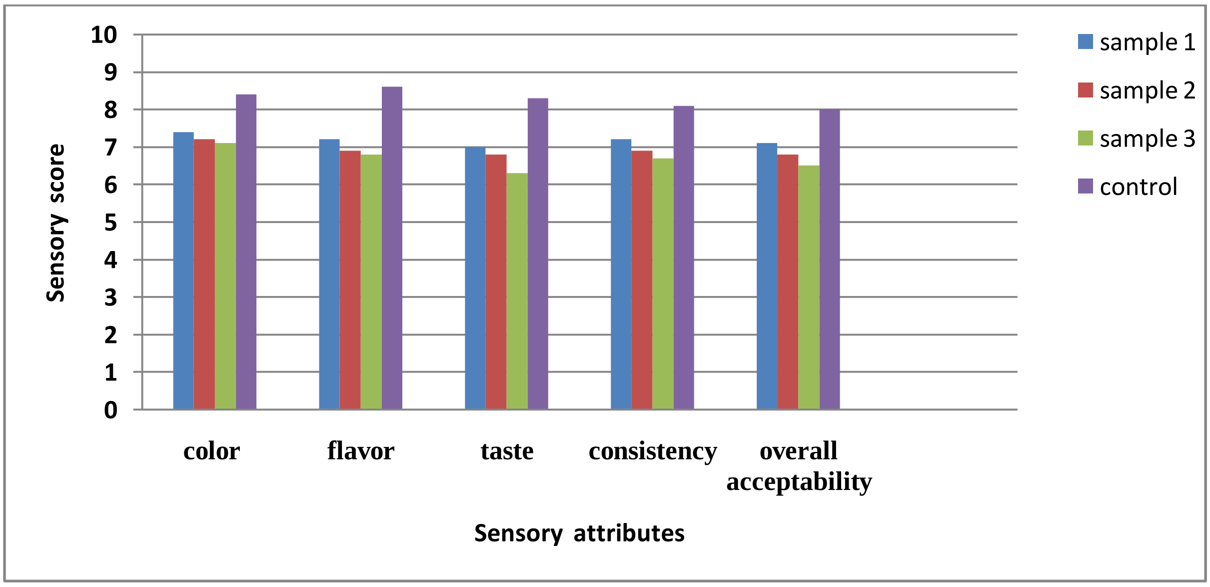

Figure 4.2: Proportion of preference for complementary foods

4.6 Microbiological quality of weaning foods

Microbial analysis was conducted on freshly prepared samples to determine if the blends are wholesome for consumption. The results on the microbial load in the formulated blends are presented in table 4.5. The total number of viable bacteria count in formulated weaning blends were 1.5, 1.0 and 1.2 (cfu/ml) at 10^{-3} dillution. However comparatively higher count was obtained for yeasts and molds 2.0, 1.8 and 1.7(cfu/ml) at 10^{-4} dillution. The result revealed that all the diets are free from *Salmonella spp.* when packets were opened. Total viable count and *salmonella spp* may indicate processing and product handling hygiene.

Generally a food product for consumption should have microbial count below $1x10^5$cfu/ml. The international microbiological standard recommends a bacteria contaminants limit of less than 10^6 cfu/ml for food. The low counts of the examined foods indicated adequate thermal process, good quality of raw materials, high standard of personal hygiene and as a result of the good different processing conditions under which the production of foods were carried out.

Table 4.5. Microbial evaluation of the formulations

Weaning food sample	**Total viable count (CFU/ml)**	**Yeasts and molds count (CFU/ml))**	***Salmonella*/ml**
S_1	1.5×10^3	2.0×10^4	nd
S_2	1.0×10^3	1.5×10^4	nd
S_3	1.2×10^3	1.7×10^4	nd
Limits	100000	100000	nil

nd – not detected

As shown in Table 4.5, all the formulations meet the set standards for processed cereal based foods for infants and young children specification for microbial quality. The developed complementary foods therefore meet these requirements.

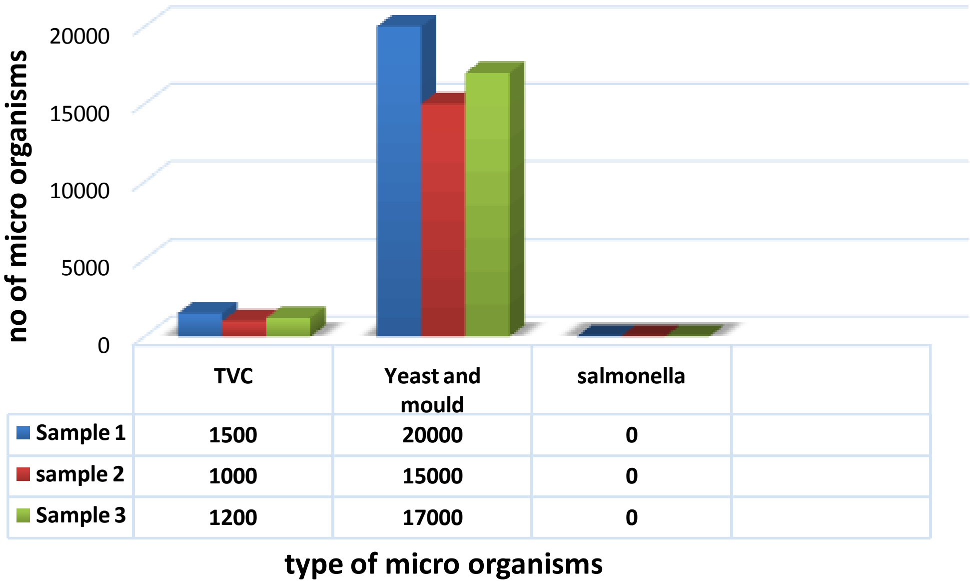

Fig 4.3: Microbial load in formulations

4.7 Shelf life study of weaning foods:

The products were packaged in a medium density polyethylene bags and stored at ambient temperature of 25^{0}C for periodic analysis. The moisture content, protein and fat content of packaged product were measured over 2 months period. Some of the organoleptic quality likes color, flavor was also observed during this storage period. Results are summarized in the table 4.6:

Table 4.6: Shelf life study of formulated weaning foods

Storage periods (days)	Sample code	Observations		
		Moisture	Protein	fat
0	S_1	7.7±.01	17.90±.09	8.1±.03
	S_2	6.8±.03	19.2±.01	7.8±.06
	S_3	7.52±.02	18.6±.02	7.67±.01
30	S_1	7.78±.05	17.87±.07	7.98±.02
	S_2	6.87±.07	19.17±.01	7.72±.06
	S_3	7.57±0.4	18.56±.02	7.61±.04
60	S_1	7.8±.03	17.8±.02	7.95±.07
	S_2	6.9±.09	19.12±.03	7.7±.08
	S_3	7.6±.06	18.49±.05	7.58±.04

There was significant difference between the moisture, protein and fat contents before and after the 60 days evaluation period. No off flavor and off color was developed during the storage periods.

In the table 10 it is shown that the moisture content of the formulated weaning foods slightly increased gradually with time, the change in moisture content could be due to moisture absorption from environment

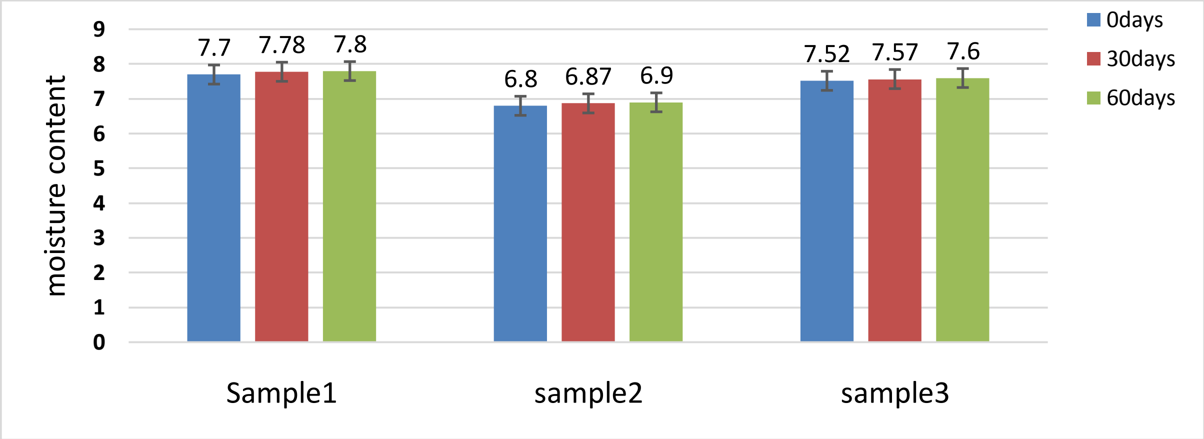

Fig 4.4: Change in moisture during storage period. Values are mean ±SD.

A depletion of protein for S_1, S_2 and for S_3 was observed. This change in protein content could be due to increase in moisture content in the formulations and instrumental error. This change however still meet the recommended protein content for weaning food.

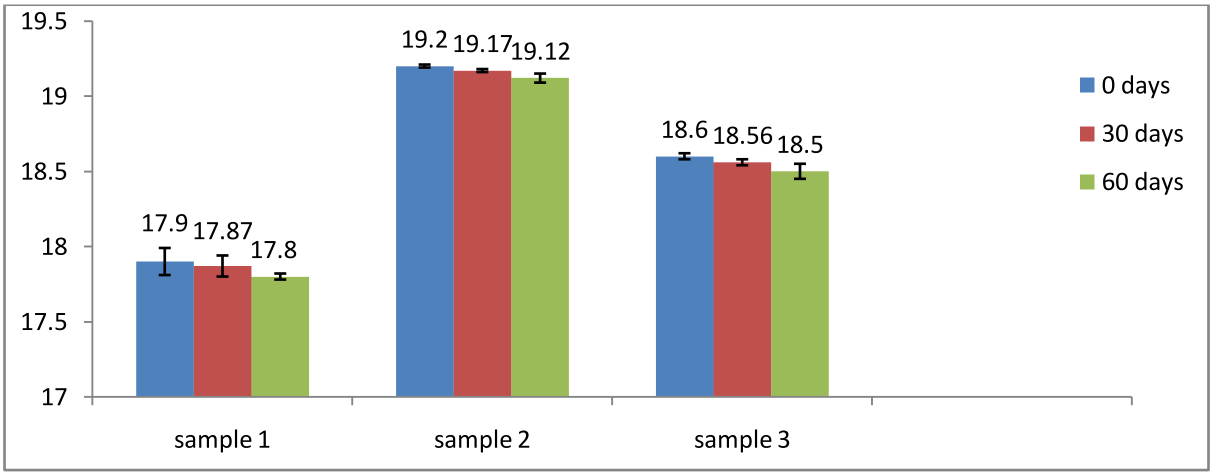

Fig 4.5: Change in protein during storage period. Values are mean ±SD.

The fat content was also decreased slightly with an increase in storage period. After 60 days of storage, the fat content decreased from an initial range (7.67 to 8.1) to (7.58 to 7.95) after 60 days.

However fat content might be decreased due to increase moisture content, partial oxidation and instrumental error.

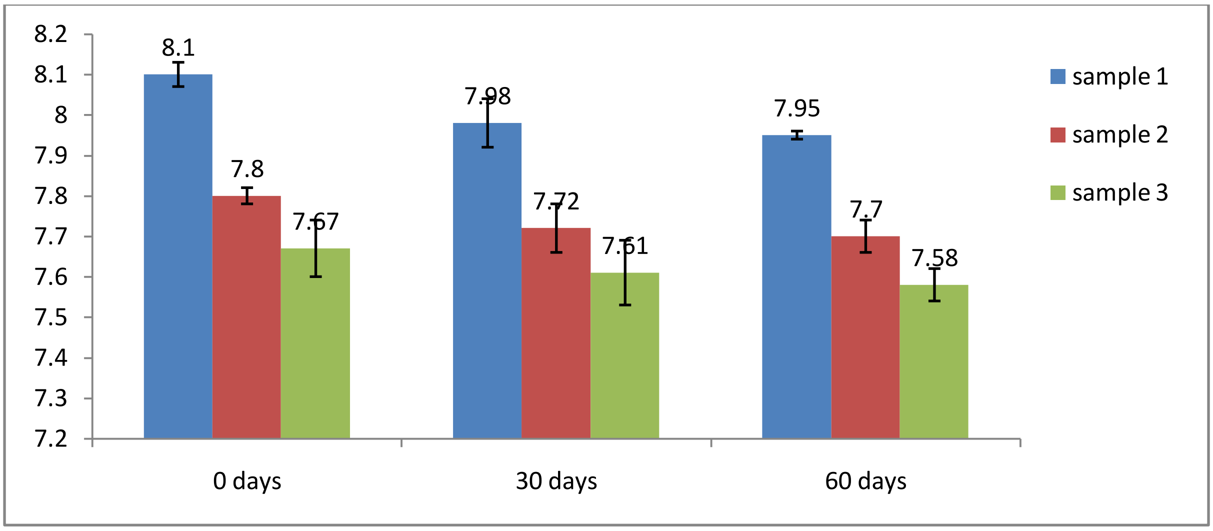

Fig 4.6: Fat changes during storage period. Values are mean ±SD.

Chapter 5

SUMMARY AND CONCLUSION

Children in the developing countries are the most vulnerable and affected by malnutrition. Inappropriate calorie intake and faulty or absence of complementary feeding after six months of age is considered as one of the major factor for the child malnutrition. The weaning formulations in the present study were based on commonly consumed, low cost food materials locally available in market. Addition of egg in the cereal and pulse based weaning foods can be served as an excellent source of protein and minerals.

From the results it was clear that the local formulations compared favorably well with the commercial products as recommended dietary allowance for infants. From the microbial point of view, the prepared weaning food showed satisfactory results with regards to microbial food safety.

The study was a part of the effort to provide home based complementary foods that can be more cost effective to the low income families. Besides it will be potentially suitable for use as weaning foods, both at the home and commercial levels. The fact that this formula is inexpensive, easily available and nutritious could make them effective in solving some of the nutrition problems facing infant and children. The product could be served in the form of porridge with water/milk.

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APPENDICES

Appendix I:

Statistical analysis of sensory evaluation for color

Table 1.1 Analysis of variance (ANOVA) for color of weaning food

		Sum of squares	Degree of freedom	Mean square	F-ratio	Significance
Color	Between Groups	10.675	3	3.558	13.774	.000
	Within Groups	9.3	36	.258		
	Total	19.975	39			

Table 1.2. Duncan's Multiple Range Test (DMRT) value for color

Color

Sample		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	3.00	10	7.1000	
	2.00	10	7.2000	
	1.00	10	7.4000	
	4.00	10		8.4000
	Sig.		0.221	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix II:

Statistical analysis of sensory evaluation for flavor

Table 2.1 Analysis of variance (ANOVA) for flavor of weaning food

		Sum of squares	Degree of freedom	Mean square	F-ratio	Significance
Flavor	Between Groups	20.875	3	6.958	20.040	.000
	Within Groups	12.500	36	0.347		
	Total	33.375	39			

Table 2.2 Duncan's Multiple Range Test (DMRT) value for flavor

Flavor

Sample		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	3.00	10	6.8000	
	2.00	10	6.9000	
	1.00	10	7.2000	
	4.00	10		8.6000
	Sig.		0.160	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix III:

Statistical analysis of sensory evaluation for taste

Table 3.1 Analysis of variance (ANOVA) for taste of weaning food

		Sum of squares	Degree of freedom	Mean square	F-ratio	Significance
Taste	Between Groups	25.875	3	8.525	43.732	.000
	Within Groups	7.100	36	0.197		
	Total	32.975	39			

Table 3.2 Duncan's Multiple Range Test (DMRT) value for taste

Taste

Sample		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	3.00	10	6.3000		
	2.00	10		6.9000	
	1.00	10		7.2000	
	4.00	10			8.5000
	Sig.		1.000	0.140	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix IV:

Statistical analysis of sensory evaluation for Consistency

Table 4.1 Analysis of variance (ANOVA) for consistency of weaning food

		Sum of squares	Degree of freedom	Mean square	F-ratio	Significance
Taste	Between Groups	11.600	3	3.867	10.875	.000
	Within Groups	12.800	36	0.356		
	Total	24.400	39			

Table 4.2 Duncan's Multiple Range Test (DMRT) value for consistency

Consistency

Sample		N	Subset for alpha = 0.05	
			1	2
Duncan[a]	3.00	10	6.7000	
	2.00	10	6.9000	
	1.00	10	7.1000	
	4.00	10		8.1000
	Sig.		0.165	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix V:

Statistical analysis of sensory evaluation for overall acceptability

Table 5.1 Analysis of variance (ANOVA) for overall acceptability of weaning food

		Sum of squares	Degree of freedom	Mean Square	F- ratio	Significance
Overall acceptability	Between Groups	12.600	3	4.200	11.631	.000
	Within Groups	13.000	36	0.361		
	Total	25.600	39			

Table 5.2 Duncan's Multiple Range Test (DMRT) value for overall acceptability

Overall acceptability

Sample		N	Subset for alpha = 0.05		
			1	2	3
Duncan[a]	3.00	10	6.5000		
	2.00	10	6.8000	6.8000	
	1.00	10		7.1000	
	4.00	10			8.0000
	Sig.		0.272	0.272	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix VI

6.1 Hedonic rating test of prepared weaning foods

Name of the tester ………………………………………….. Date………/……/2017

Please taste the sample and give numerical score ranging from (1-9) in the appropriate space.

Sensory attributes	**Sample code**			
	S_1	**S_2**	**S_3**	**S_4**
Color				
Flavor				
Taste				
Consistency				
Overall acceptability				

Hedonic scale used

9= Like extremely

8= Like very much

7= Like moderately

6= Like slightly

5= Neither like nor dislike

4= Dislike slightly

3= Dislike moderately

2= Dislike very much

1= Dislike extremely

____

Signature