EFFECT OF PROCESSING ON NUTRITIONAL AND ANTI-NUTRITIONAL COMPONENTS OF RED KIDNEY BEAN (*Phaseolus vulgaris)*

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

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Approval Letter

This ***dissertation*** entitled ***Effect of processing on nutritional and anti-nutritional components of red kidney bean (Phaseolus vulgaris)*** presented by **Junika Baral** has been accepted as the partial fulfillment of the requirements for the **Bachelor degree in Science in Nutrition and Dietetics.**

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Junika Baral

Abstract

Red Kidney Bean ***(****Phaseolus vulgaris****)*** was collected from Itahari municipality, located in Sunsari district in Province No.1 on the month of January 2022. The main aim of this study was to determine the effect of processing on nutritional (Iron, Calcium, Potassium, Sodium and Ascorbic acid) and anti-nutritional components (oxalate, phytate, polyphenol and tannin) of red kidney bean. The effect of different treatments as soaking (12 h), germination (48 h and 96 h), dehulling (12 h soaking and dehulled), raw open cooked (55 min at 100ºC), soaked open cooked (12 h soaking and open cooking), raw autoclaved (15 min at 121ºC), soaked autoclaved (12 h soaking and autoclaved) on the anti-nutritional components of red kidney bean were studied at Central Campus of Technology.

The mean value of tannin, polyphenol, phytate and oxalate content in raw red kidney bean were found to be 480.43 mg/100 g, 770.34 mg/100 g, 630.69 mg/100 g and 250.09 mg/100 g respectively on dry basis. Maximum reduction of anti-nutrients: Tannin (74%), Polyphenol (69%) and Phytate (72%) were found when red kidney bean was soaked and dehulled. Maximum reduction of oxalate (69%) were found when red kidney bean was soaked and autoclaved. The reduction percentage by soaking was less effective method compared to other methods to reduce tannin, phytate, oxalate and polyphenols. Soaked autoclaving was the most effective method for the reduction of anti-nutrients of red kidney bean in case of cooking treatments. Dehulling of red kidney bean is concluded as most effective among the processing techniques. However, soaking was done prior dehulling, so the resulting reduction in anti-nutrient was because of combined effect of soaking and dehulling rather than the singlet process. Hence, combined effect of treatments was more effective than single process. However, the processing methods such as soaking, dehulling, germination, raw open cooking, raw autoclaving, soaked open cooking and soaked autoclaving reduced the anti-nutrients of kidney bean significantly ($p<0.05$).

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Abbreviations	Full form
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ANC	Anti-nutritional Component
CCT	Central Campus Technology
D.F.	Degree of freedom
FAO	Food and Agriculture Organization
LSD	Least Significant Difference
S.D	Standard Deviation
USDA	United States Department of Agriculture

Part I

Introduction

1.1 General introduction

The term legumes have been mainly derived from Latin word ‘legumen’ which means the seeds that are harvested in pods. In some regions, legumes are considered as pulses, pea, or member of beans family. The edible seeds of legumes play an important role in diet providing essential nutrients with medium to high calories value (Sindhumathi *et al*., 2019).

Legumes are broad diversity of crops that are included in flowering plants producing seeds in pods that are often cultured for food and feeds. Legumes ranked as 3rd largest family of flowering plants having more than 19500 species and over 750 genera (Abbas and Ahmad, 2018) . Legumes represent a major source of nutrients, including valuable but incompletely balance protein, particularly in vegetarians’ diet (Ghadge *et al.*, 2008).

Kidney bean (*Phaseolus vulgaris*) is an important leguminous human food cultivated in arid and semi-arid areas of Pakistan. Both raw and cooked food legumes are consumed traditionally either alone or combined with cereals and other food groups. It is well established that the proteins of food legumes and cereals are nutritionally complementary in respect of Sulphur-containing amino acids and lysine, and a balanced blend or mixture of both grains has a greater nutritional value than either ingredients alone (Yasmin *et al*., 2008a). To avoid predation, sedentary species (plants, fungi, and bacteria) synthesize a range of low and high molecular weight compounds. These secondary metabolites play a role in defense against herbivores, insects, pathogens, or adverse growing conditions. Many of these compounds may be labeled as anti-nutrients in the human diet due to their occurrence in fresh foods and processed foodstuffs (khokhar and Aptenten., 2003a).

The terms anti-nutrient or natural toxicant have been widely employed to describe plant defense metabolites in the food and nutrition literature. The observed biological effects vary greatly, depending upon the structures of the individual compounds, which can range from high molecular-weight proteins to simple amino acids and oligosaccharides. Consumption of raw legumes leads to nausea, vomiting, and diarrhea. More chronic effects of protein anti-nutrients include pancreatitis and some forms intestinal cancer although such effects have not been proven unequivocally.

A number of anti-nutrients have been shown to possess beneficial properties, for example, anticancer, antimicrobial). Such compounds are of increasing interest in the fields of biochemistry, medicine, pharmacology, and nutrition. Physical and chemical methods employed to reduce or remove anti-nutritional factors include soaking, cooking, germination, fermentation, selective extraction, irradiation, and enzymic treatment (khokhar and Apenten., 2003b).

1.2 Statement of the problem

Kidney bean is a nutritious legume eaten all over the world. Cooked food legumes are consumed traditionally either alone or combined with cereals and other food groups. The scientific validation of the traditional processing methods in terms of food safety and quality has not been attempted. In Nepal, there is poor documentation and very few researches were done previously in anti-nutritional content of red kidney bean. Due to poor documentation and poor research there is less knowledge about the household treatment to reduce anti-nutrients of red kidney bean. A number of treatment methods like soaking, open cooking, pressure cooking, germination and dehulling are used for the lowering its anti-nutrients and toxicity, the information on comparative effectiveness of these methods are still the subject matter of research. The documentation of the processing methods which are effective in reducing these factors may significantly contribute in reducing health risk that are associated with consumption of red kidney bean. As a result, to enhance the reduction of anti-nutritional components by house hold treatments towards improving the nutritional properties of red kidney beans are more than validated**.**

1.3 Objectives

1.3.1 General objectives

The general objective of this work is to study the effect of processing methods on nutritional and anti-nutritional components of red kidney bean.

1.3.2 Specific objectives

The specific objectives were as follows:

1. To determine the tannin, oxalate, phytate and polyphenol content of red kidney bean.
2. To determine the physical and chemical properties of red kidney bean.

3. To determine iron, calcium, sodium and potassium in red kidney bean.
4. To determine the effect of reduction pattern of anti-nutrients in red kidney beans by processing methods such as soaking, open cooking, pressure cooking, germination, and dehulling.

1.4 Significance of the study

Legumes are rich sources of carbohydrates, proteins, fats, minerals, fiber, antioxidants and vitamins; these beans are considered low in fat and are cholesterol free but some legumes are rich in oil such as soybean (Hayat *et al.*, 2014) . Due to its high protein contents these are considered as meat of poor man, the researchers working on identifying and evaluating legumes as an alternative protein source of crop in future (MartínCabrejas, 2019) . Kidney beans are regarded as an important source of protein and minerals for livestock feed production, as well as, potential raw materials for processing into human food (Shimelis and Rakshit., 2007a). Reducing antinutritional component has dual benefits i.e. enhanced mineral absorption as well as improves the utilization. Thus, this study specifically determines the content of nutrients and anti-nutrients in red kidney bean and effect of various processing methods to reduce those anti-nutrients. The results of this study might help in the establishment of the effective and optimized way for the use of red kidney bean in household level and industrial levels, which can help to improve its production and utilization potentials.

1.5 Limitations of the study

i. The anti-nutrients present in red kidney bean such as hemagglutinin and trypsin inhibitor was not determined.
ii. During germination, variation was done only on time factor.

Part II

Literature Review

2.1 The origin and distribution of red kidney bean

Common bean or red kidney bean is a major grain legume crop present all over the world, and third in importance after soybean and peanut. This is one of the most ancient crops, and the ancestors of *P.vulgaris* appear to have spread in both North and South from a region centered in Ecuador and Northern Peru. Kidney beans then were brought to Europe and Africa during the sixteenth century by visiting Spanish and Portuguese explorers (Jati *et al.*, 2013) . It is derived from common bean ancestor; usually dark red in color and kidney shaped. Also known as common bean, snap dragon, navy bean, pinto bean, rajma and surkh lobia. Leading producing countries are Brazil, United States, India, China, Turkey and Ethopia (Shehzad *et al.*, 2015).

It is the most widely produced and consumed food legume in Africa, India, Latin America and Mexico (Shimelis and Rakshit, 2007b). It grows optimally at temperatures of 18 to 24°C. Cultivated under rain fed conditions the crop requires a minimum of 400 to 500 mm of rain during the growing season, but an annual total of 600 to 650 mm is considered ideal. Beans prefer an optimum soil pH (H_2O) of 5.8 to 6.5, and are very sensitive to acidic (pH (H_2O) < 5.2) soils (acid saturation above 10 %).They will also not grow well in soils that are compacted, too alkaline or poorly drained (Liebenberg, 2002).

2.2 Classification and nomenclature

The scientific name of red kidney bean is *phaseolus vulgaris*. According to USDA, the Taxonomy hierarchy of red kidney bean is given below:

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Rosidae

Order: Fabales

Family: Fabaceae / Leguminosae – Pea family

Genus: *Phaseolus* L. – bean

Species: *Phaseolus vulgaris* L. – kidney bean Source:(U.S.D.A)

2.3 Physiology and morphology of red kidney bean (Structure of kidney bean)

Phaseolous vulgaris is a herbaceous annual plant and grown throught the world for their edible seeds. Kidney bean varieties form an erect bush of 25 to 60cm tall and pole or running varieties can form vines of 2 to 3-meter long. All cultivars of red kidney beans have green or purple leaves, which are divided into 3oval, smooth-edged leaflets, each of 6 to 14 cm long and 3 to 10 cm wide. The white, pink or purple flowers are about 1 cm long, and the pods would be 8 to 20 cm long and 1 to 1.5 cm long. These may be green, yellow, black or purple in color depending on cultivar and each contains 4-5 beans. The kidney beans are smooth, plump, kidney shaped and up to 1.5 cm long (Nassar *et al.,* 2010).

Habitat: Terrestrial

Flower petal color: Blue to purple, pink to red, white

Leaf type: The leaves are compound (made up of two or more discrete leaflets).

Leaf arrangement: Alternate: there is one leaf per node along the stem.

Leaf blade edges: The edge of the leaf blade is entire (has no teeth or lobes).

Flower symmetry: There is only one way to evenly divide the flower (the flower is bilaterally symmetrical).

Number of sepals, petals or tepals: There are five petals, sepals, or tepals in the flower.

Fruit type (general): The fruit is dry but does not split open when ripe. The fruit of kidney bean is a simple dehiscent legume which develop from a single carpel, cylindrical, constricted between the seeds, splitting along both sutures at maturity (Nassar *et al*., 2010).

Inflorescence: Flowers few to many, racemose or fascicled on axillary peduncles, white, yellow, red, purple, papilionaceous, closely subtended by 2 bracts like an outer calyx which are sometimes deciduous; lobes of calyx equaling or exceeding its tube; keel coiled, being the distinctive mark of the genus; stamens 9 and 1; style bearded longitudinally (Nassar *et al*., 2010).

Pods: Pod slender, curved or somewhat straight, long, about 13 cm in length with prominent beak, narrower, about 0.8-1.0 cm in width, usually 4-5 seeded and glabrous (Nassar *et al.,* 2010).

The fruit and the seed: Fruit of kidney bean plant is green in color and turned into yellow brown when matured. It is simple dehiscent legume (pod), splitting along both sutures at maturity. Seeds are red in color, relatively large in size, kidney in shape and hilum usually white. Seed length about 1.6 cm, breadth about 0.7 cm and thickness about 0.6 cm. Embryo is relatively large and dicotyledonous. Endosperm is absent (Nassar *et al.,* 2010).

A kidney-shaped bean has the following structures:

Testa: The protective layer on the outside is called a testa which is usually very thin but tough. Within the surface of the testa, there is a tiny opening called the micropyle, which is where water can enter and start the germination process, breaking the dormant stage (Nassar *et al.,* 2010).

Cotyledons: The cotyledons are the two large parts of the bean seed that takes up most of the space within the bean. These serve as food storage for the young bean plant until it grows large enough to support itself (Nassar *et al.,* 2010). The development of cotyledons are of two types:

Hypocotyl – part of the stem, between the cotyledons and the foot. It is found below the cotyledons and above the radicle.

Epicotyl – part of stem above the cotyledons and below the plumule.

Embryo: The embryo is the infant plant made up of two parts: the radicle, or the first root, and the plumule, or the first leaves. When water enters the micropyle, the radicle starts growing and moves down and out through the micropyle into the soil below and the plumule starts growing upwards (Nassar *et al.*, 2010).

Hilum: The hilum, or scar, on a bean is the site where the bean originally attached to the fruit of the plant.

The stem is erect, green in color, ribbed, cylindrical and solid. Plant not more than 60 cm in height (determinate bush type). Stem nodes are few (6-8 in number). Branching starts at the second internode from the base then continuous upwards. Noteworthy that, the lateral branches play a vital role in yield production. They are mainly responsible of flowering (Nassar *et al.*, 2010). The general structure of kidney bean fruit is shown in figure 2.1.

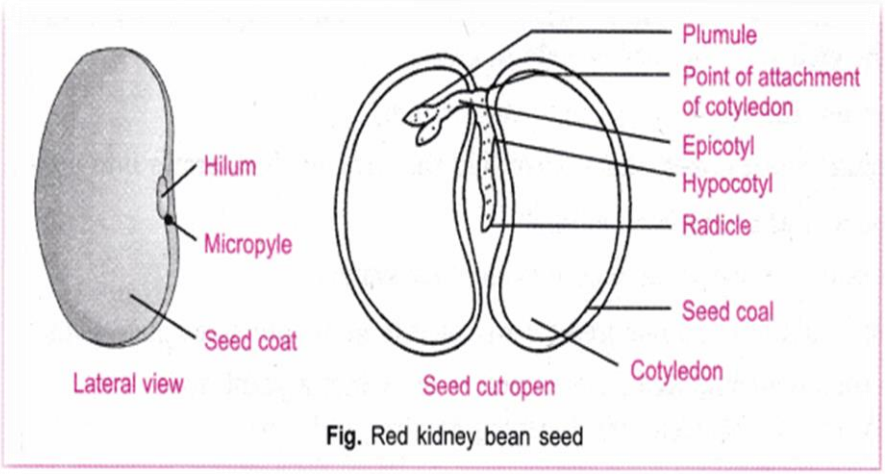

Fig.2.1 structure of red kidney bean

2.4 Chemical and nutritional composition of red kidney bean

Red kidney beans are splendid sources of energy, proteins, carbohydrates minerals and vitamins (Rehman and Shah, 2004). These are renowned for their higher dietary fiber, minerals and protein contents. The flour and protein essence these red kidney beans depicted vital functional features (Tang, 2008). The merit of this bean is its great caloric index and

protein quantity. The scientists have provoked that small amount of phenolic compounds and phytates (available in legumes) may be defensive against cardio vascular disease (CVD) and cancer. Beans have two or three folds more amounts of protein than cereals and provide a suitable path for eliminating protein malnutrition. Thus the potential for beans to be used as nutraceuticals and functional food is thus very promising. The nutraceutical components of food support health and also provide basic nutrients. Various studies have been proved that consuming red kidney beans reduced the risks of diabetes and obesity (Shehzad *et al.*, 2015).

Table 2.1 Constituents of whole red kidney bean

Constituents	**Amount percentage of dry matter**
Proximate	
Moisture	12.39±4.60
Ash	3.90 ± 0.12
Crude Fat	1.30±0.33
Crude protein	21.83±2.80
Crude fibre	4.51±0.72
Carbohydrate	60.65±2.21
Mineral	
Ca (mg/100gm)	143
Fe (mg/100gm)	6.8
Na (mg/100gm)	18
K (mg/100gm)	1324

Source: (Sasanam *et al.*, 2011)

2.5 Anti-nutritional factor

Anti-nutritional factors (ANFs) are defined as biological components present in foods that can reduce nutrient utilization or food uptake, which leads to impaired gastrointestinal functions and metabolic performance (Nagraj *et al.*, 2020). The terms anti-nutrient or natural

toxicant have been widely employed to describe plant defense metabolites in the food and nutrition literature. The observed biological effects vary greatly, depending upon the structures of the individual compounds, which can range from high molecular-weight proteins to simple amino acids and oligosaccharides. The structures of the anti-nutrients and their chemical properties, especially their heat- lability, dictate which physical processes will be most effective in their reduction or removal, thereby minimizing adverse biological effects (Khokhar and Apenten., 2003c).

They can reduce the nutritional value of a plant by causing a deficiency in an essential nutrient or preventing through digestion when consumed by humans or animals (Prathibha *et al.*, 1995). Several anti-nutritional factors are present in root and tuber crops and are partially neutralized during ordinary cooking (Bhandari and Kawabata, 2005). The remaining anti-nutrients can, however, be responsible for the development of serious gastric distress and may interfere with digestion of nutrients, which inevitably results in chronic deficits in absorption of nutrients. Anti-nutritional factors include cyanogen glycosides, saponin, phytate, enzyme inhibitors (trypsin and amylase inhibitors), oxalate and total polyphenols (Brune., 1989).

2.6 Anti-nutritional factors in red kidney bean

2..6.1 Tannin

The term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyl) to form strong complexes with various macromolecules (Khanal *et al.*, 2004). Tannins are phenolic compounds, which consist of molecular weights greater than 500 Da. One of the properties of these compounds is that they can precipitate proteins. Tannins are secondary compounds, which are formed in plant leaves, fruits and bark (Timotheo and Lauer, 2018).

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 (Ali *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 (D'Mello, 2000). Tannins accumulate mainly in the bran section of the legumes. When ingested, tannins form

complexes with proteins, which cause inactivation of many digestive enzymes and decrease protein digestibility (Joye, 2019).

The dark color and astringent taste of food is often ascribed to tannins. They can have a large influence on the nutritive value of many foods eaten by humans such as vegetables, fruits, chocolate, tea, alcoholic and nonalcoholic beverages, etc. Tannins are phenolic plant secondary compounds and are widely distributed in the plant kingdom, especially in pulses (Arias *et al.*, 2004). Foods rich in tannins are considered to be of low nutritional value because they precipitate proteins, inhibiting digestive enzymes and Fe absorption and affect the utilization of vitamins and minerals from meals (Tinkílíç and Uyaník, 2001).

Tannins are very important commercial products. However, their chemistry is very complex and diverse. They can be classified into two groups, the proanthocyanins (or condensed tannins) and the polyesters of gallic acid and (or) hexahydroxy diphenic acid (hydrolysable tannins, respectively, Gallo- and ellagitannins) (Özacar and ŞENGİL, 2002). Condensed tannins are derivatives of flavanols and hydrolysable tannins are esters of a sugar, usually glucose (Adamczyk *et al.*, 2017). Tannin contents were 610 mg/100 g of raw unprocessed red kidney beans (Yasmin *et al.*, 2008a). The structure of hydrolysable tannin and condensed tannin is shown in Fig 2.2.

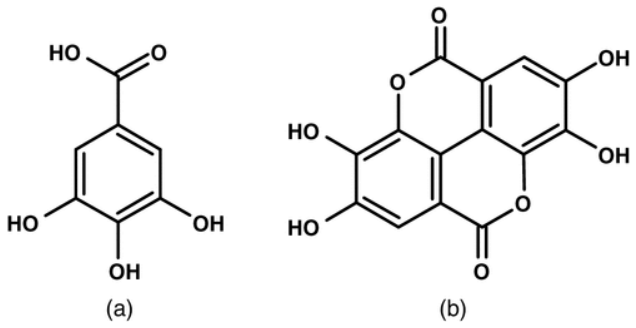

Fig 2.2 Structure of hydrolysable tannin (a) and condensed tannin (b)

2.6.2 Phytic acid

Phytates or phytic acids occur naturally in the plant kingdom. Phytate is generally known as myo-inositol-1,2, 3,4,5,6-hexakis dihydrogen phosphate, which is present in foods at various levels ranging from 0.1 to 6.0% (Gupta *et al.*, 2015). Phytic acid is a secondary compound, which concentrates naturally in plant seeds, mainly in legumes, peanuts, cereals, and oilseeds

and generally found in all plant-based foods (Lolas, 1976). Phytic acid hinders the activity of enzymes, which are necessary for protein degradation in the small intestine and stomach (Kies *et al.*, 2006).

Generally, phytic acids affect the bioavailability of minerals and has a strong effect on infants, pregnant and lactating women when large portions of cereal-based foods are consumed (Al Hasan *et al.*, 2016; Chan *et al.*, 2007). During germination of seeds, some native enzymes are activated, which degrade the phytic acid (Kaukovirta-Norja *et al.*, 2004; Larsson and Sandberg, 1992).

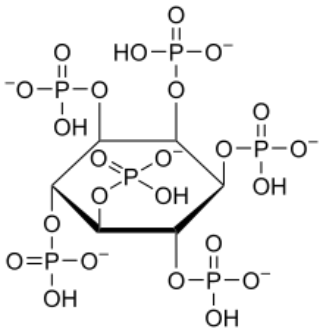

Fig.2.3 Structure of Phytate

In wheat and rice, which are generally recognized as monocotyledon crops, phytates are present in the bran or aleurone layer and can be easily separated during milling. On the other hand, in dicotyledons such as legumes, oilseeds and nuts, phytates are found in close association with proteins, which reduces ease of separation by a simple processing method like milling (Sinha and Khare, 2017).

Phytic acid is generally a negatively-charged structure, which generally binds with positively charged metal ions such as zinc, iron, magnesium and calcium to make complexes and reduce the bioavailability of these ions through lower absorption rates. Mainly due to this chelating property, phytic acid is considered as a most effective anti-nutrient in foods, and a cause of mineral ions deficiencies in animal and human nutrition (Bora, 2014; Grases *et al.*, 2017). The level of phytic acid in unprocessed samples was 610 mg/100 g (Yasmin *et al.*, 2008b).

2.6.3 Oxalate

Oxalates are anti-nutrient compounds present in vegetables such as spinach, chard, beet or rhubarb. These compounds are a strong organic acid with the ability to form water-soluble

salts by binding to minerals such as sodium or potassium, as well as water insoluble salts by binding to calcium, iron or zinc (Lo *et al.*, 2018). Oxalate is an anti-nutrient which under ordinary conditions is restricted to 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).

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

Due to the solubility of oxalate in water, culinary processes, such as boiling and steaming allow to reduce considerably the content of these compounds (Chai and Liebman, 2005). Oxalate content in raw red kidney bean was found to be in the range of 98.86 – 117.01 mg/100 g (Shi *et al.*, 2018).

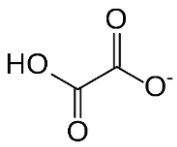

Fig.2.4 Structure of Oxalate

2.6.4 Polyphenols

Phenolic acids are classified as aromatic secondary metabolites of plant (Tomás-Barberán and Espín, 2001) and universally present in plants kingdoms (Dai and Mumper, 2010). They are generally referred to as phenols that have carboxylic acid functionality. These compounds are important for growth and reproduction of plants and are synthesized as a response to environmental factors such as moderate chilling and pollution (Kefeli *et al.*, 2003). Naturally phenolic acids consist of two different carbon structures: (1) the hydroxycinnamic and (2) hydroxybenzoic structure. Hydroxycinnamic acids are formed as simple esters with hydroxyl-carboxylic acid (Mandal *et al.*, 2010) and their derivatives have more antioxidants ability as compared to hydrooxbenzoic acid derivatives. Hydroxycinnamic acid derivatives has more potential for delocalization of the phenoxyl radical (Steenkamp *et al.*, 2013).

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). 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 re-arrangements (Singh *et al.*, 2017). Polyphenols are reported to be present in higher amounts in colored and darker legume varieties than in pale varieties (Salunkhe *et al.*, 1983).

Phenolic acids are usually included in study due to their beneficial role against oxidative damage and degenerative diseases such as inflammation, cancer and cardiovascular diseases (Battisti *et al.*, 2008). They have gained more importance due to their antioxidant properties and used in food products (Dai and Mumper, 2010). Phenolic acids also have unique taste, flavor and health-promoting effects found in fruits and vegetables (Tomás-Barberán and Espín, 2001).

Polyphenols inhibit several digestive enzymes, lower protein as well as starch digestibility and hinder mineral adsorption from the diet. For human consumption food legumes in India are processed in a variety of ways depending upon taste and cultural preferences which are known to affect the level of the anti-nutrients (Subbulakshmi *et al.*, 1976). The total polyphenol contents in unprocessed red kidney bean were 216 mg/100 g (Yasmin *et al*., 2008a).

2.6.5 Trypsin inhibitor

Trypsin inhibitors are a group of serine protease enzymes. They reduce the biological activity of the digestive enzymes trypsin and chymotrypsin. Trypsin is a proteolytic enzyme which is important for the digestion of proteins in living organisms. It is a globular protein with a molecular weight of 24-kDa with 220 residues. This enzyme protein is produced in its inactive form called 'trypsinogen' in the pancreas which is then activated during digestion as it enters the small intestine (Walsh *et al.*, 1964). Trypsin is an enzyme involved in the breakdown of wide range of proteins, primarily as part of digestion in humans and other animals such as mono-gastric and young ruminants. When trypsin inhibitor is consumed, it acts as an irreversible and competitive substrate (Silverman *et al.*, 2001).The ingestion of trypsin inhibitors can result in the formation of an irreversible trypsin enzyme- trypsin inhibitor complex which leads to a trypsin enzyme drop in the intestine, interfering in protein digestibility process (Cabrera-Orozco *et al.*, 2013).

Legumes trypsin inhibitors (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. Soya bean 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 (Van Der Ven *et al.*, 2005).

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

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

2.6.6 Saponin

Saponins are commonly considered as non-volatile, surface active secondary metabolites, which are broadly dispersed in nature but found principally in plants. Saponins are steroids or triterpenes and contain a sugar moiety in their structure. They are naturally produced as foam producing triterpene or glycosides by many plant species, including groundnut, lupin, oil seeds. (Kiranmayi, 2014). Saponins are plant-derived secondary compounds, which are found in more than 100 families of wild and cultivated plants that belong to the Magnoliophyta division. Magnoliophyta can be divided into two key classes: Liliopsida and Magnoliopsida, which contain majority of species that produce saponins (Vincken *et al.*, 2007). Saponins have a property of being able to interact with the cholesterol group of erythrocyte membranes, which leads to hemolysis (Fleck *et al.*, 2019).

Previous studies have reported that saponins also showed inhibitory activities of digestive enzymes such as amylase, glucosidase, trypsin, chymotrypsin and lipase, which can cause indigestion-related health disorders (H. Ali *et al.*, 2006; Birari and Bhutani, 2007; Ercan and El, 2016).Saponins are not readily hydrolyzed by the human digestive enzymes, therefore gastrointestinal digestion can be severely impaired (Amin *et al.*, 2011). Previous studies have demonstrated that animal metabolism and health could be affected by saponins in different ways. The effects include bloating in ruminants, reduced nutrient absorption, decreased liver cholesterol and overall growth rate, and reduced intestinal absorption of many nutrients through binding of saponins to the small intestine cells (Addisu and Assefa, 2016; Kregiel *et al.*, 2017).

It should be noted that the low levels of saponins in legumes may not be injurious to health but could become toxic when consumed at higher concentrations in the diet (Muzquiz *et al.*, 2004). Saponins are also considered as factors that reduce absorption of vitamins. It has been suggested that saponins can form complexes with various sterols that have similar structures as fat soluble vitamins, which would interfere with sterol activity and absorption (Cheeke, 1971).

2.6.7 Lectin

Lectins and hemagglutinins are a form of sugar-binding proteins, which easily attach to red blood cells to cause agglutination. These anti-nutrients are mainly found in foods, which are consumed in raw forms (Hamid *et al.*, 2013). 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).

Phytohemagglutinin is a tetrameric glycoprotein with a molecular mass of 120 kDa, which is found in kidney beans and also consists of two diverse subunits (Lajolo and Genovese, 2002) Cereals and legumes generally contain lectins, which are glycoproteins. In addition, transport and hydrolytic functions of the enterocyte would be impaired by consumption of foods that contain lectins (Krupa, 2008).

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)

Purified lectins from beans or soybeans impaired rat growth, induced enlargement of the small intestine, caused damage to the epithelium of the small intestine, and stimulated hypertrophy and hyperplasia of the pancreas. Lectins impair nutrient absorption by binding to intestinal epithelial cells, and also cause damages in the intestinal tract, which allow bacterial population to come into contact with the blood stream (Muramoto, 2017).

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 amount of lectin present in unprocessed roba variety of red kidney bean was found to be 1.92 g/kg PHA (Shimelis and Rakshit., 2007a).

2.6.8 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). Humans do not have the enzyme α-galactosidase to cleave the α-galactosyl linkage and the intact oligosaccharide is not absorbed by the digestive tract. These oligosaccharides accumulate in the large intestine where the α-galactosidase containing intestinal bacteria degrade them and subsequent anaerobic fermentation results in production of H_2, CO_2 and traces of CH_4. These gases cause abdominal discomfort due to a flatus effect and sometimes result in diarrhea (Stoddard *et al.*, 2010). Due to these effects, these oligosaccharides are known as flatus-producing carbohydrates (Sefa-Dedeh and Stanley, 1979).

Traditional methods of processing grain legumes prior to their consumption significantly reduced the level of oligosaccharides (Hill, 2003), and the flatulence side effect of legume oligosaccharides can be minimized in different ways such as processing, plant breeding, incorporation of microbial or plant α-galactosidase, or by changing the water in which beans are boiled one or more times (Stoddard *et al.*, 2010; Venter and Eyssen, 2001).

2.7 Different methods for the reduction of anti-nutritional 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).

Several factors influence the nutritional and anti-nutritional content of food. These include the genetic make-up of the plant, the soil in which it is grown, use of fertilizer, prevailing weather, maturity at harvest, packaging, storage conditions and method utilized for processing (Agiang *et al.*, 2010). Different effective processing has been done to reduce the anti-nutritional factor in red kidney beans.

2.7.1 Soaking

Soaking could be one of the processes to remove soluble anti-nutritional factors, which can be eliminated with the discarded soaking solution. However, some metabolic reactions can take place during soaking which will affect some of the constituent compounds (Verde *et al.*, 1992).Soaking is an attractive method for removing anti- nutrient content of foods because it also reduces cooking time. Soaking also enhances release of enzymes (e.g. endogenous phytases), which are present in plant foods like almonds and other nuts and grains. Soaking generally provides essential moist conditions in nuts, grains and other edible seeds, which are required for their germination and associated reductions in level of enzyme inhibitors as well as other anti-nutrients to enhance digestibility and nutritional value (Kumari, 2018).

Soaking process decreases the monosaccharide, disaccharides, and oligosaccharides in kidney beans. Change in the carbohydrate composition was seen less when the cooking water not drained off (Vidal-Valverde *et al.*, 1994). Vidal-Valverde *et al.* (1994) reported retention of 98-99% of trypsin inhibitor activity in many cultivars of Phaseolus vulgaris after the seeds were soaked in water for 18 h. Dhurandhar and Chang (1990) soaked navy and red kidney beans for 16 h in water at ambient temperature and both showed insignificant decreases in trypsin inhibitor activity. However, the reduction of anti-nutrients may depend on the type of legume. In kidney beans, soybeans and faba beans, soaking reduces protease inhibitors only very slightly. Not only is soaking useful for legumes, leafy vegetables can also be soaked to reduce some of their calcium oxalate. Soaking is typically used in combination with other methods, such as sprouting, fermenting and cooking (Arnarson, 2017).

Soaking of red kidney bean in water for 9 h reduces phytic acid by 0.8%, phenols by 12,8%, cyanide by 7.7% (Yasmin *et al*., 2008b). Soaking of faba bean in water for 12 hours, significantly reduced phytic acid content by 32.7% . Kataria *et al.* (1988) reported that soaking of black gram in plain water for 18 h reduced polyphenols by 10%. Soaking mung bean for 18 h in distilled water reduced phenols by 7% (Kataria *et al.*, 1989). Soaking the seeds for 12 h reduced the polyphenol contents of both the legumes significantly: 48% in chickpea and 47% in black gram (Jood *et al.*, 1987).

Soaking the legumes seeds in distilled water significantly decrease ($p<0.05$) the total oxalate content in range from 17.4% - 51.89%. 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).

2.7.2 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). This process however reduce/remove some anti-nutritional components (ANCs) such as tannins, saponins and total phenolics but is liable to increase the level of phytic acid, trypsin inhibitor, chymotrypsin inhibitor and α-amylase inhibitor. This may be due to higher concentration of these ANCs in pulse cotyledon as compared with the hull . Further, dehulling removes embryo and gummy layer present between hull and cotyledons (Goyal *et al.*, 2010), which may also be responsible for changes in ANC concentration. The wet dehulling method involves soaking of pulses in water for 6–8 h during which some water-soluble ANCs may leach out, though it is not quantified (Vishwakarma *et al.*, 2018).

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 (Wang *et al.*, 2009), faba bean and kidney bean . 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). 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). 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), faba bean and kidney bean (Alonso *et al*., 2000a).

2.7.3 Cooking

It is probably the oldest treatment for making legumes edible. Usually it includes a prior soaking of the seeds and subsequent cooking in boiling water until they become tender. Addition of mineral salts to the soaking and or cooking media can produce a reduction in the cooking time (Prodanov *et al.*, 2004; Van Buren, 1986). In general, cooking produces denaturation of proteins and their diffusion into cooking water (Haytowitz and Matthews, 1983), inactivation of heat sensitive factors, such as trypsin inhibitors (Frias *et al.*, 2000), decrease of phytic acid and a-galactoside contents (El-Adawy, 2002).

Prior to consumption, the legumes are generally subjected to three types of cooking: ordinary cooking, pressure-cooking or autoclaving and microwave cooking. The traditional method for home preparation of most legume seeds consists of a water soaking period (usually overnight) followed by cooking of the rehydrated seeds including or after discarding the soaking water. Legume seeds become edible after prolonged boiling in water or after a shorter time when a pressure cooker is used (Abdel-Gawad, 1993). Soaking and/or cooking can cause considerable losses of essential nutrients such as water-soluble minerals, vitamins and starch, due to their high solubility and thermal instability (Abdel-Rahman, 1983; El-Adawy, 2002).

There are two types of cooking generally practiced traditionally as well as industrially they are:

a) Open

b) Pressure

Pressure cooking and boiling resulted in significant destruction in the anti-nutrients like phytates, tannins and trypsin inhibitors. Among them pressure cooking preserves more nutrient as compared to open cooking/normal cooking (Deol and Bains, 2010).

2.7.3.1 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*., 2008a).

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). The reduction in polyphenol and tannin of presoaked cooked red kidney bean is 61% and 82-93% (Yasmin *et al.*, 2008a). 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).

2.7.3.2 Autoclaving

Cooking under pressure is what autoclaving implies. This procedure reduces the amount of time it takes to cook. The thermolabile, 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).

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).

Less than 10% of total tannin decomposed during cooking, while up to 50% were leached to the cooking liquor (Ziena *et al.*, 1991). Sharma and Sehgal (1992)reported that significant reduction in tannins (76-81%) after cooking of two faba bean cultivars. Tannin content in lentils increased after cooking (Vidal-Valverde *et al.*, 1994). Rehman and Shah (2005) stated that tannin content of black grams, red kidney bean and white kidney bean significantly reduced after ordinary cooking and pressure cooking at 121 ^{0}C for 20 min, respectively.

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. Similarly, a loss of total oxalate contents of 76% in white bean, 59% in soybean and 40% in red bean due to cooking was found by (Akhtar *et al.*, 2011). Loss of soluble oxalates in cooking water was considered to be the primary factor contributing to total oxalates reduction (Akhtar *et al.*, 2011).

2.7.4 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 hydrolysing 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, protein and carbohydrates. Germination process is studied extensively for degradation of ANCs in pulses. However, the extent of degradation depends on the type of pulses, type of ANCs and germination conditions. It is suggested that the proteases are responsible for inactivation of proteinaceous ANCs such as enzyme inhibitors and lectins. During germination, phytic acid is hydrolysed by an endogenous enzyme, phytase, into inorganic phosphorus, the biologically available form, for plant growth and development. The phytic acid present in pulses therefore converts into a soluble form, and due to this phenomenon, the reduction in phytic acid content of germinated pulses has been demonstrated by many researchers (Camacho *et al.*, 1992).

Germination modifies the quantitative and qualitative phenolic composition of pulses (López-Amorós *et al.*, 2006). This process has shown up to 20.8% reduction in total cyanide

content in kidney bean (Yasmin *et al*., 2008b). It also reduces the content of enzyme inhibitors such as trypsin inhibitors, α-amylase inhibitors and chymotrypsin inhibitors in pulses (Alonso *et al*., 2000b).

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 (Paul *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). Sharma and Sehgal (1992) reported that 48 hours germination of two faba bean varieties (VH-131 and WF) reduced tannin content by 90 and 91%, respectively. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. Similar losses of phytic acid during soaking and germination have been reported by (Grewal and Jood, 2006).

The reduction of Tannin was 47%, 51% and 60.3% after 2 days, 4 days and 6 days of germination for SML cultivar. Tannin content was reduced by 66.9%, 75% and 78% after 2, 4 and 6 days, respectively. Reduction in tannin content after germination may be attributed to the leaching out effect during hydration (Beleia *et al.*, 1993). 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/100g which decreased to 308 mg/100 g during 18 h germination and 341 mg/100 g 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 and same has been previously investigated by Murugkar *et al*. (2013) and Pal *et al*. (2016). Similarly, sprouting was also considerably effective in decreasing the polyphenol contents of both the legumes (19-28% in chickpea and 17-20% in black gram) (Jood *et al*., 1987).

2.7.5 Combination of several processing methods

Legumes contains different anti-nutritional factors. Eliminating the anti-nutritional factors by single processing method only gave partial detoxification and that the use of one method of processing may not affect the desired removal of the anti-nutritional factors. Therefore, combination of two or more methods is required. According to Effiong and Umoren (2011) ,soaking the seeds in water prior to cooking was more effective in improving the nutritional value of the legumes than cooking alone of legumes. They reasoned that soaking prior to cooking may have open up more surface area for heat penetration.

2.7.6 General uses of kidney bean

Red kidney 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. It is mainly consumed as curry with rice.
c. Some types of these dry beans or the remaining husk can be used to make an "extract." This extract is used as medicine.
d. Kidney bean flour is used in making papad, dosa, idli, vada etc.
e. The crop is also utilized as fodder and green manure.

2.8 Health benefits of kidney bean

Health benefits of beans are manifold. Their low glycemic index (class III) helps maintain healthy blood glucose levels and healthy weight levels. Beans are rich in folate that plays a role in reduction of birth defects and maintain healthy vascular heart condition. The abundance of iron and other vitamins and minerals, such as B_1, B_3, and pantothenic acid, potassium, copper, and phosphorus, in beans can promote overall health (Zhang *et al.*, 2008).

It has been reported that phenolic acids present in red kidney beans have anticancer characteristic (Duranti, 2006; Nyau, 2014). The beneficial actions of phenolic acids and flavonoids in preventing CVD are due to their antioxidant activity (Heim *et al.*, 2002), prevention of atherosclerosis (Tripoli *et al.*, 2007), effects on platelet aggregation (Lamuela-Raventós *et al.,* 2005). Phenolic compounds and their bacterial fermented products show beneficial effects in colon. The diets rich in flavonoids also effect the microbial composition of gut flora (Gee and Johnson, 2001).

2.9 Physical properties of kidney bean

2.9.1 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 (Unal *et al.*, 2008). By using the 1000 kernel weight, a producer can account for seed size variations when calculating seeding rates, calibrating seed drills and estimating shattering and combine losses (Miller and McLelland, 2001).

2.9.2 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 (Ashwin Kumar, 2017).

2.9.3 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).

Part III

Materials and methods

3.1 Materials

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

3.1.1 Collection of kidney bean

Red kidney bean of unknown history was purchased from Itahari municipality at Sunsari district, Province No. 1, Nepal on the month of January.

3.1.2 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 3.1

Table 3.1 List of chemicals used

Chemicals	
Sulphuric acid	Folin-ciocalteu reagent
Oxalic acid	Tannic acid solution
Boric acid	Methanol
Sodium hydroxide solution	Acetic acid
Ammonium hydroxide solution	Gallic acid
Sodium carbonate solution	Calcium chloride solution
Ammonium thiocyanate solution	Ethanol
Hydrochloric acid	Potassium permanganate solution
Iron chloride solution	Indicators(Phenolphthalein,Methylorange)

3.1.3 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 3.2

Table 3.2 List of Equipments used

Equipments	
Weighing machine	Spectrophotometer
Distillation set	Desiccator
Soxhlet apparatus	Centrifuge
Titration apparatus	Incubator
Hot air oven	Mortar and pestle
Thermometer	Measuring cylinder
Water bath	Funnel
Autoclave	Muffle furnace

3.2 Methodology

The general outline for processing of red kidney bean is presented in Fig 3.1

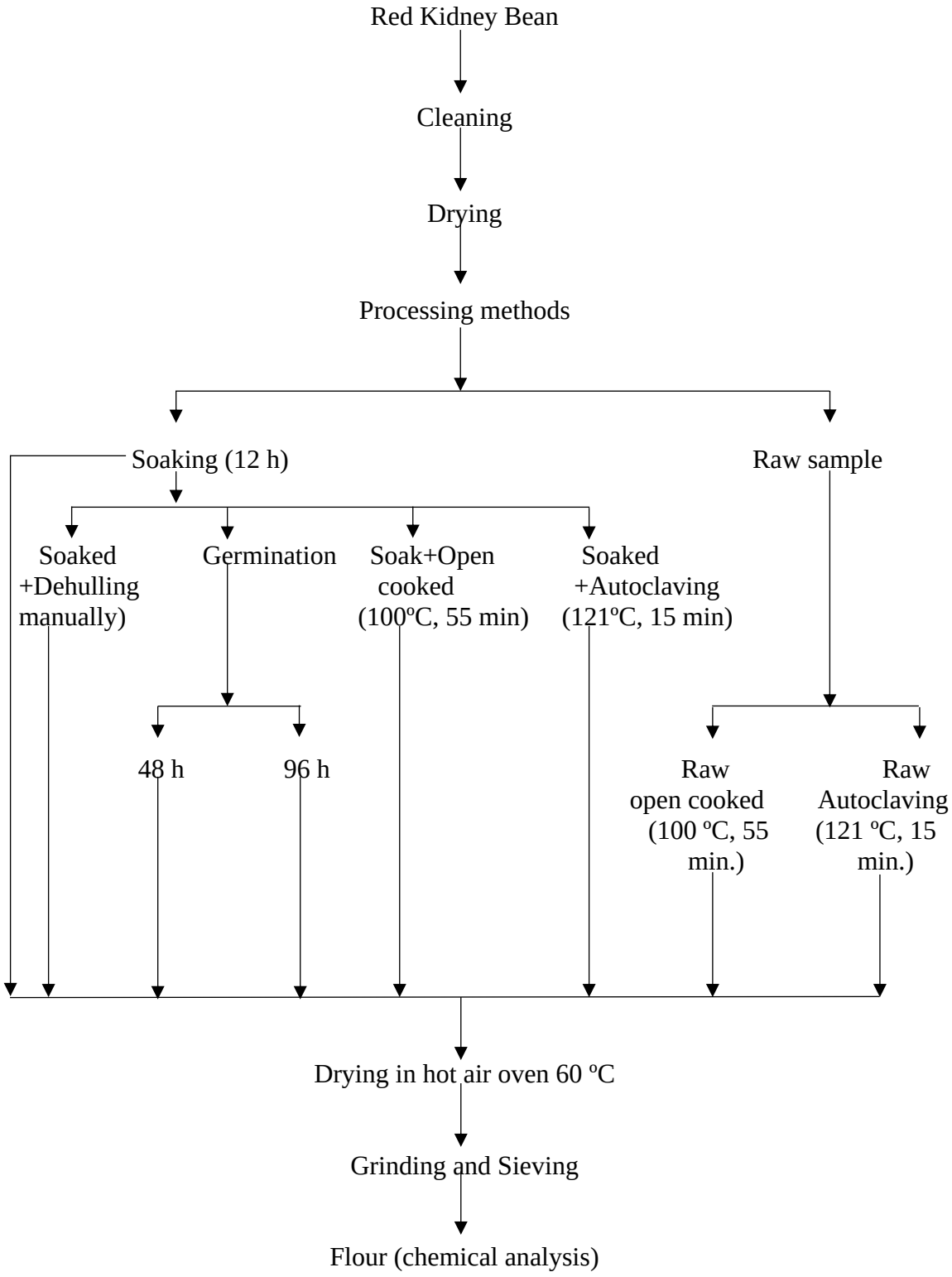

Fig.3.1 General Flowsheet for processing of red kidney bean.

3.3 Processing methods

3.3.1 Soaking

Red kidney bean seeds were soaked in the room temperature (15-18ºC) for 12 h in distilled water. The proportion of seed to soaking solution was 1:5 (w/v). The soaking solution was drained off after soaking time and the seeds were rinsed twice in distilled water, seeds air-dried, and then kept in oven at 60°C for complete drying. Samples were ground to pass through 30-mesh screen. The ground samples were kept in air-tight bottles and stored at 4°C for subsequent analysis (Yasmin *et al*., 2008a).

3.3.2 Dehulling

Seeds were soaked in distilled water for 12 h at 15-18ºC. A seed to water ratio of 1:5(w/v) was used. The soaked water was discarded. The soaked seeds were rinsed twice in distilled water and then dehulled using hand. The dehulled sample were dried in hot air oven maintained at 60°C. Thus, samples were grinded in grinder and sieved using 0.5 mm size mesh and stored in plastic containers at room temperature until required for further analysis (Jood *et al.*, 1987).

3.3.3 Germination

Red kidney bean seeds were soaked in distilled water (1:5 w/v) for 12 h at (15-18ºC). The water was drained off, and the seeds transferred to a moisture adherent flax cloth (tarts) to germinate for 4 days in the dark. Every 24 h, the seeds were moistened with distilled water and carefully shaken. After 4 days of germination, the sprouts and the seeds were ground and dried in an air oven at 60°C for analysis (Yasmin *et al*., 2008a).

3.3.4 Raw open cooked

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

3.3.5 Soaked open cooked

Similarly, the soaked seeds after rinsing in distilled water were put in round-mouthed tall beakers fitted with condensers connected to running water. After adding distilled water (two times the weight of soaked seeds), the samples were cooked on a hot plate until they became soft as felt between fingers, rinsed twice by using distilled water and finally, samples were grinded in grinder and sieved using 0.5 mm sieve and stored in plastic containers at room temperature until required for further analysis (Jood *et al.*, 1987).

3.3.6 Raw pressure cooked

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

3.3.7 Soaked pressure cooked

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

3.4 Analytical methods

3.4.1 Proximate Analysis

The proximate analysis carried out are as follows.

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 red kidney 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 kidney bean

3.4.2.1 Thousand kernel weight

The 1000 kernel weight of kidney bean was determined by measuring the weight of 1000 kernels of red kidney 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 seeds were allowed to flow freely into the measuring bushel. After the bushel was filled, the excess material was leveled off with gentle zigzag strokes using the standard seed buro

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

3.4.2.3 Length by breadth ratio

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

3.4.3 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)

3.4.4 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 kidney 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).

3.4.5 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).

3.4.6 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 100^0C 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 (60^0C) to faint violet color that persisted for at least 15s which is equivalent for 2.2 mg of oxalate (Patel and Dutta, 2018).

3.4.7 Determination of ascorbic acid

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

$$\text{Vitamin C (mg per 100g)} = \frac{\text{Titer} \times \text{Dye factor} \times \text{Volume made up} \times 100}{\text{Aliquot of extract taken(ml)} \times \text{weight of sample(gm)}}$$

3.4.8 Determination of iron

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

$$\text{Iron(mg/100g)} = \frac{\text{Absorbance of sample} \times 0.1 \times \text{Total volume of ash solution} \times 100}{\text{Absorbanceofstandard} \times 5 \times \text{Wtofsampletakenforashing}}$$

3.4.9 Determination of calcium

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

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

3.4.10 Determination of potassium

Potassium solution is automized in an oxy- hydrogen or oxy- acetylene flame. The flame excites atoms of potassium causing them to emit radiations at specific wavelengths. The amount of radiation emitted is measured by the emission flame photometer(768nm). Under standard conditions, the amount of emission is proportional to the concentration of potassium in the sample solution (Rai and KC, 2007)

3.4.11 Determination of sodium

Sodium solution is automized in an oxy-hydrogen or oxy-acetylene flame. The flame excites atoms of sodium causing them to emit radiations at specific wavelengths. The amount of radiation emitted is measured by the emission flame photometer(768nm). Under standard conditions, the amount of emission is proportional to the concentration of sodium in the sample solution (Rai and KC, 2007).

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 techniques were subjected to analysis of variance (ANOVA) and considered at 95% confidence level using statistical software GenStat. Means of the data were compared by using Fisher's protected LSD method at 5% level of significance.

Part IV

Results and discussion

Red kidney beans (*Phaseolus vulgaris* L.), are splendid sources of energy, proteins, carbohydrates minerals and vitamins (Rehman and shah., 2004). These are renowned for their higher dietary fiber, minerals and protein contents (Tang, 2008). Different processing methods were carried out i.e., soaking, dehulling, germination, and cooking (open and pressure cooking). Thus obtained processed samples were analyzed to study the effect of different processing methods on its nutrients and anti-nutrients by single and combination of processing methods.

4.1 Physical properties of red kidney bean

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

Table 4.1 Physical properties of red kidney bean

Physical properties	Red Kidney bean seed
l/b ratio	2.05 ±0.02
bulk density(kg/hl)	70.73 ±0.22
1000kernel weight (g)	623.52±0.39

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

Altuntas and Demirtola (2007) found that the thousand kernel weight of kidney bean seeds was 709.80g in which our data was lesser than their findings because of different variety. The value of l/b ratio of raw kidney bean seed was found to be 2.05 which means the red kidney bean seeds was bold in nature. The value of length breadth ratio was observed from 1.64 – 2.49 in which our obtained data was in range. Bulk density of red kidney bean was found to be 70.73 Bulk density was found to be in the range of 72-87kg/hl in which our obtained data was slightly low. However, the values are lower than reported by other authors. The bulk density of a material depends on the solids density and the geometry, size and surface properties of the individual particles (Wani *et al.*, 2014).

4.2 Proximate composition of raw red kidney bean

The proximate composition of raw red kidney bean is given in Table 4.2.

Table 4.2 Proximate composition of raw red kidney bean (dry basis)

Proximate constituents	Composition (%)
Moisture	11.06±0.13
Protein*	27.43±0.10
Ash*	4.41±0.03
Fat*	1.60±0.08
Crude Fiber*	4.26±0.09
Carbohydrate*	62.30±1.87

[Values presented are the average of triplicates determination ± standard deviation. *Represents values in dry basis.]

Moisture content of raw red kidney bean was found to be 11.06%. Moisture content of raw sample of kidney bean variety was found to be in the range of 10.1-12.7% (Khatoon and Prakash, 2004). The value was found to be 12.39 by (Sasanam *et al.*, 2011) and 7.32 by (Olanipekun *et al.*, 2015). The protein content in the kidney bean was found to be 27.43% which was similar to the data found by (Khattab and Arntfield, 2009) i.e. 28.12%.The protein content in common bean range from 21.1–39.4% (Salunkhe *et al.*, 1986).The data was found to be 23.6% by (Duke, 2012), (Fan and Sosulski, 1974), (Wolf, 1988) but the value was 21.83 by (Sasanam *et al.*, 2011). The ash content of raw red kidney bean was found to be 4.41% which is comparable to the data found by (Khattab and Arntfield, 2009; Sasanam *et al.*, 2011) i.e. 3.9% , similarly Bhagya *et al.* (2007) found 3.6%. It was found that the fat content of raw red kidney bean was 1.60% which was similar data found by (Chaudhary and Sharma, 2013) i.e. 1.5%. The crude fiber content of raw red kidney bean was found to be 4.26 % which is comparable to the data obtained by (Hossain and Becker, 2001) i.e. 4.0%. Carbohydrate was found to be the abundant component, nearly about 62.30% which was similar to the data obtained by (Sasanam *et al.*, 2011) i.e. 60.65%.

4.3 Distribution of anti- nutrients in raw red kidney bean

The mean values of different anti-nutrients determined are presented in Table 4.3

Table 4.3 Distribution of anti- nutrients in raw red kidney bean (mg/100 g).

Anti-nutrients	Values in dry basis (mg/100 g)
Tannin	480.43±5.11
Polyphenol	770.34±4.88
Phytate	630.69±6.48
Oxalate	250.09±2.09

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

The tannin content in the raw red kidney bean was found 480.43 mg/100 g which is greater than the data obtained by (Alonso *et al*., 2000a) i.e. 359 mg/100 g but is less than data obtained by (Yasmin *et al*.,2008a) i.e. 610 mg/100g. Polyphenol content was found to be 770.34 mg/100 g which is higher than the findings of (Alonso *et al*., 2000b) i.e. 207 mg/100 g, i.e. (Yasmin *et al*., 2008a) i.e. 216mg/100 g. Aknod *et al.* (2011) found that polyphenol content in 29 varieties of kidney beans was ranged between 587-1414 mg/100 g so the above obtained data was within range. The phytate content in the raw red kidney bean was found 630.69 mg/100 g which is very similar to the value obtained by (Yasmin *et al.*, 2008a) i.e. 610mg/100 g and (Margier *et al.*, 2018) i.e. 627.33 mg/100 g, but higher than the range obtained by (Rasha Mohamed *et al.*, 2011) i.e.330-550 mg/100 g. The Oxalate content in the kidney bean was found to be 250.09 mg/100 g which was higher than the range obtained by (Shi *et al.*, 2018) i.e. 98.86 – 117.01 mg/100 g. According to different research it is concluded that anti-nutrients value are not absolute they vary according to variety and/or cultivar, climatic conditions, locations, irrigation condition, types of soil and year during which they are grown which was also discussed by (Bassiri and Nahapetian, 1977).

4.4 Mineral Composition of raw red kidney bean

The mean values of different minerals determined are presented in Table 4.4

Table 4.4 Distribution of minerals in raw red kidney bean on dry basis (mg/100 g).

Minerals	**mg/100g**
Iron	7.2±0.29
Calcium	138.7±2.06
Potassium	1133.4±3.08
Sodium	12.7±1.72

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

The iron content in raw red kidney bean was found 7.2 mg/100 g which is similar to the data obtained by (Barampama and Simard, 1993) i.e. 7.12 mg/100g and slightly higher than data obtained by (Barampama and Simard, 1995) i.e. 6.97 mg/100 g. The calcium content in raw red kidney bean was found to be 138.7 mg/100g which is comparable to the data obtained by (Barampama and Simard, 1995) i.e. 139.58 mg/100 g. The amount of calcium was found in the range of 55-200 mg/100 g in mung bean by (Dahiya *et al.*, 2015). The potassium content in raw red kidney bean was found to be 1133.4 mg/100g which is higher than the data obtained by (Barampama and Simard, 1995) i.e. 1084 mg/100 g. The sodium content in raw red kidney bean was found to be 12.7 mg/100 g which is lower than the data obtained by (Audu and Aremu, 2011) i.e. 33 mg/100 g.

4.5 Ascorbic acid content of raw red kidney bean

The mean values of ascorbic acid content of raw red kidney bean was determined on the basis of dry matter and found to 4.21±0.28 mg/100 g. Kambabazi *et al.* (2021) found that ascorbic acid content in red kidney bean was 2.76 mg/100 g which is lower than our finding.

4.6 Effect of processing methods on tannin content of red kidney bean

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

4.6.1 Effect of soaking

Tannin content of raw red kidney bean was determined and the value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 480.43 mg/100 g to 353.18 mg/100 g after soaking i.e., 26.48% reduction.

The result obtained in this study is similar with result obtained by (Shimelis and Rakshit., 2007a) , he reported that the reduction of 25% after 12 h of soaking of kidney bean . In our case, there was slightly higher reduction. But the reduction of tannin in kidney bean after 12 h soaking was found to be 24.2% by (Alonso *et al*., 2000a), which was slightly lower reduction than the values obtained in this study. Abbas and Ahmad (2018) reported that there was 39.4% reduction in the tannin content after soaking for 18 h . The loss of tannin content after soaking may be attributed to leaching out into soaking water under the concentration gradient (Kataria *et al.*, 1988).

4.6.2 Effect of dehulling

Tannin content of red kidney bean was found to be significantly reduced ($p<0.05$) from 480.43 mg/100 g to 124.56 mg/100 g i.e., 74.07% reduction after dehulling process. This study shows that highest reduction of tannin in red kidney bean was seen in dehulled sample.

Deshpande *et al.* (1982) reported that dehulling the seeds reduced the tannin by 85.4%, 72.7%, 93.3% in light red kidney bean, dark red kidney bean, small red kidney bean, the value obtained in this study was found to be similar with dark red kidney bean. 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). Removal of seed coats lowered the tannin content of beans by 68–95% (Deshpande *et al.*, 1982).

4.6.3 Effect of germination

The tannin content of raw red kidney bean was determined and the value obtained showed that there is significant reduction ($p<0.05$) in tannin content, which is reduced from 480.43 mg/100 g to 230.43 mg/100 g and 159.27 mg/100 g after 48 h and 96 h of germination i.e., 52.04% and 66.85% reduction.

(Yasmin *et al.*, 2008a) reported the loss of tannin content by 68.6% in 96 h of germination in red kidney bean which is similar to this study. Similarly Alonso *et al.* (2000a) found that there was 63.8% reduction of tannin in 48 h which was higher than the data obtained in this study. A reduction of 74% and 76% in 48 h and 96 h in roba variety of kidney bean was found by (Shimelis and Rakshit, 2007a) which is higher than this study. Reduction in tannin content after germination may be attributed to the leaching out effect during hydration which was reported by (Kataria *et al.*, 1989).

The tannin content of different processing treatments is given in Fig. 4.1

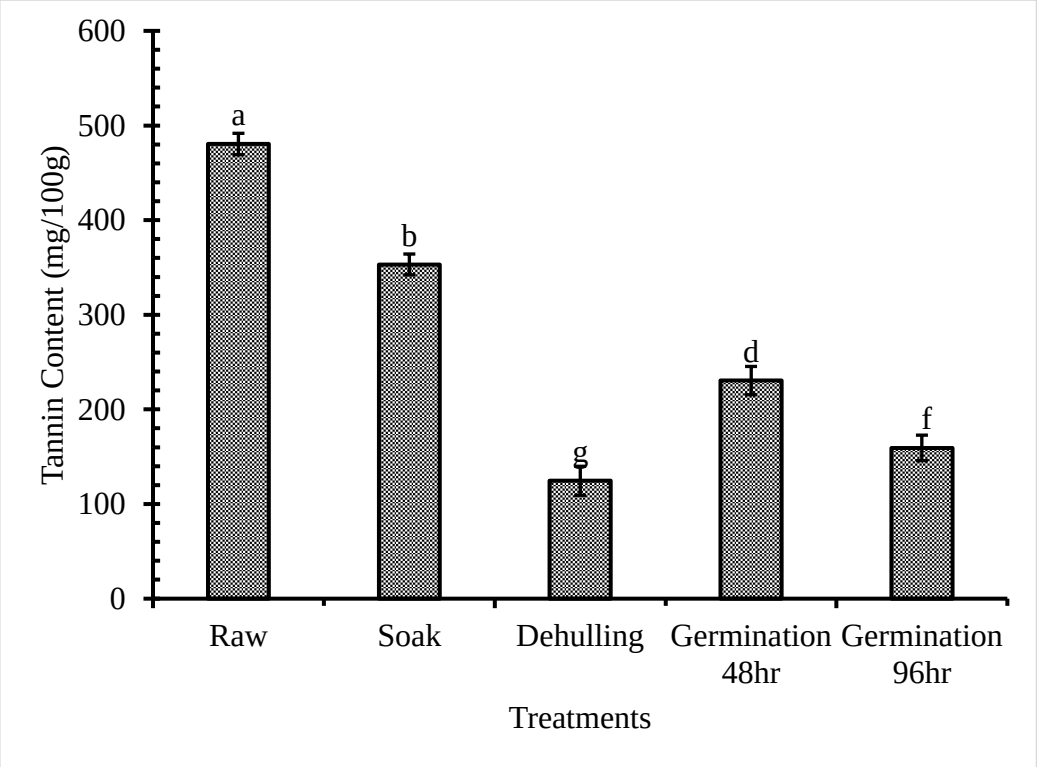

Fig 4.1 Effect of processing method on tannin content

[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.].

4.6.4 Effect of cooking

The effect of open cooking for 55 min and autoclaving at 15 psig for 15 min on total tannin content of red kidney bean was studied. Cooking shows significant decrease ($p<0.05$) in tannin content of red kidney bean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. Different cooking method reduce tannin content on varying extent, from 480.43 mg/100 g to 281.41 mg/100 g ,257.48 mg/100 g, 197.53 mg/100 g and 181.71 mg/100 g respectively for samples of raw open cooked, raw autoclave, soaked open cooked, soaked autoclave respectively. This research results that soaked autoclaving reduced 62.17% of tannin content which is the most effective method, followed by soaked open cooked 58.88% reduction, raw autoclaving 46.41% reduction and raw open cooked 41.43% reduction.

The effect of cooking methods on tannin content is presented in Fig.4.2

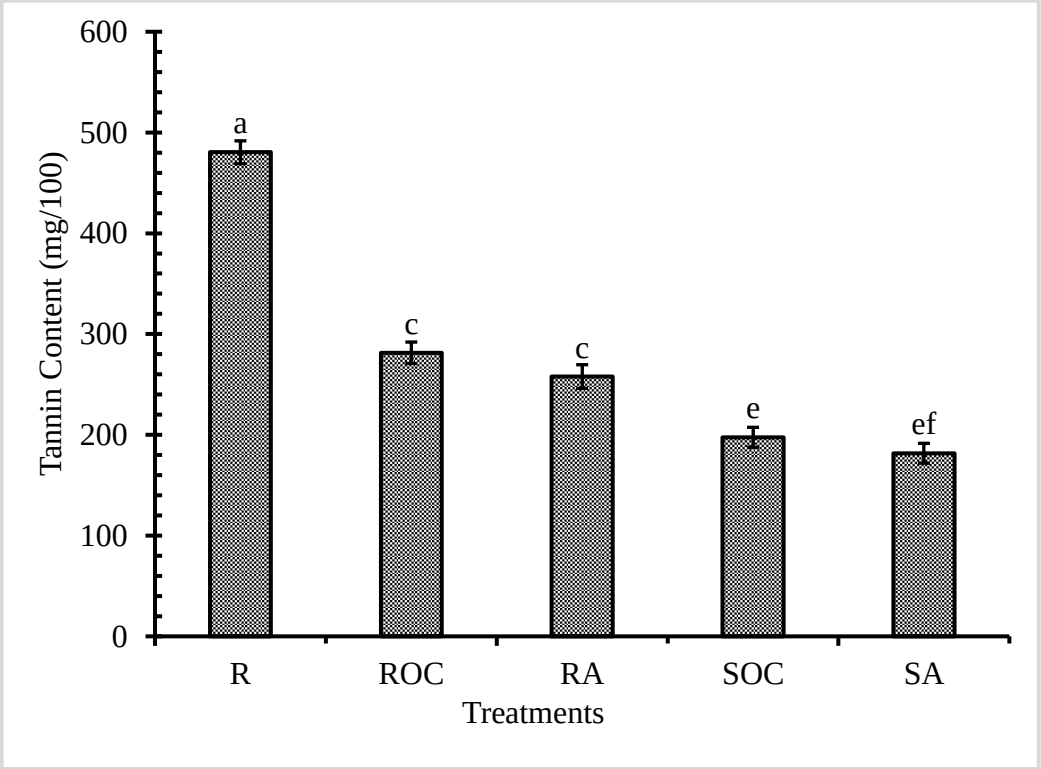

Fig.4.2 Effect of cooking methods on tannin content

[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. R, ROC, SOC, RA and SA are the samples of red kidney bean representing raw, raw open cooked, soaked open cooked, raw autoclaved and soaked autoclaved].

(Shimelis and Rakshit, 2007a) found 75%, 72%, 70% and 34% reduction of tannin in soaked autoclave, raw autoclave, soaked open cooking, and raw open cooking in which the data obtained in this study is lower except for raw open cooking which was found higher in this study. Ali *et al*. (2014) studied effect of cooking in tannin content in different varieties of faba bean ranges from 37.6-78%, where he found maximum reduction during pressure-cooking than open cooking which was similar to the data obtained in this study. The tannin content of chickpea is reduced by 48% after cooking (Alajaji and El-Adawy, 2006). Rehman and Shah. (2005) stated that tannin content of black grams, red kidney bean and white kidney bean significantly reduced after ordinary cooking and pressure-cooking at 121^0C for 20 min, respectively. Cooking and autoclaving reduce tannins by 27- 35% and 50-72% respectively (Shimelis and Rakshit, 2007a).

4.5 Effect of processing method on Polyphenols content of red kidney bean

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

4.5.1 Effect of soaking

The effect of soaking on polyphenols content of red kidney bean was studied and the value obtained showed that there is significant reduction ($p<0.05$) in polyphenols content. The result shows reduction from 770.34 mg/100 g to 526.63 mg/100 g after soaking the kidney bean for 12 h i.e., 31.77% reduction.

Alonso *et al.* (2000a) reported that the reduction of polyphenols of soaked red kidney bean seeds was 20.7% which was lower to the data obtained in this research. Similarly, Yasmin *et al.* (2008b) reported 13% reduction of polyphenol which was also lower than this research. Rehman and Shah. (2005) reported a significant reduction in the polyphenol contents of different legumes with various soaking processes.

4.5.2 Effect of Dehulling

Polyphenols content of red kidney bean was found to be significantly reduced ($p<0.05$) from 770.34 mg /100 g to 235.17 mg/100 g i.e., 69.47% reduction after dehulling process.

According to Alonso *et al.* (2000a) the reduction of polyphenols content in red kidney bean and faba bean was 90.8% and 81.6% which was found higher than the data obtained in this study. During dehulling, 60- 70% of polyphenols was decreased (Ma and Bliss, 1978) in which our finding was within the range.

4.5.3 Effect of germination

The effect of germination on polyphenol content of red kidney bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in polyphenol content, which was reduced from 770.34 mg/100 g to 395.53 mg/100 g and 265.27 mg/100 g after 48 h and 96 h of germination i.e., 48.66% and 65.56% reduction.

According to Alonso *et al.* (2000a) there was 40.6% and 53.1% reduction in polyphenol content in kidney beans after 48 h and 72 h of germination which was lower than our findings. Similarly, Yasmin *et al.* (2008a) reported that there was 54.5% reduction of polyphenol content after 96 h of germination. Grewal and Jood (2006) found that the polyphenol content of asha cultivar of mung bean seeds was reduced by 32% after germination. 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).

The polyphenols content of different processing treatments is given in figure 4.3

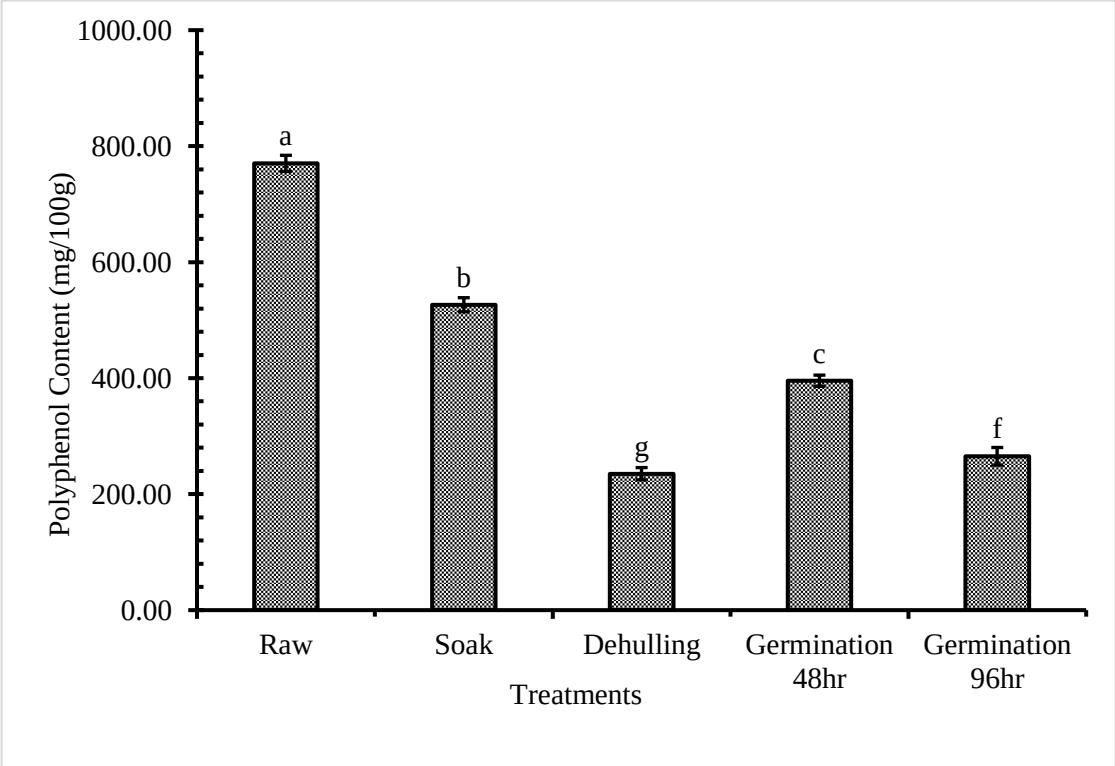

Fig.4.3 Effect of processing method on polyphenol content

[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].

4.5.4 Effect of cooking

The effect of cooking on polyphenol content of red kidney bean was studied. It shows significant reduction ($p<0.05$) on polyphenol content range from 770.34 mg/100 g to 395.81 mg/100 g, 375.54 mg/100 g, 355.73 mg/100 g, and 333.86 mg/100 g for samples of raw open cooking, soaked open cooking, raw autoclaving and soaked autoclaving respectively. This research finding results that soaked autoclaving reduced 56.66% of polyphenol content which is the most effective method, followed by raw autoclaving 53.82% reduction, soaked open cooked 51.25% reduction and raw open cooked 48.61% reduction.

The effect of cooking methods on polyphenol content is given in Fig.4.4

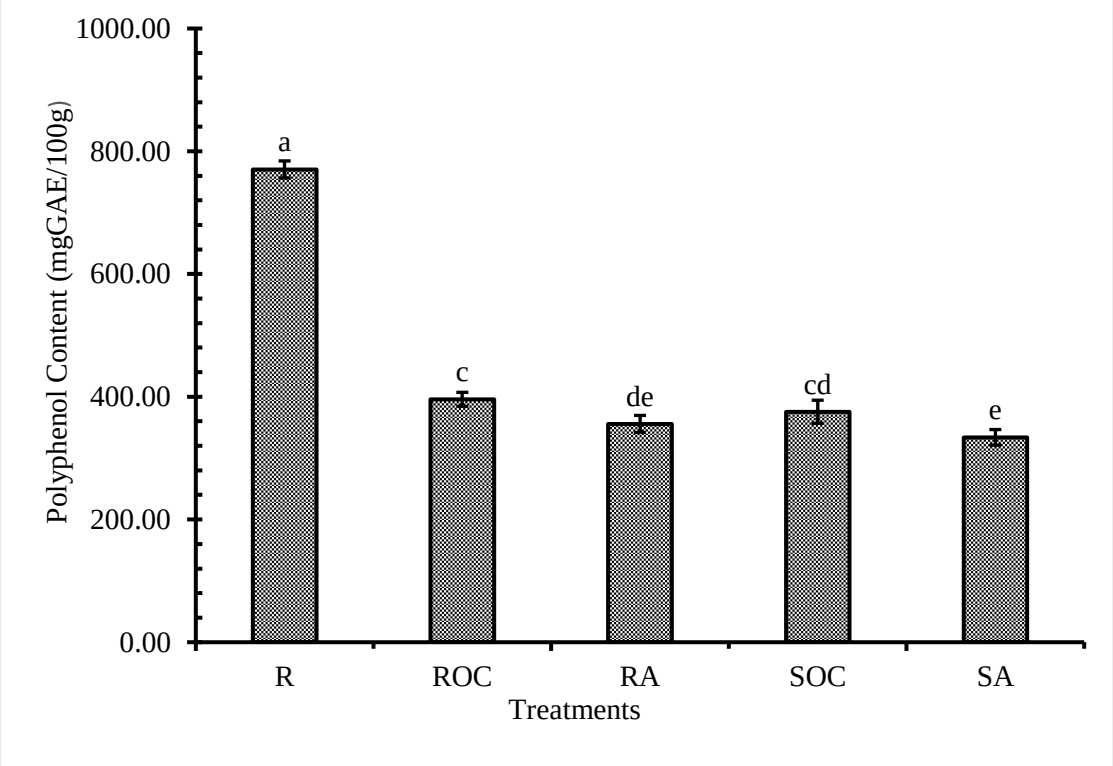

Fig.4.4 Effect of cooking methods on polyphenol content

[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. R, ROC, SOC, RA and SA are the samples of red kidney bean

representing raw, raw open cooked, soaked open cooked, raw autoclaved and soaked autoclaved].

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.*, 1989). Adebooye and Singh (2007) found that cooking of the whole grain resulted in statistically significant losses ($p \leq 0.05$) in the total phenolics of the two cowpea varieties. Comparison from his study showed that cooking resulted in 19-37% losses in phenolics in raw open cooked samples of black gram of different varieties which was slightly lower than our findings, where reduction was 48.61% in red kidney bean. He also found that raw pressure cooking decreased the polyphenols of both the pulses to a greater extent than ordinary cooking: 21-31% and 22-40%, respectively in chickpea and black gram. This study shows higher reduction of polyphenol.

4.6 Effect of processing method on Phytate content of red kidney bean

The effects of soaking, germination, open cooking, autoclaving and dehulling on the phytate content in red kidney bean was studied. All the treatments significantly reduced ($p<0.05$) the phytate of the red kidney bean seeds, but to the varying extent. Dehulling 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 red kidney bean was studied and the value obtained showed that there is significant reduction ($p<0.05$) in phytate content. The result shows great reduction from 630.69 mg/100 g to 495.37 mg/100 g after soaking the red kidney bean for 12 hours i.e., 21.46% reduction.

The result obtained in this research is higher than the values obtained by (Alonso *et al.*, 2000a) i.e., 17%. Akindahunsi (2004) concluded that the red kidney beans and pinto beans soaked in distilled water for 18 h at room temperature reduced their phytate content by 51.7% and 52.7 % respectively. Phytate is reduced by 28% during 12 h soaking in black gram (Kataria *et al.*, 1988) which is slightly higher than our finding. Soaking caused a 42.82-48.91%reduction in phytic acid content. This could be due to the fact that phytic acid in dried legumes exist wholly as a water soluble salt (probably potassium phytate) (Crean and Haisman, 1963).

4.6.2 Effect of Dehulling

The effect of dehulling on phytate content of red kidney bean was studied. The value obtained showed that there is significant reduction ($p<0.05$) in phytate content, which is reduced from 630.69 mg/100 g to 172.27 mg/100 g after dehulling i.e., 72.69 % reduction. This result correlates well with an earlier report on wheat, that dehulling to get refined flours considerably reduced phytate content (Ghavidel and Prakash, 2007) where reduction ranges from 52.63–76.00 %.

Mubarak (2005) reported that 21% phytic acid was reduced after dehulling of mung bean. Similar results have been reported by (Oburuoga and Anyika, 2012) which is similar to our finding. 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

(Grewal and Jood, 2006). It has been reported that dehulled beans, particularly after soaking, reduce phytic acid in P. *vulgaris* (Ibrahim *et al.*, 2002).

4.6.3 Effect of Germination

Germination shows considerable decrease in phytate content of red kidney bean and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that germination significantly reduced ($p<0.05$) total phytate content, which reduced from 630.69 mg/100 g to 319.72 mg /100 g and 200.71 mg/100 g after 48 and 96 hour of germination i.e., 49.31% and 68.18% reduction.

The result obtained in this research is lower than the data obtained by (Chau and Cheung, 1997) i.e., 55.4%. Pal *et al.* (2016) found that there is 49% reduction of phytic acid in horse gram after 48 hour of germination which is similar to our finding. Noor *et al.* (1980) reported over 70% reduction of phytic acid in 96 hour of germination which is similar to our finding. 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.

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

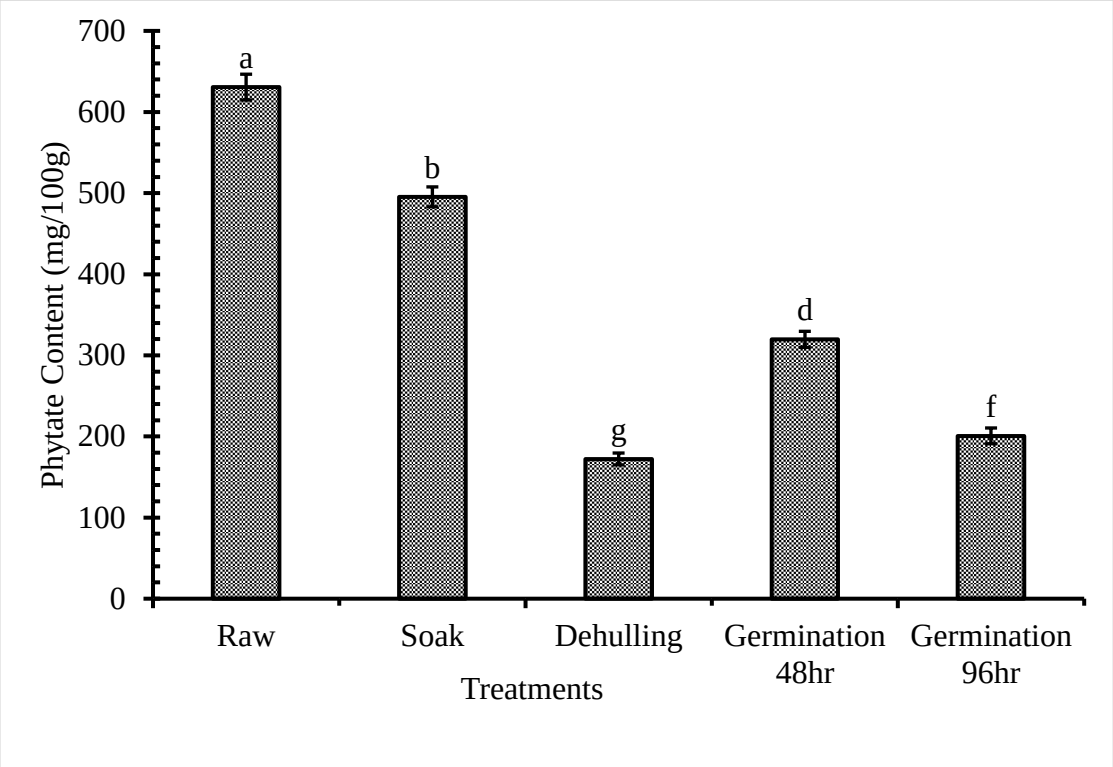

Fig.4.5 Effect of processing methods on phytate content

[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.]

4.6.4 Effect of cooking

Effect of open cooking for 55 min and pressure cooking at 15 psig for 15 min on total phytate content of red kidney bean was studied. The value obtained showed that there is significant reduction ($p<0.05$) in phytate content, which is reduced from 630.69 mg/100 g to 376.57, 475.51, 294.93 and 375.85 mg/ 100 g for raw autoclaving, raw open cooked, soaked autoclaving, soak open cooked. The findings obtained by this research result that soaked autoclave reduced 42.19 % of phytate content which is the most effective method, followed by soaked open cooking 40.41% reduction, raw autoclaved 40.29% reduction and raw open cooking 24.60% reduction. Effect of cooking methods on phytate content is given in Fig. 4.6.

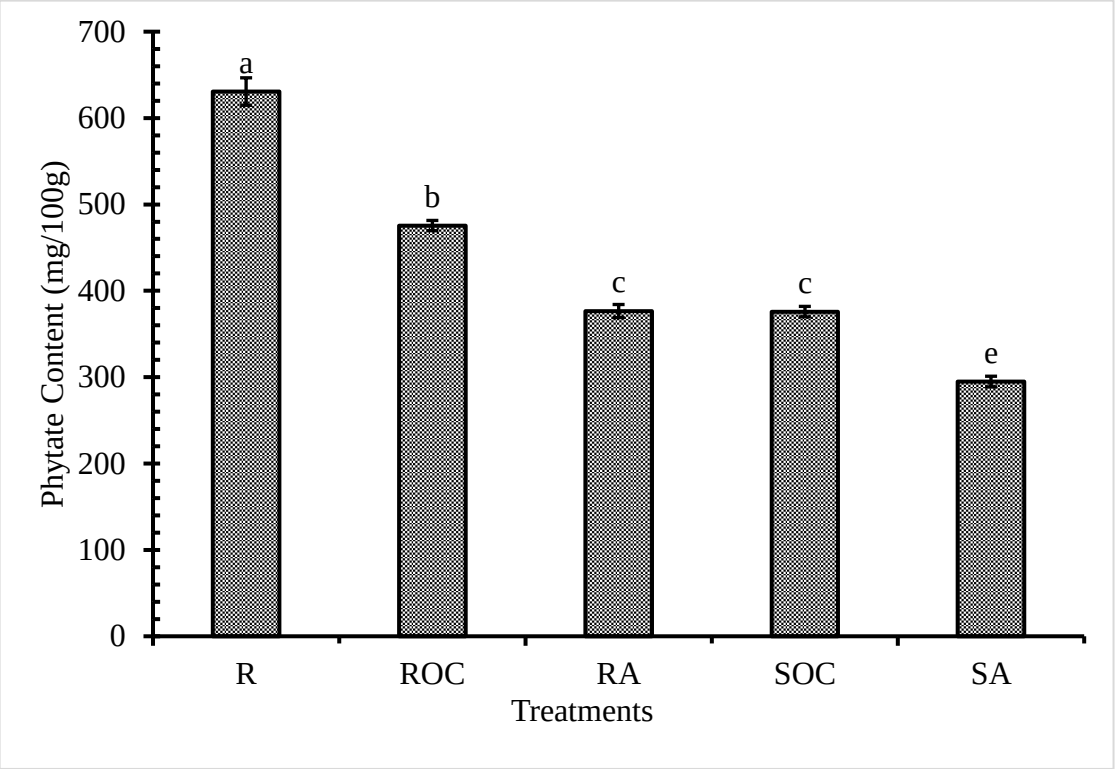

Fig.4.6 Effect of cooking methods on phytate content

[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. R, ROC, SOC, RA and SA are the samples of red kidney bean representing raw, raw open cooked, soaked open cooked, raw autoclaved and soaked autoclaved].

Alonso *et al.* (2000b) reported that reduction of phytic acid content in raw open, raw autoclaved, soak open , soak autoclaved were 26%, 60%, 61% and 62% respectively. The result obtained in this study was found to be lower than him. Rehman and Salariya. (2005) reported the reductions of 24-35% for phytic acid content when cooking was done by the ordinary boiling method and reduction of 28-51.6% phytic acid content as a result of cooking food legumes in an autoclave at 121^0C for different time periods.

4.7 Effect of processing method on oxalate content of red kidney bean

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

4.7.1 Effect of soaking

Soaking shows considerable decrease in oxalate content of red kidney bean 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 250.09 mg/100 g to 192.51mg/100 g i.e., 23.02% 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. Loss of soluble oxalates in water was considered to be the primary factor contributing to total oxalates reduction (Akhtar *et al.*, 2011).

4.7.2 Effect of Dehulling

The oxalate content of the raw kidney bean was determined and found to be 250.09 mg/100 g. Present study shows that dehulling significantly decrease ($p<0.05$) oxalate content from 250.09 mg/100 g to 152.07 mg/100 g i.e., 39.19% 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 575.60 mg/ 100 g in raw to 346.23 mg/ 100 g after dehulling of horse gram i.e., 39.85% reduction of total oxalate content.

4.7.3 Effect of germination

The effect of germination on oxalate content of red kidney bean was studied. The value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was reduced from 250.09 mg/100 g to 115.35 and 83.93 mg/100 g after germination for 48 h and 96 h i.e., 53.88 and 66.44% reduction.

Handa *et al.* (2017) reported that reduction of oxalate in 48 hour germination was 40.62% which was lower than the data obtained in this research. Suma and Urooj (2014) reported that reduction of oxalate in 72 hour germination was 47.09%. 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. During germination, oxalate oxidase gets activated which breaks down oxalic acid into carbon dioxide and hydrogen peroxide consequently releases calcium (Murugkar *et al.*, 2013).

The oxalate content of processing treatments is given in Fig.4.7

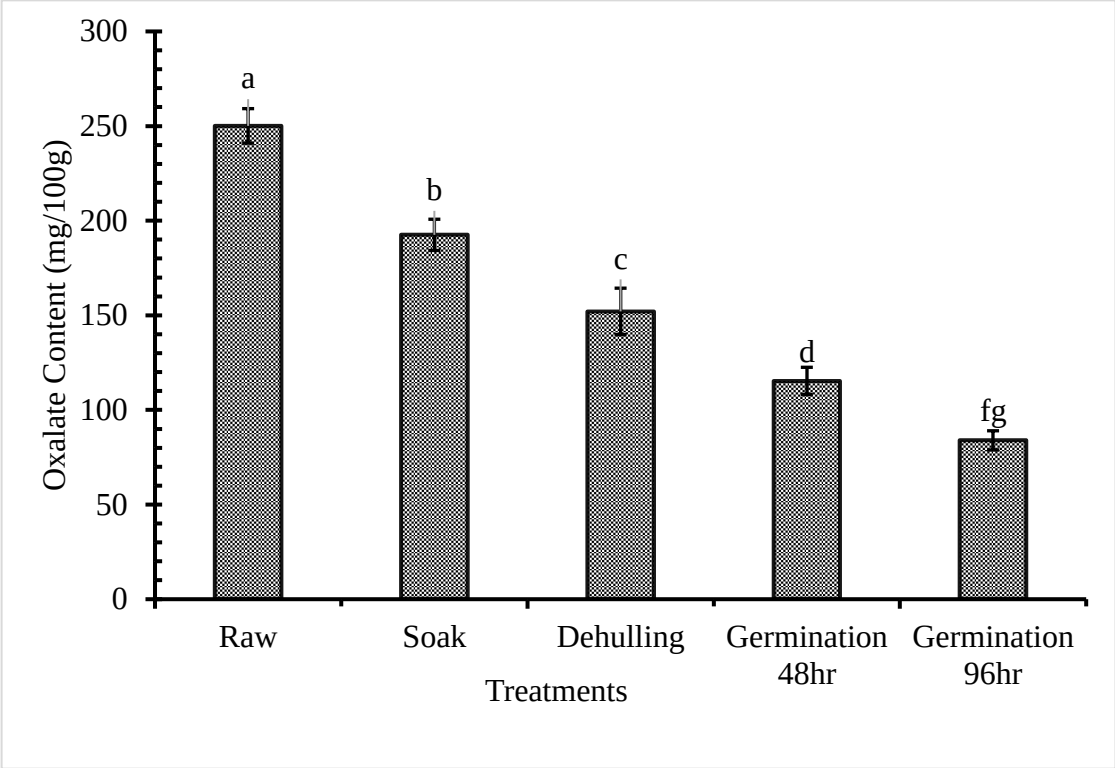

Fig.4.7 Effect of processing methods on oxalate content

[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.]

4.7.4 Effect of cooking

The effect of cooking on oxalate content of red kidney bean was studied. It shows significant reduction ($p<0.05$) on oxalate content range from 250.09 mg/100 g to 105.40 mg/100 g, 97.19 mg/100 g, 91.84 mg/100 g, and 74.37 mg/100 g for samples of raw open cooked, raw autoclaving, soaked open cooked, and soaked autoclaving respectively. These research findings result that soaked autoclaving reduced 70.26% of oxalate content which is the most effective method, followed by soak open cooking 63.28%, raw autoclaving 61.14% reduction and raw open cooked 57.86% reduction.

The effect of cooking methods on oxalate content is presented in Fig.4.8

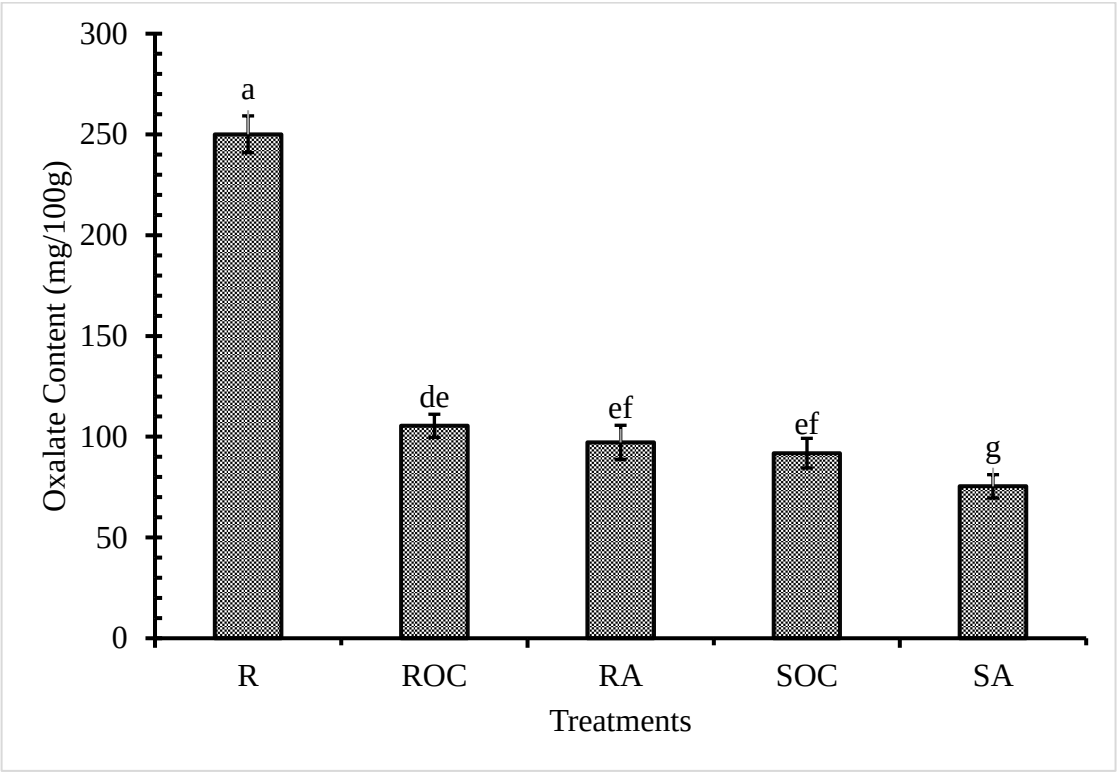

Fig.4.8 Effect of cooking methods on oxalate content

[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. R, ROC, SOC, RA and SA are the samples of red kidney bean representing raw, raw open cooked, soaked open cooked, raw autoclaved and soaked autoclaved].

According to Akhtar *et al.* (2011), he found the reductions in total oxalates as a result of cooking pre-soaked 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 where he found maximum reduction during pressure cooking than open cooking which was similar to our findings.

4.8 Comparison on proximate composition of effective treatment

Components	Raw	Dehulled	% Change
Moisture	11.06±0.13	6.41±0.41	-42.04
Protein (% db)	27.43^{a}±0.10	30.51^{b}±1.36	+11.23
Ash (% db)	4.41^{a}±0.03	3.15^{b}±0.02	-28.57
Fat (% db)	1.60^{a}±0.08	0.97^{b}±0.02	-39.38
Crude Fiber (% db)	4.26^{a}±0.09	2.06^{b}±0.09	-51.643
Carbohydrate (% db), by difference	62.30^{a}±1.87	65.11^{a}±2.43	+4.51

[Values presented are the average of triplicates determination ± standard deviation. Same figure in superscript along two rows in raw and dehulled column represent no significant difference ($p>0.05$), where + Sign represents increase in value where – sign represent decrease in value].

Moisture content of dried dehulled red kidney bean was found to be 6.41% which is comparatively lower than raw red kidney bean. The results of this study showed that dehulling resulted in significant ($p\leq 0.05$) losses of ash and mineral content i.e. from 4.41 to 3.15%. The implication of this result is that the seed coat is rich in mineral nutrients and substantial proportion of mineral nutrients are lost when red kidney bean is dehulled and the seed coat is discarded. Dehulling results in significant reduction ($p\leq 0.05$) of crude fiber content i.e. from 6.26 to 2.06%. This finding is consistent with the report of a previous study by (Attia *et al.*, 1994). Dehulling results in significant reduction ($p\leq 0.05$) of fat content i.e. from 1.60 to 0.97%. This was suspected to be due to the presence of higher amounts of fatty acids in the seed coat. But the protein content in the present study significantly increased ($p\leq 0.05$) i.e. protein content from 27.43% to 30.51 % and carbohydrate content in the present

study increased non-significantly ($p>0.05$) and was found to be increased from 62.30% to 65.11%. Increase in carbohydrate and protein content during dehulling was due to removal of mineral and anti-nutrients rich seed coat fraction along with fat rich germ part. According to Mang *et al.* (2016) the increase in protein and carbohydrate content after removal of hull may be due to the complexion of protein and carbohydrate by tannins and polyphenols present in the bean hulls.

4.9 Comparison on ultimate composition of minerals

Components	Raw (mg/100g)	Dehulled (mg/100g)	%Change
Iron	7.15^{a}±0.29	4.72^{b}±0.09	-33.99
Calcium	138.68^{a}±2.06	115.88^{b}±0.46	-16.44
Potassium	1133.44^{a}±3.08	1068.49^{b}.±0.93	-5.73
sodium	12.68^{a}±1.72	8.17^{b}±0.32	-35.57

[Values presented are the average of triplicates determination ± standard deviation. All values are expressed on dry basis. Same figure in superscript along two rows in raw and dehulled column represent no significant difference ($p>0.05$), where + Sign represents increase in value where – sign represent decrease in value].

Iron content of dehulled kidney bean was 4.72 mg/100 g. Dehulling results in significant reduction ($p<0.05$) of iron from 7.15 mg/100 g to 4.72 mg/100 g. Calcium content of dehulled kidney bean was 115.88 mg/100 g and after dehulling there is significant reduction ($p<0.05$) of calcium from 138.68 mg/100g to 115.88 mg/100 g. Dehulling results in significant reduction ($p<0.05$) of potassium from 1133.44 mg/100 g to 1068.49 mg/100g. Sodium content of dehulled kidney bean was 8.17 mg/100 g. Dehulling results in significant reduction ($p<0.05$) of sodium from 12.68 mg/100 g to 8.17 mg/100 g. Decrease in the mineral content of the samples might be as a result of dehulling of the beans. Seed coat removal has been implicated in reducing mineral content in beans (Farinde *et al.*, 2018).

4.10 Ascorbic Acid Content after dehulling

The ascorbic acid content of raw red kidney bean was 4.21 mg/100 g. After soaking for 12 hours and dehulling ascorbic acid content increases non-significantly ($p>0.05$) from 4.21 mg/100 g- 4.67 mg/100 g and the values are expressed on dry basis.

Part V

Conclusion and recommendations

5.1 Conclusions

In this study raw red kidney bean was processed with several treatments and the variations in reduction of anti-nutrients were analyzed in the lab. Based on the result and discussion following conclusions can be drawn.

1. Red kidney bean was subjected to a variety of methods, including soaking, soaking and dehulling, 48 h and 96 h of germination, raw open cooking, soaked open cooking, raw autoclaving, and soaked autoclaving, all of which significantly reduced anti-nutrients.
2. Dehulling was found to be the most effective method for the reduction of tannin (74.07%), phytate (72.69%) and polyphenols (69.47%) present in red kidney bean.
3. For the reduction of oxalate the most effective method was soaked autoclaving i.e., 69.86%.
4. All processing methods, single or combined reduce all anti-nutrients significantly ($p<0.05$).
5. In case of cooking, soaked autoclaving method was more effective than raw open cooking and raw autoclaving method in reducing the anti-nutrients
6. Dehulling reduced mineral content significantly ($p<0.05$).
7. Ascorbic acid content of the dehulled sample increases non-significantly.

5.2 Recommendations

Based on the present study, the following recommendations could be made for further study.

1. Among all the processing methods, dehulling and soaked autoclaving shows maximum reduction (if combined effect with soaking is considered), So, it is recommended to process red kidney bean through dehulling and/or soaked autoclaving for making other product from red kidney bean.
2. The effect of processing methods to reduce other antinutrients like trypsin inhibitor, hemagglutinin, lectin etc. present in red kidney bean can be studied.
3. Time and temperature of the different processing methods can be varied.

4. Effects of different combined treatments (dehulling and cooking, germination and cooking) in nutritional and anti-nutritional factors can be studied.
5. Reduction of mineral content of red kidney bean decreases significantly by dehulling.

Part VI

Summary

Kidney bean (*Phaseolus vulgaris*) is a major grain legume crop present all over the world and is commonly used for its wide health benefits. It is mainly consumed by cooking. Red kidney beans are splendid sources of energy, proteins, carbohydrates minerals vitamins and dietary fiber. Beans have two or three folds more amounts of protein than cereals and provide a suitable path for eliminating protein malnutrition. It has antioxidant, anti-microbial, anti-hypertensive, anti-melanogenesis, anti-inflammatory, immunomodulatory and anti-tumor properties. So, its use is increasing day by day all over the world. Thus the potential for beans to be used as nutraceuticals and functional food is very promising.

In the present study, eight types of processing methods were used to investigate reduction of anti-nutrient level in red kidney bean and the nutrient content of the effective method was also determined. The anti-nutrients studied were oxalate, phytate, polyphenol and tannin. The processing method include soaking, 48 h and 96 h germination, dehulling, raw open cooked, soaked open cooked, raw autoclaving and soaked autoclaving method. The ascorbic acid and minerals: iron, calcium, sodium and potassium were studied. Tannin and polyphenol were analyzed spectrophotometrically while oxalate was determined by titration with potassium permanganate and phytate was determined using ammonium thiocyanate.

The mean value of tannin, oxalate, polyphenols and phytate of raw red kidney bean were 480.43 mg/100 g, 250.09 mg/100 g, 770.34 mg/100 g, 630.69 mg/100 g respectively. All the processing methods reduced ($p<0.05$) significantly the anti-nutrients of red kidney bean where combination treatments were best seen than the single treatments. The combination treatments dehulling i.e., soaking and dehulling reduced the tannin, phytic acid and polyphenols content of red kidney bean more effectively than other processing methods. The reduction in tannin by raw open cooking and raw autoclaving were not significantly ($p>0.05$) different. The reduction in oxalate by raw autoclaving and soak open cooking were not significantly ($p>0.05$) different. The combination treatments i.e., soaked autoclaving reduced the oxalate content of red kidney bean greater than other methods. Dehulling was found to be most effective processing technique on the basis of percentage reduction. However, soaking was done prior dehulling, so the resulting reduction in anti-nutrient was because of combined effect of soaking and dehulling rather than dehulling alone. Hence

combination treatments were better than single process. Soaking and dehulling reduces anti-nutrients but it also reduces minerals like iron, calcium, sodium and potassium. However it increases ascorbic acid non-significantly.

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Appendices

Appendix A

Table. A.1 One Way ANOVA table for Tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	289594.0	36199.3	162.55	<.001
Residual	18	4008.5	222.7		
Total	26	293602.5			

Table. A. 2 Effect of different treatments on Tannin content

Treatments	Tannin (mg/100g)
Raw	480.4^{a}±11.33
Soak	353.2^{b}±11.01
Raw Open Cooking	281.4^{c}±10.64
Raw autoclaving	257.8^{c}±11.73
Germination 48hr	230.4^{d}±14.95
Soak Open Cooking	197.5^{e}±9.91
Soak Autoclaving	181.7^{ef}±9.86
Germination 96hour	159.3^{f}±13.51
Soaking and Dehulling	124.6^{g}±15.31

[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 Polyphenols

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	8	615469.2	76933.6	299.22	<.001
Residual	18	4628.0	257.1		
Total	26	620097.2			

Table. A.4 Effect of different treatments on polyphenols content

Treatments	Polyphenol (mg/100g)
Raw	770.3^{a}±13.91
Soak	526.6^{b}±12.09
Raw Open Cooking	395.8^{c}±11.35
Germination 48hr	395.5^{c}±9.72
Soak Open Cooking	375.5^{cd}±16.83
Raw Autoclaving	355.7^{de}±13.86
Soak Autoclaving	333.9^{e}±12.68
Germination 96hour	265.3^{f}±15.19
Soaking and Dehulling	235.2^{g}±10.62

[* 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 Phytate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	512373.0	64046.6	469.74	<.001
Residual	18	2454.2	136.3		
Total	26	514827.2			

Table. A. 6 Effect of different treatments on phytate content

Treatments	Phytate (mg/100g)
Raw	630.7^{a}±15.90
Soak	495.4^{b}±12.31
Raw Open Cooking	475.5^{b}±5.94
Raw Autoclaving	376.6^{c}±7.57
Soak Open Cooking	375.9^{c}±6.09
Germination 48hr	319.7^{d}±9.89
Soak Autoclaving	294.9^{e}±6.16
Germination 96hour	200.7^{f}±9.74
Soaking and Dehulling	172.3^{g}±7.31

[* 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 Oxalate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	82141.14	10267.64	106.14	<.001
Residual	18	1741.30	96.74		
Total	26	83882.45			

Table. A. 8 Effect of different treatments on oxalate content

Treatments	Oxalate (mg/100g)
Raw	250.1^{a}±9.11
Soak	192.5^{b}±8.25
Soaking and Dehulling	152.1^{c}±12.27
Germination 48hr	115.3^{d}±7.18
Raw Open Cooking	105.4^{de}±5.76
Raw Autoclaving	97.2^{ef}±8.49
Soak Open Cooking	91.8^{ef}±7.36
Germination 96hour	83.9^{fg}±5.07
Soak Autoclaving	74.4^{g}±6.48

[* 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 Paired t-test comparison of protein content of most effective method with Raw Red Kidney bean 5% level of significance

	Raw Red Kidney Bean	Dehulled sample
Mean	27.43	30.51
Variance	1.1617	3.432233333
Observations	3	3
Pearson Correlation	0.973931434	
Hypothesized Mean Difference	0	
df	2	
t Stat	-6.569355965	
P(T<=t) one-tail	0.011198014	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.022396028	
t Critical two-tail	4.30265273	

Table A.10 Paired t-test comparison of fat content of most effective method with Raw Red Kidney bean 5% level of significance

	Raw Red Kidney Bean	Dehulled Sample
Mean	1.6	0.97
Variance	0.003333333	0.000610333
Observations	3	3
Pearson Correlation	0.923108869	
Hypothesized Mean Difference	0	
df	2	
t Stat	28.24665589	
P(T<=t) one-tail	0.00062549	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00125098	
t Critical two-tail	4.30265273	

Table A.11 Paired t-test comparison of ash content of most effective method with Raw Red Kidney bean 5% level of significance

	Raw Red Kidney Bean	Dehulled Sample
Mean	4.41	3.15
Variance	0.0003	0.0003
Observations	3	3
Pearson Correlation	0.5	
Hypothesized Mean Difference	0	
df	2	
t Stat	124	
P(T<=t) one-tail	3.2515E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	6.50301E-05	
t Critical two-tail	4.30265273	

Table A.12 Paired t-test comparison of crude Fiber content of most effective method with Raw Red Kidney bean 5% level of significance

	Raw Red Kidney bean	Dehulled Sample
Mean	4.26	2.06
Variance	0.0012	0.0007
Observations	3	3
Pearson Correlation	-0.981980506	
Hypothesized Mean Difference	0	
df	2	
t Stat	64.06816471	
P(T<=t) one-tail	0.000121766	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000243532	
t Critical two-tail	4.30265273	

Table A.13 Paired t-test comparison of Carbohydrate content of most effective method with Raw Red Kidney bean 5% level of significance.

	Raw Red Kidney bean	Dehulled Sample
Mean	62.3	65.10333333
Variance	5.4999	9.061733333
Observations	3	3
Pearson Correlation	0.063360101	
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.313403719	
P(T<=t) one-tail	0.159745876	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.319491752	
t Critical two-tail	4.30265273	

Appendix B

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

Tannic acid concentration (ug)	Absorbance
0	0.00
2	0.17
4	0.31
6	0.41
8	0.62
10	0.72

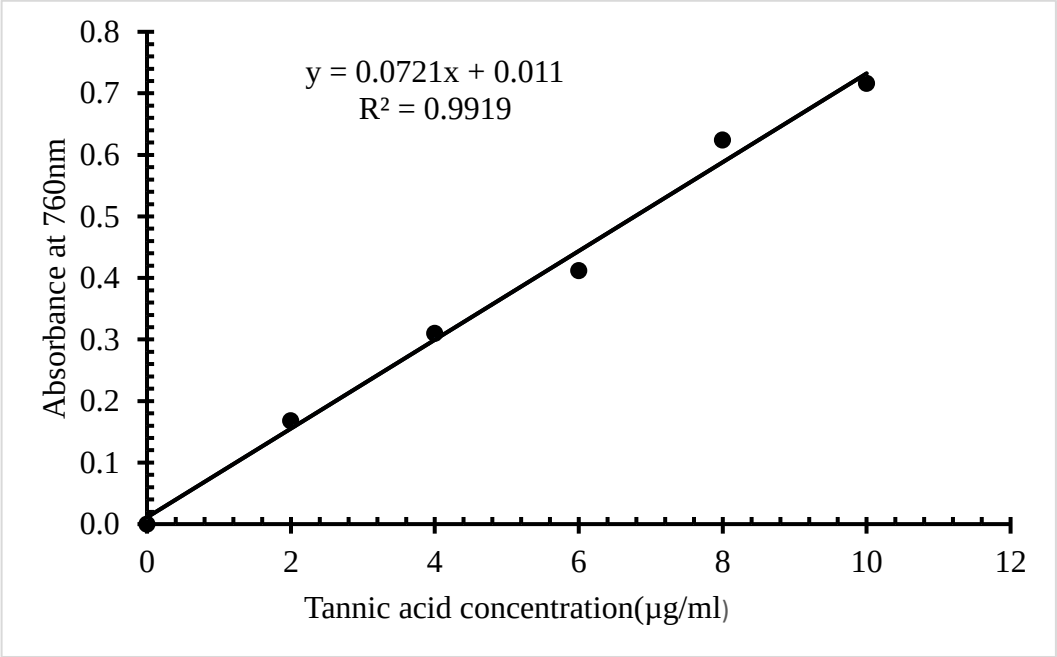

Fig. B.1 Standard curve for tannin determination

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

Gallic acid concentration µg/ml	Absorbance
0	0
50	0.623
100	0.946
150	1.651
200	1.921
250	2.624

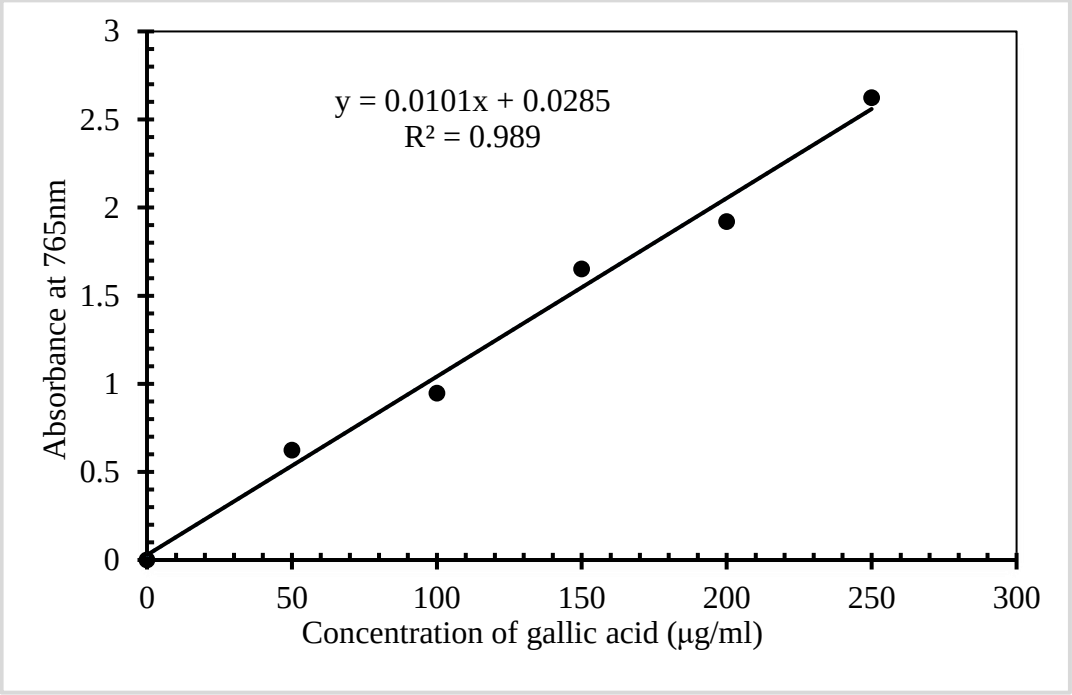

Fig B.2 Standard curve for polyphenol determination

Color Plates

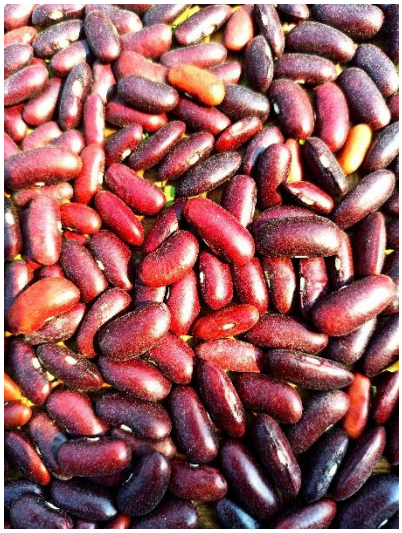

Plate 1 Raw Red Kidney bean

Plate 2 Polyphenol Extracts

Plate 3 Distillation in Kjeldahl's distillation set

Plate 4 Sample preparation for determination of tannin

Plate 5 Spectrophotometric determination of polyphenols

Plate 6 Bulk density determination