CHANGES IN PHYTOCHEMICAL PROPERTIES OF BUCKWHEAT VARIETIES ON MALTING

by

Nirmala Subedi

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment for the degree of B.Tech. in Food Technology

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Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Changes in Phytochemical Properties of Buckwheat Varieties on Malting* presented by Nirmala Subedi has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

Dissertation Committee

1. **Head of the Department _____**

   (Mr. Basanta Kumar Rai, Assoc. Prof.)

2. **External Examiner _____**

   (Mr. Birendra Kumar Yadav, Asst. Prof.)

3. **Supervisor _____**

   (Mr. Kabindra Bhattarai, Teaching Asst.)

4. **Internal Examiner _____**

   (Mrs. Mahalaxmi Pradhananga, Asst. Prof.)

May, 2018

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Contents

Abstract

This research was conducted for comparative study of the proximate composition, micro nutrients, nutritional and anti-nutritional composition on malting of buckwheat varieties (common and tartary buckwheat). Buckwheat samples were taken from NARC which were collected from Dolakha district of Nepal. Steeping of buckwheat seeds were done for 20 h in tap water and germination was carried out at 26-28°C and 85-86% RH in open environment and 24.5°C and 93.5% RH in humidity chamber for 3 days. Physical properties, germination percentage, changes in proximate composition, mineral composition, flavonoid, tannin, polyphenol content and antioxidant activity were analyzed.

The proximate composition of common and tartary buckwheat increased on malting except fat and moisture. The mineral composition of common and tartary buckwheat increased on malting except iron content. The flavonoid content, total polyphenol content and antioxidant activity of unmalted common buckwheat was 84 mg/100 g, 205.6 mg/100 g, 9.742% and tartary buckwheat was 250.9 mg/100 g, 388.6 mg/100 g, 13.229% respectively whose value increased on malting to 91.2 mg/100, 240.9 mg/100 g, 13.835% in open environment and to 97.3 mg/100 g, 248.9 mg/100 g, 18.476% in humidity chamber for common buckwheat whereas to 270 mg/100 g, 440.8 mg/100 g, 34.447% for tartary in open environment and 298.4 mg/100 g, 451 mg/100 g, 40.096% in humidity chamber. The tannin content decreased on malting from 103.6 mg/100 g to 83.57 mg/100 g and 70.69 mg/100 g for common buckwheat and from 137.10 mg/100 g to 103.6 mg/100 g and 93.34 mg/100 g for tartary buckwheat in open environment and in humidity chamber respectively.

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List of Abbreviations

Abbreviations	Full forms
ANOVA	Analysis of Varience
BLA	Blood Lactic Acid
BUN	Blood Urea Nitrogen
DPPH	2,2-diphenyl-1-picrylhydrazyl
H.C	Humidity Chamber
IDF	Insoluble Dietary Fiber
NARC	Nepal Agriculture Research Council
NO	Nitric Oxide
O.E	Open Environment
SDF	Soluble Dietary Fiber
TDF	Total Dietary Fiber
TFC	Total Flavonoid Content
TPC	Total Polyphenol Content

Part I

Introduction

1.1 General introduction

Buckwheat (*Fagopryum esculentum* Moench) is derived from Anglo-Saxon-boc (beech) and whoet (wheat) because it resembles the beach nut and whoet (wheat). It is classified as pseudocereal because of the similarity to conventional cereals in its use and chemical composition. Buckwheat has a powerful ecological adaptability that allows the plant to grow in almost all the kinds of extreme environments. Buckwheat is a dicotyledon and belongs to the family Polygonaceae. Its seeds are brown in color irregularly shaped and have four triangular surfaces (Ahmed *et al.*, 2014).

Buckwheat is a broad leafed herbaceous annual plant. Buckwheat seed is a fruit strictly as achene. The seed is covered by a hull (pericarp) and has a triangular shape and its flower can be pink, white or yellow. The exact shape, size and color of the seed may vary depending on species and varieties. The hull may be glossy or dull and brown or black or gray in color. The dehulled buckwheat seed is called groat and resembles cereal kernel in its gross chemical composition and structure. The first layer of groat is one cell thick, light green colored testa (seed coat). Under the testa there is starchy endosperm. The starchy endosperm is a one-cell aleurone layer (Przybylski and Gruczynska, 2009).

Buckwheat is one of the major honey-producing crops. Buckwheat honey is characterized by valuable therapeutic properties, dark tea-like colour and spicy flavour. Flowers of buckwheat are rich in flavonoids, with the precedence of rutin. Due to a considerable content of antioxidant compounds, buckwheat honey is valued in phytotherapy, it may be applied for the production of meals and baking products. The major producers of buckwheat are China, Russia, Ukraine, etc. Buckwheat is also cultivated in Slovenia, Brazil, Hungary, Austria, Nepal and Poland. The production of buckwheat is characterized by a high variability of crops. This lack of crop stability results from genetic properties of buckwheat and its high susceptibility to unfavourable climatic

conditions in the vegetative season. Buckwheat occupies a special place amongst cultivable crops due to its nutritional, dietetic and therapeutic properties. Grains and other parts of that plant are applied in the food, pharmaceutical, cosmetic and feed industry. In addition, its valuable components are included into a group of neutraceuticals (Wronkowska *et al.*, 2010).

The most widely grown buckwheat species include common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Buckwheat has attracted more and more attention due to its nutritional and medicinal values in recent years. Buckwheat contains many important bioactive compounds such as protein rich in essential amino acids, oil rich in essential fatty acids, starch with a low glycemic index, polyphenol compounds (including rutin, quercetin, orientin, vitexin, isovitexin and isoorientin) and many essential minerals. Studies have revealed that buckwheat can cure chronic human diseases, decrease blood cholesterin, inhibit mammary cancer, prevent gallstones and many others. It is well known that many unique physiological functions of buckwheat are mainly attributed to its high content of flavonoids, especially rutin. Quercetin is an important factor for plant secondary metabolism; it has a wide range of biological activity and is also a promising candidate for prevention and treatment of various cancers (Bai *et al.*, 2015).

Malting is the controlled germination followed by controlled drying of the kernels. The main objective of malting is to promote the development of hydrolytic enzymes, which are not present in non-germinated grain. Malting cause an improvement in protein digestibility. Malting has produced improvement in flavor profile and color. The process of malting comprises three unit operations: steeping, germination and drying (Mella, 2011). Malted grains have technological functionalities in food systems due to their increased enzyme activities. Malted buckwheat can be used in brewing. They can be used as a source of enzymes to alter other grain-based foods (Mäkinen and Arendt, 2015).

1.2 Statement of the problem

Buckwheat (*Fagopyrum* spp.) is an important crop of the Nepalese hilly areas, and are a staple food crop in the remote hills. Buckwheat fits well into tight cropping rotations in the mid hills and plain as a catch crop. Nepal has the unique climatic conditions which allows for buckwheat cultivation in some parts of the country all year round (Baniya *et al.*, 2000). Malting produces hydrolytic enzymes (responsible for converting starch into simple sugars and other hydrolytic activities), which are absent in ungerminated grains (Khoddami *et al.*, 2017). For the production of beer, malt which contains enzymes are required. These enzymes are the most important ingredients to convert the complex sugars to simple sugars in the mashing process. Malt which contains enzymes is imported for production. This will cause increase in cost of production of beer. This is the problem that needs an immediate solution from experts in the field instead of thinking import as the solution (Alemu, 2016).

The detailed study on nutritional composition and anti- nutritional constituents of Nepalese buckwheat varieties common and tartary buckwheat of different regions are lacking. Malting process transforms the grain into more palatable and nutritionally richer form. Thus this study compares the nutritional composition and anti-nutrients composition of buckwheat before and after malting which has not been studied yet. Drinking beer made from barley or other gluten containing grains can be harmful for celiac patients. The only acceptable health solution for celiac patients is a strict life-long elimination of gluten from their diet. When seeking a palatable, high-quality, and healthy gluten free raw material, the nutritive properties of buckwheat suggest that researching these pseudo cereal for malting and brewing purposes is worthwhile (Deželak *et al.*, 2014). This research can help to produce good malt that can be used for production of gluten free beer.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this dissertation is to study the changes in phytochemical properties of buckwheat varieties on malting.

1.3.2 Specific objectives

- To study the proximate composition of un-malted buckwheat of different varieties.
- To study the proximate composition of buckwheat varieties on malting in open environment and in humidity chamber.
- To compare flavonoid, tannin, polyphenol and antioxidant activity of unmalted and malted buckwheat varieties in open environment and in humidity chamber.
- To compare mineral composition of unmalted and malted buckwheat varieties in open environment and in humidity chamber.

1.4 Significance of the study

Buckwheat contains a high level of starch similar to many cereal grains, and it is known for its high levels of resistant starch (Molinari *et al.*, 2017). Buckwheat flour contains some essential nutrients at a high level. Buckwheat proteins have a high biological value (Khan *et al.*, 2013).

The usual demand for buckwheat has been increasing due to its potential health benefits. They contain different nutrients for normal functioning of body. The main criteria are to determine the nutritional composition of un-malted and malted common and tartary buckwheat of hilly region in open environment and in humidity chamber. The nutritional changes on malting of buckwheat in open environment and in humidity chamber varies. Malting has an impact on the abundance and profile of phytochemicals in cereals and pseudocereals, which in turn has an influence on the potential health effects of the finished product (Khoddami *et al*., 2017). So, this malting can have potential benefits on health. This study can also help to find out on which environment the nutritional composition of

malted buckwheat is good which can be used for the preparation of different food products or gluten free beers.

1.5 Limitations of the study

- Comparative study on oxalates, phytic acid and micronutrients at different germination time, temperature and relative humidity were not analyzed.

Part II

Literature review

2.1 Historical background of buckwheat

Yunnan province of south-west china was a diversity hotspot for buckwheat plant according to the results obtained from past two decades on centre of origin of buckwheat. This is the place where highest diversity of endemic wild relatives of common buckwheat can be found today. Several species of genus *Fagopyrum* can be found here. The region is dominated by rigged mountainous topography, climatic variability and biologically diversified ecosystem (Gondola and Papp, 2010).

From south west china buckwheat cultivation was probably distributed from south of the Himalayas to Bhutan, Nepal, northern India and northern Pakistan. Some buckwheat was also grown in Afghanistan. In Bhutan and Nepal at higher altitude buckwheat is still one of the most important crop in addition to barley and potatoes. In Nepal it was grown at altitudes over 3000 m. From china buckwheat cultivation was found to spread to Korea and Japan. It was found that buckwheat distribution came from south-west of china and from there to northern china and Siberia and from there to Russia and Ukraine and to central Europe. Buckwheat appeared in the central Europe around the year 1400, in Slovenia it was first mentioned in a document from the year 1426. From central Europe buckwheat was spread further to Western Europe. In Europe buckwheat was grown mainly in Russia, Ukraine, Belarus, Poland, Slovenia, Austria, Denmark and northern France. In the first half of this century buckwheat was also widespread in Germany and in the Czech Republic, Slovakia and to smaller extent in Portugal, Spain, Great Britain, Switzerland, Italy, Bosnia-Herzegovina, Bulgaria and Finland but later some other crops replaced it. In Europe some buckwheat is now grown as well in Hungary. It is also coming back in Czech Republic, Slovakia, and Northern Italy and in Luxembourg and in beginning to be grown as a raw material for dietetic foods in Norway. In Slovenia and in southern Austria buckwheat may have two crops in the year one sown in May and harvested in July or August. The second crop may be sown in the end of July and harvested in first days of October. From Europe

and from Asia (mainly from Japan) buckwheat was spread by Emigrants to USA, Canada, Argentina, Brazil and South Africa. Some buckwheat had been grown as well as in Australia (Kreft, 2001).

Buckwheat is generally known as hundred days crop (Ghyabre) in mustang and one of the major food crops in high hill regions of Nepal. These dependencies on buckwheat were driven due to the remoteness and agro- climatic difficulties in these parts of Nepal. Some people believed that buckwheat might have introduced in the Himalayan region during the Vedic era (1500-1200 B.C) by a hermit Bishwamitra. In this context it is a crop with 3200 to 3500's domestication history in Nepal. The average yield of buckwheat in world was 0.91, 0.85 and 0.89 t/ha in 2001,2002 and 2003 respectively (Dongol *et al.*, 2004).

China is a large producing country with a total planting area of 1 million ha and a production of 1.05 million tons. Common buckwheat is planted to 0.7 million with a production of 0.75 million tons and tartary buckwheat in 0.3 million tons. Large proportion of buckwheat production is consumed by farmers themselves. However, considerable amount of production are made available to local market (Zhang *et al*., 2004).

2.2 Buckwheat and its utilization in Nepal

Buckwheat (*Fagopyrum* spp) is one of the staple food crops of the mountain people of Nepal, where it is the sixth most important crop after maize , rice, wheat, finger millet and barley and the main crop in certain high mountain pockets where the Sherpa (people of Tibetan origin) like. To date, buckwheat has been an under- exploited, poor people's or neglected crop in Nepal. This pseudo cereal (called KUANNA = Ku =bad and ANNA = cereal in Nepali) generally receives low inputs, little care and a low priority from the farmers themselves (Baniya *et al*., 1990).

In the districts of Mustang, Manang and Dolpa buckwheat is widely cultivated and used as a major meal. In the Mustang in the foothills of Annapurna and Dhaulagiri Himalayas there is a tradition of celebrating buckwheat sowing as a community festival. They strictly follow the tradition of sowing buckwheat at the same time. The community people are

informed about the date of sowing of buckwheat by beating the community's drum. The people of mustang use all parts of buckwheat (Rajbhandari, 2004).

Buckwheat is cultivated and preferred for consumption by people of Mongolian origin (i.e. Sherpa, Thakali, Tamang, Magar, Gurung, Rai, Limbu, Tareli, etc.) living in high and mid-hills of Nepal. Kami (Mustang, Solukhumbu), Thakuri (Jumla, Humla, Mustang), Chhetri (Jumla, Humla), etc are also reported to use the crop. Common buckwheat is summer crop in high hills, autumn crop in mid hills and winter crop in plains. Tartary buckwheat is summer crop in high hills; and autumn and spring crop in mid hills and it is not grown in plains. It has an immense socio-religious and economic importance which are most important and key factors for buckwheat diversity conservation (Baniya *et al.*, 2004).

In Nepal the flour of tartaray buckwheat is used in different religious festivals by making various processed products. Some of the lama's monks (Gumba) also used it for social or religious purposes. Farmers believe that tartary buckwheat can be used to cure headache because it contains rutin which strengthens capillaries and is good for patients with hemorrhage problem. It is also used to treat gastric acidity, jaundice, cold, fever, intestinal disorders and pneumonia. In some of the areas, flour of tartary buckwheat is used for plastering bone fractures. Buckwheat is used for the preparation of baby food, bread, pancakes, thick porridge, for making sausage and other locally consumed food products. In some of the areas tender leaves and shoots are used as green vegetable/ salad. It is used for the preparation of local wine and whisky. Buckwheat is also used to produce vinegar which is usually produced by local factories and preferred by local people. Some of these products can be found in supermarkets in cities (Zhang *et al.*, 2004).

Common buckwheat is mainly used to make flour and consumed as "haba" (dhido in Nepalese), mixing with the blood of the goat. Seedlings and tender leaves are used for green vegetables. Tartary buckwheat is almost used for the animal feed and used for food in upper mustang (Nemoto *et al.*, 1995). The utilization of buckwheat in Nepal is shown in Table 2.1

Table 2.1 Local utilization of buckwheat plants, grains and its products in Nepal.

S.N	Use	Form/ Part of use	Usage
1	Food	Flour	Dhindo, Bhat, Sel, Sen-sweets.
2	Vegetables	Leaves, tender shoots	Fresh and dry vegetables pickles, soup, sausage, Dorpa-dal, salad, etc.
3	Alcoholic drinks	Fermentation and distillation	Rakshi and Jaand.
4	Medicinal use	Usually flour and grain	Tite buckwheat bread by people with the stomach problems.
5	Animal feed	Fresh and dry straw	Fresh and dry straw used as fooder.
6	Fuel	Straw	Straw used as fuel supplement.
7	Soil improvement	Plant, straw,etc.	Used as green manure and compost, as bedding material for the cattle, which is then spread to the farmland.

Source: Dongol *et al.* (2004)

2.3 Buckwheat cultivation and its production in Nepal

Buckwheat is mostly cultivated in marginal unplanned areas with sloping infertile land on which other crops cannot be grown. However, in high mountain areas, farmers grow this crop on the better land and apply adequate compost. Buckwheat planting starts in May at elevation above 2800 m and continues upto September at lower altitudes. In the southern plain area, this crop is planted from mid-November to mid-December. Depending on the altitude, buckwheat takes from two to four months to mature in Nepal. In certain mountain areas, buckwheat is planted in December as a second crop and is harvested in March. Though it is generally considered as an autumn crop, buckwheat is a summer crop in high mountain, an autumn crop in middle mountain and in winter crop in low mountain and in plain areas (Baniya, 1990).

In Nepal, buckwheat has flexible planting time. In Mustang and Manang districts, buckwheat is grown under irrigated conditions. The planting is done in late August and harvested at the end of October. In high mountain areas of Mustang, Manang, Jumla, Humla and Dolpa, tartary buckwheat is grown in summer and fields left fallow in winter. In Solukhumbu, it is sown after harvest of maize. In upper Dolpa, tartary buckwheat is grown in the area which is rainfed, steep and sloppy. Early maturing varieties of buckwheat are preferred to escape from early snowfall and cold weather (Zhang *et al*., 2004).

In Nepal, buckwheat cultivation ranges from 60 m in terai to 4500 m above sea level. *F. esculentum* is generally grown in lower altitude (terai and mid-hills) but in higher altitude *F. esculentum* is replaced with *F. tataricum* in different cropping pattern. It is cultivated in 61 out of 75 districts of Nepal Figure. Buckwheat varieties are summer crop in hill (high altitude > 1700 m), autumn and spring crop in mid-hills (600–1700 m), and winter crop in Terai. However, all seasons (summer, autumn, winter, and spring) are suitable to cultivate buckwheat in different agro ecological zones of Nepal (Luitel *et al.*, 2017).

The production and yield of buckwheat in Nepal has been increased from 2010 to 2014. In 2010/2011 buckwheat was cultivated in 10304 ha area with the production of 8841 t/yr and yield of 0.858 t/ha but its cultivation, production and yield increased in 2013/2014. In 2013/2014 buckwheat is cultivated in 10510 ha area with the production of 10355 t/yr and yield of 0.983 t/ha (Luitel *et al.*, 2017).

2.4 Varieties of buckwheat

Until now a total of six different species and sub species have been reported in Nepal. These are *Fagopyrum esculentum, F. tataricum, F. cymosum, F. megacarpum, F. tataricum Meissn*. ssp. *potanini* (Chinese type) and *F. tataricum* ssp. *potanini* (Tibetan type). Among them, *F. esculentum* (common or mithe) and *F. tataricum* (tartary or tite) are cultivated. Some important landraces of buckwheat are Gyabre, Chhendrung, Ghanbre, Jhoumbre, Chuche, Bhadule, Gore, etc (Baniya *et al.*, 2004).

Mithe Phaper, local chuchche, Local Lekhari, Bhate, (recommended variety by NARC), Dalle, Kalo, Barule, Takule, Tilkunde, Tote, Ghode, and Tite Phaper are the names of local landraces recorded from Karnali zone. Buckwheat from Jumla districts of Karnali zone from Nepal include Barule, Bharule, Chuchche, Chode, Kalo, Mithe, Seto, Tilkhude, Tite, and Tote Phaper (Luitel *et al.*, 2017).

In Nepal site, most widely distributed tartary buckwheat varieties include Dhop (grey and black in colour) in Mustang and Manang, Tite (with yellow and white seed in colour) in solukhumbu, Bharule, Chucche (with light brown grey seed colour) in Jhumla and Humla and Ghore; Syangrel and Tashung (with black, grey and brown seed colour) in Dholpa. Two cultivated species *Fagopyrum esculentum* Moench (sweet or common) and *Fagopyrum tataricum* Gaertn (bitter or tartary) and one wild species *Fagopyrum cymosum* Meissn are found in Nepal. Bitter buckwheat is grown exclusively in high mountain regions above 1500 m the sweet type is very common and is extensively cultivated in middle and low mountain and to some extent in high mountain and plain areas. The wild type is perennial and occurs in the altitudinal range of 1500 m to 3000 m mainly beside

rivers and trekking routes as a companion crop of tartary buckwheat. Dr. Ujihara has reported that most Nepalese common buckwheat varieties are of autumn type, indeterminant growth and sensitive to day – length. Bitter buckwheat is more cold tolerant, higher yielding and fills the grains better than the sweet types (Poudel, 2012).

2.5 Types of buckwheat cultivated in Nepal

2.5.1 Common buckwheat

Common buckwheat (*Fagopyrum esculentum* Moench) is a heterostylous diploid species with an indeterminate growth habit. It is an annual broadleafed plant, with a smooth, succulent stalk, a knotted single main stem that develops lateral branches. Generally, in a field population the plants only develop primary branches. The main stem is grooved, generally green, but sometimes tinged with red. Plant height varies from 30 to as much as 120 cm or more. The stems are more or less round and hollow, with a diameter of 3 to 15 mm. At the time of maturity the stems and branches turn red. The leaves are petiolate, positioned alternately on the opposite sides of the stem, heart shaped, ovate-triangular to triangular, 4 to 8 cm long. The blades are glabrous (hairless) (Gondola and Papp, 2010). The plants have short tap root and fine lateral roots producing root system (Woo *et al.*, 2010). 1000 kernel weight of common buckwheat is about 24.9 g (Kaliniewicz *et al.*, 2015). The sphericity of common buckwheat is about 70.79 % (Unal *et al.*, 2017).

Fruit is 2-4 inches long with keeled edges varies in color form silvery grey to brown or black. It is cultivated to obtain grain for human consumptions. It is also grown for livestock and poultry feeds as a green manure, smother crop to crowd out weeds and source of buckwheat honey. It has triangular seeds with black soft hull, light green to white kernel (Ratan and Kothiyal, 2011).

Flowers are white to pink and the perianth is planar (perpendicular to the pedicel) at the anthesis with the diameter about 6-7 mm. It is formed of five petaloid tepals not joined together. Flowering starts 4 to 6 weeks after sowing and goes on during 4 to 15 weeks. It is one of the oldest domesticated crops of Asia. This crops was very popular food during 17^{th} – 19^{th} century and was later abandoned during 20^{th} century in western countries because of

competition with the wheat (Cawoy *et al.*, 2009). Common buckwheat varieties are generally grown in midhill and Terai. There are altogether 19 local landraces of common buckwheat listed from Nepal (Luitel *et al.*, 2017).

2.5.2 Tartary buckwheat

Tartary buckwheat (*Fagopyrum tartaricum)* is an edible plant in the genus *Fagopyrum* of the family Polygonaceae. It has slightly bitter taste and higher rutin content than common buckwheat. Tartary buckwheat is increasing interest from food technologist and consumers for its significant as anti-hyperglycemic, anti-hyperlipidemic and anti-hypertensive functions (Li *et al*., 2009).

It is herbaceous plant characterized with colourful branches or unbranched stem reaching up to 1m, branched taproot system, petiolated leaves and triangular leaf blade with the length being almost equal with width 2–8 cm and cordate or hastate leaf bases. Inflorescences are dense spicate or corymbose. Flowers are yellow-green, 2.5 mm in diameter, pedicels are nonparticulate, perianth is 2mm long, nectaries are yellow, alternating with stamens, being homostyly that is self-pollinated flowers; stigmas are capitate. Triquetrous achene is about 5mm long, exerting more than twice the length of the persistent perianth with three deep grooves and the angles are rounded except the tip. Flowers are homomorphic, self-fertile and cleistogamous with pollination occurring before the flower opens (Luitel *et al.*, 2017).

The flowers are without fragrance. The fruit is small, ovoid, conical, brownish, grey or black in color with dull irregular faces on each of which is a deep furrow. For tartary buckwheat 1000 seed weight is 15-25 g (Leder, 2009). Every family grows tartary buckwheat in upper mustang and dolpa districts and diversity of buckwheat is very high in Manang, Dolpa, Mustang, Jumla and Solukhumbu. Bitter buckwheat is grown in marginal land and in higher altitude. It can withstand the poor, infertile and acidic soils, nutrients, moistures and heat stress with wider adaptability which is prevalent to hilly area of Nepal. These unique characteristics of buckwheat show a great potential crop in future in food-

deficit areas like high mountains which has high risk of climatic change impact (Luitel *et al.*, 2017).

2.6 Utilization of buckwheat

Buckwheat grain is grown mainly for human consumption and as animal feed, although it can also be used as a vegetable, a green manure crop, as a smother crop to crowd out weeds and as a source of buckwheat honey (Campbell, 1997).

2.6.1 Human consumption

Common buckwheat is consumed in many different preparations in different countries. In Japan it is mainly consumed as noodle soba. In Europe and North America buckwheat flour is generally mixed with wheat flour to prepare pancakes, biscuits, noodles, cereals, and is used as a meat extender. In Southeast Asia buckwheat is a staple food in many hilly areas. Here the flour is used to make unleavened bread chapattis. It is also mixed with water and fried to produce a crisp pakora. Buckwheat is used to make alcoholic drinks; the liquor prepared from Tartary buckwheat being ascribed medicinal qualities. In China it has been reported that buckwheat is used for the production of vinegar (Campbell, 1997).

2.6.2 Vegetable crop

Buckwheat is often raised as a leafy vegetable crop in many areas of the Indian subcontinent. The leafy tender shoots of the plants are harvested and dishes are prepared from them. This often augments the supply of fresh vegetables that are available at time of year. The crop is generally dual purpose as the remainder of the crop is harvested for grain and straw (Campbell, 1997).

2.6.3 Green manure and soil conditioner

Buckwheat is useful as a green manure crop for renovation of low-productivity land because it grows well on such land and produces a green manure crop in a short time (Marshall and Pomeranz 1982). When ploughed, the plant material decays rapidly, making nitrogen and mineral constituents available for the succeeding crop. The resulting humus

improves the physical condition and moisture-holding capacity of the soil. When a crop is harvested early in a year a second crop of buckwheat often can be grown and ploughed down as green manure (Campbell, 1997).

2.6.4 Feed and cover for wildlife

Sportsmen have known that buckwheat is useful as a food and cover crop for wildlife. Deer eat buckwheat and will begin foraging as soon as a few seeds have developed. The grain is also eaten by wild turkeys, pheasant, grouse, waterfowl and other birds. The crop is generally planted and not harvested so that the standing plants provide both food and cover for wildlife (Campbell, 1997).

2.6.5 Honey crop

Buckwheat is used as honey crop. The flowers of buckwheat are excellent nectar producers so, its usage as a bee pasture is very popular. The reason why buckwheat is interesting for bee keepers is that its honey production come late in the season when other nectar sources are scarce (Radics and Mikohazi, 2010).

2.6.6 Smother crop

Buckwheat has been used as a smother crop, owing to the lack of good herbicides for broad-leaved weed control. Buckwheat is generally a very good competitor as it germinates rapidly and the dense canopy that it produces soon shades the soil. Often growers will increase the seeding rate in areas where they expect more weed competition so that the canopy is developed more quickly. This rapidly smothers out most weeds, especially broadleaved ones. If the weed growth gets above the buckwheat canopy, buckwheat becomes a poor competitor. Buckwheat has been cited as being a useful crop for the control of many weeds including quack grass, Canada thistle, sow thistle, creeping jenny, leafy spurge, Russian knapweed and perennial pepper grass (Campbell, 1997).

2.7 Health benefits of buckwheat

Buckwheat is an excellent medicinal plant as well as a nutrient-abundant crop. Its flour and leaf contain large quantity of flavonoid compounds. The rutin contain reaches a proportion of 0.8-1.5% and in auto tetraploid tartary buckwheat it even reaches as high as 2.41%. Rutin contributes to a multitude of physiological functions, which can maintain the resistance of blood capillary promote the proliferation of cells and forestall the agglutination of blood cells. It can further serve as anti-inflammatory and anti-allergic, as diuretic and spasmolytic and serve for depression of cough, reduction of lipemia and for cardiac stimulation. The adequate quercetin enables tartary buckwheat to render better service to removal of phlegm, ease of cough and certain lessening of asthma (Gang *et al.*, 2001).

Buckwheat contains plentiful vitamins. Vitamin B1 can help to enhance digestive function to resist neuritis and to prevent beriberi. Vitamin B2 can enhance human body's development and is a vital element for protection against perleche, glossitis and eyeliditis. Serving to reduce lipemia and cholesterol in human body, vitamin PP is an important auxiliary medicine for the treatment of hypertension and cardiovascular diseases. It has particularly good effect on senior patients for its capacity to lower down fragility and permeability of tiny blood vessels and to restore their elasticity, thereupon is effective for protection against cerebral hemorrhage, maintenance of ocular blood circulation, and preservation and promotion of eyesight. The content of tocopherol in vitamin E is relatively higher. It is effective for prevention of oxidation and for cure of sterility and contributes to cell regeneration and to deference of aging. Tartary buckwheat contains fairly rich common elements and microelements (Mg, Ca, Se, Mo, Zn, Cr, etc.), which serve protection against coronary heart disease. Whereas compared with common medicinal herbs, it contains lower proportion of harmful elements (Co, Pb, Ba, Cd, etc.) to this disease. The microelement selenium (Se) contained in tartary buckwheat could combine with minerals into an unstable 'mineral selenium-protein' compound which helps to decompose and excrete toxins (eg, Pb, Hg, Cd, etc.) in human body. Selenium still has

anti-oxidant and immunity-regulatory functions similar to vitamin C and E, which has distinct effect on prevention of Keshan disease, Kashin-Beck disease, sterility and premature senility and on treatment of cancer as well. Tartary buckwheat food possesses apparent functions of three reductions, namely the reductions of lipemia, blood sugar and glucosuria. Some medical experts call it 'Powder of Three Reductions' consequently. Tartary buckwheat food has specially good effect on diabetes and are well preventive and curative to hyperlipemia, vascular sclerosis, cardiovascular diseases. It still possesses certain radiation-resistant property, hence an extremely curative food for radiation sufferes (Gang *et al.*, 2001).

Buckwheat is rich in dietary fiber which has a positive physiological effect in the gastrointestinal tract and also significantly influences the metabolism of other nutrients Buckwheat seeds contain no gluten so they are safe for people with celiac disease. It influences the metabolism of other nutrients. Buckwheat groats contain important resistant starch and it could be useful in preventing the colon cancer. Rutin (quercetin-3-rutinosid) is a flavonol glycoside synthesized in higher plants as a protectant against ultraviolet radiation and diseases. It also decreases the permeability of the blood vessels and has an antioedema effect, reduces the risk of arteriosclerosis, etc (Vojtíšková *et al.*, 2015).

Tartary buckwheat extracts has anti-fatigue properties which extended the exhaustive swimming time of mice, effectively inhibiting the increase of blood lactic acid (BLA), decreasing the level of blood urea nitrogen(BUN), increasing the tissue glycogen content and the activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD) of mice. Moreover, buckwheat polysaccharides can significantly stimulate cytokine secretion (differentiation inducer) and then increase cell differentiation and maturity in monocytecells. The main phenolics of buckwheat extract are rutin and quercetin. Rutin (quercetin-3-O-β-rutinoside) is the best-known glycoside derived from flavonol quercetin. Rutin has relaxing effects on smooth muscles and is effective for preventing capillary apoplexy and retinal hemorrhage, reduces high blood pressure and show antioxidant and lipid peroxidation activities. It also helps in lipid-lowering activity by decreasing the

absorption of dietary cholesterol as well as lowering plasma and hepatic cholesterol. Proanthocyanidins in the buckwheat flour reduced nitrous acid producing nitric oxide (NO) when the flour was suspended in acidified saliva or in acidic buffer solution in the presence of nitrite. The increase in the concentration of NO could improve the activity of stomach helping the digestion of ingested foods (Zhang *et al*., 2012).

2.8 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopside

Order: Caryophyllales

Family: Polygonaceae

Genus: *Fagopyrum*

Species: *esculentum*

Source: Ahmed *et al.* (2014)

2.9 Chemical composition of buckwheat

2.9.1 Carbohydrate

Starch is the major component of buckwheat endosperm which plays an important role in appearance, structure and quality of food products. It is important reserve polysaccharides and the most abundant constituent in buckwheat. It has unique properties which contribute to functionality of food products. The major components of starch are glucose polymers – amylose and amylopectin. It is the most important reserve polysaccharide. The major components of starch are glucose polymers – amylose and amylopectin (Hung *et al.*, 2009).

Amylose content in starch generally ranges from 20% to 26%. Starch granules are 2-15 mm in diameter, round, oval or polygonal in shape with a few holes and pits on the surface. Buckwheat grains also contain 0.65-0.76% reducing sugars, 0.79-1.16% oligosaccharides and 0.1 -0.2% non-starchy polysaccharides. The major component of tartary buckwheat seed is starch and may amount over 70% of the total dry weight. There appears to be little difference in starch properties and structures between common and Tartary buckwheat, and this may be due to the limited genotypes (Zhu, 2016).

2.9.2 Fiber

Dietary fiber is a part of a plant or analogous carbohydrates that is resistant to digestion and absorption in the human small intestine but is partially or completely fermented by micro-flora in the large intestine. The content of total dietary fiber in buckwheat groats may range from 5.0- 11.0%. The bran fractions obtained by milling buckwheat were especially enriched in dietary fiber 15 – 22% because the outer layer covering the seeds contains non-starch polysaccharides. The amount of total dietary fiber (TDF) in buckwheat may be affected by both variety and environmental factors during growth. The major components of TDF are cellulose, non-starch polysaccharides, resistant starch and lignins. These components are concentrated in the cell walls of the starchy endosperm, aleurone, seed coat, and hull. Smaller groats have less endosperm and, therefore, relatively more seed coat, resulting in more dietary fiber (Przybylski and Gruczynska, 2009). Very low crude fiber is found in Baljeet *et al.* (2010).

Total dietary fibre (TDF) is classified in view of its affinity to water as either insoluble dietary fibre (IDF) or soluble dietary fibre (SDF). In general, IDF includes cellulose, lignins, and certain non-cellulosic polysaccharides, while SDF includes pectins and some associated non-cellulosic polysaccharide (Christa and Smietana, 2008). The total dietary fibre content of tartary buckwheat seeds was 26% with that of soluble and insoluble fibers being 0.54% and 24% respectively (Zhu, 2016).

2.9.3 Protein and amino acids

Proteins are the main structural constituents of tissues in human body and of biologically active compounds i.e enzymes, hormones and antibodies. The major protein fractions of buckwheat grains are water-soluble and salt soluble albumins and globulins. Globulins consist of 12 -13 sub units with molecular weight from 16 – 60 kDa. A peculiar biochemical characteristic of buckwheat grain protein is a negligible fraction of prolamines and lack of alpha gliadin which enables applying buckwheat grains or buckwheat products in foodstuffs for patients suffering from affections linked with glutein intolerance. Buckwheat grains are also a source of dietetic proteins with a well-balanced amino acid composition as well as of lysine, the first limiting amino acid in cereals,(Wronkowska *et al.*, 2010).

The proteins content in common and tartary buckwheat varies from 7 to 21% depending upon the cultivar and environmental conditions during the growth. Buckwheat proteins consists of 18.2% albumin, 43.3% globulin, 0.8% prolamin, 22.7% glutelin and 5.0% other nitrogen containing components (Przybylski and Gruczynska, 2009). The protein content of tartary buckwheat is in the range 7.82%-18.94% in (Guo *et al.*, 2007).

2.9.4 Lipids

In general, lipids comprise a small part of cereals and pseudo cereals, but they have an important physiological role. Lipids also play a role in food quality as they may cause deterioration of stored seeds or flours. In both common and tartary buckwheat, lipids are concentrated in the embryo. The embryo contains most of the unsaturated fatty acids, while the hull has a high level of saturated fatty acids. One of these essential fatty acids (linoleic acid), is the major fatty acid present in buckwheat; the level of linoleic acid is particularly high in the seed coat (Wijngaard and Arendt, 2006). The lipid content in tartary buckwheat varies from 2.5%-2.8% in Ahmed *et al.* (2014), 2.83% in Qin *et al.* (2010) and 2.45% in

Bonafacciaa *et al.* (2002). The lipid content in common buckwheat varies from 1.6%-2.9% in Dogra and Awasthi (2015) and 3.16% in Sindhu and Khatkar (2016). In the whole buckwheat grain the total lipids content range from 1.5 to 4.0% and 1.2 to 4.3% for common and tartary buckwheat. The content of lipids varies by seed part and is usually in the embryo 9.6-19.7%, the endosperm 2.0-3.0% and the hulls 0.4-0.7%. Buckwheat oil contains 16-25% of saturated and 74.79% of unsaturated fatty acids. Among these fatty acids palmitic, oleic and linoleic are dominant with contribution of 15-20, 30-45 and 31-41%, respectively (Przybylski and Gruczynska, 2009).

2.9.5 Vitamins

Vitamins are a group of organic compounds that are essential in very small amounts for the normal functioning of the human body. They vary widely in their chemical and physiological functions. Thiamine (vitamin B1) is known to be strongly adhered to thiamine-binding proteins in buckwheat seeds. Tartary buckwheat has higher levels of vitamin B than in common buckwheat (Wijngaard and Arendt, 2006). Buckwheat contains higher levels of niacin, B6, vitamin K and choline. buckwheat does not contain vitamin A while carotenoids such as lutein and zeaxanthin are present in similar amounts as in other cereals (Przybylski and Gruczynska, 2009).

2.9.6 Minerals

The ash content of common buckwheat is in the range 1.4%-2.5% (Dogra and Awasthi, 2015) whereas the ash content of tartary buckwheat is in the range 1.8%-2.3% (Thakur *et al.*, 2017). The ash content of buckwheat varies from 2.0-2.2%, depending upon the variety and conditions during growth. Different parts of the buckwheat seed contain different amounts of minerals; hull, aleurone tissues and embryo are the main locations of the most of the minerals. Buckwheat seeds are a good source of many essential minerals; whereas the amounts are similar to other cereals. The mineral content in buckwheat seeds and their morphological fractions reach: 2.0-2.5% in the whole grains, 1.8-2.0% in the kernel, 2.2-3.5% in the dehulled grains, 0.80-9% in flour, and 3.4-4.2% in the hulls. In comparison with rice, wheat, corn flour, buckwheat contains the highest amounts of zinc, copper, and manganese. Trace elements, e.g. selenium or chromium, are also present in buckwheat, however at very low levels. As in other plant material, mineral content is highly influenced

by the presence of these elements in soil where the crop was produced (Przybylski and Gruczynska, 2009).

The iron content of common buckwheat is 3.4-6.4 mg/100 g (Steadman *et al.*, 2001). The iron content of tartary buckwheat is 2.47-21.5 mg/100 g (Wang Louming *et al.*, 1995). The calcium content in common buckwheat is found to be 72.7 mg/100 g (Ikeda *et al.*, 2001) and 70.14 mg/100 g (Akpoghelie *et al.*, 2016). Minerals are cofactors in antioxidative enzymes. Activity of the following enzymes is maintained by: superoxide dismutase on zinc, copper and manganese; glutathione peroxidase and thioredoxine reductase on selenium; and catalase on iron. The bioavailability of zinc, copper and potassium from buckwheat is especially high (Przybylski and Gruczynska, 2009).

2.10 Total polyphenols

Phenolic compounds are the most widely distributed secondary metabolites, ubiquitously present in the plant kingdom. Higher plants synthesize several thousand known different phenolic compounds. The ability to synthesize phenolic compounds has been selected throughout the course of evolution in different plant lineages, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time .The three major classes of phenolics are flavonoids, phenolic acids and condensed tannins. Flavonoids are compounds that possess the same C15 (C6-C3-C6) basic skeleton (Lattanzio, 2014). Three of the numerous classes of flavonoids are found in buckwheat are:

2.10.1 Flavonoids in buckwheat

Flavonoids constitute relatively diverse family of aromatic molecules that are derived from phenylalanine and malonyl-coenzyme A (coA; via fatty acid pathway). Flavonoids contain six major subgroups that are found in most of the plants: the chalcones, flavones, flavonols, flavandiols, anthocyanins and condensed tannins. Buckwheat contains several kinds of flavonoids in the seeds, leaves and stems and rutin is one of the compounds. It is an antioxidant that has many useful pharmacological effects (Panwar *et al.*, 2012).

Six flavonoids including rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin are isolated and identified in buckwheat grains Buckwheat grains also contain other kinds of flavonoids such as hyperoside (quercetin 3-O-β-D-galactoside), quercitrin (quercetin 3-O-α-rhamnoside), and catechins (Zhou *et al.*, 2016).

Flavonoids compounds are accumulated in the vacuoles of plant cells. Flavonoids give the plant a rich taste .The role of flavonoids in flowers is to provide colors which is due to the presence of anthocyanin which is attractive to plant pollinators and in leaves, these compounds are increasingly believed to promote physiological survival of the plant, protecting it from, for example, fungal pathogens and UV-radiation. Buckwheat are well known for their antioxidant activity. Dietary flavonoids like epicatechin, galate, gallic acid, quercetin-3-glucoside possess strong antioxidant activity. Flavonoid gained recent attention because of their broad biological and pharmacological activities in these order Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Saxena *et al.*, 2012). The structure of flavonoid is shown in Fig. 2.1

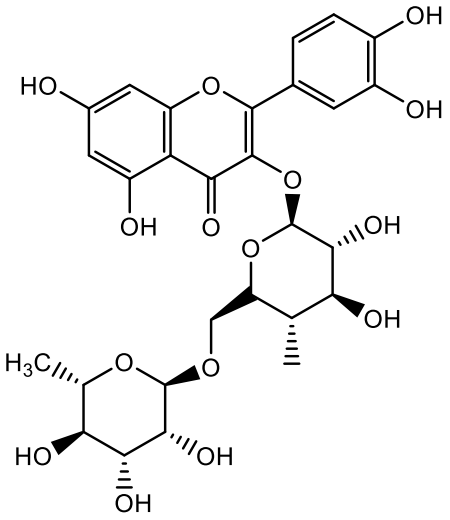

Fig. 2.1 Chemical structure of flavonoid rutin

Source: Leiber *et al.* (2012)

2.10.2 Tannins

Tannins are a unique group of phenolic compounds of relatively high molecular weight which have the ability to complex strongly with carbohydrates and proteins. The name "tannin" is derived from the French "tannin" (tanning substance) and is used for a range of natural polyphenols. In higher plants, tannins consist of two major groups of metabolites: the hydrolyzable tannins and condensed tannins. Hydrolyzable tannins are split by acids,

bases, and in some cases by hydrolytic enzymes (tannase) into sugars (usually D-glucose) or related polyols and a phenolic acid. Condensed tannins constitute one of the most ubiquitous groups of all plant phenolics (Lattanzio, 2014).

Tannins are often considered to be nutritionally undesirable. Tannins form complexes with proteins, starch, and digestive enzymes to cause a reduction in nutritional values of foods. They can cause a browning reaction in foods through the action of polyphenol oxidase by darkening reactions adversely affecting the acceptability of such foods (Chung *et al.*, 1988).

Hydrolysable tannins are usually found in lower concentrations in plants than CTs. Hydrolysable tannins are subdivided intotaragallotannins (gallic and quinic acid) and caffetannins (caffeic and quinic acid). Condensed tannins have a variety of chemical structures affecting their physical and biological properties (Hassabpour, 2011). Condensed tannins are structurally more complex than hydrolyzable tannins. They are mainly the polymerized products of flavan-3-ols and flavan-3, 4-diols, or a mixture of the two (Chung *et al.*, 1988).

Condensed tannins can help decrease the inflammation of UC patients who have been left vulnerable from a defect in GI mucin. Tannins have shown antibacterial activities against Kocuria rhizophila, Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa (Clinton and ND, 2009). The structure of tannin is shown in Fig. 2.2

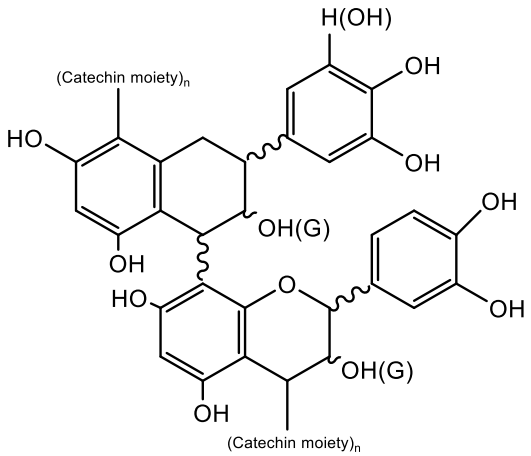

Fig. 2.2 Structure of condensed tannin.

Source: Khanbabaee and Ree (2001)

2.10.3 Rutin in buckwheat

Rutin (quercetin-3-rutinosid) is a flavonol glycoside synthesized in higher plants as a protectant against ultraviolet radiation and disease. No rutin was found in cereals and pseudocereals except buckwheat, which can be used as a good source of dietary rutin. Different parts of plants contain different concentrations of rutin. Most rutin is accumulated in the inflorescence (up to 12%, d.w.b.– dry weight basis), in stalks (0.4–1.0%, d.w.b.) and in upper leaves (8–10%, d.w.b.). Ecological factors, such as UV irradiation, may also have a great influence on rutin content (Kreft *et al*.,2006).

Rutin may be up to 300-fold more highly concentrated in groats of Tartary buckwheat than in groats of common buckwheat. The synthesis of rutin and other polyphenolic compounds may be influenced by ultraviolet rays. Modest amount of UV-B radiation may stimulate the synthesis of rutin. The highest amount of rutin was contained in the upper epidermis and the glycosidase was the most active in the lower epidermis. In leaves situated higher on the plant there was more rutin than in the lower ones. More rutin is synthesised at higher temperature (24.5°C daytime, and 18°C at night) in comparison to lower temperature (18°C daytime, and 12°C at night) (Kreft e*t al*., 2003).

Rutin has a wide range of pharmacological properties (e.g. antioxidative activity) that have been exploited in human medicine and nutrition. It is used as an antimicrobial, antifungal, and anti-allergic agent. Current research has shown its multispectrum pharmacological benefits for the treatment of various chronic diseases, such as cancer, diabetes, hypertension, and hypercholesterolemia. Rutin can induce bone formation via the differentiation of human MG-63 osteosarcoma cells. Rutin may have therapeutic potential for the treatment of neurodegenerative diseases associated with oxidative stress. Rutin might be useful as an adjuvant in radioiodine therapy, since this flavonoid increases thyroid iodide uptake without greatly affecting thyroid function. Rutin from buckwheat herb tea for the prevention of cardiovascular diseases (Al-Dhabi *et al.*, 2015).

2.11 Malting

Malting believed to be the oldest technology dating back to 10,000 BC can be defined as the limited germination of cereal grains under controlled moist conditions. Malting is controlled germination 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 which have such important effects on physical quality of beer the maltster is concerned with the both degradation of the endosperm and accumulation of the enzymes in the grains. But the growth of the germs of the embryo is an incidental to making of the malt and leads to unwanted depletion of the endosperm has progressed to only a limited extent, the maltsters terminates the growth of the embryo by drying the grain (Poudel, 2012).

Malting can be defined as the process of steeping, germination and drying (kilning) of cereal grains to advance the production of hydrolytic enzymes (responsible for converting starch into simple sugars and other hydrolytic activities), which are absent in ungerminated grains. Malting has an impact on the abundance and profile of phytochemicals in cereals and pseudocereals, which in turn has an influence on the potential health effects of the finished product (Khoddami *et al.*, 2017)

Malting is the controlled germination followed by controlled drying of the kernels. The main objective of malting is to promote the development of hydrolytic enzymes, which are not present in non-germinated grain. Malting caused an improvement in protein digestibility. Other benefits of the malting process include increased vitamin C content, phosphorus availability, and synthesis of lysine and tryptophan. Also during malting, both starch and protein are partially degraded allowing for better digestibility. Furthermore amylases are elaborated and as a result, the viscosity of gelled starch decreases. Malting has produced improvement in flavor profile and color. The process of malting comprises three unit operations: steeping, germination and drying (Mella, 2011).

2.11.1 Steeping

Steeping involves soaking buckwheat grains in water. The fundamental reason is to hydrated the grain and initiate metabolism of living tissues, which are habitually dormant when the grains are dry. For efficient production of good quality buckwheat malt, a steep out moisture level of 40-43 % is recommended (Poudel, 2012).

2.11.2 Germination

Germination refers to the psychological process culminating in the emergence of the embryo from its enclosing openings, which can include the endosperm, perisperm, testa or pericarp. The absorption of water by seed activates metabolic processes that subsequently lead to the expansion of the embryo and penetration of the radicle through the surrounding tissues (Bewley *et al.*, 2013). Germination is the beginning of the development of seed embryo in which viable seed is wetted, water is taken up, respiration, protein synthesis and other metabolic activities begin and after a certain period of time, the radicals or hypocotyls emerges through the seed coverings which mark the end of the process. Germination is such process employed to improve the nutrient composition and functional properties of legumes and cereal seeds (Rai, 2013).

Germination improves the nutritive value of cereals and has been found to decrease the levels of anti-nutrients compounds present in cereal, therefore maximizing the levels of utilizable nutrients. Germination was suggested to be a suitable technological procedure for improving the nutritional quality of cereals and other seeds. This is a consequence of enzymes activation and their involvement in the synthesis of a wide range of chemical compounds causing the enhancement of nutritional quality. Germinated seeds are rich in vitamins, minerals and are also reported to contain phytochemicals important for disease prevention. An increase in the bioavailability of minerals and vitamins has been observed due to germination. In addition, germination is a simple tool that allows enhancing the palatability and digestibility (Brajdes and Vizireanu, 2012).

Germination of seed is a complex sequence of the processes during which various requirements (temperature, aeration and moisture) should be met; water uptake is an essential and dynamic process towards rehydration and initiation of metabolic process leading to germination and is a function of the various water potentials. It is characterized by four distinct phases (Rai, 2013). The germination percentage of buckwheat at 24.5℃ is 84% (Aliyas *et al.*, 2015). Higher temperature during germination may affect germination percentage. This might be due to damage to the seed structure. Higher temperature might alter enzymatic activity and reduce quantity of amino acids available (via RNA synthesis), thereby modifying metabolic reactions that reduce embryo development and restrict seed germination (Morbeck de Oliveira *et al.*, 2013).

2.11.2.1 The imbibition phase

It is an initial phase and can take place in inert, dead and viable seeds. It is attributed to the passive water uptake and absorption by the seed colloids and into the crevices and interstices of the seed cover and tissues. Towards the end of this phase and the beginning of the transition phase, the water uptake becomes an active process as it temperature dependent there is an increase in respiration rate and in some cases it becomes light sensitive (Rai, 2013).

2.11.2.2 The transition phase

The changeover to this lag phase is not distinguishable and can take place in the dormant and non-viable seeds. It is also known as the pause phase, since in this phase the major metabolic events take place in preparation for radicle emergence. Any condition affecting the hydration level attained during imbibition may retard or even inhibit germination (Rai, 2013).

2.11.2.3 The growth phase

It occurs only in viable, non- dormant seeds. It concides with the radicle profusion and is thus associated with the establishment of cellular division and extension and a rapid increase in water uptake rate. Non-president cotyledons do not achieve growth phase of water, eventually their water content declines as degradation occurs (Rai, 2013).

2.11.2.4 Seeding establishment phase

It is marked by the depletion of stored reserves disintegration of cotyledons, increase in photosynthesis. The duration of each of these phases depends on certain in-herient properties, eg. hydrable substrate levels, seed coat permeability, seed size, oxygen uptake and on prevailing condition during hydration (Rai, 2013).

2.11.3 Kilning (drying)

During kilning water is removed from the green malt. Malt is kilned to produce a friable, stable-on-storage product, from which roots can be easily removed. Kilning consists of passing a flow of warm dry air through a bed of malt at various rates and at increasing temperatures to dry the malted grain. The survival of enzymes in malt is greatly influenced by the temperature and time of the kilning regime (Nic Phiarais and Arendt, 2015).

Common buckwheat is normally kilned at 40℃ for about 40h. All enzymatic activities were found to decrease during the kilning stage. After prolonged kilning at 40°C, inactivation of hydrolytic enzymes occurred; two-stage kilning for shorter periods is recommended (Nic Phiarais *et al*., 2005).

2.12 Physical changes during malting

During steeping the grains swell and increase in its volume by about a quarter. Space is allowed in the steep tanks to accommodate the swollen grains. The first microscopic indication of germination after casting is the appearance of chit. The white coleorhizae or root-sheath breaks through the pericarp and testa and produces from the base of the corn. In time seminal roots also called rootlets, culms, cooms or malt sprouts brusts 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. 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 enzymes partial dissolution of some cell walls. This process cytolysis begins in the compressed layer, adjunct to the scutlellum and progressively spreads through starchy endosperm towards the apex of the grains (Poudel, 2012).

2.13 Chemical changes during malting

2.13.1 Carbohydrate

The percentage of starch decreases and the composition of the remaining starch alter. The proportion of amalyase increases. The overall pentosans content of starchy endosperm declines while that of husk remain unchanged. The partial hydrolysis of the insoluble hemicellulose appears to rise to the soluble gum, which in turn when hydrolyzed further provides monosaccharides. 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 klining but the sucrose often increases in amount. Maltose also increased. The grain at the first respiratory substrate uses up raffinose before other sugars is mobilized to support the growth of embryo (Shrestha, 1995). The decrease in carbohydrate content on malting could be attributed to metabolism. The carbohydrates may have been digested into simple sugars

by amylolytic enzymes which are rapidly taken up by the growing embryo to serve as its energy source during germination as reported in Ogbonna *et al.* (2012).

During germination, there was a decrease in storage carbohydrates and an increase in total soluble and reducing sugars due to the energy needs of the growing plant. The raffinose and stacchyose contents decreased quickly during the first 24 hr of germination and almost disappeared after 48 hr of the process (Colmenares *et al.*, 1990). The content of reducing sugar increased dramatically due to the hydrolysis of carbohydrates by the activation of amylase during the germination process. As a result, the taste and digestibility of buckwheat can be improved because of the increase of reducing sugar (Zhang *et al*., 2015).

Germination may increase or decrease in carbohydrate content. The decrease in carbohydrate content might be due to active respiration process during soaking and germination. On the other hand, total sugar, reducing and non-reducing sugar contents increased after germination, this is might be due to increase the activities of α-amylase and β-amylase enzymes (Devrajan *et al.*, 2017).

2.13.2 Protein

One of the most important chemical changes that occur during germination is the degradation of the protein and their conversion into soluble peptides and amino acids to provide substrates for the plant's development which can result in the changes in protein content and size distribution, as well as protein properties without any chemical modifications. The germination process was also one of methods used to improve the functionality of seed protein. During germination the content of crude protein increased and protein was degraded to increase the soluble protein content and free amino acids (Li and Xu, 2015). The increase in protein content after malting was found in buckwheat and amaranth (Omary *et al.*, 2012). Similar increment was also found in Chauhan and Singh (2013). The increase in proteins might be attributed to the dry weight losses through respiration during malting (Singh *et al.*, 2015).

During germination the proteins are hydrolysed with increased proteolytic activity (El-Mahdy and El-Sebaiy, 1982). The proteinases of germinating buckwheat hydrolyze storage proteins into amino acids and small peptides. During malting there was a degradation of proteins in all four fractions of buckwheat, along with the appearance of new protein bands

in the albumin and glutelin fractions. This is possibly indicative of enzyme synthesis, whereas the complete disappearance of protein bands in the globulin and glutelin fractions is most likely a result of complete protein degradation. The results concurred with changes observed in the free and total amino acid level (Nic Phiarais and Schehl, 2008).

Germination with or without light, caused an increase in non-protein nitrogen and a substantial decrease inprotein nitrogen due to the hydrolysis of storage proteins that released peptides and free amino acids (Urbano *et al.*, 2005). The amino acid patterns of protein indicate balance between synthetic and degradation process. It is believed that during germination the newly formed proteins differ in their amino acid contents from reserve protein (Chen and Thacker, 1978). The protein of the germinated was more soluble than the un-germinated. This might be due to the high proteolytic activity during germination, which will lead to an increase in the protein solubility resulting from hydrolysis of the storage proteins. Germination causes activation of intrinsic amylases, proteases, phytases and fiber-degrading enzymes, thereby increasing nutrient digestibility. The activity of intrinsic proteases in germinated grains leads to an increase in-vitro protein digestibility (Manukumar *et al.*, 2014).

2.13.3 Lipids

During germination, extensive hydrolysis of triacylglycerols occurred which demonstrated this behaviour by observing that the lypolytic potential increases markedly during the malting process. The reduction in total lipids was probably correlated with the lipolytic activity. During germination, there is a need for a large amount of energy and building materials that must be produced by respiration and other metabolic processes. In the final step of malting the total lipid content does not vary significantly. During kilning, the humidity is lowered, the germination and the structure modification are stopped, the activity of lipases is stopped too and, as a consequence, the lipid content remains unaltered (Bravi *et al.*, 2012). The decrease in fat content after malting me be due to total solid loss during soaking prior to germination or use of fat as an energy source in germination process which was used as the major source of carbon for seed growth as fatty acids are oxidized to carbon dioxide and water to generate energy for germination (Sharma *et al.*, 2016).

In the germinating seeds the stored fats are metabolized by lipase enzymes. The enzyme is the same that causes the synthesis of fats from glycerol and fatty acids. When water is diminishing, the enzyme plays synthesizing role. When the water content is increasing or seeds under germination, the enzyme play catabolizing role. The principle products formed from fats during germination have been shown to carbohydrate. Neither fatty acids nor the glycerol accumulate in large concentration during germination (Rai, 2013).

2.13.4 Vitamins

Cereal grains have been reported as rich source of certain B-vitamins, and tocols (vitamin E). Ascorbic acid has been found in pseudo-cereals i.e buckwheat. Germination has been investigated to increase the content of tocols in cereal products and an increase in vitamin E during the germination. Folic acid deficiencies have been connected to megaloblastic anemia, birth defects (neuronal tube defect), and increased risk for cardiovascular disease and certain types of cancer. Germination has been repeatedly reported as way to improve folate content. Germination increased the β-carotenes in buckwheat which is present in small amounts (Hubner and Arendt, 2013).

2.13.5 Minerals

During germination and seedling development mineral elements are released from their storage compounds in grains to be available for the growing embryo. Simultaneously the loss of dry matter (mostly of non-fibrous carbohydrates) due to respiration is an important factor in concentrating the mineral elements and bioactive compound contents (Pongrac *et al.*, 2016). The beneficial effect of germination on iron and calcium availability may probably be attributed to the decrease in phytate content as a result of germination during germination, de novo synthesis and activation of endogenous phytases with concomitant decrease in phytate content occurs in cereals and legumes (Luo *et al.*, 2013). The increase in ash content during malting reported that germination and fermentation would increase the mineral content due to an increase in fitase enzyme activity during germination. The enzyme will hydrolyze the bond between the protein-enzyme minerals become free, therefore increasing the availability of minerals (Narsih *et al.*, 2012). The increase in calcium content may be due to dry matter loss of water soluble constituents during steeping and washing (Tizazu *et al.*, 2011). The phosphorous content increases during malting (Pongrac *et al.*, 2016). The increment in phosphorus content may be due to hydrolysis of

phytate by the enzyme phytase, which is released during germination (Abdelrahaman *et al.*, 2007).

Increased mineral availability during germination may be due to increased phytase activity, which resulted in decreased content of phytate in sprouts. Antinutrients like polyphenols and saponins are also known to hinder the availability of minerals, which are catabolized during germination leading to improvement in mineral availability (Saleh *et al.*, 2013). The iron content might be decreased during malting. The decrease in iron content during malting may be due to the leaching of minerals during soaking (Kumari *et al.*, 2014).

2.13.6 Crude fiber

Crude fiber increases after malting. The increase in crude fiber after malting may be due to the synthesis of carbohydrates such as hemicellulose and cellulose (Banusha and Vasantharuba, 2013). The similar increase during malting has been found in sorghum (Elkhier and Hamid, 2008)The increase in crude fiber after malting is also found in (Chowdhury and Rahman, 2017).

2.13.7 Other changes

The tannin content decreased significantly during germination. The loss of tannins, therefore, can be attributed to leaching of tannins into the growth medium as indicated by the significant browning of supporting filter paper during germination (Elmaki *et al.*, 1999). The decrease in tannin content during malting might be due to tannins leaching out of the grain into water during soaking and germination and binding of polyphenols with other organic substances such as carbohydrate or protein (Khoddami *et al.*, 2017).

Germination increases antioxidant activity of buckwheat. The increase of antioxidant activities seemed to be related to the biochemical metabolism of buckwheat during germination, which resulted in raising the contents of antioxidant compounds such as polyphenolics (Zhang *et al.*, 2015). The antioxidant activity of common buckwheat was 7.75% which increases after malting (Jhon, 2017). The increase in antioxidant activity during malting is due to many metabolic changes during germination such as increase in the activity of the endogenous hydrolytic enzymes during germination (Alvarez-Jubete *et al.*, 2010).

The total polyphenol content may decrease or increase after malting. The decrease in TPC upon malting might be due to leaching of polyphenols into the soaking medium during the malting process. The kilning stage of the malting process performed in the present study might have led to phenolics forming insoluble complexes (via hydroxyl groups) with proteins, carbohydrates and minerals, or might have caused heat-induced polymerization or degradation, leading to a decrease in the apparent concentration of phenolics (Khoddami *et al.*, 2017). The total polyphenol content might be increased. The increase in polyphenol content during malting might be due to the the action of endogenous esterase activated during germination which can lead to the release of cell wall bound phenolic compounds (Carciochi *et al.*, 2014).

The flavonoid content increases after malting (Kreft and Jane., 2013). The increase in flavonoid content may be attributed to the bound flavonoid compounds becoming free by the action of enhanced hydrolytic enzyme activity (Martinez *et al.*, 2013). The flavonoid content has been increased from 13.66 mg/100 g to 283.43 mg/100 g during malting (Brajdes and Vizireanu, 2012).

Part III

Materials and methods

3.1 Raw materials

The Nepalese buckwheat varieties (common and tartary buckwheat) were taken from NARC which was collected from Dolakha District, Nepal.

3.2 Preparation of buckwheat malt

3.2.1 Cleaning

Seeds were screened to remove impurities such as stones, strings, weed seeds, etc. The sample was divided with sampling method. The sample was roughly screened by passing through a coarse sieve to retain large impurities and over a fine sieve to retain grain and to allow small impurities such as sands to pass through. Seeds were then washed to remove impurities which have attached with the grains.

3.2.2 Steeping

The cleaned seeds were then immersed in water in a bowl. The light material present in the sample was skimmed off. Steeping was done for 20 h in room temperature which brought moisture content to the required level of 45-50%.

3.2.3 Draining and germination

Steeped water was drained off. The steep grain was first collected in a muslin cloth and swirled in order to drain excess water. The grains were spread over the germination paper and covered with the germination paper and kept for germination in open environment at temperature 26-28°C and relative humidity 85-86% in open environment and 24.5°C and relative humidity 93.5% in humidity chamber. During germination the grains were moisturized by sprinkling water at 6 h interval and mixed gently in order to equalize temperature and to aerate the mass. The germination of buckwheat is shown in Fig. 3.1

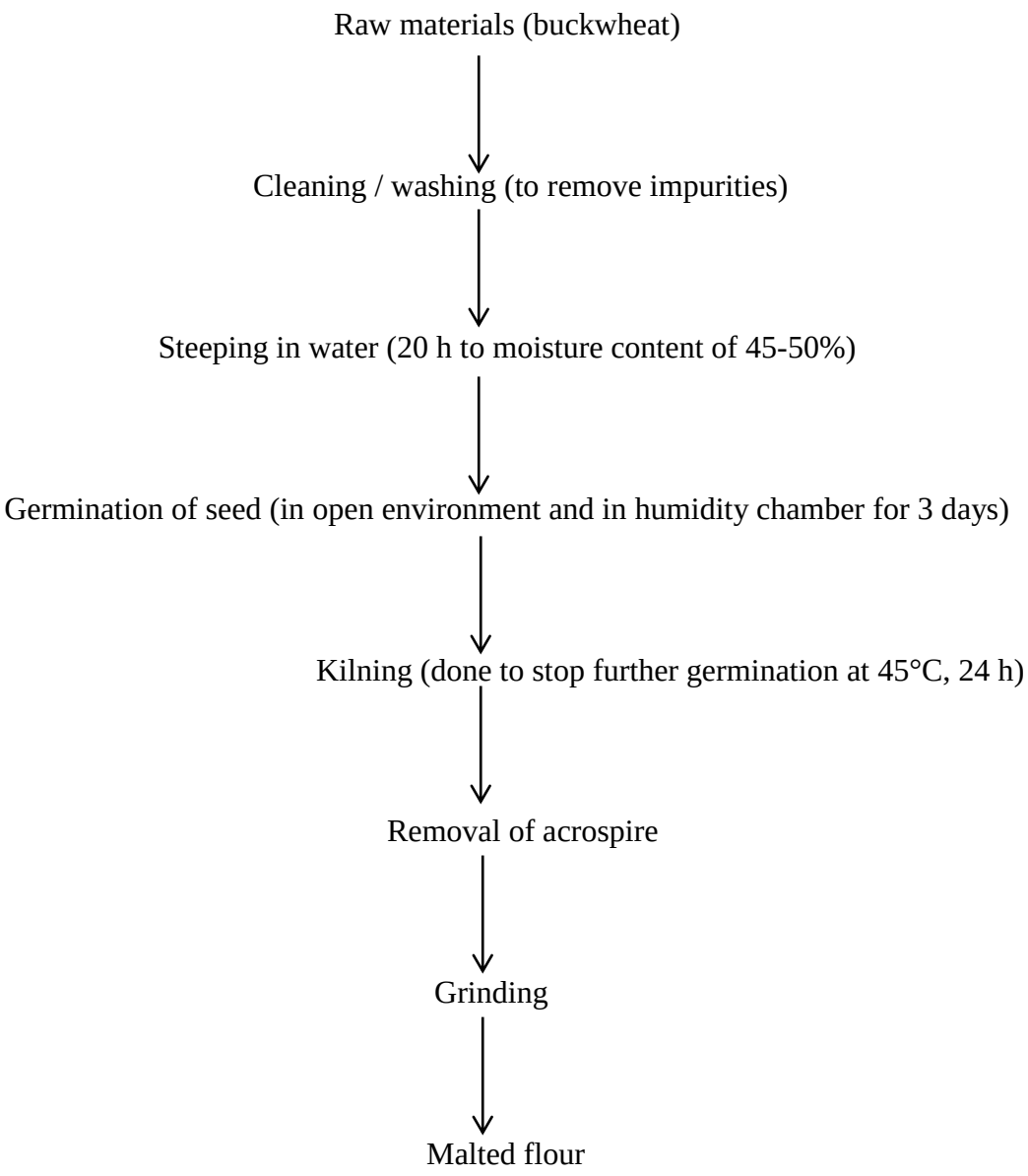

Fig. 3.1 Flow diagram showing procedure of malting

3.3.4 Kilning

The buckwheat varieties germinated in both open environment and humidity chamber was taken in cabinet dryer to stop further germination. Drying was carried out at 45°C for 24 h. The grains were then rubbed and sprouts were removed with the help of screen. The malted grains are then grind in the mixture and the sieved using ASTM 20 (0.850 mm) and ASTM 40 (425 µm) sieves. The flour is then taken for analysis.

3.3 Experimental procedure

3.3.1 Physical analysis

3.3.1.1 1000 kernel weight

The 1000 kernel weight of raw materials and final products were determined by measuring the weight of 1000 kernels of sorghum grains after selecting the appropriate sample size by quartering method (Buffo *et al.*, 1998).

3.3.1.2 Bulk density

The bulk density was measured by pouring the grains into the funnel-shaped hopper, the hopper was centered over the measuring bushel, the hopper valve was opened quickly, and the grains were allowed to flow freely into the measuring bushel. After the bushel was filled, the excess material was leveled off with gentle zigzag strokes using the standard Seedburo striking stick. The filled measuring bushel was then weighed, and the mass of grains in the bushel was determined by subtracting the mass of the measuring bushel itself (Clementson *et al.*, 2010). The bulk density (ρ) of grain was then calculated using the following expression:

$$\text{Bulk density} = \frac{\text{Mass of grain}}{\text{Volume of bushel}}$$

3.3.1.3. Sphericity

Sphericity of grain was determined as mentioned in (Simonyan *et al.*, 2007).

$$\text{Sphericity} = (lbt)^{1/3} / l$$

Where, l = length of grain

b = breadth of grain

t = thickness of grain

3.3.2 Chemical analysis

All the chemical analysis was done on wet basis and the results were presented on dry basis.

3.3.2.1 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℃ to constant weight. The difference in weight was the water that has evaporated in Ranganna (1986).

3.3.2.2 Determination of crude protein

The crude protein content of the sample was determined by estimating nitrogen content in the sample by macro Kjeldahl method using the factor 6.25 as described in Ranganna (1986).

3.3.3.3 Determination of crude fat

The crude fat content of the sample was determined by solvent extraction method as described in Ranganna (1986).

3.3.3.4 Determination of ash content

Ash content of the sample was determined using electric muffle furnace. Finely ground sample in silica dish was ashed in muffle furnace at 550±10°C until constant weight is obtained as described in Ranganna (1986).

3.3.3.5 Determination of crude fibre

The crude fibre content of the sample was determined gravimetrically by acid and alkali treatments as described in Ranganna (1986).

3.3.3.6 Determination of iron

The iron content of the sample was determined by colorimetric method as described by Ranganna (1986).

3.3.3.7 Determination of calcium

Calcium content was determined by titration method as mentioned by Ranganna (1986).

3.3.3.8 Determination of total phenolic content

Extraction

The seeds are milled to flour. 5 g of malted flour was taken and 30 ml of 80 % methanol was added to sample. The mixture was rotated in hot plate and filtered. This process was repeated for 2 times and then final filtered sample was made to 100 ml for analysis (Amorim *et al.*, 2008).

Determination

About 1 ml of extracted sample was taken in 25 ml volumetric flask. 9 ml of distilled water was added and after that 1 ml of folin-ciocatteus reagent was added and shaked well. After 5 min, 10 ml of 7% Na_2CO_3 was added and volume was made with distilled water. Incubation was done for 90 min at room temperature. Finally reading was taken at 765 nm (Mahdavi *et al.*, 2010). The polyphenol content was determined with the help of standard curve.

3.3.3.9 Determination of flavonoid (rutin) content

0.5 ml of extract was taken and 2 ml of distilled water was added. After that 0.15 ml of 5% $NaNO_2$ solution was added in it and allowed to stand for 6 min. After 6 min 0.15 ml of 10% $AlCl_3$ was added in it and again it was left for 6 min. After leaving for 6 min 2 ml of 4 % $NaNO_2$ solution was added in it and after that 0.2 ml of distilled water was added and finally it was allowed to leave for 15 min. Finally absorbance was taken at 510 nm (Samatha *et al.*, 2012). From the standard curve flavonoid content was determined.

3.3.3.10 Determination of tannin content

0.1 ml of extract was taken and 0.5 ml of Folin-Denis reagent was added in it. After that 1 ml of 0.5% Na_2CO_3 was added in it and then 10 ml of volume was made up with distilled

water. It was then allowed to stand for 30 min and readings were taken at absorbance 755 nm (Rajan *et al.*, 2011). From the standard curve tannin content was determined.

3.3.3.11 Determination of antioxidant activity

0.1 ml of extract was taken and volume was made upto 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).

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

3.3.3.12 Germination percentage

The germination percentage is calculated as reported in (Nahar *et al.*, 2009).

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds tested}} \times 100\%$$

3.4 Statistical analysis

For all chemical analysis two replicated of the same sample was used for the determination of each constituent. Mean values with standard deviation were computed. Data were subjected to analysis of variance and read at 95 % confidence level using GenStat Release 7.1 (Discovery Edition 3 developed by VSN International Limited).

Part IV

Results and discussion

Two varieties of buckwheat *mithe* and *tite phapar* (common and tartary buckwheat) were taken from NARC which was collected from Dolakha district of Nepal. Buckwheat malts were prepared by germinating at temperature 26-28°C, 85-86% RH in open environment and 24.5°C, 93.5% RH in humidity chamber. The physical properties, germination percentage, proximate composition, flavonoid, tannin, polyphenol content, antioxidant activity and mineral composition were determined and comparison was done between malted and unmalted buckwheat.

4.1 Physical properties of unmalted buckwheat varieties

The mean value of triplicates of buckwheat varieties for sphericity, 1000 kernel wt., bulk density, particle density and porosity are presented in Table 4.1

Table 4.1 Physical properties of unmalted buckwheat grains

Varieties	Sphericity	Bulk density(Kg/HL)	1000 kernel wt. (g)
Common	0.70 (0.01)	83.9 (2.81)	23.92 (0.60)
Tartary	0.58 (0.01)	69.54 (0.35)	18.13 (0.27)

* Values are the mean of triplicates and the values in bracket indicates standard deviation

The sphericity and 1000 kernel weight of common buckwheat is similar to the result as reported in Kaliniewicz *et al.* (2015) and Unal *et al.* (2017). The bulk density of common buckwheat is similar to the result as reported in Unal *et al.* (2017). The sphericity and bulk density of tartary buckwheat was 0.58 and 69.54. Similarly the 1000 kernel weight of tartary buckwheat is in the range as reported by Leder (2009).

4.2 Germination Percentage of buckwheat Varieties

Table 4.2 Germination percentage of buckwheat varieties in O.E and H.C

Varieties	Open environment (%)	Humidity chamber (%)
Common buckwheat	80.33	84.85
Tartary buckwheat	81.01	85.95

The germination percentage of common and tartary buckwheat was 80.33 % and 81.01 % in open environment and 84.85% and 85.95% in humidity chamber. The temperature in humidity chamber was 24.5°C. The germination percentage at 24.5°C is similar to the result as reported by Aliyas *et al.* (2015). Here the germination percentage is less at 27 °C than at 24.5°C. This might be due to damage to the seed structure. Higher temperature might alter enzymatic activity and reduce quantity of amino acids available (via RNA synthesis), thereby modifying metabolic reactions that reduce embryo development and restrict seed germination as reported by Morbeck de Oliveira *et al.* (2013).

4.3 Proximate composition

The proximate analysis gives data about the nutritional components of foods. The proximate composition such as moisture content , crude protein, crude fiber, crude fat and ash content of unmalted and malted common and tartary buckwheat flour in open environment and in humidity chamber were determined as per the standard procedure and results are expressed in dry basis percentage in Table 4.3 and Table 4.4

Table 4.3 Proximate composition of common buckwheat before and after malting in open environment and in humidity chamber (% dry basis except moisture)

Parameters	Before malting	Malt (open environment)	Malt (humidity chamber)
Moisture	12.8 (0.098)	7.05 (0.043)	6.62 (0.098)
Total ash	2.43 (0.010)	2.51 (0.082)	2.59 (0.008)
Crude fat	3.05 (0.01)	2.553 (0.030)	2.436 (0.032)
Crude fibre	0.963 (0.015)	1.686 (0.009)	1.910 (0.007)
Crude protein	13.59 (0.020)	14.44 (0.306)	15.28 (0.296)
Carbohydrate	79.958 (0.022)	78.917 (0.329)	77.649 (0.318)

*Values are the means of triplicate determination and the values in bracket indicates standard deviation

Table 4.4 Proximate composition of tartary buckwheat before and after malting in open environment and in humidity chamber (% dry basis except moisture)

Parameters	Before malting	Malt (open environment)	Malt (humidity chamber)
Moisture	12.5 (0.1)	5.58 (0.018)	5.01 (0.287)
Total ash	2.18 (0.010)	2.27 (0.738)	2.32 (0.045)
Crude fat	2.823 (0.020)	2.345(0.005)	2.223 (0.005)
Crude fibre	1.8 (0.1)	1.941 (0.008)	1.962 (0.016)
Crude protein	14.60 (0.444)	17.053 (0.133)	18.031(0.33)
Carbohydrate	78.585 (0.37)	76.496 (0.145)	75.335 (0.06)

* Values are the mean of triplicates and the values in bracket indicates standard deviation

4.3.1 Moisture content

The moisture content of unmalted common buckwheat was found to be 12.8% and tartary buckwheat was found to be 12.5%. The moisture content is found to be slightly higher than reported by Poudel (2012), who analyzed moisture content for common buckwheat (11.81%) and tartary buckwheat (11.14%). The moisture content of unmalted common buckwheat has found to be 11.4% as reported by Devrajan *et al.* (2017), whereas the moisture content of unmalted tartary buckwheat was in the range 10.2-11.5% as reported by Thakur *et al.* (2017). The variation in moisture content may be due to genetic and environmental factors.

The moisture content after malting of buckwheat has been decreased to 5% after drying at 40℃ as reported by Nic Phiarais *et al.* (2005). Similarly after malting the moisture content was decreased to 7.05% in open environment and in humidity chamber it was decreased to 6.62% for common buckwheat and for tartary buckwheat it was decreased to 5.58% in open environment and 5.01% in humidity chamber.

4.3.2 Ash content

The ash content of common buckwheat before malting was 2.43 % similar to the research that was conducted by Qin *et al.* (2010) and 1.4 %-2.5% by Dogra and Awasthi (2015). The ash content of un-malted tartary buckwheat is found to be 2.18 % which is similar to the report in the range of 1.8-2.3% reported by Thakur *et al.* (2017). During malting the common buckwheat percentage increased to 2.51% in open environment and 2.59% in humidity chamber and in tartary buckwheat 2.27% in open environment and 2.32% in humidity chamber. During malting ash content of buckwheat varieties seen to be increased this increase in ash content has been found in similar other findings by Chowdhury and Rahman (2017). The increased in ash content during malting is reported that germination and fermentation would increase the mineral content due to an increase in fitase enzyme activity during germination. The enzyme will hydrolyze the bond between the protein-enzyme minerals become free, therefore increasing the availability of minerals as reported in Narsih *et al.* (2012).

4.3.3 Fat content

The fat content of common buckwheat before malting was found to be 3.05%. The fat content of common buckwheat was reported to 3.06% in Zhang *et al*. (2015) and 1.6%-2.9% by Dogra and Awasthi (2015) and 3.16% in Sindhu and Khatkar (2016). The fat content of common buckwheat after malting in open environment was found to 2.553% whereas in humidity chamber was 2.436 %. The fat content of unmalted tartary buckwheat was found to be 2.823%. The fat content of unmalted tartary buckwheat has been reported to 2.45% by Bonafacciaa *et al.* (2002) and 2.5%-2.8% by Ahmed *et al.* (2014) and 2.83% by Qin *et al.* (2010) whereas malted has 2.345% in open environment and 2.223% in humidity chamber. The decrease in fat content after malting me be due to total solid loss during soaking prior to germination or use of fat as an energy source in germination process which was used as the major source of carbon for seed growth as fatty acids are oxidized to carbon dioxide and water to generate energy for germination as reported in Sharma *et al.* (2016). It has been reported that the decrease in fat content might be due to increase activity of lipase enzyme Devrajan *et al.* (2017).

4.3.4 Crude fiber

The crude fiber content of unmalted common buckwheat was found to be 0.96%. This content is found in the range of 0.77%-0.96% that has been reported by Khan *et al.* (2013). Similarly the content of very low crude fiber is also found in Baljeet *et al.* (2010). Similarly the crude fiber content of unmalted tartary buckwheat was found to be 1.8% which is similar to the result reported by Qin *et al.* (2010). The crude fiber content of malted common buckwheat was found to be 1.68% in open environment and 1.91% in humidity chamber. Here crude fiber percentage increases about 40%. This increase in fiber percentage after malting is similar to the result reported in Chowdhury and Rahman (2017). Similarly the malted tartary buckwheat has crude fiber of 1.94% in open environment and 1.96% in humidity chamber. This result is similar to the germination of finger millet as there is slightly increase in crude fiber percentage which may due to the synthesis of carbohydrates such as hemicellulose and cellulose according to Banusha and Vasantharuba (2013). The similar increase during malting has been found in sorghum as reported by Elkhier and Hamid (2008)

4.3.5 Protein content

The protein content in my studied unmalted common buckwheat was found to be 13.59%. This result is similar to the result that has reported by Sindhu and Khatkar (2016). Similarly the protein content of unmalted tartary buckwheat has found to be 14.60%. This result is similar to the result obtained in Guo *et al.* (2007) and in Qin *et al.* (2010). The protein content of malted common buckwheat is increased to 14.44% in open environment and 15.28% in humidity chamber. Here protein content has been increased after germination or malting which has been seen to be increased in other findings to that has been reported by Devrajan *et al.* (2017). Similarly the protein content of malted tartary buckwheat has also increased to 17.05% in open environment to18.03% in humidity chamber. Increase in protein content has been found in buckwheat and amaranth flour after germination as reported by Omary *et al.* (2012). Similar increment also found in Chauhan and Singh (2013). The differences in protein content in various buckwheat cultivars may be due to cultivars variability and growing conditions has reported in Qin *et al.* (2010). The increase of these proteins might be attributed to the dry weight losses through respiration during malting as reported in Singh *et al.* (2015).

4.3.6 Carbohydrate content

The carbohydrate content of unmalted common and tartary buckwheat was found to be 79.958% and 78.585% which is similar to the result as reported in Khan *et al.* (2013). The carbohydrate content of malted common buckwheat was 78.917% and 77.649% in open environment and in humidity chamber whereas malted tartary buckwheat has 76.496% and 75.335% in open environment and in humidity chamber. The decrease in carbohydrate content on malting could be attributed to metabolism. The carbohydrates may have been digested into simple sugars by amylolytic enzymes which are rapidly taken up by the growing embryo to serve as its energy source during germination as reported in Ogbonna *et al.* (2012).

4.4 Effect of malting on phytochemicals of buckwheat varieties

The flavonoid, tannin, polyphenol and antioxidant activity of unmalted and malted buckwheat varieties in open environment and in humidity chamber were determined as per

the standard procedure provided. Germination in the buckwheat grains had some effects on nutritional components and other anti-nutritional factors present in grains.

4.4.1 Flavonoid content

The flavonoid content of unmalted and malted common and tartary buckwheat in open environment and in humidity chamber is shown in Fig. 4.1

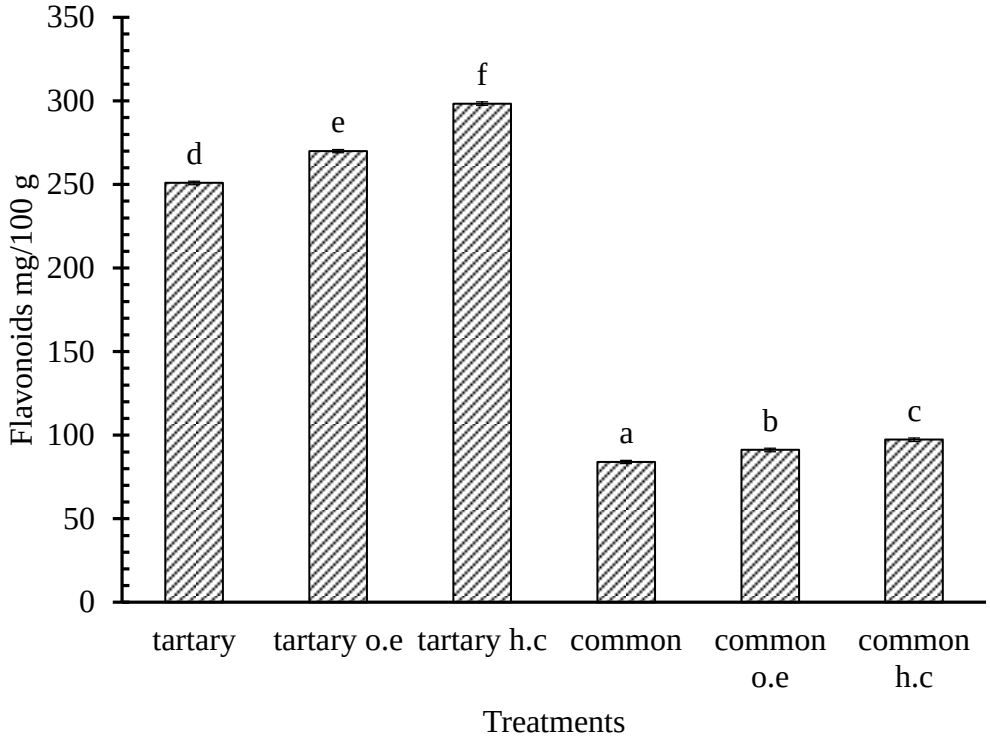

Fig. 4.1 Comparison of unmalted and malted buckwheat varieties in open environment and in humidity chamber on TFC

The flavonoid content of unmalted common buckwheat was found to be 84 mg/100 g, malted common buckwheat in open environment and in humidity chamber was found to be 91.2 mg/100 g and 97.3 mg/100 g. Similarly the flavonoid content of unmalted tartary buckwheat was found to be 250.9 mg/100 g and malted was found to be 270 mg/100 g in open environment and 298.4 mg/100 g in humidity chamber. The statistical analysis (two-way ANOVA) showed that the flavonoids content of malted buckwheat was significantly increased ($p<0.05$) during malting.

Here, the flavonoid content of unmalted common buckwheat was found to be similar to the result reported by Qin *et al.* (2010). The flavonoid content of buckwheat has been increased from 13.66 mg/100 g to 283.43 mg/100 g during malting as reported by Brajdes and Vizireanu (2012). The tartary buckwheat flavonoid content is increased by 2 folds which has been reported by Kreft and Jane (2013) which is similar to the result of this study. The increase in flavonoid content may be attributed to the bound flavonoid compounds becoming free by the action of enhanced hydrolytic enzyme activity as reported in Martinez *et al.* (2013). Another study reported that increase in flavonoid content may be due to the increase of PAL activity during the initial stage of buckwheat germination Zhang *et al*. (2015).

4.4.2 Tannin content

The tannin content of unmalted and malted common and tartary buckwheat in open environment and in humidity chamber is shown in Fig. 4.2

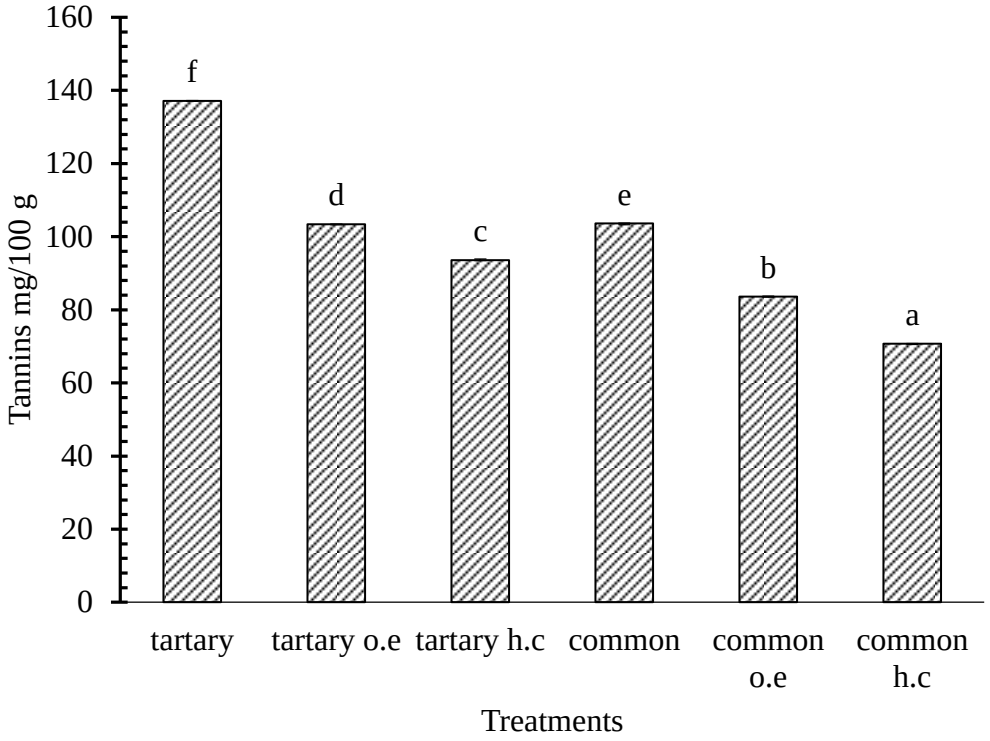

Fig. 4.2 Comparison of unmalted and malted buckwheat varieties in open environment and in humidity chamber on tannin content

The tannin content of unmalted common buckwheat was found to be 103.60 mg/100 g and malted was found to be 83.57 mg/100 g and 70.69 mg/100 g in open environment and in humidity chamber. Similarly the tannin content of unmalted tartary buckwheat was found to be 137.10 mg/100 g and malted was found to be 103.6 mg/100 g in open environment and 93.34 mg/100 g in humidity chamber. The statistical analysis (two- way ANOVA) showed that the tannin content of malted buckwheat was significantly decreased ($p<0.05$) during malting.

The tannin content of malted common and tartary buckwheat has similar result reported by Rai (2013). The decrease in tannin content during malting might be due to tannins

leaching out of the grain into water during soaking and binding of polyphenols with other organic substances such as carbohydrate or protein Khoddami *et al.* (2017) and Sharma *et al.* (2017).

4.4.3 Polyphenol content

The polyphenol content of unmalted common and tartary buckwheat in open environment and in humidity chamber is shown in Fig. 4.3

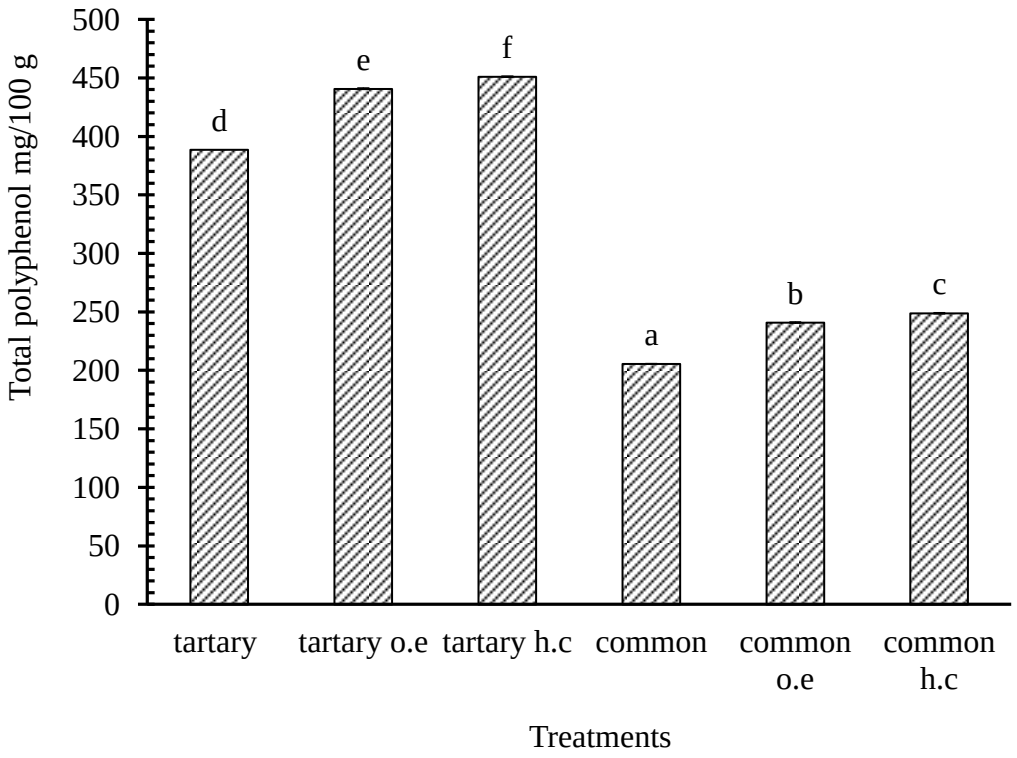

Fig. 4.3 Comparison of unmalted and malted buckwheat varieties on TPC in open environment and in humidity chamber

The polyphenol content of unmalted common buckwheat was found to be 205.628 mg/100 g and unmalted tartary buckwheat was found to be 388.59 mg/100 g. Similarly the content increases to 240.88 mg/100 g and 248.903 mg/100 g for common buckwheat and 440.766 mg/100 g and 451 mg/100 g for tartary buckwheat in open environment and humidity chamber.

Statistical analysis (two- way ANOVA) showed that the total polyphenol content increases more in humidity chamber than in open environment during malting ($p<0.05$). The content of unmalted common buckwheat polyphenol content is similar to the result reported in Vollmannová *et al.* (2013). The unmalted tartary buckwheat polyphenol is similar to the result reported in Molinari *et al.* (2017). The increase in polyphenol content after malting is found in Brajdes and Vizireanu (2012) and Zhang *et al*. (2015). The increases observed of TPC during germination can be explained by the action of endogenous esterase activated during germination which can lead to the release of cell wall bound phenolic compounds as reported in Carciochi *et al.* (2014).

4.4.4 Antioxidant activity

The antioxidant activity of unmalted and malted common and tartary buckwheat in open environment and in humidity chamber is shown in Fig. 4.4

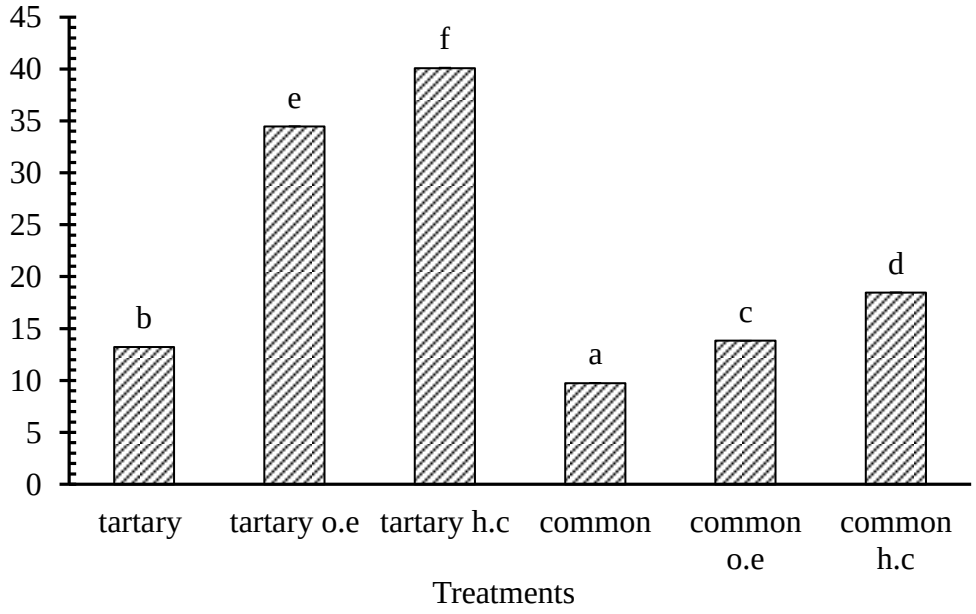

Fig. 4.4 Comparison of unmalted and malted common buckwheat on antioxidant activity in open environment and humidity chamber

The antioxidant activity of unmalted common and tartary buckwheat was found to be 9.742 % and 13.229% whereas the malted common buckwheat has 13.835% and 18.476% in open environment and humidity chamber and the malted tartary buckwheat has 34.447% and 40.096 % respectively.

Statistical analysis (two- way ANOVA) showed that the antioxidant activity increases more in humidity chamber than in open environment during malting ($p<0.05$). The unmalted common buckwheat and unmalted tartary buckwheat antioxidant activity is similar to the result that is obtained in Jhon (2017) and Ren and Sun (2014). The increased in antioxidant activity during malting may be due to increase in flavonoids content as reported in Ren and Sun (2014). The increase in antioxidant activity during malting is due to many metabolic changes during germination such as increase in the activity of the endogenous hydrolytic enzymes during germination. Other common metabolic changes include improved protein and starch digestion, increased sugar and B vitamin content and decreased levels of phytates and proteases inhibitors as reported in Alvarez-Jubete *et al.* (2010).

4.5 Mineral content

The mineral content of common and tartary buckwheat before and after malting is shown in Table 4.5 and Table 4.6

Table 4.5 Mineral composition of common buckwheat before and after malting

Parameters	Before malting	Malt (open environment)	Malt (humidity chamber)
Iron	3.656 (0.237)	3.430 (0.040)	2.416 (0.065)
Calcium	78.436 (0.045)	101.367 (0.059)	104.696 (0.019)
Phosphorous	231.552 (0.092)	415.860 (0.056)	419.166 (0.051)

*Values are means of triplicate determinations ± S.D (mg/100 g, db)

Table 4.6 Mineral composition of tartary buckwheat before and after malting

Parameters	Before malting	Malt (open environment)	Malt (humidity chamber)
Iron	6.879 (0.074)	3.297 (0.038)	3.026 (0.155)
Calcium	86.686 (0.015)	109.518 (0.027)	116.677 (0.032)
Phosphorous	323.824 (0.059)	608.165 (0.054)	611.145 (0.053)

*Values are means of triplicate determinations ± S.D (mg/100 g, db)

4.5.1 Iron content

The iron content of unmalted common buckwheat and tartary buckwheat was found to be 6.879mg/100 g and 6.879 mg/100 g. After malting its content increases to 3.430mg/100 g and 2.416 mg/100 g for common buckwheat and 3.297 mg/100 g and 3.026 mg/100 g for tartary buckwheat in open environment and in humidity chamber. The statistical analysis (two- way ANOVA) showed that the iron content of malted buckwheat was significantly decreased ($p<0.05$) during malting.

The iron content of unmalted common buckwheat is similar to the result reported by Steadman *et al.* (2001) and Nedeljkovic *et al.* (2014). The iron content of unmalted tartary buckwheat was similar to the result reported by Wang Louming *et al.* (1995) and Ikeda *et al.* (2004). The decrease in iron content after malting has been found in legumes as reported in Luo *et al.* (2013). The decrease in iron content may due to leaching of minerals during soaking as reported in soyabean by Kumari *et al.* (2014).

4.5.2 Calcium content

The calcium content of unmalted common and tartary buckwheat was 78.436 mg/100 g and 86.686 mg/100 g. After malting its content increases to 101.367 mg/100 g and 104.696

mg/100 g for common buckwheat and 109.518 mg/100 g and 116.677 mg/100 g for tartary buckwheat in open environment and in humidity chamber. The statistical analysis (two-way ANOVA) showed that the calcium content of malted buckwheat was significantly increased ($p<0.05$) during malting.

The calcium content of unmalted common buckwheat was similar to the result reported by Steadman *et al.* (2001), Ikeda *et al.* (2001) and Akpoghelie *et al.* (2016). After malting, calcium content was found to increase. The increase in calcium content percentage after germination is similar to Omary *et al.* (2012). Whereas the calcium content of unmalted tartary buckwheat found to be in range as reported in Wang Louming *et al.* (1995). The observed increase in calcium after germination might be due to loss of water-soluble constituents during steeping and washing as reported by Tizazu *et al.* (2011).

4.5.3 Phosphorous content

The phosphorous content of unmalted common and tartary buckwheat was 231.552 mg/100 g and 323.824 mg/100 g. After malting its content increases to 415.860 mg/100 g and 419.166 mg/100 g for common buckwheat and 608.165 mg/100 g and 611.145 mg/100 g for tartary buckwheat in open environment and in humidity chamber. The statistical analysis (two- way ANOVA) showed that the phosphorous content of malted buckwheat was significantly increased ($p<0.05$) during malting.

The unmalted common and tartary phosphorous content is similar to the result as reported in Wang Louming *et al.* (1995). Here the phosphorous content has increased after malting by 1.88 times. Similar increment has been found by 1.84 times as reported in Pongrac *et al.* (2016). The increment in phosphorus content may be due to hydrolysis of phytate by the enzyme phytase, which is released during germination as reported in (Abdelrahaman *et al.* (2007)).

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of analysis of common and tartary buckwheat grain and malt following conclusions were drawn.

- The content of ash, protein, crude fiber content increased during malting. But the content of moisture and fat decreased after malting.
- The flavonoid, polyphenol and antioxidant activity increased significantly on malting. The content of flavonoid, polyphenol and antioxidant is more in tartary than in common buckwheat and its content increased more in humidity chamber than in open environment.
- Tannin content decreased significantly after malting. The content decreases more in humidity chamber than in open environment.
- The mineral content calcium and phosphorous increased after malting and its content increased more in humidity chamber than in open environment whereas iron content has been found to be decreased after malting.
- On the basis of proximate, minerals, flavonoid and antioxidant content the best method for malting is in humidity at 24.5°C at 93.5% humidity as compared to open environment.

5.2 Recommendations

- Malting at 24.5°C and 93.5% RH produces good malt rich in rutin which can be used for preparation of gluten free beers in large scale.
- Malting at different germination time, temperature and relative humidity can be done and changes in composition can be studied.

Part VI

Summary

Common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tartaricum*) are two varieties of buckwheat cultivated in Nepal which are very nutritious pseudo cereals. It has given more attention due to its nutritional and medicinal values. Buckwheat varieties from Dolakha district of Nepal were collected and were cleaned and steeped for 20 h and germinated at 26-28°C and 85-86% RH in open environment and 24.5°C and 93.5% RH in humidity chamber for about 3 days. The 3 days germinated buckwheat samples were kilned in cabinet dryer at 45°C for about 24 hours until the moisture content is below 8%. The main aim of malting is to increase the amount of flavonoids (rutin) and antioxidant activity and decrease anti-nutrients such as total polyphenol and total tannins. The kilned malted grains were ground to form powder for various chemical analyses.

The germination percentage of common and tartary buckwheat was 80.33% and 81.01% in open environment and 84.85% and 85.95% in humidity chamber. The mineral content for unmalted common and tartary buckwheat was determined. The mean values for mineral composition for iron, calcium and phosphorous were found to be 3.656, 78.436 and 231.552 mg/100 g for common buckwheat and 6.879, 86.686 and 323.824 mg/100 g respectively. The mineral composition of malted common and tartary buckwheat was determined. The mean values for malted common buckwheat for iron, calcium and phosphorous content were found to be 3.430, 101.367 and 415.860 mg/100 g in open environment and 2.416, 104.696 and 419.166 mg/100 g in humidity chamber respectively. Whereas for tartary buckwheat was found to be 3.297, 109.518 and 608.165 in open environment and 3.026, 116.77 and 611.145 mg/100 g in humidity chamber respectively.

The flavonoid content of unmalted common and tartary buckwheat was determined. The unmalted common and tartary buckwheat flavonoid content was found to be 84 mg/100 g and 250.9 mg/100 g respectively. After malting its content increases to 91.2 mg/100 g and 97.3 mg/100 g for common buckwheat in open environment and humidity chamber respectively. Similarly its content increases to 270 mg/100 g and 298.4 mg/100 g

for tartary buckwheat in open environment and in humidity chamber respectively. The tannin content for common and tartary buckwheat was analyzed. The tannin content for unmalted common and tartary buckwheat was found to be 103.6 mg/100 g and 137.10 mg/100 g respectively. After malting the tannin content decreases to 83.57 mg/100 g and 70.69 mg/100 g for common buckwheat in open environment and in humidity chamber respectively. Whereas for malted tartary buckwheat its content decreases to 103.6 mg/100 g and 93.34 mg/100 g in open environment and in humidity chamber respectively.

The total polyphenol content for common and tartary buckwheat was analysed. The polyphenol content for unmalted common and tartary buckwheat were found to be 205.6 mg/100 g and 388.6 mg/100 g respectively. After malting the content of polyphenol for common buckwheat was increased to 240.9 mg/100 g and 248.9 mg/100 g in open environment and in humidity chamber respectively. Similarly the malted tartary buckwheat polyphenol increased to 440.8 mg/100 g and 451 mg/100 g in open environment and in humidity chamber respectively. The antioxidant activity of unmalted common and tartary buckwheat was found to be 9.742% and 13.229%. The percentage of antioxidant activity was increased to 13.835% and 18.476% in open environment and in humidity chamber for common buckwheat whereas for tartary buckwheat was found to be 34.447% and 40.096% in open environment and in humidity chamber respectively.

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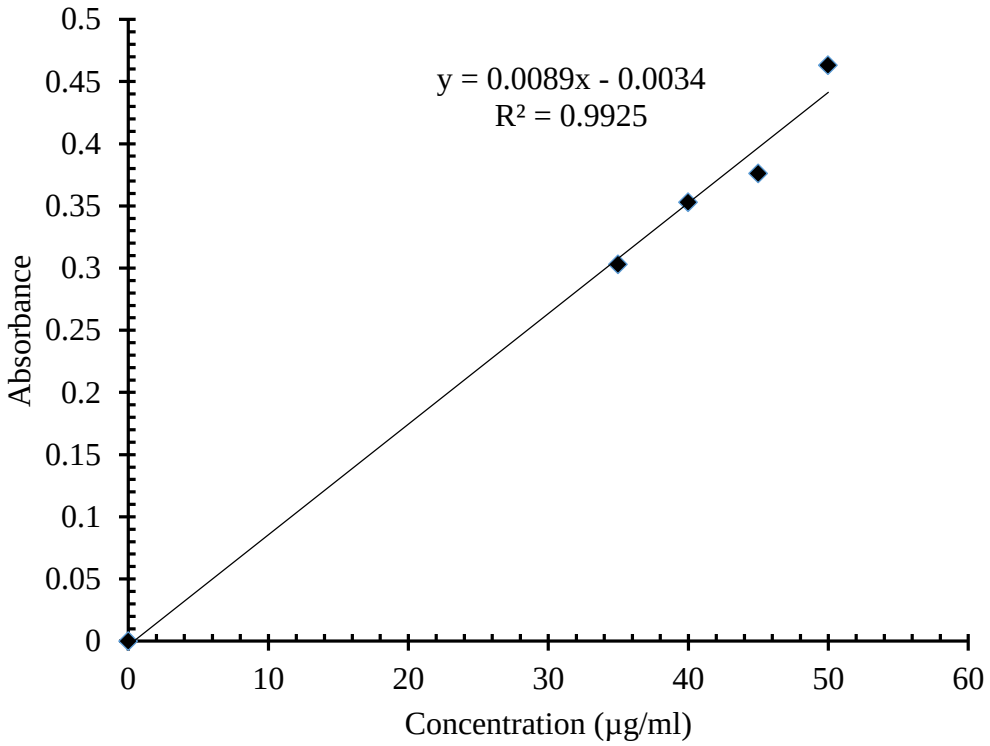
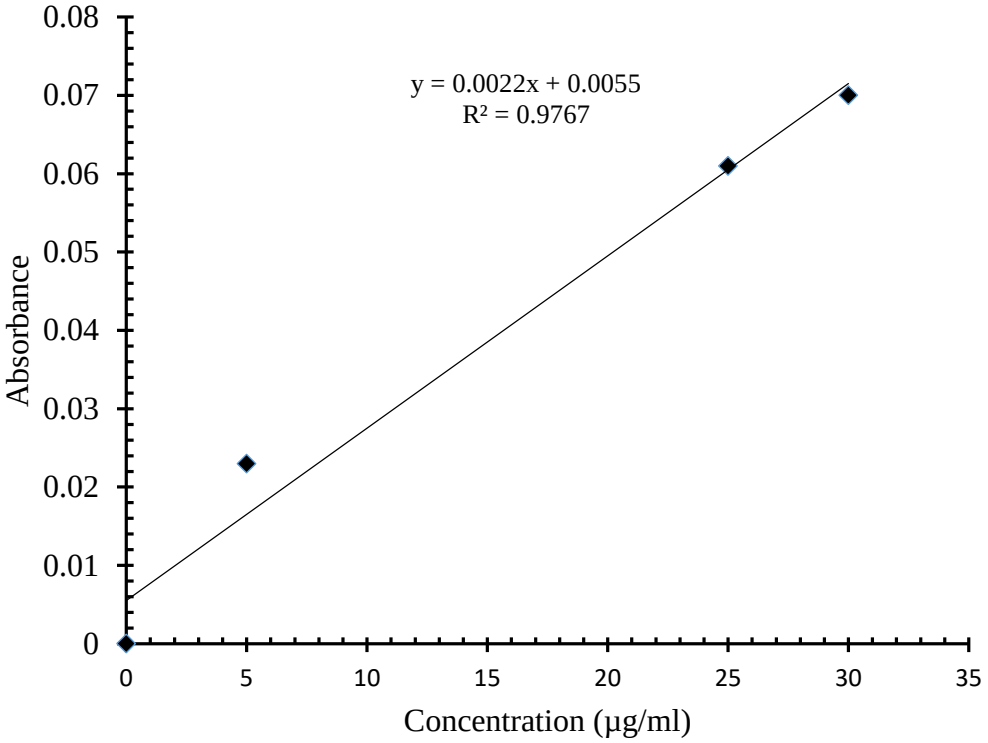
Appendices

Appendix A

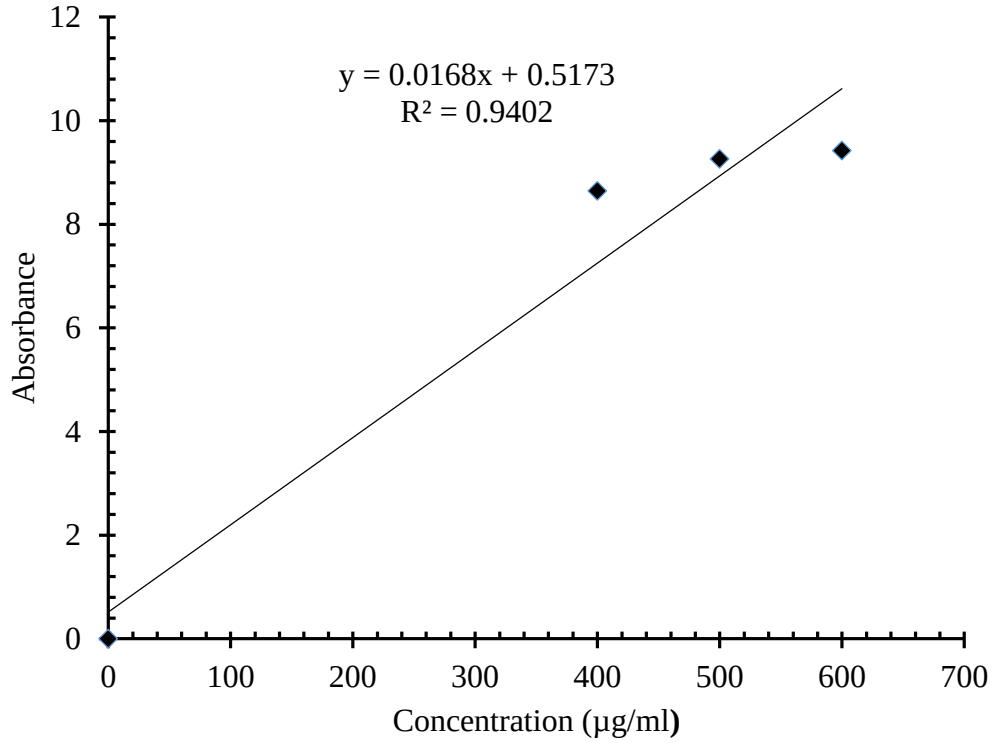
1. Standard curve for flavonoids



2. Standard curve for tannin content



3. Standard curve for Polyphenol content



Appendix B

Table B.1 ANOVA (no blocking) of flavonoid content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatments	5	1.53E+05	3.06E+04	1.73E+05	<.001	7.66E-01
Triplicate	2	1.09E+00	5.43E-01	3.06	0.092	0.5416
Residual	10	1.77E+00	1.77E-01			
Total	17	1.53E+05				

Table B.2 ANOVA (no blocking) of tannin content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatments	5	7686.467	1537.293	1.11E+05	<.001	0.2142
Triplicate	2	0.01368	0.00684	0.49	0.625	0.1514
Residual	10	0.13859	0.01386			
Total	17	7686.619				

Table B.3 ANOVA (no blocking) of polyphenol content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatments	5	1.81E+05	3.62E+04	1.85E+05	<.001	8.05E-01
Triplicate	2	9.94E-01	4.97E-01	2.54	0.128	0.569
Residual	10	1.96E+00	1.96E-01			
Total	17	1.81E+05				

Table B.4 ANOVA (no blocking) of antioxidant activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatments	5	2.36E+03	4.73E+02	4.22E+05	<.001	0.06091
Triplicate	2	2.03E-03	1.01E-03	0.9	0.436	4.31E-02
Residual	10	1.12E-02	1.12E-03			
Total	17	2.36E+03				

Table B.5 ANOVA (no blocking) of calcium content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatments	5	3.10E+03	6.20E+02	7.57E+05	<.001	0.05206
triplicate	2	7.01E-03	3.51E-03	4.28	0.045	0.03681
Residual	10	8.19E-03	8.19E-04			
Total	17	3.10E+03				

Table B.6 ANOVA (no blocking) of iron content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatments	5	37.20978	7.44196	600.95	<.001	0.2025
triplicate	2	0.06367	0.03184	2.57	0.126	0.1432
Residual	10	0.12384	0.01238			
Total	17	37.39729				

Table B.7 ANOVA (no blocking) of phosphorous content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	l.s.d
Treatment	5	3.46E+05	6.92E+04	2.29E+07	<.001	0.1001
Triplicate	2	1.70E-02	8.51E-03	2.81	0.108	0.0708
Residual	10	3.03E-02	3.03E-03			
Total	17	3.46E+05				

Table B.8 Equipment and chemicals

B.8.1 List of equipments used

Physical apparatus	**Physical apparatus**
Electric balance	Grinding arrangement
Hot plate	Bunsen burner
Spectrophotometer	Thermometer
Whatman filter paper	Vacuum filter
Soxhlet apparatus	Muffle furnace
Hot air oven	Cabinet dryer
Desiccator	Incubator
Cuvet	Humidity chamber
Glasswares (Beaker, Volumetric flask, Conical flask, Pipette, Burette, Petri dish, Porcelain basin, Crucible)	Kjeldahl digestion and distillation set Water bath

B.8.2 List of chemicals used

Chemical	Supplier/Manufacturer	Other specifications
Sodium hydroxide (NaOH)	Thermofisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCl)	Thermo Electron LLS India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermofisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merk (India) Limited	Amorphous
Oxalic acid	Merk (India) Limited	Crystal
DPPH	Himedia laboratories (india) Pvt. Ltd.	Amorphous
Methanol	Thermo Fischer Scientific India Pvt. Ltd.	Liquid
Petroleum benzene	Merk life Pvt. Ltd.	B.P. 60℃-80℃
Folin- Ciocalteau reagent	Thermofisher Scientific India Pvt. Ltd	Liquid
Sodium Carbonate (Na_2CO_3)	Qualigens fine chemicals	99.5%, LR grade

Color Plates

P. 1 Steeping of buckwheat

P. 2 Humidity chamber used for germination

P. 3 Germination of tartary buckwheat in humidity chamber

P. 4 Germination of common buckwheat in humidity chamber

P. 5 Germination of tartary buckwheat in open environment

P. 6 Germination of common buckwheat in open environment