FORMULATION OF LITO WITH MUSTARD GREEN LEAVES
(*Brassica juncea* L.)

by

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Formulation of Lito with Mustard Green Leaves (*Brassica juncea* L.)

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Approval Letter

This *dissertation* entitled ***Formuation of Lito with Mustard green leaves (Brassica juncea* L.*)*** presented by **Abishek Tiwari** has been accepted as the partial fulfillment of the requirement for the degree of **B.Sc. Nutrition and Dietetics.**

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Abishek Tiwari

Abstract

A nutritionally rich *lito* was prepared suited for children from germinated wheat, green gram, and mustard green leaves. Five formulations A, B, C, D and E were formulated varying the proportion of mustard green leaves powder i.e., Control (0%), 5%, 10%, 15% and 20% respectively while keeping the amount of cereal and pulse constant in each formulation. The mustard green was dried, and wheat and green gram were germinated for 72 hours and mixed.

The physiochemical analysis showed significant difference ($p<0.05$) in crude fiber, carbohydrate and energy content when compared to control sample A. The formulation containing 5% mustard green was selected as the best sample from sensory evaluation and statistical analysis. The protein, fat, carbohydrate, crude fiber, and total ash of the product were found to be 18.58%, 1.66%, 73.32%, 3.89% and 2.54% respectively. The food can supply 382.5 Kcal /100 gm. Germination significantly increased protein and calcium content of formulated weaning food. The iron and calcium content of the product was found to be 3.55 mg/100 gm and 50.23 mg/100 gm, respectively. The total cost for the preparation was calculated as NRs. 135 per Kg. Hence, the developed *lito* is nutritious and cost-effective weaning food.

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Abbreviation	Full form
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic acid
DFTQC	Department of Food Technology and Quality Control
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
ICMR	Indian Council of Medical Research
IDD	Iron Deficiency Disorders
MOHP	Ministry of Health and Population
NDHS	Nepal Demographic Health Survey
RDA	Recommended Daily Allowances
UNU	United Nation University
WHO	World Health Organization

Part I

Introduction

1.1 General Introduction

A lack or surplus (or imbalance) of energy, protein, and other nutrients results in measurable negative effects on tissue/body form (body shape, size, composition), body function, and clinical outcome, and is referred to as malnutrition (El-Adawy, 2002). Micronutrient deficiencies are widespread in many developing nations and are frequently brought on by insufficient food intake, poor dietary quality, low bioavailability (due to the presence of inhibitors, mode of preparation, and interactions), and/or infections. Micronutrient status can have an impact on health outcomes like child survival, growth, and development directly (for example, deficiencies of vitamin A, iodine, iron, zinc, or folic acid) or indirectly (for example, interactions with vitamin A, zinc, or iron), increasing food intake due to improved appetite, and lowering morbidity (UN, 2000).

After 6 months, an infant's need for energy and nutrients exceeds what breast milk can provide, and complementary feeding is required to fill the energy and nutrient gap (Dewey and Brown, 2003). An infant's growth may be stunted if complementary food is not offered at this age or is introduced incorrectly. In many countries, the era of complementary feeding from 6-23 months is the time of highest prevalence of growth retardation, nutritional deficiencies, and viral diseases (Dewey and Adu, 2008).The sharp increase in the prevalence of stunting in young children beginning at the age of six months is usually associated with suboptimal complementary feeding practices (Dewey, 2013).The percentage of children age 6-23 months fed unhealthy foods and sweet foods are 68.7% and 43.3% respectively (NDHS, 2022). These food promotes the risk of dental caries and obesity in children.(WHO and UNICEF, 2021).

Green leafy vegetables are one of the most valued components for their color, flavor, and therapeutic value. These serve as good sources of minerals such as calcium and iron. The leafy vegetables are less expensive, easy-to-cook and rich in several nutrients essential for human health. Parents are frequently instructed to add a grain product, such as precooked cereal, to milk feedings first before progressively introducing other solid foods, such as pureed fruit, vegetables, and meats (Kleinman, 2009). Despite the fact that some medical practitioners advise introducing foods randomly, others contend that vegetables should be introduced before fruit because the infants' inherent preference for sweet tastes (Weiffenbach, 1977). Therefore, dietary modifications to increase the consumption of green leafy vegetables that are rich in micronutrients, is essential (Punia *et al.*, 2004).

Germination is a natural process that occurs throughout the growth stage of seeds when they meet the minimum conditions for growth and development. Reserve resources, which are frequently needed for respiration and the synthesis of new cells prior to the development of the embryo, deteriorate during this time. Different studies show that germination can enhance the protein and dietary fiber content's capacity to be digested, lower the amount of tannin and phytic acid, and boost the bioavailability of minerals. The bioavailability of trace elements and minerals has also been linked to germination, which also increased the levels of calcium, copper, manganese, zinc, riboflavin, niacin, and ascorbic acid (Megat and Azrina, 2012).

Food supplied to infants and toddlers should have a high nutrient density because they do not consume enough to meet their high nutrient needs for growth and development (Daelmans *et al.*, 2013). A major global health priority is ensuring adequate nutrition between the ages of 6 and 24 months (Dewey, 2013). So, the availability and accessibility of low-cost fortified complementary foods can play a significant role in the behavioral changes required to enhance the nutritional status of infants and young children (Rivera and Lutter, 2001). Calcium is an important element, which is associated with increased bone mass during adolescence, facture and bone fragility when supplemented in children (Hallfrisch *et al.*, 2000). Iron deficiency is a serious health problem affecting a large portion of the world’s population causes anemia and related cognitive and intellectual impairment in children (Kumkum *et al.*, 2010).

Commercial fortified food products are out of reach for the poor in many developing nations. As a result, homemade complementary food are frequently used in child feeding practices (A2Z, 2010). The basic ingredients for complementary foods are typically made from locally sourced staples, but the specific foods chosen by different people vary widely due to custom, accessibility, and availability (Kuyper *et al.*, 2013). In this study, we prepared low-cost green leafy vegetable fortified complementary food for the targeted children.

1.2 Statement of the problem

Malnutrition is still a serious issue for public health. It is estimated that malnutrition contributes to roughly 45% of mortality in children under the age of five (De Groot *et al.*, 2020). According to several studies, underlying diseases including tuberculosis, malaria, diarrhea, etc., as well as a lack of access to a balanced food and poverty all lead to high levels of malnutrition (Black *et al.*, 2008; Mwanri *et al.*, 2000).

Being a developing nation, malnutrition has been Nepal's main issue. Children under the age of five are more likely to experience malnutrition. In Nepal, 10% of children under the age of five suffer from acute malnutrition, with 2% suffering from severe malnutrition and 8% from moderate malnutrition (NDHS, 2016). So, management of acute malnutrition is the most. As per the WHO decision making criteria, wasting prevalence is at a critical level in Nepal, affecting an estimated 430,000 children under five years of age at any point in time (Collins *et al.*, 2006).

There is a demand for nutritional weaning meals that are both acceptable and cheap to low-income families. According to guidelines, an excellent weaning meal must be nutrient packed, easily digested, of appropriate consistency, and cheap to the target market (FAO/WHO/UNU, 1985). Therefore, development of complementary foods based on locally available cereals and legumes has been suggested by the Integrated Child Development Scheme (ICDS) and Food and Agriculture Organization (FAO) to combat malnutrition among mothers and children of low socio-economic groups. So, this work primarily focuses on the production and quality evaluation of *Lito* using locally available raw materials.

1.3 Objectives

1.3.1 General Objective

To develop *lito* with incorporation of green leafy vegetable and its nutrient evaluation.

1.3.2 Specific Objectives

1. To carry out the proximate analysis of raw and germinated materials i.e., wheat flour, green gram, and mustard green powder.
2. To determine the sensory evaluation of formulated product.

3. To analyze physio-chemical properties of optimized product.
4. To evaluate the cost of product.

1.4 Significance of the study

The developed complementary food blend is made up of variety of food groups like cereal, pulse, and green leafy vegetables to provide wholesome nutrition to children. The significance of this study is:

This is different to other complementary foods which are made up of the addition of green leafy vegetables. The developed products also add a variety to complementary foods given to malnourished children in feeding programs. This research will lay the groundwork to produce complementary food from locally available raw materials using the traditional pretreatment technique of germination. This food is high in iron and calcium, which can help children to prevent the different kinds of serious complications like IDD, constipation, osteomalacia etc. which might be fatal during the early days of children for proper growth and development. Any factory, government agency, local agency, or other organization whose primary goal is to improve the nutritional status of children can produce balanced weaning food using this formula.

1.5 Limitation of the study

1. The analysis of vitamin and trace element content, as well as amino acid and fatty acid composition was not determined.
2. Clinical trials using albino rats were not feasible.

Part II

Literature review

2.1 Food and nutrition

Food is that which nourishes the body. Food may also be defined as anything eaten or drunk which meets the needs of energy, building, regulation, and protection of the body. In short, food is the raw material from which our bodies are made. Intake of the right kinds and amounts of food can ensure good nutrition and health, which may be evident in our appearance, efficiency and emotional well-being (Mudambi, 2007).

Nutrients are components of food that are needed by the body in adequate amounts to grow, reproduce and lead a normal, healthy life. Nutrients include water, proteins, fats, carbohydrates, minerals, and vitamins. There are several nutrients in each of the groups: proteins, fats, carbohydrates, minerals, and vitamins; hence the plural form of these words has been used. Thus there are over 40 essential nutrients supplied by food, which are used to produce literally thousands of substances necessary for life and physical fitness (Mudambi, 2007).

Adequate, optimum and good nutrition are expressions used to indicate that the supply of the essential nutrients is correct in amount and proportion. It also implies that the utilization of such nutrients in the body is such that the highest level of physical and mental health is maintained throughout the life-cycle (Mudambi, 2007). Good nutrition is a goal which can be achieved by anyone who desires it. Moderation in everything that we eat is the key to success. It is necessary to select wisely from the different foods available on the market. Always remember that no single food can be the complete health food for healthy living (Joshi, 2010).

2.2 Components of Nutrition

2.2.1 Macronutrients

Carbohydrates, proteins, and fats are the three types of macronutrients. While macronutrients are utilized in metabolism, they are broken down into their constituents. Monosaccharaides, amino acids, and fatty acids are the building blocks of macronutrients. Monosaccharaides, amino acids, and fatty acids are the building blocks of carbohydrates, proteins, and lipids. Energy is the primary purpose of carbs and lipids, which are macronutrients. Proteins, on the other hand, are required for the formation of bodily cells and tissues. They are, however, used to obtain energy during prolonged hunger. Chemical bonds provide the energy for macronutrients. This chemical energy is transformed to cellular energy and then used to power your body's essential processes (Bakanlıgi, 2015; Tasgin, 2017).

2.2.2 Micronutrients

There are around forty different micronutrients that are essential for good health. Micronutrients are divided into two classes. Most micronutrients are classed as type I, which includes iodine, iron, vitamins A and C. Deficiencies in type I micronutrients do not affect growth (i.e., the individual can have normal growth with appropriate weight and still be deficient in micronutrients) and thus deficiency in type I micronutrients is not determined by anthropometric measurement. Deficiencies in type I micronutrients will cause major illnesses such as anemia, scurvy, and impaired immunity. Type II micronutrients, including magnesium, Sulphur, nitrogen, essential amino-acids, phosphorus, zinc, potassium, sodium, and chloride, are essential for growth and tissue repair. Type II micronutrients are required only in small quantities, but the correct balance is essential for good health. A deficiency in any of the type II micronutrients will lead to growth failure, measured by stunting and wasting (Shenkin, 2006).

2.3 Nutritional status of children in Nepal

The term "nutritional status" refers to the state of the body in those areas affected by diet, the levels of nutrients in the body, and the capacity of those levels to maintain normal metabolic integrity in children under the age of five and identify the risk factors for childhood malnutrition. One of the main indicators of child survival is the nutritional status of children, which serves as a proxy indicator for evaluating the health state of the overall community. Malnutrition among children continues to be a serious public health issue in Nepal despite numerous initiatives. It has been hypothesized that extreme starvation in infancy could affect a child's ability to develop normally physiologically (Raja Lakshmi, 1990).

According to NDHS (2022), height and weight measurements were obtained for 2765 children under the age 5; the percentage with valid data for height-for-age, weight for age, weight for height and weight for age were 97%, 97% and 98% respectively. The government of Nepal's target for SDG 2.1.1 is that the prevalence of stunting (height-for-age) among children under 5 years be at or below 29% by 2022 and at or below 15% by 2030. Similarly, the target for SDG 2.2.2, the prevalence of wasting (height-for-weight) among children under 5 years is 7% by 2022 and 4% by 2030 (National Planning Commission, 2020).

The trends in nutritional status of children in Nepal is presented below.

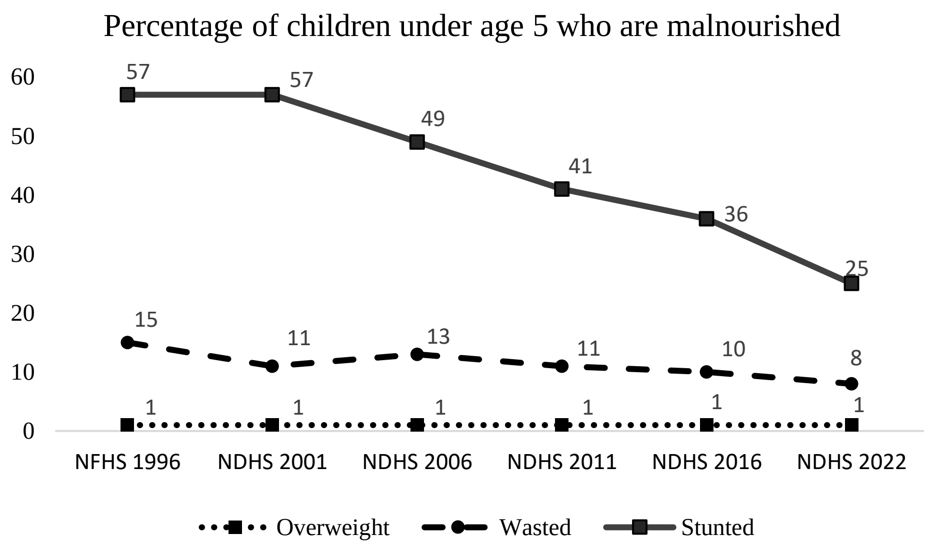

Figure 2.1 Trends in nutritional status of children in Nepal

2.4 Recommended Dietary Allowances (RDAs) for vulnerable groups

The recommended dietary allowance (RDA) is an estimate of the minimum daily average dietary intake level that covers the nutrient requirements of nearly all (97 to 98 percent) of healthy individuals (Wolfe and Miller, 2008). Consuming micronutrients and macronutrients in accordance with the RDA is essential for healthy cellular development and individual wellbeing. It is possible to design a variety of diets to satisfy known nutritional needs. To ensure intake, RDAs should be offered from a variety of foods that are agreeable and appetizing (Council, 1989). Child nutrition is vital for proper growth and development and as the sick and aged they are considered vulnerable because they have special nutritional requirement (RDAs) to achieve the required rate of growth for a period

(Hermoso *et al.*, 2010). The recommended dietary allowances for children under 3 years is presented in Table 2.1

Table 2.1 Recommended Dietary Allowances for Children <3 years.

Nutrients	0-6 months	6-12 months	1-3 years
Net Calories (Kcal/day)	530	680	1110
Proteins (g/day)	8.0	10.5	12.5
Dietary fiber (g/day)	-	-	15
Calcium (mg/day)	300	300	500
Iron (mg/day)	-	3	8
Vitamin A (mg/day)	350	350	390
Ascorbic acid (mg/day)	20	30	30
Zinc (mg/day)	-	2.5	3.3
Thiamine (mg/day)	0.2	0.4	0.7
Riboflavin (mg/day)	0.4	0.6	1.1
Niacin (mg/day)	2	5	7
Dietary folate (mg/day)	25	85	120
Vitamin B12 (mg/day)	1.2	1.2	1.2

Source: (ICMR, 2020)

2.5 Complementary food

Complementary feeding is defined as the process that begins when breast milk alone is no longer able to support the nutritional needs of newborns and additional meals and liquids are necessary in addition to breast milk (WHO, 2021). This is the most essential growth period for the child, lasting from six months to two years, as nutrition deficits can lead to chronic long-term health problems such as rickets, iron deficiency anemia, stroke, cancer, and coronary heart disease, among others (Haimi and Lerner, 2014).

The food given to children throughout their weaning stage is extremely important because an inadequate complementary diet will greatly impair the child's future growth, health, and cognitive development (National Population Commission, 2013; Oloko and Ekpo, 2018). As a result, complementary food should be healthy, sufficient, and begin on time. However, complementary feeding has been identified as one of the most frequently compromised and incorrectly practiced stages of a child's development. In civilizations all around the world, early initiation and incorrect weaning are widespread practices (Gonah and Mutambara, 2016; Salim *et al.*, 2016). While some mothers start feeding their children other foods other than breast milk immediately away, others wait until their children are more than nine months old, resulting in either over- or under-nutrition (Folasade *et al.*, 2017). When complementary feeds are introduced too early (before the age of six months), it can cause feeding difficulties, an increased risk of infection and allergies, the premature termination of breastfeeding, obesity in children and adults, and it can also affect children's feeding behavior in the long term (Kavitha *et al.*, 2014; WHO, 2009).

In Nepal, only one-third of infants are introduced to breastfeeding within one hour of birth, even though 70% are exclusively breastfed for the first six months. At six months, just 65 percent of children receive appropriate complemental food (NDHS, 2011). In many developing nations, the prevalence of nutrition rises throughout the supplemental feeding phase, which lasts from 6 to 18 months. Early nutritional deficiencies have been linked to long-term difficulties in child growth and health (Olatona *et al.*, 2017). According to a Nigerian study, 14.9% of mothers were low aware of the benefits of complementary feeding 72.1% of mothers were aware that their kid should be nursed on demand after four months of starting on other feeds (Olatona *et al.*, 2017). According to an Ethiopian study, just 25% knew when to begin complemental feeding (Guled *et al.*, 2016).

MoALD (2023) has updated standards for cereal-based complementary food for infants and young children from six months to three years of age. The moisture content in complementary food should not be over 13 percent, and protein content should not be less than 15 percent.

2.6 Dietary Fiber

Endogenous plant materials in the diet that are resistant to human-produced digestive enzymes are known as dietary fiber. A healthy diet should have enough dietary fiber. It facilitates the effective passage of food and waste through the digestive tract (Lattimer and Haub, 2010). Fiber is mostly complex carbohydrates. The two types of fiber are soluble and insoluble. While they work differently, both are needed for proper bowel function. Most fiber sources contain both kinds of fiber in varying amounts. Insoluble Fiber cannot be dissolved in water. This type of fiber attracts water to the intestines, making stools bulky and soft. It also speeds the movement of food through the digestive tract. Therefore, insoluble fiber may help prevent diverticular disease, colon cancer, hemorrhoids, and constipation. Cellulose, hemi cellulose and lignin are insoluble fibers. They produce the tough, chewy texture of wheat bran, whole grains, corn bran, and some vegetables (Jenkins *et al.,* 1978).

Numerous observational studies have found an association between consuming food high in fiber and a lower risk of type II diabetes. By assisting in the normalization of postprandial glucose response, lowering insulin levels, and reducing total insulin needs, dietary fiber is thought to play a critical role in lowering overall risk (IFIC, 2008). Dietary interventions that include fiber are thought to help since fiber aids in laxation and the promotion of normal GI function in children (Anderson *et al.*, 2009). For infants older than 6 months of age, the intake of dietary fiber is recommended. Foods that are good sources of fiber such as fruits and vegetables, plus cereals such as oatmeal, are recommended (Xinias and Mavroudi, 2015). The American Academy of Pediatrics suggests 0.5 g fiber/kg body weight/d for children over 2 years of age (Barness, 1993). With maximum limit of 35g/d (Kranz *et al.*, 2012). The upper limit of age was set due to safety concerns that consumption of excess fiber during childhood could negatively impact calorie as well as micronutrient intake by decreasing the bioavailability of minerals (Williams *et al.*, 1995) . For example, some sources of fiber that are high in phytates and oxalates can decrease the bioavailability of iron, a nutrient that is particularly important for growing children (Stewart and Schroeder, 2013).

2.7 Raw materials and their nutritive value

2.7.1 Cereals

Cereals are grains or edible seeds from the grass family, Gramineae (Bender, 2006). Cereals are important foods all throughout the world, with wheat, rice, maize, oat, barley, millets, sorghum, rye, triticale, and fonio among the most frequent. Cereals like wheat, rice, and maize are grown on massive scales and contribute to the global food supply (Rosenblueth *et al.*, 2018). These staple foods can be preserved for an extended amount of time without losing nutritional content. Cereal grains are a good source of macro- and micronutrients since they contain carbs, proteins, dietary fibers, vitamins, and minerals (Poole *et al.*, 2021).

Cereals are a good source of energy, which is especially crucial after six months, when exclusively breastfeeding is no longer sufficient to meet the infant's nutritional needs (Agostoni *et al.*, 2008). Cereals supply non-digestible carbohydrates, which are primarily responsible for the establishment of 'adult-like' microbiota through expanding the Bacteroides population (Fallani *et al.*, 2011). Clear alterations in the infant's gut microbiota have been seen during weaning with the addition of wheat, sorghum, rice, or oats into a large intestine in vitro (Gamage *et al.*, 2017). Furthermore, a higher proportion of complex carbohydrates in infant cereals has been proven in vivo to increase the fermentative activity of the intestinal microbiota of infants aged six to ten months (Bernal *et al.*, 2013). Cereals have a mild flavor and a semi-solid texture and consistency, making them suitable for the transition from milk to the acceptance of solid meals at the start of complementary feeding (Nicklaus *et al.*, 2015; Sakashita *et al.*, 2003).

2.7.1.1 Wheat

Wheat is the universal cereal of the Old World agriculture and the world’s foremost consumed crop plant followed by rice and maize (FAO, 2011). It is a staple food for 40% of the world’s population, in Europe, North America, and the western and northern parts of Asia. The demand for wheat is growing fast in new wheat growing regions of the world such as eastern and southern Africa (5.8%), West and Central Africa (4.7%), and South Asia and the Pacific (4.3%). Demand is also growing in the traditional wheat growing regions of Central Asia (5.6%), Australia (2.2%), and North Africa (2.2%) (Shiferaw *et al.*, 2013).

In 100 grams, wheat provides 341 calories and is a rich source of multiple essential nutrients, such as protein, dietary fiber, manganese, phosphorus, and niacin. Several B vitamins and other dietary minerals are in significant content (DFTQC, 2017). Since the proteins present in the wheat endosperm (gluten proteins) are especially poor in lysine, white flours are more lacking in lysine contrasted and entire grains. Supplementation with proteins from other food sources (legumes) is normally used to make up for this inadequacy, since the restriction of a solitary fundamental amino corrosive makes the others separate and become discharged, which is particularly significant during the time of development. Further, wheat is a significant hotspot for regular and bio-invigorated supplement supplementation, including dietary fiber, protein and dietary mineral (Suhasini and Malleshi, 2003). The nutrient composition of 100-gram wheat is presented in Table 2.2

Table 2.2 Nutrient composition of 100 g wheat

Parameter	Values
Moisture (g)	12.2
Protein (g)	12.7
Fat (g)	1.7
Carbohydrate (g)	69.4
Fiber (g)	1.9
Calcium (mg)	48
Iron (mg)	4.9

Source: DFTQC (2017)

2.7.2 Pulses

Pulses are the edible seeds of Leguminosae family members, and are classified by the United Nations Food and Agricultural Organization (FAO) as "Leguminosae crops harvested exclusively for their grain, including dry beans, peas, and lentils" (FAO, 1994).

Pulses contain about 21-25% protein, but only a little amount of important amino acids like methionine, tryptophan, and cysteine (Tiwari and Singh, 2012). The protein level of pulses is double that of grains. Pulse proteins were divided into two groups: albumin and globulin. Globulins are the most abundant storage proteins in pulse seeds, accounting for 35-72% of total protein, with the remaining protein consisting of albumin. Globulin proteins contain more glutamine, aspartic acid, arginine, and lysine. They are high in the amino acid lysine, which is commonly lacking in cereal grains. Pulses, on the other hand, are poor in the important amino acids' methionine and tryptophan, which are abundant in grain-based goods. Thus, when combined with cereal, pulses, and grain-based products give a higher quality protein.

2.7.2.1 Green gram

Mung bean is well known as green gram or moong bean. Green gram has been consumed as a common traditional food worldwide for more than 3500 years (Kole, 2007). Green gram is a protein-rich staple food. It contains about 23-25% protein which is almost three times that of cereals (Khattak and Bibi, 2007). Mung beans have much greater carbohydrate content (50%–60%) than soybeans, and starch is the predominant carbohydrate in the legume (Tang *et al.*, 2014). Green grams are commonly produced for human use (as dry beans or fresh sprouts). Green gram has an advantage over other legumes because of its short growing period and ability to adapt to a wide range of farming systems (Afam *et al.*, 2016).

Green gram is a high-protein food that is almost free of flatulence-causing substances. Green gram seeds are therefore preferable for feeding infants and people recovering from illness. The seeds have greater lysine content than any other legume seed (Adsule *et al.*, 1986). Sprouted green gram is an excellent source of protein, iron, and calcium as well as having a variety of therapeutic properties. Sprouted green gram-based food products offer high biological qualities, are calorie rich, have minimal dietary bulk, and are cost-effective in addressing nutritional issues (Bisht *et al.*, 2005). The nutrient composition of 100-gram green gram is presented in Table 2.3

Table 2.3 Nutrient composition of 100 g green gram

Parameter	Values
Moisture	10.4
Protein (g)	24
Fat (g)	1.3
Carbohydrate (g)	59.9
Fiber (g)	4.2
Calcium (mg)	75
Iron (mg)	3.9
Vitamin C (mg)	0

Source: DFTQC (2017)

2.7.3 Green Leafy Vegetable

Green leafy vegetables have a significant position among food crops since they offer sufficient levels of many vitamins and minerals for people. They are a useful source of minerals including calcium, iron, and phosphorus as well as vitamins like beta-carotene, ascorbic acid, riboflavin, and folic acid. There are several underused greens in nature with high nutritional content that can feed the world's expanding population (Odhav *et al.*, 2007).

Green leafy vegetables (GLV), whether they are grown locally or picked up from the wild, are a diverse source of vitamins and phytochemicals. Nutritionists are particularly interested in GLV as a source of minerals and micronutrients like iron and vitamin C that are absent from common diets. GLV are also the main sources of lutein and zeaxanthin (Mangels *et al.*, 1993; Sommerburg *et al.*, 1998) which have been identified as important eye protective agents. GLV consumption has been reported to contribute to lowering the risk of age-related cataract. These are known to contain antioxidants necessary in neutralizing free radicals which are known human chemical hazards (Mosha and Gaga, 1999).

2.7.3.1 Mustard Green

The cruciferous family includes the annual herb mustard (*Brassica juncea)*, often known as Chinese mustard, brown mustard, leaf mustard, vegetable mustard, or Oriental mustard (Lin *et al.*, 2011). Glucosinolate and their breakdown products, polyphenols (flavonoids and anthocyanin), substantial levels of dietary fiber, chlorophylls, beta-carotene and ascorbic acid, minerals, and volatile components are all found in mustard, a cheap and nutrient-dense food (Kim *et al.*, 2007). The nutrient composition of 100-gram mustard green is presented in Table 2.4

Table 2.4 Nutrient composition of 100 g mustard green (wet basis)

Parameter	Values
Protein (g)	2.86
Fat (g)	0.42
Carbohydrate (g)	4.67
Fiber (g)	3.2
Calcium (mg)	115
Iron (mg)	1.6
Vitamin A	3024 IU
Vitamin C (mg)	70

Source:(Agarwal *et al.*, 2014)

Green leafy vegetables are well known for having low calorie, carbohydrate, and glycemic index values. These qualities make them perfect for encouraging and maintaining a healthy body weight, and when combined with their high fiber content, these vegetable aid to reduce type II diabetes particularly. Additionally, eating of the most nutritionally dense foods, such as green leafy vegetables, will improve the nutritional status of both low-income rural and urban households (Chadha, 2003).

2.7.4 Germination

The beginning of the germination process involves the dry seed ingesting water, and it ends when the radicle penetrates the seed coat layers (Bewley, 1997; Weitbrecht *et al.*, 2011).

Dry seeds often absorb water in three steps. Phase I is distinguished by the early, fast water intake by a dry seed, which results in swelling and a change in form (Bewley, 1997). The rapid rehydration disturbs the membrane structure, causing low molecular weight metabolites and cellular solutes to flow out of the seed; however, the membrane shape is restored after a brief time of hydration. Numerous physiological processes, such as protein synthesis from preexisting mRNA and the restart of respiratory activities including glycolysis and the oxidative pentose phosphate respiratory pathways, are triggered during this period of water intake. Within minutes of ingestion, the restoration of respiratory activity is marked by a dramatic rise in oxygen intake and carbon dioxide output (Bewley, 1997).

Phase II of seed germination begins when the seed water absorption rate decreases and stabilizes (Bewley, 1997; Weitbrecht *et al.*, 2011). Several processes occur at this stage, including the continuation of DNA and mitochondrial repair, the creation of new mitochondria, and the production of proteins from newly created gene transcripts. Additionally, at this stage, the beginning of embryo enlargement and the weakening of seed covering layers take place (Bewley, 1997). Germination, which is defined as radicle protrusion through the seed coat, signals the conclusion of phase II and the start of phase III (post-germination stage).

Phase III is primarily distinguished by the mobilization of storage reserves that have been deposited in storage organs, such as the endosperm in cereals. This causes further increases in water absorption, which are followed by seedling growth (Nonogaki *et al.*, 2010). In addition, during the third phase of water intake, cell division, DNA synthesis, and radicle cell elongation take place.

2.8 Medicinal values of the raw materials used

2.8.1 Wheat

Wheat contains several medicinal properties. Every part of the whole-wheat grain contains nutrients that the human body requires. Wheat starch and gluten provide heat and energy; the inner bran coats, phosphates, and other mineral salts; the outer bran, the much-needed roughage the indigestible portion that aids in bowel movement; the germ, vitamins B and E; and wheat protein helps build and repair muscular tissue. The wheat germ, which is removed during the refining process, is also high in essential vitamin E, a deficiency of which can lead to heart disease. Because of the loss of vitamins and minerals in refined wheat flour, constipation and other digestive disturbances and nutritional disorders are common. As a result, whole wheat, which includes bran and wheat germ, protects against diseases such as constipation, ischemia, heart disease, diverticulum, appendicitis, obesity, and diabetes (Hadjivassiliou *et al.*, 2003).

Wheat protein, which accounts for up to 8% of the grain, has the added benefit of containing eight essential amino acids in delicately balanced proportions. When wheat protein is metabolized into health-building amino acids, it causes a complete internal rejuvenation. These amino acids support the heart and lungs, tendons and ligaments, brain, nervous system, and glandular network, as well as the heart and lungs, tendons and ligaments, brain, nervous system, and glandular network. Natural brown Wheat contains B-complex vitamins, particularly thiamin, riboflavin, and niacin, which promote youthful energy and nourishment of the skin and blood vessels. Natural brown wheat contains an abundance of minerals that help to nourish the hormonal system, heal wounds, and regulate blood pressure. Wheat also contains iron, which helps to enrich the bloodstream, as well as phosphorus and potassium, which help to maintain internal water balance. Wheat thus contributes to the restoration of internal harmony (Adams *et al.*, 2002; Alvarez *et al.*, 2000).

2.8.2 Green gram

Green gram is well known for its detoxifying properties and is used in the summer to calm the mind, treat heat exhaustion, and lessen swelling. The mung bean has been shown to be helpful in controlling gastrointestinal distress and moisturizing the skin. Mung beans are rich in bioactive phytochemicals and a variety of balanced nutrients, including protein and dietary fiber. The antioxidant, antibacterial, anti-inflammatory, and anticancer properties of mung beans are assumed to be primarily attributed to their high quantities of proteins, amino acids, oligosaccharides, and polyphenols, which also have a role in the control of lipid metabolism (Tang *et al.*, 2014).

Besides being a nutritious food, green gram also contains antinutritional factors like phenolic, trypsin inhibitors, tannin, saponins, oligosaccharides, phytic acid, and hemagglutinins. Various studies proved that cooking and processing methods could reduce these antinutritional factors and improve the availability and digestibility of nutrient (Grewal and Jood, 2006).

2.8.3 Mustard green leaves

Brassica vegetables like mustard green leaves (*Brassica juncea L.*) provide a variety of phytochemicals that are good for health, such as carotenoids, phenolic compounds, and glucosinolate. These substances are widely mentioned for their capacity to act as antioxidant detoxifiers (Wu *et al.*, 2004). This is particularly relevant to human health since an imbalance between oxidants and antioxidants in the body can result in the onset of conditions like cancer, diabetes, and cardiovascular disease (Chu *et al.*, 2002).

The carotenoids found in mustard leaves also have antioxidants and other health effects. As one of the several carotenoids found in mustard leaves, -carotene is particularly interesting for human health due to its provitamin A activity and anti-carcinogenic properties (Peto *et al.*, 1981). Retinoid, which are a component of vitamin A and are necessary for cell growth, immunity, reproduction, and vision (West and Darnton-Hill, 2008). Anthocyanin, which are water-soluble red-purple pigments that are found in the leaf tissue of certain mustard cultivars, act as antioxidants. Mustard greens are a rich source of vitamin K whether they are cooked or raw. Blood thickening, which is vital for bone and heart health, is facilitated by vitamin K. Heart problems and brittle bones are only two of the significant health problems that can result from vitamin K insufficiency (osteoporosis).

Insufficient vitamin K can harm brain cells and cause dementia, poor brain function, and Alzheimer's disease, according to numerous studies. It is advised that greens be included in the daily meal to prevent such illnesses (Lietzow, 2021; Monsalve *et al.*, 2001; Sawicka *et al.*, 2020; Sharma *et al.*, 2019).

2.9 Processing Technologies for Complementary Food

The nutritional value of cereals and legumes has been enhanced using conventional processes including soaking, boiling, germination, and fermentation. Food processing techniques including soaking, germination, and fermentation decrease phytic acid levels, increase the solubility of minerals in food, and enhance the bioavailability of minerals in cereals and legumes. By releasing exogenous and endogenous enzymes like phytase produced during processing, processing techniques lower levels of anti-nutritional organic components such as phytates, phenols, tannins, and enzyme inhibitors (El-Adawy, 2002).

2.9.1 Soaking

Soaking or steeping is a preparation for decertification of grain that allows the husk or skin to be removed more easily. When non-corticated grains are soaked in water for a brief period, the husk is easily removed. In grains and legumes, soaking increases the hydration coefficient, seed weight, total protein, ash, fat, and fiber content (El-Adawy, 2002).

The first step in the malting process is to soak the cereal grain in water. The purpose of steeping is to allow the grain to absorb enough moisture to start germination. The length of time for steeping depends on the water's temperature and level of aeration. Steeping times of 40–60 hours are advised at a temperature of 25–10–12°C for cereals. For legumes, a temperature of 20–25°C and steeping times of 16–20 hours are advised (Rosentrater and Evers, 2017).

2.9.2 Germination

Legumes and cereals that have germinated or sprouted are more palatable and nutritious, in part because certain anti-nutrients like phytase and protease inhibitors are broken down. Germination enhances the nutritional value of cereals and legumes because it is more effective than heat treatment at reducing phytic acid. The overall amount of essential amino acids in grains and legumes is also marginally increased by germination. It has been demonstrated that dehusking, germination, heating, and roasting improve the nutritional value of legumes (Kadam *et al.*, 1985).

The transformation of complex chemicals into simpler forms, the formation of vital elements, and the breakdown of nutritionally undesirable constituents are the fundamental causes of the beneficial nutritional changes that take place during sprouting as soon as seeds are moistened during soaking, their metabolic activity increases. During hydration and subsequent sprouting, many metabolic alterations take place. Enzymes break down the reserved chemical components, such as protein, carbohydrates, and lipids, into simple chemicals that are then used to develop new compounds. Increased hydrolytic enzyme activity, improvements in the quantity of total proteins, lipids, and some important amino acids, total sugars, and B-group vitamins are all brought about by sprouting, while dry matter, starch, and anti-nutrients are reduced. The only noticeable and due to the absence of starch is the higher quantity of protein, fat, fiber, and total ash. However, the metabolic benefits of sprouting include an improvement in amino acid composition, B-group vitamins, sugars, protein, and starch digestibility, as well as a reduction in phytates and protease inhibitors (Chavan *et al.*, 1989). A decrease in phytate content up to 25% was reported when wheat grains were germinated for 3 days (Azeke *et al.*, 2011). According to El Beltagy (1996); The hemagglutinin activity of mung bean seeds decreased by about 84.4% after three days of germination.

2.9.3 Drying

The amount of water in germinated grains has an impact on the seeds' quality and capacity to be stored for both long and short periods of time. Furthermore, a high moisture content is not advised for grinds intended for processing by grinding since it increases seed plasticity and makes size reduction challenging. It is advisable to dry germinated grains to a safe level to reduce their moisture content. Drying is required for secure storage because the decreased water activity prevents microbiological development (Majumder *et al.*, 2016).

During germination, and particularly during the drying treatment, the profile of volatile compounds will change (Heiniö *et al.*, 2001). Drying produces a friable, readily milled stable product that may be stored for extended periods and from which roots may easily be removed.

2.9.4 Roasting

Roasting is a dry heat cooking method in which hot air envelops the food, cooking it evenly on all sides at temperatures of at least 150 °C (300 °F) using an open flame, oven, or other heat source. Roasting can increase flavor by caramelizing and browning the surface of the meal. Dry roasting is a method of applying heat to dry foods without the use of oil or water as a carrier. Dry roasting, as opposed to other dry heat processes, is employed with foods such as nuts and seeds. Dry roasted foods are stirred throughout the cooking process to ensure equal heating (Gahlawat and Sehgal, 1992).

Roasting process render grain digestible, without the loss of nutritious component and the grains are consumed throughout the world (Srivastav *et al.*, 1994). Roasting improves color, extends shelf life, enhances flavor, and reduces the anti-nutrient factors of cereals and legumes. Moreover, roasting grains lead to denaturation of proteins, thus improving their digestibility. Roasting can improve protein digestibility (Kavitha and Parimalavalli, 2014a).

2.9.5 Milling and Sieving

The outer bran in coarse grains is fibrous, bitter, astringent, or colored. Milling of the coarse grains is therefore desirable to confer adequate consumer acceptability to them. It is obvious that over milling or very high refining must be avoided, since it removes the aleuronic layers and germ rich in protein, vitamins and minerals (Viraktamath *et al.*, 1971).

2.9.6 Blending

It is the uniform mixing of all the ingredients. It is the process of mixing two or more substances so that their individual properties are lost, and they become smooth and homogenous. The primary goal of blending is to combine or mix the constituent ingredients so that they are indistinguishable from one another, resulting in the lipid-based paste product (Amagloh *et al.*, 2012).

2.9.7 Packaging

Packaging is an essential part of processing and distributing foods. Whereas preservation is the major role of packaging, there are several functions for packaging, each of which must be understood by the food manufacturer (Coles *et al.*, 2003).

2.9.7.1 HDPE

After polyvinyl chloride and polypropylene, high-density polyethylene (HDPE) is the third-largest commodity plastic material in the world by volume. It is a thermoplastic substance made of hydrogen and carbon atoms that are linked to create high-molecular-weight goods (Kumar and Singh, 2013). Lower temperatures and atmospheric pressure are used to create HDPE in a liquid phase process. It softens between 120 and 130 degrees Celsius, making it suitable for applications including steam sterilization, hot filling, and cook-in-bag. It may be utilized in thinner gauges, which saves money, because of its increased stiffness. The retention of essential oils and smells is exceptional. In general, films made of polyethene are soft, flexible, and impact resistant. They can be challenging to open, though. The higher the density, the stronger the resistance, i.e., the lower the value of WVTR, but the oxygen transfer rate is high. They are particularly resistant to water and water vapor (Coles *et al.*, 2003).

According to Marsh and Bugusu (2007), the main advantages of HDPE are:

- Water proofness, low gas and water vapor permeability.
- Good aroma retention.
- It is heat sealable, can be oriented and made into bags.
- It is useful in wrapping meat, fish, and dried foods.

2.9.7.2 Aluminum foil

Light, air, moisture, and germs are completely barred by aluminum foil. Foil is frequently used in food and drug packaging because of this. Additionally, it is utilized to create aseptic packaging, which enables perishable foods to be stored without refrigeration. Because it is highly flexible, easily formed into thin sheets, and quickly folded, rolled, and packaged, aluminum is utilized for packaging Aluminum foil is widely used in food and pharmaceutical packaging because it creates a complete barrier against aromas, flavors, moisture, pathogens, light, and oxygen (which can cause lipids to oxidize or become rancid). Aluminum is used to create long-lasting packs (aseptic packaging) for beverages and dairy products that enable storage without refrigeration. Aluminum foil comes in a variety of thicknesses, with thinner foils used to wrap foods and thicker foils used for trays. Foil, like all aluminum packaging, acts as an excellent barrier against moisture, air, odors, light, and microorganisms. It is resistant to acidic foods and does not need to be lacquered or otherwise protected. Although aluminum is easily recyclable, foils made from recycled aluminum cannot be produced without pinhole formation in thin sheets (Scott and Brock, 2006).

2.9.7.3 Glass bottles

Glass is the primary material used to make bottles. Glass bottles come in a wide range of sizes, but the ones that are most frequently encountered are between around 10 ml and five liters. Although plastic is replacing glass bottles and jars as the preferred packaging material for condiments and oils, glass bottles and jars still have advantages over other materials since they can be recycled and reused. Glass containers are simple to wash, sterilize, and reuse. Glass packaging for your goods conveys quality and substance and has a high level of shelf appeal (Coles *et al.*, 2003)**.**

Natural, toxicologically inert raw materials are used exclusively in the production of glass. The principal components sodium and potassium silicates are nontoxic and chemically very inert. Silicate and cation transfer into food is minimal, and even if it does, it is toxicologically inconsequential because the cations that are often present are not hazardous. In glass-bottled food products, hardly no signs of harmful migrants coming from the glass are discovered (Schrenk, 2014).

Part III

Materials and Methods

3.1 Materials

3.1.1 Wheat

Wheat was collected from Dharan market. The scientific name of wheat is '*Triticum compactum'* It is recognized as '*gahu*' locally.

3.1.2 Green gram

Green grams were collected from Dharan market. It is locally known as '*mung daal.'* The scientific name of green gram is '*Vigna radiata.'*

3.1.3 Mustard green

Mustard green was collected from Dharan market. It is locally known as '*rayo saag.'* The scientific name of mustard green is '*Brassica juncea L.'*

3.1.4 Packaging material

HDPE was used as packaging material for the packaging of the product.

3.2 Chemicals, reagents, and equipment used

3.2.1 Chemicals and reagents

Analytical grade reagents from different suppliers were used for all experiments. H2SO4 (97-99% assay, Qualigens), NaOH (97% assay, Merck), HCl (35% assay, Merck), boric acid (99.5% assay (powder), Merck), Petroleum ether (assay > 99%, Thermo Fisher Scientific), Indicators (Phenolphthalein, Methyl red, Bromocresol green), Potassium permanganate (99% assay, SDFCL), Methanol (assay >99%, Emplura), Ethanol (assay >99%, Fischer Scientific), Oxalic acid (assay>99%, Merck), Ammonium oxalate (assay>99%, Qualigens).

3.2.2 Glassware and equipment

Standardized and calibrated glassware and equipment were used.

- Hot air oven
- Spectrophotometer (UV-VIS single Beam Spectrophotometer MODEL NO-291

- Soxhlet apparatus (Y.P. scientific glass work)
- Centrifuge (Y.P. scientific glass work)
- Electronic balance (AMPUT Electronic Balance Model No-457B, Sensitivity ± 0.01)
- Thermometer
- Heating mantle (Burner)
- Incubator (Y.P. scientific glass work)
- Water bath (Intake serological water bath)
- Chromatography chamber
- Atomizer

Standardized glassware such as petri dish, burette, pipette, test tubes, volumetric flask, beaker, funnel, conical flask, measuring cylinder, crucible, etc. were used.

3.3 Method

3.3.1 Processing of Raw Materials

3.3.1.1 Wheat

Wheat was sorted, cleaned, and soaked in water for 12 hours at 22°C and 60% humidity and then drained. It was spread on a wet muslin cloth and covered by a wet muslin cloth. It was kept for germination at 22°C.Water was sprinkled on the layer at 3-to-4-hour intervals. The grain germinated for 3 days. It was then dried in cabinet drier at 55°C for 3 hours and 70°C for 1 hour until moisture sufficiently reduced to about 5%. The germinated part was removed, then roasted and milled into grits and packed in air tight plastic bags (Tehseen *et al.*, 2014).

3.3.1.2 Green gram

The grains were first screened to remove impurities, after which they were cleaned, washed, and left to soak in extra water. The larger trays were used because the grains almost doubled in volume. The recommended steeping time for the grains is 12 hours at room temperature with additional water, which was changed after 4 and 8 hours. The grains should germinate for three days at room temperature following steeping. To stop further germination, the germinated grains should then be dried in a cabinet dryer. Drying took place for (7-8) hours at 60 degrees Celsius. The cotyledons and husk were taken off the dried grains after they had been dehusked. It was lightly roasted and dry milled to enhance the flavor and taste. To obtain the grits, dry milling and sieving were performed, and they were then packaged in airtight containers (Rana *et al.*, 2015).

3.3.1.3 Mustard Green

Mustard green was sorted and thoroughly washed in running water to remove adhering dirt. The washed mustard green was spread as a single layer on aluminum trays and dried in a tray drier at 60°C for 5-6 hours. The dried mustard was be grounded to fine powder in electric grinder and sieved by using thin mesh .This was done by following the procedure of Prasad *et al.* (2016).

3.3.2 Formulation

3.3.2.1 Basis of formulation

The preparation of diet was done based on specification of formulation of malted sorghum flour. The flour is made from two parts cereals–sorghum, one-part cowpea flour (Elemo *et al.*, 2011). The pulses and grains were cleaned, germinated, and roasted well (separately) and ground into fine flour (separately). The flour was stored in an airtight container (Sanjay, 2016).

3.3.2.2 Calculation of amounts of ingredients

The amounts of ingredients were calculated on dry weight basis, for the formulation of Super flour. Cereals were taken as staple source of food; legumes were taken as source of plant protein and green leafy vegetable were taken as a source of vitamins and minerals. Finally, from the calculation, five different products were prepared taking Super flour as a basis i.e., cereals: legumes = 2:1 ratio. Cereals and legumes germinated, and green leafy vegetables were dried. Mustard green leaf powder was not added in the product which served as control. Then five different products were prepared varying the amount of green leafy vegetable powder in each product i.e., Control, 5%, 10%, 15%, and 20% keeping the number of cereals and legumes constant. Table 3.1 shows the amount of ingredients in each product in grams.

Table 3.1 Formulae mixes on dry basis.

Ingredients	A	B	C	D	E
Wheat	66.7	63.3	60	56.7	53.3
Green gram	33.3	31.7	30	28.3	26.7
Mustard Green	0	5	10	15	20
Total	100	100	100	100	100

3.3.3 Product Preparation

The calculated amount of ingredients for five different products were calculated on a dry basis. Flowchart diagram of different ingredients used for the preparation of *lito* is shown in Fig 3.1.

3.3.3.1 Grinding and milling

All the roasted cereals and legumes were ground using the grinder available in laboratory of Central Campus of Technology, Dharan.

3.3.3.2 Sieving of the ground powder product

All the grounded flour was sieve using 300µ sieve.

3.3.3.3 Mixing

The calculated amounts of ingredients were weighed according to the formulation and mixed homogenously.

3.3.3.4 Packaging

After completion of proper mixing, the product was packed immediately in airtight plastic bags, then it was repacked in experimental packaging material High Density Polyethylene (HDPE). The package was kept at room temperature.

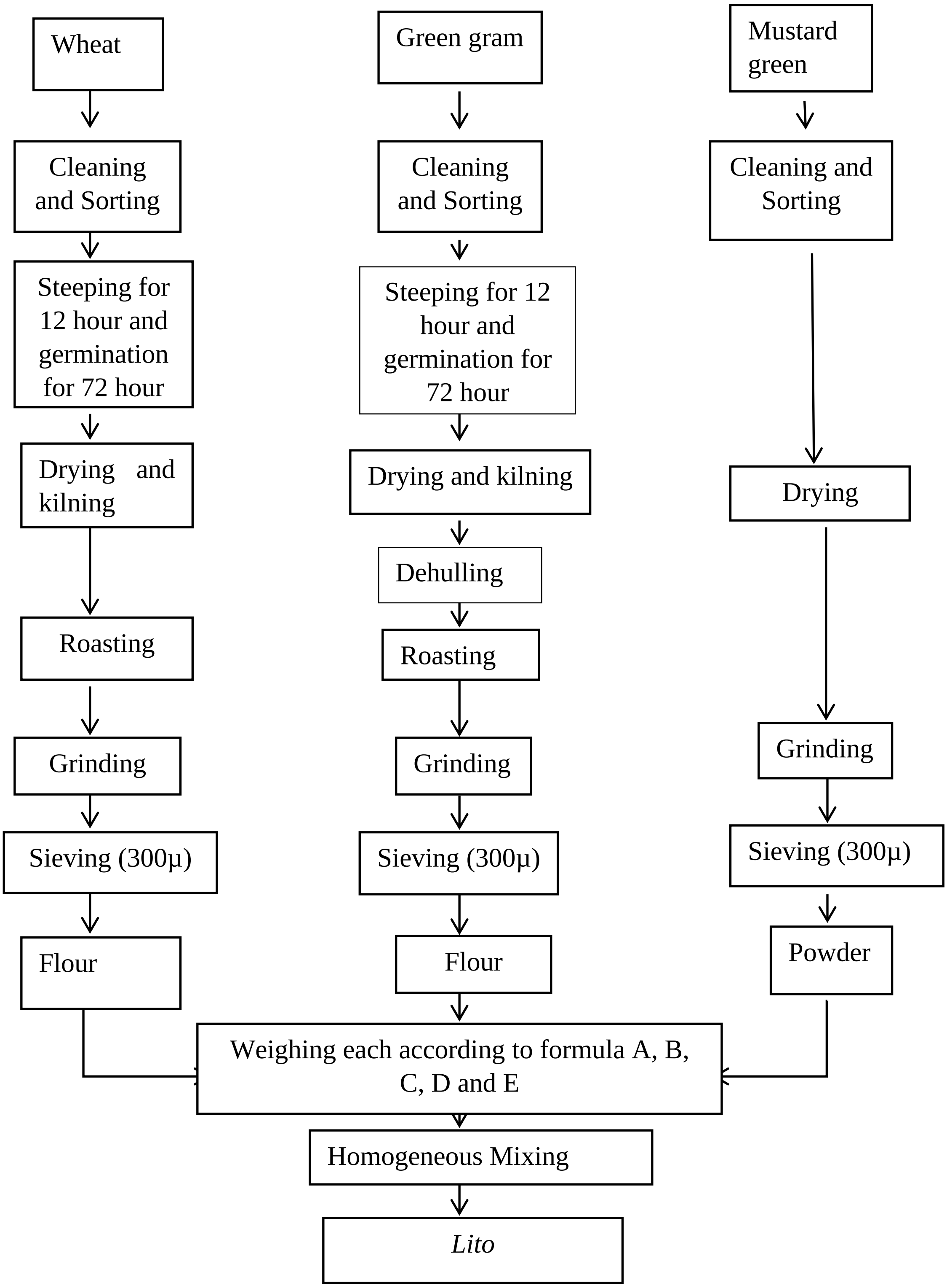

Fig 3.1 Outline for the preparation of *Lito* (Pilot Plant Scale)

3.3.4 Evaluation of prepared product

3.3.4.1 Sensory evaluation

Sensory evaluation was performed by 9-point hedonic scoring (9 = like extremely, 1 = dislike extremely) for color, flavor, taste, texture, and overall acceptance. The evaluation was carried out by 10 semi-panelists comprising of teachers and students of Central Campus of Technology including 4 females and 6 males. Sensory evaluation was carried out in individual booths with adequate light and free from obnoxious odors. Each panelist was provided with five samples coded random numbers and an evaluation card (Appendix A). They were provided with portable water for rinsing between samples. Verbal communication among the panelists was prohibited. They were asked to evaluate the samples individually using a score card.

3.3.4.2 Physio-chemical Analysis of Product

3.3.4.2.1 Moisture Content

Moisture content was determined by using hot air oven (ambassador, working temperature 0 to 300ºC as per KC and Rai (2007).

3.3.4.2.2 Crude Fat

The fat content was determined by Soxhlet method as per KC and Rai (2007).

3.3.4.2.3 Crude Protein

The crude protein was determined by using Kjeldahl’s method as per KC and Rai (2007).

3.3.4.2.4 Crude Fiber

Crude fiber was determined as per KC and Rai (2007).

3.3.3.2.5 Total ash

Total ash content was determined by ashing in electric muffle furnace (ambassador, working temperature 900ºC, UK) as per KC and Rai (2007).

3.3.3.2.6 Total Carbohydrate

Total carbohydrate shall be determined by difference method as per as Rangana (2001).

3.3.3.2.7 Calcium

Calcium content was determined by volumetric method as per KC and Rai (2007).

3.3.3.2.8 Iron

Iron content was determined by colorimetric method as per KC and Rai (2007).

3.3.3.2.9 Determination of Energy Value

One of the methods specified by the FDA was employed. This uses the general factors of 4, 4 and 9 calories per gram of protein, total carbohydrate, and total fat, respectively, to calculate the calorie content of food (Bassey *et al.*, 2013).

Total energy = energy from carbohydrate + energy from protein + energy from fat

3.4 Data analysis

Data on analysis of sensory analysis were tabulated for comparison and were graphically represented using Microsoft excel-2010.The data obtained in the course of experiment was analyzed by applying statistical tools -2 way ANOVA (no blocking) using the statistical software GenStat Release 7.1 (Discovery Edition 3 developed by VSN International Limited) which is developed by Lawes Agricultural Trust (1995) to statistical analysis and the scores given by the panelist shall be analyzed by a two way ANOVA at 5% level of significance.

Part IV

Results and discussions

4.1 Proximate analysis of raw sample

Proximate analysis provides inexpensive but vital information, particularly in the nutritional and biochemical point of view. Because of the generic nature of the test used for the determination, the term crude is frequently used as a modifier; for example, crude protein, crude fat, and crude fiber, etc. As a result, proximate constituent is only one category of compound found in biological material (Acharya and Karki, 2008).

The proximate analysis of raw wheat, green gram and dried mustard green powder was conducted. The result is tabulated in Table 4.1.

Table 4.1 Analysis of raw sample

Parameter	Wheat	Green Gram	Mustard green
Moisture (%)	9.78±0.095	9.44±0.075	7.41±0.07
Protein (% db.)	11.38±0.348	24.94±0.512	29.5±0.370
Fat (% db.)	1.87±0.092	1.71±0.087	3.13±0.150
Ash content (% db.)	1.95±0.072	4.53±0.231	10.40±0.238
Crude fiber (% db.)	2.76±0.370	5.73±0.356	16.76±0.349
Carbohydrate (% db.)	82.04±0.177	63.07±0.743	40.19±0.123
Calcium (mg/100 g)	28.48±0.721	70.76±0.935	12.25±0.429
Iron (mg/100 g)	3.24±0.315	6.68±0.576	1.17±0.234

(The values are the means of triplicate samples ±Standard deviation.)

Table 4.1 shows the proximate and ultimate composition of the wheat, green gram, and mustard green on dry basis. The proximate and ultimate composition of wheat is similar to that reported in Steve (2012) and Kavitha and Parimalavalli (2014b). The proximate and ultimate composition of green gram is similar to that reported in Abbas and Shah (2007) and Paul *et al.* (2011). Similarly, Tian and Deng (2020) satisfy the proximate and ultimate composition of mustard green as listed above.

4.2 Proximate analysis of germinated sample

The nutritional composition of cereal and legume flours is affected by germination. Germination enhanced the amount of moisture and protein in cereal and pulse flours. Germination has significant effects on the ash, fat, fiber, carbohydrate, and energy content (Kavitha and Parimalavalli, 2014b).

The proximate analysis of raw wheat, green gram and dried mustard green powder was conducted. The result is tabulated in Table 4.2

Table 4.2 Analysis of germinated sample

Parameter	Wheat	Green gram
Moisture (%)	7.73±0.105	6.14±0.055
Protein (% db.)	13.52±0.271	27.14±0.39
Fat (% db.)	1.63±0.140	1.58±0.07
Ash content (% db.)	1.59±0.065	3.59±0.163
Crude fiber (% db.)	2.41±0.284	4.92±0.383
Carbohydrate (% db.)	80.85±0.520	62.71±0.569
Calcium (mg/100 g)	41.40±0.531	78.22±0.435
Iron (mg/100 g)	2.58±0.520	5.97±0.858

(The values are the means of triplicate samples± represents standard deviation)

The protein content of germinated wheat and green grams was increased in comparison to that of raw samples. This increase was due to the use of seed components during the germination process. This result was in agreement with those reported in El Beltagy

(1996). Increment of protein was also attributed to loss of dry matter through respiration and microbial spoilage (Mugendi *et al.*, 2010).

The fat content of germinated samples was lower than raw samples. Depletion of stored fat that promoted the catabolic activities of the seeds during sprouting may be the cause of the decrease in fat content (Onimawo and Asugo, 2004). Kwon (1994) suggested that to produce energy for sprouting and certain structural components, fatty acids are oxidized to CO2 and H2O in young seedlings.

The crude fiber content of germinated wheat and green gram decreased in comparison to that of raw samples. This may be due to hydrolyzing of dietary fibers while sprouting (Chandrasiri *et al.*, 2016).

The ash content of the raw samples was higher than that of germinated sample. The decrease in ash content signifies loss in minerals due to rootlet and washing in water to eliminate the sour smell during the period of germination (Tatsadjieu *et al.*, 2004). The reduction in ash content might be due to the leaching out of both macro and micro elements into the soaking water (D'souza, 2013).

The carbohydrate content of germinated wheat and green gram was decreased in comparison to that of raw samples. The reduction of carbohydrate content might be due to their utilization as an energy source to start germination (El-Adawy, 1986).

Compared to the iron content of the raw samples, the iron content of the germinated samples is lower. Iron leaching into the soaking media could be the cause of the reduction after soaking (Saharan *et al.*, 2001).The calcium content of the grains increased after germination in comparison to the raw samples. Similar finding has been found on the work done by Oloyo (2004).

4.3 Organoleptic quality of formulated products

The prepared five Super flour formulae were subjected to sensory evaluation. The samples were provided to ten semi-trained panelists. The semi trained panelists evaluated various parameters of *Lito* namely color, flavor, taste, texture, and overall acceptability. The panelists were requested to provide scores in the score sheets as per their perception. Data was analyzed statistically, and the best product was found out.

4.3.1 Color

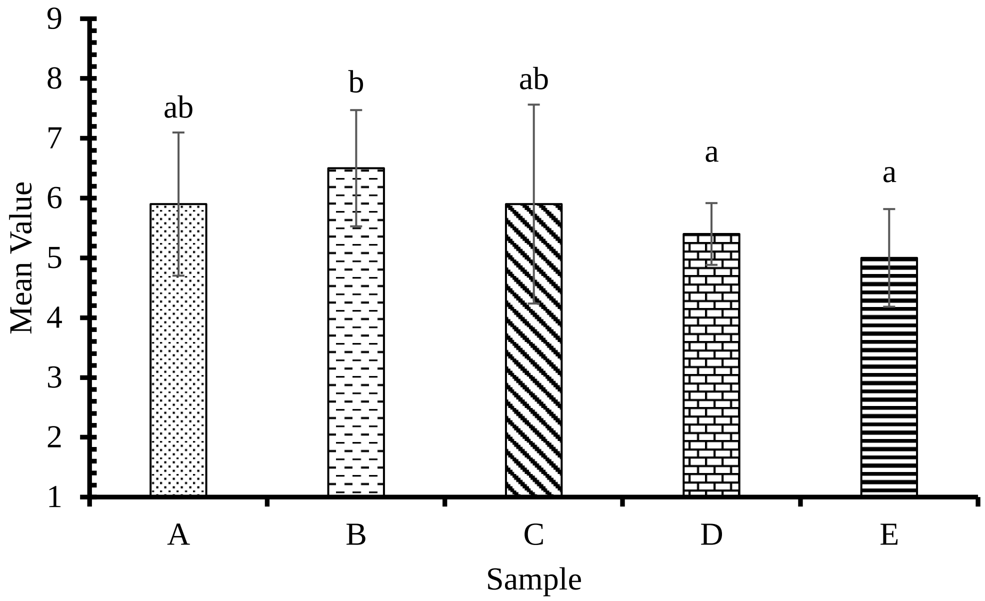

Fig 4.1 Average sensory score for color for five different formulas

The average sensory score for color was 5.9, 6.5, 5.9, 5.4 and 6.8 for A, B, C, D and E, respectively. The analysis of variance showed that color B was significant difference ($p<0.05$) from sample D and E. Sample B had less concentration of mustard green powder so, less green was observed and acceptable by most panelist.

Galla *et al.* (2017) reported that spinach-based products, that is, biscuit, have already been reported for sensory acceptability where in researchers documented better consumer acceptability scores at 5% spinach powder supplementation. Gupta and Prakash (2011b) reported incorporation of greens at 12% levels significantly brought down the sensory scores of all products, mostly attributable to the dark green color of leaves that affected color and appearance of the prepared products adversely.

4.3.2 Flavor

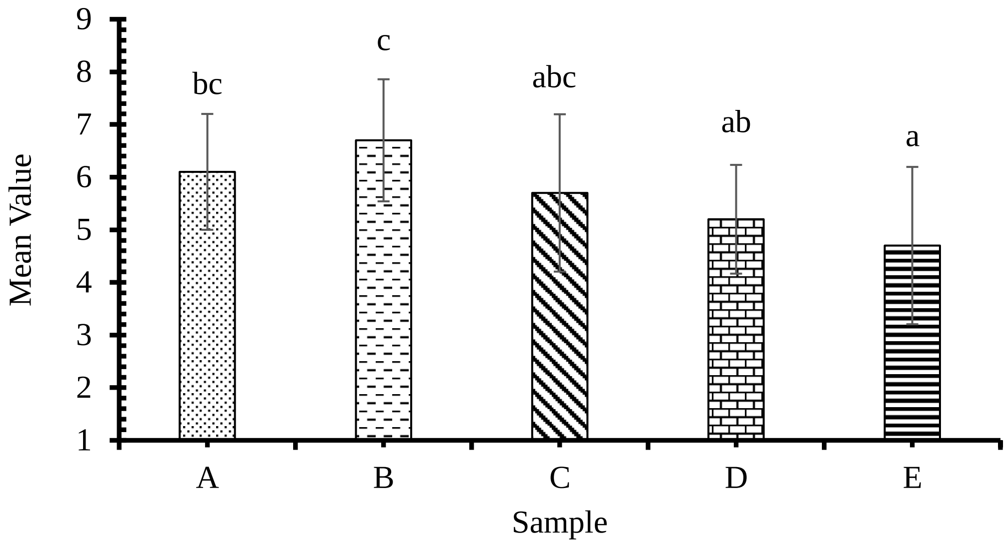

Fig 4.2 Average sensory score for flavor for five different formulas

The average sensory score for flavor was 6.1, 6.7, 5.7, 5.2 and 4.7 and for A, B, C, D and E, respectively. Sample B was significant difference ($p<0.05$) from D and E. Sample B was found to score higher (6.7) among the samples and acceptable by all.

In comparison to other formulations, sample B had an acceptable flavor. This may be because none of the ingredients could fade off the flavor of other ingredients. All the ingredients could contribute equally to flavor of the *lito*.

4.3.3 Texture

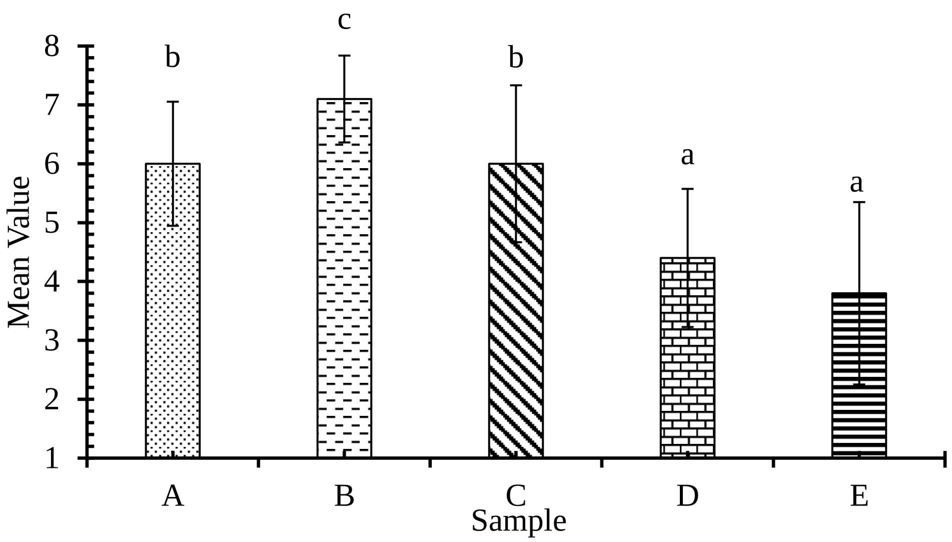

Fig 4.3 Average sensory score for texture for five different formulas

The average sensory score for texture were 6, 7.1, 6, 4.4 and 3.8 for A, B, C, D and E, respectively. The analysis of variance showed that in case of texture, Sample B was significantly different ($p<0.05$) from D and E. Sample B was significantly different ($p<0.05$) from sample A and sample C. Sample B was accepted by most panelist as it showed greater texture than other.

The product incorporated with cauliflower leaves had greater texture at level of 5% of incorporation but the average acceptability scores for texture decreased at the 10% incorporation of green leafy vegetable (Joshi and Mathur, 2015).

4.3.4 Taste

The average sensory score for taste were 5.7, 7.4, 6.1, 4.4 and 3.6 for A, B, C, D and E, respectively. The analysis of variance showed that in case of taste. Sample B was significantly different ($p<0.05$) from sample A, D and E. Sample C was significantly different ($p<0.05$) from sample D and E. Also, sample A was significantly different ($p<0.05$) from sample B and sample D and E. Sample B was reported to be superior significantly from other samples.

According to Shere *et al.* (2018), the results pertaining to taste were not encouraging as the spinach imparts its vegetable taste to the product therefore, taste parameter obtained fewer score.

Fig 4.4 shows the histogram for mean sensory scores of taste attribute.

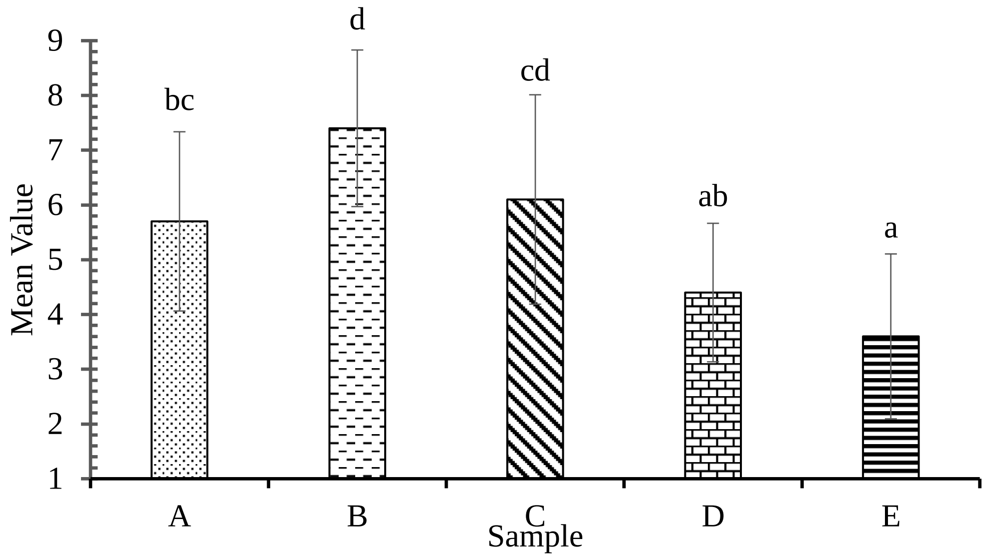

Fig 4.4 Average sensory score for taste for five different formulas

4.3.5 Overall acceptability

The average sensory score for taste were 5.9, 7.7, 6.2, 5 and 4.1 for A, B, C, D and E, respectively. The analysis of variance showed that in case of overall acceptability, Sample B was significantly different ($p<0.05$) from sample A, C, D and E. Sample A was significantly different ($p<0.05$) from sample B and E. Also, sample C was significantly different ($p<0.05$) from sample B and sample D and E. Sample D was significantly different from sample B and E. From the above score, Sample B was reported to have better acceptability.

Khan *et al.* (2015) states that highly acceptable quality chapattis can be prepared using 5% dehydrated spinach powder and addition of beyond 5% levels of dehydrated spinach powder significantly reduced the overall acceptance of the chapattis. Similarly, Gupta and Prakash (2011a) states that sensory evaluation of products incorporated with different levels of dehydrated *Amaranthus paniculatus* and *Peucedanum graveolens* greens revealed that they could be incorporated in traditional products at lower levels of 4% with no detrimental effects on sensory quality. Fig 4.5 shows the histogram for mean sensory scores of overall acceptability attribute.

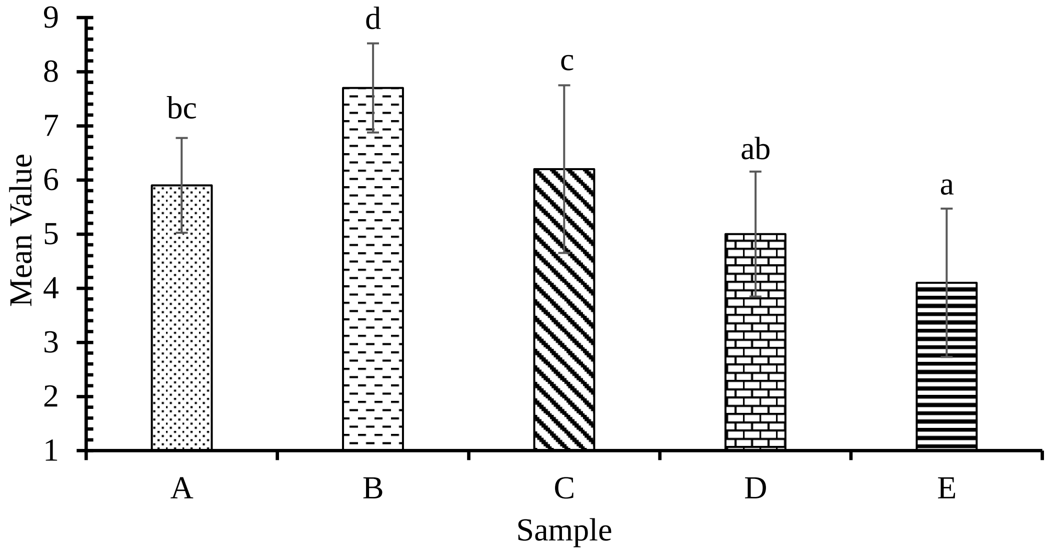

Fig 4.5 Average sensory score for overall acceptability for five different formulas

4.4 Analysis of different formulation of *Lito*

The proximate and ultimate analysis of different formulation of *lito* is mentioned in Table 4.3.

Table 4.3 Analysis of *Lito*

Parameters	A	B	C	D	E
Moisture (%)	5.49^{a}±0.342	5.37^{b}±0.282	5.10^{a}±0.220	6.16^{b}±0.175	6.07^{b}±0.172
Protein (% db.)	18.1^{a}±0.190	18.58^{a}±0.197	19.20^{b}±0.245	19.77^{c}±0.338	20.35^{d}±0.276
Fat (% db.)	1.60^{a}±0.115	1.66^{ab}±0.106	1.76^{abc}±0.141	1.84^{bc}±0.155	1.91^{c}±0.105
Ash content (% db.)	2.37^{a}±0.245	2.54^{a}±0.185	3.07^{b}±0.177	3.47^{bc}±0.247	3.88^{c}±0.378
Crude fiber (% db.)	3.20^{a}±0.208	3.89^{b}±0.149	4.59^{c}±0.337	5.27^{d}±0.240	5.95^{e}±0.211
Carbohydrate (% db.)	74.70^{e}±0.497	73.32^{d}±0.333	71.37^{c}±0.540	69.66^{b}±0.315	67.90^{a}±0.453
Calcium (mg/100 g)	51.88^{d}±0.576	50.24^{c}±0.820	49.53^{bc}±0.176	48.72^{b}±0.965	45.98^{a}±0.430
Iron (mg/100 g)	3.58^{ab}±0.381	3.55^{b}±0.128	3.45^{ab}±0.198	3.32^{a}±0.281	3.20^{a}±0.270
Energy (Kcal/100 g)	385.7^{e}±2.20	382.5^{d}±1.29	378.16^{c}±2.46	374.22^{b}±0.315	370.22^{a}±0.804

(The given values are the means of triplicate samples. Figure after ± sign represents standard deviation.)

There was significant difference ($p<0.05$) in moisture content of sample B, D and E while no significant difference was observed in sample C when compared to control sample A. In case of protein, significant difference ($p<0.05$) was seen in sample C, D and E while sample B wasn't significantly different from control sample A. In case of fat, sample A was significant different ($p<0.05$) from sample D and sample E whereas no significant different was seen in sample B and C. There was significant difference ($p<0.05$) in ash content of sample C, D and E whereas Sample B wasn't significantly different when compared to control sample A. In case of Calcium, Sample A was significantly different ($p<0.05$) from Sample B, C, D and E. Iron wasn't significantly different from other sample when compared to control sample A. There were significant differences ($p<0.05$) in crude fiber, carbohydrate and energy when compared to control sample A.

Calculated values for total energy provided by the blends ranged from 385.7 to 370.22 kcal/100 g dry matter and were significantly different ($p>0.05$). These values are similar to the acceptable and typical energy levels of 375 kcal/100 g dry weight provided by industrially processed weaning foods and predicted energy levels (Heimendinger *et al.*, 1989).

From sensory analysis point of view, product B was found to be superior in terms of color, flavor, taste, texture, overall acceptability. It contained 18.58 grams of protein. Infants and young children require more protein as they get older. The amount of protein needed to meet their daily dietary needs is 9.1 g for 6 to 8 months, 9.6 g for 9 to 11 months, and 10.9 g for 12 to 23 months (in grams per day). Infants and young children get most of the daily protein they need from breast milk. The quantity of protein required from complementary foods is 1.9 g/day at 6-8 months (21%), 4.0 g/day at 9-11 months (42%), and 6.2 g/day (57%) at 12-23 months when typical breast-milk intake is considered (Dewey, 2001; Michaelsen, 2000; WHO, 2003).

According to DFTQC (2017), weaning food must have a soft texture, a high concentration of nutrients, or at least 75 kcal/100 g with the addition of water and 12% of calories from protein, using sources of vegetable protein. According to Imtiaz *et al.* (2011), weaning food prepared from germinated wheat flour and mung seed flour had moisture of 5.15%, protein of 23.97%, fat of 1.33%, total ash of 2.87%, carbohydrate of 65.08%, crude fiber of 1.65% and energy of 377.16 Kcal. In Cerelac, protein-derived kcals was 14.8 (Mahgoub, 1999). According to the Indian Council of Medical Research (Achaya *et al.*, 1981), the recommended calorie requirement for pre-scholar is 7.1% in total mixed diets. The result indicated that formulation B was adequate for weaning purposes.

4.5 Reconstitution of *Lito*

It was done by following the procedure followed by Hussain and Kaul (2019) where 35 g of porridge mix gave the best results in 100 ml of milk and 5 g of sugar.

4.6 Cost Evaluation

The cost of the best formulated *Lito* analyzed from sensory evaluation was NRs. 185 per Kg (Calculation is given in Appendix C).

Nestle Cerelac Fortified Baby Cereal with Milk, Multigrain and Fruits costs NRs. 382 per 300g which is overly expensive for the people with low income.

Thus, there was a vast difference in the price of weaning food self-prepared and baby food available in the market. The prepared mustard green fortified *Lito* cost around NRs. 93 for 500 g while the market value of *Lito* is about NRs. 140. Also, baby foods are also overly expensive in comparison to home prepared. Hence, the prepared mustard green fortified *lito* is cheap and affordable for everyone.

Part V

Conclusion and recommendations

5.1 Conclusion

From the above result and discussions, it can be concluded that:

a. *Lito* was prepared from germinated wheat and green gram varying the proportion of dried mustard green leaves powder in different formulations i.e., Control, 5%, 10%, 15% and 20%.
b. Different chemical analysis was analyzed such as moisture, crude protein, crude fat, ash content, crude fiber, iron, calcium, carbohydrate, and energy content. There was an increment in protein, ash content and calcium whereas carbohydrate, iron and fiber content decreased as compared to raw sample.
c. From the sensory evaluation and statistical analysis of the product, product-B was found to be the best among all; it contained 5% of mustard green powder.
d. The cost of best *lito* was found to be NRs. 134.9 per kg.

5.2 Recommendations

a. Phytochemicals and other anti-nutritional factors such as phytic acid and tannin can be observed.
b. Study on fatty acid composition and amino acid profile of the prepared products can be studied.
c. Other formulations of the *lito* can also be made.

Part VI

Summary

About one in ten children under the age of five suffer from acute childhood malnutrition, especially those who reside in developing nations where conditions of extreme poverty are common. In children under the age of five, malnutrition often starts during the phase when they switch from breast milk to a solid diet. One or a combination of variables, such as improper complemental feeding techniques after 6 months of age, poor digestion or absorption, and higher metabolic needs, may be the precise cause of this growth failure. The ideal weaning meal should be economical, readily available at home, clean, simple to digest, and most importantly, bioavailable. It should also be high in calories and protein, with an acceptable number of trace elements like iron, calcium, vitamins, etc.

Lito is a weaning food that is given to weaned infants. It is made from cereals and legumes in the ratio of 2:1 with the addition of mustard green. The cereals and legumes germinated for 2 days, and mustard green was dried in the cabinet drier for 6-7 hours. Five different products of *Lito* were made from the germinated cereals and legumes varying the amount of green leafy vegetable in each product while keeping the ratio of cereals and legumes (2:1) constant. The raw materials were processed, and the products were prepared in the laboratory and sensory evaluation was performed by ten semi trained panelists. Based on results from sensory evaluation product B containing 5% mustard green powder was taken for further chemical analysis. The analysis includes the proximate analysis of the product. The protein, fat, carbohydrate, crude fiber, and total ash of the product were found to be 18.58%, 1.66%, 73.32%, 3.89% and 2.54% respectively. The product can supply 382.5 kcal/ 100 g. The physiochemical analysis showed significant difference ($p<0.05$) in crude fiber, carbohydrate and energy content when compared to control sample A.

This study where *Lito* has been prepared from locally available food which contains important nutrients required for weaned infants which is nutritious and cost effective. If further researched the production of weaning food using different locally available nutritious food could be possible in Nepal.

PART - VII

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Appendices

Appendix A

Sensory evaluation card

Sensory analysis of *Lito*

Name of Panellist: Date:

Name of the product: Green leafy vegetable incorporated *Lito*

Dear panellist, you are provided five samples of Green leafy vegetable incorporated *Lito* on each proportion with variation on Green leafy vegetable content. Please evaluate the following samples of Super flour and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample as shown below:

Judge the characteristics on the 1-9 scale as below:

Like extremely- 9 | Like slightly- 6 | Dislike moderately- 3

Like very much- 8 | Neither like nor dislike- 5 | Dislike very much- 2

Like moderately- 7 | Dislike slightly- 4 | Dislike extremely- 1

Parameters	Sample Code				
	A	B	C	D	E
Color					
Flavour					
Texture					
Taste					
Overall acceptability					

Any comments:

Signature:

Appendix B

1. Sensory evaluation of the product

Table B.1.1 Two-way ANOVA for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	12.920	3.230	3.14	0.026
Panellist	9	17.620	1.958	1.90	0.083
Residual	36	37.080	1.030		
Total	49	67.620			

Table B.1.2 Two-way ANOVA for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	24.080	6.020	3.99	0.009
Panellist	9	18.480	2.053	1.36	0.242
Residual	36	54.320	1.509		
Total	49	96.880			

Table B.1.3 Two-way ANOVA for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	77.5200	17.8800	18.67	<.001
Panellist	9	30.4200	3.3800	3.53	0.003
Residual	36	34.4800	0.9578		
Total	49	136.4200			

Table B.1.4 Two-way ANOVA for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	88.120	22.030	9.93	<.001
Panellist	9	30.320	3.369	1.52	0.179
Residual	36	79.880	2.219		
Total	49	198.320			

Table B.1.5 Two-way ANOVA for overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	73.080	18.270	15.62	<.001
Panellist	9	21.380	2.376	2.03	0.064
Residual	36	42.120	1.170		
Total	49	136.580			

5 Analysis of different formulation of samples

Table B.2.1 One way ANOVA for moisture

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	2.49364	0.62341	10.18	0.001
Residual	10	0.61233	0.06123		
Total	14	3.10597			

Since, $p<0.05$, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.2 One way ANOVA for protein

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	29.48287	2.37072	36.32	<.001
Residual	10	0.65267	0.06527		
Total	14	10.1355			

Since, $p<0.05$, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.3 One way ANOVA for crude fat

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	0.19569	0.04892	3.04	0.070
Residual	10	0.16087	0.01609		
Total	14	0.35656			

Since, $p<0.05$, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.4 One way ANOVA for ash content

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	4.75264	1.18816	17.98	<.001
Residual	10	0.66100	0.06610		
Total	14	5.41364			

Since, p<0.05, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.5 One way ANOVA for crude fiber

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	14.24209	3.56052	63.06	<.001
Residual	10	0.56460	0.05646		
Total	14	14.80669			

Since, p<0.05, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.6 One way ANOVA for carbohydrate

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	89.5554	22.3888	117.24	<.001
Residual	10	1.9097	0.1910		
Total	14	91.4651			

Since, p<0.05, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.7 One way ANOVA for calcium

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	56.8965	14.2241	33.00	<.001
Residual	10	4.3097	0.4310		
Total	14	61.2062			

Since, p<0.05, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.8 One way ANOVA for iron

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	0.84731	0.21183	2.60	0.101
Residual	10	0.81527	0.08153		
Total	14	1.66257			

Since, p<0.05, there is a significant difference between the samples, so LSD testing is necessary.

Table B.2.9 One way ANOVA for energy

Source of variation	d.f.	s.s	m.s	v.r	F pr.
Sample	4	464.669	116.167	40.04	<.001
Residual	10	29.012	2.901		
Total	14	493.682			

Since, p<0.05, there is a significant difference between the samples, so LSD testing is necessary.

Appendix C

Table C.1 Cost calculation of the product

Particulars	Cost (NRs/Kg)	Weight (g)	Cost (NRs/Kg)
Wheat	70	633	44.31
Green gram	180	317	57.06
Mustard green	120	50	6
Total raw materials			107.37
Processing and labor cost (Ten percent of total material cost)			10.73
Profit (10% of total cost)			16.8
Grand Total			134.9

The cost of raw materials varies with season and time.

List of Plates

P1 Formulated samples of different compositions

P2 Protein Determination through Kjeldahl method