لا يوجد نص يمكن قراءته بشكل طبيعي من الصورة المقدمة.
PREPARATION AND EVALUATION OF LEGUME BASED WEANING FOODS

By

Ahmad Bilal
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NATIONAL INSTITUTE OF FOOD SCIENCE & TECHNOLOGY
FACULTY OF FOOD, NUTRITION AND HOME SCIENCES
UNIVERSITY OF AGRICULTURE
FAISALABAD
2017
DECLARATION

I hereby declare that the work presented in this thesis “Preparation and evaluation of legume based weaning foods” is my own effort except where other acknowledged and that the thesis is my own composition. No part of the thesis has previously been presented for any other degree. University may take action if information is incorrect at any stage.

Ahmad Bilal
2006-ag-1317
To,

The Controller of Examinations,
University of Agriculture,
Faisalabad.

We, the supervisory committee, certify that the contents and form of thesis submitted by Mr. Ahmad Bilal, Reg. No. 2006-ag-1317 have been found satisfactory and recommend that it be processed for evaluation by external examiner(s) for the award of degree:

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HAZRAT MUHAMMAD ﷺ
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ABSTRACT

Protein energy malnutrition (PEM) is the leading nutritional problem worldwide especially in infants. It refers to the macronutrient deficiency resulting from an inadequate intake of protein and energy. Legumes are locally available, cheaper source of protein and can complement cereals to prepare weaning foods to combat PEM. Among the legumes, cowpea and mungbean have good protein and amino acid profile, but may contain certain anti-nutritional components. Roasting was employed to increase the nutritional profile of the product and to reduce anti-nutritional factors in the product. In the present study, roasted cowpea and mungbean flours were analyzed for anti-nutritional factors (hemagglutinin & trypsin inhibitor activities and phytate content) and were mixed with wheat flour to make composite flour. The proximate analysis of mungbean depicted higher moisture and ash contents (5.93 and 4.02%) as compared to cowpea (3.37 and 3.35%), whereas crude protein, fat & fiber contents in cowpea were higher (23.10, 2.05 & 3.57%) than mungbean (22.45, 1.34 & 3.07%), respectively. The mineral profile explicated that potassium, calcium, sodium, iron and zinc were 28.19, 387.51, 61.20, 78.33 and 45.75 mg/100g in cowpea while 10.46, 7.55, 5.31, 1.28 and 2.17 mg/100g in mungbean respectively. After roasting, phytate contents presented decrement in cowpea and mungbean up to 67.03 and 71.12% as compared to raw counterparts, respectively. Likewise, the percent decrease in hemagglutinin-lectin and trypsin inhibitor activities were 68.85 & 78.47% and 79.81 & 77.00% in roasted cowpea & mungbean as compared to raw versions, accordingly. Amongst composite flours, T0 (100% Wheat flour) had highest moisture content 12.22%, T3 (30% cowpea) showed highest protein content 13.14% while crude fat, crude fiber and ash contents were maximum in T9 (15% cowpea & 15% mungbean) as 1.69, 1.12 and 1.29%, correspondingly. Furthermore, maximum nitrogen free extract (NFE) value was observed in T5 (20% mungbean) as 76.32% and highest water absorption & oil absorption capacity was noticed in T3 1.44 mL/g & T8 (10% cowpea & 10% mungbean) 1.16 mL/g, respectively. Additionally, the emulsion capacity was highest in T8 as 75.75% while emulsion stability was maximum in treatment; T2 (20% cowpea) i.e. 53.33%. Highest foaming stability was observed in T8 as 68.67%, whereas highest foam capacity (44.74%) was reported in T3. In the product development phase, nine weaning foods were prepared using different ratios of cowpea and mungbean. Three most acceptable weaning food prototypes; W2= Roasted cowpea (20%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%), W5= Roasted mungbean (20%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%), W8= Roasted cowpea & mungbean (10:10%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%) were selected on the basis of sensory response with overall acceptability scored as 7.67, 7.66 and 7.92 and were renamed as W1S, W2S and W3S, respectively however, Wc= soybean (20%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%) was run as control. Amongst the selected weaning foods, W3S had more moisture content (4.58%) while higher crude protein was found in W3S (17.01%). On the other hand, fat, fiber and ash were highest in WC as 11.11, 4.57 and 2.67% respectively. W3S showed higher loose and packed bulk density values as well as reconstitution index, viscosity and gross calorific value. Regarding growth study parameters, protein efficiency ratio (PER), net protein ratio (NPR) and relative net protein ratio (RNPR) were viewed maximum in W3 as 2.65, 3.34 and 53.18 among the prepared treatments and found relatively comparable with Wc (2.84, 6.28 and 100), correspondingly. In nitrogen balance study, true digestibility (TD), biological value (BV) and net protein utilization (NPU) were highest in W3S as 80.31, 85.09 and 82.67% i.e. quite equivalent to Wc values (91.25, 91.84 & 89.37%), respectively. The acceptability appraisal of prepared infant formulations was conducted through short term infant feeding trails with the help of nursing mothers. The acceptability scores were ranged from 3.18 to 4.07 (out of 5) from day 1 to day 15. Conclusively, the selected legume based weaning foods presented comparable characteristics to that of existing soy based equivalents in terms of protein content, energy value and acceptability test as well as its impact on growth & nitrogen balance aspects.
CHAPTER 1

INTRODUCTION

Malnutrition is a growing impediment for human health in Pakistan i.e., especially prevalent amongst the children and women. Of the leading nutritional problems, under-nutrition in terms of protein energy malnutrition (PEM) is of notable importance due to high dependence on monotonous starchy staples. Amongst the various segments of population, children are considered as the most vulnerable to PEM owing to their high reliance on protein for energy. In this context, three national nutritional surveys have already been conducted during the past two decades (Binagwaho et al., 1998; Stipanuk, 2006; WHO, 2007). In Pakistan, 9.6 million children under-five are reported as malnourished (Global Nutrition Report, 2015). However, according to Global Nutrition Report (2016), 45% of the Pakistani children are estimated as stunted. The child malnutrition is strongly linked with mental & physical disabilities leading to poor educational attainment alongside, leads to higher morbidity and mortality rate in the long run (Chirwa and Ngalawa, 2008; Cheah et al., 2010). The poor socio-economic status and food insecurity are the possible determinants that not only impact negatively on the nutritional status of mothers but also liable for frequent infections among the children over and above the feasibility of health care services (Cheah et al., 2010; Linnemayr et al., 2008).

No doubt, human milk is considered as complete food for infants up to six months of age that fulfills all the nutritional requirements for optimal physical and mental development of the body. Moreover, it contains immunological & bioactive peptides, PUFA and readily bioaccessible forms of minerals like iron & zinc. However, with the progression in age, complementary/weaning foods facilitate nursing mothers to introduce semi-solid food in the diet plan of their babies besides breast feeding to cope up the increasing body needs while shifting from six months to two years of age. In this regard, complementary foods should supplement higher demand for protein that increases up to three folds from six months to two years and ought to meet the calorific intake of infants that widen up to 548 kcal by 23rd month. Thus, weaning/complementary foods are regarded essential for infant health predominantly at early years of life followed by increase in tissue mass in later life. Insufficient access to weaning foods is likely to cause nutrient deficiency in infants. Commercially, complementary foods are available but many of them are unable to fulfill protein requirements, energy density and micronutrients concentration & quality. Hence,
easily digestible, energy dense and nutritionally balanced, rich in macro- & micro-nutrients, complementary foods are necessitated for proper growth and survival of infants against PEM & hidden hunger (micronutrient malnutrition). In this connection, legumes, commonly called as “daals” in the sub-continent region, have received immense attention being an important source of micronutrients and low-cost vegetable proteins in contrast to animal products i.e. considered as less favorable choice (Jirapa et al., 2001; Daelmans and Saadeh, 2003; Bond et al., 2005; Mennella et al., 2006; Chang et al., 2008; Dewey and Adu-Afarwuah, 2008; Melo et al., 2008; Sai-Ut et al., 2009; Olivia and Ardythe, 2013).

The cereal-legume based complementary foods have gained substantial attentions in the recent time. This is because cereal flour contains sulfur containing amino acids (S-AAs) however, deficient in lysine. In this context, wheat is a choice cereal owing to large quantity of gluten. However, legume flour serve as an imperative part in human nutrition being rich in protein content and quality, supplying ample amounts of lysine as well as micronutrients but insufficient in S-AAs (Badifu and Aka, 2001; Egli et al., 2003; Mubarak, 2005; Ghasemzadeh and Ghavidel, 2011; Olapade and Oluwole, 2013). Thus, to meet the dietary requirements, cereal based weaning foods ought to be supplemented with legumes but their role seems limited due to numerous reasons such as presence of antinutrients including phytates and trypsin inhibitors, hindering minerals availability like iron & zinc and low digestibility of protein & starch (Kamchan et al., 2004; Seena et al., 2006; Khattab and Arntfield, 2009). In order to overcome these side-effects, different processing procedures; roasting, germination, boiling and de-husking are employed. Amongst these processing methods, roasting improves color, enhances flavor, extends shelf life, reduces anti-nutrients in legumes and denatures protein resultantly improving protein digestibility & quality of legumes (Seena et al., 2006; Boye et al., 2010a).

In the development of infant formulations, not only the recipe but also functional and nutritional properties are necessary measures to reach a well-balanced complementary food (Nasirpour et al., 2006). The legume flours have functional and emulsifying properties that differ based on quantity & quality of protein, whereas foaming characteristics vary in terms of preparation methods (Meng and Ma, 2002). Other functional attributes of legume proteins include solubility and fat & water absorption capacity. Some of the functional attributes of legume derived proteins are analogous to already existing & commonly available soy/whey based weaning foods (Boye et al., 2010a).
The utilization of indigenous legumes in complementary foods could combat child malnutrition in the country. Pakistan is agriculture-based state, where varied types of leguminous plants are cultivated such as mungbean, cowpea, chickpea, lentils, kidney beans, broad bean, etc. The nutritive significance of legumes is based on the existence of certain amino acids and their physiological exploitation after digestion & absorption. Mungbean (Vigna radiate) is the main legume consumed by sub-continent and consumed in different forms such as daal, savory food or sweet snack (Dahiya et al., 2013). The protein content in mungbean is accounted up to 20 to 30% along with balanced amino acid picture except methionine i.e. considered as the first limiting amino acid (Alsohaimy et al., 2007). Besides, it is rich in iron content and ensures high palatability and taste hence preferred in the formulation of weaning foods (Imtiaz et al., 2011). The processing and consumption suitability of mungbean mainly depends on physical and chemical properties that vary in different climatic zones (Bisht et al., 2005; Makeen et al., 2007). Likewise, cowpea (Vigna unguiculata), commonly known as cow gram, lobia, china pea and black-eyed bean, is also an appropriate source of dietary protein with high bioavailability, rich in soluble & insoluble dietary fiber as well as minerals & vitamins. In the Indo-Pakistan sub-continent, cowpea is widely used in culinary dishes (Papalamprou et al., 2009; Appiah et al., 2011; Ashraduzzaman et al., 2011; Ashogbon and Akintayo, 2013). The protein content of chickpea is quite similar to mungbean, whereas anti-nutritional substances are on the lower side in mungbean (Dahiya et al., 2013).

In the course of infancy, optimum nutrition promises proper growth and health alongside, secures from infections and disease incidences. In this perspective, weaning/complementary feeding is gaining the interest of scientific fraternity (Mennella et al., 2006; Melo et al., 2008). Inadequate provision of complementary foods or inappropriate feeding practices may be the possible causes of macro- & micro-nutrient malnutrition in the country. In Pakistan, it is the need of hour to explore the nutritional worth of some indigenous legumes to design infant formulas that could mitigate the existing PEM, considering the affordability of the low income segments of the masses as well. Purposely, in the current investigation, cowpea and mungbean based weaning formulations were prepared and bioassessed using Sprague Dawley rats in order to find out their suitability. Lastly, the infant’s acceptability response was also evaluated. The objectives of the instant study design are mentioned herein;

**Objectives**
• Evaluate the impact of roasting process on the physicochemical properties and anti-nutritional factors of cowpea and mungbean

• Develop and analyze the legume based weaning foods using physicochemical and infant acceptability tests

• Bioassessment of protein quality of the selected weaning food formulations using Sprague Dawley rats
CHAPTER 2

REVIEW OF LITERATURE

Among the developing world, the escalating population growth, food & nutritional insecurity and rising costs for food and health care are the major threats responsible for prevailing protein energy malnutrition (PEM). Under such circumstances, indigenous legume sources are attaining popularity due to their distinctive protein nature, contributing essential amino acids like lysine *i.e.* lacking in wheat. Besides, leguminous plants are comprised of antioxidants and dietary fiber that help to avert numerous physiological dysfunctions. In the present scrutiny, composite flour blends were prepared using two types of roasted legumes; cowpea and mungbean. Additionally, three treatments of these composite flours were used for the preparation of weaning foods after analyzing their functional properties. Finally, the prepared weaning foods were subjected to physicochemical profiling and biological evaluation.

2.1. Malnutrition

2.2. Legumes; a prefatory outlook

2.2.1. Origin and cultivation

2.2.2. Nutritional aspects

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2.1. Malnutrition

Imbalance, either excess or poor intake of energy or nutrients refers to malnutrition. It is generally divided in two broad categories *i.e.* under- and over-nutrition. Under-nutrition covers wasting, stunting, underweight and micronutrient deficiencies. It is most widely recognized form of malnutrition. On the other hand, over-nutrition results in weight gain, and obesity mediated disorders (Hoffer, 2001).

In numerous parts of the world, malnutrition especially PEM has become one of the most severe nutritional problems (Iqbal *et al.*, 2006). Approximately 1.9 billion adults are
overweight while 462 million people are underweight in the world. In the developing nations, under-nutrition of infants and child under-five has come out as severe concerns among poor families. Being a severe global disparity, malnutrition has resulted in approx. 3.5 million deaths per annum in children under-five that accounts up to 45% of deaths (Roux et al., 2010; Rytter, 2014). According to WHO, 41, 159 and 50 million children under-five are underweight, stunted and wasted, respectively (WHO, 2016). In Pakistan, vicious cycle of malnutrition caused 35% of deaths in children under-five. Nationally, around 31.5% children under-five were underweight, predominantly in rural areas i.e. 33.3%. Domestically, stunting contributed approx. 43.7% with high percentage in rural areas (46.3%) comparatively to urban areas (36.9%). At national level, wasting among children was 15.1% with more prevalence in rural area 16.1% than urban areas 12.7% (GOP, 2011).

PEM is a type of malnutrition that has become a serious health issue both in under-developing and developing states (Brabin and Coulter, 2003; FAO, 2004; Grover and Ee, 2009). World widely, approx. 925 million people were suffering from PEM till the year 2010 (Hildick, 2012). In children, PEM is clinically defined as $<70\%$ weight to height ratio and/or swelling of feet or wasting of muscle mass that are the characteristics of kwashiorkor or marasmus (Schaible and Kaufmann, 2007). In low income economies, PEM is responsible for up to 50 to 60% of mortalities under-five due to the occurrence of various associated morbidities (Ubesie et al., 2012). PEM is also a serious issue in critically-ill patients and their situation deteriorated during the stay in the hospital. The malnutrition is associated with many chronic outcomes that may lead to increase in hospitalization or may prove fatal (Isabel et al., 2003). The consequences of malnutrition are often severe, leading to poor quality of life (Smolinaer et al., 2008).

Food insecurity, poverty, low educational level, climate & environmental changes and inequalities in terms of food distributional networks are the major influential factors resulting in poor energy and nutrients intakes ultimately leads to malnutrition (Bain et al., 2013). Diminishing protein supplies continues to be the foremost nutritional threat all over the globe that ultimately affects food and nutritional security. The poor economic condition and improper dietary habits and feeding practices are supposed to be the major determinants of this threat (Sachs and McArthur, 2005).

In order to improve nutritional status, multi-sectorial approaches are necessitated including food & agriculture, health, water, sanitation & hygiene, education, employment and social
protection. Food fortification, supplementation and enrichment are established strategies that could be employed to mitigate malnutrition (Boius et al., 2009). Food fortification is one of the most substantial interventions to alleviate malnutrition as it has proven as a cost effective promising strategy. Thus, fortification or supplementation of diet with different legumes such as cowpea, chickpea and mungbean are commonly practiced to improve the quality of diet ultimately improving overall health status or lowering malnutrition associated disease burden (Boye et al., 2010b).

Optimum nutrition and health care during early childhood is essential for child survival by overcoming malnutrition (Lutter and Dewey, 2003). Complementary feeding is the inclusion of semisolid to solid food in the dietary regimen of infants besides breastfeeding. It is usually offered at the age of 6 months and continues till 2 years because during this phase, the infant needs could not be compensated solely on breast milk thus complementary feeding could suppress the incidences of malnutrition (Daelmans and Saadeh, 2003). PEM normally occurs in children when they shift from liquid based foods to semisolid foods. As infants are growing rapidly during this period hence hypercaloric and nutritionally balanced weaning foods are include besides breast milk (Asma et al., 2006). The availability of sufficient amount of nutritious food is a viable solution of malnutrition (Black et al., 2008). In the developing economies, cereal flour is the basic ingredient of 70% of the weaning food formulations being staple, cheap and easily available but it is a poor source of protein in contrast to legumes. In this scenario, the researchers are encouraging the use of indigenous legumes, cultivated in the poor income countries, to formulate weaning foods (Ijarotimi, 2008). To overcome the menace of PEM in infants, numerous weaning food prototypes need to formulate using maize, cowpea, mungbean and other legumes in order to improve protein value and overall quality of weaning food.

2.2. Legumes; a prefatory outlook

2.2.1. Origin and cultivation

Cowpea (Vigna unguiculata) is a legume having a place in the family Fabaceae. It is commonly named as china pea, southern pea or cow gram. Cowpea is one of the important food crops i.e. often consumed in tropical and subtropical countries. It was first cultivated in Africa with different sizes and shapes of the seed (Appiah et al., 2011; Ashogbon and Akintayo, 2013). It is widely grown in India and West Indies in the form of immature pods and mature seeds (Sreerama et al., 2012). In India, the crop was introduced during the Neolithic period and in Europe since the prehistoric times; 8th century B.C. (Tosti and Negri
Later, cowpea was brought to West Indies by the Spanish people during 16th century and was taken to the USA somewhere around 17th century. At the same time, it was introduced in South America as well (Timko et al., 2007). Now, Nigeria is the major cultivar of cowpea, where around 2.1 million tons of cowpea produced annually, that accounts up to 61% of the cultivation in Africa and 58% of the globe. Considering FAO estimates, it is reported that dry cowpea has covered upto 10 million hectares of the globe, producing approx. 4 million metric tons, annually. Singh et al. (2006) estimated the worldwide production of cowpea grain *i.e.* approx. 4.5 million, covering 12-14 million hectares. In Pakistan, production of cowpea was estimated up to 553 tons with an area of 257 hectares during 2003-04. The best conditions for the cultivation of cowpea include; high temperatures ~20-35 °C, pH of soil 5.5 (acidic) to 8.3 (alkaline) and heavy clay to sand textured soil besides, it is drought resistant crop (Valenzuel and Smith, 2002).

Mungbean (*Vigna Radiata*) is commonly known as green bean, cultivated normally for its palatable seeds that could be utilized after cooking, fermenting, roasting, milling or sprouting. During milling, they are separated from husk and cooked. It is used for cooking purposes mainly in preparing soups, curries, noodles, bread and sweets. Seeds are mostly roasted with spices. Left over leaves, stalks and husks are used as fodder (Arain, 2012).

Mungbean is basically an ancient crop of Asia. Seeds of mungbean are available in different sizes, shapes and colors. It is also known as green gram belonging to family Leguminosae. Like most other legumes, mungbeans are lacking in sulfur containing amino acids, whereas uncooked beans may contain trypsin inhibitors (Madar et al., 2002)

Mungbean is adapted to agro-climatic conditions of the sub-continent being suitable for its cultivation. Mungbean is mainly cultivated during dry season from October to April. Drought is mainly responsible for limiting the production of mungbean. During water stressed condition, decrease in the yield of mungbean is expected (Uddin *et al.*, 2013). Mungbean is one of the important Kharif crops of Pakistan *i.e.* widely grown in Southern Punjab and Sindh province. Punjab is a major province where mungbean is cultivated, covering 88% of the area with total production up to 85%. Major areas from mungbean cultivation are Layyah, Bhakar, Mianwali and Rawalpindi. Varieties of mungbean mainly grown in Pakistan include NM-2006; 20 maunds/acre (Arain, 2012). Area utilized for mungbean cultivation in Malakand Division of Pakistan *i.e.* about 1643 hectares, 1158 kg production and 700 kg/hectares yield. Though, the national average yield is up to 450 kg/hectares (Ghafoor and Arshad, 2011).
2.2.2. Nutritional aspect

Leguminous plant are a good source of protein, energy, vitamins, minerals and dietary fiber; mandatory components for human wellbeing (Jacobs and Gallaher, 2004). It plays an imperative role in the cropping system of the developing economies. It actively participates in achieving food and nutritional security for consumers as well as producers. In regions, where animal protein sources are lacking owing to religious norms or price constraints, legumes are considered essential to fulfill protein needs. Current investigations have brought the health benefits of legumes in the lime light and encouraged their use in diet to improve the vascular health and to overcome the high risk of diabetes (Flight and Clifton, 2006). Moreover, legumes help in achieving the goals like reduction in hunger & poverty, improving nutrition & health and enhancing ecosystem resilience (Akibode and Maredia, 2012). Numerous leguminous crops including chickpea, lentil, etc. comprises of approx. 17 to 30% of protein, carrying different types and proportions of essential amino acids thus considered as a good protein source at relatively low price (Boye et al., 2010b).

The health prespectives of cowpea are vital, being consumed by millions of low income people as their staple diet. Total protein in cowpea is 2 to 4 folds higher than cereal and tuber crops while fat contents are relatively low. Furthermore, it possesses considerable amounts of folic acid; preventive against spinal cord defects in fetus and B-complex vitamins; thiamin, riboflavin, niacin, vitamin B₆ & pantothenic acid. Alongside, it is also a chief source of vitamin A and C. Cowpea is rich source of potassium and also contains considerable proportions of magnesium, phosphorus & calcium. In addition, it possesses some traces of micro-minerals such as iron, zinc, selenium, copper, manganese and sodium (Hall et al., 2003; Asare et al., 2013). Additionally, it contains starch, dietary fiber and phytochemicals. Mallillin et al. (2008) reported the nutritional composition of cowpea as approx. 22.3% protein, 67% carbohydrates, 3.2% fat and 34% dietary fiber. The crude protein among different varieties and cultivars of cowpea varies from 18 to 35%. In a comparative study, Olalekan and Bosede (2010) assessed the iron content of cowpea, jack beans and pigeon pea comparable i.e. up to 0.8 mg/g. Another reseachere groups determined the digestibility of cowpea based diet as 87%. Further, they found positive nitrogen balance as 0.5 and net protein retention as 0.7 (Rangel et al., 2004). Thus, cowpea exhibits considerable quantity of dietary fiber, macronutrient and micronutrient. It is necessary for body growth and maintenance of pregnant women (Osunbitan et al., 2016).
Mungbean consists of 24% protein, 63% carbohydrate, 16% dietary fiber and 1% fat. Mungbean are easily digestible alongside contains higher protein content ranging from 22 to 24% (Madar et al., 2002). Germination of mungbean increase mineral content including potassium, calcium, phosphorous, magnesium, iron and manganese. However, it lowers the level of starch and reducing sugars by 8.78 and 36.1%, respectively. The mungbean protein is an important source of essential nutrients like total aromatic amino acids, valine, isoleucine and leucine but deficit in threonine, lysine, tryptophan and sulfur amino acids (Mubarak, 2005).

Cowpea is a nutritious plant that ensures high quality protein at relatively lesser price when compared with animal proteins and thus considered as a useful crop for developing countries (Hamid et al., 2015). Cowpea is low in fat, whereas regarded as a good source of protein, dietary fiber, micronutrients and phytochemicals. Cowpea is a part of diet of many population groups as in Africa. They are well-known, economical and readily accessible source of dietary protein. In the developing nations, weaning foods for children is being employed as a preventive measure against PEM (Mamiro et al., 2011).

2.2.3. Therapeutic potency

Whole and dehulled cowpea has been analyzed for their physicochemical and proximate properties. The result showed that dehulled cowpea had higher protein content. Cowpea possesses some anti-nutritional elements, putting it in the underutilized group of legumes. Research revealed that numerous processing procedures could be employed to decrease the negativities associated with anti-nutrients. However, these anti-nutrients possess certain physiological benefits. Earlier, the polyphenols were considered to be anti-nutritional components, on account of their interface with proteins digestion and now it is established that these moieties possess anti-tumor potency (Odedeji and Oyeleke, 2011).

Cowpea exhibits a range of benefits owing to its healthy nutritional profile. Cowpeas extracts have anticancer properties owing to the presence of polyphenols. Both whole seeds and its coat are rich source of free and bound phenolics. In a study, Guiterrez-Uribe et al. (2011) assessed the impact of phenolics in whole cowpeas to down-regulate mammary cancer cells. The phenolic extract of the whole seeds at a concentration of approximately 100 mg gallic acid equivalent (GAE) inhibited 65% proliferation of hormone-dependent mammary cancer cells. Extracts of the seed coats inhibited cell proliferation but to a significantly lesser extent hence indicated synergistic effects of phenolics & other
phytochemicals. Moreover, cowpea along with other ingredients can be used to fortify weaning foods. In this regard, different flour blends could be prepared using specific ratios of raw ingredients to develop weaning foods. The outcomes of nutritional researches have discovered that it is a beneficial ingredient for improving overall health (Bassey et al., 2013).

There are beneficial health effects and serve as an anti-diabetic agent, this is because mungbean is mainly considered for its low glycemic index owing to the presence of approx. 8% resistant starch along with high amylose content. In this connection, various researches were conducted to prove its efficacy. Cooked noodles prepared from mungbeans have also shown their lower starch digestibility and glycemic index. Feeding of mungbeans for 5 weeks decreased the fatty acid level and non-fasting plasma glucose. Mungbean starch demonstrated lower plasma triacylglycerol and adipocyte volume. Conclusively, use of starch from mungbeans may be useful for modulating both lipid and glucose metabolism. The mechanism behind low cholesterol level is related to mungbean starch that decreases fatty acid synthase activity or manages plasma leptin concentration hence preventing weight gain and fat deposition in the adipose tissues (Madar et al., 2002).

Earlier, a study was performed that demonstrated nutritional attributes of mungbean. Results indicated that mungbean is a fabulous source of protein and iron, varied from 14.6 to 33 g/100g and 5.9 to 7.6 mg/100g, respectively. V. radiata and V. mungo, varieties of mungbeans are widely utilized for remedial purposes owing to its antioxidant activity or therapeutic potential being appreciated since ancient times (Sharma and Mishra, 2009). Besides their well-known antihypertensive and antidiabetic properties, V. radiata is known for the treatment of various ailments including hepatitis, gastritis, etc. and later in 2009, it is considered as an anti-oncogenic agent. On the other hand, V. mungo is considered for its hypolipidemic perspectives. Recent reviews on mungbeans have revealed their beneficial nature attributed to the abundance of macronutrients; protein & sugar and micronutrient like minerals (Suneja et al., 2011).

Dietary fiber from mungbeans sprouts showed positive effect in lowering blood cholesterol levels. When mungbean is used as fiber enriched source, a substantial reduction was observed in plasma cholesterol level with an increase in total short chain fatty acids. This data suggests that mungbean sprouts contain dietary fiber. Mungbeans exhibit metal binding ability and free radical scavenging potential by which cells are protected from
redox-mediated oxidative damage. It has antioxidant activity \textit{i.e.} comparable with vitamin E (Madar \textit{et al.}, 2002).

Earlier researches have uncovered that these legumes are rich sources of protein hence not only improve nutrition but suppresses protein hunger, a standout point to deal with the menace amongst the low-income states. As animals are expensive source of protein, individuals are focusing towards plant protein. In this respect, cowpea and mungbean are considered as the best sources to substitute animal proteins, especially for mothers with low incomes. Cereals fortified with legumes have been used as weaning diet since long times for example, nut-ogi (corngruel-peanut), soya-ogi (corn gruel- soya bean), ogi- melon (corn gruel- melon seed) and cowpea-ogi (Butt and Batool, 2010).

\textbf{2.3. Anti-nutritional factors}

Legumes possess many beneficial nutritional components, mainly protein & mineral profile but bioavailability of minerals suppresses owing to the presence of anti-nutrients like phytic acid. Such anti-nutrients are produced during maturation of plant seeds (Loewus, 2002). Inositol phosphate contents in different legumes vary according to genotype, type of soil, climate and year. Two types of myo-inositol (IP\textsubscript{6} and IP\textsubscript{5}) are mainly reported anti-nutritional factors in legumes. Phytic acid binds with minerals (Fe, Zn, Ca & Cu), forming insoluble complexes that render them unavailable for digestion and absorption leading to poor bioavailability of minerals. It also make complex by binding with protein resultantly lowering its solubility within the body. Furthermore, phytic acid interacts with digestive enzymes mainly \textit{\alpha}-amylase, trypsin, pepsin, chymotrypsin and lipase hence reduce the nutrient digestibility. Conclusively, binding phytates to protein, starch or mineral moieties could modify digestibility, functionality and solubility of these nutrients (Konietzny and Greiner, 2003).

Two types of protease inhibitors namely Bowman-Birk and Kunitz are present in legume seeds. They could mainly down-regulate the activity of enzymes; trypsin or chymotrypsin resultantly affecting digestion. They are heat labile due to which their levels can be reduced before consumption by cooking (Greiner and Domoney, 2004). Trypsin inhibitor has the ability to destroy proteolytic activity of trypsin (Liener, 1994). Decrease in growth in response to trypsin inhibitors is mainly due to its inhibitory action on intestinal enzymes involved in protein digestion or existence of this inhibitor in a diet carrying free amino acids (Lajolo and Genovese, 2002).
The \( \alpha \)-galactosides, mainly oligosaccharides, related to another class of anti-nutritional components involved in flatulence. Mammals suffer from flatulence because there is no \( \alpha \)-galactosidase enzyme present in their intestine that is mainly involved in the breakdown of these compounds. If such legumes are consumed by human, they passed to large intestine where fermentation occurs due to intestinal bacteria, resulting in the formation of hydrogen, carbon dioxide and methane gas, leading to diarrheal and abdominal pain. Higher contents of these compounds could reduce the ability of small intestine to absorb such nutrients by changing the osmotic pressure of small intestine. However, their digestibility could be improved by exogenous \( \alpha \)-galactosidases (Rochfort and Panozzo, 2007). Sprouting of cowpea decreases starch oligosaccharide, resulting in loss after 48 hr due to its breakdown into simple sugars by galactosidase enzyme (Devi et al., 2015). Soaking removes stachyose and raffinose from 50 to 57\% at 60 °C by hydrolyzing into smaller units by the action of galactosidase or by leeching. The reduction to such an extent could appreciably control the discomfort related to flatulence (Kon, 1979).

Other common anti-nutrients in legumes are protein in nature such as enzyme inhibitors; \( \alpha \)-amylases and pancreatic proteases (Lajolo et al., 2004). The \( \alpha \)-amylase inhibitors are present in many legumes or beans and have more inhibition activity on human salivary amylase as compared to pancreatic amylase. High intake of \( \alpha \)-amylase could effect on metabolism and results in enlargement of pancreas and small intestine. Lectins are glycoproteins that form complexes with certain sugars and aglycoproteins on cellular layer of gastrointestinal tract and interfere with breakdown & absorption of nutrients. Different types of lectins have different interactions on agglutination, digestion, mitogenesis, blood group and toxicity. Lectins cause alteration to hormonal system as well as cause depletion of nutrients resulting in suppressed growth rate, increase in depression and higher incidences of disease & death. Higher intake of lectins from beans results in depletion of skeletal muscles of body, glucogen and lipid. Saponins form strong physical linkages with constituents or mucosal cell membranes of digestive system. These physiological interactions can affect the uptake of nutrients (Lajolo and Genovese, 2002).

Despite of the existence of anti-nutrients in leguminous plants, their level could be suppressed via different types of processings. Besides, processing also improves nutritional profile of legumes by increasing digestibility of carbohydrates and proteins (Naveeda and Jamuna, 2004). In this context, various types of processing techniques include hydration, boiling, germination and roasting (Shimelis and Rakhshit, 2007).
Some of the processing techniques that have been used to minimize the extent of anti-nutrients include roasting, autoclaving, heat processing such as cooking, fermentation, dehulling, germination and non-heating processing methods like imbibition (Rehman and Shah, 2005). Most of these anti-nutritional components are heat labile, the lectins and protease inhibitors could easily be removed during cooking thus associated adversities could be avoided on consumption (Rochfort and Panozzo, 2007). However, phytic acid, saponins and tannins are relatively heat stable but their level could be minimized by fermentation, germination, soaking and dehulling. In dehulling, seed coat is removed ultimately removing tannins i.e. existing in seed coat abundantly (Wood and Harden, 2006).

Previously, Preet and Punia (2000) focused that germination and dehulling impact negatively on anti-nutritional properties of cowpea. Resultantly, each process was found to lower the phytates as well as phenolics in cowpea. However, dehulling procedure was reported more active in eliminating polyphenolic substances, whereas soaking was found more effective in lowering the level of anti-nutrients; oligosaccharides, protease inhibitors and tannins because they are leached into the soaking medium (Saxena et al., 2003). The soaking medium and time matters a lot, on which extent of anti-nutrients leaching depends. The soaking medium employed could be bicarbonate solution, salt solution or water. For example, Wang et al. (2008) investigated the influence of steaming, soaking and boiling on the anti-nutrients present in cowpea. They noticed that steaming+soaking combined technique as more efficient in decreasing the action of trypsin inhibitors. Additionally, fermentation is another technique employed to reduce the anti-nutrients; tannins or phytates to significantly lower levels (Reyes et al., 2004). Combination of pressure and heat is required to eliminate allergic reactions of lupin; an allergenic proteins i.e. stable towards heat (Guillamon et al., 2008).

Moreover, it is stated that germination significantly influence on the compositional characteristics of cereal or legume flours and is more effective as compared to cooking in decreasing the level of raffinose, stachyose and phytic acid. Previously, Ghavidel and Prakash (2007) revealed the efficacy of dehulling and germination against anti-nutrient moieties of cowpea. They found dehulling more effective in contrast to germination in reducing phytic acids or tannins. Cooking methods such as microwave cooking, autoclaving and boiling are considered more effective than germination in reducing the level of saponins, tannins, hemagglutinin activity and trypsin inhibitors (Adawy, 2002). Later, Kalpanadevi and Mohan (2013) assessed the influence of hydration, germination,
autoclaving & cooking and combination of these techniques against anti-nutrients reduction in cowpea. They reported that autoclaving and cooking are more effective procedures in lowering phytic acids, phenolics, tannins, hydrogen cyanide and trypsin inhibitors relative to germination and hydration.

Autoclaving was found more efficient when compared with extrusion, microwaving and boiling in decreasing lupin allergenicity (Alvarez et al., 2005). Likewise, another study was carried out to assess the impact of various processing techniques including soaking, germination, autoclaving, boiling, toasting, roasting and microwaving on functional & nutritional attributes along with anti-nutritional constituents of mungbean seeds. Results indicated autoclaving and germination as better processes as compared to other methods in increasing in vitro protein digestibility. It was due to protein denaturation, destruction of trypsin inhibitors and reduction of phytic acid and tannin (Mubarak, 2005).

In general, roasting is very useful to reduce anti-nutrients, present in cereal and leguminous crops. Roasting improves color, enhances flavor, increases shelf stability and lowers anti-nutritional factors. Additionally, roasting of grains may leads to denaturation of proteins, improving their digestibility.

2.4. Legume composite flours
Composite flour blends are the mixtures of numerous flours particularly from cereals and legumes. In this context, wheat flour is the most commonly used cereal in weaning food formulations to achieve specific functional and nutritional characteristics. In an attempt to help the third world countries to decrease their import, FAO in 1957 started a study on the technological possibility of the utilization of composite flours in different products.

The utilization of composite flours from cereals, legumes and tubers is therefore expected to enhance the consumption of native crops ultimately responsible to enhance the nutritive quality of the diet. Composite flours are therefore beneficial in the sense because intrinsic deficiencies of essential amino acids in wheat flour (lysine, tryptophan and threonine) could be supplemented from other sources like legumes. Commonly, composite flours are employed in bakery products to develop nutritionally rich end products to improve quality of life. In order to formulate composite flour, it is important to know that up to what level of cowpea supplementation to wheat flour influence the desired quality of the product. Previously, a study was carried to study the impact of cowpea or/and cassava flour substitution to wheat flour, considering chemical, physical, nutritional and sensory qualities
of the product. Cassava and cowpea were incorporated into flours and used to replace wheat flour in different ratios alongside 100% wheat flour was run as control. The addition of cowpea flour is reported to increase protein quantity (Adekunlea and Marya, 2014).

In a study conducted by Ahmed and Campbell (2012), the supplementation of cowpea @ 5, 15, & 20% in wheat flour showed significant variations with respect to moisture, protein and fiber as compared to control. In this study, the protein level in wheat+cowpea flour blends was found to be varying from 6.1 to 9.9%. Increment in the levels of cowpea flour resulted in improvement in water absorption of composite flours. In another study, wheat flour was supplemented with mungbean in different proportions i.e. 5, 10, 15 and 20 that influenced the macro-components largely by increasing the level of supplementation (Riaz et al., 2007). Likewise, another research found that cowpeas or plantain flours at different proportions increased the protein content depending on the amount of cowpea or plantain flour added (Onwulata et al., 2015). Similarly, nutritional profile of composite flour prepared by wheat and cowpea blends was studied for their proximate composition as well as functional properties. Crude protein was found to be significantly high by increasing the level of cowpea flour (Olapade and Oluwole, 2013; Butt et al., 2011).

Infants are the most vulnerable segment of the population facing malnutrition thus improvement in their diet is necessitated. Weaning/complementary food is the first semi-solid food introduces to the infants, after or in-conjunction with breast feeding hence, it is essential to improve its quality. The composite flour blends are prepared for complementary foods to improve the energy and protein value. Earlier, Imtiaz et al. (2011) evaluated the weaning foods prepared from composite flours, including wheat and mungbean in different proportions. Five treatments were prepared; F1: 68% wheat flour+12% mungbean, F2: 56% wheat flour+24% mungbean flour, F3: 44% wheat flour+36% mungbean flour, F4: 32% wheat flour+48% mungbean flour and F5: 20% wheat flour+80% mungbean flour. They found

Table 2.1. Legume based weaning foods

<table>
<thead>
<tr>
<th>Legumes</th>
<th>Cereal-legume blends</th>
<th>References</th>
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<tbody>
<tr>
<td>Cheap source of high quality protein e.g. cowpea, whereas cereals possess poor amino acid profile</td>
<td>Blend is complementary</td>
<td>(Kataria et al., 1989; Ijarotimi, 2008; Butt and Batool, 2010; Hamid et al., 2015)</td>
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</tbody>
</table>
Rich source of minerals like iron *e.g.* mungbean and cowpea

Combat hidden hunger

(Onweluzo and Nwabugwu 2009; Olalekan and Bosede, 2010; Intiaz *et al.*, 2011)

Good functional attributes

Enhance antioxidant status of the body

(Madar *et al.*, 2002; Nasirpour *et al.*, 2006; Sharma and Mishra, 2009)

Rich in lysine and deficient in S-containing amino acids, whereas cereals deficient in lysine and rich in S-containing amino acids

Balance nutritional value of cereal-legume based weaning foods

(Kataria *et al.*, 1989; Amuna *et al.*, 2000; Badifu and Aka, 2001; Madar *et al.*, 2002; Egli *et al.*, 2003; Mubarak, 2005; Ghasemzadeh and Ghavidel, 2011; Olapade and Oluwole, 2013)

Processing procedures like roasting, boiling, autoclaving, microwaving, soaking and sprouting improve nutritional value of legumes

(i) Suppress anti-nutritional factors; phytates, saponins, tannins, amylase inhibitors and oligosaccharides

(ii) Improve bioavailability, palatability and digestibility of essential nutrients


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that F₃ and F₂ having 24 and 36% mungbean flour, respectively showed optimal compositional, functional and nutritional properties.

Millet possesses high protein value while cowpea contributes essential amino acid thus weaning foods prepared by the composite flour blends showed high nutritive value (Almeida-Dominguez *et al.*, 1993). In a composite, 47% cowpea was added and evaluated for protein quality. The results indicated that weaning food prepared by adding cowpea showed better nutritive quality as it contained higher protein content *i.e.* approximately 16.8% (Bassey *et al.*, 2013). In a similar way, nutritional quality, based on protein digestibility, protein digestibility corrected amino acid score (PDCAAS), amino acid profiling or indexes and biological values, of germinated cowpea flour based weaning/complementary foods was evaluated. The germinated cowpea showed higher protein digestibility and PDCAAS while amino acid profiling did not show any significant difference. It also exhibited better sensory acceptability scores (Jirapa *et al.*, 2001). A composite weaning meal was made by using pearl millet and roasted cowpea. The objective
was to prepare a high protein diet, meeting the nutritional requirements of infants. The amino acid profile of the flour blends showed that lysine, methionine and cysteine contents were meeting the criteria of FAO as referred for infants. Thus, cowpea supplementation helps to improve amino acid value of weaning foods (Modu et al., 2010). However, the supplementation of mungbean has been also carried out in yogurt based weaning foods. The weaning food prepared with the addition of 17% mungbean showed the better results. Fat contents of the prepared weaning food were significantly higher while fiber content was within the acceptable range. The calories provided by this weaning food were significantly higher than the commercially available weaning food (Munasinghe et al., 2013).

2.5. Functional properties of legumes

Legumes are the major contributors of macronutrients particularly proteins. Functional and nutritive characteristics of legumes are the major determinants of protein quality. Functional properties effect on protein quality of legumes during processing, manufacturing, storage and consumption. Protein functionality depends on properties such as solubility, viscosity, gelatinization, foaming and oil/water absorption capacities (Ahmed et al., 2016). Physicochemical changes especially, proteins denaturation affects the subsequent product characteristics prepared from legumes. Protein influences on foaming, emulsification and oil & water absorption capacities while swelling and viscosity are related to starch granules (Abu et al., 2005). In addition, proteins perform many other subtle functions in foods. There is a need to manipulate these functional characteristics to facilitate modern food processing and product quality. Conclusively, legumes+cereal blends offer favorable physichochemical properties (Boye et al., 2010a).

Foaming is mainly based on protein content i.e. formation of interfacial protein film that entraps air bubbles in suspension hence slows down aggregation rate (Sreerama et al., 2012). Hence, foaming property of legumes is described by its whipping ability. Foaming ability is responsible to increase volume in 30 sec i.e. said as foaming ability (Lawhon et al., 1972). The foaming property of cowpea depends on the protein content and rheological properties. Cowpea contains about 24.0% protein of which globulin constitutes 66.0% while albumin is comprised of 24.9%. Similarly, glutelins in cowpea comprises of 4.70% of the protein. The main proteins responsible for the foaming property of cowpea are albumin and globulins. The foaming ability of the cowpea is affected by the denaturation
of the albumin, resulting from the high temperature during the processing. Poor storage also harms the foaming ability of the cowpea (Reddy et al., 1984; Chan and Phillips, 1994). Furthermore, pH is another factor involved in the foaming process. In this regard, foaming ability & stability of the cowpea flour is pH dependent. It has been observed that the foaming ability of cowpea is higher at alkaline pH as compared to the acidic (pH 4.0). The alkaline pH is responsible for the formation of stable layer between water and air interface ultimately achieve stability, elasticity and more intact textural characteristics. The change in pH decreases the net charges of the protein at the isoelectric point thus reduces the electrostatic repulsion (Kudre et al., 2013; Liao and Mangino, 1987).

Foam formation involves continuous dispersion of gas in liquid and entrapment of gas in thin liquid films. Gas trapped in liquid creates bubbles that increases viscosity and provides stability during displacement process (Osei-Bonsu et al., 2015). Draining and film rupture cause foams to collapse. In order to prevent collapsing, surfactants are required to oppose the draining. Therefore, the gas-liquid mixtures will foam only in the presence of surfactants to stabilize erupting bubble (Joseph, 1997). Certain processing treatments could also affect the foaming properties. For example, addition of 0.2 M NaCl in cowpea improves the foaming capacity and stability of the processed flours as well as raw cowpea (Giami, 1993). The findings of another study showed that foaming ability are related positively with source, amount & solubility of protein (Appiah et al., 2011).

Emulsion activity index is used to measure the emulsifying ability i.e. enhanced by increasing the protein content and concentration of oil. The stability of emulsion depends on type & concentration of protein, variety of legume as well as on processing method (Ihediohanma et al., 2014). Thus, proteins are ideal materials for improving emulsion stability due to their amphiphilic nature. Previously, Abu et al. (2006) reported positive relationship between oil absorption and emulsifying properties. The formation and stability of emulsion are critical factors involved in the preparation of cake, coffee whiteners and frozen desserts (Adobewale et al., 2005).

Emulsion capacity of red cowpea is approx. 35.04%, whereas emulsion stability of red cowpea ~ 96.04%. On the other hand, the emulsion capacity & stability of black cowpea were measured around 34.06 & 73.81%, respectively. Denkov et al. (2006) reported that heating and aging effect on the emulsion stability. In this study, cowpea was used in the different forms; cowpea slurry and cowpea flour. Furthermore, emulsion ability of various
products of cowpea differed with respect to heating and aging process. In this context, emulsion forming ability of cowpea flour was reported as 9.73% more than that of cowpea slurry 3.87%. The outcomes of the study showed that less protein content contributes to less emulsion capacity and vice versa (Singh et al., 2003). Likewise, water imbibition, oil holding, emulsifying and foaming capacities of 8S globulin fractions from 15 mungbean cultivars were studied. There was found a negative association between water and oil holding capacities. Anyhow, emulsion stability of 8S globulin fraction of mungbean was found to be better in contrast to soya bean 7S protein. The foaming ability is a direct indicator of foaming stability (Liu et al., 2015).

Water holding capacity is a term, defining water carrying capacity of protein, present in food materials (Kohn et al., 2016). From economic point of view, this characteristic is considered as it ultimately impact on yield or quality of food products (Kohn, 2014). During food preparation, WAC of legumes plays a major role as it also affects the other sensory and functional properties of the products. Therefore, flours prepared from different legumes possess various applications as food ingredients on the basis of their interaction with water. In a scrutiny, Sreerama et al. (2012) worked on cowpea, horse gram and chickpea and reported significant impact of these three legumes on WAC under ambient conditions. Results for water absorption capacities for all the three legumes were in the ranges of 124.60-148.10 g/100 g. Similarly, Phaseolus legume flour exhibited higher WAC and oil absorption capacity as compared to other flours prepared from red kidney bean, naval force bean, lima bean, pinto bean, dark bean, mungbean, chickpea, little red bean, lentils and bruised eye bean (Du et al., 2014). Swelling is another way for measuring the WAC of proteins present in foods like custards and dough. The imbibition of water in proteins is attributed to inadequate amount of water but do not dissolve thus cause thickening and increasing viscosity. Similarly, by adding 0.4% NaCl in cowpea flour significantly improved the emulsification capacity of raw flour. Likewise, swelling properties of mungbeans were investigated by Andrabi et al. (2015), they declared that solubility index and swelling property are directly related to temperature as increment in these attributes were noted at 50-90 °C. Conclusively, functional properties of protein predict physicochemical attributes of foods.

Variation in physicochemical properties has been reported in many research studies using different processing treatments that could affect the overall product quality. In this regard, germination, fermentation and heat treatments could alter the nutrient composition and
functional attributes of cowpea flour. Significant results for WAC were observed after heat treated cowpea flour. Likewise, germinated cowpeas revealed maximum protein solubility of about 0.39 mg/mL. Additionally, water & oil holding abilities, viscosity, foaming & emulsifying characteristics and solubility of protein in raw and heat processed cowpea flour were evaluated by Abbey and Ibeh (1998). Water & oil holding potential (3.60 and 3.20 g/g) of heat processed flour were significantly improved as compared to raw version (2.40 and 2.90 g/g). Likewise, gelation ability of raw flour was found to be increased from 16 to 18% after heat processing.

Oil holding is affected by several factors such as surface area, protein content, charge, hydrophobicity and liquidity of oil and type of method used for protein isolation (El-Adawy, 2000). Moreover, temperature also increases the fat absorption capacity by increasing the rate of protein dissociation into subunits that in turn enhances the availability of binding sites (Ghavidel and Parakash, 2006). Later, David et al. (2015) compared the functional properties of wheat flour to that of cowpea. Results for oil absorption capacity declared that values were not significantly different for both the flours. Similarly in another study, oil retention limit of cowpea seeds were found to be significantly high as compared to rice (Iwe, 2016).

Literature reviewed suggests that processing of legume flours can significantly affect the oil absorption capacity. Likewise in a study, fat absorption capacity of raw, heat treated, germinated and fermented cowpeas were investigated. Results depicted that oil absorption capacity of raw cowpea was found to be 2.8 g/g which was lesser fat absorption capacity of germinated flour 4.1 g/g, fermented flour 3.6 g/g, thermally processed flour 3.1 g/g (Sunday, 1993). In the same way, the effects of irradiation at different doses of γ-radiation such as low, medium and high were applied to analyze the functional attributes of cowpea in the form of flours and pastes. Purposely, cowpea pastes and flours were irradiated at 2, 10 and 50 kGy. Significant results were found for oil absorption capacity. Moreover, as a result of starch degradation, significant reductions were noted linearly in starch associated functional attributes for example pasting and swelling (Abu et al., 2005).
2.6. Weaning foods

An age-appropriate diet is the one that provides adequate nutrition according to specific age group. Meaningfully, weaning foods are introduced to accustom a baby to foods other than mother milk. Although, mother milk is known to be the best and complete food after birth till 6 months. It comprises of essential nutrients and immune boosting ingredients, regarded necessary for balanced health and proper growth (Temple et al., 1996). According to WHO guidelines, children should be supplied with mother milk till 2 years of age. So, weaning period starts when mother milk merely cannot meet infant’s nutritional requirements at the age of six months. Therefore, in this whole period their nutritional needs are complemented with weaning foods up to two years of age. Furthermore, infancy is a duration when physical, immunological, physiological and mental growth requires optimal nutrition at a rapid rate (Daelmans and Saadeh, 2003). So, commencement of complementary solid foods is a diversion from milk to family foods, where variety of foods, texture and flavor could easily fulfill nutritional needs and health benefits.

Timing and the types of weaning foods introduced to an infant are known to impact their growth and development during whole period of infancy. In Pakistan, weaning foods are commonly prepared from locally available resources that are mostly consumed as a part of adult’s diets. Commonly employed complementary foods consumed by Pakistani infants include homemade cereals like khitchri (a mixture of rice, lentils, and oil), dalia, suji, roti, choori, sagodana and kheer as well as biscuits, tea, rusks, bread, eggs, meat, yoghurt, potato and bananas that are convenient and easily available (Bhutta, 2000). Moreover, feeding with commercially available infant foods such as cerelac is also widely practiced in many families (Lingam et al., 2014). They all are good sources of energy but might be unable to satisfy the optimal protein requirements of the growing infants (Bhutta, 2000).

According to WHO (2007), the mean protein intake was estimated up to 2.04 g/kg/day for the first 3 months, 1.38 g/kg/day (3-5.9 months), 1.21 g/kg/day (6-11.9 months), 0.97 g/kg/day (1 year) and 0.84 g/kg/day (5 year). Furthermore, the protein RDA for infants is documented as 14 g during a period of 0 to 1 year (Modu et al., 2010). Codex Alimentarius Commission in 1981 established the global standards for required essential nutrients included in various infant formulas i.e. revised over the years. The lists of food additives that are permitted for infant formulations also cover this standard. As far as the amount of protein in processed cereal-based foods is concerned, there is currently no consensus
regarding what should be the appropriate amount of protein in infant formulas. Though, the minimum protein-to-energy ratio for infant formulas prepared using milk and cereals & water should not exceed 5.5 and 2 g/100kcal, correspondingly (Ryan and Hay, 2016).

Traditionally, weaning foods in many countries are prepared from flours of starchy staples that form thick pastes on heating due to starch gelatinization. Addition of sugar to weaning gruels is by far the most common practice to improve flavor and to encourage infants to eat. Amongst non-wheat cereals, rice is the commonest introductory food to infants. Different foods in puree form like vegetables, fruit and potatoes are also suitable as first weaning foods. Gluten free cereals should be introduced during earlier days with slow progression towards gluten containing cereals. Banana is also considered as the most popular food item i.e. soft in texture and ease in availability throughout the year (Chaudhry and Humayun, 2007).

During early childhood, consumption of balanced and nutritious weaning foods is responsible for healthy eating behaviors. Late weaning can increase the risk of various ailments as infants will become nutritionally compromised hence prone to infections. Moreover, children will not be able to learn chewing & swallowing and more likely refuse new foods. So, late weaning results in delayed growth due to inadequate intake of energy, protein, vitamins and minerals; Fe & Zn (Shamim et al., 2006). Similarly during infancy, babies develop different tastes and establish food preferences. Therefore, it is important to introduce foods of varied taste, texture and smell so that the infant learns to like and accept a wide variety of foods. Accordingly, weaning foods should be designed, considering nutritional requirement of infants as the foremost criteria. Besides, such foods should ensure the weight gain from 3 to 10 kg with the completion of first year of life (Sarwar, 2002).

The selection of weaning food is very important as poor intake of complementary foods leads to macro- and micro-nutrient deficiencies. Mostly mothers are well-aware of the additional caloric requirement for their babies after 6 months with few exemptions. Besides, in many urban areas children are mainly fed on commercial items such as milk-based ready-to-use cereals instead of any special home-prepared weaning foods. Over diluted preparations are often used probably because of high price of commercially available weaning foods. Therefore, programs related to education of mothers regarding weaning practices should be launched for proper infant growth. It has been reported through
various epidemiological studies that diarrhea and PEM are most prevalent during this crucial weaning phase of life. Infants particularly suffer from diarrheal diseases during the onset of weaning. According to estimates, exclusive dependence on mother milk for initial 6 months followed by addition of suitable complementary or weaning food during 9-12 months could save the lives of 1.3 million infants each year (Temple et al., 1996). Moreover, breast fed infants depicted 14 times less chance to die from diarrheal diseases due to presence of anti-infective and immunological factors in mother milk as compared to non-breast fed infants. Hence, during this period, children under-five have an increased risk of malnourishment (Daelmans and Saadeh, 2003). Furthermore, PEM in children is common among families facing socioeconomic constraints and hence poor food intake considerably affects the infant’s nutrition. Intake of optimal protein to energy ratio is of important consideration as it influences growth and maintenance. If the energy intake from other sources is inadequate, then body protein is utilized as an energy source besides fulfillment of basic function of growth and development. This leads to poor body resistance to diseases & infections and low quality of life, making infants vulnerable to malnutrition if problem is not tackled properly (Grover and Ee, 2009).

Infants requires nutritionally balanced, high protein and calorie dense complementary foods rather than sole reliance on mother’s milk because of growing nutritional body needs (Umeta et al., 2003). Consequently, provision of complementary foods that are comparatively energy and nutrient dense should be incorporated into a child menu right
Table 2.2. Guidelines for complementary feeding of infants

<table>
<thead>
<tr>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>First six months; exclusively on human milk</td>
<td>(Temple et al., 1996; Daelmans and Saadeh, 2003; Gibbs et al., 2011)</td>
</tr>
<tr>
<td>First two years; gradually introduce semi-solid to solid (complementary) foods in addition to breastfeeding</td>
<td>Jirapa et al., 2001; Daelmans and Saadeh, 2003; Bond et al., 2005; Mennella et al., 2006; Chang et al., 2008; Dewey and Adu-Afarwuah, 2008; Melo et al., 2008; Sai-Ut et al., 2009; Olivia and Ardythe, 2013</td>
</tr>
<tr>
<td>Initially, the complementary foods should be pasty. Later, getting thicker until child start consuming family meal</td>
<td>(Monte and Giugliani, 2004)</td>
</tr>
<tr>
<td>Complementary foods should fulfill caloric demand and based on child liking especially when sick to stimulate appetite</td>
<td>(Asma et al., 2006; Modu et al., 2010)</td>
</tr>
<tr>
<td>Complementary foods should be varied colored predominately derived from fruits, vegetable and legume sources</td>
<td>(Butt and Batool, 2010; Bassey et al., 2013)</td>
</tr>
<tr>
<td>Hygienic practices should be considered while feeding the child like washing of hands and feeding via clean spoon</td>
<td>(Monte and Giugliani, 2004)</td>
</tr>
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</table>

after 6 months till he learn to eat foods from all groups; meat & milk, fruits & vegetables, cereals, etc. (Gibbs et al., 2011).

Higher prices of animal proteins, poor availability of cost-effective healthy weaning foods, poor nursing practices and late inclusion of complementary foods normally result in malnutrition in Asia. It is therefore imperative to encourage complementary feeding by using locally available, inexpensive and nutritious complementary foods. So far, malnutrition and poor weaning habits are on upper hand among the developing economies. This in turn contributes to infant mortality, weaker immunity and poor intellectual & physical development. The complementary foods mostly are of poor nutritional quality as prevailing weaning practices are lacking in quality, contributing malnutrition (Anigo et al., 2009).

Development of cost-effective, protein rich weaning/complementary foods for infants from locally available sources could bring benefit to the developing economies. Thus, there is a dire need to develop infant formulations with an optimal nutrient profile by using high quality legume/cereal mixtures. Weaning foods especially cereal-based gruels are generally
low in protein or essential amino acids; tryptophan, lysine, etc. but high in sulfur amino acids (SAAs). On the other hand, legumes are rich in lysine content, whereas insufficient in SAAs (Amuna et al., 2000). Thus, legume fortification increases protein content of cereal-legume composite but also their protein quality. Since legumes are comparatively cheaper source of protein and iron therefore can be a good alternative for high quality proteins in the formulation of complementary foods for children in middle and low income families (Onweluzo and Nwabugwu, 2009).

In the developing states, increased interest in consuming flours from different types of legumes is on the rise. Legumes are utilized to enhance nutritional value and amino acid profile of weaning food formulations (Zanna and Milala, 2004). However, cereals are used as basic component in the preparation of weaning foods (Egli et al., 2003). Increasingly, incorporation of legumes in weaning food formulation caused significant improvement in nutritional and functional value (Nasirpour et al., 2006).

Nutritional composition and formulation of the complementary foods have attained more attention by the nutritionists and processors from the last many years than any other food product. As a result, various recipes for weaning foods are available in the industry. Literature suggests that a single cereal or legume could not provide adequate amount of all nutrients to meet the nutritional requirement of a child. Therefore, combination of cereals, with an appropriate proportion of one or more legumes should be suggested (Hofvander and Underwood, 1987). For example, five plant based locally prepared weaning foods were found to have obvious difference in their nutritional value (Fernandez et al., 2002). Likewise, in animal trail aimed to evaluate the protein present in two commercial and homemade weaning foods. Findings suggested that homemade food prepared in a ratio of 50% pasta based on wheat flour protein and 50% soybean protein-granulate showed high protein quality more or less similar to the commercial weaning food (Asemi et al., 2009). Thus, it can probably helpful to support the growth of infants and combating alarming health conditions during infancy especially amongst the developing world.

In another study, a cereal based weaning food supplemented with common legumes such as peanut and cowpea in addition to banana was analyzed for nutritional evaluation. For the developing states, banana is the fourth major staple food throughout the tropics. A
Table 2.3. Weaning food ingredients

<table>
<thead>
<tr>
<th>Ingredients for weaning foods</th>
<th>Benefits in weaning food formulations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>All legumes and pulses such as cowpea, pigeon pea, mungbean, kidney beans and groundnut</td>
<td>Mitigate protein energy malnutrition (PEM)</td>
<td>(Al-Kaisey et al., 2000)</td>
</tr>
<tr>
<td>Yoghurt supplemented with mungbean, soya bean and brown rice</td>
<td>Combat PEM</td>
<td>(Munasinghe et al., 2013)</td>
</tr>
<tr>
<td>Composite of 40% ripe banana, 47% cowpea and 13% peanut</td>
<td>Substitute for mother milk</td>
<td>(Bassey et al., 2013)</td>
</tr>
<tr>
<td>Oats with banana and rice with banana</td>
<td>High in vitro digestibility</td>
<td>(Bassey et al., 2013)</td>
</tr>
<tr>
<td>Cereal-legume blends; nut-ogi (corn gruel-peanut), soya-ogi (corn gruel-soya bean), ogi-melon (corn gruel-melon seed) and cowpea-ogi</td>
<td>Balanced nutritive value</td>
<td>(Butt and Batool, 2010)</td>
</tr>
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</table>

A composite of ripe banana (40%), cowpea (47%) and peanut (13%) was analyzed for nutrient composition after processing. This blend was compared to two popular commercial weaning foods such as oats with banana and rice with banana. *In vitro* digestibility was highest for oats+banana than other two treatments. Conclusive results for feasibility of using precooked composite from locally available crops suggested that it is an efficient substitute for mother milk (Bassey et al., 2013). Along with cereal based weaning foods, yoghurt based complementary food also possesses a strong potential for eliminating PEM by providing good quality proteins. In a research trial carried out on three yoghurt-based weaning blends supplemented with mungbean, soya bean and brown rice, showed significant results for protein quality (Munasinghe et al., 2013).

Numerous locally available legumes when mixed with normally accessible carbohydrate sources, results in comparatively inexpensive weaning foods that might reduce the PEM and improve nutritional status of infants. All legumes and pulses such as cowpea, pigeon pea, mungbean, kidney beans, and groundnut have proven suitable for supplementing weaning foods. Amongst legumes, lentil is commonly used to supplement cereal based diets to prepare protein enriched complementary foods (Al-Kaisey et al., 2000). Cowpea is a key leguminous crop grown in tropics. It is a significant source of dietary protein.
predominantly lysine i.e. considered beneficial against chronic protein deficiency (Pereira et al., 2009). Likewise, mungbean are easily digestible and are high in protein content. Germinated mungbean seeds have high levels of potassium, magnesium, calcium, phosphorous, iron and manganese (Madar et al., 2002). Several applications of cowpea and mungbean have demonstrated its potential usage in the preparation of weaning foods.

2.7. Bioevaluation of legumes

Food processing affects on protein quality, if protein efficiency ratio (PER) reaches >2 represents excellent quality (Lalles and Jansman, 1998; Ulloa et al., 2006). For analyzing protein quality, bioefficacy based on Protein Efficiency Ratio; PER, Net Protein Ratio; NPR and Relative Net Protein Ratio; RNPR was assessed, whereas nitrogen balance encompasses Net Protein Utilization; NPU, Biological Value; BV and True Digestibility; TD (Seena et al., 2005). Amount of protein in the diet influences feed consumption of experimental animals as it varies with protein level thus determines food quantity requirement to achieve normal physiologic and metabolic processes (Sakanata et al., 2000). The availability of protein is also determined by measuring the nitrogen balance and growth rates (Ekanayake et al., 2000). The protein deficiencies are directly related with the quality of the protein being consumed that affects the growth rate of infants. Different weaning food formulations have been assessed via in vitro analysis to understand the role of major nutritional components for normal metabolic functions and growth rate (Gropper et al., 2005).

Though legumes provide high protein quality, still numerous developing countries are facing protein malnutrition. Legumes could not balance protein requirements due to some limiting sulfurous amino acids. Previous Studies have shown that nutrition achieved via leguminous crops relies on processing procedures or on the existence ant-nutrients or food anti-nutrient interactions; phytates, tannins, oligosaccharides, saponins and amylase inhibitors.

Tannins interfere with protein digestibility by binding to enzymes via lysine or methionine linkages. Thus, it reduces the absorption of vitamin B12 as well. Likewise, saponin, phytic acid and polyphenols were found significantly lower after applying various cooking methods. In Indo-Pak subcontinent, mungbean has been grown since ancient times where people mostly consume cereal based diet for high nutritional value.
Table 2.4. Effect of cooking procedures on legumes

<table>
<thead>
<tr>
<th>Cooking procedures</th>
<th>Impact on nutritional value</th>
<th>References</th>
</tr>
</thead>
</table>
| Roasting           | (i) Enhances protein bioavailability and digestibility  
(ii) Improves color and flavor  
(iii) Extends shelf life  
(iv) Reduces anti-nutrients       | (Dos Anjos et al., 2016)               |
| Boiling            | (i) Improves protein quality by inactivating thermally labile anti-nutrients                  | (Dos Anjos et al., 2016)               |
| Combinations of processing treatments | Enhances efficiency                                                                                      | (Dos Anjos et al., 2016)               |

Examples

<table>
<thead>
<tr>
<th>Thermal effect on red kidney beans</th>
<th>Significant availability of S-containing amino acids</th>
<th>(Wu et al., 1996)</th>
</tr>
</thead>
</table>
| Roasting of chickpea, pigeon seeds, mungbean and soya bean | (i) Reduces phytate content  
(ii) Slight increase in total dietary fiber  
(iii) Slightly increases in in vitro protein digestibility | (Chitra et al., 1996) |
| Autoclaving of velvet bean         | Significant improvement in utilizable proteins, PER, TD, BV, and NPU | (Vadivel and Pugalenthi, 2007) |
| Cooked beans                      | Increases TD, BV and NPU values                      | (Bhagya et al., 2009) |

and protein content. It is often known as a “green pearl” being rich in protein, starch, vitamin B complex and minerals. Increase in protein digestibility of mungbeans results in reduced flatulence (Kataria et al., 1989).

Processing/cooking enhances protein digestibility on one hand, whereas reduces deleterious anti-nutrients on the other hand. Different processing treatments can potentially enhance the protein bioavailability such as roasting and boiling. Roasting of legumes and grains promotes denaturation of proteins thereby improving their digestibility. Roasting has the ability to improve color, enhance flavor and extend the shelf life of legumes and reduce anti-nutrient factors. Boiling can also improve the protein value, whereas destroys the heat sensitive anti-nutrients. The application of a single technique is generally insufficient for effective results. Consequently, combinations of processing treatments are commonly employed to obtain significant results (Dos Anjos et al., 2016).
Similarly, in a research study that highlighted the nutritional as well as anti-nutritional attributes of mungbean (*Vigna radiate* L.) by using different traditional processing methods. The conventional processes such as boiling, autoclaving, roasting, soaking, sprouting and microwaving were employed. Results showed that these anti-nutritional constituents were efficiently reduced resulting in increased palatability, bioavailability and consumers acceptance. It is therefore recommended to use roasting, microwaving, sprouting and autoclaving for mungbean to attain optimal nutrition alon with short processing time at home scale as well as in food establishments (Kavitha *et al.*, 2015).

Similarly, germination can also affect nutrient profile and secondary metabolites of mungbeans. Wongsiri *et al.* (2014) found that germination of mungbean for 24 hrs resulted in increased protein quantity however, non-significant difference was observed via 18 hrs germination period. Thus, germination of legumes can potentially enhance the protein bioavailability as well as lowering the anti-nutritional content. Positive correlation between germination time and crude protein showed that it is efficient in elevating the certain amino acid levels such as phenylalanine. In another trial carried out on young & older rats by Gilani and Sepehr (2003) using PDCASS method, it was studied that anti-nutrients impart negative response on amino acid & protein digestibilities. Moreover, protein products (n=15) were fed as main protein source (10%). In older rats, the protein digestibility was significantly less in contrast to young animals for most of the products. Anyhow, domestic processing of mungbeans also revealed marked effect on protein digestibility. Moreover, positive relationship between cooking time and protein digestibility has been observed (Kataria *et al.*, 1989).

In the same manner, influence of thermal processing was evaluated on several varieties of cowpeas. Heat treatment generally had no significant effect on amino acid concentration except for lysine. Additionally, all heat treatments reduced the concentration of trypsin inhibitors to below 2000 TIU/g. Amino acid digestibility for the nhemba cowpeas was lower than for the black-eyed cowpeas except for tryptophan. Roasting had moderate effect on amino acid digestibility in black-eyed beans and increased digestibility of several amino acids in nhemba cowpeas and the average increase was found to be 3.4 percentage units. Similarly, the impact of autoclaving, boiling and roasting procedures on anti-nutritional components of cowpeas was evaluated by Udensi *et al.* (2007). Significant reduction in trypsin inhibitors was observed after 60 min of autoclaving. Findings from this study also revealed lower levels of tannins in cowpeas after using
Table 2.5. Impact of processing procedures on cowpea seeds

<table>
<thead>
<tr>
<th>Processing procedures</th>
<th>Impact on nutritional value</th>
<th>References</th>
</tr>
</thead>
</table>
| Heat processing on different varieties of cowpeas | (i) No significant effect on amino acid concentration except for lysine  
(ii) Reduces concentration of trypsin inhibitors to below 2000 TIU/g  
(iii) Amino acid digestibility for nhemba cowpeas lower than that of black-eyed cowpeas except for tryptophan  
(iv) Moderate effect of roasting on amino acid digestibility in black-eyed beans in contrast to nhemba cowpeas | (Udensi et al., 2007)                    |
| Autoclaving (60 min) of cowpeas        | (i) Significant reduction in trypsin inhibitors  
(ii) Enhances in vitro protein digestibility | (Vijayakumari et al., 1998; Mensa-Wilmot et al., 2001) |
| Roasting (120 min) or boiling (60 min) of cowpeas | Reduces tannins  | (Mensa-Wilmot et al., 2001) |
| Thermally treated cowpea varieties    | Alternative to low cost protein source for food formulas | (Avanza et al., 2013)                    |

roasting and boiling at 120 and 60 min of respective treatments. The protein value of cereal-legume mixtures formulated with optimum nutrients was examined to determine the PER, NPR and Protein Digestibility Corrected Amino Acid Score (PDCAAS). Two preparations were made including 45-50% maize, 35-40% cowpeas and either peanut or soybeans to enhance the amino acid balance. With an adjusted level of casein at 2.5, acceptable PERs were found to be 2.1-2.4. The protein digestibility thus obtained was 87.4-92.1 and NPR ranged from 3.0-3.3. PDCAAS for the formulations was in a range of 0.72-0.82 (Mensa-Wilmot et al., 2001). In the same manner, protein digestibility of V. Sinensis and V. Aconitifolia could be enhanced by autoclaving up to 14.8 and 12.5%, respectively (Vijayakumari et al., 1998). Moreover, availability of individual amino acids from red kidney beans was assessed by using heat treatment. The thermal effect on beans revealed
significant availability of S-containing amino acids. Availability from thermally processed beans was found to be varying from 39.8 to 68.0% as compared to raw beans.

Table 2.6. Impact of processing procedures on mungbean seeds

<table>
<thead>
<tr>
<th>Processing procedures</th>
<th>Impact on nutritional value</th>
<th>References</th>
</tr>
</thead>
</table>
| Roasting, microwaving, sprouting or autoclaving of mungbean seeds | (i) Improves nutritional quality palatability, bioavailability and acceptance for consumers  
(ii) Reduces cooking time and anti-nutritional constituents                                                         | (Kavitha et al., 2015)      |
| Germination (24 hr) of mungbean seeds                      | (i) Increases crude protein content  
(ii) Enhances protein bioavailability  
(iii) Reduces anti-nutritional contents  
(iv) Efficient in elevating certain amino acid levels such as phenylalanine                                  | (Wongsiri et al., 2014)     |
| Domestic processing of mungbean seeds                      | (i) Marked effect on protein digestibility  
(ii) Cooking time positively related with protein digestibility                                                        | (Kataria et al., 1989)      |

that exhibited −18.6% (Wu et al., 1996). One of their peers, Chitra et al. (1996) also confirmed that phytic acid content was moderately reduced after roasting as compared to raw chickpea, pigeon seeds and mungbean soya bean as well as also caused a slight increment in dietary fiber content. A subtle change in protein digestibility of all the legumes was also observed as roasting indicated slight increase in it. In addition to dry heat like roasting or microwaving, pressurized boiling also resulted in decline in the polyphenol content of a cowpea variety namely *Vigna unguiculata* (L.) Walp. Anyhow, all the heat treatments (dry or wet) were effective enough in inactivating or removing the polyphenols with slight variation in responses towards *in vitro* protein digestibilities ranged from 1 up to 26% (Laurena et al., 1987).

The protein derived from vegetables is incorporated in cereal based complementary foods. In various previous researches, cereal and legume based complementary foods were widely studied. For instance, Jackson (2009) evaluated the protein quality of cereal-legume;
cowpea & navybean residues, incorporated into laboratory rat chow to assess the effect during four weeks. The outcomes of the study reported that cowpea based diet improved protein quality than that of navybean. Furthermore, cowpea: wheat in a ratio of 30:70 depicted optimal ratio to ensure protein quality. Thus, protein extracted from legumes could be applied for human consumption. As infants are nutritionally vulnerable, thus require high caloric, nutrient dense semisolid foods with high nutrient bioavailability. Traditionally, cereal porridge is considered as the first semisolid food for infants. In resource poor economies, whole grain cereals are more readily available for complementary foods as compared to refined flours (Hotz and Gibson, 2007). Nonetheless, phytates in wheat bran render it less suitable for weaning foods formulation by hindering micronutrients bioavailability. Different processing treatments can significantly increase the bioavailability of different micronutrients. Likewise, incubation of wheat bran with distilled water for 80 min at 55 °C dramatically increased its endogenous phytase activity upto 4 folds as well as reducing the phytate content by about 70% (Guo et al., 2015). In the same way, cowpea seeds such as Cuarenton and Colorado were subjected to thermal processing to analyze its effect on compositional and antinutritional components in comparison to raw counterparts. Conclusive, results suggests that thermally treated cowpea could serve as cost-effective and important protein source for weaning foods (Avanza et al., 2013).

In a study, cereal based weaning foods developed from whole/fermented & dried maize, cowpea/ground bean, soybean or melon seeds were administered to 40 weanling albino rats. The study noticed that tempe-fortified maize based weaning food was significantly proficient in nutritional value with special reference to protein quality (PER, NPR, TD, BV & NPU) hence support the infant growth during weaning duration amongst the low income countries (Egounlety, 2002). In another research study, Ijarotimi O.S (2006) reported the PER, NPU and BV of sorghum and yam bean based weaning food varied from 0.26-0.43, 79.8-83.1 and 0.62-0.73, accordingly. Later, Bhagya et al. (2009) stated higher values for TD, BV and NPU of cooked bean diet than raw equivalent. Earlier researchers, Vadivel and Pugalenthi (2007) found momentous improvement in utilizable protein value via autoclaving as compared to other processing methods. Likewise, El-Niely (2007) associated the increase in PER of legumes with radiations @ 10 kGy.
CHAPTER 3

MATERIALS AND METHODS

3.1. Procurement and preparation of raw materials

Cowpea and mungbean were acquired from Pulses Research Institute, Ayub Agricultural Research Institute, Faisalabad. The remaining ingredients in the preparation of weaning including wheat, rice, etc. were purchased locally. Analytical grade reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). Each selected legume was properly cleaned followed by roasting at 200±2 °C for two minutes in a sand bath. After the attainment of ambient temperature, the resultant samples were sieved, dehulled, ground followed by final sieving. The final flour samples were separately packed in polyethylene bags and stored under refrigeration conditions until analyzed.

3.2 Chemical analysis of legume samples

The legume samples in raw form were assessed for moisture, crude- protein, -fat & -fiber, and ash as described in detail below.

3.2.1 Moisture content

In the selected legumes, moisture content was determined according to the AACC method no “44-15A Moisture-Air-Oven Methods” (AACC, 2000). Purposely, in a pre-weighed china dish, accurately weighed 5 g sample was taken then dried in a hot air oven (at 105 °C) till constant weight. Afterwards, the sample was placed in a desiccator and moisture content was calculated after cooling as expressed in the mathematical formula given below.

\[
\text{Wt.of moisture evaporated (g)} = \text{Wt.of original sample (g)} - \text{Wt.of dried sample (g)}
\]

\[
\text{Equation # 1}
\]

\[
\text{Moisture (\%)} = \frac{\text{Wt.of moisture evaporated (g)}}{\text{Wt.of original sample (g)}} \times 100
\]

\[
\text{Equation # 2}
\]
3.2.2 Crude protein

In each legume sample, nitrogen was estimated by using Kjeldahl apparatus (model: D-40599) based on Kjeldahl method “30-25b Crude Protein-Improved Kjeldahl Method” (AACC, 2000). The sample (50 g) was placed in a Kjeldahl digestion tube for digestion using concentrated $\text{H}_2\text{SO}_4$ (25 mL) and digestion tablets until color changed to light greenish. Afterwards, the resultant sample was diluted up to 250 mL volume. The digested, diluted sample (10 mL) and 40% NaOH (10 mL) was taken in the distillation apparatus followed by liberation of NH$_3$ that was captured in % boric acid+methyl red (indicator) solution carrying beaker. As a result, ammonium borate was formed and titrated using 0.1 N $\text{H}_2\text{SO}_4$. Finally, acid volume used in titration was noted for nitrogen determination in the sample.

Nitrogen

$$\text{Nitrogen} = \frac{\text{Vol. of } 0.1 \text{ N } \text{H}_2\text{SO}_4 \text{ used (mL)} \times 0.0014 \times \text{diluted Vol. (250 mL)}}{\text{Wt. of original sample (g)} \times \text{Vol. of digested, diluted sample (10 mL)}} \times 100$$

Equation # 3

Crude protein (%)=Nitrogen (%) x factor

Equation # 4

3.2.3 Crude fat

Each legume flour sample was tested for crude fat content by running samples through Soxtec System HT2, Extraction Unit, Tecator, Hoganas, Sweden (AACC, 2000). Five grams of moisture free sample was measured and placed in extraction thimble however, 50 mL of extraction non-polar solvent (petroleum ether) was taken into pre-weighed flask. After attachment of extraction thimble and flask to Soxhlet extraction system, the extraction was carried out till the completion of 5 siphonings. Later, the solvent was evaporated through rotary apparatus. The resultant extracted fat was placed in oven at 110 °C for 1 hr and crude fat was determined using the following expression.

$$\text{Crude fat} (%) = \frac{\text{Wt. of fat (g)}}{\text{Wt. of original sample (g)}} \times 100$$

Equation # 5
3.2.4 Crude fiber

The selected legume flours were assessed for crude fiber by method no. 32-10 Crude Fiber in Flours (AACC, 2000). Ten grams of sample was treated with 1.25% H_2SO_4 (150 mL) for 30 min to digest all acid solubilized fractions. Afterwards, the filtered, washed sample was treated with alkali followed by final washing with boiled water. Lastly, the sample was dried in oven (at 110 °C) for 24 hrs. The resultant sample was cooled in a desiccator and weighed \( w_1 \). Afterwards, the resultant sample was placed in a muffle furnace for 2 hr at 550-600 °C followed by cooling in a desiccator and reweighed \( w_2 \), subtracted \( w_1 - w_2 \) to attain loss in weight on ignition. The percentage crude fiber was calculated as mentioned below.

\[
\text{Crude fiber (\%)} = \frac{\text{Loss in wt. on ignition (g)}}{\text{Wt. of original sample (g)}} \times 100
\]

Equation # 6

3.2.5 Ash content

The ash content in the selected samples was measured by the method # “08-01 Ash-Basic Method” (AACC, 2000). Purposely, the sample (5 g) was weighed into a pre-weighted porcelain crucible and charred directly on flame in crucible until there were no fumes coming out. After charring, the sample was shifted to muffle furnace (at 550-600 °C, 5 hrs) for ignition (greyish white residues). Then, the sample was cooled in a desiccator, weighed and ash content was calculated as expressed below.

\[
\text{Ash content (\%)} = \frac{\text{Wt. of ash in sample (g)}}{\text{Wt. of sample (g)}} \times 100
\]

Equation # 7

3.2.6 Nitrogen Free Extracts (NFE)

The nitrogen free extract was calculated according to the expression given below;

\[
\text{NFE (\%)} = 100 - [\text{moisture (\%)} + \text{crude protein(\%)} + \text{crude fat(\%)} + \text{crude fiber(\%)} + \text{ash(\%)}]
\]

Equation # 8
3.3. Mineral profile

The raw legume samples were probed for mineral profile (AOAC, 2006). For the purpose, wet digestion of dried sample (0.5 g) was carried out using di-acid mixture of nitric acid (HNO₃) and perchloric acid (HClO₄) in the ratio of 7:3 on hot plate till 1 to 2 mL solution left following dilution up to 100 mL. For the assessment of zinc and iron, Atomic Absorption Spectrophotometer (Varian AA240, Victoria Australia) was used, whereas calcium, potassium and sodium were analyzed using Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge, UK).

3.4. Anti-nutrients

The samples (raw & roasted) were uniformly mixed with Ca(OH)₂ solution (20% w/w) to measure the anti-nutritional factors exist in legumes. The anti-nutrients were assessed according to their respective protocols.

3.4.1. Hemagglutinin activity

Hemagglutinin (Lectin) activity was determined by Rabbit Erythrocyte Agglutination Test i.e. expressed as hemagglutinin units per milligram; HU/mg (1 HU= minimum quantity of sample required for agglutination) as indicated by Dry et al. (1983). In this test, blood cells of rabbit were washed, centrifuged to obtain clear supernatant. Later, trypsinization was done to improve the sensitivity of plant hemagglutinin by adding trypsin for 30 min at 37 °C trailed by re-washing and dilution to achieve the absorbance 0.500 at 620 nm. Afterwards, flour sample (1 g) was shaken vigorously for 5 min using hemagglutinating buffer (50 mL) followed by overnight stay at 4 °C. After centrifugation, the supernatant was diluted with successive serial dilutions using hemagglutinating buffer in 1:1 ratio followed by addition of prepared tryspinized RBCs suspension (1 mL) for 24 hrs at 4 °C to assess the ability of legume sample to agglutinate trypsinized RBCs, visual estimation was done after incubation at room temperature for one hour.

3.4.2. Trypsin inhibitor activity

Trypsin inhibitor activity was determined using substrate; benzoyl-DL-arginine-p-nitroanilide (BAPNA). The finely ground legume flour (4 g) was mixed with 0.05 M sodium phosphate buffer (pH 7.5, 40 mL) and distilled water (40 mL) using Sorvall Omni Mixer (Ivan Sorvall, Inc., Newton). The ensuing slurry was subjected to centrifugation at 15 °C, 6000 rpm for 1 hr (M-3k30, Sigma, Germany) to achieve supernatant that was diluted
until enzymatic inhibition reached within the range of 40 to 60%. The resultant extract (0.1 mL) was then incubated (at 37°C for 20 min in water bath) with 0.5 mL trypsin solution (5 mg/mL), 2 mL BAPNA (2% w/v), 1.0 mL sodium phosphate buffer (pH 7.5, 0.1 M) and 0.4 mL HCl (0.001 M) to up-regulate the generation of enzyme inhibitor complex. The incubated mixture volume (4.0 mL) was mixed with 6.0 mL of 5% trichloroacetic acid solution to inhibit the reaction. The optical density of the sample was measure at 410 nm using UV/Vis spectrophotometer alongside blank was run for comparison. The calculations were expressed as trypsin inhibitor units per milligram of sample (TIU/mg); 1TIU = increase of 0.01 in optical density units (Decker, 1977).

3.4.3. Phytates

The phytate content of selected legumes were measured following the method described by Haug and Lantszch (1983). The samples were mixed with acidic NH₄Fe(SO₄) solution of known strength and heated. The decrement in Fe value in the supernatant was a measure of phytate content, determined at 519 nm using spectrophotometer (CE 7200-7000 series, Cecil, UK). Purposely, the selected legume flour (0.06 g) was treated with 10 mL of 0.2 M/L HCL at 4 °C overnight and 0.5 mL of the resultant extract was placed in a test tube followed by the addition of 1 mL of ferric solution [0.2 g ammonium iron (III) sulphate in 100 mL of 2 M/L HCl with final volume reached up to 1000 mL using distilled water]. Then the test tube was enclosed via a stopper and incubated (30 min, using water bath). Afterwards, the sample was cooled trailed by the addition of 2 mL of (1% v/v) 2’,2'-bipyridine solution (1.0 g 2, 2’-bipyridine and 1.0 mL thioglycollic acid with final volume up to 100 mL using distilled water). Finally, the optical density was analyzed at 519 nm against blank. The phytate content was recorded using standard calibration curve of phytate phosphorous.
3.5. Treatment plan

Table: 3.1. Composite flour blends treatment plan

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wheat flour %</th>
<th>Roasted cowpea %</th>
<th>Roasted mungbean %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T1</td>
<td>90</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>T2</td>
<td>80</td>
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<td>T3</td>
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<td>T8</td>
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<td>10</td>
<td>10</td>
</tr>
<tr>
<td>T9</td>
<td>70</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

3.6. Proximate Composition

The compositional analysis of composite flours was carried out according to their standard methods as described earlier (AACC, 2000).

3.7. Functional properties of composite flour

3.7.1. Oil holding capacity

The oil holding ability of samples was determined by placing sample (0.5 g) and corn oil (6 mL) in a centrifuge tube followed by 30 min stay time and centrifugation (3000 g) for 25 min. The oil separated the sample was decanted and the tubes were reweighed to assess the oil holding ability of sample (Kaur and Singh, 2007).

3.7.2. Water imbibition ability

Purposely, each flour blend (3 g) was mixed with distilled water (25 mL) in a centrifuge tube followed by stirring and centrifugation (3000 g) for 25 min. The ensuing supernatant i.e. excess water was pipetted out and tube were reweighed (Kaur and Singh, 2007).

3.7.3. Foaming potential

The foaming ability was assessed by combining flour (1g) with distilled water (50 mL) in a graduated cylinder (capacity; 250 mL). The foam formation was expressed as mL/100mL i.e. known as foaming ability, whereas foaming stability was recorded after 60 min stay time after foam formation (Siddiq et al., 2010).
3.7.4. Emulsion characteristics

For emulsification, each flour sample (0.5 g) was mixed with distilled water (3 mL) and oil (3 mL) followed by shaking (5 min) and centrifugation (2000 g, 30 min). Afterwards, the emulsified layer volume was divided by whole slurry volume to reach emulsion capacity, expressed as mL/100mL. On the other hand, emulsion stability was measured by heating (80 °C) this slurry trailed by centrifugation (2000 g, 30 min) and again emulsified layer volume was divided by whole slurry volume and results were achieved as mL/100mL (Siddiq et al., 2010).

3.8. Preparation of weaning foods

Selected legume flours were combined with rest of ingredients (soybean, milk powder, rice flour, wheat flour, sugar, vegetable oil and multi-vitamins mixture) to formulate weaning food alongside, soy based weaning food was served as control for comparison (Appendix I).

3.9. Sensory evaluation

Weaning foods i.e. W₁ to W₉ and control were evaluated for various sensory traits by a panel of judges (Meilgaard et al., 2007). The sensory performa was based on three questions; (1) visual appearance involving color & texture; (2) product development making such as ease of preparation, smoothness & thickness and (3) eating characteristics including taste+flavor. On the basis of these traits, overall acceptability was calculated. The periodic sensory evaluation was carried out at set intervals in the Sensory Evaluation Laboratory of the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad. The panel was comprised of 10 untrained panelists, aging from 25 to 40 years including students and staff members of the department served as replicates. Soft white light was used to mask any color variation that might influence a panelist’s judgment. The weaning foods were served at room temperature in transparent cups, carrying random codes. Besides, judges were also supplied with water and unsalted crackers to clear their palate in between the evaluation. The panelists were asked to evaluate the weaning foods on 9-point hedonic scale system:

1 Dislike extremely  2 Dislike very much  3 Dislike moderately  4 Dislike slightly  
5 Neither like nor dislike  6 Like slightly  7 Like moderately  8 Like very much  9 Like extremely
3.10. Selection of best treatments

Based on sensory evaluation, most acceptable weaning foods; W₂, W₅ & W₈ were selected, renamed as W₁₅, W₂₅ & W₃₅ (Table 1) and compared with soy based weaning food as control for further assessment.

3.11. Evaluation of weaning food

3.11.1. Compositional analysis

The proximate components of weaning foods were measured as per their respective standard methods discussed earlier (AACC, 2000).

3.11.2. Calorific value

The gross energy of each weaning food on percentage or per serving basis was analyzed using Oxygen Bomb Calorimeter; C-2000, IKA WERKE (AOAC, 2006). Furthermore, energy value for protein and fat were also measured using Atwater’s expressions; 4x%Protein & 9x%Fat (Passmore and Eastwood, 1986).

3.11.3. Loose and packed/tapped bulk density

The loose bulk density was analyzed by placing legume sample (50 g) into a graduated cylinder (capacity: 100 mL), whereas packed/tapped bulk density was measured by tapping the cylinder gently several times on a laboratory bench. The result for loose & packed bulk density was calculated using expressions sample weight to volume before & after tapping and expressed as g/mL (Amandikwa et al., 2015).

3.11.4. Reconstitution index

The weaning food formulation was subjected to boiling water for 90 sec then the resultant sample were poured in a volumetric cylinder (capacity: 250 mL). For reconstitution index assessment, volume of deposited material was measured after ten minutes (Osundhunsi and Aworh, 2002).

3.11.5. Viscosity determination

The viscosity of weaning food slurry (40%) was measured using a DV-E Viscometer (LVDVE 230, Brookfield Viscometers Ltd., Harlow, UK) following the method of Thathola and Srivastava (2002). Evaluation of viscosity was done at a constant speed, 50 °C and reading was recorded after 1 min.
3.12. Bioassessment trail

The biological evaluation of legume based weaning foods was carried out by feeding the tested diets along with control to Sprague Dawley rats. Besides the treated samples, mineral-vitamin mixture in addition to sucrose, corn starch & cellulose were also incorporated in the experimental diets (Eggum, 1973).

3.12.1. Housing of rats

In animal study, Sprague Dawley rats (total = 20) were brought from the National Institute of Health (NIH), Islamabad and accommodated in the Animal Room of NIFSAT, University of Agriculture, Faisalabad (conditions: temperature; 23±2 °C & humidity; 50±5% for 12-hr light-dark cycle). The rats were split into 4 groups, carrying 5 rats in each. The respective diets were fed to each group during 10 days and spilled diets & urinary/feces outputs were collected on daily basis. At the end of experimental trial, overnight fasted rats were sacrificed followed by drying in oven.

3.12.2. Feed consumption

On daily basis, the feed consumed by each group was assessed by subtracting spilled diet out of total diet during the entire period (Wolf and Weidbrode, 2003).

3.12.3. Body weight gain

In response to protein based diet, the increase in weight of rat among each group was determined for growth index (Seena et al., 2006).

3.12.4. Protein quality evaluation

For the determiniant of growth related aspects including protein efficiency ratio (PER), net protein ratio (NPR) and relative net protein ratio (RNPR) were measured using net feed consumption and weight of rats. Furthermore, in order to determine nitrogen content or balance, true digestibility (TD), biological value (BV) and net protein utilization (NPU) were estimated employing spilled diets, urinary/feces excretions and dried rat bodies (Ingbian and Adegoke, 2007).

3.13. Infant acceptability test

The acceptability study regarding infant formulations was conducted during short time feeding trial (Thathola and Srivastava, 2002). Intentionally, weaning foods were provided to the selected nursing mothers, ten in each. The mothers were selected randomly from
Faisalabad district. The study was carried out on healthy and regular weaning food consuming infants, aged between 6 and 24 months. Furthermore, a questionnaire was supplied to mothers to record the reaction of their infants towards the weaning food during fortnight study at consistent intervals *i.e.* Day 1, 5, 10 and 15. Before conducting the trials, the mothers were given lectures regarding preparation method and asked to note feedback of their infants along with general health perspectives. Then answer the questionnaire based on five point hedonic scale as described below (Mosha and Vincent, 2004).

1. Rejected
2. Accepted only if hungry
3. Moderately accepted
4. Accepted
5. Highly accepted

### 3.14. Statistical application

The obtained data was analyzed statistically via applying statistical software (Statistix 8.1). Furthermore, level of significance (*p* value) was determined by applying ANOVA using CRD under one factor factorial except infant acceptability test *i.e.* two factor factorial design. Lastly, the separation between means were compared via Tukey’s HSD multiple comparison tests (Mason *et al*., 2003).
CHAPTER 4

RESULTS AND DISCUSSION

Protein is one of the essential components of diet performing diversified role especially in repair, growth and maintenance of human body. Among plant sources, legumes are one of the most important contributors of protein in our daily diet. Accordingly, the present research was designed to formulate protein based weaning food using indigenous legumes \textit{i.e.} cowpea and mungbean. In the tested legumes, inactivation of anti-nutrients was carried out through roasting before preparation of composite flours and weaning food. Moreover, legumes based composite flours were evaluated for their functional characteristics \textit{i.e.} oil absorption, water absorption, emulsifying and foaming properties. On the basis of sensory evaluation, three weaning foods along with control were assessed for physicochemical characteristics followed by bio-evaluation trial to study growth & nitrogen balance parameters using Sprague Dawley rats and infant acceptability test. The results and related discussion of each parameter is explained herein.

4.1. Compositional analysis of cowpea and mungbean

4.1.1. Proximate composition

The compositional analysis measures the nutritional and quality characteristics of raw material. In this perspective, cowpea and mungbean flours were assessed for varied components.

The composition after roasting revealed that moisture content was $5.97 \pm 0.145$ and $3.37 \pm 0.055\%$ in cowpea and mungbean, respectively (Table 4.1). Protein content in cowpea was $24.57 \pm 0.66\%$ while in mungbean it was $24.25 \pm 0.94\%$. The higher crude fat was documented in cowpea ($2.05 \pm 0.07\%$) as compared to mungbean ($1.34 \pm 0.03\%$). Similarly, crude fiber was $3.07 \pm 0.075$ and $3.57 \pm 0.085\%$ in cowpea and mungbean, correspondingly. Likewise, ash content in cowpea and mungbean was $3.02 \pm 0.045$ and $3.35 \pm 0.070\%$, respectively. Whereas, NFE noticed in mungbean and cowpea were $65.73 \pm 1.11$ and $61.98 \pm 0.70\%$ accordingly.

Current results regarding components analysis are in harmony with the results mentioned in previous literature; though, slight variation can be due to maturity index, variety difference, soil fertility and different environmental conditions. Proximate analysis of
cowpea was done by Anjos et al. (2012) who reported moisture, crude protein, -fat, -fiber and ash as 6.00, 24.94, 1.70, 3.75 and 3.42%, respectively in roasted cowpeas. Similarly, in a recent research, nutritional composition of two varieties of cowpeas revealed that moisture, crude protein, -fat, -fiber and ash ranged from 7.82-9.76, 20.88-26.22, 6.65-7.98, 4.91-5.49 and 3.13-3.81%, respectively (Ayogu et al., 2016). Close results were expounded by Matondi et al. (2015), they reported 26.09% protein, 3.73% fat and 6.58% ash in cowpea.

Present outcomes for mungbean are in accordance with the results of Kavitha et al. (2014). They observed moisture, crude protein, -fat, -fiber and ash as 3.42, 24.46, 1.36, 3.10 and 3.06%, respectively in flour of roasted mungbean. Later on, Padmashree et al. (2016) probed raw mungbean samples for moisture, crude protein, -fat, -fiber, ash and carbohydrates and reported values were 10.43, 24.89, 1.72, 4.23, 3.42 and 55.31%, correspondingly. Earlier, Shaheen et al. (2012) conducted the proximate assay of different legumes including mungbean and revealed moisture, crude fat, -fiber and ash as 9.74, 1.35, 2.90 and 2.91%, respectively. However, Noor et al. (2012) observed quite low amount of protein (16%) in mungbean samples. In various reports, differences in proximate composition of different legume samples such as cowpea and mungbean have been reported due to different environments and genetic makeup. Moreover, it is documented that protein may differentiate on the basis of frequency of rainfall, intensity of light, season, day length, temperature and agronomic practices (Bampidis and Christodoulou, 2011; Dahiya et al., 2013).

As a result, from nutritional aspects, tested legumes (cowpea and mungbean) had comparable nutritional value with special reference to fat, protein and fiber. Moreover, they are accessible protein source and substitutes of animal protein without obvious rise in lipid profile. It is clear from the current study that leguminous plants are good source of quality protein hence help in alleviating PEM and possess tremendous potential to be used in weaning foods.

4.2. Mineral profile

Mineral profile in the current study (Table 4.2) comprised of K, Ca, Na, Fe and Zn and their respective values were 28.19±0.89, 61.20±4.13, 45.75±1.05, 7.55±0.37 and 1.28±0.07 mg/100g for cowpea and 387.51±14.11, 78.33±2.52, 10.46±0.48, 5.31±0.81 and 2.17±0.14 mg/100g for mungbean, respectively. The results for Zn, Fe, Ca, Na and K are in conformity
with the earlier results of Owolabi et al. (2012) who measured these minerals in different cowpea samples which varied from 0.23 to 0.66, 0.48 to 2.65, 15.00 to 36.80, 0.41 to 0.60 and 16.21 to 18.69 mg/100g, respectively. Later, Osunbitan et al. (2016) determined manganese was in the range of 0.1264-0.171 mg/100g, iron 0.1314-0.2058 mg/100g, zinc 0.2302-0.252 mg/100g and copper contents were in the range of 0.0221-0.03 mg/100g. Na content was varying from 0.0573-0.237 mg/100g, whereas K, Ca, Mg and P ranged from 109 to 116, 7 to 9, 14 to 16 and 33 to 35 mg/100g, correspondingly. These varieties contain higher content of essential mineral elements (calcium, iron, zinc, manganese, phosphorus and potassium) than those tested in this study. Thus, their consumption would reduce the occurrence of nutritional deficiency and its associated health problem in infants, children and pregnant women. Earlier, Dahiya et al. (2013) noted K (363±1.5 to 414±1.1 mg/100g), Ca (81±1.2 to 114±1.0 mg/100g), Na (8.8±0.3 to 13.2±0.3 mg/100g), Zn (1.2±0.03 to 2.1±0.14 mg/100g) and Fe (3.6±0.15 to 4.6±0.10 mg/100g) in established and newly bred varieties of mungbean samples.

4.3. Anti-nutritional factors

4.3.1. Phytates

The chelating effect of phytic acid causes its complex formation with Ca, Mg, Fe and Zn, hindering their bioavailability (Vasagam and Rajkumar, 2011). From the statistical inference, it was found that phytic acid varied momentously among the processed and raw legumes (Table 4.3).

Mean values in Table 4.4 showed higher phytic acid content in untreated cowpea (13.59±0.54 mg/g) as compared to mungbean (6.51±0.82 mg/g). Roasting disassociated phytate complexes efficiently thereby minimizing these anti-nutrients in the selected legumes. More decrease in this property was noticed due to roasting in cowpea (4.48±0.36 mg/g) than that of mungbean (1.88±0.06 mg/g).

Current findings are in agreement with Padmashree et al. (2016), they reported that mungbean contains 6.65 mg/g phytates. These results are also in conformity with earlier findings of Owolabi et al. (2012), who observed that cowpea contain phytates ranging from 3.00 to 8.50 mg/100g. Anjos et al. (2012) recorded 28.33% reduction in phytate content of cowpea samples on roasting at 120 °C for 60 min. phytic acid is negatively related to absorption of minerals including P, Mg, Fe, Ca and Zn and macromolecules; protein and lipid (El-Adawy, 2003).
### Table 4.1. Proximate composition (%) of legumes samples

<table>
<thead>
<tr>
<th>Legumes</th>
<th>Moisture</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td>5.93±0.14a</td>
<td>23.10±0.66</td>
<td>2.05±0.07a</td>
<td>3.57±0.08a</td>
<td>3.35±0.07b</td>
<td>61.98±0.70b</td>
</tr>
<tr>
<td>Mungbean</td>
<td>3.37±0.05b</td>
<td>22.45±1.04</td>
<td>1.34±0.03b</td>
<td>3.07±0.07b</td>
<td>4.02±0.04a</td>
<td>65.73±1.11a</td>
</tr>
<tr>
<td>F value</td>
<td>810.44**</td>
<td>0.82ns</td>
<td>244.26**</td>
<td>59.55**</td>
<td>187.68**</td>
<td>24.32**</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a column are statistically alike (n =3)

### Table 4.2. Mineral profiling of cowpea and mungbean

<table>
<thead>
<tr>
<th>Minerals (mg/100g)</th>
<th>Cowpea</th>
<th>Mungbean</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>28.19±0.89b</td>
<td>387.51±14.11a</td>
<td>1936.04**</td>
</tr>
<tr>
<td>Calcium</td>
<td>61.20±4.13b</td>
<td>78.33±2.52a</td>
<td>37.65**</td>
</tr>
<tr>
<td>Sodium</td>
<td>45.75±1.05a</td>
<td>10.46±0.48b</td>
<td>2802.35**</td>
</tr>
<tr>
<td>Iron</td>
<td>7.55±0.37a</td>
<td>5.31±0.81b</td>
<td>19.13*</td>
</tr>
<tr>
<td>Zinc</td>
<td>1.28±0.07b</td>
<td>2.17±0.14a</td>
<td>89.89**</td>
</tr>
</tbody>
</table>

Values are expressed as means ± standard deviation (n =3)

Means sharing the same letter in a column are statistically alike
Phytic acid forms insoluble complexes with metals, lowering solubility and absorption of protein (Abdelrahaman et al., 2007). Additionally, processing procedures involving dehusking, cooking, roasting and germination were found to enhance nutritional value of legumes. Commonly, boiling of pulses is considered as the common cooking procedure at domestic level. The anti-nutritional substances in beans are reported to decrease by boiling (Vadivel and Janardhanan, 2000). The boiling procedure indicates reduction of phytic acid up to 41 and 38% in brown tepary and white beans, respectively. Likewise, the reduction up to 7, 53 and 16% was observed in cowpea, fava bean and chick pea, respectively.

4.3.2. Hemagglutinin-lectin activity

Heat sensitive hemagglutinin is a growth intoxicant even in a little amount in food hence recognized as a toxin at higher concentration (Liener, 1994).

Mean squares in Table 4.3 reported that hemagglutinin activity in legumes showed considerable decrement after heating. As evident more hemagglutinin-lectin activity were higher in mungbean (2.74±0.09 HU/mg) as compared to cowpea (0.61±0.03 HU/mg). Conclusively, it has been revealed from the current study that roasting reduced hemagglutinin-lectin significantly.

The current study is in corroboration with previous finding that observed higher reduction in mungbean 0.59±0.02 HU/mg (Thorne et al., 1983). The obtained results were consistent with earlier outcomes of Almeida et al. (2008), they showed that hemagglutinin activity varied from 0.0513 to 0.0572 HU/mg. Furthermore, significant decrease was viewed in lectin activity after heating. It is well known that germination, soaking and roasting decrease hemagglutinin activity in beans (Alonso et al., 2000). The heat treatment like microwave was found to decrease hemagglutinin activity in legume samples (El-Adawy, 2003). Hemagglutinins are sugar-protein complexes and could bound to RBCs in the body besides, could also bind with specific receptors on intestinal cells, forming lesions or abnormalities in microvillus development resultantly lowering nutrient absorptivity. Moreover, El-Adawy et al. (2000) and Mubarak (2005) worked on hemagglutinin activity in mungbean.

4.3.3. Trypsin inhibitor activity (TIA)

Trypsin inhibitors are considered as anti-nutrients due to their negative impact on growth related factors, PER and enzymes involved in digestion (Bahnassey et al., 1986). Thus,
### Table 4.3. Mean squares for anti-nutritional factors in legume samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Phytates (Raw)</th>
<th>Phytates (Roasted)</th>
<th>Haemagglutinin-lectin activity (Raw)</th>
<th>Haemagglutinin-lectin activity (Roasted)</th>
<th>Trypsin inhibitor activity (Raw)</th>
<th>Trypsin inhibitor activity (Roasted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legumes</td>
<td>1</td>
<td>75.18**</td>
<td>10.14**</td>
<td>6.81**</td>
<td>0.24**</td>
<td>175.61**</td>
<td>10.94**</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.48</td>
<td>0.06</td>
<td>0.01</td>
<td>0.00</td>
<td>0.64</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Highly significant (p<0.01)

### Table 4.4. Anti-nutritional factors of legume samples

<table>
<thead>
<tr>
<th>Legumes</th>
<th>*Phytates (mg/g)</th>
<th>**Phytates (mg/g)</th>
<th>*Haemagglutinin-lectin activity/mg</th>
<th>**Haemagglutinin-lectin activity/mg</th>
<th>*Trypsin inhibitor activity/mg</th>
<th>**Trypsin inhibitor activity/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td>13.59±0.54^a</td>
<td>4.48±0.36^a</td>
<td>0.61±0.03^b</td>
<td>0.19±0.01^b</td>
<td>7.53±0.18^b</td>
<td>1.52±0.25^b</td>
</tr>
<tr>
<td>Mungbean</td>
<td>6.51±0.82^b</td>
<td>1.88±0.06^b</td>
<td>2.74±0.09^a</td>
<td>0.59±0.02^a</td>
<td>18.35±1.12^a</td>
<td>4.22±0.41^a</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a column are statistically alike
*Raw
**Roasted
inactivation of these anti-nutrients via processing is usually practiced for eating purpose (Liener et al., 1994).

The current study explicated significant differences in TIA of cowpea and mungbean samples, prior and after thermal application. Mean values for the said trait showed maximum activity was in mungbean (18.35±1.12 TIU/mg) followed by cowpea (7.53±0.18 TIU/mg) as shown in Table 4.4.

The present results are in accordance with Olivera-Castillo et al. (2007) who also observed notable reduction in TIA in processed, dried cowpea. The highest decrement for the mentioned trait was reported in roasted cowpea followed by roasted mungbean. Previously, it has been documented that heat processing could reduce the TIA exist in rice bran (Tashiro and Ikegami, 1996). Furthermore, microwaving and dry & moist thermal processing have also been reported to reduce TIA (Deolankar and Singh, 1979; Hernandez-Infante et al., 1998). Similarly, cooking under pressure of legumes can be used at household level to minimize the TIA (Seena et al., 2006).

Conclusively, the present findings are in accordance with El-Adawy (2003), who found decrease in TIA, present in legumes based on thermal processing. Moreover, in another study, soaking (12 hr) was found to reduce TIA, phytic acid and polyphenols in dry beans up to 51, 35 and 43%, respectively (Ramakrishn et al., 2008).

4.4. Proximate analysis of composite flours

Compositional analysis of food products gives a picture of overall ingredients and percentage of various nutrients. Legumes/beans are considered as rich source of protein, dietary fiber and carbohydrate for low income countries (Rehman and Shah, 2005). The quantity and quality of protein is based on light intensity, temperature, rainfall, day length, agricultural practices and season (Bampidis and Christodoulou, 2011).

In this context, nine composite flours along with control were determined for varied macromolecules. Mean squares for compositional status presented momentous variations among composite flours (Table 4.5).

Proximate profiling demonstrated that moisture (%) was varying from 7.98±0.45 (T6) to 11.38±0.38 (T0) in different composite flours. However, crude protein depicted significant difference however, T3 (14.23±0.57%) explicated maximum value for the said parameter, whereas minimum value was that of control (10.43±0.69%). The maximum crude fat was
observed in T₃ (1.12±0.13%) that varied significantly from other composite flours and lowest was recorded in control (0.91±0.12). Furthermore, crude fiber and ash were ranged from 0.44±0.03% (T₀) to 0.53±0.24% (T₃) and 0.65±0.1% (T₀) to 1.07±0.23% (T₃), respectively whilst, NFE ranged from 72.65±1.22% (T₃) to 77.44±1.27% (T₄).

The current outcomes regarding compositional analysis are in corroboration with the findings of earlier scientists. The proximate components of legume flour blends was also assessed by Butt et al. (2011), they viewed variations in moisture, crude protein, -fat, -fiber and ash in 100% wheat flour (control) as 12.30, 10.65, 0.90, 0.46 and 0.56%, accordingly.

In T₂ (90 wheat flour and 10% cowpea) treatment, the recorded values of composition were moisture (12.10%), protein (12.41%), ash (0.82%), fat (0.99%) & fiber (0.55%). Fat, fiber, protein and ash content showed increment in repose to increase in level of cowpea flour. In T₄ (80% wheat flour and 20% cowpea), the proximate analysis depicted 11.50% moisture, 13.25% protein, 1.08% ash, 1.13% fat and 0.64% fiber. The maximum moisture level was noted in control, whereas 20% cowpea flour presented lower moisture value. 12.15%, protein, fat, fiber and ash were close to our results in composite flour samples.

Present finding are in accordance with Olapade and Oluwole (2013), they observed the compositional analysis of cowpea composite flour. Conclusively, from nutritional point of view, composite flours showed good nutritive value in terms of protein, fiber and fat. Moreover, they found it high in accessibility & protein value, serving as a substitute for meat based products, without any rise in serum cholesterol level. This is similar to what observed in the current exploration. Conclusively, legumes help in alleviating PEM amongst the developing economies.

In the nutshell, proximate composition determines the nutritional value, quality and overall acceptance of raw material. It is important to measure some of the ingredients after milling like protein and ash that either they exist or not and if present then quantity does matter. Milling significantly effects on ash content i.e. concentrated in bran portion and indicates milling performance and yield by measuring the bran contamination in flour. The existence of ash ultimately influences color of the finished product therefore miller should need to adjust it according to the end product as some of the products need lighter color while others demand for a little darker tone. Furthermore, the nutritional
Table 4.5. Mean squares for proximate composition of composite flour samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Moisture</th>
<th>Crude protein</th>
<th>Crude fat</th>
<th>Crude fiber</th>
<th>Ash</th>
<th>NFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite flours</td>
<td>9</td>
<td>2.73774**</td>
<td>4.61**</td>
<td>0.1896**</td>
<td>0.13381**</td>
<td>0.15605**</td>
<td>4.5917ns</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>0.19685</td>
<td>0.34519</td>
<td>0.00924</td>
<td>0.00059</td>
<td>0.00496</td>
<td>12.4145</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** = Highly significant \( (p<0.01) \)
* = Significant \( (p<0.05) \)
ns = Non-significant \( (p\geq0.05) \)
Table 4.6. Proximate analysis (%) of composite flours

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>T₅</th>
<th>T₆</th>
<th>T₇</th>
<th>T₈</th>
<th>T₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>11.32±0.38ᵃ</td>
<td>10.78±0.44ᵇ</td>
<td>10.24±0.32ᵃᵇᶜ</td>
<td>9.70±0.51ᵇᶜ</td>
<td>10.53±0.43ᵃᵇᶜ</td>
<td>9.40±0.31ᶜ</td>
<td>7.98±0.45ᵈ</td>
<td>10.72±0.1⁸ᵃᵇ</td>
<td>9.88±0.15ᵇᶜ</td>
<td>9.32±0.24ᶜ</td>
</tr>
<tr>
<td>Crude protein</td>
<td>10.43±0.69ᵉ</td>
<td>11.70±0.53ᵈᵉ</td>
<td>12.96±0.4ᵃᵇᶜᵈ</td>
<td>14.23±0.57ᵃ</td>
<td>11.63±0.44ᵈᵉ</td>
<td>11.86±0.53ᶜ</td>
<td>13.40±0.44ᵃᵇᶜ</td>
<td>11.39±0.57ᵈ</td>
<td>12.47±0.69ᵈ</td>
<td>14.10±0.72ᵃ</td>
</tr>
<tr>
<td>Crude fat</td>
<td>0.91±0.1²ᵈ</td>
<td>0.93±0.09ᵈ</td>
<td>1.05±0.11ᶜᵈ</td>
<td>1.12±0.13ᵇᶜᵈ</td>
<td>0.93±0.16ᵈ</td>
<td>0.92±0.13ᵈ</td>
<td>1.30±0.29ᵇᶜ</td>
<td>0.94±0.09⁵ᵈ</td>
<td>1.39±0.27ᵃᵇ</td>
<td>1.64±0.18ᵃ</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>0.44±0.03ᵉ</td>
<td>0.48±0.03ᵈᵉ</td>
<td>0.50±0.04ᵈᵉ</td>
<td>0.53±0.24ᵈ</td>
<td>0.45±0.06ᵉ</td>
<td>0.55±0.10ᵈ</td>
<td>0.79±0.16ᵇ</td>
<td>0.67±0.17ᶜ</td>
<td>0.73±0.19ᵇᶜ</td>
<td>1.12±0.14ᵃ</td>
</tr>
<tr>
<td>Ash</td>
<td>0.65±0.1ᶠ</td>
<td>0.72±0.02ᵉᶠ</td>
<td>0.91±0.07ᵈᵉ</td>
<td>1.07±0.23ᵇᶜ</td>
<td>0.68±0.18ᶠ</td>
<td>0.93±0.11ᶜᵈ</td>
<td>1.14±0.10ᵃᵇ</td>
<td>0.75±0.07ᵈᵉ</td>
<td>0.64±0.04ᶠ</td>
<td>1.29±0.18ᵃ</td>
</tr>
<tr>
<td>NFE</td>
<td>76.24±1.31</td>
<td>75.39±1.11</td>
<td>74.33±0.95</td>
<td>73.35±1.22</td>
<td>75.78±1.27</td>
<td>76.32±1.20</td>
<td>75.37±1.45</td>
<td>75.52±1.09</td>
<td>74.88±1.35</td>
<td>72.52±1.42</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a row are statistically alike (n=3)
Control=100% wheat flour; T₁= 90% wheat flour and 10% cowpea, T₂= 80% wheat flour and 20% cowpea; T₃=70% wheat flour and 30% cowpea, T₄=90% wheat flour and 10% mungbean, T₅=80% wheat flour and 20% mungbean; T₆=70% wheat flour and 30% mungbean, T₇=90% wheat flour, 5% cowpea and 5% mungbean; T₈=80% wheat flour, 10% cowpea and 10% mungbean; T₉=70% wheat flour, 15% cowpea and 15% mungbean
worth of cereal-legume flour blends could be harnessed via processing such as roasting. Therefore, the effect of roasting on nutritional quality of flour need to determine as it denatures the protein. Alongside, it impacts on fat, fiber, ash and carbohydrate ultimately energy value (Kavitha and Parimalavalli, 2014).

4.5. Functional Properties of composite flours

The functional properties of legume based food products are affected by processing. These attributes also depend on food components especially water, air and oil, whereas protein, serving as surfactants imparts remarkable functional value based on film foaming and emulsifying ability. The water-protein interaction measures the solubility, viscosity and gelling ability however, oil-gas association determines foaming and emulsifying characteristics.

Regarding functionality, protein serves as an important ingredient in the development of food products with special influence on foaming, emulsifying, solubilizing, gelling and oil holding capacities. Due to varying processing characteristics, protein either individually or in conjunction with other ingredients causes such interactions that ultimately affects on end product quality.

4.5.1. Absorption properties

Proteins are hydrophobic and hydrophilic in nature thus emulsified with water and oil in numerous food products. Means for water and oil holding ability of tested legume flour blends presented momentous variations.

Mean values for water imbibition in Figure 4.1 presented highest value by $T_3$ (1.44 mL/g) trailed by $T_6$ (1.41 mL/g) and $T_9$ (1.39 mL/g). This effect was related to polar amino acid cite of protein-water interactions. The decrement in WAC of control (1.07mL/g) is attributed to conformational alterations in protein structure. Oil and water holding abilities measure the binding ability of oil and water molecules under scarce oil and water conditions, respectively.

The oil holding ability of legume flour blends ranged from 1.07 to 1.44 mL/g. The oil binding capacity of protein derived from legumes regulates the degree of contact to oil. In this regard, $T_3$ and $T_6$ displayed strong association with lipophilic molecules than that of $T_2$ and $T_5$ treatments. This is due to non-polar moities association with oil via hydro-carbon bonds.
WAC of legumes varied between 0.6 and 4.9 mL/g, this has been observed in numerous studies. Furthermore, WAC might be affected by the variety of pulses *i.e.* pea, cowpea, lentils, mungbean or faba bean (Kaur and Singh, 2007; Boye *et al.*, 2010a). Moreover, it seems that processing techniques subjected to minimize the anti-nutrients might also affect the water holding capacity (WHC).

Solubility of protein is influenced by balance of hydrophobic protein molecules that depends on polar and non-polar amino acids arrangement ultimately affect on protein interactions with protein molecules or solvent (Boye *et al.*, 2010b).

Concentration of fiber, protein and starch controls the WAC that eventually results in swelling of food product. Swelling power of starch molecule is relatively higher in water, which when combined with the swelling capability of fiber and protein could result in improved swelling power of starch. Therefore, legumes having high fiber and protein play a significant role in swelling and WAC (Torruco-Uco and Betancur-Ancona, 2007).

The current findings regarding water holding capacity of mungbeans are in agreement with the outcomes of Suliman *et al.* (2006) that stated this parameter as 1.9 mL/g. On the other hand, Aguilera *et al.* (2009) reported cowpea value for the said attribute that was in harmony with the current findings. They noted 3.20 mL/g WAC for cowpea powders. Similarly, mungbean WAC was in accordance with outcomes of Li *et al.* (2010). They explicated 2.10 g/g WAC for mungbean powders (MBP).

The instant results of oil absorption capacity (OAC) for legumes are in agreement with the results of Suliman *et al.* (2006), Sai-Ut *et al.* (2009), Singh *et al.* (2008) and Li *et al.* (2010), they assessed oil holding capacity, varying from 1.90 to 3.42 g/g for various tested legumes. Mechanism for fat absorption includes physical entrapment of oil. Hence, moisture content, flour composition, particle size and microstructures of proteins are amongst various factors that may affect OAC. Furthermore, amount of non-polar amino acids residues and protein compositional profile is concerned, alterations in starch, protein & lipid conformation might be due to different oil retention characteristics of legume proteins (Lazou and Krokida, 2010).

Conclusively, oil holding capacity portrays the ability of protein to physically bind with fat particles present in food formulations. In the current study, the oil binding capacity of cowpea and mungbean flours was relatively higher than wheat based treatments; this indicates that their inclusion would be useful for the preparation of such foods where oil
Table 4.7. Mean squares for functional properties of composite flour samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>WAC</th>
<th>OAC</th>
<th>F Capacity</th>
<th>F Stability</th>
<th>E Capacity</th>
<th>E Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite flours</td>
<td>9</td>
<td>0.03165**</td>
<td>0.01452**</td>
<td>331.968**</td>
<td>172.213**</td>
<td>128.955**</td>
<td>104.445**</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>0.00082</td>
<td>0.00046</td>
<td>1.119</td>
<td>1.675</td>
<td>0.700</td>
<td>1.117</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Highly significant ($p<0.01$)

WAC= Water absorption capacity
OAC= Oil absorption capacity
F Capacity = Foaming Capacity
F Stability = Foaming Stability
E Capacity = Emulsion Capacity
E Stability = Emulsion Stability
Control=100% wheat flour; T1= 90% wheat flour and 10% cowpea, T2= 80% wheat flour and 20% cowpea; T3=70% wheat flour and 30% cowpea, T4=90% wheat flour and 10% mungbean, T5=80% wheat flour and 20% mungbean; T6=70% wheat flour and 30% mungbean, T7=90% wheat flour, 5% cowpea and 5% mungbean; T8=80% wheat flour, 10% cowpea and 10% mungbean; T9=70% wheat flour, 15% cowpea and 15% mungbean

WAC= Water absorption capacity; OAC= Oil absorption capacity

Figure 4.1: Water and oil absorption capacity of composite flour samples (n=3)
holding capacity is required for instance bakery items. Likewise, the water holding potential characterizes the capacity of flour to associate with water molecules as in case of dough. In the present work, the selected legumes showed an upper hand in water holding potential as compared to wheat flour thus they could be beneficial in the preparation of bakery products where hydration is required to overcome handling issues. Furthermore, water holding capacity of cereal-legume flour blends is positively linked with texture of the food products. As a result, it could be stated that water and oil absorption abilities could ultimately influence on flow, elasticity, plasticity or mechanical strength of the end product (Makri et al., 2005; David et al., 2007; Yellavila et al., 2015).

4.5.2. Foaming properties

Commonly, foaming expansion/capacity of proteins represents increase in volume of protein solution due to inclusion of air. However, foaming stability (FS) assesses the ability of food to retain air bubbles after foaming i.e. quantitatively estimated as separated volume of foam and protein solution after sometime or decrease in foam volume (Boye et al., 2010b).

Whipping ability of legume proteins influences the foaming properties. Food foam represents thin liquid film coated gas bubbles, generated by whipping of air in solution or suspension phase (Sikorski, 2002). Mean values for FC depicted momentous difference while, legume flour blends affected non-significantly on FS.

The maximum FC was exhibited by T₃ (44.74%) followed by T₉ (43.31%) and T₆ (42.06%) whilst, the minimum in control (12.92%) as depicted in Figure 4.2. Highest FS was found in T₈ (68.67%) whereas, lowest was recorded in control (42.12 %) as elucidated in Figure 4.2. It was found that mungbean form denser foam in response to more air-water interactions. The highest value of FC was recorded for T₃ with the increase in hydration, whereas lower values were recorded in T₄ and T₀. However, the said trait reduced markably in T₁ due to protein denaturation or loss of elasticity (because of the formation of disulfide linkages).

Reduction in surface tension yields flexible protein and better foaming ability as compared to aggregated protein structure i.e. responsible for poor denaturation ability (Akintayo et al., 1998). Different proteins have different abilities for foam development and stability. Foaming potential is influenced by diffusion properties of proteins into air-water interface by unbinding its configuration, whereas FS is the potential of thick cohesive layers of protein to surround air bubbles (Damodaran, 1997). Thermal treatments have detrimental effect on foaming properties of legume based foods.
The current findings regarding FC for mungbean are in arrangement with earlier findings of Suliman et al. (2006). For cowpea, the present results are similar to that of Aguilera et al. (2009), they portrayed the value of mentioned attribute as 24%.

Furthermore, Mortuza et al. (2009) observed FC for broad beans as 57.19% that was higher due to variety or cultivar differences. Additionally, Sai-Ut et al. (2009) showed FC as 33% for kidney beans. It was viewed that FS of legume flour blends are in accordance with previous researchers that clarified FS in Ts 68.67% and control 42.12% (Suliman et al., 2006; Mortuza et al., 2009). The foaming property of protein derived from pulses showed influence isolates & concentrates composition, other legume components like polysaccharides and by degree of protein denaturation on processing (Boye et al., 2010a).

In this regard, Alamanou and Doxastakis (1997) depicted higher value of FC for lupin (a protein) obtained via ultrafiltration in contrast to isoelectric precipitation. Furthermore, legume polysaccharides achieved via ultrafiltration get adsorbed in protein molecules forming complexes. Resultantly, stable foam forms due to increase in steric repulsion forces among the adjacent bubbles.

Later, Makri and Doxastakis (2006) worked on protein concentrate derived from dry beans and found its dependence on method and pH condition during extraction. In another study, Pozani et al. (2002) explained that heat treatment may improve foaming ability and stability of protein extracted from legumes. It is associated with denaturation of protein during preparation process (Boye et al., 2010a).

Foam capacity is the measure of protein to slow down coalescence by maintaining air bubbles in suspension. It is defined as the ability to produce foam or dispersion of foam in continuous phase after vigorous shaking in the presence of soluble surfactant. As this property is associated with protein, resultantly it is found higher in cowpea-legume flour blends as compared to wheat sample (Yellavila et al., 2015). Hence, protein enriched flours could be employed in the preparation of light density & aerated food products such as weaning foods.

4.5.3. Emulsion properties

Water or oil holding capacities are elaborated as amount of water or oil bind to protein mass, reflecting protein ability to leak from food product during manufacturing and storage procedures. Protein serves as surfactants, not only involve in forming but also help in stabilizing emulsion by repelling oil droplets via electrostatic forces.
Control=100% wheat flour; T1= 90% wheat flour and 10% cowpea, T2= 80% wheat flour and 20% cowpea; T3=70% wheat flour and 30% cowpea, T4=90% wheat flour and 10% mungbean, T5=80% wheat flour and 20% mungbean; T6=70% wheat flour and 30% mungbean, T7=90% wheat flour, 5% cowpea and 5% mungbean; T8=80% wheat flour, 10% cowpea and 10% mungbean; T9=70% wheat flour, 15% cowpea and 15% mungbean

F Capacity = Foaming Capacity; F Stability = Foaming Stability

**Figure 4.2:** Foaming capacity and stability of composite flour samples (n=3)
The mean values regarding emulsion capacity (EC) and stability (ES) presented significant difference among legume flour blends. Legume composites depicted EC in the range of 51.23 to 75.75% with highest value observed in T8. Moreover, non-significant variations were noticed between cowpea and mungbean. For ES, maximum value was reported in T2 as 53.33% trailed by control 38.38% and T1 36.85%. Though, the lowest value was that of T6 i.e. 33.17% as presented in Figure 4.3. ES of protein indicates antagonistic response to emulsion collapse. Emulsion characteristics of protein vary on the basis of hydrophobic nature, structural stability, molar mass and conditions like pH, temperature & ionic strength. Thermal treatment facilitates protein linkage with non-polar solvents resultantly intensifies emulsion ability of hydrophobic units of proteins.

The T9 value pertaining to EC is in accordance with Suliman et al. (2006) that was 75.3%, whereas control value for the similar trait were between 22.9 and 48.80% according to Aguilera et al. (2009) and Sai-Ut et al. (2009). The ES for legume composite flours is supported by Suliman et al. (2006), Singh et al. (2008), Sai-Ut et al. (2009) and Butt and Batool (2010). These researchers reported 41.4% ES for control whilst, legume flour blends were ranged from 21 to 36.33%. Besides, Makri et al. (2005) viewed enhancement in emulsification with processing time.

Emulsifying capacity is the ability of an emulsifier (protein) to hold maximum extent of discrete phase within the continuous phase, whereas emulsion stability describes the capacity of surface active agents (protein) to hold water and oil phases of emulsion for some time under certain conditions (Makri et al., 2005). Thus, protein in composite flour has the ability to stabilize emulsion by repelling oil droplets, electrostatically. The emulsion capacity increases by incorporating legume flour in wheat flour i.e. rich in protein as viewed in the instant scrutiny. Instead, emulsion stability improves when rigid globular protein structure forms, creating strong cohesive forces to prevent any mechanical deformation. During food processing, the increment in emulsion properties is related with protein functionality i.e. viewed in many of the commercial food products such as cake, coffee whiteners meat products, frozen desserts, mayonnaise and salad dressing. Additionally, flour containing higher emulsion capacity could stabilize emulsions in soups (Adebowale et al., 2005; Chandra et al., 2015). In this study, decrease in emulsion stability could be related with some of the proteins that may get denatured over roasting procedures as supported by A´lvarez et al. (2007).
Figure 4.3: Emulsion capacity and Stability of composite flour samples (n=3)

Control=100% wheat flour; T1= 90% wheat flour and 10% cowpea; T2= 80% wheat flour and 20% cowpea; T3=70% wheat flour and 30% cowpea, T4=90% wheat flour and 10% mungbean, T5=80% wheat flour and 20% mungbean; T6=70% wheat flour and 30% mungbean, T7=90% wheat flour, 5% cowpea and 5% mungbean; T8=80% wheat flour, 10% cowpea and 10% mungbean; T9=70% wheat flour, 15% cowpea and 15% mungbean

E Capacity = Emulsion Capacity; E Stability = Emulsion Stability
4.6. Weaning foods

Infant foods are gaining important, being nutritious and easily accessible & adequate source of energy that could complement their rising needs of the body. The recommendations for optimal weaning foods include; nutrient dense, easy to digest, stable and cost-effective (Bond et al., 2005). Previous researchers highlighted the importance of legumes over cereals in infant formulas for the development of immune responses and physical & mental growth. The nutritional value of infant formulations could be optimized by incorporating multiple nutrients sources to meet the energy needs of the growing body (Asma et al., 2006).

In this milieu, nine complementary foods, varying in cowpea and mungbean proportion were prepared along with control. Afterwards, one treatment from each group of weaning foods was selected on the basis of sensory evaluation along with control. The three weaning foods were tested for proximate analysis, gross energy value, reconstitution index, biological evaluation and infant acceptability trial.

4.7. Sensory evaluation of weaning foods

Sensory evaluation of infant foods is an important aspect to assess the consumer acceptance. The product development is a systematic hedonic criterion based on observation and documentation of product attributes by the selected judges. The sensory evaluation was based on following characteristics: appearance; color & texture, facilitate making of product; smoothness, ease of preparation & thickness and eating acceptability sweetness, taste & flavor. Based on these parameters, overall acceptability by a panel of ten in members was carried out using a 9-point hedonic scale; 1 (minimum score) for extreme disliking to 9 (maximum score) for extreme liking. The mean square values concerning sensory attributes noted momentous variations within the weaning food samples (Table 4.8).

The scores for sensory aspects (Table 4.9) were found to vary from 7.29±0.13 to 7.80±0.25 for color, 6.83±0.16 to 7.44±0.09 for texture, 7.14±0.16 to 7.86±0.10 for smoothness, 6.74±0.17 to 7.64±0.11 for ease of preparation, 7.13±0.21 to 7.63±0.23 for thickness, 7.02±0.11 to 7.67±0.27 for sweetness, 7.14±0.13 to 7.65±0.24 for taste, 7.21±0.11 to 7.82±0.23 for flavor and 7.12±0.17 to 7.92±0.27 for overall evaluation/acceptability.

Infant foods as dry mix showed uniform, light golden appearance; the paste consistency was not affected by the incorporation of legumes. The samples showed decrement with respect to thickness. Likewise, overall evaluation/acceptability among the treatments was varying significantly. Nonetheless, maximum scores were allotted to T8 (10% cowpea+10% mungbean)
in contrast to other treated diets.

Sensory response based on appearance and taste contributes product acceptance (Jaros et al., 2000). Furthermore, the way of presentation also influences the scoring pattern. Consumer acceptance relies on ingredient optimization and nutritional value. Furthermore, sensory perception is imperative to select a weaning food. The selection of weaning food is based on human senses: eyes, nose, tongue and touch that play a combined effect (Bodyfelt et al., 2008).

The results of present assessment are comparable to the verdicts of Olapade et al. (2012) in their trial, the addition of cowpea resulted in significant impact on the sensory scores of the bread. The cowpea flour supplementation @ 10% produced bread with high acceptability.

Indigenous resources and technologies are used to improve protein quality and quantity. Preparation of weaning foods from legumes have numerous positive traits such as favorable consistency, organoleptic acceptability, decrease in bulk, improvement in shelf life, partial or complete exclusion of anti-nutrients, short cooking time and enhanced nutritional worth (Kannan et al., 2001). Conclusively, the present scrutiny explicated systemic scoring for sensory attributes besides some constraints; e.g. the initial scoring was based on adults however infants response was assessed later using infant acceptability test conducted with the help of nursing mothers. The sensory outcomes depicted that legume based infant foods have considerable acceptance owing to tempting taste and look.

4.8. Analysis of selected weaning foods

Based on sensory evaluation W8 which contained mixture of 10% cowpea 10% mungbean scored highest as compared to rest of the treatments. Thus W8 (20% equal mixture of cowpea and mungbean), W2 (20% cowpea), W5 (20% mungbean) were selected for further analyses with new names; W3S, W1S and W2S, accordingly.

4.8.1. Proximate analysis

Mean squares regarding weaning food composition presented non-momentous deviations for crude protein, -fat, -fiber, ash and NFE (Table 4.10). The results obviously portrayed that weaning foods were nutrient dense with crude protein 14.09±0.27 to 17.01±0.19%, crude fat 9.13±0.65 to 11.11±0.36%, crude fiber 1.73±0.26 to 4.57±0.37%, ash 1.23±0.21 to 2.67±0.39% and NFE 63.74±1.02 to 68.26±0.99% (Table 4.11). The compositional analysis of weaning foods explicated ample quantities of desired nutrients to meet the FAO criteria i.e. complementary foods should contain 10-25% of fat, 15% of protein and dietary fiber not
Table 4.8. Mean squares for sensory response of weaning foods

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Color</th>
<th>Texture</th>
<th>Ease of preparation</th>
<th>Smoothness</th>
<th>Thickness</th>
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<td>0.48982**</td>
<td>0.52401**</td>
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</table>

<table>
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<tr>
<th>SOV</th>
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<th>Taste</th>
<th>Flavor</th>
<th>Overall acceptability</th>
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</thead>
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<tr>
<td>Total</td>
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<td></td>
</tr>
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</table>

**Highly significant ($p<0.01$)

*Significant ($p<0.05$)
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<th>Parameter*</th>
<th>W₁</th>
<th>W₂</th>
<th>W₃</th>
<th>W₄</th>
<th>W₅</th>
<th>W₆</th>
<th>W₇</th>
<th>W₈</th>
<th>W₉</th>
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<tr>
<td><strong>Color</strong></td>
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<td><strong>Texture</strong></td>
<td>6.97±0.11</td>
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<td>6.83±0.16</td>
<td>7.36±0.15</td>
<td>6.95±0.11</td>
<td>6.98±0.08</td>
<td>7.41±0.07</td>
<td>7.01±0.12</td>
<td>7.44±0.09</td>
</tr>
<tr>
<td><strong>Ease of preparation</strong></td>
<td>6.92±0.13</td>
<td>7.31±0.15</td>
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<td>7.25±0.19</td>
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<tr>
<td><strong>Smoothness</strong></td>
<td>7.25±0.15</td>
<td>7.51±0.18</td>
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<td>7.26±0.12</td>
<td>7.65±0.22</td>
<td>7.14±0.16</td>
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<td>7.48±0.09</td>
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<tr>
<td><strong>Sweetness</strong></td>
<td>7.12±0.11</td>
<td>7.46±0.22</td>
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<td><strong>Taste</strong></td>
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<tr>
<td><strong>Flavor</strong></td>
<td>7.21±0.11</td>
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<td>7.28±0.23</td>
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<tr>
<td><strong>Overall acceptability</strong></td>
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<td>7.67±0.24</td>
<td>7.12±0.17</td>
<td>7.24±0.12</td>
<td>7.66±0.26</td>
<td>7.14±0.22</td>
<td>7.42±0.13</td>
<td>7.92±0.27</td>
<td>7.39±0.21</td>
<td>7.58±0.24</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a row are statistically alike (n=10)

*Sensory evaluation using Hedonic scale. W₁= Roasted cowpea (10%)+wheat flour (40%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₂= Roasted cowpea (20%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₃= Roasted cowpea (30%)+wheat flour (20%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₄= Roasted mungbean (10%)+wheat flour (40%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₅= Roasted mungbean (20%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₆= Roasted mungbean (30%)+wheat flour (20%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₇= Roasted cowpea & mungbean (5:5%)+wheat flour (40%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₈= Roasted cowpea & mungbean (10:10%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₉= Roasted cowpea (15%)+wheat flour (20%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%); W₁₀= soybean (20%)+wheat flour (30%)+rice flour (13%)+sugar (10%)+vegetable oil (10%)+milk powder (16%)+vitamin-mineral mix (1%).
Currently, Gibbs et al. (2011) stated that legumes and cereals are the commonly employed basic ingredients in the preparation of weaning foods. World widely, animal foods are not preferred owing to economic and religious constraints. In this scenario, multi-mix technology based on composite flour along with whole milk powder is commercially and commonly employed in the designing of infant formulations (Mosha and Vicent, 2004).

The present study results are in parallel to the conclusions achieved by Ikujenlola (2008) and Ikujenlola and Adurotuye (2014). They illustrated that crude protein, -fat, ash, crude fiber and moisture in infant foods were divergent from 7.23 to 17.90, 3.45 to 18.10, 2.0 to 4.25, 2.05 to 5.0 & 4.0 to 9.0%, correspondingly. Previously, protein among different maize and soy flour based weaning foods was ranged from 9.49±0.1 to 19.70±0.4% (Osundahunsi and Aworh, 2002). The current findings are also in close proximity with the work of Modu et al. (2010). They reported that protein, moisture, fat, ash, fiber and carbohydrates in cowpea based supplemented foods were 14.96, 4.70, 4.60, 2.50, 5.60 and 67.64% accordingly.

Combination of legume and cereal based formulation exhibited crude protein from 15.0±0.1 to 16.0±0.3%, crude fiber 3.4±0.5 to 4.0±0.4%, fat 11.0±0.3 to 12.4±0.6%, ash 2.0±0.1 to 2.6±0.2% and carbohydrates 60.0±1 to 60.0±0.6% (Owino et al., 2007). Afterwards, Onabanjo et al. (2008) found comparable results as observed in the instant research. They found mean values for composition (moisture, ash, crude protein, -fat and -fiber) of weaning foods were 4.14, 4.74, 14.58, 10.67 and 2.11%, accordingly.

Later, Munasinghe et al. (2012) formulated three extruded weaning foods with different ratios of brown rice, soybean, mungbean, powdered milk and yoghurt to accomplish the daily requirement of vitamins and minerals for children (1-3 years older). The selected weaning formulations were compared with existing commercial weaning food. They demonstrated crude protein, -fat, ash and crude fiber in weaning foods, ranged from 15.22 to 16.19, 12.38 to 12.48, 1.66 to 1.70 and 0.54 to 0.93% respectively.

Work by Imtiaz et al. (2011) is also in close proximity to the current research, where proximate composition of infant formulations developed from germinated wheat+mungbean flour was analyzed. The moisture, protein, ash, fiber, fat and carbohydrates contents were reported as 5.26, 17.35, 2.60, 1.86, 1.23 and 61.73%, accordingly.
4.8.2. Calorific value

The protein and fat are the key nutrients required for infant formulations to achieve body needs, alleviating malnutrition. The mean squares for energy value (%, per serving and protein & fat) depicted non-momentous variations among the weaning foods (Table 4.12 and 4.14). The energy value for weaning formulations was achieved from combustion, whereas energy obtained from protein and fat was calculated using Atwater’s expression (Passmore and Eastwood, 1986).

Mean values for gross energy were 397.88±21.61, 398.04±22.81, 398.31±28.31, and 398.69±24.02 kcal/100g for W2S (mungbean based food), WC (soy control based food), W1S (cowpea based food) and W3S (cowpea + mungbean based food) samples, respectively. Maximum energy value obtained from fat varied from 127.71±9.49 to 130.16±9.36 kcal/100g trailed by protein from 58.42±5.01 to 58.91±4.38 kcal/100g as described in Table 4.13. Formulations in the current study were suitable for delivering 121.11±11.25, 122.13±9.47, 122.38±7.70 and 123.72 ± 10.08 kcal gross energy per single meal for W2S, WC, W1S and W3S, respectively from which 37.01±3.14 to 37.78±3.63 kcal/100g energy was acquired from fat whilst, protein contributed about 16.48±1.31 to 16.58±1.49 kcal (Table 4.15).

From the current estimation, it was deduced that weaning preparations using protein rich legumes have the potential to meet energy needs. The consumption of meal on daily basis or single serving was adjusted based on infant age or energy need. The current results are in accordance with the findings of Modu et al. (2010), it was estimated that the energy value of cowpea based supplemented weaning food was about 371.80 kcal/100g. Likewise, the energy value of mungbean based weaning food was about 376.60 kcal/100g (Imtiaz et al., 2011).

The calorific value of weaning supplements based on cereal and legume combinations are ranged from 372 to 397 kcal/100g (Baskaran and Bhattacharaya, 2004). The current results are corroborated by the findings of Tizazu et al. (2010), they viewed that calorific value of infant foods varied from 369.85 to 371.93 kcal/100g. In another scrutiny, calorific count of legume, sorghum and oil seed based weaning food was ranged from 405.8-413.2 kcal/100g. It was inferred that weaning formulations in the instant
### Table 4.10. Mean squares for proximate composition of complementary food samples

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<tr>
<th>SOV</th>
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<th>Moisture</th>
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<th>NFE</th>
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<td>1.19081**</td>
<td>4.69450**</td>
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<td>8</td>
<td>0.09123</td>
<td>0.09928</td>
<td>0.1994</td>
<td>0.10329</td>
<td>0.12082</td>
<td>0.6384</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Highly significant (p<0.01)
* = Significant (p<0.05)

### Table 4.11. Proximate analysis (%) of weaning foods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>W1S</th>
<th>W2S</th>
<th>W3S</th>
<th>WC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3.10±0.14b</td>
<td>4.58±0.24a</td>
<td>4.20±0.35a</td>
<td>3.82±0.41ab</td>
</tr>
<tr>
<td>Crude protein</td>
<td>16.06±0.45b</td>
<td>15.16±0.30c</td>
<td>17.01±0.19a</td>
<td>14.09±0.27d</td>
</tr>
<tr>
<td>Crude fat</td>
<td>9.13±0.65b</td>
<td>10.15±0.28ab</td>
<td>9.25±0.41b</td>
<td>11.11±0.36a</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.22±0.41b</td>
<td>1.73±0.26b</td>
<td>2.02±0.21b</td>
<td>4.57±0.37a</td>
</tr>
<tr>
<td>Ash</td>
<td>1.23±0.21c</td>
<td>2.56±0.36ab</td>
<td>1.69±0.40bc</td>
<td>2.67±0.39a</td>
</tr>
<tr>
<td>NFE</td>
<td>68.26±0.99a</td>
<td>65.82±0.27b</td>
<td>65.84±1.34ab</td>
<td>63.74±1.02c</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a row are statistically alike (n=3)
W_S = Selected weaning food
W1S = Cowpea based weaning food; W2S=Mungbean based weaning food; W3S=Cowpea+mungbean based weaning food; WC =soy based weaning food
Table 4.12. Mean squares for percent calorific value of weaning food samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Gross energy</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning foods</td>
<td>3</td>
<td>0.377&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>3.149&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.1277&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>591.42</td>
<td>106.69</td>
<td>20.05</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>ns</sup> Non-significant (p≥0.05)

Table 4.13. Percent calorific value of weaning food samples

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>Gross energy (kcal/100g)</th>
<th>Fat (kcal/100g)</th>
<th>Protein (kcal/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W&lt;sub&gt;1S&lt;/sub&gt;</td>
<td>398.31±28.31</td>
<td>128.94±10.38</td>
<td>58.65±3.48</td>
</tr>
<tr>
<td>W&lt;sub&gt;2S&lt;/sub&gt;</td>
<td>397.88±21.61</td>
<td>127.71±9.49</td>
<td>58.42±5.01</td>
</tr>
<tr>
<td>W&lt;sub&gt;3S&lt;/sub&gt;</td>
<td>398.69±24.02</td>
<td>129.38±11.89</td>
<td>58.91±4.38</td>
</tr>
<tr>
<td>W&lt;sub&gt;C&lt;/sub&gt;</td>
<td>398.04±22.81</td>
<td>130.16±9.36</td>
<td>58.56±4.88</td>
</tr>
</tbody>
</table>

(n=3)

W<sub>S</sub>= Selected weaning food
W<sub>1S</sub>= Cowpea based weaning food; W<sub>2S</sub>=Mungbean based weaning food; W<sub>3S</sub>=Cowpea+mungbean based weaning food; W<sub>C</sub>=soy based weaning food
Table 4.14. Mean squares for per meal calorific value of weaning food samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Gross value</th>
<th>Fat</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning foods</td>
<td>3</td>
<td>3.46&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.303&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.005&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>94.28</td>
<td>12.45</td>
<td>2.069</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>ns</sup> Non-significant (p≥0.05)

Table 4.15. Per meal calorific value of weaning food samples

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>Gross value (kcal)</th>
<th>Fat (kcal)</th>
<th>Protein (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W₁S</td>
<td>122.38±7.70</td>
<td>38.69±2.83</td>
<td>16.61±1.21</td>
</tr>
<tr>
<td>W₂S</td>
<td>121.11±11.25</td>
<td>38.53±3.89</td>
<td>16.63±1.68</td>
</tr>
<tr>
<td>W₃S</td>
<td>123.72±10.08</td>
<td>37.44±3.49</td>
<td>16.58±1.49</td>
</tr>
<tr>
<td>W₅</td>
<td>122.13±9.47</td>
<td>37.78±3.63</td>
<td>16.50±1.37</td>
</tr>
</tbody>
</table>

(n=3)
Wₖ= Selected weaning food
W₁S= Cowpea based weaning food; W₂S=Mungbean based weaning food; W₃S=Cowpea+mungbean based weaning food; W₅=soy based weaning food
investigation are in harmony with the strategies of FAO/WHO to achieve energy needs (Asma et al., 2006).

Conclusively, the estimation of end product is very important, as it gives an idea that what we are eating and how this is going to impact on our overall health. Numerous ingredients are employed in the making of weaning foods for instance wheat flour, soy/cowpea/mungbean flour, rice flour, milk powder, sugar, vegetable oil and multivitamins mixture. Thus, compositional analysis of end product gives estimation that either each component of the resultant foods is fulfilling RDA/calorific count of a specific group or not.

4.8.3. Reconstitution index

The idea about reconstitution index is the ease for preparing weaning foods. The pre-processed infant foods are considered instant that can be prepared using warm water without pro-longed cooking. Mean squares in Table 4.16 portrays momentous differences in reconstitution index of numerous legumes based weaning foods.

Mean values (Table 4.17) for the said trait were 56.56±0.83, 55.44±0.86 and 54.36±0.75 mL for W3S, W1S and W2S, correspondingly while 53.83±0.74 mL for control. The weaning formulations in the current study were prepared via drum drying that influence the physicochemical behavior, improving reconstitution index of the finished product. The resultant formulations were having uniformity, resultantly facilitate spoon feeding to infants (FAO/WHO, 2006).

Earlier study revealed that steam blanching to maize flour improve reconstitution index of weaning foods (Adeyemi et al., 1988). Moreover, the reconstitution index of cereal-legume mixture ranged between 60 and 80 mL (Sanni et al., 2001). Additionally, fermented cereals-soy based weaning foods possess reconstitution index of 46 to 56 mL (Onilude et al., 1999).

4.8.4. Viscosity

The viscosity is responsible for acceptability of final product. Furthermore, pasting properties or consistency is preferred in baby food formulations in contrast to watery mouthfeel in less gummy infant foods. Mean squares in terms of complementary foods indicated non-momentous variations as shown in Table 4.16. The mean values for viscosity were 1.97±1.10, 1.97±1.11, 1.98±1.10 and 1.99±1.11 Pa.s for Wc, W2S, W1S and W3S, correspondingly.
The mothers usually favor ease in spoonable feeding to their infants, ranged between 1000 and 3000 cP. The increase in viscosity ultimately reduces the amount consumed by infants; lower the viscosity; lesser the reconstituted prototype bulk (Mosha and Svanberg, 1993). The low viscosity of fruit based weaning food results in less swelling of starch (Bukusuba et al., 2008).

Typically, less consistency and low caloric infant porridges were fed to infants via bottles that may lead to poor growth and development. In contrary, thick, energy dense porridges were administered via spoon that is also safer from diarrheal infections beyond meeting energy requirements (Islam et al., 2008).

The starch is regarded as the basic ingredient in the preparation of weaning foods, this starch may face numerous alteration during processing or paste formation. The improvement in nutritional value along with proper viscosity is the desired features in the development of complementary foods (Suhasini and Malleshi, 2003). The present study results are supported by Zanna and Milala (2004), they found the said attribute to be varying between 1630 and 2056 cP for cowpea based formulation. Furthermore, viscosity of sorghum based weaning formulation was in the range of 2700-2900 cP (Chakravarthi and Kapoor, 2003; Lalude and Fashakin, 2006).

Earlier study demonstrated that viscosity varies from low to high and thin to thick. The high viscosity of amylose rich porridge was energy dense but indigestible, whereas thin porridge was found as poor energy sources unable to fulfill the energy needs of the infants (Stephenson et al., 1994).

A former study based on complementary feeding was performed involving many developing countries like India, Tanzania and Peru. The study concluded that alterations in viscosity of energy rich weaning foods unable to improve energy value because of digestibility issues. Furthermore, the study suggested that by incorporating oil or peanut butter type matrix to weaning foods is a better option to fulfill the desired energy needs @ 4.18 kJ/g but compromises the stability of weaning foods on the other hand (Paul et al., 2008).

4.8.5. Bulk density (loose and packed)

The bulk density determines mass: volume i.e. inversely related to size of particle thereby influences on packing design. The increase in bulk density, the greater the packing advantage as more amount of flour could be packed in a constant volume. In infant
Table 4.16. Mean Squares for reconstitution index and viscosity of weaning food samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Reconstitution index (mL)</th>
<th>Viscosity (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning foods</td>
<td>3</td>
<td>4.39668*</td>
<td>0.00029ns</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.9675</td>
<td>0.01130</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = Non-significant (p≥0.05)
* = Significant (p<0.05)

Table 4.17. Reconstitution index and viscosity of weaning food samples

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>Reconstitution index (mL)</th>
<th>Viscosity (Pa.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1S</td>
<td>55.44±0.86(^a)</td>
<td>1.98±1.10</td>
</tr>
<tr>
<td>W2S</td>
<td>54.36±0.75(^ab)</td>
<td>1.97±1.11</td>
</tr>
<tr>
<td>W3S</td>
<td>56.56±0.83(^a)</td>
<td>1.99±1.11</td>
</tr>
<tr>
<td>Wc</td>
<td>53.83±0.74(^b)</td>
<td>1.97±1.10</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a column are statistically alike (n=3)

WS= Selected weaning food
W1S= Cowpea based weaning food; W2S=Mungbean based weaning food; W3S=Cowpea+mungbean based weaning food; Wc=soy based weaning food
### Table 4.18. Mean squares for loose and packed bulk density of weaning food samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Bulk density (loose)</th>
<th>Bulk density (packed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning foods</td>
<td>3</td>
<td>494.88(^{ns})</td>
<td>3231.30(^{ns})</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>2318.98</td>
<td>1771.99</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{ns}\) Non-significant (\(p \geq 0.05\))

### Table 4.19. Loose and packed Bulk density of weaning food samples

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>Bulk density (loose) (kg/m(^3))</th>
<th>Bulk density (packed) (kg/m(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(W_{1S})</td>
<td>510.11±27.95</td>
<td>570.30±27.37</td>
</tr>
<tr>
<td>(W_{2S})</td>
<td>540.05±34.83</td>
<td>590.14±28.43</td>
</tr>
<tr>
<td>(W_{3S})</td>
<td>590.27±37.71</td>
<td>660.23±29.37</td>
</tr>
<tr>
<td>(W_{C})</td>
<td>460.19±11.54</td>
<td>520.08±18.62</td>
</tr>
</tbody>
</table>

\(n=3\)

\(W_{6}=\) Selected weaning food
\(W_{1S}=\) Cowpea based weaning food; \(W_{2S}=\) Mungbean based weaning food; \(W_{3S}=\) Cowpea+mungbean based weaning food; \(W_{C}=\) Soy based weaning food
feeding formulas, low bulk density is desirable \textit{i.e.} characterized by low protein and moisture content (David \textit{et al.}, 2007). In this regard, \( W_C \) showed minimum moisture and protein content thus lower bulk density.

Among the developing states, cereal or starch based weaning formulations are commonly used. The bulk density is thought as a critical parameter because it depicts the suitability of such foods for infants in contrast to dry mixes. Mean squares regarding this parameter inferred non-momentous variations within the treatments (Table 4.18). However, means values Table 4.19 for packed and loose density were ranged from 460.19±11.54 to 520.08±18.62 and 590.27±37.71 to 660.23±29.37 kg/m\(^3\), correspondingly. The bulk density of the current study prepared legume based infant foods is in agreement with the bulk density found by Mitzener \textit{et al.} (1984) \textit{i.e.} 0.75 g/mL.

4.9. Bioassessment of the selected weaning foods

The nutritive value of legumes based infant foods were assessed for protein quality involving rats (Sprague Dawley). The animal models are an affirmative approach to test the treated diets; cowpea, mungbean and their blend against soybean as standard diet. Intentionally, growth parameters; Protein Efficiency Ratio (PER), Net Protein Ratio (NPR) and Relative Net Protein Ratio (RNPR), whereas nitrogen balance based on true digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) were assessed.

4.9.1. Growth parameters

Mean squares in Table 4.21 for growth parameters \textit{i.e.} PER, NPR and RNPR presented momentous variations amongst the tested diets including cowpea, mungbean and their blends, compared against standard diet.

4.9.2. Protein efficiency ratio (PER)

Protein plays an important role in growth, development & maintenance and a source of energy. The proteins from dietary sources get converted to amino acids resultantly absorbed within the cells. Maximum PER amongst the tested diets was observed in \( W_C \) (2.84±0.36), trailed by \( W_{38} \) (2.65±0.20) and \( W_{15} \) (2.28±0.10). Nonetheless, the minimum value for PER was 2.22±0.14 in \( W_{25} \). The rats fed on legume based complementary foods showed better growth. The present results displayed obvious up-regulation in PER of diets designed by soy and cowpea+mungbean blend.
**Table 4.20. Mean squares for growth and nitrogen balance study parameters of experimental diets**

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>PER</th>
<th>NPR</th>
<th>RNPR</th>
<th>TD</th>
<th>BV</th>
<th>NPU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning</td>
<td>3</td>
<td>0.44146**</td>
<td>12.505**</td>
<td>770.271**</td>
<td>391.370**</td>
<td>604.81**</td>
<td>134.687**</td>
</tr>
<tr>
<td>foods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>0.02</td>
<td>0.0598</td>
<td>23.913</td>
<td>22.147</td>
<td>20.828</td>
<td>19.08</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Highly significant (p<0.01)*

**Table 4.21. Growth study parameters of experimental diets**

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>PER</th>
<th>NPR</th>
<th>RNPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>W₁S</td>
<td>2.28±0.10b</td>
<td>3.11±0.13b</td>
<td>77.07±4.54c</td>
</tr>
<tr>
<td>W₂S</td>
<td>2.22±0.14b</td>
<td>2.95±0.21b</td>
<td>68.66±4.04c</td>
</tr>
<tr>
<td>W₃S</td>
<td>2.65±0.20a</td>
<td>3.34±0.26b</td>
<td>86.25±5.08b</td>
</tr>
<tr>
<td>W₃C</td>
<td>2.84±0.36a</td>
<td>6.28±0.34a</td>
<td>97.57±5.74a</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a column are statistically alike (n=5)

W₁S = Cowpea based weaning food; W₂S=Mungbean based weaning food; W₃S=Cowpea+mungbean based weaning food; W₃C=soy based weaning food

**Table 4.22. Nitrogen balance study parameters of experimental diets**

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>TD (%)</th>
<th>BV (%)</th>
<th>NPU (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W₁S</td>
<td>76.82±3.46bc</td>
<td>79.05±3.59b</td>
<td>75.02±2.24bc</td>
</tr>
<tr>
<td>W₂S</td>
<td>70.07±3.96c</td>
<td>65.97±3.44c</td>
<td>70.11±2.47c</td>
</tr>
<tr>
<td>W₃S</td>
<td>80.31±3.54b</td>
<td>85.09±4.52ab</td>
<td>82.67±2.15ab</td>
</tr>
<tr>
<td>W₃C</td>
<td>91.25±5.45a</td>
<td>91.84±5.08a</td>
<td>89.37±3.63a</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a column are statistically alike (n=5)

W₁S = Selected weaning food
W₂S = Cowpea based weaning food; W₃S=Mungbean based weaning food; W₃S=Cowpea+mungbean based weaning food; W₃C=soy based weaning food
PER is related with essential amino acids or digestibility of these amino acids by humans or test animals. Currently, biological markers are widely studied in nutrition based projects. FAO/WHO has recommended to measure protein quality in order to reach nutritional value of legumes. Becker (2007) measured PER for short time in tested animals by measuring gain in weight per unit of protein. It estimates protein in weaning formulations (Gropper et al., 2005).

Furthermore, bioassessment of protein containing legume based infant foods is vital to determine the nutritional worth of the food. Growth based study is a promising approach to appraise the nutritive value of protein for human use (Gigliotti et al., 2008). The outcomes of recent study concerning PER of W1S and W3S are in accord to the study conducted by Oluwatufunmi et al. (2015) on MAC (maize and cowpea based weaning food) and MABC (maize, bambara and cowpea based weaning food), that revealed PER values as 2.30 and 2.42, respectively.

4.9.3. Net protein ratio (NPR) and Relative net protein ration (RNPR)

NPR measures the dietary protein intake. WC displayed maximum NPR value as 6.28±0.34 in contrast to 3.34±0.26, 3.11±0.13 and 2.95±0.21 for W3S, W1S and W2S, correspondingly. However, soy protein diets showed increase in growth as compared to tested legume based complementary diets (Table 4.20).

The RNPR is a comparable attribute to NPR but outcomes are stated as ratio between NPRs of test protein to that of standard reference protein i.e. soy regarded as 100 (Table 4.20). The RNPR of legume rich weaning foods were ranged from 68.66±4.04 to 97.57±5.74 in comparison to soy.

The present investigation concerning W1S are in agreement to the work done by Wilmot et al. (2001), they found NPR as 3.3 of cowpea soy+maize+amylase based infant formulation. Furthermore, NPR of cowpea soy+toasted maize weaning formulations presented NPR as 3.3.

4.9.4. Nitrogen balance study

Mean squares regarding nitrogen balance involving TD, BV and NPU demonstrated momentous differences amongst the legume based weaning foods (Table 4.21).
4.9.4.1. True Digestibility (TD)

The TD determined using feed intake, dried animal bodies and urinary & fecal nitrogen. Amongst the experimental diets, maximum TD value was that of WC 91.25±5.45% trailed by W3S, W1S and W2S as 80.31±3.54, 76.82±3.46 and 70.07±3.96%, correspondingly (Table 4.22).

Earlier researchers stated that improvement in digestibility identifies decrease in fecal nitrogen along with higher nitrogen retention in the dried rat bodies. The minimum TD in W2S might be due to loss of protein while processing that ultimately alters textural characteristics. This hinders enzymatic digestion ultimately lowers TD value in the tested animals. The digestibility of protein relies on limiting and essential amino acids present in protein based sample (Mepba and Achinewhu, 2003).

The outcomes of the current study regarding TD of W1S are also endorsed by Oluwatufunmi et al. (2015), they demonstrated that TD for cowpea+maize based weaning formulation was 89.59%. In W3S, they found TD as 92.15% in maize+Bambara+cowpea based infant preparations. Additionally, skim milk+plantain+cowpea based weaning foods presented 71.40% TD value.

4.9.4.2. Biological value (BV)

It estimates the ability of protein to support growth by retaining nitrogen in the body. It assesses protein absorption from food and becomes the part of body. Mean values inferred maximum BV by WC 91.84±5.08%, whereas values noted for W3S, W1S and W2S were 85.09±4.52, 79.05±3.59 and 65.97±3.44%, correspondingly. However, soy based standard weaning formulas presented BV as 91.84±5.08% (Table 4.22).

Thermal treatment improves the accessibility of protein as obvious from bioindicators assessed in the present study. The BV for W3S was higher than other counterparts owing to optimal protein digestibility. These determinants are linked with digestion & absorption of protein therefore affects the quantity of dietary nitrogen wasted during digestion through feces.

Furthermore, cellular bioactivity and bioavailability of amino acids influences BV. The findings regarding instant investigation of W3S are in close proximity to the study carried out by Oluwatufunmi et al. (2015), they revealed BV as 97.18% in cowpea+maize based infant foods.
4.9.4.3. Net protein utilization (NPU)

Means concerning NPU in Table 4.22 reported maximum value as 89.37±3.63% for Wc, followed by 82.67±2.15, 75.02±2.24 and 70.11±2.47% for W3S, W1S and W2S, correspondingly. NPU gives an indication of protein and BV of the amino acids bioavailable from food (Marrion et al., 2003; Becker, 2007). It determines the retention of tested protein stated as percent nitrogen absorption. The low value of NPU was that of W2 might be due to increase in excretion and relative suppression in retention of nitrogen in the body. The NPU value for W3S is in harmony with the findings of Oluwatufunmi et al. (2015), they elucidated the said parameter as 86.44% in cowpea+maize based weaning food. The value of W1S is correlated with results mentioned by Olapade et al. (2015), they indicated NPU as 75.38% in plantain+cowpea based weaning formulation. In conclusion, growth and nitrogen balance presented that legume protein possesses good nutritive value i.e. considered beneficial for weaning food preparation.

The nutrients provision or relative proportion in infant foods is considered crucial for growth. Semi-solid foods are introduced after six months in order to meet the basic nutritional requirements of infants with progression in their age. At this age, the breast milk is unable to fill up the gap of rising energy and nutrient needs. In order to meet these needs, fruit & vegetables and staple cereal based semi-solid foods are considered. Furthermore, multiple ingredients with different moieties and varied protein & energy value are optimally added to achieve healthy immune system and required body mass (Vadivel and Pugalenthi, 2010).

In the present study, protein quality aspects i.e. PER, NPR, RNPR, TD, BV and NPU were tested to incorporate various ingredients in weaning foods. The weaning food should be comprised of adequate essential amino acids to improve the health of pre-schoolers. Previously, in vivo protein digestibility of cowpea, mungbean and their blends was found higher than other legumes. Additionally, these sources are cost-effective, available and substitute of animal protein thereby these sources should be utilized to curtail prevailing PEM in the country.

4.10. Infant acceptability test

Besides growth and health, weaning food acceptance involving infants is crucial as it estimates behavioural response, feeding frequency and liking & disliking based on facial
expressions that could normally be noted by the nursing mothers (Schwartz et al., 2011).

Means squares regarding infant acceptability test displayed non-momentous variations in weaning foods, whereas evident fluctuations were observed during study period (Table 4.23). Considering evaluation, 5-point hedonic scale carrying questionnaire (Appendix-II) regarding infant acceptability by involving nursing mothers was previously described by Mosha and Vincent (2004).

The scores allocated to prepared weaning foods by nursing mothers considering the infants behaviour showed variance from 3.04±0.42 to 3.22±0.23 at initiation of the study plan while 3.24±0.28 to 3.45±0.21 on 5th day, 3.43±0.26 to 3.68±0.25 on 10th day and 3.77±0.34 to 4.07±0.38 on 15th day in W1S, W2S, W3S and WC, accordingly (Table 4.24). The significant response was observed for infant acceptability test throughout the study period. Overall acceptability lagged behind as 3.15±0.08 at start and reached to 3.98±0.06 (maximum value) at the termination of the study done (Table 4.25). Conclusively, the overall acceptability showed an inclining trend with the passage of time, this is because infants were adapted to their respective weaning food.

The rise in acceptability showed that taste was acceptable to infants as scores followed a linear increment as the time passes. Furthermore, no deleterious impact was noticed in infants during the trial.

The frequency of consumption of infants gives a better picture regarding sensory response results (Temelli et al., 2004). The short term infant foods could be altered based on liking/disliking for newly designed food.

Previously, Birch et al. (1998) measured that infant acceptability augments if they consume weaning foods frequently or give positive facial expressions during adaptation period. Similarly, Maier et al. (2007) demonstrated that infant acceptability enhances if the foods are having better paste stickiness in contrast to thicker gruels (Opendi and Muyonga, 1999).

Conclusively, texture and taste are regarded as the main determinants for infant acceptance for newly developed food. It is anticipated that new food acceptance depends mainly on taste of the targeted population. Furthermore, socio-economical issues also influence the infant acceptability and sustainability regarding weaning foods. Globally, weaning foods are the markers for infant health thus social infrastructure aneed to be
Table 4.23. Mean squares for infant acceptability scores of weaning food samples

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Infant acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning foods</td>
<td>3</td>
<td>0.43307&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Period (days)</td>
<td>3</td>
<td>4.85676**</td>
</tr>
<tr>
<td>Interaction (weaning food x days)</td>
<td>9</td>
<td>0.02828&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Error</td>
<td>144</td>
<td>0.29299</td>
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<tr>
<td>Total</td>
<td>159</td>
<td></td>
</tr>
</tbody>
</table>

*ns* Non-significant (*p*≥0.05)
**Highly significant (*p*<0.01)

Table 4.24. Infant acceptability scores of weaning food samples

<table>
<thead>
<tr>
<th>Weaning foods</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; Day</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Day</th>
<th>10&lt;sup&gt;th&lt;/sup&gt; Day</th>
<th>15&lt;sup&gt;th&lt;/sup&gt; Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>W&lt;sub&gt;1&lt;/sub&gt;S</td>
<td>3.18±0.31</td>
<td>3.29±0.15</td>
<td>3.58±0.21</td>
<td>4.03±0.21</td>
</tr>
<tr>
<td>W&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>3.07±0.42</td>
<td>3.24±0.28</td>
<td>3.43±0.26</td>
<td>3.77±0.34</td>
</tr>
<tr>
<td>W&lt;sub&gt;3&lt;/sub&gt;S</td>
<td>3.22±0.23</td>
<td>3.45±0.21</td>
<td>3.65±0.27</td>
<td>4.07±0.38</td>
</tr>
<tr>
<td>W&lt;sub&gt;C&lt;/sub&gt;</td>
<td>3.19±0.28</td>
<td>3.45±0.19</td>
<td>3.68±0.25</td>
<td>4.06±0.26</td>
</tr>
</tbody>
</table>

(n=10)
W<sub>S</sub>=Selected weaning food
W<sub>1</sub>S=Cowpea based weaning food; W<sub>2</sub>S=Mungbean based weaning food; W<sub>3</sub>S=Cowpea+mungbean based weaning food; W<sub>C</sub>=soy based weaning food

Table 4.25. Infant acceptability during the study period

<table>
<thead>
<tr>
<th>Day</th>
<th>Infant acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>3.15±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.36±0.10&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>10&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.58±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15&lt;sup&gt;th&lt;/sup&gt;</td>
<td>3.98±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means sharing the same letter in a column are statistically alike (n=10)
considered to introduce such healthy versions amongst the developing economies. Lastly, multiple dietary sources, predominantly indigenous legumes should need to be re-introduced to address the malnutrition mediated challenges, faced by Pakistan.
CHAPTER 5

SUMMARY

Amongst the developing states, large population segments, predominantly infants and children under-five are suffering from protein-energy insufficiencies hence considered as the most susceptible segments of the population. Recent nutritional surveys have clearly demonstrated a wide gap between protein demand and supply. In order to overcome the threat, there is a dire necessity to explore new protein sources to bridge the growing gap with the progression in age. Accordingly, numerous studies are being conducted to measure the ability of legumes as an efficient source of dietary protein. In the current study, development and bioassessment of weaning foods using varied indigenous legumes i.e. cowpea and mungbean were carried out. Based on sensory response, the best selected weaning foods were further evaluated via animal study.

Proximate profiling measures the nutritive value or varied components of the food. The compositional analysis of mungbean and cowpea showed that moisture content was 5.93±0.14 and 3.37±0.05%, respectively. Crude protein, fat & fiber contents in cowpea were higher (23.10±0.66, 2.05±0.07 & 3.57±0.08%) than mungbean (22.45±1.04, 1.34±0.03 & 3.07±0.07%), accordingly. However, ash content was higher in mungbean 4.02±0.04% than cowpea 3.35±0.07%. The mineral assessment showed that potassium, calcium, sodium, iron and zinc were pronouncedly higher 28.19±0.89, 387.51±14.11, 61.20±4.13, 78.33±2.52 & 45.75±1.05 mg/100g in cowpea in contrast to mungbean 10.46±0.48, 7.55±0.37, 5.31±0.81, 1.28±0.07 & 2.17±0.14 mg/100g, respectively.

The anti-nutrients in legumes are responsible for hindering the bioavailability of numerous micronutrients. In contrast, the processing procedures like roasting have the potential to dissociate phytate complex, alongside down-regulates the activities associated with hemagglutinin-lectin and trypsin inhibitor, resultanty overcome the anti-nutritional aspects. In this context, the phytates presented significant decrement to 4.48±0.36 mg/g in roasted cowpea and 1.88±0.06 mg/g in roasted mungbean as compared to raw equivalents; 13.59±0.54 and 6.51±0.82 mg/g, respectively. Furthermore, the hemagglutinin lectin activity was decreased momentarily from 0.61±0.03 (raw) to 0.19±0.01 Hg/mg in roasted cowpea and 2.74±0.09 (raw) to 0.59±0.02 Hg/mg in roasted mungbean, whereas trypsin inhibitor activity demonstrated obvious variations among values as 7.53±0.18 & 1.52±0.25 TIU/mg in raw & heat-treated cowpea and 18.35±1.12 & 4.22±0.41 TIU/mg in raw & heat-
treated mungbean, correspondingly. In order to assess the effect of processing conditions, the compositional analysis was carried out for various composite flour treatments; control (100 wheat flour), T1 (90 wheat flour & 10% cowpea), T2 (80 wheat flour & 20% cowpea), T3 (70 wheat flour & 30% cowpea), T4 (90 wheat flour & 10% mungbean), T5 (80 wheat flour & 20% mungbean), T6 (70 wheat flour & 30% mungbean), T7 (90 wheat flour +5% cowpea & 5% mungbean), T8 (80 wheat flour + 10% cowpea & 10% mungbean) and T9 (70 wheat flour +15% cowpea & 15% mungbean). All the compositional characteristics indicated significant difference with respect to treatments. The maximum moisture content was reported in control (11.32%) whilst, lowest in T6 (7.98%). The maximum crude protein and -fat was found in T3 as 14.23 and 1.05% as compared to minimum in control (10.43 and 0.91%), respectively. Furthermore, the range for crude fiber was from 0.53 to 0.44% and ash content varied from 1.07 to 0.65%. In addition, NFE was ranged lowest (T4) to highest (T2) as 72.87 to 77.44%, correspondingly.

The functional properties including water & oil holding capacity, emulsification and foaming stability of cereal-legume or protein enriched flour blends play an imperative part towards the textural quality of the end products. In this connection, the functional attributes responded momentously regarding treatments. The maximum water & oil absorption capacities were attributed by T3 1.44 and T8 1.16 mL/g while minimum values were noted in control (1.07 & 0.91 mL/g), respectively. The tested composite flours exhibited better emulsion capacity & -stability with maximum value achieved by T8 (75.75 & 38.75%) while minimum by control (51.23 & 18.15%), accordingly. Similarly, maximum foaming stability & -capacity was recorded in T8 (68.67 & 38.75%), whereas lowest in control (42.12 & 18.15%), respectively. Briefly, the resultant legume based weaning foods depicted uniform texture, light golden color and better paste consistency.

Sensory quality of food is one of the important elements to assess the consumer likeness and acceptability. In the present study, statistical inferences differed significantly within the weaning foods, considering traits; color, texture, ease of preparation, smoothness, thickness, sweetness, taste, flavor and overall acceptability. Furthermore, scores for sensory response of all treatments remained within acceptable limits showing their suitability for industrial application. However, maximum scores were attained by W8 (7.92±0.27) followed by W2 (7.67±0.24) and W5 (7.66±0.26) for overall acceptability. Resultantly, these three treatments were selected, renamed as W1S (20% cowpea), W2S
(20% mungbean) & W3S (10% cowpea & 10% mungbean) and further tested against already existing soy based weaning food prototype (control; Wc).

The final product analyses give a better understanding regarding its nutritional worth and impact on overall health status. The mean squares pertaining to proximate compositional attributes indicated significant variations as a function of treatments. The moisture showed variation from 3.10±0.14% (W1S) to 4.58±0.24% (W2S), crude protein 14.09±0.27% (Wc) to 17.01±0.19% (W3S), crude fat 9.13±0.65% (W1S) to 11.11±0.36% (Wc), crude fiber 1.73±0.26% (W2S) to 4.57±0.37% (Wc), ash 1.23±0.21% (W1S) to 2.67±0.39% (Wc) and NFE 63.74±1.02 (Wc) to 68.26±0.99% (W1S). The mean squares relating gross energy value, viscosity and bulk density exhibited non-significant variations among the samples, whereas reconstitution index were varying significantly with values reported as 55.44±0.86, 54.36±0.57, 56.56±0.83 and 53.83±0.74 mL for W1S & W2S, W3S & Wc respectively.

Parameters associated with protein bioassessment were affected momentously via tested diets using Sprague Dawley rats as model animals. Regarding growth parameters, protein efficiency ratio (PER), net protein ratio (NPR) & relative net protein ratio (RNPR) were viewed maximum in Wc 2.84±0.36, 6.28±0.34 & 100 followed by W3S 2.65±0.20, 3.34±0.26 & 53.18, whereas lowest was recorded in W2S 2.22±0.10, 2.95±0.21 & 46.97, correspondingly. Similar trend was viewed in nitrogen balance study, true digestibility (TD), biological value (BV) and net protein utilization (NPU) were highest in Wc 91.25±5.45, 91.84±5.08 & 89.37±3.63% trailed by W3S as 80.31±3.54, 85.09±4.52 and 82.67±2.15% while minimum was found in W2S 70.07±3.96 65.97±3.44 & 70.11±2.47%, accordingly.

The acceptability of prepared infant foods was assessed via short term feeding, involving nursing mothers. The acceptability scores were in the range of 3.18 to 4.07 out of total five hedonic score (maximum) during day 1 to 15.

It is assumed from the current exploration that the weaning food containing cowpea and mungbean, each @ 10% (W3S) presented better with respect to sensory response, growth study parameters and nitrogen balance study hence it could be employed in weaning food formulations, effectively. In the nutshell, information obtained from the current research is beneficial for researchers to comprehend compositional, functional and nutritional aspects.
of legume based weaning foods.

The cereal and legumes, cultivated in Pakistan have splendid nutritional worth to improve infant nutrition besides the prevention of morbidities. Thus, these crops should be utilized to formulate nutritious, acceptable and cost-effective complementary foods as these have clearly demonstrated their favorable comparison with already existing proprietary weaning foods in terms of protein-energy value and growth & nitrogen balance study. Furthermore, roasting ensures optimal provision of nutrients along with significant decrement of anti-nutrients to tolerable limits in flour blends for the preparation of weaning foods. The quantitative analyses of the study could be used to update the existing dietary guidelines. For future works, it is suggested that the resultant formulated foods should be assessed for the cooking-nutrient and cooking-shelf stability linkages along with bioavailability studies. Additionally, it gives the food manufacturers a new path for improving the already available food formulas on the basis of acceptability tests as taste is the foremost priority for successful marketing of novel foods. Domestically, such studies would go a long way in guiding mothers to prepare nutritious & healthy weaning foods for their babies from locally exiting cereals and legumes with far less cost as that of commercially available formulas.
RECOMMENDATIONS

- Legumes *i.e.* cowpea and mungbean should be incorporated in weaning formulations to meet the protein needs of the infants

- Besides cowpea and mungbean, the feasibility of using other low cost and high protein quality legumes in waning foods should be investigated to overcome protein-energy malnutrition

- A detailed shelf-life study of the newly developed prototype must be conducted to emphasize the commercial significance

- Pre-soaking of cowpea and mungbean under proper conditions need to be probed to overcome flatulence in association with stachyose and raffinose

- In addition to roasting procedure, other innovative methods to reduce anti-nutritional factors for the preparation of weaning foods must be explored

- Appropriate packaging must be assessed to ensure minimal alterations in the physicochemical and sensory characteristics of waning foods

- A cost analysis of legume based weaning foods should be conducted in comparison to traditional weaning foods to ascertain economic benefits

- Therapeutic efficacy of indigenous legumes ought to be probed to address the prevailing malnutrition amongst the infants and children under-five

- Nutrition support programs involving dieticians should be launched to create awareness regarding legume based weaning foods amongst the low-income countries

- Community based trials should be conducted for further evidence generation
LITERATURE CITED


Mitzener, K., N. Scrimshaw and R. Morgan. 1984. Improving the nutritional status of children during the weaning period. HOVIPREP (Home and village prepared weaning foods).


## Appendix-I

### Weaning food formulations (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W5</th>
<th>W6</th>
<th>W7</th>
<th>W8</th>
<th>W9</th>
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<td>Roasted mungbean</td>
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<td>20</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Roasted cowpea+mungbean</td>
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<td>-</td>
<td>5+5</td>
<td>10+10</td>
<td>15+15</td>
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</table>
Appendix-II

Sensory evaluation of legume based weaning food

Directions

Take the samples one by one in your mouth and evaluate them for the following parameters on Hedonic scale. Hedonic scale is given at the end of this form. It is very important to rinse your mouth thoroughly with distilled water before taking each sample in your mouth.

Name of Judge: ________________________________________

Age/Sex: ______________________________________________

Signature: ______________________________________________

Date: _________________________________________________

Sensory evaluation performa of legume based weaning food

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>$T_2$</th>
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<td>Overall acceptability</td>
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</tbody>
</table>

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
Appendix-III

Performa for infant acceptability of weaning food

Date of beginning of trial

Rate of Linking for Complementary Food by your infant (1\textsuperscript{st} Day)

- Rejected
- Accepted only if Hunger
- Moderately Accepted
- Accepted
- Highly Accepted

Rate of Linking for Complementary Food by your infant (5\textsuperscript{th} Day)

- Rejected
- Accepted only if Hunger
- Moderately Accepted
- Accepted
- Highly Accepted

Rate of Linking for Complementary Food by your infant (10\textsuperscript{th} Day)

- Rejected
- Accepted only if Hunger
- Moderately Accepted
- Accepted
- Highly Accepted

Rate of Linking for Complementary Food by your infant (15\textsuperscript{th} Day)

- Rejected
- Accepted only if Hunger
- Moderately Accepted
- Accepted
- Highly Accepted

Name _____________________
Location ___________________
Food Code ________________

1=Rejected
2=accepted only if hunger
3=Moderately accepted
4=Accepted
5=Highly accepted

Directions:
- Please mark each box regarding question on respective day
- Prepare food with warm measured with the graduated cup given with food
- Note down on separated sheet if any diet related health consequences appears in your infant