EFFECTS OF PROCESSING TECHNIQUES ON ANTI-NUTRITIONAL FACTORS OF BLACK GRAM (*Vigna mungo*)

by

Tripti Ranabhat

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2020

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

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Tripti Ranabhat

Department of Food Technology

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Institute of Science and Technology

Tribhuvan University, Nepal

January, 2020

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Effect of processing techniques on antinutritional factors of black gram (Vigna mungo)* presented by Tripti Ranabhat has been accepted as the partial fulfillment of the requirements for the B.Tech. Degree in Food Technology.

Dissertation Committee

1. Head of Department _____

(Mr. Basanta Kumar Rai, Prof.)

2. External Examiner _____

(Mr. ……..…...)

3. Supervisor _____

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

4. Internal Examiner _____

(Mr……..)

January, 2020.

Acknowledgements

I would like to express deep gratitude to my guide Mrs. Babita Adhikari Dahal for her precious advice, valuable suggestions, guidance, encouragement, motivation and constructive comments, which enabled me to carry out this thesis successfully.

I am also indebted to Prof. Dr. Dhan Bahadur Karki, Campus Chief, Central Campus of Technology, Hattisar, Dharan, for his kind co-operation and suggestion in providing an opportunity and facilities to perform this work successfully. I am also equally thankful to Prof. Basanta Kumar Rai for his generosity and co-operation.

Many thanks and gratitude is expressed to all the teachers and staff members, librarian for their support. My special thanks goes to Mr. Prajwal Bhandari, Head Technical Assistant, CCT, Hatisar, Dharan and Mr. Sachin Basnet for making my entire study in an enthusiastic and passionate environment.

Above all, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never reached this far, and this work would have never seen the light of the day.

Date of submission: January, 2020.

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(Tripti Ranabhat)

Abstract

The main aim of my study is to determine the effect of processing techniques on anti-nutritional factors of *Vigna mungo*. The effect of different treatments (soaking, germination, dehulling, raw open cooked, soaked open cooked, raw pressure cooked, soaked pressure cooked) on the anti-nutrient (Oxalate, phytate, polyphenol and tannin) contents of raw black gram were studied at Central Campus of Technology.

The mean value of Oxalate, phytate, polyphenol and tannin content in raw black gram were found to be 397, 150.8, 1278.18 and 507.97 mg/100 g respectively. Maximum reduction of anti-nutrients: Oxalate (46.59%), Phytate (69.23%), Polyphenol (72.03%) and Tannin (60.54%) were found when black gram was soaked and dehulled. Soaked pressure cooked was second most effective in terms of anti-nutrients removal. Soaked pressure cooked showed significant reduction ($p<0.05$) of anti-nutrient: Oxalate (59.69%), Phytate (61.46%), Polyphenol (44.177%) and Tannin (48.82%) which is the second most effective method. The reduction percentage by soaking for 24 hour and germination for 48 hours were found to be less effective method compared to dehulled and cooked. Hence, Dehulling of black gram 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. Similarly, other processing methods (soaking, germination, raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked) were also effective in reducing the anti-nutrients.

Contents

List of tables

List of figures

List of Plates

List of abbreviation

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

Part I

Introduction

1.1 General introduction

Mankind depends on a very limited number of crops to meet the needs of staple diets and on a very limited number of major non-food crops to meet associated needs. In general, a small number of varieties occupy large areas for these cultivated species (Williams and Wandzilak, 1989). Diversification of production and consumption habits to include a broader range of plant species, in particular those currently identified as 'underutilized', can contribute significantly to improved health and nutrition, livelihoods, household food security and ecological sustainability. In particular, these plant species offer enormous potential for contributing to the achievement of millennium development goal particularly in combating hidden hunger and offering medicinal and income generation options. They are also closely tied to cultural traditions, and therefore have an important role in supporting social diversity (Jaenicke and Hoschle-Zeledon, 2006).

Food legumes are rich and less expensive sources of proteins in human diet in several developing countries. Biological utilization of pulses is limited due to deficient Sulphur containing amino acids (Elias *et al.*, 1964) and the presence of antinutrients including phytic acid, saponins, polyphenols, enzyme inhibitors, Lectins etc (Salunkhe, 1982).

Pulses are known to form the important source of protein and other dietary constituents in Indian diet. Black gram (*Vigna mungo*. L.) has occupied an important place in human nutrition as rich source of protein in the diet of consumers of India and Western diet. Proteins of black gram are more easily digestible and are almost as good as meat and very good sources of phosphoric acid and vitamins. It also consists of satisfactory amount of sulfur containing amino acids (FAO, 1973). In India black gram is consumed in the form dhal. It is used as source for microorganisms in fermented foods. It is also preferred in the preparation of many fermented foods like idli, dosa, wada etc. Although it is a good source of protein, presence of antinutritional factors is one of the main drawbacks limiting the nutritional and food qualities of the legumes (Liener, 1994) Black gram contains some antinutritional factors such as protease inhibitors, amylase inhibitors, polyphenols, phytic acid, tannic acid etc. (U. Singh and Jambunathan, 1981).

The anti-nutritional factors may be defined as those substances generated in the natural feed stuffs by the normal metabolism of species and by different mechanisms (e.g.,

inactivation of some nutrients, diminution of the digestive process or metabolic utilization of the feed) which exert effects contrary to optimum nutrition (Cheeke and Shull, 1985). In order to reduce the effect of anti-nutrients, which may have some health-hazard potentials, proper processing before consumption is necessary. Cooking improves digestibility, promote palatability, improves keeping quality, and makes root crops safer to eat (Anoma and Thamilini, 2016)

1.2 Statement of the problem

In spite of the fact that, black gram has the significant potential for being staple legumes, scientific validation of traditional processing methods in terms of food quality and safety has not been attempted. Although 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 about the processing methods those are effective in reducing these factors may significantly contribute in reducing health risk that are associated with consumption of black gram. Hence efforts to enhance the reduction of anti-nutrition by house hold treatments towards improving the nutritional properties of black gram which is practiced worldwide is more than justified.

1.3 Objectives

1.3.1 General objectives

The general objective of this work is to study the capability of different processing techniques (soaking, open cooking, pressure cooking, germination and dehulling) to reduce anti-nutritional factors of black gram with emphasis on choosing the best.

1.3.2 Specific objectives

The specific objectives were as follows:

1. To determine the tannin, oxalate, phytate and polyphenol content of raw black gram.
2. To process black gram with various treatments namely soaking, open cooking, pressure cooking, germination, and dehulling.
3. To determine the effect of reduction pattern of anti-nutrients in processed black gram.
4. To conclude the type and the level of treatment that could potentially reduce the highest amount of anti-nutrients.

1.4 Justification of work

In the present world, interest has been increased in search of additional foods to meet the demand of overly increased population (Siddhuraju *et al.*, 2000). Pulses are the major source of proteins and other nutrients in the diets of malnourished areas of the world. They are also good sources of slowly digestible starch, the most desirable form of dietary starch that is completely, but more slowly, digested in the small intestine, and attenuates postprandial plasma glucose and insulin levels (Jenkins *et al.*, 1981). Seeds, sprouts and green pods are edible and much appreciated for their high digestibility and lack of flatulence induction (Fery, 2002; Jansen, 2006). Black gram is an important legume in the tropical and subtropical developing countries. It is rich carbohydrate in the form of starch and protein content. It is consumed in many processed forms in the industry and also as livestock feed.

This study specifically determines anti-nutritional contents of raw black gram and the effect of various processing 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 black gram in household level and also may provide opportunities to promote and support use of black gram into processed foods like noodles and cookies in the industrial scale which can help to improve its production and utilization potentials.

1.5 Limitations of the study

1. Only one variety of black was taken for study.
2. Only few processing methods were performed due to lack of time period.
3. Simple titrimetric method was used to determine phytate due to unavailability of sophisticated chromatographic techniques.

Part II

Literature review

2.1 The origin and distribution of black gram

Vigna mungo originated from central Asia and India from where it was domesticated. It is now found in many tropical areas of Asia, Africa and Madagascar. It is cultivated in the USA and Australia as a fodder crop (Arora and Mauria, 1989; Jansen, 2006). It is generally found in lowlands but can grow up to 1800 m above sea level provided there is neither frost nor prolonged cloudiness (Akgul, 2017; Arora and Mauria, 1989). Optimal growth conditions are average day temperatures ranging from 25°C to 35°C and annual rainfall of 600-1000 mm. It has poor tolerance of wet tropical climates but in high rainfall areas it can be grown during the dry period on residual moisture. It grows better on rich black vertisols or loamy soils, well-drained soils with a pH 6-7 (Arora and Mauria, 1989; Baligar and Fageria, 2007). It can withstand acidic soils (down to pH 4.5) if lime and gypsum are added to the soil (Baligar and Fageria, 2007). It is drought-tolerant and thus suitable for semi-arid areas (Arora and Mauria, 1989).

2.2 Classification and nomenclature

The scientific name of black gram is *Vigna mungo*. According to USDA, the Taxonomy hierarchy of black gram is given below:

Kingdom:	Plantae-Plants
Subkingdom:	Tracheobionta-Vascular plants
Super division:	Spermatophyta-Seed plants
Division:	Magnoliopsida-Flowering plants
Class:	Magnoliopsida-Dicotyledons
Subclass:	Rosidae
Order:	Fabales
Family:	Fabaceae/Leguminosae-Pea family
Genus:	*Vigna savi* (cowpea)
Species:	*Vigna mungo* (L.) Hepper-Black gram

Source: USDA (n.d.)

2.3 Physiology and morphology of Black gram (Structure of black gram)

Vigna mungo is densely hairy, erect, suberect or trailing and annual herb with taproot producing branched root system having smooth and rounded nodules. The plant grows 30 to 100 cm. Leaves are trifoliate having 3 ovate or rhombic ovate leaflets and measures 4 to 10 cm by 2.5 to 5 cm. Flowers are small, axillary and bright yellow in color. Fruit is a cylindrical and upright legume pods. Pods are cylindrical, narrow measuring 4 to 6 cm long. Each pod possesses 4 to 10 seeds in ellipsoid shape and usually black or mottled or grey black in color.

Habitat

Tropical

Habit

Annual, herbaceous, 30-100 cm in height, erect, semi erect to trailing or spreading types, plant densely hairy.

Leaves

Trifoliate, alternate, stipulate, stipules narrow and falcate, petiolate, pulvinate, stipellate, stipple small and flat, leaflets ovate, entire acute, sparsely hairy on both surfaces, palmately reticulate.

Inflorescence

Axillary raceme with the flowers congested at the top of the peduncle, flowers five to six, bracteate, bracteolate, bracteoles pedicellate, bisexual hypogynous, zygomorphic, complete, pentamerous.

Pod

Mature pod is puff to brown color, 6-8 mm long (shorter than mug bean) round, erect with long and dense hairs and short hooked beak.

Seed

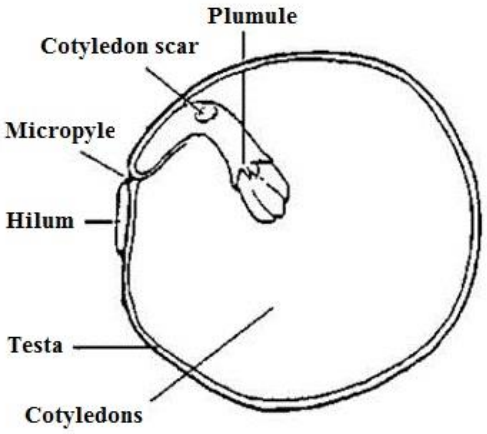
Oblong with square ends, black Hilum white and concave, seed coat surface is smooth. Cotyledons white in color.

Structure of fruit

Legumes are plant in the family Leguminosae or a fruit of these specific plants. A legume fruit is simple dry fruit that develops from a simple carpel and usually dehisces on two sides. Among them Black gram is a well-known legume.

Black gram mature seeds have three major components: the seed coat, the cotyledons, and the embryo. The seed coat accounts for 7-15% of the whole seed mass. Cotyledons are about 85% of the seed mass and the embryo constitutes of 1-4%. The external structure of the seed are the testa, hilum, micropyle, and raphe. The testa is the outer most part of the seed and covers almost all of the seed surface. The hilum is an oval scar on the seed coat where the seed was attached to the stalk. The micropyle is a small opening in the seed coat next to the hilum. The raphe is a ridge on the side of the hilum opposite the micropyle. When the seed coat is removed from grain, the remaining part is the embryonic structure. The embryonic structure consists of two cotyledons (or seed leaves) and a short axis above and below them. The two cotyledons are not physically attached to each other except at the axis and a weak protection provided by the seed coat. Thus, the seed is unusually vulnerable to breakage. The outermost layer of the seed coat is the cuticle, and it can be smooth or rough. Both the micropyle and hilum have been related to the permeability of the testa and to water absorption.

It is an erect, suberect or trailing, densely hairy, annual bush. The tap root produces a branched root system with smooth, rounded nodules. The pods are narrow, cylindrical and up to six cm long. The plant grows 30–100 cm with large hairy leaves and 4–6 cm seed pods(Anon., 2006). The general structure of black gram fruit is shown in figure 2.1.



Source: Patel *et al.* (n.d.)

Fig. 2.1 Structure of Black gram Fruit

2.4 Chemical and nutritional composition of black gram

Whole black gram is a rich source of protein, fiber, several vitamins and essential minerals such as calcium and iron (N. R. Reddy *et al.*, 1982). Processing of black gram into dehusked

cotyledon essentially involves the removal of seed coat, germ, aleurone layer and plumule, and these fractions may consist of a variety of nutrients. Incorporation of black gram flour was reported to improve the quality of buffalo meat burgers (Modi *et al.*, 2004) and beef sausages (Bhattacharya *et al.*, 2004) and the nutritional quality of biscuit (M. Patel and Rao, 1995). Foods rich in nutraceuticals and dietary fiber are gaining importance because of their health benefits. Polyphenols, carotenoids and dietary fiber have a role in prevention of cardiovascular disease, cancer and diabetes (Lario *et al.*, 2004; Scalbert *et al.*, 2005). Black gram lipids were shown to have cholesterol-reducing effect in both humans and experimental animals (Devi and Kurup, 1972).

Nutritional value of Black gram according to Economics division, DOA is given in table 1.

Components	**Amount**
Proximate	
Protein%	20-25
Fat%	1.3
Ash%	3.40
Crude fiber%	4.2
Starch%	40-47
Vitamins	
Vitamin A (IU)	300
Vitamin B (mg/100 g)	0.52-0.66
Vitamin B (mg/100 g)	0.29-0.22
Niacin (mg/100 g)	2
Vitamin C (mg/100 g)	5
Minerals	
Iron (mg/100 g)	7.8
Calcium (mg/100 g)	145

Source:(Anonymous)

2.5 Anti-nutritional factor

Foods are complex substances that contain many chemical compounds, more than 50 of which are required to nourish the body. These nutrients include water, proteins, lipids, carbohydrates, minerals and vitamins. Additionally, most plant foods also consist of thousands of natural compounds, depending on the situation may have beneficial or deleterious effect on consuming them. These compounds, with the exception of nutrients,

are referred to as allelochemicals. Anti-nutritional factors may be regarded as the class of these compounds that are generally not lethal. They diminish animal productivity but may also cause toxicity during the periods of scarcity or confinement when the food rich in 19 these substances is consumed by animals in large quantities (Rosenthal and Janzen, 1979). Anti-nutrients are potentially harmful and give rise to a genuine concern for human health in that they prevent digestion and absorption of vitamins, minerals and other nutrients. 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 (Brune, 1989). Anti-nutritional factors include cyanogen glycosides, saponin, phytate, enzyme inhibitors (trypsin and amylase inhibitors), oxalate and total polyphenols.

2.6 Antinutritional factors in black gram

2.6.1 Tannins

Tannins are water soluble phenolic compounds having molecular weights between 500 to 3000 giving the usual phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and proteins. 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.*, 1993). 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 (Tinko and Uyano, 2001).

They are readily form indigestible complexes with proteins and other macro-molecules under specific environmental conditions. Tannins had been reported to affect protein digestibility, adversely influencing the bioavailability of non-haem iron leading to poor iron and calcium absorption, also carbohydrate is affected leading to reduced energy value of a

diet containing tannin (Adeparusi, 2001). This results in growth depression, in all probability owing to enzyme resistant substrates formed by interaction between tannins and protein/starch. Digestibility of the substrates is compromised by interaction between tannin and the enzymes (S. S. Deshpande and Salunke, 1986).

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) (Mahmut and Ayhan, 2002). Condensed tannins are derivatives of flavanols and hydrolysable tannins are esters of a sugar, usually glucose (Bartosz *et al.*, 2017). The co-occurrence of both kinds of tannins in the same plant or plant tissue is often observed. Tannins are found in the leaves, fruits, barks, roots and wood of trees (Mahmut and Ayhan, 2002). The tannin content in raw Black gram is about 861 mg/100 g (P. U. Rao and Deosthale, 1982) The structure of hydrolysable tannin and condensed tannin is shown in Fig. 2.5.

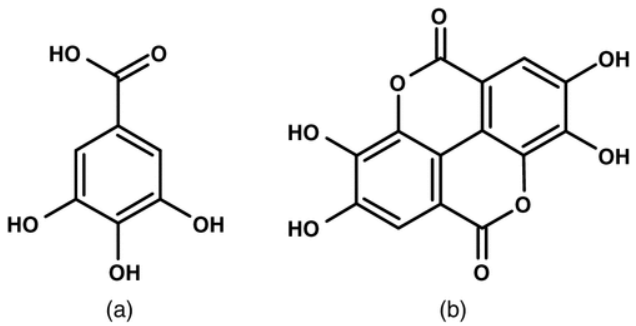


Fig. 2.5 Structure of hydrolysable tannin (a) and condensed tannin (b)

2.6.2 Phytate

Phytic acid, also known as inositol hexakisphosphate or phytate when in the salt form, is the storage form of phosphate in many plant tissues, especially seeds and grains. Phytic acid is not digested by humans, and is therefore not a dietary source of inositol or phosphate. In fact, because phytic acid is a good metal chelator, it is believed to have a negative nutritional impact on strongly chelating metals necessary for good health (e.g., iron and calcium) and could prevent their absorption by the intestine. For this reason and because phytic acid is

thought to have a positive dietary impact as an antioxidant to prevent carcinogenesis, determining the phytic acid content of foods is of interest (Phesatcha *et al.*, 2012).

Phytate is found in all seeds; mature legumes and cereal grains have the highest concentration. Phytates do not have a characteristic absorption spectrum. A brief history of phytate isolation is presented along with a description of its chemical structure. Phytates are important because they are the major phosphorus form in mature plant seeds, not absorbed from the GI tract, and hydrolyzed only slightly in human and animal intestines.

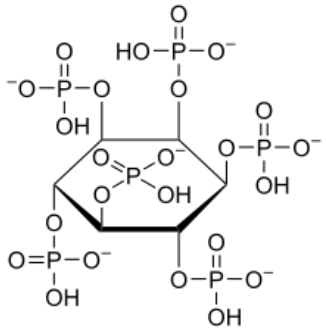


Fig. 2.6.2 Structure of Phytate

Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5% (w/w) (Loewus, 2001). The phosphorus bound to phytate is not typically bio-available to any animal that is non-ruminant. Ruminant animals, such as cows and sheep, chew, swallow, and then regurgitate their food. This regurgitated food is known as cud and is chewed a second time. Due to an enzyme located in their first stomach chamber, the rumen, these animals are able to separate, and process the phosphorus in phytates. Humans and other non-ruminant animals are unable to do so (Maga, 1982). Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn2+, Fe2+/3+, Ca2+, Mg2+, Mn2+, and Cu2+. Whether or not high levels of consumption of phytate-containing foods will result in mineral deficiency will depend on what else is being consumed. In areas of the world where cereal proteins are a major and predominant dietary factor, the associated phytate intake is a cause for concern (Sparvoli and Cominelli, 2014). The phytate content in raw Black gram is about 1147.03 mg/100 g (Kakati *et al.*, 2010).

2.6.3 Oxalate

Legumes, nuts, and different types of grain-based flours are commonly consumed throughout the world. Soybeans and other legumes such as lentils, red kidney beans, and white beans have been previously analyzed for oxalate (Fox *et al.*, 2015; Massey *et al.*,

2001). The oxalate content of nuts has been reported to be relatively high (Massey *et al.*, 2001) and there are published values in the literature for almonds, cashews, hazelnuts, peanuts, pecans, pistachios, and walnuts (Hodgkinson, 1977). However, comprehensive reports of oxalate concentrations in either legumes or nuts have not been published. In addition, there are few reported data on the oxalate contents of different types of flour products.

High temperature is known to cause the calcium oxalate-containing cells (raphides) to collapse, leading to the breakdown of oxalate structure.

Thermal degradation

$$CaC_2O_4.nH_2O(s) \rightarrow CaC_2O_4\ (s) + nH_2O\ (g)$$

$$CaC_2O_4(s) + \text{heat} \rightarrow CaCO_3(s) + CO\ (g)$$

$$CaCO_3(s) \rightarrow CaO(s) + CO_2\ (g)$$

The presence of Na+ ions was found to increase the decomposition rate and reduce the activation energy of the above reaction. The Na+ ions act as a catalyst for the decomposition reaction (Schempf *et al.*, 1965).

About 75% of all kidney stones are composed primarily of calcium oxalate (Williams and Wandzilak, 1989) and hyperoxaluria is a primary risk factor for this disorder (Goldfarb, 1988). Urinary oxalate originates from a combination of absorbed dietary oxalate and endogenous formation from oxalate precursors such as ascorbic acid and glyoxylate (Williams and Wandzilak, 1989).

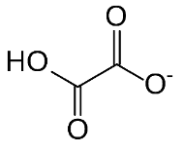


Fig. 2.6.3 Structure of Oxalate

Oxalic acid and its salts occur as end products of metabolism in a number of plant tissues. When these plants are eaten, they may have an adverse effect because oxalates bind calcium and other minerals. While oxalic acid is a normal end product of mammalian metabolism, the consumption of additional oxalic acid may cause stone formation in the urinary tract when the acid is excreted in the urine. Soaking and cooking of foodstuffs, high in oxalate will reduce the oxalate content by leaching. The mean daily intake of oxalate in English diets has been calculated to be 70-150 mg, with tea appearing to contribute the greatest proportion of oxalate in these diets; rhubarb, spinach and beet are other common high oxalate-content

foods. Vegetarians who consume greater amounts of vegetables will have a higher intake of oxalates, which may reduce calcium availability. This may be an increased risk factor for women, who require greater amounts of calcium in the diet. In humans, diets low in calcium and high in oxalates are not recommended but the occasional consumption of high oxalate foods as part of a nutritious diet does not pose any particular problem (Morrison and Savage, 1999). Oxalate content in raw black gram (Vigna mungo) was found in range from 543 to 690 mg/100g (Deraniyagala and Gunawardena, 1999).

2.6.4 Polyphenols

Polyphenols, an important group of antinutritional compounds commonly present in food legumes, could be appreciably reduced by common methods of domestic processing and cooking (Jood *et al.*, 1987). 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 antinutrients (Subbulakshmi *et al.*, 1976). Jood and Chauhan (1987); (Jood *et al.*, 1987) reports the effect of various domestic treatments including soaking, cooking (ordinary and pressure cooking), sprouting and cooking of sprouts on the polyphenol contents of some important cultivars of two major Indian food legumes namely, chickpea and black gram. Polyphenol contents of chickpea as well as black gram varieties ranged between 815 and 875 and between 842 and 892 mg /100 g, respectively (Jood *et al.*, 1987).

Soaking the seeds for 12 h reduced the polyphenol contents of both the legumes significantly 48% in chickpea and 47% in black gram. When the soaked seeds were cooked, there was a significant decline in the level of polyphenols of both chickpea and black gram cultivars: 9-12% in chickpea and 11-15% in black gram (Jood *et al.*, 1987).

Cooking of soaked seeds appeared to be a little more advantageous than cooking of unsoaked seeds, but the differences between the two treatments were not statistically significant and autoclaving decreased the polyphenols of both the pulses to a greater extent than ordinary cooking: 21-31% and 22-30%, respectively in chickpea and black gram (Jood *et al.*, 1987).

Sprouting was also considerably effective in decreasing the polyphenol contents of both the legumes (19-28% in chickpea and 17-20% in black gram). Cooking of sprouts had relatively little effect (Jood *et al.*, 1987). The reduction of total phenolics compounds during germination may be attributed to the presence of polyphenol-oxidase and enzymatic hydrolysis (D. S. S. Rao and Deosthale, 1981).

Three-fold reduction in total phenol content of variety Shekher- 2 was noted after 24 hr. (64.09%) and 48 hr. (66.94%) sprouted seeds as compare to raw seed (Jood *et al.*, 1987). More phenolics in the seed coat of S-1552 than C-152 as decortication resulted in such drastic reduction in the phenolic content. Comparison showed that 24.4 and 53.6% of the total phenolics in the uncooked whole grain of C-152 and S-1552, respectively, were lost because of decortication (Adebooye and Singh, 2007).

2.6.5 Trypsin inhibitor

Grain legumes are known to possess a number of antinutritional factors. Among these, the most studied is trypsin inhibitor (TI), since it is ubiquitously present in the plant kingdom (Ryan, 1981).

The trypsin inhibitor content in raw Black gram is about 2463.25 mg/100 g (Kakati *et al.*, 2010). Trypsin inhibitor has adverse effects on the pancreas (Montagnac *et al.*, 2009).

2.6.6 Saponin

Saponins are amphiphilic compounds, with the presence of a lipid-soluble aglycone and water-soluble chain(s) in their structure. It is found in plant tissues that are most vulnerable to fungal or bacterial attack or insect predation (Cheok *et al.*, 2014). They exhibit surfactant properties as a result they show foaming action upon shaking in an aqueous solution. Saponins are divided into two groups: Steroidal saponins, which occur as glycosides in certain pastures plants and triterpenoid saponins, which occur in soybean (Das. and TK, 2012).

Certain evidences show that saponins provide neuro protective effects on attenuation of central nervous system disorders, such as Parkinson's disease, stroke, Huntington's disease and Alzheimer's disease, along some in-vivo studies showing saponins have tumor-inhibitory effects and antifungal activity (Jiayi, 2016.). The presence of saponins in legumes has attracted considerable interest owing to health benefits while having adverse sensory characteristics (Omizu, 2011).

2.6.7 Lectin

Lectins are a unique group of sugar binding proteins of non-immune origin, able to agglutinate cells and/or precipitate glycoconjugates (Goldstein *et al.*, 1980). Though lectins display a wide variety of unique and interesting properties (Lis and Sharon 1981). The

common functions of lectins have been questioned due to their ubiquitous nature and varied chemical and physical properties (Hankins and Shannon, 1978; S. S. Singh and Rao, 1991). Lectins have been reported in black gram (Singh and Rao 1991; Reddy *et al.*, 1982; Sharma and Salahuddin 1993). Black gram (Vigna mungo L. Hepper) seeds contain two galactose-specific lectins, BGL-I and BGL-II. BGL-I. They were stable between pH 3·5 and 7·5. They were inactivated at 50 °C.

2.7 Methods of reduction of anti-nutrients

There are many chemical and physical processes employed in domestically as well as in industrially to eliminate or to reduce the antinutritional factors. Some basic processing techniques include soaking, cooking, autoclaving, fermenting, germination etc. individually at many occasions a combination of the above methods are used for effective elimination or the reduction of anti-nutritional factors (Misra, 2012).

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).

Processing method is one of the most common and widely used methods in the reduction of anti-nutrients from foods. Foods processing is aimed at reducing the toxic substances in food, increasing the palatability of foods, developing aroma, increasing the shelf life of foods, and minimizing the postharvest loses. There are different kinds of processing method that are effective in reducing anti-nutritional factors in plant foods. These may include; extruder cooking, germination, roasting, socking, boiling, fermentation radiation (Tilahun *et al.*, 2009).

The term "food processing" covers an enormous field, from simple boiling to the use of irradiation. The types of cooking methods differ in countries around the world and also vary with the ethnic background of the family. Processing (cooking) can be both beneficial and detrimental to nutrient composition of foods. It is known that processing techniques may decrease the food value of some nutrients (Nestares *et al.*, 1996) : for example, there is some inevitable leaching of nutrients into the cooking water during processing. The cooking water may or may not be discarded, depending upon cultural and personal preference. Washing and peeling result in the loss of many water-soluble vitamins, since these are more concentrated in the peel and outer layers. With careful control of the processes, nutrient

losses can be minimized without affecting palatability. Different effective processing has been done to reduce the anti-nutritional factor in black gram (Vigna mungo).

2.7.1 Soaking

Soaking is one of the processes used to remove soluble antinutritional factors, which can be eliminated with the discarded soaking liquors, but some metabolic reactions can take place during soaking affecting the content of some compounds (Vidal-Valverde and Frias, 1992). Soaking, is an integral part of traditional methods of processing, saving energy cost by shortening cooking time, offers an additional advantage of rendering the grain nutritionally superior by removing certain anti-nutritional factors like phytic acid, saponin and polyphenols (Kataria *et al.*, 1988a; Kataria *et al.*, 1988b). The decrease of these anti-nutrient contents during soaking may be attributed to leaching out into soaking water under the influence of the concentration gradient.

Many authors cited that soaking the bean prior cooking reduced the cooking time (Taiwo, 1998). Soaking allow the water to disperse in the protein fraction and starch granules which facilitate the protein denaturation and starch gelatinization, which soften the texture of beans (Siddiq and Uebersax, 2012). Soaking the beans in water for 12-14 hours shown no effect on trypsin inhibitor of beans but it causes reduction in oligosaccharides, sucrose content with instance reduction in stachyose and reffinose in chickpea (Egounlety and Aworh, 2003). Phytate is water soluble so the legumes that soaked in water for overnight shown considerable removal of phytates in water in addition to that it also enhances the naturally occurring phytase (Kumar *et al.*, 2010). The loss in phytase may due to the leached down of phytase ion in soaking liquid under the influence of difference in chemical potential which manage the diffusion rate (S. Deshpande and Cheryan, 1984). Soaking the peas in distilled water showed an increase in trypsin inhibitor (Wang *et al.*, 2008). Soaking legumes in simple tap water not reduce the tannin contents (Taiwo, 1998).

Soaking of faba bean in water for 12 hours, significantly reduced phytic acid content by 32.7% (Alonso *et al.*, 2000). A reduction by 28% in phytic acid in black gram Kataria *et al.* (1988a) and by 30% in mungbean. Kataria *et al.* (1989) when soaked in water for 18 hours. Soaking in distilled water or other solution generally affects polyphenol and tannins in grain legumes. Alonso *et al.* (2000) reported that tannin and polyphenols in faba bean seeds were reduced by 47.7 and 4.85%, respectively, after soaking in double-deionized water for 12

hours. Kataria *et al.* (1988a) reported that soaking of black gram in plain water for 18 hours reduced polyphenols by 10%. Soaking mungbean for 18 hours 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

In many countries of the world grain legumes are initially 34 processed by hull (seed coat) removal and splitting (Siegel and Fawcett, 1976). Removal of the hull (dehulling) facilitates a reduction of fiber and tannin contents and improvement in appearance, texture, cooking quality, palatability and digestibility of the grain (Kon *et al.*, 1973).

Since most tannins are located in the testa, physical removal of the testa reduced tannin content. Dehulling significantly ($P\leq 0.05$) reduced tannin content. For cultivar SML tannins were reduced by 54%. The reduction was 35%, 43% and 59% for cultivars Shambat 616, SML 85/1/1 and Shambat 00104, respectively. Phytic acid increased by 4.7%, 5.8%, 6.6% and 7.7% for cultivar SML 85/1/1, Shambat 00104, SML and Shambat 616, respectively. Similar results were reported by Alonso *et al.* (2000). The oxalic acid content in raw samples varied from 456.69 (VL Gahat 8) to 596.44 (HPK4) mg/100 g whereas after dehulling, a highly significant decrease in amount of oxalic acid content was found. Lowest oxalic acid content (318.56 mg/100 g) was recorded in genotype HPK 2 and highest (451.20 mg/100 g) in VLG-31 (Pal *et al.*, 2016).

2.7.3 Cooking

Cooking generally inactivates heat sensitive factors such as trypsin and chymotrypsin inhibitors and volatile compounds. Cooking of beans may be done with or without soaking which typically reduce cooking time and energy cost**.**

Vidal-Valverde *et al.* (1994) reported that cooking of lentils after soaking in distilled water reduced phytic acid by 39% while cooking after soaking in sodium bicarbonate

resulted in 29% reduction. Kataria *et al.* (1988a) found that pressure cooking of soaked seeds of black gram reduced phytic acid by 33%, whereas that of unsoaked seeds resulted in a reduction of 8%. In the whole grain, C-152 lost 72.2% phytate after cooking while S-1552 lost 70.3%. Z. U. Rehman and Salariya (2005), reported that reductions of 21–27% for tannins and 24–35% for phytic acid content when cooking was done by the ordinary boiling method. They also reported that reduction in these antinutrients was significantly higher on cooking in an autoclave at 121 ºC. Reduction in tannin and phytic acid content was 33.1–45.7 and 28.0– 51.6%, respectively, as a result of cooking food legumes in an autoclave at 121 ºC for different time periods.

Cooking beans in water with or without pressure increases the protein quality, the protein and carbohydrate digestibility and inactivates protease and α-amylase inhibitors (Bressani, 1993a, 1993b). Cooking when done under controlled time and temperature improve protein quality of food grain legumes (Bressani, 1993a). An interesting aspect of this study is that the different samples were cooked with regulated amount of water such that no water was drained after cooking.

Two types of cooking are generally practiced traditionally as well as industrially there 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). 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). Z. 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 ºC for 20 min, respectively.

Cooking of soaked seeds appeared to be a little more advantageous than cooking of unsoaked seeds, but the differences between the two treatments were not statistically significant and autoclaving decreased the polyphenols of both the pulses to a greater extent than ordinary cooking: 21-31% and 22-30%, respectively in chickpea and black gram (Jood

et al., 1987). 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). 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 has been documented to be an effective treatment to remove anti-nutritional factors in legumes and mobilizing secondary metabolic compounds, which are thought to function as reserve nutrients (e.g. phytate and raffinose oligosaccharides) (Vidal-Valverde *et al.*, 1994). Germination and fermentation increase vitamins and reduce flatulence factors and phytic acid (Bressani, 1993a).

Germination is the most effective process for the reduction of phytic acid in legumes. N. R. Reddy *et al.* (1978) noticed that phytic acid was hydrolyzed during germination resulting in an increase in available inorganic phosphorus. A. Sharma and Sehgal (1992a)reported that 48 hours germination of two faba bean varieties (VH-131 and WF) reduced tannin content by 90 and 91%, respectively. A decrease in phytic acid content after germination for lentils was reported by Vidal-Valverde. *et al.* (1994) for faba bean by Alonso et al. (2000) for black gram and mung bean by Kataria *et al.* (1989). 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). Khattab *et al.* (2009) reported that 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 exists wholly as a water-soluble salt presumably as potassium phytate (Crean and Haisman, 1963).

The reduction of Tannin was 47, 51 and 60.3% after 2, 4 and 6 days of germination for SML cultivar. Shambat 0010 shows the highest levels of reduction after germination; its 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/100 g which decreased to 308 mg/100 g during 18 h germination and 341 mg/100 g during 12 hours 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). Oxalate content in raw seeds of variety Shekher-2 and Uradsadabahar was recorded 1.74 mg/g and 1.81 mg/g. Minor reduction was observed after 24 hr sprouted seeds of Shekher-2 (0.58%) and Uradsadabahr (1.11%) (Kumari and Verma, 2015).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 antinutritional factors. Eliminating the antinutritional 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 antinutritional 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.

2.8 Black gram utilization and products

Black gram is the basis of products, including food, flour, animal feed, starches, protein concentrates and preparing fast foods. Black gram flour can be used in many food formulations, and its functional and chemical properties are affected by the processing methods. All in all, different processing techniques are primary steps which leads to the product formulation. Black gram can be utilized for coating as biodegradable film comprising the properties of native starch. It is used in preparation of halwa and imarti. In south India, the husked dal is ground into a fine paste and allowed to ferment and then mixed with equal quantity of rice flour to make dosa and idly (Siegel and Fawcett, 1976)

Black gram is best source of protein and carbohydrate from which different kinds of products are prepared by various processors. Some of its useful products are listed as follows:

i. Dal
ii. Uttapa
iii. Dosa
iv. Idaly
v. Vada
vi. Dal makhhani
vii. Papad
viii. Maseura
ix. Urad dal laddu
x. Ulundhu kali (as cake)
xi. Black gram curry
xii. Khaman Dhokla
xiii. Black gram flour cake

2.9 Importance of black gram

Black gram is the third most important legumes in south Asia. It is originated in India, where it has been in cultivation from ancient time. Total black gram production is 2 million tones which is cheapest source of protein in many communities. It is consumed in the form of dal (whole or split, husked or unhusked or parched). Black gram plays an important role in many cultural dishes in India in particular and Asia in common. The dishes include bread, soups, stews, curries, and other side dishes. And it is considered as an indispensable ingredient of exotic cuisines. This excellent bean is very rich in nutrients especially protein, various vitamins, essential minerals, fiber, and antioxidants that all help improve the internal health, the hair, and skin health in various different ways. Due to these nutritious contents, black gram gradually becomes popular in the dishes all over the world. Being rich in protein and phosphoric acid it is an important part in our diet and animal feed, it helps in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen (Salunkhe, 1982)

Black gram has medical properties which helps to heal rheumatic pains, stiff shoulder and contracted knees. It also helps to reduce cholesterol which ultimately improves cardiovascular health and support blood circulation due to high level of magnesium and folate.

Some health benefits of black gram are:

- It improves digestion.
- It is good for diabetics.
- It reduces pain.
- It is good for skin, heart.
- It boosts bone mineral density.
- It is diuretic.
- Energy booster.

Part III

Materials and methods

3.1 Materials

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

3.1.1 Collection of raw materials

The chief ingredient of research, black gram of single variety (*Vigna mungo* dhankute) was collected from Dhankuta.

3.1.2 Chemicals

- H_2SO_4 (Qualigens, 97-99% assay)
- NaOH (Merck, 97% assay)
- HCl (Merck, 35% assay)
- Boric acid (Merck, Powder, 99.5% assay)
- Petroleum ether (Thermo Fisher Scientific, BP 60-80 ^oC)
- Acetone (Qualigens)
- Indicators (Phenolphthalein, Methyl orange)
- $KMnO_4$ (SDFCL, 99% assay)
- Na_2CO_3 (Thermo Electron, anhydrous)
- Folin-denis reagent
- Picric acid, Ferric-chloride (Merck)
- Ammonium thiocyanate (Qualigens, 99% assay), etc.

3.1.3 Glassware and utensils

Petri dish, burette, pipette, test tubes, volumetric flask, bucket, funnel, conical flask, measuring cylinder, crucible, etc.

3.1.4 Equipment

- Hot air oven
- Spectrophotometer (UV-VIS Single Beam Spectrophotometer MODEL NO-291)
- Desiccator
- Soxhlet apparatus
- Electronic balance (AMPUT Electronic Balance Model No-457B, Sensitivity ±0.01
- Thermometer
- Heating mantle (burner)
- Water bath (Intake Serological Wath Bath)

3.2 Methodology

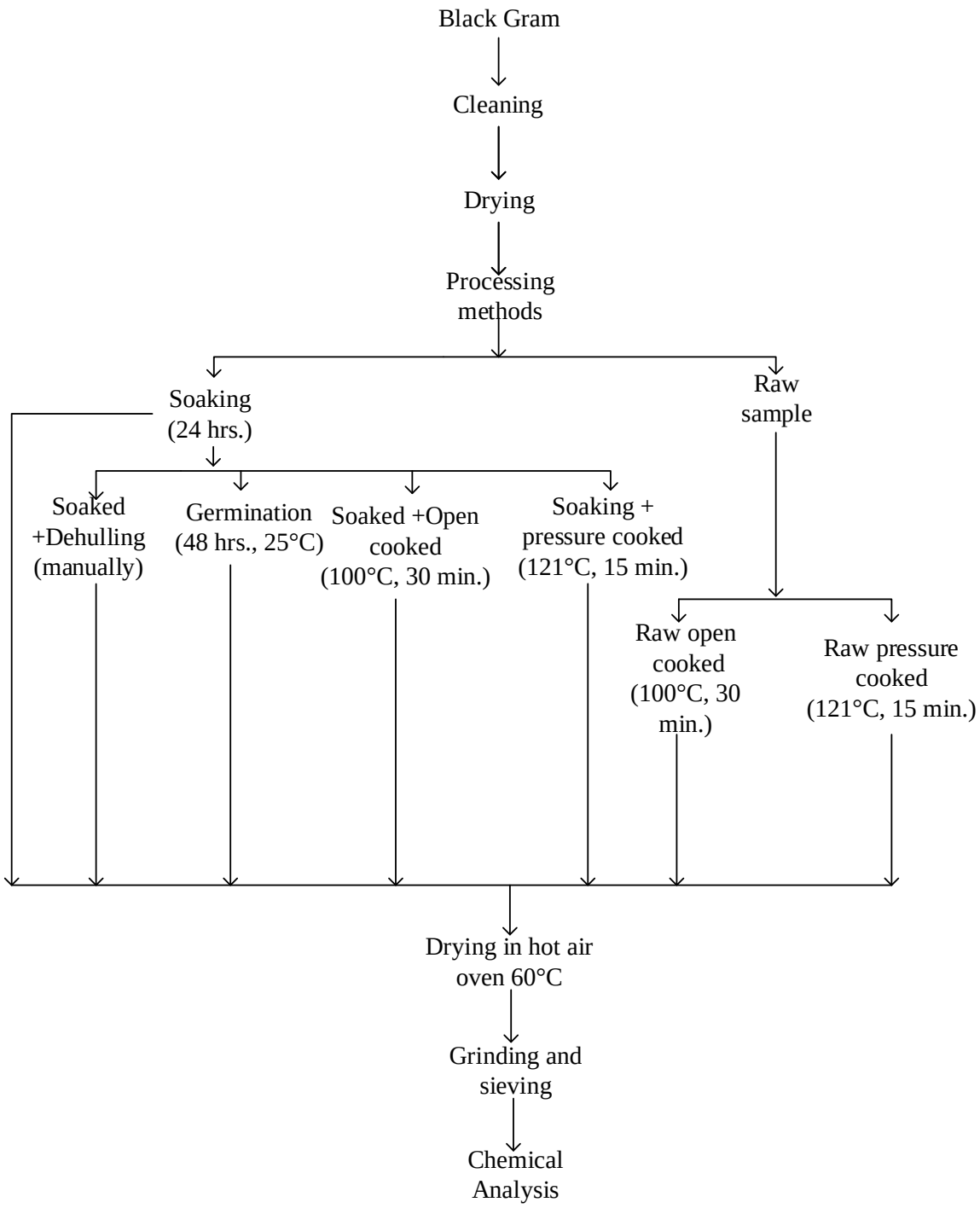


Fig. 3.1 General Flowsheet for processing of black gram

3.3 Processing methods

3.3.1 Soaking

Seeds were soaked in plain water for 12 h at 37 °C. A seed to water ratio of 1:5 (wt: vol) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried in hot air oven maintained at 55° C (except those used for cooking and germination). 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).

3.3.2 Dehulling

Seeds were soaked in plain water for 12 h at 37 ºC. A seed to water ratio of 1:5(wt.: vol) was used. The unimbibed 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 55º 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

The soaked seeds were germinated in sterile Petri dishes lined with wet filter paper in a BOD incubator kept at 25 °C. To obtain a sprout measuring 1.5 to 2.5 cm, the usual size consumed, the seeds of chickpea and black gram varieties were germinated for 68 h and 48 h, respectively. The sprouts were rinsed in distilled water and dried at 55 °C or the rinsed sprouts were cooked till soft, mashed and dried at 55 °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).

3.3.4 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 tap water (seven times the weight of dry seeds), the samples were cooked on a hot plate until they became soft as felt between fingers 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 tap water (three times the weight of dry seeds), the samples were cooked on a hot plate until they became soft as felt between fingers 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 (wt: vol) was used. The cooked samples were mashed and then dried at 55 °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, dry seeds to water ratio of 1:2 (wt: vol) was used. The cooked samples were mashed and then dried at 55 °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 of given sample of Black gram was done by following the procedure as mentioned by (KC and Rai, 2007).

- **Moisture content:** Moisture content were determined by using hot air oven.
- **Crude fat:** Crude fat was determined by using Soxhlet apparatus.
- **Crude protein:** Crude protein was determined by micro Kjeldahl apparatus.
- **Total ash:** Total ash was determined by electric muffle furnace.
- **Crude fiber:** Crude fiber was determined by recovering of ash free residue after sequential treatment with 1.25% sulfuric acid and 1.25% sodium hydroxide and by ashing the retentate.
- **Total carbohydrate**: It was determined by difference method.
  Total carbohydrate% =100- (moisture+ protein +fat + crude fiber+ ash).

3.4.2 Determination of Oxalate

0.1 g of sample was weighed and mixed with 30 ml of 1 M HCL. Each mixture was then shaken in a water bath at 100º C for 30 minutes. To each mixture was added 0.5 ml of 5% Calcium chloride and thoroughly mixed to precipitate out calcium oxalate. The suspension was centrifuged at 3000 rpm for 15 minutes and the supernatant was separated. The pellet was washed twice with 2 ml of 0.35 M NH_4OH then dissolved on 0.5 M H_2SO_4. The solution was then titrated with standard solution of 0.1 M $KMNO_4$ with temperature (60ºC) to faint violet color that persisted for at least 15 seconds which is equivalent for 2.2 mg of oxalate.

3.4.3 Determination of polyphenols

Total phenolic content was determined calorimetrically, using the Folin-Ciocalteu reagents, as described by Singleton *et al.* (1999). The mixture was stirred and allowed to stand for 30 min. the absorbance at 765 nm was measured using a model UV-VIS Single Beam Spectrophotometer. A blank sample consisting of water and reagents was used as reference. The results were expressed as milligrams of gallic acid equivalents per grams powder (mg GAE/g powder) by reference to gallic acid equivalents.

3.4.4 Determination of Phytate

Young and Greaves (1940) was used for the determination of Phytic acid content. 0.2 g of the sample was weighed into 250 ml conical flask. It was soaked in 100 ml of 20% concentrated HCL for 3 hours, the sample was then filtered 50 ml of the filtrate was placed in a 250 ml beaker and 100 ml beaker and 100 ml distilled water added to the sample. Then 10 ml of 0.3% ammonium thiocyanate solution 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 that contained 0.00195 g iron per 1 ml.

Percentage of phytic acid is calculated by formula:

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

3.4.5 Determination of Tannins

Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of phosphor-tungsto-molybdic acid by tannin-like compounds in alkaline condition. The measurement is done at 760 nm. Tannin was quantitatively determined as reported in the manual of food quality control (AOAC, 1990).

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 (Twelfth Edition developed by VSN International Limited).

Part IV

Results and discussion

Whole black gram is a rich source of protein, fiber, several vitamins, calcium, iron, (Talakatta *et al.*, 2012), fructose, non-reducing oligosaccharides, sucrose, raffinose, stachyose, verbascose, ajugose (Suneja *et al.*, 2011). Black gram is an important subsidiary food and rich source of protein and carbohydrate in the diet of many developing as well as developed countries. Unfortunately, the nutritional quality of black gram is subdued by the presence of inherent anti-nutritional factors mainly phytate, polyphenols, tannin, etc.

4.1 Nutritional composition

Proximate composition of raw Black gram as obtained in the laboratory are tabulated in the Table 4.1

Table 4.1 Proximate composition of raw black gram (dry basis)

Proximate constituents	Composition (%)
Moisture (%)	11.41±1.32
Protein (%) *	26.2±0.34
Ash (%) *	4.71±0.12
Fat (%) *	1.625±0.03
Crude Fiber (%) *	6.227±0.65
Carbohydrate (%) *	61.238±3.4

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

Moisture content of raw black gram was found to be 11.41% and the value was similar to the result obtained by Suneja *et al.* (2011), where the moisture level of different varieties of raw black gram is in the range of 7.9-12.6%. Protein was found to be abundant component, nearly about 26.2%. Carbohydrate was found to be the next abundant component, nearly about 56.6%. The value of crude fiber may be different with different varieties of black gram,

nearly about 2.9-7%. Ash content of raw black gram was found to be 4.71%, and the lipid extract obtained were quite low, 1.625%.

4.2 Distribution of anti- nutrients in raw Black gram

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

Table 4.2 Distribution of anti- nutrients in raw black gram (mg/100 g).

Anti-nutrients	Values (mg/100 g)
Oxalate	397±1.45
Phytate	150.8±0.148
Polyphenol	1278.18±2.034
Tannin	507.97±1.783

*Values presented are the average of triplicate determination ± standard deviation

Oxalate content in raw black gram (Vigna mungo) was found in range from 543 to 690 mg/100g by Deraniyagala and Gunawardena (1999) which was higher than value obtained by us. Phytate content in average was found to be 150.8 mg which was found to be very less than value determined by Kakati *et al.* (2010), 1147.03 mg/100 g. Polyphenols in black gram is the most variable a,ntinutrients where its value ranges from 842 to 892 mg /100 gin study conducted by Jood *et al.* (1987) The polyphenol content in our sample of raw black gram was found to be 1278.18 mg/100 g which was higher than that their findings. Average tannin content in our sample was found to be 507.97 mg/100 g which was lower than findings from D. S. S. Rao and Deosthale (1981) experiment 861 mg/100 g. According to different research it is concluded that antinutrients 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).

4.3 Effect of different processing method on oxalate content of Black gram

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

4.3.1 Effect of Soaking

Soaking shows considerable decrease in oxalate content of black gram and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that soaking significantly reduced ($p<0.05$) total oxalate content, which reduced from 397 mg/ 100 g to 310 mg /100 g.

Our results obtained tally in line with result obtained by Shi *et al.* (2018) where he found significant reduction ($p<0.05$) in oxalate during soaking i.e. 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%). He concludes that Soaking the legumes seeds in distilled water significantly decrease ($p<0.05$) the total oxalate content in range from 17.4%- 51.89%. Loss of soluble oxalates in water was considered to be the primary factor contributing to total oxalates reduction Akhtar *et al.* (2011).

4.3.2 Effect of Germination

Germinated samples showed a significant ($P \leq 0.05$) decrease in the oxalic acid content over the raw and dehulled samples, which is reduced from 397 mg/ 100 g to 280 mg/100 g after germination (29.47% reduction).

Near about similar results were observed by Murugkar *et al.* (2013) and Pal *et al.* (2016). He 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 hour of germination. Our result obtained was slightly similar to that of his research i.e. decrease from 397-280 mg/100 g which is 29.47% reduction during 48 hours 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).

4.3.3 Effect of Dehulling

Dehulling shows considerable decrease in oxalate content of black gram and has been documented to be an effective treatment to remove anti-nutritional factors in legumes. This result shows that dehulling significantly reduced ($p<0.05$) total oxalate content, which reduced from 397 mg/ 100 g to 212 mg /100 g i.e. 46.59% reduction during dehulling.

Our result obtained tally with Pal *et al.* (2016), he found a highly significant decrease in amount of oxalic acid content range from 587.84 mg/ 100 g in raw to 373.75 mg/ 100 g after dehulling i.e. 36.42% reduction of total oxalate content. In our research 46.62% reduction of oxalate content was found in dehulled sample.

The oxalate content of different processing treatments is given in figure 4.1.

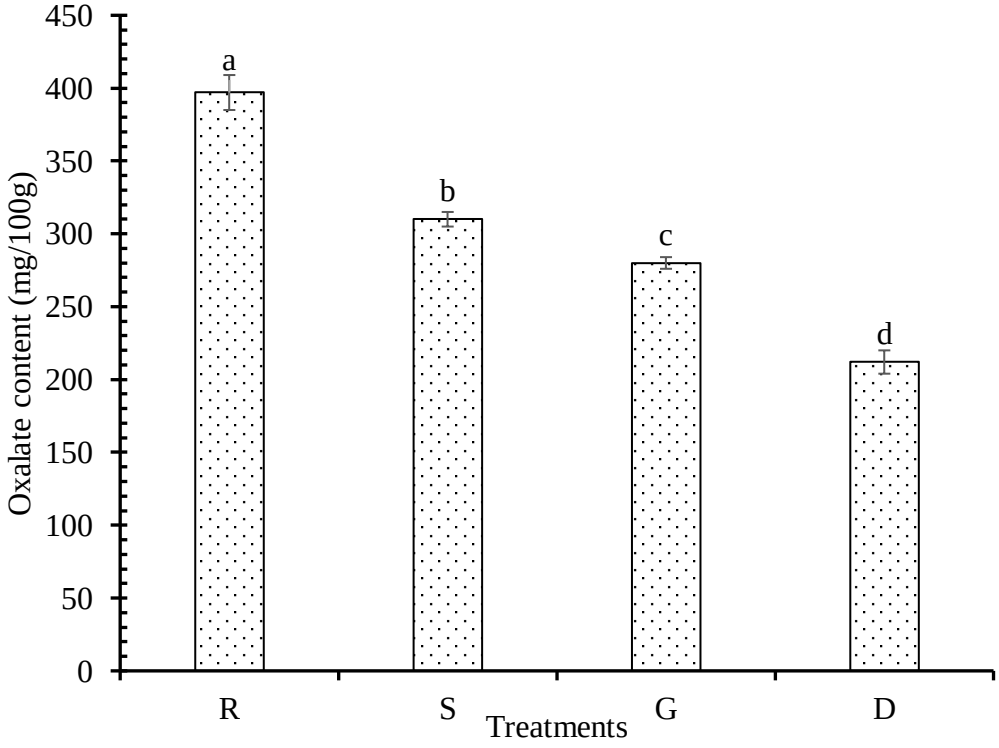


Fig: 4.1 Effect of different processing method on oxalate content

[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, S, G and D are the samples of black gram representing raw, soaked, germinated and dehulled respectively].

4.3.4 Effect of Cooking

Effect of cooking shows significant reduction ($p<0.05$) on oxalate content range from 397 mg/100 g to 232 mg/100 g, 200 mg/100 g, 200 mg/100 g, and 160 mg/100 g for samples of raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked respectively. Our findings result that soaked pressure cooked reduced 59.69% of oxalate content which is the most effective method, followed by soaked open cooked and raw pressure cooked 49.62% reduction and raw open cooked 41.56% reduction. The effect of cooking methods on oxalate content is presented in Fig. 4.2.

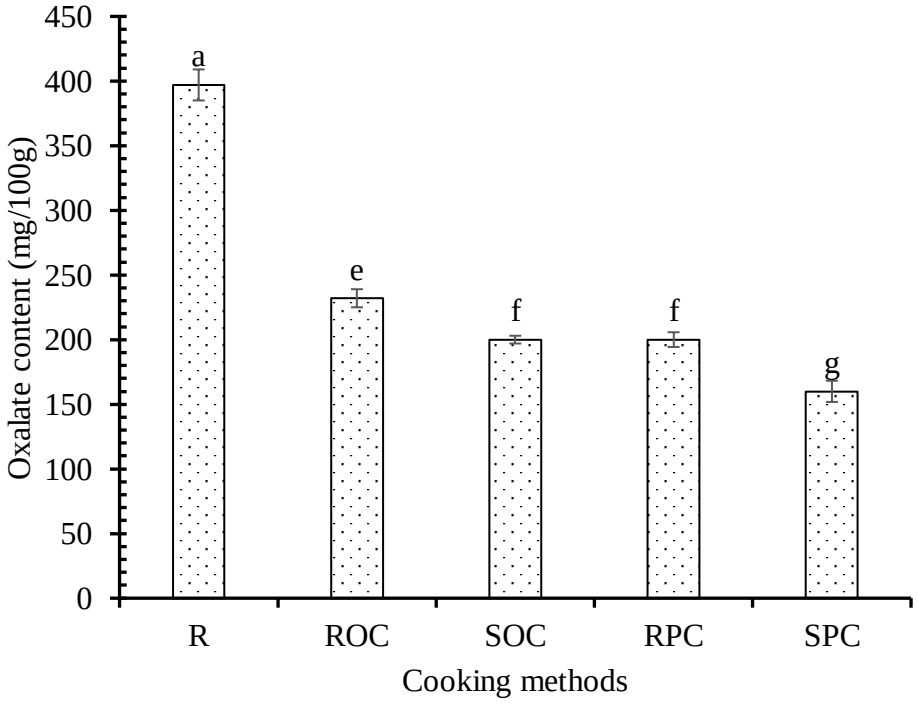


Fig. 4.2 Effect of cooking methods on oxalate content

[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, RPC and SPC are the samples of black gram representing raw, raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked].

According to Akhtar *et al.* (2011), he found 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 where he found maximum reduction during pressure cooking than

open cooking which was similar to our findings. Our findings were slightly similar to his research which range from 397 mg/100 g to 160 mg/ 100 g for soaked pressure cooked i.e. 59.689% reduction. Loss of soluble oxalate in water was considered to be the primary factor contributing to total oxalate reduction (Akhtar *et al.*, 2011).

4.4 Effect of different processing method on Phytate content of Black gram

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

4.4.1 Effect of Soaking

Effect of soaking on phytate content of black gram was studied and the value obtained showed that there is significant reduction ($p<0.05$) in phytate content. Our result shows great reduction range from 150.8 mg/100 g to 116.02 mg /100 g after soaking for 12 hours (23.06% reduction). Similar result was obtained by (Alonso et al., 2000), he found that soaking of faba bean in water for 12 hours, significantly reduced phytic acid content by 32.7%. Also, the result obtained by Kataria *et al.* (1988a) shows similar value which is reduced by 28% during 12 hours soaking in black gram.

Phytate is water soluble so the legumes that soaked in water for overnight shown considerable removal of phytates in water in addition to that it also enhances the naturally occurring phytase (Kumar *et al.*, 2010). The loss in phytate may be due to the leached down of phytate ion in soaking liquid under the influence of difference in chemical potential which manage the diffusion rate (S. Deshpande and Cheryan, 1984). The phytate content of black gram may vary depending on the variety, growth, season, soil conditions, time of harvest and many factors. Differences observed in the reported values and the values determined in this study could be attributed to these factors.

4.4.2 Effect of Germination

Germination shows considerable decrease in phytate content of black gram 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 150.8 mg/ 100 g to 80.21 mg /100 g. Our results obtained tally in line with result obtained by Grewal et al. (2006), where he found significant reduction in phytate during germination i.e. 42.82-48.91% reduction.

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). Khattab *et al.* (2009) reported that similar reduction was obtained during soaking and germination. This could be due to the fact that phytic acid in dried legumes exists wholly as a water-soluble salt presumably as potassium phytate (Crean and Haisman, 1963).

4.4.3 Effect of Dehulling

Effect of dehulling on phytate content of black gram was studied. The value obtained showed that there is significant reduction ($p<0.05$) in phytate content, which is reduced 116.02 mg/ 100 g to 46.4 mg/100 g after dehulling (69.23% 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 %.

During research conducted by Cummings (1976), he reported that phytic acid forms complexes with the seat coat fractions which is the reason for decrease in phytic acid. Literature data also suggest that milling lowers the phytic acid content of rice. This may be attributed to phytic acid accumulating in aleurone layer and globoids which are removed during the polishing of rice which also supports our present findings R. Reddy and Salunkhe (1980).

The phytate content of different processing treatments is given in figure 4.3.

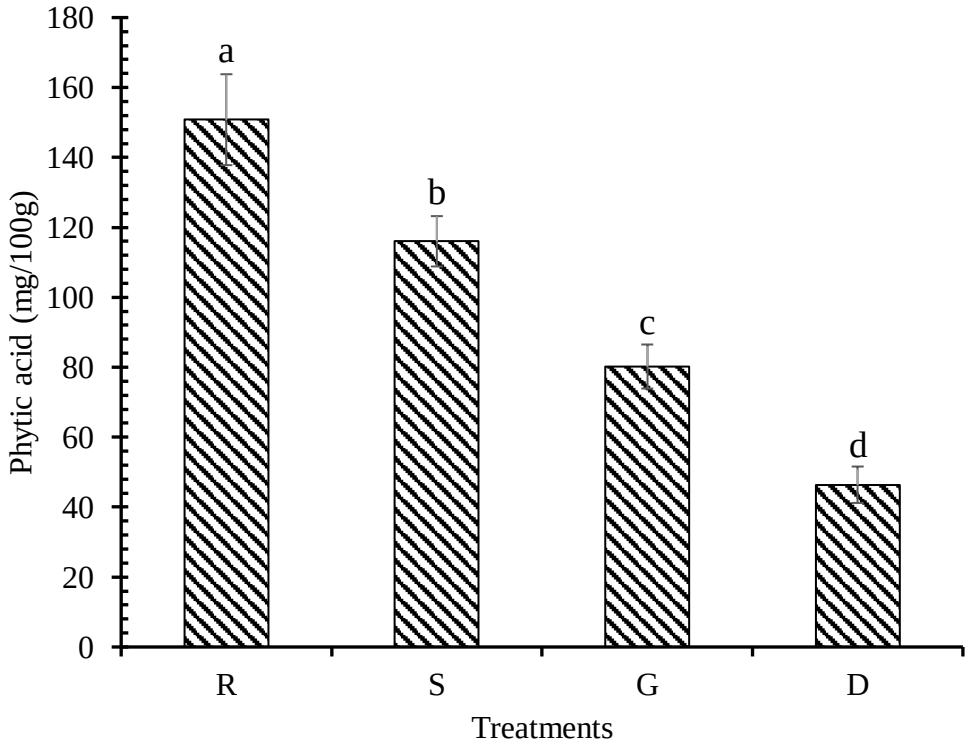


Fig 4.3 Effect of different processing method on phytate content

[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, S, G and D are the samples of black gram representing raw, soaked, germinated and dehulled respectively].

4.4.4 Effect of Cooking

Effect of open cooking for 30 min and pressure cooking at 15 psig for 15 min on total phytate content of Black gram was studied. An interesting aspect of this study is that the different samples were cooked with regulated amount of water such that no water was drained after cooking. The value obtained showed that there is significant reduction ($p<0.05$) in phytate content, which is reduced from 150.8 mg/100 g to 104.42, 69.61, 69.61 and 58.12 mg/ 100 g for raw open cooked, soaked open cooked, raw pressure cooked, soaked pressure cooked respectively. The effect of cooking methods on phytate content is given in Fig. 4.4.

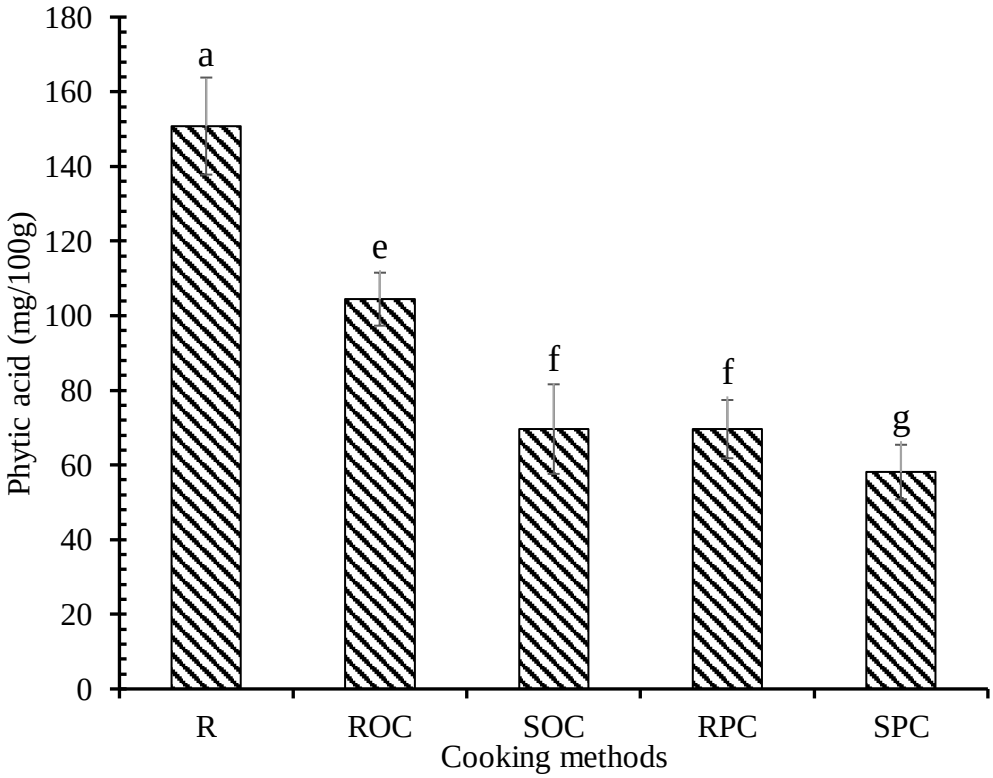


Fig. 4.4 Effect of cooking methods on phytate content

[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, RPC and SPC are the samples of black gram representing raw, raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked].

Similar results were obtained by Kataria *et al.* (1988a), he found that pressure cooking of soaked seeds of black gram reduced phytic acid by 33%, whereas that of unsoaked seeds resulted in a reduction of 8%. Our findings were found to be greater reduction compared to his finding i.e. 30.75% reduction for raw open cooked, 53.84% reduction for both soaked

open cooked and raw pressure cooked, 61.46% reduction for soaked pressure-cooked sample. Z. U. Rehman and Salariya (2005), reported that 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 ºC for different time periods.

4.5 Effect of different processing method on Polyphenols content of black gram

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

4.5.1 Effect of Soaking

Polyphenol content of raw black gram was determined and found to be 1278.18 mg/100 g. Present study show that soaking significantly decreased ($p<0.05$) polyphenol content from (1278.18 mg/ 100 g to 762.3 mg/ 100 g) i.e. 40.36% reduction.

During research conducted by Jood *et al.* (1987), he reported that Soaking the seeds for 12 h reduced the polyphenol contents of both the legumes significantly 48% in chickpea and 47% in black gram. Our result obtained shows slightly lower reduction as compared to this research. The loss of polyphenols during soaking may be due to leaching out of soluble polyphenolic compounds in soaking water which was discussed by Kumari and Verma (2015).

4.5.2 Effect of Germination

Polyphenols content of raw black gram was determined and the value obtained showed that there is significant reduction ($p<0.05$) in polyphenols content, which is reduced from 1278.18 mg/100 g to 1155.23 mg/100 g after germination (9.62% reduction).

During research conducted by Jood *et al.* (1987), he reported that Sprouting was considerably effective in decreasing the polyphenol contents of both the legumes (19-28% in chickpea and 17-20% in black gram). Our result obtained was slightly lower than his findings i.e. 9.62% reduction. D. S. S. Rao and Deosthale (1981) concluded that the reduction of total phenolics compounds during germination may be attributed to the presence of polyphenol-oxidase and enzymatic hydrolysis.

4.5.3 Effect of Dehulling

Polyphenols content of black gram was found to be significantly reduced ($p<0.05$) from 1278.18 mg /100 g to 357.54 mg/100 g (72.03% reduction) after dehulling process. Polyphenols are not uniformly distributed all over black gram, it varies with different parts. Higher amount of polyphenols was reported in seed coat which was found to be 13,466 mg GAE /100 g and in cotyledon was 382.04 mg GAE/ 100 g according to Jood *et al.* (1987).

More phenolics in the seed coat of S-1552 than C-152 as decortication resulted in such drastic reduction in the phenolic content. Comparison showed that 24.4 and 53.6% of the total phenolics in the uncooked whole grain of C-152 and S-1552, respectively, were lost because of decortication (Adebooye and Singh, 2007). Dehulling removes seed coat and with its removal maximum amount of polyphenols concentrated at seed coat is reduced which justified our result which showed maximum reduction in polyphenol content of dehulled sample.

The polyphenols content of different processing treatments is given in figure 4.5.

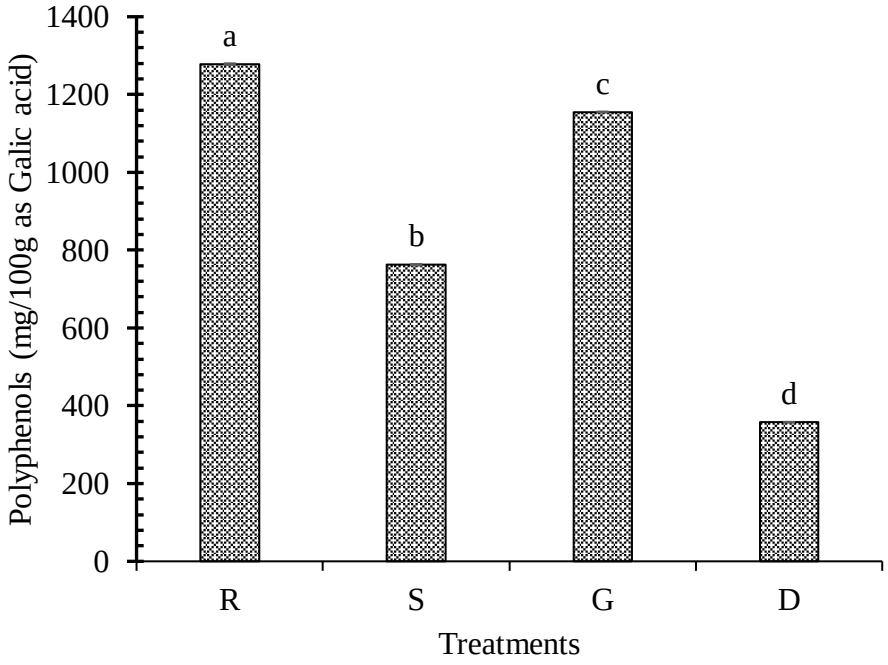


Fig 4.5 Effect of different processing method on polyphenol content

[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. R, S, G and D are the samples of black gram representing raw, soaked, germinated and dehulled respectively].

4.5.4 Effect of Cooking

Effect of open cooking for 30 min and pressure cooking at 15 psig for 15 min on total polyphenol content of Black gram was studied. An interesting aspect of this study is that the different samples were cooked with regulated amount of water such that no water was drained after cooking. The value obtained showed that there is significant reduction ($p<0.05$) in polyphenol content, which is reduced from 1278.18 mg/100 g to 649.87, 610.186, 734.943, 713.525 mg/ 100 g for raw open cooked, soaked open cooked, raw pressure cooked, soaked pressure cooked respectively. Effect of cooking methods on polyphenol content is given in Fig. 4.6.

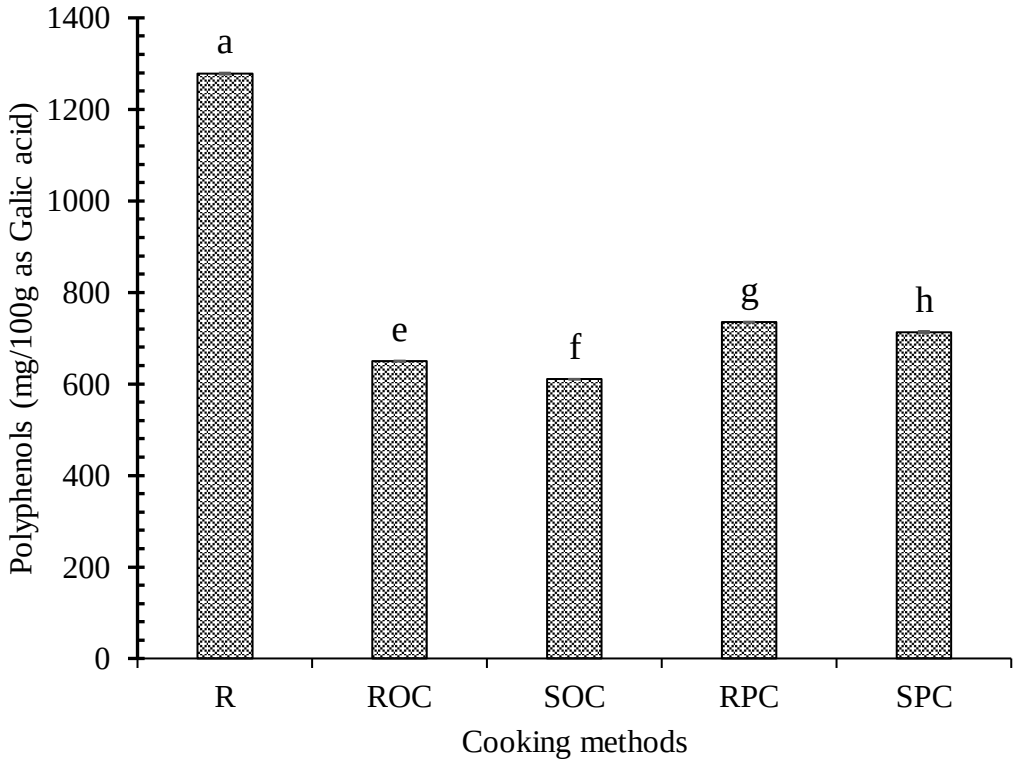


Fig. 4.6 Effect of cooking methods on polyphenol content

[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, RPC and SPC are the samples of black gram representing raw, raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked].

Similar results was obtained by Adebooye and Singh (2007), he 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 49.16%. 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 which was slightly lower than our findings, where reduction was 42.50%. According to Jood *et al.* (1987), he found that cooking of soaked seeds appeared to be a little more advantageous than cooking of unsoaked seeds, which shows significant difference ($p<0.05$) in polyphenol reduction among open cooking and pressure cooking as in line with our findings. Therefore, the losses of phenolics because of cooking were suspected to be due to outright destruction or breakdown as noted by (Zhang and Hamauzu, 2004) or conversion of phenolics to other products during cooking, or it could also be attributed to possible labile nature of phenolics and its subsequent escape as vapor during cooking (Adebooye and Singh, 2007).

4.6 Effect of different processing method on Tannin content of black gram

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

4.6.1 Effect of Soaking

Tannin content of raw black gram was determined and the value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 507.97 mg/100 g to 385.506 mg/100 g after soaking (24.109 % reduction).

Effect of soaking on faba bean was studied by Ibrahim and Rahim (1987) found that there is significant reduction ($p<0.05$) in tannin content of that bean where reduction of 29.02 % tannin was observed during 12 hours of soaking. In our case there was slightly lower reduction than that in faba bean. Similar data was obtained by Alonso et al. (2000) where tannin in faba seeds were reduced by 47.7% after soaking for 12 hours. 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 Germination

Effect of germination on tannin content of black gram was studied. The value obtained shows that there is significant reduction ($p<0.05$) in tannin content, which is reduced from 507.97 mg/ 100 g to 290.56 mg/100 g after germination (42.80 % reduction).

Our result obtained tally in line with result obtained by Ibrahim and Rahim (1987), he reported that the reduction of 47, 51 and 60.3 % after 24, 48 and 72 hours of germination for SML cultivar where our result also fall under this research i.e. 42.80% reduction. . Reduction in tannin content after germination may be attributed to the leaching out effect during hydration which was reported by (Beleia *et al.*, 1993).

4.6.3 Effect of Dehulling

The tannin content of Raw black gram was determined. Our present study found that dehulling significantly decreased ($p<0.05$) tannin content from 507.97 mg/ 100 g to 200.45 mg/ 100 g after dehulling i.e. 60.54 % reduction.

During research conducted by Ibrahim and Rahim (1987), he found that dehulling significantly ($P\leq 0.05$) reduced tannin content. For cultivar SML tannins were reduced by

54%. He found that the reduction of 35%, 43% and 59% for cultivars Shambat 616, SML 85/1/1 and Shambat 00104, respectively for dehulled faba beans. Our result obtained was slightly higher than this research which was 60.54% reduction. Since most tannins are located in the testa, physical removal of the testa reduced tannin content.

The tannin content of different processing treatments is given in Fig. 4.7.

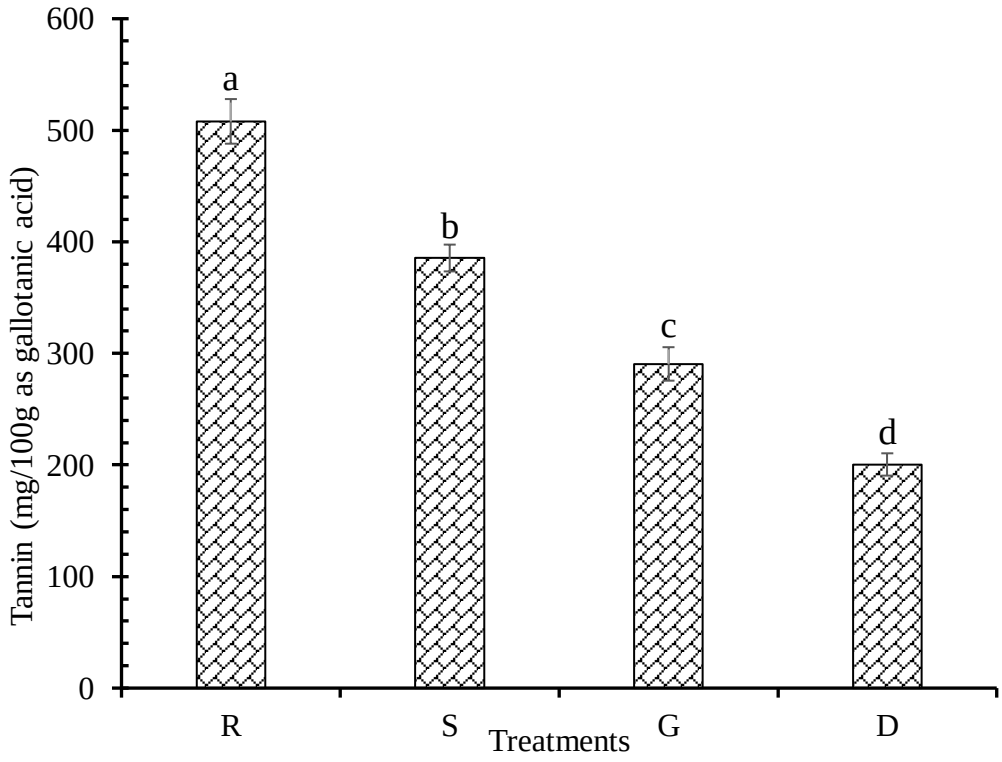


Fig 4.7 Effect of different processing method on tannin content

[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. R, S, G and D are the samples of black gram representing raw, soaked, germinated and dehulled respectively].

4.6.4 Effect of Cooking

Cooking shows significant decrease ($p<0.05$) in tannin content of black gram 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 507.97 mg/100 g to 373.79 mg/100 g ,310.41 mg/100 g, 343.73 mg/100 g and 260 mg/100 g respectively for samples of raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked. Out of which maximum reduction of 48.82% was found to be in soaked pressure-cooked followed by soaked open cooked 38.89%, raw pressure-cooked 32.33%, raw open cooked 26.41%. Effect of cooking methods on tannin content is presented in Fig. 4.8.

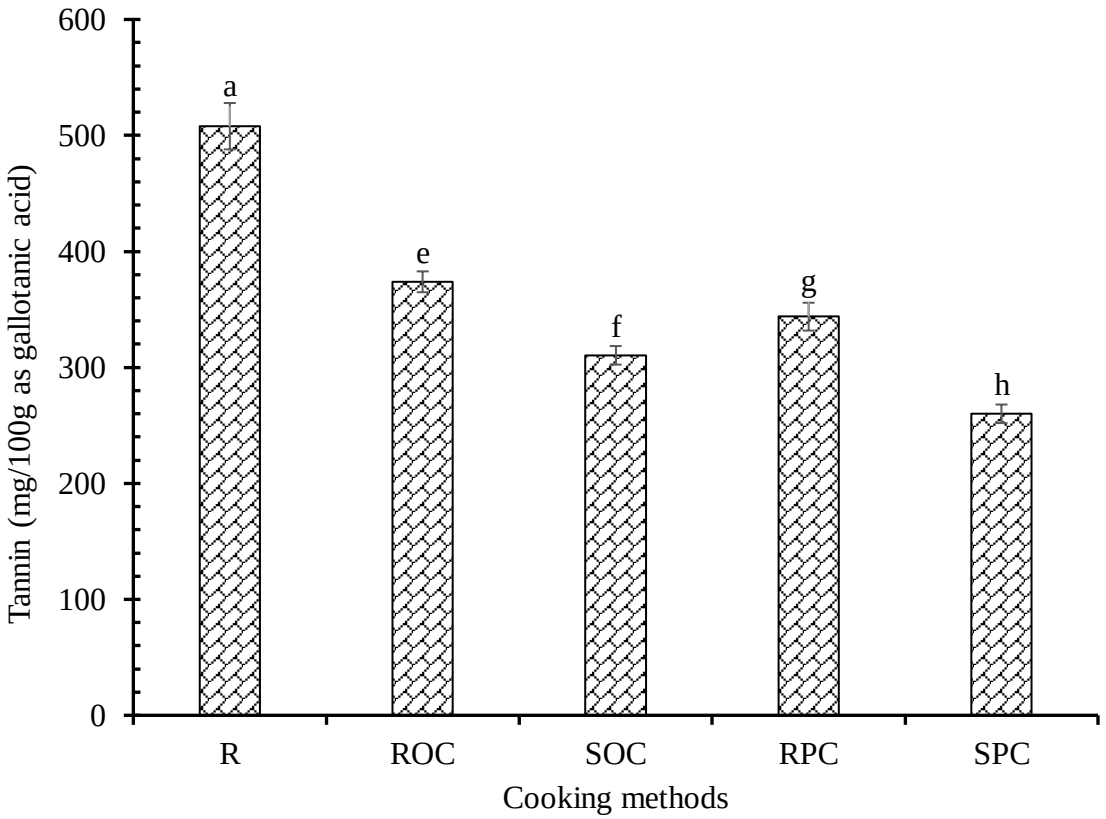


Fig. 4.8 Effect of cooking methods on tannin content

[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, RPC and SPC are the samples of black gram representing raw, raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure cooked].

Ibrahim and Rahim (1987) 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 our findings. Z. U. Rehman and Salariya (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 ºC for 20 min, respectively. About 30% of total tannin decomposed during cooking (Ziena *et al.*, 1991). A. Sharma and Sehgal (1992a) reported that significant reduction in tannins (76-81%) after cooking of two faba bean cultivars.

4.7 Comparison on Proximate composition of best effective treatment

Components	Raw	Dehulled	% Change
Moisture (%)	11.41±0.23	4.02±0.16	-
Protein (%) *	26.2^{a}±0.34	29.12^{b}±0.28	+11.145
Ash (%) *	4.71^{a}±0.12	3.13^{b}±0.08	-29.94
Fat (%) *	1.625^{a}±0.03	1.05^{b}±0.08	-35.384
Crude Fiber (%) *	6.227^{a}±0.65	3.507^{b}±0.29	-43.701
Carbohydrate (%), by difference*	61.238^{a}±3.4	63.193^{a}±4.5	+3.192

[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 ($p>0.05$)

*Represents values in dry basis. Where + Sign represents increase in value where – sign represent decrease in value].

Moisture content of dried dehulled black gram was found to be 4.02% which is comparatively lower than raw black gram. Our results showed that decortication resulted in significant ($p\leq 0.05$) losses of ash and mineral content i.e. from 4.71% to 3.13%. The implication of this result is that the seed coat is rich in mineral nutrients and substantial proportion of mineral nutrients are lost when cowpea is decorticated and the seed coat is discarded. Decortification results in significant reduction ($p\leq 0.05$) of crude fiber content i.e. from 6.227% to 3.507%. Chetia (1991) reported that the decrease in the crude fiber content can be attributed to the dilution effect on nutrients in processed and cooked samples with the increase in the moisture content. Similar results was also reported by Rajaram and Janardhan (1990) in Vigna spp. This finding is consistent with the report of a previous study by Attia *et al.* (1994) which stated that decortication of chickpea resulted in significant losses of dietary fiber, Ca, Mg, Zn and K. Decortification results in significant reduction ($p\leq 0.05$) of fat content i.e. from 1.625% to 1.05%. This was suspected to be due to the presence of higher amounts of fatty acids in the seed coat and germ which was removed during dehulling.

But the protein and carbohydrate content in the present study significantly increased ($p \leq 0.05$) i.e. protein content from 26.62% to 29.12% and carbohydrate content was found to be increased from 61.23% to 63.193%, which was consistent with the values earlier reported by Mang *et al.* (2016), where he found crude protein content of mucuna (*Mucuna pruriens* L.) seeds was increased from 28.38%-36.96% and carbohydrate content was also found to be increased from 20.93% to 25.11%. Increase in carbohydrate and protein content during dehulling was due to removal of mineral and antinutrients 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 complextion of protein and carbohydrate by tannins and polyphenols present in the bean hulls.

Part V

Conclusions and recommendation

5.1 Conclusions

In this study raw black gram was processed with several treatments and the variations in reduction of anti-nutrients were analyzed in the lab. Within the scope of the present work following conclusions can be drawn.

1 The mean value of oxalate, phytate, polyphenol and tannin content in raw black gram were found to be 397, 150.8, 1278.18 and 507.97 mg/100 g respectively on the dry basis.

2 Maximum reduction of anti-nutrients such as phytate (69.23%), polyphenols (72.03%) and tannin (60.54%) were found by dehulling except for oxalate which is reduced by soaked pressure cooked (59.69%).

3 All processing methods, single or combined reduce all anti-nutrients significantly ($p<0.05$).

4 In case of cooking, soaked and pressure-cooking method was more effective than raw open cooking method in reducing the anti-nutrients.

5 Overall viewing, dehulled and soaked pressure-cooked method was found to be most effective in reducing the anti-nutrients of black gram.

5.2 Recommendations

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

1. Reduction trend of anti-nutrients of black gram shows maximum reduction on dehulling and in pressure cooking (if combined effect with soaking is considered). So, it is recommended to process black gram through dehulling and/or soaking pressure cooking for making other product from black gram.

2. Effects of different combined treatments (dehulling and cooking, germination and cooking) in anti-nutritional factors can be studied.

3. Reduction pattern of anti-nutritional factors on varying processing time in different methods can be studied.

4. Effect of different processing techniques in other anti-nutritional factors like trypsin inhibitors, saponin which are abundant in black gram can be studied.

Part VI

Summary

Black gram is one of the most nutritious beans and is commonly used in India for its wide health benefits. It is both, consumed by cooking and used in Ayurvedic medicine, and is equally beneficial when used either way. It offers a ton of health benefits. Black gram can improve your digestion as it is filled with fibers that help with the bulking up and movement of your stool. It can therefore be used to combat both, constipation and diarrhea. Black gram is one of the most vital varieties of pulse in the Indian subcontinent, and has a lot of nutrients in it. Although they are not that common, they are fairly easy to procure all over the world.

In the present study, seven types of processing methods were used to investigate reduction of anti-nutrient level in black gram. The anti-nutrient studied were oxalate, phytate, polyphenol and tannin. The processing method include soaking, germination, dehulling, raw open cooked, soaked open cooked, raw pressure cooked and soaked pressure-cooked method. 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 oxalate, phytate, polyphenols and tannin content of raw black gram were 397 mg/ 100 g, 150.8 mg/100 g, 1278.18 mg/100 g and 507.97 mg/100 g respectively. All processing method reduced significantly ($p<0.05$) the anti-nutrients content of black gram along with the combination method which was more effective than singlet treatment. The reduction in phytate of soaked open cooked and raw pressure cooked were not significantly different ($p>0.05$). Similarly, decrease in oxalate level by soaked open cooked and raw pressure were not significantly different($p>0.05$). 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. Therefore, combination-processing method was more effective than singlet method.

References

Adebooye and Singh. (2007). Effect of cooking on the profile of phenolics, tannins, phytate, amino acid and mineral nutrients of whole- grain and decortificated vegetable cowpea (Vigna unguiculata L. Walp). *J. Food Qual.* . **30** (1101-1120).

Adeparusi. (2001). Effect of processing on the nutrients and antinutrients of lima bean (Phaseolus lunatus L.), flour. *Food/Nahrung*. **4.**, 94-96. (2009). Effect of processing on some physicochemical and antinutritional factors of taro (Colocasia esculenta (l.) schott.) grown in Ethiopia. M.Sc. (Food Science and Nutrition) Thesis. Addis Ababa Univ., Ethopia.

Agiang, Umoh, Essien and Eteng. (2010). Nutrient Changes and Antinutrient Contents of Beniseed and Beniseed Soup during Cooking using a Nigerian Traditional Method. *Pak. J. Biol. Sci.* **13** (20), 1011-1015.

Akgul. (2017). Green Port / Eco Port Project - Applications and Procedures in Turkey. **95**, 1042-1063. [doi:10.1088/1755-1315/95/4/042063].

Akhtar, Israr, Bhatty and Ali. (2011). Effect of cooking on soluble and insoluble oxalate contents in selected Pakistani vegetables and beans. *Int. J. Food Properties*. **14**, 241-249.

Alonso, Aguirre and Marzo. (2000). Effects of of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba bean and kidney bean. *Food Chem.* **68**, 159-1633.

Anoma and Thamilini. (2016). Roots and Tuber Crops as Functional Foods: A Review on Phytochemical Constituents and Their Potential Health Benefits. *Int. J. Food Sci.* **2016**, 15. [doi:10.1155/2016/3631647].

Anon. (2006). "Post Harvest Profile of Black Gram". Government of India, (Ministry of Agriculture)

Anonymous. Economics division, DOA. Retrieved from https://www.doa.gov.lk/FCRDI/index.php/en/crop/44-blackgram-e. [Accessed 23 Feb., 2018].

AOAC. (1990). "Official Methods of Analysis" (15th ed.). Arlington, VA, USA. Association of Official Analytical Chemists. pp. 912-918.

Arias, Ortiz and Mozos. (1993). Phenolic compounds and pyrimidine glycoside determination in Vicia narbonensis seed. *J. Agric. Food Chem.* **41**, 2329-2333. [Cited in M. Muzquiz, C. Carmen, G. D. Hill, M. M. Pedrosa and B. Carmen. (2004). "Recent Advances of Research in Antinutritional Factors in Legume Seeds and Oilseed". EAAP Publication. Toledo, Spain].

Arora and Mauria. (1989). Record from Proseabase. van der Maesen, L. J. G.; Somaatmadja, S. (Eds). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. *Food Agric. Org. U.S.*

Attia, El-Tabey Shehata, Aman and Hamza. (1994). Effect of cooking and decortication on the physical properties, the chemical composition and the nutritive value of chickpea (Cicer arietinum L.). *Food Chem.* **50**, 125-131.

Baligar and Fageria. (2007). Agronomy and physiology of tropical cover crops. *J. Plant Nutr*. **8** (8), 1287-1339.

Bartosz, Judy, Veikko, Sylwia and Aino. (2017). Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances. *Food Chem.* **6** (5), 610-614.

Bassiri and Nahapetian. (1977). Differences in concentrations and interrelationships of phytate, phosphorus, magnesium, calcium, zinc, and iron in wheat varieties grown under dry land and irrigated conditions. *J. Agric. Food Chem.* **25**, 1118.

Beleia, Thu Thao and Ida. (1993). Lowering phytic phosphorus by hydration of soybean. *J. Food Sci*. **58**, 375-378.

Bhandari and Kawabata. (2005). Assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam (Dioscorea spp) tubers of Nepal. *Food Chem.* **85.**, 281-287.

Bhattacharya, Latha and Bhat. (2004). Controlled stress rheological measurement of blackgram flour dispersions. **63**, 135-139. [doi:10.1016/S0260-8774(03)00291-7].

Bressani. (1993a). Grain quality of common beans. *Food Reviews Int.* **9**, 217-297.

Bressani. (1993b). Grain quality of common beans. *Food Reviews Int.* **9**, 217-297.

Brune. (1989). Iron absorption and phenolic compounds: importance of different phenolic structures. *Eur. J. Clinical Nutr.* **43**, 547-558.

Cheeke and Shull. (1985). "Natural toxicants in feeds and poisonous plants". Westport (Conn.) : Avi. [ISBN 0870554824].

Cheok, Salman and Sulaiman. (2014). Extraction and quantification of saponins. *A review. Food Res. Int.* **59**, 16-40.

Chetia. (1991). Biochemical compositions of matured seeds of field bean (Dolichos lablab L.) in relation to cooking. M.Sc. (Agri.) Thesis. Assam Agricultural Univ., Jorhat.

Crean and Haisman. (1963). The interaction between phytic acid and divalent cations during the cooking of dried peas. *J. food sci. and agric.* **14** (11), 824-833. [doi:10.1002/d.y2740141109].

Cummings. (1976). What is fiber? In "Fiber in Human Nutrition" [Press release]. Plenum Press. Retrieved from Spillar, G.A. Amen, R.J.

Das. and TK. (2012). Saponin: Role in animal system. Veterinary World. **5.** (4.), 248-254.

Deol and Bains. (2010). Effect of household cooking methods on nutritional and anti nutritional factors in green cowpea (Vigna unguiculata) pods. *J. Food Sci. Technol.* **47** (5), 579-581. [doi10.1007/s13197-010-0112-3].

Deraniyagala and Gunawardena. (1999). Effects of cooking on the mineral and oxalate content of some legumes. *Vidyodaya J. Food Sci.* **8**, 39-49.

Deshpande and Cheryan. (1984). Effects of Phytic Acid, Divalent Cations, and Their Interactions on α-Amylase Activity. *J.Food. Sci.* **49**, 516-519.

Deshpande and Salunke. (1986). Interactions of tannic acid and catechin with legume starches. *J. Food Sci.* **47.** (4), 2080-2081. [doi:10.1080/10408398609527441].

Devi and Kurup. (1972). Hypolipidaemic activity of Phaseolus mungo (blackgram) in rats fed a high-fat-high-cholesterol diet: Isolation of a protein and polysaccharide fraction. *J. Food Sci.* **15** (2), 223-230.

Effiong and Umoren. (2011). Effect of Multi-processing Techniques on the Chemical Composition of Horse Eye Bean (Mucuna urens). *Asian J. Animal Sci.* **5** (5), 340-348.

Egounlety and Aworh. (2003). Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. Walp) and groundbean (Macrotyloma geocarpa Harms). *J. Food Eng.* **56**, 249-254.

Elias, Colindres and Bressani. (1964). The nutritive value of eight varieties of cowpea (Vigna sinensis). *J Food Sci.* **29**, 118-122.

FAO. (1973). Energy and protein requirements : report of a Joint FAO/WHO ad hoc expert committee [meeting held in Rome from 22 March to 2 April 1971]. Geneva: World Health Organization.

Fery. (2002). New opportunities in Vigna [Press release]. ASHS Press, Alexandria. Retrieved from J. Janick and A. Whipkey (eds.).

Fox, Uniacke-Lowe, McSweeny and O'Mahony. (2015). "Dairy Chemistry and Biochemistry" (2nd ed.). Springer International. Switzerland.

Ghavidel and Prakash. (2007). The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *LWT Food Sci. Technol.* **40**, 1292-1299. [doi:10.1016/j.lwt.2006.08.002]

Goldfarb. (1988). Dietary factors in the pathogenesis and prophylaxis of calcium nephrolithiasis. *Kidney Int.* **34.**, 544-555.

Goldstein, Hughes, Monsingny, Osawa and Sharon. (1980). What should be called a lectin. **285** (66).

Grewal and Jood. (2006). Effect of processing treatments on antinutritional contents of green gram. *J. Food Chemi.* **30** (5), 535-546. [doi:10.1111/j.1745-4514.2006.00080].

Hankins and Shannon. (1978). The physical and enzymatic properties of a phytochem-agglutinin from mungbeans. *J. Biol. Chem.* **253** 7791-7797.

Hodgkinson. (1977). "Oxalic Acid in Biology and Medicine".Academic Press, New York.

Ibrahim and Rahim. (1987). Effect of processing on anti-nutritional factors and in vitro protein digestibility of faba bean(Vicia faba). *Department Food Sci. Technol.*, 128.

Jaenicke and Hoschle-Zeledon. (2006). "Strategic Framework for Underutilized Plant Species Research and Development with Special Reference to Asia and the Pacific, and to Sub-Saharan Africa". International Centre for Underutilized Crops. Rome.

Jansen. (2006). Record from Protabase. Brink, M. & Belay, G. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. *Wageningen University & Research*.

Jenkins, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins and Goff. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr.* **34** (3), 362-366. [doi:10.1093/ajcn/34.3.362].

Jiayi. (2016.). Analytical method development for quantification of total.

Jood and Chauhan. (1987). Polyphenols of chickpea and blackgram as affected by domesting processing and cooking methods. *Journal of the science of Food and Agriculture*. **39** (2), 145-149.

Jood, Chauhan and Kapoor. (1987). Polyphenols of Chickpea and Blackgram as Affected by Domestic Processing and Cooking Methods. *J. Sci. Food Agric.* **39**, 145-149.

Kakati, C., Kotoki and Saikia. (2010). Effect of traditional methods of processing on the nutrient contents and some antinutritional factors in newly developed cultivars of green gram [Vigna radiata (L.) Wilezek] and black gram [Vigna mungo (L.) Hepper] of Assam, India. *Int. Food Res. J.* **17**, 377-384.

Kataria, Chauhan and Gandhi. (1988a). Effect of domestic processing and cooking on the anti-nutrients of blackgram. *Food Chem.s.* **30**.

Kataria, Chauhan and Gandhi. (1988b). Effect of domestic processing and cooking on the anti-nutrients of blackgram. *Food Chem.* **30**.

Kataria, Chauhan and Puria. (1989). Anti-nutrients and protein digestibility (in vitro) of Mung bean as affected by domestic processing and cooking. *Food chem.* **32**, 9-17.

KC and Rai. (2007). Basic food analysis handbook (1st ed ed.). Mrs. Maya K.C. Kathmandu, Nepal: .

Khattab, Arntfield and Nyachoti. (2009). Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation. *LWT - Food Sci. Technol.* **42**, 1107-1112. [doi:10.1016/j.lwt.2009.02.008].

Kon, Brown, Ohanneson and Booth. (1973). Split peeled beans preparation and some properties. *J. Food Sci.* **38**, 496.

Kumar, Sinha, Makkar and Becker. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chem.* **120**, 945-959.

Kumari and Verma. (2015). Effect of sprouting time on antinutritional factors in Vigna mungo varieties. **15**, 701-703.

Lario, Sendra, Garcia-Perez, Fuentes, Sayas-Barbara and Fernandez-Lopez. (2004). Preparation of high dietary fibre powder from lemon juice by products. *Innovative Food Sci. Emerging Technol.* **5**, 113-117.

Liener. (1994). Implications of antinutritional components in soybean foods. *Crit Rev Food Sci Nutr.* **34** (1), 31-67. [doi:10.1080/10408399409527649].

Lis and Sharon (1981). Lectins in higher plants; in The Biochemistry of plants (a comprehensive treatise. *A Marcus*. 6 371-447. New York: Academic Press.

Loewus. (2001). Biosynthesis of phytate in food grains and seeds. *In*.). pp. 53-61.

Maga. (1982). Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *Am. Chem. Soc.* **30** (1), 1-9. [doi:10.1021/jf00109a001].

Mahmut and Ayhan. (2002). The use of tannins from Turkish acorns (valonia) in water treatment as a coagulant and coagulant aid. *Turkish J. Eng. Environ. Sci.* **26** (3), 255-263.

Mang, Nijintang, Abdou, Scher, Bernard and Mbofung. (2016). Dehulling reduces toxicity and improves in vivo biological value of proteins in vegetal milk derived from two mucuna (Mucuna pruriens L.)seeds varieties. *J. Food Sci. Technol.* **53** (6), 2548-2557. [doi:10.1007/s13197-016-2211-2].

Massey, Palmer and Horner. (2001). Oxalate content of soybean seeds (glycine max: leguminosae), soyfoods, and other edible legumes. *J. Agric. Food Chem.* **49**, 4262-4266.

Misra. (2012). Anti Nutritive Bioactive Compounds Present In Unconventional Pulses and Legumes. *Res. J. Pharm. , Biol. Chem. Sci.* **3**, 586-597.

Modi, Mahendrakar, Rao and Nm. (2004). Quality of buffalo meat burger containing legume flours as binders. *Meat sci.* **66**, 143-149. [doi:10.1016/S0309-1740(03)00078-0].

Montagnac, Davis and Tanumihardjo. (2009). Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comp. Rev*. **8** (3), 181-194.

Morrison and Savage. (1999). "Oxalate content of foods and its effect on humans". Vol. 8.

Murugkar, Gulati and Gupta. (2013). Effect of sprouting on physical properties and functional and nutritional components of multi-nutrient mixes. *Int J Food Nutr Sci*. **2** (2), 2-15.

Nestares, Lopez-Frias, Barrionuevo and Urbano. (1996). Nutritional Assessment of Raw and Processed Chickpea (Cicer arietinum L.) Protein in Growing Rats. *J. Agric. Food Chem. 1996, 44, 2760−2765*. **44**, 2760-2765.

Omizu. (2011). Determination of saponin contents in raw soybean and fermented soybean foods of India. *J. Scientific Ind. Res.* **70** (7), 533-538.

Pal, Bhartiya, ArunKumar, Kant, Aditya and Bisht. (2016). Impact of dehulling and germination on nutrients, antinutrients, and antioxidant properties in horsegram. *J. Food Sci Technol.* **53** (1), 337-347. [doi:10.1007/s13197-015-2037-3].

Patel, Devraja, Sharma and Singh. (n.d.). Introduction to cereal grains, legumes and oilseeds, structure and composition of cereal grains, legumes and oilseeds. Retrieved from http://ecoursesonline.iasri.res.in/mod/resource/view.php?id=5905. (Last update 21 September 2012). [Accessed 11 December, 2019.].

Patel and Rao. (1995). Effect of Untreated, Roasted and Germinated Black Gram (Phaseolus mungo) Flours on the Physicochemical and Biscuit (Cookie) Making Characteristics of Soft Wheat Flour. *J. Cereal Sci.* **22**, 285-291. [doi:10.1006/jcrs.1995.0065].

Phesatcha, Tukkeeree and Rohrer. (2012). Determination of Phytic Acid in Soybeans and Black Sesame Seeds. Thermo Fisher Scientific Inc. Retrieved from www.thermoscientific.com. [Accessed 6 March,2019].

Prathibha, Bala and Leelama. (1995). Enzyme inhibitors in tuber crops and their thermal stability. *Plant Foods Human Nutr.* **48**, 247-257. .

R. Reddy and Salunkhe. (1980). "Effect of fermentation on phytate phosphorus and mineral content in black gram and rice". Vol. 45.

Rajaram and Janardhan. (1990). Chemical Analysis and Nutritional Evaluation of Certain underexploited Vigna spp. *Food Sci. Nutri.* **42**, 213-221.

Rao and Deosthale. (1981). Mineral composition of four Indian food legumes. *J. Food Sci.* **46**, 1962-1963.

Rao and Deosthale. (1982). Tannin content of pulses: Varietal differences and effects of germination and cooking. *John Wiley & Sons, Ltd*. **33** (10), 1013-1016. 10.1002/jsfa.2740331012.

Reddy, Balakrishnan and Salunkhe. (1978). Phytate, phosphorous and mineral changes during germination and cooking of blackgram (Phaseolus mungo) seeds. *J. Food Sci.* . **43**, 540-543.

Reddy, Salunkhe and Sathe. (1982). Biochemistry of blackgram (Phaseolus mungo): a Review. *CRC Crit. Rev. Food Sci. Nutr.* . **15**, 49-114.

Rehman and Salariya. (2005). The effects of hydrothermal processing on antinutrients, protein and starch digestibility of food legumes. *Int. J. Food Sci. Technol.* **40**, 695-700.

Rehman and Shah. (2005). Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chem.* **91**, 327-331.

Rosenthal and Janzen. (1979). . *In:* "Herbivores: Their Interaction With Secondary Plant Metabolites". " (Vol. 41).). New york. Academic Press.

Ryan. (1981). "Proteinase inhibitors. In: The biochemistry of plants.". Vol. 6. Stumpf, P. K. . Academic Press, New York.

Salunkhe. (1982). Legumes in human nutrition. *Current Science*. **51**, 387-394.

Scalbert, Manach, Morand and Remesy. (2005). Dietary polyphenols and the prevention of diseases. *Cri. Review Food Sci. Nutr.* **45**, 287-306.

Schempf, Freeberg and Angelon. (1965). "Effect of sodium ion impurity on thermal decomposition reaction of calcium oxalate as studied by absorption infrared spectrometric and thermoanalysis techniques. In: "Analytical Chemistry"" (13 ed ed.). Vol. 37.

Sharma and Sehgal. (1992). "Effect of domestic processing, cooking and germination on the trypsin inhibitor activity and tannin content of faba bean (Vicia faba)". Vol. 42.

Sharma and Sehgal. (1992a). Effect of domestic processing, cooking and germination on the trypsin inhibitor activity and tannin content of faba bean (Vicia faba L.). *Plant Foods Hum. Nutr*. **42** (20), 127-133.

Shi, Arntfield and Nickerson. (2018). Changes in levels of phytic acid, lectins and oxalates during soaking and cooking of Canadian pulses. *Food Res. Inter.* **56**. [doi:10.1016/j.foodres.2018.02.056].

Siddhuraju, Becker and Makkar. (2000). Studies on the Nutritional Composition and Antinutritional Factors of Three Different Germplasm Seed Materials of an Under-Utilized Tropical Legume, Mucuna pruriens Var. Utilis. *Am.Chem. Soc.* **48** (12), 6048-6060. [doi:10.1021/jf0006630].

Siddiq and Uebersax. (2012). Dry Beans and Pulses Production, Processing, and Nutrition. Wiley Online Library.

Siegel and Fawcett. (1976). "Food legume processing and utilization.". IDRC-TSI. Inter. Development Res. Center. Ottawa, Canada.

Singh and Jambunathan. (1981). Studies on Desi and Kabull Chickpea (Cicer arietinum L.) Cultivars: Levels of Protease Inhibitors, Levels of Polyphenolic Compounds and in vitro Protein Digestibility. *J. Food Sci.* **46** (5), 1364-1367. [doi:10.1111/j.1365-2621.1981.tb04176.x].

Singh and Rao. (1991). A monomelic protein with hemagglutinating activity from seeds of Vignamungo (Phaseolus mungo). *Indian J. Biochem. Biophys*. **28**, 439-443.

Singleton, Orthofer and Lamuela-Raventos. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Oxidants and Antioxidants. **299**, 152-178. [doi:10.1016/S0076-6879(99)99017-1.].

Sparvoli and Cominelli. (2014). Phytate transport by MRPs. *In:* (Vol. 22).). pp. 19-38. [ISBN 978-3-319-06510-6].

Subbulakshmi, Kumar and Venkataraman. (1976). Effect of germination on the carbohydrates, proteins, trypsin inhibitor and haemoglutinins in horse gram and moth bean. *Nutr. Rep. Int.* **13**, 19-31.

Suneja, Grewal, Gupta and Kaur. (2011). Levels of nutritional constituents and antinutritional factors in black gram (Vigna mungo L. Hepper). *Food Res. Int.* **44**, 621-628. [doi:10.1016/j.foodres.2010.12.020].

Taiwo. (1998). The potential of cowpea as human food in Nigeria. *Technovation*. **18**, 469-481.

Talakatta, Pratape and Ummiti. (2012). Nutrient distribution, phenolic acid composition, antioxidant and alpha-glucosidase inhibitory potentials of black gram (Vigna mungo L.) and its milled by-products. *Food Res. Int.* **46**, 370–377. [doi:10.1016/j.foodres.2011.12.026].

Tilahun, Fisseha and Sebsebe. (2009). An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. *J. Ethnobiology and Ethnomedicine*. **5**.

Tinko and Uyano. (2001). Spectrophotometric determination of the tannin contents of various turkish black tea, beer and wine samples. *Intl. J. Food Sci. Nutr.* **52**, 289-294.

Vidal-Valverde and Frias. (1992). Changes in carbohydrates during germination of lentils. *L. Lebensm uters Forsch.* **194**, 461-464.

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

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

Wang, Hatcher and Gawalko. (2008). Effect of variety and processing on nutrients and certain anti-nutrients in field peas (Pisum sativum). *Food Chem.* **111**, 132-138.

Williams and Wandzilak. (1989). Oxalate synthesis, transport and the hyperoxaluricsyndromes. *J. Urology* **141**, 742-747.

Young and Greaves. (1940). Influence of variety and treatment on phytic acid content of wheat. *J. Food Res.* **5**, 103-105.

Zhang and Hamauzu. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chem*. **88**, 503-509.

Ziena, Youssef and EL Mahdy. (1991). Amino acid composition and some anti-nutritional factors of cooked faba bean (Medammis); Effect of cooking temperature and time. *J. Food Sci.* **56** (5), 1347-1352.

Appendices

Appendix A

Table. A. 1 One Way ANOVA table for Oxalate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	123113.62	17587.66	721.55	<.001
Residual	16	390.00	24.38		
Total	23	123503.62			

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

Treatments	Oxalate (mg/100g)
Raw sample	397^{a}±12
Soaked sample	310^{b}±5
Germinated sample	280^{c}±4
Soaked and Dehulled sample	212^{d}±8
Raw open cooked	232^{e}±7
Soaked Open Cooked	200^{f}±3
Raw Pressure Cooked	200^{f}±5.7
Soaked Pressure Cooked	160^{g}±8.2

[* Values presented are the average of triplicate ± standard deviation.

Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A. 3 One Way ANOVA table for Phytate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	7	25047.9525	3578.2789	7321.29	<.001
Residual	16	7.8200	0.4887		
Total	23	25055.7725			

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

Treatments	Phytate (mg/100g)
Raw sample	150.8^{a}±13
Soaked sample	116.02^{b}±7.2
Germinated sample	80.21^{c}±6.3
Soaked and Dehulled sample	46.4^{d}±5.2
Raw open cooked	104.42^{e}±7.1
Soaked Open Cooked	69.61^{f}±12
Raw Pressure Cooked	69.61^{f}±7.8
Soaked Pressure Cooked	58.12^{g}±7.3

[* Values presented are the average of triplicate ± standard deviation.

Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A. 5 One Way ANOVA table for Polyphenols

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	1857000	265300	147900	<.001
Residual	16	28.69	1.793		
Total	23	1857028.69			

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

Treatments	Polyphenols (mg/100g)
Raw sample	1278.18^{a}±2
Soaked sample	762.3^{b}±1.8
Germinated sample	1155.23^{c}±1
Soaked and Dehulled sample	357.54^{d}±1
Raw open cooked	649.87^{e}±1
Soaked Open Cooked	610.186^{f}±0.81
Raw Pressure Cooked	734.943^{g}±1
Soaked Pressure Cooked	713.525^{h}±2

[* Values presented are the average of triplicate ± standard deviation.

Means in the same column with different superscript are significantly different ($p<0.05$) where values with same superscript within a column are not significantly different.]

Table. A. 7 One Way ANOVA table for Tannins

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	7	232767.787	33252.541	21688.41	<.001
Residual	16	24.531	1.533		
Total	23	232792.318			

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

Treatments	Tannin (mg/100g)
Raw sample	507.97^{a}±20
Soaked sample	385.50^{b}±12
Germinated sample	290.56^{c}±15
Soaked and Dehulled sample	200.45^{d}±10
Raw open cooked	373.80^{e}±9
Soaked Open Cooked	310.41^{f}±8
Raw Pressure Cooked	343.73^{g}±12
Soaked Pressure Cooked	260^{h}±8

[* 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 Black Gram 5% level of significance

	Raw Black Gram	**Dehulled sample**
Mean	26.2	29.12
Variance	0.1156	0.0784
Observations	3	3
Pearson Correlation	1	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-84.2931393	
P(T<=t) one-tail	7.03548E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00014071	
t Critical two-tail	4.30265273	

Table A. 10 Paired t-test comparison of Ash content of most effective method with Raw Black Gram at 5% level of significance

	Raw Black Gram	**Dehulled Sample**
Mean	4.71	3.13
Variance	0.0144	0.0064
Observations	3	3
Pearson Correlation	1	
Hypothesized Mean Difference	0	
Df	2	
t Stat	68.4160069	
P(T<=t) one-tail	0.000106786	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000213573	
t Critical two-tail	4.30265273	

Table A. 11 Paired t-test comparison of Fat content of most effective method with Raw Black Gram 5% level of significance

	Raw Black Gram	**Dehulled sample**
Mean	1.625	1.05
Variance	0.0009	0.0064
Observations	3	3
Pearson Correlation	-1	
Hypothesized Mean Difference	0	
Df	2	
t Stat	9.053902	
P(T<=t) one-tail	0.00599	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.01198	
t Critical two-tail	4.302653	

Table A. 12 Paired t-test comparison of Crude fiber content of most effective method with Raw Black Gram 5% level of significance

	Raw Black Gram	**Dehulled sample**
Mean	6.227	3.507
Variance	0.4225	0.0841
Observations	3	3
Pearson Correlation	1	
Hypothesized Mean Difference	0	
Df	2	
t Stat	13.0866061	
P(T<=t) one-tail	0.002894225	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.00578845	
t Critical two-tail	4.30265273	

Table A. 13 Paired t-test comparison of carbohydrate content of most effective method with Raw Black Gram 5% level of significance

	Raw Black Gram	**Dehulled Black Gram**
Mean	61.238	63.193
Variance	11.56	20.25
Observations	3	3
Pearson Correlation	-1	
Hypothesized Mean Difference	0	
Df	2	
t Stat	0.428627763	
P(T<=t) one-tail	0.354972069	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.709944138	
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
20	0.076
40	0.187
60	0.221
80	0.321
100	0.418

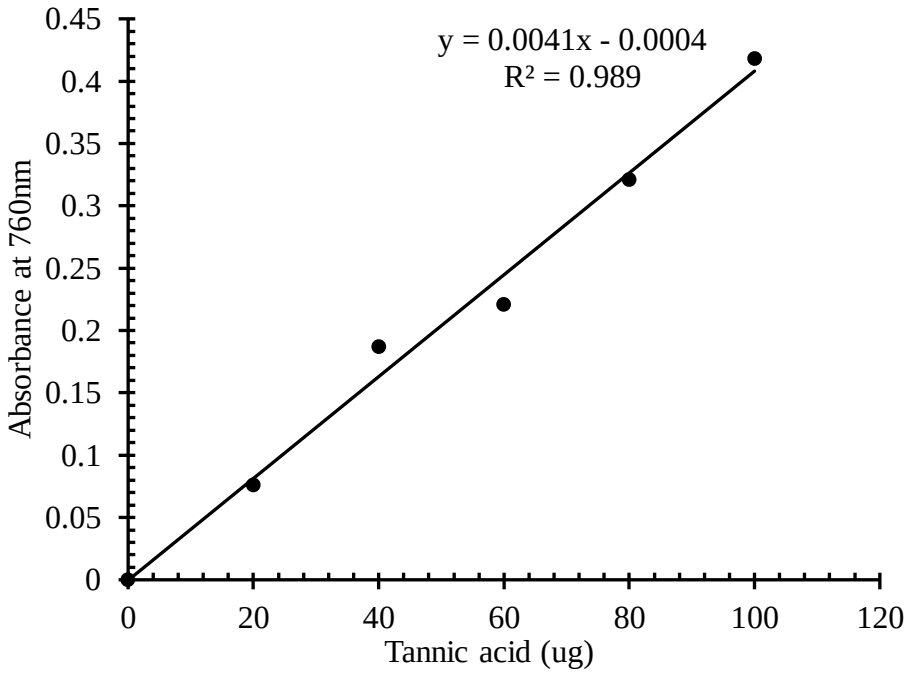


Fig. B.1 Standard curve for tannin determination

Table.B.2 Standard curve data for polyphenols as Gallic acid

Gallic acid Concentration (ug/ml)	Absorbance
0	0
10	0.726
40	1.523
80	2.68

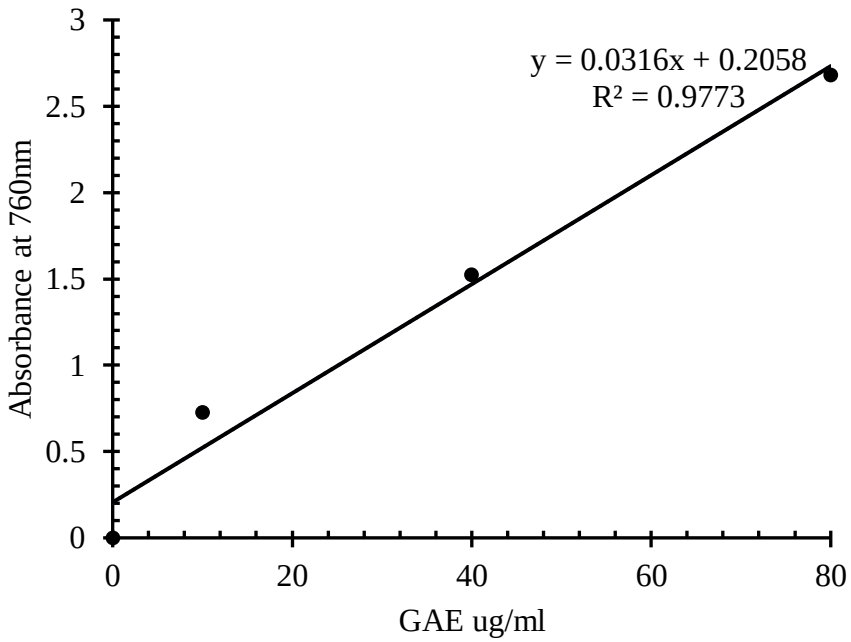


Fig. B.2 Standard curve for polyphenols determination

Color Plates

Plate 1 Soaked black gam

Plate 2 Dehulled sample

Plate 3 Raw open cooked sample

Plate 4 Soaked open cooked

Plate 5 Germinated sample

Plate 6 Dried germinated sample

Plate 7 Sample Preparation

Plate 7 Spectrophotometric determination of polyphenols and tannins

Plate 9 Distillation in Kjeldahl's distillation set

Plate 10 Titration for protein determination