EFFECT OF GERMINATION AND ROASTING ON NUTRITIONAL AND ANTI NUTRITIONAL COMPONENTS OF SESAME (*Sesamum indicum*) SEEDS (BLACK)

by

Sadiksha Bhattarai

Department of Nutrition and Dietetics

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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Effect of Germination and Roasting on Nutritional and Antinutritional Components of Sesame (*Sesamum Indicum*) Seeds (Black)

A dissertation submitted to the Nutrition and Dietetics department in Tribhuvan University in the partial fulfillment of the requirements for the Bachelor degree in Nutrition & Dietetics

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Sadiksha Bhattarai

Department of Nutrition and Dietetics

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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Tribhuvan University

Institute of Science and Technology

Department of Nutrition and Dietetics

Central Campus of Technology, Dharan

Approval Letter

This ***dissertation*** entitled ***Effect of germination and roasting on nutritional and antinutritional components of sesame (Sesamum indicum) seeds (black)*** presented by **Sadiksha Bhattarai** has been accepted as the partial fulfillment of the requirement for the degree of **B.Sc. Nutrition and Dietetics.**

Dissertation Committee

1. **Head of the Department** _____
   (Mr. Kabindra Bhattarai, Asst. Prof.)
2. **External Examiner** _____
   (Birendra Kumar Yadav, Asst. Prof.)
3. **Supervisor** _____
   (Mr. Devraj Acharya., Asst. Prof.)
4. **Internal Examiner** _____
   (Yadav KC, Asst. Prof)

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Abstract

The nutritional and antinutritional component of germinated and roasted sesame seeds were evaluated. The seeds were soaked overnight and germinated for five days. Roasting of the seeds were carried out in round, deep, open aluminum pan (*karai*) with constant stirring till the seeds imparted aroma and slight change in color. The proximate, phytochemicals and antinutrient content of raw, germinated and roasted seeds were analyzed and compared to suggest the effective and efficient method to process sesame seeds for their maximum consumption. The optimum germinated sample was compared with roasted sesame seeds.

There was significant increase ($p < 0.05$) in phytochemicals and decrease in antinutrients in both germinated and roasted samples. On three days of germination, oxalate and phytate was reduced by 52 %, 59.3 % respectively. Total phenolic content, total flavonoid content and DPPH radical scavenging activity was increased by 171.6 %, 134.8 %, and to 78.38 % respectively on three days of germination. Roasting reduced antinutrients (oxalate, phytate) by 29 %, and 45 % respectively while TPC, TFC and DPPH scavenging activity was increased by 57 %, 11.07 % and to 36.73 % respectively. No significant difference was seen in proximate composition of raw and roasted sesame seeds whereas there was significant increase ($p < 0.05$) in protein content and decrease in fat and carbohydrate content of germinated sesame seeds. From the statistical analysis it was found that germinated sesame seeds had highest nutritional content and lowest antinutritional content when compared to both roasted and raw seed. Thus, this study conclude that third day germinated sesame seed helps to enhance the nutritional value when compared to common household roasting method.

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List of Abbreviations

Abbreviation	Full form
FAO	Food and Agriculture Organization
WHO	World Health Organization
IU	International Unit
g	Gram
kg	Kilogram
mg	Milligram
µg	Microgram
min.	Minutes
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
mn	meganewton
ANOVA	Analysis of Variance
°C	Degree Celsius
AOAC	Association of Analytical Chemist
CCT	Central Campus of Technology
S.D.	Standard Deviation
LSD	Least Significant Difference
D.F	Degree of Freedom
CE	Catechin Equivalent
QE	Quercetin Equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
CHD	Coronary Heart Disease
IUPAC	International Union of Pure and Applied Chemistry

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Part I

Introduction

1.1 Background

Sesame (*Sesamum indicum*), otherwise known as sesamum or benniseed, member of the family Pedaliaceae, is one of the most ancient oilseeds crop known to mankind. Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (Sabahelkhier *et al.*, 2008). Sesame is grown primarily for its oil-rich seeds. Before seeds were appreciated for their ability to add nutty flavor or garnish foods, they were primarily used for oil and wine (Gandhi, 2009). After the extraction of oil, the cake is mostly used for livestock feed or often as manure. Its color varies from cream-white to charcoal-black but it is mainly white or black. Other colors of some sesame seed varieties include, yellow, red or brown (Naturland, 2002). In Nepal, the notable colors for sesame seed are white, light brown and black. Global production of sesame seed is estimated by FAO at 3.15 metric tons per year (2001) having risen from 1.4 metric tons in the early 1960's. However only a small proportion of the global sesame harvest enters international trade. For the most part, the oil is expressed locally and used locally for cooking or the seeds themselves are eaten, particularly after being fried (T.Y.Tunde-Akintunde *et al.*, 2012).

Sesame seed is rich in fat, protein, carbohydrates, fiber and some minerals. The oil seed is renowned for its stability because it strongly resists oxidative rancidity even after long exposure to air (Agrisystems, 2010). The oil fraction shows a remarkable stability to oxidation. This could be attributed to endogenous antioxidants namely lignin's and tocopherols (Elleuch *et al.*, 2007). The seed is rich in protein and the protein of the seed has amino acid profile with good nutritional value similar to soybean (NAERLS, 2010). The sesame seeds have some potential nutraceutical compounds such as phenolic and tocopherols with antioxidant activity that have significant effect on reducing blood pressure, lipid profile and degeneration of vessels impact reducing chronic diseases (Jannat *et al.*, 2010). Sesame is a rich source of calcium (approx. 1 %) and phosphorous (approx. 0.7 %). Sesame contains ample amounts of oleic (43 %), linoleic (35 %), palmitic (11 %) and stearic acid (7 %) which together comprise 96 % of the total fatty acids (Saydut *et al.*, 2008). Though sesame seeds have a wide range of health and commercial benefits, they have some anti-nutritional properties. Sesame seeds contain a high amount of the

phytate and oxalate which is an anti-nutrient. Another disadvantage of the seed is that it produces allergic reactions in some people.

However, the decrease in the levels of antinutritional factors like phytate and oxalates to a safe limits may be caused by thermal degradation, soaking in distilled water, germination, and extraction of methanol (Yasmin *et al.*, 2008). According to (Hahm *et al.*, 2009), distinct times of germination influences in the nutritional quality of sesame seeds (*Sesamum indicum*). According to (Abderrahim *et al.*, 2012), the germination increases the quantity of phenolic compounds and antioxidant capacity in grains. Roasting of the seeds also enhances phytochemical properties(Özdemir *et al.*, 2001);(Heru Rizki *et al.*, 2015) and decreases antinutritional value (Adeyemi *et al.*, 2011);(O.M *et al.*, 2020b). Thus, the effect of processing technique brings changes in the antinutritional and nutritional factors of sesame seed. The correct processing technique to reduce anti-nutritional factors efficiently and enhance the phytochemical properties need further analysis.

1.2 Statement of problem

Sesame seeds have been widely employed in culinary as well as traditional medicines for their nutritive, preventive, and curative properties. Sesame is an important source of phytonutrients such as omega-6 fatty acids, flavonoid, phenolic anti-oxidants, vitamins, and dietary fiber with potential anti-cancer as well as health promoting properties. Sesame increases the recycling of vitamin E, improves liver function and protects against alcohol induced oxidative stress (Shasmitha, 2015). In Nepal, the seeds are roasted and used for various culinary processes. Studies have shown germination of seeds enhances the therapeutic potential of the seeds. Roasting in oven has helped to enhance the phytochemical and reduce antinutritional property of seeds. In our country open pan roasting is commonly used and thus the effect of this roasting techniques is still the subject of research. There are limited studies based on comparison of nutritional and anti- nutritional properties of germinated and roasted seeds. Therefore, comparing common household processing techniques (open pan roasting) with germination will figure out which technique is more effective in enhancing nutritional property of seeds and fulfils the research gap.

1.3 Objectives of study

1.3.1 General objectives

The general objective of my study was to study the effect of roasting and germination on nutritional and anti-nutritional component of sesame seed.

1.3.2 Specific objectives

- To determine nutritional composition of sesame seeds.
- To determine effect of variation in germination time on antinutritional and phytochemical composition of sesame seeds.
- To determine effect of common roasting technique in household on antinutritional and phytochemical composition of sesame seeds.
- To determine the most effective treatment between roasting and germination.

1.4 Significance of study

Sesame seeds contains a large group of fat-soluble antioxidants (sesamin, sesamolin and tocopherols), which play an important role on health-promoting effects (Nascimento *et al.*, 2012), acting especially against oxidative processes in cells. Sesame seeds are consumed commonly by roasting and least used method of consumption is germination. Germinated sesame seeds present an excellent source of sesamol, a potent natural antioxidant, and a-tocopherol , the most active form of vitamin E (Hahm *et al.*, 2009). The significant reduction in anti-nutrients such as phytate and oxalate is seen in germinated seed as the time progressed (Maria and Victoria, 2018.) Roasting reduces the moisture content, develops pleasant flavor and makes the seed more acceptable for consumption (H. Rizki *et al.*, 2016). Folasade and Akinoso (2014) also reported significant reduction in phytate and oxalate content of sesame seed during germination, roasting and cooking. This study determines anti-nutritional content and phytochemical properties of germinated sesame seeds based on germination time and comparison with roasted seeds (processed using common household technique). The documentation about the processing methods that are effective in enhancing the nutritional properties will help in establishment of effective and optimized way for use of sesame seeds in household level.

1.5 Limitations

- Only one variety of sesame seed was used for research work.
- Fatty acid composition of sesame seed was not determined.
- Only methanolic extract was studied.

Part II

Literature Review

2.1 Sesame

Sesame *(Sesamum indicum*), also known as sesamum, gingelly, beniseed, sim-sim and *til* is perhaps the oldest oilseed known and used by human beings (Joshi, 1961). It has been cultivated for centuries, particularly in Asia and Africa, for its high content of edible oil and protein. The crop is now grown in a wide range of environments, extending from the semi-arid tropics and subtropics to temperate regions. Consequently, the crop has a large diversity in cultivators and cultural systems. Although considered to have originated in central Africa most probably Ethiopia, many believe that there is convincing evidence to show that sesame originated in India (Bedigian and Harlan, 1986). Sesame was widely dispersed by people both westward and eastward, reaching China and Japan which themselves became secondary distribution centers.

Fig.2.1 Sesame pods and seeds

Sesame is an important cash crop for small and marginal farmers in several developing countries. It is cultivated for seed which contains 38-54 % oil of very high quality and 18-25 % protein. The great diversity of sesame types, their wide environmental adaptation and considerable range of seed oil content and characteristics make an exceptional gene pool which must be harnessed to produce better cultivators to extend the range and profitability of sesame growing (Hegde, 2012). The genus Sesamum, one among the 13 genera of the family Pedaliaceae, comprises about 40 species. Many occur in Africa (18 exclusively), 8

occur in India- Sri Lanka region (five exclusively). The Australian records are probably due to imports by Chinese immigrants in the mid-nineteenth century (MR, 1996).

There are many varieties and strains of S. indicium which differ considerably in size, form, growth, color of flowers, seed size, color and composition. Sesame is typically and erect, branches annual, occasionally perennial, 0.5-2m in height, with will developed root system, multiflowered, whose fruit is a capsule containing a number of small oleaginous seed. Sesame seeds are small, ovulate slightly flattened with testa of variable color varying from black, white, yellow, reddish brown, grey, dark grey, olive green and dark brown. Seed weight is around 3g/1000 seeds. The seeds mature 4-6 weeks after fertilization.

2.1.1 Production of sesame seed

Sesame is a traditional oilseed crop of Nepal. It is grown since long time back especially in Terai and *besi* of mid hills. The area under sesame is limited to certain pocket of Terai and mid hills covering about 4000 hectors of land in spite of its high market value. It is mainly grown in monsoon season and also grown in spring season after rice called as *Baisakh til* in eastern Terai. The major constrains for sesame production in Nepal are unavailability of high yielding varieties, instability in yield, low tolerance to water logging and low resistance to disease (Ghimire, 2000).

2.1.2 Chemical composition

Sesame seed has high food value due to its high content of oil and protein. The composition is markedly influenced by genetic and environmental factors (L. Kinman and M. Stark, 1954). The seeds contain 6–7 % moisture, 17–32 % protein, 48–55 % oil, 14–16 % sugar, 6–8 % fiber and 5–7 % ash. The hull content averages about 17% of sesame seed, and contains large quantities of oxalic acids, calcium, other minerals and crude fiber (Hedge, 2012).The proximate composition of whole sesame seeds is given in Table 2.1.

Table 2.1 Nutritional composition of whole sesame seed

Constituent (%)	**Joshi 1961**	**Smith 1971**	**Gopalanet.al 1982**	**Weiss 1983**
Moisture	5.8	8.0	5.3	5.4
Protein	19.3	22.0	18.3	18.6
Fat	51.0	43.0	43.3	49.1
Carbohydrate	21.2	21.0	25.0	21.6
Ash	5.7	6.0	5.2	5.3

Source:(Gopalan *et al.*, 1982; Joshi, 1961; Smith, 1971; Weiss, 2000)

Basically, all seeds contain some kind of stored energy used as a fuel by the young plant in the first phase of its life. This energy is commonly stored in form of proteins, carbohydrates, or fat.

2.2 Uses of sesame seed

Sesame seed has been used for food, industrial, nutraceutical, and pharmaceutical uses. Sesame oil, queen of oils is an ingredient of variety of food. It is used as a substitute of olive oil, as a salad oil and for cooking fish and vegetables in many parts of the world. Aqua hulled, double washed and dried sesame seed are used on hamburger buns. Roasted natural sesame seeds are used in preparation of bread, bread sticks, cookies, chocolates and ice-creams. Mechanically hulled sesame seeds are the basis for candies and creamy, sweet wholesome tahini (spread). The antioxidant property of refined sesame oil contributing its greater shelf life makes it suitable for food industry (Prasad M N *et al.*, 2012).

Dehulled sesame seeds are sweet and oleaginous and are used directly in different types of foods in various parts of the world. They are used in the manufacture of traditional confections such as halva, laddu and pickles. They are also eaten whole after roasting (Hedge, 2012). Sesame flour is an edible, creamy and light brown powder from sesame seed. Sesame floor has high protein, high level of methionine and tryptophan, and 10-12% sesame oil. Sesame seed contains three times more calcium than a comparable measure of milk (B. Morris, 2002).

Sesame oil is used as a solvent for intramuscular and has nutritive, anti-inflammatory, and moisturizing properties and has been used as a laxative. The leaves are rich in a gummy matter and when mixed with water from rich bland mucilage that is used in the treatment of infant cholera, diarrhea, dysentery. Cephalin from sesame seed has hemostatic activity. Historically, fiber is used as an anti-diabetic, antitumor, antiulcer, cancer preventive, cardioprotective and laxative. Myristic acid has cancer preventive capability and is found in sesame seeds. Nutritionally sesame seeds are rich in oils, protein and micronutrient such as minerals, lignins, tocopherol and phytosterol. Sesame seed exerts many health benefits such as hypo cholesterolemic effect, anti-cancer activity, oxidative stress attenuation and blood pressure reduction (Elleuch *et al.*, 2011).

The nutraceutical uses as noted by (Prasad M N *et al.*, 2012) are regulating cholesterol, neurological role, helps in blood pressure regulation and has antioxidant property. The major lignin sesamin present in sesame seed is mainly related to lipid metabolism through a series of biochemical action in both human and animals (Matsumura *et al.*, 1998). The sesame oil which is rich in polyunsaturated fatty acid, sesamin and vitamin E greatly reduces hypertension when compared to blood pressure lowering drug (Prasad M N *et al.*, 2012). The important antioxidants sesamolin and sesame maintains the fats including low density lipoproteins which causes arteriosclerosis and are believed to promote the integrity of body tissue. This anti-oxidants lignin has shown hypocholesterolemia and immunomodulatory effect (Chavali *et al.*, 1997). This antioxidant acts as a defense against reactive oxygen species. Dietary intake of alpha tocopherol reduces photo carcinogenesis induced by ultraviolet light (Balan *et al.*, 2009). According to traditional system of medicine sesame is known to cure bleeding dysentery, burns, ear pain, headache and impotency. In Ayurveda, sesame oil is regarded as an anti-bacterial mouthwash and it can be applied to nostrils to relieve anxiety and insomnia. The pain associated premenstrual syndrome can be overcome by applying the oil on to the abdominal region (Moazzami and Kamal-Eldin, 2006).

2.2.1 Pharmacological uses

Sesame oil is a pharmaceutic aid used as a solvent for intramuscular injection and has been used as laxative. It contains flavonoids which has hypoglycemic activity. Sesame oil contains large amount of linoleate in triglyceride form which selectively inhibited malignant melanoma growth (B. Morris, 2002). Sesame seed consumption appears to increase plasma gamma-tocopherol and enhanced vitamin E activity which are believed to prevent cancer

and heart disease (Cooney *et al.*, 2001). Sesamin remained at 90% of the original level after roasting (Yoshida *et al.*, 2001). Sesame seed contains lecithin which has antioxidant and hepatoprotective activity and ranges. Lecithin is also likely effective for reducing hepatic steatosis in long term parenteral nutrition patients and a successful treatment for dermatitis and dry skin (B. Morris, 2002)

2.3 Nutritional Factors

2.3.1 Lipids

Sesame seeds contain more oil than many other oilseeds. Oil content varies with genetic and environmental factors. A wide range of the oil content, from 37–63 %, has been reported in sesame seed (Lyon, 1972). Oil content in seeds also varies considerably among different varieties and also with growing seasons (Lyon, 1972).

The average content of oil was 55.0% in white-seed strains and that of black-seed strains was 47.8% (Tashiro *et al.*, 1990), although the content can vary considerably depending on the species and cultivation conditions. Fatty acids in the oil are mainly oleic (18:1=39.1%) and linoleic (18:2=40.0%) acids, with palmitic (16:0=9.4%) and stearic (18:0=4.76%) acids in smaller amounts, and linolenic (18:3=0.46%) acid in trace amount (Numa and Tanabe, 1984).

The lipids of sesame seeds are mostly composed of neutral triglycerides with small quantities of phosphatides (0.03–0.13 % with lecithin: cephalin ratio of 52: 46). The glycerides are mixed type, principally oleo-dilinoleo, linoleo-dioleo triglycerides and triglycerides with one radical of a saturated fatty acid combined with one radical each of oleic and linoleic acids (Lyon, 1972). Sesame seed contains high levels of sesamin and γ-tocopherol compared with α- and δ- tocopherol, and their concentration is influenced by genetic, environmental and geographical factors (Williamson *et al.*, 2008).

Sesame oil contains about 80 % unsaturated fatty acids. Oleic and linoleic acids are the major fatty acids and are present in approximately equal amounts (Lyon, 1972). The saturated fatty acids account for less than 20 % of the total fatty acids. Palmitic and stearic acids are the major saturated fatty acids in sesame oil. About 44 and 42 % of linoleic and oleic acids and 13% saturated fatty acids are found in sesame oil (Smith, 1971). Arachidic

and linolenic acids are present in very small quantities and are least affected from year to year (Were *et al.*, 2006).

2.3.2 Proteins

Sesame seed contains 17–32 % protein with an average of about 25 % (Lyon, 1972). Protein content tends to decline with increase in productivity level (Caliskan *et al.*, 2004). The proteins in the seed are located mostly in the outer layers of the seed. Based on their solubility, sesame proteins have been classified as albumin (8.6 %), globulins (67.3 %), prolamin (1.3 %) and glutelin (6.9 %) fractions (Rivas R *et al.*, 1981). The essential amino acid composition of sesame seed proteins indicates that sesame proteins are rich in sulphur-containing amino acids, particularly methionine (Smith, 1971) and also tryptophan (Johnson *et al.*, 1979). Tryptophan, which is limiting in many oilseed proteins, is adequate in sesame.

The high level of sulphur-containing amino acids in sesame seed proteins is unique. It suggests that sesame protein should be more widely used as a supplement for methionine and tryptophan and should be an excellent protein source for baby and weaning foods. The use of sesame seed protein would eliminate the problems encountered when foods are supplemented with free methionine, which is unstable (Peter, 2012).

2.3.3 Carbohydrates

Sesame seeds contain 14–25 % carbohydrates. The seeds contain about 5 % sugars, most of which are of reducing type. Defatted sesame meal contains more sugars (Taha *et al.*, 1987). The crude fiber is present mostly in husk or seed coat. Sesame seed consist of D-glucose, D-galactose, D-fructose and planteose (Peter, 2012).

2.3.4 Minerals

Sesame seed is a good source of certain minerals, particularly calcium, phosphorus and iron. The seeds contain a total of 4–7 % minerals. Deosthale (1981) reported 1 % calcium and 0.7 % phosphorus in the seeds. It also contains sodium and potassium. Calcium is mostly present in the seed coat which is lost during dehulling. Further, the bioavailability of calcium from sesame is less than that from milk or bread probably because of the high concentration of oxalate and phytate in the seed. Poneros-Schneier and Erdman Jr (2006) reported the bioavailability of calcium from some food products, relative to $CaCO_3$ as non-fat dry milk 100 %; whole wheat bread 95 %; almond powder 60 %; sesame seeds 65 %; and spinach 47

%. Sesame grown on selenium-rich soils also contains high selenium, although most of it is present in the hulls (J.E and R.R, 1985). Maria and Victoria (2018) reported the values of calcium and iron in sesame seed is 439.25 ± 1.00 and 6.42 ± 0.02 mg/100 g respectively. Similarly the (Hahm *et al.*, 2009) reported their values as 421 ± 30 and 6.19 ± 0.51 mg/100 g respectively. The result obtained was quite similar to the values to the mentioned values. According to (Folasade and Akinoso, 2014), calcium and iron in sesame seed is 464.97 ± 0.68 and 6.42 ± 0.02 mg/100 g respectively.

2.3.5 Vitamins

Sesame seeds are an important source of certain vitamins, particularly niacin, folic acid and tocopherol (Weiss, 2000). The vitamin A content of seeds is, however, very low. Vitamin E group includes several tocopherols, isomers and derivatives that differ in their biological activity. Sesame oil is rich in tocopherols. However, the proportion of δ-tocopherols is more than that of α-tocopherols. Therefore, the vitamin E activity of sesame oil is less than that of sunflower oil (Weiss, 2000).

2.4 Phytochemicals and antioxidant

Phytochemical refers to every naturally occurring chemical present in plants. In plants, phytochemicals act as a natural defense system for host plants and provide color, aroma and flavor. There is a wide distribution of biologically active constituents throughout the plant kingdom, particularly in plants used as animal feeding stuff and in human nutrition. Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. Phytochemicals found in plant foods have both adverse effect and health benefit. For example, phytate, lectins, phenolic compounds, saponin and enzymes inhibitors have been shown to reduce the availability of nutrient and cause growth inhibition, while phytoestrogens and lignans have been linked with infertility problems. However, phytate, lectins, phenolic compounds, amylase inhibitors and saponins have known to reduce the blood glucose responses to starchy food and /or the plasma cholesterol and triglycerides. In addition, phytate, phenolics, saponins, protease inhibitors, phyto estrogens and lignin have been related to reduce cancer risk.

2.4.1 Antioxidants

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).

Many degenerative diseases are caused by the excessive production of free radicals. These free radicals can be scavenged by antioxidants. Many plants, including fruits and vegetables, are natural antioxidants owing to the presence of phenolic and flavonoid compounds that exert antioxidant capacity. Phenolic compounds such as flavonoids have many benefits including antioxidant, antibacterial, and antidiabetic activities (Ruslan *et al.*, 2018). The consumption of antioxidants in a diet is essential to avoid the oxidative stress. Recently studies are associating flavonoids, an antioxidant compound, with a decrease in cancer risk and cardiovascular disease (Žilić *et al.*, 2014).

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 Goyal, 2014).The DPPH assay method is based on the reduction of DPPH, a stable free radical.

2.4.2 Phenols

Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol, which is also called carbolic acid, C_6H_5OH. In plants, the phenolic units are esterified or methylated and are submitted to conjugation, which means that the natural phenols are mostly found in the glycoside form instead of the aglycone form. This property of undergoing conjugation with other molecules enables it to scavenge free radicals and thus inhibit the oxidative mechanisms that can lead to degenerative diseases such as cancer (Egbuna and Ifemeje, 2015). Phenols are reported antitumor agents and exhibit antiviral and antimicrobial activities (Robbins, 1980), hypotensive effects (Matsubara *et al.*, 1985) and antioxidant properties (Robak and Gryglewski, 1988).

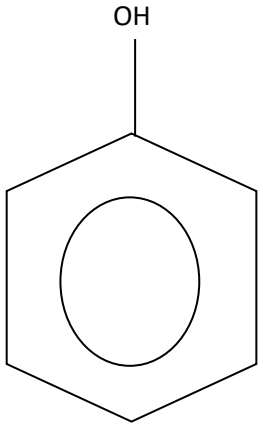

Fig.2.2 Structure of phenol

Several phenolics (e.g. chlorogenic acid, gallic acid, caffeic acid, tannic acid, catechin) have been shown to inhibit the mutagenic effects of both direct-acting carcinogens (e.g. benzo(a)pyrene diol epoxide) and carcinogens that require metabolic activation (e.g. aflatoxin B) and to trap nitrite, thus reducing the nitrosating species and preventing the endogenous formation of carcinogenic nitrosamines (H.F. and M.P., 1984).

The total phenolic content (TPC) of the methanolic sesame oil extracts was reported 26.00 ± 0.14 mg GAE/g of extract. Sesame oil extract contained higher TPC compared to other commonly available vegetable oils. TPC of sunflower, corn, rapeseed, and soy oils are 12.0, 12.6, 13.1, and 14.8mg GAE /g of methanolic extract, respectively (Siger *et al.*, 2008) . Presence of bioactive components especially, phenolic antioxidants are attributable to better oxidative stability of oils. Moreover, the bioactive components present in oils may serve as important bioactive that help reducing the oxidative stress in the human body. The total phenolic content was significantly ($p < 0.05$) higher (5 times) in the black than that in the white sesame seed hulls (Shahidi *et al.*, 2006b).

2.4.3 Total flavonoid content

Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids. Flavonoids are now considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic

applications. This is attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function. Research on flavonoids received an added impulse with the discovery of the low cardiovascular mortality rate and also prevention of CHD (Panche *et al.*, 2016). 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 flavanols.

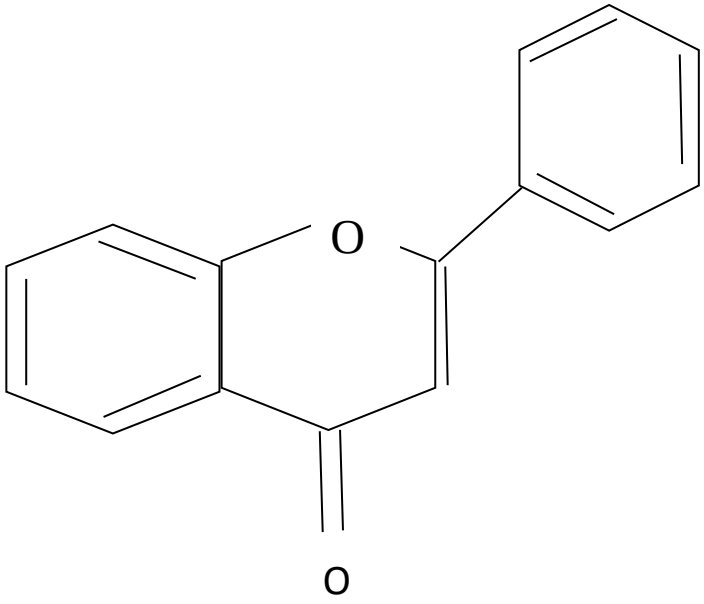

Fig.2.3 Structure of flavonoids

The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds (for instance alkaloids) mean that many animals, including humans, ingest significant quantities in their diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". In vitro studies show that flavonoids also have anti-allergic, anti-inflammatory, anti-microbial, anti-cancer and anti-diarrheal activities. In vitro, flavonoids have antiviral activity against several viruses, among them poliovirus. At very high concentration, flavonoids chelate metals such as iron and zinc and reduce the absorption of these nutrients. They also inhibit digestive enzymes and may also precipitate proteins (Egbuna and Ifemeje, 2015). The flavonoid content in raw sesame seed was reported to be 126.78 ± 1.61 mg RE/100 g (Thummakomma *et al.*, 2018). Ruslan *et al.* (2018) reported TFC in different extracts of the two varieties of sesame seeds expressed in terms of QE, and

the values ranged from 0.29 to 4.29 g QE/100 g. The highest TFC was 4.29 g QE/100 g, while the lowest TFC was 0.29 g QE/100 g.

2.5 Antinutritional Factors

2.5.1 Oxalate

The anti-nutritional factor that is of primary concern is oxalate. Oxalate is the di-anion with the formula $(C_2O_4)^{2-}$. Many metal ions form insoluble precipitates with oxalate, a prominent example being calcium oxalate, the primary constituent of the most common kind of kidney stones. Oxalate occurs in many plants, where it is synthesized via the incomplete oxidation of carbohydrates (Egbuna and Ifemeje, 2015). Oxalate 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). Oxalate and its salts are extensively spread in numerous plant tissues as the end products of metabolism. Oxalate content in foodstuffs has long been a concern in human diets, due to the negative health effects connected to a high intake of oxalate. Incidences of kidney stones, hypocalcemia and hyposideremia (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).

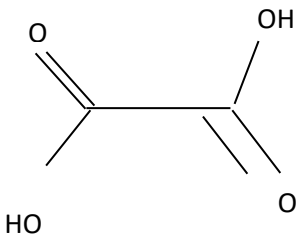

Fig. 2.4 Structure of oxalate

In the body, oxalate combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. Iron oxalate crystals cause significant oxidative damage and diminish iron stores needed for red blood cell formation whereas many kidney stones result from calcium crystals (Egbuna and Ifemeje, 2015).

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). Consumption of foods high in oxalate 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 and Haron, 2014). Folasade and Akinoso (2014) stated 154.00 ± 3.60 mg/100 g in raw black sesame seed. It occurs in the outer layer of thin hull and practically all of it can be removed by decortication (Lyon, 1972). According to (Olagunju and Ifesan, 2013), the oxalate content of black sesame seed was reported to be 1.5mg/g.

2.5.2 Phytate

Phytate (myoinositol 1,2,3,4,5,6, hexakis-dihydrogen phosphate; PA) is present in foods in concentrations ranging from 0.1 to 6.0 %. It is found as crystalline globoid inside protein bodies in the cotyledon of legumes or oilseeds or in the bran region of the cereal grains (N. R. Reddy *et al.*, 1982). Salts of phytate, designated as phytates, are found in plants, animals and soil. Phytate is ubiquitous among plant seeds and grains, comprising 0.5 to 5 % (w/w). It is primarily present as a salt of the mono- and divalent cations K^+, Mg^{2+}, and Ca^{2+} which accumulates in the seeds during the ripening period. Phytate is regarded as the primary storage form of both phosphate and inositol in plant seeds and grains (Loewus, 2001). Because phytate is a naturally occurring compound formed during maturation of plant seeds and grains, it is a common constituent of plant-derived foods (N. Reddy, 2001).

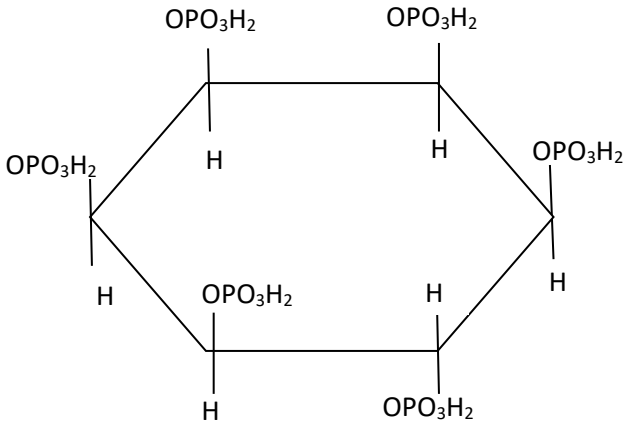

Fig.2.5 Structure of phytate

The major concern about the presence of phytate in the diet is its negative effect on mineral uptake. Minerals of concern in this regard would include Zn^{2+}, Fe^{2+} / $^{3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}(Vucenik and Shamsuddin, 2003). Its highly negatively charged structure at a wide range of pH values makes it very reactive with other positively charged ions such as minerals, forming insoluble complexes which are less available for digestion and absorption in the small intestine. This is the main reason why phytate has traditionally been considered as an antinutrient. The adverse effect of phytate in mineral availability depends on a number of factors including the concentration of phytate and the strength of its binding with different minerals. For example, zinc (Zn) forms one of the strongest mineral complexes with phytate (Evans and Martin, 1988). Sesame meal contains 1.0-1.3% as phosphorous of phytate. The analysis of anti-nutritional content of sesame varieties showed that variety Tate had the highest phytate content (435.45 mg/100 g) whereas variety Argene had the lowest (224.92 mg/100 g) (Deme *et al.*, 2017).

2.6 Methods of reduction of antinutritional factor

2.6.1 Roasting

Roasting reduces the moisture content, develops pleasant flavor and makes the seed more acceptable for consumption. Roasting is important for oily seeds prior to oil extraction. It causes desirable or undesirable changes in physical, chemical and nutritional properties of the seed. One of the desired outcomes of roasting process is increase in antioxidant activity that occurs due to formation of Millard reaction products. In food industry roasting is a process used to improve the food quality and extend shelf life of the food. The process is carried out for promoting more flavor, desired color and texture changes that ultimately increases the overall palatability. The most important condition of roasting process is time and temperature (H. Rizki *et al.*, 2016). Generally, the thermal treatment applied to foods of plant origin by heating or roasting cause's evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which may result in a greater availability of plant phenolic compounds in the matrix. Therefore, a thermal process can affect both the nutritional and bioactive characteristics of foods. Polyphenols occur in nature in free or bound forms, thus some processing methods such as boiling or heating have been shown to increase the polyphenolic content of foods (Kamalaja *et al.*, 2018).

Roasting of sesame can be done in a number of ways for sesame products production, it was reported that sesame seeds were roasted at 90°C-100°C for sesame paste production (N. Sawaya *et al.*, 1985). (El-Adawy and Mansour, 2000), studied the effect roasting methods on the nutritional properties of sesame paste, they advised roasting at 130 °C for 1 hour. The control of roasting in sesame by- products production is carried out by experienced operators as many food processing operations. On the other hand, sesame products producers considered that determination of optimum roasting conditions should be established in order to make good quality of sesame products. During the roasting, seeds become more crumble and brittle, which are typical characteristics of the roasted products (H. Rizki *et al.*, 2016). Therefore, the main goal of our work was to propose the roasting conditions optimal for obtaining high polyphenols yields and strong antioxidant activity, and also, in order to make good quality, the optimum roasting conditions should be established with the purpose of investigating the effects of roasting temperature and time on the nutritional and organoleptic quality properties.

2.6.2 Germination

Germination is a natural biological process of all superior plants by which the seed come out of its latency stage, once the minimal environmental conditions needed for its growth and development, such as humidity, temperature, nutrients, etc., are given. For the seed to germinate, there are also external factors such as humid substrate, availability of oxygen for aerobic respiration and an adequate temperature for the different metabolic processes and development of plantlet (Sangronis and Machado, 2007). During germination there are certain changes that occur as far as the quantity and type of nutrients within the seed. Those changes can vary depending on the type of vegetable, seed and the conditions of germination. Germinated seeds are good source of ascorbic acid, riboflavin, choline, thiamine, tocopherols and pantothenic acid (Bau *et al.*, 1997). Morphologically, germination is a transformation of an embryo into a seedling. Physiologically, germination is the resumption of the metabolism and growth which were earlier depressed or suspended. Biochemically germination is a sequential differentiation of oxidative and synthetic pathway and restoration of biochemical pathways typical of vegetable growth and development (Jan and Amen,1977).

Sesame seeds germinated in dark chambers maintained near 100% relative humidity at 35°C without presoaking reached >99% germination rate in 4 days with the final moisture

content stayed 2% (w/w), characterizing sesame seeds as orthodox seeds that are suitable for long term storage at low temperature and humidity under defined environment. With noticeable reduction in fat content (23%), germinated dry sesame seeds were found rich in linolenic acid, P, and Na, increasing from 0.38% (w/w), 445 mg/100 g, and 7.6 mg/100 g before germination to 0.81% (w/w), 472 mg/100 g, and 8.4 mg/100 g after germination, respectively. Sesame seeds after germination contained considerable amount of Ca (462 mg/100 g), higher than that of soybean. Germinated seeds presents an excellent source of sesamol (475 mg/100 g), a potent natural antioxidant, and a-tocopherol (32 mg/ 100 g), the most active form of vitamin E (Hahm *et al.*, 2009).

2.7 Chemical changes during germination

2.7.1 Effect of germination time on chemical composition

The germination time is also a relevant fact and could interfere in germination process (López-Amorós and Estrella, 2006). According to (Hahm *et al.*, 2009), distinct times of germination influences in the nutritional quality of sesame seeds (*Sesamum indicum*).The increase in moisture was directly proportional ($p \leq 0.05$) to the germination time, that is, how greater was the time of sesame germination, greater the water absorption by the seed (Menezes *et al.*, 2018). The same result was found by (Rusydi *et al.*, 2011) and (Amistá and Tavano, 2013) where was found a significant increase of moisture after soy, peanut and rice, and quinoa germination, respectively. This increase in moisture is explained due to seed's hydration in germination process. The more time germinating, more hydrated will be the grain (Martinez *et al.*, 2011).

In relation to lipids content, the time of germination caused a significant interference ($p \leq 0.05$) in values (Menezes *et al.*, 2018), that's means the lipids levels decreases with a higher germination time.(Rusydi *et al.*, 2011) found a significant lipids reduction during the germination process for soy, white, black, red and brown rice. This decrease could be explained due the use of lipid for the seeds growth, because according to(Hahm *et al.*, 2009) the fatty acids are used as energy for germination, and will be oxidized into carbon dioxide and water.

Protein levels on the sample were inversely proportional to germination time ($p \leq 0.05$) (Menezes *et al.*, 2018).(Rusydi *et al.*, 2011) found a significant reduction on protein quantities after soy, peanut and rice germination. This could indicate that the protein

synthesis is lower that the proteolysis in germination(Rusydi *et al.*, 2011). This decrease could be due a higher protein dilution because of the great absorption of water during the germination. However, other authors as (Martinez *et al.*, 2011) and (Ohtsubo *et al.*, 2005) identified an increase of protein content during the germination process. Martinez *et al.* (2011) justified that this increase is due to an enzymatic synthesis or other components losses.

In relation to ashes, was possible to observe a reduction on ash content with increasing germination time (Menezes *et al.*, 2018). Furthermore, (Rusydi *et al.*, 2011) and (Ruiz and Bressani, 1990) found similar results on rice and amaranth seeds evaluation, respectively. This fact could be explained for the minerals loss during the germination process, because the food is washed before the germination begins (Rusydi *et al.*, 2011). The ash reduction could be also explained due to higher absorption of water by the germinated seed, diluting the total ashes content (Menezes *et al.*, 2018).

2.7.2 Effect of germination on phytochemicals

The bioactive compounds are substances presents on foods and act as metabolic modulators capable of inhibiting the onset of degenerative diseases (Vizzoto *et al.*, 2010). According to (Abderrahim *et al.*, 2012), the germination increases the quantity of phenolic compounds and antioxidant capacity in grains. In germination method, due to biochemical process, are formed bioactive compounds with antioxidant function, such as phenolic compounds and tocopherols (Žilić *et al.*, 2014). According to (Li *et al.*, 2014), 128% increase in total flavonoid content of peanut sprouts compared to raw peanut seeds.

The phenolic compounds in white sesame seed were analyzed during the germination time and it was observed an increase directly proportional to time (Menezes *et al.*, 2018).(Xu *et al.*, 2009) found an increase of phenolic compounds in oatmeal in different germination times (12, 24, 36 and 48 hours), as (Troszynska *et al.*, 2006) when was evaluated the mung beans. This fact could be justified because the germination releases the phenolic compounds that were connected and then increases the quantity of total phenolic (Kaukovirta-Norja *et al.*, 2004). According to (Shabbir *et al.*, 2015), the increase in total phenolic content in sesame sprouts is attributed to the increase in phenolic compounds such as sesamol and alpha-tocopherol which are potent antioxidant compounds. Liu *et al.* (2011) reported change

in total phenolic content in sesame seeds from 0.51mg GAE/g in raw sesame seeds to 13.42 mg GAE/g in fifth day of germination.

In a study, (Shabbir *et al.*, 2015) reported the value of DPPH scavenging activity in germinated sesame seed of 1st day, 3rd day and 5th day were 18%, 87% and 89% respectively. Increase in the DDPH activity of sprouts is due to changes in phenolic composition during germination (Shabbir *et al.*, 2015). According to (Liu *et al.*, 2011) the radical scavenging activities during plant growth at third and fourth days of germination increased significantly. Alvarez-Jubete *et al.* (2010) reported the increase in antioxidant activity is due to many metabolic changes during germination such as increase in the activity of the endogenous hydrolytic enzymes during germination.

2.7.3 Effect of germination on antinutrients

The inherent anti nutrient (phytate and oxalate) in the seeds could be reduced to tolerable limit by processing especially by germination. The phytate content of raw sesame sample was higher than the germinated samples. There was significant decrease in the concentration of phytate as germination time progressed. Reduction in phytate content may be attributed to increased synthesis of phytase during germination and the subsequent increase in phytate degradation(Maria and Victoria, 2018). Because germination is mainly a catabolic process that supplies important nutrients to the growing plant though hydrolysis of reserve nutrients, reduction in phytate was expected as it is primary source of phosphorus and cations during the process (Archana *et al.*, 1999). Phytate content was also reduced by 59.31 and 62.39 % in whole and dehulled black sesame cultivar (Folasade and Akinoso, 2014).This reduction may be attributed to leaching out of phytate ions into soaking water under the influence of concentration gradient, such losses may be taken as a function of changed permeability of seed coat (Duhan *et al.*, 1989). Reduction in the level of phytate during germination could be attributed to leaching out during hydration as well as activation of phytase (Eskin and Wiebe, 1983).

Plant seeds utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability, nutritional value and the mineral composition. Reduction of phytates could therefore favor enhanced absorption of the proteins as germination would reduce the immobilization effects of phytate and other antinutritional factors (Olagunju and Ifesan, 2013). According to (Olagunju and Ifesan, 2013), phytate and oxalate was reduced

by 49 % and 51.4% respectively, after four days of germination of sesame seeds. Similarly, there were significant decrease in oxalate concentrations as germination time progressed compared to raw sesame. Decrease in oxalate during germination could be as a result of the activation of oxalate oxidase which breakdown oxalic acid into carbon dioxide and hydrogen peroxide and consequently releasing calcium (Pal *et al.*, 2016).

2.8 Chemical changes during roasting

According to (Folasade and Akinoso, 2014), no significant change was observed in crude fiber content when seeds were toasted for 25 minutes in an oven. According to (Adeyemi *et al.*, 2011) reduction in oxalate content (39%) when the seeds were roasted for five minutes. According to (O.M *et al.*, 2020b) , roasting of sesame seeds for 1hour in an oven at 120 ℃ showed reduction of oxalate content by 42% due to thermo-liable nature of oxalate. (Folasade and Akinoso, 2014) reported 57% reduction in oxalate content when sesame seeds were roasted for 25 min in toaster oven at 160℃. The only factor that could account for the lower concentrations of phytate and oxalate in roasted sesame was the heat applied as these anti-nutrients are thermo labile in nature(O.M *et al.*, 2020b). (Folasade and Akinoso, 2014) also reported significant reduction in phytate and oxalate content of sesame seed during germination, roasting and cooking.

Roasting caused a significant reduction in phytate contents in both brown and white sesame seeds (the reductions were 23.1 and 28.6 %, respectively). Also, tehineh (sesame seeds were dehulled and roasted during the preparation) contained a high level of phytate (3.7%) (Embaby, 2010). Similarly, a significant reduction of phytate contents by thermal processing (roasting, cooking, autoclaving and microwave) has been observed in other plant foodstuff (Fagbemi *et al.*, 2005; Frontela *et al.*, 2008; Habiba, 2002; Wang *et al.*, 2008). The apparent decrease in phytate content during thermal processing may be partly due either to the formation of insoluble complexes between phytate and other components, such as phytate-protein and phytate-protein-mineral complexes or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphate (Siddhuraju and Becker, 2001). On the other hand, some authors reported that phytate contents were unaffected or increased after heat treatments (Embaby, 2010; Martin-Cabrejas *et al.*, 2008; Yagoub, 2007).

Kamalaja *et al.* (2018) reported 24 % increase in flavonoid content when sesame seeds were roasted using domestic processing technique. Increase in total flavonoid content could

be with breaking of binds in the structure of SDG lignan complex and also other polyphenolic compounds in selected nuts and oil seeds with heat treatment at roasting process (Kamalaja *et al.*, 2018). Yunusa *et al.* (2015) reported increase in flavonoid content of almonds when roasted for 5 minutes at 150℃. Total antioxidant reflects presence of naturally occurring and neo-formed antioxidant constituents in oils obtained from either roasted or raw sesame seeds. Roasting caused a clear increase in antioxidant activity. This activity gradually increased during roasting reaching to an apparent maximum within 120 min, and there was a decrease in the antioxidant activity of samples after roasting time of 180 min. The content of phenolic and flavonoids compounds of sesame seeds increased with increase in roasting time, the highest levels of phenolic compounds and flavonoids were obtained with a roasting temperature of 150°C and a roasting time of 90 min with the values 3.9mg gallic acid/g dry matter and 0.14 mg quercetin/g dry matter, and start to decreased until arrive to 1.22 mg gallic acid/g dry matter and 0.08mg quercetin/g dry matter respectively(Heru Rizki *et al.*, 2015).

The increased production of phenolic compounds during roasting in this study may be related to the increased generation of Maillard reaction products during roasting (Özdemir *et al.*, 2001). The technique measures the Maillard reaction products that contain phenolic structures in addition to naturally occurring phenolic compounds (Durmaz and Alpaslan, 2007), generally, the thermal treatment applied to foods of plant origin by heating or roasting causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which may result in a greater availability of plant phenolic compounds in the matrix. Therefore, a thermal process can affect both the nutritional and bioactive characteristics of foods (Heru Rizki *et al.*, 2015).

Part III

Materials and Methods

3.1 Materials

Sesame was bought from the local market of Damak, Jhapa, Nepal. It is locally known as *til*.

3.2 Method

The procedure applied for germination and roasting of sesame seed is as follows. The flowsheet of processing of the sample is present in Fig. 3.1

3.2.1 Cleaning

The sesame seed sample was first cleaned screening to remove impurities such as stones, strings, weed seeds, broken corn etc. and then by winnowing with *nanglo* (flat round woven bamboo tray) to remove dusts, husk, immature grains and other light particles. Then shifting, hand picking and finally washing with water was done to remove adhering dust and finer impurities. Then the seeds were sun dried to obtain cleaned and dried sesame seeds(Maria and Victoria, 2018).

3.2.2 Steeping

Cleaned seeds were transferred to the plastic containers and water was added enough to soak the seeds. Light materials present in the sample were skimmed off. Agitation was done to clean the seed. The grain was steeped for 12 h at room temperature (28 ± 3ºC) and drained to remove the excess water (Maria and Victoria, 2018).

3.2.3 Germination

The steeped seeds were first collected in a muslin cloth and swirled in order to drain excess water and then kept for germination at ambient temperature of average 28 ± 3ºC and 85% RH which was calculated with the help of dry bulb and wet bulb temperature recorded in the thermometer of quality control laboratory. The seeds were spread thinly on a piece of cloth for germination to take place. The germination process was closely monitored to prevent discontinuity of germination and mold growth which was achieved by constant wetting and intermittent uniform spreading of the germinating seedlings. Germination was carried out for 5 days. Only husk remained out of kernel at 6^{th} day, thus germination was terminated at 6^{th} day. The first day sample was taken after 24 h of germination. After that other samples were taken at an interval of 24 h for up to 120 h to determine phenol, flavonoid, DPPH radical scavenging activity, phytate and oxalate.

3.2.4 Drying

Different samples of germinated sesame seeds were taken each day for five days and were dried to stop further germination. Drying was carried out in a cabinet drier at 50 ± 5°C until moisture content was less than 10 % which was determined using a moisture meter (Maria and Victoria, 2018).

3.2.5 Roasting

Five hundred grams of the seeds were roasted in a preheated *karai* (bowl shaped frying pan with two handles) and dry roasted with periodic shaking for even roasting. The time taken for roasting was in range of 2.36-2.47 min and temperature was in range of 148 ± 5 °C. The roasted seeds were then cooled and kept ready for extraction of analysis samples for antinutrients, total flavonoids, total phenols and antioxidant activity levels. The sesame seeds were roasted in a *karai* till the pleasant aroma came and changed the color to slight brownish (Kamalaja *et al.*, 2018).

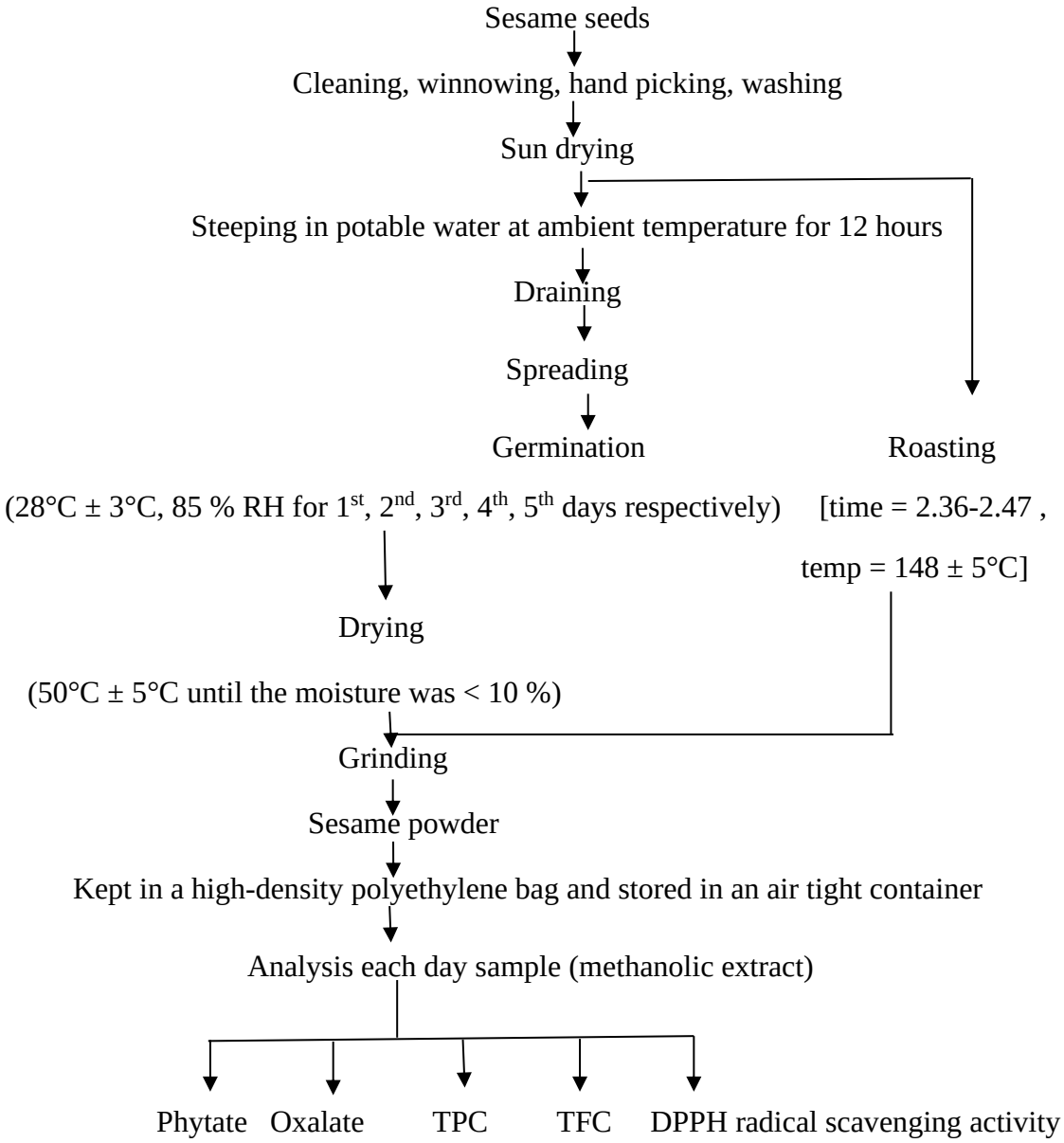

Fig. 3.1 General flowchart of processing of sesame seeds

Source-(Maria and Victoria, 2018)

3.3 Experimental procedure

3.3.1 Proximate analysis

3.3.1.1 Determination of moisture content

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

3.3.1.2 Determination of protein content

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

$$\text{Nitrogen (\%)} = \frac{(\text{Sample titre} - \text{Blank Titre}) \times \text{Normality of HCl} \times 14 \times 100}{\text{weight of sample} \times 100}$$

3.3.1.3 Determination of ash content

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

3.3.1.4 Determination of crude fat

The fat content of the samples was determined as described in (S., 1986). The calculated data were presented as gram per 100 g on dry basis.

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

3.3.1.5 Determination of crude fiber

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

3.3.1.6 Determination of carbohydrate

Total carbohydrate content of the samples was determined by difference method.

Carbohydrate (%) = 100 – [sum of protein, total ash, fiber and fat].

3.3.1.7 Determination of Energy value

One of the methods specified by FDA was employed. This uses the general factors of 4, 4 and 9 calories per gram of protein, total carbohydrate, and total fat, respectively, to calculate the calorie content of food (Bassey *et al.*, 2013).

Energy value per 100g = [carbohydrate×4+protein×4+Fat×9] Kcal

3.3.2 Ultimate analysis

3.3.2.1 Determination of iron

Iron in the sample will be determined by converting all the iron into ferric form using oxidizing agents like potassium per sulphate or hydrogen per oxide and treating thereafter with potassium thiocyanate to form a red ferric thiocyanate which is measured colorimetrically at 480 nm (Rai and K.C., 2007).

3.3.2.2 Determination of calcium

Calcium content will be determined by volumetric method using sulphuric acid and titrating with potassium per manganate (Rai and K.C., 2007)

3.3.3 Determination of antinutrient

3.3.3.1 Phytate

The phytate was extracted with trichloroacetic acid and precipitated as ferric salt. The iron content of the precipitate was determined colorimetrically and the phytate phosphorous content was calculated from this value assuming a constant 4Fe: 6P molecular ratio in the precipitate. The iron present in the test from the standard curve was used to calculate the phytate content as per the equation (Sadashivam and Manickam, 2016)

$$\text{Phytate P mg/100g sample} = \frac{\mu g\ Fe \times 15}{\text{Weight of sample}}$$

3.3.3.2 Oxalate

Oxalate was determined as modified version of (AOAC, 2005). 5g sample was taken which was treated with tungstophosphoric acid and ammonium hydroxide. The precipitate thus obtained was then treated with sulphuric acid and titrated with standard potassium permanganate and until the pink color persisted for 30 seconds.

The calculation was done as:

1 mL of 0.002 M $KMnO_4$ = 0.45 mg anhydrous oxalic acid.

3.3.4 Determination of phytochemical components

3.3.4.1 Total phenolic content

Phenols reacts with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produces blue colored complex (molybdenum blue). Extraction of sample was done in ethanol. Standard curve of catechol was plotted and concentration of phenol in test sample was expressed as mg CE per 100g material (Sadashivam and Manickam, 2016).

3.3.4.2 Total flavonoids content

Total flavonoid content was determined using a modified Aluminum chloride assay method as described by (Barek *et al.*, 2015). 2 mL of solution was pipetted out in a test tube in which 0.2 mL of 5% Sodium Nitrate ($NaNO_3$) was mixed and stood for 5 minutes. 0.2 mL of 5% Aluminum Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This was followed by addition of 2 mL of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5mL. The absorbance was measured after 15 minutes at 510 nm against a reagent blank. The test result was correlated with standard curve of Quercetin (20, 40, 60, 80, 100 µg/mL) and the total flavonoid content was expressed as mg QE/g of dry weight.

3.3.4.3 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 = $1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100\%$

3.3.5 Statistical analysis

Analysis was carried out in triplicate. Data on analysis of phenols, flavonoids, antioxidant activity, oxalic acid and phytate were tabulated for comparison and were graphically represented using Microsoft excel-2016. Data were statistically processed by Gene stat version 12.1.0.3338 for analysis of variance (ANOVA). Means of the data were compared by using Fisher's Unprotected LSD method at 5% level of significance. T-test was performed in MS-Excel to compare best germinated sample with roasted sample.

Part IV

Results and Discussion

Sesamum indicum-was collected from the local market of Damak, Jhapa. The sample was first cleaned, washed and steeped for 12 hours and then germinated at 28 ± 3°C for 24 h, 48 h, 96 h, 120 h. Germinated samples were dried in a cabinet dryer for desired moisture content value less than 10 %. The germinated sample having maximum nutritional property and minimum anti nutritional factor and phytochemical was then compared with open pan roasted sample. Germinated and roasted sample were comparatively studied for quantitative analysis of oxalate, phytate, TPC, TFC and DPPH radical scavenging activity.

4.1 Proximate composition

The proximate analysis gives data about the nutritional components of foods. The proximate composition such as moisture content, crude protein, crude fiber, crude fat and ash content of raw sesame seeds were determined and results are expressed percentage in dry basis in Table 4.1

Table 4.1 Proximate composition of raw sesame seeds.

Parameters	**Value (%)**
Moisture content	5.26 ± 0.43
Crude protein	23.24 ± 0.82
Crude fat	49.25 ± 1.54
Crude fiber	8.50 ± 0.77
Ash content	6.02 ± 0.49
Carbohydrate	7.73 ± 0.57

Values are the mean ± S.D. of three determinations. All values are expressed on dry basis

The result was in accordance to (Hedge, 2012), seeds contain 6–7 % moisture, 17–32 % protein, 48–55 % oil, 14–16 % sugar, 6–8 % fiber and 5–7 % ash. The hull content averages about 17 % of sesame seed, and contains large quantities of oxalic acids, calcium, other minerals and crude fiber. (Gopalan *et al.*, 1982) reported raw sesame seeds contain 5.3 % moisture, 18.3 %

protein, 43.3 % fat, and 5.2 % ash. Fagbemi *et al.* (2005) reported 4.6 % moisture, 6.4 % ash, 4.59 % crude fiber, 59.97 % crude fat, 12.35 % crude protein and 12.85 % carbohydrate.

4.2 Mineral composition of sesame seed

The raw sesame seeds were quantitatively analyzed for calcium and iron content. The mean value of calcium and iron content is demonstrated in Table 4.2.

Table 4.2 Iron and calcium content of raw sesame seeds

Minerals	Value (mg/100 g)
Calcium	510 ± 0.13
Iron	5.13 ± 1.32

The mean values of calcium and iron content was found to be 510 ± 0.13 mg/100 g and 5.13 ± 1.32 mg/100 g on the basis of dry weight. Maria and Victoria (2018) reported the values of calcium and iron in sesame seed is 439.25 ± 1.00 and 6.42 ± 0.02 mg/100 g respectively. Similarly the (Hahm *et al.*, 2009) reported their values as 421 ± 30 and 6.19 ± 0.51 mg/100 g respectively. The result obtained was quite similar to the values to the mentioned values. According to (Folasade and Akinoso, 2014), calcium and iron in sesame seed is 464.97 ± 0.68 and 6.42 ± 0.02 mg/100 g respectively.

4.3 Effect of processing on antinutrients and phytochemicals

4.3.1 Germination

4.3.1.1 Phytate

Phytate is an antinutritional factor present in sesame seed which reduces the bioavailability of nutrients from the seed. The sesame seeds were germinated for five days. The change in phytate content was analyzed in each day of germinated sample as well as in raw sample. The reduction in phytate content is demonstrated in Fig. 4.3.

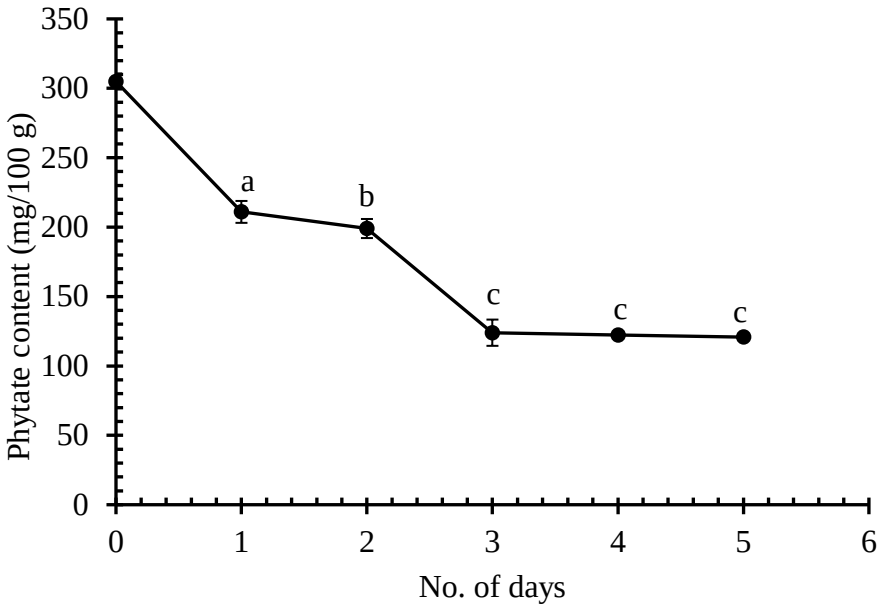

Fig.4.3 Effect of germination on phytate content of sesame seed.

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5 % level of significance]

The mean value of phytate content in raw sesame seed was found to be 304.9 ± 5.78 mg/100 g on the basis of dry matter. Phytate content was reduced on the progressive days of germination. The mean value of progressive days of germination was 210.97 ± 7.89 mg/100 g, 198.99 ± 6.87 mg/100 g, 123.93 ± 9.45 mg/100 g, 122.93 ± 1.55 mg/100 g and 120.76 ± 0.56 mg/100 g on the basis of dry matter on 1st, 2nd, 3rd, 4th and 5th days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between phytate content in the first three days germinated samples ($p < 0.05$) while there was no significant difference between third, fourth and fifth germination days. The mean value showed that third day onwards germination had least significant difference in phytate content. Phytate content was reduced by 59.3 % on germination of the seeds for three days.

The phytate content of raw sesame seed was in accordance to (Deme *et al.*, 2017), 285 mg/100 g phytate in raw seeds. Folasade and Akinoso (2014) suggested that the amount of phytate depends on the seed size. (Lyon, 1972) reported that phytate content of sesame variety was affected by genotype. According to (Folasade and Akinoso, 2014), phytate content decreased significantly ($p < 0.05$) 26.60 % in whole sesame seeds of white cultivar,

respectively. Phytate content was also reduced by 59.31 and 62.39 % in whole and dehulled black sesame cultivar (Folasade and Akinoso, 2014).This reduction may be attributed to leaching out of phytate ions into soaking water under the influence of concentration gradient, such losses may be taken as a function of changed permeability of seed coat (Duhan *et al.*, 1989). Reduction in the level of phytate during germination could be attributed to leaching out during hydration as well as activation of phytase (Eskin and Wiebe, 1983).

Plant seeds utilize phytate as a source of inorganic phosphate during germination and thus tend to increase palatability, nutritional value and the mineral composition. Reduction of phytates could therefore favor enhanced absorption of the proteins as germination would reduce the immobilization effects of phytate and other antinutritional factors (Olagunju and Ifesan, 2013). According to (Olagunju and Ifesan, 2013), phytate was reduced by 49% after four days of germination of sesame seeds.

4.3.1.2 Oxalate content

Oxalate is a potential antinutrient present in sesame seed. The sesame seeds were germinated for five days. The change in oxalate content was analyzed in each day of germinated sample as well as in raw sample. The reduction in oxalate content is demonstrated in Fig. 4.4.

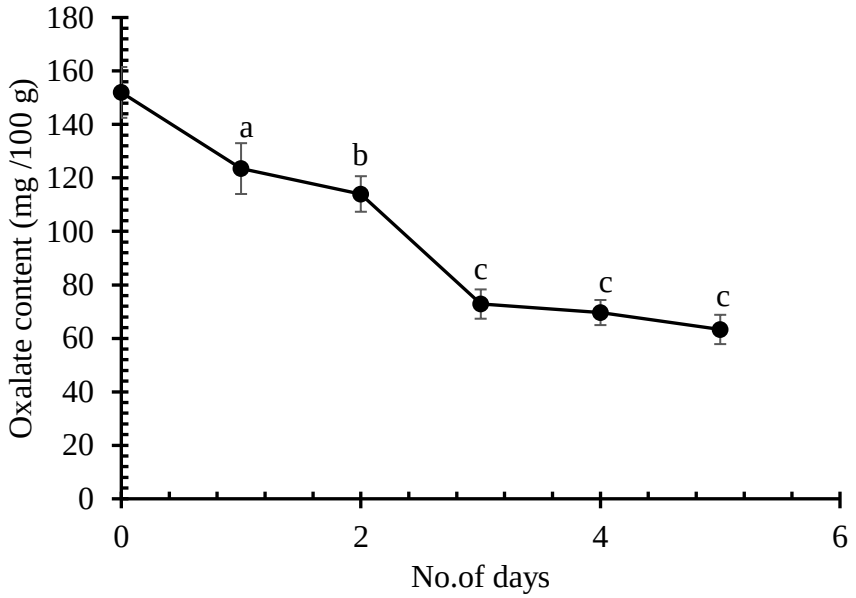

Fig.4.4 Effect of germination on oxalate content of sesame seeds

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5 % level of significance]

The mean value of oxalate content of raw sesame seed was observed 151.99 ± 9.50 mg/100 g on the basis of dry matter. Oxalate content was reduced as the days of germination was increased. The mean value of progressive days of germination was 123.5 ± 9.50 mg/100 g, 114 ± 6.65 mg/100 g, 72.83 ± 5.48 mg/100 g, 69.66 ± 4.69 mg/100 g and 63.33 ± 5.48 mg/100 g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between oxalate content in the first three days germinated samples ($p < 0.05$) while there was no significant difference between third, fourth and fifth germination days. The mean value showed that third day onwards germination had least significant difference in oxalate content. Oxalate content was reduced by 52 % on germination of the seeds for three days.

This result was in accordance to (Menezes *et al.*, 2018), the effect of germination on inactivation of oxalate in white and black sesame cultivars showed that oxalate decreased significantly ($p < 0.05$) by 46.79 % and 51.80 % of whole and dehulled seed of white seed respectively. (Menezes *et al.*, 2018) also reported the oxalate content decreased by 51.47 and 53.30% of whole and dehulled black seed respectively. Decrease in oxalate during germination could be as a result of the activation of oxalate oxidase which breakdown oxalic acid into carbon dioxide and hydrogen peroxide and consequently releasing calcium (Pal *et al.*, 2016). According to (Olagunju and Ifesan, 2013), germination of sesame seed for four days resulted in 51.4 % reduction in oxalate content in black sesame seed compared to raw seeds.

4.3.1.3 Total phenolic content

Phenolic compounds are widely distributed in the plant kingdom. These compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules (Shahidi *et al.*, 2006a). The sesame seeds were germinated for five days. The change in total phenolic content was analyzed in each day of germinated sample as well as in raw sample. The increment in total phenolic content is demonstrated in Fig. 4.5.

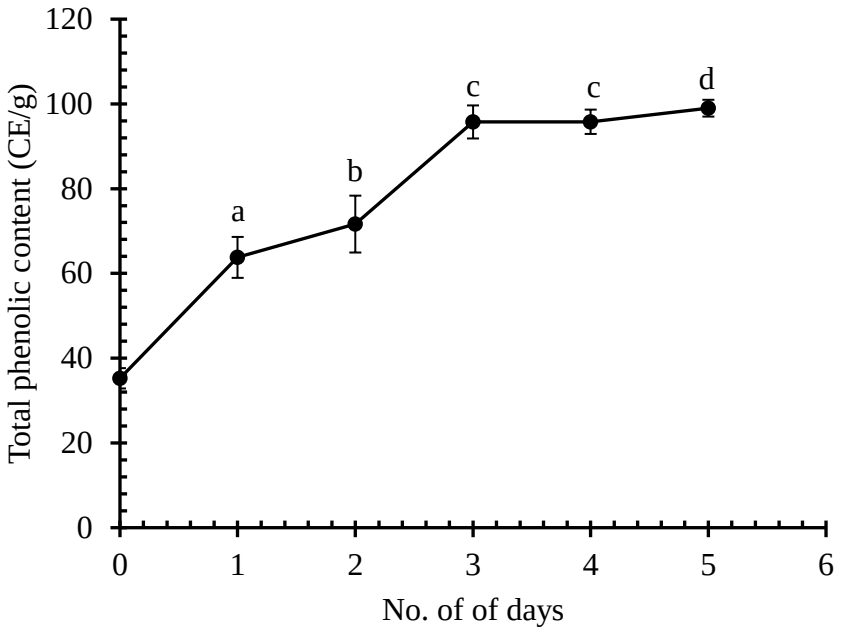

Fig.4.5 Effect of germination on TPC of sesame seeds

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p>0.05$) at 5 % level of significance]

The mean value of raw sesame seed was observed 35.25 ± 2.40 mg CE/g of methanolic extract on the basis of dry matter. The mean value of progressive days of germination was 63.78 ± 4.83 mg CE/g, 71.64 ± 6.71 mg CE/g, 95.74 ± 3.90 mg CE/g, 95.78 ± 2.87 mg CE/g and 98.99 ± 1.98 mg CE /g on the basis of dry matter on 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between total phenol content in the first three days germinated samples ($p < 0.05$) while there was no significant difference between third, fourth germination days. Meanwhile fourth- and fifth-day sample showed significant difference between the sample. The mean value showed that third day and fourth day had least significant difference in total phenolic content. Total phenolic content was increased by 171.6 % on germination of the seeds for three days and slight increment (179.6%) on fifth day of germination.

According to (Shabbir *et al.*, 2015), the total phenolic content of sesame seeds was increased in fifth day of germination. The increase in total phenolic content in sesame sprouts is attributed to the increase in phenolic compounds such as sesamol and alpha-tocopherol which are potent antioxidant compounds. Liu *et al.* (2011) reported increase in total phenolic content in sesame

seeds in five days of germination. The percentage of change in phenolic content was extremely high compared to the observed value in this study. Menezes *et al.* (2018) reported similar change in percentage of total phenolic content in three days white sesame sprouts (of about 200 %). Xu *et al.* (2009) found an increase of phenolic compounds in oatmeal in different germination times (12, 24, 36 and 48 hours) and (Troszynska *et al.*, 2006) reported increase in phenolic content with germination when mung beans were evaluated.

4.3.1.4 Total flavonoid content (TFC)

Sesame seeds contain flavonoids and other phenolic compounds that can act as antioxidants (Ruslan *et al.*, 2018). The flavonoid content of raw sesame seed was determined using quercetin as standard solution. The sesame seeds were germinated for five days. The change in total flavonoid content was analyzed in each day of germinated sample as well as in raw sample. The increment in total flavonoid content is demonstrated in Fig. 4.6.

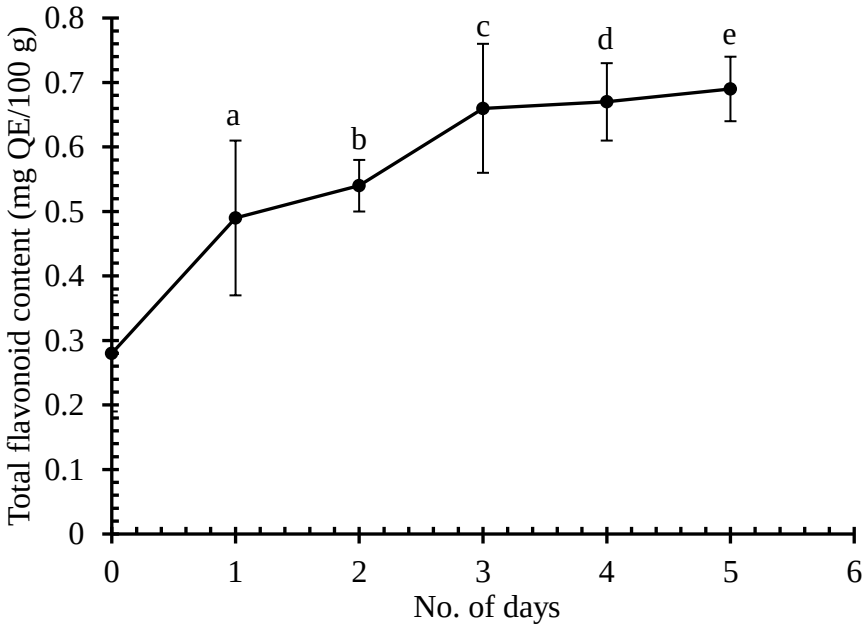

Fig.4.6 Effect of germination on TFC of sesame seeds.

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5% level of significance]

The mean value of flavonoid content of raw sesame seed was observed 0.28 ± 0.09 mg QE/100g on the basis of dry matter. The mean value of total flavonoid content in progressive

days of germination was 0.49 ± 0.12 mg QE/100 g, 0.54 ± 0.04 mg QE/100 g, 0.66 ± 0.10 mg QE/100 g, 0.67 ± 0.01 mg QE/100 g and 0.69 ± 0.01 mg QE/100 g on the basis of dry matter on 1st, 2nd, 3rd, 4th and 5th days of germination respectively. The analysis of variance (Appendix B) showed that there was significant difference between the total flavonoid content in the different days germinated samples ($p < 0.05$). Total flavonoid content was increased by 134.8 % on germination of the seeds on third day and slight increment (146.2 %) on fifth day of germination.

The results was in accordance to (Li *et al.*, 2014), 128% increase in total flavonoid content of peanut sprouts compared to raw peanut seeds.

4.3.1.5 DPPH radical scavenging activity

The DPPH radical is a stable organic free radical with an adsorption peak at 517 nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. The sesame seeds were germinated for five days. The change in DPPH radical scavenging activity was analyzed in each day of germinated sample as well as in raw sample. The increment in DPPH radical scavenging activity is demonstrated in Fig. 4.7.

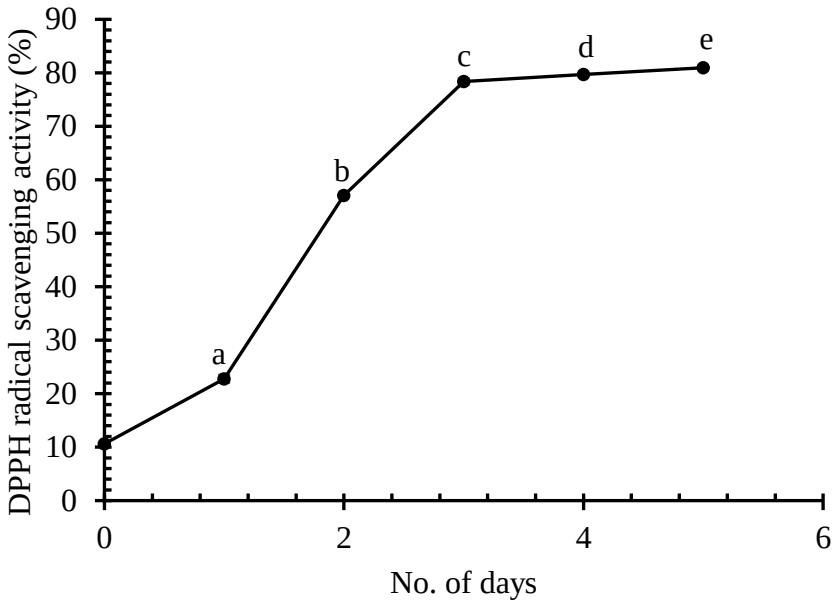

Fig.4.7 Effect of germination on DPPH radical scavenging activity of sesame seeds

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5% level of significance]

The mean value of DPPH scavenging activity in raw sesame seed was observed 10.62 ± 0.06% on the basis of dry matter. The mean value of progressive days of germination are 22.79 ± 0.77 %, 57.08 ± 0.08 %, 78.38 ± 0.57 %, 79.62 ± 0.04 %, 80.95 ± 0.04 % respectively. The analysis of variance (Appendix B), showed that significance difference ($p < 0.05$) in DPPH scavenging activity in each day of germinated samples. DPPH scavenging activity was directly proportional to number of days of germination.

The result was in accordance to (Shabbir *et al.,* 2015), the value of DPPH scavenging activity in germinated sesame seed of 1st day, 3rd day and 5th day were 18%, 87% and 89% respectively. Increase in the DDPH activity of sprouts is due to changes in phenolic composition during germination (Shabbir *et al.,* 2015). According to (Liu *et al.,* 2011) the radical scavenging activities during plant growth at third and fourth days of germination increased significantly. Alvarez-Jubete *et al.* (2010) reported the increase in antioxidant activity is due to many metabolic changes during germination such as increase in the activity of the endogenous hydrolytic enzymes during germination.

4.3.2 Roasting

4.3.2.1 Phytate

Sesame seeds were roasted following household roasting technique in an open pan with periodic stirring. The seeds were roasted in three batches and average time and temperature were noted. The roasted seeds were analyzed for antinutritional and phytochemical properties. The mean phytate content of roasted sesame seed was 167.20 ± 0.21 mg/100 g. The phytate content of sesame seed was reduced by 45 % compared to raw sesame seeds.

According to (Adeyemi *et al.,* 2011), roasting of sesame seeds for five minutes resulted in 54.7% reduction in phytate content. According to (Maria and Victoria, 2018), open pan roasting at 75-85°C for 20 minutes showed higher reduction in phytate content compared to microwave roasted sesame seed. Maria and Victoria (2018) reported 38.9 % reduction in phytate content upon roasting of sesame seeds and suggested the lower concentrations of phytate in roasted sesame was because these antinutrients are thermo labile in nature.

4.3.2.2 Oxalate content

Sesame seeds were roasted following household roasting technique in an open pan with continuous stirring. The seeds were roasted in three batches and average time and temperature were noted. The roasted seeds were analyzed for antinutritional and phytochemical properties. The mean value of oxalate content of roasted sesame seed was 107.66 ± 0.29 mg/100 g. The percentage of reduction of oxalate content was found to be 29 %.

According to (Adeyemi *et al.*, 2011) higher reduction in oxalate content (39%) when the seeds were roasted for five minutes. According to (O.M *et al.*, 2020a), roasting of sesame seeds for 1hour in an oven at 120℃ showed reduction of oxalate content by 42 % due to thermo-liable nature of oxalate. Folasade and Akinoso (2014) reported 57 % reduction in oxalate content when sesame seeds were roasted for 25 min in toaster oven at 160℃.

4.3.2.3 Total phenolic content

Sesame seeds were roasted following household roasting technique in an open pan with continuous stirring. The seeds were roasted in three batches and average time and temperature were noted. The roasted seeds were analyzed for antinutritional and phytochemical properties. The mean value of total phenol content of roasted sesame seed was found to be 55.38 ± 0.13 mg catechin equivalent/g. The total phenolic content initially in raw sample was 35.25 ± 0.02 mg which remarkably increased by 57 % upon roasting.

The result was in accordance to (Heru Rizki *et al.*, 2015) who reported increase in phenolic compounds with increase in time of roasting when seeds were roasted in oven for 90 min at 150℃. The increased production of phenolic compounds during roasting may be related to the increased generation of Maillard reaction products during roasting (Özdemir *et al.*, 2001). Kamalaja *et al.* (2018) reported increase in phenolic content of sesame seeds when sesame seeds were roasted following domestic procedure. Durmaz and Alpaslan (2007) reported, the thermal treatment applied to foods of plant origin by heating or roasting causes evaporation of intracellular water, triggering chemical reactions that can change the lignocellulosic structure and promotes protein denaturation, which may result in a greater availability of plant phenolic compounds in the matrix. Therefore, a thermal process can affect both the nutritional and bioactive characteristics of foods.

4.3.2.4 Total flavonoid content

Sesame seeds were roasted following household roasting technique in an open pan with continuous stirring. The seeds were roasted in three batches and average time and temperature were noted. The roasted seeds were analyzed for antinutritional and phytochemical properties. The mean value of total flavonoid content of roasted sesame seed was found to be 3.11 mg QE/g. The total flavonoid content initially in raw sample was 2.8 mg QE/g which remarkably increased by 11.07 % upon roasting.

The result was in accordance to (Heru Rizki *et al.*, 2015) who reported increase in flavonoids content with increase in time of roasting when seeds were roasted in oven for 90 min at 150℃. Kamalaja *et al.* (2018) reported 24 % increase in flavonoid content when sesame seeds were roasted using domestic processing technique. Increase in total flavonoid content could be with breaking of binds in the structure of SDG lignan complex and also other polyphenolic compounds in selected nuts and oil seeds with heat treatment at roasting process (Kamalaja *et al.*, 2018). Yunusa *et al.* (2015) reported increase in flavonoid content of almonds when roasted for 5 minutes at 150℃.

4.3.2.5 DPPH free radical scavenging activity

Sesame seeds were roasted following household roasting technique in an open pan with continuous stirring. The seeds were roasted in three batches and average time and temperature were noted. The roasted seeds were analyzed for antinutritional and phytochemical properties. The mean value of DPPH radical scavenging activity of roasted sesame seeds was found to be 36.73 %. The DPPH radical scavenging activity of roasted sesame was increased by 245.8 % compared to raw sesame seeds.

Total antioxidant reflects presence of naturally occurring and neo-formed antioxidant constituents in oils obtained from either roasted or raw sesame seeds. The result was in accordance with (Heru Rizki *et al.*, 2015), who reported increase in DPPH radical scavenging activity with roasting of sesame seeds. S *et al.* (2006) reported as the concentration of phenolic compounds or degree of hydroxylation of phenolic compounds increases, the DPPH radical scavenging activity also increases. The result was in accordance to (Lawal *et al.*, 2019) 40 % increase in antioxidant activity on roasting sesame seeds for 10 minutes at 120℃.

4.4 Comparison of optimum germinated and roasted sample

From the analysis of antinutrients and phytochemicals in sesame sprouts up to 5th day of germination the third day sample showed significant difference ($p < 0.05$) and a higher mean value compared to day 1 and day 2 sample. Though some of the parameters showed significant difference in 4^{th} and 5^{th} day sample but the mean value showed smaller difference compared to third day. Thus, third day sample was considered optimum considering reduced anti-nutritional and enhanced phytochemical properties compared to raw and time convenient compared to fourth and fifth day of germination.

4.4.1 Proximate composition

The proximate values of raw, optimum germinated and roasted sesame seeds were analyzed. The concentration of each parameters of proximate composition is presented in Fig. 4.8.

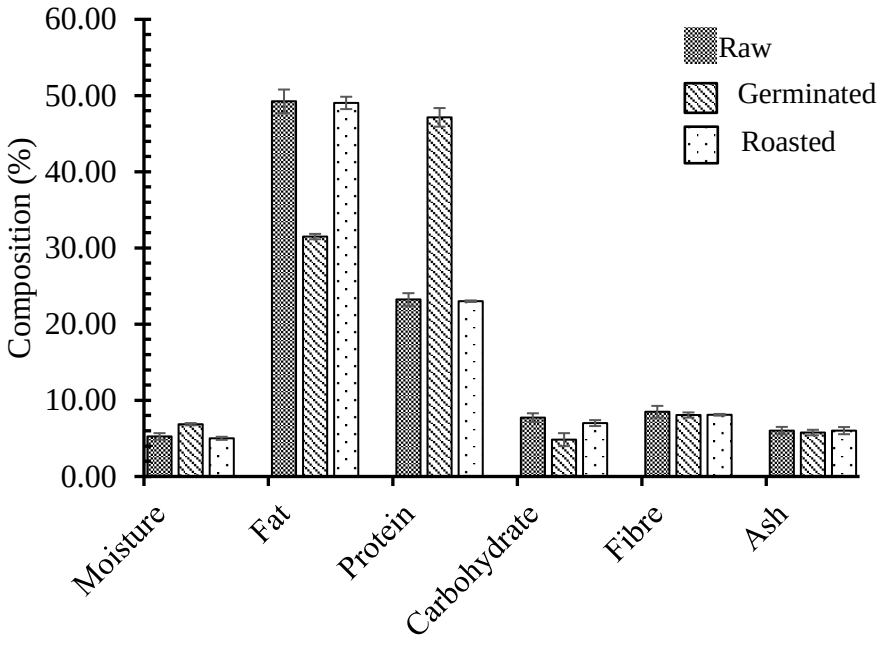

Fig 4.8 Effect of processing techniques on proximate composition

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5 % level of significance]

The mean value of moisture content of raw sesame seeds was 5.26 ± 0.43 % on the basis of dry weight. The moisture content of germinated and roasted sesame seeds was 6.86 ± 0.15 % and 5.01 ± 0.20 % on the basis of dry weight respectively. The analysis of variance showed

significant difference between raw and germinated sample ($p < 0.05$). According to (Menezes *et al.*, 2018), as the days of germination increased, the moisture content of the seeds increased. The same result was found by (Rusydi *et al.*, 2011) and (Amistá and Tavano, 2013), significant increase of moisture after soy, peanut and rice, and quinoa germination, respectively. This increase in moisture is explained due to seed's hydration in germination process. Increase in the time of germination, increases moisture content of seeds (Martinez *et al.*, 2011).

The mean value of crude protein content of raw, germinated, roasted sesame seeds was 23.24 ± 0.82, 47.14 ± 1.22 and 23.01 ± 0.01 % respectively on the basis of dry weight. Germinated sesame seeds showed highest percentage of crude protein compared to raw and roasted seeds. The analysis of variance showed significant difference between raw and germinated sample ($p < 0.05$) in terms of protein content. Maria and Victoria (2018) reported increase in protein content of white sesame seeds with increase in germination time. The significant increase in protein content seen in germinated samples compared to raw seed is attributed to increased water activity as a result of hormonal or compositional change during degradation of other components (H, 2010).

The mean value of crude fat content of raw, germinated and roasted sesame seeds was 49.25 ± 1.54, 31.49 ± 0.35 and 49.03 ± 0.81 % respectively on the basis of dry weight. The analysis of variance showed significant decrease ($p < 0.05$) in crude fat in germinated seeds compared to raw sesame seeds. The result was in accordance to (Liu *et al.*, 2011), significant decrease in crude fat during germination. The crude fat content was 57 %, 54 %, 44 % and 20 % of raw, 1^{st}, 2^{nd}, 3^{rd} and 5^{th} days sprout respectively. The sesame sprouts were rich in polyunsaturated fatty acids with oleic and linolenic being the major fatty acids. Hahm *et al.* (2009) reported reduction in fat content as germination progressed, 23% reduction in four days of germination as the fatty acids are used as energy for germination, and will be oxidized into carbon dioxide and water (Menezes *et al.*, 2018) reported in relation to lipids content, the time of germination caused a significant interference ($p \leq 0.05$) in values, that means the lipids levels decreases with a higher germination time.

The mean value of crude fiber content of raw, germinated and roasted sesame seeds was 8.50 ± 0.77, 8.07 ± 0.34 and 8.10 ± 0.11 % respectively on the basis of dry weight. The analysis of variance showed there was no significant difference in fiber content ($p < 0.05$) between raw, optimum germinated and roasted seeds. According to (Folasade and Akinoso, 2014), no

significant change was observed in crude fiber content when seeds were toasted for 25 minutes in an oven.

The mean value of ash content of raw, germinated and roasted sesame seeds was 6.02 ± 0.49, 5.77 ± 0.30 and 6.02 ± 0.47 % respectively on the basis of dry weight. According to (Hahm *et al.*, 2009), no significant change in ash content during germination was reported. (Shabbir *et al.*, 2015) also reported no significant change in ash content during germination.

The mean value of carbohydrate content of raw, germinated and roasted seeds was 7.73 ± 0.57, 4.84 ± 0.85 and 7.01 ± 0.39 % respectively on the basis of dry weight. (Maria and Victoria, 2018) reported significant decrease in carbohydrate content of white sesame seeds with germination time. The carbohydrate content in sprouts decreased continuously with germination time, from 13.1% to 9.6 % at 24 h of germination compared to 5.4 % at 96 h. This observation could be due to the utilization of fat and carbohydrate for biochemical activities of the germinating seeds (Maria and Victoria, 2018).

No significant difference was observed in proxiimate composition of raw and roasted sesame seeds. Paired t-test of germinated and roasted seasame seeds showed significant difference (t-tabulated < t- calculated) in terms of carbohydrate, protein, fat and moisture. Optimum germinated seeds had higher protein content and lower fat and carbohydrate content compared to raw and roasted sesame seeds.

4.4.2 Oxalate

The mean value of oxalate content of roasted sesame seed was compared with oxalate content of raw and optimum germinated sample. The change in oxalate content in two processing techniques compared to raw is demonstrated in Fig. 4.9.

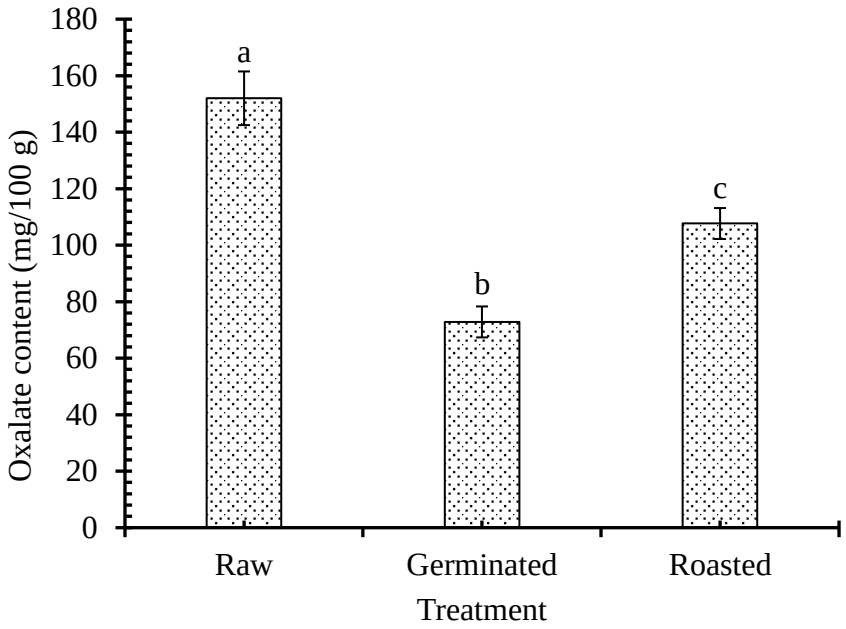

Fig.4.9 Effect of treatments on oxalate content.

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5% level of significance]

The mean value of oxalate content of raw sesame seeds was 151.99 ± 0.15 mg/100 g, three days germinated seeds was 72.83 ± 0.07 mg/100 g and roasted seeds was 107.66 ± 0.29 mg/100 g on the basis of dry matter. The analysis of variance (Appendix B) showed significant difference between the three samples in context to oxalate content ($p < 0.05$).

Significant difference between oxalate content of germinated and roasted sesame seeds was observed. T-test (Appendix C) for oxalate content showed that tabulated value (4.3) is less than calculated value (5.49). Germination showed greater reduction in oxalate content compared with roasting.

Germinated seeds showed lower oxalate content compared to roasted sesame seeds. The result was in accordance to (Maria and Victoria, 2018) 51.9 % reduction in oxalate content when seeds were germinated for and 32 % reduction when sesame seeds were roasted.

4.4.3 Phytate

The mean value of phytate content of roasted sesame seed was compared with phytate content of raw and optimum germinated sample. The change in phytate content in two processing techniques compared to raw is demonstrated in Fig. 4.10.

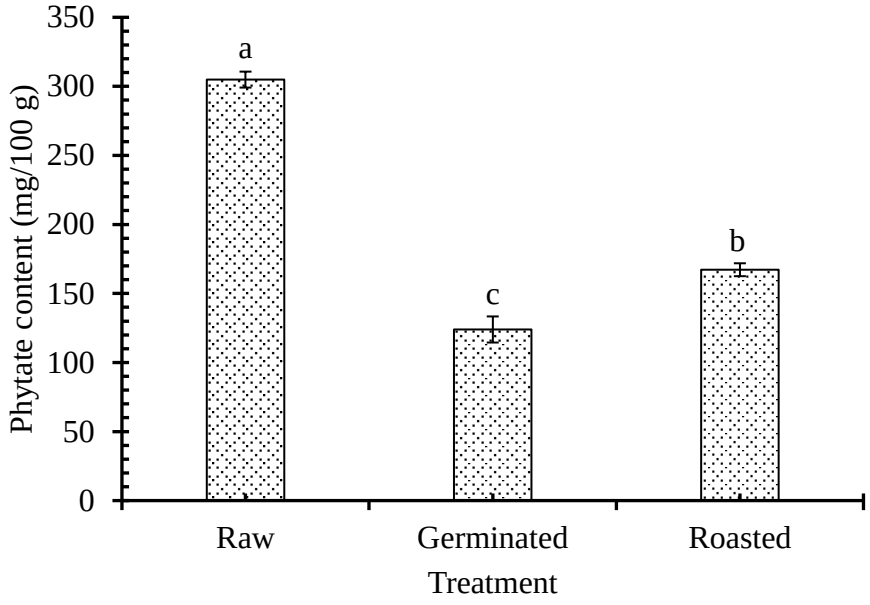

Fig.4.10 Effect of treatments on phytate content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5% level of significance]

The mean value of phytate of raw sesame seeds was 304.9 ± 2.38 mg/100 g, three days germinated seeds was 123.93 ± 0.69 mg/100 g and roasted seeds was 167.20 ± 0.21 mg/100 g on the basis of dry matter. The analysis of variance (Appendix B) showed significant difference between the three samples in context to phytate content ($p < 0.05$).

Significant difference between phytate content of germinated and roasted sesame seeds was observed. T-test (Appendix C) for phytate content showed that tabulated value (4.3) is less than calculated value (91.8). Germination of sesame seeds showed maximum reduction in phytate content compared to roasting.

Germinated seeds showed lower phytate content compared to roasted sesame seeds. The result was in accordance to (Maria and Victoria, 2018), 59.2 % reduction in phytate content

when seeds were germinated for and 18.3 % reduction when sesame seeds were roasted. Reduction in the level of phytic acid during germination could be attributed to leaching out during hydration as well as activation of phytase (Eskin and Wiebe, 1983).

4.4.4 Total phenolic content

The mean value of total phenolic content of roasted sesame seed was compared with raw and optimum germinated sample. The change in total phenolic content in two processing techniques compared to raw is demonstrated in Fig. 4.11.

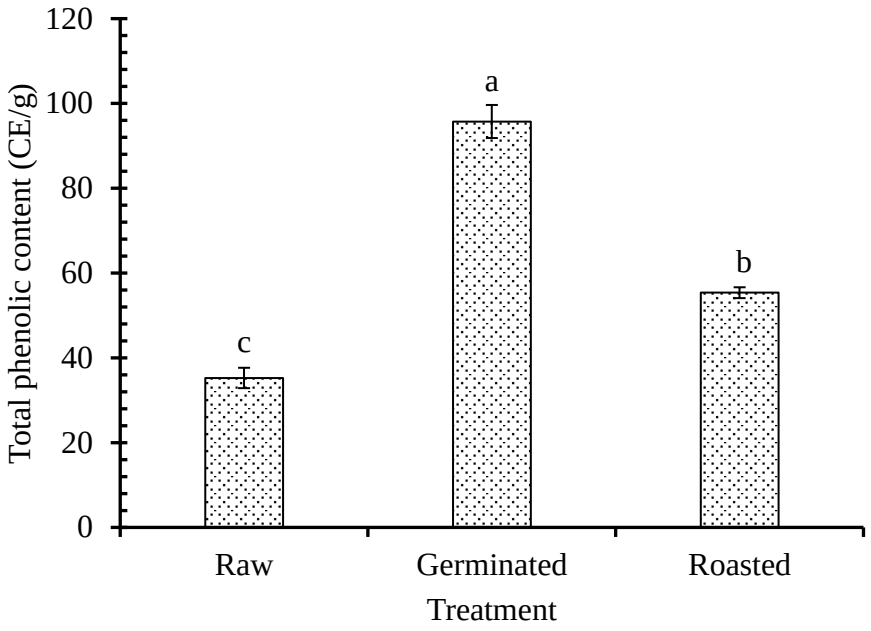

Fig. 4.11 Effect of treatment on total phenolic content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5% level of significance]

The mean value of total phenolic content of raw sesame seeds was 35.25 ± 0.02 mg CE/g, three days germinated seeds was 95.74 ± 0.39 mg CE/g and roasted seeds was 55.36 ± 0.13 mg CE/g on the basis of dry matter. The analysis of variance (Appendix B) showed significant difference between the three samples in context to total phenolic content ($p < 0.05$).

Significant difference was observed in total phenolic content of germinated and roasted sesame seeds. T-test (Appendix C) for total phenolic content showed that tabulated value (4.3)

is less than calculated value (134.26). Germination of sesame seeds showed maximum increment in total phenolic content compared to roasting.

The total phenolic content of germinated seeds was reported higher compared to roasted sesame seeds. The result was in accordance to (Khatkhar *et al.*, 2016) germination of fenugreek seeds resulted in higher total phenolic content compared to roasted seeds.

4.4.5 Total flavonoid content

The mean value of total flavonoid content of roasted sesame seed was compared with raw and optimum germinated sample. The change in total flavonoid content in two processing techniques compared to raw is demonstrated in Fig. 4.12.

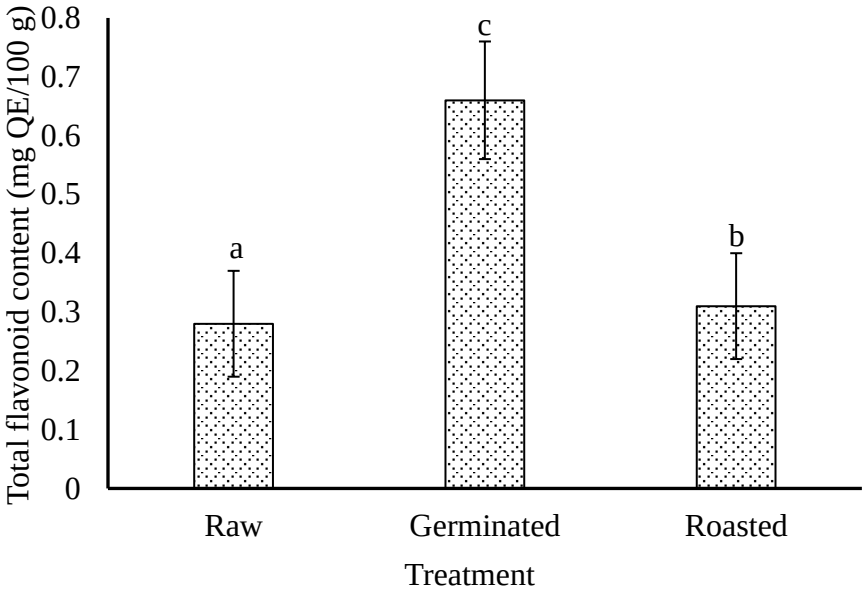

Fig.4.12 Effect of treatment on total flavonoid content

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5% level of significance]

The mean value of total flavonoid content of raw sesame seeds was 2.8 mg QE/g, three days germinated seeds was 6.6 ± 0.01 mg QE/g and roasted seeds was 3.11 mg QE/g on the basis of dry matter. The analysis of variance (Appendix B) showed significant difference between the three samples in context to flavonoid content ($p < 0.05$).

Significant difference in total flavonoid content was observed between germinated and roasted sesame seeds. T-test (Appendix C) for total flavonoid content showed that tabulated value (4.3) is less than calculated value (313.5). Germination of sesame seeds showed maximum increment in total flavonoid content compared to roasting.

The total flavonoid content of germinated seeds was reported higher compared to roasted sesame seeds. The result was in accordance to (Khatkhar *et al.*, 2016) germination of fenugreek seeds resulted in higher total flavonoid content compared to roasted seeds.

4.4.6 DPPH radical scavenging activity

The mean value of DPPH radical scavenging activity of roasted sesame seed was compared with raw and optimum germinated sample. The change in DPPH radical scavenging activity in two processing techniques compared to raw is demonstrated in Fig. 4.13.

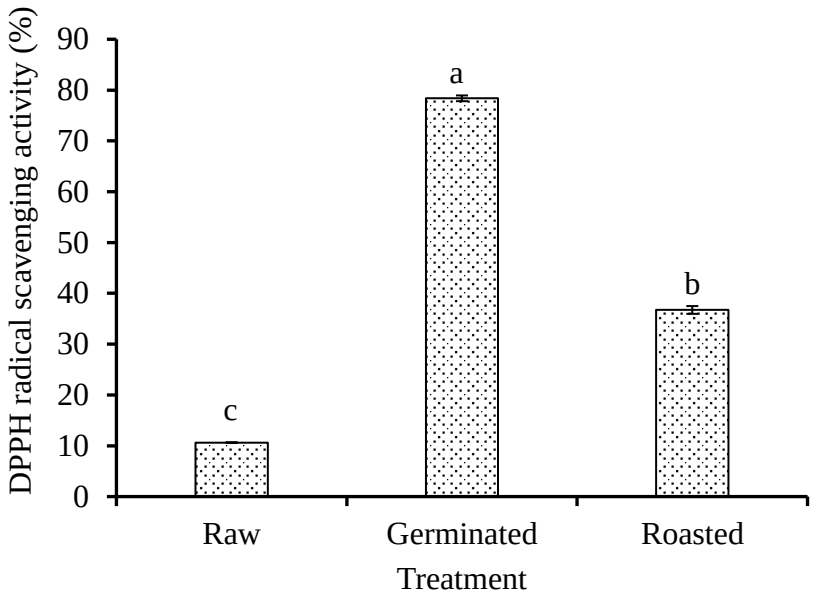

Fig.4.13 Effect of processing treatment on DPPH radical scavenging activity

[Plotted values are means of triplicates. Vertical error bars represent ± standard deviations. Values on top of the bars bearing similar superscript are not significantly different ($p > 0.05$) at 5 % level of significance]

The mean value of DPPH radical scavenging activity of raw sesame seeds was 10.62 %, three days germinated seeds was 78.38 % and roasted seeds was 36.73 % on the basis of dry matter. The analysis of variance (Appendix B) showed significant difference between the three samples in context to DPPH radical scavenging activity ($p < 0.05$).

Significant difference in DPPH scavenging activity was observed between germinated and raw sesame seeds. T-test (Appendix C) for DPPH radical scavenging activity showed that tabulated value (4.3) is less than calculated value (20.37). Germination of sesame seeds showed maximum increment in DPPH radical scavenging activity compared to roasting.

The DPPH radical scavenging activity of germinated seeds was reported higher compared to roasted sesame seeds. The result was in accordance to (Khatkhar *et al.*, 2016) germination of fenugreek seeds resulted in higher DPPH radical scavenging activity compared to roasted seeds.

Part V

Conclusions and Recommendations

5.1 Conclusions

In this study, sesame seed was germinated for five days. Roasting in open pan similar to domestic settings was also performed. The raw, germinated and roasted sesame seed were analyzed for nutritional and anti-nutritional properties.

- The raw sesame seeds were high in crude protein (23.24 ± 0.82 %) and crude fat (49.25 ± 1.54 %). Iron and calcium content were 5.13 ± 1.32 mg/100 g and 510 ± 0.13 mg/100 g respectively.
- Oxalate and phytate content decreased significantly upon germination whereas phytochemicals (TPC and TFC) and DPPH radical scavenging activity increased significantly. Significant change in crude fat, crude protein and carbohydrate content were noted in germinated seeds.
- Anti-nutrients (oxalate and phytate) decreased significantly upon roasting of the seeds following common household technique and TPC, TFC and DPPH radical scavenging activity increased significantly.
- Comparing germinated sample with roasted sample maximum reduction in antinutrients and maximum increment in phytochemicals and antioxidants was noted in germinated samples. Among different germinated samples third day sample was best on the basis of maximum nutritional and minimum anti-nutritional factors.

5.2 Recommendations

- Maximum reduction of antinutritional factors and increase in phytochemical properties of sesame seeds was found in 3 days of germination compared to 2.41 min of roasting time.
- Germination followed by roasting can be packaged in proper packaging material to increase commercial market of sesame seed with optimum phytochemical properties and minimum antinutritional factors.

Part VI

Summary

Sesame seeds were collected from Damak, Jhapa and germinated for five days. Each day germinated sample along with raw seeds were analyzed for antinutrients (phytate and oxalate) and phytochemicals (phenols, flavonoids) and DPPH radical scavenging activity. The optimum germinated sample was selected based on minimum antinutrients and maximum phytochemical properties. Raw seeds were roasted in three batches in 2.5 mm thick aluminum open vessel by continuously stirring. The time and temperature of roasting of the seeds were noted. Time taken for roasting was 2.41 min and temperature was 148°C in average. Sensory evaluation was performed to judge the roasting of the seeds. The roasted seeds were also analyzed for its antinutrients and phytochemical property.

The antinutrients of raw sesame seeds were found to be 151.99 ± 0.15 mg/100 g oxalate and mg/100 g phytate. Analysis of variance (ANOVA) of each day germinated seeds up to five days showed significant difference ($p < 0.05$) between raw and germinated seeds in terms of both antinutrients and phytochemicals. Seeds germinated for 72 hours (three days) had the highest significant loss of antinutrients and thereafter the antinutrients of the seeds did not result in significant reduction ($p > 0.05$). Roasting of the seeds also resulted in significant reduction of antinutrients ($p < 0.05$). Performing t-test of the roasted and optimum germinated sample (i.e., third day) significant difference in antinutrient property was observed (t-tabulated < t-calculated).

The total phenolic content and total flavonoid content which both contribute to the antioxidant and phytochemical property of the seed was found to be 35.25 ± 0.02 CE/g and 2.81 mg QE/g respectively in raw sample. The phytochemical properties showed significant increase ($p < 0.05$) in each day germinated samples. Roasting of the seeds also lead to the increment of total phenolic content and total flavonoid content by 57 % and 14.28 % respectively. T-test of roasted and optimum germinated sample showed significant difference in phenol and flavonoid content (t-tabulated < t-calculated).

DPPH radical scavenging activity of raw sesame seed was found to be 10.62 % which increased to 80.95 % upon five days of germination and 36.73 % during roasting. ANOVA of each day germinated sample showed significant difference ($p < 0.05$) in DPPH scavenging activity among each other. Comparison of optimum germinated sample with roasted sample

statistically using t-test showed significant difference (t-tabulated < t-calculated) in context to DPPH scavenging activity.

This study showed that significant difference between germination and roasting of sesame seeds in terms of antinutrients and phytochemical property. The effective treatment for processing of sesame seed as suggested by the results of the study is germination. Though household roasting techniques are convenient in terms of time, roasted seeds are of lower nutritional value compared to germinated seeds. Germination of seeds for longer period increases the phytochemicals for some extent but three days (72 hours) of germination is considered optimum in terms of higher nutritional value, lower antinutritional property and time factor. Thus, compared to conventional roasting techniques commonly used in household setting, germination of the seeds leads to the nutritional enhancement of the sesame seeds.

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Appendices

Appendix A

1. Standard curve of catechin for phenolic content

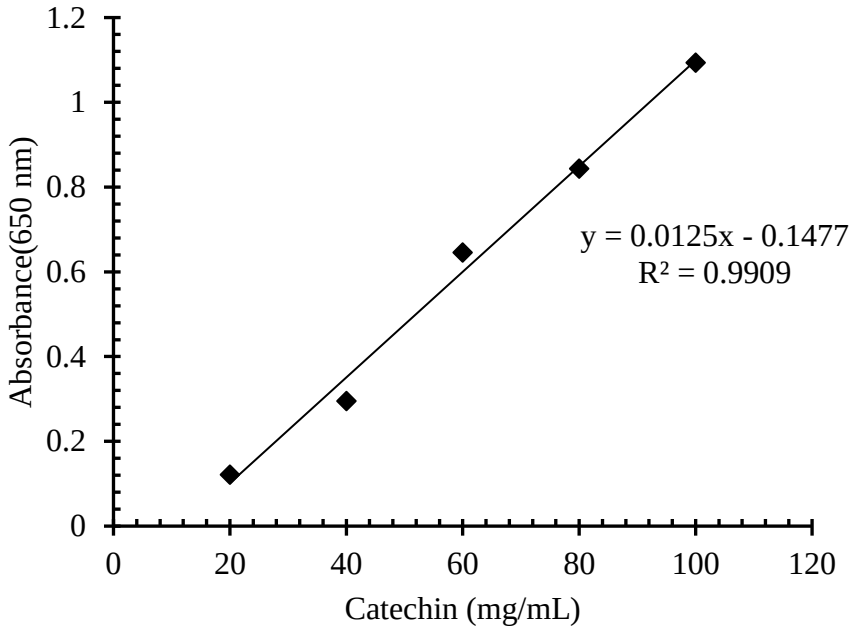

Fig. A1 Standard curve of catechin for TPC

2. Standard curve of quercetin for total flavonoid content

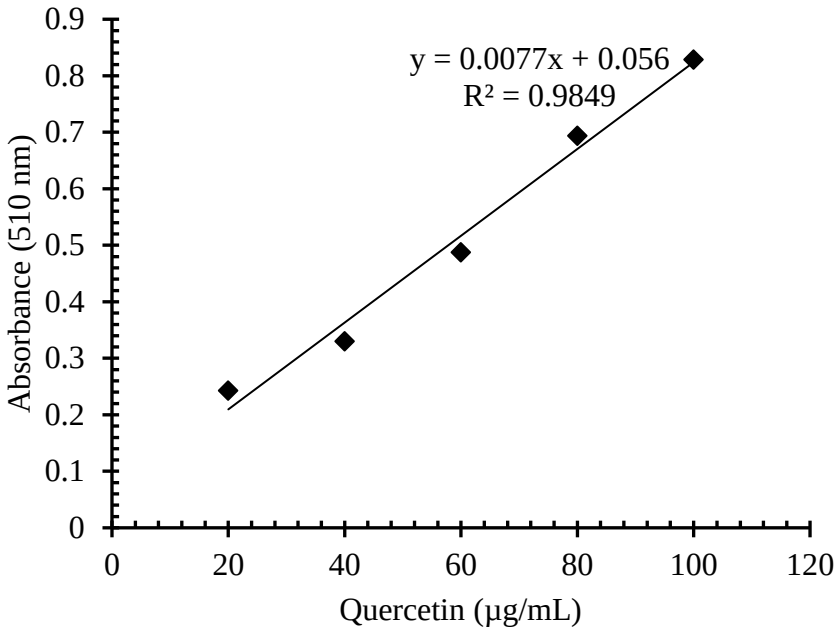

Fig.A2. Standard curve of quercetin for TFC

3. Standard curve of $FeCl_3$ for phytate

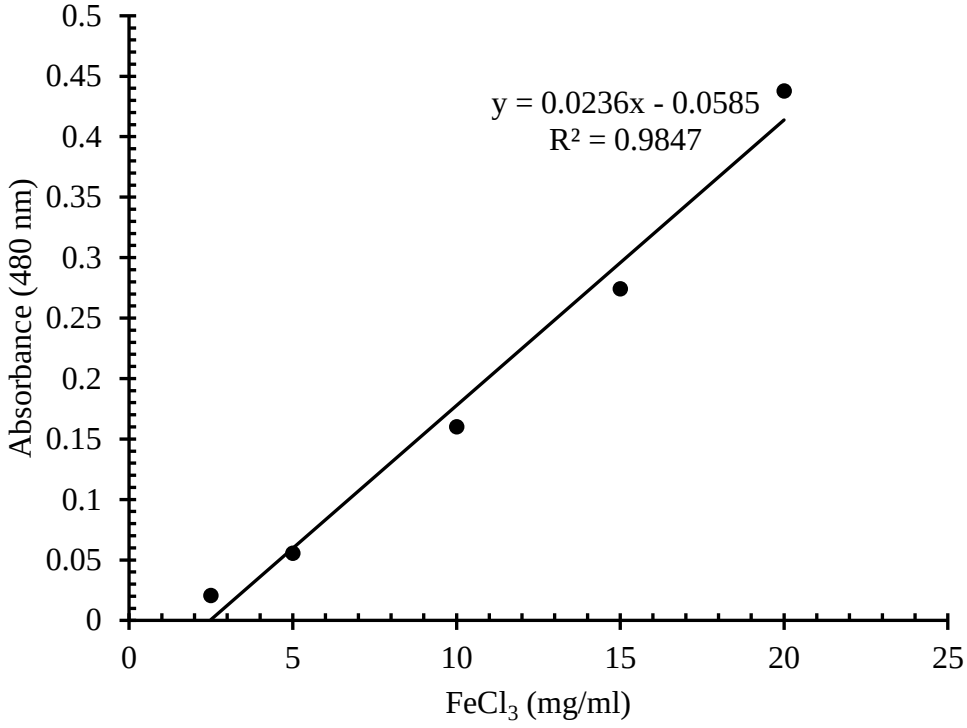

Fig.A.3 Standard curve of $FeCl_3$ for Phytate.

Appendix B

TableB.1 One- way ANOVA table for phytate

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean squares**	**Variance ratio**	**F probability ratio**
Treatments	2	55578.32	26789.16	12945.36	<.001
Residual	6	12.416	2.069		
Total	8	53590.737			

Table B.2 Effect of different treatment on phytate

Treatment	**Phytate content (mg/100 g)**
Raw	304.9 ± 5.78
D1	210.97 ± 7.89 [a]
D2	198.99 ± 6.87 [b]
D3	123.93 ± 9.45 [c]
D4	122.23 ± 1.55 [c]
D5	120.76 ± 0.56 [c]
Roasted	167.20 ± 4.67 [a´]

[* Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p<0.05$) whereas values with same superscript within a column are not significantly different. In the table D=day of germination, numbers represent the respective day]

Table B.3 One- way ANOVA table for oxalate

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Treatments	2	9445.44	4722.77	235.5	<.001
Residual	6	120.33	20.05		
Total	8	9565.86			

Table B.4 Effect of different treatment on oxalate.

Treatment-Time	Oxalate content (mg/100 g)
Raw	151.99 ± 9.50
D1	123.5 ± 9.50 [a]
D2	114 ± 6.65 [b]
D3	72.83 ± 5.48 [c]
D4	69.66 ± 4.69 [c]
D5	63.33 ± 5.48 [c]
Roasted	107.66 ± 5.48 [a´]

[* Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p < 0.05$) whereas values with same superscript within a column are not significantly different. In the table D = day of germination, numbers represent the respective day]

Table B.5 One - way ANOVA for Total Phenolic Content

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Treatments	2	5694.05	2847.02591	49931.15	<.001
Residual	6	0.342	0.05702		
Total	8	5694.39			

Table B.6 Effect of different treatment on TPC

Treatment-Time	TPC (mg CE/g)
Raw	35.25 ± 2.40
D1	63.78 ± 4.83 [a]
D2	71.64 ± 6.71 [b]
D3	95.74 ± 3.90 [c]
D4	95.78 ± 2.87 [c]
D5	98.99 ± 1.98 [d]
Roasted	55.36 ± 1.28 [a´]

[* Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p < 0.05$) whereas values with same superscript within a column are not significantly different. In the table D = day of germination, numbers represent the respective day]

Table B.7 One- way ANOVA table for total flavonoid content

Source of variation	**Degree of freedom**	**Sum of squares**	**Mean squares**	**Variance ratio**	**F probability ratio**
Treatments	2	0.2657	1.329×10^{-1}	1.930×10^{5}	< 0.001
Residual	6	4.130×10^{-6}	6.883×10^{-7}		
Total	8	2.657×10^{-1}			

Table B.8 Effect of different treatment on TFC

Treatment-Time	**TFC (mg QE/100 g)**
Raw	0.28 ± 0.09
D1	0.49 ± 0.12 [a]
D2	0.54 ± 0.04 [b]
D3	0.66 ±0.10 [c]
D4	0.67 ± 0.06 [d]
D5	0.69 ± 0.05 [e]
Roasted	0.31 ± 0.09 [a´]

[* Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p < 0.05$) whereas values with same superscript within a column are not significantly different. In the table D = day of germination, numbers represent the respective day]

Table B.9 One-way ANOVA table for DPPH scavenging activity

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Treatments	2	7009.0299	3504.5150	11567.09	< 0.001
Residual	6	1.8178	0.3030		
Total	8	7010.8478			

Table B.10 Effect of different treatment on DPPH scavenging activity

Treatment	DPPH scavenging activity (%)
Raw	10.62 ± 0.06
D1	22.79 ± 0.77 [a]
D2	57.08 ± 0.08 [b]
D3	78.38 ± 0.57 [c]
D4	79.62 ± 0.04 [d]
D5	80.95 ± 0.04 [e]
Roasted	36.73 ± 0.77 [a´]

[* Values presented are the average of triplicate ± standard deviation. Means in the same column with different superscript are significantly different ($p < 0.05$) whereas values with same superscript within a column are not significantly different. In the table D = day of germination, numbers represent the respective day]

Appendix C

Comparison between optimum germinated and roasted samples

Table C.1A t-test for moisture content

	Germinated	**Roasted**
Mean	7.365398563	5.0128655
Variance	0.022869607	0.041539
Observations	3	3
Pearson Correlation	-0.90276127	
Hypothesized Mean Difference	0	
Df	2	
t Stat	11.75982449	
P(T<=t) one-tail	0.003576751	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.007153502	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.1B t-test for fat content

	Germinated	**Roasted**
Mean	31.48678887	49.02761
Variance	0.124596773	0.6634981
Observations	3	3
Pearson Correlation	-0.993370753	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-26.0584246	
P(T<=t) one-tail	0.000734709	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.001469419	
t Critical two-tail	4.30265273	

t-tabulated< t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.1C t-test for protein content

	Germinated	**Roasted**
Mean	47.13746851	23.011654
Variance	1.494060509	0.0106751
Observations	3	3
Pearson Correlation	-0.55725087	
Hypothesized Mean Difference	0	
Df	2	
t Stat	32.57582812	
P(T<=t) one-tail	0.000470507	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000941013	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.1D t-test for carbohydrate content

	Germinated	**Roasted**
Mean	4.84032212	7.0143705
Variance	0.720629293	0.152647913
Observations	3	3
Pearson Correlation	0.487872191	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-14.72614155	
P(T<=t) one-tail	0.002289817	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.004579633	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.1E t-test for fiber content

	Germinated	**Roasted**
Mean	8.066469063	8.1001057
Variance	0.116802552	0.0129516
Observations	3	3
Pearson Correlation	-0.032028604	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-0.160207452	
P(T<=t) one-tail	0.4437181	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.887436199	
t Critical two-tail	4.30265273	

t-tabulated > t calculated, there is no significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.1F t-test for ash content

	Germinated	**Roasted**
Mean	5.768951435	6.0169257
Variance	0.088909445	0.2189121
Observations	3	3
Pearson Correlation	-0.893785841	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-0.575385297	
P(T<=t) one-tail	0.31156947	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.623138939	
t Critical two-tail	4.30265273	

t-tabulated > t calculated, there is no significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.2A t-test for Oxalate

	Germinated	**Roasted**
Mean	72.8303333	107.6626667
Variance	30.0833333	30.08333333
Observation	3	3
Pearson correlation	-1	
Hypothesized Mean Difference	0	
Df	2	
t- stat	-5.49984211	
P(T<=t) one tail	0.01575286	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.03150573	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.2B t-test for Phytate

	Germinated	**Roasted**
Mean	123.9286667	167.2018333
Variance	0.479600333	0.047632263
Observation	3	3
Pearson correlation	-0.461070726	
Hypothesized Mean Difference	0	
Df	2	
t- stat	-91.80024929	
P(T<=t) one tail	5.93205E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000118641	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.2C t-test for Total Phenolic Content

	Germinated	**Roasted**
Mean	95.73796667	55.35523333
Variance	0.153821823	0.016621173
Observation	3	3
Pearson correlation	-0.998140573	
Hypothesized Mean Difference	0	
Df	2	
t- stat	134.2657841	
P(T<=t) one tail	2.77334E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	5.54668E-05	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.2D t-test for Total Flavonoid Content

	Germinated	**Roasted**
Mean	6.251787759	2.947232206
Variance	0.000122768	5.2615E-05
Observation	3	3
Pearson correlation	-0.981980506	
Hypothesized Mean Difference	0	
Df	2	
t- stat	313.546965	
P(T<=t) one tail	5.08579E-06	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	1.01716E-05	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Table C.2E t-test for DPPH scavenging activity

	Germinated	**Roasted**
Mean	78.38	34.72
Variance	0.3229	9.9408
Observation	3	3
Pearson correlation	-0.978893343	
Hypothesized Mean Difference	0	
Df	2	
t- stat	20.37778093	
P(T<=t) one tail	0.00119975	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.002399501	
t Critical two-tail	4.30265273	

t-tabulated < t calculated, there is significant difference between the optimum germinated sample (third day) and roasted sample.

Appendix D

Chemicals and Equipment

1. Chemicals

H_2SO_4 (Qualigens, 97-99% assay), NaOH (Merck, 97% assay), HCl (Merck, 35% assay), Boric acid (Merck, Powder, 99.5% assay), Petroleum benzene (Thermo Fisher Scientific), Indicators (Phenolphthalein, Methyl red, Bromocresol green) , Potassium permanganate (SDFCL, 99% assay) , Na_2CO_3 (Thermo Electron, anhydrous) ,Sodium nitrate ,Quercetin, Ferric-chloride (Merck), Aluminum Chloride, Catechin, Methanol, Ammonia solution(Fisher Scientific 25%), Folin-Ciocalteau reagent, Ethanol, Oxalic acid(Merck), Trichloroacetic acid, Potassium thiocyanate(Thermo Fisher Scientific India Pvt. Ltd.), Ammonium oxalate (Qualigens Fine chemical 99%), DPPH (HiMedia Laboratories Pvt. Ltd), potassium persulphate (GlaxoSmithKline Pharmaceuticals Ltd.), Nitric acid (Fisher Scientific India Pvt. Ltd), Ferric nitrate , Potassium thiocyanide, Sodium sulphate, Ferric nitrate, Calcium chloride, Silver nitrate, acetic acid.

2. Equipment

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

Appendix E

Color plates

Plate 1 Determination of protein by Kjeldahl method

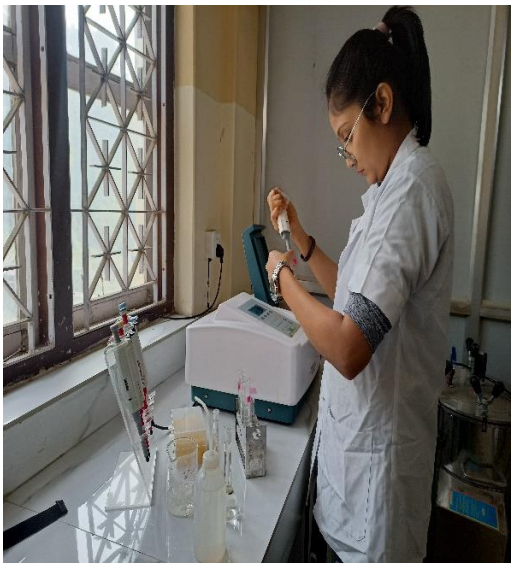

Plate 2 Spectrometric determination of flavonoids

Plate 3 Sample preparation