FORMULATION OF PORRIDGE FROM GERMINATED BARLEY (*Hordeum vulgare*), PADDY (*Oryza sativa*) & GREEN GRAM *(Vigna radiata)* AND ITS NUTRITIONAL EVALUATION

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2023

Formulation of Porridge from Germinated Barley *(Hordeum vulgare),* Paddy *(Oryza sativa)* & Green gram *(Vigna radiata)* and Its Nutritional Evaluation

A dissertation submitted to the Department of Nutrition and Dietetics, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of Bsc. Nutrition and dietetics

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July, 2023

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Approval Letter

This ***dissertation*** entitled ***Formulation of Porridge from Germinated Barley (Hordeum vulgare), Paddy (Oryza sativa), & Green Gram (Vigna radiata) and its nutritional evaluation presented*** by **Kala Khadka** has been accepted as the partial fulfillment of the requirements for **Bachelor degree in Nutrition and Dietetics.**

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Acknowledgement

First and foremost, I would like to express my gratitude to my research supervisor Mr. Sabin Bahadur Khatri; Teaching Assistant of Central Campus of Technology, for the valuable discussions, efforts, encouragement, patience, understanding and continuous effort throughout this study. His guidance helped me in all the time of research and writing of this thesis.

Furthermore, I am very much indebted to Prof. Dr. Dil Kumar Limbu, Campus Chief, Central Campus of Technology, for providing all the necessary facilities for my dissertation. From deepest of my heart I would like to thank HOD of Nutrition and Dietetics, Asst. Prof. Mr. Kabindra Bhattarai for providing me with all the necessary facilities for the research.

Many thanks and gratitude are expressed to all the teachers and staff members. My special thanks go to Mr. Sachin Basnet, Miss Sharon Bhandari, Mr. Mahesh Shrestha and Mr. Suman Magar, for making my entire study in an enthusiastic and passionate environment.

I am always grateful to my supportive friends Mandira Khadka, Aashish Khadka, Rushna Basnet, Junu Tamang, Soniya Rai and Dipen Shrestha for their valuable time and assistance who were available for me at any time.

Above all, I am indebted to my parents without whose constant encouragement, inspiration and affection, I would never reach this far, and this work would have never seen the light of the day.

Date of submission: July, 2023

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Abstract

Porridge is a convenient food not just for weaning infants, but also for the elderly and convalescents due to its easy digestibility. Protein energy malnutrition is still a major problem in all the segments of population. Sixteen products were formulated varying the proportion of cereals and pulses using design expert software. The grains selected were cleaned, washed, soaked, germinated, dried, roasted and then stored in air tight containers. The roasted grains where then mixed homogenously according to the required proportion and then milled. The milled grains where then sieved and the grits obtained were kept in separate air tight containers. Green gram was germinated for several days and tannin and phytic acid content was checked each day.

It was found that tannin and phytic acid content was reduced in the germinated sample when compared to the raw sample. The tannin and phytic acid content of green gram were reduced by 35.45% and 33.71% after 72 hours of germination respectively. From the sensory evaluation and statistical analysis of the sixteen products, the formulation containing 30 g of barley, 20 g of rice and 50 g of green gram was found to be the best. The protein, fat, carbohydrate, crude fiber, and total ash of the product were found to be 27.8%, 2.5%, 64.29%, 3.3% and 2.2% respectively. The food can supply 390.86 kcal/100 g. The iron and calcium content of the product were found to be 3.7 mg/100 g and 83.8 mg/100 g respectively. The total cost for the preparation was calculated as NRs. 125 per kg. Hence, the prepared porridge is nutritious and cost effective weaning food and germination was considered as one of the effective method for reduction of antinutritional factors.

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List of Abbreviations

Abbreviations	Full form
RDA	Recommended Dietary Allowance
NDHS	Nepal Demographic and Health Survey
%	Percent
ANOVA	Analysis of Variance
WHO	World Health Organization
LSD	Least Significant Difference
G	Gram
d.f.	Degree of Freedom
m.s.	Mean Squares
Mg	Milligram
^{o}C	Degree Celsius
s.s.	Sum of Squares
v.r.	Variance Ratio
µg	Microgram
CCT	Central Campus of Technology
$FeCl_3$	Ferric Chloride
Fig.	Figure
H	Hour
Kcal	Kilocalorie

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Part I

Introduction

1.1 General introduction

Porridge is a convenient food not just for weaning infants, but also for the elderly and convalescents due to its easy digestibility (Rhim *et al.*, 2011) . It is a traditional food in much of Northern Europe and Russia. It was also commonly used as prison food for inmates in the UK prison system and so "doing porridge" became a slang term for a sentence in prison. In India, Dalia is actually the blended course granules of wheat and various types of pulses. In developing countries breakfast meals for both adults and infants are based on local staple diet made from cereals, legumes, roots, cassava and potatoes tubers (Rana *et al.*).

The protein digestibility and biological value of rice have been reported to be higher than those of the other major cereals (i.e. wheat, corn and barley). It also represent an interesting source of proteins for the development of protein-enriched ingredients for the formulation and manufacture of nutritional products (Amagliani *et al.*, 2017). Barley also contains β-glucans which is also regarded as important functional ingredients for the cereal food industry (Brennan and Cleary, 2005).

Cereals are limited in essential amino acids such as lysine even though rich in threonine and tryptophan, while most oil seeds and legumes are rich in lysine and deficient in sulphur containing amino acids. Therefore, the combination of cereals and pulses in formulation of dalia gives a nutritious food containing all the amino acids. Porridge produced from cereals is eaten in many parts of world particularly in developing countries where they are part of the basic diet (Ocheme and Chinma, 2008). Depending on the proportion of the cereals and liquid, two types of porridges are prepared for consumption that can be easily distinguished: thick and thin porridges. Thick porridges are solid-like and can be consumed with spoon or hand whereas thin porridge or gruel is taken in by drinking as having fluid or semi-fluid consistency. The infants are usually given thin porridge as a complementary meal.

Legumes are an excellent source of protein, dietary fiber, micronutrients and bioactive compounds and also source of resistant starch which is broken down by bacteria

in the large intestine to produce short-chain fatty acid (such as butyrate) used by intestinal cells for food energy. Pulses contain no cholesterol, little fat and sodium like other plant-based foods (Prasad *et al.*, 2016).

Cereals and pulses are the major source of protein in the diet. A judiciously mixing of pulses with cereals was in practice long before food scientists and nutritionists understood the nutritional importance of this practice. The protein quality of pulses for food is low; however, when mixed with cereals total dietary quality can exceed 70% of milk protein. In the diet, nearly 85% of protein is supplied by plant and plant products. In this context, pulses are important and cheaper source of protein when compared to animal protein besides this; they are also good source of minerals and vitamins in the daily diets of the people, especially of low-income groups. In addition to this, pulses have been reported to reduce the levels of cholesterol and blood glucose. On this context, dietary importance of pulses is having global appreciation and recognition (Eggum and Beames, 1983).

Germination is a natural process that occurs during growth period of seeds where they meet the minimum condition for growth and development. During this period, reserve materials are degraded which is commonly used for respiration and synthesis of new cells prior to developing embryo. Different studies found that effect of germination on legumes can improve digestibility of protein content and dietary fiber, reduce tannin and phytic acid content and also increase mineral bioavailability. Germination was also reported to be associated with bioavailability of trace elements and minerals and also improved calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content (Megat Rusydi and Azrina, 2012).

1.2 Statement of the problem

Protein Energy Malnutrition (PEM) generally occurs during the crucial transitional phase when children are weaned from liquid to semi-solid or fully adult foods. During this period, because of their rapid growth, children need nutritionally balanced, calorie-dense supplementary foods in addition to mother's milk (Wondimu and Malleshi, 1996).

Under nutrition is one of the major problems confronting infants and young children in developing countries such as Nepal. The problem arises from two factors: an inadequacy in the supply of food needed for infants and children; and ineffective utilization

of such foods available. The weaning period is a critical one in child feeding; appropriate complementation during this period is essential to the nutritional well-being of the child (Alnwick *et al.*, 1988). Poverty and food insecurity seriously constrain accessibility of nutritious diets, including high protein quality, adequate micronutrient content and bioavailability, essential fatty acids, low anti-nutrient content, and high nutrient density (Baskota, 2019).

Nepal being a developing country, malnutrition has been its major problem. The trend of malnutrition is higher in under five children. Acute malnutrition affects 11 % of children aged below five years in Nepal, where 2.6 % are severely malnourished and 8.3% are moderately malnourished (Case, 2012). One of the reasons for this is inappropriate initiation and correct method of doing complementary feeding practices after 6 months of age. The desirable weaning food should be rich in calories, protein and adequate amount of trace elements like iron, calcium, vitamins etc. and also inexpensive, home available, clean, easily digestible and the most importantly bio-available (Dop and Benbouzin, 1999).

(Adsule *et al.*, 1986) reported that nutritious baby foods have been developed by blending cereals and legumes with or without skim milk powder. Inclusion of various legumes in the diet has been shown to improve the condition of children suffering from protein malnutrition and to promote growth in underweight children who are less seriously malnourished. The nutritional quality of green gram is relatively better than most of the other cereals. Among the legumes, green gram has relatively low amounts of protease inhibitors, polyphenols and flatus-producing oligosaccharides and no hem-agglutinating and amylase inhibitor activities. While soaking and germination of green gram improves the availability of nutrients and has beneficial effects on its digestibility and nutritional quality. Green gram has high protein content and is rich in lysine. Therefore, green gram can be blended with cereals to improve the nutritional quality of cereals-based products like bread, chapatti (unleavened bread), and confectionary products. Green gram, particularly after germination, can be used in the preparation of cheap and nutritious weaning foods.

During this time, special foods are rarely available for the children. Consequently, they have to depend on the same types of foods as those eaten by adults. In the poor countries, these foods are mainly starchy tubers like cassava and sweet potato, or cereals

like maize, rice, wheat, sorghum, and millet. Industrial manufacture of cereal-based weaning foods often includes operations intended to reduce processes modify the starch structures and hence results in lower water-binding in the gruels. However, such sophisticated technologies make rather make expensive products even when low-cost alternatives are developed, and in poor countries these products are normally only available to urban children of higher income families (Mosha and Svanberg, 1983).

1.3 Objectives

1.3.1 General Objectives

The general objective of the work is to prepare porridge from germinated barley, paddy and green gram and its nutritional evaluation.

1.3.2 Specific Objectives

- To formulate and evaluate porridge using varying proportion of germinated rice, barley and green gram.
- To determine sensory and nutritional properties of prepared porridge.

1.4 Significance of the study

The need for subsidized weaning foods must be recognized by the country so that these foods are made available to low-income groups. This can be achieved in part through the creation of low-cost production units at the peasant association level; such units could be planned and administered by the national organizations themselves. This formula will be beneficial especially to children of low-income group. This study will provide a basis for the preparation of weaning food from locally available. This weaning food could be effective in terms of digestibility and bioavailability for growing children. It is easy to prepare and is quick, thus, saves cooking time and require few cooking skills. The weaning food protein quality is also increased by the supplementation of cereal and pulses. Thus, it could be a complete food for growing children. So this work primarily focuses as the alternative to industrial processes for reducing the dietary bulk of weaning foods using locally available raw materials that is inexpensive, easily digestible and bioavailable which is due to the increased use of improved traditional food preparation procedures that will also modify starch structures (Alnwick *et al.*, 1988).

1.5 Limitations of the study

- Analysis of amino acid could not be performed.
- Phytochemical analysis of cereal grain could not be done due to time constraints.

Part II

Literature review

2.1 Food and Nutrition

Food has been a basic part of our existence. Through the centuries we have acquired a wealth of information about the use of food to ensure growth of children and youth, to maintain good health through life, and to meet special needs of pregnancy and lactation to use it to recover from illness. Food is that which nourishes the body. Food may also be defined as anything eaten or drunk, which meets the needs for 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).

Nutrition has been defined as food at work in the body. Nutrition includes everything that happens to food from the time it is eaten until it is used for various functions in the body. The study of the science of nutrition deals with what nutrients we need, how much we need, why we need these, and where we can get them. Nutrition is the result of the kinds of foods supplied to the body and how the body uses the food supplied (Mudambi, 2007). Good nutrition is associated with human well-being. Right from the pregnancy to birth, during the infancy and in adulthood good nutrition plays a vital role in physical and mental well-being of human being including brain functioning, immune system and physical activities, which ultimately leads to increase overall productivity of human being. Good nutrition flows throughout the life cycle and across the generations, so it plays a central role in the country's overall development (Haddad *et al.*, 2015).

2.2 Weaning

The introduction to solid feeding and the gradual replacement of milk by solid food as the main source of nutrition is the process known as weaning (Foote and Marriott, 2003). The term weaning is commonly used to refer to the termination of lactation. According to this broader definition, the weaning process is initiated with the first intake of non-milk foods and culminates in the complete cessation of suckling. The weaning process is therefore bracketed by two distinct events that may be separated by days, months or even years.

From an individual perspective these are important milestones in development. In practice these milestones may be difficult to determine in observational studies and are not always particularly meaningful in terms of energy budgets and reproductive constraints (Humphrey, 2010).

For the majority of infants weaning should commence between 4-6 months of age. The introduction of new foods in a gradual process using a few pureed semisolid foods followed by an increasing familiarization with a greater range of tastes and textures and the beginning of self-feeding should take at least six months. From 1 year of age a child should be capable of participating in family meals and eating at least some family foods. The nutritional content of weaning foods becomes of increasing importance as infancy progresses. The most pertinent concerns are the nutrient densities of the foods and the bioavailability of essential micronutrients therein (Foote and Marriott, 2003).

Unwillingness of the infant to eat while exerting favourite to beverage rather than eating, allergic reactions and health problems with child including vomiting, abdominal colic and diarrhoea may ascend due to wrong feeding practices adopted by mothers. However, it was estimated that, there were 10 million annual deaths of under-five year old children. Over one third of under-five mortality is caused by malnourishment related to inadequate complementary feeding during weaning practice. Initiate safe and nutritionally adequate complementary foods at 6 month is vital to achieve optimal growth, development and health of the children. Weaning is often beneficial because it reduces early infant mortality. Proper weaning practice transforms the baby into a time of dietary independence from breastfeeding and confirms appropriate growth and development of the child. Proper weaning practice takes into consideration dietary need, right timing, adequate food consistency, frequency and hygiene. In addition, if weaning practice is not properly done, failure to succeed can be a complication which can lead to infant mortality and morbidity (Ibrahim *et al.*, 2017).

Weaning is the process by which a baby slowly gets used to eating family or adult foods and relies less and less on breast milk. The process varies from culture to culture and is often regulated by the child's individual needs. Healthy babies of weaning age are growing and developing very fast, so great care has to be taken to see that they are getting enough of the right kind of food. During weaning babies move about more, and become

more independent of their mothers. They start to come into greater contact with germs in the environment. At the same time the way in which a baby's body is protected against germs changes. When babies are very small they still have protection (immunity) received from their mothers during pregnancy. But after about 4- 5 months this protection has gone, and babies start to develop their own immunity as they come into contact with germs in the environment. Because of this change babies are very likely to get infectious illnesses from the age of about 4-5 months especially if they are not breast-fed. This is why any food prepared for babies should be stored and fed to them in very hygienic ways (Armstrong, 1989).

Babies who are malnourished may get worse during the weaning period, and babies may become malnourished for the first time during weaning. Poor feeding and illness stop many children of weaning age growing well. This shows up on the growth chart as poor weight gain, or in more serious cases, as weight loss (Armstrong, 1989). Appropriate complementary feeding during the weaning period is a complex aspect of child feeding, and is critical to nutritional well- being. In infancy, growth is more rapid than at any period of life; nutritive requirements per unit of body weight are also greater. Good food sources of energy, protein, calcium, and iron are particularly important during this time. On the basis of body weight, children require twice as much protein, calcium, and iron as do adults (Alnwick *et al.*, 1988).

Any item besides breast milk that is given to the infant in any manner represents a weaning food (Brown, 1978). In Nepal, complementary foods have traditionally been of low caloric density and low protein content, containing little or no fat, and often limited in micronutrients. Such foods are not well suited as supplements to the breastfed infant's diet. Moreover, supplementary feeding is usually initiated too late and in quantities that are inadequate. As a result of all these factors, infants are commonly half-starved from 6-12 months of age (Alnwick *et al.*, 1988).

2.3 Nutritional requirement of weaning food

A nutritionally adequate weaning diet is essential for achieving optimal growth in the first year. Growth in the first year influences both the well-being of the child and the long-term health of the adult (Sajilata *et al.*, 2002). Infancy and childhood are both critical periods of rapid physical growth and cognitive and emotional development. It is also well recognized

that nutrition in these age groups is not only an important factor for the normal development but also fundamental for future health status. Infants are considered a vulnerable group because they have a relatively high requirement of nutrients per unit body weight during a sensitive period of rapid growth and development. In addition, recent research has shown that infancy is a critical period, setting the foundation of long-term health and reduced risk for chronic diseases such as cardiovascular disease, dyslipidemia, obesity, and osteoporosis. Some of the main physiological considerations include weaning, increased growth rate, occurring brain development, and the still immature function of kidneys and the gastrointestinal system (Grammatikaki and Huybrechts, 2016).

The requirement of nutrients of infants aged 1-3 years are energy 1060 kcal, protein 16.7 g/day, fat 27 g/day, iron 9 mg/day, calcium 600 mg/day (Sesikeran, 2010). Table 2.1 shows recommended dietary allowances for children from 6 months to 3 years.

Table 2.1 Recommended Dietary Allowances for children from 6 months to 3 years

Nutrients	**(6-12) months**	**(1-3) years**
Body weight	8.4	12.9
Net calories (kcal/kg)	80 kcal/kg	1060
Proteins (g/kg)	1.69	16.7
Visible fat (g)	19	27
Calcium (mg)	500	600
Iron (mg)	5	9
Vitamin A (µg)		
Retinol	350	400
β carotene	2800	3200
Zinc (mg)	-	5
Magnesium (mg)	45	50
Thiamine (mg)	0.2	0.5
Riboflavin (mg)	0.6	0.8
Niacin (mg)	650 µ/kg	8
Pyridoxine (mg)	0.4	0.9
Vitamin B12 (µg)	0.2	0.2-1.0
Ascorbic acid (mg)	25	40
Dietary folate (µg)	25	80

Source: (Sesikeran, 2010)

2.4 Ingredients used in porridge preparation

2.4.1 Cereals

Cereals belong to the Graminae family, commonly known as grasses. Most of these plants are perennial; however, all commercial cereals are annual. Cereals are the main food source for mankind. By far they provide most of the calories, proteins, B-vitamins, and minerals. The inhabitants of developing countries have a higher dependency on cereal-based foods because they are cheaper compared to animal foods. A cereal is any grass cultivated for the edible components of its grain composed of the endosperm, germ, and bran. Cereal grains are grown in greater quantities and provide more food energy

worldwide than any other type of crop and are therefore staple crops. In their natural form (as in whole grain), cereals are a rich source of vitamins, minerals, carbohydrates, fats, oils, and protein. When refined by the removal of the bran and germ, the remaining endosperm is mostly carbohydrate (Serna-Saldivar, 2016).

Cereals contain water-soluble fiber, such as β-glucan and arabinoxylan, oligosaccharides, such as galacto and fructo-oligosaccharides and resistant starch, which have been suggested to fulfil the prebiotic concept. Cereals contain water-soluble fibre, such as β-glucan and arabinoxylan, oligosaccharides, such as galacto- and fructo-oligosaccharides and resistant starch, which have been suggested to fulfil the prebiotic concept (Charalampopoulos *et al.*, 2002).

2.4.1.1 Barley

Barley (Hordeum vulgare) is an ancient and important cereal grain crop. Barley was presumably first used as a human food but evolved primarily into a feed, malting and brewing grain due in part to the rise in prominence of wheat and rice. In recent times, about two-thirds of the barley crop has been used for feed, one-third for malting and about 2% for food directly. However, throughout its history, it has remained a major food source for some cultures principally in Asia and northern Africa. Most production of barley takes place in Europe (62%), followed by Asia (15%) and America (13%). Figure from 1991 show that the use of barley for food in 12 countries in Europe averaged around 0.3%, while the use in e.g. China, India and Ethiopia was above 60%. It is used worldwide for animal feed and human food, with its main use for human consumption being the production of alcoholic drinks (though proportions vary greatly for cultural reasons in different countries) (Newman and Newman, 2008).

2.4.1.2 Paddy

Paddy (*Oryza sativa* L.) is the most important cereal crop in the developing world and is the staple food of over half the world's population (Juliano, 1993). During the last 12,000 years or so, the long process of cultivation, domestication, dispersal and diversification is also a facet of human history for the rice-eating peoples of Africa and Asia. Today, human activities have led to the growing of paddy in more than 100 countries of the world (Smith and Dilday, 2002).

It is the staple food for over half the world's population, mainly in Asian countries, where it provides a considerable proportion of the protein intake for millions of people. The protein digestibility and biological value of rice have been reported to be higher than those of the other major cereals (i.e., wheat, corn and barley). Paddy represents an interesting source of proteins for the development of protein-enriched ingredients for the formulation and manufacture of nutritional products (Amagliani *et al.*, 2017).

2.4.2 Pulses

Pulses belong to the family leguminosae. The family leguminosae is made up of many species which are cultivated all over the world. Pulses have a high protein content, the value is about twice that in cereal and several times that in root tuber, so they can help to improve the protein intake of meals in which cereals and root tubers in combination with pulses are eaten. Pulse when eaten with cereals, can also help to increase the protein quality of meal (Ofuya and Akhidue, 2005).

Pulses are an important source of nutrition for billions of people around the world. s. Pulse grains are an excellent source of protein, carbohydrates, dietary fibre, vitamins, minerals and phytochemicals. Large number of people in the world consumes pulses as staple food in combination with cereals and depends on them for meeting their protein requirement. The high lysine and folate content makes pulses perfect for making the composite flours with cereals. Pulses contain approximately 21–25% protein; however have limiting amount of essential amino acids such as methionine, tryptophan and cysteine. The protein content in pulses is almost double than that found in cereals. Pulses can provide adequate minerals required to fulfil nutritional requirement(Singh, 2017). Legume seeds generally contain 20% to 30% protein and are lysine rich, complementing the nutritional profiles of cereals and tubers in the diet (Graham and Vance, 2003).

2.4.2.1 Green gram

Mung bean is well known as green gram or mung bean. Mung bean 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). Green gram can be an excellent source of protein with higher digestibility. Green gram is an excellent source of protein and is almost free from flatulence-causing factors. Because of this, green gram seeds are preferred for feeding

babies and those convalescing. The seeds contain a higher proportion of lysine than any other legume seeds (Adsule *et al.*, 1986).

2.5 Nutritive value of cereals and pulses

(Ghavidel and Prakash, 2010) studied that germinated dehulled legumes can be used in combination with cereals and vegetables for producing composite weaning mixes, which will prove to be beneficial especially for young children in developing countries, because of their low cost and ease of preparation.

(Sadana and Chabra, 2004) conducted a research on development and sensory evaluation of low cost weaning food formulation which showed that germinated and supplemented grain flour weaning food formulations were more acceptable as compared to control products prepared from ungerminated grain flour.

A study done by Nutrition Collaborative Research Program during the market analysis of complementary foods in Nepal showed the nutritional value of Sarbottam Pitho were Moisture 3.53%, Protein 14.72%, Fat 7.4%, Ash 1.92%, Carbohydrate 73% and Energy 400 Kcal (Magnani *et al.*, 2012).

(Kanu *et al.*, 2009) conducted a research on production and evaluation of breakfast cereal-based porridge mixed with sesame and pigeon peas for adults. It was found that the formulated products contained (10-13) % protein, (75-80)% carbohydrate and (132.2-477.8) Kcal energy. It showed that foodstuff blends can be of high nutritional value and a balanced status than their individual components.

It was found that protein content was slightly decreased in the roasted weaning food because of the heat denaturation of proteins and more decreased in the malted weaning food because of the leaching out of the soluble protein and removal of rootlets, thereby reducing the total dry matter during malting (Kshirsagar, 1994).

A research which was conducted on composite weaning mixes showed the nutritional characteristics of formulated weaning food which contained (4.2-4.3) % moisture, (20-23) % protein, (0.8-1.5) % fat, (1.73-2.43) % ash and (3.45-7.49) % crude fiber (Ghavidel and Prakash, 2010).

2.6 Germination

Germination is a complex process during which the seed must quickly recover physically from maturation drying, resume a sustained intensity of metabolism, complete essential cellular events to allow for the embryo to emerge, and prepare for subsequent seedling growth (Nonogaki *et al.*, 2010).

Germination of pulses and cereals is one of the traditional methods of food processing, which is extensively used in the preparation of weaning and geriatric foods. Germination generally improves the nutritional quality of foods by increasing their nutrient content and digestibility. The content of vitamin C is particularly enhanced during the process of germination. Germination can bring about a twofold increase in bioavailability of iron. Most weaning foods are prepared from cereals or starchy roots, commonly reconstituted with water. Such foods become highly viscous when reconstituted and are difficult to feed to infants. Due to the small stomach capacity of infants, they cannot consume adequate amounts of bulky foods, resulting in inadequate intakes of vital nutrients (Luo *et al.*, 2014).

Germination has been found to change the appearance, flavor, and taste of the grain as well as their nutritional value. The products might be consumed in form of sprouts or further processed, e.g., dried or roasted (Hübner and Arendt, 2013). Seed germination depends on both internal and external conditions. The most important external factors include right temperature, water, oxygen or air and sometimes light or darkness. Various plants require different variables for successful seed germination. Often this depends on the individual seed variety and is closely linked to the ecological conditions of a plant's natural habitat. For some seeds, their future germination response is affected by environmental conditions during seed formation; most often these responses are types of seed dormancy (Raven *et al.*, 2005).

Germination increases the activity of endogenous phytase activity in cereals, legumes, and oil seeds through de novo synthesis, activation of intrinsic phytase, or both. Tropical cereals such as maize and sorghum have a lower endogenous phytase activity than do rye, 14 wheat, triticale, buckwheat, and barley. Hence, a mixture of cereal flours prepared from germinated and ungerminated cereals will promote some phytate hydrolysis when prepared as porridge for infant and young child feeding. The rate of phytate

hydrolysis varies with the species and variety as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57°C), solubility of phytate, and the presence of certain inhibitors (Egli *et al.*, 2002).

Germination has often been proposed as a simple processing method by which the nutrient composition and certain functional properties of seeds might be improved and by which the quality of a cereal can be improved for both digestibility and physiological function. During germination, enzymatic activity and bioactive compounds increased within the seed. Germination is induced by rehydration of the seed, which increases both respiration and metabolic activity that allow the mobilization of primary and secondary metabolites and improves the nutritional and functional qualities by changing chemical compositions and eliminating antinutritional factors (I. Hussain and Uddin, 2012).

2.7 Antinutritional factors

Anti-nutritional factors are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods. Antinutrients in plant foods are responsible for deleterious effects related to the absorption of nutrients and micronutrients. These anti-nutritional factors are also known as ‘secondary metabolites’ in plants and they have been shown to be highly biologically active. These secondary metabolites are secondary compound produced as side products of processes leading to the synthesis of primary metabolites (Gemede and Ratta, 2014).

The possibility now exists to eliminate anti-nutrients entirely using genetic engineering; but, since these compounds may also have beneficial effects, such genetic modifications could make the foods more nutritious but not improve people's health (Welch and Graham, 2004). Many traditional methods of food preparation such as fermentation, cooking, and malting increase the nutritive quality of plant foods through reducing certain anti-nutrients such as phytic acid, polyphenols, and oxalic acid (Hotz and Gibson, 2007).

2.7.1 Tannin

Tannin is an astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannins are known to be responsible for decreased feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. If tannin concentration in the diet

becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed [9]. Tannins also form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the antinutritional effects of tannin containing foods (Gemede and Ratta, 2014).

Humans also consume a number of other foods containing considerable amounts of condensed tannins, especially in beverages, i.e., cider, tea, cocoa, and red wine. When fed at levels that commonly occur in cereals and legumes (approximately 1 to 2%), tannins have depressed the growth rate and resulted in poor feed efficiency ratio and increase in the amount of food required per unit weight gain. Other deleterious effects of tannins include damage to the mucosal lining of the GI tract, alteration in the excretion of certain cations, and increased excretion of proteins and essential amino acids. High dietary levels (about 5%) can cause death (Deshpande *et al.*, 1986).

Most legumes contain tannins. Red-colored beans contain the most tannins, and white colored beans have the least. Condensed tannins inhibit digestion by binding to consumed plant proteins and making them more difficult to digest, and by interfering with protein absorption and digestive enzymes. Tannins form insoluble complexes with proteins, carbohydrates and lipids leading to a reduction in digestibility of these nutrients. Many tannin-consuming animals secrete a tannin-binding protein (mucin) in their saliva. Tanninbinding capacity of salivary mucin is directly related to its proline content. Salivary proline-rich proteins (PRPs) are sometimes used to inactivate tannins. One reason is that they inactivate tannins to a greater extent than do dietary proteins resulting in reduced fecal nitrogen losses. PRPs additionally contain non-specific nitrogen and non-essential amino acids making them more convenient than valuable dietary protein (Shimada, 2006).

2.7.2 Phytic acid

Phytic acid (PA, myo-inositol hexakisphosphate, IP6) is a natural plant compound with a unique structure that is responsible for its characteristic properties. Phytate is the calcium salt of PA and phytin is the calcium/magnesium salt of PA. Complete hydrolysis of phytic acid results in inositol and inorganic phosphates (Oatway *et al.*, 2001).

Phytic acid is found in most cereal grains, legumes, nuts, oilseeds, tubers, pollen, spores, and organic soils. It acts as the primary phosphorus reserve accounting for up to 85% of the total phosphorus (P) in cereals and legumes (1). In cereal grains, oilseeds, and

legumes, phytate P constitutes the major portion (60–97%) of total P, and in roots and tubers 21–25% of total P may occur as phytates. Phytic acid is deposited in seeds during the seed development (Oatway *et al.*, 2001). The most concentrated sources tend to be oil seeds, whole grains and legumes. Roots, tubers, and other vegetables may also contain phytic acid, but usually in lower amounts Phytic acid is isolated in the aleurone layer in most grains, making it more concentrated in the bran. In legumes, it's found in the cotyledon layer (Nissar *et al.*, 2017).

Phytic acid, mostly as phytate, is found within the hulls of seeds, including nuts, grains and pulses. In-home food preparation techniques can break down the phytic acid in all of these foods. Simply cooking the food will reduce the phytic acid to some degree. More effective methods are soaking in an acid medium, sprouting and lactic acid fermentation such as in sourdough and pickling (Reddy *et al.*, 1989). Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc, although the binding of calcium with phytic acid is pH-dependent (Dendougui and Schwedt, 2004). The binding of phytic acid with iron is more complex, although there certainly is a strong binding affinity, molecules like phenols and tannins also influence the binding (Prom-u-thai *et al.*, 2006). When iron and zinc bind to phytic acid they form insoluble precipitates and are far less absorbable in the intestines. This process can therefore contribute to iron and zinc deficiencies in people whose diets rely on these foods for their mineral intake, such as those in developing countries and vegetarians (Association, 2003).

2.7.3 Oxalates

Oxalate is dianion with the formula $C_2O_4^{2-}$, also written $(COO)_2^{-2}$. Oxalates occur in many plants where it is synthesized by incomplete oxidation of carbohydrate (Philip, 2012).

Oxalates are found most commonly in dark coloured fruits and vegetables like berries, spinach and also cereals and legumes like wheat, rye, soybean, tofu, lentils, kidney beans. Consumption of high oxalate foods exerts a negative effect on calcium and iron absorption in the body (Chai and Liebman, 2005). Oxalates can be found in all plant foods; however, certain plants contain very high levels and people who are prone to kidney stone formation are advised not to eat these foods. Oxalates that is bound to calcium travels as a waste product from the blood to kidney and excreted from the body in the urine. Consumption of high oxalates binds to calcium in body and forms crystal resulting in

kidney stone. Oxalate is also an end-product of metabolism in the liver. Some amino acids and carbohydrates are degraded to oxalates (Savage and Klunklin, 2018).

2.7.4 Trypsin inhibitor

Trypsin inhibitor inhibits the function of trypsin enzyme, causes pancreatic hypertrophy and dietary loss of cysteine. Trypsin inhibitors are proteins that interfere with nutrient absorption by reducing the activity of proteolytic enzymes trypsin and chymotrypsin. The amount and activity of trypsin inhibitors in the diet has been shown to be inversely related to the availability of energy and protein. Proteases are enzymes (e.g., trypsin and chymotrypsin) in human gastric juices that usually break down protein. Trypsin helps to regulate secretions from the pancreas. When trypsin is inhibited by protease inhibitors, the pancreas does not receive the signals. Protease inhibitors are found in nearly all cereal grains and legumes. Trypsin inhibitors in soybean give rise to inactivation and loss of trypsin in the small intestine, thus triggering the release of cholecytokinin and induce pancreatic synthesis of excess trypsin and burden on sulphur containing amino acids requirement of the body. The presence of protease 20 inhibitors in food decreases the appar-ent nutritional quality of proteins in the diet by affecting the ability of body digestive enzymes to degrade dietary protein, and thus limiting the intake of amino acids needed to con-struct new proteins. However, in certain situations the effects of inhibitors on protein digestion might be advantageous, e.g. by improving the intact absorption of some therapeutic proteins such as orally delivered insulin (Yamamoto *et al.*, 1994).

2.8 Ways to reduce anti-nutrients in food

Anti-nutrients are endogenous components in plants that may disturb digestion and/or alter biochemical, physiological, and immunological responses in organisms using the plants as nutrient sources (Krogdahl and Bakke, 2015). Food processing techniques are used to enhance nutritional quality, improve the digestibility and bioavailability of food nutrients with reducing anti-nutrients (Sarita and Singh, 2016). Some of the ways to reduce anti-nutrients in food are;

2.8.1 Soaking

Soaking of grains is popular and household food processing technique. It is used for reducing antinutritional compounds like phytic acid and phytase activity to improve bioavailability of minerals. It is found that combination of different processing like

dehulling, soaking and cooking decreased in significant amount of antinutrients like polyphenols, phytate and increase the protein digestibility in vitro and improve the bioavailability of minerals such as iron and zinc (Sarita and Singh, 2016).

Beans and other legumes are often soaked in water overnight to improve their nutritional value (Fernandes *et al.*, 2010). Most of the anti-nutrients in these foods are found in the skin. Since many anti-nutrients are water-soluble, they simply dissolve when foods are soaked. In legumes, soaking has been found to decrease phytate, protease inhibitors, lectins, tannins and calcium oxalate. For example, a 12-hour soak reduced the phytate content of peas by up to 9% (Bishnoi *et al.*, 1994). Another study found that soaking pigeon peas for 6-18 hours decreased lectins by 38-50%, tannins by 13-25% and protease inhibitors by 28-30% (Onwuka, 2006). However, the reduction of anti-nutrients may depend on the type of legume. In kidney beans, soybeans and fava beans, soaking reduces protease inhibitors only very slightly. Not only is soaking useful for legumes, leafy vegetables can also be soaked to reduce some of their calcium oxalate. Soaking is typically used in combination with other methods, such as sprouting, fermenting and cooking (Awuchi and Okpala, 2022).

2.8.2 Germination

Sprouting is a period in the life cycle of plants when they start emerging from the seed. This natural process is also known as germination. This process increases the availability of nutrients in seeds, grains and legumes. Sprouting takes a few days. During sprouting, changes take place within the seed that lead to the degradation of anti-nutrients such as phytate and protease inhibitors. Sprouting has been shown to reduce phytate by 37-81% in various types of grains and legumes. There also seems to be a slight decrease in lectins and protease inhibitors during sprouting (Bau *et al.*, 1997).

2.8.3 Fermentation

Fermentation is an ancient method originally used to preserve food. It is a natural process that occurs when microorganisms, such as bacteria or yeasts, start digesting carbs in food. Although food that becomes fermented by accident is most often considered spoiled, controlled fermentation is widely used in food production. Food products that are processed by fermentation include yogurt, cheese, wine, beer, coffee, cocoa and soy sauce. Another good example of fermented food is sourdough bread. Making of sourdough

effectively degrades anti-nutrients in the grains, leading to increased availability of nutrients (Leenhardt *et al.*, 2005).

Fermentation is widely used in food preservation, provides many varieties of food products with different flavors and texture, and improves the nutritional properties of raw food significantly (Sarita and Singh, 2016). In fact, sourdough fermentation is more effective at reducing anti-nutrients in grains than yeast fermentation in typical bread (Lopez *et al.*, 2003). In various grains and legumes, fermentation effectively degrades phytate and lectins. For example, fermenting pre-soaked brown beans for 48 hours caused an 88% reduction in phytate (Gustafsson and Sandberg, 1995).

2.8.4 Boiling

High heat, especially when boiling, can degrade anti-nutrients like lectins, tannins and protease inhibitors (Egbe and Akinyele, 1990). One study showed that boiling pigeon peas for 80 minutes reduced protease inhibitors by 70%, lectin by 79% and tannin by 69%.Additionally, calcium oxalate is reduced by 19-87% in boiled green leafy vegetables. Steaming and baking are not as effective. In contrast, phytate is heat-resistant and not as easily degraded with boiling. The cooking time required depends on the type of anti-nutrient, food plant and the cooking method. Generally, a longer cooking time results in greater reductions of anti-nutrients (Awuchi and Okpala, 2022).

2.8.5 Roasting

Roasting can improve protein digestibility. Roasting is an important unit operation in processing of grain for making Sarbottam Pitho due to its significant effect on the odor in the final products (Mridula *et al.*, 2008). Heat can kill or inactivate potentially harmful organisms including bacteria and viruses. Roasting reduces the amount of aflatoxins produced by fungi (Samarajeewa *et al.*, 1990). The goal of roasting is to improve sensory qualities and achieve inactivation of destructive enzymes which improves the storage and nutritional quality of the product (Rackis *et al.*, 1986). Sade reported that during roasting total phenols and tannins decrease (Sade, 2009). Malik observed the reduction in mineral contents during roasting; he said that might be due to the loss of nutrients while heating at high temperature. It should be noted that, the drying effect of roasting reduces the moisture content of the flour. Reduced moisture allows a larger concentration of solids by weight, resulting in an increased viscosity (Malik *et al.*, 2002).

2.9 Method of processing raw materials

2.9.1 Barley

The barley should be first sorted and cleaned and then washed with water for 5 times and soaked in water for 8 hours. Excess water should be drained; seeds should be tied in a muslin cloth. These seeds should be germinated at room temperature for 48 hours and oven-dried. The germinated barley should be roasted and grounded into flour by using the electric grinder (Pandy and Singh, 2019).

2.9.2 Paddy

The seeds should be washed and soaked for 8 hours. The soaked seeds should be placed in a dark condition at room temperature (32±5°C). Germination should be allowed to proceed for 48 hours. The germinated seeds should be dried in the oven at 55°C to 65°C for a total of about 10 to 12 hours after which the seeds should be cleaned of sprouts and hulls by rubbing. Dehulling should be done and roasted. The seeds should then be milled to yield flour. The flour should then be packed in airtight container (Marero *et al.*, 1988).

2.9.3 Green gram

The grains have to be screened to remove impurities and then the cleaned grains should be washed and soaked in excess water. The grains almost double in volume hence the trays of bigger sizes should be taken. Soaking should be done for 10-15 hour. At the end of soaking period, the soaked grains should be spread for germination. Water has to be sprinkled during germination to keep the grains moist. The germination has to be done for 24 hours at 30±2 ^{o}C. The sprouts should be then dried to stop the germination in hot air oven at 55^{o}C for 20 hours. The dried grains should be dehusked and the husk and cotyledons has to be removed. To improve the taste and flavor it has to be roasted under mild heat and then dry milled (Bijili Sanjay, 2006). Sieving should be done before standardizing the size of grits. The sample should be then packed in airtight plastic container. The container has to be stored at room temperature until further use. After that weighing, blending and cooking process should be done as required (Rana *et al.*, 2015)

2.10 Technology for the preparation of porridge

Traditional treatments such as soaking, cooking, germinating have been used to improve nutritional quality of the cereals and legumes. Processing of food such as soaking, germination and fermentation leads to reduction in phytic acid and increases of the mineral

solubility in foods and also improves the bioavailability of the minerals in cereals and legumes (El-Adawy, 2002a).

2.10.1 Soaking or steeping

Soaking or steeping is a pretreatment for decertification of grain facilitate the removal of the husk or skin. Non- corticated grains are soaked in water for a short time lead them to easy husk removal. Soaking process increases hydration coefficient, seed weight, total protein, ash, fat, fiber of cereals and legumes. All anti-nutritional factors such as phytic acid, tannin, trypsin inhibitor and hemagglutinin activity decreases during soaking in 0.5% sodium bicarbonate (El-Adawy, 2002a).

Selected grain is 'steeped' usually by immersing in water, for a period chosen to achieve a particular moisture level. The water is drained from the grain, which germinates. Time period for steeping depends on temperature and degree of aeration of the steep water. A temperature of (10-12) ℃ is recommended with steeping times of (40- 60) hours. A temperature of 20- 25℃ is recommended with steeping times of (16- 20)hours for legumes (Kent, 1994).

2.10.2 Germination

Germination as a method of processing is commonly employed to improve the palatability and digestibility of legumes. Germination or sprouting of legumes and cereals increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytase and protease inhibitors. Germination was more effective in reducing phytic acid than heat treatment, and therefore it improves the nutritional quality of cereals and legumes. Germination also slightly increases the total essential amino acids in cereals and legumes. Dehusking, germination, cooking and roasting have been shown to produce beneficial effects on nutritional quality of legumes (Kadam *et al.*, 1985). Certain tannins and polyphenols are reduced as a result of formation of polyphenol complex with proteins and the gradual degradation of oligosaccharides, thus facilitating in iron absorption (Camacho *et al.*, 1992).

The desirable nutritional changes that occur during sprouting are mainly due to the breakdown of complex compounds into simpler form, transformation into essential constituents and breakdown of nutritionally undesirable constituents. The metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Complex

biochemical changes occur during hydration and subsequent sprouting. The reserve chemical constituents, such as protein, starch and lipids are broken down by enzymes into simple compounds that are used to make new compounds. Sprouting causes increased activity of hydrolytic enzymes, improvements in the content of total proteins, fat and certain essential amino acids, total sugars, B- group vitamins and a decrease in dry matter, starch and anti- nutrients. The increased content of protein, fat, fiber and total ash are only apparent and attributable to the disappearance of starch. However, improvement in amino acid composition, B- group vitamins, sugars, protein and starch digestibility's, and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process (Kadam *et al.*, 1985).

2.10.3 Drying

Drying produce a friable, readily milled stable product that may be stored for long period, and from which roots may easily be removed. In drying green malt, the removal of moisture at low temperature allows the maximum survival of enzymes and the least development of aroma and color. Diastatic enzyme survives if the green malt is dried in a rapid air- flow at 40°C, to not less than 10% moisture (Hough *et al.*, 2012). Drying is necessary for safe storage, because it inhibits microbial growth due to low water activity (Dziki and Gawlik-Dziki, 2019).

2.10.4 Roasting

Roasting is a cooking method that uses dry heat where hot air envelops the food, cooking it evenly on all sides with temperature of at least 150°C (300°F) from an open flame, oven, or another heat source. Roasting can enhance flavor through caramelization and Maillard browning on the surface of the food. Dry roasting is a process by which heat is applied to dry food stuffs without the use of oil, or water as a carrier. Unlike other dry heat methods, dry roasting is used with foods such as nuts and seeds. Dry roasted foods are stirred as they are roasted to ensure even heating (Gahlawat and Sehgal, 1994). Roasted grains exhibit improved texture, enhanced crispiness and volume due to puffing (Sharma *et al.*, 2011).

Roasting reduces the moisture content, thereby concentrating the food value. Roasting also enhance acceptability by imparting a nutty flavor to the food. Most of the antinutritional factors or toxic effects of legumes (trypsin inhibitor, hem-agglutinin, goitrogenic agents, cyanogenic glucosides, alkaloids, etc.) are partially or fully eliminated

by roasting. Roasted millet had the lowest tannin and polyphenol content by 51% and 48% respectively (Sade, 2009). Similarly, on roasting, in vitro protein and starch digestibility of weaning foods increased by (15-21)% and (16-19)% respectively. Roasting also improved in vitro iron availability by (12- 19)% (Gahlawat and Sehgal, 1994).

2.10.5 Milling and sieving

The outer bran in coarse grain 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).

2.10.6 Blending

It is the homogenous mixing of the entire ingredient. It is the process of combining two or more ingredients together so that they lose their individual characteristics and become smooth and uniform. The main objective of blending is to combine or mix so that the constitute parts are indistinguishable from one another resulting into the lipid-based paste product (Amagloh *et al.*, 2012).

2.10.7 Packaging

Packaging is the technology of enclosing or protecting products for distribution, storage, sale and use. Packaging also refers to the process of designing, evaluating and producing packages. Packaging can be described as a coordinated system of preparing goods for transport, warehousing, logistics, sale, and end use. Packaging contains, protects, preserves, transports, informs, and sells (Soroka, 2002). 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)

Part III

Materials and methods

3.1 Materials

3.1.1 Barley

Barley (*Hordeum vulgar*), which was of local variety, was collected from Dharan market. It is locally known as '*jau*'.

3.1.2 Paddy

Paddy (*Oryza sativa*), which was of local variety, was collected from Dharan market. It is locally known as '*dhan*'.

3.1.3 Green gram

Green gram (*Vigna radiata*), which was of local variety, was collected from Dharan market. It is locally known as 'mung daal'.

3.2 Chemicals, reagents and equipment used

3.2.1 Chemicals and reagents

3.2.2 Glassware and equipment

Standardized and calibrated glassware and equipment were used.

- Hot air oven
- Spectrophotometer
- Incubator
- Centrifuge
- Soxhlet apparatus
- Water bath
- Electronic balance
- Heating mantle (burner)
- Colorimeter

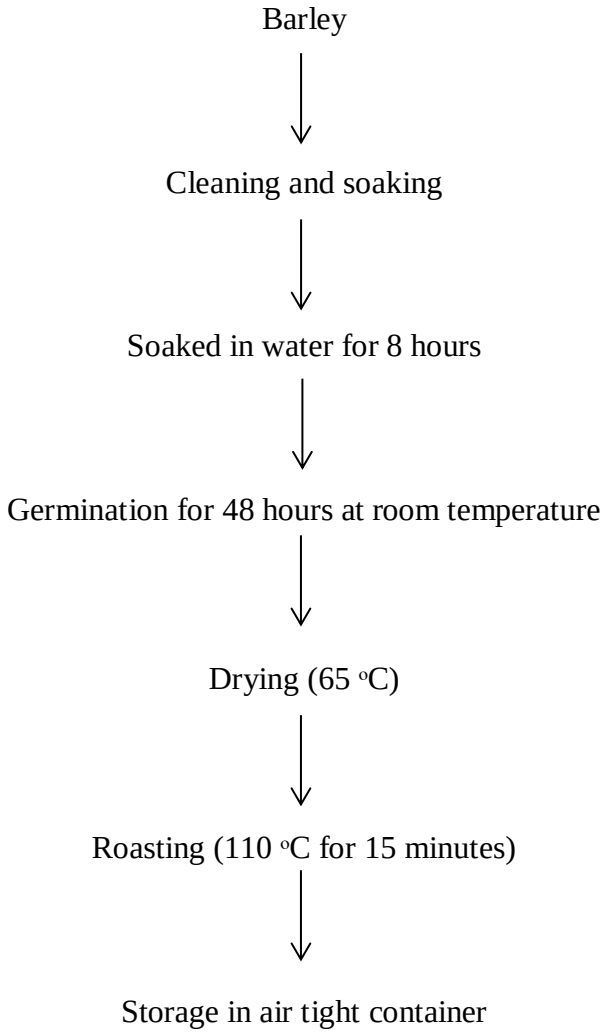
Standardized glassware such as burette, petriplates, test tubes, conical flask, volumetric flask, crucible, measuring cylinder, beaker, funnel, pipette, etc. was used.

3.3 Method

3.3.1 Processing of raw materials

3.3.1.1 Barley

Barley was sorted first and cleaned and then washed and soaked in water for 8 hours. Excess water was drained and seeds were tied in a muslin cloth. These seeds were germinated at room temperature for 48 hours and oven-dried. The germinated barley was roasted and grounded into flour by using the electric grinder and then stored in air tight container (Pandy and Singh, 2019). Fig 3.1 shows the flow diagram for the processing of barley.

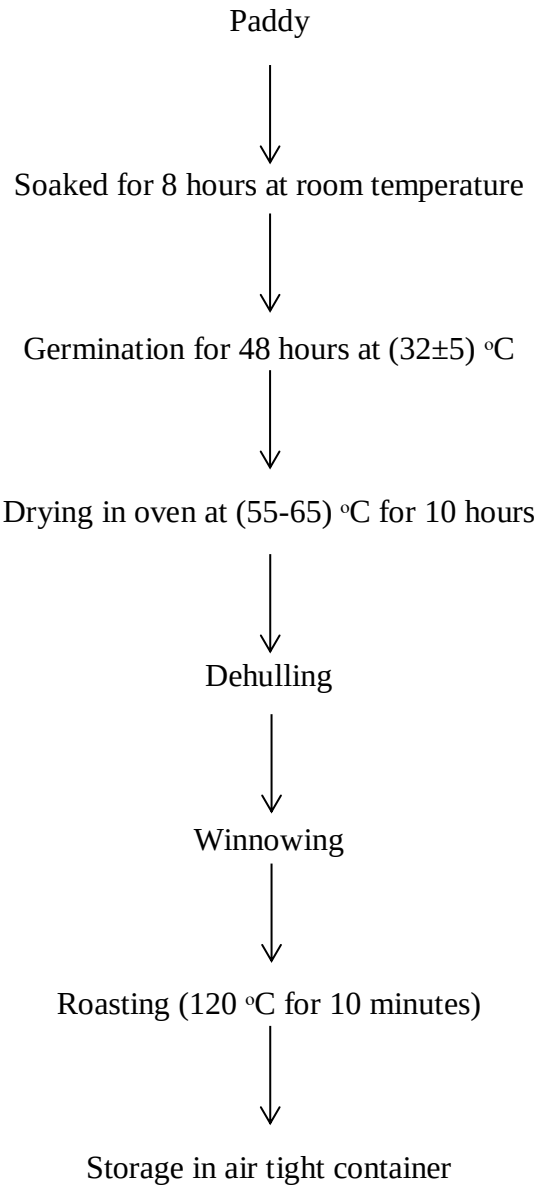


Source: (Pandy and Singh, 2019)

Fig 3.1 Flowchart for processing of barley grain

3.3.1.2 Paddy

The seeds were washed and soaked for 8 hours at room temperature. The soaked seeds were placed in a dark condition at room temperature (32±5°C). Germination was allowed to proceed for 48 hours. The germinated seeds were dried in the oven at 55°C to 65°C for a total of about 10 hours after which the seeds were cleaned of sprouts and hulls by rubbing. Dehulling, followed by winnowing should be done and roasted. The seeds were then be milled to yield flour. The flour was then be packed in airtight container (Marero *et al.*, 1988). Fig 3.2 shows the flow diagram for the processing of paddy.



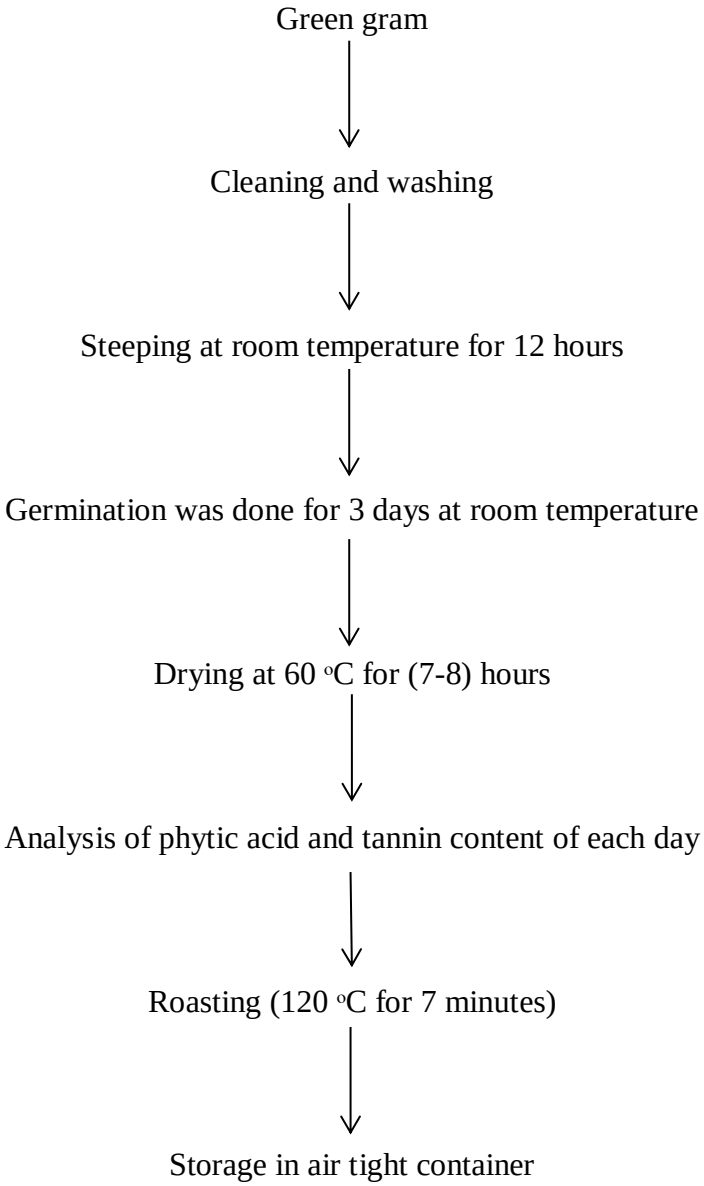
Source: (Marero *et al.*, 1988)

Fig.3.2 Flow chart for processing of paddy

3.3.1.3 Green gram

The grains were screened to remove impurities and then the cleaned grains were washed and soaked in excess water. The grains almost double in volume hence the tray of bigger size was taken. The grains were steeped for 12 hours in excess water at room temperature, which was changed after 4 and 8 hours. After steeping, the grains were germinated for three days at room temperature. Then, the germinated grains were dried in cabinet drier to stop further germination. Drying was carried out at 60°C for (7-8) hours. The dried grains were dehusked and the husk and cotyledons were removed. After drying, phytic acid and tannin content of green gram was done each

day. To improve the taste and flavor, it was roasted under mild heat and then dry milled. Dry milling and sieving were done to obtain the grits and then be packed in air tight container (Rana *et al.*, 2015). Fig 3.3 shows the flow diagram for the processing of green gram.



Source: (Rana *et al.*, 2015)

Fig.3.3 Flowchart for processing of green gram

3.5 Research design

Using the Design expert version 10, sixteen formulations were made which are then coded alphabetically as given in the Table 3.1.

Table 3.1 Sample code for different formulation

Ingredients	Barley (%)	Rice (%)	Green gram (%)
A	28.45	22.68	48.87
B	31.2	25	43.79
C	25.75	24.25	50
D	32.14	22.07	45.78
E	32.15	22.07	45.78
F	38.56	20	41.44
G	33.78	25	41.22
H	38.56	20	41.44
I	25.75	24.25	50
J	35.11	22.19	42.69
K	28.76	25	46.25
L	34.87	20	45.13
M	32.15	22.07	45.78
N	36.78	23.22	40
O	36.78	23.22	40
P	30	20	50

3.4 Formulation

For the formulation of multigrain porridge, the amounts of ingredients were calculated on dry weight basis. Legume was taken as the source of protein and the cereals as source of carbohydrate. The pulses and cereals were cleaned, soaked, germinated, dried and roasted well (separately) and then stored in an air tight container. The stored roasted grains were then taken in required amount as per the formulations. Then the grains were mixed and milled together. Grits were sieved and then stored in an airtight container separately and used for further work.

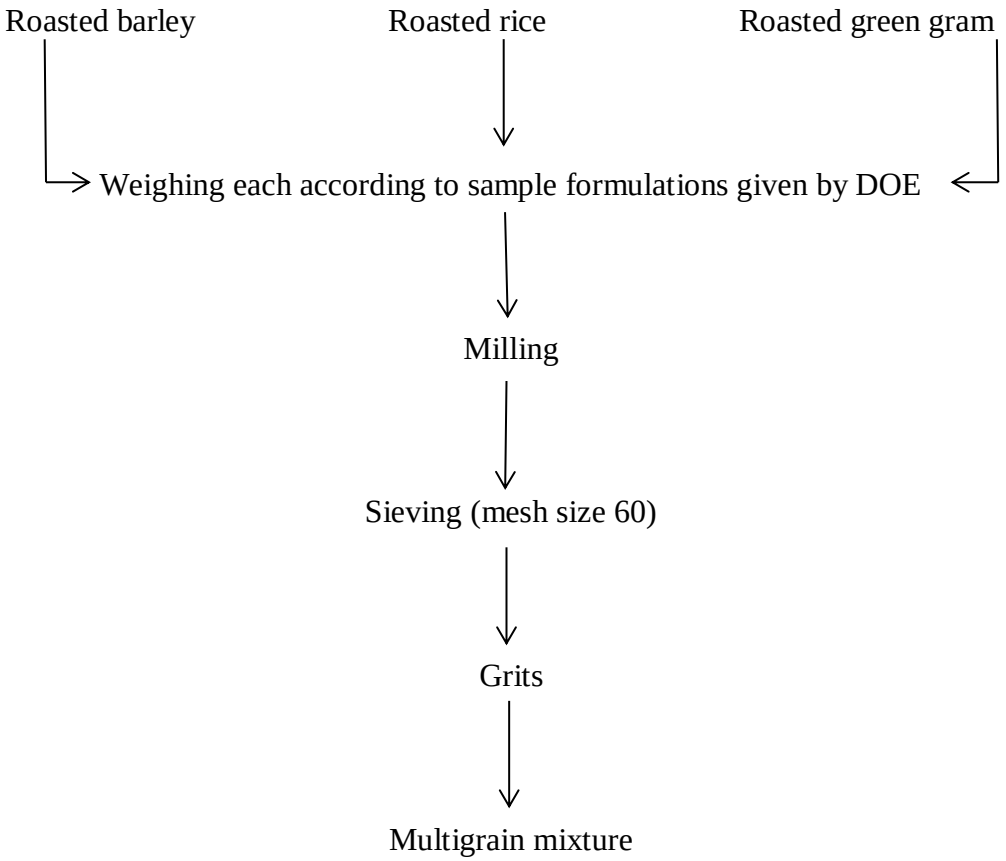


Fig.3.4 Process flow diagram for the preparation of multigrain porridge

3.6 Sensory evaluation

Sensory evaluation was performed by 9-point hedonic scoring test (9=like extremely and 1=dislike extremely) for color, flavor, taste, texture and overall acceptance. The evaluation was carried out by 11 panelists who were the students of Central Campus of Technology, Dharan. Each panelist was provided with samples coded random numbers and evaluation card (Appendix A). They were provided with potable water for rinsing between the samples. Verbal communication among the panelist was prohibited. They were asked to evaluate the samples individually using score card.

3.7 Chemical analysis

3.7.1 Proximate analysis

3.7.1.1 Determination of moisture content

The moisture content was determined by using hot air oven. 5g of sample weighted and heated in an insulated oven at 110 C to constant weight. The difference in weight was the water that has evaporated as (Ranganna, 1986). The results were expressed in terms of percentage.

3.7.1.2 Determination of protein

Crude protein was determined by the kjeldahl method, total protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Ranganna, 1986). The calculated data were presented per 100 g on dry basis.

$$\text{Nitrogen \%} = \frac{(\text{Sample titre-Blank titre}) \times \text{Normality of HCl} \times 14 \times 10}{\text{Weight of sample} \times 100}$$

3.7.1.3 Determination of crude fat

The fat content of the samples was determined as described in (Ranganna, 1986). The calculated data were presented as gram per 100 g on dry basis.

$$\text{\% Crude fat} = \frac{\text{weight of ether soluble material}}{\text{Weight of sample}} \times 100$$

3.7.1.4 Determination of total ash

The ash content was determined by incinerating the beans (5 g) in a muffle furnace at 525oC for 4-6 hours (Ranganna, 1986). The calculated data were presented as g/100 g on dry basis.

3.7.1.5 Determination of crude fiber

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of (Ranganna, 1986). The calculated data were presented as g/100 g on dry basis.

$$\text{\% Crude Fiber} = \frac{\text{Loss of weight noted}}{\text{Weight of sample taken}} \times 100$$

3.7.1.6 Determination of carbohydrate

Total carbohydrate content of the samples was determined by the difference method.

Carbohydrate (%) = 100- [sum of protein, total ash, fiber and fat]

3.7.1.7 Determination of energy value

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

Energy value per 100g= [carbohydrate* 4 + protein* 4 + fat* 9] kcal

3.7.2 Ultimate analysis

3.7.2.1 Determination of calcium

Calcium was precipitated as calcium oxalate. The precipitate was dissolved in hot dilute sulphuric acid and titrated with standard potassium permanganate (Ranganna, 1986).

$$\text{Calcium}\left(\frac{\text{mg}}{100}\right)=\frac{\text{Titre}\times 0.2\times\text{Total volume of ash solution}\times 100}{\text{Volume taken for estimation}\times\text{Weight of sample taken for ashing}}$$

3.7.2.2 Determination of iron

Iron in the sample was determined by converting all the iron into ferric form using oxidizing agents like potassium per sulphate or hydrogen per oxide and treating therewith potassium thiocyanate to form a red ferric thiocyanate which was measured calorimetrically at 480nm (Ranganna, 1986).

$$\text{Iron}\left(\frac{\text{mg}}{100}\right)=\frac{\text{Absorbance of sample}\times 0.1\times\text{Total volume of ash solution}\times 100}{\text{Absorbance of standard}\times 5\times\text{Weight of sample taken for ashing}}$$

3.8 Determination of antinutritional factors

3.8.1 Determination of tannin

Colorimetric estimation of tannins is based on the blue color formed by the reduction of the Folin-ciocalteu reagent by tannin-like compounds in alkaline condition. The green gram seed weighing 0.5 g was boiled for 30 min with 40 ml of water. Then it was cooled and was transferred to a 50 ml volumetric flask and diluted to mark. It was then shaked well and filtered. 0 to 1 ml aliquots of the standard tannic acid solution were taken in test tube and 7.5 ml water was added to each. Then, 0.5 ml Folin-ciocalteu reagent and 1 ml Na_2CO_3 solution was added and volume was made to 10 ml. After then, color was measured after 30 min at 760 nm against experimental blank adjusted to 0 absorbency (Ranganna, 1986).

3.8.2 Determination of phytic acid

The phytate was extracted with trichloroacetic acid and was precipitated as ferric salt. The iron content of the precipitate was determined colorimetrically and phytate phosphorus content was calculated from that value assuming a constant 4Fe:6P

molecular ratio in the precipitate (Sadasivam and Manickam, 1992). Result was presented as phytate mg per 100 g sample.

$$\text{Phytate P}\left(\frac{\text{mg}}{100\text{g}}\right)\text{sample}=\frac{\mu\text{g}\times 15}{\text{Weight of sample}}$$

3.9 Statistical analysis

Analysis was carried out in triplicate. Data on analysis of tannin, phytic acid and sensory analysis were tabulated for comparison and were graphically represented using Microsoft excel-2016. Data were statistically processed by Gene stat version 12.1.0.3338 for analysis of variance (ANOVA). Means of the data were compared by using Fisher's Unprotected LSD method at 5% level of significance.

3.10 Reconstitution of multigrain porridge mix

On the basis of preliminary study, 35 g of porridge mix gave the best results in 100 ml of milk and 5 g of sugar (A. Hussain and Kaul, 2019).

3.10 Cost evaluation

From the sensory analysis, the best product was found and its cost of production was calculated per 100 g of the product.

Part IV

Results and discussion

Porridge was prepared from germinated barley, rice and green gram. This study focused on the formulation of multigrain porridge from the cheap and locally available cereals as a carbohydrate source, legumes as a protein source followed by the household traditional method of pre-treatment and germination. Germination of the legumes was done until the antinutritional factor i.e. tannin and phytic acid in order to improve the nutritional quality and bioavailability.

4.1 Proximate analysis of raw samples

Proximate analysis gives very important information, particularly from the nutritional and biochemical points of views. The results normally expressed in percentage and because of the fairly general nature of test employed for the determination, the term crude is usually used as a modifier; for instant, crude protein, crude fat and crude fiber, etc. Therefore, proximate constituent represents only a category of compounds present in biological material (Acharya and Karki, 2008).

Table 4.1 The proximate analysis of raw sample

Parameters	**Barley (%)**	**Paddy (%)**	**Green gram (%)**
Moisture (% wb)	11.1±0.31	12.9±0.2	9.4±0.1
Crude protein (%db)	11.9±0.15	7.6±0.34	22.7±0.25
Crude fat (%db)	3.7±0.25	1.3±0.2	1.2±0.05
Ash (%db)	2.3±0.06	1.2±0.26	3.7±0.22
Crude fiber (%db)	4.5±0.1	1.8±0.21	4.2±0.3
Carbohydrate (%db)	77.4±0.22	87.9±0.26	68±0.04

(Values are the mean ± sd of three determinations. All values are expressed on dry basis.)

Table 4.1 shows the proximate composition of barley, paddy and green gram. The proximate composition of barley is similar to that reported in (Abeshu and Abrha, 2017) and (del Carmen Robles-Ramírez *et al.*, 2020). The proximate composition of rice is similar to that reported in (Verma and Srivastav, 2017) and (Jamal *et al.*, 2016).

Also, the proximate composition of green gram was similar to that reported in (Paul *et al.*, 2011).

4.2 Proximate analysis of germinated samples

Germination affects the nutritional composition of cereal and legume flours. Germination increased moisture and protein content in cereal and pulse flours. Germination significantly affect ash, fat, fiber, carbohydrate and energy content (Kavitha and Parimalavalli, 2014). Table 4.2 shows the proximate analysis of germinated samples.

Table 4.2 The proximate analysis of germinated samples

Parameters	**Barley (%)**	**Paddy (%)**	**Green gram (%)**
Moisture (%wb)	3.9±0.09	4.3±0.51	3.8±0.45
Crude protein (%db)	15.5±0.43	9.7±0.45	25.5±0.5
Crude fat (%db)	2.9±0.25	0.8±0.03	1.1±0.03
Ash (%db)	2.2±0.01	0.97±0.09	3.1±0.05
Crude fiber (%db)	3.8±0.05	1.07±0.04	3.3±0.05
Carbohydrate (%db)	75.4±0.29	83.2±0.15	66.8±0.25

(Values are the mean ± sd of three determinations. All values are expressed on dry basis.)

Table 4.2 shows the proximate composition of germinated barley, paddy and green gram. The proximate composition of barley is similar to that reported in (Rico *et al.*, 2020). The proximate composition of germinated rice is similar to that reported in (Abubakar *et al.*, 2018).

The moisture content of barley, paddy and green gram decreased after germination and drying process. (Oyenuga, 1968) reported that low moisture content of food samples is a desirable phenomenon, since the microbial activity is reduced. Low moisture content in food samples increased the storage period of food products (Alozie *et al.*, 2009).

The protein content of germinated barley, paddy and green gram increased in comparison to raw samples. This observation agreed with other scientific findings that

processing techniques such as germination improved the nutritinal quality of the food products, particualrly in terms of protein content (Enujiugha *et al.*, 2003).

The fat content of germinated barley, paddy and green gram decreased as comparison to raw samples. Other similar findings has been found in work done by (Kavitha and Parimalavalli, 2014).

The ash content is higher in raw materials than in germinated samples. The decrease in ash content represents loss in minerals due to rootlet and washing in water to reduce sour smell during the period of germination (Tatsadjieu *et al.*, 2004).

The crude fibe content is decreased in germinated samples than in raw. Similar findings can be seen in work done by (Kavitha and Parimalavalli, 2014).

The carbohydrate content of germinated sample can be seen lower than that of raw materials. It could be due to the utilization of carbohydrate for biochemical activities of the germinating seeds (Wang *et al.*, 1997).

4.3 Evaluation of tannin and phytic acid content in germinated green gram

4.3.1 Phytic acid

The green gram was germinated for three days. The change in phytic acid content was checked in each day of germinated sample. The phytic acid content was reduced by germination which is shown in figure 4.1.

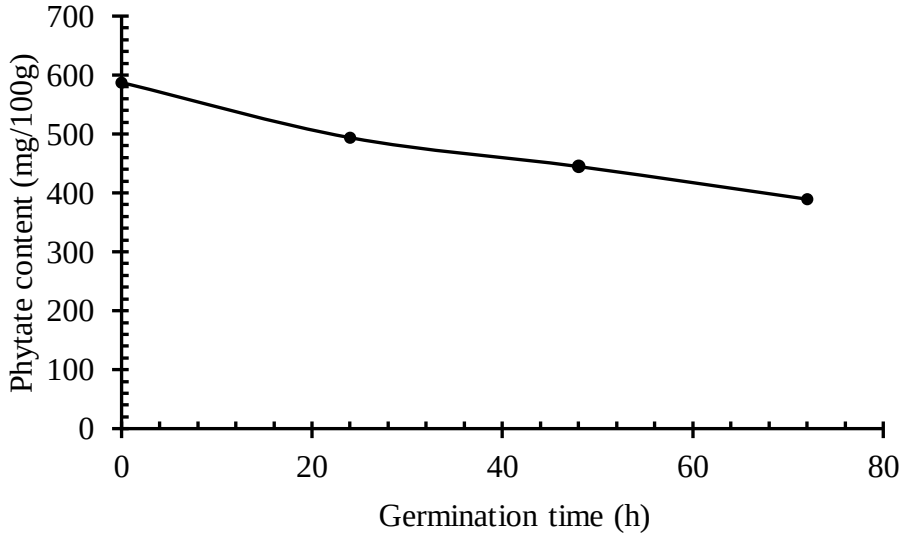


Fig 4.1 Effect of germination on phytate content of sprouted green grams

Phytic acid is also known as a major storage form of phosphorus in legumes. Germination also resulted in significant ($p \leq 0.05$) loss of phytic acid in green gram cultivars. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase (Grewal and Jood, 2006). Plant seed use phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value (Wang *et al.*, 1997). The analysis of variance (appendix B) showed that there was a significant differemce between the three consecutive germination days ($p<0.05$). Phytic acid content of raw green gram was 587.3 mg which declined to 389.3mg/100g during 72 hours of germination. Phytic acid was reduced by 15.93%, 24.28% and 33.71% at 24, 48 and 72 hours of germination respectively.

Results showed substantial decrease in the concentration of phytic acid in the seeds processed by germination and are in agreement with the earlier studies. The results are well in agreement with those of (Rasha Mohamed *et al.*, 2011). Phytic acid content of mung bean seeds were significantly ($p < 0.05$) reduced by germination (Vijayakumari *et al.*, 1998). A loss of 15.4% phytic acid content of mung beans has been reported by (Rasha Mohamed *et al.*, 2011) on 24 hours of germination. Phytic acid content of raw green gram was 635 mg, which declined 522-428 mg/100 g during 72 hours of germination the maximum reduction was recorded for 60 hours for 33.5ºC (380 mg/100 g) minimum for 48 hours and 30ºC (421 mg/100 g) for experimental values. Several legumes are known to contain phytase enzyme and its activity varies widely (I. Hussain *et al.*, 2011).

4.3.2 Tannin

The green gram was germinated for three days. The change in tannin content was checked in each day of germinated sample. The tannin content was reduced by germination which was shown in figure 4.2.

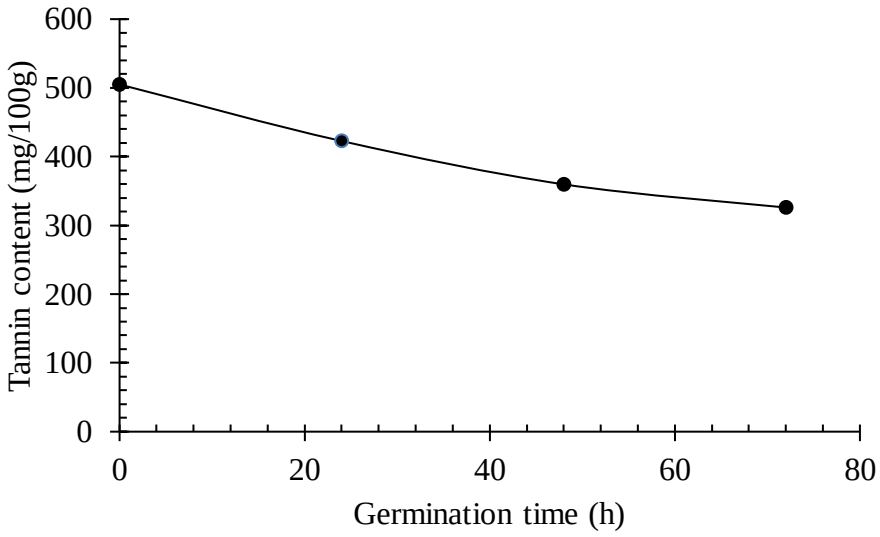


Fig 4.2 Effect of germination on tannin content of sprouted green gram

The analysis of variance (Appendix-B) showed that there was significant difference between three consecutive germination days ($p<0.05$). The tannin content of raw green gram was 505 mg tannic acid per 100g dry weight. Tannin content was reduced by 16.29%, 28.77% and 35.45%, at 24, 48 and 72 hours respectively.

Results showed substantial decrease in the concentration of tannic acid in the seeds processed by germination and are in agreement with the earlier studies. The higher reduction of tannin could have been due to the tannin activity during germination. These results agree well with those reported (El-Adawy, 2002b). (I. Hussain *et al.,* 2011) showed that reduction of 395.8 mg of tannic acid (around 39%) was lost during germination. Similar results were obtained by (Vijayakumari *et al.*, 1998) for Vigna aconitifolia and Vigna sinensis. (Khandelwal *et al.,* 2010) showed that reduction of tannin content by 22-59% during germination. Cooking of raw pulse brought about a 70% decrease in their tannin content. Loss of tannin during germination may be due to enzymatic degradation. Overnight soaking of mung bean in water causes 25% of tannin was lost. When germination was continued for 48 h, a further 10% loss of tannin was observed in green gram (Rao and Deosthale, 1982).

4.4 Cooking of the porridge

Among the sixteen formulation, thirteen formulations i.e. A, B, C, D, F, G, J, K, L, N and P were cooked for sensory analysis. 35 g of different formulations of multigrain grits were taken and cooked with 100 ml of milk and 5 g of sugar. The cooked products were then taken for sensory anaysis.

4.5 Sensory evaluation of different formulations of multigrain porridge

The sixteen formulations i.e. A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P which was cooked and then provided to 11 panelists i.e. students of Central Campus of Technolog. The panelists evaluated for various parameters of the product namely color, flavor, taste, texture and overall acceptability. The panelists were requested to provide scores in the score as per their perception. Data was analyze statistically and the best product was found.

4.5.1 Color

The average sensory score for color was 4.73, 5.27, 5.36, 5.36, 5.36, 4.45, 5.45, 4.45, 5.36, 5.73, 5.55, 5.45, 5.36, 5.36, 5.36 and 7.91 for A, B, C, D, E, F, G, H, I, J, K, L, M, N, Oand P respectively. The analyis of variance showed that there was no significant difference ($p>0.05$) between the formulations A, B, C, D, E, G, I, L, M, N and O. However, they are significantly different with sample F, H, J, K and P. Similarly, there is no significant difference between the sample J and K. Sample P was found significantly different with all the samples in terms of color and was found to score higher (7.91) among the samples.

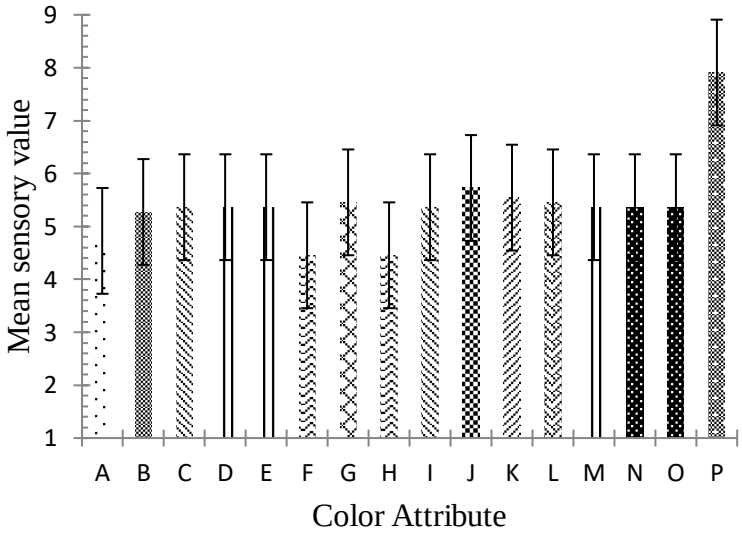


Fig 4.3 Mean sensory score of the samples for color attribute

4.5.2 Flavor

The average sensory score for flavor was 5.636, 6.091, 5.364, 5.273, 5.273, 4.636, 5.455, 4.636, 5.364, 5.091, 5.636, 4.545, 5.273, 5.455, 5.455 and 8.182 for A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P respectively. The analysis of variance showed

that there was no significant difference (p>0.05) between the formulations A, C, D, E, F, G, H, I, J, K, M and N. Sample P was found significantly different with all the samples in terms of flavor and was found to score higher (8.182) among the samples.

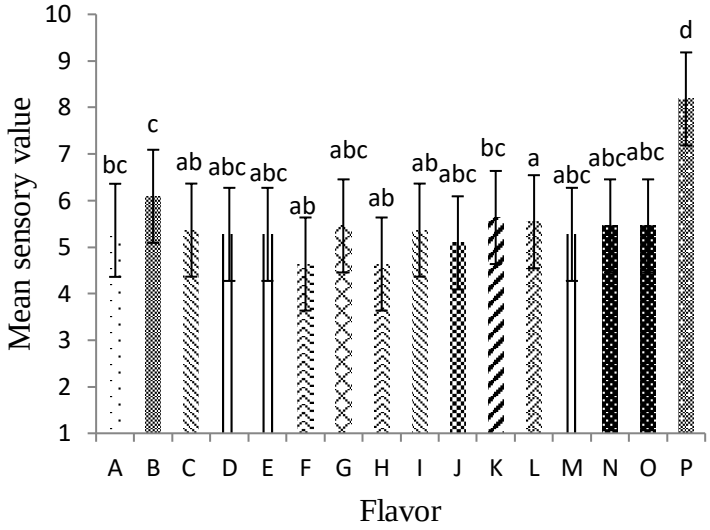


Fig 4.4 Mean sensory score of the sample for flavor attribute

4.5.3 Texture

The average sensory score for texture was 5.636, 5.818, 5.182, 5.727, 5.727, 5.909, 4.727, 5.909, 5.182, 5.091, 5.000, 4.727, 5.727, 5.818, 5.818 and 8.091 for A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P respectively. The analysis of variance showed that there is no significant difference (p>0.05) between the formulations A, B, D, E, M, N and O. Similarly, there was no significant difference between the sample C, I and J. Likewise, there was no significant difference between the sample G and L. There was significant difference between the sample G, K and P. However, sample P was found significantly different with all the samples in terms of texture and was found higher (8.091) among the samples.

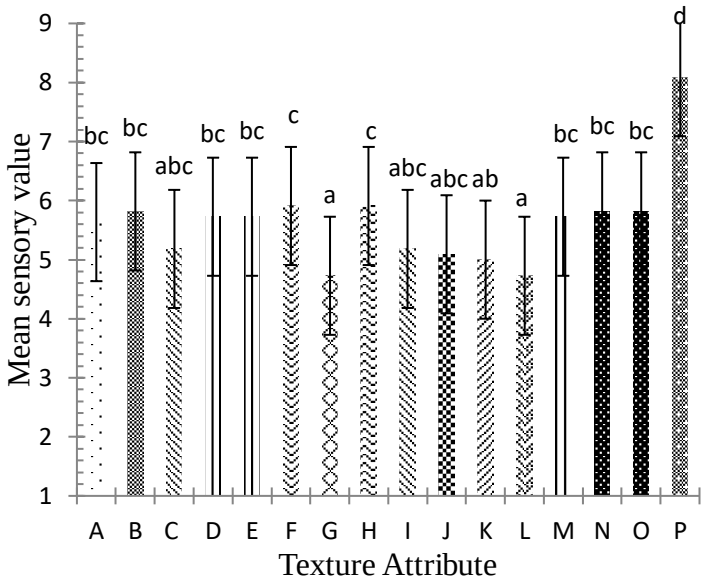


Fig 4.5 Mean sensory score of the sample for texture attribute

4.5.4 Taste

The average sensory score for taste was 5.364, 5.364, 4.545, 5.727, 5.727, 5.727, 5.091, 5.727, 4.545, 5.727, 4.909, 4.636, 5.727, 5.33, 5.33 and 8.091 for A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P respectively. The analysis of variance showed that there is no significant difference between the formualtions A, B, G, K, N and O. However, they are significantly different ($p<0.05$) with sample C, D, E, F,H, I, J, L, M and P. Sample P was found significantly different with all the samples in terms of taste and was found higher (8.091) among the samples.

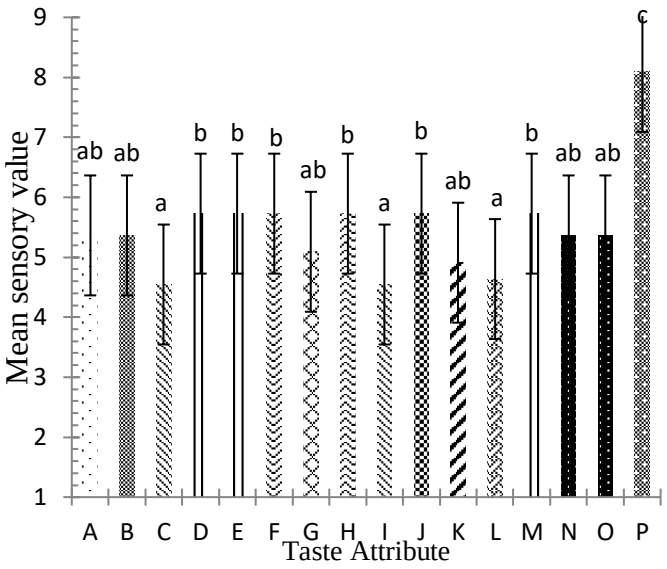


Fig 4.6 Mean sensory score of the sample for taste attribute

4.5.5 Overall acceptance

The average sensory score for overall acceptability was 5.273, 5.545, 4.727, 5.636, 5.636, 5.364, 5.091, 5.727, 4.545, 5.364, 5.364, 5.364, 5.727, 5.545, 5.545 and 7.818 for A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P respectively. Statistical analysis showed that the mean score value for overall acceptability of sample A, F, G, H, J, K and L was not found to be significantly different ($p<0.05$) to each other. Similarly, there was no significant difference between the sample B, D, E, M and N. Sample P has got the highest value of mean sensory score.

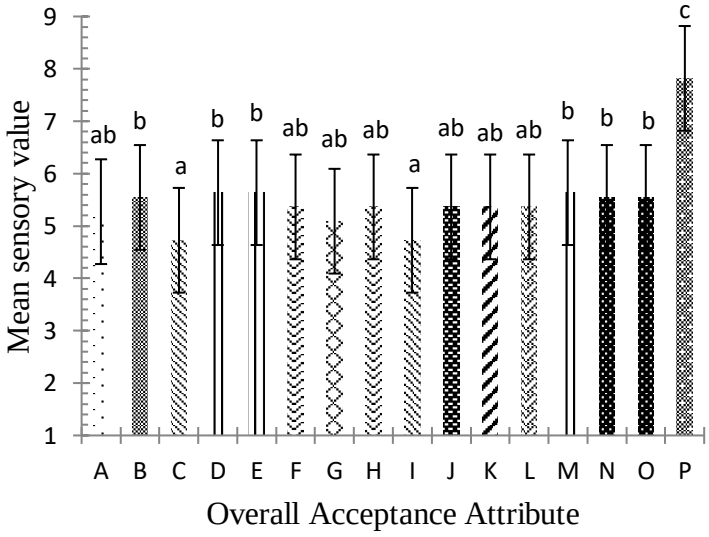


Fig 4.7 Mean sensory score of the sample for overall acceptance attribute

4.6 Analysis of the final product

Analysis of the sample P i.e. in the ratio of 30:20:50 was selected best from sensory analysis.

The result is tabulated in table 4.3

Table 4.3 Analysis of multigrain porridge

Parameters	Values
Moisture (% wb)	4.5±0.1
Crude protein ((%db)	27.8±0.55
Crude fat (%db)	2.5±0.11
Ash content (%db)	2.2±0.08
Crude fiber (%db)	3.3±0.17
Carbohydrate (%db)	64.29±0.31
Iron (mg)	3.7±0.15
Calcium (mg)	83.8±0.2
Energy (kcal)	390.8±1.21

(Values are the mean ± sd of three determinations. All values are expressed on dry basis.)

Similar nutritional value was found by Nutrition Collaborative Research Program during the market analyis of complementary foods in Nepal where the analysis of Sarbottam Pitho showed moisture 3.53%, protein 14.72%, fat 7.4%, ash 1.92%, carbohydrate 73% and energy 400 kcal (Magnani *et al.*, 2012). Weaning food from cereals, legumes and fruit had moisture of 4.26%, protein of 16.8%, fat of 7.25%, total ash of 3%, carbohydrate of 60%, crude fiber of 3.2% and energy of 401.28 kcal (Harmayani *et al.*, 2019). 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 (Imtiaz *et al.*, 2011).

4.7 Cost evaluation

From the statistical analysis the best product was found as sample P and its cost of prodduction was calculated per 100g of the product. The estimated cost calculation is shown in table 4.4

Table 4.4 Cost evaluation of the best sample P

Raw materials	Price/kg	Amount of materials	Price
Barley	50	3	150
Rice	50	3	150
Green gram	200	3 kg	600
Price/kg			125
Price/100 g			12.5

The cost of the product was calculated to be NRs. 12.5/ 100g excluding labor cost, packaging cost and taxes.

Part V

Conclusions and recommendation

5.1 Conclusions

On the basis of this study following conclusions were drawn.

a. Germination time of green gram for reduction of phytic acid and tannin was 72 hours.
b. Tannin content in green gram was reduced by 16.29%, 28.77% and 35.45% at 24, 48 and 72 hours respectively.
c. Phytic acid content in green gram was reduced by 15.93%, 24.28% and 33.71% at 24, 48 and 72 hours respectively.
d. The sensory evaluation showed that the formulation P i.e. the formulation having barley, rice and green gram in the ratio 30:20:50 was the best among the sixteen formulations in terms of color, flavor, taste, texture and overall acceptability.

5.2 Recommendations

The following recommendation can be drawn from conclusion.

a. Study on fatty acid composition and amino acid profile of the prepared product can be studied.

Part VI

Summary

Porridge is a convenient food not just for weaning infants, but also for the elderly and convalescents due to its easy digestibility. The cereals and legumes were germinated. The germination of green gram was carried out till the anti-nutritional factors i.e. tannin and phytic acid were significantly reduced to the least. Barley and paddy was germinated for 24 hours and green gram was germinated for 24, 48 and 72 hours. The phytate content was found to be decreased from 587 to 493.7, 444.7 and 389.3 mg/100g on consecutive days of germination. Tannin content was found to be decreased from 505.0 to 422.7, 359.7 and 326.0 mg tannic acid per 100g on consecutive days of germination.

Sixteen different products were made from the germinated cereals and legumes varying the proportion of each grain. The raw materials were processed and the products were prepared in the laboratory. The sensory evaluation of the selected sixteen samples was performed by eleven panelists who were the students from Central Campus of Technology. On the basis of sensory evaluation, the product P containing 30 g barley, 20 g rice and 50 g green gram was taken for further chemical analysis of the product. The protein, fat, carbohydrate, crude fiber and total ash of the product were found to be 27.8%, 2.5%, 64.29%, 3.3% and 2.2% respectively. The diet can supply 390.86 kcal/100 g. The iron and calcium content of the product was found to be 3.7 mg/100 g and 83.8 mg/100 g respectively.

This study where porridge has been prepared from locally available food which contains important nutrients required for weaning children could be effective in terms of digestibility, bioavailability and physiological function. If further researched the production of weaning food using different locally available nutritious food could be possible in Nepal at a very minimum cost.

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Appendices

Appendix A

A.1 Sensory analysis score card

Date:

Sensory analysis of weaning food (multigrain porridge)

Name of the panelist:

Name of the product: weaning food for infants (multigrain porridge)

Type of product: weaning food

Dear panelist, you are given sixteen samples of multigrain porridge, please conduct the sensory analysis based on the following parameter using the table given;

Sample code	Color	Flavor	Texture	Taste	Overall Acceptance
A					
B					
C					
D					
F					
G					
J					
K					
L					
N					
P					

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Comments (if any)

_____

Signature

Appendix B

1. Standard curve for phytate content

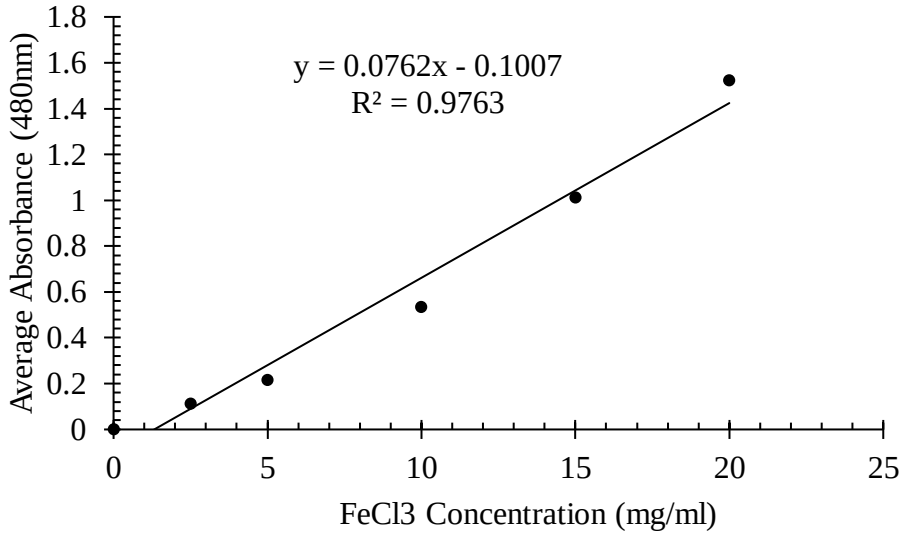

Fig. B.1 Standard curve for phytate content

2. Standard curve for tannin content

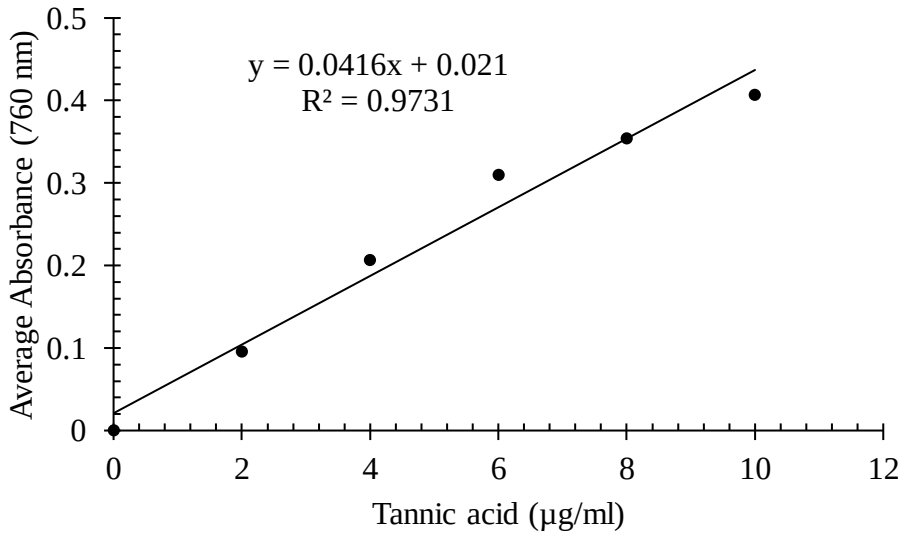

Fig.B.2 Standard curve for tannin content

Appendix C

1. Tannin and phytic acid content in germinated green gram

Table C.1.1 ANOVA for tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
No. of days	3	55791.33	18597.11	1923.84	<.001
Residual	8	77.33	9.67		
Total	11	55868.67			

Since ($p<0.05$), there is a significant difference between the samples in different germination days so LSD testing is necessary.

Table C.1.2 LSD of means

No. of days	Mean	Column A	LSD	d.f.
3D*	326.0±0.15	A	5.854	8
2D*	359.7±0.36	B		
1D*	422.7±0.51	C		
R*	505.0±0.7	D		

(*=Significantly different)

Table C.1.3 ANOVA for phytic acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
No. of days	3	48515	16171.67	758.05	<.001
Residual	8	170.67	21.33		
Total	11	48685.67			

Since ($p<0.05$), there is a significant difference between the samples in different germination days so LSD testing is necessary.

Table C.1.4 LSD of means

No. of days	Mean	Column A	LSD	d.f.
3D*	389.3±0.25	A	8.70	8
2D*	444.7±0.2	B		
1D*	493.7±0.12	C		
R*	587.3±0.17	D		

(*=Significantly different)

Appendix-D

1. Sensory evaluation of the product

Table D.1.1 One-way ANNOVA for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Formulation	10	84.231	8.423	5.26	<.001
Panelist	10	23.868	2.387	1.07	0.388
Residual	100	160.132	1.601		
Total	120	268.231			

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

Table D.1.2 LSD for color

Sample	Mean	Column 1	LSD
A	4.727	BC	
B	5.273	BC	
C	5.364	BC	
D	5.364	BC	
F	4.455	C	1.071
G	5.455	BC	
J	5.727	B	
K	5.545	B	
L	5.455	BC	
N	5.364	BC	
P	7.909	A	

Table D.1.3 One-way ANNOVA for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Formulation	10	103.504	10.350	7.06	<.001
Panelist	10	21.322	2.132	0.94	0.502
Residual	100	146.678	1.467		
Total	120	271.504			

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

TABLE D.1.4 LSD for flavor

Sample	Mean	Column 1	LSD
A	5.636	BC	
B	6.091	C	
C	5.364	AB	
D	5.273	ABC	
F	4.636	AB	
G	5.455	ABC	0.6725
J	5.091	ABC	
K	5.636	BC	
L	5.545	A	
N	5.455	ABC	
P	8.18	D	

Table D.1.5 One-way ANNOVA for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Formulation	10	96.017	9.602	8.37	<.001
Panelist	10	38.017	3.802	1.98	0.042
Residual	100	114.711	1.147		
Total	120	248.744			

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

Table D.1.6 LSD for texture

Sample	Mean	Column 1	LSD
A	5.636	BC	
B	5.818	BC	
C	5.182	ABC	
D	5.727	BC	
F	5.909	C	0.9061
G	4.727	A	
J	5.091	ABC	
K	5.000	AB	
L	4.727	A	
N	5.818	BC	
P	8.091	D	

Table D.1.7 One-way ANNOVA for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Formulation	10	100.066	10.007	9.21	<.001
Panelist	10	27.521	2.752	1.45	0.168
Residual	100	108.661	1.087		
Total	120	236.248			

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

Table D.1.8 LSD for taste

Sample	Mean	Column 1	LSD
A	5.364	AB	
B	5.364	AB	
C	4.545	A	
D	5.727	B	0.8818
F	5.727	B	
G	5.091	AB	
J	5.727	B	
K	4.909	AB	
L	4.636	A	
N	5.364	AB	
P	8.091	C	

Table D.1.9 One-way ANNOVA for overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Formulation	10	68.8099	6.8810	10.89	<.001
Panelist	10	35.9008	3.5901	2.99	0.002
Residual	100	63.1901	0.6319		
Total	120	167.9008			

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

Table D.1.10 LSD for overall acceptability

Sample	Mean	Column 1	LSD
A	5.273	AB	
B	5.545	B	
C	4.727	A	
D	5.636	B	
F	5.364	AB	0.6725
G	5.091	AB	
J	5.364	AB	
K	5.364	AB	
L	5.364	AB	
N	5.545	B	
P	7.818	C	

List of plates

P1 Reading absorbance on spectrophotometer