EFFECT OF DIFFERENT TREATMENT CONDITIONS ON BIOACTIVE AND ANTINUTRITIONAL COMPONENTS OF SOYBEAN SEEDS

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Approval Letter

This ***dissertation*** entitled ***Effect of Different Treatment Conditions on Bioactive and Anti-nutritional Components of Soybean Seeds*** presented by ***Apar Adhikari*** has been accepted as the partial fulfilment of the requirement for the ***B. Tech. degree in Food Technology***

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(Apar Adhikari)

Abstract

The present work aims to determine the effect of different treatment conditions on bioactive and anti-nutritional components of yellow soybean. The effect of different treatments as soaking (12 h), germination (48 h), roasting (15 min at 160ºC), cooking (20 min at 100ºC) and soaked cooking (12 h soaking and cooking 20 min at 100ºC) on the bioactive and anti-nutritional components as oxalate, phytate, saponin, polyphenol, tannin and antioxidant activity of raw soybean was determined.

The mean value of oxalate, phytate, tannin, saponin, polyphenol and antioxidant activity of raw soybean were found 233.58 mg/100 g, 1008.54 mg/100g, 158.34 mg/100g, 1591.34 mg/100g, 243.67 mg GAE/100g and 18.82% inhibition respectively. The maximum reduction of antinutrients: phytate (45.57 %) and tannin (46.67 %) but also the increase in antioxidant activity (14.82 %) was noted when the soybean seeds were germinated. Similarly, the maximum reduction of oxalate (60.21 %) and total phenolic content (45.72 %) was noted on soaked cooking whereas maximum reduction of saponins (25.28 %) as noted when soybean seeds were roasted. The other treatments such as soaking, cooking and roasting also significantly reduced ($p<0.05$) the antinutritional and bioactive components but the percentage reduction was less than other treatment conditions. Overall, the best processing method was found to be germination as it not only significantly reduced ($p<0.05$) anti-nutrients but also enhanced antioxidant activity with several other health benefits Hence, the results suggest that germination could be a preferred processing technique in the food industry for producing healthier soybean-based products, offering potential benefits for both consumers and food manufacturers.

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List of Abbreviations

Abbreviation	Full form
ANF	Antinutritional factor
ANOVA	Analysis of variance
AOAC	Association of Analytical Chemist
CCT	Central Campus of Technology
db	Dry basis
FAO	Food and Agriculture Organization
GAE	Gallic acid equivalent
HSD	Honestly significant difference
LSD	Least significance difference
wb	Wet basis

Part I

Introduction

1.1 General introduction

Soybean (*Glycine max*) belongs to the Leguminosae family and the Papilionoidea subfamily. It is an affordable and high-quality protein source for both humans and animals (Alabi and Anuonye, 2007). Soybeans are available in many forms, such as soy flour, tofu, soy sauce, and soy paste. The diverse nutrient content in soybean seeds, including flavonoids, polyunsaturated fatty acids, folate, and vitamin C, provides numerous health benefits, such as antioxidant, anti-inflammatory, and anti-obesity effects (Huang *et al.*, 2016). As a result, there is a growing demand for protein-rich foods in developing countries due to their affordability, availability, and accessibility to the population (Tao and Li, 2018). Legumes like soybeans have been shown to provide high-quality proteins, making them a viable alternative to animal-based protein sources (Fabiyi and Hamidu, 2011). Soybeans have also been proven to be an alternative milk source for those who are lactose intolerant. Additionally, their high isoflavone content contributes to beneficial phytochemical properties (Hendrich and Murphy, 2001). To get the desired texture, soybeans require significantly more boiling time than other legumes. This increased cooking time most likely leads to their widespread use in processed meals in East Asian nations. Given its nutritious importance, a variety of approaches have been developed to increase soybean intake (Sugano, 2005).

Anti-nutritional factors are naturally occurring substances found in foods due to the normal metabolic processes of organisms. These substances can reduce the effectiveness of nutrients, hinder digestion, or interfere with metabolic utilization, resulting in adverse nutritional effects (Akande *et al.*, 2010). Soybeans contain anti-nutritional components such as phytic acid and tannin. These substances, particularly phytic acid, act as powerful chelating agents by forming insoluble phytates, reducing the bioavailability of essential divalent cations such as calcium, iron, and zinc (Reddy and Salunkhe, 1981).

Soybeans are commonly used as a supplement in infant formula, which remains inaccessible to poor or rural populations. Locally, soybeans are roasted, ground, and added to infant porridge or cereal blends. In households, soybeans are typically processed through methods such as roasting, boiling, soaking, drying, extrusion, salt treatment, fermentation,

germination, and urea treatment before consumption (Akande and Fabiyi, 2010). Intense food processing at high temperatures can cause chemical changes like lipid oxidation and non-enzymatic browning, which can negatively impact the retention of nutrients and bioactive compounds (Djikeng *et al.*, 2018). Lipid oxidation and non-enzymatic browning can reduce the nutritional quality of food by causing the loss of essential amino acids, fatty acids, vitamins, and available carbohydrates, as well as decreasing protein digestibility. These reactions can also alter the organoleptic properties of the food (Cuvelier and Maillard, 2012).

1.2 Statement of the problem

Legumes are rich sources of carbohydrates, proteins, fats, minerals, fibre, antioxidants and vitamins; these beans are considered low in fat and are cholesterol free but some legumes are rich in oil such as soybean (Hayat *et al.*, 2014). Due to its high protein contents these are considered as meat of poor man, the researchers working on identifying and evaluating legumes as an alternative protein source of crop in future (Martín-Cabrejas and Benefits, 2019). Soybean are high in protein and a decent source of both carbs and fat. They are a rich source of various vitamins, minerals, and beneficial plant compounds such as isoflavones (Manandhar, 2021). Different treatment techniques, such as soaking, cooking, germination, and roasting, can be employed to reduce the antinutrients; however, study is still needed to determine how beneficial these techniques are in comparison. The health concerns associated with soy consumption may be significantly decreased with the documentation of treatment conditions that effectively remove the antinutrients found in soy. Therefore, it is more than justified to try to increase soy's nutritious qualities by treating it at home to lower its antinutrient content.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this work was to study the effect of treatment conditions on the bioactive and anti-nutritional components of soybeans.

1.3.2 Specific objectives

The specific objectives of this dissertation work were to:

a. To determine the chemical composition and bioactive components of raw soybean.
b. To determine the influence of different treatment conditions on the concentration of antinutritional factors (e.g., oxalate, phytate, tannin, saponin) and bioactive components (e.g., total polyphenols, antioxidant activity) of soybean seeds.
c. To determine the most effective treatment condition for the reduction of antinutritional factors.

1.4 Significance of the study

Soybeans are cost-effective source of macronutrients that can help to manage protein-energy malnutrition and improve nutrition for vulnerable populations in developing countries. In industrialized nations, it might enhance the nutritional value of functional meals (Etiosa *et al.*, 2018). Antinutritional factors such as oxalates, phytates, tannin and saponin present in the soybean can interfere with the absorption of biomolecules and hamper their bioavailability to the human beings and monogastric animals (Ram *et al.*, 2020). This study helps to determine the bioactive components and anti-nutrients in soybean and effect of different treatment conditions to reduce those anti-nutrients. This study might help in the establishment of the effective and optimized way for the use of soybean in household level and industrial levels, which can help to improve its production and utilization potentials.

1.5 Limitations of the study

a. The important antinutrients found in soybean, such as lectins, vicine, haemagluttins, and trypsin inhibitors were not determined.
b. Only one variety of yellow soybean was taken for study due to time limitation.

Part II

Literature review

2.1 General introduction of soybean

Soybean (*Glycine max*), which belongs to the Fabaceae family and is grown for its many applications as an edible bean, including soy milk, meat, flour, oil, and more, is one of the most nutritious legumes (Sylvia *et al.*, 2023). It is recognized as an oilseed containing several nutrients, including protein, carbohydrate vitamins and minerals (Liu and Liu, 1997). It contains high-quality protein, little saturated fat, no cholesterol, and a lot of nutritional fiber. Soybean is an excellent source of several vitamins and minerals, including riboflavin, folacin, calcium, iron, phosphorus, zinc, and magnesium (Ogunlakin *et al.*, 2015). Anti-nutritional elements including phytic acid and tannin are found in soybeans. These anti-nutritional elements, particularly phytates, are strong chelating agents that cause insoluble phytates to develop, which lowers the bioavailability of divalent cations like calcium, iron, and zinc (Reddy and Salunkhe, 1981).

2.2 Distribution and production of soybean

Based on current research, soybeans were first recognized in the northern areas of China in about 2000 B.C. as one of the most important farmed legumes and one of the five sacred grains of Chinese culture. It was initially cultivated there for its seeds about 1100 B.C. From this primordial location, soybeans spread to countries in Southeast Asia, including Korea, Japan, and Southern China. Soybeans were first introduced to North America and Europe as a crop for fodder (Caldwell *et al.*, 1973).

An ancient crop, soybean is Nepal's fifth most significant legume crop. It is farmed in tropical to temperate regions at elevations between 500 and 3000 meters, mostly in the mid-hills as an intercrop or mixed crop with maize in the summer. The economic significance of soybean seeds for the oil and animal feed sectors has led to an increase in their cultivation in terai and inner terai in recent years (Durbar, 2005).

2.3 Classification and nomenclature of soybean

The taxonomic nomenclature of soybean is as follows:

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Subfamily	Papilionaceae
Genus	*Glycine*
Species	*max* (L.) Merr.

Source: Henny *et al.* (2007)

2.4 Structure of soybean seed

An upright annual plant, soybeans have a hairy stem that can grow up to 130 cm in height, depending on the conditions. The lateral roots and taproot of a soybean plant are often indistinguishable from one another. The symbiotic association between the nitrogen-fixing *Bradyrhizobium* bacteria and the soybean plant produces the root nodules that define the root system (Miladinović and Đorđević, 2011).

The soybean seed can have various shapes but is typically oval and contains an embryo within a seed coat, with minimal endosperm tissue. The hilum, a seed scar, is prominently visible on the seed coat's surface. It is oval and forms when the seed separates from the ovary. The micropyle, a tiny hole created during seed development, is found at one end of the hilum. Sometimes, the hypocotyl-radicle axis above the micropyle can be seen through the seed coat. The seed coat itself has eight to ten cell layers: the outermost epidermis made of palisade cells, followed by the loosely packed hypodermis, and the inner parenchyma tissue consisting of six to eight layers of thin-walled, flattened cells (Norman, 1978).

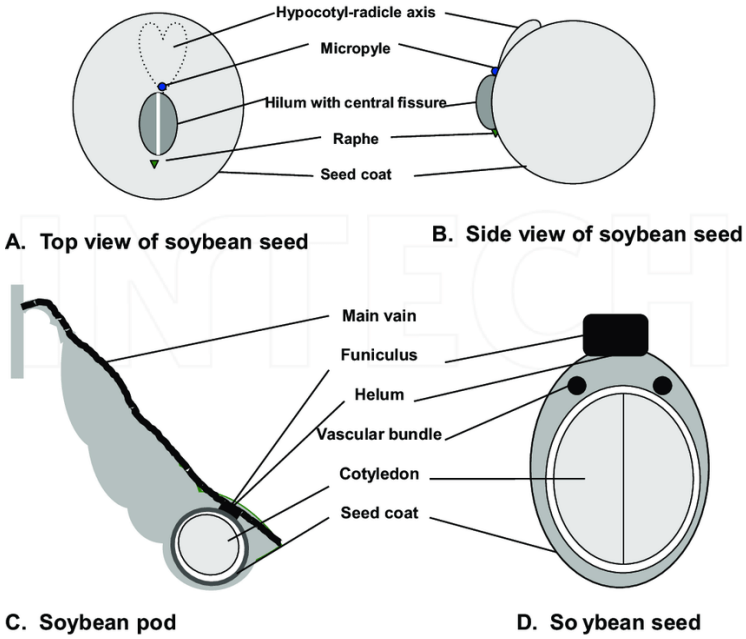

Figure 2.1 Structures of soybean seed and pod. (A) Top view of soybean seed. (B) Side view of soybean seed. (C) Soybean pod with seeds inside. (D) Soybean seed.

Source: Ohyama *et al.* (2017)

2.5 Chemical composition of soybean

Table 2.1 gives the chemical constituents of whole soybean seed.

Table 2.1 Chemical constituents of soybean per 100 g.

Component	Content (g/100 g)[a]	Mineral	Content (mg/100 g)[a]	Vitamin	Content (per 100 g)[a]
Moisture	12.5 (11.7)	Na	1 (1)	Retinol (μg)	0 (0)
Protein	35.3 (33.0)	K	1900 (1800)	Carotene (μg)	6 (7)
Fat	20.25 (21.1)	Ca	240 (230)	Retinol Eq. (μg)	1 (1)
Carbohydrate	28.2 (30.8)	Mg	220 (230)	Vitamin D (μg)	0 (0)
Ash	5.0 (4.8	P	580 (480)	Vitamin E (mg)	3.6 (3.4)
Dietary fiber	19.0 (21.7)	Fe	9.4 (8.6)	Vitamin K (mg)	18 (34)
		Zn	3.2 (4.5)	Vitamin B_1 (mg)	0.83 (0.88)
		Cu	0.98 (0.97)	Vitamin B_2 (mg)	0.30 (0.30)
		Mn	1.90 (-)	Niacin (mg)	2.2 (2.1)
				Vitamin B_6 (mg)	0.53 (0.46)
				Vitamin B_{12} (μg)	0 (0)
				Folic acid (μg)	230 (220)
				Vitamin C (mg)	Tr (Tr)

[a] Data from Resources Council, Science and Technology Agency, Japan, *Standard Tables of Food Comp. in Japan* 5th rev. ed., Printing Bureau of Ministry of Finance, Tokyo, 2000.

[b] Values in parentheses are for products in the United States.

Tr = trace Source: Sugano (2005)

2.6 Antinutritional factors

Chemicals known as "anti-nutritional factors" are those produced in natural food and/or feedstuffs by a species' normal metabolism. These compounds can act against optimal nutrition through a variety of mechanisms, such as the inactivation of certain nutrients, a decrease in the digestive process, or the metabolic utilization of food or feed (Soetan and Oyewole, 2009). These anti-nutritional elements have been demonstrated to be extremely physiologically active; they are sometimes referred to as "secondary metabolites" in plants. The mechanisms leading to the production of primary metabolites yield these secondary

metabolites as byproducts. The widespread presence of a wide variety of natural compounds in tropical plants that can have harmful effects on humans and animals and that act to decrease nutrient utilization and/or food intake are known as anti-nutritional factors. This is one of the main factors limiting the wider food utilization of many tropical plants (Shanthakumari *et al.*, 2008).

Antinutrients are substances that have been developed by plants for a variety of purposes, including self-defense. Among other biological purposes, these substances limit the body's ability to fully utilize certain nutrients, such as proteins, vitamins, and minerals, which prevents food from being used to its full potential and lowers its nutritional value. When ingested at the proper levels, several plant compounds have been demonstrated to either be beneficial or detrimental to human and animal health (Ugwu and Oranye, 2006).

2.7 Anti-nutritional factors present in soybean

2.7.1 Phytate

Phytate, or myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate), is a significant phosphorus-storage form found in the fully matured seeds of monocot and dicot plants. It typically accounts for more than 80% of the soluble myo-inositol phosphate in seeds and about 75% of the total phosphorus in seeds (Dorsch *et al.*, 2003). One of the most prevalent kinds of antinutrients, phytate is present in high amounts in soybeans and can also be found in seeds, nuts, legumes, and grains (Al-Wahsh *et al.*, 2005). Phytate can create insoluble complexes that are not absorbed by the gut, which reduces their bioavailability. It can also bind strongly to iron, zinc, and, to a lesser extent, calcium, magnesium, and potassium (de Melo Ribeiro *et al.*, 2019). Phytic acid and iron have a more complicated binding relationship because, despite their significant attraction to one another, other compounds like phenols and tannins also affect the binding (Prom-u-thai *et al.*, 2006).

Iron and zinc are much less absorbable in the intestines when they bind to phytic acid and form insoluble precipitates. Therefore, in individuals whose diets depend on these items for their mineral intake—such as those in impoverished nations and vegetarians—this process may contribute to iron and zinc shortages (Baskota, 2019). Digestive enzymes are inhibited by the phytotic acid-protein complex. Even though home cooking techniques might weaken phytic acid. The phytic acid in the meal will be somewhat reduced just by boiling it. Soaking

in an acidic media, fermenting lactic acid, and sprouting are more efficacious techniques (Chukwuebuka and Chinenye, 2015).

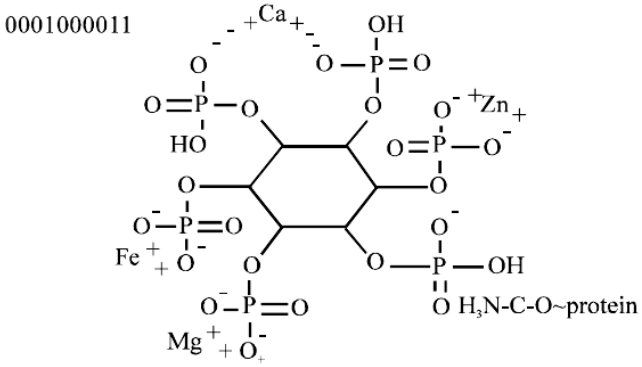

Fig.2.2 Structure of phytic acid with different possibilities to chelate with cations

Source: Singh *et al.* (2018)

2.7.2 Oxalate

Oxalate is dianion with the formula (C2O4) -2, also written ((COO)2) -2 (Dean, 2012).

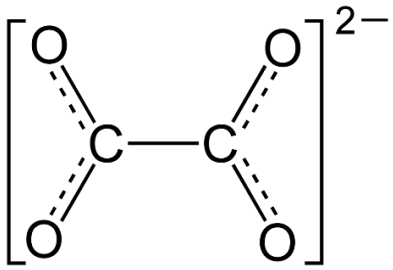

Fig.2.3 Molecular structure of oxalate

Source: Lo *et al.* (2018)

Oxalates are found most commonly in dark-coloured fruits and vegetables like berries, and spinach and also in cereals and legumes like wheat, rye, soybean, tofu, lentils, and kidney beans. Consumption of high-oxalate foods exerts a negative effect on calcium and iron absorption in the body (Chai and Liebman, 2005). Oxalates are present in clinically meaningful amounts in about 75% of all flowering plants, including soy. Oxalates bind to calcium and other minerals in the small intestine, reducing absorption and potentially leading to deficiencies (D'Adamo and Sahin, 2014).

Most people can induct normal amounts of oxalate-rich foods, while individuals with specific conditions, such as enteric and primary hyperoxaluria, need to lower their oxalate admission. In sensitive people, even limited quantities of oxalates can result in burning in the eyes, ears, mouth, and throat; enormous amounts may cause abdominal pain, muscle weakness, nausea, and diarrhoea (Oburuoga and Anyika, 2012). According to criteria established by researchers, soy foods that have undergone more processing, such as tofu, soymilk, and soy sauce, tend to be low oxalate, while more minimally processed soy foods, such as soy flour, textured vegetable (soy) protein, soy nuts, edamame, and soy nut butter, are inclined to be high oxalate (Al-Wahsh *et al.*, 2005). The oxalate content of seeds from 11 cultivars of soybean showed relatively high levels of total oxalate from 0.67 to 3.5 g/100 g of dry weight (Massey *et al.*, 2001).

2.7.3 Tannins

The word tannin is very old and reflects a traditional innovation. Tanning was the word utilized in logical writing to describe the process of transforming raw animal hides or skins into durable, non-putrescible leathers by utilizing plant extracts from various plant parts. Tannin is an astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids which have molecular weights ranging from 500 to over 3000 (Gemede *et al.*, 2014).

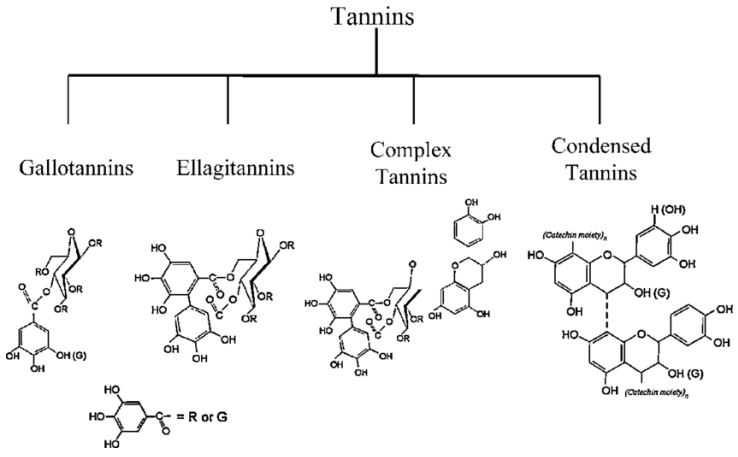

Fig. 2.4 Main chemical structures of the tannins

Source : Aguilar *et al.* (2007)

In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca2+) and iron (II) (Fe2+) to form crystals of the corresponding oxalates which are then excreted

in urine as minute crystals. Oxalate crystals can be razor-sharp and may cause damage to various tissues. The sharp crystals cause damage due to their physical structure, but any contact with the crystals also increases inflammation. Iron oxalate crystals cause significant oxidative damage and diminish iron stores needed for red blood cell formation whereas many kidney stones result from calcium crystals (Chukwuebuka and Chinenye, 2015). Most legumes contain tannins. Red-coloured beans contain the most tannins, and white-coloured beans have the least. Condensed tannins inhibit digestion by binding to consumed plant proteins and making them more difficult to digest, and by interfering with protein absorption and digestive enzymes. Tannins form insoluble complexes with proteins, carbohydrates, and lipids leading to a reduction in digestibility of these nutrients. Many tannin-consuming animals secrete a tannin-binding protein (mucin) in their saliva. Tannin tannin-binding capacity of salivary mucin is directly related to its proline content. Salivary proline-rich proteins (PRPs) are sometimes used to inactivate tannins. One reason is that they inactivate tannins to a greater extent than do dietary proteins resulting in reduced faecal nitrogen losses. PRPs additionally contain non-specific nitrogen and nonessential amino acids making them more convenient than valuable dietary protein (Shimada, 2006). Foods rich in tannins are considered to be of low nutritional value because they precipitate proteins, inhibiting digestive enzymes and Fe absorption and affect the utilization of vitamins and minerals from meals (Tinkílíç, 2001). The raw soybeans contain 1.93 mg/g tannin and it decreases on further processing (Adeyemo and Onilude, 2013).

2.7.4 Saponins

Soy and other legumes are rich in saponins, a class of bioactive compounds found in many plant species that protects plants against pathogens, pests, and foraging animals due to antimicrobial, antifungal, antiparasitic, insecticidal, and antifeedant properties. In general, saponins have many properties that can have positive or negative effects on humans and various animals (Moses *et al.*, 2014). Saponins are amphiphilic, heat-stable, glycosidic compounds that are comprised of one or more oligosaccharide moieties connected to a triterpenoid or steroidal aglycone. The aglycone is very hydrophobic, and the sugar chains are extremely hydrophilic; these properties provide these molecules with magnificent foaming and emulsifying properties (Liener, 1994). Saponins are plant-derived secondary compounds, which are found in more than 100 families of wild and cultivated plants that belong to the Magnoliophyta division. Magnoliophyta can be divided into two key classes:

Liliopsida and Magnoliopsida, which contain the majority of species that produce saponins (Vincken *et al.*, 2007).

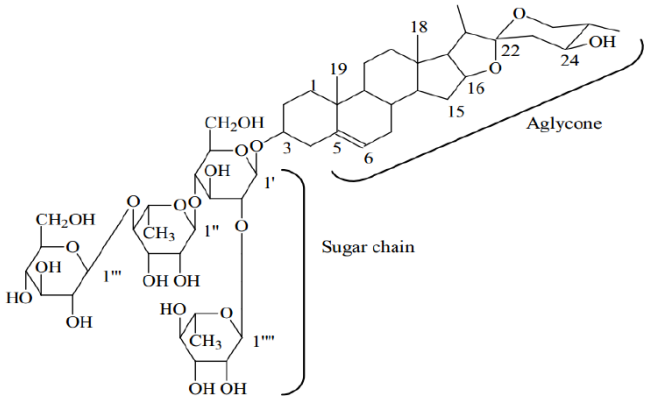

Fig.2.5 Structure of saponin

Source: Moghimipour and Handali (2014)

Saponins are also considered as factors that reduce the absorption of vitamins. It has been suggested that saponins can form complexes with various sterols that have similar structures as fat-soluble vitamins, which would interfere with sterol activity and absorption (Cheeke, 1971). Because dietary saponins rupture erythrocytes and liberate hemoglobin, they are extremely hazardous to animals with low body temperatures. It was discovered that saponins decreased nutrient conversion efficiency and utilization in ruminants (Cheeke, 1989; Sen *et al.*, 1998). The content of total saponin in the seed hypocotyl fraction of soybean ranged from 0.62–6.16%. The content of saponin in soybean seed was more greatly dependent on the variety (Shiraiwa *et al.*, 1991).

2.8 Bioactive components

2.8.1 Flavonoids

Flavonoids are phenolic chemicals found naturally in plants, vegetables, and flowers. Fifteen kinds of chemicals make up the flavonoid family: flavones, flavanols, flavanones, chalcones, and isoflavones. While flavonoids are present everywhere, isoflavones are only found in legumes, especially soybeans (Boue *et al.*, 2003). It is generally recognized that flavonoids are the most abundant and best-researched phenolic chemicals found in plant diets, making them some of the most potent plant antioxidants (Bravo, 1998). The total flavonoid contents found in soybean was 191.70 ± 8.73 mg.QE/g (Sharma and Giri, 2022).

2.8.2 Polyphenols

Pentose phosphate, phenylpropanoid, and shikimic acid routes are the main pathways used to synthesize phenolic chemicals, which are widely distributed bioactive secondary metabolites found in all higher plants (Balasundram *et al.*, 2006). The simplest of the class is phenol, which is also called carbolic acid, C6H5OH. In plants, the phenolic units are esterified or methylated and are submitted to conjugation, which means that the natural phenols are mostly found in the glycoside form instead of the aglycone form. This property of undergoing conjugation with other molecules enables it to scavenge free radicals and thus 8 inhibit the oxidative mechanisms that can lead to degenerative diseases such as cancer (Chukwuebuka and Chinenye, 2015). Chronic ingestion of phenol can lead to nausea, vomiting, headaches, abdominal pain, sore throat, mouth ulcers, and dark urine may occur, as well as respiratory and cardiovascular effects. In animals and humans, after ingestion, natural phenols become part of the xenobiotic metabolism (Harding *et al.*, 1988).

2.8.3 Antioxidants

Antioxidants are substances that can postpone, slow down, or stop the oxidation-induced development of rancidity or other taste degradation. Chemical processes called oxidation occur when electrons are transferred from one material to an oxidizing agent. Antioxidants have two ways to slow these reactions: they can react with intermediaries to directly stop the oxidation reaction, or they can react with the oxidizing agent to stop the oxidation reaction from happening (Pokorny, 2007).

It has been demonstrated that soybean flour is a basic source of many antioxidant chemicals, including phospholipids, tocopherols, amino acids, peptides, and isoflavone glycosides and their derivatives. Sulfhydryl compounds and aromatic amines may have some antioxidant effects. (Hayes *et al.*, 1977). Soybeans, defatted soy flour, soy protein concentrates, and soy isolates possess appreciable antioxidant activity in lipid-aqueous systems. The antioxidant properties of soybeans, defatted soy flour, and soy protein concentrates are due primarily to polyphenolic compounds (Pratt and Birac, 1979).

2.9 Different treatment conditions

The nutritional and anti-nutritional value of food is influenced by several variables. These comprise the plant's genetic composition, the soil in which it is produced, fertilizer use, the

weather during harvest, packing, storage conditions, and the processing technique used (Agiang *et al.*, 2010).

Cereals and legumes are rich sources of both macro- and micronutrients as well as anti-nutritional elements. Edible crops include major anti-nutritional components such as tannins, saponins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitors, and goitrogens. Due to a decrease in nutrient bioavailability, anti-nutritional substances mix with nutrients to become the main cause of worry (Samtiya *et al.*, 2020). Several reported soybean processing methods are intended to increase the nutritious qualities and eliminate anti-nutritional factors (ANFs). These consist of the following: alkaline treatment, extraction, roasting, cooking, and fermenting (Ari *et al.*, 2017).

2.9.1 Soaking

Soaking is a unit process in which materials are submerged in water to facilitate fermentation, seed softening, or sprouting. It has been noted that soaking, a popular local treatment method, affects the characteristics of legume seeds (Roy *et al.*, 2010). Soaking is capable of eliminating soluble antinutritional elements from a solution, but it can also cause metabolic events that influence certain chemical substances (Grieshop and Fahey, 2001). Many constituents break down into simpler compounds throughout the soaking process, changing the texture, flavor, fragrance, and taste of the final product (Liener, 1994). Through hydrolysis and passive diffusion, soaking lowers the phytate content of grains and legumes, making it a practical way to boost mineral availability. It can be used as a germination or fermentation pretreatment. Whole almonds have higher levels of phytic acid, according to research (Shivani Kumari, 2018).

According to the research conducted by Gadzama (2022), increasing the soaking duration led to a greater reduction in anti-nutritional factors (ANFs). Soaking achieved the highest reduction rates, with decreases of 90.89% for phytates, 46.54% for protease inhibitors, 57.14% for tannins, 19.23% for oxalates, and 60.16% for saponins. Additionally, the research conducted by Chauhan *et al.* (2022) shows that the protein content increased from 24.67% to 26.15%, reflecting an enhancement in nutritional value. The fat content remained almost unchanged, with a slight decrease from 16.36% to 16.34%. Antioxidant activity showed a significant improvement, rising from 27.69% to 32.09%. Additionally, the total

phenolic components, important for their antioxidant properties, increased from 56.56 mg GAE/100g to 58.59 mg GAE/100g.

2.9.2 Germination

Seed germination process can be divided into three phases. The first phase, occurring shortly after water absorption (imbibition) within the first day, involves the seed swelling as cell walls and internal compounds like proteins and starches absorb water. This phase also includes repairing DNA and breaking down large molecules. The second phase, from one to two days after imbibition, focuses on activating ATP production through processes such as glycolysis and the Krebs cycle. This stage also includes using stored mRNA to synthesize proteins. The final phase, starting after two to three days, is marked by the emergence of the radicle, the embryonic root of the seedling, which signifies the beginning of plant growth (Damaris *et al.*, 2019). Soybean sprouts are easily digested and rich in protein and minerals. They have been a staple in diets across China and other Asian nations for years, often serving as a vegetable substitute when fresh fruits and vegetables are scarce, particularly in rural parts of China where seasonal produce isn't always available year-round. However, the production of soybean sprouts faces challenges such as reduced yield, quality issues, and the occurrence of rot (Lee *et al.*, 1999).

Seed germination is a highly effective method for enhancing the nutritional profile of grain seeds, thereby enabling their use in the development of diverse food products. This process not only increases the bioavailability of essential minerals, vitamins, and dietary fibers but also enhances their overall nutritional value. These improvements are crucial for promoting both health and nutrition (Warle *et al.*, 2015). The research conducted by Kayembe (2011) shows that the germination process significantly enhances the nutritional profile of seeds, as evidenced by an increase in protein content from 39.10% in raw seeds to 43.40% in germinated seeds. Similarly, fat content also shows an increase from 15.80% to 19.18% after germination. Similarly, Ogunlakin *et al.* (2015) found that the tannin content decreased from 36.67 mg/100 g to 16.67 mg/100 g. Phytates drop from 65.00 mg/100 g to 31.67 mg/100 g. Trypsin inhibitors reduce from 12.60 mg/100 g to 4.13 mg/100 g, and oxalates decrease from 25.00 mg/100 g to 15.00 mg/100 g.

2.9.3 Cooking

Cooking is one of the oldest methods for preparing legumes, usually involving soaking the seeds first and then boiling them until they soften. Adding mineral salts to the soaking or cooking water can shorten the cooking time. This process denatures proteins, causing them to diffuse into the cooking water, inactivates heat-sensitive factors such as trypsin inhibitors, and reduces the levels of phytic acid and α-galactosides (Prodanov *et al.*, 2004). Heat treatment of pulses mainly enhances their protein quality by initially reducing toxic activities from proteinaceous toxins like trypsin inhibitors and haemagglutinins. However, with continued heating, protein quality decreases due to the Maillard browning reaction, which makes lysine unavailable. Although early cooking improves digestibility and reduces toxins, the relationship between increased digestibility and specific toxin detoxification remains unclear (Walker and Kochhar, 1982).

Soaking and cooking mung bean seeds led to a substantial decrease in tannins, with reductions of 45.5% in boiled seeds, respectively. In faba beans, cooking increased tannin accessibility and decreased tannin/catechin ratios. Soaking and cooking five types of legumes (white kidney bean, red kidney bean, lentil, chickpea, and white gram) significantly reduced phytic acid and tannin contents. The greatest reductions in phytic acid (78%) and tannins (66%) were achieved by soaking in sodium bicarbonate followed by cooking (Ragee *et al.*, 2013).

2.9.4 Roasting

Roasting is one of the most commonly used methods for soybean processing. This method denatures proteins and decreases the activity of anti-nutritional factors like trypsin inhibitors, lectins, and protease inhibitors. Additionally, heat processing improves the availability of essential amino acids, making the protein more digestible. Numerous studies demonstrate the positive impact of these heat processing techniques on soybean digestibility (Asghar *et al.*, 2024). Roasting soybeans improves their physical properties, such as color, odor, flavor, and texture, while also altering their nutritional and chemical profiles. This process enhances antioxidant potential, with roasted black soybeans exhibiting higher antioxidant activity than unroasted ones. The increased presence of bioactive components contributes to reducing the risk of cancer, cardiovascular diseases, obesity, and diabetes. Similar benefits have been

observed in dry-roasted and boiled peanuts, which show improved phytochemical composition (Özcan and Uslu, 2024).

The research conducted by Özcan and Uslu (2024) shows that the treatment of soybeans at 150°C for 10 minutes resulted in significant changes in their chemical composition and antioxidant properties compared to the untreated control. Specifically, the moisture content decreased marginally from 10.92% to 10.74%, while the oil content increased from 11.85% to 13.57%. Furthermore, there was a notable enhancement in antioxidant activity, rising from 2.92% in the control to 3.69% in the treated soybeans. However, the total phenolic content exhibited a reduction, decreasing from 12.24 mg/100g to 9.74 mg/100g. Similarly D'souza (2013) found that the roasting of field beans led to significant changes in their nutritional composition compared to the raw, untreated control. The moisture content drastically decreased from 5.23% to 1.93%, indicating a substantial reduction in water content due to the roasting process. Protein content exhibited a minor decrease from 22.41% to 21.86%, and lipid content increased slightly from 1.92% to 1.32%. The levels of phytic acid showed a substantial decrease from 501.33 mg to 298.47 mg, indicating a reduction in anti-nutritional factors. Also, D'souza (2013) found tannin content decreased from 0.51% to 0.74%.

Part III

3. Materials and methods

3.1 Raw materials, equipment and chemicals

Yellow soybean seeds were collected from the local market of Dharan. All the equipment and chemicals required for this research were used from laboratory of Central campus of Technology.

3.2 Methods

The general outline for the treatment conditions of soybean seeds is presented in the following flowchart.

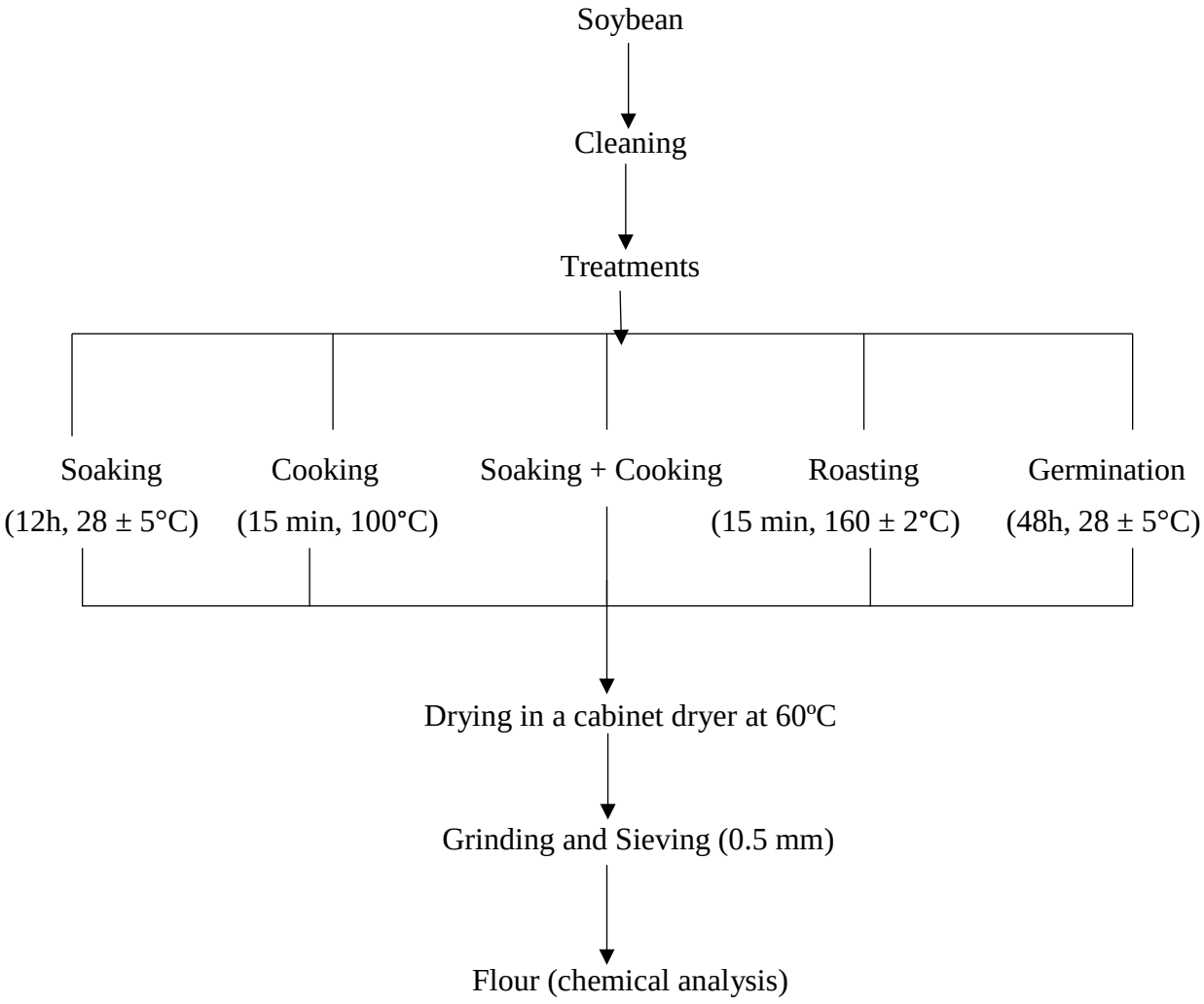

Fig. 3.1 General flowsheet for treatments of soybean

3.2.1 Experimental procedure

3.2.1.1 Soaking

Soybean seeds (600 g) were soaked in tap water at a 1:5 (w/v) ratio at room temperature (28 ± 5°C) for 12 hours, following the method of Kaur and Kapoor (1990) with slight modifications. After soaking, 200 g of the seeds were rinsed with water and dried in a cabinet dryer at 60°C till 10% moisture content was achieved. The dried seeds were then ground into a powder using a 0.5 mm sieve and stored in airtight polypropylene (PP) bags for further analysis.

3.2.1.2 Germination

The soaked soybean seeds (200 g) were germinated at room temperature (28 ± 5°C) for 48 hr following the method of Kaur and Kapoor (1990). After germination, the sprouted seeds were dried in a cabinet dryer at 60°C till 10% moisture content was achieved. The sprouted seeds were then ground into a powder using a 0.5 mm sieve and stored in airtight polypropylene (PP) bags for further analysis.

3.2.1.3 Cooking

Raw soybean seeds (200 g) were cooked following the method of Jood *et al.* (1987) with slight modifications. Briefly, 200g of the seeds were rinsed with water and placed in a beaker containing an amount of water equal to twice the weight of the seeds. The seeds were then cooked at 100°C on a hot plate for 15 minutes as they became soft to the touch. After cooking, the seeds were rinsed with water and dried in a cabinet dryer at 60°C till 10% moisture content was achieved. The dried seeds were subsequently ground into a powder using a 0.5 mm sieve and stored in airtight polypropylene (PP) bags for further analysis.

3.2.1.4 Soaked cooking

Soaked cooking was done according to Jood *et al.* (1987) with slight modifications. Briefly, 200 g of soaked seed were placed in a beaker containing an amount of water equal to twice the weight of the seeds. The seeds were then cooked at 100°C on a hot plate for 15 minutes as they became soft to the touch. After cooking, the seeds were rinsed with water and dried in a cabinet dryer at 60°C till 10% moisture content was achieved. The dried seeds were

subsequently ground into a powder using a 0.5 mm sieve and stored in airtight polypropylene (PP) bags for further analysis.

3.2.1.5 Roasting

Roasting of soybean seeds (200 g) was done on trays with sand at 160°C for 15 min following the methods of Kavitha *et al.* (2015). The roasted seeds were cooled and then ground into a powder using a 0.5 mm sieve and stored in airtight polypropylene (PP) bags for further analysis.

3.2.2 Analytical methods

3.2.2.1 Moisture content

The moisture content was determined by using the hot air oven method as described by Ranganna (1986).

3.2.2.2 Protein content

Crude protein was determined by micro Kjeldahl method, and total protein was calculated by multiplying the nitrogen content by a factor of 6.25 (Ranganna, 1986).

3.2.2.3 Fat content

The fat content of the samples was determined by using the soxhlet apparatus as described in Ranganna (1986).

3.2.2.4 Ash content

The ash content was determined as described in Ranganna (1986) by dry ashing method.

3.2.2.5 Crude fiber content

Crude fiber content was determined as described in AOAC (2005).

3.2.2.6 Carbohydrate content

The total carbohydrate content of the samples was determined by different methods. Carbohydrate (%) = 100 – [protein + total ash + fiber + moisture + fat]

3.2.2.7 Determination of oxalate content

The sample weighing 0.1 g was mixed with 30 ml of 1 M HCL. Each mixture was then shaken in a water bath at 100ºC for 30 min. To each mixture, 0.5 ml of 5% $CaCl_2$ and thoroughly mixed to precipitate out calcium oxalate. The suspension was centrifuged for 15 min and the supernatant was separated. The pellet was washed twice with 2 ml of 0.35 M NH_4OH and then dissolved on 0.5 M H_2SO_4. The solution was then titrated with a standard solution of 0.1 M $KMnO_4$ with temperature (60ºC) to faint violet color that persisted for at least 15 s which is equivalent to 2.2 mg of oxalate (Patel and Dutta, 2018).

3.2.2.8 Determination of phytate content

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 of distilled water was added to the sample. Then, 10 ml of 0.3% ammonium thiocyanate solution was added as an indicator and titrated with a standard iron (III) chloride solution which contained 0.00195 g iron per 1 ml (Emmanuel and Deborah, 2018).

$$\% \text{ Phytic acid} = \frac{\text{Titer value} \times 0.00195 \times 1.19 \times 100}{2} \quad \textbf{……} \quad \textbf{(3.3)}$$

3.2.2.9 Determination of tannin content

Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of the Folin-Ciocalteu reagent by tannin-like compounds in alkaline conditions. The mung 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 shaken well and filtered. 0 to 1 ml aliquots of the standard tannic acid solution were taken in a 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 were added and volume was made to 10 ml. After that, color was measured after 30 min at 760 nm against an experimental blank adjusted to 0 absorbency (Ranganna, 1986).

3.2.2.10 Determination of polyphenol

The fresh grind sample weighing 1 g was extracted in 25 ml methanol; extracts were subjected to shaking in a rotary shaker at room temperature for 24 h. The extract was filtered through Whatman paper no. 1 filter paper and the filtrate was stored at (4±2) ºC until use.

Then, 0.5 ml methanol solution of the concentrated solution was mixed with 2.5 ml of FC reagent, and 5 min later, 2.5 ml Na_2CO_3 (7.5%w/v) was added. The mixed sample was incubated in an incubator at 45ºC for 45 min. The absorbance was measured at 765 nm against the 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 34 calibration plots and expressed as mg of gallic acid equivalent (GAE) of phenol/100 g of dry sample (Singleton, 1999).

3.2.2.11 Determination of saponin

The spectrophotometric method was used for saponin analysis (Brunner, 1984). 1 g of the finely ground sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken for 2 h to ensure uniform mixing. Thereafter the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker, 20 ml of 40% saturated solution of magnesium carbonate was added and the mixture made up to 250 ml in a 250 ml standard flask. The mixture obtained with saturated $MgCO_3$ was again filtered through a Whatmann No. 1 filter paper to obtain a clear colorless solution. One millilitre of the colorless solution was pipette into a 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red color to develop. 0–10 ppm standard saponin was prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$. The absorbance of the sample, as well as the standard saponin solution, was read after color development on a spectrophotometer at a wavelength of 380 nm.

$$\text{Saponin} = \frac{\text{Absorbance of sample} \times \text{dil. factor} \times \text{gradient of standard graph}}{\text{sample weight} \times 10{,}000} \quad \textbf{…… (3.4)}$$

3.2.2.12 Determination of DPPH radical scavenging activity

Antioxidant activity was measured as radical scavenging activity against 1, 1-diphenyl-1picryl hydrazyl radical (DPPH) as described by Singh *et al.* (2008) with slight modification. Different dilutions of the extracts were made using 80% methanol. Methanolic extract was used for the determination of antioxidant activity. 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 ml of methanol**.**

Freshly prepared sample of 1 ml was taken in test tubes and 4 ml of DPPH of added. After incubation in dark for 30 min, the absorbance of the solution was read spectrophotometrically at 517 nm. The absorbance of DPPH solution without sample addition was read. The difference in absorbance of DPPH solution and DPPH solution + sample was calculated. The decrease in absorbance with sample addition was used for. Finally, percentage scavenging activity will be determined using following equation

$$\% \text{ S.A.} = \frac{\text{Ac-As}}{\text{Ac}} \times 100\% \quad \textbf{……..……. (3.5)}$$

Ac represents absorbance of blank, and As stands for absorbance of the test sample.

3.3 Statistical analysis

All chemical analyses employed triplicate samples to determine each component. Mean values and standard deviations were calculated. Data on processing procedures were analyzed using ANOVA with a level of confidence of 95% using IBM SPSS Statistics Version 27. Means of the data were compared by using Tukey's HSD method at 5% level of significance. Microsoft Excel (2019) was used to create the graphs and for the general calculations.

Part IV

Results and discussions

Soybean (*Glycine max*) was collected from the local market of various treatment conditions were used, including soaking, germination, roasting, cooking, and soaked cooking. Samples were tested to see how different treatment conditions affect bioactive components and anti-nutrients, both individually and in combination.

4.1 Proximate composition of soybean

The proximate composition of raw soybean is given in Table 4.1.

Table 4.1 Proximate composition of raw soybean

Parameters	**Values (%)**
Moisture	9.67±0.15
Crude protein (db)	40.31±0.46
Crude fat (db)	22.53±0.84
Crude fiber (db)	7.33±0.24
Crude ash (db)	6.16±0.23
Carbohydrate (db) (by difference method)	23.67±0.68

Data are presented as the mean of triplicate analysis (dry basis) ± standard deviation.

The proximate analysis of soybean for moisture content (%), crude protein (% db), crude fat (% db), crude fiber (% db), crude ash (% db) and carbohydrate (% db) was found to be 9.67%, 40.31%, 22.53%, 7.33%, 6.16% and 23.67% respectively.

This result is a bit different to that of the data reported by Khadka *et al.* (2024) i.e moisture 8.89%, protein 40.2% (db), fat 20.42% (db), crude fiber 4.55% (db), crude ash 4.43% (db) and carbohydrate 30.4% (db) and the data reported by (Sharma and Giri, 2022) i.e moisture 10.37%, protein 39.9% (db), fat 24.73% (db), crude ash 3.12% (db), crude fiber 6.08% (db) and carbohydrate 16.86% (db). Soybean moisture, protein, and fat content can vary based

on maturity stage, climatic conditions, variety, storage, and packaging material. Environmental conditions, variety, and experimental errors can also cause carbohydrate content, crude fiber, and ash content variations (Pokharel, 2022).

4.2 Antinutrients and bioactive components present in soybean

The mean values of different antinutrients determined are presented in Table 4.2.

Table 4.2 Distribution of antinutrients and bioactive components in raw soybean (mg/100 g).

Anti-nutrients and Bioactive components	Values in dry basis
Oxalate (mg/100g)	233.58
Phytate (mg/100g)	1008.54
Tannin (mg/100g)	158.34
Saponin (mg/100g)	1591.34
Total phenolic content (mg GAE/100g)	243.67
Antioxidant activity (% inhibition)	18.82

Data are presented as the mean of triplicate analysis (dry basis) ± standard deviation.

The analysis of anti-nutrient and bioactive component in the sample provides valuable insights and are comparable with existing literature. The oxalate content measured at 233.58 mg/100g. This result is similar to the result observed by Judprasong *et al.* (2006) i.e. 204 mg/100 g but less than the result by Shi *et al.* (2018) i.e. 370.49 mg/100g. The phytate content of 1008.54 mg/100g is higher than previously reported values by Ogunlakin *et al.* (2015), yet it aligns with findings from the research by Yang *et al.* (2014) i.e. 1180 mg/100g and by Sharma *et al.* (2013) i.e. 1480 mg/100gm emphasizing the influence of sample preparation and analysis methods. The tannin content was found to be 158.34 mg/100g, which is lower than the values reported by Sylvia *et al.* (2023) i.e. 191.25 mg/100g and S. Kumari *et al.* (2015) i.e. 312 mg/100 g, indicating variability in tannin levels across different samples. The saponin content, at 1591.34 mg/100g, is slightly higher than the values reported by Zhou *et al.* (2017) i.e. 1300mg/100g but lower than the findings of Sharma *et al.* (2013)

i.e. 1820 mg/100 g, indicating that saponin levels may differ depending on environmental factors and sample processing. The total phenolic content was measured at 243.67 mg GAE/100g which was similar to the result found by Sorour *et al.* (2018) i.e. 249.40 mg GAE/100 g. Also, Bursać *et al.* (2017) found 205 mg GAE/100 g phenolic content whereas Zhou *et al.* (2017) found 280 mg GAE/100 g phenolic contents in soybean. Lastly, the antioxidant activity was recorded at 18.82% inhibition, highlighting the sample's potential to mitigate oxidative stress. This result closely aligns with the antioxidant activity of Hardee variety of soybean reported by Chaturvedi *et al.* (2012) i.e. around 20%. Similarly, Yang *et al.* (2014) reported 16.7% DPPH scavenging activity in yellow soybean.

Overall, these variations in anti-nutrient and bioactive components underscore the influence of factors such as cultivar differences, growing conditions, and analytical methods, consistent with the conclusions of prior research.

4.4 Effect of different treatment conditions on oxalate content of soybean

The effects of soaking, roasting, cooking, soaked cooking and germination on the oxalate content in soybeans were studied. All the treatments significantly reduced ($p<0.05$) the oxalate content of soybean but to a varying extent. The combination treatment, i.e., soaked cooking, had a more pronounced effect than other treatments in reducing oxalate contents.

The oxalate content of soybean on different treatment conditions is given in Fig. 4.1.

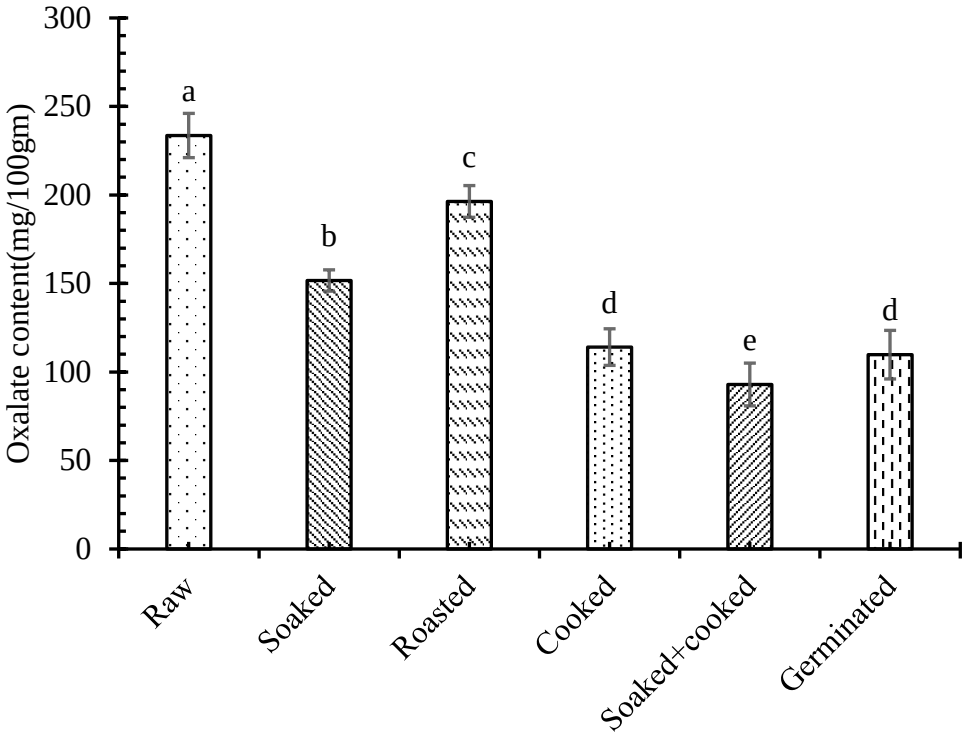

Fig 4.1 Effect of different treatment conditions on oxalate content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscript not significantly different ($p>0.05$) at 5% level of significance.].

4.4.1 Effect of soaking

Soaking showed a considerable decrease in the oxalate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) total oxalate content which reduced from 233.58 mg/100 g to 151.67mg/100 g i.e., 35.06% reduction.

The decrease in total oxalate contents might be due to the leaching of soluble oxalates during the soaking process Shi *et al.* (2018). Data regarding the effect of soaking on total oxalate contents in soybean was scarce. The reduction pattern of oxalate in this study is comparable to the study of Gadzama (2022) which shows 3.85% to 19.23% reduction in oxalate content of soybean after 12-72h of soaking. This result was quite lower than the study of Shi *et al.* (2018) who reported a significant reduction of oxalate content i.e. 51.89% after soaking only for 4 hrs in distilled water. Such difference in the result may be due to the

difference in oxalate content present in raw soybeans due to different varieties of soybeans, geographical differences, and differences in processing conditions (time, temp., etc.).

4.4.2 Effect of cooking

Cooking showed a considerable decrease in the oxalate content of soybeans, and it has been documented to be an effective treatment for removing anti-nutritional factors in legumes. This result shows that cooking significantly reduced ($p<0.05$) total oxalate content which reduced from 233.58 mg/100 g to 114.06 mg/100 g i.e., a 51.17% reduction.

The decrease in oxalate content following cooking might be attributed to the degradation or leaching of oxalates during the cooking process. Data on the effect of cooking on oxalate content in soybean seeds, however, is more comprehensive compared to the limited data available for other processing methods. The reduction pattern observed in this study aligns with the findings of Sylvia *et al.* (2023), which documented a 54.9% decrease in oxalate content after 20 minutes of boiling. This reduction is also consistent with the results of Akhtar *et al.* (2011), which reported a similar 55% reduction after 2 h of cooking. Small differences in the percentage reduction of oxalate content may be due to various factors, including differences in the initial oxalate levels in raw soybean, which can vary due to genetic variation among soybean cultivars, environmental conditions during growth, and specific processing conditions such as cooking time, temperature, and methods.

4.4.3 Effect of soaked cooking

Soaked cooking showed a considerable decrease in the oxalate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaked cooking significantly reduced ($p<0.05$) total oxalate content which reduced from 233.58 mg/100 g to 92.94 mg/100 g i.e., 60.21% reduction.

The observed reduction in oxalate content in beans following different cooking methods can be attributed to the degradation or leaching of oxalates during the soaking and cooking process. In this study, soaked cooking led to a 60.21% reduction which is similar to the result reported by Quinteros *et al.* (2003) from traditional cooking method i.e. 59.61% reduction in oxalate content of soybean following the same procedure of soaking overnight and cooking. These findings were higher than a previous study by Sylvia *et al.* (2023) which

reported 48% decrease in oxalate content when soaked, de-hulled, boiled for 20 min, and dried.

4.4.4 Effect of germination

Germination showed a considerable decrease in the oxalate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that germination significantly reduced ($p<0.05$) total oxalate content which reduced from 233.58 mg/100 g to 109.78 mg/100 g i.e., 53.01% reduction. Ogunlakin *et al.* (2015) found 46% reduction in the oxalate content of soybean when germinated for 2 days whereas 60% reduction was found when it was germinated for 4 days. Also, Dobhal and Raghuvanshi (2023) found 50% reduction on the oxalate content after 72 h of reduction. Small differences in the percentage reduction of oxalate content may be due to various factors, including differences in the initial oxalate levels in raw soybean, which can vary due to genetic variation among soybean cultivars, environmental conditions during growth, and specific processing conditions such as germination time, temperature, and methods.

4.4.5 Effect of roasting

Roasting showed a considerable decrease in the oxalate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that roasting significantly reduced ($p<0.05$) total oxalate content which reduced from 233.58 mg/100 g to 196.34 mg/100 g i.e., 15.94% reduction.

The process of roasting, which involves dry heat cooking at high temperatures, can break down oxalates and make them less available. However, the extent of oxalate reduction can vary depending on the food type and roasting conditions (e.g., temperature and duration). Olapade and Ajayi (2016) found 19.04% reduction of oxalate content when roasted for 10 minutes at 190°C. Also, Pokharel (2022) found significant reduction of 14.41% reduction of oxalate content while roasting green gram at 160°C for 15 min.

4.5 Effect of different treatment conditions on phytate content of soybean

The effects of soaking, roasting, cooking, soaked cooking and germination on the phytate content in soybeans were studied. All the treatments significantly reduced ($p<0.05$) the phytate content of soybean but to a varying extent. The combination treatment, i.e., soaked cooking, had a more pronounced effect than other treatments in reducing phytate contents.

The phytate content of soybean on different treatment conditions is given in Fig. 4.2

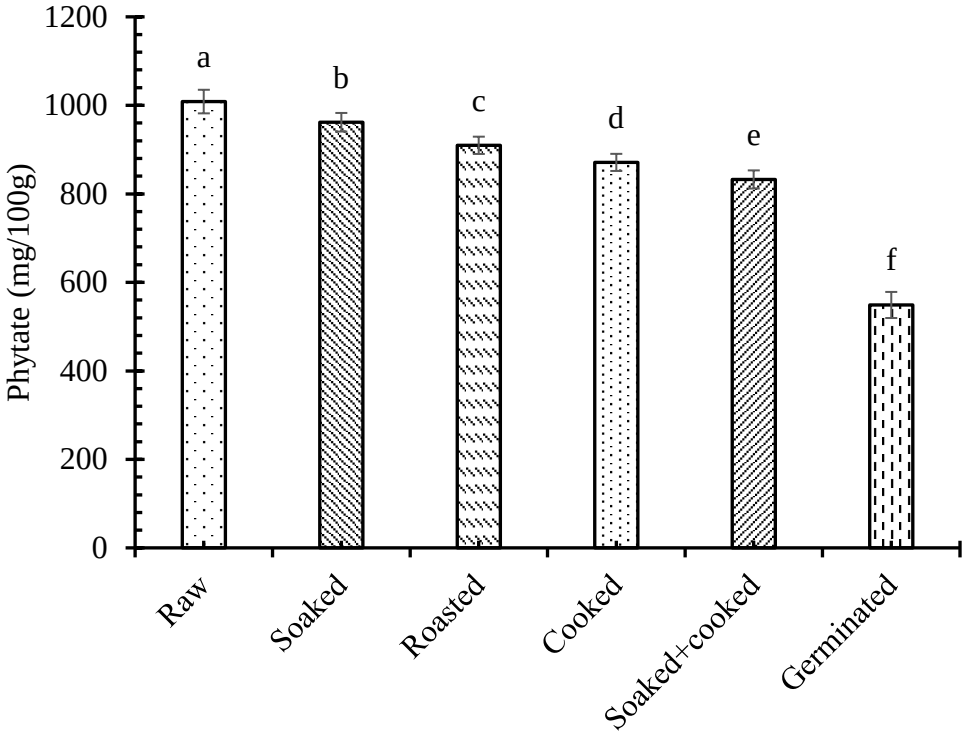

Fig 4.2 Effect of different treatment conditions on phytate content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscript not significantly different ($p>0.05$) at 5% level of significance.].

4.5.1 Effect of soaking

Soaking showed a considerate decrease in the phytate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) phytate content which reduced from 1008.54 mg/100 g to 961.83 mg/100 g i.e., 4.63% reduction.

The loss in phytates during soaking of the tested samples may be due to leaching of phytate ions into the soaking water. Shi *et al.* (2018) found 2.8% reduction on phytic acid content of soybean when soaked for 4h which is quite lower than this result which may be due lower soaking time. Alonso *et al.* (2000) found 5.6% reduction on phytic acid of kidney bean content when soaked for 12 h.

4.5.2 Effect of roasting

Roasting showed a considerable decrease in the phytate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) phytate content which reduced from 1008.54 mg/100 g to 909.68 mg/100 g i.e., 9.80% reduction.

Agume *et al.* (2017) found 11.53% reduction on soybean when roasted for 10 min at 110°C which is similar to the finding of this result. Similarly, reduction of 13.4% was found by Chauhan *et al.* (2022) when seeds were roasted on a hot plate at 180°C for 10 s. Slight difference in phytate content reduction maybe due to the difference in geographical variations and processing conditions.

4.5.3 Effect of cooking

Cooking showed a considerable decrease in the phytate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) phytate content which reduced from 1008.54 mg/100 g to 871.07 mg/100 g i.e., 13.63% reduction.

Phytic acid concentration of legume seeds decreases after cooking, either owing to leaching, heat breakdown, or formation of insoluble compounds with other components including protein and minerals. Only 3.42% reduction of phytate of soybean was noted by Shi *et al.* (2018) while boiling. In contrast Kavitha *et al.* (2015), found 21.1% reduction on phytate of mung bean when boiled. These differences may be due to difference in varieties, geographical regions and processing conditions.

4.5.4 Effect of soaked cooking

Soaked cooking showed a considerable decrease in the phytate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) phytate content which reduced from 1008.54 mg/100 g to 832.52 mg/100 g i.e., 17.45% reduction.

Soaked cooking showed more prominent result than soaking and cooking done separately. Kavitha *et al.* (2015) found 31.3% reduction of phytate content of mung bean when soaked and cooked. Alonso *et al.* (2000) found 26% reduction on phytate of red kidney

bean when soaked and cooked. These differences may be due to difference in varieties, geographical regions and processing conditions.

4.5.5 Effect of germination

Germination showed a considerable decrease in the oxalate content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) phytate which reduced from 1008.54 mg/100 g to 548.91 mg/100 g i.e., 45.57% reduction.

According to Luo *et al.* (2014) increased activity of the phytase enzyme during germination treatment may lower phytic acid levels. Egli *et al.* (2002) reported 50.09% phytate reduction on soybean when germinated. Similarly, Ogunlakin *et al.* (2015) found 51.27% reduction on phytate content of soybean when germinated for 2 days.

4.6 Effect of different treatment conditions on saponin content of soybean

The effects of soaking, roasting, cooking, soaked cooking and germination on the saponin content in soybeans were studied. All the treatments significantly reduced ($p<0.05$) the saponin content of soybean but to a varying extent. All these treatments showed the saponin content significantly ($p<0.05$) different from each other. The dry heat treatment, i.e., roasting had a more pronounced effect than other treatments in reducing saponin contents.

The saponin content of soybean on different treatment conditions is given in Fig. 4.3.

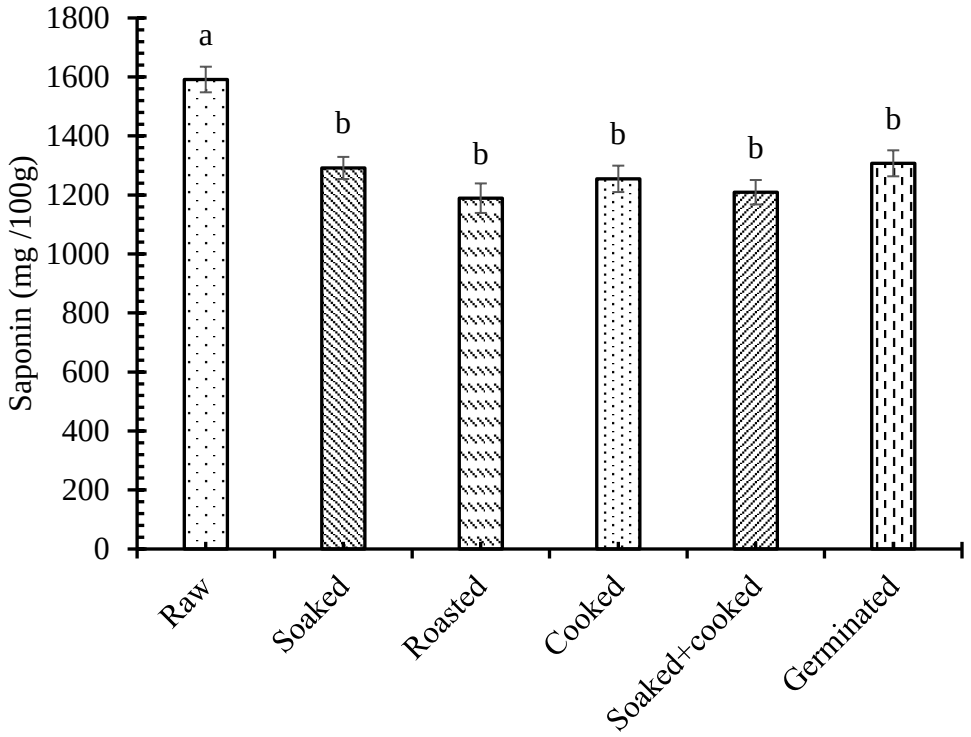

Fig 4.3 Effect of different treatment conditions on saponin content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscript are significantly different ($p<0.05$) at 5% level of significance].

4.6.1 Effect of soaking

Soaking showed a considerate decrease in the saponin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) saponin content which reduced from 1591.34 mg/100 g to 1291.54 mg/100 g i.e., 18.84% reduction.

Similar result was found by Adeleke *et al.* (2017) in Bambara groundnut commercial seeds while soaking i.e. 17% reduction in 12h. But Sharma *et al.* (2013) found 29% of reduction on saponin content of soybean which is a bit higher than this result. The difference could be attributed to differences in seed coat permeability of the samples which affects the rate of leaching. According to Katiyar *et al.* (1989) repeated washing with water counteracts the unfavorable effect of saponins, making the meal more appealing by lessening its bitterness.

4.6.2 Effect of roasting

Roasting showed a considerate decrease in the saponin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that roasting significantly reduced ($p<0.05$) saponin content which reduced from 1591.34 mg/100 g to 1189.08 mg/100 g i.e., 25.28% reduction.

Shi *et al.* (2004) noted that Saponins may deteriorate during roasting due to their temperature sensitivity. Zhou *et al.* (2017) found about 30% of reduction on saponins of yellow soybean which is comparable this result. Pokharel (2022) found 17.35% reduction when mung bean was roasted.

4.6.3 Effect of cooking

Cooking showed a considerate decrease in the saponin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that cooking significantly reduced ($p<0.05$) saponin content which reduced from 1591.34 mg/100 g to 1254.61 mg/100 g i.e., 21.16% reduction.

Ruiz *et al.* (1996) found similar reduction on lentils i.e. 20.36% reduction after 30 minutes of boiling. Similarly, 28% reduction of saponin was found by Adeleke *et al.* (2017) in Bambara groundnut commercial when boiled for 30 min.

4.6.4 Effect of soaked cooking

Soaked cooking showed a considerate decrease in the saponin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaked cooking significantly reduced ($p<0.05$) saponin content which reduced from 1591.34 mg/100 g to 1209.27 mg/100 g i.e., 24.01% reduction.

Kataria *et al.* (1989) found only 9-14% of reduction on saponin after soaked cooking. These differences in the percentage reduction of saponin content may be due to various factors, including differences in the initial saponin levels in raw soybean, which can vary due to genetic variation among soybean cultivars, environmental conditions during growth, and specific processing conditions such as processing time, temperature, and methods.

4.6.5 Effect of germination

Germination showed a considerate decrease in the saponin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that germination significantly reduced ($p<0.05$) saponin content which reduced from 1591.34 mg/100 g to 1307.29 mg/100 g i.e., 17.85% reduction.

Kataria *et al.* (1989) found 5-22% reduction on different varieties of amphidiploids when germinated. The possibility of enzymic degradation as an explanation for the loss of saponin during germination has been suggested by Kataria *et al.* (1988), although it is not proven.

4.7 Effect of different treatment conditions on tannin content of soybean

The effects of soaking, roasting, cooking, soaked cooking and germination on the tannin content in soybeans were studied. All the treatments significantly reduced ($p<0.05$) the tannin content of soybean but to a varying extent. All these treatments showed the tannin content significantly different ($p<0.05$) from each other. The combination treatment, i.e., soaked cooking, had a more pronounced effect than other treatments in reducing tannin contents.

The tannin content of soybean on different treatment conditions is given in Fig. 4.4

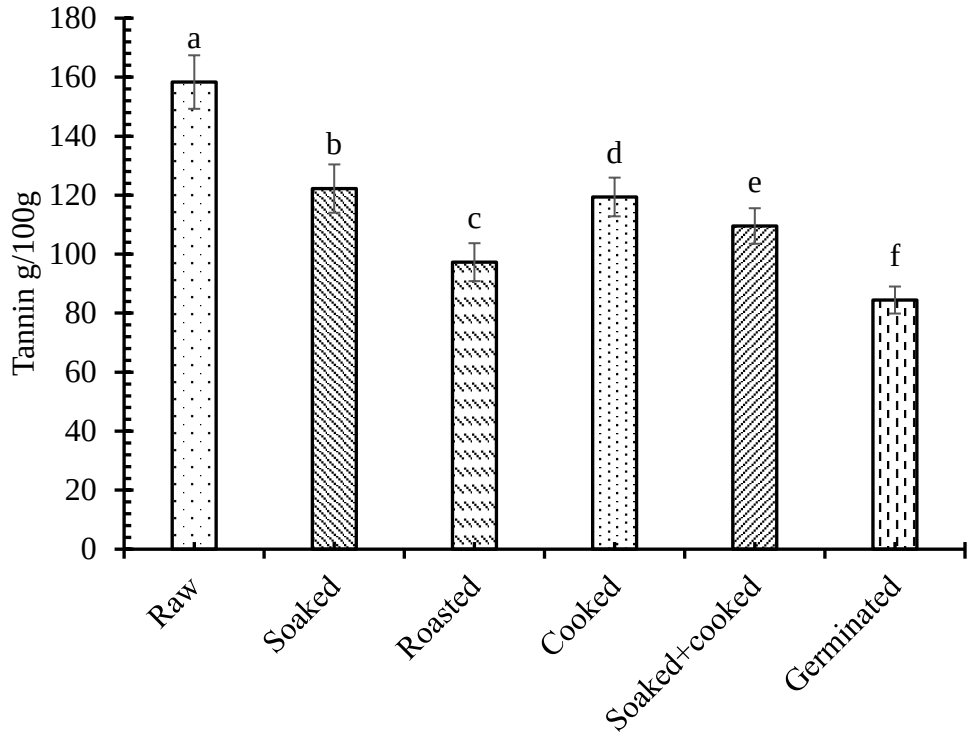

Fig 4.4 Effect of different treatment conditions on tannin content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscript are significantly different ($p<0.05$) at 5% level of significance].

4.7.1 Effect of soaking

Soaking showed a considerate decrease in the tannin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) tannin content which reduced from 158.34 mg/100 g to 122.23 mg/100 g i.e., 22.81% reduction.

Similar reduction i.e. 22.8% was found in chickpea when soaked for 12h by Sorour *et al.* (2018) but reduction on soybean was 15.7% when soaked for 24h. Egounlety and Aworh (2003) found 54.60% reduction on tannin of soybean when soaked for 12-14 hrs. Also, Sharma *et al.* (2013) found 14.7% reduction on tannin content of soybean when soaked on distilled water. Water soluble tannins may be reduced by leaching from seed coverings into the liquid media. These differences in result may be due to difference in varieties, geographical regions and processing conditions.

4.7.2 Effect of roasting

Roasting showed a considerate decrease in the tannin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that roasting significantly reduced ($p<0.05$) tannin content which reduced from 158.34 mg/100 g to 97.29 mg/100 g i.e., 38.56% reduction. Chauhan *et al.* (2022) found 33.59% reduction when roasted at 180°C for 10 seconds. The loss of components during high-temperature treatments may be the cause of tannin losses after dry heat treatments. Additionally, the breakdown of tannins or their interaction with other seed constituents, including proteins, to produce insoluble complexes, might be the cause of the tannin loss (Embaby, 2010).

4.7.3 Effect of cooking

Cooking showed a considerate decrease in the tannin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This

result shows that cooking significantly reduced ($p<0.05$) tannin content which reduced from 158.34 mg/100 g to 119.38 mg/100 g i.e., 24.61% reduction.

Near about results were reported by Yang *et al.* (2014) who found 22.50% reduction of tannin after boiling. Similarly, Kaur *et al.* (2020) reported the reduction of tannin content of rice bean in range of 18.75-27.67% but Chisowa (2022) found 46.6% reduction of tannin content when soybean seeds were boiled for 30 min which is higher than this result. Because legume seeds have a high tannin coating, a prior study found that prolonged boiling significantly reduced the tannin content of the seeds. Furthermore, because tannins are thermally unstable and water soluble, they prefer to dissolve in aqueous environments and deteriorate during boiling, which results in the decrease in tannin content (Rakić *et al.*, 2007; Reddy and Pierson, 1994).

4.7.4 Effect of soaked cooking

Soaked cooking showed a considerate decrease in the tannin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaked cooking significantly reduced ($p<0.05$) tannin content which reduced from 158.34 mg/100 g to 109.54 mg/100 g i.e., 30.82% reduction.

Exact data for reduction of tannin when soaked and cooked couldn't be found but similar result was found in mung bean by Kavitha *et al.* (2015) who reported 31.3% reduction of tannin content. The tannin concentration decreases as they boil because they degrade during boiling and prefer to dissolve in water when soaked.

4.7.5 Effect of germination

Germination showed a considerate decrease in the tannin content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that germination significantly reduced ($p<0.05$) tannin content which reduced from 158.34 mg/100 g to 84.45 mg/100 g i.e., 46.67% reduction.

Chauhan *et al.* (2022) found similar reduction pattern on the tannin concentration of soybean seeds i.e. 47.22%. Also, Sorour *et al.* (2018) found 47.90% reduction when soybean seeds were germinated for 72h. The increased activity of polyphenol oxidase and other catabolic enzymes may be the cause of the reduction in tannin concentration in legumes during germination. Enzymes that have been activated cause different components to be

hydrolyzed during germination. This post-germination decrease in tannin concentration might be related to enzymatic hydrolysis (Deshpande and Cheryan, 1983; Khandelwal *et al.*, 2010).

4.8 Effect of different treatment conditions on total phenolic content of soybean

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

The total phenolic content of soybean on different treatment conditions is given in Fig. 4.5.

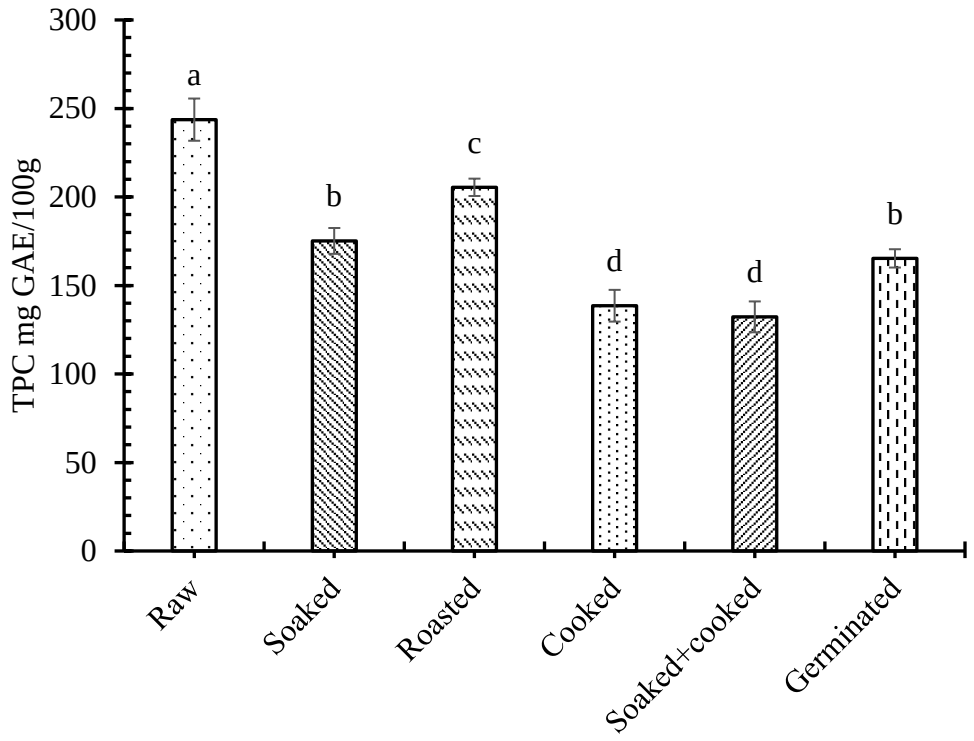

Fig 4.5 Effect of different treatment conditions on total phenolic content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscript are significantly different ($p<0.05$) at 5% level of significance].

4.8.1 Effect of soaking

Soaking showed a considerate decrease in the total phenolic content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) total phenolic content i.e. from 243.67 mg GAE/100 g to 175.15 mg GAE/100 g i.e., 28.12% reduction.

Soaking legumes can result in a partial reduction in phenol content due to the release of water-soluble phenolic chemicals into the water. This process is triggered by enzymes such as phenol oxidases, which catalyze the oxidation of phenolic substances, resulting in conversion or degradation (Patterson *et al.*, 2017; Siah *et al.*, 2019). Reduction of 30.1% total phenols on soybean was found by Sharma *et al.* (2013) which is similar to this result. Also, Patterson *et al.* (2017) found 20.7% reduction on total phenolic content of red kidney beans when soaking.

4.8.2 Effect of roasting

Roasting showed a considerate decrease in the total phenolic content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that roasting significantly reduced ($p<0.05$) total phenolic content i.e. from 243.67 mg GAE/100 g to 205.44 mg GAE/100 g i.e., 15.69% reduction.

Zhou *et al.* (2017) found similar result on yellow soybean when roasting i.e. 14.28 % reduction of polyphenol content. Similarly, 13.16% reduction on polyphenols was found on black gram by Le *et al.* (2023). The polyphenols' chemical reactivity may change as a result of roasting, which uses dry heat.

4.8.3 Effect of cooking

Cooking showed a considerate decrease in the total phenolic content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that cooking significantly reduced ($p<0.05$) total phenolic content i.e. from 243.67 mg GAE/100 g to 138.55 mg GAE/100 g i.e., 43.14% reduction.

Cooking can cause phenolic compounds to alter in different ways and to become sensitive to heat. Elevated temperatures have the potential to degrade or modify the structure of phenolic compounds, resulting in a decrease in their concentration. Since many phenolic compounds are water-soluble, they can seep into the cooking liquid while boiling or

simmering, two common water-based cooking techniques. This may cause some of the food's phenolic components to be lost Patterson *et al.* (2017). Reduction of 42% phenolic contents was observed by Kaur *et al.* (2020) when rice bean were boiled for 15 minutes. Also, Yang *et al.* (2014) found 31.44% reduction of phenolic content when boiling for 30 min.

4.8.4 Effect of soaked cooking

Soaked cooking showed a considerate decrease in the total phenolic content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaked cooking significantly reduced ($p<0.05$) total phenolic content i.e. from 243.67 mg GAE/100 g to 132.26 mg GAE/100 g i.e., 45.72% reduction.

Reduced extractability as a result of the seeds' altered chemical reactivity may account for a reduction in the quantity of polyphenols extracted from cooked seeds (Kataria *et al.*, 1989). Sharma *et al.* (2013) found 43.1% reduction of total phenols when cooked which was similar to this result. Similarly, Pokharel (2022) and Baral (2022) found 46.78% reduction on mung bean and 51.25% reduction on red kidney bean respectively.

4.8.5 Effect of germination

Germination showed a considerate decrease in the total phenolic content of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that germination significantly reduced ($p<0.05$) total phenolic content i.e. from 243.67 mg GAE/100 g to 165.3 mg GAE/100 g i.e., 32.16% reduction.

Enzymatic hydrolysis and the presence of polyphenol oxidase may have contributed to the decrease in the concentration of phenolic compounds during germination. Losses might also be partially explained by polyphenols leaching into the water before germination (Jood *et al.*, 1987; Paramjyothi and Mulimani, 1996). Sorour *et al.* (2018) found 25.28% reduction when soybean was germinated for 72h. Kaur *et al.* (2020) found 43.10% reduction on polyphenols when rice bean was germinated. These differences in result may be due to difference in varieties, geographical regions and germinating conditions such as time, temp., etc.

4.9 Effect of different treatment conditions on antioxidant activity of soybean

The effects of soaking, roasting, cooking, soaked cooking and germination on the antioxidant activity of soybeans were studied. Antioxidant activity was significantly ($p<0.05$) reduced when roasted, cooked, soaked+cooked and germinated. The combination treatment i.e. soaked cooking had more pronounced effect on reducing the antioxidant activity of soybean.

The antioxidant activity of soybean on different treatment conditions is given in Fig. 4.5.

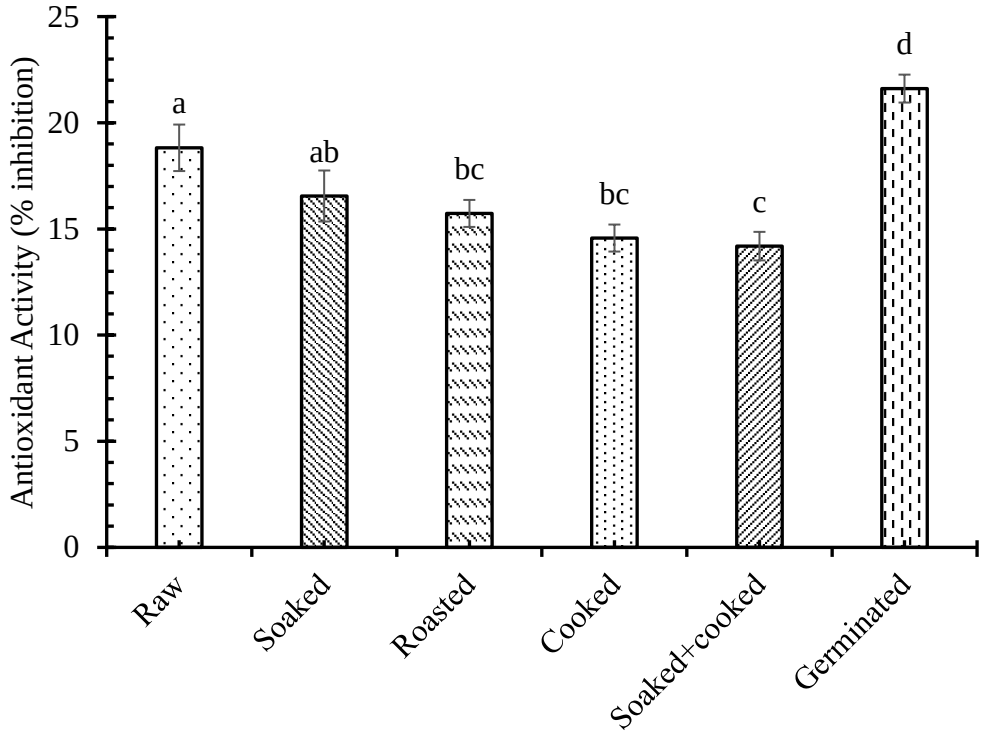

Fig 4.6 Effect of different treatment conditions on antioxidant activity

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscript are significantly different ($p<0.05$) at 5% level of significance].

4.9.1 Effect of soaking

Soaking showed a considerate decrease in the antioxidant activity of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) antioxidant activity i.e. from 18.82% inhibition to 16.55% inhibition i.e., 12.06% reduction. Data regarding the antioxidant activity of soyabean soaking was hard to find. Boateng *et al.* (2008) found

11.83% reduction on antioxidant activity of soybean when soaked which is similar to this result.

4.9.2 Effect of roasting

Roasting showed a considerate decrease in the antioxidant activity of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that roasting significantly reduced ($p<0.05$) antioxidant activity i.e. from 18.82% inhibition to 15.73% inhibition i.e., 16.42% reduction. Yang *et al.* (2014) found near about results i.e. 19.92% reduction on antioxidant activity when roasted. Also, Boateng *et al.* (2008) found 10.04% reduction on antioxidant activity of black eyed peas.

4.9.3 Effect of cooking

Cooking showed a considerate decrease in the antioxidant activity of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that cooking significantly reduced ($p<0.05$) antioxidant activity i.e. from 18.82% inhibition to 14.57% inhibition i.e., 22.58% reduction. Yang *et al.* (2014) found 26.55% reduction on antioxidant activity when cooked.

4.9.4 Effect of soaked cooking

Soaked cooking showed a considerate decrease in the antioxidant activity of soybean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaked cooking significantly reduced ($p<0.05$) antioxidant activity i.e. from % inhibition to 14.19% inhibition i.e., 24.60% reduction.

4.9.5 Effect of germination

Germination showed a considerate increase in the antioxidant activity of soybean which is in contradiction to the result of other processing methods. This result shows that germination significantly increased ($p<0.05$) antioxidant activity i.e. from 18.82% inhibition to 21.61% inhibition i.e., 14.82% increment. Kaur *et al.* (2020) found similar result i.e. 18.72% increment on the antioxidant activity of rice bean. Also, 10.15% increment on antioxidant activity was found by Chauhan *et al.* (2022).

Part V

Conclusions and Recommendations

5.1 Conclusions

The research conducted on the treatment of raw soybeans revealed that a range of techniques, such as soaking, roasting, germination, raw cooking and soaked cooking can significantly lower ($p<0.05$) the anti-nutrient levels in soybeans. Furthermore, the nutritional content of soybean was significantly ($p<0.05$) boosted by the processing procedures. Based on the result and discussion following conclusions can be drawn.

1. Soybean seeds were subjected to a variety of treatment conditions, including soaking, germination, roasting, cooking and soaked cooking, all of which significantly reduced ($p<0.05$) antinutritional components.
2. Germination increased the antioxidant activity by 14.82 % but significantly reduced ($p<0.05$) all the other bioactive components and antinutrients. Germination was found to be the most effective treatment to reduce phytate (45.57 %) and tannin (46.67 %).
3. Roasting was found to be the most effective method to reduce saponin content in soybean i.e. 25.28 %.
4. The combination treatment, soaked cooking was found to be the most effective treatment to reduce bioactive components and antinutrients such as oxalate (60.21 %), total phenolic content (45.72 %) and antioxidant activity (24.60 %).
5. Overall, the best processing method was found to be germination as it not only significantly reduced ($p<0.05$) anti-nutrients but also enhanced antioxidant activity with several other health benefits.

5.2 Recommendations

Based on the findings of the current study, the following recommendations for future research might be made.

1. Future research should look at the impact of processing on additional antinutritional components found in soybeans, such as trypsin inhibitors, lectins, flavonoids, etc.

2. The effects on bioactive and anti-nutritional aspects of various treatments (dehulling, autoclaving, fermentation) as well as combination treatments (soaking and roasting, dehulling and cooking, germination and cooking) may be investigated.
3. Research can be done by varying other factors like time, temperature, etc.
4. The effect on the changes on proximate compositions after these treatments can be studied.

Part VI

Summary

Soybean (*Glycine max*) is a legume that belongs to the Fabaceae family and is highly valued for its rich nutritional content, especially its high protein levels. Originating in East Asia, it has become a globally significant crop, serving as a fundamental source of protein for both humans and animals. The seeds of soybeans are packed with nutrients, including essential amino acids, unsaturated fatty acids, and various vitamins. Additionally, soybeans provide health benefits due to their bioactive components, such as flavonoids and isoflavones, which exhibit antioxidant and anti-inflammatory properties. With diverse applications, from food products like tofu and soy milk to animal feed and industrial uses, soybeans play a critical role in meeting nutritional needs globally.

In this study, several treatment conditions were employed to investigate the reduction of anti-nutritional factors in soybeans. The methods included soaking, germination, roasting, raw cooking and soaked cooking. In order to improve the nutritional value and digestibility of soybeans, important anti-nutritional substances like oxalates, tannins, phytates, and polyphenols were reduced. Spectrophotometry and titration were two of the techniques used in chemical studies to determine the concentrations of these substances both before and after processing. These treated soybean samples were also examined to see whether their antioxidant activity had changed in any way.

Soybean seeds were subjected to various processing methods i.e. soaking, germination, roasting, raw cooking and soaked cooking all of which significantly reduced ($p<0.05$) anti-nutrients. Germination significantly ($p<0.05$) increased antioxidant activity by 14.82%, though it significantly decreased ($p<0.05$) other anti-nutrients and bioactive components. Roasting effectively reduced saponin content by 25.28%, while soaked cooking was the most effective for reducing oxalate (60.21%) and total phenolic content (45.72%), despite lowering antioxidant activity (24.60%). Germination proved the most effective for reducing phytate (45.57%) and tannin (46.67%).

Overall, germination was concluded as the best processing method, as it not only significantly reduced anti-nutrients but also enhanced antioxidant activity, offering several health benefits.

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Appendices

Appendix A

Table A.1 ANOVA for oxalate

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		45851.315	5	9170.263	1098235.091	<0.001
	Linear Term	Contrast	32960.416	1	32960.416	3947355.248	<0.001
		Deviation	12890.899	4	3222.725	385955.052	<0.001
Within Groups			0.100	12	0.008		
Total			45851.415	17			

Table A.2 Tukey HSD test for oxalate

Soybean samples		N	Subset for alpha = 0.05				
			1	2	3	4	5
Tukey HSD[a]	Soaked+Cooked	3	92.94				
	Germinated	3		109.78			
	Cooked	3		114.06			
	Soaked	3			151.67		
	Roasted	3				196.34	
	Raw	3					233.48
	Sig.		1.000	0.073	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.3 ANOVA for phytate

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		397784.500	5	79556.900	53037.933	<0.001
	Linear Term	Contrast	318241.071	1	318241.071	212160.714	<0.001
		Deviation	79543.429	4	19885.857	13257.238	<0.001
Within Groups			18.000	12	1.500		
Total			397802.500	17			

Table A.4 Tukey HSD test for phytate

Soybean samples		N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	Germinated	3	548.91					
	Soaked+Cooked	3		832.52				
	Cooked	3			871.07			
	Roasted	3				909.68		
	Soaked	3					961.83	
	Raw	3						1008.54
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.5 ANOVA for tannin

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		9580.043	5	1916.009	2873.941	<0.001
	Linear Term	Contrast	6223.145	1	6223.145	9334.485	<0.001
		Deviation	3356.897	4	839.224	1258.805	<0.001
Within Groups			8.000	12	0.667		
Total			9588.043	17			

Table A.6 Tukey HSD test for tannin

Soybean samples		N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	Roasted	3		97.29				
	Germinated	3	84.85					
	Soaked+Cooked	3			110.34			
	Cooked	3				119.38		
	Soaked	3					122.24	
	Raw	3						158.34
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.7 ANOVA for saponin

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		321867.330	5	64373.466	33.686	<0.001
	Linear Term	Contrast	109924.215	1	109924.215	57.523	<0.001
		Deviation	211943.115	4	52985.779	27.727	<0.001
Within Groups			22931.596	12	1910.966		
Total			344798.926	17			

Table A.8 Tukey HSD test for saponin

Soybean samples		N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	Roasted	3	1189.0800	
	Soaked+Cooked	3	1209.2700	
	Cooked	3	1254.6100	
	Soaked	3	1291.5400	
	Germinated	3	1307.2900	
	Raw	3		1591.3400
	Sig.		0.054	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.9 ANOVA for polyphenols

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		26618.380	5	5323.676	79.269	<0.001
	Linear Term	Contrast	14786.536	1	14786.536	220.169	<0.001
		Deviation	11831.844	4	2957.961	44.043	<0.001
Within Groups			805.920	12	67.160		
Total			27424.300	17			

Table A.10 Tukey HSD test for polyphenols

Soybean samples		N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Soaked+Cooked	3	132.26			
	Cooked	3	138.55			
	Germinated	3		165.30		
	Soaked	3		175.15		
	Roasted	3			205.44	
	Raw	3				243.66
	Sig.		0.928	0.687	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table A.11 ANOVA for antioxidants

			Sum of Squares	df	Mean Square	F	Sig.
Between Groups	(Combined)		120.402	5	24.080	33.341	<0.001
	Linear Term	Contrast	1.397	1	1.397	1.935	0.190
		Deviation	119.005	4	29.751	41.192	<0.001
Within Groups			8.667	12	0.722		
Total			129.069	17			

Table A.12 Tukey HSD test for antioxidants

Soybean samples		N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Soaked+Cooked	3	14.1900			
	Cooked	3	14.5700	14.5700		
	Roasted	3	15.7300	15.7300		
	Soaked	3		16.5500	16.5500	
	Raw	3			18.8200	
	Germinated	3				21.6100
	Sig.		0.297	0.115	0.058	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix B

Standard curve for tannin

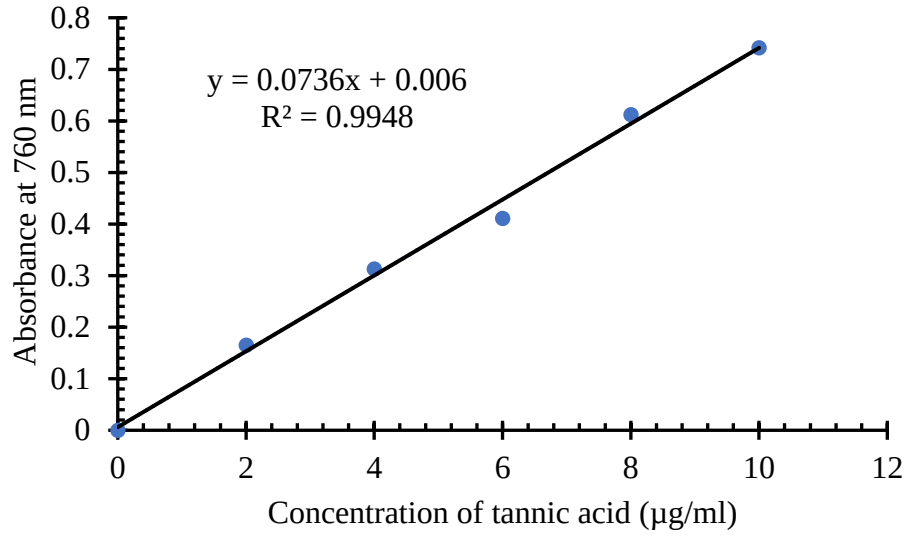

Fig. B.1 Standard curve for tannin determination

Standard curve for total phenolic content

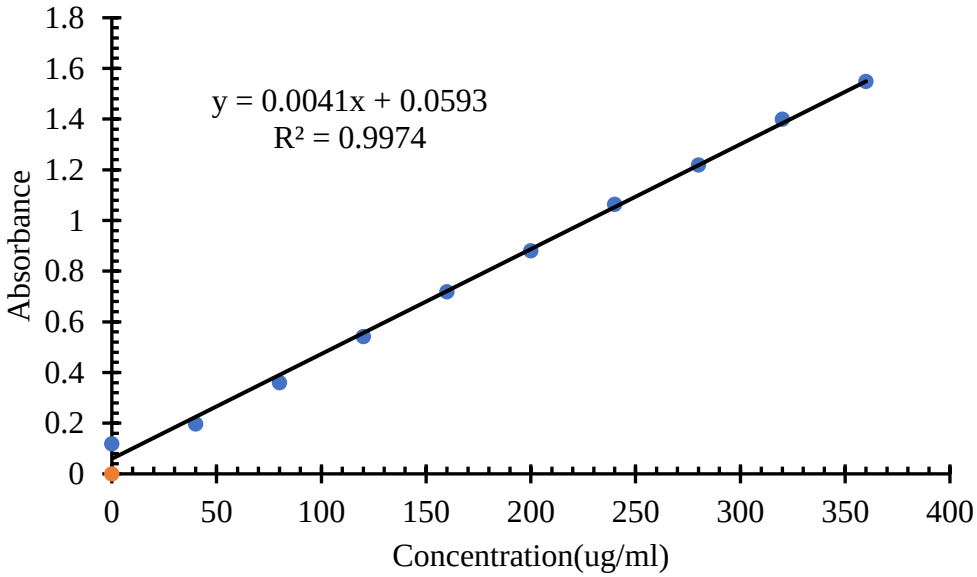

Fig. B.2 Standard curve for total polyphenol content determination

Standard curve for saponin content

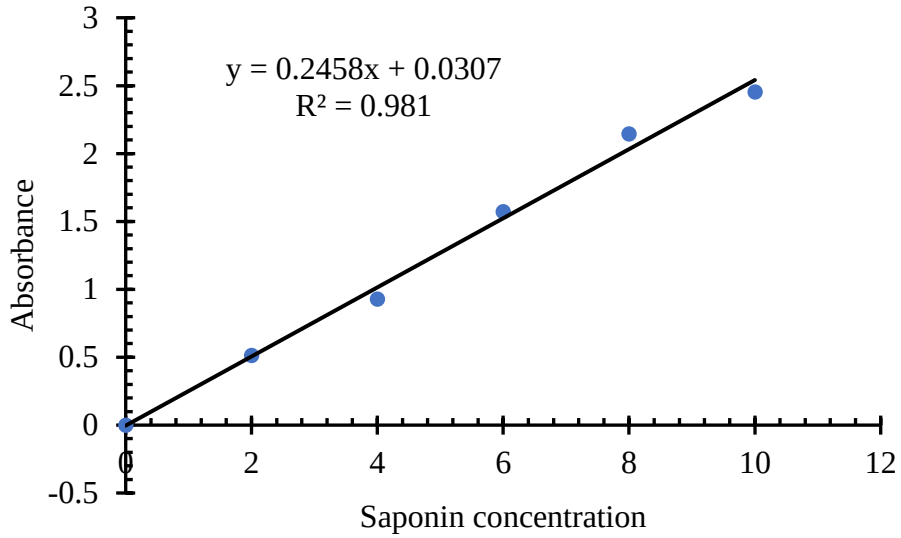

Fig. B.3 Standard curve for saponin content determination

Appendix C

Table C.1 List of chemicals used.

Chemical	Supplier/Manufacturer	Other Specifications
Sodium hydroxide (NaOH)	Thermo Fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCL)	Thermo Fisher Scientific India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt. Ltd.	97%, LR grade
Boric acid	Merck (India) Limited	Amorphous
Oxalic acid	Merck (India) Limited	Crystal
DiNitroSalicylic Acid	Himedia Laboratories Pvt. Ltd.	98%, LR grade
Sodium Carbonate (Na_2CO_3)	Qualigens fine chemicals	99.5%, LR grade
Petroleum ether	Merck life Pvt. Ltd.	B.P. 60°C-80°C
Phosphoric acid	Loba chemicals	56-60%
Phenolphthalein indicator	Merck life pvt. Ltd.	pH 8.2-9.8
Potassium	Merck life Pvt. Ltd.	99% Assay
Sodium sulphite	Thermo Fisher Scientific India Pvt. Ltd.	99%, fused flakes
Dil. Ammonia	Fisher Scientific	25% NH_3
Methanol	Thermo Fisher Scientific India Pvt. Ltd.	99% Assay
Potassium Permanganate	Avantor Performance Materials ltd.	99% Assay
Potassium thiocyanate	Thermo Fisher Scientific India Pvt. Ltd.	97% Assay
Metaphosphoric acid	S.D. fine chemicals Ltd.	66% HPO_3, 40% NaPO
L-ascorbic acid	S.D. fine chemicals Ltd.	99% Assay

Tannic acid	Avlcare Laboratories Pvt. Ltd.	Analytical Reagent
Ammonium oxalate	Qualigens fine chemicals	99% Assay
Nitric acid	Fisher Scientific India Pvt. Ltd.	68-75% Assay
DPPH	HiMedia Laboratories Pvt. Ltd.	

Appendix D

Table D.1 List of physical apparatus used.

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

Color Plates

Fig. C.1 Soxhlet apparatus

Fig. C.2 Protein content determination

Fig. C.3 Spectrophotometric determination of TPC.

Fig. C.4 Raw and soaked soybeans