EFFECT OF GERMINATION TIME ON ANTI-NUTRIENTS AND PHYTOCHEMICAL PROPERTIES OF BLACK- EYED BEAN *(Vigna Unguiculata)*

by

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Effect of Germination Time on Anti-Nutrients and Phytochemical Properties of Black- Eyed Bean *(Vigna Unguiculata)*

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Approval Letter

The ***dissertation*** entitled "***Effect of Germination Time on Anti-Nutrients and Phytochemical Properties of Black- Eyed Bean (Vigna Unguiculata)***" presented by **Rushna Basnet** has been accepted as the partial fulfillment of the requirements for **Bachelor degree in Nutrition and Dietetics**.

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Rushna Basnet

Abstract

Vigna unguiculata (black- eyed bean) also known as cowpea, is a widely cultivated legume in Asia, Central and South America, and Africa. It is a staple food that provides human nutrition with large amounts of proteins, carbohydrates, dietary fibers, vitamins of the β complex, essential minerals, and a small quantity of lipids. High protein and carbohydrate contents with relatively low-fat and a complementary amino acid pattern to cereal grains make black- eyed bean an essential nutritional food in the human diet. The main aim of this research work was to determine the effect of germination time on black- eyed bean's anti-nutritional and phytochemical properties. Black-eyed was bought from the local market of Dharan, Nepal and then cleaned and steeped in water for 12 hours at 28 ± 3ºC, followed by draining and germination for consecutive 5 days at ambient temperature (28 ± 3°C, 90% RH). Then, the germinated legumes were dried in a cabinet dryer at 50 ± 5ºC for 16-18 hours, and dried samples were then taken for quantitative analysis of different components (protein, tannin, phytate, oxalate, flavonoid, total phenolic content and DPPH radical scavenging activity).

The mean value of proximate composition of Black- eyed bean were found to be 27.0%, 0.86%, 3.41%, 4.42% and 64.25% on dry weight basis for protein, fat, ash, fiber and carbohydrate content respectively. The mean value of phytate, oxalate, flavonoid and tannin content in raw Black- eyed bean were found to be 819.8 mg/100g, 170.54 mg/100 g, 105.56 mg/100 g, and 199.9 mg/100 g based on dry weight respectively. Germination time showed significant reduction ($p< 0.05$) in phytate, tannin and flavonoid content of black- eyed bean for consecutive five days of germination. Germination time also showed a significant reduction ($p< 0.05$) in oxalate content in the first 3 days of germination, but there was no significant difference in oxalate content of black- eyed bean in third, fourth and fifth days of germination. Significant reduction ($p<0.05$) of TPC in black- eyed bean i.e., by 72.26 % was also found after consecutive five days of germination i.e., from 732.2 mg GAE/100g to 203.1 mg GAE /100g based on dry matter. The increase in germination time from day 1 to day 5 of black- eyed bean showed a significant increase ($p< 0.05$) in protein content from 27.03 to 39.54%. Germination time also showed significant increment ($p<0.05$) in DPPH radical scavenging activity of black-eyed bean i.e., from 41.6% to 74.55% respectively. Hence, germination was considered an effective method for reducing antinutritional factors and increasing the antioxidant and nutritional properties.

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List of Abbreviations

Abbreviations	Full form
%	Percentage
µg	Microgram
ANOVA	Analysis of Variance
CCT	Central campus of technology
d.f.	Degree of freedom
DB	Dry basis
DFTQC	Department of food technology and quality control
DPPH	Diphenyl picryl hydrazyl
FeCL3	Ferric chloride
fig.	Figure
G	Gram
GAE	Gallic acid equivalent
hr	Hour
LSD	Least significant difference
MC	Moisture content
Mg	Milligram
QE	Quercetin equivalent
TAE	Tannin acid equivalent
TFC	Total flavonoid content
TPC	Total phenolic content

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PART I

Introduction

1.1 General introduction

Vigna unguiculata (cowpea) is a widely cultivated legume in Asia, Central and South America, and Africa. It is a staple food that provides human nutrition with large amounts of proteins, carbohydrates, dietary fibers, vitamins of the β complex, essential minerals, and a small quantity of lipids (Sombié *et al.*, 2018). Cowpea is a legume which is eaten as a high-quality plant protein in different parts of the world. High protein and carbohydrate contents with relatively low-fat and a complementary amino acid pattern to cereal grains make cowpea an essential nutritional food in the human diet. Cowpea has gained more attention recently from consumers and researchers worldwide as a result of their exerted health-beneficial properties, including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory, and anti-hypertensive (Jayathilake *et al.*, 2018).

Germination is a complex process during which the seed should quickly recover physically from maturation drying, resume a sustained intensity of metabolism process, completes the critical cellular events to allow the embryo to emerge, and prepare for subsequent seedling growth. Early following the start of imbibition of the dry seed, there is a re-establishment of metabolism; restitution of the chemical and structural integrity of cells requires the co-participation of synthetic and protective events. Protein synthesis and respiratory activity initially involve components stored within the mature dry seed, although transcription and translation 6 commence early during imbibition, as shown by transciptome and metabolome analyses (Nonogaki *et al.*, 2010).

Compounds or substances which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects are referred to as antinutrients or antinutritional factors. Seeds of legumes and other plant sources contain in their raw state wide varieties of antinutrients which are potentially toxic. The major antinutrients includes: toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohemagglutinin), protease inhibitors, chlorogenic acid and amylase inhibitors. These antinutrients pose a major constraint in the use of plant protein sources in livestock feeds without adequate and effective processing. The level or concentration of these anti- nutrients in plant protein sources vary with the species of plant, cultivar and post-harvest treatments (processing methods) (Akande *et al.*, 2010).

Phytochemical refers to every naturally occurring chemical present in plants. In plants, phytochemicals act as a natural defense system for host plants and provide color, aroma, and flavor. There is a wide distribution of biologically active constituents throughout the plant 11 kingdom, particularly in plants used for animal feeding and human nutrition. Phytochemicals are present in various plants and are used as essential components of human and animal diets. More than 4000 of these compounds have been discovered, and scientists are expected to find out many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs, and spice. Several phytochemicals are known, some of which include: alkaloids, saponins, glycosides, anthraquinones, steroids, and terpenoids (Rowland, 1999).

1.2 Statement of the problem

Pulses are significant protein sources and other nutrients in our diet. Among legumes, a black-eyed bean is generally consumed during illness in Nepal's present scenario. A diet containing antinutritional factors impairs the absorption of nutrients like minerals and reduces bioavailability. Reducing anti-nutritional factors has dual benefits, i.e., enhanced mineral absorption and improved utilization. The sprouts of germinated pulses are a rich source of vitamin C (Khyade and Jagtap, 2016).

Cowpeas are extensively grown in many countries worldwide since they are an essential source of vegetable protein. The utilization of vegetable protein is gaining increasing attention due to the world's need for more low-cost dietary proteins, particularly for low-income countries. The high cost and limited availability of animal proteins in developing countries have directed interest toward several seed and legume proteins as potential sources of vegetable protein for food use. However, besides the nutritional components, cowpeas contain anti-nutritional factors that must be removed or eliminated to improve their nutritional quality and organoleptic acceptability (Wang *et al.*, 1997).

Germination is the initial stage of a plant's growth, during which the primary root and stem emerge. This process activates the seed's enzymatic system, leading to the mobilization of reserve nutrients required for plant growth (Kumar *et al.*, 2022). During germination, anti-nutritional factors (ANFs) present in pulses are broken down. Proteases play a role in inactivating proteinaceous ANFs such as enzyme inhibitors and lectins. Phytic acid, an antinutrient that binds to essential minerals, is degraded by the enzyme phytase, leading to

increased availability of inorganic phosphorus, which is biologically accessible for plant growth. As a result, the phytic acid content of germinated pulses decreases (Camacho *et al.*, 1992). Germination also modifies the phenolic composition of pulses. It leads to a reduction in total cyanide content, enzyme inhibitors (trypsin, α-amylase, and chymotrypsin inhibitors), and tannins. Additionally, polyphenol content decreases during germination, which is attributed to the presence of polyphenol oxidase and enzymatic hydrolysis (R. Sinha and Kawatra, 2003a).

This study mainly focuses on reducing the anti-nutritional factors in black-eyed beans by varying germination days. Antinutrients are chemicals that have been evolved by plants for their defense, among other biological functions, and reduce the maximum utilization of nutrients, especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in food and decreasing the nutritive value (H. F. Gemede and Ratta, 2014). Antinutrients reduce nutrient intake, digestion, absorption, and utilization of nutrients and may produce other adverse effects on health (Akande *et al.*, 2010).

1.3 Objectives

1.3.1 General objectives

The general objective of the dissertation work was to study effect of germination time on antinutrients and phytochemical properties of black- eyed bean (cowpea).

1.3.2 Specific objectives

The specific objectives of this dissertation work are:

a) To determine the proximate composition of black- eyed bean (cowpea).
b) To determine the antinutrients present in black eyed bean as tannin, phytate and oxalate and phytochemical properties such as phenolic content and anti-oxidant activities.
c) To determine the reduction pattern of anti- nutrients and effect on phytochemical properties of germinated black- eyed bean.

1.4 Significance of the study

This study specifically determines the content of antinutrients and phytochemicals in black-eyed beans and the effect of germination time on reducing those antinutrients. The results of this study might help establish a practical and optimized way to use black-eyed beans at the

household and industrial levels. The metabolic changes during the different germination stages influence the bioavailability of essential nutrients and improve the palatability, digestibility, and availability of certain nutrients. During germination, several enzymes become active; vitamins are increased, whereas there is a reduction in phytates and tannins (P. M. Mehta and Bedi, 1993). Sprouting or controlled germination of legumes increases protein and carbohydrate digestibility, enhances some of their vitamin contents, reduces antinutritional factors, and improves their overall nutritional quality. As sprouting proceeds, the ratio of essential to non-essential amino acids changes, providing more essential amino acids. Sprouted seeds have more maltose, therefore, improving the digestibility of carbohydrates (Uppal and Bains, 2012b).

1.5 Limitations of the study

The study has the following limitation:

1. Analysis of anti-nutrients like trypsin inhibitor, lectin, haemagglutinin etc. could not be performed due to time constraints.

PART II

Literature Review

2.1 Pulses

Legumes are plants or fruits of the Fabaceae family (Leguminosae). Legumes are grown agriculturally, mainly for their grain seed called a pulse. Fruits of legumes are dry and develop from simple carpels, dehiscing on both sides along a seam. Grain legumes are cultivated for their seeds. Seeds are used primarily for human and animal consumption or for the production of industrial oils. Grain legumes include beans, lentils, peas, and peanuts (Baskota, 2019).

Legumes (also known as pulses) are generally regarded as a good source of proteins and carbohydrates in the human diet and also a popular staple diet for a large group of the world population. Legume seeds have almost twice the amount of proteins as cereals which are five rich in all essential amino acids except sulfur-containing amino acids. Starch present in legumes is slowly digestible, providing a longer-lasting source of energy (low glycemic load) (Chaudhary *et al.*, 2015).

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

2.2 Black-eyed bean

The cowpea alternatively known as cowpea, bachapin bean, black-eyed pea, southern, crowder pea, china pea, and cow gram; in Afrikaans: akkerboon, swartbekboon, koertjie; in Zulu: isihlumaya; in Venda: munawa (plant), nawa (fruits) imbumba, indumba; in Shangaan: dinaba, munaoa, tinyawa (J. E. Smith *et al.*, 1999). It is also known internationally as lubia, niebe coupe, or frijol. However, they are all species of Vigna unguiculata (L) Walp., which in older references may be identified as Vigna sinensis (L) (Nkaa *et al.*, 2014).

The cowpeas *Vigna unguiculata (L.) Walp*. are an annual herbaceous legume grown for its edible seeds or fodder. Cultivated cowpeas are herbaceous annuals that are either erect, prostrate, or climbing annuals with a tap root, and virtually all are glabrous. They are mostly grown for grain, but a small proportion (about 10%) are grown as green leafy vegetables and fodder in Africa or as fresh pods in eastern Asia. It is an essential food source and is estimated to be the main source of protein for more than 200 million population in sub-Saharan Africa and is in the list of top ten fresh vegetables in the People's Republic of China (hereafter "China") (Kpoviessi *et al.*, 2019).

Taxonomy of black- eyed bean

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Sub-family: Faboideae
Tribe: Phaseoleae
Sub-tribe: Phaseolinae
Genus: Vigna
Section: Catiang
Species: unguiculata
Botanical varieties: 1. *Vigna unguiculata unguiculata var. unguiculata*
2. *Vigna unguiculata unguiculata var. spontanea*

Source: (Maréchal, 1978)

2.2.1 Center of origin and diversity

Cowpeas *(Vigna unguiculata (L.) Walp)* are one of the most ancient food source of human and has probably been used as a crop plant since Neolithic times. Some literature indicates that cowpea was introduced to the Indian subcontinent from Africa approximately 2000 to 3500 years ago, while others state that before 300 BC, cowpeas reached Europe and possibly North Africa from Asia. Cowpea is now cultivated throughout the tropics and subtropics and has become a major part of the diet of about 110 million people. Cowpea is believed to have first cultivated from by some workers in West Africa because both cultivated and wild

species abound in the region. Others believe that it originated in Southern Africa (Boukar *et al.*, 2015).

Several hypotheses have been proposed for the domestication of cowpea in different parts of sub-Saharan (Ba *et al.*, 2004). . It is likely that cowpea was domesticated only once, probably in West Africa about 2000 B.C., as stated by Padulosi[1] and Ng (1997), and that the progenitor of cultivated cowpea was the wild cowpea V. unguiculata var. spontanae (Pasquet, 1999). The greatest genetic diversity in wild relatives of cowpea has been found in southern Africa in a region encompassing Namibia from the west, across Botswana, Zambia, Zimbabwe, and Mozambique to the east, and South Africa and Swaziland to the south. The South African Transvaal may have been the center of speciation of Vigna unguiculata due to the presence there of the most primitive subspecies (Padulosi[1] and Ng, 1997).

2.2.2 Distribution of cowpea

Cowpeas are grown as warm-season-adapted annuals in tropical and subtropical zones in all countries in sub-Saharan Africa and in Asia, South America, Central America, the Caribbean, the United States, and around the Mediterranean Sea. In subtropical zones, temperatures are only suitable for cowpea in the summer, whereas temperatures are suitable year-round in tropical zones (Hall, 2001). The vast majority of the world's cowpea production (over 95%) takes place in sub Saharan Africa (B. B. Singh and Matsui, 2002). Asia is the second largest producing region, representing less than 3% of the global production on average over the 1993-2014 period, most of it being cropped in Myanmar (Nourhane and Amel, 2021).

In Africa, cowpea can be cultivated up to 1800 m altitude but is mainly grown in the lowlands. The center of maximum diversity of cultivated cowpeas and landraces is found in West Africa in a region comprising the Sudan savannah zone of Nigeria, central Burkina Faso, Ghana, Togo, northern Benin, and the north-western part of Cameroon(Padulosi[1] and Ng, 1997). Substantial cowpea cultivation also occurs in the semi-arid Sahelian zone, which is a transition zone between the Sahara desert in the north and the Sudan savannah zone in the south. Significant cowpea production also occurs in the northern Guinea savannah zone, and the forest and southern Guinea savannah zones of West Africa, the United Republic of Tanzania, and Uganda, and some cowpeas are cultivated in central, southern, and north-

eastern Africa. The wild relatives of cowpea are widely distributed across sub-Saharan Africa. They occupy a range of habitats to an elevation of 2600 m. Vigna monantha has been found in Somalia on the coastal plain from Hobyo to Bender Bayla (OCDE, 2016).

In Asia, cowpea ranks as one of the top ten fresh vegetables. It is cultivated across a broad geographic range, except for some permanently cold regions. According to FAO statistics, Myanmar is the main cowpea producer in Asia (Nourhane and Amel, 2021). The estimated annual cultivation area in Asia in total is 1 million ha, with China alone making up roughly one-fifth of the world's fresh pod production with over 1.5 million tonnes (OCDE, 2016).

2.2.3 Structure of cowpea

The cowpea (*Vigna unguiculata (L.) Walp.)* is an annual herbaceous legume cultivated for its edible seeds or for fodder. Cultivated cowpeas are herbaceous annuals that are either erect, prostrate or climbing annuals with a tap root and virtually all are glabrous. They are mostly grown for grain but a small proportion (about 10%) are grown as green leafy vegetables and fodder in Africa or as fresh pods in eastern Asia (Kpoviessi *et al.*, 2019).

- **Root**: cowpea has a strong taproot and many spreading lateral roots in surface soil (Nourhane and Amel, 2021).
- **Leaves**: The first pair of leaves is basic and opposite while the rest are arranged in an alternate patterns and are trifoliate. The leaves are usually dark green in colour. Leaves exhibit considerable variation in size (6 to 16 x 4 to 11 cm) and shape (linear- lanceolate to ovate). The leaf petiole is 5 to 25 cm long (Nourhane and Amel, 2021)
- **Stems**: Striate, smooth or slightly hairy with some purple shades (Nourhane and Amel, 2021).
- **Inflorescence**: Flowers are arranged in racemose or intermediate inflorescences at the distal ends of 5 to 60 cm long peduncles. Flowers are borne in alternate pairs, with usually only two to a few flowers per inflorescence. Flowers are conspicuous, self-pollinating, borne on short pedicels and the corollas may be white, dirty yellow, pink, pale blue or purple in colour. The flower is large (standard is 2-3 cm in diameter), with a straight keel, diadelphous stamens (one

free and nine fused), a sessile ovary with many ovules, and a style that is bearded along the inside and ends in an oblique stigma (Nourhane and Amel, 2021).

- **Fruit and seeds**: Seeds vary considerably in size, shape and colour. Pods occur in pairs forming a V, mostly pending and vertical, but they can be erect. They are cylindrical, 2-6 cm long and 3-12 mm broad and contain 8-20 seeds. Seeds can be white, pink brown or black. The seeds are relatively large (2 to 12 mm long) and weigh 5 to 30 g/100 seeds. The testa may be smooth or wrinkled; white, green, buff, red, brown, black, speckled, blotched, eyed (hilum white, surrounded by a dark ring) or mottled in colour. Fruit: pods that vary in size, shape, colour and texture. They may be erect, crescent-shaped or coiled. Usually yellow when ripe, but may also be brown or purple in colour (Nourhane and Amel, 2021).

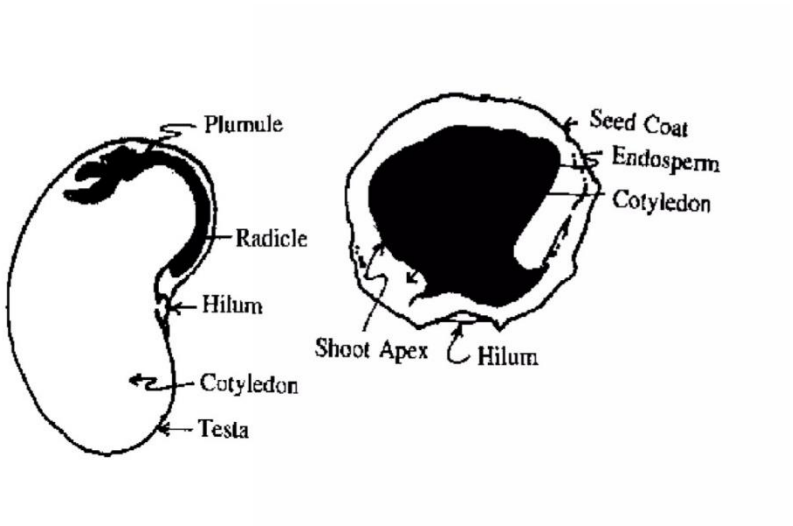

Fig.2.1 Structure of cowpea

2.2.4 Production of cowpea

It is estimated that the annual world cowpea crop is grown on 12.5 million ha, and the total grain production is 3 million tons, although only a small proportion enters the international trade. Central and West Africa is the leading region for producing cowpea in the world; this area produces around 64% of the estimated 3 million tons of cowpea seed produced annually. Brazil and Nigeria are the world's two top producers of cowpeas. Other countries in Africa, e.g., Nigeria, West Africa, Senegal, Ghana, Mali, and Burkina Faso. Ghana, Niger, and Cameroon are significant producers. The major production areas elsewhere in the world are Asia (India, Myanmar) and the Americas (USA, Brazil, West Indies). Cowpea production is

widely distributed throughout the tropics. However, Central and West Africa account for more than 64 % of the area with about 8 million ha, followed by about 2.4 million ha in Central and South America, 1.3 million ha in Asia and 0. 80 million ha in East and Central Africa. Cowpeas can be regarded as the fulcrum of sustainable farming in semiarid lands. This applies to West and Central Africa (Siddiq and Uebersax, 2022).

In Nepal, Cowpea is given less importance among the grain legumes and is considered a minor crop. Cowpea cultivation covers about 3657 ha with a production of 36321 t and productivity of only 9.93 t ha-1 in Nepal (Chaulagain *et al.*, 2023). The production scenario of cowpea in developed countries is better than that of underdeveloped countries due to the use of responsive input efficiency and production technology. The underdeveloped countries have low production due to low input and labor-intensive production systems.

2.3 Chemical composition of Cowpea

Cowpea has been promoted as a high-quality protein constituent of the daily diet among economically depressed societies in developing countries, with the aim of reducing the high prevalence of protein and energy malnutrition (Animasaun *et al.*, 2015; Elharadallou *et al.*, 2015; Santos and Boiteux, 2013). Nutritionally, cowpea grain is more or less same as other pulses, with relatively low-fat content and high total protein concentration. Cowpea is considered as a nutrient-dense food with low energy density (Khalid and Elharadallou, 2013; Kirse and Karklina, 2015). The following Table 2.1 gives a general idea about the chemical constituents of cowpea (whole):

Table 2.1 Chemical constituents of cowpea

Constituents	**%**
Moisture	13.4
Carbohydrate	54.5
Protein	24.1
Fat	1
Minerals	3.2
Fiber	3.8
Calcium	77(mg)
Iron	12(mg)

(Source: DFTQC 2017)

2.4 Physical properties of black-eyed bean

2.4.1 Thousand kernel weight

The 1000 kernel weight is a proportion of seed size. It is the load in grams of 1,000 seeds. Seed size and the thousand kernel weight can fluctuate starting with one harvest then onto the next, between variety of a similar yield and even from one year to another or from one field to another of a similar variety. As a result of this variety in seed size, the quantity of seeds in plant is additionally exceptionally factors (Unal *et al.*, 2008). By using the 1000 kernel weight, a producer can account for seed size variations when calculating seeding rates, calibrating seed drills and estimating shattering and combine losses (Miller and McLelland, 2001).

2.4.2 l/b ratio

The l/b ratio is defined as the ratio of length to breadth of the grain. It is used to determine the shape of the individual grain. The value of l/b ratio above 3 is generally considered as slender and below 3 is generally considered as bold (Rather *et al.*, 2016).

2.4.3 Bulk density

Bulk density is defined as the weight per standard volume measured in a standard manner. It is also known as ‘test weight’, ‘bushel weight’ or ‘specific weight’. The factor that affects the bulk density are insect infestation, excessive foreign matter and moisture content. Bulk

density is required for the design of storage, transport and separation systems. It has also been used to determine the dielectric properties of cereal grains (Kruszelnicka, 2021).

2.5 Phytochemicals and antioxidant

2.5.1 Phytochemical

Phytochemical refers to every naturally occurring chemical present in plants. In plants, phytochemicals act as a natural defense system for host plants and provide color, aroma, and flavor. There is a wide distribution of biologically active constituents throughout the plant kingdom, particularly in plants used for animal feeding and human nutrition. Phytochemicals are present in various plants and are used as essential components of human and animal diets. More than 4000 of these compounds have been discovered, and scientists are expected to find out many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs, and spice (Rowland, 1999).

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

2.5.2 Antioxidants

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

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

2.6 Anti- nutritional factors

Anti-nutritional factors (ANFs) are defined as biological components present in foods that can reduce nutrient utilization or food uptake, which leads to impaired gastrointestinal functions and metabolic performance (Nagraj *et al.*, 2020). Anti-nutritional factors are principally connected with mixtures or substances of the normal or engineered beginning, which meddle with the absorption of nutrients, act to lessen nutrient intake, digestion, and usage, and may create other antagonistic outcomes. Antinutrients are, as often as possible, identified with plant-based, crude, or vegan diets and are normally integrated into plants (Habtamu Fekadu Gemede *et al.*, 2014). A portion of the normal manifestations displayed by an enormous number of antinutrients in the body can be sickness, swelling, cerebral pains, rashes, nutritional deficiencies, and so forth. Then again, such synthetic mixtures can be obviously worthwhile to mankind when consumed admirably. In fact, plants, for their own protection, essentially use antinutrients (Essack *et al.*, 2017).

The sensitivity to antinutrients varies widely between individuals, but adequate food processing is initially recommended to reduce these factors. A person cannot eliminate antinutrients once they have been introduced to the body (K. O. Soetan and Oyewole, 2009). Most of the secondary metabolites, acting as antinutrients, bring out very harmful biological responses, while some of them are mostly applied in nutrition and as pharmacologically active agents (K.O. Soetan, 2008). Most antinutrients are found in grains, beans, legumes, and nuts, but they can also be found in leaves, roots, and fruits of certain varieties of plants. The main antinutrients found in plant-based foods are phytates, tannins, lectins, oxalates, polyphenols, saponins, etc. (Popova and Mihaylova, 2019).

2.6.1 Phytic acid

Phytic acid (known as inositol hexakisphosphate (IP6), inositol polyphosphate, or phytate in salt form) is a saturated cyclic acid and the principal storage form of phosphorus in several plant tissues, mainly bran and seeds. It can be found in grains and cereals (Baskota, 2019).

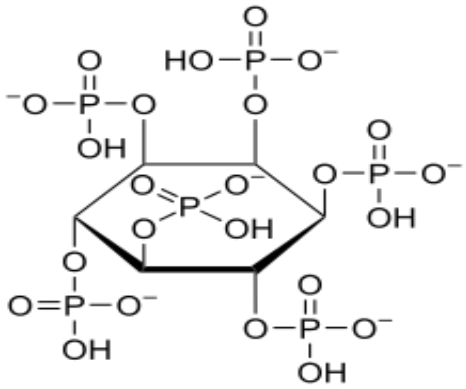

Fig.2.2 Structure of Phytic acid

Phytic acid, mostly as phytate, is found within the hulls of seeds, including nuts, grains, and pulses. Simply cooking the food can decrease the phytic acid to some degree. More effective methods include soaking in an acidic medium, Lactic acid fermentation, such as sourdough, pickling, and sprouting (Fardet, 2010).

Phytic acid has a high binding affinity for essential minerals such as iron, calcium, and zinc; however, the binding of calcium with phytic acid is pH-dependent (Dendougui and Schwedt, 2004). The binding ability of phytic acid with iron is more complex, and although there certainly is a strong binding affinity, other molecules like phenols and tannins also influence the binding (Prom-u-thai *et al.*, 2006). When zinc and iron bind to phytic acid, they form insoluble precipitates that are less absorbable in our intestine. This process therefore contributes to zinc and iron deficiencies in individuals whose diets rely on these foods for their mineral intakes, such as those in developing countries and vegetarians (Baskota, 2019).

Phytic acid not only grabs onto or chelates essential minerals but also can inhibit enzymes needed for food digestion, including pepsin, which is necessary for the protein breakdown in the stomach, and amylase, which is needed for the starch breakdown into sugar. As required for protein digestion in the small intestine, phytate also inhibits trypsin. Although indigestible for many animals, phytic acid and its metabolites, as they occur in seeds and grains, have various important roles for the seedling plant. Mostly, phytic acid functions as a phosphorus store, an energy store, a source of cations, and a source of myoinositol (a cell wall precursor). In plant seeds, phytic acid is the major form of phosphorus storage (Fardet, 2010).

In animal cells, myoinositol polyphosphates are ubiquitous, and phytic acid (myoinositol hexakisphosphate) is the most abundant, with its concentration ranging from 10 to 100 μM in mammalian cells, depending on type of cell and developmental stage (Sasakawa *et al.*, 1995). Studies examining the effects of phytic acid show that it is essential for regulating vital cellular functions. Both in vivo and in vitro experiments have shown striking anticancer (preventive and therapeutic) effects of phytic acid. Research shows anticarcinogenic effects, albeit to a lesser extent, and that it inhibits cancer. In addition to reducing cell proliferation, phytic acid increases the differentiation of malignant cells, often resulting in reversion to the normal phenotype (Shamsuddin, 2002). The phytate content present in black-eyed beans is 833 mg/100g (Deol and Bains, 2010).

2.6.2 Tannin

The word tannin is very old and reflects a traditional innovation. Tanning was the word utilized in logical writing to describe the process of transforming raw animal hides or skins into durable, non-putrescible leathers by utilizing plant extracts from various plant parts. The tannins in plants are astringent, bitter polyphenolic compounds that bind protein and other organic compounds, such as amino acids and alkaloids, which have molecular weights ranging from 500 to 3000 (Habtamu Fekadu Gemede *et al.*, 2014) .

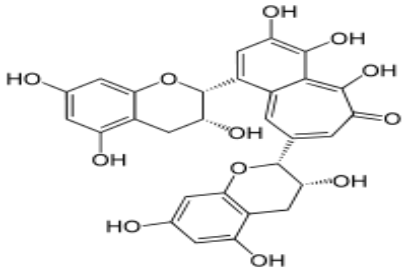

Fig.2.3 Structure of tannin

Tannins are heat stable, and they diminish protein digestibility in animals and humans, presumably by either making protein partially inaccessible or hindering digestive enzymes and increasing fecal nitrogen (Ali *et al.*, 2014). The tannin is widely distributed in several species of plants, where it plays a role in protection from predation, perhaps also as pesticides, and helps in regulating the growth of plant. Astringency from tannins is what causes a dry, puckery sensation in the mouth after consuming unripe fruit, tea or red wine.

Likewise, the modification or destruction of tannins with time plays an critical role when determining harvesting times (McGee, 2007).

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

2.6.3 Flavonoid

Flavonoids are the largest group of phenolic compounds and have a basic skeleton composed of three rings (C6-C3-C6). They are classified into six major classes according to their substitution pattern in the B-and C- rings, which are flavan-3-ols, anthocyanins, flavones, isoflavones, flavanones, and flavonols (Harborne and Baxter, 1999). The flavonoid polymers are also known as proanthocyanidins. Flavonoids occur as plant secondary metabolites that are involved in pigmentation, antioxidants, antimicrobials, anti-stressors, and UV irradiation protection (Vaya and Aviram, 2001). There are more than 4000 flavonoids described so far within plant parts that are normally consumed by humans, as well as 650 flavones and 1030 flavanols (Ghasemzadeh *et al.*, 2010). Flavonoids are found in almost all plant-based food and beverages, but the levels vary depending on the degree of ripeness of fruits, variety, and processing. Most flavonoids enhance the potency of vitamin C (ascorbic acid) and function as antioxidants. The antioxidant activity of flavonoids is believed to be due to their ability to act as free radical acceptors and complex metal ions (Hertog *et al.*, 1992).

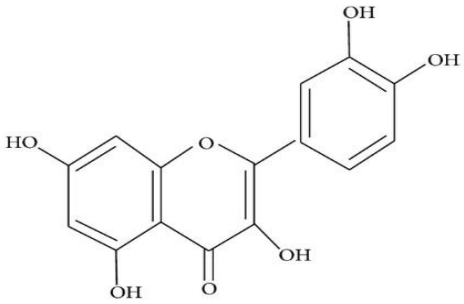

Fig.2.4 Structure of flavonoid

Previous studies showed that the ingestion of flavonoids reduces the risk of cardiovascular diseases, metabolic disorders, and certain types of cancer. Physiologically, flavonoids reduce oxidative stress, inhibit low-density lipoproteins oxidation, and inhibit platelet aggregation, as well as acting as vasodilators in the bloodstream. Free radicals are constantly generated, resulting in extensive damage to tissues and leading to various disease conditions such as cancer, Alzheimer's, renal diseases, cardiac abnormalities, etc. Medicinal plants with antioxidant properties play a vital function in exhibiting beneficial effects and are employed as an alternative source of medicine to mitigate the disease associated with oxidative stress. Flavonoids have existed for over one billion years and possess a wide spectrum of biological activities that might be able to influence processes that are dysregulated in a disease (David *et al.*, 2016).

2.6.4 Oxalate

Oxalate is dianion with the formula (C2O4) -2, also written as ((COO)2) -2. Oxalates occur in many plants where it is synthesized by incomplete oxidation of carbohydrate (Dean, 2012).

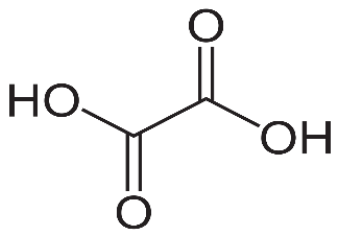

Fig.2.5 Structure of oxalate

Oxalate is an anti-nutrient that, under ordinary conditions, is restricted to isolate compartments. However, when it is handled and additionally processed, it comes into contact

with the nutrients in the gastrointestinal tract (Noonan and Savage, 1999). When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. In ruminants, oxalic acid is of only minor importance as an anti-nutritive factor since ruminal microflora can probably metabolize soluble oxalates to less significantly insoluble calcium oxalate. While the importance of the anti-nutritive activity of oxalic acid has been recognized for more than fifty years, it might be a subject of interest to nutritionists in the future (Oladimeji *et al.*, 2000).

2.6.5 Polyphenol

Polyphenols, which include more than 8000 compounds, are a family of natural compounds widely distributed in the outer layers of plant as suspected from their protective function in the plants (Manach *et al.*, 2004). Phenolic compounds are extensively dispensed bioactive secondary metabolites existing in all higher plants that are primarily synthesized by means of the shikimic acid, pentose phosphate and phenylpropanoid pathways (Balasundram *et al.*, 2006). It is assessed for that more than 8000 phenolic compounds have been isolated and described in flora (Ouchemoukh *et al.*, 2017). Phenolic compounds influence the sensory properties of foods and tannins primarily contribute to the astringency of food sources (Landete, 2012).

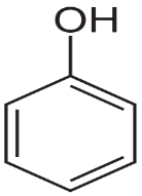

Fig.2.6 Structure of phenol

Polyphenols occur in all plant foods and contribute to the beneficial health effects of vegetables and fruit (Balch and Balch, 2000). They range from simple molecules, such as phenolic acid, to highly polymerized compounds, such as tannins. Phenolic acids account for about one-third of the total intake of polyphenols in the human diet. These compounds are capable of removing free radicals, chelating, metal catalysts, active antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases (Oboh, 2006). As a result, they neutralize free radicals formed during the normal physiological functioning of the human

body (Burns *et al.*, 2001). The antioxidant activity of phenols is due to their redox properties through which they act as hydrogen donors, singlet oxygen quenchers, and reducing and metal chelating agents. There is a highly positive relationship between total phenols and the antioxidant activity of many plant materials (Gülçin *et al.*, 2004).

In recent years, much attention has been paid by nutritionists to dietary polyphenols due to their potent antioxidative effects and their credible effects in the prevention of various oxidative stress associated diseases. The oxidation process is one of the most important ways to produce free radicals in food and even in living systems. Free radicals cause many human eight diseases like cancer, Alzheimer's, cardiac, kidney, and liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders, and aging (Halliwell and Gutteridge, 1990). The polyphenols content in black-eyed beans is 778 mg/100g (Punia, 2000).

2.7 Impact of anti -nutrients on health

Antinutritional factors have potential adverse effects on human health. As the legumes contain wide range of antinutritional factors such as tannins, phytates, oxalates, saponin and lectins. Phytate has negative impact on the bioavailability of minerals usually divalent and trivalent ions of minerals such as Zn, Mg, Ca, Cu and Mn. Whenever higher level of phytate consumption results in minerals deficiency (Habtamu Fekadu Gemede *et al.,* 2014). Oxalate is the salt of oxalic acid calcium oxalate mostly distributed in plants. Oxalic acid formed strong bonds with minerals such as potassium, calcium and magnesium. Some oxalic salts are soluble whereas others are insoluble the insoluble salts solidify in the urinary tract when accumulate in kidney it forms kidney stones (Nachbar *et al.*, 1980). When oxalic acid is digested in gastrointestinal tract it comes in contact with nutrients (Bsc and Bsc, 1999)this than bind the minerals and make them unavailable. Saponin is extremely toxic to cold blooded animals. In dietary plants saponin impart a bitter taste and astringency when consumed have bitter taste and irritate throat it also reduced the bioavailability of nutrients, reduced the enzyme activity and affect digestibility of protein (Liener, 1974).Some studies shown that saponin have inverse relationship with renal stone (Loewus, 2002).Beyond its adverse effect on health recent researches shows it possesses anticarcinogenic, immunostimulatory 13 and hypocholesterolemia properties. To inhibit dental carries saponin diet be used it also used in the treatment of hypercaliuria and as an antidote of lead poisoning (Habtamu Fekadu Gemede *et al.*, 2014). Washing repeatedly by water reduce the adverse effects and enhance the palatability by reducing the bitterness property (Katiyar *et al.*, 1989).

2.8 Ways to reduce anti-nutrients in food

Nutrients in plants are not always easily digested. This is because plants may contain antinutrients. These are plant compounds that reduce the absorption of nutrients from the digestive system. They are of a particular concern in societies that base their diets largely on grains and legumes (Savage and Klunklin, 2018). By using various methods alone or in combinations, it is possible to reduce the level of anti-nutrients in foods (Reddy and Pierson, 1994).

There are several factors that affects the content of nutritional and antinutritional factors present in legumes. The intrinsic factors includes varieties, cultivars, biotypes, etc. and extrinsic factors includes soil, use of fertilizer, maturity at harvest, storage condition, packaging and method used for processing, etc. that affects antinutritional factors present in beans (Nikolopoulou and Grigorakis, 2008). Simple ways to reduce the amount of antinutrients in foods are:

2.8.1 Soaking

Soaking is one of the processes used to remove soluble anti-nutritional factors, which can be eliminated with the discarded soaking liquors, but some metabolic reactions can take place during soaking affecting the content of some compounds (Vidal-Valverde *et al.*, 1994) . Soaking, is an integral part of traditional methods of processing, saving energy cost by shortening cooking time, offers an additional advantage of rendering the grain nutritionally superior by removing certain anti-nutritional factors like phytic acid, saponin and polyphenols (Kataria *et al.*, 1989). The decrease of these anti-nutrient contents during soaking may be attributed to leaching out into soaking water under the influence of the concentration gradient.

Soaking allows the water to spread in the protein fraction and starch granules allowing protein denaturation and starch gelatinization to occur, softening the texture of beans (Siddiq and Uebersax, 2022). Beans and other legumes are often soaked in water overnight to improve their nutritional value (Fernandes *et al.*, 2010). Most of the antinutrients in these foods are found in the skin. Since many antinutrients are water-soluble, they simply dissolve when foods are soaked. In legumes, soaking has been found to decrease phytate, protease inhibitors, lectins, tannins and calcium oxalate. For example, a 12-hour soak reduced the phytate content of peas by up to 9% (Bishnoi *et al.*, 1994). Another study found that soaking

pigeon peas for 6-18 hours decreased lectins by 38-50%, tannins by 13-25% and protease inhibitors by 28-30% (Onwuka, 2006). However, the reduction of antinutrients may depend on the type of legume. In kidney beans, soybeans and fava beans, soaking reduces protease inhibitors only very slightly. Not only is soaking useful for legumes, leafy vegetables can also be soaked to reduce some of their calcium oxalate. Soaking is typically used in combination with other methods, such as sprouting, fermenting and cooking (R. R. Adhikari, 2021).

Phenolic content of cowpea was found to be 144.66 mg GAE/100 g which decreased to 69.54 mg GAE/100 g after soaking. Flavonoid content of cowpea flour showed a decrease from 15.57 mg QE/100g to 13.13 mg QE/100g (Chipurura and Foods, 2018). The phytic acid content of raw cowpea decreased from 836.0 mg/100g to 752.66 mg/100g during soaking (R Sinha and Kawatra, 2003b). The soaking process caused a significant reduction in soluble oxalates in peas (36.51 – 47.62%), lentils (26.66 – 48.79%), fava beans (45.34 – 45.82%), chickpeas (29.92– 35.53%), beans (36.56 – 39.65%) and soybean (56.29%) (Shi *et al.*, 2018).

2.8.2 Germination

Germination is the first stage of a plant's growth during which the primary root and stem come out. In this stage, the reserve nutrients required for plant growth are mobilized by hydrolyzing proteins and carbohydrates to obtain the required substrates for the seed development. The seed enzymatic system is activated during its germination. It is considered one of the most effective processing methods for improving the nutritional quality of pulses, enhancing the digestibility of nutrients as protein and carbohydrates (Kumar *et al.*, 2022). For the breakdown of anti-nutritional chemicals in pulses, the germination process has been widely researched. The degree of deterioration, on the other hand, is dependent on the type of pulses, the type of ANFs, and the germination conditions. Proteases are thought to be responsible for the inactivation of proteinaceous ANFs such enzyme inhibitors and lectins. Phytic acid is digested by an endogenous enzyme called phytase during germination into inorganic phosphorus, which is the biologically accessible form for plant growth and development. As a result, the phytic acid in pulses transforms to a soluble form, and several researchers have documented the drop in phytic acid content of germinated pulses as a result of this occurrence (Camacho *et al.*, 1992).

The most effective method for reducing phytic acid in legumes is germination. Phytic acid was degraded during germination, leading to an increase in inorganic phosphorus availability (Virginia *et al.*, 2012). The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. A decrease in phytic acid content after germination for lentils was reported by Vidal-Valverde *et al.* (2002), for fava bean by Alonso *et al.* (2000), for black gram and mung bean by (Kataria *et al.*, 1989). Because phytic acid's chelating capacity is diminished, lowering its levels enhanced the availability of minerals in the digestive system of animals (Akande and Fabiyi, 2010).

Germination modifies the quantitative and qualitative phenolic composition of pulses. This process has shown up to 20.8% reduction in total cyanide content in kidney bean (Yasmin *et al.*, 2008). It also reduces the content of enzyme inhibitors such as trypsin inhibitors, α-amylase inhibitors and chymotrypsin inhibitors in pulses (Alonso *et al.*, 2000). Phytic acid content of raw cowpeas was 836.0mg/100gm, which declined to 731.33, 620.00, 526.33 and 436.00 mg/100 g during 24, 48, 60 and 72 hours of germination, respectively. Germination of cowpea seeds led to a significant decrease in polyphenol content; the reduction was greater when germination was carried out for a longer time. Germination for 72 hours resulted in about a 32.5 percent reduction in the polyphenol content of cowpea seeds. Decrease in polyphenol content during germination may have been a function of the presence of polyphenol oxidase and enzymatic hydrolysis.(R Sinha and Kawatra, 2003b). Germination caused a significant reduction in tannin contents and the percentages of reduction were 12.63 and 25.82% after 24 and 48 h of germination, respectively (Ibrahim *et al.*, 2002).

2.8.3 Fermentation

Fermentation is a metabolic process that allows sugars to be metabolized for energy while also improving mineral absorption from plant-based diets. Because cereals are difficult to ingest in their natural/raw forms, fermentation is one of the processing processes used to make cereal grains digestible while also improving the nutritional content and safety elements of these foods (Galati *et al.*, 2014).Although food that becomes fermented by accident is most often considered spoiled, controlled fermentation is widely used in food production. Food products that are processed by fermentation include yogurt, cheese, wine, beer, coffee, cocoa and soy sauce. Another good example of fermented food is sourdough bread. Making of sourdough effectively degrades antinutrients in the grains, leading to

increased availability of nutrients (Leenhardt *et al.*, 2005) . In fact, sourdough fermentation is more effective at reducing antinutrients in grains than yeast fermentation in typical bread (Lopez *et al.*, 2003).

Fermentation is such an important process, which significantly lowers the content of anti-nutrients such as phytic acid, tannins, and polyphenols of cereals (Simwaka *et al.*, 2017). Phytic acid generally forms complexes with metal cations such as iron, zinc, calcium, and proteins in grains. Enzymes destroy these complexes, which necessitate a pH that is maintained through fermentation. As a result, phytic acid concentration is reduced, and soluble iron, zinc, and calcium are liberated, enhancing the nutritional value of dietary grains (Gibson *et al.*, 2010). As the fermentation (LAB) period of maize flour is increased, the significant reductions in anti-nutrients, including tannin, polyphenol, phytate and trypsin inhibitor activity were observed (Ogodo *et al.*, 2019).

In various grains and legumes, fermentation effectively degrades phytate and lectins. For example, fermenting pre-soaked brown beans for 48 hours caused an 88% reduction in phytate (Gustafsson and Sandberg, 1995). The fermentation by microorganisms significantly decreased the level of cyanide, tannins, phytate, oxalate and saponins by 86, 73, 72, 61, and 92%, respectively in the cassava products (Etsuyankpa *et al.*, 2015).

2.8.4 Boiling

High heat, especially when boiling, can degrade antinutrients like lectins, tannins and protease inhibitors (Egbe and Akinyele, 1990).One study showed that boiling pigeon peas for 80 minutes reduced protease inhibitors by 70%, lectin by 79% and tannin by 69%.Additionally, calcium oxalate is reduced by 19-87% in boiled green leafy vegetables. Steaming and baking are not as effective. In contrast, phytate is heat-resistant and not as easily degraded with boiling. The cooking time required depends on the type of antinutrient, food plant and the cooking method. Generally, a longer cooking time results in greater reductions of antinutrients (R. R. Adhikari, 2021) .On boiling of cowpea, saponin, TPC, flavonoid and tannin is reduced by 39.27%, 36.97%, 17.80% and 59.78% respectively (Chipurura and Foods, 2018).

2.8.5 Roasting

Roasting is a cooking technique that uses dry heat to roast food evenly on all sides at temperatures of at least 150°C from an open flame, oven, or other heat source. Protein

digestibility can be improved by roasting. Bacteria and viruses can be killed or rendered inactive by heat. The amount of aflatoxins produced by fungi is reduced when they are roasted (Samarajeewa *et al.*, 1990). The goal of roasting is to improve sensory qualities and achieve inactivation of destructive enzymes which improves the storage and nutritional quality of the product (Rackis *et al.*, 1986). Legumes after roasting are used in a variety of snacking recipes that are popular among the impoverished. According to studies, roasting did not lead to a significant reduction in different amino acids When compared to the values indicated by cooked horse gram, roasting improved the rats' growth rate and digestibility (Rana, 2022).

A significant decrease of phytates and condensed tannin contents was recorded for roasted varieties of lentils i.e., reduction up to 63.01% and 41.41% respectively for phytates and condensed tannin contents at 140°C for 30 min (Attou *et al.*, 2020).Similarly, reduction in phytic acid and tannin of chickpea was reported up to 56% and 57% respectively (Pokharel, 2022).Roasting of lima bean seeds helps in the reduction of phytic acid (40%), tannin (30%) and trypsin inhibitor (98%) (El-Gohery, 2021).Roasting of black bean seeds reduce the polyphenols and saponin by 8% and 20% respectively (LE *et al.*, 2021).

Roasting reduced the phytate content by up to 62.35% following the same trend of reduction by boiling (68.43% at 60 min). The reduction in phytate by roasting may be due to the heat labile nature of phytic acid. Roasting was equally effective in reducing haemagglutinin content by 67.50% and the tannin content up to 75% at 120 min (Udensi *et al.*, 2007).

2.9 Health benefits of cowpea

Cowpea (Vigna unguiculata) is a legume consumed as a high-quality plant protein source in many parts of the world. High protein and carbohydrate contents with relatively low fat content and complementary amino acid pattern to that of cereal grains make cowpea an important nutritional food in the human diet (Jayathilake *et al.*, 2018). Cowpea is considered as an incredible source of many other health-promoting components, such as soluble and insoluble dietary fiber, phenolic compounds, minerals, and many other functional compounds including B group vitamins (Mudryj *et al.*, 2012). Thus, cowpea contributes a lot in improving quality of human health by offering a number of health benefits. The epidemiological evidences indicate that the consumption of cowpea provides protective

effects for several chronic diseases like, gastrointestinal disorders, cardiovascular diseases, hypercholesterolemia, obesity, diabetes and several types of cancer (Barnes *et al.*, 2015; Chon, 2013; Frota *et al.*, 2008; Jayanthakali *et al.*, 2018; Trehan *et al.*, 2015a).

In addition, literature evidences also show functional ingredients in cowpea to aid in weight loss, improve digestion and strengthen blood circulation (Perera *et al.*, 2016; Trehan *et al.*, 2015b). All these beneficial effects exerted by cowpea are attributed to the presence of phytochemicals, resistant starch, dietary fiber and low fat content along with beneficial unsaturated fatty acids. The low glycemic index of cowpea is attributed to the action of resistant starch and dietary fiber which attenuate insulin responses and reduce hunger (Liyanage, 2018).

2.10 Malting of cowpea

Malting is the controlled germination process followed by drying and terminating the growth of the embryo, which activates the enzymes of the resting grains resulting the conversion of cereal starch into fermentable sugar and other particles, partial hydrolysis of protein and macromolecules into micro molecules (Rosentrater and Evers, 2017). Germination is done for the purpose of developing an active enzyme content which will later convert starches in the malted barley and in other cereals grains into sugars, which can be easily fermented during fermentation step (R. R. Adhikari, 2021).

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

2.10.1 Outline of Malting

Malting is controlled germination process which produces a complement of enzymes which are able to convert cereal starches (endosperm) to fermentable sugars, to secure an adequate

supply of amino acids and other minor nutrient for yeast and modify the quality of the micro molecules (Evers and Rosentrater, 2018).

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

2.10.1.1 Malting operation

The sequence of operation in malting is as follows:

- The collection of stocks of suitable grains or legumes.
- The storage of the pulses until it is required.
- Steeping the pulses in water, germination of the pulses.
- Drying and curing on a kiln.

Steeping an arranged so that sufficient moisture outers the grain to initiate germination. The moisture content of 42-46 % (Wet weight basic) that is eventually achieved is sufficient to support growth and biochemical alteration in the grains during the malting period, without, however, allowing excessive growth. Since growth, results in the production of largely unwanted rootlets and loses in dry weight due to grain respiration. A balance must be struck between achieving sufficient growth to adequately alter the barley into malt but without excessive growth that would reduce the quality of malt eventually produced. In newer malting processes, the wetted grain may be drained and aerated at intervals and germination may commence before the grain contains sufficient to malt adequately. Thus, the steeping and germination tend to merge (Hough *et al.*, 2012)

Germination traditionally carried out in darkness at relatively low temperature 12- 15°C for choice but this could not be easily controlled. Originally it was processed in autumn, winter and spring to take advantage of the cool weather but never malting have temperature controlled atmosphere. Regulating the moisture and temperature of the grain controls the 17

intensity of germination process. The changes occurring in germination that are essential in converting barley into malt are collectively termed as “modification” and may be summarized as follows. First may hydrolytic enzymes appear and increase in amount, adding to those that are already present in the barley. These enzymes began to catalyze the hydrolytic degradation of the reserve substances at the starchy and endosperm and in the particular cell wall are partially or completely degraded resulting in loss of storage. Consequently, simple roller may readily crush dry malt in contrast to dry barley. Gummy polysaccharides are also degraded during malting so that the work derived from malt has a low viscosity compared with extracts of raw barley. Simple water-soluble product of hydrolysis accumulates in the grain during malting (R. R. Adhikari, 2021).

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

Cabinet drier consist of an insulated cabinet fitted with shallow mesh or perforated trays, each of which carries a thin layer of food. Hot air is circulated through the cabinet tray. A system of duct and baffles is used to direct air, over and/ or through each tray, to promote uniform air distribution either horizontally between the trays of food materials or vertically through the trays and food (Fellows, 2009). Air heaters may be direct gas burners, steam coil exchangers or electrical resistance heaters. The air is blown past the heaters and thus heated air is used for dying. It is relatively cheap to build and maintain, flexible in design, and produces variable product quality due to relatively poor control. It is used singly or in groups, mainly for small- scale production (1-20 ton/day) of dried fruits and vegetables (B. G. Smith *et al.*, 2007).

2.10.2 Chemical changes during germination

The percentage of starch decreases and the composition of the remaining starch alter. The proportion of amylase increases. The overall pentosans content of starchy endosperm declines while that of husk remain unchanged. The partial hydrolysis of the insoluble

hemicellulose appears to give rise to the soluble gum, which in turn, when hydrolyzed, 18 further provides mono-saccharides. The quantity of simple sugars alters dramatically, those produced by the hydrolysis of polysaccharides on the one hand and those consumed by the living parts of the grains on the other hand. The amount of sugar declines during kilning but the sucrose often increases in amount. Maltose is also increased. The grain as the first respiratory substrate uses up raffinose before other sugars is mobilized to support the growth of embryo (Shrestha, 1995). Total free amino acid content was significantly affected ($p>0.05$) by germination time and watering regime (B. Adhikari and Rai, 2021).

During malting the reduction in phytate content as well as increase in α- amylase activity and the sweetness in the malt flours occurs (B. Adhikari and Rai, 2021). The complex organic phosphates of aleurone layer and starchy endosperm are hydrolyzed to yield inorganic phosphates and ultimate product of hydrolysis of phytic acid, are known to increase. Vitamins like riboflavin, pantothenic acid, pyridoxine, pyridoxal, and pyridoxamine group increases in the malting while others do not. Ascorbic acid also alters during germination but is completely destroyed during kilning. There is reduction in the quantity of inorganic materials during malting because the materials move to the roots and some is lost by leaching in the steep liquor (Shrestha, 1995). The statistical analysis done by (Friend *et al.*, 1995) shows there is no significant effect of malting of grain on the acidity.

2.10.2.1 Changes in nutritional composition during germination

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

F.N. Ehirim *et al.* (2018a) reported increase in moisture content of cowpea from 5.63 to 6.72 % after 5 days of germination. After entry of water in seed coat, seed swelling starts and initiates sprouting. During sprouting, the water intake of seed varies. The percent increase was highest in 120h. According to (Murugkar and Jha, 2009) their was an increase in moisture 54 to 56.1 % after 48 h of sprouting in soybean. Likewise (Uwaegbute *et al.*, 2000) also observed that the moisture increased from 15.6 to 17.6 % after sprouting cowpea. Usually during sprouting, seeds absorb water by a process called imbibitions (Nonogaki *et al.*, 2010).

Ash contents, calculated on moisture free basis, increased with increase in sprouting time. The ash content slightly decreased with 24 h germination and thereafter with 48 and 72 h germination it become at par with control. After 96 h germination the ash content reaches maximum level (Shah *et al.*, 2011).

El-Adawy *et al.* (2003) reported significant increase in ash content during sprouting in mungbean, pea and lentil seed. The decrease in crude fat and carbohydrate contents during sprouting may have led to the apparent increase observed in ash and other chemical components. Ash contents, calculated on moisture free basis, increased with increase in sprouting time. The ash content slightly increased but were not significantly difference ($p<0.05$). After 120h germination the ash content reaches maximum level (F.N. Ehirim *et al.*, 2018a) .

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

M. B. Mehta *et al.* (2007) have found that there is increase in protein content of cowpea after 28h of sprouting. Uppal and Bains (2012a) observed crude protein increase from 8 to 11% after sprouting. This increase in protein content may be attributed to loss in dry matter, particularly carbohydrate through respiration.

Chaudhary *et al.* (2015) reported the significant loss of lipid during the germination period.Badshah *et al.* (1991) and Chung *et al.* (1989) also noted significant losses in lipid content during canola sprouting. The crude fat concentration decreased with sprouting time in cowpea. It decreased from 3.02% to 1.51% (F. N. Ehirim *et al.*, 2018b). The decrease in fat content of seed could be due to total solid loss during soaking prior to germination as mentioned by Wang *et al.* (1997) or use of fat as an energy source in sprouting process (El-Adawy *et al.*, 2003).

Chung *et al.* (1989)reported that in barley (but not in canola) sprouting was associated with significant increase in crude fiber from 3.75% in unsprouted barley to 6% in 5 days sprouts due to synthesis of structural carbohydrates such as cellulose and hemicellulose, a major constituent of cell walls. Crude fibre content was significantly increased ($p<0.05$). The values increased from 4.14% to 5.08 in cowpea (F. N. Ehirim *et al.*, 2018b). This increment may be due to emergence of plumule and radical during germination (Berry *et al.*, 1988).

F.N. Ehirim *et al.* (2018a) reported that the carbohydrate content of sprouted cowpea decreased from 64.01 to 47.87%.Uppal and Bains (2012a) also reported 5.6 % decrease and Jirapa *et al.* (2001) reported 2.34 % decrease in carbohydrate content after 24 h of sprouting in cowpea. Vidal-Valverde *et al.* (2002) explained that during sprouting, carbohydrate was used as source of energy for embryonic growth which could explain the changes of carbohydrate content after sprouting.

2.10.2.2 Change in antioxidant activity during germination

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

h of germination in moth. Fouad and Rehab (2015) reported the higher DPPH radical scavenging activity in germinated lentils (49.26-62.19%) than raw lentil seeds (40.76%).

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

2.10.3 Physical changes during malting

During steeping, the grains swell and increase its volume by about a quarter. Space is allowed in the steep tanks to accommodate the swollen grain. The first microscopic indication of germination after casting is the appearance of the 'chit'. The white coleorhizae or root-sheath that breaks through the pericarp and testa and produce from the base of the corn. In time seminal roots also called rootlets, culms, cooms, or malt sprouts bursts, through root sheath and form a tough at end of the grain, at the same time the first 'leaf- seat' or coleoptiles. Variously called by maltsters the 'acrospires', 'spire', 'blade', penetrates the apex between pericarp and the husk. In conventional malting practice, the malt is kilned and growth terminated before the acrospires grows beyond the end of the grain (Hough and Hough, 1991).

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

As these hydrolytic breakdown processes precede alterations may be detected in protoplasm of cells of the aleuronic layer of columnar cells between the compressed cells endosperm and the scutellum. The products of the hydrolysis of endosperm are absorbed into the scutellum. The products of the hydrolysis of endosperm are absorbed into the scutellum epithelium and are transported through the scutellum into the embryo to provide necessary nutrients. As germination, proceeds the cells of epithelium tend to separate and elongate so forming a 'pile' which projects into the solubilized part of the endosperm. This alteration in

similar form greatly increases the surface area of the cells and makes the epithelium a more efficient absorptive organ (Hough and Hough, 1991).

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

PART III

Materials and Methods

3.1 Materials

3.1.1 Cowpea

Cowpea (*Vigna uniguiculata*) of uniguiculata(bundle lobia) variety was purchased from the local market of Dharan, Nepal.

3.1.2 Equipment and Chemicals

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

Table 3.1 List of chemicals

Chemical Specification	**Supplier/ Manufacturer**	**Other Specification**
Sodium Hydroxide (NaOH)	Thermo Fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric Acid (HCl)	Thermo Fisher Scientific India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck (India) Limited	Amorphous
Potassium Permanganate	Avantor Performance Materials Ltd.	99% Assay
Potassium Thiocyanate	Thermo Fisher Scientific India Pvt. Ltd.	97% Assay
Tannic acid	Avarice Laboratories Pvt. Ltd.	Analytical Reagent
Nitric acid	Fisher Scientific India Pvt. Ltd.	68-75% Assay
DPPH	Hi Media Laboratories Pvt. Ltd	
Quercetin	Avarice Laboratories Pvt. Ltd.	Analytical Reagent
Gallic acid	Avarice Laboratories Pvt. Ltd.	Analytical Reagent

Table 3.2 List of equipment used

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

3.2 Method

3.2.1 Flow chart for Malting Cowpea

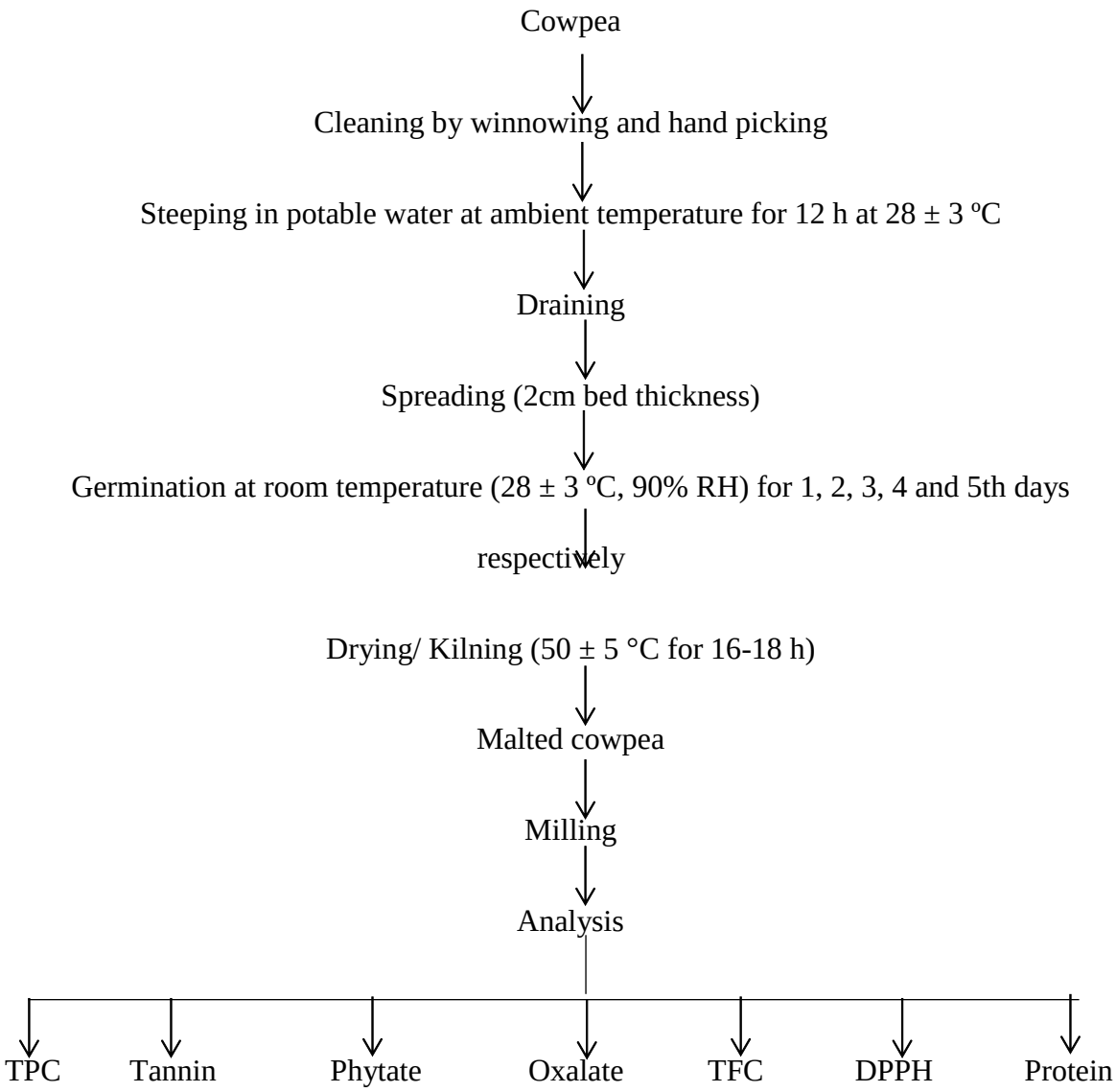

Fig.3.1 Flow diagram of experimental work

3.2.2 Cleaning

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

3.2.3 Steeping

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

3.2.4 Germination

The steeped cowpea seeds were first collected in a muslin cloth and swirled in order to drain excess water and then kept for germination at ambient temperature of average 28ºC. The drying out of grains is prevented by moistening muslin cloth and spraying the potable water at the interval of every 24 h. The pulses bed was turned and mixed from time to time to aerate the mass and to equalize the temperature and moisture during germination. The first test sample was taken after 24 h of germination. After that other samples were taken at an interval of 24 h for upto 120 h to determine DPPH radical scavenging activity, phytate, tannin, oxalate, phenols and flavonoid.

3.2.5 Drying/kilning

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

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

3.3 Experimental procedure

3.3.1 Proximate analysis

3.3.1.1 Determination of moisture content

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

3.3.1.2 Determination of crude protein

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

$$\text{Nitrogen\%} = \frac{(\text{sample titer-blank titer}) \times \text{normality of Hcl} \times 14 \times 100}{(\text{wt of sample} \times 100)}$$

3.3.1.3 Determination of ash content

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

3.3.1.4 Determination of crude fat

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

$$\text{\% crude fat} = \frac{\text{wt. of ether soluble material} \times 100}{\text{wt. of sample}}$$

3.3.1.5 Determination of crude fiber

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

$$\text{\% crude fiber} = \frac{\text{loss in wt noted}}{\text{wt. of sample taken}} \times 100$$

3.3.1.6 Determination of carbohydrate

Total carbohydrate content of the samples was determined by difference method as mentioned in Ranganna (1986).

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

3.3.2 Ultimate analysis

3.3.2.1 Determination of iron

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

$$\text{Iron}\left(\frac{\text{mg}}{100\text{g}}\right)=\frac{\text{absorbance of sample}\times 0.1\times\text{total volume of ash solution}\times 100}{(\text{absorbance of standard}\times 5\times\text{wt. of sample taken for ashing})}$$

3.3.2.2 Determination of calcium

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

$$\text{Calcium}\left(\frac{\text{mg}}{100\text{g}}\right)=\frac{\text{titer}\times 0.2\times\text{total volume of ash solution}\times 100}{(\text{volume taken for estimation}\times\text{wt. of sample taken for ashing})}$$

3.4 Preparation of extract

Plant materials were extracted as per Ahmad *et al.* (2014) with slight modification.10 g of powdered samples were steeped in 80% methanol (100 ml) for 12 h at room temperature. They were then filtered using Whatman No.1 filter paper. Finally, extracts were transferred to brown coloured glass bottles, sealed by using bottle caps and stored at 4 ± 2ºC until analysis. The extract concentration was determined by evaporating in rotary vacuum evaporator.

3.5 Determination of physical properties

3.5.1 Thousand kernel weight

The 1000 kernel weight of cowpea was determined by measuring the weight of 1000 kernels of cowpea seeds after selecting the appropriate sample size by quartering method (Imran *et al.*, 2016) .

3.5.2 Bulk density

The bulk density was measured by pouring the seeds 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).

3.5.3 Length by breadth ratio

Length by breadth ratio of cowpea seed was determined as per (Işık *et al.*, 2008).

3.6 Qualitative Analysis for Phytochemicals

The plant extracts were screened for the presence of the phytochemical classes by using the standard following methods (Jaradat *et al.*, 2015).

a) Test for protein

- Ninhydrin test: Boil 2 ml of 0.2% Ninhydrin solution with the entire plant crude extract, appeared violet color indicate the presence of proteins and amino acids.

b) Test for carbohydrates

- Iodine test: 2 ml of iodine solution mixed with crude plant extract. Purple or dark blue colors prove the presence of the carbohydrate.

c) Test for Phenols and Tannins

- Two milliliters of 2% solution of FeCl3 mixed with crude extract Black or blue-green color indicated the presence of tannins and phenols.

d) Test for Flavonoids

- Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of diluted acid to solution, this result indicated the presence of flavonoids.

3.7 Quantitative Analysis of Phytochemicals

3.7.1 Total phenolic content

TPC was determined using the Folin–Ciocalteu method with slight modifications (Singleton *et al.*, 1999). The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin- Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na2CO3 aqueous solution. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at wave length=765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GAE/ 100 g of dry sample).

3.7.2 Total flavonoid content

Total flavonoid content was determined using a modified aluminum chloride assay method as described by (Barek *et al.*, 2015). 2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate (NaNO3) was mixed and stand for5 minutes. 0.2 ml of 5% Aluminum Chloride (AlCl3) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5ml. The absorbance was measured after 15 minutes at 510nm against a reagent blank. The total flavonoid content is expressed as mg QE/ 100g of dry weight.

3.7.3 Total tannin content

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na2CO3solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. Absorbance for test and standard solutions were measured against the blank at 725 nm with

an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of TAE / 100g of extract (Miean and Mohamed, 2001; Ribarova *et al.*, 2005; R. Singh *et al.*, 1970).

3.7.4 Oxalate

The oxalate content was determined by the method of Day and underwood (Day and Underwood, 1991). All 7 samples of cowpea powder (1g from each sample) was mixed with 75 ml of 3M sulphuric acid (H2SO4) in a conical flask and stirred for 1 hour using a magnetic stirrer. The mixture was allowed filtering and a 25 ml of aliquots of the filtrate was titrated against 0.05M Potassium Permanganate (KMnO4) solution until violent color persisted at least for 30 seconds. The oxalate content of the sample was determined using the following equation.

1 ml 0.05 KMnO4 = 2.2 mg oxalate

3.7.5 Free Radical Scavenging Activity Using (DPPH)

Extract(100µL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5mL). The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/ VI'S spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula (Hatano *et al.*, 1988).

$$\%\text{of inhibition} = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control).

I%=percentage of inhibition

3.7.6 Phytate

The sample weighing 0.2 g was placed in a 250 ml conical flask. It was soaked in 100 ml of 20% concentrated HCl for 3 h, the sample was then filtered. 50 ml of the filtrate was placed in a 250 ml beaker and 100 ml distilled water was added to the sample. Then, 10 ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution which contained 0.00195 g iron per 1 ml (Emmanuel and Deborah, 2018).

$$\% \text{ phytic acid} = \frac{\text{titer value} \times 0.00195 \times 1.19 \times 100}{2}$$

PART IV

Results and Discussion

The cowpea was purchased from local market of Dharan, Sunsari. It was handpicked, winnowed and then washed. The sample was steeped for 12 h and then germinated at 28 ± 3°C for 1st, 2nd, 3rd, 4th and 5th days respectively. Thus, germinated samples were dried at 50±5 °C in cabinet dryer for 16-18 h. The sample was used for further quantitative analysis of protein, tannin, phytate, oxalate, TFC, TPC and DPPH scavenging activity.

4.1 Proximate composition

The proximate composition of raw black- eyed bean was determined. The results obtained are given in Table 4.1.

Table 4.1 The proximate composition of cowpea

Parameters	**Values (%)**
Crude protein (dry basis)	27.07 ± 0.12
Crude fat (dry basis)	0.86 ± 0.05
Ash content (dry basis)	3.41 ± 0.15
Crude fibre (dry basis)	4.42 ± 0.15
Carbohydrate (dry basis)	64.25 ± 0.35
Moisture	13.06 ± 0.05

Values presented are the average of triplicates determination ± standard deviation.

Table 4.1 shows the proximate composition of cowpea according to which the protein, fat, ash, fiber and carbohydrate content of the cowpea were 27.0%, 0.86%, 3.41%, 4.42% and 64.25% respectively on dry weight basis. Similar data was found by Uriyo (2001) in which ash and fat content were found to be 3.2% and 0.7% while carbohydrate and protein content were 57.2% and 20.6% which are lower than the data obtained in this research. Meanwhile, the data also differed with studies done by F.N. Ehirim *et al.* (2018a) in case of protein content and fat content were protein and fat content were found to be 20.16% and 3.02% while ash, crude fiber and carbohydrate were found to be 3.07, 4.14 and 64.01% which is similar to the data obtained in this research. However, the composition of sample may vary according to genus, species, growing conditions and many more factors (Fried *et al.*, 2008).So, this composition may be agreeable.

4.2 Mineral composition of cowpea

Table 4.2 Mineral composition of cowpea

Minerals	Values (mg/100g dry basis)
Iron	10.86 ± 0.30
Calcium	76.2 ± 1.21

[Values presented are the average of triplicates determination ± standard deviation.]

The iron content in raw cowpea was found to be 10.86 mg/100g and calcium content was found to be 76.2 mg/ 100g which is similar to the data obtained by DFTQC (2017) i.e. 12 and 77 mg/100g. Similar data was obtained by (Omenna *et al.*, 2016) in which iron content in cowpea was found to be 10.51 mg/100g. According to (F. N. Ehirim *et al.*, 2018b) calcium content in cowpea was found to be 30.30 mg/100g which is much lower than the data obtained in this study. The amount of calcium and iron content was found in the range of (105.99- 138.18) mg/ 100g and (4.85- 6.75) mg/ 100 g in different varieties of cowpea (Devi *et al.*, 2015). (Ghavidel and Prakash, 2007) found 6.5 mg/100g iron which is lower than the data obtained in this study and 87 mg/ 100g calcium in cowpea which is higher than the data obtained in this study.

4.3 Physical properties of cowpea

The physical properties of cowpea were determined. The results obtained are presented in Table 4.3.

Table 4.3 Physical properties of cowpea

Physical properties	Cowpea seed
l/b ratio	1.43 ± 0.07
Bulk density (kg/hl)	77.52 ± 0.19
1000 kernel weight (g)	96.1 ± 0.74

[Values presented are the average of triplicates determination ± standard deviation.]

(Hamid *et al.*, 2016) found that the thousand kernel weight of cowpea seeds was 98.82g in which our data was comparable to their findings. The value of l/b ratio of raw cowpea seed was found to be 1.43 which is similar to their findings while bulk density of cowpea was found to be 77.52 kg/hl which is slightly less than their findings. The bulk density of a

material depends on the solids density and the geometry, size and surface properties of the individual particles (Wani *et al.*, 2017).

Knowledge of physical properties is imperative for the design of equipments which are used for processing of seeds in the industries which involves harvesting, threshing, cleaning, separation, transportation and packaging. The dimensions of cowpea beans and their 1000-seed weight give indication of the space the flour would occupy as well as their bulkiness (Hamid *et al.*, 2016).

4.4 Effect of germination in protein content of cowpea

The cowpea was germinated for five days. The change in protein content was analyzed in each day of germinated sample as well as in raw sample. The increment in protein content is demonstrated in Fig. 4.1.

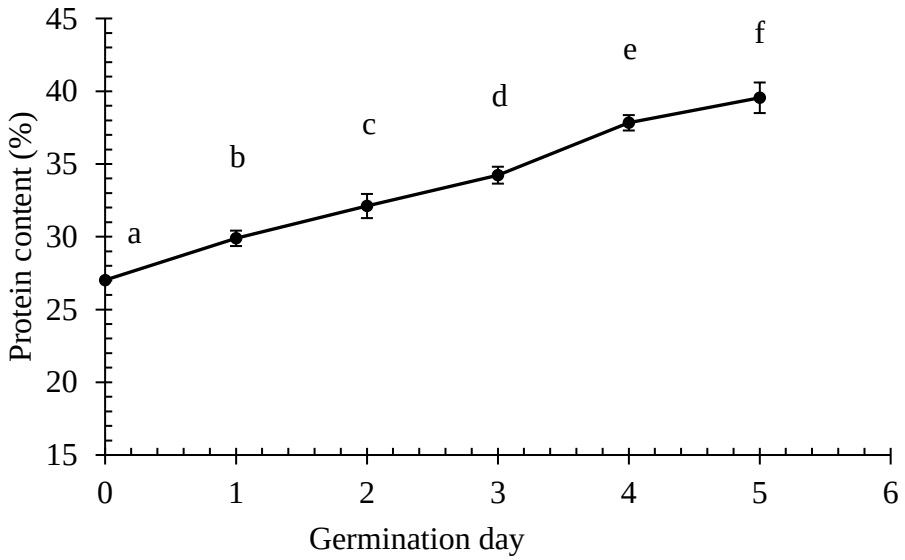

Fig.4.1 Effect of germination time in protein content

The mean value of protein content in raw cowpea was found to be 27.07±0.12% on the basis of dry matter. Protein content was increased on the progressive days of germination. The mean value of progressive days of germination was 29.89±0.53%, 32.11±0.83%, 34.23±0.58%, 37.83±0.53% and 39.55±1.05% on the basis of dry matter on 1st, 2nd, 3rd, 4th and 5th days of germination i.e. from 27.07% - 39.55% respectively. The analysis of variance (Appendix B) showed that there was significant difference between protein content in the different days of germinated samples ($p < 0.05$).

Results showed substantial increase in the protein content of cowpea seeds processed by germination and are in agreement with the earlier studies. These results are higher with those of F.N. Ehirim *et al.* (2018a) who reported increase in protein content of cowpea from 20.16 - 34.05% respectively. M. B. Mehta *et al.* (2007) have found that there is increase in protein content of cowpea after 28h of sprouting. Uppal and Bains (2012a) observed crude protein increase from 8 to 11% after sprouting. Germination increased the protein content by 26.2 and 33.4% in two varieties of cowpea as reported by (Akinlosotu and Akinyele, 1991). Opuku *et al.* (1981) reported an increase in protein content on sprouting ranging from 14 to 40%.

The increase in protein content may be attributed to loss in dry matter, particularly carbohydrate through respiration (Uppal and Bains, 2012a). Also, Bau *et al.* (1997) assumed that the increased was due to synthesis of enzyme proteins (for example, proteases) by germinating seed or other constituents. A further explanation was done by Nonogaki *et al.* (2010) where they noted that protein synthesis occurred during inhibitions and that hormonal changes play an important role in achieving the completion of germination.

Jimenez *et al.* (1985) revealed that the protein digestibility increased as the germination period advanced and obtained better values when autoclaved. Punia (2000) and Trugo *et al.* (2000) observed a progressive increase in protein digestibility as the germination advanced.

4.5 Effect of germination in phytochemicals of cowpea

4.5.1 Effect of gemination in total phenolic content

The cowpea was germinated for five days. The change in total phenolic content was analyzed in each day of germinated sample as well as in raw sample. The reduction in Total phenolic content is shown in Fig. 4.2.

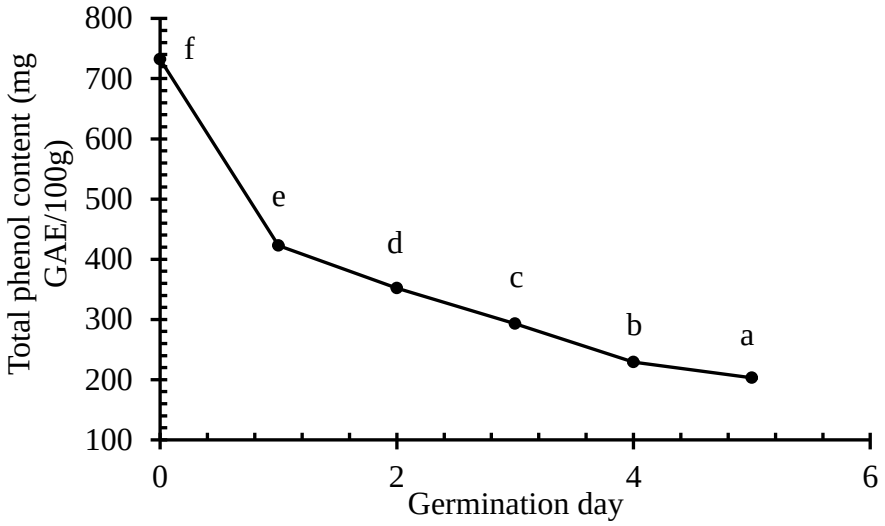

Fig.4.2 Effect of germination in Total phenol content

The mean value of TPC in raw cowpea was observed 732.2±1.08 mg GAE/ 100g on the basis of dry matter. The mean value of progressive days of germination was 422.9±1.15 mg GAE/100g, 352.2±0.45 mg GAE/ 100g, 293.0±0.77 mg GAE/ 100g, 229.01±1.77 mg GAE/ 100g and 203.1±1.53 mg GAE/ 100g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between total phenol content in different days of germinated samples ($p < 0.05$).

Results showed substantial decrease in the concentration of TPC in the seeds processed by germination and are in agreement with the earlier studies. Our results are well in agreement with those of (Sorour *et al.*, 2018) according to which TPC content in cowpea decreased from 763.42±1.66 mg GAE/ 100g to 328.55±0.51mg GAE/ 100 g(56.9%) on 72 hours of germination. According to research conducted by Preet and Punia (2000), TPC content of cowpea seeds was reduced by 65% after 48 hours of germination which is higher than the data obtained by our research. The reduction in phenolic compounds content during germination may have been a function of the presence of polyphenol oxidase and enzymatic hydrolysis (Jood *et al.*, 1987; Paramjyothi and Mulimani, 1996).

Sorour *et al.* (2018) also reported decrease in TPC content of soyabean, chickpea and faba bean by 25.2%, 43% and 21.4% after 72 hours of germination which is lower than the data obtained by our research. According to research conducted by Jood *et al.* (1987), they

found that the polyphenol content of asha cultivar of mung bean seeds was reduced by 32% after germination for 48 hours which is lower than the data obtained by our research.

4.5.2 Effect of germination in DPPH free radical scavenging activity of cowpea

The cowpea was germinated for five days. The change in DPPH radical scavenging activity was analyzed in each day of germinated sample as well as in raw sample. The increment in DPPH radical scavenging activity is demonstrated in Fig. 4.3.

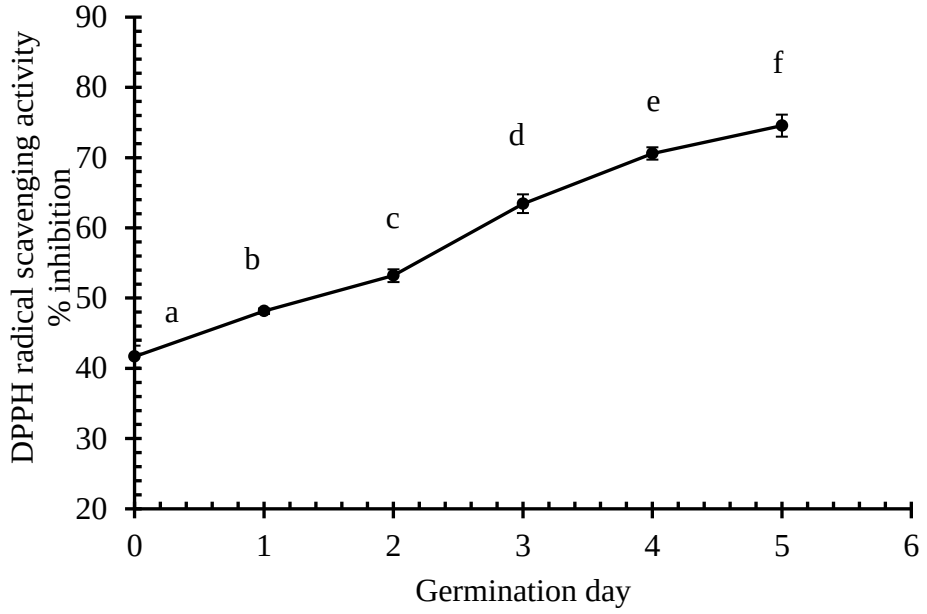

Fig.4.3 Effect of germination time on DPPH radical scavenging activity

The mean value of DPPH scavenging activity in raw cowpea was observed 41.6±1.56 % on the basis of dry matter. The mean value of progressive days of germination was 48.13±0.39 %, 53.19±0.91 %, 63.44±1.33%, 70.59±0.88% and 74.55±1.57% on 1st, 2nd, 3rd, 4th and 5th days of germination respectively. The analysis of variance (Appendix B), showed that significance difference ($p < 0.05$) in DPPH scavenging activity in each day of germinated samples. DPPH scavenging activity was directly proportional to number of days of germination.

The result was in accordance to Khyade and Jagtap (2016) , the value of DPPH radical scavenging activity of cowpea was increased by 8.33% raw value with 48 hours of germination i.e. from 40.09±3.63 to 49.837±0.34. Likewise, the % DPPH inhibition of yellow mustard was increased by 14.81 % on sprouting. The increase in % DPPH inhibition

by chickpea sprouts was 11.23 % and black gram by 9.5%. This signifies the importance of sprouting as a convenient method to improve the antioxidant activity of seeds.

Abderrahim *et al.* (2012) reported the increase in antioxidant activity is due to many metabolic changes during germination such as increase in the activity of the endogenous hydrolytic enzymes during germination. Khyade and Jagtap (2016) also observed that seeds on sprouting showed an increase in % DPPH inhibition which implies that the ability of the components in sprouts (antioxidants) to scavenge free radicals increases after the germination process, thus giving sprouts a significant physiological role to help quench free radicals and ward off degenerative diseases. Germination enhances the antioxidant capacity of the soluble extracts in germinated edible seeds and sprouts when compared with raw seeds. This effect could be attributed to the increase of certain antioxidant compounds such as antioxidant and vitamins (Gan *et al.*, 2017).

4.6 Effect of germination on tannin content

The cowpea was germinated for five days. The change in tannin content was analyzed in each day of germinated sample as well as in raw sample. The reduction in tannin content is shown in Fig. 4.4.

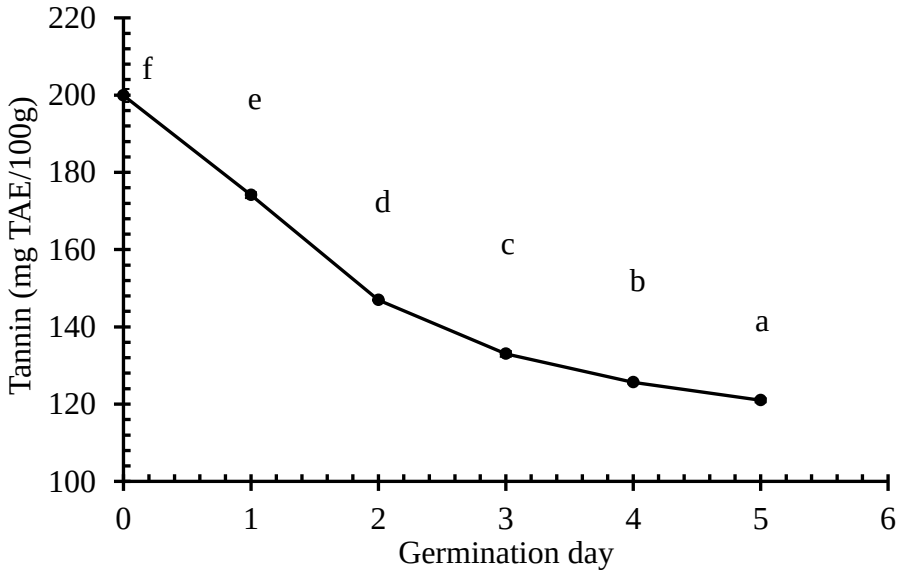

Fig.4.4 Effect of germination time on Tannin content

The mean value of tannin content in raw cowpea was found to be 199.9±1.69 mg/100 g on the basis of dry matter. Tannin content was reduced on the progressive days of

germination. The mean value of progressive days of germination was 174.1±0.767 mg/100g, 146.9±0.08 mg/ 100g, 133.0±0.75 mg/ 100g, 125.6±0.27 mg/100g and 121.0±0.53 mg/ 100g on the basis of dry matter after 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of germination i.e.,12.9%, 26.51%, 33.46%, 37.16% and 39.46% respectively. The analysis of variance (Appendix B) showed that there was significant difference between tannin content in the different days of germinated samples ($p < 0.05$).

(Ibrahim *et al.*, 2002) reported reduction in tannin contents from 210.17 mg/100g to 183.63 mg/100g and 155.91 mg/100g i.e., by 12.63% and 25.82% after 24 and 48 h of germination in cowpea which is similar to the data obtained in this study. (Ghavidel and Prakash, 2007) reported the loss of tannin content by 27.65% after 24 hours of germination which is greater than the data obtained in this study. Similarly (Sorour *et al.*, 2018) found that there was 48.4, 61.7, and 64.8% reduction of tannin in 24, 48 and 72 hours of germination which was higher than the data obtained by our research. A reduction of 32%, 66%, 71% and 76% in 24, 48, 72, and 96 hours in cowpea was found by (Kalpanadevi and Mohan, 2013) which is higher than thus study. Increased activity of polyphenol oxidase and other catabolic enzymes may be responsible for the reduction in tannin content of pulses after germination. Activated enzymes cause the hydrolysis of different components during germination. Enzymatic hydrolysis could be blame for the decrement in tannin content following germination(Deshpande *et al.*, 1986; Khandelwal *et al.*, 2010).

4.7 Effect of germination on phytate content

The cowpea was germinated for five days. The change in phytate content was analyzed in each day of germinated sample as well as in raw sample. The reduction in phytate content is demonstrated in Fig. 4.5.

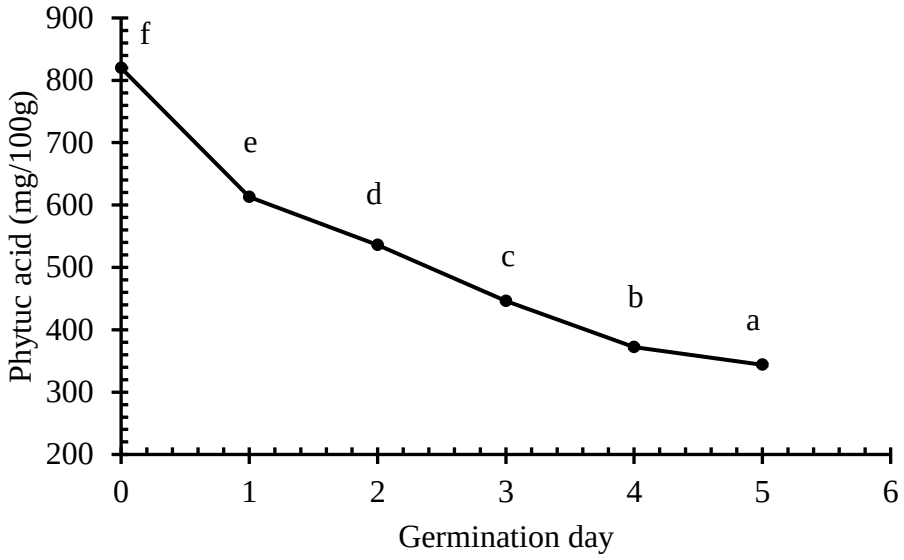

Fig.4.5 Effect of germination time on phytic acid content

The mean value of phytate content in raw cowpea was found to be 819.8 ± 3.06 mg/100 g on the basis of dry matter. Phytate content was reduced on the progressive days of germination. The mean value of progressive days of germination was 612.9 ± 1.91 mg/100 g, 536.1 ± 1.70 mg/100 g, 446.2 ± 2.13 mg/100 g, 372.2 ± 2.81 mg/100 g and 343.8 ± 2.53 mg/100g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of germination i.e., by 25.23%, 34.6%, 45.57%, 54.59% and 58.06% respectively. The analysis of variance (Appendix B) showed that there was significant difference between phytate content in the different days of germinated samples ($p < 0.05$).

Our results showed substantial decrease in the concentration of phytic acid in the seeds processed by germination and are in agreement with the earlier studies. Our results are well comparable with R. Sinha and Kawatra (2003a) , which showed 47.8% reduction in phytate content after 72 hours of germination. Ibrahim *et al.* (2002) also reported reduction of phytate content and the reduction level increased from 17.4 to 22.9%, as the germination time increased from 24 to 48 h which is lower than the data obtained from our study. Kalpanadevi and Mohan (2013) also reported decrease in phytate content of cowpea by 38%, 79%, 86% and 95% after 24, 48, 72 and 96 hours of germination which is too much greater than the data obtained in this research. According to Sorour *et al.* (2018), reduction in phytate concentration ranged from (37.8-54.6)% in cowpea during various germination period.

Uppal and Bains (2012a) also observed 43.19 % decrease in phytic acid in cowpea after sprouting for 24 h which is greater than the value obtained in this research.

The decrease in phytic acid in pulses revealed that an increase in phytate hydrolysis during germination resulted in the liberation of inorganic phosphates from organic phosphorus containing compounds for plant growth (phytate). Phytic acid degradation during germination could be related to an increase in endogenous phytase activity for its usage as a source of inorganic phosphate during germination (Egli *et al.*, 2002; Eskin and Wiebe, 1983; Tabekhia and Luh, 1980). Since phytic acid has been considered to be one of the factors responsible for reducing minerals bioavailability, its reduction during germination may enhance the nutritional quality of beans. Because phytic acid is thought to be one of the reasons that reduce mineral bioavailability, decreasing it during germination could improve the nutritional quality of beans.

4.8 Effect of germination on flavonoid content

The cowpea was germinated for five days. The change in flavonoid content was analyzed in each day of germinated sample as well as in raw sample. The reduction in flavonoid content is demonstrated in Fig.4.6.

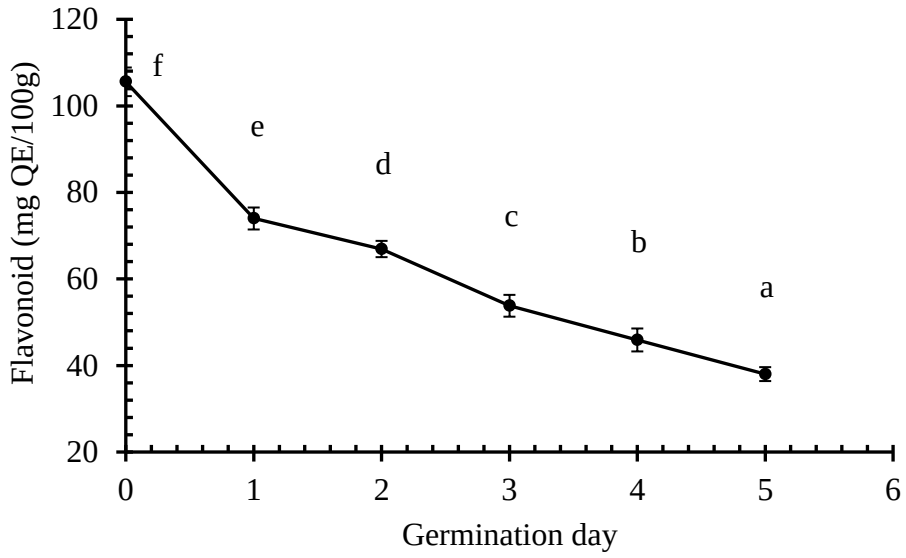

Fig.4.6 Effect of germination time on Flavonoid content

The mean value of flavonoid content in raw cowpea was found to be 105.56±3.31 mg QE/100 g on the basis of dry matter. Flavonoid content was reduced on the progressive days

of germination. The mean value of progressive days of germination was 73.97±2.54 mg QE/100 g, 66.92±1.88 mg QE/100 g, 53.82±2.52 mg QE/100 g,45.94±2.65 mg QE/100 g and 38.01±1.61 mg QE/100 g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of germination i.e., by 29.92%, 36.60%, 49.01%, 56.47% and 63.99% respectively. The analysis of variance (Appendix B) showed that there was significant difference between flavonoid content in the different days of germinated samples ($p < 0.05$).

Our results showed substantial decrease in the concentration of flavonoid in the seeds processed by germination and are in agreement with the earlier studies. (Parvez *et al.*, 2019) also reported reduction in flavonoid content upto 38-61% in 24, 48 and 72 hours which is higher than the data obtained in this study.(James *et al.*, 2020) also reported reduction in flavonoid content of cowpea by 24.57% in 96 hours of germination which is lower than the data obtained in this study. This reduction might have originated from diffusion of phenolic content into water during soaking. Observed that germination of heated cowpea seeds at 12 hours, 24hours, 36 hours and 48 hours caused reduction ranged from 8.52 -53.97%. (Parvez *et al.*, 2019). F.N. Ehirim *et al.* (2018a) reported reduction in flavonoid content of cowpea by upto 91% after 120 hours of germination which is much greater than the data obtained in this study.

4.9 Effect of germination on oxalate content

The cowpea was germinated for five days. The change in oxalate content was analyzed in each day of germinated sample as well as in raw sample. The reduction in oxalate content is demonstrated in Fig. 4.7.

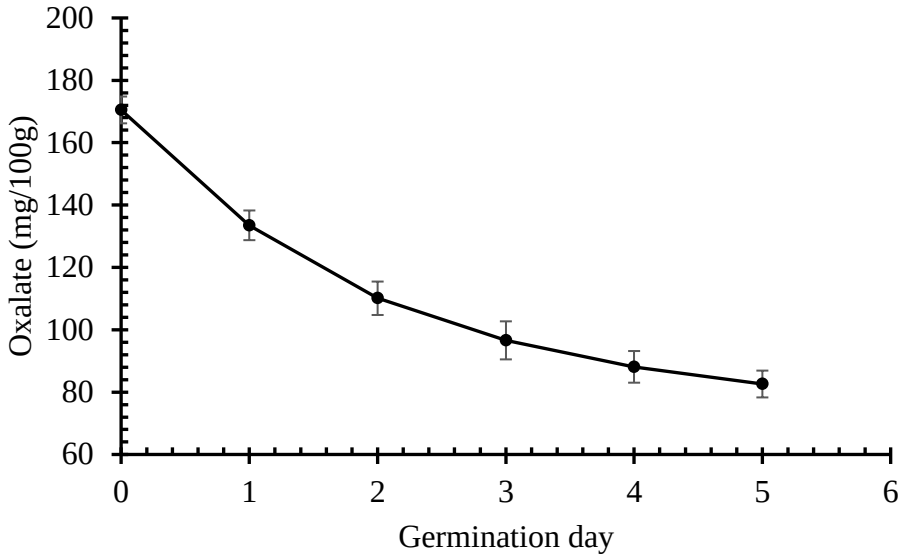

Fig.4.7 Effect of germination time on oxalate content

The mean value of oxalate content of raw cowpea was observed 170.54 ± 4.3 mg/100 g on the basis of dry matter. Oxalate content was reduced as the days of germination was increased. The mean value of progressive days of germination was 133.48 ± 5.08 mg/100 g, 110.13 ± 6.1 mg/100 g, 96.6 ± 5.36 mg/100 g, 88.12 ± 4.76 mg/100 g and 82.36± 4.3 on the basis of dry matter on 1st, 2nd, 3rd, 4th and 5th days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between oxalate content in the first three days of germinated samples ($p < 0.05$) while there was no significant difference between third, fourth and fifth germination days.

Our results showed substantial decrease in the concentration of oxalate content in the seeds processed by germination and are in agreement with the earlier studies. Our results are well in agreement with those of (Oloyo, 2004), oxalate content decreased significantly($p< 0.05$) from 15.4 to 2.06 g/100 g with 5 days of germination in pigeon pea. (Mittal *et al.*, 2012) reported the reduction of oxalate content by 58.97 % in chickpea with germination. Handa *et al.* (2017) also reported the 36.75 % reduction of oxalate content in horsegram by 24 h of germination time.

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

PART V

Conclusions and Recommendation

5.1 Conclusions

On the basis of this study following conclusions were drawn.

- Cowpea is a nutritious legume that contains moisture, crude protein, crude fat, ash, crude fiber, and carbohydrates. It also provides iron and calcium.
- During the germination process, certain components in cowpea changed. Tannin content, phytate content, flavonoid content, and oxalate content decreased. Total phenolic content was also reduced. On the other hand, the protein content increased after germination.
- Additionally, the DPPH radical scavenging activity, which indicates antioxidant potential, increased as the germination time progressed.
- Thus, germination affects the composition of cowpea, with reductions in certain compounds and an increase in protein content and antioxidant activity.

5.2 Recommendations

The following recommendation can be drawn from conclusion:

- Germination of cowpea upto 5 days bring desirable change in protein content thus increasing the nutritional quality of cowpea providing better food source for consumption on the daily basis.
- Research can be done on the effect of germination time to reduce other antinutrients like trypsin inhibitor, hemagglutinin, lectin etc. as well as on amylase activity, minerals and vitamin c content of cowpea.

PART VI

Summary

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

For this study the cowpea was bought from the local market of Dharan. The cowpea legumes were soaked for 12 hours at room temperature (28 ± 3℃), germinated at different time i.e., upto 5 days. After germination the cowpeas were dried at 50 ± 5°C for (16-18) h to obtain the desired final moisture content. The prepared cowpea malt samples were then taken for analysis. Analysis of chemical and functional properties were carried out for all samples.

The phytate content were found to be decreased from 819.8 to 612.9, 536.1, 446.2, 372.2, and 343.8 mg /100g on consecutive days of germination. Tannin content were found to be decreased from 199.9 to 174.1, 146.9, 133.0, 125.6 and 121.0 mg tannic acid/100g on consecutive days of germination. Oxalate content were found to be decreased from 170.54 to 133.48, 110.13, 96.6, 88.12 mg/100g and 82.6 mg/ 100g on consecutive days of germination. Flavonoid content were found to be decreased from 105.56 to 73.97, 66.92, 53.82, 45.94 and 38.01 mg QE/100g on consecutive days of germination. While protein content was increased from 27.02 to 29.89, 32.11, 34.23, 37.83 and 39.55 % on consecutive days of germination. DPPH radical scavenging activity was found to be increased from 41.6 to 48.13, 53.19, 63.44, 70.59 and 74.55 % on consecutive days of germination. So, germination improved the nutritional quality of cowpea.

References

Abderrahim, F., Huanatico, E., Repo-Carrasco-Valencia, R., Arribas, S. M., Gonzalez, M. C. and Condezo-Hoyos, L. (2012). Effect of germination on total phenolic compounds, total antioxidant capacity, Maillard reaction products and oxidative stress markers in Canihua *(Chenopodium pallidicaule). Int. J. Agric. Sci.* . **56** (2), 410-417.

Acharya, D. R. and Karki, D. B. (2008). Study on brewing quality of naked barley *(Hodeum Vulgare L.). J. Food Sci.Technol.Nepal*. **4**, 47-51.

Adhikari, B. and Rai, S. (2021). Processing impact on nutritional, antinutritional and phytochemical of fenugreek seeds *(Trigonella foenum-graecum L.). Himalayan J. Sci.Technol.* **5** (01), 36-44.

Adhikari, R. R. (2021). Effect of germination time on enzymatic, antinutritional and phyto-chemical properties of kwati (A Nepalese Indigenous Food). Bsc. Nutrition and Dietetics Thesis. Tribhuvan Univ., Nepal.

Ahmad, W., Khan, I., Khan, M. A., Ahmad, M., Subhan, F. and Karim, N. A. (2014). Evaluation of antidiabetic and antihyperlipidemic activity of *Artemisia indica linn* (aeriel parts) in Streptozotocin induced diabetic rats. *J. Ethnopharmacol.* **151** (1), 618-623.

Akande, K. E., Doma, U. D., Agu, H. O. and Adamu, H. M. (2010). Major antinutrients found in plant protein sources: their effect on nutrition. *Pak. J. Nutr.* **9** (8), 827-832.

Akande, K. E. and Fabiyi, E. F. (2010). Effect of processing methods on some antinutritional factors in legume seeds for poultry feeding. *Intl. J. Poultry Sci.* **9** (10), 996-1001.

Akinlosotu, A. and Akinyele, I. O. (1991). The effect of germination on the oligosaccharide and nutrient content of cowpeas *(Vigna unguiculata). Food chem.* **39** (2), 157-165.

Ali, E., Awadelkareem, A. M., Gasim, S. and Yousif, N. E. (2014). Nutritional composition and anti-nutrients of two faba bean *(Vicia faba L.). Int. J. Adv. Res.* **2** (12), 538-544.

Alonso, R., Aguirre, A. and Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in- vitro digestibility of protein and starch in faba and kidney beans. *Food chem.* **68** (2), 159-165.

Animasaun, D. A., Oyedeji, S., Azeez, Y. K., Mustapha, O. T. and Azeez, M. A. (2015). Genetic variability study among ten cultivars of cowpea (*Vigna unguiculata L. Walp*) using morpho-agronomic traits and nutritional composition. *J. Agric. Sci.* **10** (2), 119-130.

Attou, A., Bouderoua, K. and Cheriguene, A. (2020). Effects of roasting process on nutritional and antinutritional factors of two lentils varieties *(Lens culinaris. Medik)* cultivated in Algeria. *South Asian J. Exp. Biol.* **10** (6), 445-454. [doi:10.38150/sajeb.10(6).p445-454].

Ba, F. S., Pasquet, R. S. and Gepts, P. (2004). Genetic diversity in cowpea *[Vigna unguiculata (L.) Walp.]* as revealed by RAPD markers. *Gen. Res. Crop Evol.* **51**, 539-550.

Badshah, A., Zeb, A. and Sattar, A. (1991). Effect of soaking, germination and autoclaving on selected nutrients of rapeseed. *Pak. J. Sci. Ind. Res.* **34**, 446-448.

Balasundram, N., Sundram, K. and Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food chem.* **99** (1), 191-203.

Balch, J. F. and Balch, P. A. (2000). Prescription for nutritional healing. *Penguin Putnam Inc. Vemonia amygdalina used by wild chimpanzees. Tetrahedron*. **48**, 625-630.

Barek, M. L., Hasmadi, M., Zaleha, A. Z. and Fadzelly, A. B. M. (2015). Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented peas from Sabah snake grass *(Clinacanthus nutans Lind.)* leaves. *Intl. Food Res. J.* **22** (2), 661.

Barnes, M. J., Uruakpa, F. O. and Udenigwe, C. C. (2015). Influence of cowpea *(Vigna unguiculata)* peptides on insulin resistance. *J. Nutr. Health Food Sci.* **3** (2), 1-3. [doi:10.15226/jnhfs.2015.00144].

Baskota, N. (2019). Effect of germination on anti-nutritional factors of cereal & legumes and their malt use in sarbottam pitho for infants. Bsc. Nutrition and Dietetics Thesis. Tribhuvan Univ., Nepal.

Bau, H. M., Villaume, C., Nicolas, J. P. and Méjean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean *(Glycine max)* seeds. *J. Sci. Food Agric.* **73** (1), 1-9.

Berry, S. K., Ibrahim, R. H. and Karim, N. A. (1988). Effect of germination on the biological quality of winged beans *(P. tetragonolobus)*. *Nutr. Reports Intl.* **37** (6), 1237-1243.

Bishnoi, S., Khetarpaul, N. and Yadav, R. K. (1994). Effect of domestic processing and cooking methods on phytic acid and polyphenol contents of pea cultivars *(Pisum sativum)*. *J. Plant Food Human Nutr*. **45**, 381-388.

Boukar, O., Fatokun, C. A., Roberts, P. A., Abberton, M., Huynh, B. L., Close, T. J., Kyei-Boahen, S., Higgins, T. J. V. and Ehlers, J. D. (2015). Cowpea. *In:* "Grain legumes".). pp. 219-250. Springer.

Bsc, S. N. and Bsc, G. S. (1999). Oxalate content of foods and its effect on humans. *Asia Pacific J. Clin. Nutr.* **8** (1), 64-74.

Burns, J., Gardner, P. T., Matthews, D., Duthie, G. G., Lean, J. and Crozier, A. (2001). Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *J. Agric. Food Chem.* **49** (12), 5797-5808.

Camacho, L., Sierra, C., Campos, R., Guzman, E. and Marcus, D. (1992). Nutritional changes caused by the germination of legumes commonly eaten in Chile. *Archivos Latinoamericanos Nutri.* **42** (3), 283-290.

Chaudhary, R., Oluyemisi, A. E., Shrestha, A. K. and Adhikari, B. M. (2015). Effect of germination on biochemical and nutritional quality of Kwati. *J. Food Process. Preserv.* **39** (6), 1509-1517.

Chaulagain, A., Adhikari, B. B. and Poudel, A. (2023). Performance study of varieties and plant geometry on productivity of cowpea *(Vigna unguiculata (L.) Walp)* at Bharatpur, Chitwan, Nepal. *World New Nat. Sci.* **46**, 79-86.

Chipurura, B. J. I. J. o. N., Functional Foods and Foods, N. (2018). Effect of soaking, dehulling and boiling on protein, polyphenolic and antinutrient content of cowpeas (Vigna unguiculata L. Walp).

Chon, S. (2013). Total polyphenols and bioactivity of seeds and sprouts in several legumes. *Curr. Pharm. Des.* **19** (34), 6112-6124.

Chung, T. Y., Nwokolo, E. N. and Sim, J. S. (1989). Compositional and digestibility changes in sprouted barley and canola seeds. *Plant Food Human Nutr.* **39**, 267-278.

Clementson, C. L., Ileleji, K. E. and Rosentrater, K. A. (2010). Evaluation of measurement procedures used to determine the bulk density of distillers dried grains with solubles (DDGS). *Transactions ASABE*. **53** (2), 485-490. [doi: 10.13031/2013.29557].

David, A. V. A., Arulmoli, R. and Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacogn. Rev.* **10** (20), 84-89. [doi: 10.4103/0973-7847.194044].

Day, R. A. and Underwood, A. L. (1991). "Quantitative analysis". Vol. 27. Prentice Hall NJ.

Dean, P. A. W. (2012). The oxalate dianion, C2O42–: planar or nonplanar? *J. Chem. Educ.* **89** (3), 417-418.

Dendougui, F. and Schwedt, G. (2004). In vitro analysis of binding capacities of calcium to phytic acid in different food samples. *Eur. Food Res. Technol.* **219**, 409-415.

Deol, J. K. and Bains, K. (2010). Effect of household cooking methods on nutritional and anti nutritional factors in green cowpea *(Vigna unguiculata)* pods. *J. Food Sci. Technol.* **47**, 579-581.

Deshpande, S. S., Cheryan, M., Salunkhe, D. K. and Luh, B. S. (1986). Tannin analysis of food products. *Crit. Rev. Food Sci. Nutr.* **24** (4), 401-449.

Devi, C. B., Kushwaha, A. and Kumar, A. (2015). Sprouting characteristics and associated changes in nutritional composition of cowpea *(Vigna unguiculata). J. Food Sci. Technol.* **52**, 6821-6827.

Doss, A. and Anand, S. P. (2012). Preliminary phytochemical screening of Asteracantha longifolia and Pergularia daemia. *World App. Sci. J.* **18** (2), 233-235. [doi: 10.5829/idosi. wasj. 2012. 18. 02. 1136].

Egbe, I. A. and Akinyele, I. O. (1990). Effect of cooking on the antinutritional factors of lima beans (Phaseolus lunatus). *Food Chem.* **35** (2), 81-87.

Egli, I., Davidsson, L., Juillerat, M. A., Barclay, D. and Hurrell, R. F. (2002). The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *J. Food Sci.* **67** (9), 3484-3488.

Ehirim, F. N., Ezeji, C. N., Onugha, F. C. and Nwogu, O. G. (2018a). Effect of sprouting time on the nutrient and anti-Nutrient properties of cowpea *(Vigna Unguiculata). IOSR J. Environ. Sci. Toxicol. Food Technol. (IOSR-JESTFT).* **12** (7 Ver. II (July. 2018)), 1-8. DOI:[10.9790/2402-1207020108].

Ehirim, F. N., Ezeji, C. N., Onugha, F. C. and Nwogu, O. G. (2018b). Effect of sprouting time on the nutrient and anti-nutrient properties of cowpea *(Vigna Unguiculata). J. Environ. Sci. Toxicol. Food. Technol.* **7** (12), 01-08.

El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A. and El-Beltagy, A. E. (2003). Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds. *Plant Food Human Nutr.* **58**, 1-13.

El-Gohery, S. S. (2021). Effect of different treatments on nutritional value of lima bean *(Phaseolus lunatus)* and its utilization in biscuit manufacture. *J. Food Nutr. Sci.* **12** (4), 372-391.

Elharadallou, S. B., Khalid, I. I., Gobouri, A. A. and Abdel-Hafez, S. H. (2015). Amino acid composition of cowpea *(Vigna ungiculata L. Walp)* flour and its protein isolates. *Food Nutr. Sci.* **6** (09), 790.

Emmanuel, E. and Deborah, S. (2018). Phytochemical and anti-nutritional studies on some commonly consumed fruits in lokoja, kogi state of Nigeria. **2** (3), 1-5.

Eskin, N. A. M. and Wiebe, S. (1983). Changes in phytase activity and phytate during germination of two fababean cultivars. *J. Food Sci.* **48** (1), 270-271.

Essack, H., Odhav, B. and Mellem, J. J. (2017). Screening of traditional South African leafy vegetables for specific anti-nutritional factors before and after processing. *J. Food Sci. Technol.* **37**, 462-471.

Etsuyankpa, M. B., Gimba, C. E., Agbaji, E. B., Omoniyi, K. I., Ndamitso, M. M. and Mathew, J. T. (2015). Assessment of the effects of microbial fermentation on selected anti-nutrients in the products of four local cassava varieties from Niger state, Nigeria. *Am. J. Food Sci. Technol.* **3** (3), 89-96.

Evers, A. D. and Rosentrater, K. A. (2018). "Kent's Technology of Cereals: An Introduction for Students of Food Science and Agriculture" (4th ed. ed.). Elsevier. [0081005296].

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutr. Res. Rev.* **23** (1), 65-134.

Fellows, P. J. (2009). "Food processing technology: principles and practice" (3rd ed. ed.). Elsevier. [1845696344].

Fernandes, A. C., Nishida, W. and da Costa Proença, R. P. (2010). Influence of soaking on the nutritional quality of common beans *(Phaseolus vulgaris L.)* cooked with or without the soaking water: a review. *Int. J. Food Sci. Technol.* **45** (11), 2209-2218.

Fouad, A. A. and Rehab, F. M. (2015). Effect of germination time on proximate analysis, bioactive compounds and antioxidant activity of lentil *(Lens culinaris Medik*.) sprouts. *Acta Scientiarum Polonorum Technologia Alimentaria*

14 (3), 233-246.

Fried, G., Norton, L. R. and Reboud, X. (2008). Environmental and management factors determining weed species composition and diversity in France. *Agric. Eco. Environ.* **128** (1-2), 68-76.

Friend, C. P., Ross, R. G., Waniska, R. D. and Rooney, L. W. (1995). Effects of additives in wheat flour tortillas. *Cereal Food World*. **40** (7), 494-497.

Frota, K. M. G., Mendonça, S., Saldiva, P. H. N., Cruz, R. J. and Arêas, J. A. G. (2008). Cholesterol-lowering properties of whole cowpea seed and its protein isolate in hamsters. *J. Food Sci.* **73** (9), H235-H240.

Galati, A., Oguntoyinbo, F. A., Moschetti, G., Crescimanno, M. and Settanni, L. (2014). The cereal market and the role of fermentation in cereal-based food production in Africa. *Food Rev. Int.* **30** (4), 317-337.

Gan, R. Y., Lui, W. Y., Wu, K., Chan, C. L., Dai, S. H., Sui, Z. Q., Corke, H. J. T. i. F. S. and Technology. (2017). Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. *Trend Food Sci. Technol.* **59**, 1-14.

Gemede, H. F. and Ratta, N. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. *Int. J. Nutr . Food Sci.* **3** (4), 284-289.

Gemede, H. F., Ratta, N. J. I. j. o. n. and sciences, f. (2014). Antinutritional factors in plant foods: Potential health benefits and adverse effects. **3** (4), 284-289.

Gernah, D. I., Ariahu, C. C. and Ingbian, E. K. (2011). Effects of malting and lactic fermentation on some chemical and functional properties of maize *(Zea mays)*. *Am. J. Food Technol.* **6** (5), 404-412.

Ghasemzadeh, A., Jaafar, H. Z. E. and Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger *(Zingiber officinale Roscoe)*. *Mol.* **15** (6), 4324-4333. [doi:10.3390/molecules15064324].

Ghavidel, R. A. and Prakash, J. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *Food Sci. Technol.* **40** (7), 1292-1299.

Gibson, R. S., Bailey, K. B., Gibbs, M. and Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food Nutr. Bull.* **31** (2_suppl2), S134-S146.

Greenwood, C. T. and Thomson, J. (1959). A comparison of the starches from barley and malted barley. *J. Inst. Brewing*. **65** (4), 346-353.

Gujral, H. S., Angurala, M., Sharma, P. and Singh, J. (2011). Phenolic content and antioxidant activity of germinated and cooked pulses. *Int. J. Food Properties*. **14** (6), 1366-1374.

Gülçin, I., Küfrevioğlu, Ö. İ., Oktay, M. and Büyükokuroğlu, M. E. (2004). Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle *(Urtica dioica L.)*. *J. Ethnopharmacol.* **90** (2-3), 205-215.

Gustafsson, E. L. and Sandberg, A. S. (1995). Phytate reduction in brown beans (Phaseolus vulgaris L.). *J. Food Sci.* **60** (1), 149-152.

Hall, A. E. (2001). Crop physiological responses to temperature and climatic zones. Crop Responses to Environment: CRC Press LLC, Boca Raton, Florida.

Halliwell, B. and Gutteridge, J. M. C. (1990). [1] Role of free radicals and catalytic metal ions in human disease: An overview. *Method Enzymol.* **186**, 1-85. [doi:10.1016/0076-6879(90)86093-B].

Hamid, S., Muzaffar, S., Wani, I. A., Masoodi, F. A. and Bhat, M. M. (2016). Physical and cooking characteristics of two cowpea cultivars grown in temperate Indian climate. *J. Saudi Soc. Agric. Sci.* **15** (2), 127-134.

Handa, V., Kumar, V., Panghal, A., Suri, S. and Kaur, J. (2017). Effect of soaking and germination on physicochemical and functional attributes of horsegram flour. *J. Food Sci. Technol*. **54**, 4229-4239.

Harborne, J. B. and Baxter, H. (1999). "The handbook of natural flavonoids. Volume 1 and Volume 2". John Wiley and Sons. [[ISBN 047195893X]].

Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.* **36** (6), 2090-2097.

Hayes, D. P. (2005). The protective role of fruits and vegetables against radiation-induced cancer. *Nutr. Rev.* **63** (9), 303-311.

Hertog, M. G. L., Hollman, P. C. H. and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agric. Food Chem.* **40** (12), 2379-2383.

Hough, J. S., Briggs, D. E., Stevens, R. and Young, T. W. (2012). "Malting and Brewing Science: volume II hopped wort and beer" (2nd ed.). Vol. 2. Springer. [1461517990].

Hough, J. S. and Hough, J. S. (1991). "The biotechnology of malting and brewing". Vol. 1. Cambridge University Press. [0521395534].

Ibrahim, S. S., Habiba, R. A., Shatta, A. A. and Embaby, H. E. (2002). Effect of soaking, germination, cooking and fermentation on antinutritional factors in cowpeas. *Food/Nahrung*. **46** (2), 92-95.

Imran, ï., Khan, A. A., Inam, I. and Ahmad, F. (2016). Yield and yield attributes of Mungbean *(Vigna radiata L.)* cultivars as affected by phosphorous levels under different tillage systems. *Cogent Food Agric.* **2** (1), 1151982.

Işık, E., İzli, N., Tekin, Y. and Ünal, H. İ. (2008). Geometric and mechanical properties of mung bean *(vigna radiata l.)* grain: Effect of moisture. *Int. J. Food Prop.* [doi:10.1080/10942910701573024].

James, S., Nwabueze, T. U., Ndife, J., Onwuka, G. I. and Usman, M. A. A. (2020). Influence of fermentation and germination on some bioactive components of selected lesser legumes indigenous to Nigeria. *J. Agric. Food Res.* **2**, 100086.

Jaradat, N., Hussen, F. and Al Ali, A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of Ephedra alata Decne. *J. Mater. Environ. Sci*. **6** (6), 1771-1778.

Jayanthakali, C., Visvanath, R., Deen, A., Bangamuwage, R., Jayawardana, B. C., Nammi, S. and Liyanage, R. (2018). Cowpea: an overview on its nutritional facts and health benefits. *J. Sci. Food Agric.* **98** (13), 4793-4806. [doi:10.1002/jsfa.9074].

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B. C., Nammi, S. and Liyanage, R. (2018). Cowpea: an overview on its nutritional facts and health benefits. *J. Sci. Food Agric.* **98** (13), 4793-4806. [doi:10.1002/jsfa.9074].

Jimenez, M. J., Elias, L. G., Bressani, R., Navarrete, D. A., Gomez-Brenes, R. and Molina, M. R. (1985). Biochemical and nutritional studies of germinated soybean seeds. *Arch. latinoam. Nutr.* **35** (3), 480-490.

Jirapa, P., Normah, H., Zamaliah, M. M., Asmah, R. and Mohamad, K. (2001). Nutritional quality of germinated cowpea flour *(Vigna unguiculata)* and its application in home prepared powdered weaning foods. *Plant Food Human Nutr.* **56**, 203-216.

Jood, S., Chauhan, B. M. and Kapoor, A. C. (1987). Polyphenols of chickpea and blackgram as affected by domestic processing and cooking methods. *J. Sci. Food Agric.* **39** (2), 145-149.

Kalpanadevi, V. and Mohan, V. R. (2013). Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, *Vigna unguiculata (L.) Walp subsp. unguiculata*. *LWT-Food. Sci. Technol.* **51** (2), 455-461.

Kataria, A., Chauhan, B. M. and Punia, D. (1989). Antinutrients and protein digestibility (in vitro) of mungbean as affected by domestic processing and cooking. *Food Chem.* **32** (1), 9-17.

Katiyar, D. C., Khan, M. Y., Banerji, R., Misra, G. and Nigam, S. K. (1989). Studies on mahua *(Bassia latifolia)* seed cake saponin (mowrin) in cattle. *Indian J. Animal Nutr.* **6** (1), 13-17.

Kaushik, G., Satya, S. and Naik, S. N. (2010). Effect of domestic processing techniques on the nutritional quality of the soybean. *Mediterranean J. Nutr. Metab.* **3** (1), 39-46.

Khalid, I. I. and Elharadallou, S. B. (2013). Functional properties of Cowpea *(Vigna ungiculata L. Walp)*, and Lupin *(Lupinus termis)* flour and protein isolates. *J. Nutr. Food Sci.* **3** (6), 1.

Khandelwal, S., Udipi, S. A. and Ghugre, P. (2010). Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. *Food Res. Int.* **43** (2), 526-530.

Khatoon, N. and Prakash, J. (2006). Nutrient retention in microwave cooked germinated legumes. *Food Chem.* **97** (1), 115-121.

Khyade, V. B. and Jagtap, S. G. (2016). Sprouting exert significant influence on the antioxidant activity in selected pulses (black gram, cowpea, desi chickpea and yellow mustard). *World Sci. News*. (35), 73-86.

Kirse, A. and Karklina, D. (2015). Integrated evaluation of cowpea *(Vigna unguiculata (L.) Walp.)* and maple pea *(Pisum sativum var. arvense L.)* spreads. *Agron. Res.* **13** (4), 956-968.

Kpoviessi, A. D., Agbahoungba, S., Agoyi, E. E., Chougourou, D. C. and Assogbadjo, A. E. (2019). Resistance of cowpea to Cowpea bruchid *(Callosobruchus maculatus Fab.)*: Knowledge level on the genetic advances. *J. Plant. Breed. Crop. Sci.* **11** (8), 185-195.

Kruszelnicka, W. (2021). Study of selected physical-mechanical properties of corn grains important from the point of view of mechanical processing systems designing. *Mater.* **14** (6), 1467.

Kumar, Y., Basu, S., Goswami, D., Devi, M., Shivhare, U. S. and Vishwakarma, R. K. (2022). Anti-nutritional compounds in pulses: Implications and alleviation methods. *Legume Sci.* **4** (2), e111.

Landete, J. M. (2012). Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. *Crit. Rev. Food Sci. Nutr.* **52** (10), 936-948.

LE, N. L., LE, T. T. H., Nguyen, N. T. M. and Vu, L. T. K. (2021). Impact of different treatments on chemical composition, physical, anti-nutritional, antioxidant

characteristics and in vitro starch digestibility of green-kernel black bean flours. *Food Sci. Technol.* **42**. [doi:10.1590/fst.31321].

Leaves, L. and Leaves, L. (2014). Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides. *Am. J. Ethnomed.* **1** (4), 244-249.

Leenhardt, F., Levrat-Verny, M. A., Chanliaud, E. and Rémésy, C. (2005). Moderate decrease of pH by sourdough fermentation is sufficient to reduce phytate content of whole wheat flour through endogenous phytase activity. *J. Agric. Food Chem.* **53** (1), 98-102.

Liener, I. E. (1974). Phytohemagglutinins. Their nutritional significance. *J. Agric. Food Chem.* **22** (1), 17-22.

Liyanage, R. (2018). Cowpea: an overview on its nutritional facts and health benefits. *J. Food Sci. Agric.* **98** (13).

Loewus, F. (2002). Biosynthesis of phytate in food grains and seeds. *Food Phytate*

53-61.

Lopez, H. W., Duclos, V., Coudray, C., Krespine, V., Feillet-Coudray, C., Messager, A., Demigné, C. and Rémésy, C. (2003). Making bread with sourdough improves mineral bioavailability from reconstituted whole wheat flour in rats. *Nutr.* **19** (6), 524-530. [doi:10.1016/S0899-9007(02)01079-1].

Manach, C., Scalbert, A., Morand, C., Rémésy, C. and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **79** (5), 727-747.

Maréchal, R. (1978). Etude taxonomique d'un groupe complexe d'espèces des genres Phaseolus et Vigna *(Papilionaceae)* sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. *Boissiera*. **28**, 1-273.

McGee, H. (2007). "On food and cooking: the science and lore of the kitchen". Simon and Schuster. [1416556370].

Mehta, M. B., Mehta, B., Bapodra, A. H. and Joshi, H. D. (2007). Effect of germination and heat processing on protein, riboflavin, vit-C and niacin content in peas, cowpea, redgram and wheat. *Asian. J. Home. Sci.* **2** (1/2), 34-38.

Mehta, P. M. and Bedi, B. (1993). In vitro protein and starch digestibility in selected germinated legume flours. *Indian J. Nutr. Diet*. **30**, 149-153.

Mekky, R. H., Thabet, M. M., Rodríguez-Pérez, C., Elnaggar, D. M. Y., Mahrous, E. A., Segura-Carretero, A. and Abdel-Sattar, E. (2020). Comparative metabolite profiling

and antioxidant potentials of seeds and sprouts of three Egyptian cultivars of Vicia faba L. *Food Res. Int.* **136**, 109537.

Miean, K. H. and Mohamed, S. (2001). Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* **49** (6), 3106-3112.

Miller, N. G. and McLelland, M. (2001). Using 1,000 kernel weight for calculating seeding rates and harvest losses.

Mittal, R., Nagi, H. P. S., Sharma, P. and Sharma, S. (2012). Effect of processing on chemical composition and antinutritional factors in chickpea flour. *J. Food Sci. Eng.* **2** (3), 180.

Mudryj, A. N., Yu, N., Hartman, T. J., Mitchell, D. C., Lawrence, F. R. and Aukema, H. M. (2012). Pulse consumption in Canadian adults influences nutrient intakes. *Br. J. Nutr.* **108** (S1), S27-S36.

Murugkar, D. A. and Jha, K. (2009). Effect of sprouting on nutritional and functional characteristics of soybean. *J. Food Sci. Technol.* **46** (3), 240-243.

Nachbar, M. S., JD, O. and JO, T. (1980). Lectins in the US Diet. Isolation and characterization of a lectin from the tomato *(Lycopersicon esculentum)*. *Phys. Rev.* (47), 77-780.

Nagraj, G. S., Chouksey, A., Jaiswal, S. and Jaiswal, A. K. (2020). Broccoli. *In:* "Nutritional Composition and Antioxidant Properties of Fruits and Vegetables".). pp. 5-17. Elsevier.

Nikolopoulou, D. and Grigorakis, K. (2008). Nutritional and antinutritional composition of legumes and factors affecting it. *Food Sci. Technol: New Res. LV Greco. MN Bruno, eds. Nova: New York*. 105-170.

Nkaa, F. A. l., Nwokeocha, O. W. and Ihuoma, O. (2014). Effect of phosphorus fertilizer on growth and yield of cowpea *(Vigna unguiculata)*. *J. Phar. Biol. Sci.* **9** (5), 74-82.

Nonogaki, H., Bassel, G. W. and Bewley, J. D. (2010). Germination—still a mystery. *Plant Sci.* **179** (6), 574-581.

Noonan, S. C. and Savage, G. P. (1999). Oxalic acid and its effects on humans. *Asia Pacific J. Clin. Nutr.* **8** (1), 64-74.

Nourhane, D. and Amel, Z. (2021). Salinity effect on germination and Proline synthesis of the leguminous species. Thesis. Univ. center of Abdalhafid boussouf-MILA,

Obizoba, I. C. (1991). Effect of sprouting on the nitrogenous constituents and mineral composition of pigeon pea *(Cajanus cajan)* seeds. *Plant Food Human Nutr.* **41**, 21-26.

Oboh, G. (2006). Antioxidant properties of some commonly consumed and underutilized tropical legumes. *Eur. Food Res. Technol.* **224**, 61-65.

OCDE, O. (2016). "Safety Assessment of Transgenic Organisms in the Environment". OECD Publishing. [9264253017].

Ogodo, A. C., Agwaranze, D. I., Aliba, N. V., Kalu, A. C. and Nwaneri, C. B. (2019). Fermentation by lactic acid bacteria consortium and its effect on anti-nutritional factors in maize flour. *J. Biol. Sci.* **19** (1), 17-23.

Ohtsubo, K., Suzuki, K., Yasui, Y. and Kasumi, T. (2005). Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. *J. Food Composition Anal.* **18** (4), 303-316.

Oladimeji, M. O., Akindahunsi, A. A. and Okafor, A. F. (2000). Investigation of the bioavailability of zinc and calcium from some tropical tubers. *Nahrung*. **44** (2), 136-137.

Oloyo, R. A. (2004). Chemical and nutritional quality changes in germinating seeds of *Cajanus cajan L*. *Food Chem.* **85** (4), 497-502.

Omenna, E. C., Olanipekun, O. T. and Kolade, R. O. (2016). Effect of boiling, pressure cooking and germination on the nutritional and antinutrients content of cowpea *(Vigna unguiculata)*. *ISABB J. Food Agric. Sci.* **6** (1), 1-8.

Onwuka, G. I. (2006). Soaking, boiling and antinutritional factors in pigeon peas (Cajanus cajan) and cowpeas *(Vigna unguiculata)*. *J. Food Process. Presv.* **30** (5), 616-630.

Opuku, A. R., Ohenhen, S. O. and Ejiofor, N. (1981). Nutrient composition of millet *(Pennisetum thyphoides)* grains and malts. *J. Agric. Food Chem.* **29**, 1247-1248.

Ouchemoukh, S., Amessis-Ouchemoukh, N., Gómez-Romero, M., Aboud, F., Giuseppe, A., Fernández-Gutiérrez, A. and Segura-Carretero, A. (2017). Characterisation of phenolic compounds in Algerian honeys by RP-HPLC coupled to electrospray time-of-flight mass spectrometry. *LWT-Food Sci. Technol.* **85**, 460-469.

Padulosi[1], S. and Ng, N. Q. (1997). Origin, taxonomy, and morphology of *Vigna unguiculata (L.) Walp*. *Adv. Cowpea Res.*, 1.

Pal, R., Bhartiya, A., ArunKumar, R., Kant, L., Aditya, J. and Bisht, J. k. (2016). Impact of dehulling and germination on nutrients, antinutrients, and antioxidant properties in horsegram. *J. Food Sci. Technol.* **53** (1), 337-347.

Parameswaran, K. P. and Sadasivam, S. (1994). Changes in the carbohydrates and nitrogenous components during germination of proso millet, *Panicum miliaceum*. *Plant Food Human Nutr.* **45**, 97-102.

Paramjyothi, S. and Mulimani, V. H. (1996). Effect of sprouting, cooking and dehulling on polyphenols of redgram *(Cajanus cajan L).*

Parvez, R., Singh, K., Yadav, V. and Singh, L. (2019). Effect of various processing treatments on total flavonoid content of different varieties of cowpea. *Pharm. Innov. J.* **8** (6), 203-210.

Pasquet, R. S. (1999). Genetic relationships among subspecies of *Vigna unguiculata (L.) Walp*. based on allozyme variation. *Theor. Appl. Genetics*. **98**, 1104-1119.

Perera, O. S., Liyanage, R., Weththasinghe, P., Jayawardana, B. C., Vidanarachchi, J. K., Fernando, P. and Sivakanesan, R. (2016). Modulating effects of cowpea incorporated diets on serum lipids and serum antioxidant activity in Wistar rats. *J. National Sci. Fdn. SRL.* **44** (1).

Pokharel, U. (2022). Effect of processing methods on antinuritional factors present in green gram (Mung Bean) B. Tech Thesis. Tribhuvan Univ., Nepal.

Pokorny, J. (2007). Antioxidants in food preservation. *In:* "Handbook of food preservation".). pp. 277-304. CRC press. [0429191081].

Popova, A. and Mihaylova, D. (2019). Antinutrients in plant-based foods: A review. *The Open Biotechnol. J.* **13** (1).

Prasad, P., Kochhar, A. and Javed, M. (2016). Development of ready-to-eat supplementary foods using germinated cereal-pulse mix, potato flour and green leafy vegetable for malnourished children. *Nutr. Food Sci.*

Preet, K. and Punia, D. (2000). Antinutrients and digestibility (in vitro) of soaked, dehulled and germinated cowpeas. *Nutr. Health*. **14** (2), 109-117.

Prom-u-thai, C., Huang, L., Glahn, R. P., Welch, R. M., Fukai, S., Rerkasem, B. J. J. o. t. S. o. F. and Agriculture. (2006). Iron (Fe) bioavailability and the distribution of anti-Fe nutrition biochemicals in the unpolished, polished grain and bran fraction of five rice genotypes. **86** (8), 1209-1215.

Punia, K. P. D. (2000). Proximate composition, phytic acid, polyphenols and digestibility (in vitro) of four brown cowpea varieties. *Int. J. Food Sci. Nutr.* **51** (3), 189-193.

Rackis, J. J., Wolf, W. J. and Baker, E. C. (1986). Protease inhibitors in plant foods: content and inactivation. *Nutr. Toxicol. Sig. Enzyme Inhibitors Food*. 299-347.

Rana, S. (2022). Effect of various processing treatments on *Macrotyloma uniflorum* (Horse gram). *The. Pharma. Innov. J.* **11** (6), 638-643.

Ranganna, S. (1986). "Handbook of analysis and quality control for fruit and vegetable products". Tata McGraw-Hill Education. [0074518518].

Rather, T. A., Malik, M. A. and Dar, A. H. (2016). Physical, milling, cooking, and pasting characteristics of different rice varieties grown in the valley of Kashmir India. *Cogent Food Agric.* **2** (1), 1178694.

Reddy, N. R. and Pierson, M. D. (1994). Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Res. Int.* **27** (3), 281-290.

Ribarova, F., Atanassova, M., Marinova, D., Ribarova, F. and Atanassova, M. (2005). Total phenolics and flavonoids in Bulgarian fruits and vegetables. *JU. Chem. Metal*. **40**, 255-260.

Rosentrater, K. A. and Evers, A. D. (2017). "Kent's technology of cereals: An introduction for students of food science and agriculture". Woodhead Publishing. [0081005326].

Rowland, I. (1999). Optimal nutrition: fibre and phytochemicals. *Proc. Nutr. Soc.* **58** (2), 415-419.

Samarajeewa, U., Sen, A. C., Cohen, M. D. and Wei, C. I. (1990). Detoxification of aflatoxins in foods and feeds by physical and chemical methods. *J. Food Protection*. **53** (6), 489-501.

Santos, C. A. F. and Boiteux, L. S. (2013). Breeding biofortified cowpea lines for semi-arid tropical areas by combining higher seed protein and mineral levels.

Sasakawa, N., Sharif, M. and Hanley, M. R. (1995). Metabolism and biological activities of inositol pentakisphosphate and inositol hexakisphosphate. *Biochem. Pharmacol.* **50** (2), 137-146.

Savage, G. and Klunklin, W. (2018). Oxalates are found in many different European and Asian foods-effects of cooking and processing. *J. Food Res.* **7** (3), 76-81.

Shah, S. A., Zeb, A., Masood, T., Noreen, N., Abbas, S. J., Samiullah, M., Alim, M. A. and Muhammad, A. (2011). Effects of sprouting time on biochemical and nutritional qualities ofMungbean varieties. *Afr. J. Agric. Res.* **6** (22), 5091-5098.

Shamsuddin, A. M. (2002). Anti-cancer function of phytic acid. *Int. J. Food Sci. Technol.* **37** (7), 769-782.

Shi, L., Arntfield, S. D. and Nickerson, M. (2018). Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. *Food Res. Int.* **107**, 660-668.

Shimada, T. (2006). Salivary proteins as a defense against dietary tannins. *J. Chem. Ecol.* **32**, 1149-1163.

Shrestha, R. (1995). To study and compare the finger millet (kodo) malt with barley malt (European variety and local variety) on the brewing aspects. . *B. Tech. Dissertation. Tribhuvan Univ. Nepal*.

Siddiq, M. and Uebersax, M. A. (2022). "Dry Beans and Pulses: Production, Processing, and Nutrition". John Wiley & Sons. [[ISSN: 1119777119]].

Simwaka, J. E., Chamba, M. V. M., Huiming, Z., Masamba, K. G. and Luo, Y. (2017). Effect of fermentation on physicochemical and antinutritional factors of complementary foods from millet, sorghum, pumpkin and amaranth seed flours. *Int. Food Res. J.* **24** (5), 1869-1879.

Singh, B. B. and Matsui, T. (2002). Cowpea varieties for drought tolerance. *Challenges opportunities for enhancing sustainable cowpea production*. 287-300.

Singh, R., Verma, P. K. and Singh, G. (1970). Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*. *J. Complement. Med. Res.* **1** (2), 101-101.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-ciocalteu Reagent. *In:* "Methods in enzymology" (Vol. 299).). pp. 152-178. Elsevier. [[ISBN: 0076-6879]].

Sinha, R. and Kawatra, A. (2003a). Effect of processing on phytic acid and polyphenol contents of cowpeas *[Vigna unguiculata (L) Walp]*. *Plant Food Human Nutr.* **58**, 1-8.

Sinha, R. and Kawatra, A. J. P. F. f. H. N. (2003b). Effect of processing on phytic acid and polyphenol contents of cowpeas [Vigna unguiculata (L) Walp]. **58**, 1-8.

Smith, B. G., James, B. J. and Ho, C. A. L. (2007). Microstructural characteristics of dried carrot pieces and real time observations during their exposure to moisture. *Int. J. Food Eng.* **3** (4).

Smith, J. E., Korsten, L. and Aveling, T. A. S. (1999). Infection Process of *Colletotrichum dematium* on Cowpea Stems. *Mycol. Res.* **103** (2), 230-234. [doi:10.1017/S0953756298006868].

Soetan, K. O. (2008). Pharmacological and other beneficial effects of antinutritional factors in plants-A review. *Afr. J. Biotechnol.* **7** (25).

Soetan, K. O. and Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African J. Food Sci.* **3** (9), 223-232.

Sombié, P. A. E. D., Compaoré, M., Coulibaly, A. Y., Ouédraogo, J. T., Tignégré, J. B. D. L. S. and Kiendrébéogo, M. (2018). Antioxidant and phytochemical studies of 31 cowpeas *(Vigna unguiculata (L. Walp.))* genotypes from Burkina Faso. *Food*. **7** (9), 143.

Sorour, M. A. H., Galel, H. A. E., Mehanni, A. H. E. and Ahmed, W. K. (2018). Polyphenols, Tannins and Phytate contents in some Egyptian legumes as affected by soaking and germination process. *J. Sohag. Agric. Sci.* **3** (1), 94-111.

Tabekhia, M. M. and Luh, B. S. (1980). Effect of germination, cooking, and canning on phosphorus and phytate retention in dry beans. *J. Food Sci.* **45** (2), 406-408.

Traoré, T., Mouquet, C., Icard-Vernière, C., Traore, A. S. and Trèche, S. (2004). Changes in nutrient composition, phytate and cyanide contents and α-amylase activity during cereal malting in small production units in Ouagadougou *(Burkina Faso)*. *Food Chem.* **88** (1), 105-114.

Trehan, I., Benzoni, N. S., Wang, A. Z., Bollinger, L. B., Ngoma, T. N., Chimimba, U. K., Stephenson, K. B., Agapova, S. E., Maleta, K. M. and Manary, M. (2015a). Common beans and cowpeas as complementary foods to reduce environmental enteric dysfunction and stunting in Malawian children: study protocol for two randomized controlled trials. *Trials*. **16** (1), 1-12.

Trehan, I., Benzoni, N. S., Wang, A. Z., Bollinger, L. B., Ngoma, T. N., Chimimba, U. K., Stephenson, K. B., Agapova, S. E., Maleta, K. M. and Manary, M. J. (2015b). Common beans and cowpeas as complementary foods to reduce environmental enteric dysfunction and stunting in Malawian children: study protocol for two randomized controlled trials. *Trials*. **16** (1), 1-12.

Trugo, L. C., Donangelo, C. M., Trugo, N. M. F. and Bach Knudsen, K. E. (2000). Effect of heat treatment on nutritional quality of germinated legume seeds. *J. Agric. Food Chem.* **48** (6), 2082-2086.

Udensi, E. A., Ekwu, F. C. and Isinguzo, J. N. (2007). Antinutrient Factors of Vegetable Cowpea *(Sesquipedalis)* Seeds During Thermal Processing. *Pak. J. Nutr.* **6** (2), 194-197.

Unal, H., Isık, E., Izli, N. and Tekin, Y. J. I. J. o. F. P. (2008). Geometric and mechanical properties of mung bean (Vigna radiata L.) grain: Effect of moisture. **11** (3), 585-599.

Uppal, V. and Bains, K. (2012a). Effect of germination periods and hydrothermal treatments on In vitro protein and starch digestibility of germinated legumes. *J. Food Sci. Technol.* **49**, 184-191.

Uppal, V. and Bains, K. (2012b). Effect of germination periods and hydrothermal treatments on In vitro protein and starch digestibility of germinated legumes. *J. Food Sci.Technol.* **49** (2), 184-191.

Urbano, G., López-Jurado, M., Ławomir Frejnagel, S., Gómez-Villalva, E., Porres, J. M., Frías, J., Vidal-Valverde, C. and Aranda, P. (2005). Nutritional assessment of raw and germinated pea *(Pisum sativum L.)* protein and carbohydrate by In vitro and In vivo techniques. *Food chem.* **21** (2), 230-239.

Uriyo, M. G. (2001). Changes in enzyme activities during germination of cowpeas *(Vigna unguiculata, cv. California blackeye)*. *Food chem.* **73** (1), 7-10.

Uwaegbute, A. C., Iroegbu, C. U. and Eke, O. (2000). Chemical and sensory evaluation of germinated cowpeas *(Vigna unguiculata)* and their products. *Food chem.* **68** (2), 141-146.

Vaya, J. and Aviram, M. (2001). Nutritional antioxidants mechanisms of action, analyses of activities and medical applications. *Curr. Med. Chem-Immunol. Endocrine Metab. Agent*. **1** (1), 99-117.

Vidal-Valverde, C., Frias, J., Estrella, I., Gorospe, M. J., Ruiz, R. and Bacon, J. (1994). Effect of processing on some antinutritional factors of lentils. *J. Agric. Food Chem.* **42** (10), 2291-2295.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Lambein, F. and Kuo, Y. H. (2002). New functional legume foods by germination: Effect on the nutritive value of beans, lentils and peas. *Eur. Food Res. Technol.* **215**, 472-477.

Virginia, P., Swati, V. and Ajit, P. (2012). Effect of cooking and processing methods on oxalate content of green leafy vegetables and pulses. *Asian J. Food Agro. Ind.* **5** (4), 311-314.

Wang, N., Lewis, M. J., Brennan, J. G. and Westby, A. (1997). Effect of processing methods on nutrients and anti-nutritional factors in cowpea. *Food Chem.* **58** (1-2), 59-68.

Wani, I. A., Sogi, D. S., Wani, A. A. and Gill, B. S. (2017). Physical and cooking characteristics of some Indian kidney bean *(Phaseolus vulgaris L.)* cultivars. *J. Saudi Soc. Agric. Sci.* **16** (1), 7-15.

Yasmin, A., Zeb, A., Khalil, A. W., Paracha, G. M. D. and Khattak, A. B. (2008). Effect of processing on anti-nutritional factors of red kidney bean *(Phaseolus vulgaris)* grains. *Food Bioprocess. Technol.* **1** (4), 415-419.

Appendices

Appendix A

1. Standard curve for total phenol content

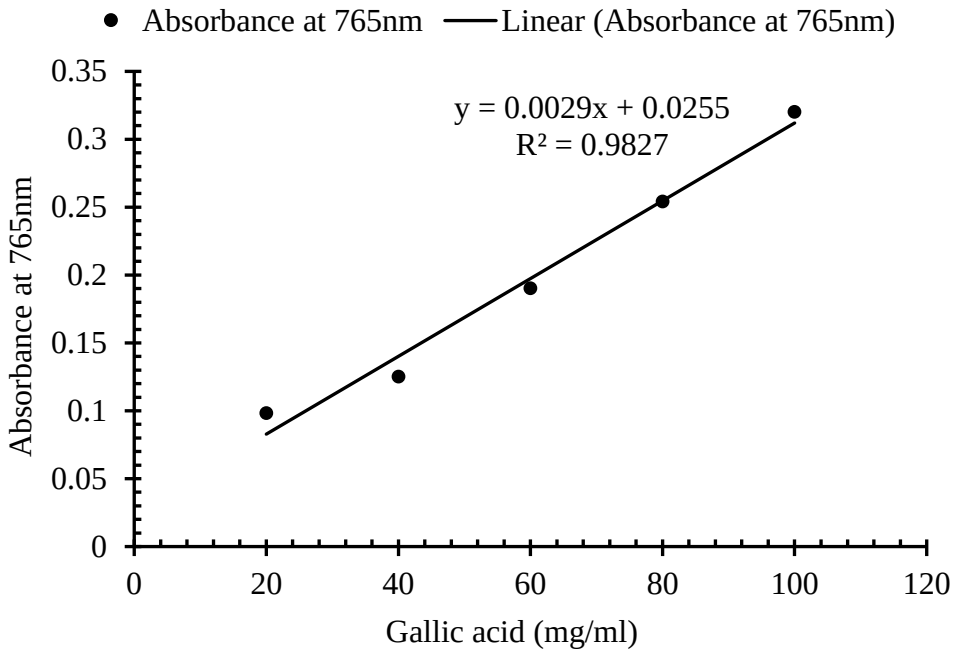

Fig. A.1 Standard curve of gallic acid for total phenol content

2.Standard curve for tannin content

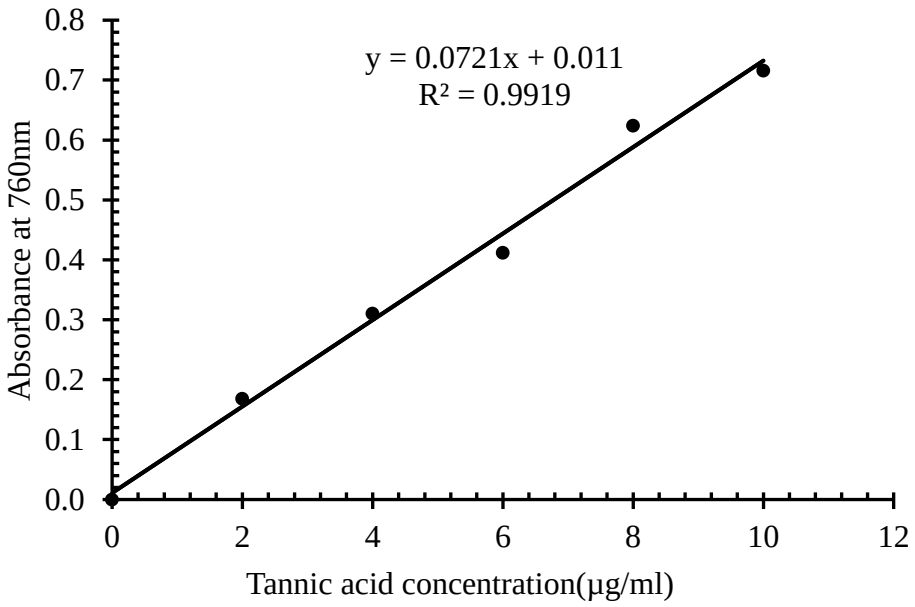

Fig. A.2 Standard curve of tannic acid for tannin content

3.Standard curve for total flavonoid content

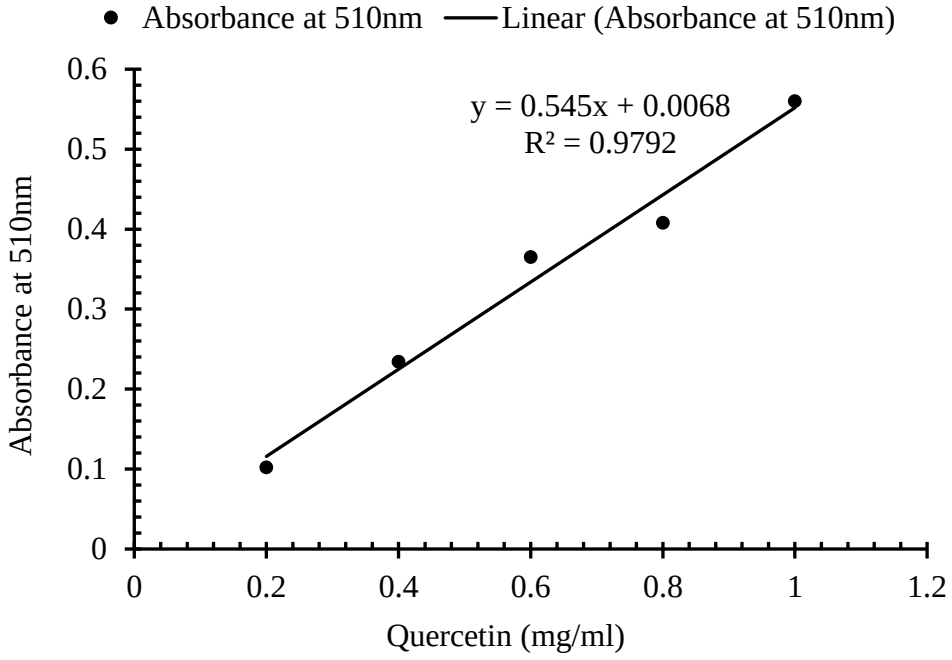

Fig. A.3 Standard curve of quercetin for total flavonoid content

Appendix B

Table B.1 ANOVA for protein content

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
No of days	5	338.4139	67.6828	148.59	<.001
Residual	12	5.4661	0.4555		
Total	17	343.8801			

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

Table B.2 LSD of means for protein

No of days	Mean	Column A	l. s. d	d. f
Raw*	27.02±0.12	A	1.201	12
D1*	29.89±0.53	B		
D2*	32.11±0.83	C		
D3*	34.23±0.58	D		
D4*	37.83±0.53	E		
D5*	39.55±1.05	F		

(*= significantly different)

Table B.3 ANOVA for TPC content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
No of days	5	565610.019	113122.004	20463.75	<.001
Residual	12	66.335	5.523		
Total	17	565676.345			

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

Table B.4 LSD of means for TPC content

No of days	Mean	Column A	L. s. d	d. f
D5*	203.1±1.53	A	4.183	12
D4*	229.01±1.77	B		
D3*	293.0±0.77	C		
D2*	352.2±0.45	D		
D1*	422.9±1.15	E		
Raw*	732.2±1.08	F		

(* = significantly different)

Table B.5 ANOVA table for DPPH

Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio	F probability ratio
No of days	5	2541.209	508.242	359.69	<.001
Residual	12	16.956	1.413		
Total	17	2558.165			

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

Table B.6 LSD of mean for DPPH

No of days	Mean	Column A	L.s. d	d. f
Raw*	41.67±1.56	A	2.115	12
D1*	48.13±0.39	B		
D2*	53.19±0.91	C		
D3*	63.44±1.33	D		
D4*	70.59±0.88	E		
D5*	74.55±1.57	F		

(*= significantly different)

Table B.7 ANOVA table for Tannin

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
No of days	5	14425.8761	2885.1752	3931.23	<.001
Residual	12	8.8069	0.7339		
Total	17	14434.6830			

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

Table B.8 LSD of means for Tannin

Treatment	Mean	Column A	L. s. d	d. f
D5*	121.0±0.53	A	1.524	12
D4*	125.6±0.27	B		
D3*	133.0±0.75	C		
D2*	146.9±0.08	D		
D1*	174.1±0.76	E		
Raw*	199.9±1.69	F		

(*= significantly different)

Table B.9 ANOVA for Phytic acid

Source of variation	Degree of freedom	Sum of square	Mean square	Variance ratio	F probability ratio
No of days	5	471102.382	94220.476	16327.30	<.001
Residual	12	69.249	5.771		
Total	17	471171.631			

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

Table B.10 LSD for means of phytic acid

No of days	Mean	Column A	L. s. d	d. f
D5*	343.8±2.53	A	4.274	12
D4*	372.2±2.81	B		
D3*	446.2±2.13	C		
D2*	536.1±1.70	D		
D1*	612.9±1.91	E		
Raw*	819.8±3.02	F		

(*= significantly different)

Table B.11 ANOVA for flavonoid content

Source of variation	Degree of freedom	Sum of square	Mean squares	Variance ratio	F probability ratio
Treatment	5	8821.750	1764.350	284.92	<.001
Residual	12	74.310	6.193		
Total	17	8896.060			

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

Table B.12 LSD for means of flavonoid

Treatment	Mean	Column A	l. s. d	d. f.
D5*	38.01±1.61	A	4.427	12
D4*	45.94±2.65	B		
D3*	53.82±2.52	C		
D2*	66.92±1.88	D		
D1*	73.97±2.54	E		
Raw*	105.56±3.31	F		

(*= significantly different)

Table B.13 ANOVA for oxalate

Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio	F probability ratio
No of days	5	16645.94	3329.19	131.91	<.001
Residual	12	302.86	25.24		
Total	17	16948.80			

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

Table B.14 LSD of means for oxalate

No of days	Mean	Column A	L. s. d	d. f.
D5	82.6± 4.3	A	8.94	12
D4	88.1± 5.08	AB		
D3*	96.6± 6.1	B		
D2*	110.1± 5.36	C		
D1*	133.5± 4.76	D		
Raw*	170.5± 4.30	E		

(*= significantly different)

List of Plates

P1 Spectrophotometric determination of polyphenols

P2 Titration for oxalate determination

P3 Prepared sample for analysis

P4 Germinated sample kept for digestion for analysis of protein