CHANGES IN NUTRITIONAL COMPOSITIONS OF FABA BEANS (*Vicia faba*) UNDER DIFFERENT PROCESSING METHODS

by

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

by

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Approval Letter

The ***dissertation*** entitled "***Changes in Nutritional Compositions of Faba beans (Vicia faba) Under Different Processing Methods***" presented by **Aashish Khadka** has been accepted as the partial fulfillment of the requirements for **Bachelor degree in Nutrition and Dietetics**.

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Sincerely, Aashish Khadka.

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Abstract

Faba beans are known for their high protein and iron content. They also contain bio-active compounds that have several health benefits. But their consumption is limited due to the presence of antinutritional factors that may impair nutrient absorption. This study was aimed to investigate the effect of various processing techniques, including soaking, dehulling, germination for 96 hours, open cooking, and autoclaving, on the nutritional composition (crude protein and iron content), and phytochemical content (total phenolic content and total flavonoid content), and antinutritional constituents (tannins and phytic acid) of faba beans (*Vicia faba*). The research employed a randomized experimental design, with faba beans subjected to different processing methods.

The mean value of crude protein was found to be 26.50g/100g and iron content to be 5.67mg/100g. The mean value of phytic acid, tannin, total phenolic content, and total flavonoid content was 953.3mg/100g, 217.37mg/100g, 391.8mg/100g and 439.3mg/100g, respectively. The results revealed that germination for 96 hours led to a significant reduction ($p<0.05$) in tannin and phytic acid levels by 66% and 75%, respectively. This prolonged germination period also resulted in significant increases ($p<0.05$) in crude protein (+19.62%), iron content (+6.75%), and total flavonoids (+30.3%). These findings highlight the efficacy of germination for 96 hours in improving the nutritional and phytochemical profile of faba beans. These processing techniques demonstrated their potential in reducing antinutrients and enhancing the nutritional quality of faba beans. However, it is worth noting that certain processing techniques resulted in a decrease in phenols and flavonoids, important bioactive components associated with various health benefits. Therefore, a balance must be struck to minimize the loss of beneficial phytochemicals while effectively reducing antinutritional constituents.

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List of Abbreviations

Abbreviation	Full form
ANFs	Anti-nutritional factors
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemist
CCT	Central Campus of Technology
CCT	Central Campus of Technology
D.F.	Degree of freedom
FAO	Food and Agriculture Organization
G24	Germination for 24 hours
G48	Germination for 48 hours
G72	Germination for 72 hours
G96	Germination for 96 hours
GAE	Gallic acid equivalent
L-DOPA	Dihydroxypehnylalanine
LSD	Least significance difference
M.S.	Mean squares
QE	Quercetin equivalent
RA	Raw autoclaving
ROC	Raw open cooking
S.D.	Standard Deviation
S.S.	Sum of squares
TFC	Total flavonoid content
TPC	Total polyphenol content
USDA	United States Department of Agriculture
V.R.	Variance ratio

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Part I

Introduction

1.1 General Introduction

Legumes belong to the family Leguminosae and consist of oilseeds such as soybeans, peanuts, clover, mesquite, and pulses, including the dry grains of peas, chickpeas, lentils, peas, beans, and lupins. Production and use of legumes date back to ancient cultures in Asia, the Middle East, South America, and North Africa. They are cultivated throughout the world for their seeds, harvested and marketed as primary products. Grain legumes are grouped into pulses and oilseeds. The pulses are different from the leguminous oilseeds, which are primarily utilized for oil (Schneider, 2002). Notable amongst legume species are chickpeas (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), lentil (*Lens culinaris*), mung bean (*Vigna radiata*), soybean (*Glycine max*), winged bean (*Psophocarpus tetragonoloba*), cowpea (*Vigna unguiculata*), pea (*Pisum sativum*), groundnut (*Arachis hypogaea*), and black gram (*Vigna mungo*), to mention a few. Some of the most important legumes in the world are peas, beans, peanuts, soybeans, and chickpea (Reyes-Moreno *et al.*, 2004).

Legumes have a special place in the diet of humans, because they contain nearly 2–3 times more protein than cereals (Reyes-Moreno *et al.*, 2004). Legumes are also excellent sources of complex carbohydrates and have been reported as beneficial for cardiovascular diseases and diabetes by some researchers, due to the large amounts of water-soluble fiber and a large content of phenolics. Legumes are also a good source of vitamins (thiamine, riboflavin, niacin, vitamin B6, and folic acid) and certain minerals (Ca, Fe, Cu, Zn, P, K, and Mg), and are an excellent source of polyunsaturated fatty acids, linoleic and linolenic acids (Augustin and Klein, 1989).

Legume crops, a sustainable source of high-protein food, are grown widely throughout the world. Among legumes, faba bean (*Vicia faba*), also known as field bean, broad bean, and horse bean, is one of the oldest crops cultivated worldwide (Mínguez and Rubiales, 2021). Faba beans (Vicia faba) are a winter-sowing grain legume with high-protein content utilized for human consumption and animal feed (Sudheesh *et al.*, 2019). Faba bean is one of the seventh world's most important pulse crops, followed by chickpea, cowpea, pea, pigeon pea, and lentil. Faba bean seeds are cultivated in more than sixty countries. East and North Africa, southern Europe, West, and South Asia, South and North America, and

Australia. Mediterranean countries, Ethiopia, Egypt, China, Afghanistan, India, Northern Europe, and Northern Africa are major producers of faba bean (Rahate *et al.*, 2021). Faba bean cultivars can be divided into two main types: broad beans and field beans. Broad bean cultivars are large, flattened seeds with a one hundred seed weight between 100-200g, and field bean cultivars are small round seeds with a one hundred seed weight between 40-80g (Crépon *et al.*, 2010). Faba beans are considered as an important crop from an ecological, nutritional, and economical point of view. It is a multiuse crop providing various ecosystem services, that is, cultivated primarily as a food source for human population residing in Asia and Africa, as animal feed/silage in the European region, and fixation of atmospheric nitrogen in agricultural soils thereby significantly reducing the application of synthetic fertilizers (Zhou *et al.*, 2018).

Nutritionally, mature seeds of faba bean are rich in proteins (26.1%), carbohydrates (58.3%), and dietary fiber (25.0%) (Larrick *et al.*, 2022). Faba bean also contains a variety of bioactive compounds, for example, total phenolics, and flavonoids with demonstrated antioxidant activity (Valente *et al.*, 2018). Faba bean contains different antinutritional factors such as lectins, saponins, trypsin inhibitor, phytic acids, condensed tannins, and favism inducing factors that negatively affected its biological value (Revilla, 2015).

Faba beans can be used as a vegetable, green or dried, fresh, or cooked/canned. It is a common breakfast food in the Middle East, Mediterranean region, China, and Ethiopia. The most popular dishes of faba bean are Medamis (stewed beans), Falafel (deep fried cotyledon paste, with some vegetables and spices), Bissara (cotyledon paste), and Nabet soup prepared from boiled germinated faba beans (A. K. Singh *et al.*, 2013).

Antinutritional factors are compound or substance which generated by normal metabolism of species in natural food stuffs and act to reduce nutrient intake, digestion, absorption and utilization and produces many other adverse effects (Kumar, 1992). Legumes contain various types of antinutritional compounds including phytic acid, tannins, polyphenols, trypsin, chymotrypsin, a-amylase inhibitors and hemagglutinin activity (Udensi *et al.*, 2007). These types of compounds also shown certain biochemical and physiological effects on human and animals, such as enlargement of pancreases and growth retardation (Liener, 1989). These constituents also have an influence on appetite,

absorption of nutrients, metabolism and on the bioavailability of certain minerals (Frolich, 1995).

Chemical and physical methods usually used to remove or reduce antinutritional factors include soaking, cooking, autoclaving, microwave cooking, extrusion, germination, fermentation, irradiation, and enzymatic treatments.

1.2 Statement of the problem

Faba beans (*Vicia faba*), a significant source of protein, dietary fiber, vitamins, and minerals, have garnered increasing attention for their potential health benefits. However, despite their nutritional richness and versatility in culinary applications, faba beans have remained underutilized in many parts of the world. Limited awareness, cultural preferences, and the perception of anti-nutritional factors in faba beans have contributed to their underutilization. The nutritional composition of faba beans can be significantly influenced by various processing methods. Understanding how these processing techniques affect the nutritional profile of faba beans is vital to maximizing their potential as a nutritious and sustainable food source. Given the lack of comprehensive research investigating the impact of different processing methods on the nutritional composition of faba beans, there exists a notable knowledge gap. Therefore, this study aims to address this gap by examining the changes in the nutritional composition of faba beans under various processing techniques, contributing valuable insights to the understanding of their nutritional value and potential health benefits.

1.3 Objective

1.3.1 General objective

- The general objective of this work was to study the effect of different processing methods on nutritional composition of faba beans.

1.3.2 Specific objective

The specific objectives of this study were as follows.

- To study nutritional and anti-nutritional properties of faba beans.
- To study changes in nutritional and phytochemical composition of faba beans under different processing techniques.

- To study changes in anti-nutritional factors of faba beans under different processing methods.
- To find out the most effective processing methods for the overall enhancement of nutritional quality of faba beans.

1.4 Significance of study

Faba beans are winter sowing crops that are rich in protein and other bio-active compounds. However, they also contain significant amount of anti-nutrients that can hinder their consumption. This study holds significant importance as it aims to investigate the effects of various pre-treatments, namely soaking, dehulling, cooking on the nutritional composition and reduction of anti-nutritional factors in faba beans. By analyzing the changes in the nutritional compositions, the study seeks to provide insights into optimizing processing techniques to enhance the functional and nutritional value of faba beans. Additionally, the study explores the reduction of anti-nutritional factors, specifically tannins and phytic acid, through different processing methods. By investigating the effectiveness of different pre-treatments in reducing these anti-nutritional factors, this study aims to improve the safety and nutritional value of faba beans, making them more accessible and beneficial to consumers. The outcomes of this study can have far-reaching implications for the food industry, public health, and dietary recommendations, promoting the consumption of faba beans as a sustainable and nutrient-rich food source.

1.5 Limitations of study

1. Important antinutrients present in faba beans like lectins, vicine, haemagluttins and trypsin inhibitors were not determined.
2. Processing techniques like fermentation and soaked cooking were not done during this study.

Part II

Literature Review

2.1 The origin and distribution of faba beans

Faba bean is assigned to the Central Asian, Mediterranean, and South American centers of Diversity and believed to be a native to North Africa and southwest Asia, and extensively cultivated elsewhere (Tanno and Willcox, 2006). (Cubero, 1974) postulated a Near Eastern center of origin, with four radii to Europe along the North African coast to Spain, along the Nile to Ethiopia, and from Mesopotamia to India (Hawtin and Hebblethwaite, 1983). Secondary centers of diversity are postulated in Afghanistan and Ethiopia. However, (Hajjar and Hodgkin, 2007) reported the origin to be Central Asia. The Chinese used them for food almost 5,000 years ago, and they were cultivated by the Egyptians 3,000 years ago, by the Hebrews in biblical times, and a little later by the Greeks and Romans (Mihailovic *et al.*, 2005). Probably, it was introduced by Europeans as a garden crop into India during the Sultanic period (1206–1555), during which its cultivation has been mentioned (Akbar, 2000; Khatoon Naqvi, 1984). The wild progenitor and the exact origin of faba bean remain unknown.

The original wild species of faba beans is unknown. It has been cultivated in the Mediterranean area since prehistoric times and it is now cultivated across the whole of Europe and in many temperate and subtropical parts of North America, South America, Asia, Africa and Australia. It is sometimes grown at high altitudes over 2000 meter in the tropics (Kirk, 2004).

The world production of faba beans was 5.43 million metric tons in 2019, which represented about 25% increase compared with 4.35 million metric tons in 1990. Regionally, Asia leads with 33.55% of total faba bean production globally, followed by Europe (EU) and Africa, with 29.36% and 27.04% share, respectively. China was the leading producer of faba beans, followed by Ethiopia; these two countries represented about 50% of the total global production whereas among EU, the United Kingdom and France were among the top five producers. Also, in 2019, Australia was the leading exporter of faba beans with 265,543 metric tons or nearly 30% of total exports, followed by the United Kingdom, Lithuania, Egypt, and Latvia. Egypt led importers with 309,355

metric tons or 40.48% of total global imports, followed by Norway, Germany, Saudi Arabia, and France (FAO, 2020).

2.2 Classifications and nomenclature

The scientific name of faba bean is Vicia faba. According to USDA, the Taxonomy hierarchy of faba beans is given below:

Kingdom: Plantae

Division: Mangaliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Genus: *Vicia*

Species*: V. faba* (Source: USDA)

2.3 Physiology and morphology of faba bean

The Faba bean is an annual herb with coarse and upright stems, unbranched 0.3 to 2 m tall, with one hollower stem from the base. The leaves are alternate, pinnate and consist of 2 to 6 leaflets each up to 8 cm long and unlike most other members of the Genus; it is without tendrils or with rudimentary tendrils. The plant flowers profusely but only a small proportion of the flowers produce pods. Flowers are large, white with dark purple markings, borne on short pedicels in clusters of 1-5 on each axillary raceme usually between the 5 and 10th node; 1-4 pods develop from each flower cluster, and growth is indeterminate though determinate mutants are available (Hanelt and Mettin, 1989). About 30% of the plants in a population are cross-fertilized and the main insect pollinators are bumblebees. There is a robust tap root with profusely branched secondary roots. Based on seed size, two subspecies were recognized, paucijuga and faba. V. Faba has a diploid (2n) chromosome number of twelve, meaning that each cell in the plant has twelve

chromosomes (6 homologous pairs). Five pairs are acrocentric chromosomes and 1 pair is metacentric (Alghamdi, 2009; Hanelt and Mettin, 1989).

Habitat: Terrestrial

Flower petal color: white with dark purple color markings.

Leaf type: leaves are compound type.

Leaf arrangement: alternate, pinnate without tendrils or with rudimentary tendrils.

Stems: hollow, unbranched, and up to 0.3-2m long.

Roots: robust tap root with profusely branched secondary roots.

Pods: each flower cluster produce 1-4 pods. Pods are generally bright green and fibrous with a leathery texture, sometimes covered in a downy coating. Each pod contains 2-8 flat green beans.

Seeds: seeds are medium sized, rounded and dimpled and are usually brown to olive green in color.

Inflorescence: the inflorescences are borne on short axillary racemes, and they bear between 1 and 6 papillionaceous flowers. These flowers are large up to 3-4cm long, white, or white with black/dark purple spots.

Pollination types: 30% of plants in a population are cross fertilized and the main insect's pollinator is bumblebees.

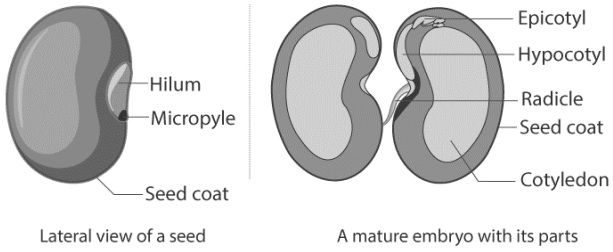

Fig.2.1 Structure of faba beans

2.4 Faba beans status in Nepal

It is mainly grown in warm places in Nepal. Faba bean is a good source of mineral nutrients, vitamins, and numerous bioactive compounds. Equally important is the contribution of faba bean in maintaining the sustainability of agricultural systems, as it is highly efficient in the symbiotic fixation of atmospheric nitrogen. They are winter season legumes (Dhakal, 2021). The current status of faba beans in Nepal is shown in the table below with total faba bean production in table 2.1 and production in province level in table 2.2.

Table 2.1 Total faba beans production in Nepal as for 2020/21

Commodity	**Area (in hectare)**	**Production (in metric tons)**
Faba beans	1,409	8,593

Source: (Sanjel, 2022)

Table 2.2 Production of faba beans in Province Level as for 2020/21

Province	**Area (in hectare)**	**Production (in metric tons)**
Koshi	259	1,657
Madhesh Province	727	3,424
Bagmati	162	1,986
Gandaki	119	533
Lumbini	104	632
Karnali	36	331
Sudurpaschim	2	10

Source :(Sanjel, 2022)

2.5 Chemical and nutritional composition of faba beans

Faba bean, also known as broad bean, is an important nutrient-rich legume, especially, high amount of lysine-rich protein, complex carbohydrates, dietary fiber, non-nutrient secondary metabolites, and bioactive compounds (antioxidants, phenols, and γ-aminobutyric acid), which have several reported health benefits (Khazaei *et al.*, 2019; Liu *et al.*, 2022). Dry legumes, including faba bean seeds, also contain high levels of polyphenol compounds that confer high levels of antioxidant activity (AA). These compounds correlate to several biological activities, including anti-aging, anti-inflammation, apoptosis, antioxidation, anticancer, antiatherosclerosis, cardiovascular

protection, and other chronic diseases. Thus, faba bean seeds could be an excellent potential functional food source (Amarowicz and Shahidi, 2017; Kwon *et al.*, 2018; S. D. Siah *et al.*, 2019). The chemical constituents of mature faba beans is shown in Table 2.3.

Table 2.3 Constituents of whole faba beans (mature)

Composition	**Per 100g (dry basis)**
Moisture	10.98 g
Protein	26.12 g
Total lipid	1.53 g
Ash	3.08 g
Carbohydrate by difference	58.28 g
Total dietary fiber	25 g
Calcium	103mg
Iron	6.7mg
Magnesium	192mg
Phosphorus	421mg
Potassium	1062mg
Sodium	13mg
Zinc	3.14mg
Vitamin C	1.4mg
Niacin	2.832mg
Folate	423µg
Vitamin A	53IU
Vitamin K	9µg

Source: (Larrick *et al.*, 2022)

2.6 Anti-nutritional factor

Faba beans have a number of antinutrients present in mature seeds. These include phytates, vicine, convicine, saponins, lectins, oligosaccharides (raffinose, stachyose), condensed tannins, and trypsin inhibitors and protease inhibitors (Mayer Labba *et al.*, 2021). Vicine and convicine are the main antinutritional compounds present in faba beans that are known to cause hemolytic anemia (called favism). Favism is one of the prime reasons for restricted use of faba beans (Luzzatto and Arese, 2018). The digestibility of legume-based proteins is correlated with the presence of protease inhibitors, which are known to reduce

the digestibility of proteins thereby leading to cause pancreatic hypertrophy. Trypsin inhibitors in faba bean are comparatively lower than other legume crops such as soybean, chickpea, and lentil (Sharma and Sehgal, 1992). Recently, major progress on reducing vicine/convicine and seed coat tannins in faba bean has been recently achieved through plant breeding interventions, for example, gene discovery (Khazaei *et al.*, 2019). Phytic acid present in faba beans is reported to negatively influence the bioavailability of minerals and alter the absorption of proteins due to phytate–mineral–protein complexes. Phytate is a chelating agent considered to be responsible for reduction in the bioavailability of divalent cations (Luo *et al.*, 2009). Some of the anti-nutrients present in faba beans are:

2.6.1 Tannins

Tannins are polyphenolic compounds of high molecular weight which interact with and precipitate proteins; they are classified into two groups according to their reaction with hydrolytic agents: hydrolysable and condensed tannins. The former is easily eliminated under the action of hydrolytic agents such as gastric juice (Cabrera and Martin, 1986). Tannins are divided into two major classes: condensed tannins, which are flavonoid-based polymers; and hydrolysable tannins, which are polygalloyl esters. Higher plants may produce any combination of condensed and hydrolysable tannins, although some chemotaxonomic patterns have not. The condensed tannins do not undergo hydrolysis in acid or base. However, in hot alcohol the flavanoid polymer is oxidatively cleaved to yield colored anthocyanidins; thus, the condensed tannins are often called "proanthocyanidins". The hydrolysable tannins are hydrolyzed in acid, in base or by esterases (tannase) to yield the parent polyol and the phenolic acids. Glucose is the most common alcohol, but hydrolysable tannins containing diverse alcohols such as hamamelose and quinic acid have been reported. If gallic acid is the only phenolic acid produced upon hydrolysis, the tannin is a gallotannin (Hagerman, 1992).

The tannin content in whole faba bean is associated with the color of the seed coat and ranges from 0.75 to 2% (Reddy *et al.*, 1985). Tannins are located mainly in the seed coat (Testa) of dry beans (Bond, 1976; Griffiths, 1981) Therefore, the physical removal of the seed coat by dehulling procedures will decrease the tannin levels in the resulting product. In this way, the nutritional value of the faba protein can be better utilized (Reddy *et al.*, 1985; Sosulski and Dabrowski, 1984). The structure of hydrolysable and condensed tannin is shown in Fig 2.2.

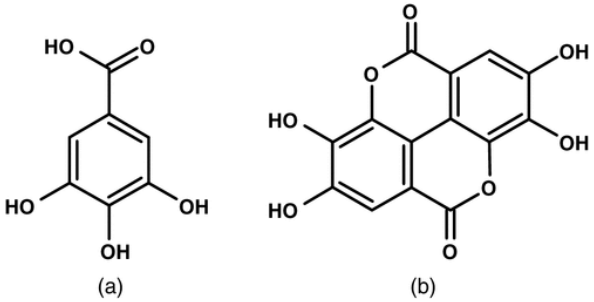

Fig.2.2 Structure of hydrolysable tannin (a) and condensed tannin (b)

2.6.2 Phytic acids

Phytic acid (myoinositol, 1, 2, 3, 4, 5, 6 hexakis-dihydrogen phosphate) and phytate are widespread in plant seed grains (also including cereals), roots and tubers. Phytic acid is generally regarded as the primary storage form of both phosphate and inositol in seeds. Phytic acid phosphorus constitutes the major portion of total phosphorus in several seeds and grains. It accounts for 50–80% of the total phosphorus in different cereals. The phytic acid content is influenced by cultivar, climatic conditions, and year. The accumulation site of phytic acid in monocotyledonous seeds (wheat, barley, rice, etc.) is the aleurone layer, particularly the aleurone grain. Aleurone grain contains two types of inclusions: (a) globoids containing high amount of phytates, and (b) protein carbohydrate bodies. Corn differs from other cereals as more than 80% of phytic acid is concentrated in germ. The phytic acid content of cereals varies from 0.5 to 2.0%. Because most of the phytic acid is in the outer parts of the kernel the different products of milling contain different levels of phytates. Bran is a product having a high phytic acid content, low extraction white flours contain low phytic acid quantities. If protein concentrates or isolates are prepared from cereals or other raw materials, such products contain also phytic acid in quantities depending on the raw material and method of processing. The association of phytate with proteins begins in seeds during ripening, when phytate accumulates in the protein-rich aleurone layer of cereals and protein bodies of legumes. Although the fine structure of phytate-rich particles in plants has been intensively studied, the nature of the interaction of proteins in such organelles with phytic acid is practically unknown (Hídvégi and Lásztity, 2002). Phytic acid, known as inositol hexakisphosphate, can exist as a free acid, a calcium salt of phytic acid (phytate) or a calcium/magnesium salt of phytic acid (phytin) based on the pH and metal ions present. Phytic acid is distributed uniformly through the dicotyledonous seeds, and it represents the primary storage form of phosphate and inositol.

Phytic acid has a high density of negatively charged phosphate groups forming very stable complexes with mineral ions which makes their intestinal uptake unavailable. Furthermore, phytates have been described to complex with proteins at different pH ranges, which may alter the protein structure, resulting in lower protein solubility, enzymatic activity and proteolytic digestibility (Rosa-Sibakov *et al.*, 2018). The structure of phytate is shown in Fig 2.3.

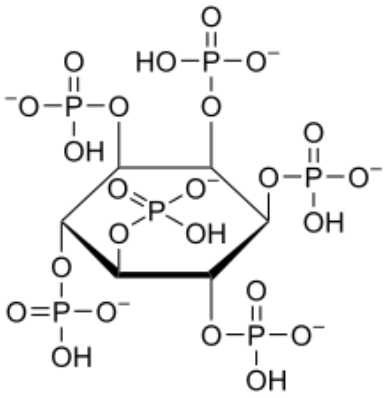

Fig.2.3 Structure of phytate

2.6.3 Oxalate

Oxalic acid forms water-soluble salts with Na+, K+ and NH4 + ions; it also binds with Ca2+, Fe2+ and Mg2+, rendering these minerals unavailable to animals (Bsc and Bsc, 1999). Oxalates are anti-nutrient compounds present in vegetables such as spinach, chard, beet, or rhubarb. These compounds are a strong organic acid with the ability to form water-soluble salts by binding to minerals such as sodium or potassium, as well as water-insoluble salts by binding to calcium, iron or zinc (Lo *et al.*, 2018). Oxalate is an anti-nutrient which under ordinary conditions is restricted to isolated compartments. However, when it is handled and additionally processed, it meets the nutrients in the gastrointestinal tract (Bsc and Bsc, 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 metabolize soluble oxalates, and to a 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).

A salt formed from oxalic acid is known as an oxalate: for instance, calcium oxalate, which has been viewed as generally distributed in plants. Strong bonds are formed between oxalic acid, and different minerals, such as sodium, calcium, magnesium, and potassium. This compound blend brings about the development of oxalate salts. Some oxalate salts, such as sodium and potassium, are soluble, whereas calcium oxalate salts are basically insoluble. The insoluble calcium oxalate has the tendency to precipitate (or solidify) in the kidneys or in the urinary tract, subsequently forming sharp-edged calcium oxalate crystals when the levels are sufficiently high. These crystals play a role to the formation of kidney stones formation in the urinary tract when the acid is excreted in the urine (Liebman and Al-Wahsh, 2011).

Due to the solubility of oxalate in water, culinary processes, such as boiling and steaming allow to reduce considerably the content of these compounds (Chai and Liebman, 2005). The structure of oxalate is shown in Fig 2.4.

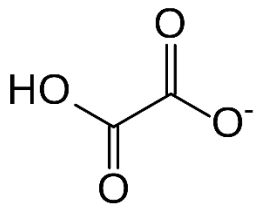

Fig.2.4 Structure of oxalate

2.6.4 Trypsin inhibitor

Trypsin inhibitors are a group of serine protease enzymes. They reduce the biological activity of the digestive enzymes' trypsin and chymotrypsin. Trypsin is a proteolytic enzyme which is important for the digestion of proteins in living organisms. It is a globular protein with a molecular weight of 24-kDa with 220 residues. This enzyme protein is produced in its inactive form called 'trypsinogen' in the pancreas which is then activated during digestion as it enters the small intestine(Walsh *et al.*, 1964). Trypsin is an enzyme involved in the breakdown of a wide range of proteins, primarily as part of digestion in humans and other animals such as mono-gastric and young ruminants. When trypsin inhibitor is consumed, it acts as an irreversible and competitive substrate (Silverman *et al.*, 2001). The ingestion of trypsin inhibitors can result in the formation of an irreversible

trypsin enzyme- trypsin inhibitor complex which leads to a trypsin enzyme drop in the intestine, interfering in protein digestibility process (Cabrera-Orozco *et al.*, 2013).

It competes with proteins to bind to trypsin and therefore renders it unavailable to bind with proteins for the digestion process. Thus, trypsin inhibitor is considered an anti-nutritional factor or ANF. Additionally, trypsin inhibitor partially meddles with chymotrypsin function (Vagadia *et al.*, 2017).

The presence of trypsin inhibitor has been found to result in delayed growth as well as metabolic and digestive diseases (Coscueta *et al.*, 2017). Additionally, pancreatic hypertrophy is a common occurrence with trypsin inhibitor consumption (Hwang *et al.*, 1977). The presence of trypsin inhibitor in a product reduces the protein efficiency and therefore results in the consumers body not being able to efficiently and fully utilize the protein (Klomklao *et al.*, 2011).

2.6.5 Lectin

Lectins are known as the carbohydrate binding proteins present in plants and animals which are capable of binding with the sugar moieties present in glycoproteins and glycolipids. Favin is the lectin which is isolated from faba beans. Lectins cover a wide category of proteins which have a common property of binding specific saccharides. It has been found that there are three binding sites in lectins. Two are for divalent metal ions and the third one is for specific saccharides. A covalent contact with saccharide and non-covalent contact with two peptide loops, one of which contains several metal ion ligands. The natural function of lectins is unknown. Each protomer has approximately 230 amino acid residues that contain binding sites for manganese and calcium along with a specific carbohydrate. It has also been found that chronic diseases like cancer and other coronary diseases can be prevented by consumption of anti-nutrients on a regular basis. Their chemical structure, concentration, time of exposure, interaction with other nutrients, all these factors are responsible for striking harmony between deleterious and beneficial effects (Ramírez-Ojeda *et al.*, 2018).

2.6.6 Protease inhibitor

Protease inhibitors (PI) are principally responsible for the lower digestibility of proteins in animals and humans. The classification of protease inhibitor is based on amino acid sequence, position and nature of reactive site and number of disulphide bridges. They are

responsible for the defense mechanism of the cell i.e., plant tissues against cell rupture and insects, pests. Also, its other physiological role is to control protein hydrolysis during germination. It is responsible for the inactivation of protein digesting enzymes from sources such as trypsin, chymotrypsin, pronase, thrombin, and papain. Enzymes such as pepsin, carboxypeptidase A and B, subtilisin are not affected by protease inhibitor. The trypsin inhibitor activity of faba beans is found to be 2285 units/g dry meal. If we look for the mechanism of trypsin-trypsin inhibitor complex formation, it has been reported that it is noncompetitive type of inhibition, in which the complex is not formed at the active site of the enzyme, as formed in competitive inhibition. The main difference between competitive and non-competitive inhibition is that the complex is formed at the active site in competitive inhibition and on sites other than active sites in non-competitive inhibition. In recent times, the approach towards faba beans has changed due to their high protein content and its cheap cost. For human consumption, a great scope in textured protein products has risen keeping the lysine rich protein content of faba bean in mind. The inactivation of enzymes occurs by the formation of complex between trypsin inhibitor and trypsin enzyme. The complex formed is very much stable (low energy) hence, both the compounds have very high affinity towards each other (association constant of 10^{10}), where association constant or binding constant is the inverse of dissociation constant. As studies were carried out on the mechanism of action, it was observed that the complex formation is due to reactive amino acid sequence. It may be single headed or double headed inhibitor. For double headed inhibitors, complexes with both trypsin and chymotrypsin were formed i.e., trypsin was inhibited by PI-chymotrypsin complex and vice versa (Hussein, 1982). The amount of trypsin indicator present in faba beans is about 2.24-2.77TIU/mg, which is lower than that present in chickpeas i.e., 7.65-8.98TIU/mg (Khan *et al.*, 2015).

2.6.7 Amino acid pyrimidine derivatives: A cause of Favism

Consumption of faba beans is associated with the development of favism. The highest incidence of favism disease is reported in the Mediterranean region. It is generally understood that the causative agents of this disease (vicine, divicine, convicine, alkaloids, and aglycones) are derived directly from the faba bean or their digestive metabolites (Oliveira *et al.*, 2000). Susceptible subjects demonstrate a biological deficiency of glucose-6-phosphate dehydrogenase (G6PD), which plays a key role in the pentose monophosphate

shunt pathway and is highly active in the red blood cells (RBCs). NADPH (nicotinamide adenine dinucleotide phosphate hydrogen) provides reduced glutathione, which eliminates free radicals that cause oxidative damage. When reduced glutathione is limited, active enzymes and functional proteins are damaged by prevailing oxidants (Luzzatto and Arese, 2018). Individuals deficient in G6PD are at risk of hemolytic anemia due to oxidative stress, which is accumulated by consumption of faba bean proteins—vicine, divicine, and convicine. This condition results in damage to RBC, which limits the transport of iron. Clinical symptoms are expressed as hemolytic anemia with yellow jaundice (hemoglobin metabolized to bilirubin), fatigue, and lack of energy, affected breathing with weak and rapid pulse (Oliveira *et al.*, 2000). Appropriate preparation and processing procedures have consistently shown that typical heat treatments (boiling or roasting) used to soften the texture are sufficient to denature proteins and reduce alkaloids, thus reduced the toxicity of faba beans (Dhull *et al.*, 2022).

2.7 Different processing methods

Besides traditional cooking of faba beans, dehulling, soaking, germination, fermentation, extrusion cooking, and enzymes treatment are some of the common treatment and processing methods used.

2.7.1 Dehulling

Dehulling, a commonly used process for legumes, can detach the hull or seed coat from whole grain. Dehulling results in an increase in protein and amino acid content, whereas fat, reducing sugar, and crude fiber contents are decreased. Dehulling also resulted in increased level of phytic acid while tannins and polyphenols were decreased (Alonso *et al.*, 2000). Pretreatments such as soaking and roasting improved the hull recoveries (Anderson *et al.*, 1994). An improvement of in vitro protein and starch digestibility after dehulling is achieved, which could be attributable to the partial loss of some antinutrients, for example, phytic acid, condensed tannins, and polyphenols (Alonso *et al.*, 2000).

2.7.2 Soaking

Soluble antinutritional compounds in faba beans can be removed by soaking and discarding the soaking water or solution (Anderson *et al.*, 1994). (Kader, 1995) studied some factors (temperature, concentration of sodium bicarbonate, protein content, size, and density of seeds) affecting the rate of water absorption during soaking of faba beans. The

water absorption rate increased with temperature and decreased with sodium bicarbonate addition. Soaking is shown to reduce ash content and trypsin inhibitor activity of faba beans. In one another study, soaking of faba beans followed by dehulling increased total protein content, with no effect on crude fat and crude fiber, whereas sugars, ash content, tannins, phytic acid, and trypsin inhibitors were decreased (Bakr, 1996). (Alonso *et al.*, 2000) reported that phenolic content and antioxidant activities of faba bean also decreased significantly after soaking, which can be due to leaching of these bioactive compounds from hull into the soaking medium.

2.7.3 Cooking and Autoclaving

Soaking and open-kettle cooking have been utilized as the most common processing methods in home preparation of faba beans. (Anderson *et al.*, 1994) cooked faba beans to a soft texture by boiling in deionized water for 40 min, which resulted in significant reduction of ash content and slight reduction of total proteins. (Kmiecik *et al.*, 2000) also observed reduction of ash content after blanching and cooking. Cooking was not shown to affect the chemical composition and mineral content of faba beans, but antinutritional factors, such as tannins and phytic acid, were reduced (Osman *et al.*, 2014). 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 (Adhikari, 2021).

2.7.4 Germination

Sprouting is a period in the life cycle of plants when they start emerging from the seed. This natural process is also known as germination. This process increases the availability of nutrients in seeds, grains, and legumes. Sprouting takes a few days. During sprouting, changes take place within the seed that lead to the degradation of antinutrients such as phytate and protease inhibitors. Sprouting has been shown to reduce phytate by 37-81% in 14 various types of grains and legumes. There also seems to be a slight decrease in lectins and protease inhibitors during sprouting (Bau *et al.*, 1997).

Germination has been investigated widely as a suitable processing treatment to obtain nutrient-rich faba bean flour. Germination resulted in a slight decrease in starch (15%), greater reduction in phytates and α-galactosides (45% and 94%, respectively), and improved dietary fiber appreciably (Hamza *et al.*, 1987; Vidal-Valverde *et al.*, 1998). About 6% increase was observed for calcium content (in germinated versus cooked samples), which could be related to the decrease in phytic acid. The improved bioavailability of calcium was also attributed to reduction in hemicelluloses content, which can interfere in its bioavailability. The phytic acid serves as a reserve of phosphorus, which is liberated by phytase action during germination thereby improving phosphorus bioavailability (Vidal-Valverde *et al.*, 1998).

2.7.5 Roasting

Roasting has been utilized traditionally for faba bean processing at home for production of flour for further application. Low roasting temperature produced low protein content compared with high roasting temperatures. Roasting has been shown to be most effective method to reduce trypsin inhibitor activity (Anderson *et al.*, 1994). (Hamza *et al.*, 1987) reported that the patterns of proteins in standard page were not affected by roasting. Also, the standard page showed a degradation of high molecular weight proteins to smaller subunits during roasting. Phytic acid content was reduced during roasting, which might be due to formation of insoluble phytins between phytic acid and some minerals. (Vidal-Valverde *et al.*, 1998) reported that dry heating causes noticeable reduction in most nutrients (soluble sugars, starch, dietary fiber, and calcium) and antinutritional factors (α-galactosides and phytic acid) of faba bean. Roasting was shown to initially decrease the antioxidant capacity of faba beans; however, roasting at 150°C for prolonged time (≥60 min) generated new phenolic compounds, which increased the antioxidant capacity (S. Siah *et al.*, 2014).

2.7.6 Extrusion

Extrusion processing of legumes has been used to remove the antinutritional factors and improve the physical (palatability and texture) and chemical (starch gelatinization, protein, and starch digestibility) properties. Extrusion of faba beans has been reported to have minimal effect on the nutritional value, that is, protein, lipids, and ash contents (Adamidou *et al.*, 2011; Nosworthy *et al.*, 2018). Extrusion processing at different preconditioning and dryer temperatures resulted in slight reduction in the total as well as soluble and insoluble

non starch polysaccharides in faba beans (Adamidou *et al.,* 2011). Extrusion processing resulted in an increase in starch level, which might be due to compositional changes in RS and easier hydrolysis of extruded starch granules by amylases during analysis. Starch was gelatinized after heat treatment (extrusion) in which crystalline structure of starch was disrupted and starch granules ruptured under high temperature, making them more accessible and readily hydrolysable by enzymes (Alonso *et al.,* 2000). Further, it was observed that preconditioning and extrusion eliminated the activities of trypsin, chymotrypsin, α-amylase inhibitors, and hemagglutinating activity without affecting the protein level. Also, thermal processing was most effective in improving the protein and starch digestibility (Francis *et al.,* 2001) and destruction of amylase and protease inhibitors. However, irrespective of preconditioning and dryer temperature, phytic acid and total tannin level were reduced after extrusion (Adamidou *et al.*, 2011), whereas (Francis *et al.*, 2001) reported that no effect was observed on the bioavailability of minerals bound by phytic acids.

(Gu *et al.,* 2020) employed extrusion processing to process flours from faba, lima, pinto, and red kidney beans. FBF had higher protein and crude fiber and lower non fiber carbohydrate content compared with flours from three other bean types. Higher protein content in FBF made it suitable for developing plant-based protein products (Liu *et al.*, 2022; Osman *et al.*, 2014; Rosa-Sibakov *et al.*, 2016).

2.7.7 Fermentation

Fermentation is one of the simple and inexpensive processing techniques to achieve desirable changes in the seed composition and improve palatability, and it is used throughout the world and particularly in developing countries. Age-old technology such as lactic acid fermentation has been proposed for processing of more digestible and palatable foodstuffs. Lactic acid fermentation is used as a major method for processing and preserving vegetables, cereals, and legumes. It is a desirable method for processing and preserving food because of its low cost, low energy requirements, and high yield with acceptable and diversified flavors for human consumption. Several experiments have demonstrated that fermentation of legumes enhances their nutritive value, reduces some antinutritional natural compounds such as phytic acid, trypsin inhibitor, and oligosaccharides, and exerts beneficial effects on protein digestibility and biological value of legumes. Thus, fermentation is an effective treatment for lowering antinutritional factors

in beans, and it could enhance nutritional quality. On this respect, no information has been found on the effect of lactic acid and natural fermentation in the content of dietary fiber (DF) of beans and the composition of their fractions (soluble and insoluble) (Martín-Cabrejas *et al.*, 2004).

2.8 General uses of vicia faba beans

Faba beans have various general uses in culinary and non-culinary contexts. Here are some of the common uses of faba beans:

2.8.1 Culinary Uses:

- Ingredient in Salads: Faba beans can be blanched, peeled, and added to salads for a fresh and nutritious component.
- Soups and Stews: They can be used in hearty soups and stews to add texture and flavor.
- Side Dishes: Faba beans can be cooked and served as a flavorful side dish alongside the main course.
- Purees and Dips: They can be mashed or pureed to create spreads and dips, such as broad bean hummus.
- Stir-Fries and Sautés: Faba beans can be included in stir-fries and sautéed dishes, offering a unique taste and texture.

2.8.2 Traditional Dishes:

- Traditional Mediterranean and Middle Eastern Cuisine: Faba beans are commonly used in traditional dishes like ful medames (Egyptian broad bean stew) and falafel (Middle Eastern fritters).
- EFieldh Cuisine: Faba beans are a key ingredient in traditional English dishes like "faba beans with bacon" or "broad bean and mint soup."
- Ingredient in Baked Goods: Faba bean flour: Ground faba beans can be used as an alternative flour in gluten-free baking, adding a unique flavor and nutritional value to bread, muffins, and other baked goods.

2.8.3 Animal Feed:

- Faba beans can be used as a nutritious component in animal feed formulations, particularly for livestock such as poultry and swine.

2.8.4 Green Manure and Cover Crop:

- Faba beans are often grown as a cover crop or green manure. They help improve soil fertility by fixing nitrogen from the air into the soil, thereby enriching it for subsequent crops.

2.9 Health benefits of faba beans

Faba beans are rich sources of lysine rich proteins, carbohydrates, minerals, vitamins, and numerous bioactive compounds. It is also a good source of l-3,4-dihydroxyphenylalanine (L-DOPA), which is a precursor of dopamine and can be potentially utilized for Parkinson's disease treatment. Faba beans contain almost twice the protein content as that in cereal grains with globulins (60%), albumins (20%), glutelin's (15%), and prolamins (8%) (Rahate *et al.*, 2021). In comparison with other beans such as lima, pinto, and red kidney beans, faba bean flour (FBF) had highest protein content of 29.76% (Alonso *et al.*, 2000; Gu *et al.*, 2020).

(A. K. Singh *et al.*, 2013) reported that faba bean is a very good source of dietary fiber, including both soluble and insoluble dietary fiber. Highest dietary fiber content was found in FBF in comparison with flours from lima, pinto, and red kidney beans (Gu *et al.*, 2020).

Diverse minerals (sodium, potassium, calcium, copper, zinc, iron, manganese, magnesium, phosphorus, and sulfur) are reported to be present in faba bean (Khalil and Mansour, 1995; Luo *et al.*, 2009; Nosworthy *et al.*, 2018). The high potassium (1,062 mg/100 g) and low sodium (13 mg/100 g) contents in mature faba bean seed are optimum for people suffering from hypertension and on a low-sodium diet (Larrick *et al.*, 2022). Faba bean is a good source of folate, an essential cofactor involved in the synthesis of pyrimidines, purines, and amino acids (Hefni *et al.*, 2015). Flavonoids, recognized as bioactive compounds having anti-inflammatory and antidiabetic properties, are also reported in faba beans, which also exhibits anti-oxidants properties (Zhang *et al.*, 2019).

Part III

Materials and methods

3.1 Materials

All chemicals used were reagent grade unless specified otherwise and distilled water was used throughout the work. All operations were performed at room temperature unless otherwise stated.

3.2 Collection of Vicia faba bean

Faba beans (*Vicia faba)* of single variety was purchased from Bhatbhateni, Itahari-5, Koshi Province.

3.3 Chemicals and Equipment

All chemicals required for this research were used from the laboratory of Central campus of Technology. The list of chemicals used for this work is shown in Table 3.1.

Table 3.1 Chemicals used for analysis

Chemical Supplier	Manufacturer	Other Specifications
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
Sulphuric acid	Fisher Scientific India Pvt. Ltd	97% Assay
Hydrochloric acid	Fisher Scientific India Pvt. Ltd	35-37% Assay
Quercetin	Avarice Laboratories Pvt. Ltd	Analytical reagent
Gallic acid	Avarice Laboratories Pvt. Ltd	Analytical reagent
L-ascorbic acid	S.D. fine chemicals Ltd	99% Assay

3.4 Equipment

All equipment required for the research were used from the laboratory of central campus off technology. The list of equipment's used for this work is shown in Table 3.2.

Table 3.2 List of equipment

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
Rotary vacuum evaporator	OEM manufacturer, India

3.5 Methodology

3.5.1 Outline of experimental procedures

The faba bean sample, which was purchased from Bhatbhateni, a local supermarket of Itahari was cleaned with water and left to dry in the room environment. The sample was then soaked in water for 12 hours. The required amount of soaked sample was taken for dehulling, and the remaining were left for further germination. With regular water splash in every two hours, the germination was done for 4 days at a recorded temperature of 21ºC and relative humidity of 85% in the month of November and December. For cooked sample, raw faba beans were open cooked in twice the volume of water until they were soft, which was for about 55 minutes. Similarly, autoclaving was done at 1.05kg/cm^2 for 15 minutes. The samples were then kept in a cabinet drier at 60°C until it was dry enough to crush into powder form. The powdered samples were steeped in 80% methanol for 12 hours at room temperature and filtered using Whatman No.1 filter paper and was concentrated in rotary vacuum evaporator. The plant extract was then stored in the refrigerator and further analysis were done. The general flowsheet of processing of faba beans is shown in Fig 3.1.

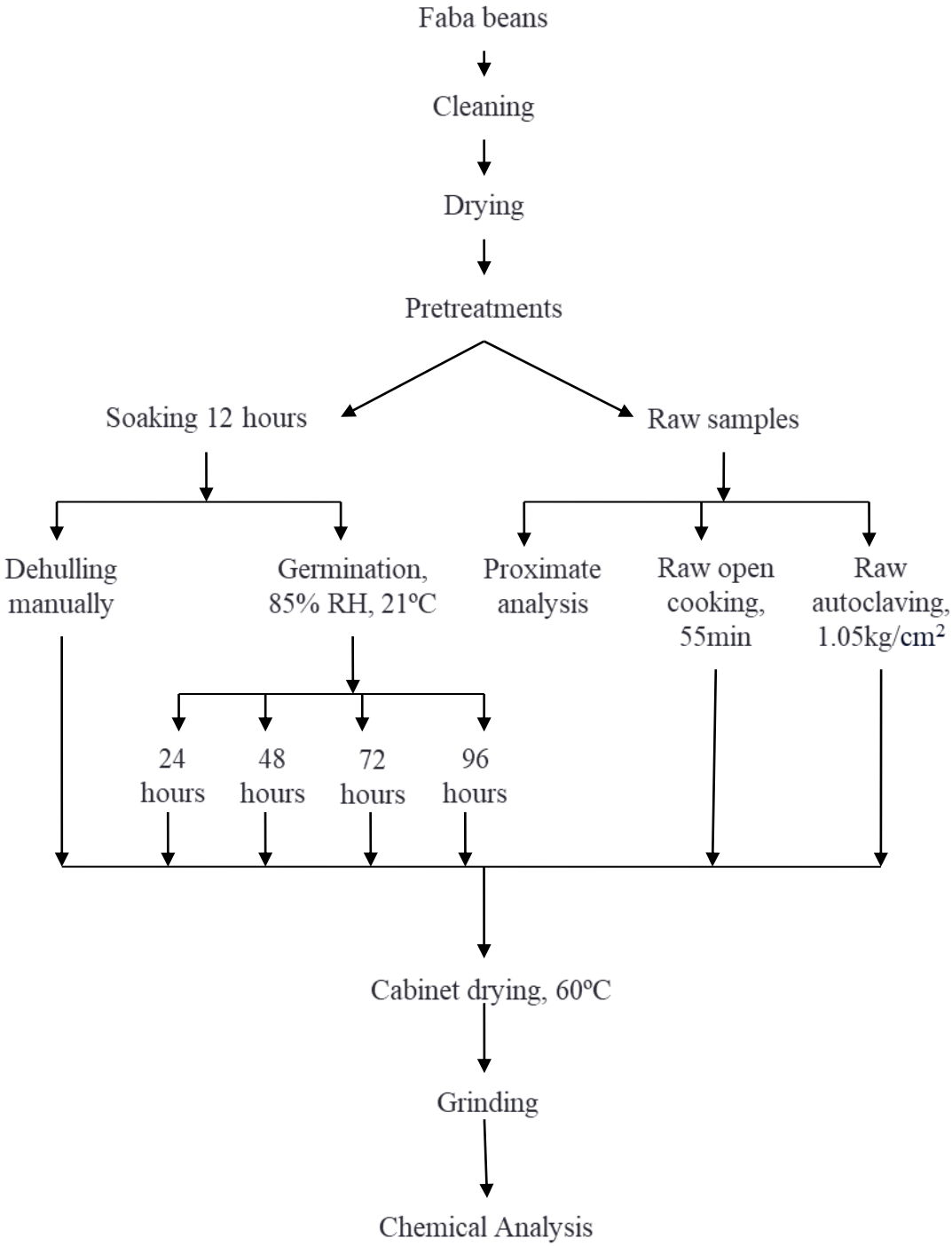

Fig.3.1 General flowsheet for processing of faba beans

3.6 Processing methods

3.6.1 Soaking

Faba beans were soaked in the room temperature of 16°C for 12h in distilled water. The proportion of seed to soaking solution was 1:5(w/v). The soaking solution was drained off after soaking time, seeds airdried, and then kept at oven at 60°C for complete drying. Samples were ground to pass through a 30-mesh screen. The ground samples were kept in plastic bags and stored at room temperature for subsequent analysis (Yasmin *et al.*, 2008).

3.6.2 Dehulling

Seeds were soaked at distilled water for 12h at 16°C. A seed to water ratio of 1:5(w/v) was used. The imbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dehulled using hand. The dehulled sample were dried in hot air oven maintained at 60°C. Samples were grinded in grinder and sieving using 0.5mm size mesh and stored in plastic bags at room temperature until required for further analysis (Sudesh Jood *et al.*, 1987a).

3.6.3 Raw open cooked

The raw faba beans were rinsed in distilled water and put in round-mouthed tall beakers fitted with condensers connected to running water. After adding distilled water (three times the weight of dry seeds), the samples were cooked on a hot plate until they became soft felt between fingers and finally, samples were grinded in a grinder and sieving using 0.5mm size mesh and stored in plastic containers at room temperature until required for further analysis (Sudesh Jood *et al.*, 1987a).

3.6.4 Raw autoclaved

The raw seeds were cooked at 1.05 kg/cm^2 pressure for 15 min in an autoclave. For this, dry seeds to water ratio of 1:2 (w/v) was used. The cooked samples were mashed and then dried at 60°C. Thus, samples were grinded in grinder and sieving using 0.5mm size mesh and stored in plastic bags at room temperature until required for further analysis (Sudesh Jood *et al.*, 1987a).

3.6.5 Germination

The faba bean seeds were soaked in distilled water (1:5, w/v) for 12h at room temperature. The water was drained off, and the seeds transferred to a moisture adherent flax cloth to germinate for 4 days in the dark at 21°C and 85% R.h. Every 2 hours, the seeds were

moistened with distilled water and carefully taken. After 4 days of germination, the sprouts and the seeds were ground and dried in an oven for 60°C for analysis (Yasmin *et al.*, 2008).

3.7 Analytical methods

3.7.1 Proximate analysis

Proximate analysis was done by following different methods which are stated below:

3.7.1.1 Moisture content

The moisture content was determined by using the 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 (Ranganna, 1986).

3.7.1.2 Protein content

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

$$\text{Nitrogen (\%)} = \frac{(\text{sample titre-blank titre}) \times \text{Noramality of HCL} \times 14 \times 100}{\text{Wt of sample} \times 100}$$

$$\text{\% of crude protein} = \text{Nitrogen} \times 6.25$$

3.7.1.3 Fat content

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

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

3.7.1.4 Ash content

The ash content was determined by incinerating the faba beans (5 g) in a muffle furnace at 525ºC (Ranganna, 1986). The calculated data were presented as gram per 100gm on dry basis.

$$\text{\% of total ash} = \frac{(\text{wt of ash})}{\text{wt of sample taken}} \times 100$$

3.7.1.5 Crude fiber content

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 gram per 100gm on dry basis.

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

3.7.1.6 Carbohydrate content

Total carbohydrate content of the samples was determined by difference method. Carbohydrate (%) = 100 – [sum of protein, total ash, fiber, moisture, and fat].

3.7.2 Physical properties of faba beans

The objective of this study was to investigate physical properties, namely, thousand seed mass, geometric mean diameter, sphericity, surface area, volume of seed, bulk density of faba bean seeds. In determination of physical measurements of faba beans, the followings were the tools and equipment employed:

- Weights of the samples were determined by using a precision electronic balance reading to an accuracy of 0.01gm.
- To determine the average size of the seed, 15 seeds were randomly picked out of 1000 seeds samples for faba beans, and their three principal dimensions (lengths, width, and thickness) were measured using a digital vernier caliper with an accuracy of 0.01 mm.
- Determination of the mean diameters (Da), geometric diameters (Dg), and the sphericity (ψ) was done by adopting (Joshi *et al.*, 1993).

The physical dimensions (length, thickness and width) of faba beans is shown in Fig 3.2.

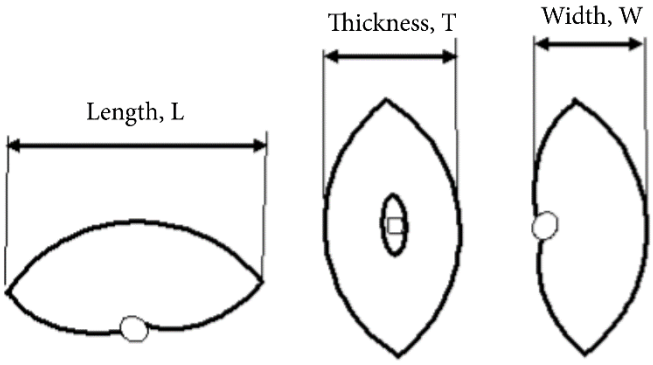

Fig.3.2 Faba beans principal dimensions

3.7.2.2 Bulk Density Measurement

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 seeds 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 seed buro 32 striking sticks. The filled measuring bushel was then weighed, and the mass of seeds in the bushel was determined by subtracting the mass of the measuring bushel itself (Clementson *et al.,* 2010).

3.7.2.3 Thousand kernel weight

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

3.7.2.4 Length by breadth ratio

Length by breadth ratio of faba bean seed was determined according to (Unal *et al.*, 2008).

3.7.2.5 Surface Area

The surface area (Sa) of faba bean seed was found by analogy with a sphere of geometric mean diameter for different values of moisture content using the following relationship (Deshpande *et al.,* 1993).

3.7.3 Phytochemicals Quantitative Analysis

3.7.3.1 Determination of 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).

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

3.7.3.2 Determination of tannin

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

3.7.3.3 Determination of polyphenol

The fresh grind sample weighing 1 g was extracted in 25 ml methanol; extracts were subjected to shaking in water bath shaker at room temperature for 24 h. The extract was filtered through Whatman paper no. 1 filter paper and filtrate were stored at (4±2) ºC until use. Then, 0.5 ml methanol solution of the concentrated solution was mixed with 2.5ml of FC reagent, and 5 min later, 2.5 ml Na_2CO_3 (7.5%w/v) were added. The mixed sample was incubated in an incubator at 45ºC for 45 min. The absorbance was measured at 765 nm against reagent blank. A standard calibration plot was generated using a known concentration of gallic acid. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg of gallic acid equivalent (GAE) of phenol/100 g of dry sample (Singleton *et al.*, 1999).

3.7.3.4 Determination of 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 for 5 min. 0.2 ml Aluminum Chloride was pipetted out, mixed in the tube, and allowed to stand for 15 min. This followed the addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally the volume was made up to 5ml. The absorbance was measured after 15 min at 510 nm against a reagent blank. Quercetin was taken as standard, and the results was expressed as mg of Quercetin equivalents (QE) per g of the dried extracts.

3.7.4 Ultimate Analysis

3.7.4.1 Determination of Ascorbic acid

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

$$\text{Vitamin C}\left(\frac{\text{mg}}{100\text{g}}\right)=\frac{\text{titer}\times\text{dye factor}\times\text{volume made up}\times 100}{\text{Aliquot of extract taken(ml)}\times\text{weight of sample(gm)}}$$

3.7.4.2 Determination of iron

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

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

3.7.4.3 Determination of calcium

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

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

3.7.5 Preparation of sample extract

Sample extract was prepared according to (Ahmad *et al.*, 2014) where 10gm of powdered sample was steeped in 80% methanol and left for 12 hours. After 12 hours, the liquid was filtered using Whatman. 1 filter paper. The supernatant was collected in a translucent container and stored in a cool and dry place. The required concentration of the extract was achieved by using rotatory vacuum evaporator.

3.8 Statistical analysis

For all chemical analysis, triplicates of the sample were used for determination of each constituent. Mean values with standard deviation were computed. Data on processing different techniques were subjected to analysis of variance (ANOVA) and considered at 95% confidence level using statistical software GenStat. Means of the data were compared by using Fisher's protected LSD method at 5% level of significance.

Part IV

Result and Discussion

4.1 Physical properties of faba beans

The physical properties of faba beans were determined and the result were shown in the given table. Average means of the three principal dimensions (length, width, and thickness) and standard errors for faba bean seeds were determined in the moisture content of 12.94% as indicated in Table 4.2. Also, other physical dimensions like 1000 kernel mass, surface area, sphericity, bulk density, and l/b ratio are shown in the same table. The seed dimensions of faba beans is shown in Table 4.1.

Table 4.1 Seed dimensions of faba beans

Physical properties	**Value**
Length(cm)	0.760±0.084
Width(cm)	0.5867±0.063
Thickness(cm)	0.5667±0.081
Mean diameter(cm)	0.6378±0.067
Geometric mean diameter(cm)	0.6322±0.066
Surface area(cm^2)	1.2556±0.096
Sphericity(%)	83.18±0.02
Bulk density(kg/m^3)	824±0.22
l/b ratio	1.29±0.066
1000 kernel mass(g)	401±1.2

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

The bulk density of the faba beans was found to be 824kg/m^3 and the 1000 kernel mass was found to be 401gm. Bulk density was greater than the result obtained by Fraser (1978) at 795kg/m^3 whereas 1000 kernel mass was less than their result. It could be because of the variation in moisture content as the moisture content of our sample was 12.94% as compared to given researcher' s sample moisture content which was 20%. Increase in moisture content decreases the bulk density of the grains while 1000 kernel mass increase with rise in moisture content. Study conducted by Altuntas (2007) on faba beans found l/b ratio, sphericity, surface area, geometric mean diameter and 1000 kernel weight to be 1.47, 83.18%, 4.29cm^2, 1.1 cm and 1140g respectively. These values were higher than the result

obtained in our study. It is also noteworthy that external environment factors like temperature, humidity, storage conditions can affect the physical dimensions of grain and the different cultivars within same species can also have different seed dimensions.

Faba beans cultivars can be divided into two types on the basis of 1000 kernel weight. They are broad beans and field beans. Broad beans are cultivars which have 1000 kernel weight between 1000g to 2000g, while field beans are those cultivars which have 1000 kernel weight between 400g to 800g (Crépon *et al.*, 2010). Since, 1000 kernel mass of our sample was 401g, our cultivar is most certainly field beans.

4.2 Proximate composition of raw faba beans

The proximate composition of raw faba beans is shown in table 4.2. Moisture content was found to be 12.94%. All the values are expressed in percentage basis. All the analysis are performed on the basis of procedure given by (Ranganna, 1986). The proximate composition of raw faba beans is shown in Table 4.2.

Table 4.2 Proximate composition of raw faba beans (dry basis)

Proximate constituents	**Composition in % (dry basis)**
Moisture content	12.94±0.66
Crude Protein	21.75±0.08
Ash	3.60±0.17
Crude Fat	0.92±0.24
Crude fiber	23.73±0.66
Carbohydrate	50.74±0.70

[Values presented are the average of triplicates determination ± standard deviation. Where + Sign represents increase in value whereas, – sign represent decrease in value.]

4.3 Mineral composition of faba beans

Iron was determined colorimetrically at 480nm by converting all the iron into ferric form using oxidizing agents and further treating with potassium thiocyanate to form red ferric thiocyanate. However, calcium was measured by titration method. The precipitate of calcium oxalate was dissolved in a hot dilute sulphuric acid and titrated against standard potassium permanganate solution. The mean value of iron content in raw faba bean was found to be 5.667mg/100g DW. The mean value of calcium content was found to be 218mg/100g. The mineral composition of faba beans is shown in Table 4.3.

Table 4.3 Minerals constituents of faba beans

Minerals	**mg/100g**
Iron*	5.667±0.07
Calcium*	218±4.02

[Values presented are the average of triplicates determination ± standard deviation. *Represents values in dry basis. Where + Sign represents increase in value where – sign represent decrease in value.]

4.4 Ascorbic acid content of faba beans

The mean value of ascorbic content was found to be 2.12±0.56.

4.5 Distribution of phytochemicals in faba beans

Faba beans also contain phenolic compounds, including flavonoids, which contribute to their antioxidant and health-promoting properties. The specific content of these compounds in faba beans can vary depending on factors such as cultivar, growing conditions, and processing methods applied. The phytochemicals constituents of raw faba beans is shown in Table 4.4.

Table 4.4 Phytochemicals in raw faba beans

Phytochemicals	**Value(mg/100g)**
Flavonoids	439.3±9.07
Polyphenol	391.8±3.09

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

The mean value of polyphenol content of raw faba bean was found to be 391.8mg/100g. The result obtained in our study was similar to that of Rahate (2021), who in their study reported polyphenol content in faba beans to be 392mg/100g. Another study done by Millar (2019) found polyphenol content in raw faba beans to be 387.5mg/100g which was almost similar to the result obtained in our study. The mean value of total flavonoid content of faba beans was found to be 439.3 mg/100g which was similar to the finding of Saleh (2019) i.e., 431mg/100g.

4.6 Distribution of anti-nutrients in faba beans

Tannins and phytic acid can have antinutrient effects, but processing methods like soaking, dehulling, germination, and cooking can help reduce their levels. The specific content of these compounds in faba beans can vary depending on factors such as cultivar, growing conditions, and processing methods applied. The anti-nutrients content of raw faba beans is shown in Table 4.5.

Table 4.5 Anti-nutrients in faba beans

Anti-nutrients	Value (mg/100g)
Tannin	217.37±8.6
Phytic acid	953.3±5.77

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

The mean value of tannin content in faba beans was found 217.37mg/100g which was similar to that of result obtained by Sharma and Sehgal (1992) i.e., 226 mg/100g. The result obtained in this study was also within the range given by Cabrera and Martin (1986), who in their study mentioned that tannin content in faba beans ranges from 181-354 mg/100g. The mean value of phytic acid content of raw faba bean was 953.3mg/100g. The result obtained in our study was almost similar to that of Millar (2019), who in their study found that the phytic acid content in raw faba was 980mg/100g. Another study by Luo (2009) showed the phytic acid content in faba beans to be 836mg/100g, which was less than the value obtained in our study. This can be due to the different variety of species, growing conditions, and processing methods applied to faba beans.

4.7 Effect of processing method on the crude protein content

The effects of soaking, germination, cooking and dehulling on the crude protein content in faba bean was studied. All the treatments significantly changed ($p<0.05$) the protein content of the faba bean, but to the varying extent. Germination for 96 hours had the most pronounced effect in the increment of protein content whereas autoclaving had the most pronounced effect in the reduction of crude protein content of faba beans.

4.7.1 Effect of soaking

Crude protein content of raw faba bean was determined, and the value obtained showed that there was no significant reduction ($p<0.05$) in protein content, which was decreased from 26.50g/100 g to 26.42g/100 g after soaking i.e., 0.30% reduction.

The result obtained in this study is similar to result obtained by Alonso (2000) who in their study reported 0.37% reduction in protein content after 12 hours of soaking of faba beans. Soaking legumes can result in a minor reduction in crude protein content due to protein leaching and loss of water-soluble proteins, the overall impact on protein content is relatively small (Alonso *et al.*, 2000).

4.7.2 Effect of dehulling

Crude protein content of dehulled faba beans was determined, and the value obtained showed that there was significant increment ($p<0.05$) in protein content, which was increased from 26.50g/100g to 31.10g/100g after dehulling i.e., 17.35% increment.

The result obtained in our study is almost similar to that of Alonso (2000), who in their study reported a 15.8% increment in the protein content of faba beans after dehulling. Another study by Meijer (1994) reported a 14.1% increment in the protein content after dehulling faba beans which was almost similar to the result obtained in our study. Because of their greater weight, cotyledons of faba beans contribute the major amount of protein to the whole seed. The hull of faba seed has significantly more weight than the whole grain. Therefore dehulling more significantly increased protein content in faba seeds (Alonso *et al.*, 2000).

4.7.3 Effect of germination

The value of crude protein after germinating faba beans was determined, and the value showed that there was significant increment ($p<0.005$) in the protein content, which increased from 26.50g/100g to 27.20mg/100g, 28.77g/100g, 30.40g/100g and 31.70g/100g after 24 hours, 48hours, 72 hours and 96 hours of germination respectively i.e., 2.64%, 8.56%, 14.71% and 19.62% increment respectively.

Our study showed increasing trend in the content of protein as days of germination progressed which was accordance with the result obtained by Alonso (2000), who in their study mentioned protein content of faba beans increased with the process of germination. Our result, regarding the effect of germination on the protein content of faba bean after 96 hours was in accordance with Shah (2011), who in their study reported protein content of faba beans increased by 19.25-26.8% after 96 hours of germination. This is due to the utilization of carbohydrates as a source of energy during germination, presumably caused by starch digestion through amylolytic enzymes (Alonso *et al.*, 2000).

4.7.4 Effect of cooking

The effect of cooking on crude protein content of faba beans was determined. The mean value of crude protein was significantly reduced ($p<0.005$) from 26.50g/100g to 26.25g/100g and 24.87g/100g after open cooking and autoclaving respectively i.e. 0.94% and 6.15% reduction, respectively.

According to the study of Dhull (2022), there was reduction in protein content of faba beans by 0.90% on open cooking and by 6.25% on autoclaving. These results were similar with the value obtained in our study. Another research by Pedrosa (2021) showed there was 7% loss of protein content during autoclaving which correlates with our result. During cooking, some of the soluble proteins in legumes can leach into the cooking water. If the cooking water is discarded, a portion of the protein content is lost along with it. (Pedrosa *et al.*, 2021). The effect of different processing methods on crude protein content of faba beans is shown in Table 4.6.

Table 4.6 Effect of processing methods on crude protein content

Processing methods	**Crude protein (mg/100g)**	**Changes**
Raw	26.50±0.04a	
Soaking	26.42±0.4a	-0.30%
Dehulling*	31.10±0.26b	+17.35%
Germination, 24h	27.20±0.30a	+2.64%
Germination, 48h*	28.77±0.85c	+8.56%
Germination, 72h	30.40±0.43b	+14.71%
Germination, 96h*	31.70±0.20d	+19.62%
Raw open cooking*	26.25±0.05e	-0.94%
Raw autoclaving*	24.87±0.04f	-6.15%

[* = significantly different]

4.8 Effect of processing method on iron content

The effects of soaking, germination, cooking and dehulling on the iron content of faba bean was studied. All the treatments significantly changed ($p<0.05$) the iron content of the faba bean, but to the varying extent. Germination for 96 hours had the most significant effect in the increment of iron content whereas dehulling had the most significant effect on the reduction of iron content.

4.8.1 Effect of soaking

The mean value of iron content of faba beans after 12 hours of soaking was found to be significantly reduced ($p<0.005$) from 5.667 mg/100g to 4.917mg/100g i.e., 13.23% reduction.

Study done by Luo (2013) showed 16.1% reduction in iron content of faba beans after 12 hours of soaking which was almost similar with the result obtained in our study. Another study done by Khalil (2001) reported a 12% reduction in the iron content after 12 hours of soaking which was similar to our study. Soaking legumes in water may result in the leaching of water-soluble nutrients, including a small amount of iron. Some iron may dissolve into the soaking water, leading to a minor reduction in the iron content of the legumes (Khalil and Mansour, 1995).

4.8.2 Effect of dehulling

The mean value of iron content of faba beans after dehulling was found to be significantly reduced ($p<0.005$) from 5.6670mg/100g to 3.333mg/100g i.e., 41% reduction.

Luo (2010) reported 35.2% reduction in iron content of faba beans after dehulling. This finding was almost similar with the result obtained in our study. According to (U. Singh *et al.*, 1992), iron is mainly found in the outer portions of endosperm of cotyledons due to which significant amount is loss during dehulling.

4.8.3 Effect of germination

The effect of germination on iron content of faba bean was studied. The value obtained showed that there was significant increment ($p<0.05$) in iron content, which was increased from 5.667mg/100g to 5.7mg/100g, 5.767mg/100g, 5.930mg/100g and 6.050mg/100g after 24, 48, 72 and 96 hours of germination respectively i.e., 0.58 %, 1.7%, 4.64% and 6.75% increment respectively.

Our study showed an increasing trend in the content of iron as days of germination progressed which is an accordance with the result obtained by Echendu (2009) , who in their study, mentioned it is expected that iron content increased differently in different legumes during germination. A study done by Luo (2013) reported a 10% increment in the iron content of faba beans after 96 hours of germination which was almost similar with the result obtained in our study. The increase in the iron content during germination might be

because of hydrolytic enzyme releasing more free iron from organic complexes (Obizoba and Atii, 1994). Germinating legumes in water may result in the leaching of water-soluble nutrients, including a small amount of iron (Khalil and Mansour, 1995).

4.8.4 Effect of cooking

The effect of cooking on iron content of faba beans was studied and the value obtained showed significant reduction ($p<0.005$) in iron content from 5.667mg/100g to 5.467 mg/100g and 5.183mg/100g on open cooking and autoclaving respectively i.e., 3.52% and 8.5% reduction, respectively.

The study done by Dhull (2022) reported open cooking and autoclaving significantly reduced the iron content of faba beans by 3.44% and 12% respectively. Similar result was obtained in our study. Luo (2013) reported autoclaving reduces the iron content of faba beans by 6.7% which is almost similar with the result obtained in our study. Another research by Khalil (2001) showed there was significant reduction in the iron content of faba beans on cooking and autoclaving which was accordance to the result obtained in our study. During cooking, especially when using water-based methods like boiling or simmering, some water-soluble iron can leach out into the cooking liquid. The degree of leaching depends on factors such as cooking time, temperature, pH, and the presence of other substances that may enhance or inhibit iron leaching (Obizoba and Atii, 1994). The effect of different processing methods on iron content of faba beans is shown in Table 4.7.

Table 4.7 Effect of processing methods on iron content

Processing methods	**Value(mg/100g)**	**Changes**
Raw	5.667±0.15a	
Soaking*	4.917±0.07b	-13.23%
Dehulling*	3.333±0.11c	-41%
Germination, 24h	5.700±0.08d	+0.58%
Germination, 48h	5.767±0.11d	+1.7%
Germination, 72h	5.930±0.03e	+4.64%
Germination, 96h	6.050±0.05e	+6.75%
Raw open cooking*	5.467±0.15f	-3.44%
Raw autoclaving*	5.183±0.07g	-12%

[* = significantly different]

4.9 Effect of processing method on phytic acid content

The effects of soaking, germination, cooking, and dehulling on the phytic acid content in faba bean was studied. All the treatments except dehulling significantly reduced ($p<0.05$) the phytate of the faba bean seeds, but to the varying extent. Germination for 96 hours had a more pronounced effect than other treatments in reduction of phytic acid contents.

4.9.1 Effect of soaking

The mean value of phytic acid content of faba beans after 12 hours of soaking was found to be significantly reduced ($p<0.005$) from 953.3mg/100g to 600.4mg/100g i.e., 37% reduction.

The result by Khalil (2001) showed significant reduction in phytic acid content of faba beans after soaking for 12 hours by 27% which is almost similar with the result obtained in our study. Research by Luo (2009) showed, when faba beans were soaked for 12 hours, phytic acid content decreased by 36% which was approximate with the result obtained in our study. The decrease in phytic acid content has been reported by different workers to be a result of a leaching out effect during hydration (Beleia *et al.*, 1993).

4.9.2 Effect of dehulling

The mean value of phytic acid content of faba beans after dehulling was found to be significantly increased ($p<0.005$) from 953.3mg/100g to 1058.9/100g i.e., 11% increment.

The research done by Alonso (2000) showed there was significant increase in the phytic acid content of faba beans by 15%, which was almost similar with the result obtained in our study. Another research by Rahate (2021) found a 9.6% increment in phytic acid content of faba beans after dehulling which was more approximate with the result obtained in our study. Since phytates are mainly located in the cotyledons, the physical removal of testa by dehulling increase phytate content by higher extent (Alonso *et al.*, 2000).

4.9.3 Effect of germination

The effect of germination on phytic acid content of faba bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in phytic acid content, which was reduced from 953.3mg/100g to 897mg/100g, 336mg/100g, 279.5mg/100g and

237.7mg/100g after 24, 48, 72 and 96 hours of germination respectively i.e., 6%, 64%, 70.68% and 75% reduction respectively.

The research done by Sharma and Sehgal (1992) showed there was significant reduction in phytic acid content of faba beans after 24 hours of germination, which was 8.46% reduction. Another study by Alonso (2000) reported a 59% reduction in phytic acid content after 48 hours of germination. Another study by Rahate (2021) reported 65% and 72% reduction in phytic acid of faba beans after 72 hours and 96 hours of germination which was almost similar with the result of our study. The decrease in phytic acid is a consequence of the increase in phytase activity during germination. Phytase breaks down the phosphate groups that are bound to the phytic acid molecule. This hydrolysis reaction converts phytic acid into inositol and inorganic phosphate. The removal of these phosphate groups from phytic acid results in a reduction of its ability to form insoluble complexes with minerals (Bau *et al.*, 1997).

4.9.4 Effect of cooking

The effect of cooking on iron content of faba beans was studied and the value obtained showed significant reduction ($p<0.005$) in phytic acid content from 953.3mg/100g to 439.7mg/100g and 391mg/100g on open cooking and autoclaving respectively i.e., 53.87% and 59% reduction, respectively.

The study done by Khalil (2001) reported 61% and 63% reduction in phytic acid content by open cooking and autoclaving faba beans respectively. These values were almost similar with the result obtained in our study. Cooking at high temperatures can lead to the thermal degradation of phytic acid. Heat breaks down the molecular structure of phytic acid, resulting in its partial or complete hydrolysis. As a result, the levels of phytic acid decrease during cooking, making the minerals bound to it more bioavailable for absorption (Dhull *et al.*, 2022). The effect of different processing methods on phytic acid content of faba beans is shown in Table 4.8.

Table 4.8 Effect of processing methods on phytic acid

Processing methods	Value (mg/100g)	Changes
Raw*	953.3±5.77a	
Soaking*	600.4±11.07b	-37%
Dehulling*	1058.9±31.03c	+11%
Germination, 24h*	897±9.050d	-6%
Germination, 48h*	336.0±5.56e	-64%
Germination, 72h*	279.5±7.26f	-70.68%
Germination, 96h*	237.7±6.65g	-75%
Raw open cooking*	439.7±9.07h	-53.87%
Raw autoclaving*	391±6.55i	-59%

(* = significantly different)

4.10 Effect of processing method on tannin content

The effects of soaking, germination, cooking and dehulling on the tannin content in faba bean was studied. All the treatments significantly reduced ($p<0.05$) the tannin content of the faba bean, but to the varying extent. Dehulling had a more pronounced effect than other treatments in reduction of tannin contents.

4.10.1 Effect of soaking

Tannin content of soaked faba bean was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 217.37mg/100 g to 119.7 mg/100 g after 12 hours soaking i.e., 45% reduction.

Our result corelates with the result obtained by Rahate (2021) who in their study found 47% reduction in tannin content after soaking faba beans for 12 hours. Another study by Patterson (2017) found tannin content of faba beans reduced by 47.7% after 12 hours of soaking which was also similar with the result obtained in our study. The reduction in the tannin content after soaking is due to the leaching out of tannin after soaking (Alonso *et al.*, 2000).

4.10.2 Effect of dehulling

Tannin content of dehulled faba bean was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 217.37mg/100 g to 61.71mg/100 g after dehulling i.e., 71.6% reduction.

Sharma and Sehgal (1992) reported a 70.5% reduction in the tannin content of faba beans after dehulling which was similar with the result obtained in our study. Another study by Van Der Poel (1991) reported tannin content in faba beans reduced by 40 to 85% after dehulling which was accordance with the result obtained in our study. Since tannins are mainly located in the testa, the physical removal of testa by dehulling significantly decreased the level of tannins in dehulled faba beans (Alonso *et al.*, 2000).

4.10.3 Effect of germination

The tannin content of faba bean after germination was determined, and the value obtained showed that there is significant reduction ($p<0.05$) in tannin content, which is reduced from 217.37 mg/100 g to 97.7mg/100g, 91mg/100gm, 86.2mg/100g and 69.1mg/100g after 24, 48, 72 and 96 hours of germination i.e., 55%, 58%, 60% and 66% reduction.

Rahate (2021) reported tannin content of faba beans reduced by 53.4%, 59% and 60% after 24, 48 and 72 hours of germination respectively which was similar to the result obtained in our study. Yasmin (2008) reported the loss of tannin content by 68.6% after 96 hours of germination which was similar with the result obtained in this study. Reduction in tannin content after germination may be attributed to the leaching out effect during hydration which was reported by (Kataria *et al.*, 1989).

4.10.4 Effect of cooking

The effect of cooking on total tannin content of faba bean was studied. Cooking shows significant reduction ($p<0.05$) in tannin content of faba bean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. The open cooking and autoclaving method of cooking significantly reduced tannin content of faba beans from 217.37mg/100g to 86.66mg/100g and 76.43mg/100g respectively i.e., 60.1% and 65% reduction respectively.

The value obtained in our study was similar to the result obtained by Dhull (2022) who in their study reported 54% and 60% reduction in the tannin content of faba beans after

open cooking and autoclaving respectively. Another study done by Patterson (2017) reported tannin content of faba beans after open cooking and autoclaving reduced by 76% and 93% respectively which was also similar with the result obtained in this study. Cooking at high temperatures can lead to the degradation or denaturation of tannins. Heat can break down the molecular structure of tannins, resulting in a decrease in their concentration. Boiling, simmering, or baking foods can contribute to the leaching of tannin and reduce its content (Pedrosa *et al.,* 2021). The effect of different processing methods on tannin content of faba beans is shown in Table 4.9.

Table 4.9 Effect of processing methods on tannin content

Processing methods	**Value (mg/100g)**	**Changes**
Raw*	217.37±8.6a	
Soaking*	119.70±13.1b	-45%
Dehulling*	61.71±4.70c	-71.6%
Germination, 24h*	97.7±1.572d	-55%
Germination, 48h*	91.0±1.48e	-58%
Germination, 72h	86.2±1.6f	-60%
Germination, 96h*	69.1±3.4g	-66%
Raw open cooking*	86.6±9.6f	-60.1%
Raw autoclaving*	76.4±16.4h	-65%

[* = significantly different]

4.11 Effect of processing on total phenolic content

The effects of soaking, germination, cooking and dehulling on the total phenolic content of faba bean was studied. All the treatments significantly reduced ($p<0.05$) the total flavonoid content of the faba bean, but to the varying extent. Dehulling had a more pronounced effect than other treatments in reduction of total phenolic contents.

4.11.1 Effect of soaking

Total phenolic content of soaked faba bean was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in total phenolic content, which was reduced from 391.8mg/100 g to 369.5mg/100 g after 12 hours soaking i.e., 5.6% reduction.

Our result was similar with the result obtained by Patterson (2017) , who in their study reported total phenolic content of faba beans reduced by 4.85% after 12 hours of soaking.

Alonso (2000), in their study found total phenolic content of faba beans reduced by 8.6% after 12 hours of soaking which was also similar with the result obtained in our study. During soaking, some water-soluble phenolic compounds may leach out into the soaking water. This leaching process can result in a partial reduction in the phenol content of the legume being soaked (Patterson *et al.*, 2017). Soaking can activate enzymes naturally present in the legumes, including phenol oxidases. These enzymes can catalyze the oxidation of phenolic compounds, leading to their conversion into other forms or degradation. Consequently, the phenol content may decrease during soaking due to enzymatic activity (S. D. Siah *et al.*, 2019).

4.11.2 Effect of dehulling

Total phenolic content of dehulled faba bean was determined, and the value obtained showed that there was significant reduction ($p<0.005$) in total phenolic content, which was reduced from 391.8mg/100g to 75.2mg/100g after dehulling i.e., 80% reduction.

The reduction observed in our study was similar with the result obtained by Patterson (2017) who in their study found dehulling of faba beans significantly reduced total phenolic content by 80%. Another research by Alonso (2000) found dehulling the faba beans reduced total phenolic content by 81.6% which was almost similar with the result obtained in our study. Dehulling legumes typically leads to a reduction in the total phenolic content. The seed coat of legumes is known to contain a significant portion of phenolic compounds. By removing the seed coat through dehulling, the overall phenolic content of the legume is decreased (Alonso *et al.*, 2000).

4.11.3 Effect of germination

The total phenolic content of faba bean after germination was determined, and the value obtained showed that there is significant reduction ($p<0.05$) in total phenolic content, which is reduced from 391.8 mg/100 g to 356.3mg/100g, 353mg/100gm, 307.8mg/100g and 272.7mg/100g after 24, 48, 72 and 96 hours of germination i.e., 9.06%, 10%, 21% and 30.3% reduction respectively.

Wei (2022) reported in their study, the polyphenol content of faba beans reduced by 7.4%, 9.1% and 11.17% after 24, 48 and 72 hours of germination respectively. These results were almost similar with the result obtained in our study. Similarly, Yasmin (2008) reported that there was 54.5% reduction of polyphenol content after 96 hours of

germination which was approximate than the result obtained in this study. Before germination, soaking is also done and some loss of polyphenol during soaking is also expected because of its leaching into the soaking water. Further decrease in polyphenols during germination may be ascribed to the presence of polyphenol oxidase and enzymic hydrolysis (S. Jood *et al.*, 1987b).

4.11.4 Effect of cooking

The effect of cooking total phenolic content of faba bean was studied. Cooking shows significant decrease ($p<0.05$) in total phenolic content of faba bean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. Open cooking and autoclaving method of cooking significantly reduced ($p<0.05$) total phenolic content of faba beans from 391.8mg/100g to 110.2mg/100g and 123.5mg/100g respectively i.e., 71.8% and 68.4% reduction respectively.

Siah (2014) found in their study that the total phenolic content of faba beans after open cooking decreased by 68% which was little almost similar with the result obtained in our study. Pedrosa (2021) reported total phenolic content of faba beans after autoclaving at 15 psig for 15 mins reduced by 35-65%. Our result lies within this range. Phenolic compounds can be sensitive to heat and undergo various changes during cooking. High temperatures can cause degradation or structural modifications of phenolic compounds, leading to a reduction in their content. Many phenolic compounds are water-soluble, meaning they can be leached into the cooking liquid during cooking methods involving water, such as boiling or simmering. This can result in a partial loss of phenolic compounds from the food (Patterson *et al.*, 2017). The effect of different processing methods on the total phenolic content of faba beans is shown in Table 4.10.

Table 4.10 Effect of processing methods on total phenolic content

Processing methods	Value (mg/100g)	Changes
Raw*	391.8±3.90a	
Soaking*	369.5±2.76b	-5.6%
Dehulling*	75.2±2.11c	-80%
Germination, 24h*	356.3±10.25d	-9.06%
Germination, 48h*	353.0±9.22e	-10%
Germination, 72h*	307.8±2.57f	-21%
Germination, 96h*	272.7±9.81g	-30.3%
Raw open cooking*	110.2±4.36h	-71.8%
Raw autoclaving*	123.5±2.57i	-68.4%

[*= significantly different]

4.12 Effect of processing on total flavonoid content

The effects of soaking, germination, cooking and dehulling on the total flavonoid content of faba bean was studied. All the treatments significantly changed ($p<0.05$) the total flavonoid content of the faba bean, but to the varying extent. Dehulling had the most pronounced effect than other treatments in reduction of total flavonoid content whereas germination for 96 hours had more pronounced effect than other treatments in increasing total flavonoid contents.

4.12.1 Effect of soaking

Total flavonoid content of soaked faba bean was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in total flavonoid content, which was reduced from 439.3mg/100 g to 311.9mg/100 g after 12 hours soaking i.e., 29% reduction.

Meital (2023) in her study reported that soaking faba beans for 12 hours decreased the total flavonoid content by 19 to 36% . The result obtained in our study was 29% which lies in this range. Another study done by Afify (2012) on the effect of soaking on flavonoid content of sorghum found flavonoid content of legumes decreased by 26% after 12 hours of soaking which was also almost similar with the result obtained in our study. The phenolic components leached out during germination which decreases the flavonoid contents after soaking (Alonso *et al.*, 2000).

4.12.2 Effect of dehulling

Total flavonoid content of dehulled faba bean was determined, and the value showed significant reduction in total flavonoid content after dehulling from 439.3mg/100g to 237.5mg/100g i.e., 45.9% reduction.

Pal (2016) reported in their study that total flavonoids of legumes significantly reduced after dehulling by 41% which was almost similar with the result obtained in this study. Another research by Oghbaei (2017) reported 42% reduction in flavonoid content of green gram after dehulling which was also approximate with the result obtained in our study. The seed coat or hull of legumes and grains is known to contain a significant portion of flavonoids. Dehulling removes this outer layer, resulting in a reduction in the overall flavonoid content of the food (Pal *et al.*, 2016).

4.12.3 Effect of germination

The total flavonoid content of faba bean after germination was determined, and the value obtained showed that there is significant increment ($p<0.05$) in total flavonoid content, which is increased from 439.3 mg/100 g to 551.2mg/100g, 578.3mg/100gm, 616.1mg/100g and 663.4mg/100g after 24, 48, 72 and 96 hours of germination i.e., 25.47%, 31.57%, 40.2% and 51% increment respectively.

Saleh (2019) in their study on the effect of germination on total flavonoid content of legumes found increment in flavonoid content by 42.5% and 52,6% after 72 and 96 hours of germination which was similar with the result of our study, Another study by Swieca (2015) found flavonoid content of legumes significantly increased by 31.25%, 56.25%, 71% and 81.25% after 24, 48, 72 and 96 hours of germination respectively which was almost similar with the result obtained in our study. Germination can result in the conversion of flavonoid glycosides, which are inactive forms of flavonoids, into their aglycone forms, which are the active and more bioavailable compounds. This enzymatic conversion during germination can enhance the availability and health benefits of flavonoids (Świeca and Gawlik-Dziki, 2015).

4.12.4 Effect of cooking

The effect of cooking on total flavonoid content of faba bean was studied. Cooking shows significant decrease ($p<0.05$) in total flavonoid content of faba bean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. The

open cooking and autoclaving method of cooking significantly reduced total flavonoid content of faba beans from 439.3mg/100g to 251.8mg/100g and 132.5mg/100g respectively i.e., 42.68% and 69.83% reduction, respectively.

Siah (2014) reported reduction in total flavonoid content of faba beans by 46.74% and 74.5% after open cooking and autoclaving respectively which was almost similar with the result obtained in our study. Another study by Meital (2023) reported 56% and 65% reduction in total flavonoid content after open cooking and autoclaving which was almost similar with the result obtained in our study. High temperatures can cause degradation and structural modifications of flavonoid compounds, leading to a reduction in their content. The extent of degradation can depend on factors such as cooking temperature and duration (Meital *et al.,* 2023). The effect of different processing methods on total flavonoid content of faba beans is shown in Table 4.11.

Table 4.11 Effect of processing on total flavonoid content

Treatments	**Mean**	**Changes**
Raw*	439.3± 9.74a	
Soaking*	311.9 ±8.88b	-29%
Dehulling*	237.5± 6.682c	-45.9%
Germination, 24h*	551.2 ±11.1d	+25.47%
Germination, 48h*	578.3 ±3.371e	+31.57%
Germination, 72h*	616.1 ±2.53f	+40.2%
Germination, 96h*	663.4 ±7.51g	+51%
Raw open cooking*	251.8 ±9.46h	-42.68%
Raw autoclaving*	132.5±2.77i	-69.83%

[* = significantly different]

Part V

Conclusion and Recommendation

5.1 Conclusion

The study on the processing of raw faba beans demonstrated that a variety of methods, including soaking, dehulling, germination for different durations, raw cooking, and autoclaving, can significantly reduce ($p<0.05$) the levels of anti-nutrients in faba beans. Also, processing methods significantly increased ($p<0.05$) nutrient content of faba beans. Following conclusions can be drawn from this study:

- Out of all the processing methods, dehulling had the most pronounced effect on reducing tannin content from 217.37mg/100g to 61.71mg/100g i.e., 71.6% reduction.
- Germination for 96 hours had the most pronounced effect on increasing protein content from 26.5mg/100g to 31.7mg/100g i.e.,+19.62% and iron content from 26.5mg/100g to 6.05mg/100g i.e., +6.75%. Additionally, the study revealed that germination for 96 hours decreased phytic acid from 953.3mg/100g to 237.7mg/100g i.e., 75% reduction.
- Out of all processing methods, dehulling had the most pronounced effect on reduction of total phenolic content from 391.8mg/100g to 75.2mg/100g i.e., 80% reduction.
- Out of all processing methods, open cooking had the most pronounced effect on the reduction of total flavonoid content from 439.3mg/100g to 132.5mg/100g i.e., 69.83%. Germination for 96 hours had the most pronounced effect on increasing total flavonoid content from 439.3mg/100g to 663.4mg/100g i.e., +51% increment.
- Overall, the best processing method was found to be germination for 96 hours as it not only significantly reduced anti-nutrients but also enhanced protein and iron content with enhancement of flavonoids content which exhibit antioxidant properties along with several other health benefits.

5.2 Recommendation

Based on the data and findings presented in the study on processing raw faba beans, the following recommendations can be made:

- Considering the effectiveness of dehulling in reducing tannin, it is recommended to dehull faba beans before consumption or further processing. This step can significantly decrease the levels of this anti-nutrient, improving the overall nutritional quality.
- Germination for 96 hours proved to be highly effective in reducing phytic acid and tannin and increasing iron content and protein content. Therefore, incorporating germinated faba beans into the diet can be recommended to enhance iron bioavailability and reduce the negative impact of phytic acid.
- Depending on the desired nutritional outcome, different cooking methods can be employed. Raw autoclaving was found to be more effective than raw open cooking in reducing tannin, and phytic acid.
- Further research can be done to investigate the impact of these pre-treatments on anti-nutritional factors present in faba beans like lectins, haemagluttins, saponins, and other anti-nutritional factors.
- Study on effect of these processing methods on other nutritional components of faba beans like vitamins and minerals is recommended.

Part VI

Summary

Soaking legumes and grains can help reduce antinutrients like phytic acid and tannins by enzymatic activity, leaching, and microbial action. Removing the outer husk or seed coat of legumes and grains through dehulling can effectively reduce antinutrients concentrated in these layers, such as phytic acid and tannins. The process of germination, achieved through soaking and allowing seeds to sprout, activates enzymes that degrade antinutrients. Germination significantly reduces antinutrients like phytic acid, and tannins. Different cooking methods, such as boiling, autoclaving, or other forms of cooking, can help reduce antinutrients. Cooking degrades antinutrients through heat, water, and enzymatic action, resulting in decreased levels of substances like phytic acid and tannins.

Soaking legumes and grains can lead to slight changes in protein content. While soaking may cause some loss of protein due to leaching into the soaking water, the overall impact on protein content is generally minimal. As for iron, soaking can help improve its bioavailability by reducing the levels of antinutrients that inhibit iron absorption. Cooking generally has a minimal effect on protein content unless excessive cooking times or high temperatures are employed, which may lead to protein denaturation. While some iron may be lost through leaching into cooking water, particularly in the case of boiling, cooking can also improve iron bioavailability by degrading antinutrients and making iron more accessible for absorption.

The sample used for analysis was purchased from a local convenience store which was cleaned and made into powdered form for further analysis. The ground sample was treated differently according to different procedure required for analysis of specific nutritional and anti-nutritional components.

Crude protein was increased from 26.50mg/100g to 26,7mg/100g, 31.1mg/100g, 27.20mg/100g, 28.77mg/100g, 30.4mg/100g and 31.70mg/100g on soaking, dehulling, and germination for 24, 48, 72 and 96 hours respectively. However, cooking decreases the protein content from 26.50mg/100g to 26.25mg/100g and 24.87mg/100g on open cooking and autoclaving respectively. Iron content of faba beans was increased from 5.667mg/100g to 5.7mg/100g, 5.767mg/100g, 5.930mg/100g and 6.050mg/100g on germination for 24, 48, 72 and 96 hours respectively. However, iron content was decreased from

5.667mg/100g to 4.917mg/100g, 3.333mg/100g, 5.467mg/100g and 5.183mg/100g on soaking, dehulling, open cooking and autoclaving respectively.

Phytic acid was decreased from 953.3mg/100g to 600.4mg/100g, 439.7mg/100g, 391mg/100g, 897mg/100g, 336mg/100g, 279.5mg/100g and 237.7mg/100g on soaking, open cooking, autoclaving, germination for 24, 48, 72 and 96 hours respectively. Tannin content was decreased from 217.37mg/100g to 119.7mg/100g, 61.71mg/100g, 86.66mg/100g, 76.43mg/100g, 97.7mg/100g, 91mg/100g, 86.2mg/100g and 69.1mg/100g on soaking, dehulling, open cooking, autoclaving, germination for 24, 48, 72 and 96 hours respectively.

Total phenolic content was decreased from 391.8mg/100g to 369.5mg/100g, 75.2mg/100g, 110.24mg/100g, 123.5mg/100g, 356.3mg/100g, 353mg/100g, 307.8mg/100g and 272.7mg/100g on soaking, dehulling, open cooking, autoclaving, germination for 24, 48, 72 and 96 hours respectively. Total flavonoid content decreased from 439.3mg/100g to 311.9mg/100g, 237.5mg/100g, 251.8mg/100g, 132.5mg/100g on soaking, dehulling, open cooking and autoclaving respectively. However, germination increased TFC from 439.3mg/100g to 551.2mg/100g, 578.3mg/100g, 616.1mg/100g and 663.4mg/100g on germinating for 24, 48, 72 and 96 hours respectively.

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Appendices

Appendix A

1 Standard curve for tannin content

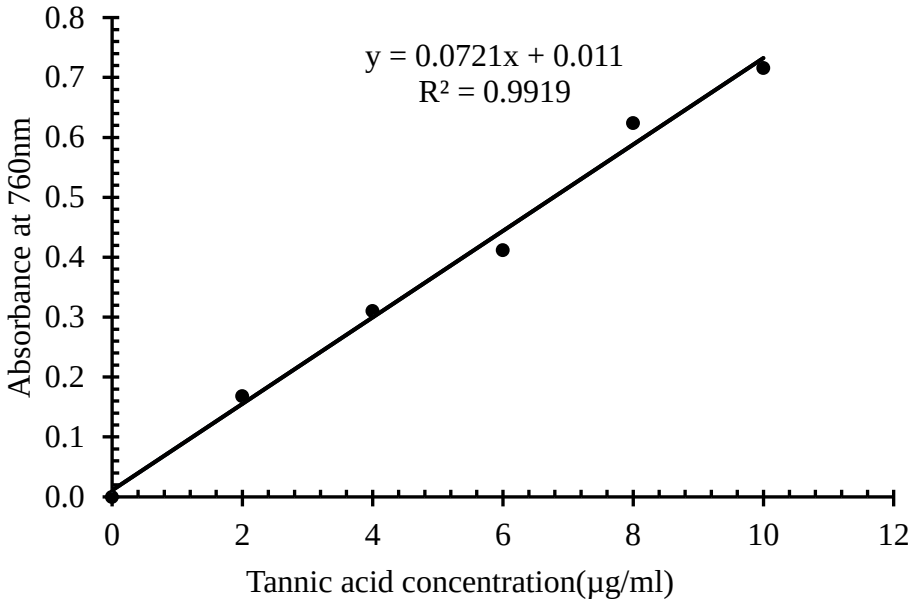

Fig. A.1 Standard curve for tannin content

2 Standard curve for total phenolic content

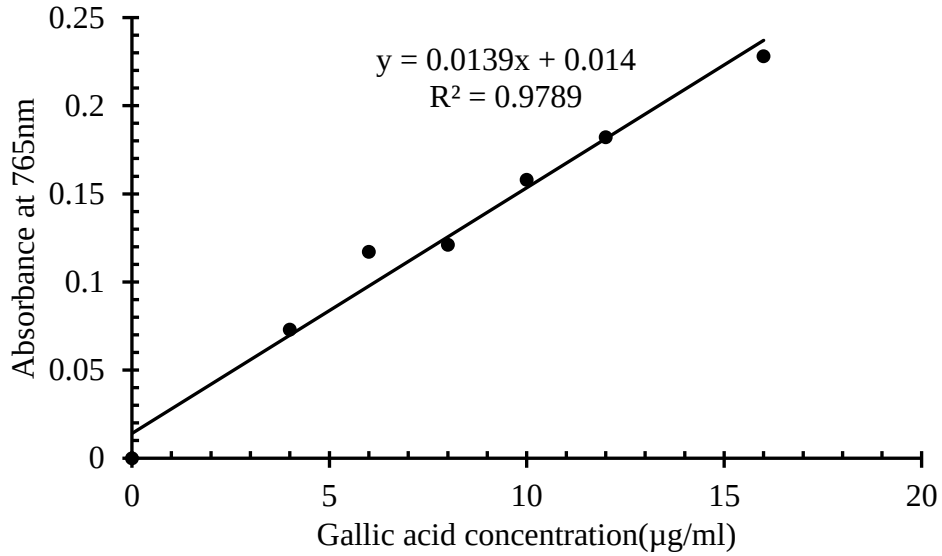

Fig. A.2 Standard curve of gallic acid for total phenol content

3 Standard curve for flavonoid

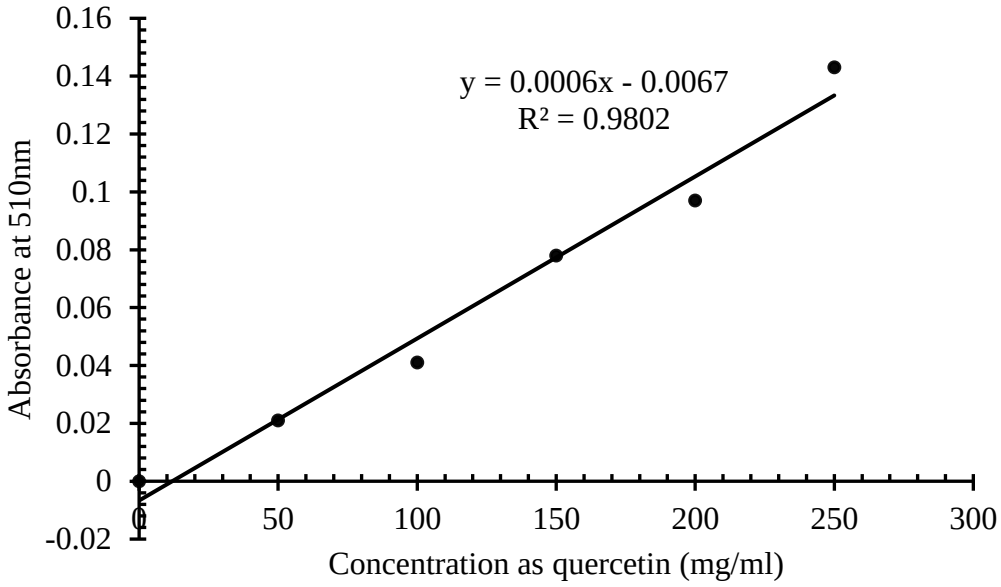

Fig. A.3 Standard curve of quercetin for total flavonoid content

Appendix B

Table B.1 ANOVA for crude protein

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F Ratio	F Probability
Treatment	8	140.4674	17.5584	117.21	< .001
Residual	18	2.6965	0.1498		
Total	26	143.1639			

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

Table B.2 LSD of means for crude protein.

Treatments	Mean	Column A	LSD	d.f.
Raw	26.50±0.400	A	0.727	18
Soaked	26.7±0.2646	A		
Dehulled	31.10±0.2646	B		
Raw Open cooked*	26.25±0.0577	C		
Raw Autoclaving*	24.87±0.04	D		
Germination 24h	27.20±0.3000	A		
Germination 48h*	28.77±0.8505	E		
Germination 72h*	30.40±0.4359	F		
Germination 96h*	31.70±0.2000	G		

(* = significantly different)

Table B.3 ANOVA for Iron content

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	Variance Ratio	F Probability ratio
Treatment	8	16.55047	2.0688	192.75	< 0.001
Residual	18	0.1932	0.01073		
Total	26	16.74367			

Since there is a significant difference between the samples in different treatments, LSD testing is necessary.

Table B.4 LSD of means for iron content.

Treatment	Mean	Column	LSD	DF
Raw*	5.667±0.152	A	0.1777	18
Soaked*	4.917±0.076	B		
Dehulled*	3.333±0.115	C		
Raw Open cooking*	5.467±0.152	D		
Raw Autoclaving	5.183±0.076	E		
Germination 24h	5.700±0.085	F		
Germination 48h	5.767±0.119	F		
Germination 72h	5.930±0.030	G		
Germination 96h*	6.050±0.050	G		

(* = significantly different)

Table B.5 ANOVA for phytic acid content

Source of Variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	8	2375838	296979.8	4375.53	<.001
Residual	18	2906.3	161.5		
Total	26	2378745			

Since p<0.005, there is a significant difference between the samples in different treatments, so LSD testing is necessary.

Table B.6 LSD of means for phytic acid

Treatments	Mean	Column A	LSD	df
Raw*	953.3±5.77	A	12.98	18
Soaked*	600.4±11.07	B		
Dehulled*	1058.9±31.03	C		
Raw open cooking*	439.7±9.07	D		
Raw autoclaving*	391±6.557	E		
Germination 24h*	897±9.050	F		
Germination 48h*	336.0±5.56	G		
Germination 72h*	279.5±7.26	H		
Germination 96h*	237.7±6.65	I		

(* = significantly different)

Table B.7 ANOVA for tannin content

Source of Variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatments	8	52768.15	6596.02	89.6	<0.01
Residual	18	1325.09	73.62		
Total	26	54093.24			

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

Table B.8 LSD of means for tannin

Treatment	Mean	Column A	LSD	d.f.
Raw*	217.37±8.6	A	14.72	18
Soaked*	119.70±13.1	B		
Dehulled*	61.71±4.70	C		
Raw open cooking*	86.66±9.6	D		
Raw autoclaving*	76.43±16.7	E		
Germination 24h*	97.7±1.572	F		
Germination 48h*	91.0±1.48	G		
Germination 72h	86.2±1.6	HI		
Germination 96h*	69.1±3.4	I		

(*= significantly different)

Table B.9 ANOVA for total phenolic content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	8	374668.7	46833.58	1222.34	<0.001
Residual	18	689.66	38.31		
Total	26	375358.3			

Since p<0.005, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.10 LSD of means for total phenolic content

Treatment	Mean	Column A	LSD	d.f.
Raw*	391.8±3.090	A	10.62	18
Soaked*	369.5±2.376	B		
Dehulled*	75.2±2.911	C		
Raw open cooking*	110.2±4.136	D		
Raw autoclaving*	123.5±2.557	E		
Germination 24h*	356.3±10.425	F		
Germination 48h*	353.0±9.272	G		
Germination 72h*	307.8±2.577	H		
Germination 96h*	272.7±9.810	I		

(*= Significantly different)

Table B.11 ANOVA for flavonoids content

Source of variation	Degree of freedom	Sum of squares	Mean square	Variance ratio	F probability ratio
Treatment	8	888771.4	111096.4	2004.3	<0.001
Residual	18	997.72	55.43		
Total	26	889769.1			

Since p<0.005, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.12 LSD of means for total flavonoid content

Treatments	Mean	Column A	LSD	d.f.
Raw*	439.3± 9.074	A	12.77	18
Soaked*	311.9 ±8.838	B		
Dehulled*	237.5± 6.682	C		
Raw open cooking*	251.8 ±9.406	D		
Raw autoclaving*	132.5±2.775	E		
Germination 24h*	551.2 ±11.1	F		
Germination 48h*	578.3 ±3.371	G		
Germination 72h*	616.1 ±2.53	H		
Germination 96h*	663.4 ±7.51	I		

(*= Significantly different)

Color Plates

Plate 1 Physical dimensions measurement

Plate 2 Sample kept at water bath for phytic acid analysis

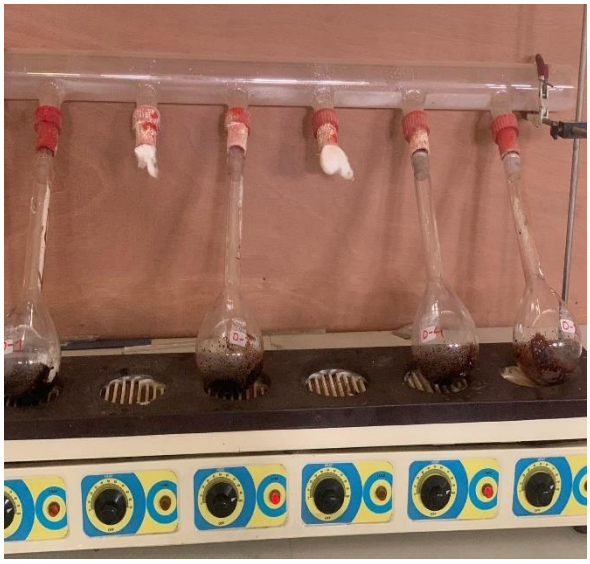

Plate 3 Germinated sample kept for digestion for analysis of protein

Plate 4 Methanolic extraction using rotatory evaporator