EFFECT OF PROCESSING ON PHYTOCHEMICALS PRESENT IN HORSEGRAM (*Macrotyloma Uniflorum*)

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

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Approval Letter

This *dissertation* entitled *"Effect of Processing on Phytochemicals Present in Horsegram (Macrotyloma uniflorum)"* presented by Pratima Sherpa has been accepted as the partial fulfillment of the requirement for the Bachelor degree in Nutrition and Dietetics.

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Abstract

The aim of this research work was to determine the effect of processing on phytochemicals present in horsegram. The effect of different processing techniques on phytochemicals such as tannin, oxalate, phytate and polyphenol in horsegram seeds were studied. Horsegram (*Macrotyloma uniflorum*) was collected from local market of Dharan sub-metropolitan, located in Sunsari district in Province No. 1 on the month of May 2022. The species used for the study was imported from India.

The mean value of tannin, oxalate, phytate and polyphenol in raw horsegram seeds were found to be 740.12 mg/100g, 462.65 mg/100g, 1076.67 mg/100g and 1254.90 mg/100g respectively on dry basis. The maximum reduction of phytochemicals: tannin (68.53%), phytate (65.43%) and polyphenols (61.12%) were found when the horsegram sample was soaked and dehulled. Maximum reduction of oxalate (63.04%) was found when horsegram was subjected to soaking and autoclaving. The reduction percentage of phytochemicals due to roasting was found lowest when compared to other processing methods. Soaked autoclaving reduced maximum phytochemicals present in horsegram seeds in case of cooking treatments. Soaking and dehulling of horsegram was found to be the treatment that reduced maximum phytochemicals from horsegram seeds. Soaking was done prior to dehulling, so the reduction of phytochemicals was due to the combined effect of soaking and dehulling. Hence, combined techniques were more effective than single processes. Meanwhile, all the processing methods such as soaking, dehulling, germination, roasting, raw open cooking, raw autoclaving, soaked open cooking and soaked autoclaving reduced the phytochemicals from horsegram significantly ($p<0.05$).

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Abbreviations	Full form
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemists
ANF	Anti-nutritional Factors
CCT	Central Campus Technology
D.F.	Degree of freedom
LSD	Least Significant Difference
S.D.	Standard Deviation
USDA	United States Department of Agriculture
NAS	US National Academy of Sciences
IT IS	Integrated Taxonomic Information System
DFTQC	Department of Food Technology and Quality Control
GAE	Gallic Acid Equivalent
FC	Folin-ciocalteu
RH	Relative humidity
Etc.	Et cetera
i.e.	Id est

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Part 1

Introduction

1.1 General introduction

Pulses are considered as important crops in the world due to their nutritional quality. Nepal contributes about 0.4% of world pulse area and production (Akibode and Maredia, 2012). Soyabean, common field beans, lentil, black-gram, green-gram, horse-gram, cowpea and broad bean are some of the important pulse crops grown in many parts of Nepal. Majority of pulse area and production are confined to terai and inner terai (Rimal and Gurung, 2017). Requirement of protein per day is 60gm for which the availability of protein from pulses is around 8g per capita per day. Legumes are recognized as the best source of vegetable protein (Kumar *et al.*, 2002). Presence of anti-nutritional factors limits the consumption of these underutilized legumes as regular food. In order to overcome these hazards, traditional and modern food processing methods are adopted (P. Vandarkuzhali, 2017).

Horsegram (*Macrotyloma uniflorum*, previously *Dolichos biflorus*) an underutilized legume of tropics and subtropics is grown mostly under dry-land agriculture (Jain *et al.*, 2012). Horsegram is popularly known as 'Gahat'. It is an important source of protein and identified as one of the potential food sources for future by the US National Academy of Sciences (Bhartiya *et al.*, 2015; NAS, 1978).

Horsegram is also consumed as medicinal food in many parts of Nepal. It is given to children suffering from mumps and is considered invaluable for getting rid of the kidney stones because it is a diuretic (Bhartiya *et al.*, 2015). The seeds of *Macrotyloma uniflorum* contain phytochemicals such as alkaloid, phenolic acid, tannin, flavonoids etc (Senthilkumar, 2022). Use of raw horse gram and its flour is well known in recent days, but maximum utilization is lacking due tannin, trypsin inhibitor, phytic acid and oxalate which interfere with the bioavailability of nutrients (Kumar *et al.*, 2002). Different processing techniques are applied to enhance the nutritional quality of legumes. The phytochemicals are lost significantly during processing (Senthilkumar, 2022). The physical methods of processing of legumes include soaking, boiling, cooking, autoclaving, roasting, dehulling and germination (Jain *et al.*, 2012).

Notable progression has been achieved through cooking, germination, soaking and partial hydrolysis of proteolytic enzyme to reduce the anti-nutrients and to improvise the nutritive value and functional qualities of legumes (Oloyo, 2004). These processing methods resulted in decreased level of polyphenols, oxalic acid and phytic acid present in the horsegram seeds (Sudha *et al.,* 1995).

1.2 Statement of problem

Horsegram (*Macrotyloma uniflorum*) commonly known as "*Gahat"* is an unexploited tropical grain legume, a cheap source of protein and a good source of calcium, iron, and molybdenum. Despite the presence of proper nutrients, horse gram is an underutilized crop (Moktan and Ojha, 2016). Horsegram is a legume rich in phytochemicals (Senthilkumar, 2022). There is a concern about high intake of foods that are rich in phytochemicals due to their increased burden on the body's tolerance to potentially harmful compounds. For example, phytic acid, lectins, phenolic compounds and tannins, saponins, enzyme inhibitors, cyanogenic glycosides and glucosinolates have shown to reduce the availability of certain nutrients and impair growth. Some compounds such as phytoestrogens and lignans have also been linked to induction of infertility in humans (Shahidi, 1997). The phytochemical components in plants have been reported to reduce the absorption of various minerals like calcium, iron, zinc, phosphorus and magnesium by forming soluble or insoluble salt, eventually leading to decreased digestibility of protein (Emmambux and Taylor, 2003). So, this study focuses on evaluating the changes in phytochemical content of horsegram seeds after processing so that it can be processed in best way possible to increase it's utilization.

1.3 Objectives

1.3.1 General objective

The objective of this dissertation is to evaluate the effects of processing on phytochemicals present in the underutilized legume horsegram.

1.3.2 Specific objectives

The specific objective of this dissertation work is:

a. To study the physical parameters of horsegram.

b. To determine the phytochemical content: tannin, oxalate, phytate and polyphenol in horsegram.
c. To process horsegram seeds with various treatment methods, namely: soaking, germination, roasting, dehulling and cooking to observe their impact on the phytochemical profile.
d. To determine the reduction pattern of phytochemicals in processed horsegram.
e. To find out the type and level of treatment that could potentially reduce maximum amount of phytochemicals present in horsegram.

1.4 Significance of the study

Horsegram is cultivated in India, Nepal, Malaysia, Mayanmar, Mauritius and Sri Lanka for food purposes and in Australia and Africa primarily for fodder purposes (Asha *et al.*, 2006). The limited use of dry seeds of horsegram is due to its poor cooking quality. However, it is consumed as soups and sprouts in many countries (Sudha *et al.*, 1995). It is a drought-tolerant plant. Being a leguminous crop, it adds nitrogen to the soils where it grows, thus improving the soil fertility (Chahota *et al.*, 2013).

It is known to contain many medicinal and therapeutic benefits, although many of them are yet to be proven scientifically. Horsegram is used as ayurvedic medicine to treat edema, piles, renal stones, and so on (Chahota *et al.*, 2013). This study will determine the effect of processing techniques on the phytochemical profile. The result of this study might help in the establishment of effective and optimized way for usage of horsegram in household and industrial scale. Likewise, the study of physical parameters of the seeds will be highly useful in optimization of post-harvest processing operations as well as to design and develop related processing equipment for horse gram.

1.5 Limitations of the study

Limitations of the present study:

- Trypsin inhibitor and hemagglutinin were not determined due to time constraints.

Part 2

Literature Review

2.1 Nomenclature of horsegram

Horsegram is alternatively known as gahat, kulthi, kulath, kollu and many other names. It is a pulse and fodder crop native to Southeast Asia and tropical Africa, but the center of origin of cultivated species is considered to be southern India (Kumar and Rana, 2013). The name Macrotyloma is derived from the Greek words ‘makros’ meaning large, ‘tylos’ meaning knob and ‘loma’ meaning margin, in reference to knobby statures on the pods (Blumenthal and Staples, 1993). Formally, the horsegram was included to the genus *Dolichos* following Linneus. (Verdcourt, 1970) assigned the horsegram to the genus *Macrotyloma*. Now, horsegram is known as *Macrotyloma uniflorum*.

The classification of horsegram is as follows:

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Superdivision: Magnoliophyta

Class: Magnoliopsida

Superorder: Rosanae

Order: Fabales

Family: Fabaceae

Sub-family: Faboideae

Genus: *Macrotyloma*

Species: *M. uniflorum*

Source: (ITIS, 2022)

2.2 Distribution of horsegram

The region of maximum genetic diversity of horsegram is considered to be in the Old World tropics, especially the southern part of India and the Himalayas (Zeven *et al.*, 1982). Studies consider horsegram as a plant native to African countries and some believe that it was domesticated in India, where its cultivation is known since prehistoric times and it is still an important cultivated crop (Pant *et al.*, 2020). Nowadays horsegram is cultivated as a low-grade pulse crop in many Southeast Asian countries, such as India, Bangladesh, Myanmar, Sri Lanka and Bhutan. It is also grown as forage and green manure in many tropical countries, especially in Australia and Africa (Chahota *et al.*, 2013).

Horsegram is extremely drought resistant crop. Moderately warm, dry climatic conditions are suitable for its optimum growth. Horsegram can be cultivated up to an altitude of 1000 m above sea level. The temperature range of (25–30)℃ and relative humidity between (50–80)% is optimum for its growth (Raghav *et al.*, 2020). Heavy rains during the initial stages of crop growth affect nodule formation owing to poor aeration in the soil. A well distributed rainfall of about 800 mm is sufficient for its successful cultivation, but it performs well even under low rainfall areas. Horsegram is mostly grown in regions with less than 900mm of annual rainfall, although it is capable of being grown with as little as 380mm (Banham and Fuller, 2014). It is generally grown on lateritic soil (poor in fertility) in south India. The crop can be grown on wide range of soils from light to heavy soil which are free from alkalinity (Krishna and Kowligi, 2013).

Horsegram is able to tolerate low soil fertility and salinity but not water logged soil, making it an important crop in the drier areas (Bhardwaj and Yadav, 2014). It does not demand much effort, and can be used as green manure to strengthen depleted soils (Bhardwaj and Yadav, 2014). It is often planted on fallow fields and is seen as an essential component of crop rotation (Banham and Fuller, 2014). The bean can be successfully intercropped with cereals. It also does well as an understory crop in orchards and plantations. It takes an estimated amount of (120-180) days for the plant to reach maturity (Banham and Fuller, 2014). Once the leaves shrivel and the pods turn light brown, the plants can be uprooted (Bhardwaj and Yadav, 2014). They are then dried in the field or under cover, and threshed to remove the pods. The yield from one acre ranges from (275-400) kg of seeds (Bhardwaj and Yadav, 2014).

2.3 Structure of horsegram

Legumes are plant in the family Leguminosae or a fruit of these specific plants (Van der Maesen and Somaatmadja, 1989). A legume fruit is simple dry fruit that develops from a simple carpel and usually dehisces on two sides. Horsegram is an under exploited legume that can be of great use. Mature seeds of horsegram 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 structures of the seed are the testa, hilum, micropyle, and raphe. The testa is the outer most part of the seed and covers almost entire seed surface. The hilum is an oval scar on the seed coat where the seeds attach to the stalk. The micropyle is a small opening in the seed coat next to the hilum. The raphe is a ridge on side of the hilum opposite to the micropyle. When the seed coat is removed from grain, the remaining part is 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, it can be smooth or rough. Both the micropyle and hilum have been related to the permeability of the testa and to water absorption (Ranabhat, 2020).

Horsegram is a perennial climbing plant with a rhizome, growing to a height of about 60cm. The stem sprouts from the rhizome each year. It is clad in varying amounts of whitish hairs and bears alternate, trifoliate leaves with petioles up to 7cm long. The leaflets are obovate or elliptical, and up to 7cm long. The flowers are borne in twos or threes in the leaf axils, and are typical of the bean family with banner, wings and keel. They are cream, yellowish or green, often with a purple blotch inside. These are followed by linear-oblong, upcurving pods up to 8 cm long, containing up to ten reddish-brown, speckled or black seeds (Wikipedia, 2022).

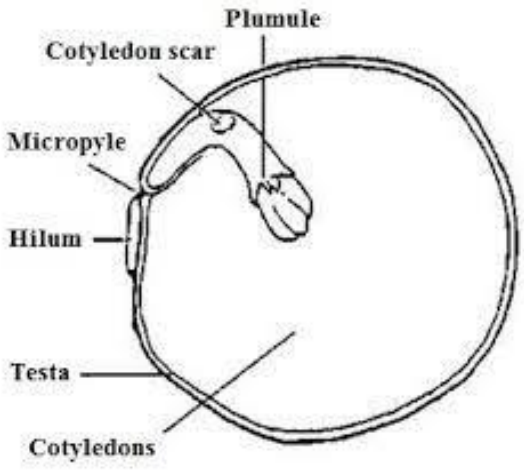

Figure 2.1 Structure of horsegram seed

2.4 Production of horsegram

Horsegram is widely grown pulse species in India. It is amongst the most ubiquitous archaeological pulse finds, indicating that it has been of widespread importance since the Neolithic period. It is especially important on the Indian states of Tamil Nadu, Karnataka and Andhra Pradesh (Fuller and Murphy, 2018). Tamil Nadu and Andhra Pradesh together account for nearly 90% of the total Indian acreage under this crop. Annual yields of horsegram are low given its area of production, which may be due in part to its use on fields with poor agronomic conditions, but this may also reflect in part a bias against research on and improvement efforts devoted to this crop. It is also cultivated, on a smaller scale, in Pakistan, Bangladesh, Nepal, and Myanmar (Spate and Learmonth, 2017). It is reported to be grown in the northwest Himalayas up to 2000 meters and in the eastern Himalayas (Sikkim) up to at least 1000 meters (Atkinson, 1980) and in recent times in Australia, Taiwan and the Philippines as a fodder crop. It was introduced in colonial Southeast Asia as a fodder crop (Burkill, 1966).

Although horsegram is largely cultivated in Southern India, people of Nepal are familiar with it and use it in many of their traditional dishes (Raizada and Gram, 2014). The reason for why this pulse is not already extensively intergraded in Nepalese agriculture is simply due to the fact that there has been no need to do so with such a large exporter close by. However, with climate change worsening its low input and resistance may be valuable and demanded.

Horsegram is a lentil that has significant value to hillside Nepalese farmers which hold the potential of exporting it to Canada. In Nepal, it is generally grown in the hilly area. In 2018/19, in Nepal, it is grown in 6,111 ha with production and productivity of 5,754 Mt and 0.94 Mt/ha respectively (MoALD, 2020).

2.5 Chemical composition of horsegram

Table 2.1 Chemical constituents of whole horsegram.

Constituents	**Per 100gm**
Moisture (g)	11.8
Protein (g)	22
Fat (g)	0.5
Carbohydrate (g)	57.2
Minerals (g)	3.2
Fiber (g)	5.3
Calcium (mg)	287
Phosphorous (mg)	311
Iron (mg)	6.77
Carotene (mg)	71
Thiamine (mg)	0.42
Riboflavin (mg)	0.2
Niacin (mg)	1.5
Potassium (mg)	726
Magnesium (mg)	156

Source: DFTQC, 2017

2.6 Health benefits of horsegram

Horsegram has long history as traditional medicine to cure many diseases, still it is neglected for its remedial potential. Traditional texts describe its use as traditional medicine for curing kidney stones, asthma, bronchitis, leucoderma, urinary discharges, heart diseases and piles (Yadava and Vyas, 1994). It also has anthelmintic activity which can be used as dietary food for infants to eradicate worms (Philip *et al.*, 2009). It is

supposed to have unique property of dissolving kidney stones, therefore, it is given to prevent or cure urinary stones (Singla and Kumar, 1985).

The extract of horsegram exerts a hypolipidaemic and hypoglycaemic action (Senthil, 2009) and has also been found beneficial in urinary troubles, acid peptic disorder (gastritis), constipation, sun-burn, kidney stone, female diseases (leucorrhoea, menstrual troubles, bleeding during pregnancy, postpartum excessive discharges), colic caused by wind, piles, rheumatism, hemorrhagic disease, intestinal worms etc. (Pati and Bhattacharjee, 2013). It is prescribed for people suffering from jaundice, water retention, iron deficiencies and is also helpful for maintaining body temperature in the winter season (Ramesh *et al.*, 2011).

Horsegram seed are rich source of dietary antioxidants (Siddhuraju and Manian, 2007) as well as has antidiabetic effect (Gupta *et al.*, 2011). Extracts from horsegram seeds reported to have significant activity against *B. subtilis, S. aureus, E. coli, and P. aeruginosa* (Gupta *et al.*, 2005). Horsegram used as medicine to treat hiccups, worms and in the treatment of bacterial and fungal infections (Kawsar *et al.*, 2008). It has functional ingredients against hypercholesterolemia and obesity (Kumar *et al.*, 2013). Horsegram has nutraceutical properties as it reduces the risk of intestinal diseases, diabetes, coronary heart disease, prevention of dental caries etc. due to presence of bioactive compounds (Prasad and Singh, 2014). Horsegram seed proteins exhibit free radical scavenging capacities which can be used as a food supplement, natural antioxidant and useful as therapeutics for health benefits of human (Petchiammal and Hopper, 2014).

The seeds and sprouts of horsegram are excellent examples of ‘functional food’ as it has role in lowering the risk of various diseases and exerting health promoting effects in addition to its nutritive value (Ramesh *et al.*, 2011). Horsegram flour is rich in protein, calcium and dietary fiber, after simple processing like soaking and drying or roasting eliminates the anti-nutritional content hence suitably processed horsegram flour could be used in the preparation of various food products (Thirukkumar and Sindhumathi, 2014).

2.7 Physical properties of horsegram

2.7.1 Thousand kernel weight

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

2.7.2 l/b ratio

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

2.7.3 Bulk density

Bulk density is defined as the weight per standard volume measured in a standard manner. It is also known as ‘test weight’, ‘bushel weight’ or ‘specific weight’. The factors that affect bulk density are insect infestation, excessive foreign matter and moisture content. Bulk density is required for the design of storage, transport and separation systems. It has also been used to determine the dielectric properties of cereal grains (Kumar *et al.*, 2017).

2.8 Phytochemicals

Phytochemicals are chemicals of plant origin (Breslin, 2017). These are chemicals produced by plants through primary or secondary metabolism (Harborne *et al.*, 1999). They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators (Russell *et al.*, 2007).

2.9 Phytochemicals present in horsegram

2.9.1 Tannin

Tannin is an astringent, polyphenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids (Chung *et al.*, 1989).

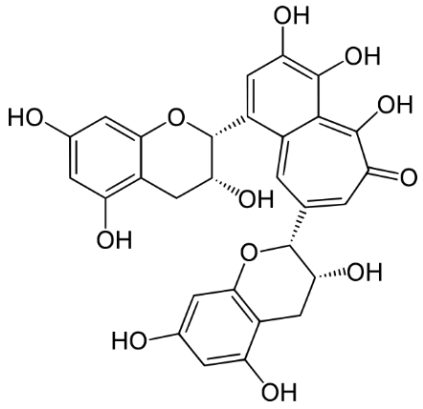

Figure 2.2 Structure of tannin

The term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyl) to form strong complexes with various macromolecules (Khanal *et al.*, 2004). The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and might help in regulating plant growth. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripe fruit or red wine or tea. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times (McGee, 2007).

Most legumes contain tannins. Red-colored beans contain the most tannin, and white colored beans have the least. Condensed tannins inhibit digestion by binding to consumed plant proteins and making them more difficult to digest, and by interfering with protein absorption and digestive enzymes. Tannins form insoluble complexes with proteins, carbohydrates and lipids leading to a reduction in digestibility of these nutrients. Many tannin-consuming animals secrete a tannin-binding protein (mucin) in their saliva. Tannin binding capacity of salivary mucin is directly related to its proline content. Salivary proline rich proteins (PRPs) are sometimes used to inactivate tannins. One reason is that they

inactivate tannins to a greater extent than dietary proteins resulting in reduced fecal nitrogen losses. PRPs additionally contain non-specific nitrogen and non-essential amino acids making them more convenient than valuable dietary protein (Shimada, 2006).

2.9.2 Phytic acid

Phytic acid (inositol hexakisphosphate (IP6), inositol polyphosphate, phytate- when in salt form), is a saturated cyclic acid and the principal storage form of phosphorus in many plant tissues, especially bran and seeds. It can be found in cereals and grains (Baskota, 2019).

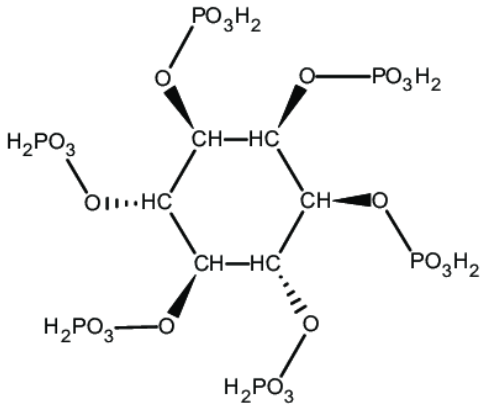

Figure 2.3 Structure of phytate

Phytic acid has a strong binding affinity to significant minerals, like calcium, iron, and zinc, albeit the binding of calcium with phytic acid is p^H-dependent (Dendougui and Schwedt, 2004). The binding of phytic acid with iron is more complex, despite the fact that there absolutely is a strong binding affinity, molecules like phenols and tannins additionally impact the binding (Prom-u-thai *et al.*, 2006). When iron and zinc bind to phytic acid they form insoluble precipitates and are undeniably less absorbable in the digestive tracts. This process can consequently contribute to iron and zinc deficiencies in people whose diets depend on these food varieties for their mineral intake, such as those in developing nations and vegetarians (Baskota, 2019).

Phytic acid not only binds or chelates vital minerals, but also inhibits enzymes that help us digest our food, such as pepsin, which helps for the breakdown of proteins in the stomach, and amylase, needed for the breakdown of starch into sugar. Trypsin, required for protein digestion in the small intestine, is additionally hindered by phytate (Bindu *et al.*, 2017). Phytic acid present in raw horsegram is 10.2±0.4 mg/g (Jade, 2019).

2.9.3 Oxalate

Oxalate is dianion with the formula $(C_2O_4)^{-2}$ also written as $((COO)_2)^{-2}$. Oxalates occur in many plants where it is synthesized by incomplete oxidation of carbohydrate (Dean, 2012).

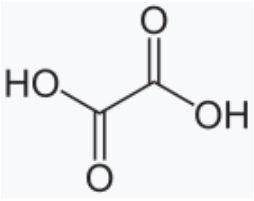

Figure 2.4 Structure of oxalate

A salt formed from oxalic acid is known as an oxalate: for instance, calcium oxalate, which has been viewed as generally distributed in plants. Oxalates are found most commonly in dark-coloured fruits and vegetables like berries, spinach and also cereals and legumes like wheat, rye, soybean, tofu, lentils, and kidney beans. Consumption of high oxalate foods exerts a negative effect on calcium and iron absorption in the body (Chai and Liebman, 2005). Strong bonds are formed between oxalic acid, and different minerals, such as sodium, calcium, magnesium and potassium. This compound blend brings about the development of oxalate salts. Some oxalate salts, such as sodium and potassium, are soluble, whereas calcium oxalate salts are basically insoluble. The insoluble calcium oxalate has the tendency to precipitate (or solidify) in the kidneys or in the urinary tract, subsequently forming sharp-edged calcium oxalate crystals when the levels are sufficiently high. These crystals play a role in the formation of kidney stones in urinary tract when the acid is excreted in the urine (Liebman and Al-Wahsh, 2011).

Most people can induct normal amounts of oxalate rich foods, while individuals with specific conditions, such as enteric and primary hyperoxaluria, need to lower their oxalate admission. In sensitive people, even limited quantities of oxalates can result in burning in the eyes, ears, mouth, and throat; enormous amounts may cause abdominal pain, muscle weakness, nausea, and diarrhea (Popova and Mihaylova, 2019). Oxalic acid content present in raw horsegram is about 5.08 mg/g (Sudha *et al.*, 1995).

2.9.4 Polyphenols

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

Food legumes chiefly contain phenolic acids, flavonoids and condensed tannins among different realized phenolic compounds (Amarowicz and Pegg, 2008). These compounds are distributed differently in the seed coat (mainly flavonoids) and the cotyledon (mainly contain non-flavonoids such as hydroxycinnamic and hydroxybenzoic acids (Shahidi and Ambigaipalan, 2015). Processing mostly leads to the reduction of phenolic compounds in legumes attributable to chemical rearrangements (Singh *et al.*, 2017). Polyphenols are reported to be present in higher amounts in colored and darker legume varieties than in pale varieties (Salunkhe *et al.*, 1983).

Polyphenols inhibit several digestive enzymes, lower protein as well as starch digestibility and prevent mineral absorption from the diet. For human utilization, food legumes are processed in a variety of ways relying on taste and cultural preferences which are known to influence the level of the anti-nutrient (Subbulakshmi *et al.*, 1976).

2.9.5 Trypsin inhibitors

Trypsin inhibitor (TI) is a protein and a sort of serine protease inhibitor (serpin) that decreases the biological activity of trypsin by controlling the activation and synergist responses of proteins. Trypsin is an enzyme involved in the breakdown of wide range of proteins, primarily as part of digestion in humans and other animals such as mono-gastric and young ruminants. When trypsin inhibitor is consumed, it acts as an irreversible and competitive substrate (Silverman *et al.*, 2001). It competes with proteins to bind to trypsin and therefore renders it unavailable to bind with proteins for the digestion process. Thus, trypsin inhibitor is considered an anti-nutritional factor or ANF. Additionally, trypsin inhibitor partially meddles with chymotrypsin function (Vagadia *et al.*, 2017).

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

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

Legumes TIs are classified in 2 families according to their molecular size: Kunitz (KTIs), with molecular weights around 20 kDa and Bowman-Birk (BBTIs) of approximately 8 kDa. Horsegram and soyabean has both families' trypsin inhibitor whereas mung bean, cowpea, lentil, etc. have only BBTIs family trypsin inhibitor. Two disulphide bond is present in KTI but seven disulphide bond is present in BBTI (Van Der Ven *et al.*, 2005). Trypsin inhibitor present in horsegram is 11.57 units/mg (Pal *et al.*, 2016).

2.9.6 Haemagglutinin

Haemagglutinin are cell agglutinating sugar specific proteins, widely distributed in leguminous plants and sometimes referred as phytoagglutinins or lectins (Srilakshmi, 2003). Excess consumption of lectins can cause severe intestinal damage, nutrient deficiencies and they can bind to erythrocytes simultaneously with immune factors, causing hemagglutination and anemia (Bhartiya *et al.,* 2015). However, oral administration of low doses can have many beneficial effects on digestive efficiency, the immune system and the body's endocrine system with beneficial consequences for general metabolism (Zhang *et al.,* 2008). In horsegram, D. biflorus agglutinin (DBA) is an important dietary lectin, identified as an allergen (Pramod *et al.*, 2006) and retarded growth is observed in rats fed on this lectin (Manage *et al.,* 1972). Horsegram seeds are rich in lectins and DBA differentially expresses in seeds, stems, leaves and roots (Beran *et al.,* 2007). Preliminary soaking prior to autoclaving or cooking is required for complete elimination of the toxicity of lectins (Jain *et al.*, 2009).

2.10 Different processing techniques

There are various methods by which legumes can be processed. Several processing techniques such as fermentation, germination, dehulling, cooking, soaking etc. are used to improve the nutritional quality and palatability of food. These legumes are also processed to increase the bioavailability of nutrients (Samtiya *et al.,* 2020).

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

2.10.1 Soaking

Soaking is one of the processes used to remove soluble anti-nutritional factors, which can be eliminated with the discarded soaking liquors, but some metabolic reactions can take place during soaking affecting the content of some compounds (Vidal-Valverde *et al.*, 1994). Soaking, is an integral part of traditional methods of processing, saving energy cost by shortening cooking time, offers an additional advantage of rendering the grain nutritionally superior by removing certain compounds like phytic acid, saponin and

polyphenols (Kataria *et al.*, 1989a). The decrease of these anti-nutrient contents during soaking may be attributed to leaching out into soaking water under the influence of the concentration gradient.

Soaking allows the water to spread in the protein fraction and starch granules allowing protein denaturation and starch gelatinization to occur, softening the texture of beans (Uebersax and Siddiq, 2012). Because phytate is water soluble, soaking in water overnight results in significant phytate elimination in the water, as well as an increase in naturally occurring phytase. Phenolic content of horsegram was found to be 248.2 mg GAE/100g which decreased to 170.12 mg GAE/100g after soaking. Tannin content of horsegram flour showed a decrease from 316.0g/100g to 204.1g/100g. The oxalate content of raw horsegram was 466 mg/100g which decreased to 308 mg/100g during soaking (Handa *et al.*, 2017).

Phytic acid in horsegram seeds reduced by 18.1%, tannin content of whole horsegram reduced by 22.55% after soaking, whereas the oxalate content lowered by 23.32% (Ojha *et al.*, 2020). The soaking process caused a significant reduction in soluble oxalates in peas (36.5–7.62)%, lentils (26.66–48.79)%, fava beans (45.34–45.82)%, chickpeas (29.92–35.53)%, beans (36.56–39.65)% and soybean 56.29% (Shi *et al.*, 2018).

2.10.2 Dehulling

Dehulling is the process of removing seed coat from pulses, and it is one of the key postharvest processes for improving the palatability of food grains. It does, however, result in a loss of nutrients and dietary fiber. Dehulling also eliminates the embryo and sticky layer that exists between the hull and the cotyledons (Kumar *et al.*, 2022). Legume grains may be classified as easy-to-dehull and hard-to-dehull. Legume grains such as pigeon pea, mung bean and horsegram belong to the hard-to-dehull group because of the presence of mucilage and gum forming a strong bond between the hulls and the cotyledons (Ramakrishnaiah and Kurien, 1983).

Dehulling has been reported to reduce tannins, phytic acid and trypsin inhibitor activity but lectin activity was not changed. In addition, dehulling has been reported to improve the palatability and taste of some legume seeds, such as chickpea (Luo and Xie, 2013).

Tannins are mainly located in the seed coat which is significantly reduced after dehulling. Dehulling decreases the level of condensed tannins (Deshpande *et al.*, 1982).

After dehulling, the content of myristic, palmitic, stearic, oleic, and linolenic acids reduced while the content of linolenic acid increased. In raw horse gram seed, dehulling was found to be most successful in lowering tannins (89.46–92.99)% and phytic acid (52.63–60.00)% concentration (Patel *et al.*, 2016). Dehulling of pulses has also been reported to decrease the polyphenols (Tajoddin *et al.*, 2010). A significant decrease in oxalate was also found in different varieties of horse gram (Alonso *et al.*, 2000).

2.10.3 Germination

Germination is the first stage of a plant's growth during which the primary root and stem come out. In this stage, the reserve nutrients required for plant growth are mobilized by hydrolyzing proteins and carbohydrates to obtain the required substrates for the seed development. The seed enzymatic system is activated during it’s germination. It is considered one of the most effective processing methods for improving the nutritional quality of pulses, enhancing the digestibility of nutrients as protein and carbohydrates (Kumar *et al.*, 2022). For the breakdown of chemical components in pulses, the germination process has been widely researched. The degree of deterioration, on the other hand, is dependent on the type of pulses, the type of ANFs, and the germination conditions. Proteases are thought to be responsible for the inactivation of proteinaceous ANFs such enzyme inhibitors and lectins. Phytic acid is digested by an endogenous enzyme called phytase during germination into inorganic phosphorus, which is the biologically accessible form for plant growth and development. As a result, the phytic acid in pulses transforms to a soluble form, and several researchers have documented the drop in phytic acid content of germinated pulses as a result of this occurrence (Camacho *et al.*, 1992).

The most effective method for reducing phytic acid in legumes is germination. Phytic acid was degraded during germination, leading in an increase in inorganic phosphorus availability (Virginia *et al.*, 2012). The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase. A decrease in phytic acid content after germination for lentils was reported by (Vidal-Valverde *et al.*, 1994) for faba bean by (Alonso *et al.*, 2000) for black gram and mung bean by (Kataria *et al.*, 1989a). Because of sprouting phytic acid's chelating capacity is diminished which lowers its levels and

enhances the availability of minerals in the digestive system of animals (Akande and Fabiyi, 2010).

Germination modifies the quantitative and qualitative phenolic composition of pulses. This process has shown up to 20.8% reduction in total cyanide content in kidney bean (Akande and Fabiyi, 2010). It also reduces the content of enzyme inhibitors such as trypsin inhibitors, α-amylase inhibitors and chymotrypsin inhibitors in pulses (Alonso *et al.*, 2000). On sprouting of horsegram, tannin, phytic acid and oxalate content reduced by 54.6%, 61.6% and 61.66% respectively (Ojha *et al.*, 2020).

2.10.4 Cooking

Pulses are generally consumed after hydrothermal processing/cooking/roasting. These are usually cooked in boiling water, and their cooking time is affected by the seed composition, structure, size, etc. Low molecular weight compounds are leached out into cooking water when cooking is done in water. Cooking (boiling, autoclaving and microwave cooking) is very effective in reducing trypsin inhibitors, haemagglutinin activity, tannins and saponins (El-Adawy, 2002). Cooking process has been reported to decrease both water and acid-extractable phytate phosphorus in pulses, which may be due to formation of insoluble complex of phytate phosphorus with other components (Kumar *et al.*, 1979).

Generally, there are two types of cooking practiced traditionally as well as industrially as open cooking and pressure cooking. Both methods result in the destruction of anti-nutrients. But pressure cooking preserves more nutrients as compared to open cooking (Deol and Bains, 2010). Similarly, (Kataria *et al.* 1989a) have also reported that pressure-cooking was more effective than ordinary cooking in reducing the amount of phytochemicals.

2.10.4.1 Open cooking

Heat sensitive factors like trypsin and chymotrypsin inhibitors, as well as volatile chemicals are often inactivated by cooking. Reduction of tannins content after cooking in various pulses such as lentil, cowpea, mung bean and kidney bean may be due to the binding of tannins with proteins (Kaur *et al.*, 2020) and other organic substances during

cooking (Kumar *et al.*, 2022). Besides tannins, cooking also causes destruction of polyphenols (Yasmin *et al.*, 2008).

Reduction of tannin (48%), phytic acid (30%) and hemagglutinin (100%) and trypsin inhibitor (82%) was reported when chick pea was open cooked for 90 min at 100℃ (Alajaji and El-Adawy, 2006). Presoaked cooking of seeds is more advantageous than unsoaked cooking in the reduction of phytochemical components. The reductions in total oxalates as a result of cooking presoaked seeds was, (30.83-41.45)%, (34.45-54.16)%, (31.85-45.81)%, (33.48-39.72)%, (37.81-44.96)% and 66.15% for peas, lentils, faba beans, chick peas, common beans and soy bean respectively. Loss of soluble oxalate in water was considered to be the primary factor contributing to total oxalate reduction (Akhtar *et al.*, 2011).

2.10.4.2 Autoclaving

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

Temperature, heating time, particle size, and moisture content all influence the degree of heat inactivation. In terms of reducing phytochemical components, presoaking seeds before autoclaving is preferable than unsoaked seeds. Presoaked autoclaved mung bean samples have decreased phytic acid and tannin by 34% and 44%, respectively, but unsoaked autoclaved mung bean samples have reduced phytic acid and tannin by 32% and 40%, respectively (Singh *et al.*, 2015). It has been reported that autoclaving of faba beans shows the reduction of trypsin inhibitor (84%), phytic acid (23%), tannin (30%) and lectin (75-100) % (Luo and Xie, 2013).

2.10.5 Roasting

Roasting is a cooking technique that uses dry heat to roast food evenly on all sides at temperatures of at least 150°C from an open flame, oven, or other heat source. Protein digestibility can be improved by roasting. Bacteria and viruses can be killed or rendered

inactive by heat. The amount of aflatoxins produced by fungi is reduced when they are roasted (Samarajeewa *et al.*, 1990). The goal of roasting is to improve sensory qualities and achieve inactivation of destructive enzymes which improves the storage and nutritional quality of the product (Rackis *et al.*, 1986). The influence of roasting on properties of horse gram was studied (Sawant *et al.*, 2015). Legumes after roasting are used in a variety of snacking recipes that are popular among the impoverished. According to studies, roasting did not lead to a significant reduction in different amino acids (Bhardwaj and Yadav, 2015). When compared to the values indicated by cooked horse gram, roasting improved the rats' growth rate and digestibility (Shagun, 2022).

A significant decrease of phytates and condensed tannin contents was recorded for roasted varieties of lentils i.e., reduction up to 63.01% and 41.41% respectively for phytates and condensed tannin contents at 140°C for 30 min (Attou *et al.*, 2020). Similarly, reduction in phytic acid and tannin of chickpea was reported up to 56% and 57% respectively (Yadav and Bhatnagar, 2017). Roasting of lima bean seeds helps in the reduction of phytic acid (40%), tannin (30%) and trypsin inhibitor (98%) (El-Gohery, 2021). Roasting of black bean seeds reduce the polyphenols and saponin by 8% and 20% respectively (Ngoc *et al.*, 2021). Roasting reduced phytate content in horsegram by 22.4%, tannin content decreased by 28% and the amount of oxalate lowered by 22.36% (Ojha *et al.*, 2020).

2.11General uses of horsegram

It is a legume that is grown in South India since the Neolithic age. Horsegram is imported to Nepal from India. It has high potential of becoming a very important food source in future as it is drought resistant and has nitrogen fixation capacities (Chand *et al.*, 2016). Horsegram is used in following ways in Nepal:

- Horsegram is enjoyed in a variety of recipes usually as dal.
- Horsegram is rich in nutrients, including protein and fiber so it is used in weight loss diets.
- It is used as medicine for diabetes, heart disease, kidney stones, constipation, urinary discharges, menstrual disturbances, diarrhea, digestion, etc.
- It is used as fodder for livestock and is also used as green manure.

Part 3

Materials and methods

3.1 Materials

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

3.1.1 Collection of horsegram

Horse gram (*Macrotyloma uniflorum*) was collected from local market of Dharan sub-metropolitan, located in Sunsari district in Province No. 1 on the month of May 2022.

3.1.2 Equipment

All equipment required for the research were used from laboratory of Central Campus of Technology.

List of equipment are in appendix A1.

3.1.3 Chemicals

All chemicals required for this research were used from laboratory of Central Campus of Technology.

List of chemicals are in appendix A2.

3.2 Methodology

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

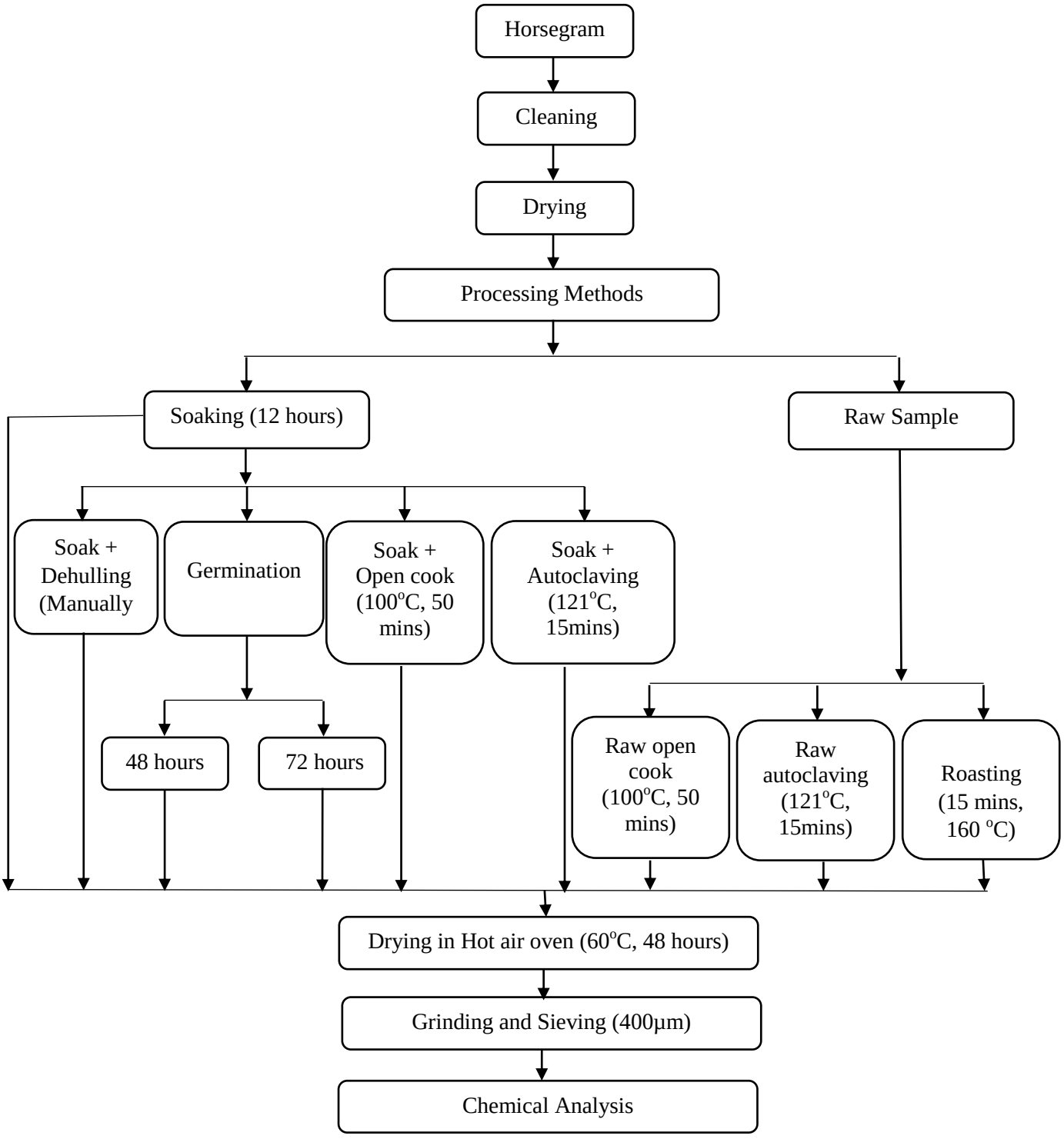

Figure 3.1 General flowsheet for processing of horsegram

3.3 Processing methods

3.3.1 Soaking

Seeds weighing 100g were soaked in tap water at ratio 1:10 (w/v) at room temperature for 12 hours. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in hot air oven at 60℃ for 48 hours. Dried sample was ground in a grinder until it became fine powder, sieved by using a mesh (400µm) and packed in high density polyethylene bags (50 µm) for further analysis (Deepinder Kaur and Kapoor, 1990).

3.3.2 Open cooking

The samples were cooked in controlled amount of water, and no water was drained after cooking. Seeds weighing 100g (12 h in tap water) was cooked in beakers with a seed to water ratio of 1:5 and 1:6 (w/v) for soaked and unsoaked seeds, respectively. The water was allowed to boil before the addition of seeds. The seeds were cooked until soft as felt between fingers (50 minutes). The cooked samples were dried in hot air oven maintained at 60℃ for 48 hours and then it was finely ground, sieved through a 400µm mesh and stored (Deepinder Kaur and Kapoor, 1990).

3.3.3 Autoclaving

The seeds weighing 100g (soaked for 12 hours) and unsoaked seeds weighing 100g were autoclaved for 15 min at 121ºC under 15 lb/in. The ratio of seed to water was 1:5 (w/v) for unsoaked seeds and 1:4 (w/v) for soaked seeds. The autoclaved seeds were then dried at 60℃ for 48 hours, finely ground, sieved and stored for analysis (Deepinder Kaur and Kapoor, 1990).

3.3.4 Germination

The seeds weighing 100g were soaked overnight in fresh water for 12 hours. Then, the seeds were rinsed and the water was drained. The seeds were then kept in a muslin cloth and swirled in order to drain excess water. Seeds were allowed to sprout until the sprouts become 1 cm length i.e., for about 48 h. The seeds were stored at (28 ± 3) °C and RH 80% for 4 days. The sprouted samples were dried in hot air oven at 60°C for 48 hours, finely

ground, sieved and stored in high density polyethylene bags (50 µm) for further analysis (Deepinder Kaur and Kapoor, 1990).

3.3.5 Roasting

Roasting of horsegram (100g) was done on a pan at 160°C for 15 min. The roasted seeds were dried in hot air oven at 60ºC for 48 hours, finely ground, sieved and stored in high density polyethylene bags (50 µm) for further analysis (Yanez *et al.*, 1986).

3.3.6 Dehulling

Hulls of horsegram (100g) were removed manually after soaking the seeds for 12 hours in distilled water (1:10, w/v). The dehulled seeds were dried at 60°C in hot air oven for 48 hours, finely ground, sieved and stored for analysis (Singh *et al.*, 2015).

3.4 Analytical methods

3.4.1 Proximate analysis of horsegram

3.4.1.1 Moisture content

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

3.4.1.2 Crude Protein

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

$$\text{Nitrogen \%} = \frac{(\text{sample titre-black titre}) \times \text{Normality of HCl} \times 14 \times 100}{\text{Weight of sample} \times 100}$$

3.4.1.3 Determination of ash content

The ash content was determined by incinerating the seeds (5g) in a muffle furnace at 525ºC for 4-6 hours (Ranganna, 1986). The calculated data were presented as g/100g on dry basis.

3.4.1.4 Determination of crude fat

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

$$\% \text{ Crude fat} = \frac{\text{Weight of ether soluble materials} \times 100}{\text{Weight of sample}}$$

3.4.1.5 Determination of crude fiber

Crude fiber was determined by using chemical process, the sample was treated with boiling dilute sulphuric acid, boiling sodium hydroxide and then with alcohol as standard method of (Ranganna, 1986). The calculated data were presented as g/100g on dry basis.

$$\% \text{ Crude fiber} = \frac{\text{(loss in weight noted)}}{\text{Weight of sample taken}} \text{x}100$$

3.4.1.6 Determination of carbohydrate

Total carbohydrate content of the samples was determined by difference method (AOAC, 1975).

$$\text{Carbohydrate (\%)} = 100 - [\text{Sum of protein, total ash, fiber and fat}].$$

3.4.2 Physical analysis of horsegram

3.4.2.1 Thousand kernel weight

The 1000 kernel weight of horsegram was determined by measuring the weight of 1000 kernels of horsegram seeds after selecting the appropriate sample size by quartering method as stated in (Buffo *et al.*, 1998).

3.4.2.2 Bulk density

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

$$\text{Bulk density} = \frac{\text{Mass of grain}}{\text{Volume of bushel}}$$

3.4.2.3 Length by breadth ratio

Length by breadth ratio of horsegram seed was determined by using a digital Vernier caliper with a least count of 0.01 mm. The length and breadth of seed were measured at the widest part. Ten random sample were taken in triplicate and mean value was calculated (Unal *et al.*, 2008).

3.4.3 Determination of oxalate

The oxalate content was determined by the method of Day and underwood (Day and Underwood, 1986). A sample of horsegram powder (1g) was mixed with 75 ml of 3M sulphuric acid (H_2SO_4) in a conical flask and stirred for 1 hour using a magnetic stirrer. The mixture was allowed filtering and a 25 ml of aliquots of the filtrate was titrated against 0.05M Potassium Permanganate ($KMnO_4$) solution until violent color persisted at least for 30 seconds. The oxalate content of the sample was determined using the following equation.

$$1 \text{ ml } 0.05 \ KMnO_4 = 2.2 \text{ mg oxalate}$$

3.4.4 Determination of phytate

Sample weighing 0.2g was placed in a 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 distilled water was added to the sample. Then, 10 ml

of 0.3% ammonium thiocyanate solution was added as an indicator and titrated with standard iron (III) chloride solution which contained 0.00195g iron per 1 ml (Ekpa and Sani, 2018).

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

3.4.5 Determination of tannin

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

The horsegram sample weighing 0.5 g was boiled for 30 min with 40 ml of water. Then it was cooled and was transferred to a 50 ml volumetric flask and diluted to mark. It was then shook well and filtered. 0 to 1 ml aliquots of the standard tannic acid solution were taken in test tube and 7.5 ml water was added to each. Then, 0.5 ml folin-ciocalteu reagent and 1 ml Na_2CO_3 solution was added and volume was made to 10 ml. After that, color was measured after 30 min at 760 nm against experimental blank adjusted to 0 absorbency (Ranganna, 1986).

3.4.6 Determination of polyphenol

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

3.4.7 Determination of iron

Iron in the sample was determined by converting all the iron into ferric by using oxidizing agents like potassium persulphate or hydrogen peroxide and treating thereafter with

potassium thiocyanate to form a red ferric thiocyanate which was measured calorimetrically at 480 nm (Ranganna, 1986).

$$\text{Iron (mg/100g)} = \frac{\text{Absorbance of sample} \times 0.1 \times \text{Total volume of ash solution} \times 100}{\text{Absorbance of standard} \times 5 \times \text{Weight of sample taken for ashing}}$$

3.4.8 Determination of calcium

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

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

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

Part 4

Result and discussion

Horse gram (*Macrotyloma uniflorum*) was collected from local market of Dharan, located in Sunsari district. The collected sample was imported from India. It was subjected to various processing techniques i.e., soaking, dehulling, germination, roasting and cooking (open and pressure cooking). Then, the obtained processed samples were analyzed to study the effect of treatments on the phytochemicals present in horsegram by single and combination methods.

4.1 Physical properties of horsegram

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

Table 4.1 Physical properties of horsegram

Physical properties	**Horsegram seeds**
l/b ratio	1.45 ± 0.05
Bulk density (kg/hl)	85.83 ± 0.76
1000 kernel weight (g)	32.96 ± 0.18

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

Length to breadth ratio of raw horsegram was found to be 1.45±0.05. The seeds of horsegram are bold in nature (Rather *et al.*, 2016). The l/b ratio of horsegram varieties ranged from 1.41-1.65 (Patil and Kasturiba, 2018) in which our data was within the range. The study conducted by (Bhokre *et al.*, 2015) showed similar values. Bulk density and thousand kernel weight of horsegram was found to be 85.83±0.76kg/hl and 32.96±0.18g respectively which was within the range found by (Vashishth *et al.*, 2020) i.e.(81-90.1) kg/hl for bulk density and (30.32-49.11) g for thousand kernel weight. Bulk density of horsegram was reported to be 85.5 kg/hl (Rajwade *et al.*, 2014) to which our data was very similar. Density values are useful in designing storage bins (Nalladurai *et al.*, 2002).

(Rajwade *et al.*, 2014) found that the thousand kernel weight of horsegram was 29.8 g in comparison to which our data was slightly higher. The reason for this difference may be due to varietal divergence. Low thousand kernel weight could be indicative of the presence of immature, damaged, and unfilled grains (Adu-Kwarteng *et al.*, 2003).

4.2 Proximate composition of horsegram

Table 4.2 Proximate composition of raw horsegram

Parameters	**Values (%)**
Moisture	11.49 ± 0.27
Protein (dry basis)	26.96 ± 1.11
Fat (dry basis)	0.90 ± 0.12
Crude fiber (dry basis)	3.53 ± 0.34
Ash (dry basis)	3.79 ± 0.18
Carbohydrate (dry basis)	64.82 ± 1.37

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

The moisture content in horsegram was found to be 11.49%. Similar data was found by (Chavan *et al.*, 2010) i.e. 12%. Meanwhile, the data differed with studies done by (Patil and Kasturiba, 2018) and (Sreerama *et al.*, 2008) which resulted in 7.4% and 8.33% moisture respectively. The protein content found in horsegram was 26.96% which is comparable to (Patil and Kasturiba, 2018). They found 24.51% protein in horsegram. The value was higher than 22.50% protein obtained by (Sreerama *et al.*, 2008). (Kaundal and Kumar, 2020) found 28.8% protein in horsegram which is very similar to our research. According to (Prasad and Singh, 2015), protein content of horsegram varieties ranged from (17.9 -25.3)%. 0.90% fat was found in horsegram. Another study by (Kaundal and Kumar, 2020) resulted in slightly higher amount of fats as 1.9% . 0.58% of fat was reported by (Bhartiya *et al.*, 2015). It was found that the fiber content in raw horsegram was 3.53%. This data was lower than the values found by (Patil and Kasturiba, 2018) and (Bhartiya *et*

al., 2015) i.e. 4.41% and 5.3% respectively. The ash content in horsegram resulted to be 3.79% which was parallel to 3.47% found by (Pagar *et al.*, 2021). Similar result was found by (Bhartiya *et al.*, 2015). The ash content in horsegram was discovered to be 0.08% (Chavan *et al.*, 2010) in comparison to which other studies found higher ash percentage. Carbohydrate is the most abundant nutritional content in horsegram which was observed to be 64.82%. This data was greater than the range (51.9 -60.9) % carbohydrate found for various varieties of horsegram (Prasad and Singh, 2015). Similar studies on horsegram by (Pagar *et al.*, 2021) and (Bhartiya *et al.*, 2015) discovered 58.86% and 57.2% carbohydrate respectively.

4.3 Phytochemicals in horsegram

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

Table 4.3 Distribution of phytochemicals in raw horsegram (mg/100 g).

Phytochemicals	**Values in dry basis (mg/100 g)**
Tannin	740.12 ± 1.84
Oxalate	462.65 ± 12.64
Phytate	1076.67 ± 5.77
Polyphenol	1254.90 ± 5.66

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

The tannin content obtained from raw horsegram was 740.12 mg/100g which is greater than data given by (Mishra and Pathan, 2019) i.e. 651 mg/100g. While comparing to the obtained value, higher tannin content of 1175.10 mg/100g was observed by (Ojha *et al.*, 2020). The data was within the range of (763.7 – 895.9) mg/100g tannin for various kinds of horsegram (Sudha *et al.*, 1995). Oxalate content was found to be 462.65 mg/100g. The result was within the range of (374 – 610) mg/100g oxalate for different varieties of horsegram (Sudha *et al.*, 1995), but higher than the findings of (Ojha *et al.*, 2020) i.e. 313 mg/100g. According to (Bhartiya *et al.*, 2015), average value of oxalate found in various

types of horsegram was 523.14 mg/100g. Different contents of total oxalate measured from legume seeds can be attributed to variety, growth, season, soil conditions and harvest time (Akhtar *et al.*, 2011). The phytate content found in horsegram was 1076.67 mg/100 g. Similar value was obtained by (Sreerama *et al.*, 2012) i.e. 1020.4 mg/100g. Phytic acid in horsegram varied from (778 -1203) mg/100g depending on variation (Pal *et al.*, 2016). Polyphenol content obtained from horsegram was 1254.90 mg/100g. (Sreerama *et al.*, 2012) reported a slightly greater value of polyphenol i.e. 1430 mg/100g. Polyphenol as high as 4653 mg/100g was observed in a variety of horsegram (Moktan and Ojha, 2016). From the result, it was observed that horsegram is rich in polyphenols and the results were in accordance of (Kawsar *et al.*, 2008). The total amount of these components in horsegram were much higher than those found in other legumes (Mishra and Pathan, 2019).

4.4 Iron and calcium content in horsegram

Table 4.4 Iron and calcium content in horsegram

Minerals	**Values (mg/100 g)**
Iron	10.12 ± 0.21
Calcium	291.2 ± 2.08

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

The iron content in raw horsegram was found to be 10.12 mg/100g which is similar to the data obtained by (Kadam *et al.*, 1985) i.e. 11 mg/100g and slightly higher than data given by (Khatun *et al.*, 2013) i.e., (5.89 - 7.44) mg/100g. The outcome discovered in this study was comparable to results found by (Morris *et al.*, 2011), they obtained (6.83 - 9.29) mg/100g iron in various samples of different varieties of horsegram. Similar data was obtained by (Pal *et al.*, 2016) which ranged from (5.17 - 9.78) mg/100g. Horse gram is a rich source of iron. (Kadwe, 1974) reported that horsegram contained a higher amount of iron than any other legume generally consumed. The calcium content in raw horsegram was found to be 291.2 mg/100g which is within the range of data obtained by (Gopalan *et al.*, 1971) i.e. (244 – 312) mg/100g. The amount of calcium was found in the range of

(136.83 – 652.02) mg/100g in horsegram (Pal *et al.*, 2016). (Kadam *et al.*, 1985) found 105 mg/100g calcium content in horsegram.

4.5 Effect of processing on tannin content

The effect of different processing methods namely soaking, germination, roasting, cooking and dehulling on the tannin content in horsegram was studied. All the treatments significantly reduced ($p<0.05$) tannin present in horsegram seeds to varying extent. Dehulling reduced maximum amount of tannin present in horsegram.

4.5.1 Effect of soaking

Tannin content of raw horsegram was determined which was found to be 740.12 mg/100 g and the value obtained for soaked horsegram sample showed that there was significant reduction ($p<0.05$) in tannin content. Tannin content reduced from 740.12 mg/100 g to 618.53 mg/100 g after soaking i.e., 16.43% reduction.

The obtained data from this study was in alignment to value obtained by (Handa *et al.*, 2017), they found that soaking reduced tannin content in horsegram by 16.78%. Tannin content of horsegram flour showed a decrease of 33.26% with an increase in the soaking time. Another research found 25.95% reduction in tannin content (Sarvani *et al.*, 2020) which was a contrast to what this research discovered. (Ojha *et al.*, 2020) reported that there was 22.55% reduction in the tannin content after soaking for 24 hours. The decline in tannin content is mainly due to the fact that these compounds are predominantly present in seed coats and are water soluble and subsequently leach into the liquid medium (Reddy and Pierson, 1994).

4.5.2 Effect of germination

The tannin content of germinated horsegram sample were determined and the value obtained showed that there is significant reduction ($p<0.05$) of tannin content after germination. The tannin content of germinated horsegram reduced from 740.12 mg/100 g to 449.76 mg/100g (39.23% reduction) and 365.42 mg/100gm (50.63% reduction) after 48 and 72 hours of germination respectively.

The value obtained in this study had similarities with the results obtained by (P. Vandarkuzhali, 2017), where 33.08% and 50.87% decrease in the tannin content after 48 and 72 hours of germination was observed. (Rizvi *et al.*, 2022) found slightly lower percentage of decrease in tannin content after 48 hours of germination i.e. 25.11%. According to (Handa *et al.*, 2017), 49.81% reduction in tannin content was found when horsegram was germinated for 72 hours. The study results are in good agreement with (El-Adawy, 2002) for germinated chickpeas. The decrease in tannin during soaking and germination may be due to the activation of enzymes in germinated seeds, which further breaks the proteins, fats, and starches into less complex structures (Vandarkuzhali, 2016).

4.5.3 Effect of dehulling

Tannin content of horsegram was found to be significantly reduced ($p<0.05$) from 740.12 mg/100 g to 232.89 mg/100 g (68.53% reduction) after dehulling. This study suggests that highest reduction of tannin present in horsegram was due to dehulling as highest percentage of decline of tannins was seen in dehulled sample.

There was 68.53% lower tannin content in dehulled horsegram when compared to raw unprocessed seeds. Similar findings have been reported by (Sudha *et al.*, 1995) with up to (60.13 - 71.81) % reduction in tannin content after dehulling. Reduction of tannin content in horse gram was found to be 59.43%, 61.95% and 60.66% in VLGahat15, HPK2 and VLGahat8 varieties respectively (Bhartiya *et al.*, 2015). Dehulling significantly reduced the tannin content of horsegram as previously observed by (Ghavidel and Prakash, 2007) in cowpea, chickpea, green gram and lentil. These findings indicate that dehulling could remove a large portion of tannins. After dehulling, there was little tannin detectable in cotyledons, indicating that most of it was present in seed coat. (Rao and Prabhavathi, 1982) also reported similar results for some decorticated legumes.

4.5.4 Effect of roasting

Tannin content reduced from 740.12 mg/100 g to 595.54 mg/100 g after roasting i.e. 19.53% reduction. Present study shows that roasting significantly reduced ($p<0.05$) tannin content.

Similar result was reported by (K. G. Singh *et al.*, 2015). They found a significant decrease in tannin content after roasting of lentils i.e., 16.9% reduction. (Ojha *et al.*, 2020)

concludes that roasting of horsegram seeds reduces tannin content by 28% which is higher when compared to data obtained in this study. (Asma *et al.*, 2020) reported roasting of lentils can reduce tannin up to 41%. Heat-induced tannin complex formation reduces the concentration of assayable tannins in the roasted seeds. The roasting treatment caused up to 50% decreases in the tannin concentrations in the seeds of various cultivars of cowpea (Plahar *et al.*, 1997). Tannin is heat stable compound so roasting has less effect in reducing tannins from the beans than other treatments.

4.5.5 Effect of cooking

The effect of open cooking for 50 min and autoclaving at 15 psi for 15 min on total tannin content of horsegram seed was studied. In this study, all samples were cooked with regulated amount of water and the seeds were not subjected to washing after cooking. The values obtained shows that cooking significantly reduces ($p<0.05$) tannin concentration. Tannin content reduced to varying extent, from 740.12 mg/100g to 401.57 mg/100g, 355.78 mg/100g, 293.13 mg/100g and 260.60 mg/100g when subjected to raw open cooking, raw autoclaving, soaked open cooking and soaked autoclaving respectively. This research discovered that soaked autoclaving reduced 64.79% of tannin content which is the maximum reduction among all the cooking methods that reduced tannin in horsegram seeds, followed by soaked open cooking with 60.26% reduction, raw autoclaving 51.88% reduction and raw open cooking 47.21% reduction.

The data obtained in this study complied with the study by (Vashishth *et al.*, 2021), they found that the reduction in tannins ranged from (51 – 66)% when subjected to various cooking method. A result similar to our data was found by (Abbas and Ahmad, 2018) as they found 48.04% lower tannin in raw open cooked seeds of horsegram. Cooking of soaked legumes in pressure cooker at 15 psi for 15 min displayed (25.23 -50.09)% reduction in tannin content of legumes (Huma *et al.*, 2008). Our study found very similar result for raw autoclaved samples of horsegram. According to (Nergiz and Gökgöz, 2007) beans that are cooked after soaking show more reduction in tannin content compared to that which are cooked without pre-soaking which was found to be true for our samples. Study on effects of cooking on hard-to-cook legumes by (Ojo, 2022) showed (64.69 - 78.46)% lower tannin concentration in open cooked samples when cooked for a long period of time. (Mishra and Pathan, 2019) reported 63.72% reduction in tannin content in

raw autoclaved sample of horsegram which was higher when compared to this study. The smaller reduction in our study might be due to varietal differences and differences in cooking time period. Tannin content of horse gram was found higher than other commonly used legumes, both in raw and cooked samples (Mishra and Pathan, 2019).

The effect of different processing techniques on tannin content is presented in Fig.4.1.

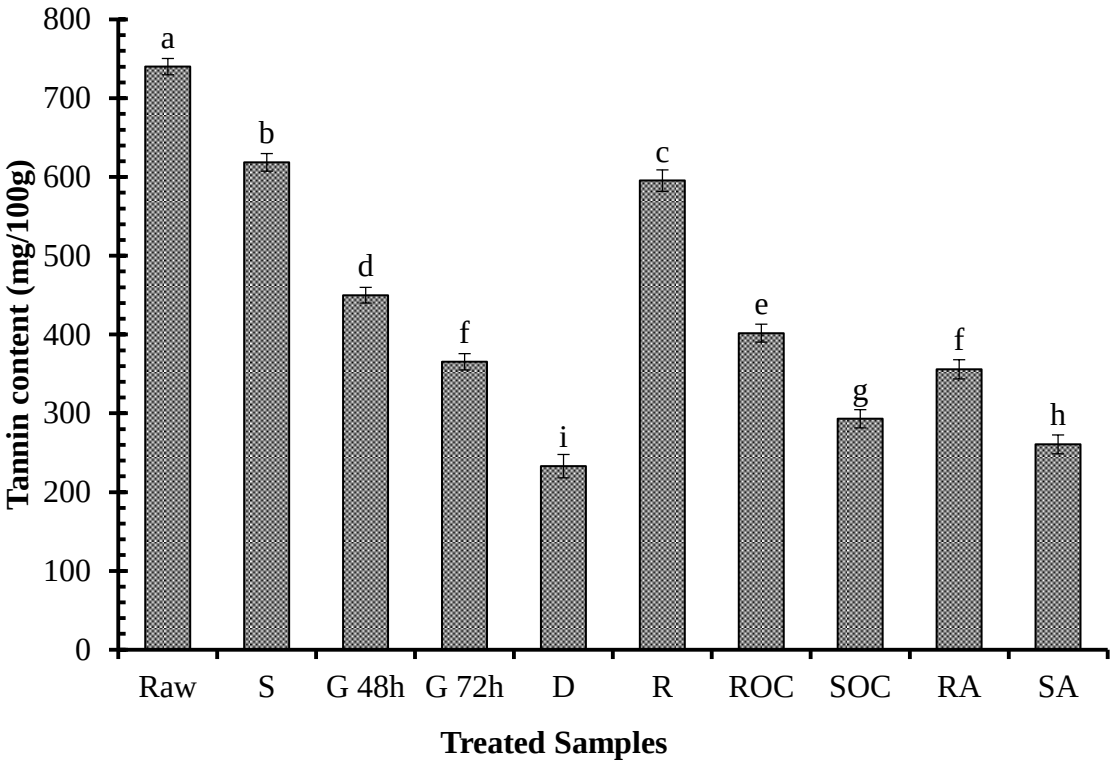

Figure 4.1 Effect of different processing techniques on tannin content

[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.

Where: S- Soak, G- Germination, D- Dehulling, R- Roasting, ROC- Raw Open Cooking, SOC- Soak Open Cooking, RA- Raw Autoclaving, SA- Soak Autoclaving]

4.6 Effect of processing on oxalate content

The effects of soaking, germination, dehulling, roasting, open cooking and autoclaving on the oxalate content in horsegram was studied. All the treatments significantly reduced

($p<0.05$) oxalate concentration in horsegram seeds to varying extent. The combination treatment i.e., soaked autoclaving had most pronounced effect in oxalate content reduction.

4.6.1 Effect of soaking

Effect of soaking on oxalate content of horsegram seed was studied. The result obtained displayed a significant reduction ($p<0.05$) in oxalate content. The result shows reduction from 462.65mg/100 g to 352.82mg/100 g after soaking for 12 hours i.e., 23.74% reduction.

Research by (Handa *et al.*, 2017) supports our study as they found that soaking for 12 hours significantly decreased the oxalate concentration in horsegram by 26.82%. The result obtained in this research was alike with result obtained by (Ojha *et al.*, 2020), where they detected 23.32% reduction of oxalate in horsegram. Similar result for reduction in oxalic acid content of soaked grains was reported by (Brudzyński and Salamon, 2011). The losses of total oxalate content ranged from (22.51–31.44)% in peas, (26.02–37.89)% in lentils, (17.40–37.08)% in fava beans, (26.86–29.09)% in chickpeas, (21.47–34.30)% in common beans and 51.89% in soybean (Shi *et al.*, 2018). Loss of oxalates in various foods is likely due to their leaching loss in cooking water (Judprasong *et al.*, 2006).

4.6.2 Effect of germination

The effect of germination on oxalate concentration in horsegram was observed. The value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was reduction from 462.65 mg/100g to 345.02 mg/100g (25.43% reduction) and 232.64 mg/100g (49.72% reduction) after 48 hours and 72 hours germination period respectively.

The data that resulted from this study is similar to result obtained in the research by (Moktan and Ojha, 2016), they found 45.59% reduction in oxalate during 72 hours germination of horsegram seeds. (Pal *et al.*, 2016) found that oxalate content decreased by (15.77 - 23.29)% in various varieties of horsegram when subjected to germination for 48 hours. Similar finding have been reported by (Murugkar *et al.*, 2013). The result obtained in this research almost tally in line with data obtained by (Patel and Dutta, 2018) which stated germination of finger millet caused 54.36% reduction in oxalate content. During germination, oxalate oxidase gets activated which breaks down oxalic acid into carbon dioxide and hydrogen peroxide consequently releasing calcium (Murugkar *et al.*, 2013).

4.6.3 Effect of dehulling

The oxalate content of the dehulled horsegram was determined and found to be 330 mg/100 g. Present study shows that dehulling of horsegram can significantly decrease ($p<0.05$) oxalate content. Oxalate concentration declined from 462.65 mg/100g to 328.33 mg/100g i.e., 50.65% reduction.

The result obtained in this research is higher than the data given by (Pal *et al.*, 2016), they found that the percentage of oxalic acid content declined from 456.69 mg/100g in raw to 301.56 mg/100g after dehulling of horse gram i.e., 33.86% reduction of total oxalate content. (Sudha *et al.*, 1995) reported similar finding as they discovered (33.51 - 48.81) % reduction of oxalate in different varieties of horsegram when dehulled. In case of this research, the difference in data could be due to variational differences. These findings indicate that dehulling could remove a large portion of oxalic acid. Similar findings have been reported by (Gad *et al.*, 1982).

4.6.4 Effect of roasting

Horsegram seeds were roasted and oxalate concentration for roasted sample was established. It was found that the oxalate content lowered from 462.65 mg/100g to 375.32 mg/100g i.e., 18.88% reduction. This result shows that roasting significantly reduced ($p<0.05$) oxalate content in horsegram.

It has been reported that the oxalate content of horsegram decreased by 22.36% (Ojha *et al.*, 2020) which was slightly higher when compared to this study. It could to due to varietal differences. Another study concluded with 46.15% lower oxalate in chickpea grains after roasting (Mittal *et al.*, 2012). It has been reported that the oxalate content of bambara groundnut is reduced by (8 – 10)% after roasting of groundnut for 15 min at 130℃ (Adegunwa *et al.*, 2014). Roasting did not had as pronounced effect as other treatments in reduction of oxalates in legumes (Judprasong *et al.*, 2006).

4.6.5 Effect of cooking

The effect of cooking on oxalate content in horsegram was studied. It shows significant reduction ($p<0.05$) that ranged from 462.65 mg/100g to 213.01 mg/100g, 198.6 mg/100g, 186.47 mg/100g and 171 mg/100 g for raw open cooked, raw autoclaved, soaked open

cooked, and soaked autoclaved samples respectively. These findings suggest that soaked autoclaving had the most pronounced effect which reduced 63.04% of oxalate content, followed by soak open cooking with 59.69% reduction, raw autoclaving (57.07% reduction) and raw open cooking (53.96% reduction).

Our study shows similar results to (Vashishth *et al.*, 2021) as they reported 63.93% and 78.59% reduction of oxalate in two different varieties of horsegram when pressure cooked. According to (Judprasong *et al.*, 2006), open cooking of legumes lowered oxalate content by (20 – 50)% to which our data was comparable. Similar result for the reduction in oxalic acid during processing was reported for wattle seeds (Ee and Yates, 2013). The results were also in accordance with reported results for various pulses (Shi *et al.*, 2018). According to (Akhtar *et al.*, 2011), the reductions in total oxalates as a result of cooking pre-soaked seeds were, (30.83-41.45)%, (34.45-54.16)%, (31.85-45.81)%, (33.48-39.72)%, (37.81-44.96)% and 66.15% for peas, lentils, faba beans, chick peas, common beans and soy bean respectively, where he found maximum reduction during pressure cooking than open cooking which was similar to our findings. The oxalate implicated as both water-soluble and heat-sensitive as a result of which significant reduction has been observed in processed grains. The reduction in oxalic acid on processing can be attributed to the hydro-thermal effect which produces the conformational changes (Vashishth *et al.*, 2021).

The effect of different processing techniques on oxalate content is presented in Fig.4.2.

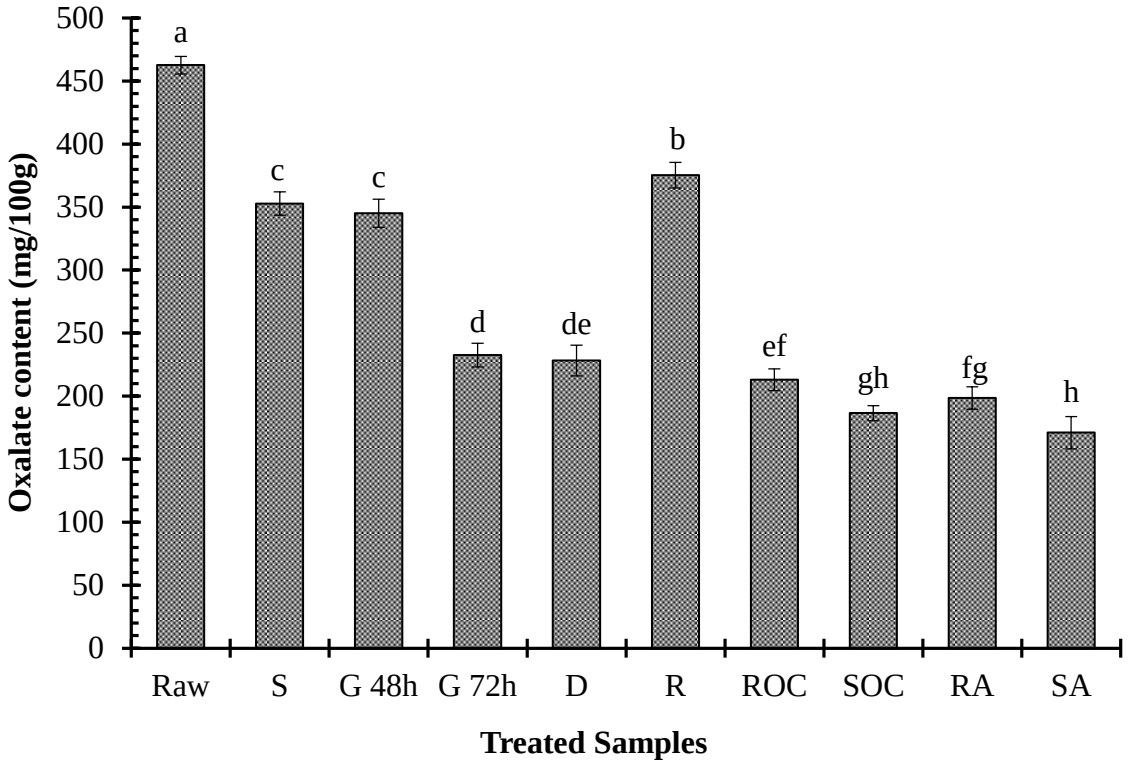

Figure 4.2 Effect of different processing techniques on oxalate content

[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.

Where: S- Soak, G- Germination, D- Dehulling, R- Roasting, ROC- Raw Open Cooking, SOC- Soak Open Cooking, RA- Raw Autoclaving, SA- Soak Autoclaving]

4.7 Effect of processing on phytate content

The effects of soaking, germination, roasting, open cooking, autoclaving and dehulling on the phytate content in horsegram was studied. All the processing methods significantly reduced ($p<0.05$) the phytate of the horsegram seeds up to varying extent. Dehulling had the most pronounced effect in lowering phytate content.

4.7.1 Effect of soaking

Effect of soaking on phytate content of horsegram was examined and the values obtained showed that there is significant reduction ($p<0.05$) in phytate content. The result shows

decline of phytate levels from 1076.67 mg/100g to 881.76 mg/100g after soaking for 12 hours (18.10% reduction).

The result obtained in this research is greater than the values observed by (Rizvi *et al.*, 2022), they found that soaking reduced phytate content by 12.73% in horsegram seeds. Similarly, after soaking for 24 hours, phytate concentration decreased by 26.04% (Sarvani *et al.*, 2020) which was higher than the values found in this study. This dissimilarity could be due to different processing periods. (Kataria *et al.*, 1989b) reported that the reduction of phytate in mung bean after soaking was 17% which was similar to the data obtained in this research. The loss of phytic acid in the soaked seeds may be because of leaching out of phytate ions into soaking water under the influence of concentration gradient which governs the rate of diffusion (Grewal and Jood, 2006).

4.7.2 Effect of germination

Germination has been documented to remove maximum amount of phytochemicals in legumes and displayed considerable deplete in phytate content of horsegram seeds. The result acquired in this research presented significant reduction ($p<0.05$) in phytate concentration after germination. Horsegram samples when germinated for 48 hours and 72 hours prevailed phytate reduction from 1076.67 mg/100g to 670.87 mg/100g (37.69%) and 390 mg/100g (63.77%) respectively.

(Vandarkuzhali, 2017) reported 60.23% reduction of phytic acid in 72 hours of germination to which our results were comparable. The results obtained in the current study were comparable with the findings of (Pagar *et al.*, 2021) who reported 39.66% reduction in phytic acid content in germinated horse gram. Meanwhile, the data in this research is higher than the data obtained by (Moktan and Ojha, 2016), they found that there was 43.69% reduction of phytic acid in horse gram after soaking for 12 hours and 72 hours of germination. According to (Rizvi *et al.*, 2022), the reduction in phytate concentration ranged from (28.16-40.50)% in horsegram during various germination period. Similar values were observed by (Sarvani *et al.*, 2020) with 41.67% reduction. The loss of phytic acid during germination may be caused by hydrolytic activity of the enzyme phytase to inositol and free phosphate (Urbano *et al.*, 2000).

4.7.3 Effect of dehulling

The effect of dehulling on phytate content of horsegram was studied. The value obtained showed that there is significant reduction ($p<0.05$) in phytate content, which is reduction from 1076.67 mg/100g to 372.17 mg/100g after dehulling i.e., 65.43% reduction. Dehulled samples had the lowest amount of phytate content when compared to other samples.

(Pal *et al.*, 2016) reported that reduction in phytic acid ranged from (32.26-61.51)% after dehulling of different varieties of horsegram, and the data that was obtained is comparable to the given range. This result correlate well with an earlier report on wheat which implies dehulling aids to get refined flours with considerably reduced levels phytate content (Ma *et al.*, 2005). A study similar to this research by (Ghavidel and Prakash, 2007) figured out that the impact of dehulling on phytic acid of legume seeds were above 50%. The loss of phytic acid in dehulled samples may be due to the removal of husk as it contained relatively higher concentration of phytic acid when compared to whole grain, and therefore, the removal of husk accounted for significantly lower phytic acid content in dehulled samples (Grewal and Jood, 2006).

4.7.4 Effect of roasting

The effect of roasting on phytate content of horsegram was studied. The value obtained showed that there was significant reduction ($p<0.05$) in phytate content, which was reduction from 1076.67 mg/100g to 824 mg/100g after roasting (23.47% reduction).

Decrease in phytate content was found to be 18.75% when horsegram seeds were roasted for 15 minutes (Sarvani *et al.*, 2020), which is similar to what we discovered. Similarly, reduction in phytic acid of chickpea was reported up to 23.91% (Chitra *et al.*, 1996) which is alike to the data obtained in this research. (Ojha *et al.*, 2020) reported that roasting of horsegram seeds reduced phytate by 22.4% which was similar to the results obtained in our research. (Singh *et al.*, 2015) found that roasting of mung bean seeds reduced phytic acid by 29%. Denaturation and formation of insoluble complexes of phytate during roasting may be responsible for the reduction of phytate content in horsegram flour (Siddhuraju and Becker, 2001).

4.7.5 Effect of cooking

The effect of open cooking and autoclaving on phytate content in horsegram was studied. The water was not drained after cooking. Results show significant reduction ($p<0.05$) of phytate content. Phytic acid levels declined from 1076.67 mg/100g to 754.16 mg/100g, 765.77 mg/100g, 684.55 mg/100g, and 649.74 mg/100g for raw open cooked, soaked open cooked, raw autoclaved and soaked autoclaved samples respectively. The findings obtained in this research indicated that soaked autoclaving reduced 39.65% of phytate, followed by raw autoclaving with 36.39% reduction, raw open cooked (29.95% reduction) and soaked open cooking (28.88% reduction).

Reduction in phytic acid content was observed to various extents depending upon the cooking conditions. (Sarvani *et al.*, 2020) discovered 32.29% lower phytate content in horsegram when the samples were autoclaved which was akin to the findings in our research. The obtained data in our study was comparable to the values obtained by (Vashishth *et al.*, 2021), where they found slightly greater reduction for soaked autoclaved samples, which were 38.81% and 45.47% for two different varieties of horsegram. Accordingly, 28.93% decline was observed by (Alajaji and El-Adawy, 2006) in raw open cooked chickpea grains. (Sharma and Sehgal, 1992) found 15.50% reduction in phytate content when soaked horsegram were cooked for 5 minutes. (24.0–35.1)% reduction in phytic acid content was obtained for boiled legumes and (28.0–51.6)% in legumes autoclaved at 121℃ for different time periods (Zia-ur-Rehman *et al.*, 2003). Reduction in phytate was significantly higher while cooking was done under pressure. These results are consistent with the findings of other works (Sharma and Sehgal, 1992; U. Singh, 1993). Meanwhile, the reduction of phytic acid after boiling was greater than after autoclaving of raw mung bean (Mubarak, 2005).

The effect of different processing techniques on phytate content is presented in Fig.4.3.

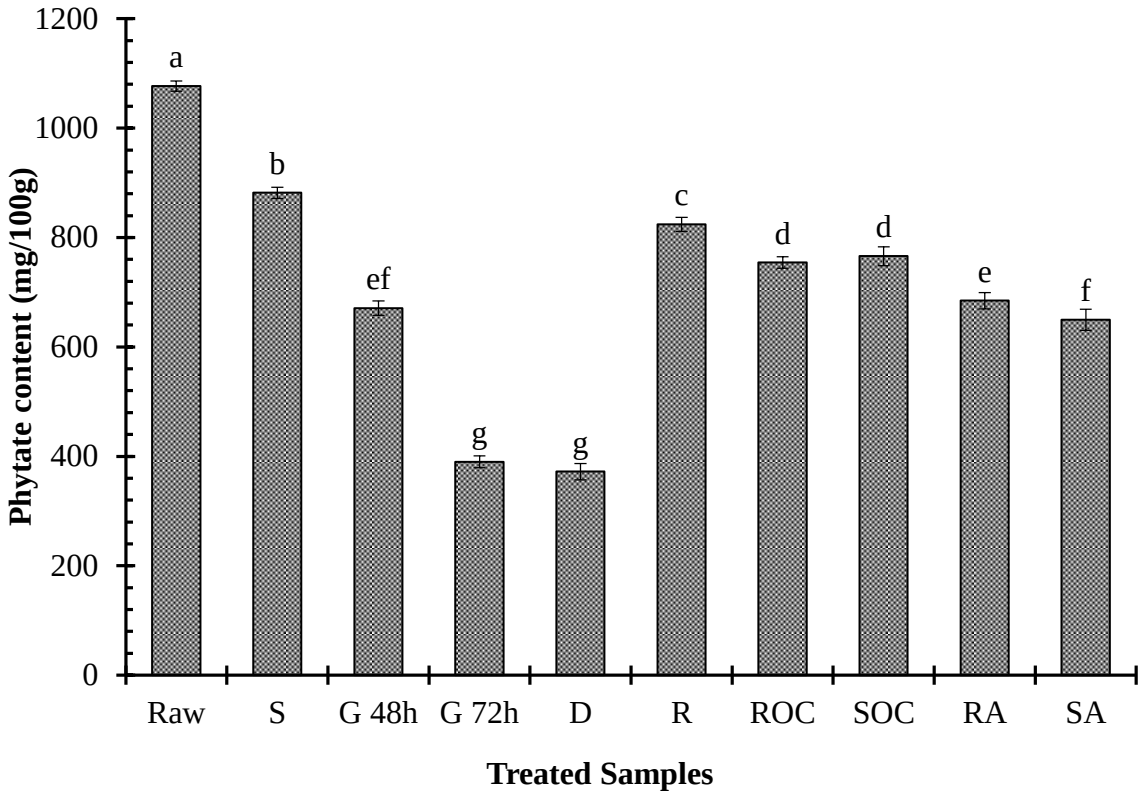

Figure 4.3 Effect of different processing techniques on phytate content

[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.

Where: S- Soak, G- Germination, D- Dehulling, R- Roasting, ROC- Raw Open Cooking, SOC- Soak Open Cooking, RA- Raw Autoclaving, SA- Soak Autoclaving]

4.8 Effect of processing on polyphenol content

The effects of soaking, germination, roasting, open cooking, autoclaving and dehulling on the polyphenol content in horsegram was studied. All the treatments significantly reduced ($p<0.05$) the polyphenols of the horsegram seeds up to the varying extent. Dehulling reduced maximum polyphenol content from horsegram seeds.

4.8.1 Effect of soaking

The effect of soaking on polyphenol content in horsegram was studied and the value obtained showed that there is significant reduction ($p<0.05$) in polyphenol content. The

result shows reduction from 1254.90 mg/100g to 909.8 mg/100g after soaking for 12 hours i.e., 27.50% reduction.

The research conducted by (Ojha *et al.*, 2020) found that the reduction of polyphenols in soaked horsegram was 19.5% which was lower than the data obtained in this research. This contrast could be due to varietal difference. Leaching out of soluble phenolic components in water might be responsible for the loss of phenolic components in soaked horsegram (Ojha *et al.*, 2020). (Grewal and Jood, 2006) reported that the polyphenol content of green gram seeds reduced by 23% after soaking for 18 h. (Rehman and Shah, 2005) reported a significant reduction in the polyphenol content of different legumes while soaking was applied.

4.8.2 Effect of germination

The effect of germination on polyphenol content of horsegram was studied. The value obtained shows that there was significant reduction ($p<0.05$) in polyphenol content, which was reduction from 1254.90 mg/100 g to 762.75 mg/100g (39.22% reduction) and 525.25 mg/100g (57.19% reduction) after 48 hours and 72 hours of germination period respectively.

Germination significantly decreased polyphenols which was in accordance with an earlier report on horsegram by (Satwadhar *et al.*, 1981). According to (Pal *et al.*, 2016) there was 67.21% and 69.57% reduction in polyphenol content in different varieties of horsegram after germination which was greater than our findings. (Kadam *et al.*, 1985) reported slightly lower reduction of polyphenol content which was 31.25% reduction. Similarly, (Grewal and Jood, 2006) found that the polyphenol content of mung bean seeds reduced by 32% after germination. Before germination, soaking was done and some loss of polyphenol during soaking is expected because of its leaching into the water. The rate of reduction of polyphenol content increased with incubation time and sprout age (Luo *et al.*, 2013). Further decrease in polyphenols during germination may be ascribed to the presence of polyphenol oxidase and enzymic hydrolysis (Jood *et al.*, 1987).

4.8.3 Effect of dehulling

Dehulling shows highest reduction of polyphenol content present in horsegram seeds. This result shows that dehulling significantly reduced ($p<0.05$) total polyphenol content from 1254.90 mg/100g to 468.77 mg/100g i.e., 62.64% reduction.

According to (Pal *et al.*, 2016), the reduction of polyphenol content in horsegram of different varieties after dehulling was (23.84-41.89)% which was lower than the data obtained in this research. Difference could be due to variation. Dehulling of soaked cowpea seeds resulted in a reduction of (70–71)% of polyphenols. Similar studies by (Sharma and Sehgal, 1992) reported that dehulling of soaked seeds significantly reduced polyphenol content in faba bean.

4.8.4 Effect of roasting

The effect of roasting on polyphenols in horsegram was studied. The value obtained showed that there was significant reduction ($p<0.05$) in polyphenol content, which was reduction from 1254.90 mg/100g to 960.59 mg/100g after roasting i.e., 22.66% reduction.

(Ojha *et al.*, 2020) reported that roasting of horsegram seeds reduced the polyphenol content by 28.3% which is higher than the data obtained in this research. Roasting which involves dry heat could bring about a change in chemical reactivity of the polyphenols. Thermal degradation of phenolic compounds during roasting decreased these components (Randhir *et al.*, 2008; Zhang *et al.*, 2010; Zhu *et al.*, 2010). Roasting decreased the polyphenol content of black bean only by 8% which is very low when compared to this research (Ngoc *et al.*, 2021). Also, roasting of mung bean seeds reduced the polyphenol content by 17% (Mendoza *et al.*, 1988).

4.8.5 Effect of cooking

The effect of cooking on polyphenol content of horsegram was studied. It shows significant reduction ($p<0.05$) of polyphenol content that reduced from 1254.90 mg/100g to 507.87mg/100g, 492.96mg/100g, 784.31mg/100g and 745.09mg/100g for samples of raw autoclaving, soaked autoclaving, raw open cooking and soaked open cooking respectively. The findings in this research display that soaked autoclaving reduced 60.72% of polyphenol content which is the most effective method, followed by raw autoclaving

(59.53% reduction), soaked open cooking (40.63% reduction) and raw open cooking (37.50% reduction).

The reduction in polyphenol content was 42.5% when soaked horsegram seeds were cooked for 10 minutes (Kadam *et al.*, 1985). The polyphenol content in the cooked horse gram pretreated with the soak solution was 35% less than that in the untreated cooked samples (Satwadhar *et al.*, 1981). According to (Mishra and Pathan, 2019), polyphenol decreased by 62.46% when raw horsegram was autoclaved, likewise polyphenols declined by 59.23%, 44.67% and 40.86% in mung bean, pigeon pea and red lentil samples when autoclaved. The effect of pressure cooking was greater when the period of pressure cooking was extended. Lower amount of polyphenols recovered from cooked seeds could be due to their reduced extractability due to their changed chemical reactivity (Kataria *et al.*, 1989a). Pressure cooking of soaked seeds decreased polyphenols to a larger extent as compared to the seeds which were ordinarily cooked after soaking, similar outcome was reported by (Grewal and Jood, 2006).

The effect of different processing techniques on polyphenol content is presented in Fig.4.4.

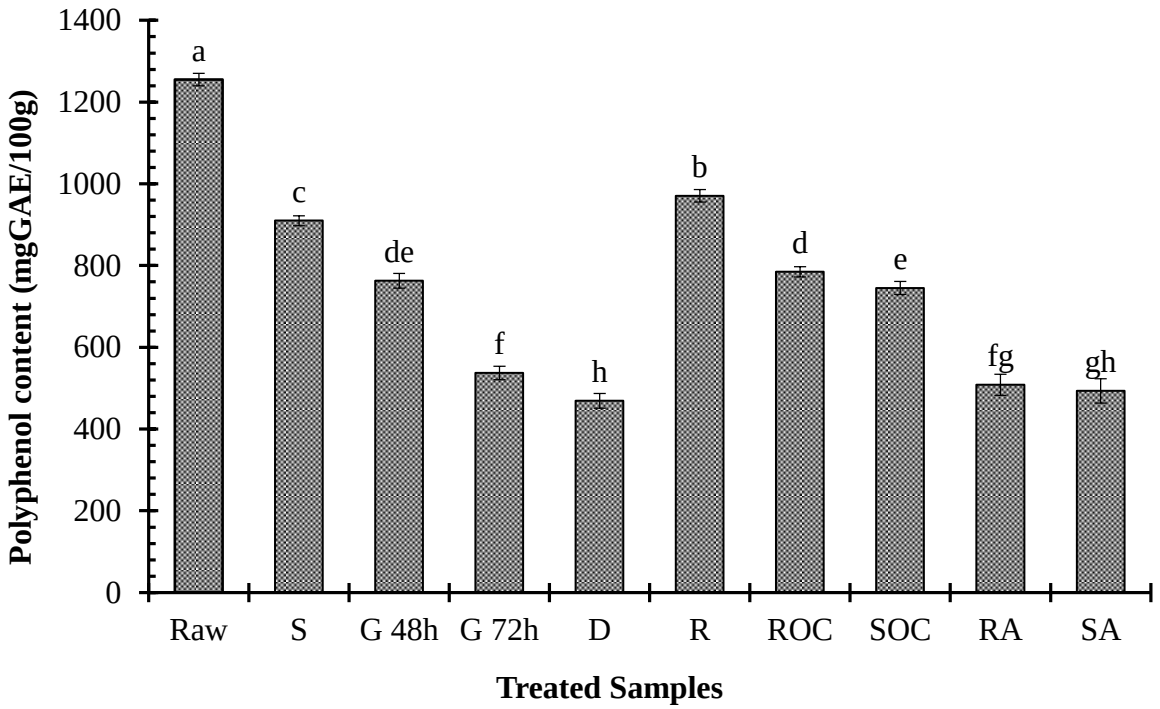

Figure 4.4 Effect of different processing techniques on polyphenol content

[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.

Where: S- Soak, G- Germination, D- Dehulling, R- Roasting, ROC- Raw Open Cooking, SOC- Soak Open Cooking, RA- Raw Autoclaving, SA- Soak Autoclaving]

4.9 Proximate composition of best effective processing technique

Table 4.5 Proximate composition of best effective processing technique

Components	Raw	Dehulled	% Change
Moisture %	11.49 ± 0.27	7.53 ± 0.18	- 34.46%
Protein % (dry basis)	26.96^{a} ± 1.11	28.84^{b} ± 1.72	+ 6.97%
Ash % (dry basis)	3.79^{a} ± 0.18	1.99^{b} ± 0.04	- 47.49%
Fat % (dry basis)	0.90^{a} ± 0.12	1.08^{b} ± 0.09	+ 20%
Crude fiber % (dry basis)	3.53^{a} ± 0.34	1.36^{b} ± 0.29	- 61.47%
Carbohydrate % (dry basis)	64.82^{a} ± 1.37	66.73^{a} ± 2.43	+ 2.95%

[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 ($p>0.05$)]

Moisture content of dehulled horsegram sample was found to be 7.53% which is lower than the raw horsegram samples. The data resulted from this study suggests that dehulling can cause significant ($p\leq0.05$) loss of ash and mineral content i.e. from 3.79% to 1.99%. The implication of this result is that the seed coat is rich in mineral nutrients and their substantial proportion is lost when seeds are dehulled and seed coats are discarded. Dehulling results in significant reduction ($p\leq0.05$) of crude fiber content i.e. from 3.53% to 1.36%. This finding is consistent with the report of a previous study by (Attia *et al.*, 1994). Dehulling resulted in significant increase ($p\leq0.05$) of fat content i.e. from 0.9% to 1.08%. Total fat content in horsegram was affected by dehulling. Fat content increased significantly ($p\leq0.05$) after dehulling (Pal *et al.*, 2016). Fat levels improved significantly

due to removal of hull portion and concentration of endosperm. The results are comparable with findings of (Sreerama *et al.*, 2012) for horsegram. The protein content in this study significantly increased ($p \leq 0.05$) i.e. from 26.96% to 28.84%. Similar results were found after dehulling by (Pal *et al.*, 2016). These results were also comparable with findings of (Sudha *et al.*, 1995). Meanwhile, carbohydrate content in the present study increased non-significantly ($p > 0.05$) from 64.82% to 66.73%. Increase in carbohydrate and protein content during dehulling was due to removal of mineral and phytochemical rich seed coat fraction along with fat rich germ part. According to (Mang *et al.*, 2016), the increase in protein and carbohydrate content after removal of hull may be due to the complexion of protein and carbohydrate by tannins and polyphenols present in the bean hulls.

4.10 Iron and calcium content of best effective processing technique

Table 4.6 Iron and calcium content of best effective processing technique

Components	Raw (mg/100g)	Dehulled (mg/100g)	%Change
Iron	$10.12^{a} \pm 0.21$	$7.30^{b} \pm 0.36$	- 27.82%
Calcium	$291.2^{a} \pm 2.08$	$163.33^{b} \pm 1.52$	- 56.09%

[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 ($p > 0.05$)]

Decortication or dehulling is the process of removing the outer hull of the pulse (Hall *et al.*, 2017). Iron content of dehulled horsegram was 7.30 mg/100g. Dehulling resulted in significant reduction ($p < 0.05$) of iron from 10.12 to 7.30 mg/100g. Calcium content of dehulled horsegram was 163.33 mg/100g. Dehulling caused significant reduction ($p < 0.05$) of calcium from 291.2 to 163.33 mg/100g. After dehulling, highly significant decrease in amount of iron and calcium content of horsegram was discovered (Pal *et al.*, 2016). (Wang *et al.*, 2009) reported reductions in calcium, iron, and some other minerals after dehulling of lentils. Similar observations were reported in peas subjected to dehulling (Wang *et al.*, 2008). (Mubarak, 2005) observed small reductions in minerals of dehulled mung beans, whereas (Attia *et al.*, 1994) observed reduction in minerals except phosphorus after dehulling of chickpea. Decline in iron and calcium levels after dehulling was observed,

which may be contributed to presence of these minerals in hull portion. The results are in accordance with an earlier report on pearl millet (Dave *et al.*, 2008).

Part 5

Conclusion and recommendation

5.1 Conclusion

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

1. All processing methods, single or combined reduce all phytochemicals significantly ($p<0.05$).
2. Dehulling reduced maximum tannin (68.53%), phytate (65.43%) and polyphenols (62.64%) present in horsegram. The most effective method to reduce oxalate content of horsegram was soaked autoclaving i.e. 63.04%.
3. In case of cooking, soaked autoclaving resulted in highest reduction of phytochemicals in horsegram seeds. However, cooking did not had as pronounced effect as some of other treatment methods.
4. Dehulling reduced iron and calcium content significantly ($p<0.05$).

5.2 Recommendation

1. Nutraceutical evaluation of horsegram can be done.
2. The effect of processing on other phytochemicals like trypsin inhibitor, hemagglutinin, lectin etc. present in horsegram can be studied.
3. Effect of different combined treatments (dehulling and cooking, germination and cooking, etc.) on phytochemicals can be studied.

Part 6

Summary

Horsegram (*Macrotyloma uniflorum*), family Fabaceae is one of the underutilized legumes, a lesser known neglected legume mainly cultivated in Asian and African countries as a dual purpose crop. It is a drought resilient legume which embraces favorable agronomic features suitable for cultivation on dry lands under poor fertility condition. Horsegram has high significance in subsistence farming and nutritional security of poor masses in developing countries. It has nutritional value comparable to other commonly consumed pulses. Horsegram is also known for it's therapeutic properties. Traditionally, it is used to cure kidney stones, asthma, bronchitis, leucoderma, urinary discharges, heart diseases, piles etc. Besides that it possess anti-diabetic, anti-ulcer activity and also helps in dietary management of obesity due to the presence of beneficial bioactive compounds. Due to the nutritional composition and medicinal properties, it has possibilities to be exploited as functional food for health benefits.

In the present study, nine types of processing techniques were used to investigate reduction pattern of phytochemicals in horsegram. The phytochemicals studied were oxalate, phytate, polyphenol and tannin. The treatments applied were soaking, 48 and 72 hour germination, dehulling, roasting, raw open cooking, soaked open cooking, raw autoclaving and soaked autoclaving method. Iron and calcium content was also determined for both raw and best effective treatment method. Tannin and polyphenol were analyzed spectrophotometrically, oxalate was determined by titration with potassium permanganate and phytate was determined using ammonium thiocyanate.

The mean value of tannin, oxalate, polyphenols and phytate of raw horsegram seeds were 740.12 mg/100 g, 462.65 mg/100 g, 1254.90 mg/100 g, 1076.67 mg/100 g respectively. All the processing methods significantly reduced ($p<0.05$) phytochemicals present in horsegram, where combination treatments were better than the single treatments. The combination treatment dehulling i.e., soaking and dehulling reduced tannin, phytate and polyphenol content of horsegram more effectively than other treatment methods. The reduction in oxalate by soaking and germination for 48 hour were not significantly ($p>0.05$) different. The reduction in phytate by raw open cooking and soaked open cooking were not significantly ($p>0.05$) different. Also, the reduction in phytate by germination 72

hour and dehulling were not significantly ($p>0.05$) different. The combination treatment i.e., soaked autoclaving reduced the oxalate content of horsegram greater than other methods. Soaking and dehulling was found to be the most effective treatment method regarding percentage reduction of phytochemicals. Soaking was done prior to dehulling, so the resulting reduction in phytochemicals was due to combined effect of soaking and dehulling. Hence, combination treatments reduced more phytochemicals than single process. Meanwhile, soaking and dehulling also reduces minerals like iron and calcium.

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Appendices

Appendix A

Table A.1 List of equipment used

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

Table A.2 List of chemicals used

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

Appendix B

Table B.1 One Way ANOVA table for Tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	9	768791.1	85421.2	611.28	<.001
Residual	20	2794.8	139.7		
Total	29	771585.9			

Table B.2 Effect of processing on Tannin content

Treatments	Tannin (mg/100g)	% Reduction
Raw	$740.1^{a} \pm 0.43$	
Soak	$618.5^{b} \pm 11.19$	- 16.43%
Roasting	$595.5^{c} \pm 13.58$	- 19.53%
Germination 48 hour	$449.8^{d} \pm 9.98$	- 39.23%
Raw Open Cooking	$401.6^{e} \pm 11.39$	- 47.21%
Germination 72 hour	$365.4^{f} \pm 10.28$	- 50.63%
Raw Autoclaving	$355.8^{f} \pm 12.08$	- 51.88%
Soak Open Cooking	$293.1^{g} \pm 11.63$	- 60.26%
Soak Autoclaving	$260.6^{h} \pm 11.98$	- 64.79%
Soaking and Dehulling	$232.9^{i} \pm 14.81$	- 68.53%

[* 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 B.3 One Way ANOVA table for Oxalate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	9	265566.61	29507.40	309.23	<.001
Residual	20	1908.46	95.42		
Total	29	267475.07			

Table B.4 Effect of processing on Oxalate content

Treatments	Oxalate (mg/100g)	% Reduction
Raw	$462.6^{a} \pm 6.97$	
Roasting	$375.3^{b} \pm 10.28$	- 18.88%
Soak	$352.8^{c} \pm 9.28$	- 23.74%
Germination 48 hour	$345.0^{c} \pm 11.17$	- 25.43%
Germination 72 hour	$232.6^{d} \pm 9.37$	- 49.72%
Soaking and Dehulling	$228.3^{de} \pm 12.14$	- 50.65%
Raw Open Cooking	$213.0^{ef} \pm 8.66$	- 53.69%
Raw Autoclaving	$198.6^{fg} \pm 8.94$	- 57.07%
Soak Open Cooking	$186.5^{gh} \pm 6.06$	- 59.69%
Soak Autoclaving	$171.0^{h} \pm 12.74$	- 63.04%

[* 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 B.5 One Way ANOVA table for Phytate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	9	1212756.5	134750.7	720.40	<.001
Residual	20	3741.0	187.0		
Total	29	1216497.4			

Table B.6 Effect of processing on Phytate content

Treatments	Phytate (mg/100g)	% Reduction
Raw	$1076.7^{a} \pm 9.43$	
Soak	$881.8^{b} \pm 10.21$	- 18.10%
Roasting	$824.0^{c} \pm 12.75$	- 23.47%
Soak Open Cooking	$765.8^{d} \pm 17.11$	- 28.88%
Raw Open Cooking	$754.2^{d} \pm 10.39$	- 29.95%
Raw Autoclaving	$684.5^{e} \pm 14.91$	- 36.39%
Germination 48 hour	$670.9^{ef} \pm 13.10$	- 37.69%
Soak Autoclaving	$649.7^{f} \pm 19.45$	- 39.65%
Germination 72 hour	$390.0^{g} \pm 10.92$	- 63.77%
Soaking and Dehulling	$372.2^{g} \pm 14.93$	- 65.43%

[* 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 B.7 One Way ANOVA table for Polyphenol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	9	2049584.3	227731.6	643.71	<.001
Residual	20	7075.6	353.8		
Total	29	2056659.9			

Table B.8 Effect of processing on Polyphenol content

Treatments	Polyphenol (mgGAE/100g)	% Reduction
Raw	1254.9^{a} ± 15.36	
Roasting	970.6^{b} ± 15.33	- 22.66%
Soak	909.8^{c} ± 12.18	- 27.50%
Raw Open Cooking	784.3^{d} ± 12.51	- 40.63%
Germination 48 hour	762.8^{de} ± 18.06	- 39.22%
Soak Open Cooking	745.1^{e} ± 16.23	- 37.50%
Germination 72 hour	537.2^{f} ± 16.45	- 57.19%
Raw Autoclaving	507.9^{fg} ± 25.95	- 59.53%
Soak Autoclaving	493.0^{gh} ± 29.82	- 60.77%
Soaking and Dehulling	468.9^{h} ± 17.94	- 62.64%

[* 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 B.9 Paired t-test comparison of protein content of most effective method with raw horsegram 5% level of significance

	Raw horsegram	Dehulled horsegram
Mean	26.96	28.84
Variance	1.2229	2.9632
Observations	3	3
Pearson Correlation	0.999999404	
Hypothesized Mean Difference	0	
Df	2	
t Stat	5.290006114	
P(T<=t) one-tail	0.01696322	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.033926439	
t Critical two-tail	4.30265273	

Table B.10 Paired t-test comparison of ash content of most effective method with raw horsegram 5% level of significance

	Raw horsegram	Dehulled horsegram
Mean	3.79	1.99
Variance	0.0279	0.0016
Observations	3	3
Pearson Correlation	0.628618557	
Hypothesized Mean Difference	0	
Df	2	
t Stat	21.4630731	
P(T<=t) one-tail	0.001081869	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002163739	
t Critical two-tail	4.30265273	

Table B.11 Paired t-test comparison of fat content of most effective method with raw horsegram 5% level of significance

	Raw horsegram	Dehulled horsegram
Mean	0.9	1.08
Variance	0.0133	0.0075
Observations	3	3
Pearson Correlation	0.97622104	
Hypothesized Mean Difference	0	
Df	2	
t Stat	8.646920305	
P(T<=t) one-tail	0.006556007	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.013112015	
t Critical two-tail	4.30265273	

Table B.12 Paired t-test comparison of fiber content of most effective method with raw horsegram 5% level of significance

	Raw horsegram	Dehulled horsegram
Mean	3.53	1.36
Variance	0.1123	0.0853
Observations	3	3
Pearson Correlation	0.995674649	
Hypothesized Mean Difference	0	
Df	2	
t Stat	72.33333333	
P(T<=t) one-tail	9.55363E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000191073	
t Critical two-tail	4.30265273	

Table B.13 Paired t-test comparison of carbohydrate content of most effective method with raw horsegram 5% level of significance

	Raw horsegram	Dehulled horsegram
Mean	64.82	66.73
Variance	1.8804	5.9049
Observations	3	3
Pearson Correlation	0.89697428	
Hypothesized Mean Difference	0	
Df	2	
t Stat	2.460678313	
P(T<=t) one-tail	0.066495359	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.132990718	
t Critical two-tail	4.30265273	

Appendix C

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

Tannic acid concentration (µg/ml)	Absorbance
0	0
2	0.15
4	0.33
6	0.43
8	0.67
10	0.83

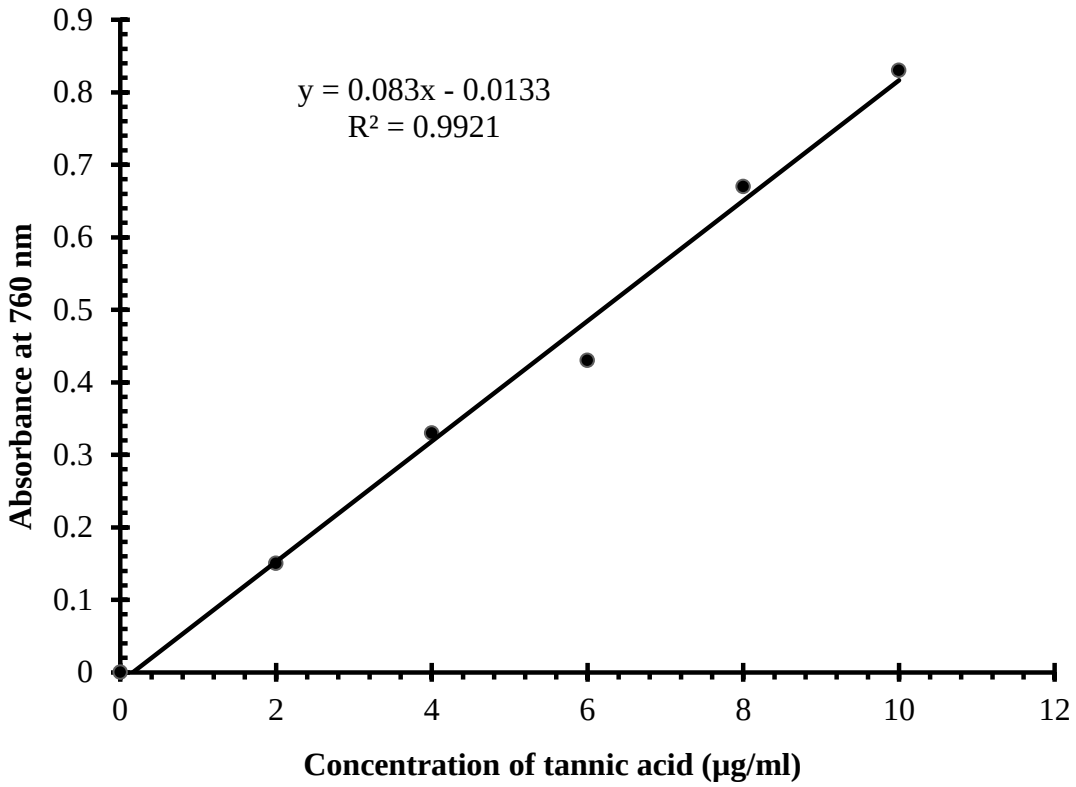

Figure C.1 Standard curve for tannin determination

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

Gallic acid concentration (µg/ml)	Absorbance
0	0
50	0.535
100	0.921
150	1.481
200	1.92
250	2.629

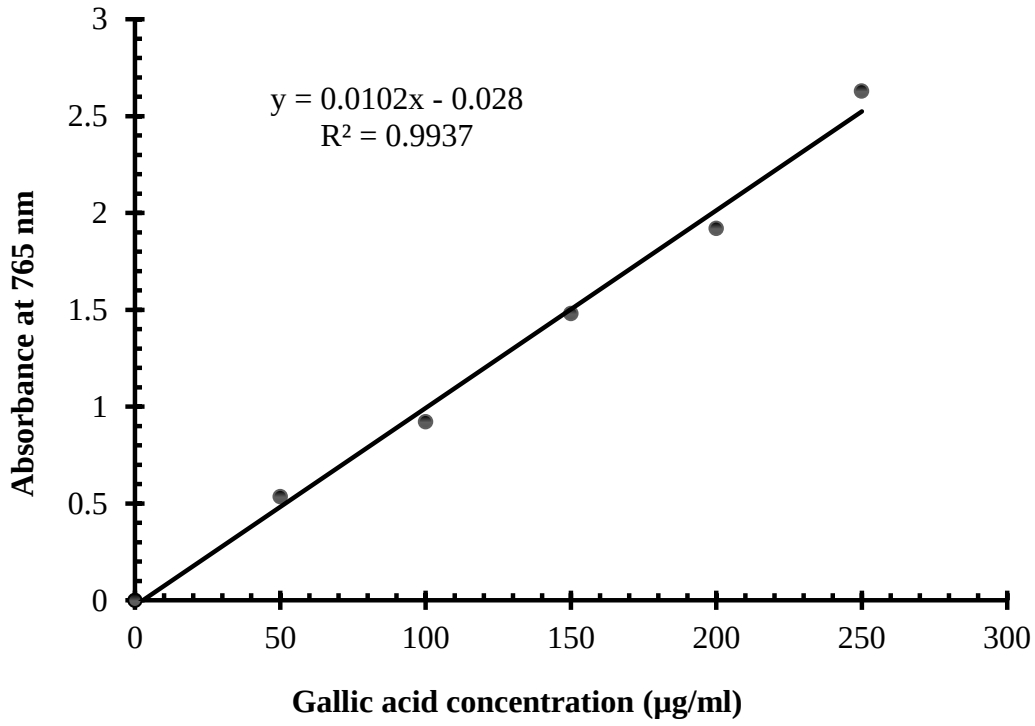

Figure C.2 Standard curve for polyphenol determination

Color plates

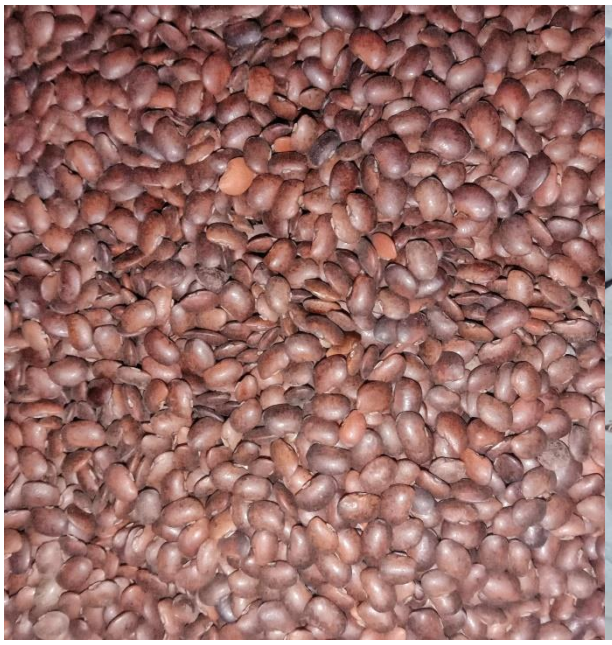

Plate 1. Raw horsegram

Plate 2. Germinated horsegram

Plate 3. Drying samples in hot air oven

Plate 4. Grinded and sieved samples

Plate 5. Extracts for determination of phytic acid

Plate 6. Distillation in Kjeldahl's distillation set