EFFECT OF GERMINATION ON ANTI-NUTRITIONAL FACTORS OF CEREAL & LEGUMES AND THEIR MALT USE IN SARBOTTAM PITHO FOR INFANTS

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

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Approval letter

This *dissertation* entitled *Effect of Germination on Anti-nutritional Factors of Cereal & Legumes and Their Malt Use in Sarbottam Pitho for Infants* presented by Niharika Baskota has been accepted as the partial fulfillment of the requirements for the Bachelor degree in Nutrition and Dietetics.

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Regards,

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Abstract

A nutritionally rich weaning food i.e. Sarbottam Pitho was prepared suited for children aged 1 to 3 years from germinated soybean, maize, wheat and apple. Five different formulations of Sarbottam Pitho A, B, C, D and E were prepared varying the amount of apple i.e. 5%, 10%, 15%, 20% and 25% respectively while the proportion of cereals and legumes were kept constant at ratio of 2:1 in each formulation. Locally available and cheap raw materials were used where cereals was used as the staple source, legumes as a protein source and apple as a source of vitamins and minerals and sweetness followed by pretreatment i.e. germination. Cereals and legumes were germinated for several days and the tannin and phytic acid content in each sample was checked each day.

It was found that the tannin and phytic acid content was reduced in the germinated sample in comparison to raw sample. On the basis of analysis of each germinated sample in each germination days, it was found that wheat had the least tannin and phytic acid content after 72 hours of germination and soybean and maize had the least tannin and phytic acid content after 96 hours of germination. Optimum germination of soybean, wheat and maize showed a reduction of tannin by 43%, 41% and 43% respectively and phytic acid by 52%, 48% and 47% respectively. From the sensory evaluation and statistical analysis of the five products, product E containing 25% apple powder was found to be the best among the all. The total cost for the preparation was calculated as NRs. 172 per Kg. Hence, the prepared Sarbottam Pitho is nutritious and cost effective weaning food which is beneficial in terms of digestibility, bioavailability and physiological function for infants.

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List of Abbreviations

Abbreviations	Full Form
AIN	Asian International Nepal
ANOVA	Analysis of Variance
CBS	Central Bureau of Statistics
DFTQC	Department of Food Technology and Quality Control
DIAAS	Digestible Indispensable Amino Acid Score
FDA	Food and Drug Administration
HDPE	High Density polyethylene
IP6	Inositol Hexaphosphate
LDL	Low Density Lipoprotein
LSD	Least Significant Difference
NDHS	Nepal Demographic and Health Survey
NPU	Net Protein Utilization
PER	Protein Efficiency Ration
PRPs	Proline Rich Proteins
UMN	United Mission to Nepal
USAID	United State Agency for International Development
WHO	World Health Organization
WVTR	Water Vapor Transmission Rate

Part I

Introduction

1.1 General Introduction

Child health in developing countries is a serious concern with a large number of children still suffering from malnourishment. South Asian region has the highest global burden of child under-nutrition, with almost 41% of children stunted, 16% wasted and 33% underweight (Siwakoti, 2014).

One of the serious problem of our country facing today is the population growth at the rate of 1.35% per year (CBS, 2068). The proportion of national population living in poverty level is 23.8% (USAID, 2014). The infant mortality rate remains 32 and under five mortality rate is 39 per thousand among the live birth (NDHS, 2016). Around 430,000 children in the country are malnourished due to lack of nourishment and of them 91,000 are suffering from malnutrition. As per the information shared at a program on malnutrition organized by the Asian International Nepal (AIN), the children fall prey to rapid malnutrition for not getting adequate food, contamination of diseases and food insecurity (Anonymous, 2015). Overall, 53% of children suffered from some degree of anemia, out of which 26% were classified as mildly anemic, 26% were moderately anemic and less than 1% were severely anemic (NDHS, 2016).There are various factors that lead to high prevalence of malnutrition in children and among them infant feeding practices is one of the most important. Whether it is breast feeding or complementary feeding, the practices adopted by mothers or caretakers have direct effect on health (Siwakoti, 2014).

Complementary feeding is another very important component of infant feeding. After 6 months, mother's milk is not sufficient for the growing child and complementary feeding should be started, timely and in adequate amounts (Siwakoti, 2014).

Weaning is the process of gradually introducing a mammal infant to what will be its adult diet and withdrawing the supply of its mother's milk. The process takes place only in mammals, as only mammals produce milk. The infant is considered to be fully weaned once it no longer receives any breast milk or bottled substitute (Siwakoti, 2014).

Baby food are specially prepared so that they are easy for the child to eat and digest. They are complementary foods and infant or child gets in addition to breast milk and are prepared from various blends of cereals, pulses, oilseed, flour and milk solids.

Weaning in human infants is a subject of controversy in terms of its initiation and correct method of doing it. The ideal age of weaning is six months. The desirable weaning food should be rich in calories and protein with adequate amount of trace elements like iron, calcium, vitamins etc. and also inexpensive, home available, clean, easily digestible and the most importantly bio-available (Siwakoti, 2014).

Bioavailability is a general term that refers to how well a nutrient can be absorbed and used by the body. It can be affected by many factors such as the presence of anti-nutrients, for example, phytates, oxalates, tannins and polyphenols in foods, a person's need, fibre, competition with other nutrients and acidity of intestinal environment (Norhaizan and Nor Faizadatul, 2009).

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

1.2 Statement of the problem

The period between weaning and the age of five is nutritionally regarded as the most vulnerable period of the life cycle because that is when rapid growth, loss of passive immunity and the development of the immune system against infection occur. The first two years of child's life are particularly important, as optimal nutrition during this period lowers morbidity and mortality, reduces the risk of chronic diseases, and fosters better development overall (WHO, 2016).

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. Sarbottam Pitho can be prepared from locally available foods which are within the accessibility of all the people and equally contain the required quantity of nutrient as well (Wagh and Deore, 2015). Right complementary feeding is highly effective in preventing various forms of

acute malnutrition, including kwashiorkor, nutritional marasmus, and several forms of wasting (Latham *et al.*, 2011).

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 (UNICEF, 2011). One of the reason 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 (UNICEF, 1987). So, this work primarily focuses on the production and evaluation of weaning food using locally available raw materials that is inexpensive, easily digestible and bioavailable.

1.3 General Objective

The general objective is to study the effect of germination on anti-nutritional factors of cereal & legumes and their malt use in sarbottam pitho for infants

1.4 Specific Objective

- To optimize germination time of maize, wheat and soybean on the basis of least tannin and phytic acid content.
- To formulate Sarbottam Pitho using optimized maize, wheat and soybean flour with incorporation of varying proportion of dried apple powder.
- To evaluate the best product on the basis of percentage of incorporated apple powder.
- To evaluate physico-chemical properties of optimized Sarbottam Pitho.
- To evaluate the cost of optimized product.

1.5 Significance of study

This formula will be beneficial specially to children of low income group. This study will provide a basis for the preparation of weaning food from locally available raw materials using traditional pretreatment technique i.e. germination. This weaning food could be effective in terms of digestibility and bioavailability for infants due to minimization of antinutritional factors. Any factory, government agencies, local agencies and others whose primary aim is to improve the nutritional status of children can produce the balanced weaning food using this formula and this work will provide the basis for the further work in this field.

1.6 Limitations

a. Clinical trials using albino rats could not be done.

b. Analysis of vitamins and trace elements, amino acid and fatty acid composition of the product could not be performed due to time constraints.

Part II

Literature Review

2.1 Weaning

The term “to wean” comes from an ancient phrase that means “to accustom to”. So, weaning refers to the period during which an infant gradually becomes accustomed to food other than milk. Weaning means addition or introduction of semi-solid foods along with continuation of breast feeding as long as possible. The term ‘Weaning’ describes the process by which baby moves or shifts from having breast milk to consuming semi-solid or solid foods with a gradual reduction in the intake of breast milk and /or baby formula (Kambli, 2014).

In the strictest sense of the word, weaning means getting a body used to drinking milk from a cup instead of sucking milk from the breast or bottle; in the broader sense, it also means getting the baby used to taking food by biting and chewing instead of only by sucking; Weaning is now discarded in favor of the phrase complementary feeding. To make weaning an easy adjustment for a baby, it should be done gradually step by step. At the beginning of the meal, when babies are extra hungry, they should be given the milk from a nipple. Otherwise, they are likely to swallow more air than usual in their hurry to satisfy their hunger. Later in the meal, when their hunger is partly satisfied, babies can drink from a cup or be fed with a bottle (Kambli, 2014).

There is no right age when a baby should be weaned. Weaning too early may cause baby at higher risk of developing digestive disorders and adverse reactions or allergies to certain foods. On the other hand, weaning too late may deprive adequate nutrition and can result in improper growth and development (Kambli, 2014).

Indicative signs for weaning are: Can sit in an upright position for feeding, shows interest in other foods, keeps putting things in the mouth, shows signs of hunger before the usual feeding times, and Keeps chewing on things. When baby is 6 months old, start offering them a wide range of foods so that they get accustomed to eating different flavors. Introduce only one food at a time as it will be easier to detect any allergic to particular food item. Foods that are given gradually for a baby are: Boiled and mashed vegetables; use vegetables like potatoes, cauliflower, carrots, and beans, etc.; Starchy foods which are rich in carbohydrates like rice, potatoes, cereals, and oats; Ripe and mashed fruits; e.g.;

banana, apple; Diluted fruit juice (1 part fruit juice to 10 parts of water); Diary products like cheese and yogurt .

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 (WHO and Fund, 1988).

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 (WHO and Fund, 1988).

Weaning can be a dangerous time for babies. In many places babies of weaning age do not grow well. They often fall ill and get more infections, especially diarrhea, than at any other time. 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 (WHO and Fund, 1988).

Weaning is a time of great changes in behavior for both the baby and the parents. Babies become more interested in the world around them and independent in their actions. Mothers at first spend almost all their time with their new babies. During weaning this change. Mothers need to go back to their usual work patterns or even take up new duties and interests (WHO and Fund, 1988). So, during weaning the close ties between mothers and their babies must gradually loosen. Babies will start to be apart from their mothers for longer and longer times. Mothers may need to rely on others in the family to take care of their babies as they return to their regular duties in, or outside, the home (WHO and Fund, 1988).

These changes in the way children are looked after during the weaning period can mean that babies are not fed properly or become upset and unhappy. For example, babies may lose their appetites when their mothers are away. Or they may be given too little food if the

person looking after them is careless or does not know what to do. If you are aware of these dangers they can usually be avoided (WHO and Fund, 1988).

Healthy growth and development is not just to do with correct feeding. Babies also need emotional stimulation and the right kind of care when they are ill. Successful weaning involves taking all these points into account (WHO and Fund, 1988).

2.2 Problems during weaning

Under-nutrition is one of the major problems confronting infants and young children in the developing countries. Malnutrition begins in infancy especially during the transition stage from breast feeding to solid diet, frequently in association with diarrheal disease. The precise cause of such growth failure is unclear, but must be due to one or a combination of factors like, insufficient dietary intake, defective digestion or absorption, increased metabolic demands etc. Traditional weaning foods are typically watery gruels of low energy density and protein content. Often, they are not consumed immediately after preparation. Unhygienic conditions of preparation and storage may lead to infection with entero-pathogenic bacteria. Weaning infant is potentially at risk in developing countries, and many nutritional problems arise with the introduction of solids. The crude preservation process, poor hygiene sanitation and inadequate knowledge of weaning food preservation introduce the risks of gastrointestinal and parasitic infection because of the heavy contamination of foodstuffs with infecting organisms. In addition, too early introduction of weaning food may lead to diarrhea through the ingestion of thin, contaminated feed with insufficient calorie and protein. Too late introduction may lead to undernutrition owing to insufficient milk intake. Thus complementary feeding begins when breast milk alone is no longer sufficient to meet the nutritional requirements of infants and therefore other foods and liquids are needed along with breast milk (Anonymous, 2002).

2.3 Nutritional requirements of the weaning infant

Infancy is the critical period of rapid physical growth and cognitive and emotional development. Infants are considered a vulnerable group because they have relatively high nutrient requirements per unit body weight. Recent research has declared infancy is the critical period in life, setting the foundation of long- term health and reduced risk for chronic diseases. Breast feeding is the preferred and recommended form of nutrition for healthy infants during the first 6 months of life providing all necessary nutrients (Gammatikaki and Huybrechts, 2016). After 6 months, supplementary feeding has to be

resorted for a baby to ensure adequate nutrient intakes in line with infant nutritional requirements to maintain the expected rate of growth, remain healthy and well nourished (Shrilakshmi, 2014).

The amount of nutrients requirement of a baby per kg body weight declines over the period of birth owing to decreasing growth rate, even though energy requirement for activity increases as the infant becomes older (Shrilakshmi, 2014). A new born baby weighs on an average 2.7 kg at birth and will be about 5.4 kg at six months and 8 kg by one year (Pawar and Dhanvijaya, 2007). The requirement of nutrients of infants aged 1-3 years are energy 1060 kcal, protein 16.7g/day, fat 27g/day, iron 9mg/day, calcium 600mg/day (ICMR, 2010).

Table 2.1 Recommended dietary allowances for children from 6 months to 3 years

Nutrients		**6- 12 months**	**1- 3 years**
Body wt.		8.4	12.9
Net calories (kcal/day)		80 kcal/kg	1060
Proteins (gm/day)		1.69 gm/kg	16.7
Visible fat (gm/day)		19	27
Calcium (mg/day)		500	600
Iron (mg/day)		5	9
Vitamin A (µg/day)	Retinol	350	400
	β- Carotene	2800	3200
Zinc (mg/day)		-	5
Magnesium (mg/day)		45	50
Thiamine (mg/day)		0.2	0.5
Riboflavin (mg/day)		0.6	0.8
Niacin (mg/day)		650 µg/kg	8
Pyridoxine (mg/day)		0.4	0.9
Vitamin B12 (µg/day)		0.2	02-1.0
Ascorbic acid (mg/day)		25	40
Dietary folate (µg/day)		25	80

Source: (ICMR 2010)

2.4 Ingredients used in weaning food and their nutritive value

2.4.1 Cereals

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. Some grains are deficient in the essential amino acid, lysine. That is why many vegetarian cultures, in order to get a balanced diet, combine their diet of grains with legumes. Many legumes, however, are deficient in the essential amino acid methionine, which grains contain. Thus, a combination of legumes with grains forms a well-balanced diet for vegetarians (Serna-Saldivar, 2010).

2.4.1.1 Maize

Maize, also known as corn, is a cereal grain which has become a staple food in many parts of the world, with total production surpassing that of wheat or rice. Maize (Zea mays) is one of the most important and widely distributed cereal crop of the world. The chief component of the maize is glutelin and prolamine (zein). The zein fraction was shown to be very low in lysine content and lacking in tryptophan. Maize protein contains excess of leucine and leucine interferes in the conversion of tryptophan to niacin and hence aggravates the pellargragenic action of maize. Whole maize is good source of thiamine, pyridoxine, pantothenic acid, fair sources of riboflavin but poor source of niacin (Serna-Saldivar, 2010). Maize contains 8.87% moisture, 12.1% protein, 4.31% fat, 70.9 % carbohydrate (DFTQC, 2012).. Anti-nutritional factors such as phytic acid, protease inhibitor, invertase inhibitor, trypsin inhibitor are found in maize. In order to minimize these antinutritional factors several pretreatment methods like incubation in water (60°C: 10hr), fermentation, milling to remove outer layer of seed, aqueous heat treatment (100°C: 10min), germination are used (FAO, 2017).

2.4.1.2 Wheat

Wheat is a grass widely cultivated for its seed, a cereal grain which is a worldwide staple food. Wheat is an important source of carbohydrates. Globally, it is the leading source of vegetal protein in human food, having a protein content of about 13%, which is relatively

high compared to other major cereals, but relatively low in protein quality for supplying essential amino acids. When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber (Shewry, 2009).

In a small part of the general population, gluten – the major part of wheat protein – can trigger coeliac disease, non-celiac gluten sensitivity, gluten ataxia and dermatitis herpetiformis.

In 100 grams, wheat provides 341 calories and is a rich source of multiple essential nutrients, such as protein, dietary fiber, manganese, phosphorus and niacin . Several B vitamins and other dietary minerals are in significant content. Wheat contains 12.2% moisture, 69.4% carbohydrates, and 1.7% fat and 12.1% protein (DFTQC, 2012). Anti-nutritional factors like phyto-haemagglutinins, phytic acid, protease inhibitor, amylase inhibitor are found and the pretreatment methods applied are aqueous heat treatment (100C: 10 min), fermentation, milling to remove outer layer of seed, germination are used (FAO, 2017).

Wheat proteins have a low quality for human nutrition, according to the new protein quality method (DIAAS) promoted by the Food and Agriculture Organization. Wheat proteins are deficient in the essential amino acid, lysine, and contain adequate amounts of the other essential amino acids, at least for adults. Because the proteins present in the wheat endosperm (gluten proteins) are particularly poor in lysine, white flours are more deficient in lysine compared with whole grains. Supplementation with proteins from other food sources (mainly legumes) is commonly used to compensate for this deficiency, since the limitation of a single essential amino acid causes the others to break down and become excreted, which is especially important during the period of growth. Further, wheat is a major source for natural and bio-fortified nutrient supplementation, including dietary fiber, protein and dietary minerals (Suhasini and Malleshi, 2003).

2.4.2 Legumes

A legume is a plant or its fruit or seed in the family Fabaceae (or Leguminosae). Legumes are grown agriculturally, primarily for their grain seed called pulse. A legume fruit is a simple dry fruit that develops from a simple carpel and usually dehisces (opens along a seam) on two sides. Grain legumes are cultivated for their seeds. The seeds are used for human and animal consumption or for the production of oils for industrial uses. Grain legumes include beans, lentils, peas, and peanuts (Whyte *et al.,* 1953).

Legumes are a significant source of protein, dietary fiber, carbohydrates and dietary minerals. Like other plant-based foods, pulses contain no cholesterol and little fat or sodium. Legumes are also an excellent source of resistant starch which is broken down by bacteria in the large intestine to produce short-chain fatty acids (such as butyrate) used by intestinal cells for food energy. Preliminary studies in humans include the potential for regular consumption of legumes in a vegetarian diet to affect metabolic syndrome. There is evidence that a portion of pulses (roughly one cup daily) in a diet may help lower blood pressure and reduce LDL cholesterol levels, though there is a concern about the quality of the supporting data (Prasad *et al.*, 2016).

2.4.2.1 Soybean

Soybean is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. It is the member of the family Leguminosae. It is well reputed pulse in hilly region of Nepal where it is cultivated mostly in a mixed crop and to less extent as pure crop under unplanned condition (Anonymous, 2017e).

Soybean is an important source of high quality, inexpensive protein and oil. Compared to other protein-rich foods such as meat, fish and eggs, soybean is by far the cheapest. It also has a superior amino acid profile compared to other source of plant protein. Soybeans are an exceptional source of essential nutrients especially protein, dietary fiber, iron, manganese, phosphorus and several B vitamins, including folate. High contents also exists for vitamin K, magnesium, zinc and potassium (WHO, 1998). Soybean contains 12.1% moisture, 33.3% protein, 15% fat, 31.3% carbohydrate, 4.3% fibre and provides 393 calorie energy (DFTQC, 2012).

For human consumption, soybeans must be cooked with "wet" heat to destroy the trypsin inhibitors (serine protease inhibitors). Raw soybeans, including the immature green form, are toxic to all monogastric animals (Khokhar and Richard, 2003). Other anti-nutritional factors present in soybean are glucosinolates, anti- vitamin B12, phytic acid, phyto-haemagglutinins. anti- vitamin A factor, anti- vitamin D factors and the pretreatment methods done are fermentation, germination, roasting at 100ºC or higher, aqueous heat treatment (100ºC: 10 min), soaking in water, incubation in water (60ºC: 10 hr) (FAO, 2017).

2.4.3 Apple

Apple is a sweet, pomaceous fruit. It is cultivated worldwide as a fruit tree, and is the most widely grown species in the genus *Malus* (Anonymous, 2017a). Delicious and crunchy, apple fruit is one of the most popular and favorite fruits among the health conscious, fitness lovers who firmly believe in the concept of "health is wealth." This wonderful fruit indeed packed with rich phyto-nutrients that in the real sense indispensable for optimal health and wellness. Certain antioxidants in apples have health promoting and disease prevention properties, and thereby, truly justifying the adage, "an apple a day keeps the doctor away" (Anonymous, 2009).

Apples are low in calories; 100 g of fresh fruit slices provide just 50 calories. They, however, contain no saturated fats or cholesterol. Apples contain 84.6% moisture, 0.2% protein, 0.5% fat, 13.3 % carbohydrate and 1% fibre (DFTQC, 2012). Nonetheless, the fruit is rich in dietary fiber, which helps prevent absorption of dietary-LDL or bad cholesterol in the gut. The fiber also saves the colon mucous membrane from exposure to toxic substances by binding to cancer-causing chemicals inside the colon. Apples are rich in antioxidant phyto-nutrients, flavonoids and polyphenolics. Some of the important flavonoids in apples are quercetin, epicatechin, and procyanidin B2. Additionally, they are also good in tartaric acid that gives tart flavor to them. Altogether, these compounds help the body protect from harmful effects of free radicals. Apple fruit contains good quantities of vitamin-C and β-carotene. Vitamin C is a powerful natural antioxidant. Consumption of foods rich in vitamin-C helps the body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals from the body. Further, apple fruit is an ideal source of B-complex vitamins such as riboflavin, thiamin, and pyridoxine (vitamin B-6). Together, these vitamins help as co-factors for enzymes in metabolism as well as in various synthetic functions inside the human body. Apples also carry small quantities of minerals like potassium, phosphorus, and calcium. Potassium is an important component of cell and body fluids helps controlling heart rate and blood pressure; thus, counters the bad influences of sodium (Anonymous, 2009). Apart from health care and nutrition, it is also known for medicinal values (Anonymous, 2004).

Apple darkens during thermal processing and storage. Accumulation of brown colour during thermal processing is due to enzymatic browning and during storage is due to non-enzymatic reaction i.e. Maillard reaction, taking place between amino group and reducing

sugar. Maillard reaction causes losses in nutritional value of food and have mutagenic effects (Burdurlu and Karadeinz, 2003).

Enzymatic browning occurs in many fruit and vegetable tissues whenever they are injured. The injury can be the result of cutting, freezing or disease. The part of the injured fruit which is exposed to air undergoes a rapid darkening. This darkening reaction results from the polyphenol oxidase (PPO) catalyzed oxidation or phenolic compounds to O-quinones which subsequently polymerize to form dark-colored pigments (Nahed, 1991).

2.5 Germination

Germination is the process by which an organism grows from a seed or similar structure. The most common example of germination is the sprouting of a seedling from a seed of an angiosperm or gymnosperm. In addition, the growth of a sporelling from a spore, such as the spores of hyphae from fungal spores, is also germination. Thus, in a general sense, germination can be thought of as anything expanding into greater being from a small existence or germ. Germination is usually the growth of a plant contained within a seed; it results in the formation of the seedling, it is also the process of reactivation of metabolic machinery of the seed resulting in the emergence of radicle and plumule. All fully developed seeds contain an embryo and, in most plant species some store of food reserves, wrapped in a seed coat. Some plants produce varying numbers of seeds that lack embryos; these are called empty seeds and never germinate. Dormant seeds are ripe seeds that do not germinate because they are subject to external environmental conditions that prevent the initiation of metabolic processes and cell growth. Under proper conditions, the seed begins to germinate and the embryonic tissues resume growth, developing towards a seedling (Anonymous, 2017c).

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,

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 a 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).

α-Amylase activity is also increased during germination of cereals. This enzyme hydrolyzes amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Gibson *et al.*, 1998). Certain tannins and other polyphenols in legumes (e.g., *Viciafaba*) and red sorghum may also be reduced during the process of germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides. Such reductions in polyphenols may facilitate iron absorption (Camacho *et al.*, 1992).

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 (Hussain and Uddin, 2012).

2.6 Antinutritional factors

Antinutrients are natural or synthetic compounds that interfere with the absorption of nutrients (Press, 2006). Antinutrients are found at some level in almost all foods for a variety of reasons. However, their levels are reduced in modern crops, probably as an outcome of the process of domestication (Project, 2008) . The possibility now exists to eliminate antinutrients 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 antinutrients such as phytic acid, polyphenols, and oxalic acid (Hotz and Gibson,

2007). Such processing methods are widely used in societies where cereals and legumes form a major part of the diet (Chavan and Kadam, 1998).

2.6.1 Tannin

A tannin (or tannoid) is an astringent, polyphenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids (Chung et al., 1998).

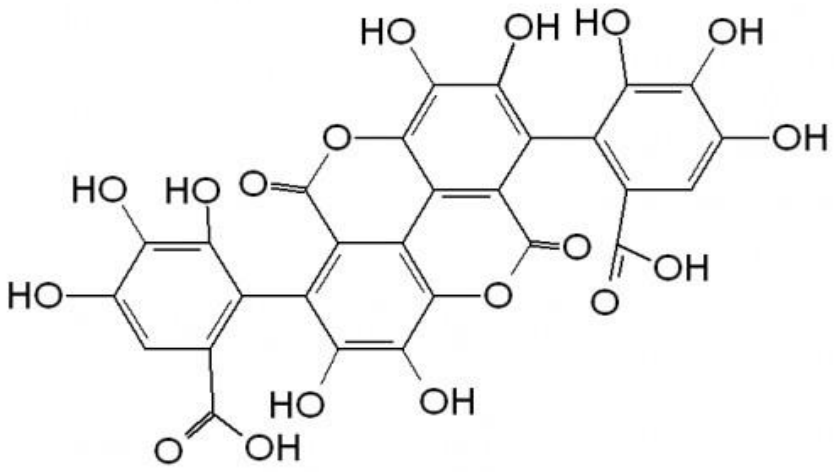

Fig 2.1 Structure of Tannin

The term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyl) to form strong complexes with various macromolecules (Chung *et al.*, 1998).

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and might help in regulating plant growth (Katie and Thorington, 2006) . The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripen fruit or red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times (McGee, 2004).

Tannins occur normally in the roots, wood, bark, leaves, and fruit of many plants, particularly in the bark of oak species. They also occur in galls, pathological growths resulting from insect attacks (Anonymous, 2017b).

In addition to their principal applications in leather manufacture and dyeing, tannins are used in the clarification of wine and beer, as a constituent to reduce viscosity of drilling mud for oil wells, and in boiler water to prevent scale formation. Because of its styptic and

a stringent property, tannin has been used to treat tonsillitis, pharyngitis, hemorrhoids, and skin eruptions; it has been administered internally to check diarrhea and intestinal bleeding and as an antidote for metallic, alkaloidal, and glycosidic poisons, with which it forms insoluble precipitates. Soluble in water, tannins form dark blue or dark green solutions with iron salts, a property utilized in the manufacture of ink (Anonymous, 2017b).

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. Tannin-binding 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).

2.6.2 Phytic acid

Phytic acid (known as inositol hexakisphosphate (IP6), inositol polyphosphate, or phytate when in salt form), is a saturated cyclic acid and the principal storage form of phosphorus in many plant tissues, especially bran and seeds. It can be found in cereals and grains (Anonymous, 2017d).

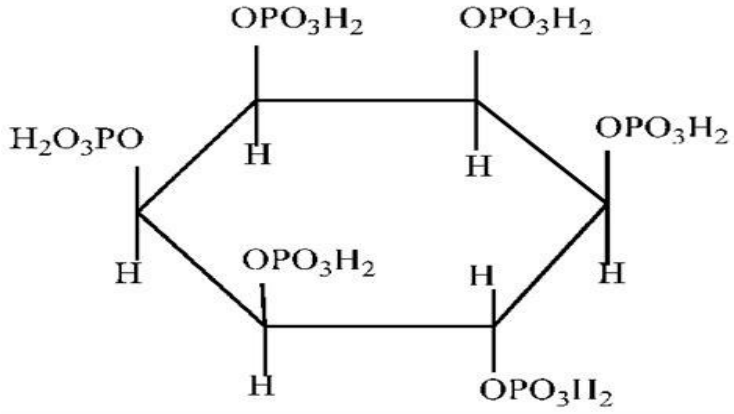

Fig 2.2 Structure of Phytic acid

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. and Canada., 2003).

Phytic acid not only grabs on to or chelates important minerals, but also inhibits enzymes that we need to digest our food, including pepsin, needed for the breakdown of proteins in the stomach, and amylase, needed for the breakdown of starch into sugar. Trypsin, needed for protein digestion in the small intestine, is also inhibited by phytates (Nagel, 2010).

Although indigestible for many animals, phytic acid and its metabolites as they occur in seeds and grains have several important roles for the seedling plant. Most notably, phytic acid functions as a phosphorus store, as an energy store, as a source of cations and as a source of myoinositol (a cell wall precursor). Phytic acid is the principal storage form of phosphorus in plant seeds (Reddy *et al.*, 1982).

In animal cells, myoinositol polyphosphates are ubiquitous, and phytic acid (myoinositol hexakisphosphate) is the most abundant, with its concentration ranging from 10 to 100 μM in mammalian cells, depending on cell type and developmental stage (Sasakawa *et al.*, 1995).

Studies examining the effects of phytic acid demonstrate that they are important in regulating vital cellular functions. Both in vivo and in vitro experiments have demonstrated striking anticancer (preventive as well as therapeutic) effects of phytic acid. Research shows anti-carcinogenic effects, albeit to a lesser extent and it acts in inhibiting

cancer. In addition to reduction in cell proliferation, phytic acid increases differentiation of malignant cells often resulting in reversion to the normal phenotype (Shamsuddin, 2002).

2.6.3 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).

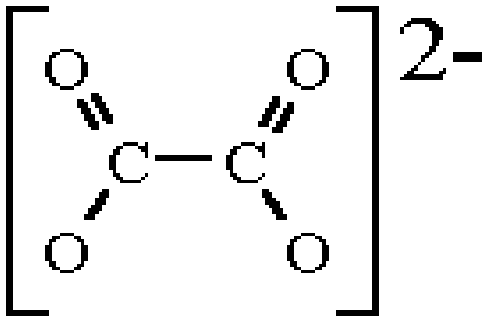

Fig 2.3 Structure of oxalate

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 Leibman, 2005). 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.from copy Oxalate is also an end-product of metabolism in the liver. Some amino acids and carbohydrates are degraded to oxalates (Savagel and Klunklin, 2018).

2.6.4 Saponins

Saponins are glycosides which are widely distributed in the plant kingdom and include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional (as soaps, fish poison, and molluscicides) and industrial applications (Taylor, 2007).

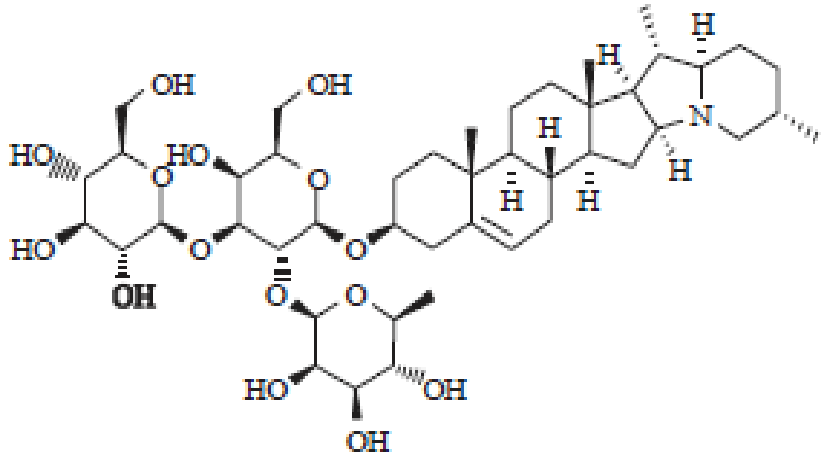

Fig 2.4 Structure of saponin

Saponins have been found in many edible legumes (lupins, lentils, and chickpeas, as well as soy, various beans, and peas) root crops (potato, yam, asparagus and allium) as well as in oats, sugar beet, tea. Saponin reduce cholesterol through the formation of an insoluble complex with cholesterol, thus preventing its absorption in the intestine. Additionally, some saponins increase the excretion of bile acids, an indirect method in decreasing cholesterol or are hydrolyzed by intestinal bacteria to diosgenin, which may exert a beneficial effect (Murphy, 2018). On the other hand saponins have the ability to hemolyze RBC. Thus saponins has been associated with both deleterious and beneficial effects (YERT, 2014).

2.6.5 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

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 pro-teins such as orally delivered insulin (Yamamoto *et al.*, 1994).

2.6.6 Ways to reduce anti-nutrients in food

Nutrients in plants are not always easily digested. This is because plants may contain antinutrients. These are plant compounds that reduce the absorption of nutrients from the digestive system. They are of a particular concern in societies that base their diets largely on grains and legumes (Arnarson, 2017). Simple ways to reduce the amount of antinutrients in foods are:

2.6.6.1 Soaking

Beans and other legumes are often soaked in water overnight to improve their nutritional value (Fernandes and Nishida, 2010). Most of the antinutrients in these foods are found in the skin. Since many antinutrients 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 antinutrients may depend on the type of legume. In kidney beans, soybeans and faba 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 (Arnarson, 2017).

2.6.6.2 Sprouting/ 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 antinutrients 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).

2.6.6.3 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 antinutrients in the grains, leading to increased availability of nutrients(Leenhardt *et al.*, 2005).In fact, sourdough fermentation is more effective at reducing antinutrients 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).

2.6.6.4 Boiling

High heat, especially when boiling, can degrade antinutrients like lectins, tannins and protease inhibitors (EgbeI 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 antinutrient, food plant and the cooking method. Generally, a longer cooking time results in greater reductions of antinutrients (Arnarson, 2017).

2.6.6.5 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 odour 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). Friedman reported reduced trypsin inhibitor activity when seed temperatures reached 90-100°C and also lipoxygenase activity was lost at temperatures of 75-80°C (Friedman, 2000). 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 lost 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).

2.7 Technology of processing of Sarbottam Pitho

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. Processing technique reduces the level of antinutritional organic factors, which including phytates, phenols, tannins and enzyme inhibitors by releasing exogenous and endogenous enzymes such as phytase enzyme formed during processing (Tarek, 2002).

2.7.1 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 themselves 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 *et al.,* 2000).

The malting process begins when the cereal grain is steeped in water. Steeping is arranged so that sufficient moisture enters the grain to initiate germination. Time period for steeping depends on temperature and degree of aeration of the steep water. A temperature of 10- 12°C is recommended with steeping times of 40- 60 hours. A temperature of 20- 25°C is recommended with steeping times of 16- 20 hours for legumes (Kent, 1994).

2.7.2 Germination

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 and Salunkhe, 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 (Chavan *et al.*, 1989).

2.7.3 Drying

Drying produce a friable, readily milled stable product that may be stored for long periods, 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.*, 1982).

2.7.4 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, 1992).

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 anti-nutritional factors or toxic effects of legumes (trypsin inhibitor, hemagglutinin, 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).

2.7.5 Milling and sieving

The outer bran in coarse grains are 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 (Viraktmath *et al.*, 1971).

2.7.6 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).

2.8 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).

2.8.1 Food packaging materials

Food packaging is packaging for food. A package provides protection, tempering resistance, and special physical, chemical or biological needs. it may bear a nutrition facts label and other information about food being offered for sale (Paine and Paine, 1992).

2.8.2 HDPE

High-density polyethylene (HDPE) is the third largest commodity plastic material in the world, after polyvinyl chloride and polypropylene in terms of volume. It is a thermoplastic material composed of carbon and hydrogen atoms joined together forming high molecular weight products(Kumar and Singh, 2013). HDPE is produced at lower temperatures and atmospheric pressure as a liquid phase process. It softens at 120- 130°C and so it can be used for hot filling, steam sterilizing or cook in the bag applications. Due to its greater rigidity, it can be used in thinner gauges thereby saving money. It has excellent retention of essential oils such as aromas. In general, the polyethene's are soft and flexible in film form with good impact resistance. However, they can be hard to open. They are very resistant to water and water vapor; the higher the density the greater the resistance, i.e. the lower the value of WVTR, but the oxygen transmission rate is high (Coles *et al.*, 2003). According to (Marsh and Bugusu, 2007) main advantages of HDPE are:

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

2.8.3 Aluminum foil

Aluminum foil provides a complete barrier to light, oxygen, moisture and bacteria. For this reason, foil is used extensively in food and pharmaceutical packaging. It is also used to make aseptic packaging that enables storage of perishable goods without refrigeration. Aluminum is used for packaging as it is highly malleable and is easily converted to thin sheets and can be easily folded, rolled and packed. Aluminum foil acts as a total barrier to light and oxygen (which causes fats to oxidize or become rancid), odors, flavor, moistness and germs, so it is used broadly in food and pharmaceutical packaging. The purpose of Aluminum is to make long life packs (aseptic packaging) for drinks and dairy goods,

which allows storing without refrigeration. Aluminum foil is made by rolling pure aluminum metal into a very thin sheet, followed by annealing to achieve dead-folding properties (a crease or fold made in the film will stay in place), which allows it to be folded tightly. Moreover, Aluminum foil is available in a wide range of thickness, with thinner foils used to wrap foods and thicker foils used for trays. Like all aluminum packaging, foil provides an excellent barrier to moisture, air, odors, light and microorganisms. It is inert to acidic foods and does not require lacquer or other protection. Although aluminum is easily recyclable, foils can't be made from recycled aluminum without pinhole formation in thin sheets (Scott and Brock, 2006).

Aluminum, when used as a component of food packaging, is in most cases covered by a polymeric film (surface coating or laminated plastic film) the level of migration of aluminum even into acidic food stuff is extremely low. There is no indication of any adverse health effects caused by aluminum in concentrations that may occur due to migration from packaging material (ILSI, 2007).

2.8.4 Glass bottles

A glass bottle is a bottle created from glass. Glass bottles can vary in size considerably but are most commonly found in sizes ranging between about 10 ml and 5 liters. Glass bottles and jars still offer advantages over other materials, though they are being increasingly displaced by plastics for packaging condiments and oils however they can be reuse and recycled. Glass bottles and jars are easy to clean, sterilize and re-use. Glass bottles and jars are available in various color choices and a multitude of design options. With high shelf appeal and wide decorating possibilities, packaging your product in glass projects quality and substance (Coles *et al.*, 2003).

Glass is entirely made from natural raw materials, which are toxicologically inert. The major constituents, i.e. sodium/ potassium silicates are nontoxic and chemically highly inert. The transfer of silicates and cations into food is marginal and even if it occurs, is toxicologically irrelevant, since the cations usually present are non- toxic. Virtually no traces of problematic migrants originating from the glass are found in glass- bottled food products (Schrenk, 2014).

Part III

Materials and methods

3.1 Materials

3.1.1 Wheat

Wheat was collected from Dharan market. It is locally known as 'gahu'.

3.1.2 Maize

Maize was collected from Ithari. It is locally known as 'makai'.

3.1.3 Soybean

Brown variety of soybean was collected from Dharan market. It is locally known as 'Nepali Bhatmas'.

3.1.4 Apple

Apple was collected from Dharan market. It is locally known as 'syau'.

3.1.5 Packaging material

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

3.2 Methods

3.2.1 Determination of Phytic acid and Tannin

3.2.1.1 Determination of Phytic acid

Phytic acid is determined colorimetrically based on extraction and precipitation as ferric salt as per Sadasivam and Manickam, (1997).

3.2.1.2 Determination of Tannin

Tannin is determined by Folin- Denis method as per Sadasivam and Manickam, (1997).

3.2.2 Processing of raw materials

3.2.2.1 Wheat

It was sorted, cleaned and soaked in water for 12 hours at 22°C and 60% humidity and then drained. It was spread on a wetted muslin cloth and covered by a wetted muslin cloth.

It was kept for germination at 22°C.Water was sprinkled on the layer at 3 to 4-hour interval. The grain was checked each day for phytic acid and tannin content from the first day of germination. It was germinated for 3 days. It was then dried in cabinet drier at 55°C for 3 hours and 70°C for 1 hour until moisture was sufficiently reduced to about 5%. The germinated part was removed, then roasted and ground into flour and packed in air tight plastic bags (Yasseen *et al.*, 2014).

3.2.2.2 Maize

Maize was cleaned to remove impurities such as stones, other grains, broken kernels, etc. The cleaned maize was steeped into water at room temperature (22°C and 60% humidity). Then the surplus water was drained off and the grain was spread at room temperature (22°C) on wetted muslin cloth and covered with wetted muslin cloth to germinate. To prevent drying out, the grain was sprinkled with water at the interval of every 3 to 4 hours. It was kept for germination. The grain was checked each day for phytic acid and tannin content from the first day of germination. It was germinated for 4 days and then first dried at 55°C unless moisture reduce about 12- 15% and then it was again dried in cabinet drier to reduce moisture to 4-6%. The rootlets were removed by agitation and screening. It was then roasted and ground in a mixture into flour. The flour was sieved and put into air tight plastic bags (Pokhrel, 2011).

3.2.2.3 Soybean

It was cleaned and soaked for 1 hour with 1% w/v sodium bicarbonate at room temperature and then drained and soaked for 12 hours in water (1.5 times water). It was then drained and spread on a wetted muslin cloth and covered by a wetted muslin cloth. It was kept for germination. The grain was checked each day for phytic acid and tannin content from the first day of germination. It was germinated for 3 days. Water was sprinkled on the layer at every 3 to 4 hours interval and kept for 4 days for germination. It was then dried in a cabinet drier at 55°C for 3 hours and 70°C for 1 hour until moisture was sufficiently reduced to about 6%. It was roasted, dehusked, splitted cotyledons were ground into flour. The flour was sieved and stored into air tight plastic bags (Pulami, 1998).

3.2.2.4 Apple

It was sorted and cleaned first. The apple peel and core were removed. Then it was cut into thin slice and dipped into 1% ascorbic acid at 60- 70°C for 15 minutes. Water was drained

and apple was dried in a cabinet drier at 50°C for 3 hours and 70°C for 1 hour. It was grinded in mixture to form powder which was stored in an air tight plastic bags (Nahed, 1991).

3.2.3 Formulation

3.2.3.1 Basis of formulation

The preparation of diet was done on the basis of specification of formulation of super flour porridge. The flour is made from two parts pulses – soybeans, one-part whole grain cereal-maize and one part another wheat. The pulses and grains were cleaned, germinated and roasted well (separately) and ground into fine flour (separately). The flour was stored in an airtight container (UMN, 2016).

3.2.3.2 Calculation of amounts of ingredients

For the formulation of Sarbottam Pitho, the amount of ingredients was calculated on dry weight basis. Legumes were taken as the source of protein and the cereals as the staple source. Apple was chosen as a source of vitamins and minerals and for sweetness. Finally, from calculation, five different products were prepared taking Sarbottam pitho as a basis i.e. cereals: legumes = 2:1 ratio. Cereals and legumes were germinated till the time the value of phytic acid and tannin on germinated ingredients were the lowest. Then five different products were prepared varying the amount of apple in each product i.e. 5%, 10%, 15%, 20% and 25% keeping the amount of cereals and legumes constant. Table 3.1 shows the amount of ingredients in each product.

Table 3.1 Formulae mixes on dry basis

Ingredients	Product-A	Product-B	Product-C	Product-D	Product-E
Wheat (g)	23.75	22.5	21.25	20	18.75
Maize (g)	23.75	22.5	21.25	20	18.75
Soybean (g)	47.5	45	42.5	40	37.5
Apple (g)	5	10	15	20	25
Total	100	100	100	100	100

3.2.4 Product Preparation

The calculated amount of ingredients for five different products were calculated on dry basis. Flow chart diagram of different ingredients used for the preparation of Sarbottam Pitho is shown in Fig 3.1.

3.2.4.1 Grinding and milling

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

3.2.4.2 Sieving of the ground powder product

All the ground flour was sieved using 300µ seive.

3.2.4.3 Mixing

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

3.2.4.4 Packaging

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

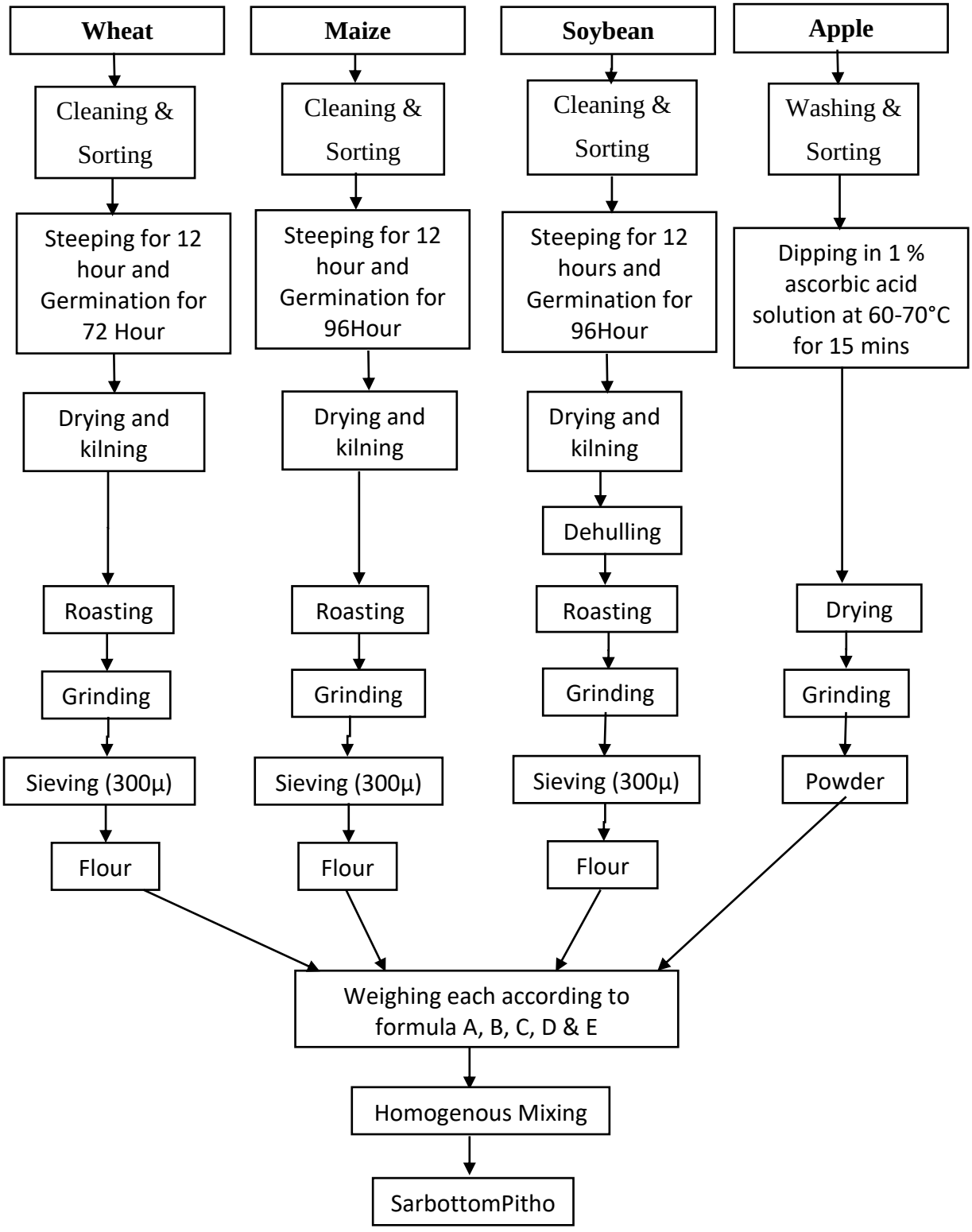

Fig 3.1 Outline for the preparation of Sarbottom Pitho (Pilot Plant Scale)

3.2.5 Evaluation of prepared Sarbottam Pitho

3.2.5.1 Sensory evaluation

Sensory evaluation was performed by 9-point hedonic scoring (9= like extremely, 1=dislike extremely) for color, flavor, taste, texture and overall acceptance. The evaluation was carried out by 12 panelists comprising of mothers of Dharan Montessori's children aged 1 to 3 years. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with 5 samples coded randomly and evaluation card (Appendix A). They were provided with portable water for rinsing between samples. Verbal communication among the panelist was prohibited. They were asked to evaluate the sample individually using a score card.

3.2.5.2 Physico-chemical analysis of product

3.2.5.2.1 Moisture content

Moisture content was determined by using hot air oven (ambassador, working temperature 0 to 300°C) as per Rangana, (2001).

3.2.5.2.2 Crude fat

The fat content was determined by Soxhlet method as per Rangana, (2001).

3.2.5.2.3 Crude protein

The crude protein was determined using kjeldahl's method as per Rangana, (2001).

3.2.5.2.4 Crude fiber

Crude fiber was determined as per Rangana, (2001).

3.2.5.2.5 Total ash

Total ash content was determined by ashing in electric muffle furnace (ambassador, working temperature 900°C, UK) as per Rangana, (2001).

3.2.5.2.6 Total carbohydrate

Total carbohydrate was determined by difference method as per Rangana, (2001).

3.2.5.3 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 gram of protein, total carbohydrate, and total fat, respectively, to calculate the calorie content of food (Bassey *et al.*, 2013).

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

3.3 Data analysis

Data on analysis of tannin and phytic acid and sensory analysis were tabulated for comparison and were graphically represented using Microsoft excel-2010. 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 LSD Fisher's Protected method at 5% level of significance.

Part IV

Results and Discussion

Sarbottam Pitho, a traditional Nepalese weaning food was prepared on a basis of ratio of cereals and legumes as ratio of 2:1 with addition of apple. This study focused on the formulation of Sarbottam Pitho for the weaning infants from the cheap and locally available cereals as a staple source, legumes as a protein source and apple as a source of vitamins and minerals and sweetness followed by the household traditional method of pretreatment, germination. Germination of the cereals and legumes was done until the antinutritional factor tannin and phytic acid was minimum in order to improve the nutritional quality and the bioavailability.

4.1 Evaluation of tannin and phytic acid content in germinated sample

4.1.1 Wheat

The wheat was germinated for five days. The change in tannin and phytic acid content was checked in each day germinated sample and the raw and germinated sample with the least tannin and phytic acid content was also analyzed. The tannin and phytic acid content was reduced by germination which is shown in the fig 4.1. The analysis of variance (Appendix B) showed that there was a significant difference between the three consecutive germination days ($p<0.05$) while there was no significant difference between third, fourth and fifth germination days. The mean value showed that the third day and onwards germination had the least significant reduction in tannin and phytic acid content. This result was in accordance to Nadeem, steeping of wheat for 24 hours following sprouting for 72 hours ranked the highest with regard to improvement in mineral extractability and decreasing antinutritional factor as phytic acid, polyphenols (Nadeem *et al.*, 2010). t-Test (Appendix B) for tannin and phytic acid showed that the tabulated value (2.77 and 2.77) was less than the calculated value (14 and 19). Hence, there was a significant difference at 5% level between raw and germinated sample.

Tannin in wheat was reduced by 43% while phytic acid was reduced by 52% by germination. According to Coulibaly, germination reduced 55% of phytic acid as compared to raw wheat sample (Coulibaly *et al.*, 2011). Similarly, Greiner showed a

marked decrease in phytic acid (54%) by germination (Greiner and Konietzny, 2006). Also, Azeke showed that germination reduced the phytic acid by 54% (Azeke *et al.*, 2011). Hussain showed 48% reduction in tannin and 55% in phytic acid after germination of 72 hours (Hussain *et al.*, 2011). Nadeem showed 58% reduction in tannin by germination (Nadeem *et al.*, 2010). 46% reduction in tannin was observed by Parmar after 72 hours of germination (Parmar *et al.*, 2017).

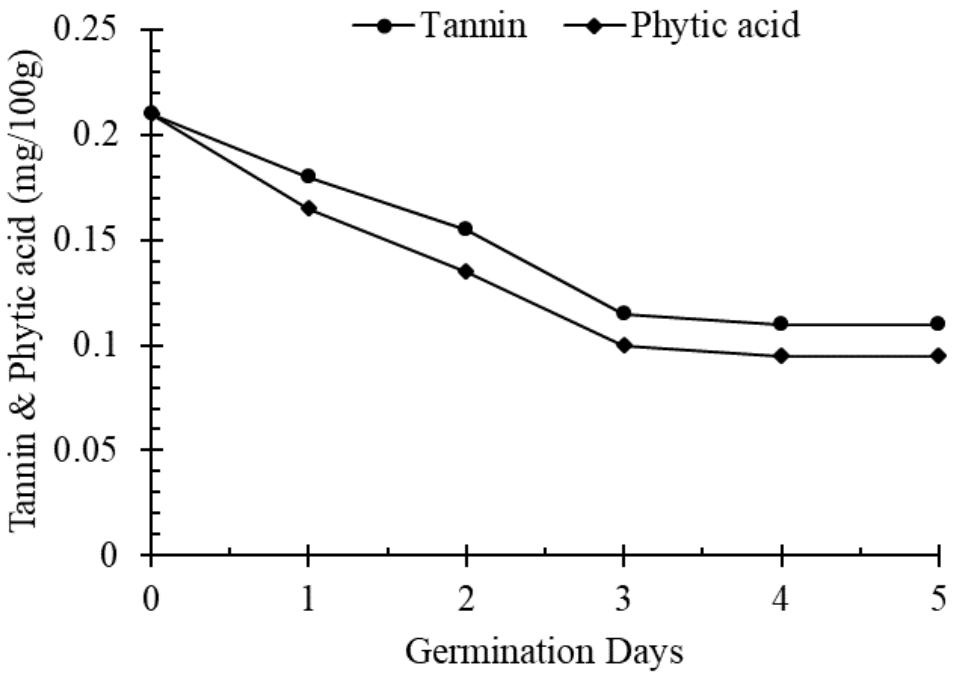

Fig 4.1 Changes in Tannin and Phytic acid by germination in wheat

4.1.2 Maize

Similarly, the maize was germinated for five days. The change in tannin and phytic acid content was checked in each day germinated sample and the raw and germinated sample of maize with least tannin and phytic acid content was also analyzed. The tannin and phytic acid content was reduced by germination which is shown in the fig 4.2. The analysis of variance (Appendix B) showed that there was a significant difference between the four consecutive germination days ($p<0.05$) while there was no significant difference between fourth and fifth germination days. The mean value showed that the fourth day and onwards germination had the least significant effect on reduction of tannin and phytic acid content. t-Test (Appendix B) for tannin and phytic acid showed that the tabulated value (2.77 and 2.77) was less than the calculated value (32 and 39). Hence, there was a significant difference at 5% level between raw and germinated sample.

Tannin was reduced by 41% and phytic acid was reduced by 48% in maize by germination. This result was in accordance to Gernah which showed 43% reduction in tannin and 52% reduction in phytic acid after 96 hour of germination (Gernah *et al.*, 2011). Similar result was obtained by Coulibaly which showed 51% reduction in phytic acid by germination in maize (Coulibaly *et al.*, 2011). Similarly, Greiner showed a marked decrease in phytic acid (51%) by germination (Greiner and Konietzny, 2006). Also Elkhalil showed 43% reduction in tannin by germination (Elkhalil *et al.*, 2003). According to Fageer, maize germinated for 96 hours had 51% reduction in phytic acid and increased in vitro protein digestibility (Fageer *et al.*, 2004). Similarly, Sokrab stated that phytic acid in maize was decreased to 51% by 4 days germination (Sokrab *et al.*, 2012)**.**

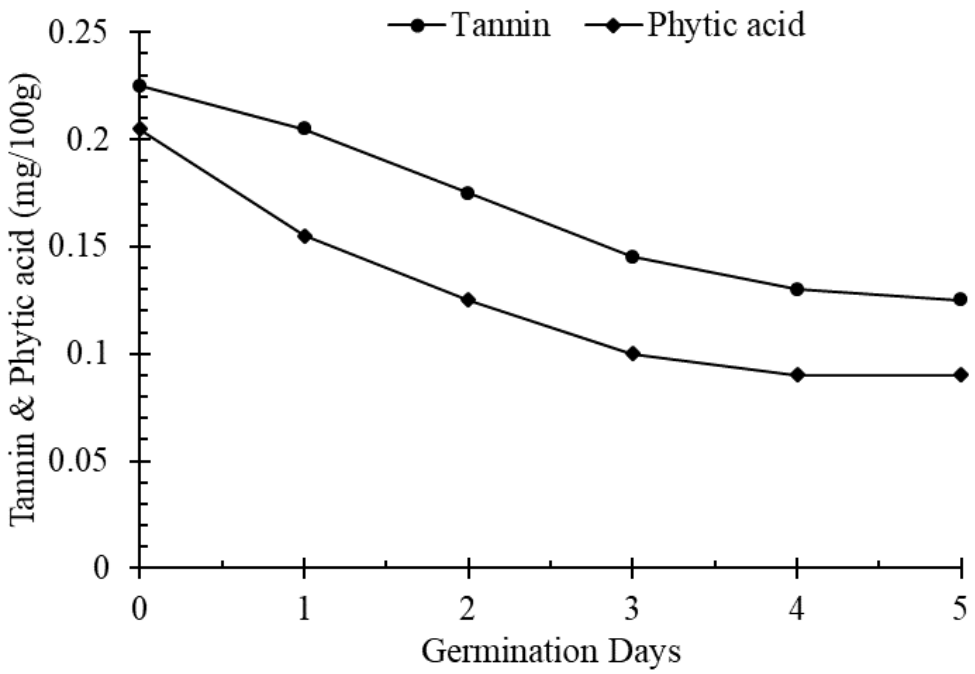

Fig 4.2 Changes in Tannin and Phytic acid by germination in maize

4.1.3 Soybean

The soybean was germinated for five days. The change in tannin and phytic acid content was checked in each day germinated sample and the raw and germinated sample of soybean with least tannin and phytic acid content was also analyzed. The tannin and phytic acid content was reduced by germination which is shown in the fig 4.3. The analysis of variance (Appendix B) showed that there was a significant difference between the four consecutive germination days ($p<0.05$) while there was no significant difference between fourth and fifth germination days ($p>0.05$) in case of tannin while in the case of phytic acid

there was a significant difference between the third consecutive germination days and no significant difference between third, fourth and fifth germination days. The mean value showed that the fourth day and onwards germination had the least significant effect on reduction of tannin and phytic acid content. t-Test (Appendix B) for tannin and phytic acid showed that the tabulated value (2.77 and 2.77) was less than the calculated value (61 and 22). Hence, there was a significant difference at 5% level between raw and germinated sample.

Tannin was reduced by 43% and phytic acid was reduced by 47% in soybean by germination. Khandelwal showed that there was 45% reduction in tannin after soaking for 24 hours followed by germination for 96 hours which was similar to this result (Khandelwal *et al.*, 2010). Ramadan showed that phytic acid in soybean was reduced by 46% soaked for 24 hours and 4 days germination (Ramadan, 2012). Kayembe showed that there was 44% reduction in tannin after 4 days germination in soybean (Kayembe, 2011). According to Egli, phytic acid was reduced by 45% after 96 hour of germination (Egli *et al.*, 2002). Similarly, Chitra showed that phytic acid was reduced by 47% in soybean by germination (Chitra *et al.*, 1996). Kayembe showed 44% reduction in tannin by germination (Kayembe, 2011). Rusydi showed 46% reduction in tannin in soybean by germination (Megat and Azrina, 2012).

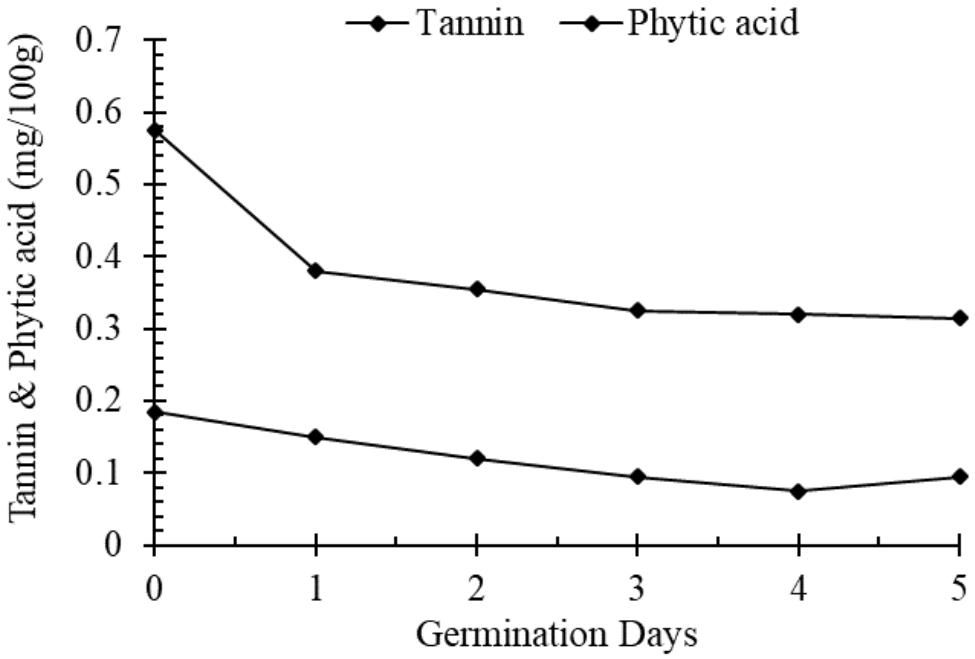

Fig 4.3 Changes in Tannin and Phytic acid by germination in soybean

4.2 Sensory evaluation of different formulation of Sarbottam Pitho

The prepared five formulations were subjected to sensory evaluation. The samples were provided to 12 panelists i.e. mothers of 1 to 3 years children. The panelists evaluated for various parameters of the product namely color, flavor, taste, texture, mouth feel and overall acceptability. The panelists were requested to provide scores in the score sheets as per their perception. Data were analyzed statistically and best product was found out.

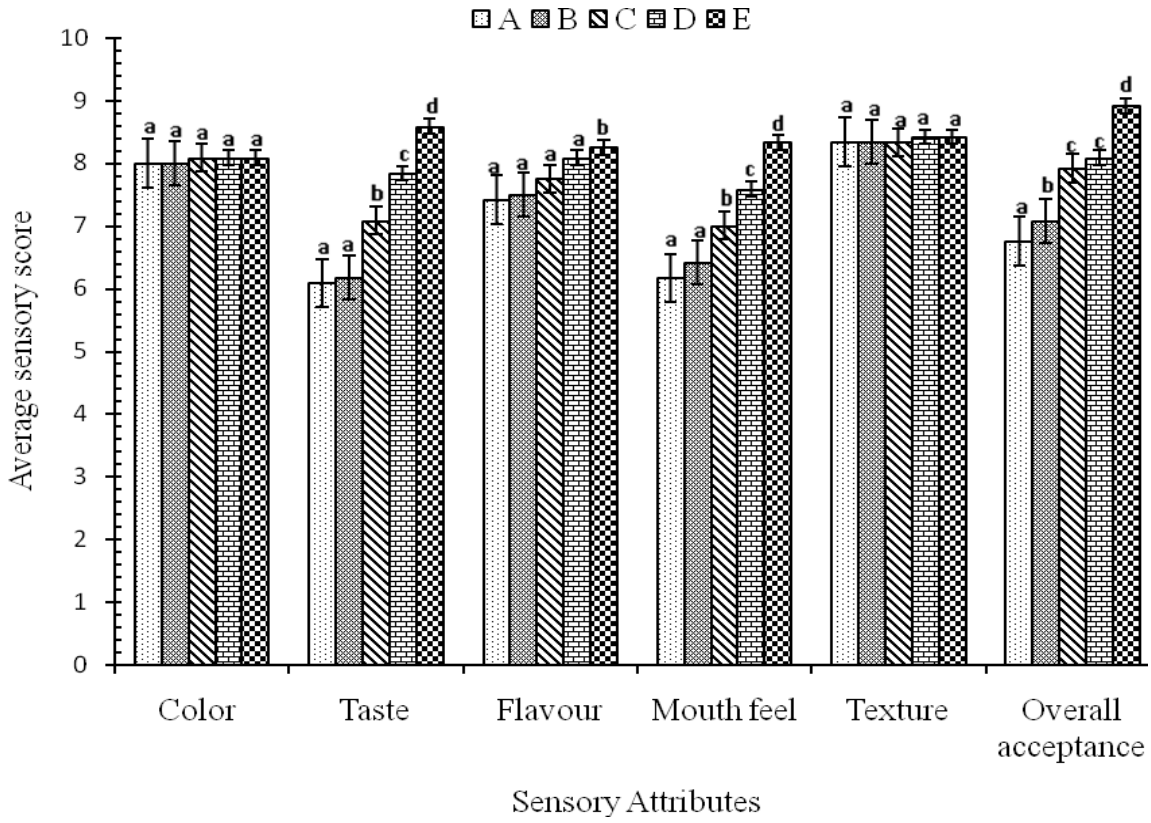

Fig 4.4 Average sensory score for five different formula

The ANOVA at 95% level of confidence ($p<0.05$) showed that the product A, B, C, D and E were significantly different from each other in sensory attributes.

4.2.1 Color

The average sensory score for color was 8, 8, 8.08, 8.08 and 8.08 for A, B, C, D and E respectively. The analysis of variance showed that there was no significant difference ($p>0.05$) between the five products.

4.2.2 Flavor

The average sensory score for flavor was 7.42, 7.5, 7.75, 8.08 and 8.25 for A, B, C, D and E respectively. In case of flavor, product E was significantly different from other four products ($p<0.05$) while the product A and B, C and D were not significantly different ($p>0.05$).

4.2.3 Taste

The average sensory score for taste was 6.08, 6.17, 7.08, 7.83 and 8.58 for A, B, C, D and E respectively. In case of taste, product C, D and E were significantly different ($p<0.05$) while the product A and B were not significantly different ($p>0.05$).

4.2.4 Texture

The average sensory score for texture was 8.33, 8.33, 8.33, 8.42 and 8.42 for A, B, C, D and E respectively. The analysis of variance showed that there was no significant difference ($p>0.05$) between the five products.

4.2.5 Mouth feel

The average sensory score for mouth feel was 6.17, 6.42, 7, 7.58 and 8.33 respectively for A, B, C, D and E respectively. The analysis of variance showed that product C, D and E were significantly different ($p<0.05$) while A and B were not significantly different ($p>0.05$).

4.2.6 Overall acceptability

The average sensory score for overall acceptance was 6.75, 7.08, 7.92, 8.08 and 8.92 for A, B, C, D and E respectively. In case of overall acceptance, product A, B and E were significantly different ($p<0.05$) while product C and D were not significantly different ($p>0.05$). Hence, from the statistical analysis the overall acceptability of product E with germinated wheat, maize, soybean and 25% apple powder were found to be superior.

Incorporation of apple powder >15% in bakery product increased the overall acceptance of product in comparison to <15% (Alsuhaibani, 2015). Also, Faisal showed that incorporation of apple powder >25% in multi-grain “Sattu” decreased the acceptance due to increase in moistness (Faisal, 2017).

4.3 Analysis of optimized Sarbottam Pitho

Analysis of the optimized Sarbottam Pitho (Sample E) found from sensory analysis was carried out. The result is tabulated in table 4.2.

Table 4.1 Analysis of Sarbottam Pitho

Parameters	Amount
Moisture (%)	3.24%
Protein (% db)	12.73%
Fat (% db)	9.30%
Total ash (% db)	2.89%
Crude fibre (% db)	2.61%
Carbohydrate (%db)	69.23%
Energy (kcal/100 gm)	411.54

Similar nutritional value was found by Nutrition Collaborative Research Program during the market analysis 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). According to Shrestha, weaning food prepared 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 (Shrestha, 1989). Similarly, weaning food prepared from multipurpose flour had moisture 4.2%, protein 18.6%, fat 8.40%, crude fiber 2.57%, carbohydrate 62.24%, ash content 3.2% (Ahmad *et al.*, 2012).

4.4 Cost Evaluation

The cost of the best formulated Sarbottam Pitho analyzed from sensory evaluation was NRs. 171 per Kg (calculation is given in Appendix D).

Sarbottam Pitho available in the market costs NRs. 140 per 500gm. Similarly, Nestle Cerelac Fortified Baby Cereal with Milk, Multigrain and Fruits costs NRs. 382 per 300gm which is highly expensive for the people with low income.

Thus, there was a vast difference in price of weaning food self-prepared and baby food available in the market. The prepared apple fortified Sarbottam Pitho cost around NRs.86 for 500 gm while the market value of Sarbottam Pitho is about NRs. 140. Also, baby foods are also highly expensive in comparison to home prepared. Hence, the prepared apple fortified Sarbottam Pitho is relatively cheap and affordable by everyone.

Part V

Conclusions and recommendations

5.1 Conclusions

From the above result and discussion, following conclusions were drawn:

a) Optimum germination time for reduction of phytic acid and tannin was 72 hours for wheat and 96 hours for maize and soybean.
b) Tannin of wheat, maize and soybean was reduced by 43%, 41% and 43% respectively and phytic acid of wheat, maize and soybean was reduced by 52%, 48% and 47% respectively during optimum germination.
c) From sensory evaluation, Sarbottam Pitho containing 25% apple powder was found to be the best.
d) The cost of best Sarbottam Pitho was found to be NRs. 171.46 per kg.

5.2 Recommendations

a) Sarbottam Pitho can be prepared by using 2:1 ratio of cereals (wheat and maize) and legumes (soybean) by malting for 72 hours for wheat and 96 hours for maize and soybean with the addition of 25% apple powder.
b) Clinical trials in the Albino rats can be done to check the efficiency of the product.
c) Study on fatty acid composition and amino acid profile of the prepared products can be studied.
d) Study on bioavailability of micronutrients after germination can also be studied.

Part VI

Summary

Acute childhood malnutrition affects about a tenth of the world's children under 5 years of age, particularly those living in circumstances of extreme poverty in developing world. Malnutrition in under 5 years is typically begins during the transition stage from breast feeding to solid diet. The precise cause of such growth failure may be due to one or a combination of factors like, inappropriate initiation and correct method of doing complementary feeding practices after 6 months of age, defective digestion or absorption, increased metabolic demands. The desirable weaning food should be rich in calories and protein with adequate amount of trace elements like iron, calcium, vitamins etc. and also inexpensive, home available, clean, easily digestible and the most importantly bio-available. So, this work primarily focuses on the production and evaluation of weaning food using locally available raw materials that has high digestibility and bio-available.

Sarbottam Pitho is a weaning food that is given to weaned infants. It is generally made from the cereals and legumes in the ratio of 2:1 with the addition of fruits. The cereals and legumes were germinated. The germination was carried out till the anti-nutritional factors i.e. tannin and phytic acid were significantly reduced to the least. The wheat was germinated for 72 hours while the maize and soybean were germinated for 96 hours. Optimum germination of wheat, maize and soybean reduced tannin by 43%, 41% and 43% respectively and phytic acid by 52%, 48% and 47% respectively.

Five different products of Sarbottam Pitho were made from the germinated cereals and legumes varying the amount of fruits in each product while keeping the ratio of cereals and legumes (2:1) constant. The raw materials were processed and the products were prepared in the laboratory and sensory evaluation was performed by 12 panelists who were the mothers of child aged 1 to 3 years. On the basis of results from sensory evaluation the product E containing 25% apple powder was taken for further chemical analysis. The analysis includes the proximate analysis of the product. The protein, fat, carbohydrate, crude fiber and total ash of the product were found to be 12.73%, 9.30%, 69.23%, 2.61% and 2.89% respectively. The diet can supply 411.54 kcal/ 100 gm. The energy contributed by protein, fat and carbohydrate were found to be 12.37%, 20.34% and 67.29% of total calories respectively.

This study where Sarbottam Pitho has been prepared from locally available food which contains important nutrients required for weaned infants, 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.

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Appendices

Appendix A

१. संवेदी मूल्यांकन कार्ड

सर्वोत्तम पिठोको संवेदी विश्लेषण

प्यानलिस्टको नाम : मिति:

उत्पादनको नाम :- सर्वोत्तम पिठो

उत्पादनको प्रकार :- विनिंग फुड

प्रिय प्यानलिस्ट तपाईहरुलाई सर्वोत्तम पिठोको ५ नमुना दिईरहेको छ, कृपया दिईरहेको तालिका प्रयोग गरि निम्न प्यारामिटरमा आधारित संवेदी बिश्लेषण गरिदिनु होला|

नमुना	रंग	फ्लेवर (स्वाद + सुगन्ध)	बनावट	स्वाद	मुखमहसुस	समग्र स्वीकृति
A						
B						
C						
D						
E						

अत्यन्तै मनपर्यो	९
धेरै मनपर्यो	८
अत्यन्तै सामान्य	७
अलिकति मनपर्यो	६
न मनपर्यो न मनपरेन	५
अलिकति मनपरेन	४
सामान्य मनपरेन	३
धेरै मनपरेन	२
अत्यन्तै मनपरेन	१

सुझाव (भएमा)

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हस्ताक्षर

Appendix- B

1. Tannin and Phytic acid content in germinated sample

Two-way ANOVA for maize

Table B.1.1 Test for Phytic acid

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Germination_time_days	4	0.00939	0.0023475	78.25	<.001
Residual	10	0.0003	0.00003		
Total	14	0.00969			

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

Table B.1.2 LSD of means

Days	Mean	Column1	l.s.d	d.f.
4*	0.09	A		
5	0.09	A		
3*	0.1	B	0.00996	10
2*	0.125	C		
1*	0.155	D		

(* = Significantly different)

Table B.1.3 Test for Tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Germination_time_days	4	0.01356	0.00339	169.5	<.001
Residual	10	0.0002	0.00002		
Total	14	0.01376			

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

Table B.1.4 LSD of means

Days	Mean	Column1	l.s.d	d.f
5	0.125	A		
4*	0.13	A		
3*	0.145	B	0.00814	10
2*	0.175	C		
1*	0.205	D		

(* = Significantly different)

Two-way ANOVA for wheat

Table B.1.5 Test for Tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Germination_time_days	4	0.01221	0.003053	305.25	<.001
Residual	10	0.0001	0.00001		
Total	14	0.01231			

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

Table B.1.6 LSD of means

Days	Mean	Column1	l.s.d.	d.f.
4	0.11	A		
5	0.11	A		
3*	0.115	A	0.005753	10
2*	0.155	B		
1*	0.18	C		

(* = Significantly different)

Table B.1.7 Test for Phytic acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Germination_time_days	4	0.01164	0.00291	72.75	<.001
Residual	10	0.0004	0.00004		
Total	14	0.01204			

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

Table B.1.8 LSD of means

Days	Mean	Column1	l.s.d.	d.f.
4	0.095	A		
5	0.095	A		
3*	0.1	A	0.01151	10
2*	0.135	B		
1*	0.165	C		

(* = Significantly different)

Two-way ANOVA for soybean

Table B.1.9 Test for Tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Germination_time_days	4	0.00921	0.002303	153.5	<.001
Residual	10	0.00015	0.000015		
Total	14	0.00936			

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

Table B.1.10 LSD for means

Days	Mean	Column1	l.s.d.	d.f.
5	0.315	A		
4*	0.32	A		
3*	0.325	B	0.00705	10
2*	0.355	C		
1*	0.38	D		

(* = Significantly different)

Table B.1.11 Test for Phytic acid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Germination_time_days	4	0.00999	0.002498	16.11	<.001
Residual	10	0.00155	0.000155		
Total	14	0.01154			

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

Table B.1.12 LSD for means

Days	Mean	Column1	l.s.d.	d.f.
4	0.075	A		
3	0.095	A		
5*	0.095	A	0.02265	10
2*	0.12	B		
1*	0.15	C		

(* = Significantly different)

2. Tannin and Phytic acid content in raw and germinated sample

t-Test: Two-Sample Assuming Equal Variances for Wheat

Table B.2.1 t-Test for Phytic acid

Column 1	**Variable 1**	**Variable 2**
Mean	0.1	0.21
Variance	0.0001	0
Observations	3	3
Pooled Variance	0.00005	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-19.05255888*	
P (T<=t) one-tail	2.2355E-05	
t Critical one-tail	2.131846782	
P (T<=t) two-tail	4.47099E-05	
t Critical two-tail	2.776445105*	

t tabulated < t calculated, there is a significant difference between the raw and germinated samples

Table B.2.2 t-Test for Tannin

Column1	**Variable 1**	**Variable 2**
Mean	0.115	0.21
Variance	0.000025	1E-04
Observations	3	3
Pooled Variance	6.25E-05	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-14.7173367*	
P (T<=t) one-tail	6.2023E-05	
t Critical one-tail	2.13184678	
P (T<=t) two-tail	0.00012405	
t Critical two-tail	2.77644511*	

t tabulated < t calculated, there is a significant difference between the raw and germinated samples

t-Test: Two-Sample Assuming Equal Variances for Maize

Table B.2.3 t-Test for Phytic acid

Column1	**Variable 1**	**Variable 2**
Mean	0.09	0.205
Variance	2.88889E-34	2.5E-05
Observations	3	3
Pooled Variance	0.0000125	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-39.83716857*	
P (T<=t) one-tail	1.18617E-06	
t Critical one-tail	2.131846782	
P (T<=t) two-tail	2.37233E-06	
t Critical two-tail	2.776445105*	

t tabulated < t calculated, there is a significant difference between the raw and germinated samples

Table B.2.4 t-Test for Tannin

Column1	**Variable 1**	**Variable 2**
Mean	0.13	0.225
Variance	0	0.000025
Observations	3	3
Pooled Variance	0.0000125	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-32.90896534*	
P (T<=t) one-tail	2.54212E-06	
t Critical one-tail	2.131846782	
P (T<=t) two-tail	5.08424E-06	
t Critical two-tail	2.776445105*	

t tabulated < t calculated, there is a significant difference between the raw and germinated samples

t-Test: Two-Sample Assuming Equal Variances for Soybean

Table B.2.5 t-Test for Phytic acid

Column1	**Variable 1**	**Variable 2**
Mean	0.095	0.185
Variance	0.000025	0.000025
Observations	3	3
Pooled Variance	0.000025	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-22.04540769*	
P (T<=t) one-tail	1.2529E-05	
t Critical one-tail	2.131846782	
P (T<=t) two-tail	2.50579E-05	
t Critical two-tail	2.776445105*	

t tabulated < t calculated, there is a significant difference between the raw and germinated samples

Table B.2.6 t-Test for Tannin

Column1	**Variable 1**	**Variable 2**
Mean	0.325	0.575
Variance	0.000025	0.000025
Observations	3	3
Pooled Variance	0.000025	
Hypothesized Mean Difference	0	
Df	4	
t Stat	-61.23724357*	
P (T<=t) one-tail	2.12955E-07	
t Critical one-tail	2.131846782	
P (T<=t) two-tail	4.25909E-07	
t Critical two-tail	2.776445105*	

t tabulated < t calculated, there is a significant difference between the raw and germinated samples

Appendix- C

1. Sensory evaluation of product

Table C.1.1 Two-way ANOVA for color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	0.1	0.025	1	0.418
Panelist	11	11.65	1.05909	42.36	<.001
Residual	44	1.1	0.025		
Total	59	12.85			

Since p>0.05, there is no significant difference between the samples so LSD testing is not required.

Table C.1.2 Two-way ANOVA for taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	55.5667	13.8917	151.55	<.001
Panelist	11	4.05	0.36818	4.02	<.001
Residual	44	4.03333	0.09167		
Total	59	63.65			

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

Table C.1.3 LSD for taste

Sample	Mean	Column1	l.s.d.
A	6.083	A	
B*	6.167	A	
C*	7.083	B	0.2491
D*	7.833	C	
E*	8.583	D	

(* = Significantly different)

Table C.1.4 Two-way ANOVA for flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	6.2667	1.5667	7.72	<.001
Panelist	11	4.4	0.4	1.97	0.056
Residual	44	8.9333	0.203		
Total	59	19.6			

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

Table C.1.5 LSD for flavor

Sample	Mean	Column1	l.s.d.
A	7.417	a	
B	7.5	a	
C	7.75	b	0.3707
D*	8.083	b	
E*	8.25	c	

(* = Significantly different)

Table C.1.6 Two-way ANOVA for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	0.1	0.025	1	0.418
Panelist	11	12.7333	1.15758	46.3	<.001
Residual	44	1.1	0.025		
Total	59	13.9333			

Since p>0.05, there is no significant difference between the samples so LSD testing is not required.

Table C.1.7 Two-way ANOVA for mouth feel

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	37.2333	9.3083	68.64	<.001
Panelist	11	8.2	0.7455	5.5	<.001
Residual	44	5.9667	0.1356		
Total	59	51.4			

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

Table C.1.8 LSD for mouth feel

Sample	Mean	Column1	l.s.d.
A	6.167	A	
B*	6.417	A	
C*	7	B	0.303
D*	7.583	C	
E*	8.333	D	

(* = Significantly different)

Table C.1.9 Two-way ANOVA for overall acceptance

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	35.33333	8.83333	91.09	<.001
Panelist	11	1.65	0.15	1.55	0.149
Residual	44	4.26667	0.09697		
Total	59	41.25			

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

Table C.1.10 LSD for overall acceptance

Product	Mean	Column1	l.s.d.
A*	6.75	a	
B*	7.083	b	
C*	7.917	c	0.2562
D	8.083	c	
E*	8.917	d	

(* = Significantly different)

Appendix D

Table D.1 Cost calculation of the product

Particulars	Cost (NRs/Kg)	Weight (gm)	Cost (NRs)
Maize	50	187.5	9.375
Wheat	30	187.5	5.625
Soybean	90	375	33.75
Apple	190	250	47.5
Total raw material		1000	96.25
Processing and labour cost (10% of total material cost)			9.625
Packaging cost			50
Profit (10% of total cost)			15.5875
Grand Total			171.4625

Cost of raw material varies with season and time

Appendix E

Photo Glimpse