EFFECT OF GERMINATION TIME ON EMZYMATIC, ANTI-NUTRITIONAL AND PHYTO-CHEMICAL PROPERTIES OF *KWATI* (A NEPALESE INDIGENIOUS FOOD)

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by

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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 Time on Enzymatic, Anti-nutritional and Phyto-chemical Properties of Kwati (A Nepalese Indigenous Food)*" presented by Rishi Raj Adhikari has been accepted as the partial fulfillment of the requirement for Bachelor degree in Nutrition and Dietetics.

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Abstract

Kwati is a Nepalese indigenous food (sprouted mixed pulses) of having traditional and religious importance in Nepal which is consumed mainly at the festival of *Janai Purnima.* A study was carried out to know the effect of germination time on biochemical, nutritional and antioxidant properties of *kwati*. Formulation for *kwati* was developed through market survey of Dharan. Legumes were first mixed in equal proportions and steeped in water over night at 28 ± 3ºC followed by draining and germination for 4 days at 28 ± 3ºC. Then, the germinated legumes were dried in cabinet dryer at 50 ± 5ºC for 16-18 hours to obtain desired moisture content of below 10% and drie samples were then taken for quantitative analysis of different components (ascorbic acid, amylase activity, tannin, phytate, oxalate, total phenolic content and DPPH radical scavenging activity).

The mean value of phytate, oxalate and tannin content in raw *kwati* were found to be 404.36 mg/100g, 170.54 mg/100g and 427.26 mg/100g dry wt respectively. Germination showed significant reduction ($p<0.05$) in phytate and tannin content for consecutive four days of germination. Significant reduction of oxalate content was found only for first three days, but there was no significant reduction of oxalate in fourth day of germination. Germination time showed a significant effect on the α–amylase activity of *kwati* malt giving a maximum value 5.08 mg maltose/ min per g dry wt. in 24 hours. Germination time of *kwati* showed significant increase ($p<0.05$) in ascorbic acid content 21.63 mg/100g on dry wt basis. DPPH radical scavenging activity was increased to 63.94 % and total phenolic content was increased by 7 % respectively on four days of germination. Hence, germination was considered as one of the effective method for reduction of antinutritional factors and to increase the antioxidant properties.

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List of Abbreviations

Abbreviation	Full Form
ANF	Antinutritional Factor
ANOVA	Analysis of Variance
ARF	Amylase Rich Food
BUN	Blood Urea Nitrogen
CCT	Central Campus of Technology
CVD	Cardio Vascular Disease
DFTQC	Department of Food Technology and Quality Control
DB	Dry Basis
Etc.	Et cetera
FAO	Food and Agricultural Organization
GI	Glycemic Index
HDL	High Density Lipoprotein
Kcal	Kilo Calorie
LDL	Low Density Lipoprotein
LSD	Least Significant Difference
MC	Moisture Content
TPC	Total phenolic content
WHO	World Health Organization

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Part I

Introduction

1.1 Background

Kwati is a curried sprouted traditional bean soup from the mountains of Nepal and parts of North East India. Generally, it is prepared from a collection of nine beans and mostly eaten in religious festivals. It is prepared by cooking a mixture of different sprouted legumes in excess water. The types of *Kwati* vary according to the availability of legumes and locality. It is also called a "hot beverage of pulses and beans" or *Kwati*. Although *Kwati* is used extensively in Nepalese community, little is known about its preparatory stages such germination, types of legumes used and nutritional changes during germination and cooking (Chaudhary *et al.*, 2015).

Food legumes are rich and less expensive sources of proteins in human diet in several developing countries. Biological utilization of pulses is limited due to deficient sulphur containing amino acids (Elías *et al.*, 1964) and the presence of antinutrients including phytic acid, saponins, polyphenols, enzyme inhibitors, lectins etc (Chitra *et al.*, 1995). Anti-nutrients are chemical substances in food that do not offer nourishment to the body e.g., phytic acid and tannins. The effect of these anti-nutrients in the body depends on the type and the concentration in which it is present in the food material. However, the presence of antinutritional factors (tannins and phytates) limits the utilization of the legumes as a main source of protein (Alonso *et al.*, 1998).

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 Rusydi and Azrina, 2012).

Sprouting is a key processing step where legumes (seeds) are soaked in water for few days, drained and leaving seeds until it germinate and begin to sprout. Sprouted foods are a convenient way to have fresh vegetables for salads. Identified as an inexpensive and effective technology, it is known to improve the nutritional quality of the seeds (Khattak *et al.*, 2007). The effects of germination on the chemical composition and biochemical constituents of seeds vary greatly with the plant species, seed varieties or cultivars and the germination conditions (temperature, light, moisture and the time of germination) (Bau *et al.*, 1997).

1.2 Statement of problem

Kwati, a mixed soup of sprouted pulses is a traditional *Newari* dish usually consumed on the festival of *Kwati Purnima (Janai Purnima)*. The ingredients used in *kwati* vary according to availability of pulses and locality also the proportion of pulses used vary accordingly. The consumption of *kwati* (mixed pulses) is mainly limited to the festival only. Several studies have reported that germination improve the nutritive value of pulses, particularly in the level of vitamins such as riboflavin, niacin, choline, biotin and ascorbic acid. Besides it brings about biochemical changes such as converting more indigestible carbohydrates into digestible and absorbable forms. Furthermore, the germination process reduces or eliminates most of the antinutrients, such as phytates, tannin, oxalates etc. There is research gap that signifies the importance of germination on *kwati* which could be the reason behind less uses in daily basis.

1.3 Objectives

1.3.1 General objectives

General objective of the study was to study the effect of variation on germination time on enzymatic activity, nutritional and anti-nutritional components of *kwati*.

1.3.2 Specific objectives

- To formulate the recipe for *kwati*.
- To study nutritional and anti-nutritional properties of raw *kwati*.
- To study the effect of variation in germination time on antinutritional, enzymatic and antioxidant properties of *kwati*.

1.4 Significance of the study

Kwati is a traditional food having large significance in Nepali culture (especially Newari) containing various types of pulses mixed together. The consumption of *kwati* is mainly limited to a festival. Pulses are rich sources of protein along with various types of antinutritional components. Diet containing antinutritional factors impairs with absorption of various nutrients like minerals also reduces the bioavailability. Reducing antinutritional factor has dual benefits i.e. enhanced mineral absorption as well as improves the utilization. The sprouts of germinated pulses are rich source of vitamin C. The outcome of this study will be helpful to overcome research gap and to increase the consumption of *kwati* at local level throughout the year by providing information about sprouting of *kwati*.

1.5 Limitations of the study

The study has the following limitations:

- During germination variation was done only on time factor.
- 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 *Kwati*

Kwati is a curried sprouted traditional bean soup from the mountains of Nepal and parts of North East India. Generally, it is prepared from a collection of nine beans and mostly eaten in religious festivals. It is prepared by cooking a mixture of different sprouted legumes in excess water. The types of *Kwati* vary according to the availability of legumes and locality. It is also called a "hot beverage of pulses and beans" or *Kwati*. Although *Kwati* is used extensively in Nepalese community, little is known about its preparatory stages such germination, types of legumes used and nutritional changes during germination and cooking (Chaudhary *et al.*, 2015).

Kwati in Nepal Bhasa literally translates to '*kwa*', meaning hot, and '*ti*', meaning soup. This "hot soup", the elders say, provided much-needed warmth to the farmers, who spent days shin-deep in muddy water, as they planted crops in the fields (to be harvested in October) just in time for Yenya Puhni, Dashain and many other festivals throughout the Valley. Generally the bond between food and festivals in the Newar community is taken for granted, as is its inextricable ties with agriculture, but getting any finite information on the *kwati* story is fraught with contrasting opinions. Food has become a ritual, without any explication just interpretation (Manander, 2019).

2.2 Pulses

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 (also known as pulses) are generally regarded as good source of proteins and carbohydrates in human diet and also a popular staple diet for a large group of world population. Legume seeds have almost twice the amount of proteins than cereals which is

rich in all essential amino acids except sulfur-containing amino acids. Starch present in legumes is slowly digestible, providing a longer lasting source of energy (low glycemic load) (Chaudhary *et al.*, 2015).

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 (P. Prasad *et al.*, 2016).

2.2.1 Chemical composition of pulses

Whole pulses are rich in carbohydrate. Besides, protein is the second most important constituent of the pulses. A very small quantity of fat is also present in the pulses. Apart from these, the pulses are rich in vitamins and minerals. (Hall *et al.*, 2017) reviewed that the protein, fat, ash and carbohydrate content in pulses generally ranges from 15-30%, <3%, 2-5% and 60-70%. (Chaudhary *et al.*, 2015) showed the protein, fat, crude fiber, ash and carbohydrate content of his three *kwati* formulations ranges from 21.6-27.6%, 6.3-9.4%, 3.1-4.6%, 2.0-3.5% and 59.2-60.8% respectively.

(Khan *et al.*, 1995) reported 80.4 mg/100g calcium content and 4.7 mg/100g iron in chickpea, (Shi *et al.*, 2016) reported calcium and iron content of green gram as 495 mg/100g and 5.41 mg/100g respectively, (Suneja *et al.*, 2011) reported calcium and iron content of black gram as 286 mg/100g and 15.67 mg/100g respectively, (S. K. Prasad and Singh, 2015) reported the iron content of 7.2 mg/100g in horse gram.

The following table gives a general idea about the chemical constituents of pulses (whole):

Table 2.1 Constituents of pulses

Name of pulse	Carbohydrate %	Protien %	Fat %	Fiber %	Ash %
Bengal gram	56.7	21.1	4	4.5	2.8
Black gram	58.8	21	1.6	4.4	3.2
Green gram	56.7	24	1.3	4.1	3.5
Horse gram	57.2	22	0.5	5.3	3.2
Dry white peas	55.7	19	1.9	4.5	2.2
Rice bean	56.6	23.6	1.1	4.5	3.5
Soybean	29.6	33.3	17.7	4.2	5
Black eyed beans	54.5	24.1	1	3.8	3.2
Red kidney bean	60.6	22.9	1.3	4.8	3.2
Lentil	59	25.1	0.7	0.7	2.1

Source: DFTQC (2012)

2.3 Phytochemicals and antioxidant

Phytochemical refers to every naturally occurring chemical present in plants. In plants, phytochemicals act as a natural defense system for host plants and provide color, aroma and flavor. There is a wide distribution of biologically active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition.

Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. More than 4000 of these compounds have been discovered and it is expected that scientists will yet discover many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs and spices (Rowland, 1999).

A number of phytochemicals are known, some of which include: alkaloids, saponins, flavonoids, glycosides, anthraquinones, steroids and terpenoids. They do not only protect the plant but have enormous physiological activities in human and animals. These include cancer prevention, antibacterial, antifungal, antioxidatie, hormonal action, enzyme stimulation and many more (Doss and Anand, 2012). Phytochemicals can have profound physiological effects, act as antioxidants, mimic body hormones and suppress development of diseases in the body (Hayes, 2005).

2.3.1 Antioxidants

Any substance which is capable of delaying, retarding or preventing the development of the rancidity or other flavors deterioration due to oxidation is called antioxidant. Oxidation reactions are chemical reactions that involve the transfer of electrons from one substance to an oxidizing agent. Antioxidants can slow these reactions either by reacting with intermediates and halting the oxidation reaction directly, or by reacting with the oxidizing agent and preventing the oxidation reaction from occurring (Pokorny, 2007).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Shekhar and Goyal, 2014). The DPPH assay method is based on the reduction of DPPH, a stable free radical.

2.3.2 Phenols

Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid, C_6H_5OH. In plants, the phenolic units are esterified or methylated and are submitted to conjugation, which means that the natural phenols are mostly found in the glycoside form instead of the aglycone form. This property of undergoing conjugation with other molecules enables it to scavenge free radicals and thus

inhibit the oxidative mechanisms that can lead to degenerative diseases such as cancer (Egbuna and Ifemeje, 2015). Phenols are reported antitumor agents and exhibit antiviral and antimicrobial activities (Robbins, 1980), hypotensive (Matsubara *et al.*, 1985) and antioxidant properties (Robak and Gryglewski, 1988).

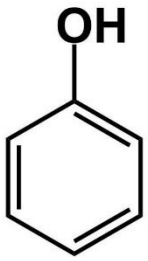

Fig.2.1 Structure of phenol

Several phenolics (e.g. chlorogenic acid, gallic acid, caffeic acid, tannic acid, catechin) have been shown to inhibit the mutagenic effects of both direct-acting carcinogens (e.g. benzo(a)pyrene diol epoxide) and carcinogens that require metabolic activation (e.g. aflatoxin B) and to trap nitrite, thus reducing the nitrosating species and preventing the endogenous formation of carcinogenic nitrosamines (H.F. and M.P., 1984).

2.3.3 Ascorbic acid

L-ascorbic acid, which is also known as vitamin C, is an important naturally occurring nutrient essential for human nutrition (Chauhan *et al.*, 1998). Vitamin C is a white crystalline compound with sour taste but no smell. Identification of its formula ($C_6H_8O_6$) was presented by group headed Professor Haworth in 1933 and it was the same group that proposed the first synthetic method for its molecular weight of 176, and melting point of 190ºC (Mottram, 1965).

Ascorbic acid has four isomers, L-ascorbic acid, D-ascorbic acid, L-arabo ascorbic acid and Disoascorbic acid. Among the four isomers, the D-forms have no biological activity and used as food additives. The presence of enediol group on ascorbic acid imparts acidic and reducing properties. It behaves as mono basic and can give salt when reacted with alkalies. Only L-ascorbic acid has important vitamin activity (Yu *et al.*, 2014). The unusual properties of L-ascorbic acid are derived from the fact that it shows acidic properties in the absence of carboxylic group, it has strong reducing properties and has one unusually stable 1, 4-lactonering (Herbert *et al.*, 1993).

2.4 Antinutritional factors

2.4.1 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 (Baskota, 2019).

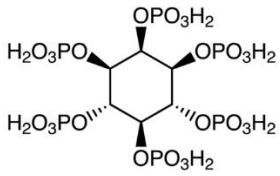

Fig.2.2 Structure of phytic acid

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 (Fardet, 2010).

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 (Chanakan *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 (Baskota, 2019).

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 phytate. 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 (Fardet, 2010).

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).

2.4.2 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.*, 1989).

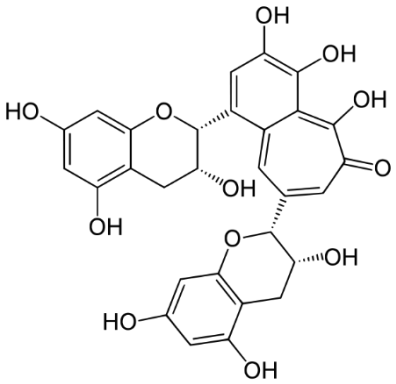

Fig.2.3 Structure of tannin

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 (Khanal *et al.*, 2004).

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. 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, 2007).

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 (Anon, 2017).

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).

2.4.2 Oxalate

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 (Dean, 2012).

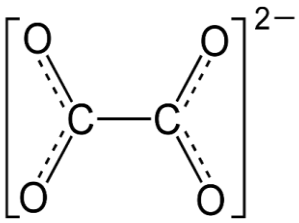

Fig.2.4 Structure of oxalate

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, and kidney beans. Consumption of high oxalate foods exerts a negative effect on calcium and iron absorption in the body (Chai and Liebman, 2005). Oxalates that are bound to calcium travels as a waste product from the blood to kidney and excreted from the body in the urine. Consumption of high oxalates binds to calcium in body and forms crystal resulting in kidney stone. Oxalate is also an end-product of metabolism in the liver. Some amino acids and carbohydrates are degraded to oxalates (Savage and Klunklin, 2018).

2.5 Impact of antinutrients on health

Antinutritional factors have potential adverse effects on human health. As the legumes contain wide range of antinutritional factors such as tannins, phytates, oxalates, saponin and lectins. Phytate has negative impact on the bioavailability of minerals usually divalent and trivalent ions of minerals such as Zn, Mg, Ca, Cu and Mn. Whenever higher level of phytate consumption results in minerals deficiency (Gemede and Ratta, 2014). Oxalate is the salt of oxalic acid calcium oxalate mostly distributed in plants. Oxalic acid formed strong bonds with minerals such as potassium, calcium and magnesium. Some oxalic salts are soluble whereas others are insoluble the insoluble salts solidify in the urinary tract when accumulate in kidney it forms kidney stones (Nachbar *et al.*, 1980). When oxalic acid is digested in gastrointestinal tract it comes in contact with nutrients (Noonan and Savage, 1999) this than bind the minerals and make them unavailable. Saponin is extremely toxic to cold blooded animals. In dietary plants saponin impart a bitter taste and astringency when consumed have bitter taste and irritate throat it also reduced the bioavailability of nutrients, reduced the enzyme activity and affect digestibility of protein (Liener, 1974). Some studies shown that saponin have inverse relationship with renal stone (Loewus and A, 2001). Beyond its adverse effect on health recent researches shows it possesses anticarcinogenic, immunostimulatory

and hypocholesterolemia properties. To inhibit dental carries saponin diet be used it also used in the treatment of hypercaliuria and as an antidote of lead poisoning(Gemede and Ratta, 2014). Washing repeatedly by water reduce the adverse effects and enhance the palatability by reducing the bitterness property (Katiyar *et al.*, 1989).

2.6 Ways to reduce antinutrients 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 (Savage and Klunklin, 2018). Simple ways to reduce the amount of antinutrients in foods are:

2.6.1 Soaking

Beans and other legumes are often soaked in water overnight to improve their nutritional value (Fernandes *et al.*, 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).

2.6.2 Germination

Sprouting is a period in the life cycle of plants when they start emerging from the seed. This natural process is also known as germination. This process increases the availability of nutrients in seeds, grains and legumes. Sprouting takes a few days. During sprouting, changes take place within the seed that lead to the degradation of 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).

2.6.3 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).

2.6.4 Boiling

High heat, especially when boiling, can degrade antinutrients like lectins, tannins and protease inhibitors (Egbe and Akinyele, 1990). One study showed that boiling pigeon peas for 80 minutes reduced protease inhibitors by 70%, lectin by 79% and tannin by 69%.Additionally, calcium oxalate is reduced by 19-87% in boiled green leafy vegetables. Steaming and baking are not as effective. In contrast, phytate is heat-resistant and not as easily degraded with boiling. The cooking time required depends on the type of antinutrient, food plant and the cooking method. Generally, a longer cooking time results in greater reductions of antinutrients (Arnarson, 2017).

2.6.5 Roasting

Roasting can improve protein digestibility. 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 (Coulibaly *et al.*, 2011). Sade reported that during roasting total phenols and tannins decrease (Sade, 2009). Malik observed the reduction in mineral contents during roasting; he said that might be due to the loss of nutrients while heating at high temperature. It should be noted that, the drying effect of roasting reduces the moisture content of the flour. Reduced moisture allows a larger concentration of solids by weight, resulting in an increased viscosity (Malik *et al.*, 2002).

2.7 Malting of *kwati*

Malting is the controlled germination process followed by drying and terminating the growth of the embryo, which activates the enzymes of the resting grains resulting the conversion of cereal starch into fermentable sugar and other particles, partial hydrolysis of protein and macromolecules into micro molecules (Evers and Rosentrater, 2018). Germination is done for the purpose of developing an active enzyme content which will later convert starches in the malted barley and in other cereals grains into sugars, which can be easily fermented during fermentation step (Potter, 1987).

It was found that malting had significant effect on dry matter content, fat content, starch content, total free amino acids content, ascorbic acid content and amylase activity except protein in which it did not had significant effect (Shrestha, 1995). An increase in reducing sugars during malting could be due to starch hydrolysis by hydrolytic enzymes such as α-amylase (Traoré *et al.*, 2004). This increased solubility could be as a result of the increase in amount of soluble sugars which directly affect the water absorption capacity of malted flour (Gernah *et al.*, 2011). Both the smaller granular size and its higher amylase content formed during the malting process is responsible for the slightly increase in gelatinization temperature (Greenwood and Thomson, 1959).

2.7.1 Outline of malting

Malting is controlled germination process which produces a complement of enzymes which are able to convert cereal starches (endosperm) to fermentable sugars, to secure an adequate supply of amino acids and other minor nutrient for yeast and modify the quality of the micro molecules (Evers and Rosentrater, 2018).

The maltster is concerned with both degradation of the endosperm and the accumulation of the enzymes in the grains. But the growth of the germs of embryo is an incidental to making of malt and leads to unwanted depletion of the endosperm material through respiration of the embryo when degradation of the endosperm has progressed to only a limited extent, the maltsters terminates the growth of embryo by drying the grain. In order that the storage of malt is possible for long period in a stable period. It has been customary for the maltsters to continue the drying, beyond that required arresting growth, by kilning (J. Hough and James, 1991).

2.7.1.1 Malting operation

The sequence of operation in malting is as follows:

- The collection of stocks of suitable grains or legumes.

- The storage of the pulses until it is required.

- Steeping the pulses in water, germination of the pulses.

- Drying and curing on a kiln.

Steeping an arranged so that sufficient moisture outers the grain to initiate germination. The moisture content of 42-46 % (Wet weight basic) that is eventually achieved is sufficient to support growth and biochemical alteration in the grains during the malting period, without, however, allowing excessive growth. Since growth, results in the production of largely unwanted rootlets and loses in dry weight due to grain respiration. A balance must be struck between achieving sufficient growth to adequately alter the barley into malt but without excessive growth that would reduce the quality of malt eventually produced. In newer malting processes, the wetted grain may be drained and aerated at intervals and germination may commence before the grain contains sufficient to malt adequately. Thus, the steeping and germination tend to merge (J. S. Hough *et al.*, 1975).

Germination traditionally carried out in darkness at relatively low temperature 12- 15°C for choice but this could not be easily controlled. Originally it was processed in autumn, winter and spring to take advantage of the cool weather but never malting have temperature-controlled atmosphere. Regulating the moisture and temperature of the grain controls the

intensity of germination process. The changes occurring in germination that are essential in converting barley into malt are collectively termed as "modification" and may be summarized as follows. First may hydrolytic enzymes appear and increase in amount, adding to those that are already present in the barley. These enzymes began to catalyze the hydrolytic degradation of the reserve substances at the starchy and endosperm and in the particular cell wall are partially or completely degraded resulting in loss of storage. Consequently, simple roller may readily crush dry malt in contrast to dry barley. Gummy polysaccharides are also degraded during malting so that the work derived from malt has a low viscosity compared with extracts of raw barley. Simple water-soluble product of hydrolysis accumulates in the grain during malting (J. S. Hough *et al.*, 1975).

Kilning, the hot air drying and cooking stages, terminates germination and produces a dry, easily milled products from which the dry, brittle, rootlets or "coolness" are easily separated. The pored products belling dry, can be stored for long periods, in addition to drying, kilning removes a raw flavor from the green malt and imparts other flavors and colors to the products, at the same time, the chemical composition of the malt is modified in particular enzyme content is reduced (J. Hough and James, 1991). Thus, formed malt is dried in a cabinet drier at 50±5ºC for 16-18 hours.

Cabinet drier consist of an insulated cabinet fitted with shallow mesh or perforated trays, each of which carries a thin layer of food. Hot air is circulated through the cabinet tray. A system of duct and baffles is used to direct air, over and/ or through each tray, to promote uniform air distribution either horizontally between the trays of food materials or vertically through the trays and food (Fellows, 2000). Air heaters may be direct gas burners, steam coil exchangers or electrical resistance heaters. The air is blown past the heaters and thus heated air is used for dying. It is relatively cheap to build and maintain, flexible in design, and produces variable product quality due to relatively poor control. It is used singly or in groups, mainly for small- scale production (1-20 ton/day) of dried fruits and vegetables (B. G. Smith *et al.*, 2007).

2.7.2 Chemical changes during malting

The percentage of starch decreases and the composition of the remaining starch alter. The proportion of amylase increases. The overall pentosans content of starchy endosperm declines while that of husk remain unchanged. The partial hydrolysis of the insoluble hemicellulose appears to give rise to the soluble gum, which in turn, when hydrolyzed,

further provides mono-saccharides. The quantity of simple sugars alters dramatically, those produced by the hydrolysis of polysaccharides on the one hand and those consumed by the living parts of the grains on the other hand. The amount of sugar declines during kilning but the sucrose often increases in amount. Maltose is also increased. The grain as the first respiratory substrate uses up raffinose before other sugars is mobilized to support the growth of embryo (Shrestha, 1995). Total free amino acid content was significantly affected ($p>0.05$) by germination time and watering regime (Sadasivam and Manickam, 2016).

During malting the reduction in phytate content as well as increase in α- amylase activity and the sweetness in the malt flours occurs (Sadasivam and Manickam, 2016). The complex organic phosphates of aleurone layer and starchy endosperm are hydrolyzed to yield inorganic phosphates and ultimate product of hydrolysis of phytic acid, are known to increase. Vitamins like riboflavin, pantothenic acid, pyridoxine, pyridoxal, and pyridoxamine group increases in the malting while others do not. Ascorbic acid also alters during germination but is completely destroyed during kilning. There is reduction in the quantity of inorganic materials during malting because the materials move to the roots and some is lost by leaching in the steep liquor (Shrestha, 1995). The statistical analysis done by (Friend *et al.*, 1995) shows there is no significant effect of malting of grain on the acidity.

2.7.2.1 Changes in nutritional composition during germination

There is significant effects of sprouting time (germination), using distilled water under room conditions, on moisture, mineral matter (ash), crude protein, crude fat, crude fiber. Initial moisture content of the seeds was 6.9% did not differ significantly. However, the absorption pattern was significantly different ($p \leq 0.05$). The moisture content of seeds was 58.1, 61.4, 80.2 and 87.2% with sprouting time of 24, 48, 72 and 96 h respectively. Effect of soaking/germination was also significant on the moisture contents of the seeds, exhibiting an upward trend with increase in sprouting time (Shah *et al.*, 2011). As germination proceeds, seed took up water from the surrounding in order for the metabolic process to start. Dry legumes absorb water rapidly, influenced by the structure of the legume. The increase in water uptake with time is due to the increasing number of cells within the seed becoming hydrated (Nonogaki *et al.*, 2010).

Ash contents, calculated on moisture free basis, increased with increase in sprouting time. The ash content slightly decreased with 24 h germination and thereafter with 48 and 72 h

germination it become at par with control. After 96 h germination the ash content reaches maximum level (Shah *et al.*, 2011). Chaudhary *et al.* (2015) also reported the significant increase in total ash content in their two formulations. T. El-Adawy *et al.* (2003) reported significant increase in ash content during sprouting in mungbean, pea and lentil seed. The decrease in crude fat and carbohydrate contents during sprouting may have led to the apparent increase observed in ash and other chemical components.

Chaudhary *et al.* (2015) reported the significant in crude protein level in all of their formulations. Increase in protein content was also noted by Camacho *et al.* (1992) during germination of beans, lentils, chickpea and pea's seeds. Ohtsubo *et al.* (2005) found an increment in crude protein of germinated brown rice. Obizoba (1991) who reported increase in % moisture, % crude protein and % ash. According to him, total nitrogen, total non-protein nitrogen; protein nitrogen, true protein nitrogen also increased with sprouting. Parameswaran and Sadasivam (1994), Khatoon and Prakash (2006), Urbano *et al.* (2005), (Ghavidel and Prakash, 2007), and (Kaushik *et al.*, 2010) also noted increase in the percent protein in germinated grains. Bau *et al.* (1997) assumed that the increase was due to synthesis of enzyme proteins (for example, proteases) by germinating seed or a compositional change following the degradation of other constituents. A further explanation was done by Nonogaki *et al.* (2010) where they noted that protein synthesis occurred during imbibitions and that hormonal changes play an important role in achieving the completion of germination.

Chaudhary *et al.* (2015) reported the significant loss of lipid during the germination period. Badshah *et al.* (1991) and Chung *et al.* (1989) also noted significant losses in lipid content during canola sprouting. The decrease in fat content of seed could be due to total solid loss during soaking prior to germination (Wang *et al.*, 1997) or use of fat as an energy source in sprouting process (T. A. El-Adawy, 2002).

Chaudhary *et al.* (2015) reported the significant increase in crude fibre content by germination in all of three formulations. Chung *et al.* (1989) reported that in barley (but not in canola) sprouting was associated with significant increase in crude fiber from 3.75% in unsprouted barley to 6% in 5 days sprouts due to synthesis of structural carbohydrates such as cellulose and hemicellulose, a major constituent of cell walls. In a similar study conducted on soybean, Jimenez *et al.* (1985) also noted an increase in the fiber and protein content of soybean seeds during sprouting.

2.7.2.2 Chnages in antinutrients content during germination

Khandelwal *et al.* (2010) showed that there was 45% reduction in tannin after soaking for 24 hours followed by germination for 96 hours in soybean. Ramadan (2012) showed that phytic acid in soybean was reduced by 46% soaked for 24 hours and 4 days germination. Kayembe (2011) showed that there was 44% reduction in tannin after 4 days germination in soybean. According to Egli, phytic acid was reduced by 45% after 96 hour of germination in soybean (Egli *et al.*, 2002). Similarly, Chitra *et al.* (1996) showed that phytic acid was reduced by 47% in soybean by germination. Megat Rusydi and Azrina (2012) showed 46% reduction in tannin in soybean by germination. Several legumes are known to contain phytase enzyme and its activity varies widely (Hussain *et al.*, 2011). In a study 13 different legumes and the authors found the rate of phytic acid hydrolysed ranging from 20-16 to 77-44% after germination for 5 days (Reddy *et al.*, 1982). Yasmin *et al.* (2008) reported the reduction of tannin and phytic acid from 6.1 mg/g to 1.9 mg/g and by 43 % respectively in 96 h of germination in red kidney bean. (Ghavidel and Prakash, 2007) reported reduction in phytic acid by 18-21 % in 5 types of legumes by germination. Mittal *et al.* (2012) reported the 58.97 % reduction of oxalate content in chickpea with germiantion. Handa *et al.* (2017) reported the 36.75 % reduction of oxalate content in horsegram by 24 h of germination. Oloyo (2004) reported the loss of total oxalate from 15.4 g/100 g to 2.06 g/100 g with 5 days of germination in pigeon pea. In pearl millet, Suma and Urooj (2014) found the 23.6 % reduction in oxalate content with 72 h germination. In legumes, complete hydrolysis of phytate did not take place during 5 days of germination LU *et al.* (1987) have demonstrated that considerable variation on the levels of phytase activity during germination exists among different cultivars of the same species. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. Similar losses of phytic acid during soaking and germination have been reported, Grewal and Jood (2006) reported that soaking caused a 42.82- 48.91% reduction in phytic acid content in mung bean, Rehman and Shah (2005) who stated that tannin content of black grams, red kidney bean and white kidney bean significantly reduced Rakić *et al.* (2007). Reductions in tannins contents were observed to various extents depending upon the germination conditions. Maximum (396 mg/100 g) reductions for tannins observed for 60 hour and 33.5ºC and minimum (522 mg/100 g) for 60 hour and 38.44ºC were obtained by the germination in mungbean seed (Hussain *et al.*, 2011).

2.7.2.3 Chnages in ascorbic acid content during germination

During sprouting (germination) several enzyme systems become active and bring about profound changes in the nutritive value of pulses. Khattak *et al.* (2007) reported a significant increase in ascorbic acid during germination from 5.7 mg to 17.5 mg per 100 g of sample in 96 h in chickpea. Ahmad and Pathak (2000) reported the 91.3 % increase in ascorbic acid content in 3 days of germination in soybean. Sangronis and Machado (2007) also showed the significant increase in ascorbic acid content of white beans by 300 % during 5 days of germination. Khyade and Jagtap (2016) reported the significant increase in ascorbic acid content of all four different types of pulses with 48 h of germination. Fernandez and Berry (1988) also reported a significant increase in ascorbic acid during germination. Riddoch *et al.* (1998) reported that many species of pulses produced significant quantities of vitamin C up to five days following germination in both light and dark although cooking caused a marked loss of ascorbate. Yang *et al.* (2001) monitored the changes in the concentration of vitamins C and E, beta-carotene, ferulic acid and vanillic acid in wheat seed over the germination period. Similarly Shah *et al.* (2011) reported that germination or malting increases the vitamin C and folic acid content of food legumes and also degrades the anti-nutrients present in these food grains. Significant increase in the content of ascorbic acid of different cereals and legumes seeds has also been reported by (Harmuth-Hoene *et al.*, 1987).

2.7.2.4 Changes in TPC during germination

The bioactive compounds are substances presents on foods and act as metabolic modulators capable of inhibiting the onset of degenerative diseases (Vizzoto *et al.*, 2010). According to (Abderrahim *et al.*, 2012), the germination increases the quantity of phenolic compounds and antioxidant capacity in grains. In germination method, due to biochemical process, are formed bioactive compounds with antioxidant function, such as phenolic compounds and tocopherols (Žilić *et al.*, 2014).

Tajoddin *et al.* (2014) reported the gradual increase in polyphenol content during 96 h of germination in all the mung bean cultivars. Shastry and John (1991) reported a progressive increase in polyphenol content in germinating *Dolichos lablab* beyond 48 h of germination. Barroga *et al.* (1985) reported an increase in polyphenol content of mung bean sprouts upon continued germination up to 120 h. Gujral *et al.* (2011) reported the increase in total phenolic content by 20.12 % and 48.1 % in 12 h and 24 h of germination respectively in moth. López-

Martínez *et al.* (2017) reported the increase in TPC from 0.75 to 1.1 g/kg and 0.8 to 1.82 g/kg upon germination at 30ºC and 40ºC, respectively, depending on the pulses. Xu *et al.* (2009) found an increase of phenolic compounds in oatmeal in different germination times (12, 24, 36 and 48 hours), as (Troszyńska *et al.*, 2006) when was evaluated the mung beans. This fact could be justified because the germination releases the phenolic compounds that were connected and then increases the quantity of total phenolic (Kaukovirta-Norja *et al.*, 2004). Gharachorloo *et al.* (2013) also reported the increase in average TPC (methanol extract) from 37.4 to 65.51 mg/kg with 120 h of germination in four different species of pulses.

2.7.2.5 Changes in antioxidant activity during germination

Khyade and Jagtap (2016) reported the increase in DPPH radical scavenging activity of black gram, chickpea, cowpea and yellow mustard by 9.5%, 11.23%, 8.33% and 14.81% from raw value with 48 hours of germination. Mekky *et al.* (2020) found the noticeable increase in DPPH radical scavenging activity of sprouts of various cultivar of fava beans than raw seeds and Okumura *et al.* (2016) also showed the same type of results. Gujral *et al.* (2011) reported the increase in DPPH radical scavenging activity by 18.75% and 32.15% with 12 h and 24 h of germination in moth. Fouad and Rehab (2015) reported the higher DPPH radical scavenging activity in germinated lentils (49.26-62.19%) than raw lentil seeds (40.76%).

Germination enhances the antioxidant capacity of the soluble extracts in germinated edible seeds and sprouts when compared with raw seeds. This effect could be attributed to the increase of certain antioxidant compounds such as antioxidant vitamins and phenolics (Gan *et al.*, 2017).

2.7.3 Physical changes during malting

During steeping, the grains swell and increase its volume by about a quarter. Space is allowed in the steep tanks to accommodate the swollen grain. The first microscopic indication of germination after casting is the appearance of the ‘chit’. The white coleorhizae or root-sheath that breaks through the pericarp and testa and produce from the base of the corn. In time seminal roots also called rootlets, culms, cooms, or malt sprouts bursts, through root sheath and form a tough at end of the grain, at the same time the first ‘leaf- seat’ or coleoptiles. Variously called by maltsters the ‘acrospires’, ‘spire’, ‘blade’, penetrates the

apex between pericarp and the husk. In conventional malting practice, the malt is kilned and growth terminated before the acrospires grows beyond the end of the grain (J. Hough and James, 1991).

Starch appears in small amounts in the embryonic structures after the onset of germination. Coincident with the appearance of this starch the first sign of the breakdown of the starchy endosperm are seen as an enzyme's partial dissolution of some cell walls. This process, cytolysis, begins in the compressed layer, adjacent to the scutellum and progressively spreads through the starchy endosperm towards the apex of the grains (Acharya and Karki, 2008).

As these hydrolytic breakdown processes precede alterations may be detected in protoplasm of cells of the aleuronic layer of columnar cells between the compressed cells endosperm and the scutellum. The products of the hydrolysis of endosperm are absorbed into the scutellum. The products of the hydrolysis of endosperm are absorbed into the scutellum epithelium and are transported through the scutellum into the embryo to provide necessary nutrients. As germination, proceeds the cells of epithelium tend to separate and elongate so forming a 'pile' which projects into the solubilized part of the endosperm. This alteration in similar form greatly increases the surface area of the cells and makes the epithelium a more efficient absorptive organ (J. Hough and James, 1991).

The softening of endosperm that occurs during malting is easily and conveniently detected by 'rubbing out' the green malt by hand. Chewing grains to see that they are 'crunchy' and devoid of hard tips may check the degree of modification of finished malt (J. Hough and James, 1991).

2.7.4 Effect of germination time on amylase activity of malt

The synthesis and activity of amylase enzymes goes simultaneously during germination and the synthesis is directly related to the moisture content, temperature and rate of respiration of germinating seeds (Getachew and Yoshihiko, 2001). At the very first stage, active gibberellin biosynthesis commences in the embryo, the level of active GAs is increased in the shoot and scutellar regions during the early stage of seed germination (Lenton *et al.*, 1994), and the GAs are transported from the embryo to the aleurone layer (Kaneko *et al.*, 2002). The α-amylase is synthesized *de novo* in the aleurone and scutellar epithelial cells

and subsequently secreted from the aleurone layer into the endosperm. The active breakdown of starch by α-amylase is initiated and the overall effect is the reduction of water holding capacity of starch matrix (Shafqat, 2013). During hydrolytic mobilization of endosperm, the metabolic activity is also increased in order to make the grain energy sufficient for the synthesis of new proteins and carbohydrate that are utilized in assembling the new botanical parts of the sprout for the self-sufficiency for photosynthesis.

Koshiba and Minamikawa (1983) reported that amylase and invertase activities were found to be increased tremendously at 24 hours and then declined gradually in *Vigna mungo* seeds. In the case of mung beans, it has been reported that highest amylase activity was found at 24 hour, while the amylase activity was decreased drastically from 48 to 96 hours due to germination. The findings indicated that amylase activity increased tremendously i.e. from 200 to 220 % at 24 hours of germination and thereafter decreased. The maximum decrease was 50 to 55 % at 96 hours of germination (Rahman *et al.*, 2007). Uriyo (2001) has also reported same results in the case of cowpea. He found that germination had a highly significant effect ($P<0.05$) on cowpea α-amylase activity. α-Amylase levels increased from 85.6 to 720.9 μ moles maltose/ ml of extract at 0 and 72 h germination time, respectively.

2.7.5 Enzyme activation during sprouting

In germination seed, enzymes may arise from two sources:

- They may be released or activated from existing proteins or

- They may be synthesized through nucleic acid-directed protein synthesis.

Enzymes of the particular interest are starch degrading enzymes, α- amylase and β-amylase. Germination leads to the production of both enzymes, with α – amylase is formed and the quantity of β-amylase increases; the β-amylases predominating in the final malt. Generally, the temperate climate cereals, mung beans, wheat and rye produce on germination high diastatic power. The hot climate cereals, sorghum, maize, millets produce much lower diastatic powers. The diastatic power developed during malting depends on following (Dahal, 2018).

- Temperature of malting;

- The moisture level;

- The % of germination, germination vigour;

- Duration of malting; and

- The cultivar employed.

β-amylase, which normally represents the minor portion of the total amylolytic activity, is present in the unfermented seed in an inactive form probably attacked in wheat at least by disulphide linkages to gluten in. Its activation is due to its release from the gluten in with the formation of active SH groups. The mediator of this reaction is unknown. Low temperature kilning schedule produce malts with greater α- amylase activity. Germination with the high watering regime gave the highest level of total β-amylase activity. They also reported that α-amylase enzyme is inactivated at the high-water regime (Dewar *et al.*, 1997).

Materials and methods

3.1 Materials

3.1.1 Raw materials

For the selection of pulses to formulate a recipe for *kwati* a structured questionnaire (Annex C) was used. Households of Dharan-14, Bijaypur (Narayanpur) were questioned to find out about kwati, no of pulses used, types of pulses used in *kwati* etc.

All the pulses were collected from local market of Dharan, Nepal. Following pulses were used to formulate the *kwati*;

- Chickpea (*Chana*)
- Balck gram (*Kalo daal*)
- Green gram (*Gota mung*)
- Red kidney beans (*Rajma*)
- Black eyed beans (*Maji bodi*)
- Soybean (*Vatmas*)
- Rice beans (*Masyang*)
- Dry white peas (*Seto matar*)
- Lentil (*Musuri*)
- Horse gram (*Gahat*)

3.1.2 Equipment and chemicals

The following equipment and chemicals used were available in Central Campus of Technology (CCT). The list of chemicals and equipment used for the analysis is shown in Table 3.1 and Table 3.2 respectively.

Table 3.1 List of chemicals used

Chemical	Supplier/Manufacturer	Other Specifications
Sodium hydroxide (NaOH)	Thermo Fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCL)	Thermo Fisher Scientific India Pvt. Ltd.	36%, LR grade

Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck (India) Limited	Amorphous
Oxalic acid	Merck (India) Limited	Crystal
DiNitroSalicylic Acid	Himedia Laboratories Pvt. Ltd.	98%, LR grade
Maltose	Merck life Pvt. Ltd	95% assay
Dextrose	Merck (India) Limited	Amorphous
Sodium Carbonate (Na_2CO_3)	Qualigens fine chemicals	99.5%, LR grade
Petroleum benzene	Merck life Pvt. Ltd.	B.P. 60ºC-80ºC
Sodium Tungstate	BDH chemicals ltd.	96% Assay
Phosphoric acid	Loba chemicals	56-60%
Phenolphthalein indicator	Merck life pvt. Ltd	pH 8.2-9.8
Potassium sodium tartarate	Merck life pvt. Ltd.	99% Assay
Sodium sulphite	Thermo Fisher Scientific India Pvt. Ltd.	99%, Fused flakes
Dil. Ammonia	Fisher Scientific	25% NH_3
Catechin		
Methanol		
Potassium Permanganate	Avantor Performance Materials ltd.	99% Assay
Potassium thiocyanate	Thermo Fisher Scientific India Pvt. Ltd.	97% Assay
Metaphosphoric acid	S.D. fine chemicals Ltd.	66% HPO_3,40% $NaPO_3$
L-ascorbic acid	S.D. fine chemicals Ltd.	99% Assay
Tannic acid	Avarice Laboratories Pvt. Ltd.	Analytical Reagent
Potassium Persulfate	GlaxoSmithKline Pharmaceuticals Ltd.	98% Assay
Sodium acetate	Qualigens fine chemicals	99-102% Assay
Ammonium oxalate	Qualigens fine chemicals	99% Assay

Nitric acid	Fisher Scientific India Pvt. Ltd.	68-75% Assay
Sulphuric acid	Fisher Scientific India Pvt. Ltd.	97% Assay
DPPH	HiMedia Laboratories Pvt. Ltd.	
Hydrochloric acid	Fisher Scientific India Pvt. Ltd.	35-37% Assay

Table 3.2 List of equipment used

Physical apparatus	Specifications
Electric balance	Phoneix instruments, India
Spectrophotometer	Labtronics, India
Soxhlet apparatus	Y.P. scientific glass work
Hot air oven	Victolab, India
Incubator	Y.P. scientific glass work
Muffle furnace	Accumax, India
Cabinet dryer	AIset YDL-2000
Colorimeter	Jenway Ltd., UK
Centrifuge	Y.P. scientific glass work
Heating mantle	Y.P. scientific glass work

3.2 Method

3.2.1 Procedure for malting *kwati*

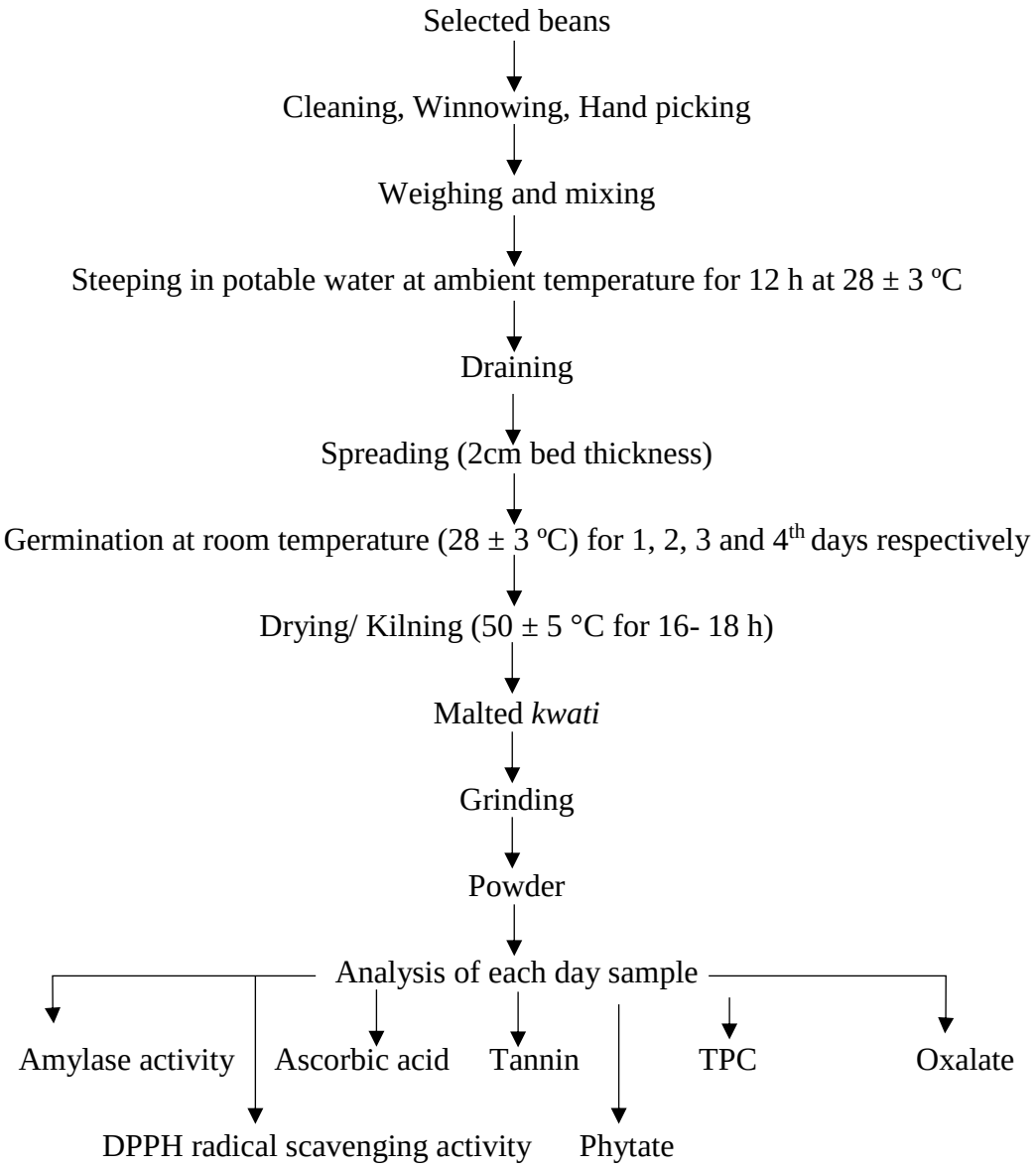

Fig. 3.1 Flow diagram of methodology

3.2.2 Cleaning

The naked bean samples were first cleaned screening to remove impurities such as stones, strings, weed seeds, broken corn etc. and then by winnowing with *nanglo* (flat round woven bamboo tray) to remove dusts, husk, immature grains and other light particles. Then shifting, hand picking.

3.2.3 Steeping

Cleaned seeds were transferred to the plastic containers and water was added 3 times that of beans. The grain was steeped for 12 h at room temperature (28 ± 3ºC) and drained to remove the excess water.

3.2.4 Germination

The steeped pulses were first collected in a muslin cloth and swirled in order to drain excess water and then kept for germination at ambient temperature of average 28ºC. The drying out of grains is prevented by moistening muslin cloth and spraying the potable water at the interval of every 24 h. The pulses bed was turned and mixed from time to time to aerate the mass and to equalize the temperature and moisture during germination. The first test sample was taken after 24 h of germination. After that other samples were taken at an interval of 24 h for upto 96 h to determine α-amylase activity, DPPH radical scavenging activity, phytate, tannin, oxalate, phenols and ascorbic acid.

3.2.5 Drying/kilning

Different samples of germinating *kwati* were taken and were dried to stop further germination. Drying was carried out in a cabinet drier at 50 ± 5°C for 16-18 h until the constant weight was obtained.

After drying, the prepared malt was packed in airtight containers.

3.3 Experimental procedure

3.3.1 Chemical analysis

3.3.1.1 Proximate analysis

3.3.1.1.1 Determination of moisture content

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

3.3.1.1.2 Determination of crude protein

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

$$\text{Nitrogen \%} = \frac{(\text{sample titre-black titre}) \times \text{Normality of HCl} \times 14 \times 100}{\text{Wt of sample} \times 100}$$

3.3.1.1.3 Determination of ash content

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

3.3.1.1.4 Determination of crude fat

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

$$\text{\% crude fat} = \frac{\text{Wt of ether soluble materials} \times 100}{\text{Wt of sample}}$$

3.3.1.1.5 Determination of crude fiber

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

$$\% \text{ Crude fiber} = \frac{(\text{loss in wt noted})}{\text{Wt of sample taken}} \times 100$$

3.3.1.1.6 Determination of carbohydrate

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

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

3.3.1.2 Ultimate analysis

3.3.1.2.1 Determination of ascorbic acid

The dichlorophenol dye, which was blue in alkaline solution and red in acid solution, was reduced by ascorbic acid to a colourless form. Result was presented in mg of ascorbic acid per 100mg (Ranganna, 1986).

$$\text{VItamin C (mg per 100g)} = \frac{\text{Titer} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract taken(ml)} \times \text{wt ofsample(g)}}$$

3.3.1.2.2 Determination of iron

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

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

3.3.1.2.3 Determination of calcium

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

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

3.3.1.3 Determination of antinutritional factors

3.3.1.3.1 Determination of phytic acid

The phytate was extracted with trichloroacetic acid and was precipitated as ferric salt. The iron content of the precipitate was determined colorimetrically and phytate phosphorus content was calculated from that value assuming a constant 4Fe:6P molecular ratio in the precipitate (Sadasivam and Manickam, 2016). Result was presented as phytate mg per 100 g sample.

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

3.3.1.3.2 Determination of tannin

The tannins were determined by Folin-Dennis method. Tannin like compounds reduce phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of that was proportional to the amount of tannins. The intensity was measured in a spectrophotometer at 700nm (Sadasivam and Manickam, 2016).

3.3.1.3.3 Determination of oxalate

Oxalate was determined as modified version of (AOAC, 2005). 5g sample was taken which was treated with tungstophosphoric acid and ammonium hydroxide. The precipitate thus obtained was then treated with sulphuric acid and titrated with standard potassium permanganate and until the pink color persisted for 30 seconds.

The calculation was done as:

$$1\text{ mL of }0.002\text{ M }KMnO_4 = 0.45\text{ mg anhydrous oxalic acid}$$

3.3.1.4 Determination of phytochemical components

3.3.1.4.1 Determination of total phenolic content

Phenols reacts with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produces blue colored complex (molybdenum blue). Extraction of sample was done in ethanol. Standard curve of catechol was plotted and concentration of phenol in test sample was expressed as mg CE per 100 g material (Sadasivam and Manickam, 2016).

3.3.1.4.2 Determination of DPPH radical scavenging activity

0.1 mL of extract was taken and volume was made up to 50 mL by 50 % methanol and after that 3 mL was taken and 1 mL of DPPH was added in it and allowed to stand for 30 min and reading was noted spectrophotometrically at 517 nm (Arab *et al.*, 2011).

$$\text{DPPH radical scavenging activity} = 1\text{-} \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100\%$$

3.3.1.5 Determination of enzyme activity

Extraction of α-amylase

The malt sample was ground with mortar and pestle and exactly one g of powdered sample was taken and diluted 100 times. The diluted sample was then filtered with ordinary filter paper. For alpha amylase extraction, 10 ml of filtered diluted sample was taken in the 50 ml conical flask, 20 mM $CaCl_2$ was added to bind the α-amylase and incubated for 10 min at 65°C (Sadasivam and Manickam, 2016).

Analysis of α-amylase

One ml of enzyme extract was mixed with 1 ml (50 mg/ml) starch and 1 ml of distilled water in test tube. It was incubated at room temperature for 10 min and the hydrolysis was stopped by heating on Bunsen flame up to boiling and holding in boiling water bath for five minutes. The same process was done for control but the enzyme was inactivated before starch addition. The reducing sugar obtained by the hydrolysis was analyzed by DNS method as described by (Sadasivam and Manickam, 2016) and Miller (Miller, 1959). Amylase activity was expressed in terms of mg of maltose produced per g dry malt per minute of incubation time.

3.3.2 Statistical analysis

Analysis were carried out in triplicate. Data on analysis of ascorbic acid, TPC, amylase activity, DPPH radical scavenging activity, tannin, oxalate and phytic acid were tabulated for comparison and were graphically represented using Microsoft excel-2016. Data were statistically processed by Gene stat version 12.1.0.3338 for analysis of variance (ANOVA).

Means of the data were compared by using Fisher's Unprotected LSD method at 5% level of significance.

Part IV

Result and discussion

The selected pulses were collected from local market of Dharan, Sunsari. The pulses were first cleaned separately and then equal amount of each pulses were weighted and mixed together. The mixed pulses (*kwati*) were steeped for 12 h and then germinated at 28 ± 3°C for 24 h, 48 h, 72h and 96 h. Thus, germinated samples were dried in cabinet dryer for desired moisture content value less than 10 %. These sample were used for further quantitative analysis of ascorbic acid, tannin, phytate, oxalate, TPC, DPPH scavenging activity and amylase activity.

4.1 Proximate composition

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

Table 4.1 The proximate composition of *kwati* (raw)

Parameters	**Values (%) DB**
Crude protein	26.9 ± 0.67
Crude fat	4.8 ± 0.08
Crude fiber	4.58 ± 0.22
Ash content	3.49 ± 0.11
Carbohydrates	60.22 ± 0.68

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

Table 4.1 shows the proximate composition of *kwati* according to which the protein, fat, crude fiber, ash and carbohydrate content of the *kwati* were 26.9, 4.8, 4.58, 3.49 and 60.22% respectively on dry weight basis.

The result was accordance with (Chaudhary *et al.*, 2015), who showed the protein, fat, crude fiber, ash and carbohydrate content of his three formulations ranges from 21.6-27.6%, 6.3-9.4%, 3.1-4.6%, 2.0-3.5% and 59.2-60.8% respectively.

4.2 Mineral composition of *kwati*

Table 4.2 Mineral composition of *kwati* (raw)

Minerals	**Values (mg/100g) DB**
Iron	9.6 ± 0.48
Calcium	123.8 ± 3.64

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

Table 4.2 shows the mineral composition of *kwati* according to which iron and calcium content of the *kwati* are 9.6 and 123.8 mg per 100g dry basis respectively on dry weight basis.

Mineral content of pulses may vary with genus, species, growing conditions and many factors. Our result might be in accordance with (Suneja *et al.*, 2011), who reported the calcium and iron content in black gram as 286 mg/100g and 45.67 mg/100g respectively. Khan *et al.* (1995) also reported the calcium and iron content in chickpea as 80.4 mg/100g and 4.7 mg/100g respectively.

4.3 Effect of germination in ascorbic acid content

During Sprouting (germination) several enzyme systems become active and bring about profound changes in the nutritive value of pulses. Vitamin C, which is present in lesser amount in dry legume seeds, increases in significant amounts after sprouting. The *kwati* was germinated for four days. The change in ascorbic acid content was analyzed in each day of germinated sample as well as in raw sample. The increment in ascorbic acid content is demonstrated in Fig. 4.1.

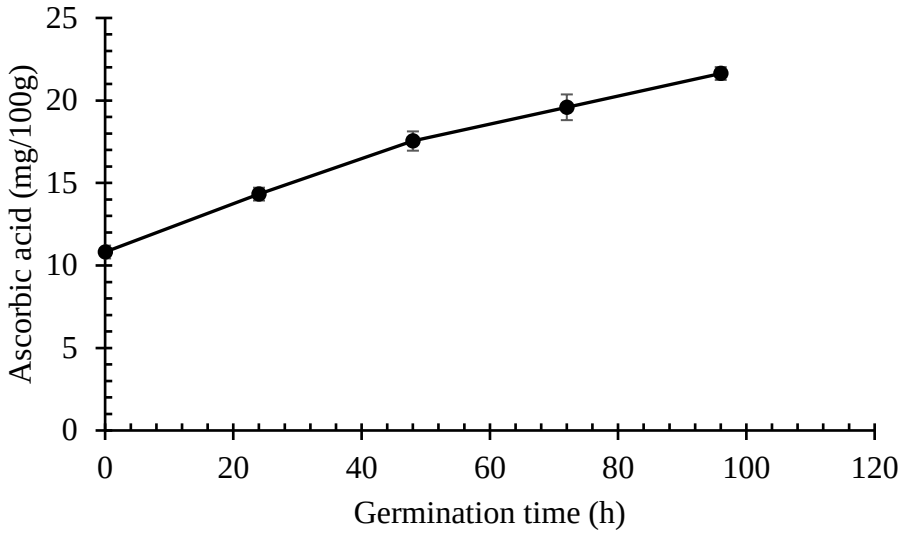

Fig. 4.1 Effect of germination on ascorbic acid content of *kwati*

The mean value of ascorbic acid content in raw *kwati* was found to be 10.81 ± 0.39 mg/100 g on the basis of dry matter. Ascorbic acid content was increased on the progressive days of germination. The mean value of progressive days of germination was 14.32 ± 0.39 mg/100 g, 17.54 ± 0.29 mg/100 g, 19.58 ± 0.78 mg/100 g, and 21.63 ± 0.39 mg/100 g on the basis of dry matter on 1st, 2nd, 3rd and 4th days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between ascorbic acid content in the different days of germinated samples ($p < 0.05$).

Results showed substantial increase in the concentration of ascorbic acid in the seeds processed by germination and are in agreement with the earlier studies. Our results are well in agreement with those of (Riddoch *et al.,* 1998) who reported that many species of pulses produced significant quantities of vitamin C up to five days following germination in both light and dark although cooking caused a marked loss of ascorbate. (Khyade and Jagtap, 2016) also reported the significant increase in ascorbic acid of all four different types of pulses with 48 h of germination. Similarly (Shah *et al.*, 2011) reported that germination or malting increases the vitamin C content of food legumes and also degrades the anti-nutrients present in these food grains. Significant increase in the content of ascorbic acid of different cereals and legumes seeds has also been reported by (Harmuth-Hoene *et al.*, 1987). (Thippeswamy *et al.*, 2015) has also found similar types of result. (Fernandez and Berry, 1988) also reported a significant increase in ascorbic acid during germination.

4.4 Effect of germination in total phenolic content

Phenolic compounds are widely distributed in the plant kingdom. These compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules (Shahidi *et al.*, 2006). The *kwati* was germinated for four days. The change in total phenolic content was analyzed in each day of germinated sample as well as in raw sample. The increment in total phenolic content is demonstrated in Fig. 4.2.

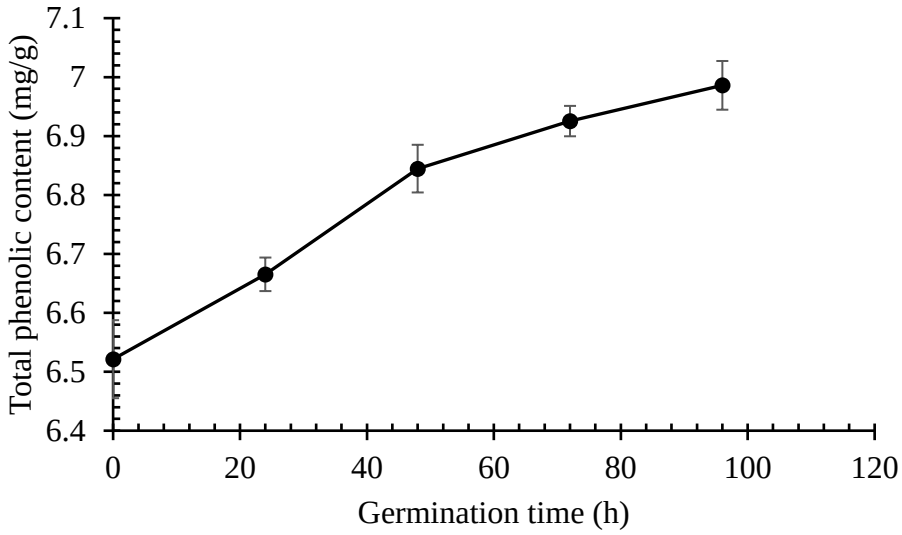

Fig. 4.2 Effect of germination on total phenolic content of *kwati*

The mean value of raw *kwati* was observed 6.52 ± 0.06 mg CE/g of methanolic extract on the basis of dry matter. The mean value of progressive days of germination was 6.66 ± 0.02 mg CE/g, 6.84 ± 0.04 mg CE/g, 6.92 ± 0.02 mg CE/g and 6.98 ± 0.04 mg CE /g on the basis of dry matter on 1st, 2nd, 3rd and 4th days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between total phenol content in the first two days of germinated samples ($p < 0.05$) while there was no significant difference between second, third and fourth germination days.

Our results showed substantial increase in the total phenolic content in the seeds processed by germination and are in agreement with the earlier studies. Our results are well in agreement with those of (López-Martínez *et al.*, 2017), who reported the increase of TPC of different pulses from 0.75-1.1 g/kg to 0.8-1.82 g/kg at 30°C and 40°C depending upon

the pulses. Tajoddin *et al.* (2014) reported the gradual increase in polyphenol content during 96 h of germination in all the mung beans cultivars. (Barroga *et al.*, 1985) also showed an increase in polyphenol content of mung bean sprouts upon continued germination up to 120 h. Xu *et al.* (2009) reported an increase in phenolic compounds in oatmeal in different germination time. The influence of germination on total phenolic contents has been investigated in many edible seeds, such as edible beans and cereal grains. Most studies found that germination can gradually accumulate soluble phenolics in germinated edible seeds and sprouts compared with raw seeds (López-Martínez *et al.*, 2017).

4.5 Effect of germination on DPPH radical scavenging activity

The DPPH radical is a stable organic free radical with an adsorption peak at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. The *kwati* was germinated for four days. The change in DPPH radical scavenging activity was analyzed in each day of germinated sample as well as in raw sample. The increment in DPPH radical scavenging activity is demonstrated in Fig. 4.3.

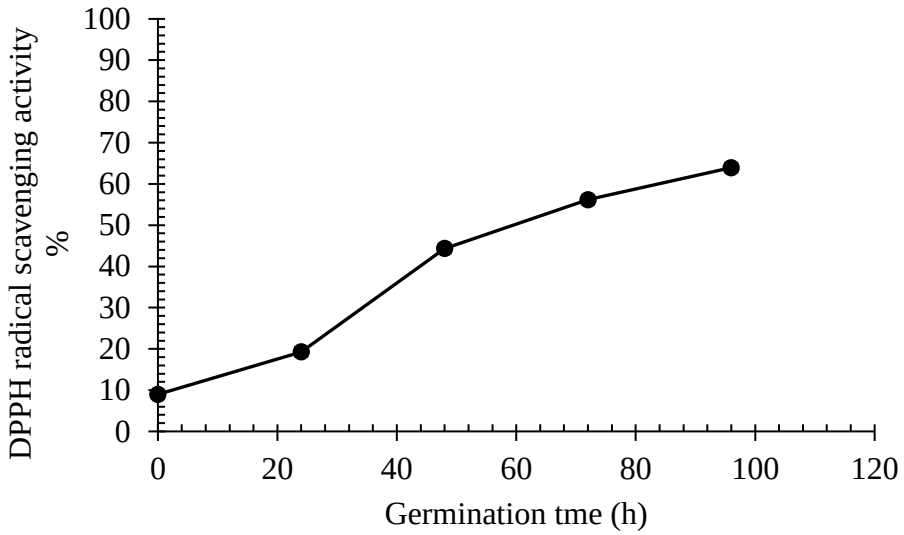

Fig. 4.3 Effect of germination on DPPH radical scavenging activity of *kwati*

The mean value of DPPH scavenging activity in raw *kwati* was observed 8.98 ± 0.19 % on the basis of dry matter. The mean value of progressive days of germination was 14.32 ± 0.39 %, 44.34 ± 0.29 %, 56.15 ± 0.54 % and 63.94 ± 0.63 % on 1st, 2nd, 3rd and 4th days of

germination respectively. The analysis of variance (Appendix B), showed that significance difference ($p < 0.05$) in DPPH scavenging activity in each day of germinated samples. DPPH scavenging activity was directly proportional to number of days of germination.

The result was accordance to (Khyade and Jagtap, 2016), the value of DPPH radical scavenging activity of black gram, chickpea, cowpea and yellow mustard were increased by 9.5%, 11.23%, 8.33% and 14.81% from raw value with 48 hours of germination. Alvarez-Jubete *et al.* (2010) reported the increase in antioxidant activity is due to many metabolic changes during germination such as increase in the activity of the endogenous hydrolytic enzymes during germination.

4.6 Effect of germination on amylase activity of malt

The *kwati* was germinated for four days. The change in amylase activity was analyzed in each day of germinated sample as well as in raw sample. The change in amylase activity is demonstrated in Fig. 4.4.

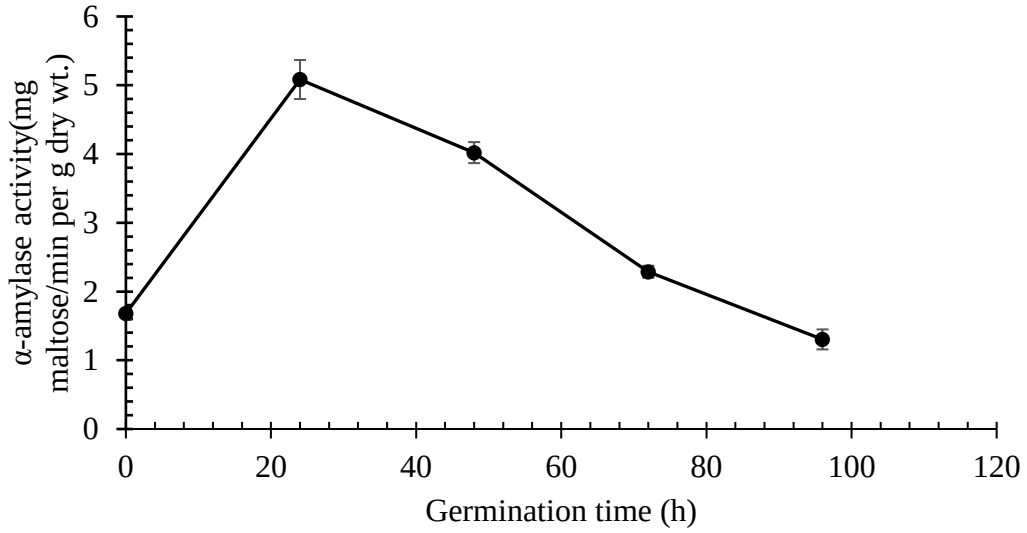

Fig. 4.4 Effect of germination on amylase activity of *kwati*

The changes of amylase activity of the *kwati* under different germination days are presented in the Fig. 4.4. Amylase activity of raw *kwati* was found to be 1.68 ± 0.09 mg maltose/min per g dry wt. During the different germination period, the highest amylase activity was found at 24 hours germination (5.08 ± 0.28 mg maltose/ min per g dry wt.), while the amylase activity was decreased drastically from 48 to 96 hour due to germination.

The mean values of amylase activity for *kwati* at successive germination period of 24 h, 48 h, 72 h and 96 h were found to be 5.08 ± 0.28, 4.02 ± 0.15, 2.28 ± 0.09 and 1.3 ± 0.14 mg maltose/ min per g dry wt. dry beans respectively. Statistical analysis showed that germination time had a significant effect ($p<0.05$) on amylase activity as shown in (Appendices B). The present findings indicated that amylase activity increased tremendously i.e. 202.3 % at 24 hours of germination and thereafter decreased to 1.3 mg maltose/ min per g dry wt. at 96 hours. The maximum decrease was 22.6 % at 96 hours of germination.

Similar result was found by (Koshiba and Minamikawa, 1983), amylase and invertase activities were found to be increased tremendously at 24 hours and then declined gradually in *Vigna mungo* seeds. Also similar result was found by (Rahman *et al.*, 2007), they indicated that amylase activity increased drastically i.e. from 200 to 220 % at 24 hours of germination and decreased suddenly. In case of mung bean, it has been reported that, appreciable increase in enzyme activity was observed (Ghavidel and Davoodi, 2011). (Uriyo, 2001) has also reported same results in the case of cowpea. He found that germination had a highly significant effect ($P<0.05$) on cowpea α-amylase activity. α-Amylase levels increased from 85.6 to 720.9 μ moles maltose/ ml of extract at 0 and 72 h germination time, respectively.

The increase in amylase activity during germination may be due to the presence of sufficient amount of moisture entrapped in the network of intact starch which would suffice to act as enzymatic reaction medium during germination. The synthesis and activity of amylase enzymes goes simultaneously during germination and the synthesis is directly related to the moisture content, temperature and rate of respiration of germinating seeds (Getachew and Yoshihiko, 2001). At the very first stage, active gibberellin biosynthesis commences in the embryo, the level of active GAs is increased in the shoot and scutellar regions during the early stage of seed germination (Lenton *et al.*, 1994), and the GAs are transported from the embryo to the aleurone layer (Kaneko *et al.*, 2002). The α-amylase is synthesized *de novo* in the aleurone and scutellar epithelial cells and subsequently secreted from the aleurone layer into the endosperm. The active breakdown of starch by α-amylase is initiated and the overall effect is the reduction of water holding capacity of starch matrix (Shafqat, 2013). During hydrolytic mobilization of endosperm, the metabolic activity is also increased in order to make the grain energy sufficient for the synthesis of new proteins and carbohydrate that are utilized in assembling the new botanical parts of the sprout for the self-sufficiency for photosynthesis.

Respiration involves the uptake of oxygen and release of carbon dioxide with the generation of energy and this respiration provokes the activation and hydration of existing enzyme (A. P. Smith, 2011). The respiratory heat energy produced in germinating seed may also have played role in increasing metabolic activities which in turn demands more energy and consequently results in higher α-amylase activity. Moreover, the synthesis and activity of amylase enzyme is proportional to the starch content (Haseltine *et al.*, 1996).

The slight decrease in α-amylase activity after 24 h and gradual decrease after 48h of germination may be due to the decrease in moisture content. As the water acts as the medium for various reactions, the decrease in water holding capacity of starch matrix as a result of enzymatic hydrolysis of endosperm and increased respiratory heat evolution in germinating seeds helps decreasing moisture content, which may have resulted in decrease in amylase activity. Furthermore, the amylase activity is directly related to the starch content (Haseltine *et al.*, 1996) but at this stage of germination, the starch content is decreased and the formed soluble sugar counterparts are rapidly utilized in synthesizing new plant parts along with respiratory loss of solutes and water. As the seedling emerges and begins to become photosynthetically active the respiration rate also starts to decline (A. P. Smith, 2011). This may have resulted in decrease in substrate concentration and reaction medium for amylase enzymes, which directly led to decrease in amylase activity.

4.7 Effect of germination on Antinutritional factors

4.7.1 Tannin

Tannin is a potential antinutrient present in pulses. The *kwati* was germinated for four days. The change in oxalate content was analyzed in each day of germinated sample as well as in raw sample. The reduction in tannin content is demonstrated in Fig. 4.5.

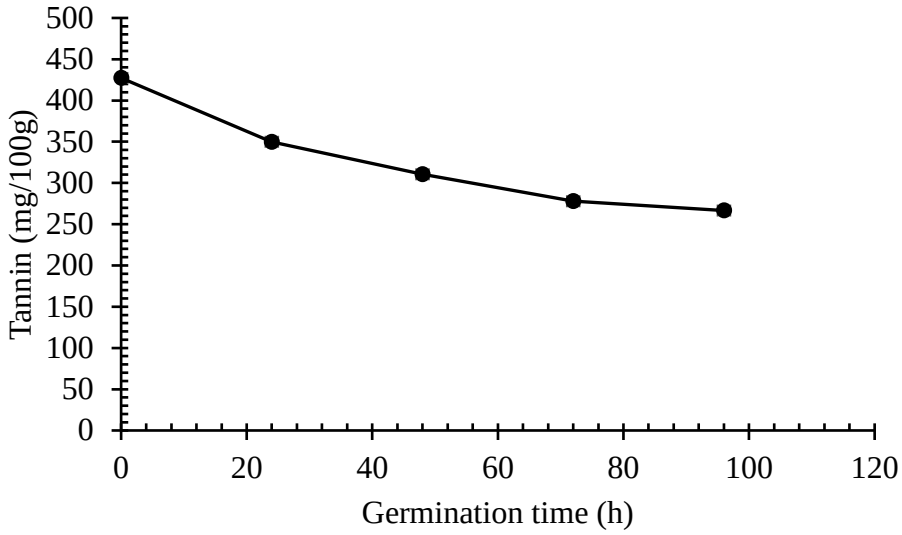

Fig. 4.5 Effect of germination on tannin content of *kwati*

The mean value of tannin content in raw *kwati* was found to be 427.26 ± 4.91 mg/100 g on the basis of dry matter. Tannin content was reduced on the progressive days of germination. The mean value of progressive days of germination was 349.93 ± 5.22 mg/100 g, 310.5 ± 3.93 mg/100 g, 277.8 ± 3.06 mg/100 g, and 266.56 ± 5.22 mg/100 g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd} and 4^{th} days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between tannin content in the different days of germinated samples ($p < 0.05$).

The results was in accordance with (Khandelwal *et al.*, 2010), 45 % decrease in tannin content after soaking for 24 h followed by germination for 98 h in soybean. Yasmin *et al.* (2008) also reported the loss of tannin content from 6.1 to 1.9 mg/g with 96 h of germination in red kidney bean. Similarly, Kayembe (2011) reported the 44 % reduction of tannin content in soybean by 96 h of germination. Rehman and Shah (2005) also reported the significant reduction in tannin by germination in the study of black gram, red kidney beans and white kidney beans.

4.7.2 Phytate

Phytate is an antinutritional factor present in pulses which reduces the bioavailability of nutrients from them. The *kwati* was germinated for four days. The change in phytate content

was analyzed in each day of germinated sample as well as in raw sample. The reduction in phytate content is demonstrated in Fig. 4.6.

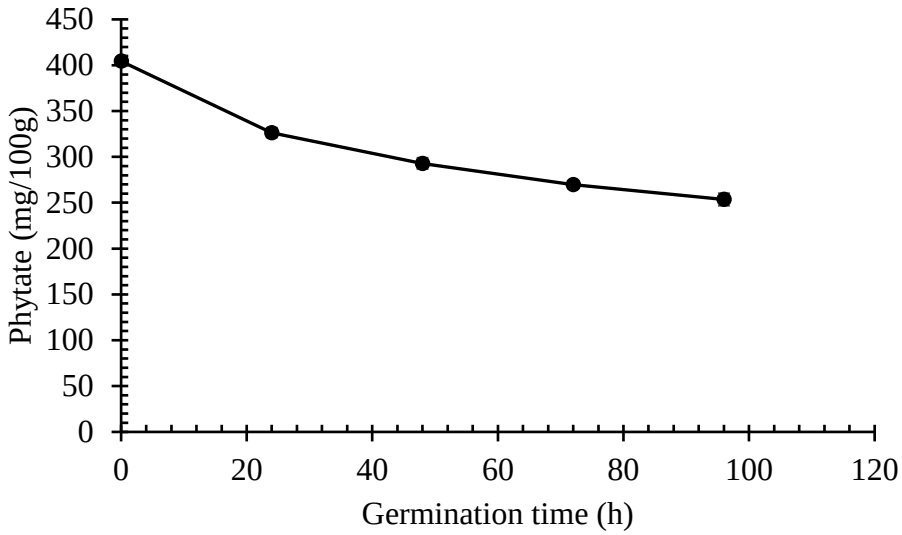

Fig. 4.6 Effect of germination on phytate content of *kwati*

The mean value of phytate content in raw *kwati* was found to be 404.36 ± 5.31 mg/100 g on the basis of dry matter. Phytate content was reduced on the progressive days of germination. The mean value of progressive days of germination was 326.46 ± 5.29 mg/100 g, 292.9 ± 6.06 mg/100 g, 269.73 ± 4.15 mg/100 g, and 253.56 ± 6.77 mg/100 g on the basis of dry matter on 1st, 2nd, 3rd and 4th days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between phytate content in the different days of germinated samples ($p < 0.05$).

Our results showed substantial decrease in the concentration of phytic acid in the seeds processed by germination and are in agreement with the earlier studies. Our results are well in agreement with those of (Ramadan, 2012), 46% reduction in phytate content after soaking for 24 h followed by 96 h of germination in soybean. Yasmin *et al.* (2008) also reported the reduction of phytate content by 43% in 96 h of germination in red kidney bean. Ghavidel and Prakash (2007) reported the reduction in phytic acid by 18-21% in 5 types of legumes by germination.

Phytic acid is water soluble and the reduction of the phytic acid during germination is attributed due to hydrolysis of the legumes by enzymes broadly designated as phytases

synthesized in germinating seedlings as reported for several plants. Plant seed utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability and nutritional value (Wang *et al.*, 1997).

4.7.3 Oxalate

Oxalate is a potential antinutrient present in pulses. The *kwati* was germinated for four days. The change in oxalate content was analyzed in each day of germinated sample as well as in raw sample. The reduction in oxalate content is demonstrated in Fig. 4.7.

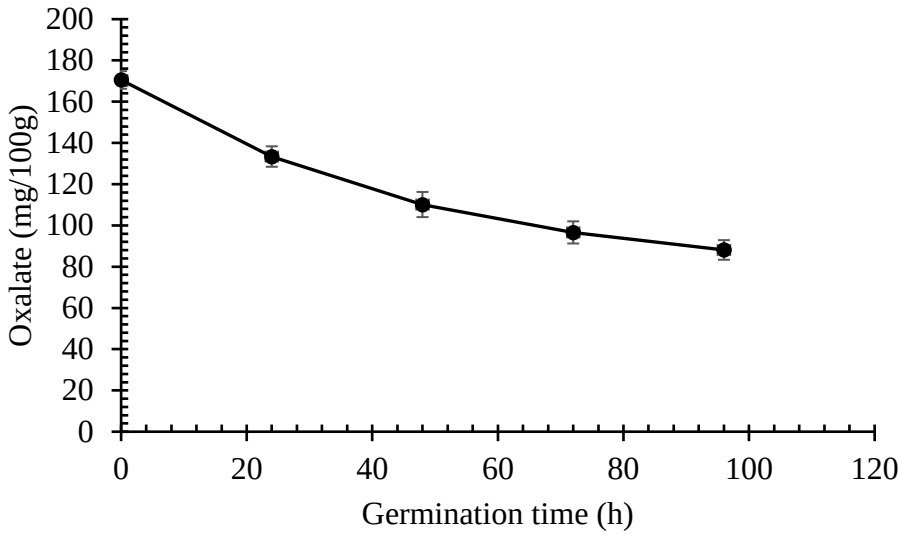

Fig. 4.7 Effect of germination on oxalate content of *kwati*

The mean value of oxalate content of raw *kwati* was observed 170.54 ± 4.3 mg/100 g on the basis of dry matter. Oxalate content was reduced as the days of germination was increased. The mean value of progressive days of germination was 133.4 ± 4.96 mg/100 g, 110.13 ± 6.1 mg/100 g, 96.6 ± 5.36 mg/100 g and 88.12 ± 4.76 mg/100 g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd} and 4^{th} days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between oxalate content in the first three days of germinated samples ($p < 0.05$) while there was no significant difference between third and fourth germination days.

Our results showed substantial decrease in the concentration of oxalate content in the seeds processed by germination and are in agreement with the earlier studies. Our results are well in agreement with those of (Oloyo, 2004), oxalate content decreased significantly ($p <$

0.05) from 15.4 to 2.06 g/100 g with 5 days of germination in pigeon pea. Mittal *et al.* (2012) reported the reduction of oxalate content by 58.97 % in chickpea with germination. Handa *et al.* (2017) also reported the 36.75 % reduction of oxalate content in horsegram by 24 h of germination time.

Decrease in oxalate during germination could be as a result of the activation of oxalate oxidase which breakdown oxalic acid into carbon dioxide and hydrogen peroxide and consequently releasing calcium (Pal *et al.*, 2016).

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of this study following conclusions were drawn.

- Nutritional composition i.e. moisture, crude protein, crude fat, ash, crude fibre and carbohydrate content of *kwati* was found to be 10.6 %, 26.9 %, 4.8 %, 3.49 %, 4.58 % and 60.22 % respectively while iron and calcium content were found to be 9.6 and 123.8 mg/100g respectively on dry basis.
- Tannin content was reduced by 18 %, 27.3 %, 34.9 % and 37.6 %respectively, phytate content was reduced by 19.2 %, 27.5 %, 33.3 % and 37.3 % respectively and oxalate content was reduced by 21.8 %, 35.3 %, 43.5 % and 48.5 % respectively.
- Alpha amylase activity was first increased till 5.08 mg maltose/ min per g dry wt. in 24 h and on further germination it was decreased gradually to 4.02, 2.28 and 1.3 mg maltose/ min per g dry wt. in 48, 76 and 96 hours.
- Ascorbic acid was increased to 14.32 mg, 17.54 mg, 19.58 mg in 24, 48 and 72 hours respectively and increased till 21.63 mg/100g in 96 hours.
- DPPH radical scavenging activity was increased to 19.27 %, 44.34 %, 56.15 % and 63.94 % respectively and TPC was increased by 2.2 %, 4.9 %, 6.1 % and 7 % respectively.

5.2 Recommendations

The following recommendation can be drawn from conclusion.

- Germination of mixed pulses (*kwati*) increases it's nutritional quality providing better food source for consumption on the daily basis.
- Formulation of different recipe for *kwati* along with its evaluation can be done via visiting different places and cultures.
- Research can be done by varying other factors like time, temperature, etc.
- Study on fatty acid profile and amino acid profile can be studied.

Part VI

Summary

Malting is the process comprising of steeping, germination and kilning. Steeping is one of the process that improve the nutritional value of the pulses by the breakdown of several complex components into simpler compounds which alter the texture and flavor. Germination is another process which improve the nutrition of the pulses as it helps in reducing starch component into simple sugars by the action of amylases, induces hydrolytic enzyme synthesis such as phytates and tannins. Tannins phytate and oxalate are known to reduce the availability of proteins, carbohydrates and minerals by forming indigestible complexes with the nutrients. The reduced tannin, phytate, oxalate and flavonoid levels due to germination could improve the availability of nutrients in the seed. The observed reduction in tannin content after germination might result from formation of hydrophobic association of tannins with seed proteins and enzymes. The last process is kilning which helps to stop germination of *kwati* and reduces the grain moisture content to desirable limit.

For this study the pulses were selected via visiting the local Dharan market and bought. The selected legumes were mixed in equal proportion and were soaked for 12 hours at room temperature, germinated at different time. After germination the mixed beans were dried at 50±5°C for 16-18 h to obtain the desired final moisture content. The prepared mixed beans malt samples were then taken for analysis. Analysis of chemical and functional properties were carried out for all samples.

Tannin content were found to be decreased from 427.26 to 349.93, 310.5, 277.8 and 266.56 mg tannic acid/100g on consecutive days of germination. The phytate content were found to be decreased from 404.36 to 326.46, 292.9, 269.73 and 253.56 mg/100g on consecutive days of germination. Oxalate content were found to be decreased from 170.54 to 133.4, 110.13, 96.6 and 88.12 mg/100g on consecutive days of germination. While amylase activity was found higher on 24h of germination and then gradually decrease. Amylase activity of raw *kwati* was found to be 1.68 units/g and 5.08, 4.02, 2.28 and 1.3 units/g for consecutive days of germination. Ascorbic acid content was found to be increased from 10.81 to 14.32, 17.54, 19.58 and 21.63 mg/100g on consecutive days of germination. DPPH radical scavenging activity was found to be increased from 8.98 % to 19.27 %, 44.34

%, 56.15 % and 63.94 % on consecutive days of germination. TPC was found to be increased from 6.52 to 6.66, 6.84, 6.92 and 6.98 mg CE/g on the consecutive days of germination. So, germination improve the nutritional quality of *kwati*.

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Appendices

Appendix A

1. Standard curve for tannin content

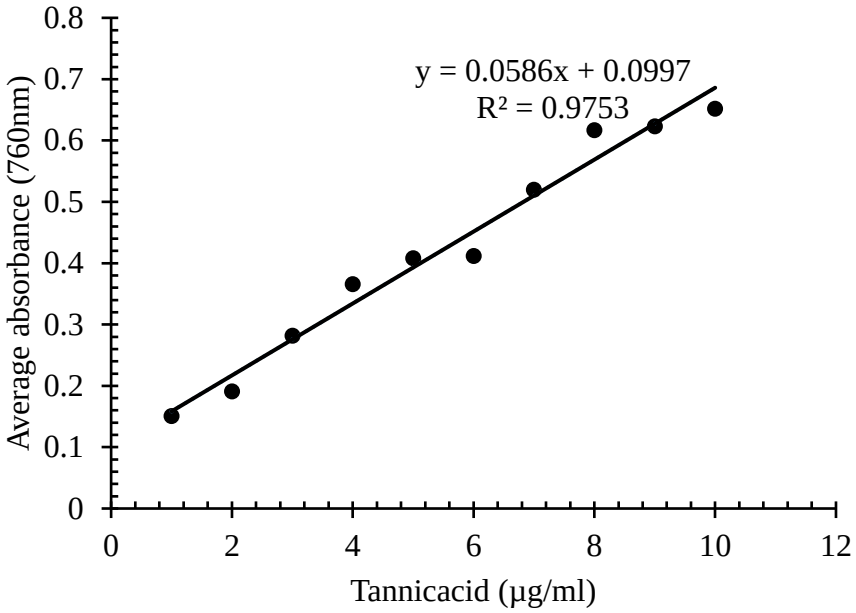

Fig. A.1 Standard curve for tannin content

2. Standard curve for phytate content

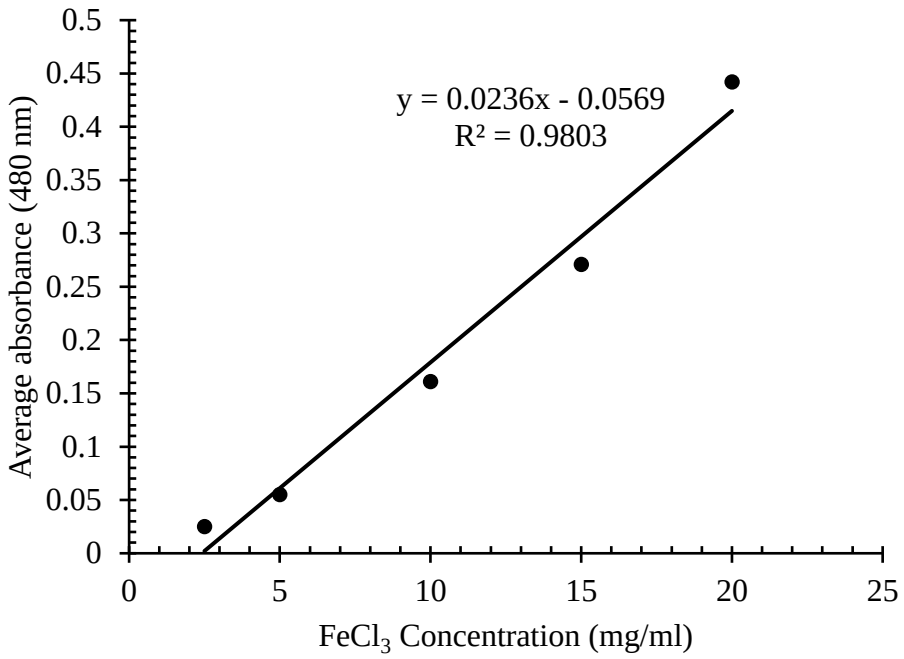

Fig. A.2 Standard curve for phytate content

3. Standard curve for amylase activity

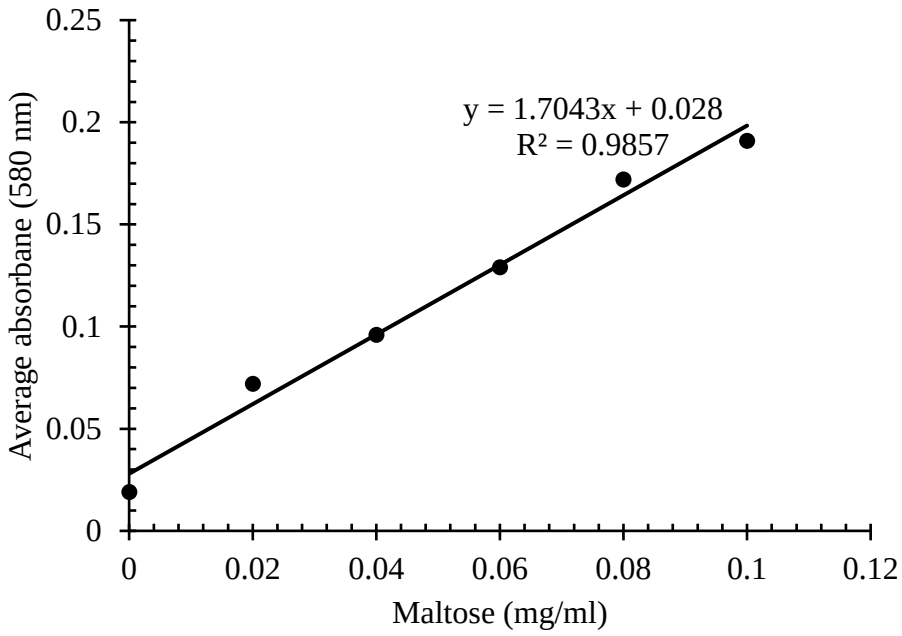

Fig. A.3 Standard curve for amylase activity

4. Standard curve for total phenol content

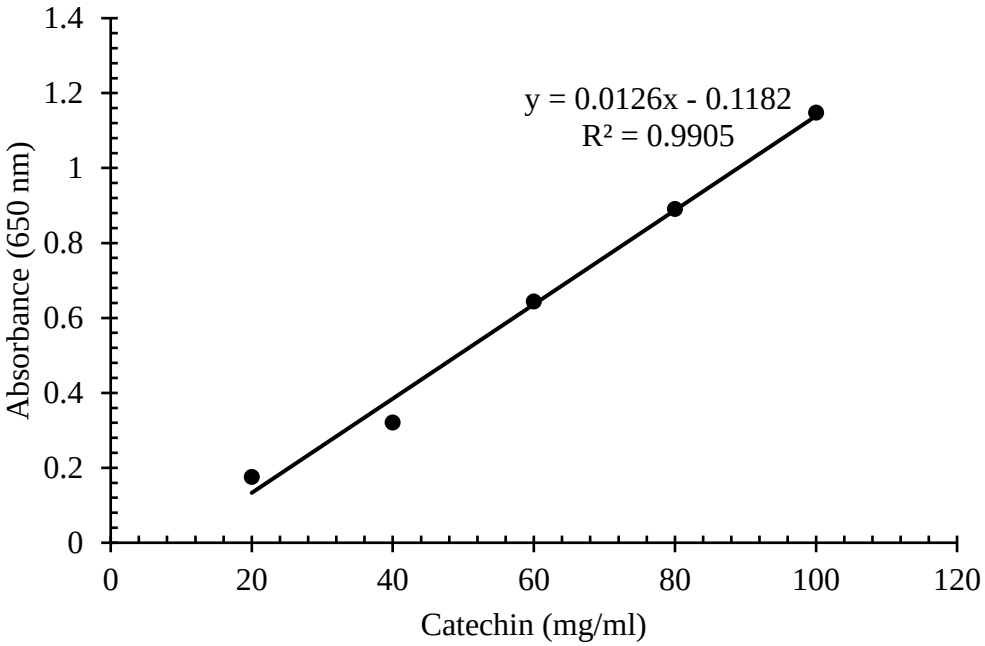

Fig. A.4 Standard curve for total phenol content

Appendix B

Table B.1 ANOVA for ascorbic acid

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	220.7913	55.1978	107.65	<.001
Residual	10	5.1275	0.5128		
Total	14	225.9188			

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

Table B.2 LSD of means for ascorbic acid

No of days	Mean	Column A	l.s.d	d.f.
C*	10.81 ± 0.39	A	1.303	10
1D*	14.32 ± 0.39	B		
2D*	17.54 ± 0.58	C		
3D*	19.58 ± 0.78	D		
4D*	21.63 ± 0.39	E		

(* = Significantly different)

Table B.3 ANOVA for total phenolic content

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	0.442297	0.110574	31.35	<.001
Residual	10	0.035275	0.003527		
Total	14	0.477572			

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

Table B.4 LSD of means for TPC

No of days	Mean	Column A	l.s.d	d.f.
C*	6.52 ± 0.06	A	0.1081	10
1D*	6.66 ± 0.02	B		
2D*	6.84 ± 0.04	C		
3D	6.92 ± 0.02	CD		
4D	6.98 ± 0.04	D		

(* = Significantly different)

Table B.5 ANOVA for DPPH scavenging activity

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	6702.5009	1675.6252	4530.59	<.001
Residual	10	3.6985	0.3698		
Total	14	6706.1994			

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

Table B.6 LSD of means for DPPH scavenging activity

No of days	Mean	Column A	l.s.d	d.f.
C*	8.98 ± 0.19	A	1.106	10
1D*	19.27 ± 0.38	B		
2D*	44.34 ± 0.29	C		
3D*	56.15 ± 0.54	D		
4D*	63.94 ± 0.69	E		

(* = Significantly different)

Table B.7 ANOVA for amylase activity

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	31.29617	7.82404	184.1	<.001
Residual	10	0.425	0.0425		
Total	14	31.72117			

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

Table B.8 LSD of means

No of days	Mean	Column A	l.s.d	d.f.
4D*	1.3 ± 0.14	A	0.3751	10
C*	1.68 ± 0.09	B		
3D*	2.28 ± 0.09	C		
2D*	4.02 ± 0.15	D		
1D*	5.08 ± 0.28	E		

(* = Significantly different)

Table B.9 ANOVA for tannin

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	50768.1	12692	345.06	<.001
Residual	10	367.82	36.78		
Total	14	51135.92			

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

Table B.10 LSD of means for tannin

No of days	Mean	Column A	l.s.d	d.f.
4D*	266.56 ± 5.22	A	11.03	10
3D*	277.8 ± 3.06	B		
2D*	310.5 ± 3.93	C		
1D*	349.93 ± 5.22	D		
C*	427.26 ± 4.91	E		

(* = Significantly different)

Table B. 11 ANOVA for phytate

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	42818.98	10704.75	185.29	<.001
Residual	10	577.73	57.77		
Total	14	43396.71			

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

Table B.12 LSD of means for phytate

No of days	Mean	Column A	l.s.d	d.f.
4D*	253.56 ± 6.77	A	13.83	10
3D*	269.73 ± 4.15	B		
2D*	292.9 ± 6.06	C		
1D*	326.46 ± 5.29	D		
C*	404.36 ± 5.31	E		

(* = Significantly different)

Table B. 13 ANOVA for oxalate

Source of variation	**Degree of freedom**	**Sum of square**	**Mean squares**	**Variance ratio**	**F probability ratio**
No of days	4	13183.49	3295.87	64.93	<.001
Residual	10	507.59	50.76		
Total	14	13691.08			

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

Table B.14 LSD of means for oxalate

No of days	Mean	Column A	l.s.d	d.f.
4D	88.12 ± 4.76	A	12.96	10
3D*	96.6 ± 5.36	A		
2D*	110.13 ± 6.1	B		
1D*	133.4 ± 4.96	C		
C*	170.54 ± 4.3	D		

(* = Significantly different)

Appendix C

Questionnaire

A) General information

Code no:

Name:

Religion: a) Hindu b) Christian c) Buddhist d) Muslim e) Others ……..

Ethnicity: a) Brahmin b) Chhetri c) Janajati d) Others …….

Household members: ……

B) Information about kwati

1) Do you celebrate Janai Purnima?

a) Yes b) No

2) Do you know about Kwati?

a) Yes b) No

3) Do you consume kwati on other days apart from janai Purnima?

a) Yes b) No

4) Do you mix pulses at home for kwati or buy already mixed from the market?

a) Mix at home b) From market

5) How many types of pulses are mixed to form kwati?

a) Less than 9 b) Exact 9 c) More than 9

6) Which types of pulses (specify) do you think is used to form kwati?

7) For how long do you soak the kwati prior germination?

a) Overnight (12 h) b) more than 12 hr

8) For how long do you generally germinate the kwati?

a) 1 day b) 2 days c) 3 days d) 4 days e) more than 4 days

List of Plates

P1 Reading absorbance on spectrophotometer

P2 Using Kjeldahl apparatus

P3 Lab work on progress