EFFECT OF ROASTING ON BIOACTIVE AND ANTINUTRITIONAL COMPONENTS OF FLAXSEED

(*Linum usitatissimum**)***

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2019

Effect of Roasting on Bioactive and Anti-nutritional Components of Flaxseed (*Linum usitatissimum*)

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

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October, 2019

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Approval Letter

This *dissertation* entitled the *Effect of Roasting on Bioactive and Anti-nutritional Components of Flaxseed (*Linum usitatissimum*)* presented by Merina Dahal has been accepted as the partial fulfillment of the requirement for the Bachelor degree in Science in Nutrition and Dietetics

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October, 2019

Acknowledgement

First and foremost I offer sincerest gratitude to my supervisor, Mrs. Babita Adhikari, who has supported me throughout my dissertation with her patience and knowledge.

I am undoubtedly grateful to Mr. Dambar Bahadur Khadka, Department Head of Nutrition and Dietetics, Central Campus of Technology. I am extremely thankful and indebted to him for sharing expertise, and sincere and valuable guidance and encouragement extended to me and also for providing me with all the necessary facilities for the research. Also, I would like to thank Prof. Dr. Dhan Bahadur Karki, Campus Chief, Central Campus of Technology, for providing the opportunity and facilities to perform this work successfully.

I want to thank my friends especially Pankaj, Shweta and Sunanta. I want to use this chance to thank all the laboratory staffs, library staffs and respected teachers who helped me a lot in completion of my dissertation.

I am also indebted to my parents for their constant encouragement, love and support, without whom this work could not have been completed. My sense of gratitude goes to everyone who directly or indirectly supported me during my research activity.

Date of submission: 2019 ……

(Merina Dahal)

Abstract

Flaxseed (*Linum usitatissimum)* collected from Morang district, Nepal was studied to explore the effect of roasting at 180ºC for 5, 10, 15 and 20 min and soaking (12 hrs followed by sun drying followed by roasting at 180ºC for 15 minutes) on its bioactive compounds (flavonoids, polyphenol and anti-oxidant activity), nutritional and anti-nutritional compounds (hydrocyanic acid and oxalate). The crude extracts of samples were prepared using 80% methanol by maceration technique for analysis of total flavonoid content, polyphenol content and free radical scavenging activity. Experimental data was analyzed using software Genstat 12th Edition.

Roasting of flaxseed had significant impact on its bioactive, nutritional and anti-nutritional components. Flavonoid content increased significantly ($p<0.001$) from 45.33±5.03 mg QE/100g to 238.16±8.8 mg QE/100g on roasting whereas there was no significant increment of flavonoid on soaking. Polyphenols content decreased significantly ($p<0.001$) on roasting and soaking followed by roasting. The highest amount of antioxidant activity was found to be in raw and 15 min roasting i.e 1.57mg AAE/mg DM. And antioxidant activity was further increased on soaking followed by roasting. Hydrocyanic acid on raw sample was 649.3±1.41mg/kg decreased significantly ($p<0.001$) to 115.2±10.41mg/kg at 15 min roasting. Similarly, oxalate content decreased significantly ($p<0.001$) on roasting. Soaking followed by roasting further decreased HCN and oxalate content significantly ($p<0.001$). Protein, fat and fiber content were decreased significantly whereas calcium content increased significantly on 15min roasting. Similarly, on soaking followed by roasting, protein, fat and calcium content were decreased and crude fiber increased significantly.

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List of Abbreviations

Abbreviation	Full form
AAE	Ascorbic Acid Equivalent
ANOVA	Analysis of variance
BP	Boiling Point
DFTQC	Department of Food Technology and Quality Control
FuFoSE	Functional Food Science in Europe
GAE	Gallic Acid Equivalent
LSD	Least Significant Difference
QE	Quercetin Equivalent
USDA	United States Department of Agriculture

Part I

Introduction

1.1 General introduction

Flax (*Linum usitatissimum)* belonging to family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste (Rubilar *et al.*, 2010). Flaxseed is also known as linseed and these terms are used interchangeably. Flaxseed is often used to describe flax when consumed by humans while linseed denotes when it is used specifically for industrial applications (Morris, 2007). Almost all parts of linseed plant are utilized for various purposes (Kajla. *et al.*, 2015). Annual production of flax was 3.06 million tons and Canada is the world's largest producer of flax (about 38% of total production) (Ganorkar & Jain, 2013). Flax is grown both for fiber and for oil, with fiber (for linen) derived from the stem of fiber varieties and oil from the seed of linseed varieties (Diederichsen & Richards, 2003).

The important flaxseed growing countries include India, China, United States, and Ethiopia (Goyal *et al.*, 2014). In Nepal, flaxseed is grown in 14,359 hectares of land area where 7,672 tons is produced annually with the production yield of 534 kg per hectare. The region of highest flaxseed production is the eastern terai with the production of 3,590 tons of flaxseed, where 7980 hectares land is used for its production (Agriculture, 2016).

Beyond its oilseed crop ability, proximate composition of flaxseed makes it more promising for its utilization in different food products. Flaxseed is one of the richest vegetarian sources of α-linolenic acid (omega 3 fatty acid) and soluble mucilage. In present era, consumer's trend towards functional food has increased significantly as health awareness rose (Ganorkar & Jain, 2013). Beside nutritional and medicinal values of flaxseed, anti-nutritional factors such as cyanate, oxalate and phytate are naturally present in it. These anti-nutritional compounds interfere with digestion, absorption and proper utilization of nutrients (Goyal *et al.*, 2014)

It need 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 *et al.*, 2016)

1.2 Statement of problem and justification

The nutritional property and functional property of flaxseed has been getting popularity among the people. Instead of its direct consumption of flaxseed, it should be processed in order to decrease certain anti nutritional factor and to increase availability of nutritional component of flaxseed. Flaxseed is being used in order to control cholesterol level, prevention of atherosclerosis and to prevent from constipation. Consumption of flaxseed 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 (WHO/FAO, 2010) including prebiotic properties of flaxseed and in its beneficial effects on coronary heart diseases, some kinds of cancer; neurological and hormonal disorders (Bassette *et al.*, 2005).

In Nepal, flaxseeds 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 flaxseed has higher concentration of anti-nutritional factor i.e hydrocyanic acid, oxalate and 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. So the optimization of flaxseed at constant temperature with varying time is carried for appropriate processing of flaxseed before consumption.

1.3 Objectives of study

1.3.1 General objective

The general objective of the study was to study the effect of roasting on bioactive and anti-nutritional components of flaxseed (*Linum usitatissimum)*

1.3.2 Specific objective

The specific objectives of the work were to:-

1) Determine the bioactive and anti-nutritional components in raw flaxseed.
2) Study effect of roasting time (5, 10, 15, 20 minutes at 180ºC) on bioactive and anti-nutritional components.
3) Optimize roasting time of the flaxseed based on reduction of anti-nutritional components.
4) Evaluate the effect of soaking (12 hour) followed by sun drying prior to roasting on bioactive and anti-nutritional components.

1.4 Significance of study

In the present world, interest has been increased in search of functional foods to decrease the risk of the evolution of diseases. Flaxseed 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 hydrocyanic acid, 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 hydrocyanic acid, 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 flaxseed as functional food ingredients and also use of flaxseed into

processed foods in the industrial scale which can help to reducing the risk of different diseases like cardiovascular diseases, cancer among increasing population.

1.5 Limitation

a) Certain compouds like lignins, saponin and phytate were not determined.
b) Only one extraction technique was used.

Part II

Literature Review

2.1 Flaxseed

Flaxseed or Linseed (*Linum usitatissimmum*), popularly known as Alas in Nepali languages, is a blue flowering rabi crop and a member of family Linaceae (Ganorkar & Jain, 2013) .The plant is native to west Asia and the Mediterranean. The spherical fruit capsules contain two seeds in each of five compartments. It varies in color dark brown to yellow (Freeman, 1995).

Flax is grown as either an oil crop or as a fibre crop, with fibre (linen) derived from the stem of fibre varieties and oil from the seed of linseed varieties. The seed of flax is flat and oval with a pointed tip, and varies in colour from dark brown to yellow. Depending on the cultivar and growing conditions, flaxseed contains 40-50% oil and meal, comprised of 23-34% protein, 4% ash, 5% viscous fibre (mucilage) and lignan precursors (9-30 mg per g of defatted meal). Annual world production of flax was 3.06 million tons in 1999-2000 and Canada is the world's largest producer of flax (about 38% of total production). Flax is currently the second most important oilseed crop in Western Canada and is grown primarily in the prairie provinces of Saskatchewan (70%), Manitoba (26%), and Alberta (4%) (Hosseinian, 2006). According to Ministry of Agriculture, (2018) the annual flaxseed production in Nepal was 7672 metric tons in the cultivable area of 14359 hector where in province I, II, III, IV, V, VI and VII flaxseed was 3502, 1678, 213, 37, 1713, 116 and 404 metric tons respectively (Agriculture, 2016).

Flaxseeds are available in two basic varieties: (i) brown; and (ii) yellow or golden. Both have similar nutritional characteristics and equal numbers of short-chainω-3 fatty acids. The exception is a type of yellow flax calledsolin(trade name Linola), which has a completely different oil profile and is very low inω-3 fatty acids (Dribnenki *et al.,* 2007). Brown flax is better known as an ingredient in paints, varnish, fiber and cattle feed (Faintuch *et al.,* 2011). Flaxseed is often used to describe flax when consumed by humans while linseed denotes when it is used specifically for industrial applications (Morris, 2007).

2.2 Classification and nomenclature

The scientific name of flaxseed is *Linum usitatissimmum*,. Flaxseed is a member of the flax family, and its taxonomic hierarchy is:

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Superorder	Rosidae
Order	Linales
Family	Linaceae
Genus	Linum L.
Species	L. usitatissimum

(USDA 2017)

2.3 History of consumption of flaxseed

As the source of linen fiber flax has been cultivated since at least 5000 BC, today it is mainly grown for its oil (Oomah, 2001). Nutritional research on flaxseed has increased its potential to be explored as a new ingredient for breads, buns, muffins and other bakery products (Chetana *et al.*, 2010). Humans have been eating flax for thousands of years. Ayurveda remains one of the most ancient and yet tradition practiced is alive widely in India, Sri Lanka and other countries that have a sound philosophical and experimental basis. Ayurveda and traditional Chinese medical system share many common approaches and have a long history of practice (Patwardhan *et al.*, 2005). Ayurvedic literature describes more than 200 herbs, minerals and fats for skin care. Flaxseed oil is believed to bring mental and physical endurance by fighting fatigue and controlling aging process. According to Ayurveda, flaxseed has properties like Madhura (balances the skin pH), Picchaila (lubricous), Balya (improves tensile strength or elasticity of the skin), Grahi (improves moisture holding capacity of skin), Tvagdoshahrit (removes skin blemishes), Vranahrit (wound healing) and useful skin disorders including dryness, undernourishment, lack of luster/glow (Goyal *et al.*, 2014).

Various medicinal uses of flaxseed are found to be described in history. About 650 B.C., Hippocrates, the father of medicine, advocated flaxseed for the relief of abdominal pains and Theophrastus recommended the use of flax mucilage as a cough remedy. In about first century A.D., Tacitus praised the virtues of flax. About 8^{th} century A.D., Charlemagne considered flax so important for the health of his subjects that he passed laws and regulations requiring its consumption. in about 15^{th} century A.D., Hildegard von Bingen used flax meal in hot compresses for the treatment of both external and internal ailments (Goyal *et al.*, 2014).

2.4 Physical and functional properties

It is essential to study the physical properties of kernels, grains and seeds which are necessary for the design of equipment to handle, transport, process and storage. Commercial utilization of flaxseed proteins in food products depend on its functional properties before its incorporation in various food products. Study of functional properties is necessary where functional properties have the role in food formulations. Bulk density is one of the functional properties where higher the bulk density is desirable as this property to reduce the paste thickness. This is an important factor in infant formulas where bulk is of concern (Anwar *et al.*, 2005).

In the study of Janaki, Surabhi and Nagarkot varieties flaxseed (*Linum usitatissimum*), it was reported that flaxseeds are in oval shape and brown in colour with smooth glossy appearance. Thousand seeds weight ranged from 5.01 to 6.05 g and seed length ranged between 4.50 to 5.45 mm per seed in three varieties. The seeds were reported to be flat and pointed and the width ranged between 0.90 to 1.45 mm (Arora & Rajni, 2006). Coskuner and Karababa (2007) analyzed some physical properties of flaxseed *(Linum usitatissimum* L.) in which the value of bulk density was found to be .66 g/cm3. On the same study it was reported that the bulk density values of 726.6–555.6 kg/$m^3$for the moisture range of 6.09–16.81% (d.b.), whereas on other study it was observed that the bulk density of 690.5–545.0 kg/m^3 for the moisture range of 6.09–16.81% (d.b.) for the commercial variety of linseed. The decrease in bulk density of flaxseed may be due to the increase in seed size with moisture content which gives rise to decrease in quantity of seeds occupying the same bulk volume (Khan *et al.*, 2016).

Similarly in another study carried to analyzed of functional properties of full fat roasted and non-roasted flax seed. There was no significant difference between full fat roasted and full fat unroasted as bulk density was 0.83 and 0.78 g/ml, water absorption capacity was 1.83 and 1.48 g/g and fat absorption capacity of 1.31 and1.20 g/g respectively. This study showed the higher fat absorption in flax flour of full fat roasted (Hussain *et al.,* 2011). On the observation of functional properties of flaxseed protein concentrate found that it has better foam stability and emulsifying capacity was 83.3% and 84.76 ml/g and emulsifying activity was 88.37% (Flores *et al.*, 2006). The improvement in functional properties may be achieved either by genetic modification, physical treatment or chemical processing Water absorption is the ability of protein to bind water imparts moisture to many food products. The difference in the water absorption capacity between the varieties may be due to the differences in the proportion of hydrophilic groups (Oomah., 1993). The study reported that higher values for water absorption capacity, may be due to reduction of polyphenols and phytates, which might have made more protein available for holding the water (Pawar *et al.*, 2001).

2.5 Nutritional composition and effect of processing

Various edible forms of flax are available in the food market—whole flaxseeds, milled flax, roasted flax and flax oil. According to its physicochemical composition, flaxseed is a multicomponent system with bio-active plant substances such as oil, protein, dietary fiber, soluble polysaccharides, lignans, phenolic compounds, vitamins (A, C and E) and mineral (P, Mg, K, Na, Fe, Cu, Mn and Zn) (Goyal *et al.*, 2014). According to food composition table by DFTQC the nutritional composition of flaxseed is 28.3g carbohydrate, 37.7 g fat, 20.3 g protein 2.4g minerals and 4.8g fiber per 100gm.

Table 2.1: Nutritional composition (%) of flaxseed

Component	Golden flax	Brown Flax
Moisture	6.73±0.03	6.52±0.04
Ash	2.84±0.01	2.63±0.01
Total lipids	37.57±0.71	38.13±1.39
Crude protein	23.24±0.06	24.42±0.11
Carbohydrates	29.61±0.76	28.29±1.45

(Sheisa *et al.*, 2013)

On a study of four varieties of flaxseed, the moisture content in raw and germinated flaxseeds varied from 6.65% to 7.29%. Germinated flaxseeds were more nutritious as compared to raw flaxseeds. Raw flaxseeds were rich in macro minerals whereas germination significantly improved the micro mineral profile of flaxseeds. Germinated seeds revealed maximum significant increase for iron, zinc and manganese. Germination process significantly reduced the cyanogenic glycosides and phytic acid. Maximum reduction for cyanogenic glycosides (82.37%) was noted in JLS-9, whereas phytic acid decline to 52.27% (Kajla *et al.*, 2017).

2.6 Dietary Fiber

Flaxseeds serve as a good source of both soluble and insoluble dietary fiber. Flaxseed holds a unique place among the oilseeds due to presence of mucilage located in outer layers of the seed (Singh *et al.*, 2011). Flaxseed mucilage has gained momentum due to its superb health benefits and potential functional properties (Mazza & Biliaderis, 1989; Susheelamma, 1987). It contains 35 – 45 % of fibre and two-third is insoluble and one third is soluble fiber. Insoluble fiber consists of cellulose, hemicellulose and lignin (Morris, 2007; Oomah., 1993). Most of the soluble fiber of flaxseed appears to be the mucilage of seed coat. It makes up 7 – 10 % of seed weight (Mazza & Biliaderis, 1989). Soluble fiber in the form of mucilaginous material consists mainly of water soluble polysaccharides; its recovery and purity vary with the extraction conditions. The water binding capacity of flaxseed mucilage is reported to be about 1600 – 3000 g of water/ 100 g of solids. High water binding capacity of flaxseed is attributed due to the presence of polysaccharides in the seed coat. Mucilage of flaxseed consists of acidic and neutral polysaccharides. The neutral fraction

constitutes L-arabinose, D-xylose and D-galactose and arabinoxylan and acidic fraction contains L-rhamnose, L-fucose, L-galactose and D-galactouronic acid. Functionally, these polysaccharides possess similar properties to guar gum (Wanasundara & Shahidi, 1997). The mucilage can be extracted by water and exhibit good foam-stability properties (Susheelamma, 1987).

Metabolism

Dietary fiber of flaxseed reaches the large intestine and is fermented by colonic micro flora with production of short chain fatty acids (SCFA), hydrogen, carbon dioxide, methane and biomass and exhibit laxative effects (Kritchevsky, 1979).

In the large intestine, both soluble and insoluble fibers have their bulking effect resulting in increasing both dry and wet weight of the colon contents and feces. Soluble fiber increases water binding, initially by the binding capacity of its macromolecules, later by increasing the mass of microbial cells. The contribution of soluble fiber to fecal weight was insignificant compared to insoluble fiber. Recent studies, however, have shown that it is of the same magnitude (Malkki, 2004).

2.7 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 isocoumarins, naphthoquinones, xanthones, stilbenes, anthraquinones, flavonoids, lignans and tannins. Among these, phenolic acids and flavonoids are more common (Dykes & Rooney, 2007). In addition to protective effect, phenolics are responsible for color, taste, organoleptic properties of the plant origin foods (Yanez *et al.*, 2004).

2.7.1 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. Flaxseed was reported to contain 8-10 g/kg total phenolic acids, about 5 g/kg of esterified phenolic acids and 3-5 g/kg of etherified phenolic acids (Oomah., 1993).

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 p-hydroxybenzoic, 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., 1993).

2.7.2 Flavonoids

Flavonoids are the polyphenols, with C_6-C_3-C_6 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-O-diglucopyranoside. Herbacetin diglucoside (HDG) are ester linked in the lignan macromolecule via 3-hydroxy-3-methylglutaric acid (HMGA) (Struijus *et al.,* 2007).

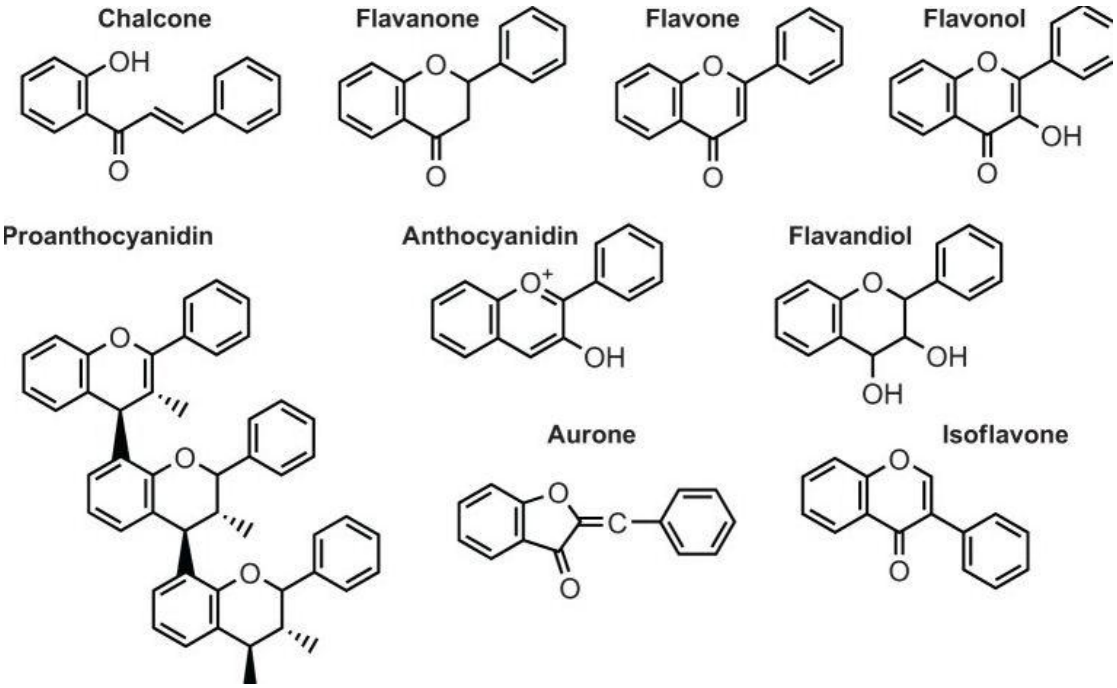

Fig 2.1: Basic structure of flavonoids

A study found that the flax contained about 35-70 mg of flavonoids/100g (Oomah, 1996). In a study the quantitative estimation of the total flavonoid in raw and roasted flaxseed powder was found to be 0.11mg, and 0.23mg per kg respectively. Thus from the quantitative estimation, it was clear that roasting was the best method of processing flaxseed and had considerably high amounts of flavonoids (Dharshini *et al.*, 2013)

2.7.3 Antioxidant and its activity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron or hydrogen from a substance to an oxidizing agent. The oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. An antioxidant can terminate these chain reactions by removing free radical intermediates and gets oxidized. It also inhibits other oxidation reactions to occur (Akande *et al.*, 2010).

Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds

like phenols and flavonoid scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases. There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. Fortunately, nature has provided us with plenty of "protecting molecules" or the so called "antioxidants" which can trap or destroy free radicals and subsequently protect us from damage due to the oxidative stress (Verma & Vinayak, 2008). Tocopherols have their own antioxidant activity, including hydrogen atom transfer at 6- hydroxyl group on the chroman ring and scavenging of singlet oxygen and other reactive species. The antioxidant activity of ascorbic acid is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and the removal of molecular oxygen (Lee *et al.*, 2004). Chen and Chu (2006) reported that flavonoids have the most potent antioxidant activity because their chemical structure contains an O-diphenolic group, a 2-3 double bond conjugated with 4-oxo function and hydroxyl groups in the position 3 and 5.

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 (Tailor. *et al.*, 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:

$$DPPH\bullet + FE \rightarrow DPPH - H + A\bullet \quad (1)$$

$$DPPH + A\bullet \rightarrow DPPH - A \quad (2)$$

$$A\bullet + A\bullet \rightarrow A - A \quad (3)$$

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 (Tailor. *et al.*, 2014).

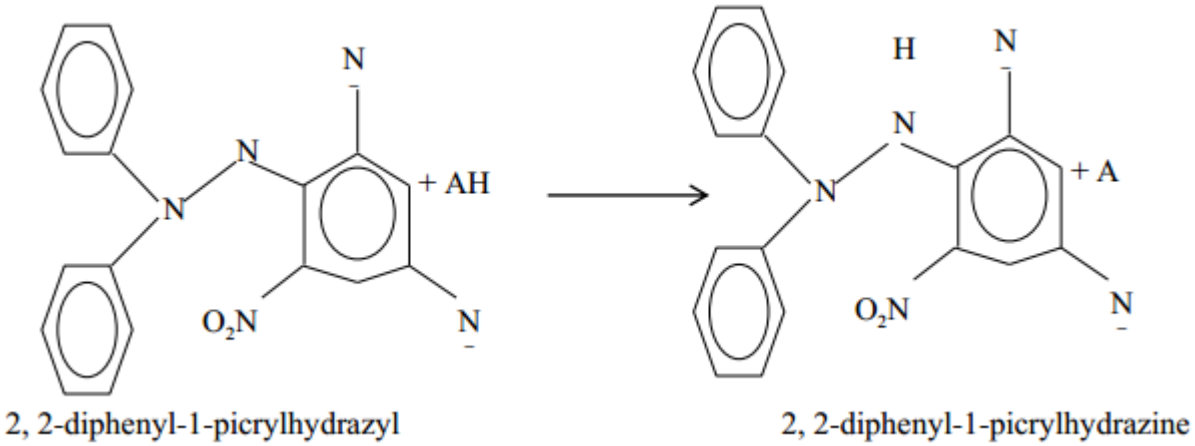

Fig 2.2: Reaction of DPPH -free radical with an antioxidant

2.7.4 Lignins

Lignans are phytoestrogens, which are abundantly available in fiber rich plants, cereals (wheat, barley, and oats), legumes (bean, lentil, soybean), vegetables (broccoli, garlic, asparagus, carrots) fruits, berries, tea and alcoholic beverages. Flaxseed contains about 75–800 times more lignans than cereal grains, legumes, fruits and vegetables (Kajla. *et al.*, 2015). Secoisolariciresinol diglycoside (SDG) is the major lignan of flaxseed, along with minor contents of matairesinol, pinoresinol, lariciresinol and isolariciresinol (Krajcova *et al.*, 2009). SDG ranges from 11.7 to 24.1 mg/g in defatted flour and 6.1 to 13.3 mg/g in whole flaxseed flour (Johnsson *et al.*, 2000).

Lignans are the diphenolic compounds synthesized by the coupling of two coniferyl alcohol residues existing in cell wall of higher plants (Toure & Xueming, 2010). Flaxseed lignans play an important role in preventing various types of cancer specially the hormone sensitive ones. Flax lignans are reported to have antioxidant property which presumably is the main reason of the anticancer activity. The lower incidences of prostate and breast cancers in Asian men and women compared to European men and women has been speculated to be due to the higher consumption of diets rich in fruits and vegetables. Various clinical studies imply that lignans prevent breast cancer by balancing the hormonal mechanisms. The lignans inhibit the aromatase activity in adipose tissue resulting in the circulation of estrogen (Kajla. *et al.*, 2015).

2.8 Anti-nutritional 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).

2.8.1 Hydrocyanic acid

Flaxseed contains cyanogenic compounds of 264–354 mg per 100 g. 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 & 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). Food Standards and Safety Authority of India (FSSAI) mentioned maximum permissible limit of hydrogen cyanide in food grains as 37.5 mg/kg (FSSAI Act, 2006). Considering the concentration of cyanogenic compounds in flaxseed as reported in literature, the daily ingestion of flaxseed indicated (30 g) may contain on average 106 mg of cyanogenic compounds, which is above the tolerable level. However it gets reduced when subjected to thermal and mechanical processes. Same study reported that cyanogenic glycoside measured as HCN/100 g flaxseeds, decreased from 20.8 to 1.0 mg/100 g after roasting (Hiremath, 2013).

Flaxseed contains cyanogenic glycosides and linamarin (acetone–cyanohydrinbeta–glucoside $C_{10}H_{17}O_6N$) in small amounts (Hall *et al.*, 2005). Whole flaxseed contains 250–550 mg/100 g cyanogenic glycosides (Mazza, 2008), of which linustatin and neolinustatin are the major components. (Park *et al.*, 2005) reported

207 and 174 mg/100 g seed of linustatin and neolinustatin, respectively in flaxseed. Upon seed damage, β-glucosidases are triggered and contribute to releasing the poisonous hydrogen cyanide (HCN). However, adequate processing of foodstuffs containing cyanogenic glycosides helps in reducing the potential risks associated with poisoning. Flaxseed meal also contains 10 mg/100 g Linatine (gammaglutamyl- 1-amino-D-proline) which induces vitamin B6 deficiency (Mazza, 2008). The linatine (a vitamin B6 antagonist) in flaxseed did not affect vitamin B6levels or metabolism in people fed up to 50 g of ground flaxseed per day. It has been reported that flaxseed depressed vitamin E levels in rats only when fed at very high levels (Ratnayake *et al.*, 1992). The cyanogenic glycosides in flaxseed raise thiocyanate levels in the blood very briefly, after which the levels drop, but even these levels are less than those of persons smoking tobacco (Kajla. *et al.*, 2015).

In a study, it was found that HCN content was removed by more than 85 % of linustatin and neolinustatin when flaxseed was heated for more than 2 h at 200 °C (Park *et al.*, 2005).

2.8.2 Oxalic acid

The anti-nutritional factor that is of primary concern is oxalic acid. Oxalic acid (ethanedioic acid, $H_2C_2O_4$) is a strongly oxidized and corrosive compound with good chelating activity, synthesized by a broad range of animals, plants and microorganisms (Stewart *et al.*, 2004). Oxalic acid and its salts are extensively spread in numerous plant tissues as the end products of metabolism. Oxalic acid content in foodstuffs has long been a concern in human diets, due to the negative health effects connected to a high intake of oxalic acid. Incidences of kidney stones, hypocalcemia and hyposideremi (low plasma levels of calcium and iron) that correspond strongly with the intake of oxalic acid that perform as an absorption inhibitor are common (Palaniswamy *et al.*, 2002).

High oxalate content in urine and blood causes several diseases such as hyperoxaluria and vitamin deficiencies. Small dose of oxalate in the body may result in pain, headaches, and twitching in muscles and cramps. Larger doses can result in a drop in blood pressure, weak, irregular heartbeat and signs of heart failure. Large

doses of oxalate may rapidly put a person in a shock-like state, causing convulsions (because of low plasma calcium), coma, and even death. The mean fatal dose for an adult is about 15 to 30 g, but the lowest reported fatal dose is merely 5 g (or about 70 mg/kg) (Tsai *et al.*, 2005). Consumption of foods high in oxalic acid in the long term can be troublesome. Healthy persons can securely consume such foods moderately, but those with gout, rheumatoid arthritis, kidney disorders, or certain forms of chronic vulvar pain (vulvodynia) are normally advised to stay away from foods high in oxalates or oxalic acid (Shimi & Haron, 2014). On flaxseed powder the oxalate content was found to be 2 to 10 mg per serving (oxalate content) while in roasted flaxseed it was found to be less than 2 mg per serving (oxalate content).The oxalate concentration was in the range of 6.43-19.40 mg/100 g for whole cooked samples, 9.03-11, 90 mg/100 g for raw soy products, and 4.36-7.99 mg/100 g for cooked ones (Shimi & Haron, 2014).

In a study it was found that oxalate content in raw flaxseed range from 2 to 10 mg/kg (Hiremath, 2013). The sample contained 1.513±0.025 mg/Kg oxalate; hence it may be considered a low oxalate food. Hui (1992) stated that intake of 5g or more oxalic acid would be fetal to humans but the negative effect can be seen at even low values. In a study the roasted pistachio and chestnuts contained very low level than that of raw samples (Ritter & Savage, 2007).

2.8.3 Phytate

Phytic acid, another anti-nutrient present in flaxseed, ranges from 23 to 33 g/kg of the flaxseed meal. Phytic acid interferes with the absorption of calcium, zinc, magnesium, copper and iron. It is a strong chelator, forming protein and mineral-phytic acid complexes and thus reducing their bioavailability (Oomah., 1993).

Phytate (also known as Inositol hexakisphosphate (InsP6)) is the salt form of phytic acid, are found in plants, animals and soil. It is primarily present as a salt of the mono- and divalent K+, Mg2+, and Ca2+ and accumulates in the seeds during the ripening period. It serves as a storage of phosphorous and minerals and accounts for 60-90% of the phosphorous in the plant. Besides phytate, other inositol phosphates are present in the seeds, however to a much lower extent. In addition, phytate has been

suggested to serve as a store of cations, of high energy phosphoryl groups, and, by chelating free iron, as a potent natural anti-oxidant (Mueller, 2001).

In cereals, phytate is located up to 80% in the aleurone layer, but is also found in the germ, while the endosperm is almost free of phytate. During the germination of seeds, phytate is hydrolysed, and phosphorous along with minerals such as calcium, magnesium and iron are liberated, becoming available for germination and development of the seedlings (Frolich *et al.*, 2011).

2.8.4 Tannins

Tannins are very important commercial products. However, their chemistry is very complex and diverse. They can be classified into two groups, the proanthocyanidins (or condensed tannins) and the polyesters of gallic acid and (or) hexahydroxydiphenic acid (hydrolysable tannins, respectively, gallo- and ellagitannins) (Mahmut & Ayhan, 2002). Condensed tannins are derivatives of flavanols and hydrolysable tannins are esters of a sugar, usually glucose (Bartosz *et al.*, 2017). The co-occurrence of both kinds of tannins in the same plant or plant tissue is often observed. Tannins are found in the leaves, fruits, barks, roots and wood of trees (Mahmut & Ayhan, 2002).

Trypsin inhibitors are anti-nutritional factors which form an indigestible complex with trypsin. This prevents trypsin activity and thus decreases trypsin concentration in the small intestine. The pancreas of the animal compensates for this by secreting more trypsin and thus increases the protein requirements of the animal for these increased secretions. Trypsin inhibitors are present in flaxseed meal; their activity level appears to be much lower than the trypsin inhibitors found in both soybean meal and canola meal. The same researcher reported the trypsin inhibitor activity (TIA) level for soybean meal is 1650 units of TIA, canola meal is 99 units of TIA and for flaxseed meal it ranges from 42 to 51 units of TIA depending on cultivar and processing (Bhatty, 1993).

Preeti and Chimmad (2010) analysed the antinutritional factors in flaxseed and recorded 325mg/100g tannins and phytic acid of 969mg/100g. Trypsin inhibitors are reported in flaxseed, though activity is insignificant as compared to soybean and canola seeds (Bhatty, 1993).

2.9 Health benefits of flaxseed

General recommendation for daily intake has been 1–3 table spoons per day for ground flaxseed or 1 table spoon for flaxseed oil. Flaxseed is emerging as one of the nutritive and functional ingredient in food products. Scientific findings are growing in support of flaxseed consumption. More studies are needed to resolve the conflicting reports regarding the health benefits, in particular the role of ALA and SDG in prostate cancer and cancer in general (Ganorkar & Jain, 2013). Consumption of 50 g of ground flaxseed daily for four weeks lead to reductions in serum total cholesterol level up to 6-9% and low density lipoprotein-cholesterol (LDL-C) up to 9-18% in healthy young adult men and women with moderately high levels of blood cholesterol of 20 women (Bierenbum *et al.*, 1993).

Flaxseed varieties contain usually about 40% of oil in the seeds. Flaxseed, besides its traditional oleo chemical uses, is now gaining recognition as a functional food ingredient for human nutrition (Oomah, 2001). Seeds of flax are the richest source of alpha-linolenic acid, lignans and other nutritional components. In term of polyphenol content, flax seeds are at the top among plant species and phenolic compounds are excellent natural antioxidants (Kasote, 2013).Consumption of flaxseed has been demonstrated to have positive health benefits including decreasing rate of tumor growth, reducing serum cholesterol level and decreasing incidence of breast, prostate and colon cancers. The health benefits of flaxseed can be credited mainly to its abundance to biologically active components (Hussain *et al.*, 2011). In particular, phenolic compounds (lignans, phenolic acid, flavonoids and phenylpropanoids) are excellent in preventing the excess of free radicals and avoiding their pathological effects (Kasote, 2013). Phenolic compounds exert their antioxidant capacity by acting as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Therefore, there is a huge interest in assessing the variability in antioxidant activity in flax seeds as these are potential ingredients in foods, as well as in the pharmaceutical and cosmetic industries. In order to better define the nutraceutical attributes of flaxseed, there is great interest in the characterization of phytochemicals and antioxidant properties of this crop (Russo & Reggiani, 2014).

Flaxseed contains n-3 fatty acids, soluble fibers, vitamin E, lignans, and other phenolic and peptide compounds which are found to exert potential diverse actions thought to benefit health e.g., anti-inflammation, vessel relaxation, antioxidant, hypo cholesterolemic, anti-carcinogenic, and attenuation of the postprandial insulin response. Ground flaxseed of 50g/day consumed over 4 weeks increased the average daily ALA plasma levels by about 10 times in healthy adults (Cunnane *et al.*, 1995). Alpha-Linolenic acid, found in flaxseeds, promote bone health by minimizing excessive bone turnover by lowering the ratio of w-3 to w-6 fatty acid in the diet. The cholesterol-lowering effects of flaxseed may be due to the activity of single or multiple components, including a-linolenic or linoleic acids, total and soluble fiber and non-protein constituents present in these seeds. As one of the richest source of plant protein, having protein content in the range of 20–25%, it is ideal for vegetarians. Flaxseed gum, a hydrocolloid, has marked swelling capacity and becomes highly viscous in aqueous solution and therefore has good water-holding capacity. Flaxseed gum also exhibits "weak gel"-like properties that can be used to replace most of the non-gelling gums for food and nonfood applications (Sudha *et al.*, 2010).

Flaxseed oil contains one of the essential fatty acids, alpha linolenic acid (Omega-3 fatty acid) which play an important role in reducing the risk of cardiovascular diseases. All the omega-3 fatty acids regulate the cholesterol, triglycerides and blood pressure, whereas alpha linolenic acid especially helps in proper growth of infants. The effect of partly ground and defatted flaxseed (*Linum usitatissimum*) on constipation patients, predominant irritable bowel syndrome was found. In a study, patients who had flaxseed (6-24 g/day) decrease in constipation and abdominal symptoms. After the open period of three months continuation, constipation and abdominal symptoms were significantly decreased (Hiremath, 2013).

2.10 Processing methods to reduce ANFs

The abundance of anti-nutritional factors and toxic influences in plants used as human foods certainly calls for concern. Therefore, ways and means of eliminating or reducing their levels to the barest minimum should be discovered (Soetan & Oyewole, 2009).

2.10.1 Heat treatment

Heat treatment is a usual process in food processing. It is an effective means of inactivating the thermo-labile ANF. This improves protein quality by inactivating anti-physiological factors, particularly trypsin inhibitor and haemagglutinins and by unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes (Alegbejo, 2013). Also dry heat was less effective than cooking (moist heat) for the improvement of growth promoting action in soybeans but the degree of inactivation is governed by temperature, duration of heating and particle size.

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).

Blanching

Noonan and Savage (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, 1996)

Extrusion

It is a form of high temperature short time (HTST) processing involving a combination of high temperature, pressure, and shear processing and is responsible for reducing the ANF content in food. Extruded amaranth grain exhibited better nutritional value than raw amaranth and the product required no additional cooking prior to consumption (Mendoza & Bressani, 1987).

2.10.2 Soaking

This process implies exposure to water and salt solutions with or without additive to encourage ANF loss. Oxalates and tannins may be removed from food by cooking in water, although this is not the most effective method. Soaking followed by wet

cooking may reduce oxalates more rapidly when compared with just wet cooking (Hotz & Gibson, 2001).

2.10.3 Germination

Germination and sprouting are commonly used processing methods for improving the eating quality of cereals and legumes. The grains are soaked with potable water, drained and finally spread in favorable condition to promote faster germination. Sprouting initiates three main types of chemical changes in the seed which include the breakdown of certain materials, transport of materials from one part of the seed to another especially from the endosperm to the embryo or from the cotyledons to the growing parts and the synthesis of new materials from the breakdown product formed. Germination of the grain has important effects on the chemical composition, nutritive value, and acceptability characteristics of products for human consumption. During seed germination, a breakdown of seed reserves, carbohydrates, and in some cases protein takes place. Germination causes an increase in several vitamins (Chen. *et al.*, 1975).

2.11 Method of 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

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 (Flores *et al.*)

2.12 Effect of different method of processing

2.12.1 Heat treatment

Heat treatment is a usual process in food processing. It is an effective means of inactivating the thermo-labile ANF. This improves protein quality by inactivating anti-physiological factors, particularly trypsin inhibitor and haemagglutinins and by unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes (Alegbejo, 2013) Also dry heat was less effective than cooking (moist heat) for the improvement of growth promoting action in soybeans but the degree of inactivation is governed by temperature, duration of heating and particle size.

In a study, flavonoids contained in the group of selected nuts and oil seeds were significantly increased on roasting. The increment of flavonoid was explained with breaking of binds in the structure of lignin complex and also other phenolic compounds (Kamalaja *et al.*, 2018). Flavonoid content of the processed flaxseed ranged from 0.005-0.12mg with roasted flaxseed having the high amount of flavonoid (0.12 mg) and with soaked flaxseed having the low amount of flavonoid (0.005 mg). Alkaloid content was high in raw flaxseed with (0.23mg) and least amount in pressure cooked with 0.05 mg. High content of total phenol was found in roasted and raw flaxseed with 47mg gallic acid equivalent and low content of total phenol was found in soaked flaxseed, with 6.34 mg gallic acid equivalent. The results of the antioxidant activity of the flaxseeds showed a positive DPPH radical scavenging activity. Raw flaxseed extracts had 35 percent; pressure cooked flaxseed extract had 69 percent and both roasted and soaked flaxseed extracts had 76 percent, which were the highest among the selected processing methods (Dharshini *et al.*, 2013). Microwaved

sunflower seeds (Helianthus annuus L.) of two varieties were extracted using n-hexane. The oilseed residue analysis revealed no changes in the contents of fiber, ash, and protein that were attributable to the roasting (Anjum *et al.*, 2006). In a study, it was found that heating process caused loss of phenolic acids (84-60 as gallic acid equivalents (mg/100g oil) contents, which confirmed that thermal treatment causes an oxidation and polymerization of phenolic compounds (Herchi *et al.*, 2016)

The behavior of antioxidant activity depending on the degree of roasting can probably be attributed to the loss of poly-phenolic compounds, and to the successive formation of other antioxidant compounds, such as Maillard reaction products, which are lost or undergone pyrolysis when more severe thermal conditions are applied. Some studies shows all of the coffee brews presented a high antioxidant activity in all tested concentrations, but the antioxidant activity decreased with roasting. At any degree of roasting, the antioxidant activity of semi-dry and natural coffees were quite similar, except for the dark natural, that showed a significantly lesser antioxidant activity than the other samples (Maris *et al.*, 2005).

Table 2.2: Chemical analysis of different flaxseed flours

	Full fat roasted	Full fat non-roasted	Partially defatted roasted	Partially defatted non roasted
Moisture%	4.23±0.16	4.53±0.12	3.96±0.10	4.13±0.09
Protein%	21.27±0.68	21.23±0.53	34.55±0.78	34.48±0.87
Fat%	38.53±1.32	38.76±1.28	5.51±0.03	5.35±0.02
Ash%	3.48±0.10	3.47±0.13	5.66±0.21	5.61±0.17
Fiber%	8.12±0.32	8.02±0.27	12.39±0.45	12.31±0.38
HCN mg/kg	20.58±0.87	145.72±5.21	25.42±0.73	197.57±7.65

(Shahzad *et al.*, 2008)

In a study of proximate analysis of roasted and unroasted sunflower oilseeds of different varieties, the oil content, protein content, fiber content and ash content in unroasted sunflower oilseed ranged from 35.8 to 38.78%, 21 to 24.94%, 7.01 to 9.50% and 5 to 5.5% respectively. Similarly, on roasted sample of 5 minutes, 10 minutes and 15 minutes, the oil content ranged from 30.25 to 38.58%, 30.02 to 37.81% and 28.732 to 36.56% respectively; the protein content ranged from 20.69 to

24.94%, 20.70 to 24.21% and 20.59 to 24.25% respectively. Also, the fibre content ranged from 7 to 9.50%, 6.79 to 9.41% and 6.71 to 9.02% ; and the ash content ranged from 4.98 to 5.50%, 4.90 to 5.48% and 4.82 to 5.45% on roasted samples of 5, 10 and 15 minutes respectively (Yunusa, 2015).

A study reported microwave roasting of flaxseed reduced the HCN content by 83.3% which may be due to deactivation of glycosidase or evaporation of HCN (Feng *et al.*, 2003). Cyanogenic glycosides are heat labile and easily destroyed by processing methods namely autoclaving, microwave roasting, pelleting and by certain detoxifying enzymes such as β-glycosidases, releasing hydrogen cyanide which can be evaporated by using steam (Cunnane *et al.*, 1993).

2.12.2 Soaking

This process implies exposure to water and salt solutions with or without additive to encourage ANF loss. Generally cereals and legumes are soaked in water overnight; phytate is water-soluble, so a considerable amount of phytate is removed into water. In addition, this process also enhances the action of naturally occurring phytate in cereals and legumes (Greiner & Konietzny, 1999).

Noonan and Savage (1999) suggested that oxalate also can be reduced by leaching in soaking solutions. Oxalates and tannins may be removed from food by cooking in water, although this is not the most effective method. Soaking followed by wet cooking may reduce oxalates more rapidly when compared with just wet cooking. As HCN and cynogenic glycosidase was found significantly lower because of its extreme solubility in water (FAO/WHO, 1965). In a study of chickpea, soaking the seeds at room temperature (for 22 h) resulted in a smaller decrease in polyphenols content, total flavonoids contents than soaking at 60˚C (for 2 h) (Segev *et al.*, 2011).

Part III

Materials and Methods

3.1 Materials required

Material:

The raw material for the study was flaxseed (*Linum usitatissimum*) collected from the local market of Morang.

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 was provided by Central Campus of Technology (CCT), Dharan

Chemicals needed:

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

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.

3.2 Methodology

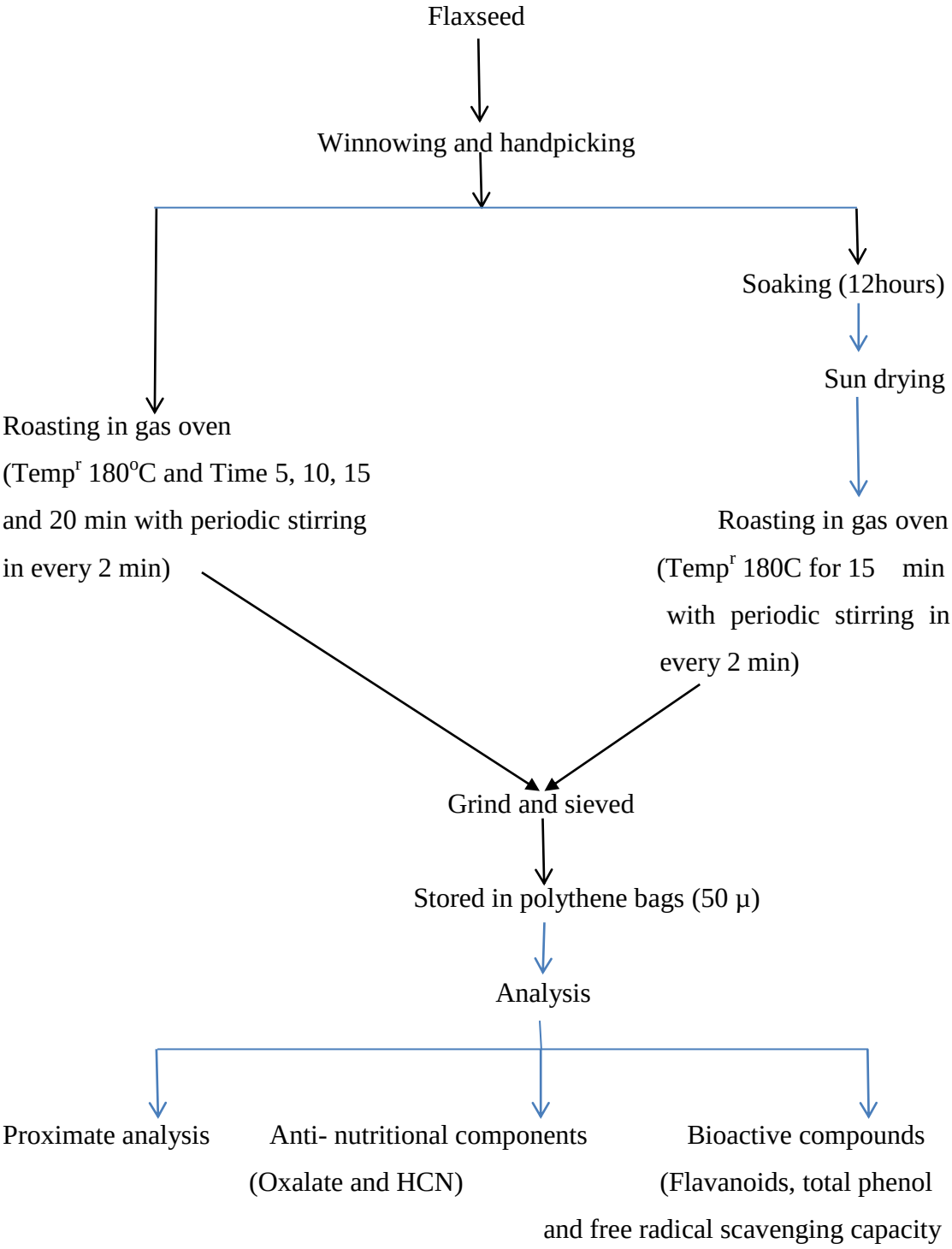

Fig 3.1: Steps of processing and analysis

3.3 Roasting of flaxseed

Flaxseed (*Linum usitatissimum*) was 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 three portions as raw sample, roasted sample and soaked sample. Roasting was done in a gas oven at 180ºC for 5, 10, 15 and 20 minutes. In each lot 50gm sample was roasted in a single layer and periodic stirring was done.

3.4 Soaking and roasting

Similarly, soaking was done for 12 hours followed by sun drying (moisture content 8.12%) and then roasted in a gas oven at 180ºC for 15 minutes.

3.5 Grinding and packaging

All the samples were powdered in grinder and sieved and packed in polythene bags of 50 µ separately. Thus, six different samples were prepared for analysis.

3.6 Preparation of methanolic extract of the samples

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%) (Karakaya, 2004).

3.7 Determination of Chemical composition

3.7.1 Determination of moisture

The moisture content of flaxseed powder was determined by heating (Raganna, 2008).

3.7.2 Determination of protein

The crude protein content of samples was determined by Kjeldahl method using factor 6.25 (Raganna, 2008).

3.7.3 Determination of fat

The fat content was determined by using standard Soxhlet apparatus using hexane (BP 65ºC) as solvent (Raganna, 2008).

3.7.4 Determination of crude fiber

The crude fiber content was determined by chemical method (Raganna, 2008).

3.7.5 Determination of total ash, calcium and iron

Total Ash, calcium content and iron content of the samples was determined by following drying ashing, volumetric and colorimetric methods respectively (Raganna, 2008).

3.8 Qualitative analysis

1) Test for total phenols

2 ml of 2% solution of $FeCl_3$ mixed with crude extract, presence of black or blue green color indicates the presence of total phenols (Jaradat *et al.*, 2015).

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).

3.9 Quantitative Analysis of nutritional component

3.9.1 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 (Berk & Hasmadi, 2015).

3.9.2 Determination of total polyphenols

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 were calculated using the standard curve equation and expressed in terms of Gallic acid equivalent (mg/ml) of dry mass (Jaradat *et al.*, 2015).

3.9.3 Determination of antioxidant activity

Antioxidant activity was measured utilizing 2, 2-diphenyl-1-1 picrylhydrazyl (DPPH) radical scavenging capacity. Free Radical Scavenging Activity Using 1,1-Diphenyl-2Picryl Hydrazyl (DPPH). DPPH, a commercial oxidizing radical is reduced by antioxidants. The disappearance of the DPPH radical absorption at a characteristic wavelength is monitored by decrease in optical density (Singh, & Jayaprakasha, 2002). Different concentrations of the methanolic extract were taken in different test tubes and the volume made to 1 ml with methanol. 4 ml of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. A control was prepared as above without any sample and methanol was used for base line correction. Changes in absorbance of samples were measured at 517 nm. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

% Free radical Scavenging Capacity = (Control OD- Sample OD)*100/Control OD

The free radical scavenging activity is also expressed by the antioxidant concentration required for a 50% DPPH reduction (IC_{50}) (Brodowska *et al.*, 2014)

3.10 Quantitative analysis of Anti nutritional factors

3.10.1 Determination of Hydrocyanic acid content

The alkaline titration method of AOAC was used for determination of hydrocyanic acid (AOAC, 2005). A sample of flaxseed powder (0.1g) was placed into Kjeldhal flask, to which 100 mL of water was added and allow standing for 2 hours. The mixture was subjected to steam distillation and 60 ml distillate was collected in 2.5% NaOH solution and diluted to 250mL. An aliquot of 100 mL was taken; to which 8 mL of ammonium hydroxides (NH_4OH) and 2 mL of 5% potassium iodide was added

and titrate with 0.02M Silver nitrate ($AgNO_3$) solution till permanent turbidity appear. The HCN content was calculated as:

1 ml $AgNO_3$ = 1.08 mg HCN

3.10.2 Determination of Oxalate content

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

1 ml 0.05 $KMnO_4$ = 2.2 mg oxalate

3.11 Statistical analysis

All analyses were carried out in triplicates. Statistical significance was established using general analysis of variance (ANOVA) models to estimate the effect of roasting and soaking on nutritional and anti-nutritional components of flaxseed (*Linum usitatissimum)*. Means were separated according Fisher's unprotected at $p<0.05$, with the help of the software Genstat 12th Edition.

Part IV

Results and Discussion

A common variety of flaxseed (*Linum usitatissimum*) was collected from the local market of Morang to study the effect of roasting and soaking on bioactive components (flavonoid, total phenol content and antioxidant activity) and anti-nutritional factors (hydrocyanic acid and oxalate). Roasting was done at 180ºC for 5, 10, 15 and 20 minutes and soaking (12 hours followed by sun drying) followed by roasting at 180ºC for 15 minutes was done. The powdered samples were analysed for the effect of roasting time on the nutritional and anti-nutritional components.

4.1 Physical properties

Flaxseed was analyzed for the physical properties which are presented in Table 4.1. The mean length of the raw flaxseed was 3.8±0.3mm and the mean bulk density of the raw flaxseed was 672±45 kg/m^3.

Table 4.1: Physical properties of flaxseed

Physical property	Mean±SD
Length	3.8±0.3mm
Bulk density	672±45 kg/m^3

4.2 Qualitative analysis for phytochemicals

Phytochemicals screening was done to understand the presence of total phenol, flavonoids, and glycosides. The results of the qualitative analysis of phytochemicals are presented below in the table 4.2**:**

Table 4.2: Qualitative analysis for phytochemicals

Phytochemicals	Methods of processing		
	Raw	Roasted	Soaked and roasted
Flavanoid	+ve	+ve	+ve
Total phenols	+ve	+ve	+ve
Glycoside	+ve	+ve	+ve

Qualitative phytochemicals analysis of the processed flaxseed revealed the presence of flavonoid, total phenols and glycoside and they were present in all raw, roasted and soaked flaxseeds.

Similar result for phytochemical analysis of flaxseed was obtained in a study (Dharshini *et al.*, 2013). The qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which signifies flaxseed as a valuable therapeutic natural source to compact dreadful infectious diseases.

4.3 Effect of roasting time on nutritional components

4.3.1 Flavonoids

Roasting significantly increased the flavonoid content of flaxseeds. Raw flaxseed contained 45.33±5.03 mg quercetin equivalent /100g dry matter of the extract. The flavonoid content increased by 21.76%, 18.91%, 94.85% and 425.37% upon roasting for 5, 10, 15 and 20 minutes respectively. The increment of total flavonoids content in the roasted flaxseed is presented in figure 4.1. The mean flavonoid content ranged from 45.33±5.03 in raw to 238.16±8.8 mg quercetin equivalent/100g dry matter of the extract in 20 minutes roasted sample. From figure 4.1, the total flavonoid content was 45.33±5.03, 55.2±0.26, 53.91±4.7, 88.33±1.588 and 238.16±8.8 mg quercetin equivalent/100g DM of the extract in raw, 5, 10, 15 and 20 minutes roasting respectively.

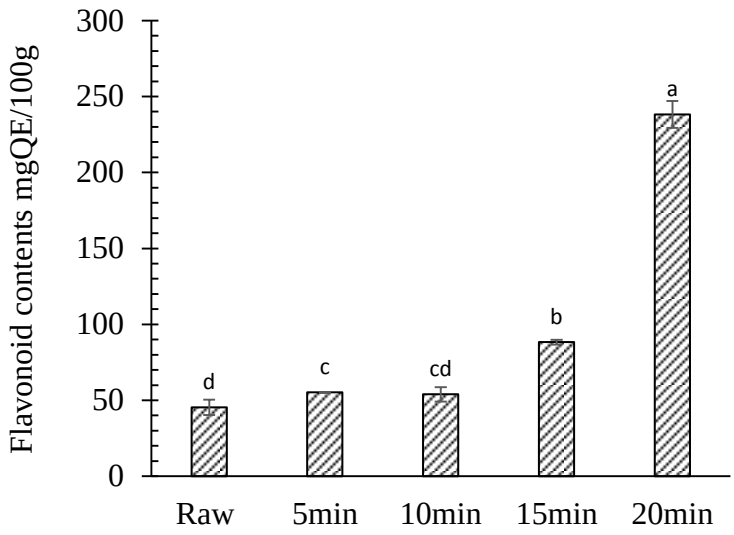

Fig 4.1: Effect of roasting on flavonoids content*

***similar alphabets do not show significant difference

The ANOVA and LSD analysis of flavonoid content is given in appendix A, which showed significant difference ($p<0.001$) among the samples. The statistical analysis showed that samples raw, 5min, 15min and 20 min roasting were significantly different ($p<0.001$) from one another whereas 10 min roasting was not significantly different from raw and 5 min roasting .

A study found that the flax contained about 35-70 mg of flavonoids/100g (Oomah, 1996). In a study the quantitative estimation of the total flavonoid in raw and roasted flaxseed powder was found to be 0.11mg, and 0.23mg per kg respectively. Thus from the quantitative estimation, it was clear that roasting was the best method of processing flaxseed and had considerably high amounts of flavonoids (Dharshini *et al.*, 2013). In a study, flavonoids contained in the group of selected nuts and oil seeds were significantly increased on roasting. The increment of flavonoid was explained with breaking of binds in the structure of lignin complex and also other phenolic compounds (Kamalaja *et al.*, 2018).

4.3.2 Polyphenols

Roasting significantly decreased the polyphenol content of the flaxseed. The polyphenol content of raw flaxseed was found to be 702.35±78.88 mg GAE/100g. After roasting, the percentage reduction of polyphenols content was noted, it was reduced by 19.43%, 28.40%, 30.66% and 40.15% on 5, 10, 15 and 20 minutes roasting respectively. It was found that the polyphenol content was reduced to 565.8±1.5, 502.9±28.07, 486.95±4.55 and 420.35±22.05 mg GAE/100g DM of the extract on 5, 10, 15 and 20 minutes roasting respectively.

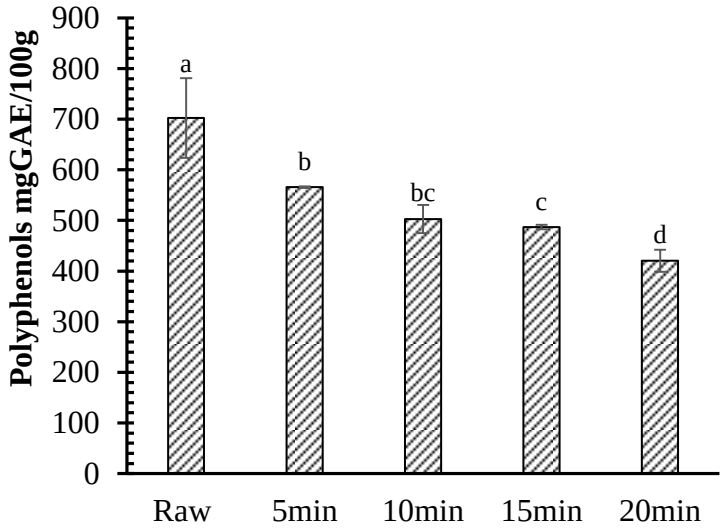

Fig 4.2: Effect of roasting time on polyphenols
*similar alphabets do not show significant difference

The ANOVA and LSD analysis of polyphenol content given in appendix A showed that there was significant difference ($p<0.001$) among the samples. The statistical analysis showed that samples raw, 5min, 15min and 20min were significantly different ($p<0.001$) from one another whereas 10min was not significantly different from 5min and 15min.

In a study, the total polyphenol content in selected flaxseed varieties ranged from 440.00 to 536.33 mg GAE/100g. Variety J-23 recorded the highest total polyphenol (536.33 mg GAE/100g) followed by Padmini variety i.e 516.66 mg GAE/100g (Hiremath, 2013). In a study, it was found that heating process caused loss of phenolic acids (84 - 60 as gallic acid equivalents (mg/100g oil) contents,

which confirmed that thermal treatment causes an oxidation and polymerization of phenolic compounds (Herchi *et al.*, 2016).

4.3.3 Free radical scavenging activity

IC_{50} concentration of methanolic extracts of flaxseed shown in table 4.3 indicates the amount of extract needed for 50% inhibition (IC_{50}) of DPPH radicals in raw and roasted samples. The antioxidant activity is inversely proportional to IC_{50} (Qusti *et al.*, 2010), which were calculated from the linear regression of the % antioxidant activity versus extracts concentration. The lowest IC50 concentration was found to be 35.13 mg dm/ml in raw flaxseed and 15min roasting. It means that 35.13 mg flaxseed extract is needed to 50% inhibit the 1 ml DDPH. As comparison with the L-ascorbic acid, 1.57 mg L-ascorbic acid is needed. IC 50 concentrations were found as 39.01 mg/ml for 5 min roasting, 38.19 mg/ml for 10 min roasting and 35.16 mg/ml for 20 min roasting. As comparing the raw and roasting samples, the highest amount of antioxidant activity was found in raw and 15 min roasting.

The IC50 value of ethanolic extract of flax seeds was 25.63 mg/ml, which means that this concentration of ethanol extract of flax seeds was required to cause 50% scavenging activity of DPPH radical (Amin & Thakur, 2014).

Table 4.3: Total antioxidant activity of different samples of flaxseed

Parameter	Total Antioxidant Activity	
	IC50 value (mg/ml)	mg ascorbic eqvt/mg dm
Raw	35.13	1.571
5min	39.01	1.414
10min	38.19	1.44
15min	35.13	1.57
20min	35.16	1.569

****Note:** IC50 value from ascorbic acid standard curve = 55.18816 mg/ml

In a study to determine the effect of thermal processing on the flavonol, rutin, and quercetin, it was shown that the radical scavenging activity decreases with decreasing amount of flavonol in the system and vice versa (Buchner *et al.*, 2006). Polyphenolic compounds have an important role in preventing lipid oxidation

and are associated with antioxidant activity (Gulcin *et al.,* 2003; Yen *et al.,* 1993). However, the total antioxidant capacity after roasting is the result of the thermal degradation of naturally occurring antioxidant compounds and the formation of new maillard reaction product having antioxidant activity. In a study it was reported that roasting nuts may destroy bioactive compound but it can also form antioxidant compound through maillard reaction (Kamalaja *et al.,* 2018).

4.4 Effect of roasting time on anti-nutritional factors

4.4.1 Hydrocyanic acid

Hydrocyanic acid content in raw flaxseed was found to be 649.3±1.41 mg/kg which decreased to 591.3±1.75mg/kg on 5 min, 213.2±4.63mg/kg on 10 min, 115.2±10.41mg/kg on 15 min and 106.6±2.76 mg/kg on 20 min roasting at temperature 180ºC. Hydrocyanic acid content in raw flaxseed was reduced by 8.93%, 67.16%, 82.25% and 83.58% on 5 min, 10 min, 15 min and 20 min roasting respectively.

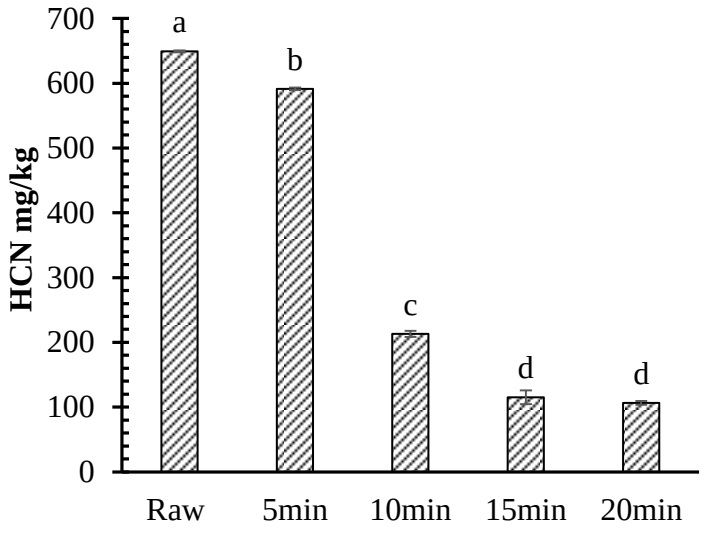

Fig.4.4: Effect of roasting time on HCN content*

*****Similar alphabets do not show significant difference

The ANOVA of HCN content given in appendix A showed that there was significant difference (P<0.001) among the samples. The statistical analysis showed

that samples raw, 5min, 10min and 15min were significantly different ($p<0.001$) from one another in term of HCN content.

In a study, it was found that HCN content was removed by more than 85 % of linustatin and neolinustatin when flaxseed was heated for more than 2 h at 200 °C (Park *et al.*, 2005). A study reported microwave roasting of flaxseed reduced the HCN content by 83.3% which may be due to deactivation of glycosidase or evaporation of HCN (Feng *et al.*, 2003). Cyanogenic glycosides are heat labile and easily destroyed by processing methods namely autoclaving, microwave roasting, pelleting and by certain detoxifying enzymes such as β-glycosidases, releasing hydrogen cyanide which can be evaporated by using steam (Cunnane *et al.*, 1993).

4.4.2 Oxalate

Oxalate content of raw flaxseed was found to be 1.513±0.025 mg/kg. The effect of roasting on the oxalate content of flaxseed is presented in fig. 4.5. There was subsequent decrease in oxalate content of roasted samples which was found to be 1.427±0.09 mg/kg on 5 min, 1.41±0.04 mg/kg on 10 min, 1.302±0.03 mg/kg on 15 min and 1.301±0.009 mg/kg on 20 min roasting.

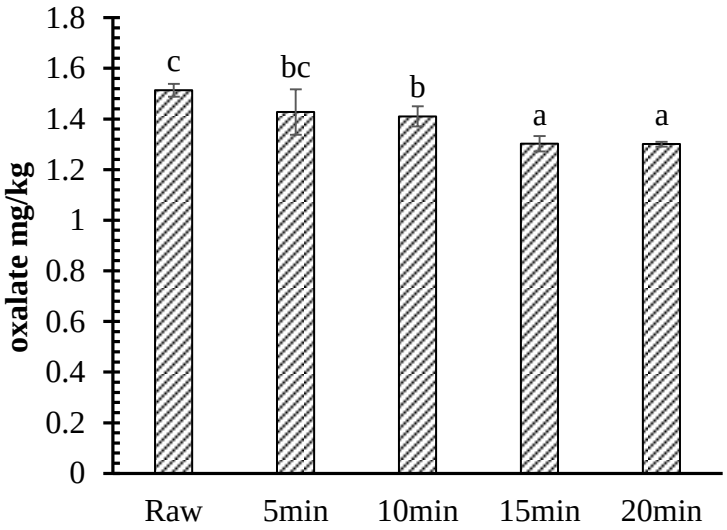

Fig 4.5: Effect of roasting time on oxalate content*

*Similar alphabets do not show significant difference

The percentage reduction of oxalate in roasted flaxseed was 5.68%, 6.8%, 13.98% and 14.05% reduction on samples 5min, 10min, 15min and 20min respectively. Placed values are means of 3 replicates. Vertical error bars represent standard deviation and index with different letters are significantly difference ($P<0.05$) by fishers unprotected. Statistical analysis showed that there was significant difference ($P<0.001$) among the samples. The statistical analysis showed that samples 15min and 20min were significantly different ($p<0.001$) from raw sample in term of oxalate content. It was found that there was no significant difference among raw sample and 5min; sample 15min and 20min whereas sample 15min and 20min was significantly different from raw sample.

In a study it was found that oxalate content in raw flaxseed range from 2 to 10 mg/kg (Hiremath, 2013). The sample contained 1.513±0.025 mg/Kg oxalate; hence it may be considered a low oxalate food. Hui (1992) stated that intake of 5g or more oxalic acid would be fatal to humans but the negative effect can be seen at even low values. In a study the roasted pistachio and chestnuts contained very low level than that of raw samples (Ritter & Savage, 2007).

4.5 Optimization of roasting time

As regard with the bioactive and anti-nutritional component analysis, the optimization study was carried out as given below:

Table 4.4: Optimized goals for bioactive and anti-nutritional components on varying time of roasting

Parameter	Goal	Optimized treatment
Total flavonoid content	Higher	20min roasting
Polyphenol content	Higher	5min roasting
Antioxidant activity	Higher	15min roasting
HCN	Lower	15 min and 20 min roasting
Oxalate content	Lower	15 min and 20min roasting

Optimization of roasting time was done based on the anti-nutritional factor reduction. The sample with 15min roasting time had significant reduction of HCN and oxalate from samples raw, 5min and 10min where there was no significant difference

between samples 15min roasting and 20 min roasting. There was no further reduction of HCN and oxalate in 20min roasted sample. So, 15 min roasting was considered as the optimized roasting time. Therefore further analysis of raw, roasting at 15min and soaking (12h followed by drying) followed by roasting was carried.

4.6 Effect of soaking followed by roasting on bioactive and anti-nutritional components

Soaking followed by roasting reduced polyphenols significantly ($p<0.001$) whereas there was no significant effect on flavonoids content. The total flavonoids were 37±12.5 mg QE/100g and the percent reduction was 18.38%. The polyphenols content were 489.55±12.9 mg GAE/ 100g of DM and the percent reduction of polyphenols was 30.29%. The lowest IC50 concentration was found to be 30.22 mg dm/ml in soaked followed by 15min roasting. The highest amount of antioxidant activity was found in soaking followed by roasting.

Similarly HCN was significantly decreased ($p<0.001$) to 83.467±5.78 mg/kg. The percent reduction of HCN on soaking was 46.23%. As HCN and cynogenic glycosidase was found significantly lower because of its extreme solubility in water (FAO/WHO, 1965). Likewise, oxalate was significantly decreased ($p<0.001$) to 0.814±0.06 mg/kg and the percent reduction of oxalate was 87.14%. Oxalate was also significantly decreased on soaked sample due to the loss of water soluble forms of oxalate i.e oxalic acid.

Table 4.5: Analysis of Variance of soaking followed by roasting (15min) with raw and 15 min roasted sample *

Parameters	Raw	Roasted (15min)	Soaked, dried and roasted(15min)	ANOVA
Flavonoid (mg QE/100g)	45.33 ± 5.03^{b}	88.33 ± 1.58^{a}	37 ± 12.5^{b}	<0.001
Polyphenols (mg GAE/100g)	702.35 ± 78.88^{a}	486.95 ± 4.55^{b}	489.55 ± 12.9^{b}	<0.001
IC 50 (mg dm/ml)	35.13	35.13	30.22	
HCN (mg/kg)	649.3 ± 1.41^{a}	115.21 ± 10.4^{b}	83.467 ± 5.78^{c}	<0.001
Oxalate (mg/kg)	1.51 ± 0.02^{c}	1.30 ± 0.03^{b}	0.814 ± 0.06^{a}	<0.001

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

4.7 Comparison of roasting and soaking followed by roasting

Table 4.5 showed that flavonoid content was significantly decreased ($p<0.001$) from 88.33±1.58 to 37±12.5, antioxidant activity was found to be increased in soaking followed by roasting, HCN was significantly decreased ($p<0.001$) from 115.21±10.4 to 83.467±5.78 and oxalate was significantly decreased ($p<0.001$) from 1.30±0.03 to 0.814±0.06 whereas no significant change in polyphenols was observed.

The statistical analysis showed significant reduction ($p<0.001$) on HCN and oxalate content. As HCN was found significantly lower because of its extreme solubility in water (FAO/WHO, 1965). Similarly oxalate was also significantly decreased on soaked sample due to the loss of water soluble forms of oxalate i.e oxalic acid.

Table 4.6: Optimized goals for bioactive and anti-nutritional components

Parameter	Goal	Optimized treatment
Total flavonoid content	Higher	15 min roasting
Polyphenols content	Higher	5min roasting
Antioxidant activity	Higher	soaking followed by roasting (15min)
HCN	Lower	soaking followed by roasting (15min)
Oxalate content	Lower	soaking followed by roasting (15min)

From the study of different parameter, 15 min roasting was found superior in terms of total flavonoids content and 5min roasting was superior regarding polyphenols whereas soaking followed by drying prior to roasting (15min) had superior regarding to antioxidant activity and anti-nutritional components analysis. So, soaking followed by roasting (15min) can be considered the best method for anti-nutritional factors reduction.

4.8 Effect of roasting and soaking followed by roasting on nutritional components

Roasting and soaking followed by roasting did not show significant change in terms of protein content. The crude protein content on raw, 15 min roasting and soaking followed by roasting were found to be 17.79±0.43g/100g, 16.33±0.33g/100g and 16±2g/100g respectively.

The crude fat content on raw, 15 min roasting and soaking followed by roasting were found to be 35.60±1g/100g, 30.70±2.6g/100g and 31.63±1.9g/100g respectively. The study showed significant reduction of fat content in roasting and soaked samples. In a study, it was found that the fat content was 38.53% in roasted flaxseed flour and 38.76% in non-roasted flaxseed flour (Hussain *et al.*, 2008). Similarly, a study on effect of roasting on physiochemical properties found that crude fat content on raw flaxseed (32.27%) was higher than roasted flaxseed flour (31.05%).

The result for decreased fat may be due to the destruction of fat during treatment process (Moknatjou *et al.*, 2015).

In the study crude fiber content was significantly decreased form 13.72±0.45g/100g to 10.88±1.1g/100g on roasting whereas there was no significant change of fiber content in raw and soaked sample. In a similar study carried by Khan *et al.* (2016), the value of crude fiber decreases on roasting i.e raw flaxseed (9.63%) to roasted flaxseed (9.34%).

Similarly, in terms of mineral analysis, the study showed soaking followed by roasting significantly reduced calcium content at $p<0.05$. The calcium content on raw, 15min roasting and soaking followed by roasting were found to be 240.5±1.5mg/100g, 245.4±1.4 mg/100g and 225.6±0.47mg/100g respectively. The iron content on raw, 15min roasting and soaking followed by roasting flaxseed were and there was not significant change on iron content during processing.

Table 4.7: Analysis of Variance of soaking followed by roasting (15min) with raw and 15 min roasting

Parameters	Raw	Roasted (15min)	Soaked, dried and roasted(15 min)	ANOVA
Moisture (ml/100g)	6.9 ± 0.1^{c}	2.7 ± 0.2^{a}	3.63 ± 0.35^{b}	<0.001
Protein (g/100g)	17.79 ± 0.43^{a}	16.33 ± 0.33^{b}	16.00 ± 2^{b}	>0.05
Fat (g/100g)	35.60 ± 1^{b}	30.70 ± 2.6^{a}	31.63 ± 1.9^{a}	<0.05
Fiber (g/100g)	13.72 ± 0.45^{b}	10.88 ± 1.1^{a}	15.57 ± 1.4^{b}	<0.05
Calcium (mg/100g)	240.5 ± 1.5^{b}	245.4 ± 1.4^{c}	225.6 ± 0.47^{a}	<0.001
Iron (mg/100g)	6.86±0.31ab	6.47±0.37a	7.13±0.15b	>0.05

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

Part V

Conclusion and recommendations

5.1 Conclusions

Based on the obtained results, following conclusions can be drawn:

1. Roasting significantly ($p<0.001$) increased flavonoid content from 45.33±5.03 mg QE/100g DM to 238.16±8.8 mg QE/100g DM
2. The polyphenols content of raw flaxseed was found to be 702.35±78.88 mg GAE/100gm DM which decreased significantly ($p<0.001$) on roasting.
3. The highest amount of antioxidant activity was found to be in raw and 15 min roasting i.e 1.57mg AAE/mg DM
4. Roasting significantly decreased hydrocyanic acid content at $p<0.001$.
5. Similarly, oxalate content was significantly decreased from 1.513±0.025 mg/kg to 1.301±0.009 mg/kg on roasting
6. On soaking followed by roasting there was no significant increment of flavonoid content whereas polyphenols was significantly ($p<0.001$) decreased. And antioxidant activity was found to be increased whereas HCN and oxalate content were significantly decreased at $p<0.001$.
7. On nutritional analysis, protein, fat and fiber content were decreased significantly whereas calcium content increased significantly on 15min roasting. Similarly, on soaking followed by roasting, protein, fat and calcium content were decreased and crude fiber increased significantly.
8. Soaking for 12h followed by roasting at 15min seems to be a promising method for anti-nutritional factors reduction.

5.2 Recommendations

Following suggestions are recommended for future work in the field of flaxseed

1. Roasting flaxseed at 180^{o}c for 15min was the best processing as majority of bioactive compound was significantly increased and maximum reduction of anti-nutritional compounds was determined whereas soaking of flaxseed prior to roasting reduced anti-nutritional compounds significantly.

2. Other components such as saponin, phytic acid, trypsin inhibitors can be determined along with which preparation and quality evaluation of flaxseed incorporated product can be done.

Part VI

Summary

Flaxseed (*Linum usitatissimum)* collected from Morang district, Nepal was studied to explore the effect of roasting at 180ºC for 5, 10, 15 and 20 min and soaking followed by roasting at 180ºC for 15 min on its bioactive compounds (flavonoids, polyphenol and anti-oxidant activity) and anti-nutritional compounds (hydrocyanic acid and oxalate). The crude extracts of samples were prepared using 80% methanol by maceration technique for analysis of total flavonoids content, polyphenols content and antioxidant activity.

Roasting of flaxseed had significant impact on its bioactive and anti-nutritional components. Flavonoid content increased significantly ($p<0.001$) from 45.33±5.03 mg QE/100g DM to 238.16±8.8 mg QE/100g DM on roasting. Polyphenol content decreased significantly ($p<0.001$) from 702.35±78.88 mg GAE/100g DM to 420.35±22.05 mg GAE/100g DM on roasting. The lowest IC50 concentration was found to be 35.13 mg dm/ml in raw flaxseed and 15min roasted samples. It means that 35.13 mg flaxseed extract is needed to 50% inhibit the 1 ml DDPH. Likewise, the statistical analysis showed significantly reduction ($p<0.001$) of HCN content on 15 min roasting and oxalate content was also significantly decreased ($p<0.001$) from 1.51±0.025 mg/kg to 1.302±0.03 on 15min roasting where there was no significant change in 20min roasting. On further processing i.e soaking followed by roasting there was no significant increment of flavonoid content whereas polyphenols was significantly ($p<0.001$) decreased. And antioxidant activity was found to be increased whereas HCN and oxalate content were significantly decreased at $p<0.001$. Similarly on nutritional analysis, Protein, fat and fiber content were decreased significantly whereas calcium content increased significantly on 15min roasting. Similarly, on soaking followed by roasting, protein, fat and calcium content were decreased significantly and fiber content was increased. Roasting flaxseed at 180^{o}c for 15min was the best processing as majority of bioactive compound was significantly increased and maximum reduction of anti-nutritional compounds was determined. And soaking followed by roasting showed significant reduction of anti-nutritional compounds in comparison to raw and roasting.

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Appendices

Appendix A

Table A.1 Analysis of Variance for Flavonoid content

Source of variation	d.f	s.s	m.s	v.r	F.pr
Sample	4	78818.84	19704.96	771.94	<0.001
Residual	10	255.26	25		
Total	14	79075.10			

Table A.2 Fisher's unprotected least significant difference test for Flavonoid content

Samples	Mean ±SD	LSD
Raw (control)	45.33±5.03	d
Roasting 5min	55.2±0.264	c
Roasting 10min	53.91±4.70	cd
Roasting 15min	88.33±1.58	b
Roasting 20min	238.16±8.80	a

Table A.3 Analysis of Variance for polyphenol content

Source of variation	d.f	s.s	m.s	v.r	F.pr
Sample	4	128185	32046	21.31	<0.001
Residual	10	15041	1504		
Total	14	143226			

Table A.4 Fisher's unprotected least significant difference test for polyphenol content

Samples	Mean ±SD	LSD
Raw (control)	702.35±78.88	a
Roasting 5min	565.8±1.519	b
Roasting 10min	502.9±28.07	bc
Roasting 15min	486.95±4.55	c
Roasting 20min	420.35±22.05	d

Table A.7: Analysis of Variance for HCN content

Source of variation	d.f	s.s	m.s	v.r	F.pr
Sample	4	839468.69	209867.17	7358.58	<0.001
Residual	10	285.20	28.52		
Total	14	839753.89			

Table A.8: Fisher's unprotected least significant difference test for HCN content

Samples	Mean ±SD	LSD
Raw (control)	649.3±1.41	a
Roasting 5min	591.3±1.75	b
Roasting 10min	213.2±4.63	c
Roasting 15min	115.2±10.41	d
Roasting 20min	106.6±2.76	d

Table A.9: Analysis of Variance for Oxalate content

Source of variation	d.f	s.s	m.s	v.r	F.pr
Sample	4	0.098359	0.024590	10.85	<0.001
Residual	10	0.022665	0.002266		
Total	14	0.121024			

Table A.10: Fisher's unprotected least significant difference test for oxalate content

Samples	Mean ±SD	LSD
Raw (control)	1.513±0.025	c
Roasting 5min	1.427±0.090	bc
Roasting 10min	1.41±0.04	b
Roasting 15min	1.302±0.03	a
Roasting 20min	1.301±0.009	a

Appendix B

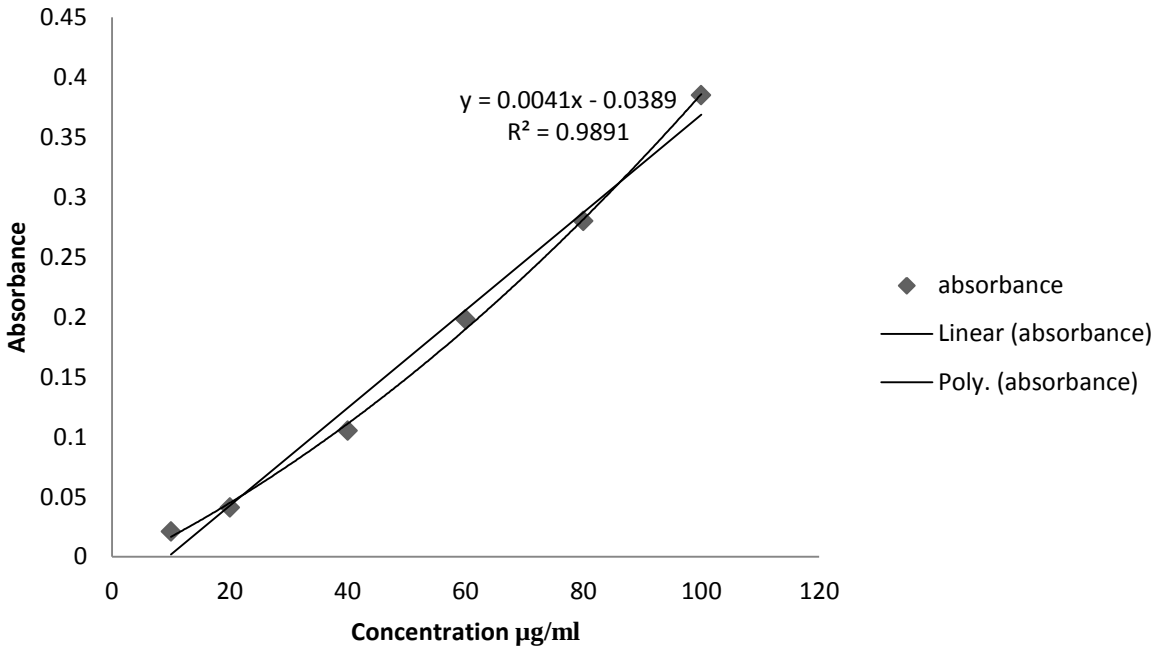

Fig. B.1: Standard curve of quercetin for flavonoids

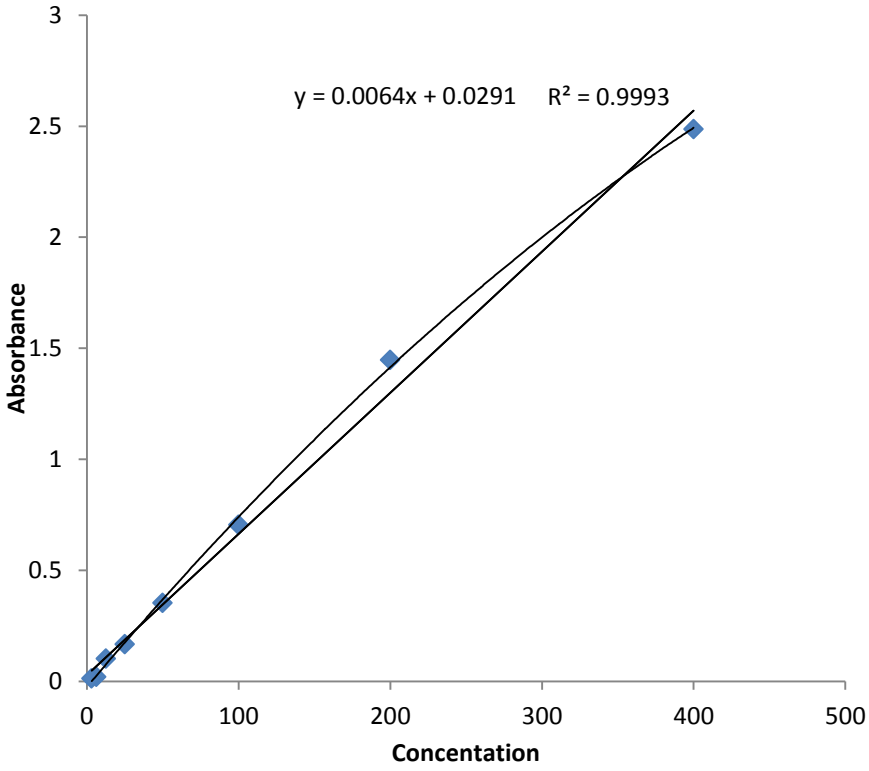

Fig. B.2: Standard curve of Gallic acid for polyphenols

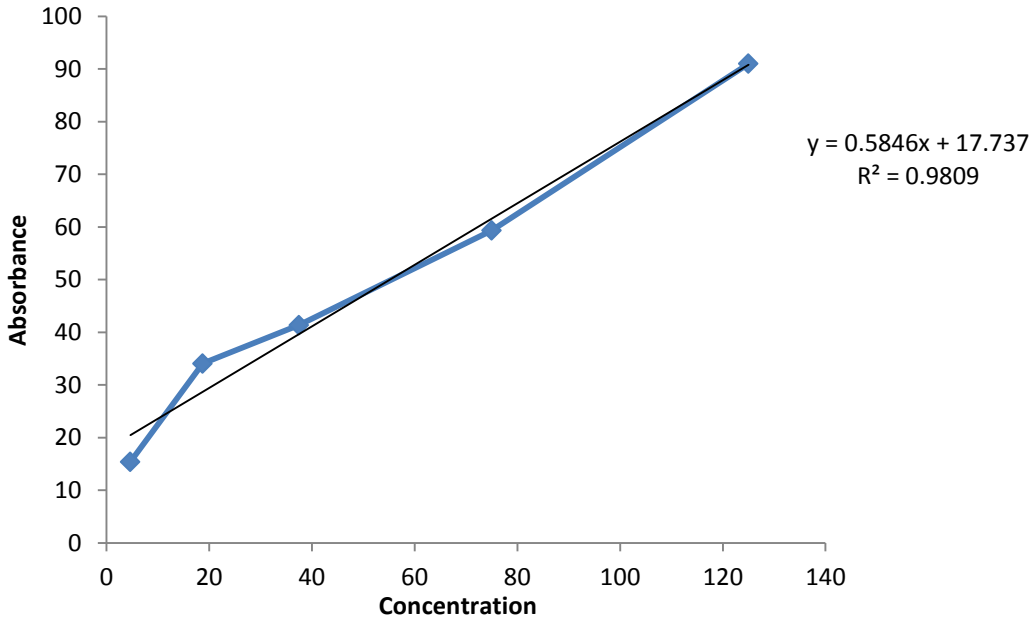

Fig. B.3: Standard curve of ascorbic acid for antioxidant activity

Appendix C

PHOTO GALLERY

Plates 1: Gas oven for roasting

Plates 2: Grinded raw flaxseed before sieving

Plates 3: Study of free radical scavenging activity of flaxseed using spectrophotometer