EFFECT OF SOAKING, GERMINATION AND ROASTING ON THE PHYTOCHEMICAL AND ANTI-NUTRITIONAL COMPONENTS OF PUMPKIN SEEDS (*Cucurbita moschata*)

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Approval letter

This ***dissertation*** entitled ***Effect of Soaking, Germination, and Roasting on the Phytochemical and Anti-nutritional Components of Pumpkin Seeds (Cucurbita moschata)*** presented by **Dipen Khadgi** has been accepted as a partial fulfillment of the requirement for the **Bachelor degree in Science in Nutrition and Dietetics**

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Date of submission: December, 2023

(Dipen Khadgi)

Abstract

Pumpkin seed (*Cucurbita moschata)* collected from Morang district, Nepal was studied to explore the effect of roasting at 200^{o}C for 5 min and 10 min, soaking for 12hrs followed by cabinet drying followed by roasting at 200^{o}C for 10 min and 48h, 96h, 144h, and 192h of germination followed by cabinet drying followed by roasting at 200^{o}C for 10 min on its bioactive compounds (phenolic acid, flavonoid and anti-oxidant activity) and anti-nutritional components (tannin, phytic acid and oxalate). The crude extracts of samples were prepared using 80% methanol by maceration technique for analysis of total phenolic content, total flavonoid content and anti- oxidant activity. Experimental data was analyzed using software Genstat 12th Edition.

The mean value of tannin, phytic acid, oxalate, phenolic content, flavonoid and DPPH activity of pumpkin seeds were 204.61 mg/100g, 22.45 mg/100g, 383.7 mg/100g and 310.4 mg/100g and 10.6 (%) respectively. All the treatments showed ($p<0.005$) significant reduction of antinutrients and increased the bioactive compounds of pumpkin seeds where both single and combination treatments were found increasing the bioactive compounds, and decreasing antinutrient components. The combination treatments i.e., germination and roasting reduced the oxalate, phytic acid content and increase flavonoid, DPPH activity more effectively than other treatment methods. However, only roasting was most effective in reducing tannin and increasing total phenolic content, so the resulting reduction in anti-nutrients and increment of bioactive compound were obtained through both single and combined treatments.

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List of Abbreviations

Abbreviation	Full form
ANFs	Anti-nutritional factors
ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemist
CCT	Central Campus of Technology
D.F.	Degree of freedom
FAO	Food and Agriculture Organization
R5	Roasting for 5 min
R10	Roasting for 10 min
SR	Soaking(12h) followed by roasting(10min)
G2R	Germination for 48h followed by roasting(10min)
G4R	Germination for 96h followed by roasting(10min)
G6R	Germination for 144h followed by roasting(10min)
G8R	Germination for 192h followed by roasting(10min)
L-DOPA	Dihydroxypehnylalanine
LSD	Least significance difference
M.S.	Mean squares

S.D.	Standard Deviation
TFC	Total flavonoid content
USDA	United States Department of Agriculture
VR	Variance ratio
DPPH	2, 2-Diphenyl-1- picrylhydrazyl

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Part I

Introduction

1.1 General introduction

Pumpkin (*Cucurbita moschata*) belongs to the family cucurbitaceae. It is a leafy green vegetable. Fruits are variable in size, color, shape, and weight. They have a moderately hard rind, with a thick, edible flesh below, and a central seed cavity. There are numerous seeds in the fruit. Most seeds are plump and tan or soft white. They are all covered with a testa that serves as a protectant around the seeds (R. W. Robinson and Decker-Walters, 1997).

Pumpkin is extensively used as vegetable, processed food and stockfeed in different parts of the world. Although very little information is available about the production statistics in India, the average yield of fruits is reported to be 25000 kg/ ha (Choudhury, 1976). The pumpkin fruits contain significant amount of seeds which is normally discarded. The seed with more than 45% oil, 30% protein (Lazos, 1986) and yield ranging between 450 and 1570 kg/ha (R. Robinson, 1975) can indeed be considered to be a potential source of edible oil and protein next to groundnut.

Pumpkin seeds are consumed directly for human consumption as a snack food in many cultures throughout the world, and the seeds are especially popular in Arabian countries, after salting and roasting (Al-Khalifa, 1996).

Pumpkin seed oil has been produced in Austria, Slovenia, and Hungary (Murkovic *et al.*, 1996). Although none of these oils has been utilized on an industrial scale, many are used as cooking oil in some countries in Africa and the Middle East (Sawaya *et al.*, 1983) and as salad oil in the south of Austria and the adjacent regions in Slovenia and Hungary (Murkovic *et al.*, 1996).

The important pumpkin growing countries include India, China, USA, Germany, Pakistan, south Africa, Mexico and Columbia. The production statistics reveal that India is one of the leading producers of pumpkin in the world (FAOSTAT, 2008). *Cucurbita moschata* is a leading crop cultivated since pre-historic time and currently most common variety of pumpkin in Asia and the United States of America. *Cucurbita moschata* is grown in almost all the

regions of India (Nath *et al.*, 1979). The main growing season is summer and rainy seasons in most parts of India. Winter pumpkins are also grown in some parts of Southern and Western India (Dhiman *et al.*, 2009)..

It needs to be processed and preserved in such a way, that its therapeutic and nutritional properties are retained the most and should have reduced anti-nutritional factors. But usually it is found to be used in the household by roasting without consideration of time and temperature of roasting. The direct and uncontrolled roasting results in varying loss of the phytochemicals and sensitive nutrients and also limited reduction of anti-nutritional factors (Khan and Saini, 2016).

1.2 Problem statement

The nutritional property and functional property of pumpkin seeds has been getting popularity among the people. Instead of its direct consumption of pumpkin seeds, it should be processed in order to decrease certain anti nutritional factor and to increase availability of nutritional component of pumpkin seeds. Pumpkin seeds are being used in order to control cholesterol level, prevention of atherosclerosis, stroke, heart attack and diabetes (Šamec *et al.*, 2022). Consumption of pumpkin seeds is beneficial for human health. Therefore, it has been considered as the source of increased interest in the field of diet and disease research due to its biologically active components.

In Nepal, pumpkin seeds are being consumed either by roasting or incorporation on certain food items. During roasting, scientific procedures are not followed, so the nutrient along with phytochemicals and their functional activities in flaxseed we consume is highly degraded. As pumpkin seeds have higher concentration of anti-nutritional factor i.e., oxalate, tannin, phytate, heat processing before consumption is necessary in order to decrease their concentration. Roasting at correct time at correct temperature is necessary in order to increase its nutritional component and decrease anti- nutritional factor. Therefore, the optimization of pumpkin seeds at constant temperature with varying time is carried for appropriate processing of pumpkin seeds before consumption

1.3 Objectives of the study

1.3.1 General objective

The general objective of this work is to study the effect of germination, soaking and roasting on the phytochemical and anti-nutritional factors of pumpkin seeds.

1.3.2 Specific objective

The specific objectives of this study are:

- To determine the phytochemical and anti-nutritional components in raw pumpkin seeds.
- To determine the effect of soaking for 12hrs followed by roasting and 48h, 96h, 144h, and 192h of germination followed by roasting on the phytochemical and anti-nutritional components of pumpkin seeds.
- To determine the effect of roasting on phytochemical and anti-nutritional components of pumpkin seeds.

1.4 Significance of the study

In the present world, interest has been increased in search of functional foods to decrease the risk of the evolution of diseases. Pumpkin seed is emerging as an important functional food ingredient because it provides oil rich in omega-3, high quality protein and soluble fiber and phenolic compounds. Roasting before consumption seems to be necessary in order to reduce the content of different anti-nutritional factors like tannin, phytate and oxalate whereas roasting at random temperature and time degrade different nutritional and bioactive components i.e., flavonoids, polyphenols. Similarly soaking followed by roasting has crucial role on reduction of anti-nutritional components as the water-soluble components like tannin, oxalic acid.

Therefore, the study was design for maximum retention of bioactive, nutritional components and reduction of anti-nutritional factors to higher extent through the effect of various processing i.e., roasting and soaking prior to roasting. The results of this study helps in the establishment of the effective and optimized way for the use of pumpkin seeds as functional food ingredients and also use of pumpkin seeds into processed foods in the industrial scale

which can help to reducing the risk of different diseases like cardiovascular diseases, cancerous diseases, diabetes among increasing population.

1.5 Limitation

- Simple titrimetric method was used to determine phytate.
- Certain compounds like lignin, saponin were not determined.

Part II

Literature Review

2.1 Origin and distribution of pumpkin seeds

Pumpkin (*Cucurbita moschata)* has been widely cultivated in many countries since ancient times. The genus Cucurbita belongs to the Cucurbitaceae family and Cucurbitales order (Armesto *et al.*, 2020). Pumpkin species originated from the American continent, from two different points of origin. One origin point includes Mexico and Central and South America; the species found here include *Cucurbita moschata*, *Cucurbita ficifolia*, *Cucurbita pepo,* and *Cucurbita mixta*. The other origin point is in South America, which includes the species Cucurbita maxima (Jacobo-Valenzuela *et al.*, 2011). The food crop *Cucurbita moschata* plays a vital role in the diet of both rural and, to some extent, urban areas in the Americas (Lira and Montes, 1992). It can be prepared via a variety of cooking methods, where it is used not only as a vegetable, but also as an ingredient in the production of bread, flour, soup, pies, and other foods (Doymaz, 2007).

Cucurbita moschata like to grow in warm tropical areas and water-rich environments, as it is not cold-resistant but is high-temperature resistant instead. It can, however, resist both drought and frost during its flowering period (Jacobo-Valenzuela *et al.*, 2011).

Production of pumpkin including squash and gourd in the world exceeded 27.6 million tons from an area of 2.04 million hectare in 2018 (Nguyen *et al.*, 2020). Production of pumpkins, squashes and gourds in 2019 was estimated above 23 million tons cultivated all over the world comprising an area of 1.54 million hectare and in Pakistan 2.7 lac tons on 26515-hectare area (Gennari *et al.*, 2019).

In many countries, such as the United States, Mexico, India, China, and Brazil, *Cucurbita moschata* is traditionally used as a medicine (Yadav *et al.*, 2010).

Pumpkin (*Cucurbita moschata),* popularly known as Pharsi in Nepali language belongs to the family Cucurbitaceae generally grown in the regions of the globe as a vegetable. These are grown-up in the tropical and sub-tropical regions and including the cucumbers and squash. Worldwide there are three types of the pumpkins are present name as "*Cucurbita pepo", "Cucurbita maxima"* and *"Cucurbita moschata*"(Lee *et al.*, 2003). For the purpose of

vegetable and medicinal pumpkins are grown throughout world. In many countries the pumpkin has been conventionally used as remedy like China, Pakistan, India, Yugoslavia, Argentina, Mexican regions, America and Brazil (Jia *et al.*, 2003). The pumpkin seeds are utilized for the cure of different diseases the herbal remedies separately or combine with medicines are used for the medical treatment. The pumpkin is the one of the famous edible plant that is utilized as the cure of many disorders due to the occurrence of many edible components and phytochemicals (Yadav *et al.*, 2010).

In USA, the pumpkins immensely used for the thanksgiving feasts and craving. The majority of the plant flora is processed into caned pumpkins. On the other hand, the considerable oval, flat shaped seeds are generally discarded as an agriculture residue. The pumpkin seeds are unique in flavored and nutty in taste and consumed salted and roasted as a snack in few regions of Mexico, Canada, United States, China and Europe. Now a days, these pumpkin seeds are selling as fermented, sprouted, baked, concentrated form of protein from pumpkins and pumpkin protein isolate, as the pumpkin seeds are rich in iron, protein, manganese, magnesium, zinc, potassium, copper, phosphorous, PUFA (polyunsaturated fatty acid), c-tocopherol and carotenoids. There is an emergent interest in unique formation of vegetable oil, and oil of the pumpkin seeds is a hopeful aspirant on this regard. Steam distillation or Cold press is done for oil extraction. The dark greenish red colored pumpkinseed oil is used for cooking, marinade and dressing of salad. It is being utilized in many chocolates, epicurean delight, cereal bars, bread, cakes, soups, pesto, muffin, pasta garnish and garnish of stew. The butter of pumpkin seeds is taken into consideration as a marvelous peanut butter alternative (Syed *et al.*, 2019).

The many food shops of United State such as Walmart, Costco and Trader Joe, promote numerous food products primarily based on pumpkin seeds, like vegetable salad, granola chunks, breads, quinoa salad, tortilla chips and cookies. The Australia, Serbia, Hungary and Slovenia produced the maximum pumpkin seeds oil. The fame of the pumpkin seeds and pumpkin seeds oil is increasing day by day in many countries in the world that is a gathering momentum slowly but surely (Syed *et al.*, 2019).

Pumpkin is extensively used as vegetable, processed food and stockfeed in different parts of the world. Although very little information is available about the production statistics in India, the average yield of fruits is reported to be 25000 kg/ ha (Choudhury, 1976). The pumpkin fruits contain significant amount of seeds which is normally discarded. The seed with

more than 45% oil, 30% protein (Lazos, 1986) and yield ranging between 450 and 1570 kg/ha (R. Robinson, 1975) can indeed be considered to be a potential source of edible oil and protein next to groundnut.

In Nepal, the trend in the yield of cucurbitaceous vegetables shows that the yields for all the crops are declining. In the year of 2012/2013 ,the yield of pumpkin was increased by 2.4% (Ghimire *et al.*, 2018).

In Nepal, pumpkin seeds can be eaten as Chutney or Dhuley achaar as a traditional pickle incorporated with other oil seeds (Adhikari, 2019).

2.2 Classification and nomenclature

The scientific name of pumpkin seeds is *Curcubita moschata.* Pumpkin seeds belong to the family Cucurbitaceae and its taxonomy hierarchy is:

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitacea
Genus	*Curcurbita*
Species	*Cucurbita moschata*

Source: USDA

2.3 Physical properties of pumpkin seed

Table 2.1 Physical properties of pumpkin seed

Parameter	Whole pumpkin seed (%)
Length (mm)	16.81
Width (mm)	8.87
Thickness (mm)	2.75
True density (kg/m^3)	1157
Bulk density (kg/m^3)	398
Geometric mean diameter, Dg (mm)	7.42
Porosity (%)	65.60
1000 seed weight (g)	202.2
Husk content (%)	26.75

(Devi *et al.*, 2018)

2.3 Chemical and nutritional composition of pumpkin seeds

Pumpkin seed is an important nutrient rich seed, especially high amount of protein, dietary fiber, essential fatty acids and bioactive components. The physical properties, chemical composition and fatty acid proportion was determined by an investigator and his collogues they found that pumpkin seeds contained 41.59% oil, 25.4% protein, 5.2 % Moisture, 25.19% carbohydrates, 5.34% fiber, and 2.49% total ash. Total phenolic compounds, total sterols, waxes and total tocopherols were 66.25 (mg gallic acid per kg oil), 1.86%, 1.56% and 882.65 (mg tocopherol per kg oil) respectively (Gohari *et al.*, 2011).

Table 2.2 Constituents of whole pumpkin seeds

Composition	**Per 100gm**
Moisture, %	5.53
Crude protein, %	28.90
Crude fat, %	31.75
Crude fiber, %	4.59
Total Carbohydrates, % (by difference)	27.86
Iron (Fe)	16.1
Manganese (Mn)	487
Zinc (Zn)	907
Copper (Cu)	124
Phosphorus (P)	848.6
Potassium (K)	404.9
Calcium (Ca)	25.7
Magnesium (Mg)	335.6
Sodium (Na)	2.2
Cobalt (Co)	0.6

Source: (Devi *et al.*, 2018)

2.4 Phytochemicals

In plants, phenols play an important role in protection against photo-oxidation and disease resistance. Phenolic compounds in general possess an aromatic ring bearing one or more hydroxyl substituents and may be found in Free State, conjugated with sugars or esters or polymerized. They are not evenly distributed in tissues or cells of plants, and can be associated with components of the cell wall such as polysaccharides and proteins (Shahidi, 2000). There are more than 8000 different known phenolic compounds with diverse structures (Robbins,

2003). In general, plant phenols on the basis of their basic structure, can be divided into different types: simple phenols, phenolic acids, coumarins and isocoumarin, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans and tannins. Among these, phenolic acids and flavonoids are more common(Dykes and Rooney, 2007). In addition to protective effect, phenolics are responsible for color, taste, organoleptic properties of the plant origin foods (Yáñez *et al.*, 2004).

2.4.1 Phenolic acids

It is well known that the phenolic acids are the derivatives of benzoic and cinnamic acid; and are generally classified into two types, hydroxybenzoic and hydroxycinnamic acid. Pumpkin seed oil has become a recognized source of phenolic compounds. It is reported that the total phenolic content (TPC) measured in the pumpkin seed oil samples ranged from 24.71 to 50.93 mg GAE/kg of oil (Andjelkovic *et al.*, 2010).

They are either in free and/or bound forms. Free phenolic acids are mainly composed of trans and cis-sinapic, o-coumaric, p-droxybenzoic, trans-p-coumaric and vanillic acids. However, most of the flaxseed phenolic acids such as phydroxybenzoic, trans-ferulic and trans-p-coumaric acids are ester bound. Among these phenolic acids, ferulic and p-coumaric acid glucosides were accumulated at high concentrations in the flaxseed (Beejmohun *et al.*, 2007). In addition, phenolic acid like caffiec acid and their glucosides were also reported in the flaxseed. Variations in phenolic acid content in flaxseed were largely attributed to seasonal effects (Oomah, 2001). The TPC of seeds of five different cultivars of pumpkin (*cucurbita moschata)*, namely Kashi Harit (KH), Narendra Upkar (NU), Pusa Vishwas (PV), Narendra Agrim (NA), and Azad Pumpkin 1 (AP1), that are grown in subtropical regions of India, scored a maximum value of 35.66mg GAE/g for KH cultivar and the corresponding value for kernel was 31.39 mg GAE/g (Singh and Kumar, 2022). These results are in agreement with the findings of Seun F Akomolafe *et al.* (2016) who reported TPC 32.90 mg GAE/g in the Nigerian variety of pumpkin seed. Another study by Seun Funmilola Akomolafe (2021) shows that the total phenolic content of raw pumpkin seeds was found to be 428 mg/100g. Another study by Saavedra *et al.* (2015) suggested that the total content of phenolics (TPC) of the seed samples was ranging from 0.95 to 3.43 mg GAE/g DW.

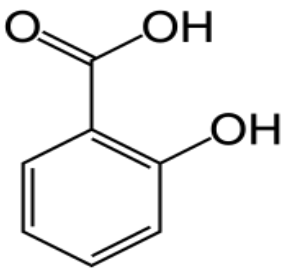

Fig 2.1: Phenolic acid

2.4.2 Flavonoid

The flavonoid content of pumpkin seeds range from 20.11-23.65 mg QE/gm (Singh and Kumar, 2022). Flavonoids are the polyphenols, with C6-C3-C6 skeleton that consists of two aromatic rings joined by a three-carbon link. Flavonoids generally include anthocyanins, flavanols, flavones, flavanones and flavonols. Depending upon growing and cultivar conditions, flaxseed possesses about 0.3-0.71g of total flavonoids per kg of flaxseed (Oomah, 2001). In the flaxseed, flavonoids are in the form of their glucoside such as herbacetin 3, 8-Odiglucopynanoside, 7-O-dimethyl ether, and kaempferol 3, 7-Odiglucopyranoside. Herbacetin diglucoside (HDG) are ester linked in the lignan macromolecule via 3-hydroxy-3-methylglutaric acid (HMGA) (Struijs *et al.*, 2007). Another study by Seun Funmilola Akomolafe (2021) found that the flavonoid content in raw pumpkin seeds was 324 mg QE/100gm.

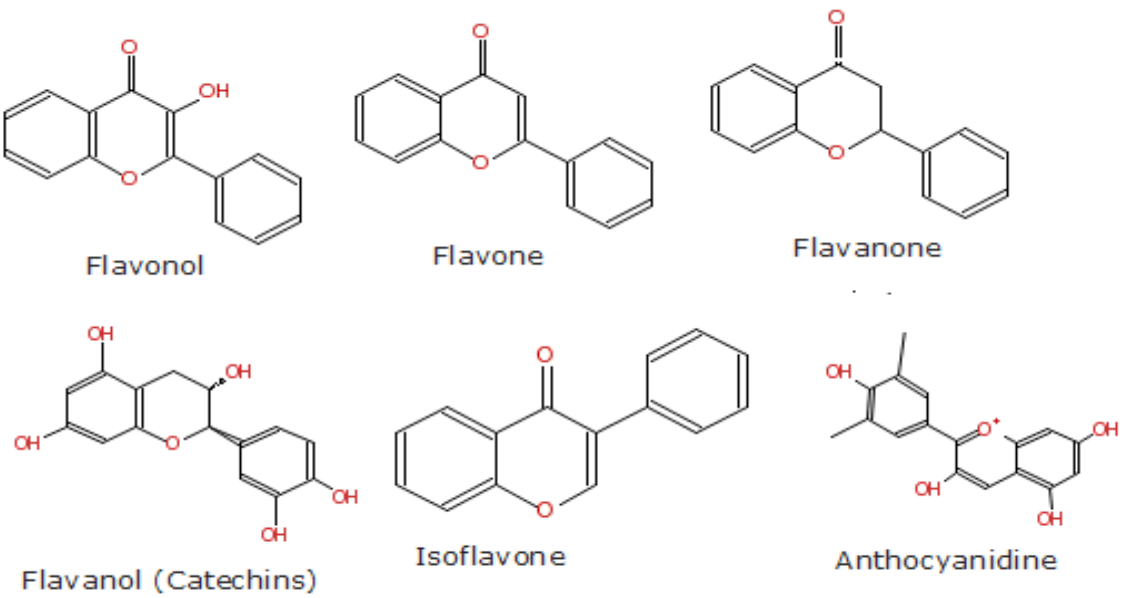

Fig.2.2 Basic structure of flavonoids

2.4.3 Antioxidant

The DPPH free radical scavenging activity of methanolic extract of pumpkin seeds varied from 49.88 to 54.15% (Singh and Kumar, 2022). Any substance which is capable of delaying, retarding or preventing the development of the rancidity or other flavors deterioration due to oxidation is called antioxidant. Oxidation reactions are chemical reactions that involve the transfer of electrons from one substance to an oxidizing agent. Antioxidants can slow these reactions either by reacting with intermediates and halting the oxidation reaction directly, or by reacting with the oxidizing agent and preventing the oxidation reaction from occurring (Pokorny, 2007).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Shekhar and Anju, 2014). The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free DPPH radical with an odd electron gives absorbance (purple color) at 517nm. When the antioxidants in plant extract react with DPPH, it is reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The scavenging of DPPH by radical scavengers can be summarized as:

DPPH • + FE DPPH – H + A• (1)

DPPH + A• DPPH – A (2)

A• + A• A – A (3)

Where FE is a scavenger of the extract and A• is a radical. The newly formed radical (A•) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Leaves and Leaves, 2014).

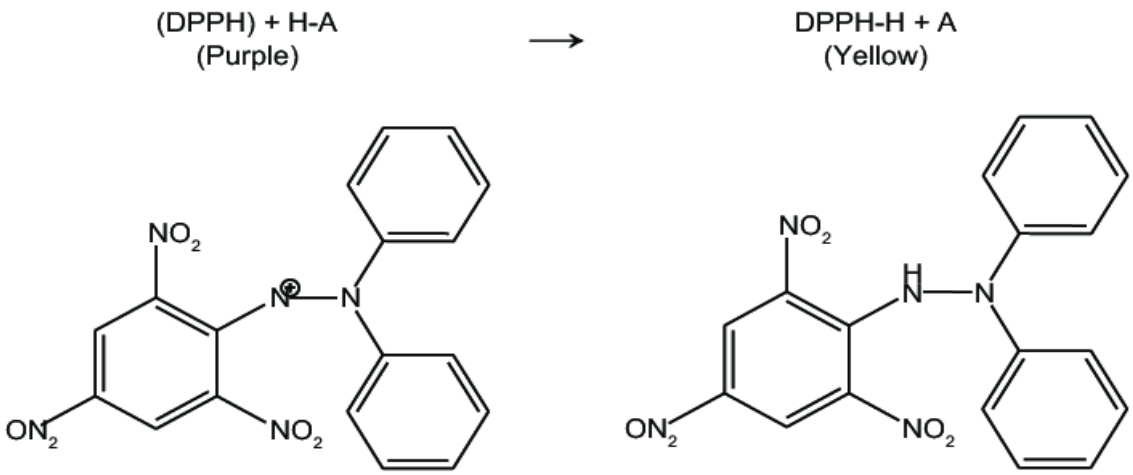

Fig.2.3 Reaction of DPPH- free radical with antioxidant

2.5 Antinutritional factors

Anti-nutrients or anti-nutritional factors are those substances generated in natural feed stuffs by the normal metabolism of species and by different mechanisms which exerts contrary to optimum nutrition. Anti-nutritional factors such as tannin, trypsin inhibitors, oxalates etc., are found in food grains (Hiremath, 2013).

Antinutritional factors in pumpkin seeds

2.5.1 Tannins

Tannins are polyphenolic compounds of high molecular weight which interact with and precipitate proteins; they are classified into two groups according to their reaction with hydrolytic agents: hydrolysable and condensed tannins. The former is easily eliminated under the action of hydrolytic agents such as gastric juice (Cabrera and Martin, 1986). Tannins are divided into two major classes: condensed tannins, which are flavonoid-based polymers; and hydrolysable tannins, which are polygalloyl esters. Higher plants may produce any combination of condensed and hydrolysable tannins, although some chemotaxonomic patterns have been not. The condensed tannins do not undergo hydrolysis in acid or base. However, in hot alcohol the flavanoid polymer is oxidatively cleaved to yield colored anthocyanidins; thus, the condensed tannins are often called "proanthocyanidins". The hydrolyzable tannins are hydrolyzed in acid, in base or by esterases (tannase) to yield the parent polyol and the phenolic acids. Glucose is the most common alcohol, but hydrolyzable tannins containing diverse alcohols such as hamamelose and quinic acid have been reported. If gallic acid is the only phenolic acid produced upon hydrolysis, the tannin is a gallotannin (Hagerman and Klucher, 1986).

Tannins are water soluble phenolic compounds having molecular weights between 500 to 3000 giving the usual phenolic reactions and having special properties such as the ability to precipitate alkaloids, gelatin and proteins. The dark color and astringent taste of food is often ascribed to tannins. They can have a large influence on the nutritive value of many foods eaten by humans such as vegetables, fruits, chocolate, tea, alcoholic and nonalcoholic beverages, etc. Tannins are phenolic plant secondary compounds and are widely distributed in the plant kingdom, especially in pulses (Arias *et al.*, 2004). Foods rich in tannins are considered to be of low nutritional value because they precipitate proteins, inhibiting digestive enzymes and Fe absorption and affect the utilization of vitamins and minerals from meals (Tinkílíç and Uyaník, 2001). The tannin content in pumpkin seeds range from 26.01-28.15 mg/100g (Singh and Kumar, 2022). Another research by (Hamed *et al.*, 2008) showed the tannin content in raw pumpkin seed was 228.31 mg/100gm.

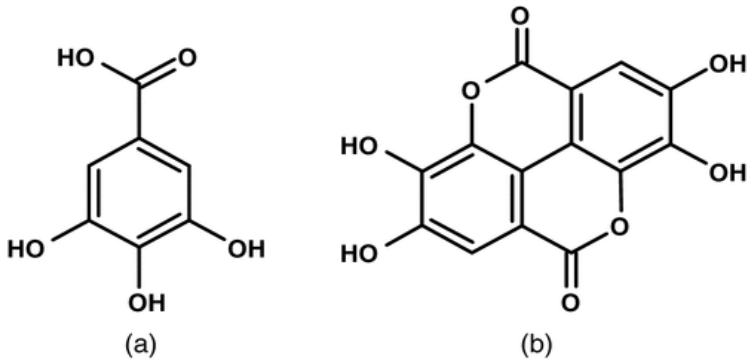

Fig.2.4 Structure of hydrolysable tannin (a) and condensed tannin (b)

2.5.2 phytate

Phytic acid (myoinositol, 1, 2, 3, 4, 5, 6 hexakis-dihydrogen phosphate) and phytate are widespread in plant seed grains (also including cereals), roots and tubers. Phytic acid is generally regarded as the primary storage form of both phosphate and inositol in seeds. Phytic acid phosphorus constitutes the major portion of total phosphorus in several seeds and grain. It accounts for 50–80% of the total phosphorus in different cereals. The phytic acid content in influenced by cultivar, climatic conditions, and year. The accumulation site of phytic acid in monocotyledonous seeds (wheat, barley, rice, etc.) is the aleurone layer, particularly the aleurone grain. Aleurone grain contains two types of inclusions: (a) globoids containing high amount of phytates, and (b) protein carbohydrate bodies. Corn differs from other cereals as more than 80% of phytic acid is concentrated in germ. Phytic acid content of cereals varies from 0.5 to 2.0%. Because most of the phytic acid is in the outer parts of the kernel the different products of milling contain different levels of phytates. Bran is the product having a high phytic acid content, low extraction white flours contain low phytic acid quantities. If protein concentrates or isolates are prepared from cereals or other raw materials, such products contain also phytic acid in quantities depending on the raw material and method of processing. The association of phytate with proteins begins in seeds during ripening, when phytate accumulates in the protein-rich aleurone layer of cereals and protein bodies of legumes. Although the fine structure of phytate-rich particles in plants has been intensively studied, the nature of the interaction of proteins in such organelles with phytic acid is practically unknown (Hídvégi and Lásztity, 2002). Phytic acid, known as inositol hexakisphosphate, can exist as a free acid, a calcium salt of phytic acid (phytate) or a calcium/magnesium salt of phytic acid (phytin) based on the pH and metal ions present. 11 Phytic acid is distributed uniformly through the dicotyledonous seeds, and it represents the primary storage form of phosphate and inositol.

Phytic acid has a high density of negatively charged phosphate groups forming very stable complexes with mineral ions which makes their intestinal uptake unavailable. Furthermore, phytates have been described to complex with proteins at different pH ranges, which may alter the protein structure, resulting in lower protein solubility, enzymatic activity and proteolytic digestibility (Rosa-Sibakov *et al.*, 2018).

Phytic acid, another anti-nutrient of pumpkin seed contains 35.06 mg/100g (A. Hussain *et al.*, 2022).Phytic acid, also known as inositol hexakisphosphate or phytate when in the salt form, is the storage form of phosphate in many plant tissues, especially seeds and grains. Phytic acid is not digested by humans, and is therefore not a dietary source of inositol or phosphate. In fact, because phytic acid is a good metal chelator, it is believed to have a negative nutritional impact on strongly chelating metals necessary for good health (e.g., iron and calcium) and could prevent their absorption by the intestine. For this reason and because phytic acid is thought to have a positive dietary impact as an antioxidant to prevent carcinogenesis, determining the phytic acid content of foods is of interest (Phescatcha *et al.*, 2012). According to Singh and Kumar (2022), the seeds of 5 different cultivars of *cucurbita moschata* scored a maximum value of 25.09mg/100g for phytate. Another research done by Hamed *et al.* (2008) showed the phytate content in raw pumpkin seeds was found to be 63.62 mg/100gm. Also, the phytic acid content in raw untreated pumpkin seed was 37.03 mg/100gm (Kindiki, 2017).

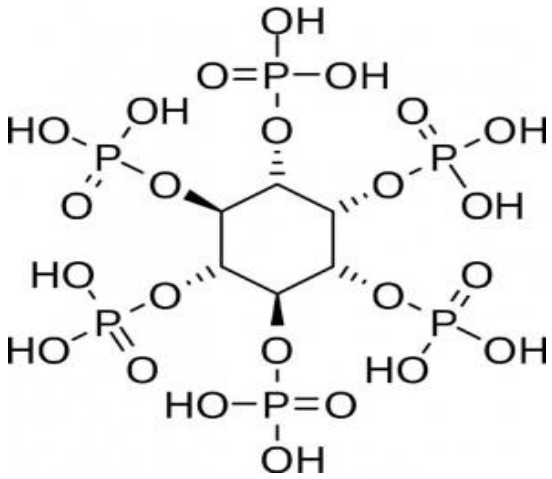

Fig.2.5 Structure of phytic acid

2.5.3 Oxalate

Oxalic acid forms water-soluble salts with Na+, K+ and NH4 + ions; it also binds with Ca2+, Fe2+ and Mg2+, rendering these minerals unavailable to animals (Bsc and Bsc, 1999). Oxalates are anti-nutrient compounds present in vegetables such as spinach, chard, beet, or rhubarb. These compounds are a strong organic acid with the ability to form water-soluble salts by binding to minerals such as sodium or potassium, as well as water-insoluble salts by binding to calcium, iron or zinc (Lo *et al.*, 2018). Oxalate is an anti-nutrient which under ordinary conditions is restricted to isolate compartments. However, when it is handled and additionally processed, it meets the nutrients in the gastrointestinal tract (Bsc and Bsc, 1999). When released, oxalic acid binds with nutrients, rendering them inaccessible to the body. If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. In ruminants, oxalic acid is of only minor importance as an anti-nutritive factor since ruminal microflora can probably metabolize soluble oxalates, and to a less significantly insoluble calcium oxalate. While the importance of the anti-nutritive activity of oxalic acid has been recognized for 12 more than fifty years it might be a subject of interest to nutritionists in the future (Oladimeji *et al.*, 2000).

Legumes, nuts, and different types of grain-based flours are commonly consumed throughout the world. Soybeans and other legumes such as lentils, red kidney beans, and white beans have been previously analyzed for oxalate (Fox *et al.*, 2015). The oxalate content of nuts has been reported to be relatively high (Massey *et al.*, 2001) and there are published values in the literature for almonds, cashews, hazelnuts, peanuts, pecans, pistachios, and walnuts (Hodgkinson, 1977). The oxalate content of pumpkin seeds range from 23.84-27.01 mg/100g (Singh and Kumar, 2022). However, comprehensive reports of oxalate concentrations in either legumes or nuts have not been published.

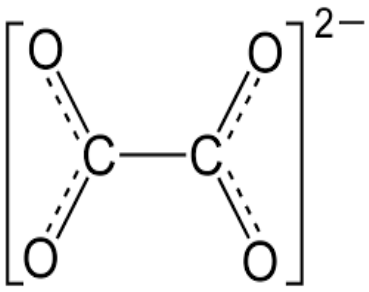

Fig.2.6 Structure of oxalate

2.5.4 Saponin

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

2.5.5 Hydrocyanic acid

The cyanide content of pumpkin seeds range from 14.21-14.95mg/100g (Singh and Kumar, 2022). Cynate are naturally present in the plants and on hydrolysis convert into hydrogen cyanide.Cynide if present in high amount per low dose repeated exposure will be toxic to human as it inhibits cytochrome Coxidase system involved in respiratory chains (Enneking and Wink, 2000).

These cyanogenic compounds are toxic to humans. It was found that ingestion of 100 mg/day may be lethal to adult individuals. However, these compounds present in seeds are instable when subjected to thermal and mechanical processes, including cooking in microwaves, autoclaving and boiling. Average tolerance of ingestion of cyanogenic compounds without adverse effects, as established by the World Health Organization (2003), is 0.11 mg/kg weight in the form of cyanogen chloride, it means that an individual weighing 60 kg may consume up to 0.66 mg of cyanogen chloride (WHO, 2003).

2.6 Methods of reduction of antinutritional factors

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

2.6.1 Soaking

Soaking is one of the processes used to remove soluble antinutritional factors, which can be eliminated with the discarded soaking liquors, but some metabolic reactions can take place during soaking affecting the content of some compounds (Vidal-Valverde *et al.,* 1994). Soaking, is an integral part of traditional methods of processing, saving energy cost by shortening cooking time, offers an additional advantage of rendering the grain nutritionally superior by removing certain anti-nutritional factors like phytic acid, saponin and polyphenols (Kataria *et al.*, 1988). 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.

2.6.2 Germination

Germination has been documented to be an effective treatment to remove anti-nutritional factors in legumes and mobilizing secondary metabolic compounds, which are thought to function as reserve nutrients (e.g. phytate and raffinose oligosaccharides) (Vidal-Valverde *et al.,* 1994). Germination and fermentation increase vitamins and reduce flatulence factors and phytic acid (Ahmed *et al.*, 2006).

2.6.3 Roasting

Roasting is a cooking method that uses dry heat, whether an open flame, oven or other heat source. Roasting usually cause caramelization or Millard browning of surface of the food, which is considered flavor enhancement. Roasting uses more indirect diffused heat (as in oven) and is suitable for slower cooking of meat in a larger, whole piece(Mbah *et al.,* 2012). Dry heat (roasting) is a widely used processing method for cereal products, fruits, and vegetables and known to improve the availability of nutrients, inactive enzymes which accelerate nutrient damage, destroy undesirable microorganisms and food contaminants. During processing, cooking and preserving of food, the application of roasting has mixed effects on its nutritive value. Roasting of flaxseed has traditionally been used to prevent gastrointestinal complications in Iran (Moknatjou *et al.*, 2015).

Heating process using electrical and gas ovens could be done by low-moderate society. Changes in chemical composition and levels of minor constituents affect the functional and nutritional characteristics of oils. Some reports suggest that retention of 23 nutrients such as vitamins in oven cooked foods is improved when the roasting time is shortened. However,

other studies indicate that nutrient retention during oven processing is not much greater than that in conventional cooking. Oven cooking has following advantage over above method i.e maximum flavor is retained, nutrients are retained, use of energy and oven temperature can be controlled, cooking can be observed (transparent oven doors) and straightforward access, adjustment or removal of items (Martínez-Flores *et al.*, 2002).

2.6.4 Blanching

Bsc and Bsc (1999) suggested mild boiling (75˚C–95˚C) is sufficient to inactivate endogenous enzymes and avoid cooking; however, the heat is minimal to eliminate oxalic acid. Typically blanching is carried out by treating the vegetables and seeds with steam or hot water for 1-10min at 75-95oC, the time/ temperature combination depend upon the types of seeds and vegetables (Cano and Jeremiah, 1996).

2.6.5 Popping

Popping involves heating of the grain in a hot pot. Popping is achieved by rapid, intense heating of grain; it makes water expand all at once; thereby expanding the grain. As expansion takes place, some of the granules are gelatinized resulting in the grain being much more available to digestive enzymes (Njoki *et al.*, 2014).

2.7 Health benefits of pumpkin seeds

Pumpkin seeds are nutritionally rich. They are rich source of proteins, fatty acids and minerals (Magnesium, Copper and Zinc). Being a good source of protein, iron and unsaturated fat, reputation of pumpkin seeds is increasing day by day (T. Amin and Thakur, 2013). The increasing market demand might be due to increasing health consciousness among the consumers. Pumpkin seeds are popular snack that can be found hulled/semi-hulled. Pumpkin seeds are fried and salted and are available in the market under the name of 'pepitos'. Fluted pumpkins seed flour has been used as a protein supplement in a variety of local foods (Giami and Bekebain, 1992).

Due to its various health benefits, it is consumed as seeds or seeds oil. It is used for the treatment of functional disorders of bladder (Widy-Tyszkiewicz *et al.*, 2012). It prevents from cardiovascular diseases (Gamonski, 2012). A study conducted by Egyptian researchers showed antihypertensive properties of pumpkin seed oil (El-Mosallamy *et al.*, 2012). Pumpkin seeds

contain anti-cancerous properties. The anticancer properties of pumpkin seeds is attributed to the presence of these lignans which acquire their anticancer properties only after they are ingested and converted by intestinal bacteria to mammalian lignans, particularly enterolactone and enterodiol (Borriello *et al.*, 1985). Similarly, it shows anti-inflammatory properties. The beta-carotene in pumpkin seeds has anti-inflammatory properties and regular consumption of pumpkin seeds can protect against joint inflammation (T. Amin and Thakur, 2013). It provides anti-diabetic effect thus helps in controlling the blood glucose. According to a study by Li *et al.* (1956), oil from un-germinated pumpkin seeds and proteins from germinated pumpkin seeds possess hypoglycemic activity. Pumpkin seeds show relief effect from anxiety . Tryptophan present in pumpkin seeds helps to relieve a person from anxiety. Tryptophan gets converted into serotonin which is a neurotransmitter and that enhances mood and promotes well-being in the brain (T. Amin and Thakur, 2013).Tryptophan also promotes sleep and thus reduces anxiety (Xu *et al.*, 1994). Pumpkin seed oil has been found to exhibit anti-hypercholesteromic effect (Al-Zuhair *et al.*, 1997). Pumpkin extracts showed a broad spectrum antimicrobial activity against bacteria (Caili *et al.*, 2006). Pumpkin seed oil has been found to exhibit anti-hypercholesteromic effect (Al-Zuhair *et al.*, 1997). The presence of unsaturated fatty acids such as oleic acid and linoleic acid in pumpkin seed reduce cholesterol level in rats (Takada *et al.*, 1994). Pectin present in pumpkin seeds are also highly responsible for reduction of cholesterol in blood. Pumpkin seeds are also used to ease the arthritis effect as γ-tocopherol present in pumpkin seed possesses anti-inflammatory properties and can be used to treat arthritis and other conditions which cause painful swelling (Jeznach *et al.*, 2012). Pumpkin seeds are a rich source of L-arginine because of which they are responsible for acting against atherosclerosis (AL-showayman, 2010).

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Part III

Materials and Methods

3.1 Materials needed

3.1.1 Equipment needed

Gas oven, Hot air oven, Centrifuge, Muffle furnace, Soxhlet Extraction apparatus, Kjeldhal set, Steam Distillation apparatus, Incubator and Spectrophotometer.

All the equipment facilities will be provided by central campus of technology (CCT), Dharan-14.

3.1.2 Chemicals needed

All chemicals used are reagent grade unless specified otherwise and distilled water will be used throughout the work. All operations will be perform at room temperature unless otherwise stated.

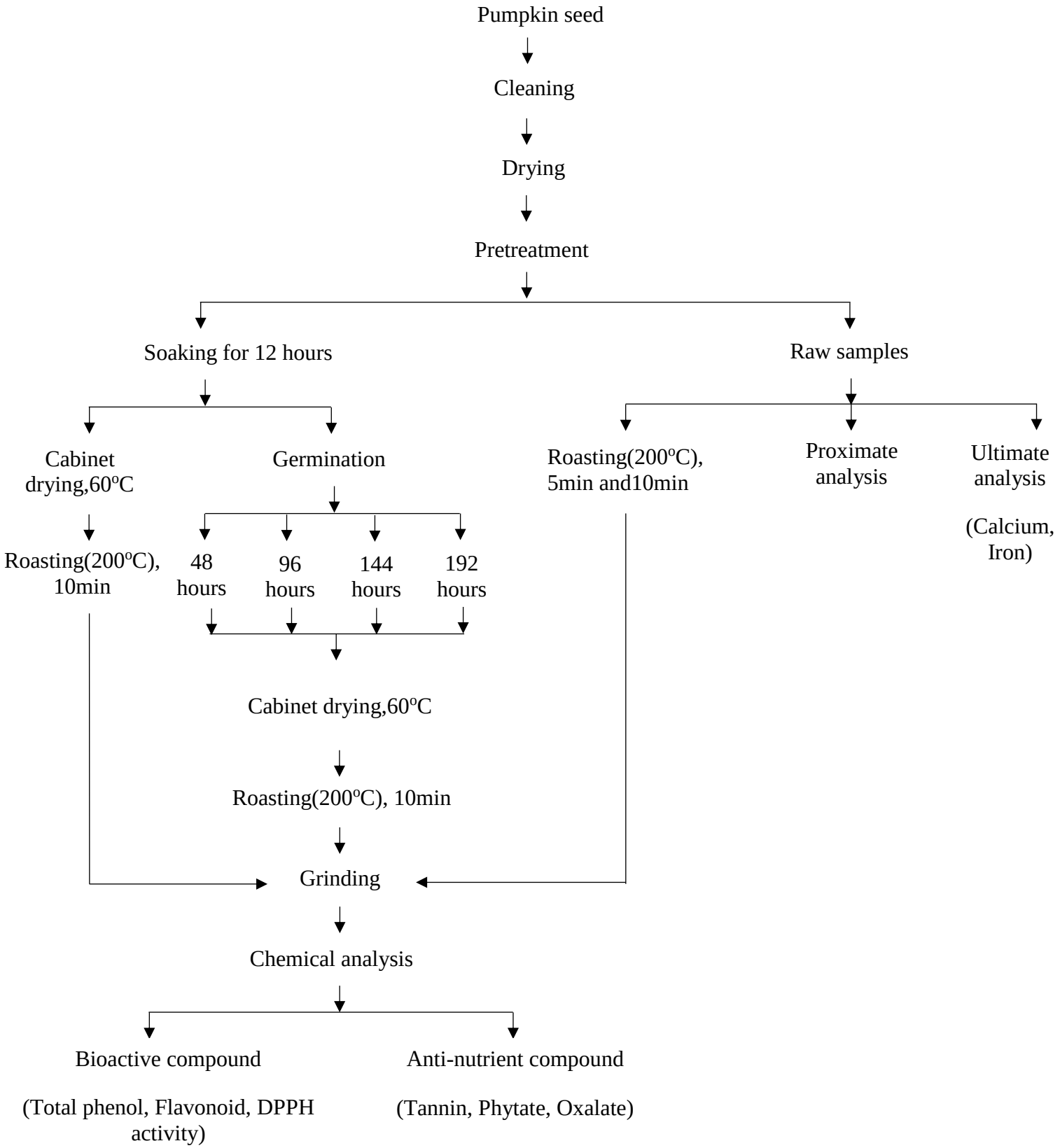
Catalyst mixture, Mixed indicator, boric acid, Sodium Hydroxide, Ammonium Hydroxide, phenolphthalein, methyl orange, ammonium oxalate, potassium persulfate, Silver Nitrate, Conc. Sulphuric Acid, Methanol, Hydrochloric acid, Potassium Permangannat, potassium ferricynide, potassium thiocyanide, hexane, Folin–Ciocalteau reagent, 1,1-Diphenyl-2Picryl Hydrazyl (DPPH), sodium carbonate, gallic acid, quercetin, aluminium chloride, potassium acetate, potassium iodide.

3.1.3 Raw material

Pumpkin seeds

A common variety of pumpkin seed (*cucurbita moschata)* was collected from the local market of Morang to study the effect of roasting, soaking followed by roasting and germination followed by roasting on bioactive components (flavonoid, total phenol content and antioxidant activity) and anti-nutritional factors (tannin, phytic acid and oxalate).

3.2 Methodology



3.3 Pretreatment Methods

3.3.1 Roasting of pumpkin seeds

Pumpkin seeds (*cucurbita moschata)* were brought from the local market of Morang district. The seeds were sorted to remove dust, foreign matter and damaged ones. The seeds were divided into four portions as raw sample, roasted sample, soaked and germinated sample. Roasting was done in a gas oven at 200^{o}C for 5 and 10 minutes. In each lot 50gm sample was roasted in a single layer and periodic stirring was done.

3.3.2 Soaked, dried and roasted

Similarly, soaking was done for 12 hours followed by drying in dryer and roasted in a gas oven at 200^{o}C for 10 minutes.

3.3.3 Germinated, dried and roasted

Similarly, germination was done for 48h, 96h, 144h and 192h followed by drying in dryer and roasted in a gas oven at 200^{o}C for 10 minutes.

3.3.4 Preparation of methanolic extract of the sample

One gram of each sample of flaxseed was ground with 30 ml of methanol (80%) in mortar and pestle for homogenization. After recovery of the homogenate, 15 ml methanol (80%) was used to wash the mortar and pestle and then pooled with the first homogenate. The mixture was refrigerated for half an hour and allowed to centrifuge at 4,500 rpm for 15 min at room temperature (27°C). Supernatant obtained by filtered using whatman filter paper was made volume up to 50 ml with methanol (80%) (Nehir El and Karakaya, 2004).

3.4 Proximate Analysis

3.4.1 Determination of moisture

Moisture content of the sample will be determined adopting AOAC (2004) method. At first weight of empty crucible with cover (previously dried at 100 °C for 1 hour) will be taken and 3 g of sample will be placed into it. Then the crucible will be placed in an air oven (thermostatically controlled) and will be dried at temperature of 105 ºC for 24 hrs. After drying, the crucible will be removed from the oven and cooled in desiccator. It will then weigh with

cover glass. The crucible will again be placed in the oven, dried for 30 minutes, taken out from the dryer, cooled in desiccator and weighed. Drying, cooling, and weighing will be performed repeatedly until the two consecutive constant weights will be attained. The moisture content of the green gram samples will be calculated by applying the following:

$$\text{Moisture Content (g \%)} = \frac{A-B}{W} \text{ x } 100$$

Where,A= initial weight of crucible and sample, B=final weight of crucible and sample

W= weight of the sample

3.4.2 Determination of protein

Protein content will be determined by the Kjeldahl method. Nitrogen content estimated by the Kjeldahl method is based on the determination of reduced nitrogen (NH2 and NH) present is the sample. The various nitrogenous compounds are converted into ammonium sulfate by boiling with conc. H2SO4. The ammonium sulfate formed is decomposed with an alkali (NaOH), and the NH3 liberated is absorbed in excess of neutral boric acid solution and then titrated with standard acid(Dhital, 2021).

$$\text{Nitrogen content} = \frac{(Ts-Tb)xNofHCLx14x100x100}{Aliquot(ml)xweightofsamplex1000}$$

Protein content = N2 content x 6.5

3.4.3 Determination of fat

Fat in the solid food sample will be estimated by dissolving the sample in organic solvent using Soxhlet apparatus followed by evaporation of solvent to obtain fat. The apparatus consists of 3 easy to fit parts, namely the extraction tube (into which sample in a thimble is kept immersed in solvent for fat extraction), the receiving flask (which receives through siphon system the solvent + extracted fat from the extraction tube and vaporizes the solvent selectively for recycling) and the condenser (which condenses the vaporized solvent onto the sample placed in the extraction tube). The recycling is done number of times (until extraction is complete) and fat is recovered by evaporating away the solvent. The percentage of crude fat of the sample will be calculated by the following equation:

$$\% \text{ Crude Fat (dry basis)} = \frac{wt\ of\ crude\ fat(g) x 100 x 100}{wt\ of\ sample(g) x dry\ matter\ \%}$$ (Dhital, 2021)

3.4.4 Determination of ash

Ash content of food stuff represents inorganic residue remaining after destruction of organic matter. Total ash will be determined by ashing the sample in the muffle furnace at 550 C for 3-4 hours (Dhital, 2021).

$$\text{Total ash} = \frac{wt\ of\ ash}{wt\ of\ sample} x100$$

3.4.5 Determination of crude fiber

Crude fiber will be determined by recovery of ash-free residue after sequential treatment of sample with 1.25% sulfuric acid and 1.25% sodium hydroxide each under standardized condition. The ash that comes along with the residue is removed by ashing in an ashless filter paper (Dhital, 2021).

3.4.6 Determination of total carbohydrate

Total carbohydrate will be determined by difference method.

Total carbohydrate (%) =100- (moisture + protein + fat + crude fiber + ash) %

3.4.7 Determination of calorific values

One of the methods specified by FDA was employed. This uses the general factors of 4, 4 calculate the calorie content of food (Bassey *et al.*, 2013).

Total energy = energy from carbohydrate + energy from protein + energy from fat

3.5 Ultimate analysis

3.5.1 Determination of iron

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

$$\text{Iron}\left(\frac{\text{mg}}{100\text{gm}}\right) = \frac{\text{Absorbance of sample} \times 0.1 \times \text{Total volume of ash solution} \times 100}{\text{Absorbance of standard} \times 5 \times \text{Wt of sample taken for ashing}}$$

3.5.2 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).

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

3.5 Qualitative analysis

3.5.1 Test for total phenols

2 ml of 2% solution of FeCl3 mixed with crude extract, presence of black or blue green color indicates the presence of total phenols (Jaradat *et al.*, 2015).

3.5.2 Test for flavonoids

Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with crude extract, intensive yellow color was formed which turned to colorless when added 2 drops of dilute acid showed the presence of flavonoids (Jaradat *et al.*, 2015).

3.6 Quantitative analysis of nutritional components

3.6.1 Determination of total phenols

0.5 ml of the extract and 1 ml of Folin-Ciocalteau reagent was mixed and incubated at room temperature for 15 minutes. Then 2.5ml of saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760nm. Also, the standard curve was prepared using 0-100 µg/ml solutions of Gallic acid in ethanol. Total phenol values are calculated using the standard curve equation and expressed in terms of Gallic acid equivalent (mg/ml) of dry mass(Jaradat *et al.*, 2015).

3.6.2 Determination of flavonoid

Aluminium chloride colorimetric method was used for determination of total flavonoids. 0.5 ml of each sample extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 450nm in triplicate. The calibration curve was prepared by preparing quercetin solutions at concentrations 10 to 100 µg/ml in methanol (Barek et al., 2015).

3.6.3 Determination of DPPH radical scavenging activity

0.1 mL of extract was taken and volume was made up to 50 mL by 50 % methanol and after that 3 mL was taken and 1 mL of DPPH was added in it and allowed to stand for 30 min and reading was noted spectrophotometrically at 517 nm (Arab *et al.*, 2011).

DPPH radical scavenging activity = 1- $\frac{absorbance\ of\ sample}{absorbance\ of\ control}$ x 100%

3.7 Quantitative analysis of anti-nutritional components

3.7.1 Determination of oxalate

The oxalate content was determined by the method of Day and underwood (Day and Underwood, 1986). A sample pumpkin seeds powder (1g) was mixed with 75 mL of 3M Sulphuric acid (H2SO4) 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 (KMnO4) solution until violent color persisted at least for 30 seconds. The oxalate content of the sample was determined using the following equation.

1 ml 0.05 KMnO4 = 2.2 mg oxalate

3.7.2 Determination of tannins

Colorimetric estimation of tannins is based on the measurement of the blue color formed by the reduction of phosphor-tungsto-molybdic acid by tannin-like compounds in alkaline condition.

The pumpkin seed weighing 0.5 g was boiled for 30 min with 40 ml of water. Then it was cooled and was transferred to a 50 ml volumetric flask and diluted to mark. It was then shaked well and filtered. 0 to 1 ml aliquots of the standard tannic acid solution were taken in test tube and 7.5 ml water was added to each. Then, 0.5 ml Folin-ciocalteu reagent and 1 ml Na2CO3 solution was added and volume was made to 10 ml. After then, color was measured after 30 min at 760 nm against experimental blank adjusted to 0 absorbency (Ranganna, 1986).

3.7.3 Determination of phytate

2 gm of fine powder sample was mixed with 50ml 3% TCA in a conical flask and shake for a while (30 sec to 1 min). The mixture was centrifuged and 10 ml of aliquot was taken and mixed with 4 ml of $FeCl_3$ and heated in water bath for 45 min and cool the content. Again, it was centrifuged for 10-15 min and clear supernatant was discarded. The precipitate thus formed was washed 2-3 times in 20-25 ml 3% TCA and heated in boiling water for 5-10 min and centrifuged and again washed with water. The precipitate was mixed with 2ml distilled water and 3ml 1.5N NaOH and made volume up to 30ml. The mixture was heated in water bath for 20 min and filtered with whatmann filter paper and washed the precipitate with 60-70ml hot distilled water and discard the filtrate. Dissolved the precipitate with 40ml hot 3.2N HNO_3 in 100ml volumetric flask and made volume up to 100ml with distilled water. 5 ml of aliquot was taken in 100ml volumetric flask and dilute to 70ml with distilled water and added 20ml of 1.5M KSCN and diluted to volume and read the color immediately (within 1 min) at 480nm (Sadasivam, 1996).

Part IV

Result and Discussion

A common variety of pumpkin seed (*Cucurbita moschata)* was collected from the local market of Morang to study the effect of roasting, soaking followed by roasting and germination followed by roasting on bioactive components (flavonoid, total phenol content and antioxidant activity) and anti-nutritional factors (tannin, phytic acid and oxalate). Roasting was done at 200^{o}C for 5 min and 10 min, soaked overnight followed by roasting at 200^{o}C for 10 min and 48h, 96h, 144h, and 192h of germination followed by roasting at 200^{o}C for 10 min was done. The powdered samples were analyzed for the effect of roasting, soaking followed by roasting and germination followed by roasting on the nutritional and anti-nutritional components.

4.1 Physical properties of pumpkin seeds

Pumpkin seed was analyzed for the physical properties which are presented in Table 4.1. The mean length of the raw pumpkin seed was 15.6±2.551 mm and the mean bulk density of the raw pumpkin seed was 402±0.624 kg/m^3.

Table 4.1 Physical properties of pumpkin seeds

physical property	Mean±SD
Length	15.6±2.551 mm
Bulk density	402±0.624 kg/m^3

4.2 Proximate composition of raw pumpkin seeds

Pumpkin seed was analyzed for the proximate composition which were expressed in percentage and presented in Table 4.2. The moisture content, protein, ash, fat, crude fiber and carbohydrate were 6.07±0.1528, 29.67±0.3927, 2.02±0.0306, 31.95±1.8583, 7.93±0.2458 and 21.53±0.995 respectively.

Table 4.2 Proximate composition of raw pumpkin seeds (dry basis)

Proximate constituents	**Composition in %**
Moisture*	6.07±0.1528
Protein*	29.67±0.3927
Ash*	2.02±0.0306
Fat*	31.95±1.8583
Crude fiber*	7.93±0.2458
Carbohydrate*	21.53±0.995

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

Moisture content of raw pumpkin seeds was found to be 6.07%. Moisture content of raw pumpkin seeds was 5.53% (Devi *et al.*, 2018). The protein content was found to be 29.67%. The protein content was in the range of 25.2% to 37.0% according to (El-Adawy and Taha, 2001). The protein content was 21.31% according to M. Z. Amin *et al.* (2019) which was slightly lower than the value obtained in this study. Ash content was found to be 2.02%. The data was lower when compared to the data of (Devi *et al.*, 2018) , which was 6.90%. The fat content of pumpkin seeds was found to be 31.95%. which is lesser than data obtained by (Gohari *et al.*, 2011), which was 41.59%. The obtained data was almost similar to that of (Devi *et al.*, 2018) which was 31.75%. Another research shows fat content in pumpkin seeds lies within range of 37.8% to 45.4 (El-Adawy and Taha, 2001). Crude fiber was found to be 7.93% which is higher than that of (Habib *et al.*, 2015), which was 2.91%. However, the data was within the range as given by (Singh and Kumar, 2022), which was 3% to 6%. Carbohydrate by difference was found to be 21.53%. The data was slightly lower when compared to data of (Devi *et al.*, 2018). However, the data lies in the range of 18% to 25%, which was the carbohydrate range obtained by (El-Adawy and Taha, 2001).

4.3 Mineral composition of pumpkin seeds

Pumpkin seed was analyzed for mineral composition which were presented in table 4.3. The iron was 9.727±0.9096 mg/100g and calcium content was 8.503±0.4676 mg/100g**.**

Table 4.3 Mineral composition of pumpkin seeds

Mineral	**mg/100gm**
Iron*	9.727±0.9096
Calcium*	8.503±0.4676

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

The mean value of iron content in raw pumpkin seeds was found to be 9.727mg/100g DW. The finding was more than that of (Syed *et al.*, 2019), which was 8.8mg/100g DW. Also the finding was high to that of 3.75mg/100g which was finding of (Elinge *et al.*, 2012).The mean value of calcium content was found to be 8.503mg/100g. The obtained result was less than the value obtained by (Elinge *et al.*, 2012), which was 9.78mg/100g, the result was also less than that of (Singh and Kumar, 2022), which was 11.73mg/100g.

4.4 Distribution of antinutrients in pumpkin seeds

Pumpkin seed was analyzed for antinutrients composition which were presented in table 4.4. The tannin content was 204.61±1.0262 mg/100g, phytic acid content was 39.17±0.3900 mg/100g and oxalate content was 22.45±1.1391 mg/100gm.

Table 4.4 Antinutrients in pumpkin seeds

Antinutrients	**Value(mg/100gm)**
Tannin	204.61±1.0262
Phytic acid	39.17±0.3900
Oxalate	22.45±1.1391

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

The value of tannin was found 204.61mg/100g which was close to that of (Hamed *et al.*, 2008) i.e. 228.31 mg/100g. The results obtained for pumpkin seeds was higher than that of reported by (El-Adawy and Taha, 2001). Another research done by (Singh and Kumar, 2022) found that tannin content in raw pumpkin seeds to be 28.15mg/100g which is very lesser than the obtained value. The mean value of phytic acid content of raw pumpkin seeds was 39.17mg/100g. The obtained value was higher than the value obtained by (Singh and Kumar, 2022) i.e. 25.09mg/100g. The obtained value was similar to the value of (Kindiki, 2017) i.e. 37.03mg/100g. The value of oxalate in raw pumpkin seeds was found to be 22.45mg/100gm which lies within the range of 23.84–27.01 mg/100 g reported by (Singh and Kumar, 2022). Another study by (Elinge *et al.*, 2012) found that the oxalate content of raw pumkin seed was 23mg/100gm which is similar to the obtained value.

4.5 Distribution of bioactive components in pumpkin seeds

Pumpkin seed was analyzed for bioactive compounds which were presented in table 4.5. The TPC was 383.7±1.528 mg GAE/100g, flavonoid was 310.4±1.385 mg QE/100g and DDPH radical scavenging capacity was 10.76 (%).

Table 4.5 Bioactive compounds in pumpkin seeds

Bioactive compounds	**Value**
Total phenolic content (mg GAE/100g)	383.7±1.528
Flavonoid (mg QE/100g)	310.4±1.385
DPPH radical scavenging capacity (%)	10.76

The mean value of TPC content of raw pumpkin seeds was found to be 383.7mg GAE/100g. The value obtained by (Seun Funmilola Akomolafe, 2021) was 428mg GAE/100g which was high as obtained in this research. Another finding by (Singh and Kumar, 2022) shows TPC of raw pumpkin seeds to be 35.66 mg GAE/100g which was higher than obtained value. However, the obtained value of TPC in our study lies within the range from 244-382 mg/100g. Another study suggested that the total content of phenolics (TPC) of the seed samples was ranging from

0.95 to 3.43 mg GAE/g DW (Saavedra *et al.*, 2015). The total flavonoid was 310.4mg QE/100g which was similar to the finding of (Seun Funmilola Akomolafe, 2021) which was 324mg/100g. (A. Hussain *et al.*, 2021) found total flavonoid content of pumpkin seeds to be 139.37mg/100g which was lesser than the result obtained in our study. The DPPH radical scavenging capacity of raw pumpkin seed was found to be 10.76% which is lesser than the value obtained by (Singh and Kumar, 2022)

4.6 Effect of pretreatment method on the total phenolic compound

The effects of roasting, (soaking and germination) followed by roasting on the total phenolic content in pumpkin seeds was studied. All the treatments significantly changed ($p<0.05$) total phenolic content of the pumpkin seeds, but to the very extend. Roasting at 200^{o}C for 10 min had the most pronounced effect in the increment of total phenolic content.

4.6.1 Effect of roasting

Total phenolic content of raw pumpkin seed was determined, and the value obtained showed that there was significant increase ($p<0.05$) in total phenolic content, which was increase from 383.7mg/100g to 491mg/100g and 383.7mg/100g to 622mg/100g after roasting at 200^{o}C for 5 min and 10 min respectively i.e., 27.7% and 62.1% increment.

Our result shows increment in total phenolic content roasted at 200^{o}C for 5min and 10min respectively. Our result was similar to the result obtained by (Peng *et al.*, 2021), who in their study reported total phenolic content of pumpkin seed increased by 56% after roasting at 200^{o}C for 10 min. Saavedra *et al.* (2015)also found that cooked squash seeds have higher TPC than fresh seeds. Studies have shown that, roasting can increase the total phenolics and flavonoids content of different seeds and grains (Bhinder *et al.*, 2019). Another study by (Seun Funmilola Akomolafe, 2021) reported, total phenolic content increased by 247% after roasting which was greater than the value obtained in our study.

4.6.2 Effect of soaking followed by roasting

Total phenolic content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in total phenolic content, which was reduced from

383.7mg/100 g to 285.5mg/100 g after 12 hours soaking followed by roasting at 200^{o}C for 10 min i.e., 25.6% reduction.

Our result was slightly lesser than the result obtained by (Dahal and Koirala, 2020), who in their study reported total phenolic content of their seed sample reduced by 30.3% after 12 hours of soaking followed by roasting. Another study by (Sharma *et al.*, 2013) reported, total phenolic content in beans reduced by 43% after 12hrs soaking followed by cooking which was greater than the value obtained in our study.

4.6.3 Effect of germination followed by roasting

Total phenolic content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in total phenolic content, which was reduced from 383.7mg/100 g to 223.4mg/100, 210.1mg/100g,186.0mg/100g and 179mg/100g after 48h, 96h, 144h and 192h of germination followed by roasting at 200^{o}C for 10 min i.e., 41.8%, 45.2%, 51.5% and 53.3% reduction.

A research done by (Khare *et al.*, 2021) reported the polyphenol content in flaxseed of one variety reduced by 55.5% which is similar to the value obtained in our study. Another research by (Alonso *et al.*, 2000) on the effect of germination time on polyphenol content of some beans found 8.6%, 9% and 20% reduction in polyphenol content after 24, 48 and 72 hours of germination respectively, which showed decrement of phenolic content after germination. Similarly, (Yasmin *et al.*, 2008) reported that there was 54.5% reduction of polyphenol content after 96 hour of germination which was similar to the result obtained in this study. Before germination, soaking is also done and some loss of polyphenol during soaking is also expected because of its leaching into the soaking water. Further decrease in polyphenols during germination may be ascribed to the presence of polyphenol oxidase and enzymic hydrolysis (Jood *et al.*, 1987). The effect of various treatments on phenolic content were shown in fig 4.5

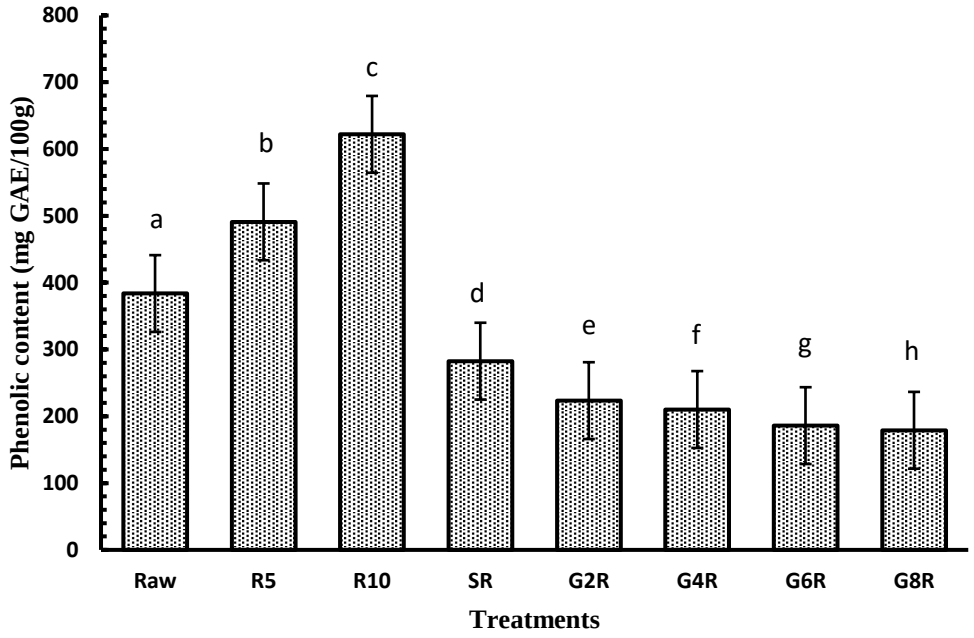


Fig 4.5 Effect of treatments on phenolic content

4.7 Effect of pretreatment method on flavonoid

The effects of roasting, (soaking and germination) followed by roasting on the total flavonoid content in pumpkin seeds was studied. All the treatments significantly changed ($p<0.05$) flavonoid content of the pumpkin seeds, but to the very extend. Germination for 8 days followed by roasting at 200^{o}C for 10 min had the most pronounced effect in the increment of total flavonoid content.

4.7.1 Effect of roasting

The flavonoid content of raw pumpkin seed was determined, and the value obtained showed that there was significant increase ($p<0.05$) in flavonoid content, which was increase from 310.3mg/100g to 460.8mg/100g and 310.3mg/100g to 584.6mg/100g after roasting at 200^{o}C for 5 min and 10 min respectively i.e., 48.5% and 88.39% increment.

Our result shows increment in flavonoid content roasted at 200^{o}C for 5min and 10min respectively. Our result was higher than that of roasting done for 5min and lesser than the result obtained by (Seun Funmilola Akomolafe, 2021), who in their study reported total phenolic content of pumpkin seed increased by 87.8% after roasting. Another study by (Peng *et al.*, 2021), the content of flavonoids in pumpkin seeds at 200°C was the highest, which was 2.81

times of the unroasted one i.e. 181% increment which is greater than the value obtained in our study. Studies have shown that, roasting can increase the total flavonoid content of different seeds and grains (Bhinder *et al.*, 2019). (Dahal and Koirala, 2020) in their study found that flavonoid content of similar seed increased by 21.76%, 18.91%, 94.85% and 425.37% upon roasting for 5, 10, 15 and 20 minutes respectively.

4.7.2 Effect of soaking followed by roasting

Total flavonoid content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in total flavonoid content, which was reduced from 310.3mg/100 g to 251.1mg/100 g after 12 hours soaking followed by roasting at 200^{o}C for 10 min i.e., 19.07% reduction.

(Dahal and Koirala, 2020) in their study reported the flavonoid content of similar type of seed reduced by 18.38% after 12 hours soaking followed by roasting at 180^{o}C for 15min which is similar to the value obtained in our study. (Meital *et al.*, 2023)in her study reported that soaking fava beans for 12 hours decreased the total flavonoid content by 19 to 36%. The result obtained in our study was 19.07% which lies in this range. Another study done by (Afify *et al.*, 2012) on the effect of soaking on flavonoid content of sorghum found flavonoid content of legumes decreased by 26% after 12 hours of soaking which was higher than the value obtained in our study. The phenolic components leached out during germination which decreases the flavonoid contents after soaking (Alonso *et al.*, 2000).

4.7.3 Effect of germination followed by roasting

Total flavonoid content of raw pumpkin seed was determined, and the value obtained showed that there was significant increment ($p<0.05$) in total flavonoid content, which was increased from 310.3mg/100 g to 695.3mg/100, 1388.5mg/100g, 1397.5mg/100g and 1881.2mg/100g after 48h, 96h, 144h, and 192h of germination followed by roasting at 200^{o}C for 10 min i.e., 124.07%, 347.47%, % and 506.25% reduction.

A research done by (Wang *et al.*, 2015) reported the flavonoid content in flaxseed of one variety increased from 243.61% to 1692.9% from 1 to 8 days of germination which greater to the value obtained in our study which indicated the increase pattern of flavonoid content after germination. (Saleh *et al.*, 2019) in their study on the effect of germination on total flavonoid content of legumes found increment in flavonoid content by 42.5% and 52,6% after 72 and 96

hours of germination which was lesser than the value obtained in our study. Another study by (Świeca and Gawlik-Dziki, 2015) found flavonoid content of legumes significantly increased by 31.25%, 56.25%, 71% and 81.25% after 24, 48,72 and 96 hours of germination respectively which was lesser than the value obtained in our study. The effect of various treatments on flavonoid content were shown in fig 4.6

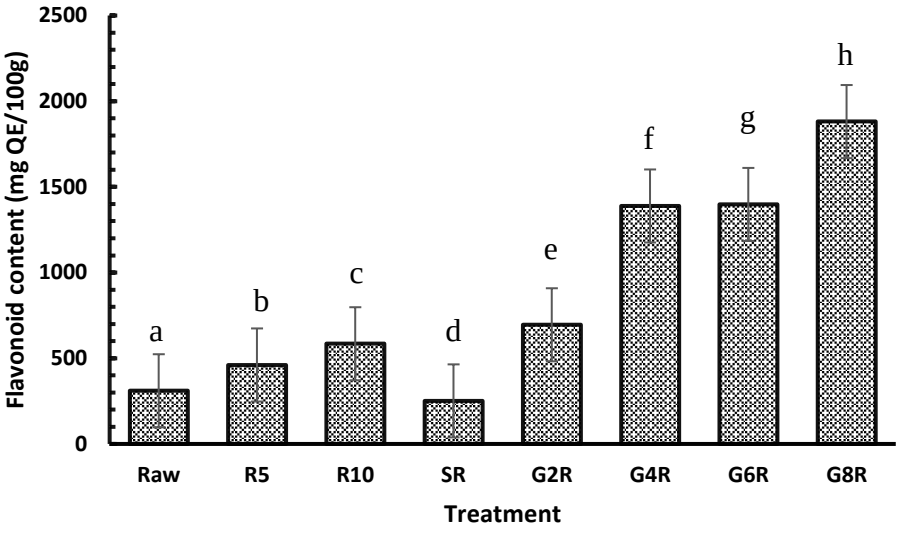


Fig. 4.6 Effect of treatment on flavonoid content

4.8 Effect of pretreatment method on oxalate content

The effects of roasting, (soaking and germination) followed by roasting on the oxalate content in pumpkin seeds was studied. All the treatments significantly changed ($p<0.05$) oxalate content of the pumpkin seeds, but to the very extend. Germination for 8 days followed by roasting at 200^{o}C for 10 min had the most pronounced effect in the decrement of oxalate content.

4.8.1 Effect of roasting

The oxalate content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was reduced from 22.55mg/100g to 20.44mg/100g and 22.55mg/100g to 18.61mg/100g after roasting at 200^{o}C for 5 min and 10 min respectively i.e.,9.35% and 17.45% increment.

Our result shows decrement in flavonoid content roasted at 200^{o}C for 5min and 10min respectively. Our result was higher than that of roasting done for 5min and lesser than the result

obtained by (Dahal and Koirala, 2020), who in their study reported oxalate content of seed reduced by 5.68% for 5min and 6.80% after roasting at 180ºC. Another study by (Mbah et al., 2012), the content of oxalate in seeds at 3.58mg/100gm after roasting for 10 min which is lower than the value obtained in our study. Another study by (P. Addo *et al.*, 2018a) reported the oxalate content of seeds reduced by 53.94% after roasting at 160ºC for 30 min which is greater than the value obtained in our study.

4.8.2 Effect of soaking followed by roasting

The oxalate content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in total oxalate content, which was reduced from 22.55mg/100 g to 16.40mg/100 g after 12 hours soaking followed by roasting at 200ºC for 10 min i.e., 27.3% reduction.

Dahal and Koirala (2020) in their study reported the oxalate content of similar type of seed reduced by 37.38% after 12 hrs soaking followed by roasting at 180ºC for 15min which is greater than the value obtained in our study. (Shi *et al.*, 2018) in their study reported that pulses soaked for 12 hours reduced the oxalate content within range of 17.4-51.89% thus obtained value in our study lies within this range. Another study done by (Handa *et al.*, 2017) on the effect of soaking on oxalate content on legumes reported the oxalate content reduced by 33.90% which is slightly similar to the value obtained in our study.

4.8.3 Effect of germination followed by roasting

The oxalate content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in oxalate content, which was increased from 22.55mg/100 g to 21.33mg/100, 12.46mg/100g, 11.54mg/100g and 11.10mg/100g after 48h, 96h, 144h, and 192h of germination followed by roasting at 200ºC for 10 min i.e., 5.41%, 44.74%, 48.82 % and 50.77% reduction.

A research done by (Echendu *et al.*, 2009) reported that oxalte content in seed reduced by 6.67% and 20% after germination for 3 and 4 days respectively which showed decrement trend of oxalate content thus obtained value in our study represented the reduction pattern of oxalate content after germination. (Handa *et al.*, 2017) in their study on the effect of germination on oxalate content of legumes reduced by 36.75%, and 37.08 after germination for 24hrs and 48hrs

which also shows reduction pattern of oxalate which was similar to the value obtained in our study. The effect of various treatments were shown in fig 4.7

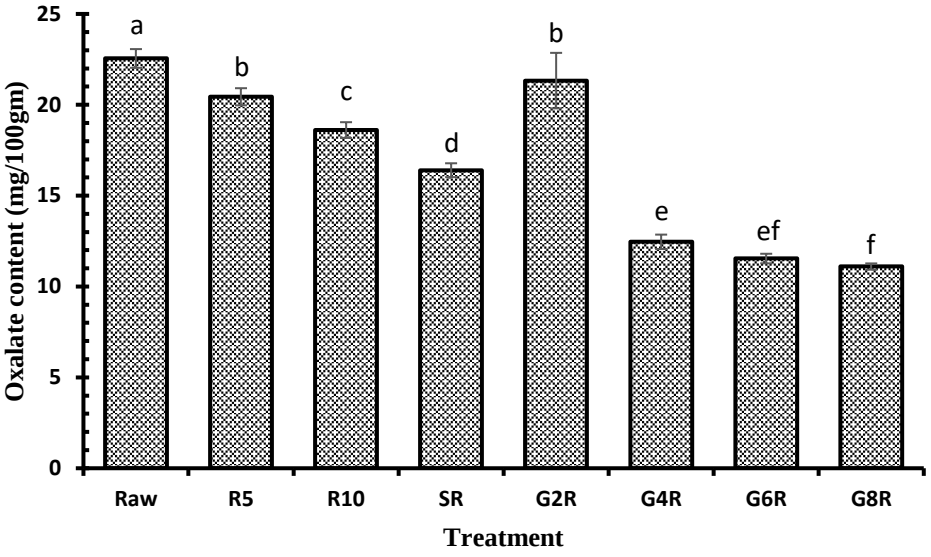


Fig. 4.7 Effect of treatment on oxalate content

4.9 Effect of pretreatment method in phytic acid

The effects of roasting, (soaking and germination) followed by roasting on the phytic acid content in pumpkin seeds was studied. All the treatments significantly changed ($p<0.05$) phytic acid content of the pumpkin seeds, but to the very extend. Germination for 8 days followed by roasting at 200^{o}C for 10 min had the most pronounced effect in the decrement of phytic acid content.

4.9.1 Effect of roasting

The phytic acid content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in phytic acid content, which was reduced from 39.34mg/100g to 36.43mg/100g and 39.34mg/100g to 32.59mg/100g after roasting at 200^{o}C for 5 min and 10 min respectively i.e.7.39% and 17.15% increment.

Our result shows decrement in phytic acid content roasted at 200^{o}C for 5min and 10min respectively. Hamed *et al.* (2008) in their study reported phytic acid content of pumpkin seed reduced by 11.77% after roasting which is higher than the value of roasting for 5min and lesser than the value of roasting for 10 obtained in our study. Another study by (B. Hussain *et al.*, 1989), the content of phytate in legumes ranged from 16% to 60% after roasting thus obtained value in our study lies within the range . Another study by (Udensi *et al.*, 2007) reported the phytate content of seeds reduced by 51.76% after roasting at 120^{o}C for 30 min which is greater than the value obtained in our study.

4.9.2 Effect of soaking followed by roasting

The phytic acid content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in phytic acid content, which was reduced from 39.34mg/100 g to 32.80mg/100 g after 12 hours soaking followed by roasting at 200^{o}C for 10 min i.e.16.62% reduction.

(Kindiki, 2017) in their study reported the phytate content of similar type of pumpkin seed reduced by 10.53% after 12 hrs soaking followed by roasting which is greater than the value obtained in our study. Another study by (Igbedioh *et al.*, 1994) reported that seeds soaked for 12 hours reduced the phytate content by 12.24% which is slightly similar to the value obtained in our study. Another study done by (Rasha Mohamed *et al.*, 2011) on the effect of soaking on phytate content on legumes reduced by 12.3% in mung bean and 15.6% in kidney bean which is slightly similar to the value obtained in our study.

4.9.3 Effect of germination followed by roasting

The phytic acid content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in phytic acid content, which was reduced from 39.34mg/100 g to 17.71mg/100, 16.44mg/100g, 15.58mg/100g and 15.03mg/100g after 48h, 96h, 144h, and 192h of germination followed by roasting at 200^{o}C for 10 min i.e., 54.98%, 58.21%, 60.39% and 61.79% reduction.

A research done by (Rai, 2022) reported the phytic acid content of flaxseed reduced on the progressive days of germination and the phytic acid content (g/kg) was found to be 10.78±0.03, 10.54±0.05, 10.12±0.07, 10.02±0.04, 9.94±0.01, 9.18±0.06 and 9.18±0.08 respectively which shows a decreasing trend in the content of phytic acid as days of germination progressed which

is similar to the finding in our study. Another finding by (Kajla *et al.*, 2017) who also reported a decrease in the content of phytic acid of flaxseed from 25.8 g/kg to 21.5 g/kg during germination. Another study by (Igbedioh *et al.*, 1994) reported reduced phytic acid content by 23.12% after 3 days germination which is lesser than the value obtained in our study.

Reduction in phytic acid in germinated flaxseed varieties might be attributed to increase in phytase activity (Arora and Rajni, 2006; Hooda and Jood, 2003). The effect of various treatments on phytic content were shown in fig 4.8

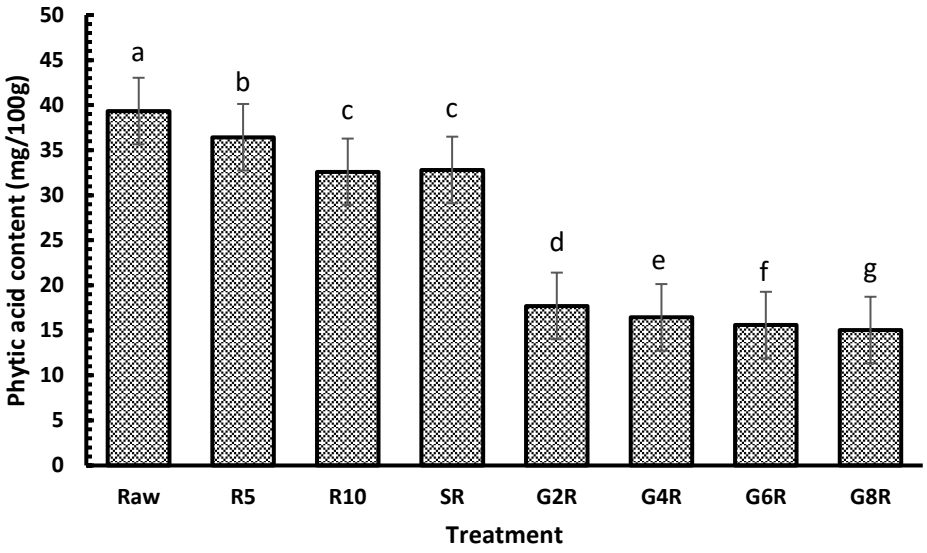


Fig 4.8 Effect of treatment in phytic acid content

4.10 Effect of pretreatment method on tannin

The effects of roasting, (soaking and germination) followed by roasting on the tannin content in pumpkin seeds was studied. All the treatments significantly changed ($p<0.05$) tannin content of the pumpkin seeds, but to the very extend. Roasting at 200^{o}C for 10 min had the most pronounced effect in the decrement of tannin content.

4.10.1 Effect of roasting

The tannin content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from

205.5mg/100g to 186.1mg/100g and 205.5mg/100g to 130.5mg/100g after roasting at 200^{o}C for 5 min and 10 min respectively i.e., 9.44% and 36.49% reduction.

Our result shows reduction in tannin content roasted at 200^{o}C for 5min and 10min respectively. Hamed *et al.* (2008) in their study reported tannin content of pumpkin seed reduced by 45.24% after roasting which is higher than the value of roasting for 5min and 10 min obtained in our study. Another study by (P. Addo *et al.*, 2018a), the content of tannin in moringa seeds reduced 37.43% after roasting for 10 min which is similar to the value obtained in our study. Another study by (P. W. Addo *et al.*, 2018b) reported the tannin content of seeds reduced by 23.85% after roasting at 160^{o}C for 30 min which is greater than the value roasted for 5min and lesser than roasted for 10min in our study.

4.10.2 Effect of soaking followed by roasting

The tannin content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in tannin content, which was reduced from 205.5mg/100 g to 153.2 mg/100 g after 12 hours soaking followed by roasting at 200^{o}C for 10 min i.e.25.45% reduction.

Vijayakumari *et al.* (1998) in their study reported the tannin content of similar type of seed reduced by 52.63% after 12 hours soaking which is greater than the value obtained in our study. Another study by (Obizoba and Atii, 1991) reported that soaked sorghum seeds reduced tannin by 32.56% which is greater than the value obtained in our study. Our result slightly corelates with the finding by (Shah, 2001) who reported tannin content reduced by 22.14% after soaking.

The reduction in the tannin content after soaking is due to the leaching out of tannin after soaking (Alonso *et al.*, 2000).

4.10.3 Effect of germination followed by roasting

The tannin content of raw pumpkin seed was determined, and the value obtained showed that there was significant reduction ($p<0.05$) in phytic acid content, which was reduced from 205.5mg/100g to 187.3mg/100, 185.4mg/100g, 184.2mg/100g and 183.7mg/100g after 48h, 96h, 144h, and 192h of germination followed by roasting at 200^{o}C for 10 min i.e., 8.85%, 9.78%, 10.36% and 10.60% reduction.

The obtained value of tannin for different interval days were in decreasing pattern which was supported by research done by (Obizoba and Atii, 1991) reported reduction of tannin content of sorghum seeds by 33.33%, 36.66%, 39.48%, 51.79% and 60.25% after 24h, 36h, 48h, 72h and 96h respectively which shows decreasing pattern of tannin content.

Another research by (Rai, 2022) reported the tannin content (g/kg) was found to be 3.34±0.09, 3.32±0.07, 3.31±0.14, 3.29±0.01, 3.28±0.08, 3.27±0.11 and 3.27±0.23 respectively. This study shows a decreasing trend in the content of tannin as days of germination progressed which supports our value obtained in our study.

Rizvi *et al.* (2022) in their study reported tannin content of legumes reduced by 18.61%, 25.10% and 28.57% after 24h, 48h and 72h of germination which also supported the reduction pattern of tannin for our study. The effect of various treatments on tannin content were shown in fig 4.9

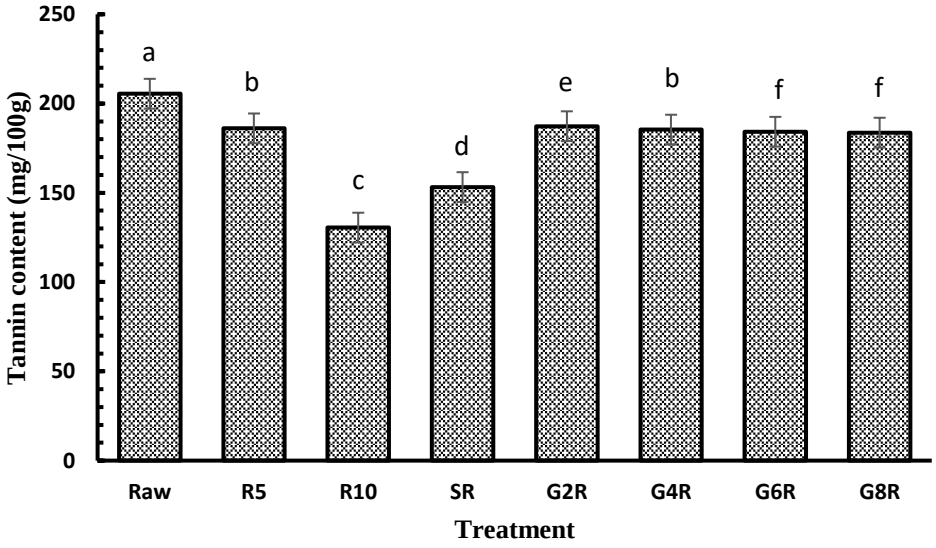


Fig 4.9 Effect of treatment on tannin content

4.11 Effect of pretreatment method on DPPH free radical scavenging capacity

The antioxidant capacity was expressed in percentage and found to be 10.76% in raw pumpkin seeds. The antioxidant capacity showed decrement in roasting for 5 min and 10 min i.e., 8.33% and 7.94% respectively. The antioxidant capacity of soaked sample followed by roasting was

found to be 8.33% which was similar to the value of roasting for 5 min. Germination for 48h, 96h, 144h, and 192h followed by roasting showed increment in the antioxidant activity i.e., 13.46%, 14.23%, 26.53% and 15.51% respectively. The effect of various treatment on DPPH radical scavenging capacity were shown in table 4.6

Table 4.6 Effect of pretreatment on DPPH free radical scavenging capacity

Treatment	Value (%)
Raw	10.76
R5	8.33
R10	7.94
SR	8.33
G2R	13.46
G4R	14.26
G6R	26.53
G8R	15.51

Table 4.7 Analysis of Variance of roasting (10min) with raw, soaked followed by roasting (10min) and germination (192 hours) followed by roasting (10min).

Parameters	Raw	Roasted (10min)	Soaked, dried and roasted (10min)	Germinated, dried and roasted (10min)	ANOVA
TPC (mg GAE/100g)	383.7±1.528	622.0±1.000^{c}	282.5±3.224^{d}	179.0±0.751^{h}	<0.001
Flavonoid (mg QE/100g)	310.4±1.385	845.7±1.155^{c}	251.1±0.843^{d}	1881.2±2.951^{h}	<0.001
DPPH activity (%)	10.76	7.94	8.33	15.51	<0.001
Oxalate (mg/100g)	22.45±1.1391	18.61± 0.4321^{c}	16.40± 0.3821^{d}	11.10± 0.1704^{f}	<0.001
Phytic acid (mg/100g)	39.17±0.3900	32.59±0.1217^{c}	32.80±0.1652^{c}	15.03±0.0243^{g}	<0.001
Tannin (mg/100g)	204.61±1.0262	186.1±0.3308^{b}	153.2±0.6943^{d}	183.7±0.5774^{f}	<0.001

*Figures with same superscript within a row are not significantly different

4.12 Comparison of roasting and soaking followed by roasting

Table 4.5 showed that total phenolic content was significantly decreased ($p<0.001$) from 622.0±1.000 to 282.5±3.224, antioxidant activity was found to be increased in soaking followed by roasting, total flavonoid was significantly decreased ($p<0.001$) from 845.7±1.155 to 251.1±0.843, oxalate content was significantly decreased from 18.61±0.4321 to 16.40±0.3821 and tannin content was significantly decreased from 186.1±0.3308 to 153.2±0.6943 and phytic acid content was changed from 32.59±0.1217 to 32.80±0.1652 whereas no any significant change in phytic acid.

4.13 Comparison of roasting and germination followed by roasting

Table 4.5 showed that total phenolic content was significantly decreased ($p<0.001$) from 622.0±1.000 to 179.0±0.751, antioxidant activity was found to be increased in germination followed by roasting, total flavonoid was significantly increased ($p<0.001$) from 845.7±1.155 to 1881.2±2.951, oxalate content was significantly decreased from 18.61±0.4321 to 11.10±0.1704 and tannin content was changed from 186.1±0.3308 to 183.7±0.5774 whereas no any significant change in tannin content and phytic acid content was changed from 32.59±0.1217 to 15.03±0.0243.

Part v

Conclusion and Recommendation

5.1 Conclusion

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

1. Pumpkin seed was subjected to a variety of methods, including roasting, soaking followed by roasting and 48h, 96h, 144h and 192h of germination followed by roasting, all of which significantly reduced antinutrients and increased bioactive compounds.
2. Roasting was found to be most effective method for the increment of total phenolic content (62.1%) and reduction of tannin (36.49%) present in pumpkin seed.
3. Germination for 192h followed by roasting was found to be most effective in increment of flavonoid (506.25%) and reduction of oxalate (50.77%), and phytic acid (61.79%) present in pumpkin seed.
4. DPPH radical scavenging capacity was found maximum on germination for 144hrs followed by roasting.

5.2 Recommendation

1. Among all the processing methods, germination followed by roasting method was the best processing as majority of bioactive compounds were significantly increased and maximum reduction of anti-nutritional compounds So, it is recommended to process pumpkin seeds through roasting and germination followed by roasting for making other product from pumpkin seeds.
2. The effect of processing methods to reduce other antinutrients like hydrocyanic acid, nitrite, etc. present in pumpkin seed can be studied.
3. Effects of other processing methods such as boiling, cooking, autoclaving, etc. in reduction of antinutrient compounds and bioavailability od bioactive compounds can be studied.

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Appendices

Appendix A

A.1 Standard curve for tannin content

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

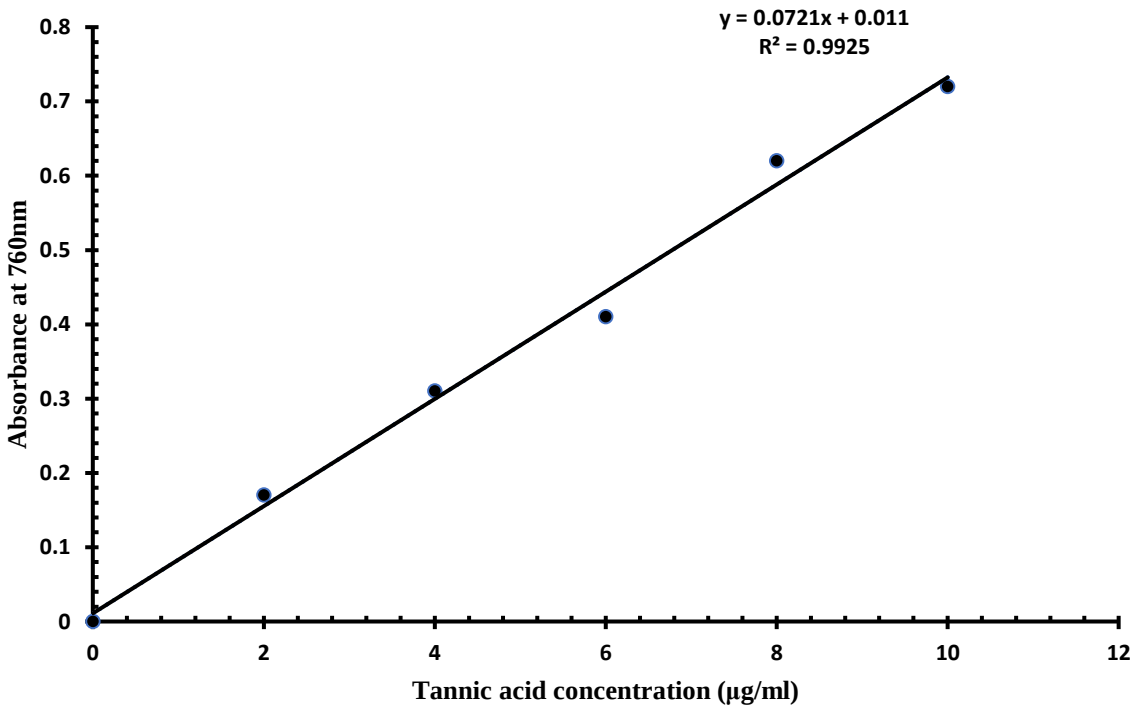

Fig A.1 Standard curve for tannin determination

A.2 Standard curve for total phenolic content

Gallic acid concentration(µg)	Absorbance
0	0
4	0.073
6	0.117
8	0.121
10	0.158
12	0.182
16	0.228

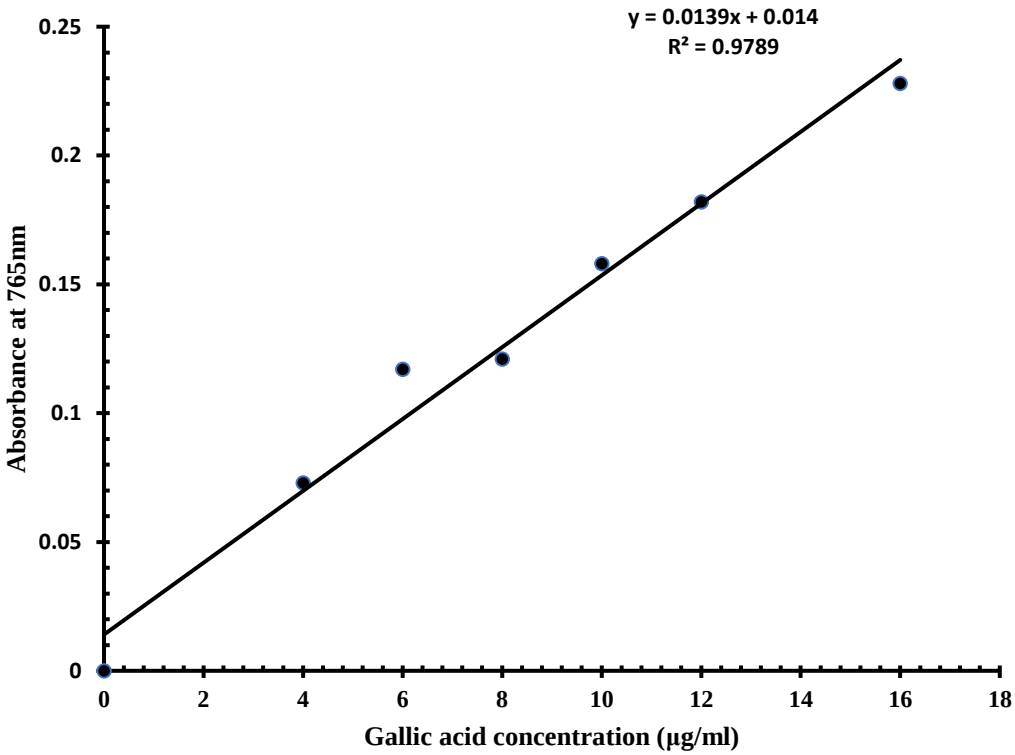

Fig A.2 Standard curve for total phenolic content

A.3 Standard curve for flavonoid

Quercetin concentration (mg)	Absorbance
0	0.000
50	0.021
100	0.057
150	0.078
200	0.098
250	0.143

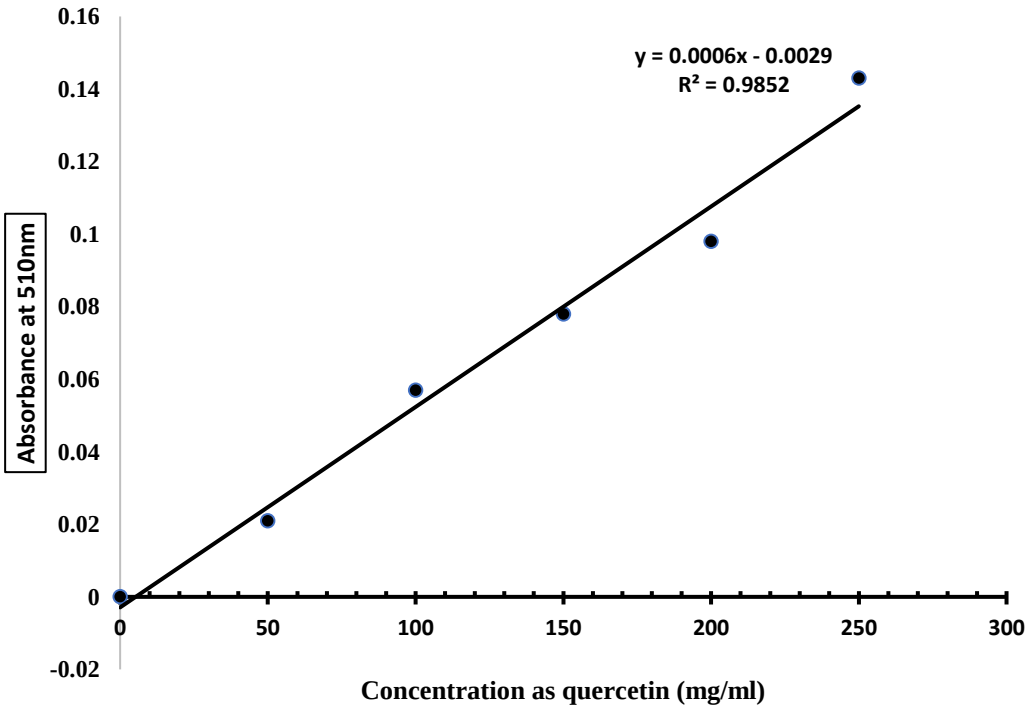

Fig A.3 Standard curve for flavonoid content

Appendix B

Table B.1 ANOVA for total phenolic content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	7	555360.973	79337.282	33328.63	<.001
Residual	16	38.087	2.380		
Total	23	555399.060			

Since p<0.005, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.2 LSD of means for total phenolic content

Treatment	Mean	Column A	l.s.d	d.f.
Raw*	383.7± 1.528	A	2.671	16
Roasting 5min*	491.0±1.000	B		
Roasting 10min*	622.0±1.000	C		
Soaking and roasting 10min*	282.5±3.224	D		
Germination 48h and roasting 10min*	223.4±1.419	E		
Germination 96h and roasting 10min*	210.1±0.859	F		
Germination 144h and roasting 10min*	186.0±1.000	G		
Germination 192h and roasting 10min*	179.0±0.751	H		

(*= Significantly different)

Table B.3 ANOVA for total flavonoid content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	7	7.366E+06	1.052E+06	4.624E+05	<.001
Residual	16	3.641E+01	2.276E+00		
Total	23	7.366E+06			

Since $p<0.005$, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.4 LSD of means for total flavonoid content

Treatment	Mean	Column A	l.s.d	d.f.
Raw*	310.3±1.528	A	2.611	16
Roasting 5min*	460.8±1.041	B		
Roasting 10min*	584.6±19.375	C		
Soaking and roasting 10min*	251.1±0.843	D		
Germination 48h and roasting 10min*	695.3±0.918	E		
Germination 96h and roasting 10min*	1388.5±1.366	F		
Germination 144h and roasting 10min*	1397.5±1.152	G		
Germination 192h and roasting 10min*	1881.2±2.951	H		

(*= Significantly different)

Table B.5 ANOVA for oxalate content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	7	447.6860	63.9551	150.06	<.001
Residual	16	6.8194	0.4262		
Total	23	454.5054			

Since $p<0.005$, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.6 LSD of means for oxalate content

Treatment	Mean	Column A	l.s.d	d.f.
Raw*	22.55±0.5145	A	1.130	16
Roasting 5min*	20.44±0.4732	B		
Roasting 10min*	18.61± 0.4321	C		
Soaking and roasting 10min*	16.40± 0.3821	D		
Germination 48h and roasting 10min*	21.33± 1.5275	B		
Germination 96h and roasting 10min*	12.46± 0.3961	E		
Germination 144h and roasting 10min*	11.54± 0.2627	EF		
Germination 192h and roasting 10min*	11.10± 0.1704	F		

(*= Significantly different)

Table B.7 ANOVA for phytic acid content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	7	2294.41567	327.77367	6497.97	<.001
Residual	16	0.80708	0.05044		
Total	23	2295.22275			

Since p<0.005, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.8 LSD of means for phytic acid content

Treatment	Mean	Column A	l.s.d	d.f.
Raw*	39.34±0.2200	A	0.3887	16
Roasting 5min*	36.43 0.3318	B		
Roasting 10min*	32.59±0.1217	C		
Soaking and roasting 10min*	32.80±0.1652	C		
Germination 48h and roasting 10min*	17.71±0.2291	D		
Germination 96h and roasting 10min*	16.44±0.2797	E		
Germination 144h and roasting 10min*	15.58±0.2676	F		
Germination 192h and roasting 10min*	15.03±0.0243	G		

(*= Significantly different)

Table B.9 ANOVA for tannin content

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	7	11701.5090	1671.6441	9800.53	<.001
Residual	16	2.7291	0.1706		
Total	23	11704.2380			

Since $p<0.005$, there is a significant difference between samples in different treatments, so LSD testing is necessary.

Table B.10 LSD of means for tannin content

Treatment	Mean	Column A	l.s.d	d.f.
Raw*	205.5±0.3347	A	0.7149	16
Roasting 5min*	186.1±0.3308	B		
Roasting 10min*	130.5±0.3855	C		
Soaking and roasting 10min*	153.2±0.6943	D		
Germination 48h and roasting 10min*	187.3±0.2000	E		
Germination 96h and roasting 10min*	185.4±0.3465	B		
Germination 144h and roasting 10min*	184.2±0.1380	F		
Germination 192h and roasting 10min*	183.7±0.5774	F		

(*= Significantly different)

Color Plates

Plate 1 Sample tested for tannin content

Plate 2 Soaked sample roasted for different chemical analysis

Plate 3 Germinated pumpkin seed sample

Plate 4 Sample kept at water bath for phytic acid analysis