EFFECT OF PROCESSING METHODS ON ANTINUTRITIONAL FACTORS PRESENT IN GREEN GRAM [MUNG BEAN]

by

Upendra Pokharel

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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Effect of Processing Methods on Antinutritional Factors Present in Green Gram [Mung bean]

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

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Upendra Pokharel

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Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Effect of Processing Methods on Anti-nutritional Factors of Green Gram* presented by Upendra Pokharel has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology.

Dissertation Committee

1. Head of the Department _____

(Mr. Basanta Kumar Rai, Prof.)

2. External Examiner _____

(Mr. Raj Kumar Rijal, Chief, FTQCO.)

3. Supervisor _____

(Mr. Pashupati Mishra, Prof.)

4. Internal Examiner _____

(Mrs. Babita Adhikari Dahal, Assoc. Prof.)

5. RMC Member _____

(Mr. Basanta Kumar Rai, Prof.)

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Date of Submission:

_____

Mr. Upendra Pokharel

Abstract

Mung bean (*Vigna radiata*) was collected from Saptari municipality, located in Saptari district in Province No. 2 on the month of March 2021. The main aim of present research work is to determine the effect of processing methods on anti-nutritional factors of mung bean of Pusa Baisakhi variety. The effect of different treatments as soaking (18 h), germination (48 h), dehulling (12 h), roasting (heated for 15 min at 160ºC), raw open cooked (20 min at 100ºC), soaked open cooked (12 h soaking and open cooked), raw autoclaved (15 min at 121ºC) and soaked autoclaved (12 h soaking and autoclaved) on the antinutrients as oxalate, phytate, saponin, polyphenol and tannin of raw mung bean were studied.

The mean value of tannin, oxalate, phytate, polyphenol and saponin in raw mung bean were found 477, 227, 627, 772 and 2618 mg/100g respectively on dry basis. The maximum reduction of antinutrients: tannin (63%) and polyphenols (53%) were found when the mung bean sample was prepared from soaking and dehulling process. The reduction percentage by soaking for 12 h and germination for 36 h was the most effective method to reduce phytate of mung bean (39%). Maximum reduction of saponin (22%) and oxalate (71%) were found when mung bean was soaked and autoclaving. The reduction percentage by roasting was less effective method compared to other method to reduce tannin, phytate, oxalate and polyphenols. Soaked autoclaving was the most effective method for the reduction of antinutrients of mung bean in case of cooking treatments. Hence, combined effect of treatments was more effective than single process. However, the processing methods as soaking, dehulling, germination, roasting, raw open cooking, raw autoclaving, soaked open cooking and soaked autoclaving reduced the antinutrients of mung bean significantly ($p<0.05$).

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Abbreviations	Full form
ANOVA	Analysis of Variance
RMC	Research Management Committee
CCT	Central Campus of Technology
D.F.	Degree of freedom
S.D.	Standard Deviation
USDA	United States Department of Agriculture
ANF	Antinutritional factor
FC	Folin-Ciocalteu

Part I

Introduction

1.1 Background

Legumes are wide variety of crops that are included in flowering plants producing seeds in pods that are much of the time refined for food and feeds. Legumes ranked as 3rd largest flowering plant family having more than 19500 species and over 750 genera (Abbas and Ahmad, 2018). They are significant source of dietary proteins and serves as major protein sources in the diets of the poor in the underdeveloped and developing countries where animal protein overall is only sometimes affordable. Furthermore, legumes have low environmental effect contrasted to other rich protein food varieties (Afam *et al.*, 2016).

Mung bean is a common pulse that is consumed worldwide, primarily in Asian countries, and has a long history of use as a traditional medicine (Hou *et al.*, 2019). Because cereals are high in sulfur-containing amino acids but low in lysine, combining the mung bean with grains has been suggested as a way to greatly improve protein quality. For good consumption, a 3:4 ratio of mung bean protein to rice protein was proposed, with the greatest chemical amino acid score. The protein digestibility of the rice-mung bean combination diet was found to be 84.4 percent of that of the rice-meat combination diet in babies, practically meeting human protein demands (Boye *et al.*, 2010).

Although legumes constitute abundant and least expensive sources of protein in human diet, their utilization is limited largely due to the presence of antinutritional compounds including trypsin inhibitors, alpha-amylase inhibitors, lectins, tannins, phytic acids, saponins, polyphenols, oxalates, chymotrypsin inhibitors, flatulence factor, hemagglutinin, cyanogenic compounds and allergens. These factors negatively affect the nutritive value of beans through direct and indirect reactions: they inhibit protein and carbohydrate digestibility; induce pathological changes in intestine and liver thus affecting metabolism; inhibit numbers of enzymes and bind nutrients making them unavailable (Deraz and Khalil, 2008).

Different studies shows that consumption of beans have high health beneficial and health implications regard to diabetes mellitus, obesity and cancer. The loss of nutrients occurs

during food preparation and processing; however, the processor should limit these losses in order to enhance nutritional quality of food. Different processing techniques are required to inactivate or remove of antinutritional factors, thus enhancing the nutritional quality of legumes. The physical methods of processing of legumes include soaking, boiling, cooking, autoclaving, dehulling and germination which significantly reduce the antinutrients present in mung bean (Abbas and Ahmad, 2018).

1.2 Statement of the problem

Mung bean is nutritious pulse eaten all over the world. It has been known to be an excellent source of protein, dietary fiber, minerals, vitamins, and significant amounts of bioactive compounds, including polyphenols, polysaccharides, and peptides, therefore, becoming a popular functional food in promoting good health (Hou *et al.*, 2019). The scientific validation of the traditional processing methods in terms of food safety and quality has not been attempted. To lower the antinutrients, different processing methods as soaking, dehulling, cooking, germination and roasting can be used but the comparative effectiveness of these methods are still the subject matter of research. The documenting of processing methods that are effective in lowering the antinutrients present in mung bean could help to greatly reduce the health risks connected with mung bean ingestion. As a result, attempts to improve the nutritional characteristics of mung bean by household treatments to reduce anti-nutrition are more than warranted.

1.3 Objectives

1.3.1 General objective

The general objective of the dissertation work was to study effect of processing methods on anti-nutritional factor of mung bean.

1.3.2 Specific objectives

The specific objectives of this dissertation work were to:

a. Determine the physical and chemical properties of mung bean.
b. Determine the antinutrients present in mung bean as tannin, phytate, saponin, oxalate and polyphenols.

c. Determine the reduction pattern of antinutrients of processed mung bean which is processed as soaking, dehulling, germination, open cooking, autoclaving and roasting.

1.4 Significance of the study

Pulses are the major sources of protein and also other nutrients in our diet. Among legumes, mung bean is generally consumed during illness at the present scenario of Nepal. Large amount of mung bean is imported from India as it has the highest production in the world (Singh *et al.*, 2013). Nowadays, production rate in Nepal is also increasing day by day mainly, in terai region of Nepal and hence consumption is also increasing due to its beneficial effect in the human body. Thus, this study specifically determines the content of antinutrients in mung bean and effect of various processing methods to reduce those antinutrients. The results of this study might help in the establishment of the effective and optimized way for the use of green gram in household level and industrial levels.

1.5 Limitations of the study

Following were the limitations of the present study:

a. Only one variety of mung bean was taken for study.
b. The antinutrients present in mung bean as trypsin inhibitor and hemagglutinin was not determined.
c. Loss of antinutrients during fermentation was not carried out.

Part II

Literature review

2.1 Nomenclature of mung bean

The mung bean alternatively known as mung bean, green gram, golden gram, moong bean, celera bean, munggo, frijol mungo, oregon pea, chickasano pea, mungboon or haricot mungo, is a plant species of legumes family. Mung bean used to be known as *Phaseolus aureus* before many *Phaseolus* species were moved to the *Vigna* genus. Now, mung bean is known as *Vigna radiata* (Heuze *et al.*, 2015).

According to USDA, the taxonomic hierarchy of mung bean is as:

Kingdom:	Plantae
Subkingdom:	Tracheobionta
Superdivision:	Spermatophyta
Division:	Magnoliophyta
Class:	Magnoliopsida
Sub-class:	Rosidae
Order:	Fabales
Family:	Fabaceae
Sub-family:	Papilionaceae
Genus:	*Vigna*
Species:	*radiata*

Source: Heuze *et al.* (2015)

2.2 Distribution of mung bean

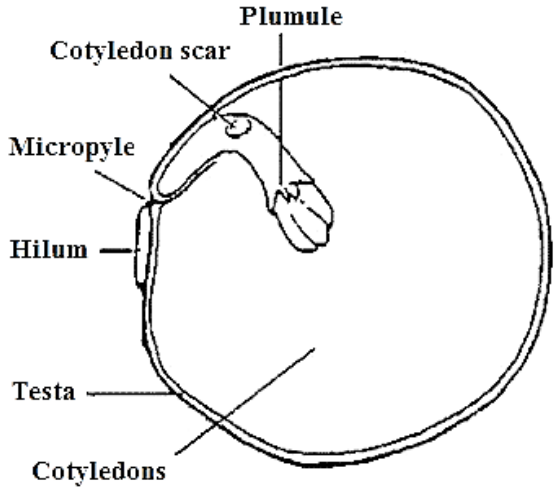
The mung bean is thought to have begun from the Indian subcontinent where it was tamed as right on time as 1500 BC. Developed mung beans were acquainted with southern and eastern Asia, Africa, Austronesia, the Americas and the West Indies. It is now widespread throughout the Tropics and is found from ocean level up to an elevation of 1850 m in the Himalayas (Lambrides and Godwin, 2006).

The mung bean is a quickly developing, warm-season legumes. It arrives at maturity rapidly under tropical and subtropical conditions where ideal temperatures are around 28-30°C and consistently above 15°C. It tends to be planted during summer and autumn. It doesn't need a lot of water (600-1000 mm precipitation/year) and is tolerant of drought. It is sensitive to waterlogging. High dampness at maturity will in general ruin the seeds that might grow prior to being harvested. The mung bean becomes on a wide scope of soils however favors very much depleted top soils or sandy soils, with a pH going from 5 to 8. It is to some degree tolerant to saline soils (Mogotsi, 2006).

2.3 Structure of green gram

The mung bean plant is a yearly, erect or semi-erect, arriving at a tallness of 0.15-1.25 m. It is slightly hairy with a well-developed root system. The stems are many-extended, now and again twining at the tips. The leaves are substitute, trifoliolate with circular to ovate leaflets, 5-18 cm long x 3-15 cm broad. The flowers (4-30) are papilionaceous, light yellow or greenish in color. The units are long, round and hollow, bristly and forthcoming. They contain 7 to 20 little, ellipsoid or cube shaped seeds. The seeds are variable in shading: they are generally green, however can likewise be yellow, olive, brown, purplish brown or dark, mottled and additionally furrowed. Seed colors and presence or nonattendance of a harsh layer are utilized to recognize various sorts of mung bean. Cultivated types are generally green or golden and can be shiny or dull depending on the presence of a texture layer. Golden gram, which has yellow seeds, low seed yield and pods that shatter at maturity, is often grown for forage or green manure. Green gram has radiant green seeds, is more productive and matures all the more consistently, with a lower propensity for cases to break (Heuze *et al.*, 2015).

The seed coat, cotyledons, and embryo are all important elements of mature seeds. The seed coat accounts for 7-15% of the total seed mass. The cotyledons make up about 85% of the seed mass, with the embryo accounting for the remaining 1-4% which is seen the Fig. 2.1. The testa, hilum, micropyle, and raphe are the seed's outer structures. The testa (smooth or harsh) is the external part of the seed and covers practically all of the seed surface. The hilum is an oval scar on the seed coat where the seed was connected to the stalk. The micropyle is a little opening in the seed coat close to the hilum. The raphe is an edge on the hilum inverse the micropyle. At the point when the seed coat is taken out from grain, the excess part is the embryonic structure. The early-stage structure comprises of two cotyledons and a short pivot above and beneath them. The two cotyledons are not actually joined to one another besides at the pivot and a frail assurance is given by the seed coat. In this manner, the seed is peculiarly vulnerable against breakage (Patel *et al.*, 2016).



Source: Patel *et al.* (2016)

Fig. 2.1 Structure of mung bean

2.4 Production of green gram

Mung bean production is mainly (90%) situated in Asia where India is the largest producer with more than 50% of world production but consumes almost its entire production. China, second largest producer, produces large amounts of mung beans, which represents 19% of its legume production. Thailand is the leading mung bean exporter which ships overseas about 60% of the domestic mung bean production (Singh *et al.*, 2013). Mung bean is an important pulse crop of Nepal. It is grown in irrigated/partially-irrigated area in the terai, inner terai and warm valleys mainly as a spring season crop in rice-wheat mung bean pattern

(Neupane *et al.*, 2003). The estimated area under mung bean is about 12000 ha with production of 6500 mt and productivity of 0.5 t/ha. Large quantity of mung bean is imported from India in Nepal as domestic production cannot meet the growing demand (Shrestha *et al.*, 2011).

2.5 Chemical composition of green gram

Table 2.1 gives the chemical constituents of whole green gram.

Table 2.1 Constituents of whole green gram

Constituents	Amount
Crude protein (% db)	24-28
Crude fiber (% db)	3-4
Crude fat (% db)	1.2-1.8
Ash (% db)	3.5-4.5
Moisture (%)	9.5-10.5
Total carbohydrate (% db)	58-62
Phosphorus (mg per 100 g)	320-330
Iron (mg per 100 g)	6-10
Calcium (mg per 100 g)	120-130
Niacin (mg per 100 g)	1-3

Source: Nwokolo and Smartt (1996)

2.6 Health benefits of mung bean

Seeds of green gram are medicinally used to treat fever, obesity and other diseases. It is useful in weakness, heat disorders and skin disorders in Ayurvedic system of medicine. The flour of green gram is used as herbal soap in India. Green gram sprouts, popular in Asian

cuisine are rich in vitamins and minerals. Recent research shows that green gram starch is a source of slowly digestible carbohydrate which is healthy for diabetic patients. It produces blood glycemic response in humans and modifies glucose and lipid metabolism favorably (Randhir *et al.*, 2004).

The green gram was recorded to be beneficial in the regulation of gastrointestinal upset and to moisturize the skin. High levels of proteins, amino acids, oligosaccharides, and polyphenols in green gram are thought to be the main contributors to the anti-melanogenesis, antioxidant, antimicrobial, anti-hypertensive, anti-inflammatory, immunomodulatory and antitumor activities of this food and are involved in the regulation of lipid metabolism (Tang *et al.*, 2014).

2.7 Physical properties of mung bean

2.7.1 Thousand kernel weight

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

2.7.2 l/b ratio

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

2.7.3 Bulk density

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

density is required for the design of storage, transport and separation systems. It has also been used to determine the dielectric properties of cereal grains (Kumar *et al.*, 2017).

2.8 Anti- nutritional factors

Food is the fundamental piece of individuals' lives. The world creates sufficient nourishment for everybody yet in addition more than 800 million individuals are as yet hitting to bed hungry. Besides, malnutrition and hunger related illnesses cause more than 60% of deaths (Lomborg, 2004). Foods are the mind-boggling substances that contain numerous synthetic mixtures which are needed to support the human body as water, proteins, fats, carbohydrate, minerals and vitamins. Antinutritional factors are principally connected with mixtures or substances of normal or engineered beginning, which meddle with the absorption of nutrients, and act to lessen nutrient intake, digestion, and usage and may create other antagonistic outcomes. Antinutrients are as often as possible identified with plant-based, crude or vegan diets and are normally integrated in plants (Gemede and Ratta, 2014). A portion of the normal manifestations displayed by an enormous number of antinutrients in the body can be sickness, swelling, cerebral pains, rashes, nutritional deficiencies, and so forth. Then again, such synthetic mixtures can be obviously worthwhile to mankind when consumed admirably. In fact, plants, for their own protection, essentially use antinutrients (Essack *et al.*, 2017).

Although people’s sensitivity to antinutrients widely differs, adequate food processing is initially recommended to reduce antinutritional factors. A person cannot eliminate antinutrients once they have been introduced to the body (Soetan and Oyewole, 2009). Most of the secondary metabolites, acting as antinutrients, elicit very harmful biological responses, while some of them are widely applied in nutrition and as pharmacologically-active agents (Soetan, 2008). Antinutrients are found in their highest concentrations in grains, beans, legumes and nuts, but can also be found in leaves, roots and fruits of certain varieties of plants. The major antinutrients found in plant-based foods are phytates, tannins, lectins, oxalates, polyphenols, saponin, etc. (Popova and Mihaylova, 2019).

2.9 Anti-nutritional factor present in mung bean

2.9.1 Tannin

The word tannin is very old and reflects a traditional innovation. Tanning was the word utilized in the 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 and Ratta, 2014). The structure of hydrolyzed tannin is shown in the Fig. 2.2.

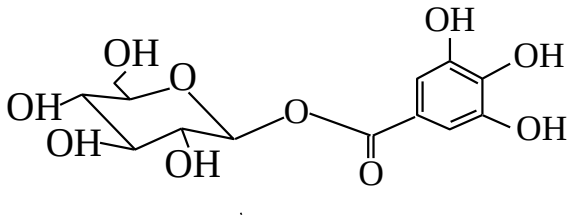


Fig. 2.2 Structure of hydrolyzed tannin

Source: Diouf *et al.* (2019)

Tannins are heat stable and they diminished protein digestibility in animals and humans, presumably by either making protein partially inaccessible or hindering digestive enzymes and increasing fecal nitrogen (Awad *et al.*, 2014). Tannins are known to be available in food products and to inhibit the activities of trypsin, chymotrypsin, amylase and lipase, decrease the protein quality of foods and meddle with dietary iron assimilation (Mello, 2000). Tannin content present in raw seeds of green gram showed a consecutive decline with dehulling, pressure cooking, soaking and germination (Kakati *et al.*, 2010). Tannins are known to be responsible for diminished feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. If tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and gastrointestinal assimilation may be depressed. Tannins also form insoluble complexes with proteins and the tannin-protein complexes may be responsible for the antinutritional impacts of tannin containing food varieties (Mueller-Harvey, 2001).

The chemistry of tannic acid is muddled because it is of natural origin and comprises of a combination of perplexing substances. Tannic acid is acquired as an amorphous fluffy or dense powder, yellowish-white to light-brown in color. It is soluble in water. The commercial tannic acid contains numerous ester linkages and is hydrolysable in the presence of acids, alkalis, or enzymes which on hydrolysis yields primarily glucose and gallic acid (Krezanoski, 1966). The tannin content present in the mung bean is 100-575 mg/100 g (Dahiya *et al.*, 2015)

2.9.2 Phytic acid

Fiber rich food sources, including both cereals and legumes, contain high level of phytate or phytic acid. Phytate, myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) is a major storage form of phosphorous in the full-grown seeds of both monocot and dicot plants and commonly represents roughly 75% of the total phosphorous and greater than 80% of soluble myo-inositol phosphate in seeds (Dorsch *et al.*, 2003). Animal and human feeds are comprised primarily of plant seed parts, seed phytic acid is generally inaccessible to monogastric animals, including humans because of the absence of phytases and it is excreted in to manure (Reddy *et al.*, 1989). Excretion of undigested phytic acid in fertilizer prompts to the eutrophication and water quality issues (Sharpley *et al.*, 1994). Phytase is a phosphatase that hydrolyses phytate to inositol and free orthophosphate (Wyss *et al.*, 1999). Fig.2.3 gives the structure of phytic acid.

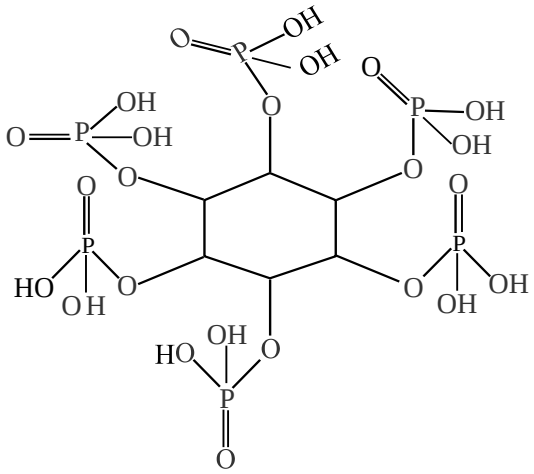


Fig. 2.3 Structure of phytic acid

Source: Aneta and Dasha (2019)

Phytic acid has a strong binding affinity to significant minerals, like calcium, iron, and zinc, albeit the binding of calcium with phytic acid is p^H-dependent (Dendougui and Schwedt, 2004). The binding of phytic acid with iron is more mind boggling, despite the fact that there absolutely is a strong binding affinity, molecules like phenols and tannins additionally impact the binding. When iron and zinc bind to phytic acid they form insoluble precipitates and are undeniably less absorbable in the digestion tracts. This process can consequently contribute to iron and zinc deficiencies in people whose diets depend on these food varieties for their mineral intake, such as those in developing nations and vegetarians (Promuthai *et al.*, 2006).

Phytic acid not only binds to or chelates vital minerals, but also inhibits enzymes that help us digest our food, such as pepsin, which helps for the breakdown of proteins in the stomach, and amylase, needed for the breakdown of starch into sugar. Trypsin, required for protein digestion in the small intestine, is additionally hindered by phytate (Bindu *et al.*, 2017). Although indigestible for many animals, phytic acid and its metabolites as they occur in seeds and grains have several important roles for the seedling plant. Most remarkably, phytic acid functions as a phosphorus store, as an energy store, as a source of cations and as a source of myoinositol (a cell wall precursor). Phytic acid is the principal storage form of phosphorus in plant seeds (Fardet, 2010). The phytic acid present in raw mung bean is 230-808 mg/100 g (Dahiya *et al.*, 2015).

2.9.3 Saponin

Saponins are normally occurring compounds that are generally distributed in all cells of legume plants. Saponins, which get their name from their capacity to form stable, soap like foams in aqueous solutions, comprise a complex and chemically diverse group of compounds (Arunasalam *et al.*, 2004). Saponins are amphiphilic, heat-stable, glycosidic compounds that are normally present in a wide variety of plant food. They comprise 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 secondary plant metabolites that contain a carbohydrate moiety (mono- or oligosaccharide) attached to an aglycone (Basu and Rastogi, 1967). Structurally, they are

composed of a lipid-soluble aglycone consisting of either a sterol or a triterpene group linked to one or more water-soluble sugar residues of different types and amounts of sugars, which occur in many plants. The structures of saponin from different plant foods are variable and depend on the types, amount of sugars, and composition of the steroid ring (Rao and Sung, 1995). The structure of soyasaponin III present in the mung bean is shown in the Fig. 2.4.

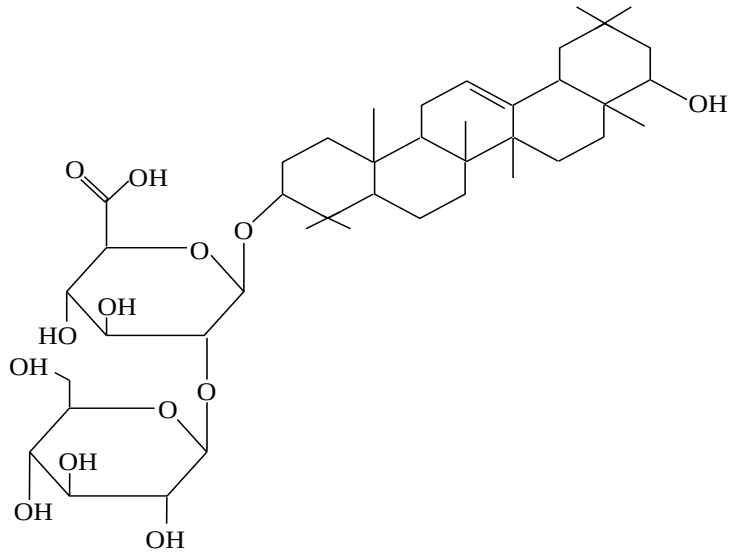


Fig. 2.4 Structure of soyasaponin III present in mung bean

Source: Shi *et al.* (2004)

Bitter taste nature of saponins are poisonous in high concentrations and can influence nutrient absorption by hindering enzymes (metabolic and digestive) and also by binding with nutrients such as iron, zinc and calcium (Sivakumaran *et al.*, 2017). Saponins are normally occurring substances with numerous biological effects. In the presence of cholesterol, saponins exhibit strong hypocholesterolemic effect. They can also prompt to hypoglycemia or impair the protein assimilation, uptake vitamins and minerals in the gut, as well as lead to the development of a leaky gut and furthermore have a hemolytic effect (Popova and Mihaylova, 2019). The content of saponin present in raw mung bean is 2848 mg/100 g (Kataria *et al.*, 1989a).

2.9.4 Oxalate

Oxalate is an anti-nutrient which under ordinary conditions is restricted to isolate compartments. However, when it is handled and additionally processed, it comes into contact with the nutrients in the gastrointestinal tract (Noonan and Savage, 1999). When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. In ruminants, oxalic acid is of only minor importance as an anti-nutritive factor since ruminal microflora can probably metabolize soluble oxalates, and to a less significantly insoluble calcium oxalate. While the importance of the anti-nutritive activity of oxalic acid has been recognized for more than fifty years it might be a subject of interest to nutritionists in the future (Oladimeji *et al.*, 2000). The structure of oxalic acid is shown in the Fig. 2.5.

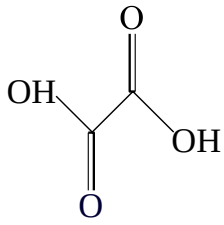


Fig. 2.5 Structure of oxalic acid

Source: Aneta and Dasha (2019)

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

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 diarrhea (Popova and Mihaylova, 2019). The oxalate content present in mung bean is 128 mg/100 g (Oburuoga and Anyika, 2012).

2.9.5 Polyphenols

Phenolic compounds are extensively dispensed bioactive secondary metabolites existing in all higher plants that are primarily synthesized by means of the shikimic acid, pentose phosphate and phenylpropanoid pathways (Balasundram *et al.*, 2006). Structurally, they have one or greater hydroxyl groups connected directly to the aromatic ring and can differ from simple molecules to highly complex polymers. Phenolic compounds are divided into subgroups of phenolic acids, flavonoids, tannins and stilbenes on the basis of quantity of phenolic hydroxyl groups connected and structural elements that hyperlink benzene rings (Singh *et al.*, 2016). It is assessed for that more than 8000 phenolic compounds have been isolated and described in flora (Ouchemoukh *et al.*, 2017). Phenolic compounds influence the sensory properties of foods and tannins primarily contribute to the astringency of food sources (Landete, 2012). The flavonoids consist of flavones, flavanols, flavanones, anthocyanidins and isoflavones. Tannins manifest in complexes with polysaccharides, proteins and alkaloids and are subdivided into hydrolysable and condensed tannins. A portion of these compounds are water soluble (phenolic acids and flavonoids), while some are insoluble (some condensed tannins). Flavonoids (60%) and phenolic acids (30%) predominantly represent phenolic compounds in our diet (Haminiuk *et al.*, 2012).

Food legumes chiefly contain phenolic acids, flavonoids and condensed tannins among different realized phenolic compounds (Amarowicz and Pegg, 2008). These compounds are distributed differently in the seed coat (mainly flavonoids) and the cotyledon (mainly contain non-flavonoids such as hydroxycinnamic and hydroxybenzoic acids) (Shahidi and Ambigaipalan, 2015). Gallic and protocatechuic acids are normal in kidney bean and mung bean. The antioxidant activity of phenolic compounds is in direct connection with their chemical structures such as number as well as position of the hydroxyl groups. Processing mostly leads to the reduction of phenolic compounds in legumes attributable to chemical

rearrangements (Singh *et al.,* 2017). Shaded (Pinto) beans have more phenolic compounds than unshaded (Cannellini) beans (Aguilera *et al.*, 2011). Polyphenols are reported to be present in higher amounts in colored and darker legume varieties than in pale varieties (Salunkhe *et al.*, 1983)

Polyphenols inhibit several digestive enzymes, lower protein as well as starch digestibility and prevent mineral adsorption from the diet. For human utilization, food legumes in India are processed in a variety of ways relying on taste and cultural preferences which are known to influence the level of the antinutrients (Subbulakshmi *et al.*, 1976). Polyphenols in mung bean were concentrated in the seed coat relatively high flavanol levels. Soaking seeds in water diminished assayable polyphenol content from 24 to 50%. Boiling for 30 min and roasting for 10 min resulted in 73% and 17% reduction of polyphenols, respectively. The lowering of polyphenols was significantly positively correlated with the lessening in protein-precipitable phenols. Mung bean sprouts had 36% less polyphenols after 48 h germination than after longer germination in which polyphenol content expanded (Barroga *et al.*, 1985). The polyphenol content present in mung bean is 285-808 mg/100 g (Dahiya *et al.*, 2015).

2.9.6 Lectin

Lectin comes from the Latin word “legere”, which signifies “to select”. It has the ability to bind carbohydrates. These days, proteins that can agglutinate red blood cells with referred sugar particularly are known to as “lectins” (Gemede and Ratta, 2014). The name “hemagglutinins” is utilized when the sugar particularity is unknown. Lectins and hemagglutinins are proteins/glycoproteins, which have no less than one non-catalytic domain that shows reversible binding to specific monosaccharides or oligosaccharides. They can bind to the carbohydrate moieties on the outer layer of erythrocytes and agglutinate the erythrocytes, without changing the properties of the carbs (Lam and Ng, 2011).

Lectins are glycoproteins broadly distributed in legumes and some specific oil seeds (including soybean) which possess an affinity for certain sugar molecules and are portrayed by their ability to combine with carbohydrate membrane receptors. Lectins have the ability to directly bind to the gastrointestinal mucosa, interacting with the enterocytes and meddling with the assimilation and transportation of 0.01% free gossypol within some low gossypol cotton nutrients (particularly carbohydrates) during digestion and causing epithelial lesions

inside the digestive tract. Despite the fact that lectins are normally announced as being labile, their stability differs between plant species, many lectins being impervious to inactivation by dry heat and requiring the presence of moisture for more complete annihilation (Hamid and Masood, 2009).

Lectins are carbohydrate binding proteins present in many plants, particularly seeds like cereals, beans, etc., in tubers like potatoes and also in animals. Lectins specifically bind carbohydrates and significantly, the carbohydrate moieties of the glycoproteins that embellish the surface of most animal cells. Dietary lectins act as protein antigens which bind to surface glycoproteins (or glycolipids) on erythrocytes or lymphocytes (Karimi *et al.*, 2012). They function as both allergens and hemagglutinins and are present in small amounts in 30% of foods, more so in a whole-grain diet. Lectins have potent in vivo effects. When consumed in excess by sensitive individuals, they can cause 3 primary physiological reactions: they can cause severe intestinal damage disrupting digestion and causing nutrient deficiencies; they can provoke IgG and IgM antibodies causing food allergies and other immune responses and they can bind to erythrocytes, simultaneously with immune factors, causing hemagglutination and anemia (Vasconcelos and Oliveira, 2004).

Lectins are a unique group of sugar binding proteins of non-immune origin, able to agglutinate cells and/or precipitate glycoconjugates. There are two major lectin presents in green gram as MBL-I and MBL-II. MBL-I was found to be a tetramer having alpha-galactosidase activity. MBL-II consisted of two monomeric lectins which were associated mainly with beta-galactosidase activity. Both MBL-I and MBL-II are D-galactose-specific lectins (Suseelan *et al.*, 1997). The hemagglutinin activity of green gram is 26.7 HU/mg (Kumar *et al.*, 2021).

2.9.7 Trypsin inhibitors

A trypsin inhibitor (TI) is a protein and a sort of serine protease inhibitor (serpin) that decreases the biological activity of trypsin by controlling the activation and synergist responses of proteins. Trypsin is an enzyme involved in the breakdown of wide range of proteins, primarily as part of digestion in humans and other animals such as mono-gastric and young ruminants. When trypsin inhibitor is consumed, it acts as an irreversible and competitive substrate (Silverman *et al.*, 2001). It competes with proteins to bind to trypsin and therefore renders it unavailable to bind with proteins for the digestion process. Thus,

trypsin inhibitor is considered an anti-nutritional factor or ANF. Additionally, trypsin inhibitor partially meddles with chymotrypsin function (Vagadia *et al.*, 2017).

Trypsinogen is idle type of trypsin, its inactive form ensures protein aspects of the body, such as the pancreas and muscles, are not broken down. It is formed in the pancreas and activated to trypsin with entero-peptidase. Chymo-trypsinogen is the inactive form of chymotrypsin and has similar functions as trypsin (Hirota *et al.*, 2006).

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

Legumes TIs are classified in 2 families according to their molecular size: Kunitz (KTIs), with molecular weights around 20 kDa and Bowman-Birk (BBTIs) of approximately 8 kDa. Soyabean has both families' trypsin inhibitor whereas mung bean, cowpea, lentil, etc. have only BBTIs family trypsin inhibitor. Two disulphide bond is present in KTI but seven disulphide bond is present in BBTI (Vanderven *et al.*, 2005). The content of trypsin inhibitor present in mung bean is 1.53 – 2.05 TIU/mg of mung bean (Aviles-Gaxiola *et al.*, 2018).

2.9.8 Flatulence factors

Legume contains some oligosaccharides such as raffinose, stachyose, verbascose and adjugose, which contain α-galactosidic bonds and are α-galactosyl derivatives of sucrose (Muzquiz *et al.*, 2012). Due to lack of α-galactosidase enzyme in human body, which is required for hydrolysis, these carbohydrates remain undigested in the human intestine and hence constitute the indigestible fibre group. However, in the colon, anaerobic fermentation of these undigested carbohydrates by the residing microflora leads to the production of gases (H_2, CO_2 and traces of CH_4), thus causing flatulence. These gases cause abdominal discomfort, and excessive consumption of these carbohydrates may lead to diarrhea. Due to these effects, these oligosaccharides are known as flatus-producing carbohydrates (Sefa-Dedeh and Stanley, 1979).

Mung beans contain soluble fiber and resistant starch, which can promote digestive health. The carbs in mung beans are less likely to cause flatulence than those of other legumes (Nair *et al.*, 2013). The key flatulence-producing raffinose family oligosaccharides in mung beans were degraded in the irradiated samples at the onset of the germination. It has been reported that that γ-irradiation at insect disinfestation dose levels improved the digestibility and nutritional quality of mung beans by reducing the content of oligosaccharides responsible for intestinal gas production (Machaiah *et al.*, 1999).

2.9.9 Allergens

Legume allergies are among the most common food-related allergies and includes peanuts, which are one of the most allergenic foods. In the case of legume allergies, the body sees certain proteins in the legume as a toxin, rather than a food. Allergic reactions can be caused by ingestion, skin contact, and even inhalation(Smits *et al.*, 2018). Legumes are implicated in many cases of food allergy. Recently, (Sasaki *et al.*, 2018) reported food allergy in 4.5% of Australian adolescents and a high frequency of peanut (2.7%) and soybean (0.1%) allergy.

Vicilin and convicilin from pea were identified as major allergens, and cross-reactivity with the major allergen from lentil (Len c 1) occurred in all 18 pea allergic patients in Spain (Sanchez-Monge *et al.*, 2004). Gly m 5 and gly m 6 are the major compounds in soyabean which is associated with severe allergic reactions. If the person is allergic to soy, he/she may be allergic to mung beans as well because of cross-reactivity (Holzhauser *et al.*, 2009).

2.10 Different methods for the reduction of antinutritional factors

Legumes and cereals contain high amounts of macronutrients and micronutrients but also anti-nutritional factors. Major anti-nutritional factors, which are found in edible crops include saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitor, and goitrogens. Anti-nutritional factors combine with nutrients and act as the major concern because of reduced nutrient bioavailability. There are various traditional methods and technologies, which can be used to reduce the levels of these anti-nutrient factors. Several processing methods such as fermentation, germination, dehulling, autoclaving, soaking etc. are used to reduce the anti-nutrient contents in foods. By using various methods alone or in combinations, it is possible to reduce the level of anti-nutrients in foods (Samtiya *et al.*, 2020).

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

2.10.1 Soaking

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

Soaking allows the water to spread in the protein fraction and starch granules allowing protein denaturation and starch gelatinization to occur, softening the texture of beans (Siddiq and Uebersax, 2012). Because phytate is water soluble, soaking beans in water overnight resulted in significant phytate elimination in the water, as well as an increase in naturally occurring phytase. The amount of phytic acid in mung beans was reduced by 18% when they were soaked for 12 h. Polyphenols were reduced by 23% after soaking, whereas the trypsin inhibitor was lowered by 7% in mung beans (Grewal and Jood, 2006). Soaking for 18 h reduces phytic acid up to 30% (Kataria *et al.*, 1989a). Soaking of mung bean for 24 h changes 28-35% reduction in the content of tannin (Kakati *et al.*, 2010). Soaking the legumes seeds in distilled water significantly decrease the total oxalate content in range from 17.4%-51.89% (Brudzynski and Salamon, 2011). The soaking process caused a significant reduction in soluble oxalates in peas (36.51 – 47.62%), lentils (26.66 – 48.79%), fava beans (45.34 – 45.82%), chickpeas (29.92– 35.53%), beans (36.56 – 39.65%) and soybean (56.29%) (Shi *et al.*, 2018).

2.10.2 Dehulling

Dehulling is the process of removing the seed coat from pulses, and it is one of the key post-harvest processes for improving the palatability of food grains. It does, however, result in a loss of nutrients and dietary fiber. Dehulling also eliminates the embryo and sticky layer that exists between the hull and the cotyledons (Kumar *et al.*, 2021). Legume grains may be classified as easy-to-dehull and hard-to-dehull. Legume grains such as pigeon pea and mung bean belong to the hard-to-dehull group because of the presence of mucilage and gum forming a strong bond between the hulls and the cotyledons (Ramakrishnaiah and Kurien, 1983).

Dehulling has been reported to reduce tannins, phytic acid and trypsin inhibitor activity but lectin activity was not changed. In addition, dehulling has been reported to improve the palatability and taste of some legume seeds, such as chickpea (Luo and Xie, 2013). Tannins are mainly located in the seed coats which is significantly reduced after dehulling. Dehulling decreases the level of condensed tannins (Deshpande *et al.*, 1982).

Dehulled mung bean tannin, phytic acid, and trypsin inhibitor levels have been reduced by 33%, 21%, and 15%, respectively (Mubarak, 2005). After dehulling, the content of myristic, palmitic, stearic, oleic, and linolenic acids reduced while the content of linolenic acid increased. Over raw horse gram seed, dehulling was most successful in lowering tannins (89.46–92.99%) and phytic acid (52.63–60.00%) concentration (Pal *et al.*, 2016). As phytates are mainly located in the cotyledons, the physical removal of testa by dehulling is reported to increase the phytic acid content of pulses, namely, lentil (Pal *et al.*, 2016), faba bean and kidney bean (Alonso *et al.*, 2000). However, a contrasting effect of dehulling on phytic acid content was also observed in green gram, cowpea and lentil (Ghavidel and Prakash, 2007). Dehulling of pulses has also been reported to decrease the polyphenols (Tajoddin *et al.*, 2010). A significant decrease in oxalate was also found in different varieties of horse gram (Alonso *et al.*, 2000).

2.10.3 Germination

Germination is the first stage of a plant's growth during which the primary root and stem come out. In this stage, the reserve nutrients required for plant growth are mobilized by hydrolyzing proteins and carbohydrates to obtain the required substrates for the seed

development. The seed enzymatic system is activated during its germination. It is considered one of the most effective processing methods for improving the nutritional quality of pulses, enhancing the digestibility of nutrients as protein and carbohydrates (Kumar *et al.*, 2021). For the breakdown of anti-nutritional chemicals in pulses, the germination process has been widely researched. The degree of deterioration, on the other hand, is dependent on the type of pulses, the type of ANFs, and the germination conditions. Proteases are thought to be responsible for the inactivation of proteinaceous ANFs such enzyme inhibitors and lectins. Phytic acid is digested by an endogenous enzyme called phytase during germination into inorganic phosphorus, which is the biologically accessible form for plant growth and development. As a result, the phytic acid in pulses transforms to a soluble form, and several researchers have documented the drop in phytic acid content of germinated pulses as a result of this occurrence (Camacho *et al.*, 1992).

The most effective method for reducing phytic acid in legumes is germination. Phytic acid was degraded during germination, leading in an increase in inorganic phosphorus availability (Virginia *et al.*, 2012). The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. A decrease in phytic acid content after germination for lentils was reported by Vidal-Valverde *et al.* (1994) for faba bean by Alonso *et al.* (2000) for black gram and mung bean by Kataria *et al.* (1989a). When compared to the whole ungerminated bean, the phytic phosphorus value of rice bean and mung bean reduced by 11.3% and 9.8%, respectively, after sprouting. Because phytic acid's chelating capacity is diminished, lowering its levels enhanced the availability of minerals in the digestive system of animals. (Akande and Fabiyi, 2010).

Germination modifies the quantitative and qualitative phenolic composition of pulses. This process has shown up to 20.8% reduction in total cyanide content in kidney bean (Yasmin *et al.*, 2008). It also reduces the content of enzyme inhibitors such as trypsin inhibitors, α-amylase inhibitors and chymotrypsin inhibitors in pulses (Alonso *et al.*, 2000). On sprouting of mung bean, tannin, phytic acid and trypsin inhibitor is reduced by 67%, 31% and 23% respectively (Mubarak, 2005).

2.10.4 Cooking

Pulses are generally consumed after hydrothermal processing/cooking/roasting. These are usually cooked in boiling water, and their cooking requirement is affected by the seed

composition, structure, size, etc. Low molecular weight compounds are leached out into cooking water when cooking is done in water. Cooking (boiling, autoclaving and microwave cooking) is very effective in reducing trypsin inhibitors, haemagglutinin activity, tannins and saponins (El-Adawy, 2002). Cooking process has been reported to decrease both water and acid-extractable phytate phosphorus in pulses, which may be due to formation of insoluble complex of phytate phosphorus with other components (Kumar *et al.*, 1978).

Generally, there are two types of cooking were practiced traditionally as well as industrially as open cooking and pressure cooking. Both methods result in the destruction of antinutrients as trypsin inhibitors, haemagglutinin activity, tannins and saponins. But pressure cooking preserves more nutrients as compared to open cooking (Deol and Bains, 2010). Similarly, Kataria *et al.* (1989b) have also reported that pressure-cooking was more effective than ordinary cooking in reducing the amount of antinutrients in black grams and mung beans.

2.10.4.1 Open cooking

Heat sensitive anti-nutritive factors like trypsin and chymotrypsin inhibitors, as well as volatile chemicals, are often inactivated by cooking. The varied samples were cooked with a controlled amount of water in this study, and no water was drained after cooking. Reduction of tannins content after cooking in various pulses such as lentil, cowpea, mung bean and kidney bean may be due to the binding of tannins with proteins (Kaur *et al.*, 2020) and other organic substances during cooking (Kumar *et al.*, 2021). Besides tannins, cooking also causes destruction of polyphenols (Yasmin *et al.*, 2008).

The sample of chick pea has the reduction of tannin (48%), phytic acid (30%) and hemagglutinin (100%) and trypsin inhibitor (82%) was reported when chick pea is open cooked for 90 min at 100ºC (Alajaji and El-Adawy, 2006). Presoaked cooking of seeds is more advantageous than unsoaked cooking in the reduction of antinutrients. The reduction in phytic acid and tannin of presoaked cooked mung bean is 20% and 15% whereas of unsoaked cooked mung bean, 30% and 25% is reduced respectively (Singh *et al.*, 2015). The reductions in total oxalates as a result of cooking presoaked seeds were, 30.83-41.45%, 34.45-54.16%, 31.85-45.81%, 33.48-39.72%, 37.81-44.96% and 66.15% for peas, lentils, faba beans, chick peas, common beans and soy bean respectively. Loss of soluble oxalate in

water was considered to be the primary factor contributing to total oxalate reduction (Akhtar *et al.*, 2011).

2.10.4.2 Autoclaving

Cooking under pressure is what autoclaving implies. This procedure reduces the amount of time it takes to cook. The thermo-labile, inhibitory compounds such as cyanogenic glycosides, saponins, terpenoids, and alkaloids could not be found after autoclaving jack beans for 30 min at 120ºC and 15 lb pressure (Akande and Fabiyi, 2010). When legumes seed is autoclaved, tannin is brought about to reduce 33-46% and 28-52% reduction in the phytic acid (Zia-ur-rehman *et al.*, 2003). Autoclaving of mung beans resulted in complete elimination of trypsin inhibitor and hemagglutinin from all samples (Mubarak, 2005).

Temperature, heating time, particle size, and moisture content all influence the degree of heat inactivation. Despite the fact that trypsin inhibitors are heat sensitive and expected to be inactivated by cooking due to denaturation (Vidal-Valverde *et al.*, 1994). The highest reduction of trypsin inhibitor activity is recorded after autoclaving (83.67%), followed by boiling (82.27%), microwave cooking (80.50%) and germination (33.95%) (Vijayakumari *et al.*, 1998). In terms of reducing antinutrients, presoaking seeds before autoclaving is preferable than unsoaked seeds. Presoaked autoclaved mung bean samples have decreased phytic acid and tannin by 34% and 44%, respectively, but unsoaked autoclaved mung bean samples have reduced phytic acid and tannin by 32% and 40%, respectively (Singh *et al.*, 2015). It has been reported that autoclaving of faba beans shows the reduction of trypsin inhibitor (84%), phytic acid (23%), tannin (30%) and lectin (75-100%) (Luo and Xie, 2013).

2.10.5 Roasting

Roasting is a cooking technique that uses dry heat to roast food evenly on all sides at temperatures of at least 150°C from an open flame, oven, or other heat source. Protein digestibility can be improved by roasting. Bacteria and viruses, for example, can be killed or rendered inactive by heat. The amount of aflatoxins produced by fungi is reduced when they are roasted (Samarajeewa *et al.*, 1990). The goal of roasting is to improve sensory qualities and achieve inactivation of destructive enzymes which improves the storage and nutritional quality of the product (Rackis *et al.*, 1986).

A significant decrease of phytates and condensed tannin contents was recorded for roasted varieties of lentils i.e., reduction up to 63.01% and 41.41% respectively for phytates and condensed tannin contents at 140°C for 30 min (Attou *et al.*, 2020). Similarly, reduction in phytic acid and tannin of chickpea was reported up to 56% and 57% respectively (Yadav and Bhatnagar, 2017). Roasting reduces phytic acid, tannin, trypsin inhibitor and polyphenols of mung bean up to 30%, 17%, 92% and 17% respectively (Singh *et al.*, 2015), (Mendoza *et al.*, 1988). Roasting of lima bean seeds helps in the reduction of phytic acid (40%), tannin (30%) and trypsin inhibitor (98%) (El-Gohery, 2021). Roasting of black bean seeds reduce the polyphenols and saponin by 8% and 20% (Ngoc *et al.*, 2021).

2.10.6 Fermentation

Fermentation is a metabolic process that allows sugars to be metabolized for energy while also improving mineral absorption from plant-based diets. Because cereals are difficult to ingest in their natural/raw forms, fermentation is one of the processing processes used to make cereal grains digestible while also improving the nutritional content and safety elements of these foods. (Galati *et al.*, 2014). Fermentation of cereals by lactic acid bacteria has been reported to increase free amino acids and their derivatives by proteolysis and by metabolic synthesis. Fermentation has been shown to improve the nutritional value of grains by increasing the content of essential amino acids such as lysine, methionine and tryptophan (Galati *et al.*, 2014).

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

The fermentation by microorganisms significantly decreased the level of cyanide, tannins, phytate, oxalate and saponins by 86, 73, 72, 61, and 92%, respectively in the cassava products (Etsuyankpa *et al.*, 2015). The antinutrients of mung bean was also reduced by

fermentation technique as the reduction of phytic acid (62%), tannin (36%) and saponin (72%) was reported (Onwurafor *et al.*, 2014).

2.11 General uses of mung bean

The domestic production of mung bean doesn't meet the demand of Nepal and hence a large volume is imported from India as it is the world's largest producer of the mung bean (Singh *et al.*, 2013). Mung bean is used in the following ways in Nepal:

a. It is mainly consumed as a thick soup (dal) prepared out of whole or split beans.
b. Seeds are used to produce bean sprouts and ingredient for salad, soup or as a vegetable.
c. It is used as medicine for diabetics, heart disease, and blood pressure.
d. Mung bean flour is used in making papad, unleavened bread, titaura (nuggets), etc.
e. The split mung bean is prepared as bhujia (fried and salted) which is a snack item in urban areas.
f. The crop is also utilized as fodder and green manure.

Part III

Materials and methods

3.1 Materials

All chemicals used were reagent grade unless specified otherwise and distilled water was used throughout the work.

3.1.1 Collection of green gram

Mung bean of Pusa Baisakhi variety was collected from Saptari municipality at Saptari district, Province No. 2, Nepal on the month of March.

3.1.2 Equipment

All equipment's required for the research were used from laboratory of Central Campus of Technology. The list of equipment's used for this work is shown in Table 3.1.

Table 3.1 List of equipment used

Physical Apparatus	
Heating arrangement	Thermometer
Weighing arrangement	Spectrophotometer
Distillation set	Water bath
Titration apparatus	Desiccator
Soxhlet apparatus	Centrifuge
Hot air oven	Mortar and pestle
Glassware and utensil	Incubator

3.1.3 Chemicals

All chemicals required for this research were used from laboratory of Central campus of Technology. The list of chemicals used for this work is shown in Table 3.2.

Table 3.2 List of chemicals used

Chemicals	
Hydrochloric acid	Tannic acid solution
Sulphuric acid	Folin-ciocalteu reagent
Oxalic acid	Standard saponin solution
Sodium hydroxide solution	Magnesium carbonate solution
Sodium carbonate solution	Methanol
Ammonium thiocyanate solution	Butanol
Iron chloride solution	Potassium permanganate solution
Ammonium hydroxide solution	Calcium chloride solution

3.2 Methodology

The general outline for processing of mung bean is presented in Fig. 3.1.

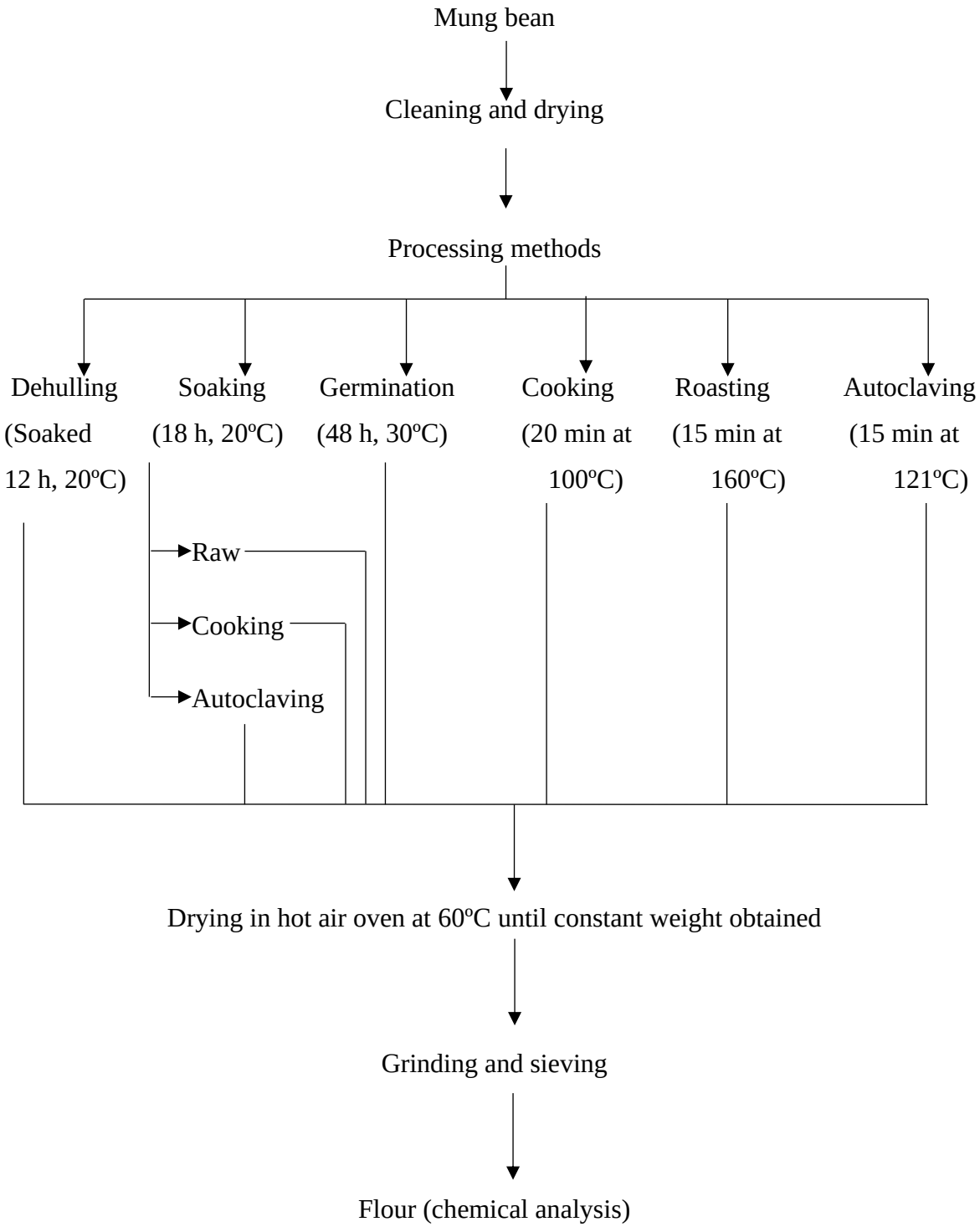


Fig. 3.1 General flowsheet for processing of mung bean

3.3 Processing methods to reduce antinutrients

3.3.1 Soaking

Seeds weighing 100 g were soaked in tap water at ratio 1:10 (w/v) at room temperature for 18 h. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in an oven at 60ºC to a constant weight. Dried samples was ground, stored in an airtight plastic container for further analysis (Singh *et al.*, 2015).

3.3.2 Open cooking

The soaked seeds weighing 100 g (12 h in tap water) was cooked in beakers with a seed to water ratio of 1:5 and 1:6 (w/v) for soaked and unsoaked seeds, respectively. The water was allowed to boil before the addition of seeds. The seeds were cooked until soft as felt between fingers (about 15 min for soaked and 20 min for unsoaked seeds). The cooked samples was then mashed and dried in a hot air oven maintained at 60ºC and then it was finely ground and stored (Singh *et al.*, 2015).

3.3.3 Autoclaving

The seeds weighing 100 g was soaked for 12 h and unsoaked seeds weighing 100g was autoclaved for 15 min at 121ºC under 15 lb/in. The ratio of seed to water was 1:5 (w/v) for unsoaked seeds and 1:4 (w/v) for soaked seeds. The autoclaved seeds was then mashed, dried at 60ºC, finely ground and stored (Singh *et al.*, 2015).

3.3.4 Germination

The seeds weighing 100 g were soaked overnight in fresh water for 12 h. After then, the seeds were rinsed and the water drained off. The seeds were then allowed to sprout in an incubator at 30°C until the sprouts become 1 cm length i.e., for about 36 h. The sprouted samples were dried in a hot air oven at 60°C, finely ground and stored in an air tight plastic container for the further analysis (Singh *et al.*, 2015).

3.3.5 Roasting

Roasting of mung bean seeds (250 g) were done on trays with sand at 160°C for 15 min. The roasted seeds was dried at 60ºC, finely ground and stored in an air tight container for further analysis (Singh *et al.*, 2015).

3.3.6 Dehulling

Hulls of mung beans (50 g) was removed manually after soaking the mung bean seeds for 12 h in distilled water (1:10, w/v). The dehulled seeds was dried at 60ºC in hot air oven and finely grounded and stored (Singh *et al.*, 2015).

3.4 Analytical methods

3.4.1 Proximate analysis of mung bean

3.4.1.1 Moisture content

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

3.4.1.2 Protein content

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

3.4.1.3 Fat content

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

3.4.1.4 Ash content

The ash content was determined by incinerating the mung beans (5 g) in a muffle furnace at 525ºC for 4-6 h (Ranganna, 1986).

3.4.1.5 Crude fiber content

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of Ranganna (1986).

3.4.1.6 Carbohydrate content

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

3.4.2 Physical analysis of mung bean

3.4.2.1 Thousand kernel weight

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

3.4.2.2 Bulk density

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

3.4.2.3 Length by breadth ratio

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

3.4.3 Determination of oxalate

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 was added 0.5 ml of 5% $CaCl_2$ and thoroughly mixed to precipitate out calcium oxalate. The suspension was centrifuged at 3000 rpm for 15 min and the supernatant was separated. The pellet was washed twice with 2 ml of 0.35 M NH_4OH then dissolved on 0.5 M H_2SO_4. The solution was then titrated with 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 for 2.2 mg of oxalate (Patel and Dutta, 2018).

3.4.4 Determination of phytate

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

$$\%\text{Phytic acid} = \frac{\text{Titer value} \times 0.00195 \times 1.19 \times 100}{2}$$

Source: Emmanuel and Deborah (2018)

3.4.5 Determination of tannin

Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of folin-ciocalteu reagent by tannin-like compounds in alkaline condition.

The 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 shaked well and filtered. 0 to 1 ml aliquots of the standard tannic acid solution were taken in test tube and 7.5 ml water was added to each. Then, 0.5 ml folin-ciocalteu reagent and 1 ml Na_2CO_3 solution was added and volume was made to 10 ml. After then, color was measured after 30 min at 760 nm against experimental blank adjusted to 0 absorbency (Ranganna, 1986).

3.4.6 Determination of polyphenol

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

calibration plot and expressed as mg of gallic acid equivalent (GAE) of phenol/100 g of dry sample (Singleton *et al.*, 1999).

3.4.7 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 Whatmann 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 milliliter 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 standard saponin solution, was read after color development on a spectrophotometer at a wavelength of 380 nm.

$$\text{Saponin} = \frac{\text{Absorbance of sample} \times \text{dil. factor} \times \text{gradient of standard graph}}{\text{sample weight} \times 10{,}000}$$

Source: Brunner (1984)

3.5 Statistical Analysis

For all chemical analysis, triplicates of the sample were used for determination of each constituent. Mean values with standard deviation were computed. Data on processing different methods were subjected to analysis of variance (ANOVA) and considered at 95% confidence level using statistical software GenStat.

Part IV

Results and discussion

Green gram (*Vigna radiata*) of Pusa Baisakhi variety was collected from saptari district and different processing methods were carried out i.e., soaking, dehulling, germination, roasting and cooking (open and pressure cooking). Then, thus obtained processed samples were analyzed to study the effect of different processing methods on its antinutrients by single and combination of processing methods.

4.1 Physical properties of mung bean

The physical properties of mung bean were determined. The results obtained are presented in Table. 4.1.

Table 4.1 Physical properties of mung bean

Physical properties	Mung bean seeds
l/b ratio	1.34 ± 0.02
Bulk density (kg/hl)	75.34 ± 0.25
1000 kernel weight (g)	17.5 ± 0.4

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

Imran *et al.* (2016) found that the thousand kernel weight of mung bean seeds of variety NM-98 was 46.96 g in which our data was much lesser than their findings because of different variety. However, the thousand kernel weight of mung beans were in range 7.2-60.1 g (Dahiya *et al.*, 2015) in which our obtained data was in range. The value of l/b ratio of raw mung bean seed was found to be 1.34 which means the mung bean seeds are bold in nature (Rather *et al.*, 2016). Similarly, Dahiya *et al.* (2015) also found that the l/b ratio and bulk density of mung bean seeds was 1.31-1.38 and 67.9-82.1 kg/hl in which our result was in range with their findings. Similar results were reported by Halil *et al.* (2008). The value of bulk density of mung bean varies according to variety, moisture content, quality and foreign matter present in the mung bean (Kumar *et al.*, 2017)

4.2 Proximate composition of mung bean

The proximate composition of raw green gram is given in Table 4.2.

Table 4.2 Proximate composition of raw mung bean

Parameters	Values (%)
Moisture	11.33±0.36
Protein (dry basis)	26.78±1.10
Fat (dry basis)	1.52±0.31
Crude fiber (dry basis)	4.78±0.23
Ash (dry basis)	3.71±0.46
Carbohydrate (dry basis) (by difference method)	51.89±1.92

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

The protein content in the mung bean was found to be 26.78% and similar data was found by Mubarak (2005) and Skylas *et al.* (2018) but Singh *et al.* (2015) found 31.34% protein whereas Nwokolo and Smartt (1996) found that the protein content in mung bean is 23.6%. The crude fiber content of raw mung bean is 4.78% which is comparable to the data obtained by Mubarak (2005) i.e. 4.63%. The crude fiber content in raw mung bean seed ranges 3.8-6.15% (Dahiya *et al.*, 2015). The ash content of raw mung bean was found to be 3.71% which is similar to the data found by Mubarak (2005) i.e. 3.76% and Singh *et al.* (2015) i.e. 3.5%. It was found that the fat content of raw mung bean was 1.52% which is in the range 0.17-5.82% given by Dahiya *et al.* (2015). The carbohydrate content of raw mung bean was found 51.89% which is similar to the data obtained by Oburuoga and Anyika (2012) i.e. 53.38% and Onwurafor *et al.* (2014) i.e. 52.54% but the value is very less than obtained by Mubarak (2005) i.e. 62.35%.

4.3 Antinutrients present in raw mung bean

The mean values of different antinutrients determined are presented in the Table 4.3.

Table 4.3 Distribution of anti- nutrients in raw green gram (mg/100 g).

Anti-nutrients	Values in dry basis (mg/100 g)
Tannin	476.81 ± 13.38
Oxalate	227.46 ± 11.67
Phytate	626.54 ± 18.5
Polyphenol	771.39 ± 15.3
Saponin	2617.59 ± 54.6

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

The tannin content in the raw mung bean was found 476.81 mg/100 g which is greater than the data obtained by Mubarak (2005) i.e. 330 mg/100 g but it is less than the value obtained by Singh *et al.* (2015) i.e. 963 mg/100 g. The Oxalate content in the mung bean was 227.46 mg/100 g which was higher than their findings i.e. 128.27 mg/100 g (Oburuoga and Anyika, 2012). The phytate in the raw mung bean was 626.54 mg/100 g which is very similar to the value obtained by Singh *et al.* (2015) i.e. 622 mg/100 g but the value is less than the range 727-940 mg/100 g obtained by Bindu *et al.* (2017). Polyphenol content was found to be 771.75 mg/100 g which is lower than the findings of Kataria *et al.* (1989a) i.e. 808 mg/100 g but it was in the range 290-820 mg/100 g given by Dahiya *et al.* (2015). The saponin content of mung bean is 2848 mg/100 g which is comparable to the values obtained by Kataria *et al.* (1989a) but the value was contradictory obtained by Sivakumaran *et al.* (2017) i.e. 1276 mg/100 g. According to different research, it is concluded that antinutrients values of mung beans varies according to variety and/or cultivar, climatic conditions, locations, irrigation condition, types of soil, year during which they are grown and storage conditions which was also discussed by Nikolopoulou and Grigorakis (2008).

4.4 Effect of different processing methods on tannin content of mung bean

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

4.4.1 Effect of soaking

The tannin content of the raw mung bean was determined and found to be 476.81 mg/100 g. Present study shows that soaking significantly decrease ($p<0.05$) tannin content from 476.81 mg/100 g to 297.21 mg/100 g i.e., 37.67% reduction.

The result obtained in this study tally in line with result obtained by Mubarak (2005), he reported that the reduction of 38.2% after 12 h of soaking of mung bean. In our case, there was slightly higher reduction. But the reduction of tannin in mung bean after 6 h, 12 h and 18 h was found to be 3%, 10% and 15.7% (Singh *et al.*, 2015) which was lower reduction than the values obtained by our study. Also, Abbas and Ahmad (2018) reported that there was 39.4% reduction in the tannin content after soaking for 18 h which is comparable to the obtained data. The loss of tannin content after soaking may be attributed to leaching out into soaking water under the concentration gradient (Kataria *et al.*, 1989b).

4.4.2 Effect of dehulling

Tannin content of green gram was found to be significantly reduced ($p<0.05$) from 476.81 mg/100 g to 174.21 mg/100 g (63.46% reduction) after dehulling process. Our study shows that highest reduction of tannin in mung bean was seen in dehulled sample.

During research conducted by Mubarak (2005), he reported that dehulling the seeds reduced the tannin in mung bean by 33.34% which is lower than the data obtained by our research. Removal of seed coats lowered the tannin content of beans by 68–95% (Deshpande *et al.*, 1982). Since, tannins are mainly located in seed coat of beans. Reduction of tannin content in horse gram was found to be 89.46-92.99% (Pal *et al.*, 2016). Oburuoga and Anyika (2012) found that the tannin was reduced 58.2% by dehulling process in mung bean seeds which is similar to the data obtained in this study.

4.4.3 Effect of germination

The tannin content of raw green gram was determined and the value obtained showed that there is significant reduction ($p<0.05$) in tannin content, which is reduced from 476.81 mg/100 g to 299.34 mg/100 g after germination i.e., 37.22% reduction.

Kakati *et al.* (2010) found that there was 39.68% reduction in tannin and 28.14% reduction in tannin content in SGC 16 and SGC 20 varieties of mung bean respectively. Reduction of tannin in mung bean was found 66.7% by Mubarak (2005) which is higher than the data obtained in this study. Reduction in tannin content after germination may be attributed to the leaching out effect during hydration which was reported by Kataria *et al.* (1989b). Singh *et al.* (2015) also found that the tannin is reduced by 65.3% after germination.

4.4.4 Effect of roasting

The effect of roasting on tannin content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 476.81 mg/100 g to 376.79 mg/100 g after roasting (20.97% reduction).

Near about similar results were observed by Singh *et al.* (2015). They found that a significant decrease in tannin content were observed by roasting of lentils i.e., 16.9% reduction. El-Gohery (2021) concludes that roasting of lima bean seeds reduces tannin content by 29.5% which is comparable to the data obtained in this study. During research conducted by Attou *et al.* (2020), they reported that roasting the seeds of lentils reduced the tannin by 41.41% which is higher reduction than the data obtained in this research. Also, the tannin content of chick pea reduced 57% by roasting (Yadav and Bhatnagar, 2017). Tannin is heat stable compound so roasting has less effect in reducing tannin from the beans than other domestic processing methods.

The tannin content of mung bean on different processing methods is shown in the Fig.4.1.

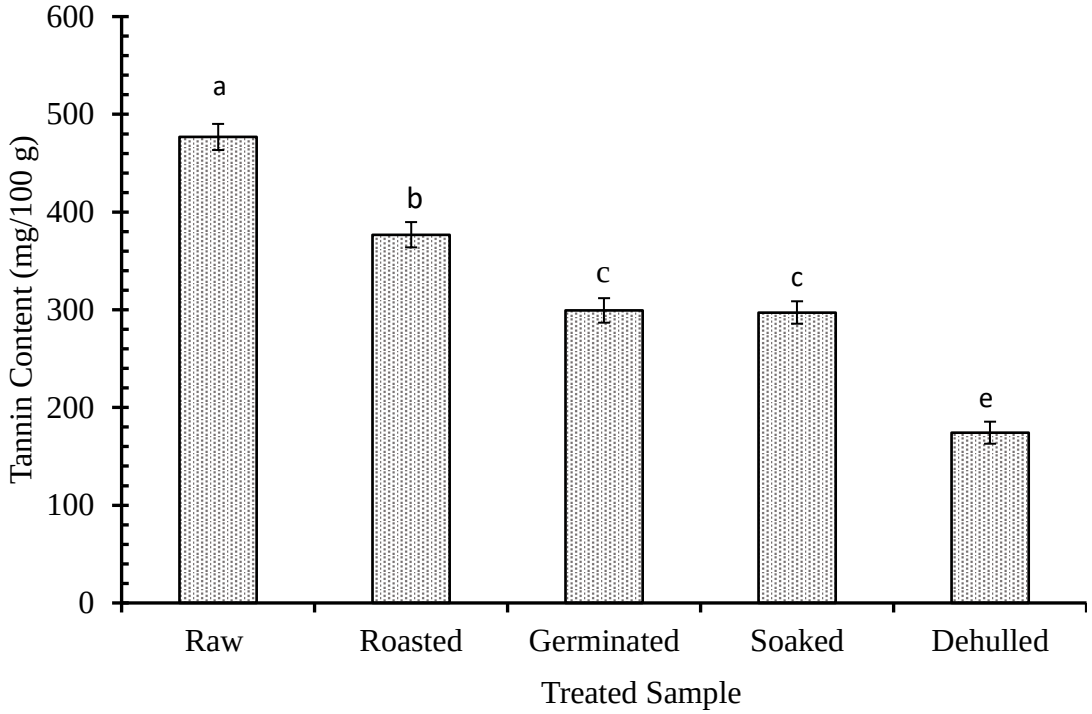


Fig. 4.1 Effect of different processing methods on tannin content

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

4.4.5 Effect of cooking

The effect of open cooking for 30 min and autoclaving at 15 psig for 15 min on total tannin content of green gram was studied. An interesting aspect of this study is that the different samples were cooked with regulated amount of water such that no water was drained after cooking. The value obtained showed that there is significant reduction ($p<0.05$) in tannin content which is reduced from 476.81 mg/100 g to 269.55 mg/100 g, 195.49 mg/100 g, 252.1 mg/100 g, 184.57 mg/100 g for raw open cooked, soaked open cooked, raw autoclaving and soaked autoclaving respectively. This research results that soaked autoclaving reduced 61.49% of tannin content which is the most effective method, followed by soaked open cooked 59% reduction, raw autoclaving 47.12% reduction and raw open cooked 43.47% reduction. The effect of cooking methods on tannin content is presented in Fig. 4.2.

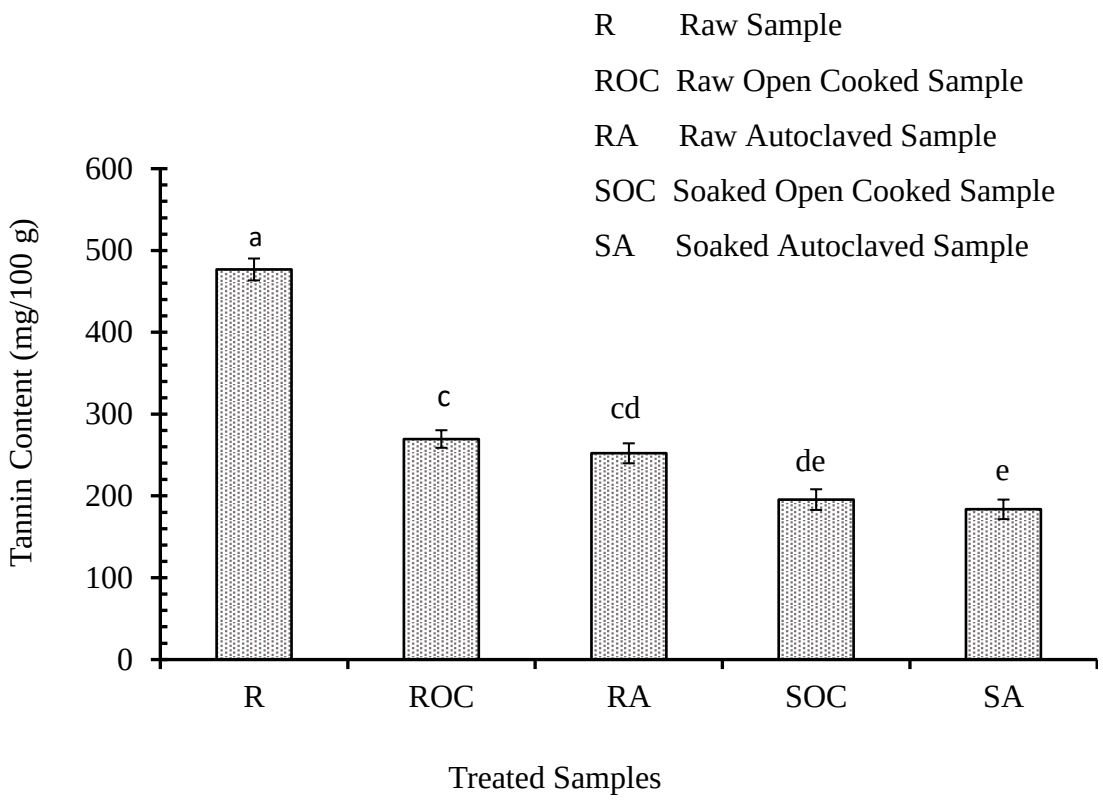


Fig. 4.2 Effect of cooking methods on tannin content

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

Mubarak (2005) studied the effect of cooking in tannin content in mung bean ranges from 45.5-55.5% reduction, where he found maximum reduction after autoclaving than open cooking which was similar to findings obtained in this research. Singh *et al.* (2015) also stated that the tannin content of mung bean significantly reduced after open cooking for 30 min at 100ºC and autoclaving for 15 min at 121ºC. The tannin content of chickpea is reduced by 48% after cooking (Alajaji and El-Adawy, 2006). Also, Awad *et al.* (2014) reported that effect of cooking in tannin content in different varieties of faba bean ranges from 37.6-78%. According to Kaur *et al.* (2020), they found that the cooking and autoclaving of rice bean reduced the tannin content by 27% and 30% respectively.

4.5 Effect of different processing methods on oxalate content of mung bean

The effects of soaking, germination, roasting, open cooking, autoclaving and dehulling on the oxalate content in green gram was studied. All the treatments significantly reduced ($p<0.05$) the oxalate of the green gram seeds, but to the varying extent. The combination treatment i.e., soaked autoclaving had most pronounced effect than other treatments in reduction of oxalate contents.

4.5.1 Effect of soaking

Soaking shows considerable decrease in oxalate content of green gram 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 227.46 mg/100 g to 172.44 mg/100 g i.e., 24.19% reduction.

Soaking the seeds in distilled water significantly decreased the contents of total oxalate in the range 17.40-51.89% (Shi *et al.*, 2018) where the obtained data in this study were in the range given by them. The results obtained in this research were similar with result obtained by Patel and Dutta (2018), where he found 19.65% reduction in finger millet. The reduction in oxalic acid during soaking and germination may be due to leaching of oxalate oxidase and oxalate decarboxylase. Similar results for reduction in oxalic acid content of soaked grains were reported by Brudzynski and Salamon (2011).

4.5.2 Effect of germination

The effect of germination on oxalate content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was reduced from 227.46 mg/100 g to 95.98 mg/100 g after germination (57.8% reduction).

The result obtained in this research tally in line with result obtained by Virginia *et al.* (2012), they found significant reduction ($p<0.05$) in oxalate during germination of green pea (65.26%). Similar results were obtained by Patel and Dutta (2018) i.e., 54.36% reduction in finger millet. Pal *et al.* (2016) found that a significant decrease in oxalate content was observed in the initial hours of germination i.e., 24 h followed by a non-significant change in the later stages and the oxalate content of raw horse gram was 466 mg/100 g which decreased to 308 mg/100 g i.e. (33.91% reduction) during 18 h germination and 341 mg/100

g i.e., (26.82% reduction) during 12 h of germination. Decrease in oxalate during germination is because of the activation of oxalate oxidase which breakdown oxalic acid into carbon dioxide and hydrogen peroxide consequently releasing calcium (Pal *et al.*, 2016).

4.5.3 Effect of dehulling

The oxalate content of the raw mung bean was determined and found to be 227.46 mg/100 g. Present study shows that soaking significantly decrease ($p<0.05$) oxalate content from 227.46 mg/100 g to 146.74 mg/100 g i.e., 35.49% reduction.

The result obtained in this research tally with the data given by Pal *et al.* (2016), they found a highly significant decrease in amount of oxalic acid content range from 456.69 mg/ 100 g in raw to 301.56 mg/ 100 g after dehulling of horse gram i.e., 33.86% reduction of total oxalate content.

4.5.4 Effect of roasting

The effect of roasting on oxalate content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was reduced from 227.46 mg/100 g to 194.69 mg/100 g after roasting (14.41% reduction).

It has been reported that the oxalate content of bambara groundnut is reduced by 8-10% after roasting of groundnut for 15 min at 130ºC in hot sand (Adegunwa *et al.*, 2014) but the findings in mung bean seeds by this research is slightly higher than their results.

The oxalate content of different processing treatments is given in Fig. 4.3.

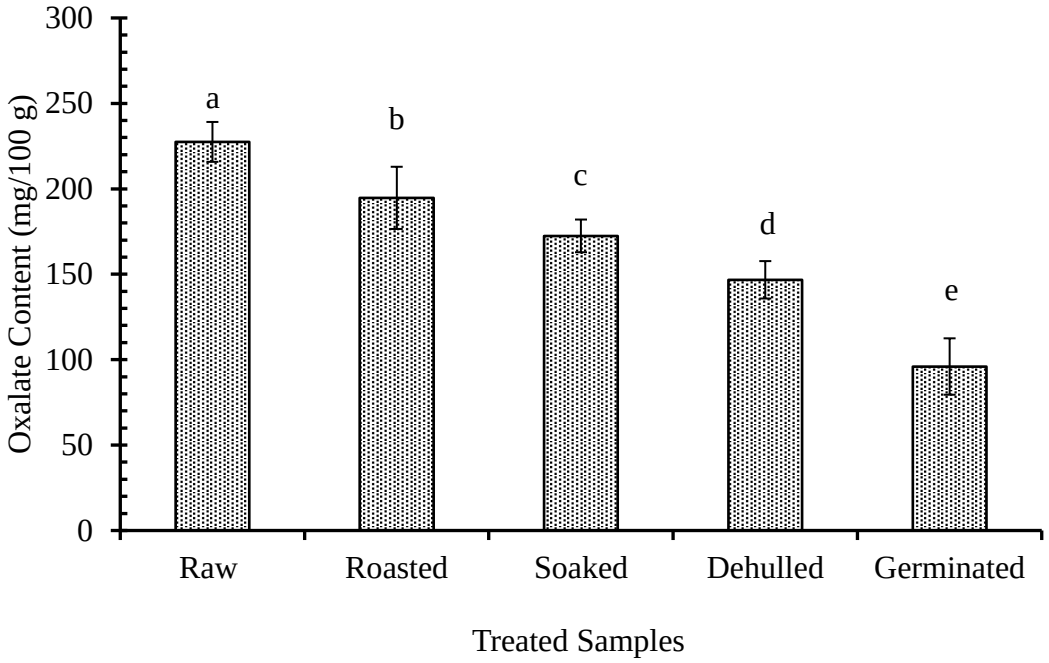


Fig. 4.3 Effect of different processing methods on oxalate content

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

4.5.5 Effect of cooking

The effect of cooking on oxalate content of mung bean was studied. It shows significant reduction ($p<0.05$) on oxalate content range from 227.46 mg/100 g to 91.68 mg/100 g, 69.39 mg/100 g, 80.65 mg/100 g, and 66.34 mg/100 g for samples of raw open cooked, soaked open cooked, raw autoclaving and soaked autoclaving respectively. This research findings result that soaked autoclaving reduced 70.83% of oxalate content which is the most effective method, followed by soaked open cooked 69.39% reduction, raw autoclaving 64.54% reduction and raw open cooked 59.69% reduction. The effect of cooking methods on oxalate content is presented in Fig. 4.4.

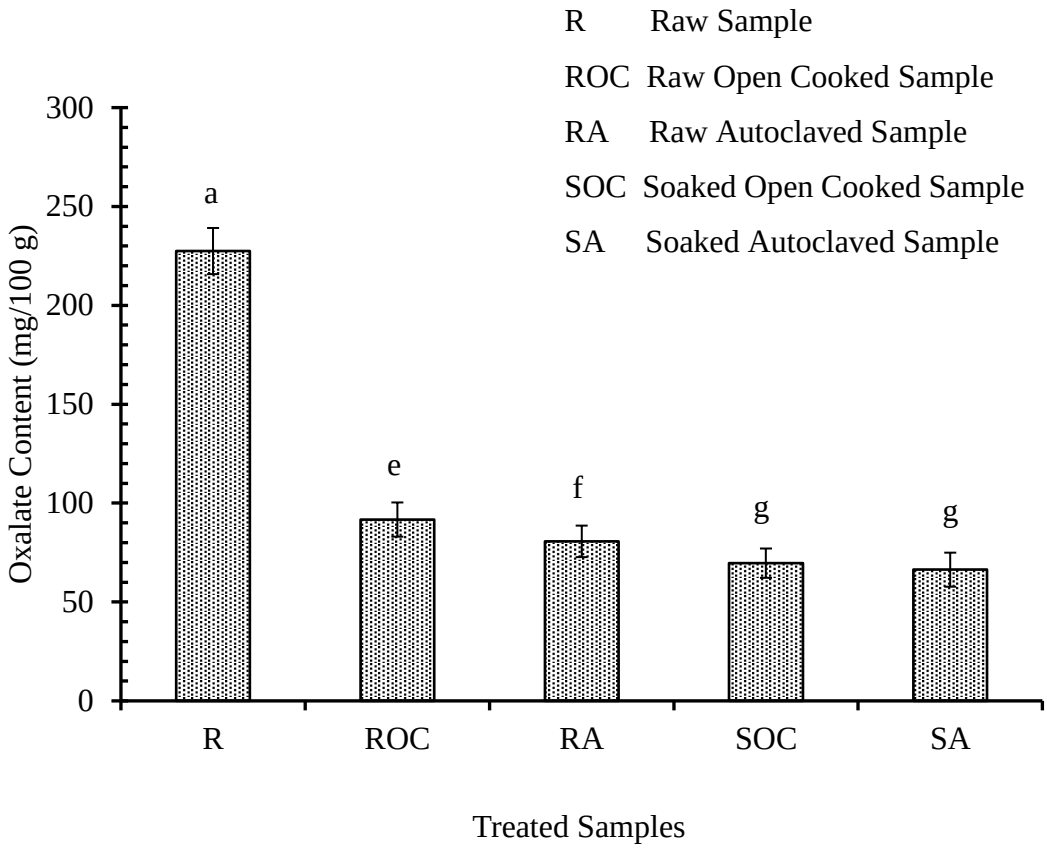


Fig. 4.4 Effect of cooking methods on oxalate content

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

According to Akhtar *et al.* (2011), they found that the reduction in the total oxalate of presoaked cooking was 66.15% of soyabean which is similar to the data obtained in this research. Loss of soluble oxalate in water was considered to be the primary factor contributing to total oxalate reduction.

4.6 Effect of different processing methods on phytate content of mung bean

The effects of soaking, germination, roasting, open cooking, autoclaving and dehulling on the phytate content in green gram was studied. All the treatments significantly reduced ($p<0.05$) the phytate of the green gram seeds, but to the varying extent. Germination had most pronounced effect than other treatments in reduction of phytate contents.

4.6.1 Effect of soaking

Effect of soaking on phytate content of green gram was studied and the value obtained showed that there is significant reduction ($p<0.05$) in phytate content. The result shows great reduction from 626.53 mg/100 g to 452.53 mg/100 g after soaking the mung bean for 18 h (27.78% reduction).

The result obtained in this research tally in line with the values obtained by Mubarak (2005), he found that soaking of mung bean in tap water reduced the phytate content by 26.7%. Kakati *et al.* (2010) reported that the reduction of phytate in SGC 16 and SGC 20 cultivar of mung bean after soaking was 17% and 21% which was similar to the data obtained in this research. Similarly, the reduction of mung bean after soaking for 6 h, 12 h and 18 h was 7%, 11% and 20% respectively (Singh *et al.*, 2015). The loss of phytic acid in the soaked seeds may be because of leaching out of phytate ions into soaking water under the influence of concentration gradient which governs the rate of diffusion (Grewal and Jood, 2006).

4.6.2 Effect of dehulling

The effect of dehulling on phytate content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in phytate content, which was reduced from 626.53 mg/100 g to 441 mg/100 g after dehulling (29.61% reduction).

From the research done by Grewal and Jood (2006), the reduction in phytate content of asha cultivar of mung bean was 24% which was similar to the data obtained in this research. On dehulling, the losses may be because of the removal of husk. As husk contained relatively higher concentration of phytic acid as compared to whole grain, and therefore, the removal of husk accounted for significantly lower phytic acid content in dehulled grains. Mubarak (2005) also reported that 21% phytic acid was reduced after dehulling of mung bean. Similar results have been reported by Oburuoga and Anyika (2012).

4.6.3 Effect of germination

Germination shows considerable decrease in phytate content of green gram and has been documented to be an effective treatment to remove phytic acid in legumes. This result shows that germination significantly reduced ($p<0.05$) total phytate content which reduced from 626.53 mg/100 g to 382.71 mg/100 g i.e., 38.91% reduction.

The result obtained in this research tally in line with the data obtained by Singh *et al.* (2015), they also found that the phytate content in the germinated sample of mung bean was reduced by 38%. Grewal and Jood (2006) reported that the reduction of phytate was 33% after germination. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase to inositol and free phosphate. In earlier studies, germination has also been reported to have a diminishing effect on the phytic acid content of various legumes like moth bean, rice bean, faba bean and pigeon pea.

4.6.4 Effect of roasting

The effect of roasting on phytate content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in phytate content, which was reduced from 626.53 mg/100 g to 487.46 mg/100 g after roasting (22.2% reduction).

A significant decrease of phytates was recorded for roasted varieties of lentils i.e., reduction up to 63.01% at 140ºC for 30 min (Attou *et al.*, 2020). Similarly, reduction in phytic acid of chickpea was reported up to 56% (Yadav and Bhatnagar, 2017) which is greater than the obtained data in this research. Singh *et al.* (2015) reported that roasting of mung bean seeds reduced by 29% which was similar to the data obtained by our research. Roasting of lima bean seeds helps in the reduction of phytic acid by 40% (El-Gohery, 2021).

The phytate content of different processing treatments is given in Fig. 4.5.

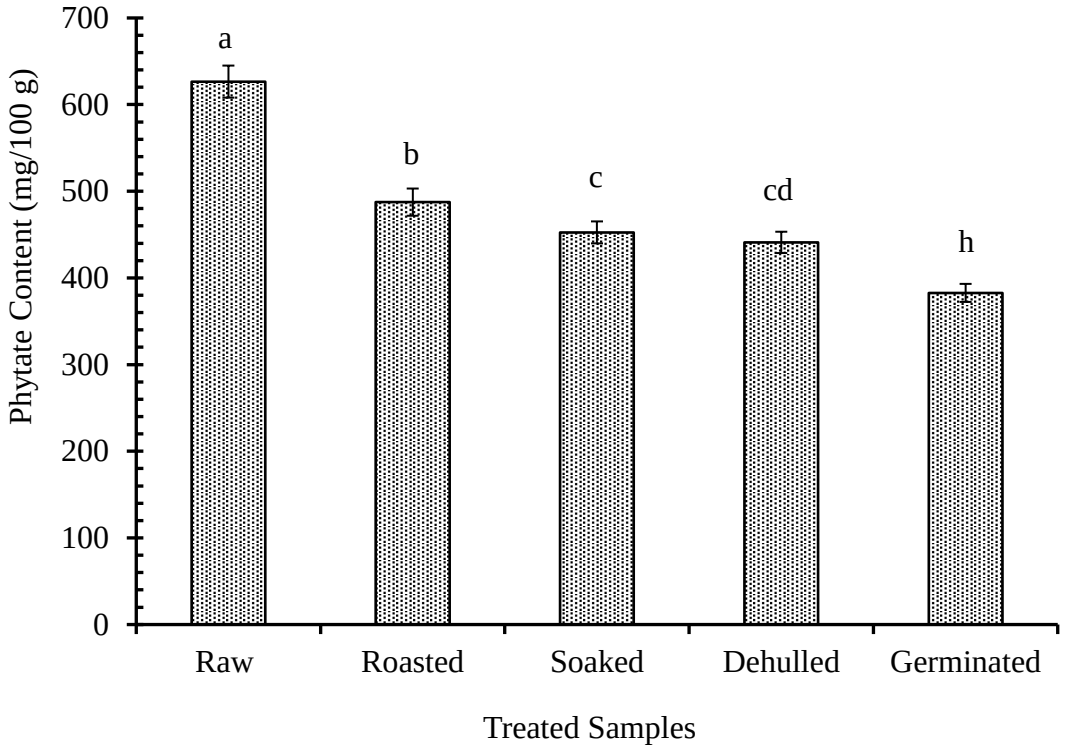


Fig. 4.5 Effect of different processing methods on phytate content

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

4.6.5 Effect of cooking

The effect of phytate content on open cooking and autoclaving of raw and soaked mung bean was studied. The water was not drained after cooking. It shows significant reduction ($p<0.05$) on phytate content range from 626.53 mg/100 g to 418.5 mg/100 g, 394.53 mg/100 g, 429.92 mg/100 g, and 406.64 mg/100 g for samples of raw open cooked, soaked open cooked, raw autoclaving and soaked autoclaving respectively. The findings obtained by this research result that soaked open cooking reduced 37.03% of phytate content which is the most effective method, followed by soaked autoclaving 35.1% reduction, raw open cooked 33.2% reduction and raw autoclaving 31.38% reduction. The effect of phytate content on cooking methods is presented in Fig.4.6.

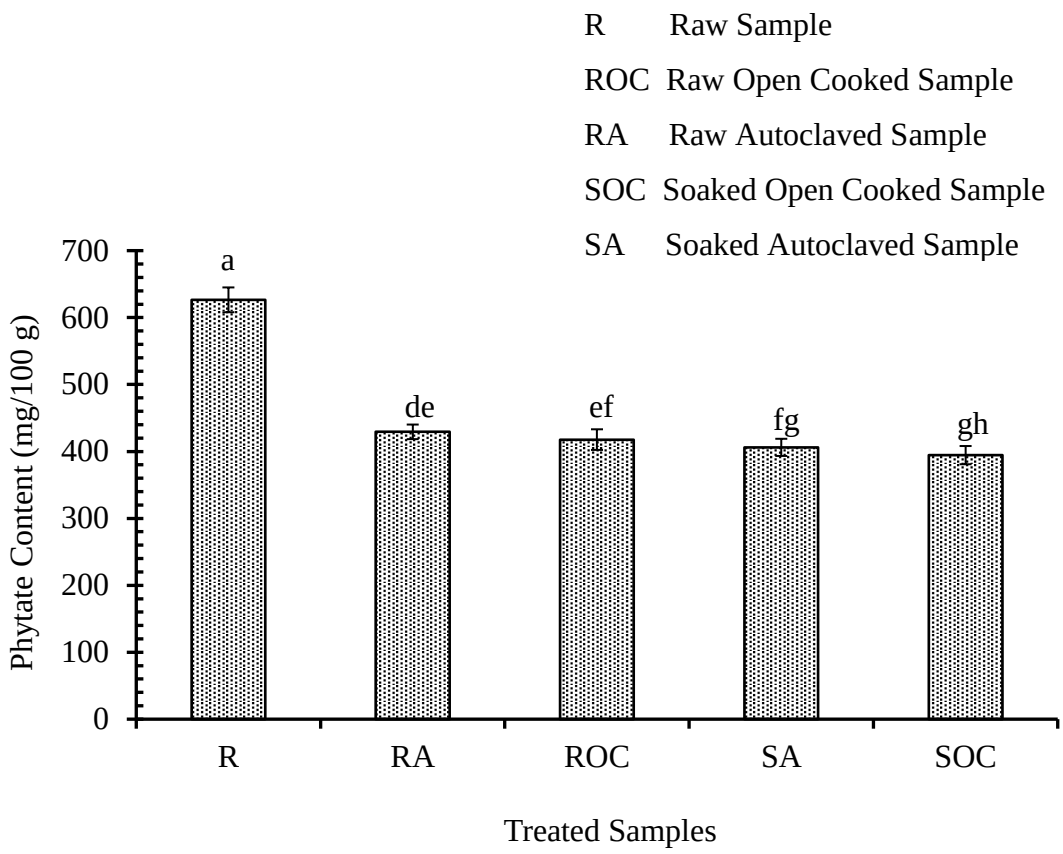


Fig. 4.6 Effect of cooking methods on phytate content

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

The reduction of phytic acid after boiling was greater than after autoclaving of raw mung bean (Mubarak, 2005) where this research also showed that soaked open cooking had higher reduction than other methods. The mung bean cultivar SGC 16 and SGC 20 on cooking the reduction of phytate was 33% and 35% which was similar to the data obtained in this research (Kakati *et al.*, 2010). The decrease might be attributed to leaching of the phytic acid into soaking water under the influence of concentration gradient, which governs the rate of diffusion. It has been reported that the reduction of soaked autoclaving and soaked open cooking was similar (31%) and raw autoclaving and raw open cooking was also similar about 21% (Singh *et al.*, 2015) but the data of this research varies with the methods of cooking.

4.7 Effect of different processing methods on polyphenols content of mung bean

The effects of soaking, germination, roasting, open cooking, autoclaving and dehulling on the polyphenol content in green gram was studied. All the treatments significantly reduced ($p<0.05$) the polyphenols of the green gram seeds, but to the varying extent. Dehulling had most pronounced effect than other treatments in reduction of polyphenols contents.

4.7.1 Effect of soaking

The effect of soaking on polyphenols content of green gram was studied and the value obtained showed that there is significant reduction ($p<0.05$) in polyphenols content. The result shows reduction from 771.39 mg/100 g to 494.79 mg/100 g after soaking the mung bean for 18 h i.e., 35.88% reduction.

The research conducted by Tajoddin *et al.* (2014), they reported that the reduction of polyphenols of soaked mung bean seeds was 32% which was similar to the data obtained in this research. The loss of polyphenols during soaking may be due to leaching out of soluble polyphenolic compounds in soaking water. Grewal and Jood (2006) also reported that the polyphenol contents of green gram seeds were reduced by 23% after soaking for 18 h which was slightly lower than data obtained in this study.

4.7.2 Effect of dehulling

Dehulling shows considerable decrease in polyphenol content of green gram and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that dehulling significantly reduced ($p<0.05$) total polyphenol content which reduced from 771.39 mg/100 g to 358.78 mg/100 g i.e., 53.48% reduction.

According to Tajoddin *et al.* (2010), the reduction of polyphenol content in mung bean of ten cultivars after dehulling was 14-52% which was within the data obtained in this research. But the asha variety of mung bean reduced the polyphenol content by only 29% after dehulling which was less than the obtained data of this research (Grewal and Jood, 2006).

4.7.3 Effect of germination

The effect of germination on polyphenol content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in polyphenol content, which was reduced from 771.39 mg/100 g to 573.49 mg/100 g after germination (25.65% reduction).

According to research conducted by Grewal and Jood (2006), they found that the polyphenol content of asha cultivar of mung bean seeds was reduced by 32% after germination which is higher than the data obtained by our research. Before germination, soaking is also done and some loss of polyphenol during soaking is also expected because of its leaching into the soaking water. Further decrease in polyphenols during germination may be ascribed to the presence of polyphenol oxidase and enzymic hydrolysis (Jood *et al.*, 1987). They reported that the polyphenols in chick pea was reduced by 23% after germination which was similar to the data obtained in this research.

4.7.4 Effect of roasting

The effect of roasting on phytate content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in phytate content, which was reduced from 771.39 mg/100 g to 598.78 mg/100 g after roasting i.e., 22.38% reduction.

During the research conducted by Mendoza *et al.* (1988), they reported that roasting of mung bean seeds reduced the polyphenol content by 17% which is similar to the data obtained in this research. Roasting which involves dry heat could bring about a change in chemical reactivity of the polyphenols. Roasting decreased the polyphenol content of black bean only by 8% which was far lesser than roasted mung bean seeds (Ngoc *et al.*, 2021).

The polyphenols content of different processing methods is given in Fig. 4.7.

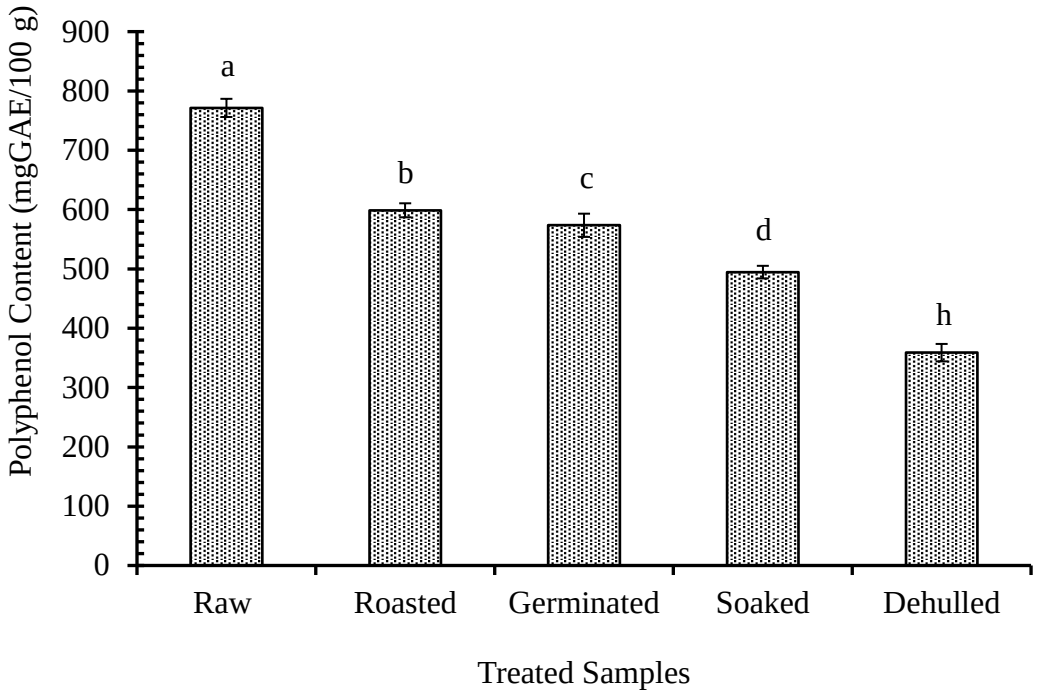


Fig. 4.7 Effect of different processing methods on polyphenol content

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

4.7.5 Effect of cooking

The effect of cooking on polyphenol content of mung bean was studied. It shows significant reduction ($p<0.05$) on polyphenol content range from 771.39 mg/100 g to 406.65 mg/100 g, 380.91 mg/100 g, 454.76 mg/100 g, and 410.6 mg/100 g for samples of raw autoclaving, soaked autoclaving, raw open cooked and soaked open cooked respectively. This research findings result that soaked autoclaving reduced 50.62% of polyphenol content which is the most effective method, followed by raw autoclaving 47.28% reduction, soaked open cooked 46.78% reduction and raw open cooked 41.05% reduction. The effect of cooking methods on polyphenol content is given in Fig. 4.8.

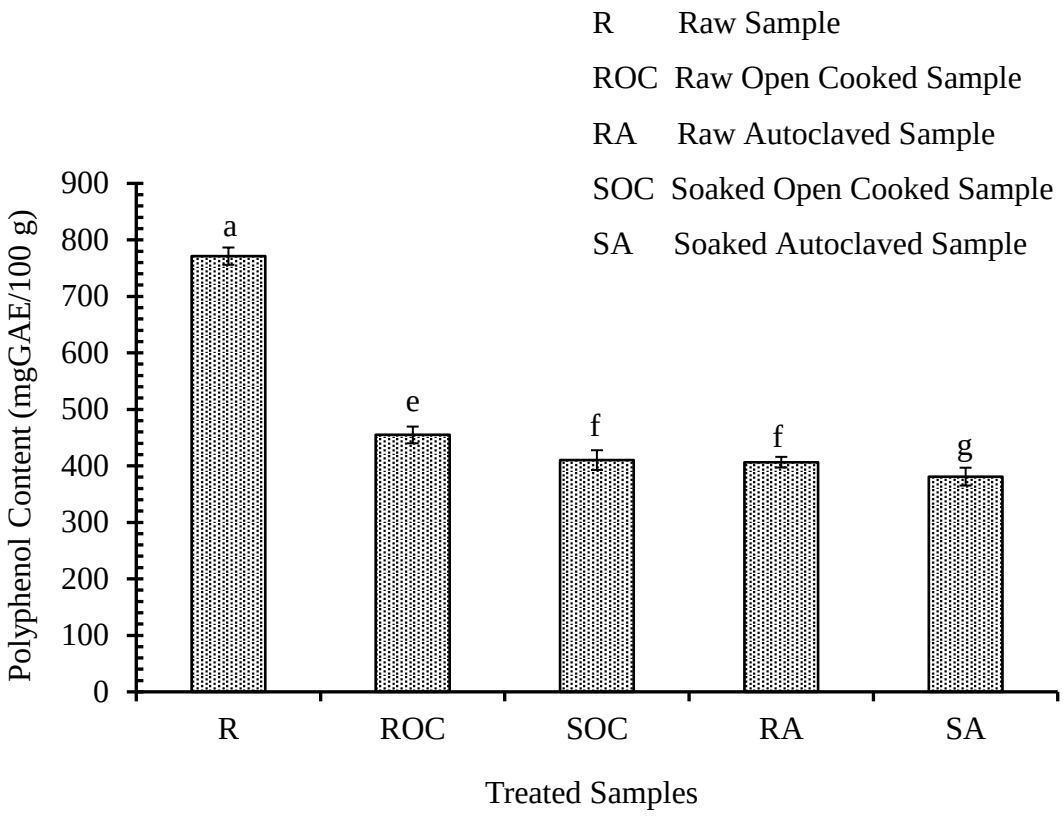


Fig. 4.8 Effect of cooking methods on polyphenol content

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

The asha variety of mung bean when cooked and autoclaving reduced the polyphenol content by 32% and 42% respectively & MHIK-25 cultivar of mung bean after cooking and autoclaving was 29% and 39% respectively (Grewal and Jood, 2006) where the obtained data in this research was slightly similar to them however the same thing was the reduction of polyphenol of autoclaved sample was greater than cooked sample. Polyphenols are reported to be present in higher amounts in colored and darker legume varieties than in pale varieties (Salunkhe *et al.*, 1983). Pressure cooking of soaked seeds for 5 min decreased polyphenols to a larger extent as compared to the seeds which were ordinarily cooked after soaking. The effect of pressure cooking was greater when the period of pressure cooking was extended. A decreased amount of polyphenols recovered from cooked seeds could be on account of reduced extractability due to their changed chemical reactivity (Kataria *et al.*, 1989b).

4.8 Effect of different processing methods on saponin content of mung bean

The effects of soaking, germination, roasting, open cooking, autoclaving and dehulling on the saponin content in green gram was studied. All the treatments significantly reduced ($p<0.05$) the saponin of the green gram seeds, but to the varying extent. The combination treatment i.e., soaked autoclaving had most pronounced effect than other treatments in reduction of phytate contents.

4.8.1 Effect of soaking

Effect of soaking on saponin content of green gram was studied and the value obtained showed that there is significant reduction ($p<0.05$) in saponin content. The result shows great reduction from 2617.59 mg/100 g to 2425.87 mg/100 g after soaking the mung bean for 18 h (7.32% reduction).

The result obtained in this research tally in line with the data of Kataria *et al.* (1989a), in which they also found 7% reduction when soaking of mung bean seeds. They also conclude that raising the time of soaking from 12 to 18 h did not influence saponin content of the seed to a significant extent. The decrease in the level of saponin in mung bean seeds during soaking may be attributed to leaching out into soaking water under the concentration gradient. Shi *et al.* (2004) also found that soaking of pigeon pea reduced to 8% of saponin content which is similar to the data of mung bean.

4.8.2 Effect of dehulling

The effect of dehulling on saponin content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in saponin content, which was reduced from 2617.59 mg/100 g to 2244.96 mg/100 g after dehulling (14.23% reduction).

According to Shi *et al.* (2004), it was reported that the reduction of saponin content was 29% after dehulling of faba beans. The data of this research was slightly lower than their findings i.e., 14%. They also reported that saponin was reduced by concentration gradient during soaking and after dehulling, was reduced by removal of seed coat.

4.8.3 Effect of germination

The effect of germination on saponin content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in saponin content, which was reduced from 2617.59 mg/100 g to 2276.54 mg/100 g after germination (13.03% reduction).

During research conducted by Kataria *et al.* (1989a), they reported that the reduction of saponin after germination of mung bean seeds was 11% which was similar to the obtained data. They also reported that enzymic degradation could be a possible explanation of the saponin loss during germination. It was reported that germination of amphidiploids of mung bean and black gram reduced saponin content by 5-16% where the data of this research was in range (Kataria *et al.*, 1989b).

4.8.4 Effect of roasting

The effect of roasting on saponin content of mung bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in saponin content, which was reduced from 2617.59 mg/100 g to 2163.51 mg/100 g after roasting i.e., 17.35% reduction.

During the research conducted by Ngoc *et al.* (2021), they reported that roasting of black bean seeds reduced the saponin content significantly by 20%. The result obtained shows slightly lower reduction as compared to this research. The decrease in saponin content of mung bean by roasting was due to thermolabile nature of saponin (Jood *et al.*, 1987).

The saponin content of different processing methods is given in Fig. 4.9.

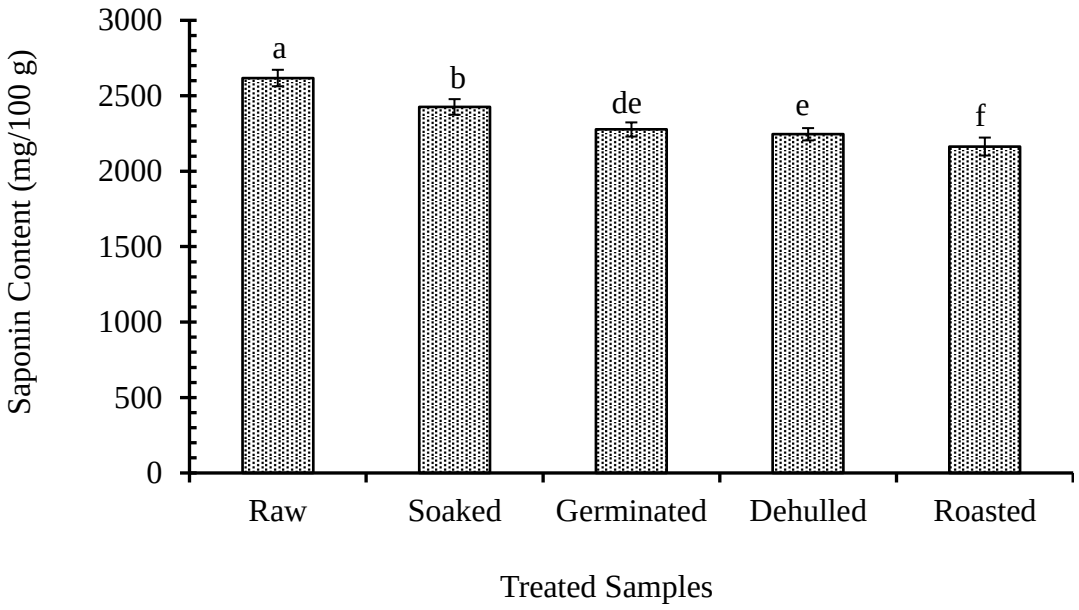


Fig. 4.9 Effect of different processing methods on saponin content

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

4.8.5 Effect of cooking

The effect of cooking on saponin content of mung bean was studied. It shows significant reduction ($p<0.05$) on saponin content range from 2617.58 mg/100 g to 2394.78 mg/100 g, 2050.29 mg/100 g, 2438.61 mg/100 g, and 2344.90 mg/100 g for samples of raw autoclaving, soaked autoclaving, raw open cooked and soaked open cooked respectively. The findings of this study results that soaked autoclaving reduced 21.67% of saponin content which is the most effective method, followed by soaked open cooking 10.42% reduction, raw autoclaving 8.51% reduction and raw open cooked 6.84% reduction. The effect of cooking methods on saponin content is given in Fig. 4.10.

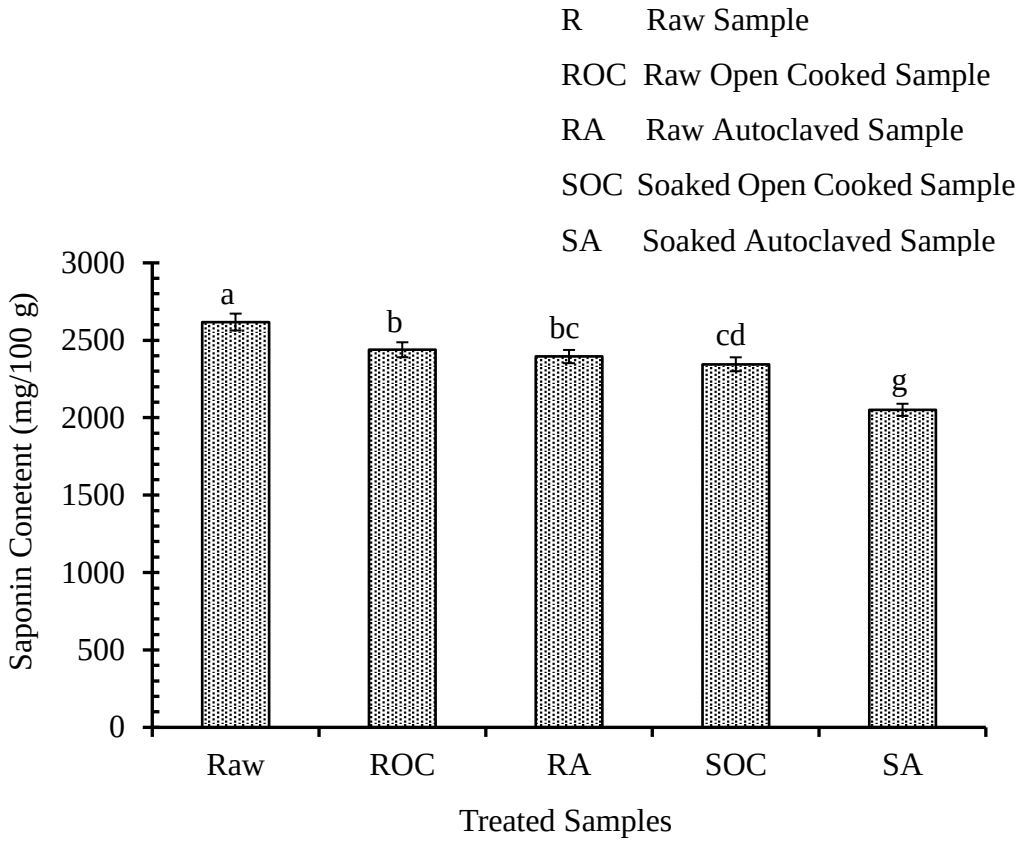


Fig. 4.10 Effect of cooking methods on saponin content

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

Kataria *et al.* (1989a) reported that the reduction of saponin of mung bean after cooking, soaked cooking, autoclaving and soaked autoclaving was 6%, 8%, 8% and 20% respectively which was similar to the obtained data of this research. Grewal and Jood (2006) conclude that the thermolabile nature of saponin and formation of a poorly extractable complex may account for the loss of saponin during cooking. The unsoaked cooking reduced saponin by 4-15%, soaked cooking reduced saponin by 9-14%, unsoaked autoclaving reduced saponin by 12-18% and soaked autoclaving reduced saponin by 23-25% of amphidiploids of black gram and green gram (Kataria *et al.*, 1989b) which was also similar to the obtained data of green gram of this research.

Part V

Conclusion and recommendations

5.1 Conclusion

Based on the results and discussion, the following conclusion can be drawn:

1) Mung bean was subjected to a variety of methods, including soaking, soaking and dehulling, germination, roasting, raw open cooking, soaked open cooking, raw autoclaving, and soaked autoclaving, all of which significantly reduced antinutrients.
2) Dehulling was the most effective method for the reduction of tannin (63%) and polyphenols (53%) present in mung bean.
3) The most effective to reduce the phytate of mung bean was germination (39%) for 48 h.
4) For the reduction of oxalate and saponin, the most effective method was soaked autoclaving i.e., 71% and 22% respectively.
5) Soaked autoclaving was the most effective method to reduce the antinutrients of mung bean in case of cooking treatments.

5.2 Recommendations

1) Among all the processing methods, soaked autoclaving method comparatively reduced the antinutrients.
2) Tannin is reduced by dehulling process whereas phytate is reduced by germination process significantly.
3) Time and temperature of the different processing methods can be varied.
4) The effect of processing methods to reduce other antinutrients like trypsin inhibitor, hemagglutinin, lectin etc. present in mung bean can be studied.

Part VI

Summary

Mung bean is nutritious beans mainly eaten in Asian countries due to its wide health benefits. Generally, it is consumed by cooking and in ayurvedic medicine however nowadays dehulled and fried mung bean is famous in Nepal. It produces blood glycemic response in humans and modifies glucose and lipid metabolism. Mung bean starch is a slowly digestible carbohydrate which is required for diabetic patients. It has antioxidant, antimicrobial, anti-hypertensive, anti-melanogenesis, anti-inflammatory, immunomodulatory and antitumor properties. So, its use is increasing day by day all over the world.

To evaluate the lowering of anti-nutrient levels in mung bean, eight different processing methods were used in this research. The processing methods include soaking, roasting, germination, open cooking, autoclaving, soaking and dehulling, soaking and open cooking & soaking and autoclaving. The antinutrients seen were tannin, oxalate, phytate, polyphenols and saponin. Tannin, polyphenols and saponin were determined by spectrophotometric methods however phytate and oxalate were determined by titration using iron chloride solution and potassium permanganate solution respectively.

The mean value of tannin, oxalate, phytate, polyphenols and saponin of raw mung bean were 477 mg/100 g, 227 mg/100 g, 627 mg/100 g, 772 mg/100 g, 2618 mg/100 g respectively. All the processing methods reduced ($p<0.05$) significantly the antinutrients of mung bean where combination treatments were best seen than the single treatments. The combination treatments dehulling i.e., soaking and dehulling reduced the tannin and polyphenols content of mung bean than other processing methods. The reduction in tannin by germination and soaking were not significantly ($p>0.05$) different. The reduction in polyphenols by soaked open cooking and raw autoclaving were not significantly ($p>0.05$) different. The reduction in oxalate by soaked open cooking and soaked autoclaving were not significantly ($p>0.05$) different which reduced the oxalate content in mung bean greater than other processing methods. The combination treatments i.e., soaked autoclaving reduced the saponin content of mung bean greater than other methods. Hence combination treatments were better than single process.

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Appendices

Appendix A

Table. A.1 One-way ANOVA table for polyphenols

Source of variation	**d. f.**	**s. s.**	**m. s.**	**v. r.**	**F pr.**
Treatments	8	424336.16	53042.02	3107.24	<.001
Residual	18	307.27	17.07		
Total	26	424643.43			

Table. A.2 Effect of different treatments of polyphenol content

Treatments	Polyphenol (mg/100 g)
Raw Sample	$771.39^{a} \pm 15.3$
Roasting	$598.79^{b} \pm 11.8$
Germination	$573.49^{c} \pm 19.6$
Soaking	$494.57^{d} \pm 10.6$
Raw Open Cooking	$454.76^{e} \pm 14.8$
Soaked Open Cooking	$410.6^{f} \pm 17.5$
Raw Autoclaving	$406.65^{f} \pm 9.4$
Soaked Autoclaving	$380.91^{g} \pm 15.8$
Dehulling	$358.78^{h} \pm 14.7$

[Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A.3 One-way ANOVA table for oxalate

Source of variation	**d. f.**	**s. s.**	**m. s.**	**v. r.**	**F pr.**
Treatments	8	85591. 47	10698.93	993.19	<.001
Residual	18	193.90	10.77		
Total	26	85785.37			

Table. A.4 Effect of different treatments of oxalate content

Treatments	Oxalate (mg/100 g)
Raw Sample	$227.5^{a} \pm 11.8$
Roasting	$194.9^{b} \pm 18.2$
Soaking	$172.8^{c} \pm 9.6$
Dehulling	$146^{d} \pm 10.9$
Germination	$95.5^{e} \pm 16.5$
Raw Open Cooking	$91.8^{e} \pm 8.7$
Raw Autoclaving	$80.3^{f} \pm 7.9$
Soaked Open Cooking	$69.7^{g} \pm 7.4$
Soaked Autoclaving	$66.3^{g} \pm 8.6$

[Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A.5 One-way ANOVA table for tannin

Source of variation	**d. f.**	**s. s.**	**m. s.**	**v. r.**	**F pr.**
Treatment	8	231877.4	28984.7	65.08	<.001
Residual	18	8016.2	445.3		
Total	26	239893.6			

Table. A.6 Effect of different treatments of tannin content

Treatments	Tannin (mg/100 g)
Raw Sample	$476.8^{a} \pm 13.4$
Roasting	$376.8^{b} \pm 12.9$
Soaking	$297.2^{c} \pm 11.5$
Germination	$299.3^{c} \pm 12.6$
Raw Open Cooking	$269.5^{c} \pm 10.7$
Raw Autoclaving	$252.1^{cd} \pm 12.2$
Soaked Open Cooking	$195.5^{de} \pm 12.7$
Soaked Autoclaving	$183.6^{e} \pm 11.9$
Dehulling	$174.2^{e} \pm 11.3$

[Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A.7 One-way ANOVA table for phytate

Source of variation	**d. f.**	**s. s.**	**m. s.**	**v. r.**	**F pr.**
Phytate	8	130573.15	16321.64	743.93	<.001
Residual	18	394.91	21.94		
Total	26	130968.06			

Table. A.8 Effect of different treatments of phytate content

Treatments	Phytate (mg/100 g)
Raw Sample	$626.5^{a} \pm 18.5$
Roasting	$487.5^{b} \pm 15.7$
Soaking	$452.5^{c} \pm 12.7$
Dehulling	$441^{cd} \pm 12.3$
Raw Autoclaving	$429.9^{de} \pm 10.9$
Raw Open Cooking	$418.5^{ef} \pm 15.4$
Soaked Autoclaving	$406.6^{fg} \pm 12.8$
Soaked Open Cooking	$394.5^{gh} \pm 13.6$
Germination	$382.7^{h} \pm 10.4$

[Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A.9 One-way ANOVA table for saponin

Source of variation	**d. f.**	**s. s.**	**m. s.**	**v. r.**	**F pr.**
Treatments	8	672416.7	84052.1	128.80	<.001
Residual	18	11746.0	652.6		
Total	26	684162.7			

Table. A.10 Effect of different treatments of saponin content

Treatments	Saponin (mg/100 g)
Raw Sample	$2617.59^{a} \pm 54.6$
Soaking	$2425.87^{b} \pm 51.9$
Raw Open Cooking	$2438.61^{b} \pm 48.4$
Raw Autoclaving	$2394.78^{bc} \pm 42.7$
Soaked Open Cooking	$2344.91^{cd} \pm 45.1$
Germination	$2276.54^{de} \pm 46.9$
Dehulling	$2244.96^{e} \pm 40.8$
Roasting	$2163.51^{f} \pm 59.4$
Soaked Autoclaving	$2050.75^{g} \pm 39.2$

[Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Appendix B

Table.B.1 Standard curve data for tannin as tannic acid

Tannic acid Concentration (ppm)	Absorbance
0	0
2	0.173
4	0.312
6	0.451
8	0.635
10	0.812

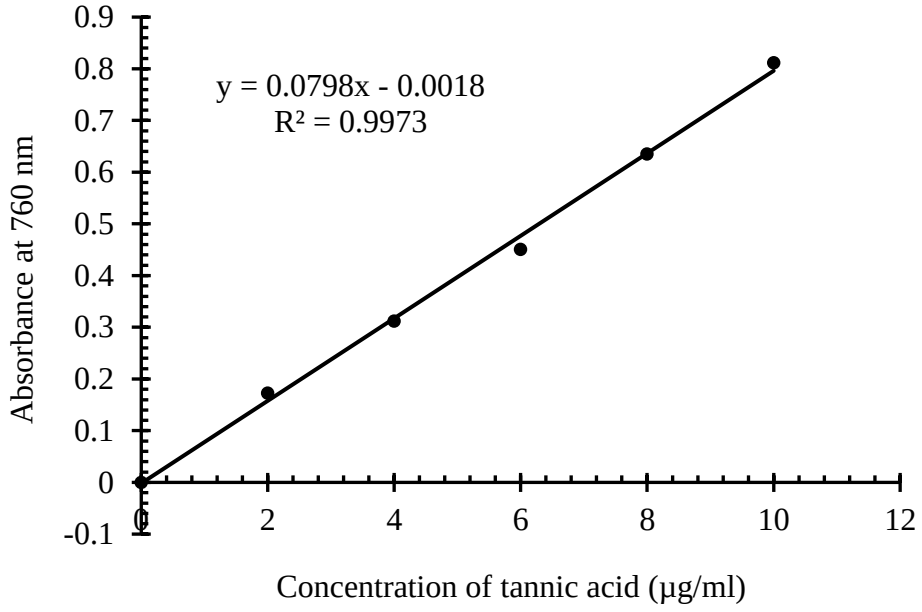

Fig. B.1 Standard curve for tannin determination

Table B.2 Standard curve data for polyphenol as gallic acid

Gallic acid concentration (ppm)	Absorbance
0	0
50	0.641
100	0.945
150	1.652
200	1.917
250	2.634

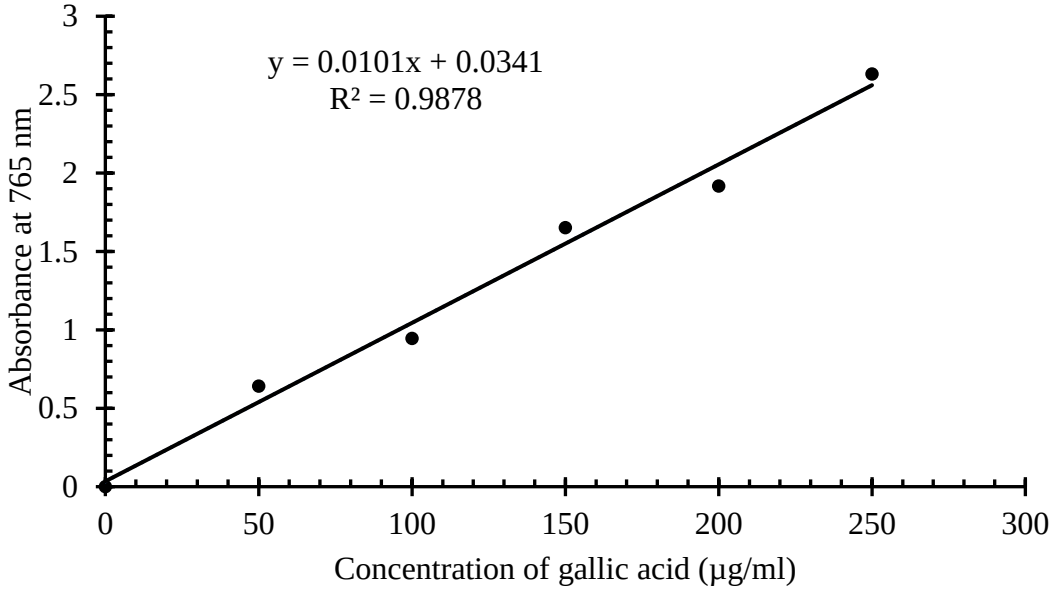

Fig B.2 Standard curve for polyphenol determination

Table.B.1 Standard curve data for saponin

Saponin Concentration (ppm)	Absorbance
0	0
2	0.506
4	0.919
6	1.732
8	2.046
10	2.354

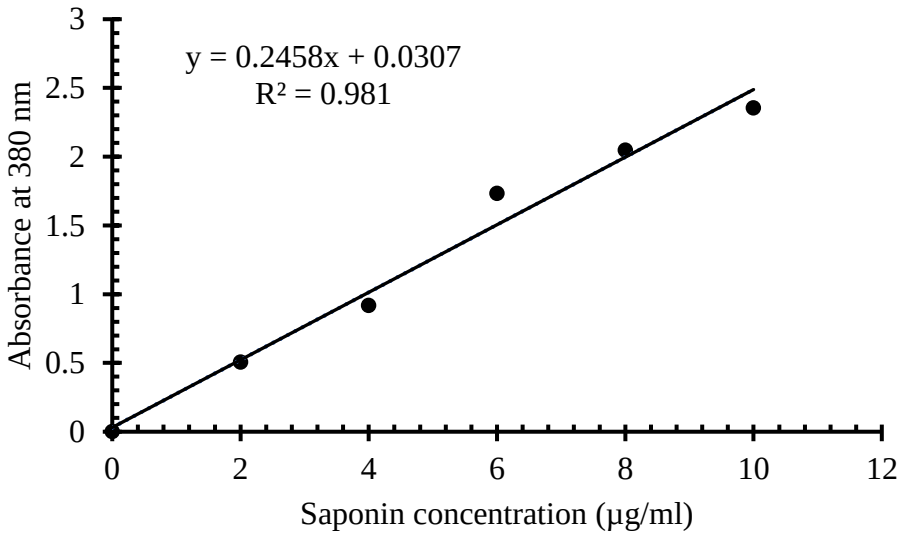

Fig B.3 Standard curve for saponin determination

Color plates

Plate 1 Soaked Mung bean

Plate 2 Dehulled Mung bean

Plate 3 Germinated Mung bean

Plate 4 Cooking of Soaked mung bean

Plate 5 Roasting of Mung bean

Plate 6 Titration for determination of phytate

Plate 7 Sample preparation for determination of polyphenols

Plate 8 Spectrophotometric determination of saponin