EFFECT OF FRYING TIME ON PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF FENUGREEK SEEDS (*Trigonella foenum-graecum*) AND CUMIN SEEDS (*Cuminum cyminum*)

by

Mandira Khadka

Department of Nutrition and Dietetics

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

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

by

Mandira Khadka

Exam roll no: 80013

T.U. Registration no: 5-2-8-128-201

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

Approval Letter

The *dissertation* entitled "*Effect of Frying Time on Phytochemical and Antioxidant Activity of Fenugreek Seeds (Trigonella foenum-graecum) and Cumin Seeds (Cuminum cyminum)*" presented by **Mandira Khadka** has been accepted as the partial fulfillment of the requirements for **Bachelor degree in Nutrition and Dietetics.**

Dissertation Committee

1. **Head of Department** — **(Mr. Kabindra Bhattarai, Asst. Prof.)**

2. **External Examiner** — **(Mr. Ananta Raj Poudel, Quality Control Officer)**

3. **Supervisor** — **(Mr. Kabindra Bhattarai, Asst. Prof.)**

4. **Internal Examiner** — **(Mr. Sabin Bdr. Khatri, Teaching Asst.)**

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Mandira Khadka

Abstract

The frying of spices is a popular technique used in Indian, Middle Eastern, and Southeast Asian cuisine to enhance the overall flavor of a dish. This study focused on the impact of frying fenugreek and cumin seeds in oil at a constant temperature and for different time intervals on their phytochemical and antioxidant activity. The spices were purchased from local market of Dharan, were winnowed, handpicked and were fried in oil at constant temperature i.e., 220°C and different frying interval (15, 30, 45, 60 sec). The fried sample was analyzed for tannin content, total phenolic content, total flavonoid content and DPPH radical scavenging activity.

The proximate composition of fenugreek seeds i.e., crude protein, crude fat, ash content, crude fiber, carbohydrate, moisture content, iron and calcium content was found to be 24.81±0.27%,4.07±0.16%,3.26±0.05%,15±0.22%,52.29±0.24%,10.76±0.24,5.076±0.11 mg/100g and 134.9±0.1mg/100g respectively and of cumin seeds was found to be16.04±0.15%,13.97±0.11,6.24±0.08%,10.22±0.23%,53.53±0.6%,9.3±1.26%,11.45±0.12 mg/100g and 1097±3.6 mg/100g respectively. All values presented are the mean± s.d of three determinations, and are expressed on dry basis. The mean value of tannin, total phenol content, total flavonoid content and antioxidant activity (IC_{50}) in raw fenugreek seed was found to be 3.96 mg tannic acid /100g, 114.22 mg GAE/100g, 75.7mgQE/100g and 339.27 µg/ml respectively and in cumin seed was found to be 2.51mg tannin acid/100g, 88.12 mgGAE/100g, 64.234 mgQE/100g and 580.67µg/ml respectively. Frying interval show significant reduction ($p<0.05$) in tannin, total phenol content, total flavonoid content and antioxidant activity of both the spices. This is a clear indication that bioactive components of spice are sensitive to heat and therefore should not be cooked when using them for medicinal purposes. The overall temperature and frying interval is believed to play a role in the spice's effectiveness, as at different frying interval, spices seems to show changes in phytochemical and antioxidant activity so, it is better to consume raw rather than frying and if fried it is better to fry for short time.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of Variance
CCT	Central Campus of Technology
CVD	Cardio Vascular Disease
d.f	Degree of Freedom
DB	Dry Basis
DFTQC	Department of Food Technology and Quality Control
DPPH	Diphenyl picryl hydrazyl
Etc.	Et cetera
$FeCl_3$	Ferric Chloride
GAE	Gallic Acid Equivalent
IC50	Inhibitory concentration
LSD	Least Significant Difference
MC	Moisture Content
QE	Quercetin Equivalent
ROS	Reactive Oxygen Species
RSA	Radical scavenging activity
TAE	Tannic Acid Equivalent
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
WHO	World Health Organization
Mg	Microgram

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Part I

Introduction

1.1 General Introduction

Spices and herbs refer to a wide range of aromatic plant products, including those made from the bark, flowers, leaves, fruit, roots, and seeds of a particular plant. Spices, excluding the leaves, can be defined as the dried components of fragrant plants (Peter and Shylaja, 2012). Spices can be used as medicine because they are natural products easily absorbed by our bodies and generally do not have any adverse effects. Herbal remedies are an important source for the discovery of new antibiotics (Okpekon *et al.*, 2004).

Spices have been widely used as food flavourings and folk medicines for thousands of years. Numerous studies have documented the antioxidant, anti-inflammatory and immunomodulatory effects of spices, which might be related to prevention and treatment of several cancers, including lung, liver, breast, stomach, colorectum, cervix, and prostate cancers (Zheng *et al.*, 2016).

Phytochemicals are bioactive plant compounds that can be used as antimicrobial, antibacterial, anticancer agents and are reported to prevent cancer, cardiovascular and inflammatory diseases. Herbs and spices are rich in phytochemicals and can be consumed or used traditionally for medical or dietary purposes since the ancient times (Guldiken *et al.*, 2018). Phytochemicals are naturally occurring substances found in plants which provide health benefits. These are known as secondary metabolites and may often be created by modified synthetic pathways from primary metabolite or share substrates of primary metabolite origin (Kabera *et al.*, 2014).

An antioxidant is a substance that inhibit or delays oxidative damage to the cells of the organisms by scavenging the free radicals such as peroxide or hydroperoxide and thus reducing the risk of degenerative diseases (Yamagishi and Matsui, 2011).

The frying of spices, also known as tempering or tadka, refers to a cooking technique commonly used in Indian, South Asian, and certain other cuisines. It involves heating oil or ghee (clarified butter) in a pan or pot and adding whole or ground spices to release their flavors and aromas (Stevenson *et al.*, 1984). It is a culinary technique that has been practiced

for centuries, revered for its ability to unlock the vibrant flavors and enticing aromas hidden within these tiny, potent ingredients (Pokorny, 1998).

Frying spices in oil while making vegetables has both positive and negative effects. The positive effects include enhanced flavor, delightful aroma, and uniform distribution of spices throughout the dish. Frying spices can elevate the taste of vegetables and make them more delicious. However, there are also potential negative effects such as the risk of burning the spices, which can result in a bitter taste. Additionally, excessive oiliness and intensified flavors may not be desirable for some individuals. It is important to carefully monitor the frying process, adjust the heat and spice quantities, and consider personal preferences to achieve a balanced and flavorful outcome (Madsen and Bertelsen, 1995).

Fenugreek (*Trigonella foenum-graecum*) belongs to the family Leguminosae. Fenugreek has been used traditionally to treat diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headaches/ migraines, diarrhea, anemia, flatulence, irregular menstrual cycles, analgesic, inflammation and arthritis, to ease labour pains and menstruation pain, and as an appetite stimulant (Vyas *et al.*, 2008). Seeds are aromatic, bitter, carminative, galactagogue and antibacterial. It constitutes 50% unavailable carbohydrates (fiber) making its highest concentration among all the natural sources of fiber. The fiber portion consists of insoluble (30%) and soluble (20%) fraction which is mostly galactomannan (Srinivasan, 2006)

Cumin (*Cuminum cyminum*) is an important and popular spice commonly known as ‘zeera’ that is used for culinary purposes due to its special aromatic effect (Fatima *et al.*, 2018) It belongs to the family known as Apiaceae. It is the most primitive cultivated herbs in Europe, Asia, and Africa (Bansal *et al.*, 2014). It is a multipurpose plant that is used worldwide for various culinary and medicinal purposes. It is usually used for imparting taste to diverse food provisions including cheese, soup, cheese, bean dishes pickle, and liquors (Mnif and Aifa, 2015).

1.2 Statement of the problem

Spices and herbs have been extensively studied in different countries because of the high antioxidant activity in certain spices and their beneficial effects on human health (Yashin *et al.*, 2017). As part of our diet, spices in addition to fruits and vegetables, could provide us with additional sources of natural antioxidants. Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing

7 compounds, tannins, alkaloids, phenolic, diterpene, and vitamins. As in recent times measurement of dietary intake of spices is gaining much significance as various phytochemicals present in spices have been recognized to have health promoting benefits and preventive role in chronic disease.

Fenugreek and cumin are an indigenous plant whose different parts have medicinal value and are commonly used as spices in kitchen. Both are versatile spices that can add flavour and depth to a wide variety of dishes and often used in spices blends such as garam masala and curry powder and as a supplement for various health benefits such as improving digestion, fenugreek in increasing milk production in breastfeeding women. Both are widely consumed by frying in oil during curries preparation. They are rich in phyto constituents and antioxidant such as phenol, flavonoid, tannin etc. Several studies reported that phyto constituents and antioxidant are good for disease prevention and overall health. But in Nepal, there is poor documentation and very few researches were done previously on effect of frying time on phytochemical and antioxidant activity of both the spices. Due to poor documentation and poor research, there is less knowledge about the best frying time of fenugreek seeds and cumin seeds in oils which has been in practised in every household since many years. Some experiments have been carried out regarding their phyto constituents and antioxidant properties.

1.3 Objective of the study

1.3.1 General objective

The general objective of this dissertation is to study effect of frying time on phytochemical and antioxidant activity of fenugreek seeds and cumin seeds.

1.3.2 Specific objective

- To extract phyto constituents of fenugreek seeds and cumin seeds by using ethanol solvent.
- To determine phytochemical constituents and antioxidant capacity of fenugreek seeds and cumin seeds.
- To study effect of frying time (15sec, 30sec, 45sec and 1min at 220° C) on phytochemical and antioxidant activity of fenugreek and cumin seeds.

1.4 Significance of the study

Spices, the predominant flavouring, colouring and aromatic agents in foods and beverages, are now gaining importance for their diversified uses. The nutritional, anti-oxidant, anti-microbial and medicinal properties of spices have far-reaching implications. In the present scenario, the anti-diabetic, anti-hyper cholesterolemic, anti-carcinogenic, anti-inflammatory effects of spices have paramount importance, as the key health issues of mankind nowadays are diabetes, cardiovascular diseases, arthritis and cancer. Spices or their active principles could be used as possible ameliorative or preventive agents for these health disorders.

This study will provide detailed information on effect of frying time on phytochemical and antioxidant activity of fenugreek seeds and cumin seeds. The outcomes of this study will be helpful to society/ community, further research purpose in academic institution. It gives strong documentation on appropriate time period for frying in oil of fenugreek seeds and cumin seeds which has been in practiced in our community since ancient times in domestic cooking.

1.5 Limitation of the study

- Only ethanolic extract was studied. Extraction in other solvent like methanol, petroleum ether, chloroform, hexane etc. could not be performed.
- Only two spices were studied.

Part II

Literature review

2.1 Background

Medicinal plants are very important for human health, it acts as an antibacterial agent against pathogenic bacteria (Zaika, 1988). Medicinal plants have been used in several indigenous herbal practices since very old times to cure several diseases. Herbal medication still continues to serve as an important health care system even today despite the greater advancements in modern medication systems in the recent years. Their long uses in the folk medicine and their safer implications in human health have generated much interest in them, especially in developing countries. It has now been established that medicines derived from plant products are safer than their synthetic counterparts (Oluyemi *et al.*, 2007). Many traditional foods, especially plant foods are reported to possess biological properties that can benefit to human health. The extracts of these plants have numerous health related effects such as antibacterial, antimutagenic anticarcinogenic, antithrombotic and vasodilator activities (Bidlack and Wang, 2000). Plants regarded for a long time as valuable source for human health maintenance (Tanaka *et al.*, 2006).

Spices refers to dried part of plant that contains volatiles oils or aromatic flavour such as, buds (cloves), bark (cinnamon), root (ginger), berries (black pepper), seeds (cumin, coriander). Spices play an important role as flavouring agents in the diet and are used throughout the world. Herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004). Spices and herbs are well known food ingredients, which enhances the flavour and aroma of the supplemented foods. Botanically, spices are one class of the aromatic plants; they are mainly present in the tropical provinces (Kirk and Sawyer, 1991).

Spices not only used for dietary purposes like aroma, colour, taste, flavour and preservations of foods but also used as a medicine in traditional system of medicine. Spices have their own unique aroma and flavour which derived from phytochemical compounds (Shekhar and Amit, 2012).

Fenugreek (*Trigonella foenum-graecum*) is an annual plant belongs to the family Leguminosae and it is the famous spices in human food. Since ancient times the green leaves and seeds of fenugreek have been used in food for many medicinal and therapeutic purposes.

It has been used to enhancement the color, flavor and texture of food materials. (Srinivasan, 2006).Fenugreek has a beneficial effect on cleansing the blood alkaloids, and as a diaphoretic, it is able to bring a sweat and help in detox the body. The alkaloids and flavonoid content of fenugreek seeds can be responsible for antinociception and anti- inflammatory effects of plant respectively (Sharara, 2017). Fenugreek seed is known to have several pharmacological attributes such as hypoglycemic, hypercholesterolemia, gastro protective, chemo-preventive, antioxidant, laxative and appetite stimulation (Shakuntala *et al.*, 2011). Fenugreek is also known for its lymphatic cleansing activity due to its vital role in supplying the cells with many nutrients and removing toxic wastes, trapped proteins and dead cells from the body. Seeds of fenugreek have many other medicinal properties such as hypocholesterolemic, antibacterial, gastric stimulant, lactation help, antidiabetic agent, hepatoprotective and anticancer (Sharara, 2017).

Fenugreek seeds are rich source of gum, fibres, alkaloids, flavonoids, volatile compounds, phenolic acids and polysaccharides (Benayad *et al.*, 2014).Due to its high content of fibre fenugreek could be used as food stabilizer, adhesive and emulsifying agent to change food texture to be more suitable for some special purposes (Murlidhar and Goswami, 2012). Dietary fiber from fenugreek blunts glucose after a meal but the mechanisms for these effects have not been fully elucidated, also fenugreek seeds contain the gum is composed of galactose and mannose and these compounds are associated with reduced glycemic effect. The hypoglycemic effect of fenugreek has been especially documented in humans and animals with type 1 and type 2 diabetes mellitus (Roberts, 2011). Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant (Cowan, 1999).

Taxonomy of fenugreek seeds

Kingdom - Plantae

Phylum - Tracheophyta

Class - Magnoliopsida

Order - Fabales

Family - Fabaceae

Genus - *Trigonella*

Species - *foenum-graecum*

Source: (Srinivasan, 2006)

Cumin (*Cuminum cyminum*) is an annual, diploid crosspollinated herb, medicinal spice, and aromatic plant, which is most widely used as a flavoring agent and food additive in different cookeries (Pandey *et al.*, 2015). The seeds, used for flavoring in pickles, soups, sausages, cheeses, also have numerous medicinal uses such as digestive system stimulant, painkiller in coughs (Agarwal *et al.*, 2010; Yan *et al.*, 2002) reported that the main active components of Cumin are cuminal and safranal 32.26% and 24.46% respectively. Cuminum cyminum seeds have showed diuretic, stomachic, astringent, carminative, fungicidal and bactericidal properties (Jirovetz *et al.*, 2003). Cumin (Cuminum cyminum L.) has broad spectrum antibacterial characteristics against gram-positive and gram-negative bacteria. It is aromatic plant used for medical preparations, food industries and as a flavor for foods (Iacobellis *et al.*, 2005).

Cumin seeds have strong aroma and special flavor because of its content of essential oil (Gachkar *et al.*, 2007). The minerals like copper, potassium, iron, manganese, magnesium, calcium, zinc, and selenium are most abundant in this spice. It additionally contains awesome measures of vitamins such as vitamin B-6, niacin, thiamin, and riboflavin. Some other indispensable anti-oxidant vitamins like vitamin C, vitamin E, and vitamin A are also present in it (Verma, 2016). C. cyminum is a significant part of chili powder and curry that is utilized to enhance the flavor in economic food items (R. P. Singh *et al.*, 2017). The seeds of this spice are utilized for carminative, diuretic, and stomachic properties. The seeds are likewise utilized broadly to quit morning sickness, jaundice, vomiting, colic, and so forth (N. Rai *et al.*, 2012). The seeds have been extensively utilized in traditional medication and also for the treatment of numerous diseases and health disorders, such as jaundice, diarrhea, toothaches, dyspepsia, and epilepsy. These medicinal assistances have usually been familiar to its rich content and potent action of active constituents such as flavonoids, terpenes, and phenols (Mnif and Aifa, 2015). These are carminative, stimulant, astringent, aromatic stomachic, and synergistic in effect (Mughal, 2022).In Algeria, it is used principally in veterinary medicine but still, it is also a traditional herbal remedy. In pregnancy, it is suspicious to reduce nausea and boost lactation. It cures the swelling of the testicles or breasts (Saini *et al.*, 2014).

C. cyminum is recognized for its astringent, stimulant, carminative, diuretic, antispasmodic properties (Dovidio and Gaertner, 2000). C. cyminum seeds consist of many phytochemicals that are recognized to have carminative, anti-flatulent, and antioxidant

properties (Madhuri *et al.*, 2014). The highest amounts of flavonoid phenolic anti-oxidants such as lutein, carotenes, and zeaxanthin are also found in seeds (Kumar *et al.*, 2021). On a global scale, there are increasing demands for cumin seed, because it is rich source for natural polyphenols and as a raw material to be incorporated into nutraceuticals, pharmaceuticals, and functional foods preparations (Gondaliya *et al.*, 2018).

Taxonomy of cumin seeds

Kingdom - Plantae

Phylum- Tracheophyta

Class - Magnoliopsida

Order - Apiales

Family - Apiaceae

Genus - *Cuminum*

Species - *cyminum*

Source; (Mughal, 2022)

2.2 Phytochemicals

Phytochemicals consist of a large group of naturally occurring non nutrient, biologically active compounds found in plants. As implied by the prefix “phyto” in the name, phytochemicals are basically produced only by plants. Phytochemicals acts as natural defense system for the host plants and in addition provide colour, aroma and flavor. Plants use phytochemicals as a natural protection from bacteria, fungi, and viruses (Ramanthan *et al.*, 1989). More than 4000 of these compounds have been discovered and it is expected that scientists will get discover many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs, and spices (Rowland, 1999).

A number of phytochemicals are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more (Doss and Anand, 2012).

Phytochemicals can have profound physiological effects, act as antioxidants, mimic body hormones and suppress development of disease in the body (Hayes, 2005).

2.2.1 Phenolics / Polyphenols

Polyphenols, which include more than 8000 compounds, are a family of natural compounds widely distributed in the outer layers of plant as suspected from their protective function in the plants (Manach *et al.*, 2004). Polyphenols occurs in all the plants foods and contribute to the beneficial health effects of vegetables and fruit (Balch and Balch, 2000). They range from simple molecules such as phenolic acid to highly polymerized compounds, such as tannins. Phenolic acids account for about one third of the total intake of polyphenols in human diet. These compounds are capable of removing free radicals, chelating, metal catalysts; active antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases (Oboh, 2006). As a result, they neutralize free radicals formed during normal physiological functioning of human body (Burns *et al.*, 2001). The antioxidant activity of phenols is due to their redox properties through which they act as hydrogen donors, singlet oxygen quenchers, reducing and metal chelating agents. There is a highly positive relationship between total phenols and antioxidant activity of many plant materials (Gülçin *et al.*, 2004).

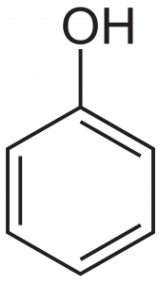

Fig 2.1 Structure of phenol

In recent years, much attention has been paid by nutritionist on the dietary polyphenols due to their potent antioxidative effects and their credible effects in the prevention of various oxidative stress associated diseases. Oxidation process is one of the most important ways for producing free radicals in food and even in living systems. Free radicals cause many human 8 diseases like cancer, Alzheimer's, cardiac, kidney and liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders and aging (Halliwell and Gutteridge, 1990).

2.2.2 Flavonoids

Flavonoids are the largest group of phenolic compounds and have a basic skeleton composed of three rings (C6-C3-C6). They are classified into six major classes according to their substitution pattern in the B-and C- rings, which are flavan-3-ols, anthocyanins, flavones, isoflavones, flavanones and flavonols (Harborne and Baxter, 1999). The flavonoid polymers are also known as proanthocyanidins. Flavonoids occur as plant secondary metabolites that are involved in pigmentation, antioxidants, antimicrobials, antistressors, and UV irradiation protection (Vaya and Aviram, 2001).. More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known (Ghasemzadeh *et al.*, 2010). Flavonoids are found in almost all plant based food and beverages, but the levels vary, depending on the degree of ripeness of fruits, variety and processing. Most flavonoids enhance the potency of vitamin C (ascorbic acid) and function as antioxidants. Antioxidant activity of flavonoids is believed to be due to their ability to act as free radical acceptor and to complex metal ions (Hertog *et al.*, 1992).

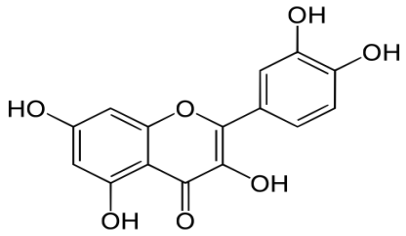

Fig 2.2 Structure of flavonoid

The previous studies showed that the ingestion of flavonoids reduces the risk of cardiovascular diseases, metabolic disorders, and certain types of cancer. These effects are due to the physiological activity of flavonoids in the reduction of oxidative stress, inhibiting low density lipoproteins oxidation and platelet aggregation, and acting as vasodilators in blood vessels. Free radicals are constantly generated resulting in extensive damage to tissues leading to various disease conditions such as cancer, Alzheimer's, renal diseases, cardiac abnormalities, etc., Medicinal plants with antioxidant properties play a vital function in exhibiting beneficial effects and employed as an alternative source of medicine to mitigate the disease associated with oxidative stress. Flavonoids have existed over one billion years and possess wide spectrum of biological activities that might be able to influence processes which are dysregulated in a disease (David *et al.*, 2016).

2.2.3 Tannins

Tannins are polyphenols sometimes called plant polyphenols although originally the name tannin was given to the plant extracts exhibiting astringency, without knowing their chemical structures (Haslam, 1989). The features distinguishing tannins from plant polyphenols of other types are basically the properties of the former: binding to proteins, basic compounds, pigments, large-molecular compounds and metallic ions, and also antioxidant activities, etc (Okuda and Ito, 2011). These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannin is soluble in water and alcohol and are found in root, bark, stem and outer layers of plant tissue. They form complexes with proteins, carbohydrates, gelatin and alkaloids. On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins and condensed tannins (Saxena *et al.*, 2013).

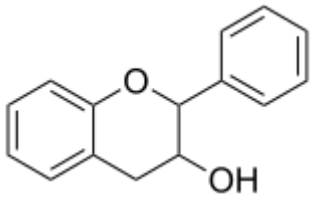

Fig 2.3 Structure of tannin

Tannins have diverse effect on biological system since they are potential metal ion chelators, protein precipitating agents and biological antioxidants. Because of varied biological roles that tannin can play and because of the enormous structural variation, it has 10 become difficult to develop models that would allow an accurate prediction of their effects in any system (Skowyra, 2014). The tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.*, 2005). Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illness such as AIDS and various cancers. The search for new compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Saxena et al., 2013).

2.2.4 Antioxidants

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell, 1995). These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol, and uric acid, are produced during normal metabolism in the body (Shi *et al.*, 1999). Other lighter antioxidants are found in the diet. Although there are several enzymes system within the body that scavenge free radicals, the principle micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and Bcarotene (Levine *et al.*, 1999). The body cannot manufacture these micronutrients, so they must be supplied in the diet.

Antioxidants are the compound that inhibit free radicals and prevent from the oxidative damage of a molecule. Oxidation can produce free radical which can damage the cells of organism leading various chronic diseases such as cancer, diabetes, cardiovascular and neurological diseases, etc. Oxidative stress can be induced by many negative factors like unhealthy diet, radiations, adverse environmental condition, and psycho-emotional stress (Devkota *et al.*, 2006; Serafini and Peluso, 2016). Abnormal production of free radicals may cause several severe human diseases such as cancer; Alzheimer's disease; cardiac, kidney, and liver diseases; fibrosis; atherosclerosis; arthritis; neurodegenerative disorders; and aging.

Several medicinal plants have been screened for their antioxidant and other biological activities (Mahesh and Satish, 2008; Martin and Ernst, 2003; Upadhyay *et al.*, 2010). Antioxidant is a chemical compound either synthesized or naturally isolated that inhibits the oxidation process happening in the body of living organisms. Oxidation is a chemical process that can generate free radicals that cause chain reactions and damage the body cells. Antioxidants are synthetic and natural and can terminate the chain reactions occurring in the body of living organisms (S. R. Rai, 2022).

2.2.4.1 Mechanism of action of antioxidants

Two principles mechanisms of action have been proposed for antioxidants (Rice-Evans and Diplock, 1993). The first is a chain- breaking mechanism by which the primary antioxidant

donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (Krinsky, 1992).

2.3 Phytochemical metabolism in human

Most phytochemicals in food exist in various forms that affect digestion and absorption. The most common are polyphenols that exist as glycosidic complexes. Some glycosides must be digested to their aglycones (unconjugated) before absorption. Other forms of phytochemicals are believed to be absorbed in the intestine without extensive digestion. Carriers are thought to be involved in the absorption of most phytochemicals. Additionally, many glycosides are neither digested nor absorbed in the small intestine. Phytochemicals that are not absorbed in the small intestine have been shown to undergo microbial alteration by the intestinal flora (Ross and Kasum, 2002). The bacteria hydrolyse the glycosides, generating aglycones which may undergo further metabolism to form various aromatic compounds (Bradlow *et al.*, 1999).

Once absorbed, most phytochemical metabolites get conjugated in the small intestine or in the liver (Rhodes, 1996). Conjugation most often involves methylation, sulfating or glucunnidation. These conjugated metabolites are then bound to plasma proteins such as albumin and are transported through the blood to various parts of the body. The amount of these conjugated metabolites in the plasma varies considerably win the type of polyphenol consumed, the food source, and the amount ingested (Kris-Etherton *et al.*, 2002). However, little is known about the metabolism of various polyphenols in the body after ingestion of specific polyphenols and which metabolites are present in plasma (Briskin, 2000).

2.4 Chemical constituents:

2.4.1 Chemical composition of fenugreek seeds

Fenugreek (*Trigonella foenum-graecum)* is a nutrient dense food rich in beneficial phytochemicals. The seeds of fenugreek contain lysine and L-tryptophan rich proteins, mucilaginous fiber and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects, may inhibit cholesterol

absorption and thought to help lower sugar levels. Therefore, fenugreek seeds are used as a traditional remedy for the treatment of diabetes and hypercholesterolemia in Indian and Chinese medicines.

Table 2.1 Chemical composition of fenugreek seeds

Constituents	**Fenugreek seeds (%)**
Carbohydrate	44.1
Fat	5.8
Protein	26.2
Fiber	7.2
Moisture	13.7
Minerals	3

Nutrient content per 100g of sample

Source: (DFTQC Food Composition Table 2017)

Table 2.2 Concentration of mineral in fenugreek seeds

Minerals & Vitamins	**Concentration (mg/100g)**
Calcium	160
Iron	6.5
Phosphorus	370
Niacin	1.1

Source: (DFTQC Food Composition Table 2017)

2.4.2 Chemical composition of cumin seeds

Cumin seeds contain essential oils, proteins, carbohydrates, minerals (iron, calcium, magnesium, and phosphorus), and vitamins (C, A, and E). They are a nutrient-dense spice that can provide many health benefits and are a good source of complete protein.

Table 2.3 Chemical composition of cumin seeds

Constituents	Cumin seeds(%)
Carbohydrate	36.6
Fat	15
Protein	18.7
Fiber	12
Moisture	11.9
Minerals	5.8

Nutrient content per 100g of sample

Source: (DFTQC Food Composition Table 2017)

Table 2.4 Concentration of mineral in cumin seeds

Minerals & Vitamins	Concentration (mg/100g)
Calcium	1080
Iron	11.7
Phosphorus	511
Niacin	2.6
Vitamin C	3

Source: (DFTQC Food Composition Table 2017)

2.5 Effect on proximate constitutes

Proximate and nutrient analyses of plants are important for determination of nutritional value. Various medicinal plant species are utilized for curing various ailments. Besides medicinal value, proximate analysis is also important, to ascertain nutritional worth of these plant as well.

Carbohydrates are considered the primary source of energy for all organisms, playing nutritional as well as structural role. High carbohydrates contents suggest suitability of the plant as feed. It is imperative to increase protein production by utilizing all the available ways and means as they play both curative and nutritive role.

Crude fibers are found in higher amount in non-starchy materials 23 and are considered good for the treatment of diseases like diabetes, gastrointestinal disorders, obesity and cancer (Ayoola *et al.*, 2010).

High ash content is an indication of the mineral stuffing available in the plant materials. Ash values ascertained high deposit of minerals in the plant tissue, already explored in the present study.

2.6 Effects of domestic cooking process on the chemical and biological properties of dietary phytochemicals

Food processing has been carried out since ancient time as a way to preserve and improve the nutritional and organoleptic properties of foods. However, it can also result with some undesired consequences such as the losses of nutrients and the formation of toxic compounds with negative effects on flavor, texture, or color (Friedman, 2015; Mogol and Gökmen, 2016; Zamora *et al.*, 2015).

On the other hand, the benefits of food processing cannot be ignored including the improvement of food safety, enhancement of nutritional value, and formation or release of natural phytochemicals with functional and bioactive properties (i.e., antioxidant or antimicrobial properties) (Nayak *et al.*, 2015; Van Boekel *et al.*, 2010). Cooking induced the formation of pyropheophytin a (Chen and Roca, 2018). The increase in dietary fibre after domestic cooking may be because of the formation of complexes between polysaccharides and proteins in the food or resistant starch in cooked potatoes (Dhingra *et al.*, 2012). Different food cooking methods at home as the final step have great influence on natural phytochemical profiles and biological properties. It can change them either in a positive or negative way (Bernhardt and Schlich, 2006). Numerous studies have been focused on the effect of cooking methods on dietary phytochemicals (Gliszczyńska-Świgło *et al.*, 2006; Miglio *et al.*, 2008; C. Zhao *et al.*, 2018).

The cooking conditions are clearly evident in inducing a series of changes in the physical properties, chemical composition and enzyme modifications of foods (Rothwell *et al.*, 2015). However, such findings on the changes in the phytochemical and biological properties that various vegetables undergo during domestic cooking were inconsistent and sometimes contradictory. For instance, (Blessington *et al.*, 2010) reported that total phenolic content and antioxidant activity are significantly increased during boiling, baking, frying and

microwaving. Foods can provide polyphenols such as flavonoids (Costa *et al.*, 2017; Faller and Fialho, 2009; Karas *et al.*, 2017; Lin and Chang, 2005), anthocyanins (Moreno *et al.*, 2010), powerful glucosinates resveratrol (Mallebrera *et al.*, 2017; Orellana-Palma *et al.*, 2017; Santhakumar *et al.*, 2018)and isothiocyanates.

2.6.1 Different domestic cooking techniques and their effects on food composition

Foods are cooked using different ways according to the traditional recipes and culinary shills of various countries for home consumption. The heat cooking methods include a wide variety of processes, i.e., boiling, steaming, frying, baking and roasting, and use of microwave ovens (Palermo *et al.*, 2014). These thermal processes can improve the sensory and textural features of the food material. Moreover, natural phytochemicals such as polyphenols, glucosinolates and carotenoids will be released by the disruption of cell walls, breakdown of complex molecular structures, and dissociation of molecular linkages between food components (Hidalgo and Zamora, 2017). Various chemical and physical changes may occur during cooking, and especially the proximate composition of the food is altered. The cooking methods may greatly affect the content of food nutrients, for example, frying seems to have a great influence on the nutritional quality. Numerous biochemical reactions take place in the hot medium and several new chemical compounds are produced. The crude fat content is significantly increased during frying (increases of 4.07 ± 0.15 g/100 g dry weight) as the cooking oil is absorbed during this process (Tian *et al.*, 2016a; Tian *et al.*, 2016b). The cooking oils are obtained from oilseeds (i.e mustard, sunflower, and cottonseed), food legumes (i.e., soybean and peanut), nuts (i.e., almond,) or fruit pulp (i.e., olives) (Ganesan *et al.*, 2019). The hydrolysis of cooking oil is observed at the beginning of cooking, in which the acid value of the oil is enhanced due to the production of free fatty acids from triglycerides. Besides the changes in the fat content, other components of the foods are also known to be affected. According to (Agbo *et al.*, 2015), the highest fibre content in the fried samples increased significantly to 1.93 ± 0.06 g/100 g fresh weight and was higher than that of the uncooked sample. On the other hand, the content of ash was decreased appreciably after frying (Bethke and Jansky, 2008)

2.6.2 Phenolic compounds

Phenolic compounds are known to be affected by food processing, and specifically by thermal treatments. The total phenolic content was decreased after boiling, steaming, microwaving, baking, and frying (Ezekiel *et al.*, 2013). This decrease may be due to water-

soluble phenols leaching into the cooking water and the structural changes of phenolics that occurs during heat processing (Kita *et al.*, 2013). Furthermore, the phenolic compounds participate in the interplay of the Maillard reaction, which results in an increase in the level of Maillard reaction products and decrease of phenolic level (Perla *et al.*, 2012). Most researchers concluded that thermal treatments could lead to the reduction of phenolic content (Gonçalves *et al.*, 2010). In another study, as compared for before and after the frying treatments resulted with an increase by 46.12% in the phenolic contents, respectively (Blessington *et al.*, 2010). The increase may be attributed to the thermal action which induces the breakdown of vegetables structure, and as a result improves the extractability of phenolic compounds from the cellular matrix and stimulates the release of dietary fiber-bound polyphenols forming the free phenolic compounds (Ruiz-Rodriguez *et al.*, 2008).

2.6.2.1 Flavonoids

The cooking processes, such as boiling and extrusion, resulted in a significant reduction in flavanols (i.e., Quercetin and Kaempferol) in foods. The decrease in total flavonoid content is caused by the destruction of flavonoids while treated with high temperatures (Sharma *et al.*, 2016). Flavonoids share a basic structure of diphenylpropanes (C6-C3-C6) parent depending on the oxidation level of the central pyran ring. Due to an increase in the temperature and shear, the structural rings start to degrade and thus flavonoid content in the product is decreased. The increase in screw speed can result with an increase in the medium temperature, and thus the product temperature Therefore, the combined effect of screw speed and temperature ultimately led to the loss of flavonoids. On the other hand, the highest loss of flavonoids was observed during frying/microwaving (Barakat and Rohn, 2014). Frying and/or microwaving were the most dramatic treatments which cause the leaching of flavonoids into the frying oil, and then a following thermal degradation (Moreno *et al.*, 2010; Vallejo *et al.*, 2003).

Anthocyanins are one of the most important classes of flavonoids and they are known to be highly unstable and easily affected by environmental factors, such as temperature, pH, oxygen, and light. After frying, steaming, boiling, microwaving and baking, total anthocyanin content was significantly decreased. Their greatest losses were caused by frying, air-frying and stir-frying with 57.06%, 44.53%, and 83.15%, respectively (Brown *et al.*, 2008).

2.6.2.2 Ascorbic acid

Wide differences may occur in the ascorbic acid content of foods because of variations in variety, cultivar, genetics, maturity stage, fertilization and environmental growing conditions on field. The ascorbic acid concentration was reduced by processing conditions and cooking methods in vegetables. The highest decrease was observed after boiling owing to its great water solubility (Bureau *et al.*, 2015). Thermal treatments can accelerate oxidation of ascorbic acid to dehydroascorbic acid, followed by the hydrolysis to 2, 3-diketogulonic acid and eventually polymerization to other nutritionally inactive components (Chuah *et al.*, 2008). A decrease was observed in the ascorbic acid content in green and red peppers after stir-frying on account of high temperatures, long cooking times and enzymatic oxidation during preparation and cooking processes and frequent stirring that expose the materials to atmospheric oxidation (Somsub *et al.*, 2008).

2.6.2.3 Antioxidant activity

Spices are rich in important phytochemicals, such as ascorbic acid, mineral elements, flavonoids, carotenoids, and other compounds. These phytochemicals have a variety of biological properties including antioxidant, anti-inflammation, anti-mutagenicity, anti-carcinogenicity, and anti-aging activities (Ke *et al.*, 2015; Rajendran *et al.*, 2014), among which, antioxidant activity is a foundation of anti-cancer, anti-inflammation and anti-aging properties (Cai *et al.*, 2004). Phytochemicals are influenced by cooking methods, and as a result the antioxidant activity is also affected during cooking procedures (Ezekiel *et al.*, 2013; Lemos *et al.*, 2015).

Some studies reported that the antioxidant activity was related with the presence of phenolic constituents because of their abilities to scavenge free radicals (Ravichandran *et al.*, 2012). The antioxidant activity modestly reduced after boiling, and the decrease might be due to the leaching of antioxidant compounds into the cooking water (Giallourou *et al.*, 2016; Girgin and El, 2015). Firstly, heating may induce numerous chemical reactions, such as Maillard reaction, caramelization, Strecker degradation and hydrolysis of esters and glycosides which result in the production of new antioxidant (Kita *et al.*, 2013). Maillard reaction occurs between an amino group and a sugar moiety, and can result with the formation of new substances which have been associated with increased antioxidant activity (Manzocco *et al.*, 2000). Secondly, the increased antioxidant activity on baked samples can be attributed to the heat leading to the denaturation of the endogenous enzymes, degrading

the antioxidants (Kamiloglu *et al.*, 2014). Generally speaking, the changes in the antioxidant activity during cooking arise from the changes in phytochemicals according to most of the studies.

2.6.3 Frying

Frying is one of the oldest food processing methods. Its popularity is related to the ease and speed of food preparation and sensory characteristics, such as unique flavor and taste (Ngadi and Xue, 2016). It is a cheap and fast process of simultaneous heat and mass transfer that changes the sensory and nutritional characteristics, as result of complex interactions between food and oil (Ziaiifar *et al.*, 2008). Frying is an efficient cooking method because it is a result of high temperature and fast heat transfer (Sanibal). The oil which the food is immersed, acts like a heat transferring compound. The process has a preserving action caused by thermal destruction of microorganisms, enzymes and reduction of water activity on the surface of the food (Fellows, 2009).

Changes in food and oil depends on the characteristics of the food, oil type, surface/volume ratio of the oil, rate of air incorporation of into the oil, temperature, heating process, length of immersion and the kind of material the frying container is made of. Additionally, the longer the oil is used, greater is the induction of adverse reactions. Extended exposure of oil to high temperatures and atmospheric air can generate highly oxidized, potentially toxic products (Del Ré and Jorge, 2006). In foods, some reactions that affect the nutritional quality may occur (ANS *et al.*, 1999). The frying process relies on high temperatures and can changes the structure of labile nutrients, such as proteins, vitamins and antioxidants. Some water-soluble molecules, such as ascorbic acid can be lost during the water evaporation (Corissin and Jorge, 2005).

2.6.3.1 Physical and chemical changes in fried foods

The conditions to which food are submitted during the frying process initiate physical and chemical changes that depend on the composition of the food, and affect the development of color, flavor, and taste, besides changing food texture. Table 2.5 summarizes the physical and chemical changes in food during the frying process.

Table 2.5 Main changes in the composition of foods during the frying process

Component	Changes during frying
Fat	Increased concentration and change in composition
Water	Significant loss
Reducing sugar	Maillard reaction
Starch	Gelatinization
Protein	Alternation of the compounds
Amino acids	Formation of heterocyclic flavoring substances
Flavoring substance	Formed by oxidative and Maillard reaction. Interaction with frying oil.
Vitamins	Moderate loss
Minerals	Small loss
Antioxidant	Moderate loss

Source: (Pokorny, 1998)

2.6.3.2 Compounds formed during the frying process

The frying of spices is typically done at the beginning of cooking a dish or added towards the end to enhance the flavor of a finished dish. It is important to be careful not to burn the spices while frying, as this can result in a bitter taste and unpleasant aroma.

Changes in food and oil depends on the characteristics of the food, oil type, surface/volume ratio of the oil, rate of air incorporation of into the oil, temperature, heating process, length of immersion and the kind of material the frying container is made of. Additionally, the longer the oil is used, greater is the induction of adverse reactions. Extended exposure of oil to high temperatures and atmospheric air can generate highly oxidized, potentially toxic products.

Table 2.6 Main groups of compounds formed in oils during the frying process

Types of change	**Causative agent**	**New compound formed**
Hydrolytic	Moisture	Free fatty acid Diacylglycerol Monoacylglycerol
Oxidative	Air	Oxidized monomers Oxidized dimers and polymers Volatile compounds (aldehydes, ketones, hydrocarbons) Sterol oxides
Thermal	Temperature	Dimers and non-polar polymers Cyclic monomers Trans isomers and position isomers

Source: (Navas Sánchez, 2005)

Part III

Materials and methods

3.1 Materials

3.1.1 Raw materials

Fenugreek seeds (*Trigonella foenum-graecum)* and cumin seeds (*Cuminum cyminum)* were purchased from the local market of Dharan**,** Nepal.

3.1.2 Equipment and chemical

All chemicals, glassware and equipment required were used from the laboratory of Central Campus of Technology, Dharan. Details of chemicals and equipment are given in Appendix C (Table C.1 and Table C.2 respectively)

3.2 Method

3.2.1 Outline of experimental procedure

The samples (fenugreek and cumin seeds) were purchased from the local market of Dharan, Nepal. They were winnowed, handpicked and deep fried in sunflower oil at constant temperature(220°C) and different time interval (15, 30, 45 and 60 sec). The samples were cooled and oil was removed by wiping with the tissue paper and then milled into powder. Samples were steeped in 80% ethanol for 12 hours at room temperature and filtered using Whatman No.1 filter paper and was concentrated in rotary vacuum evaporator. The extract concentration was determined by evaporating 5ml of extract (at 80°C) to dryness and measuring the weight. The extract was then stored in refrigerator and further analysis were done. (W. K. I. Ahmad *et al.*, 2014b).

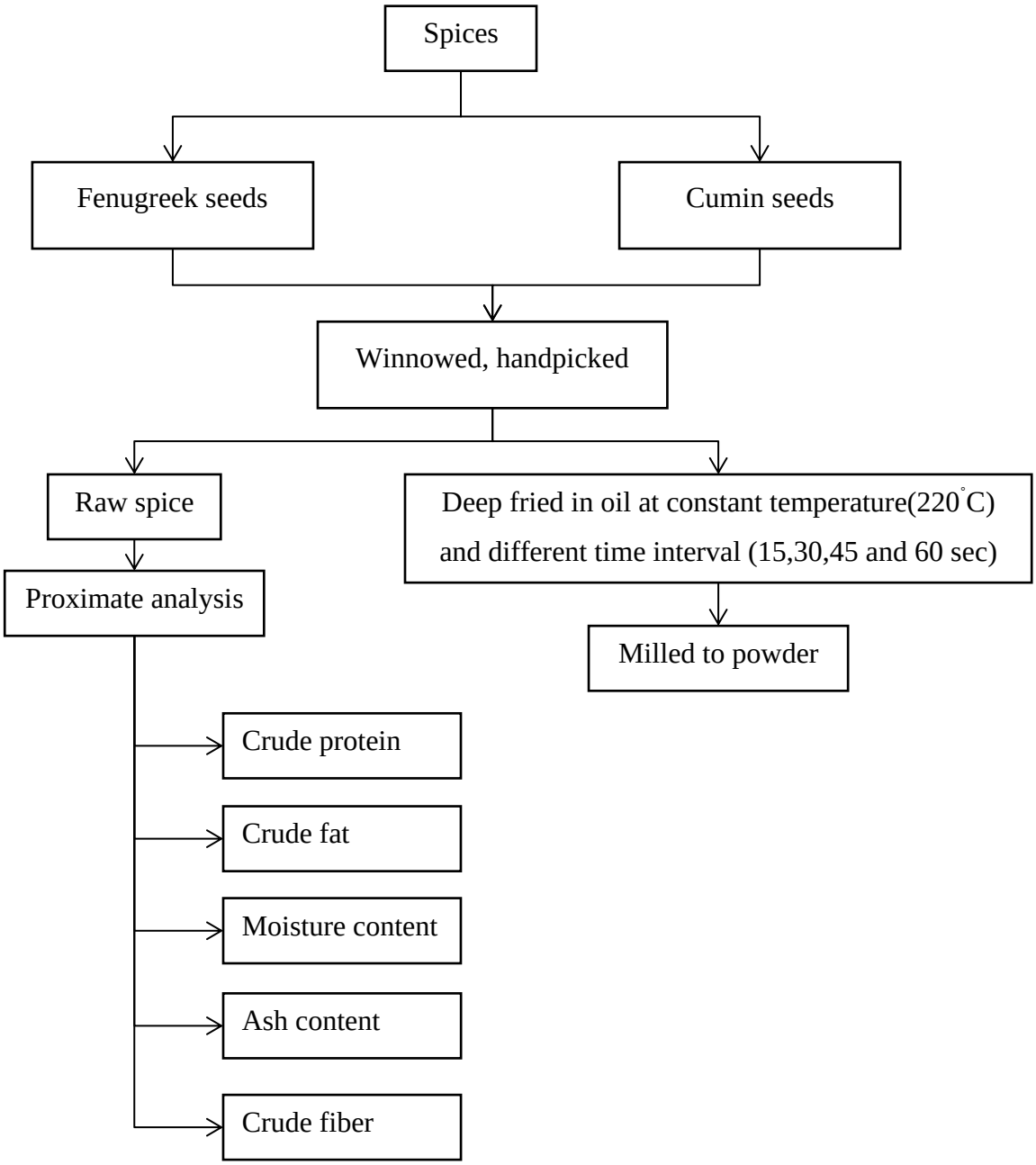

Fig 3.1 Flow diagram of sample preparation and proximate analysis

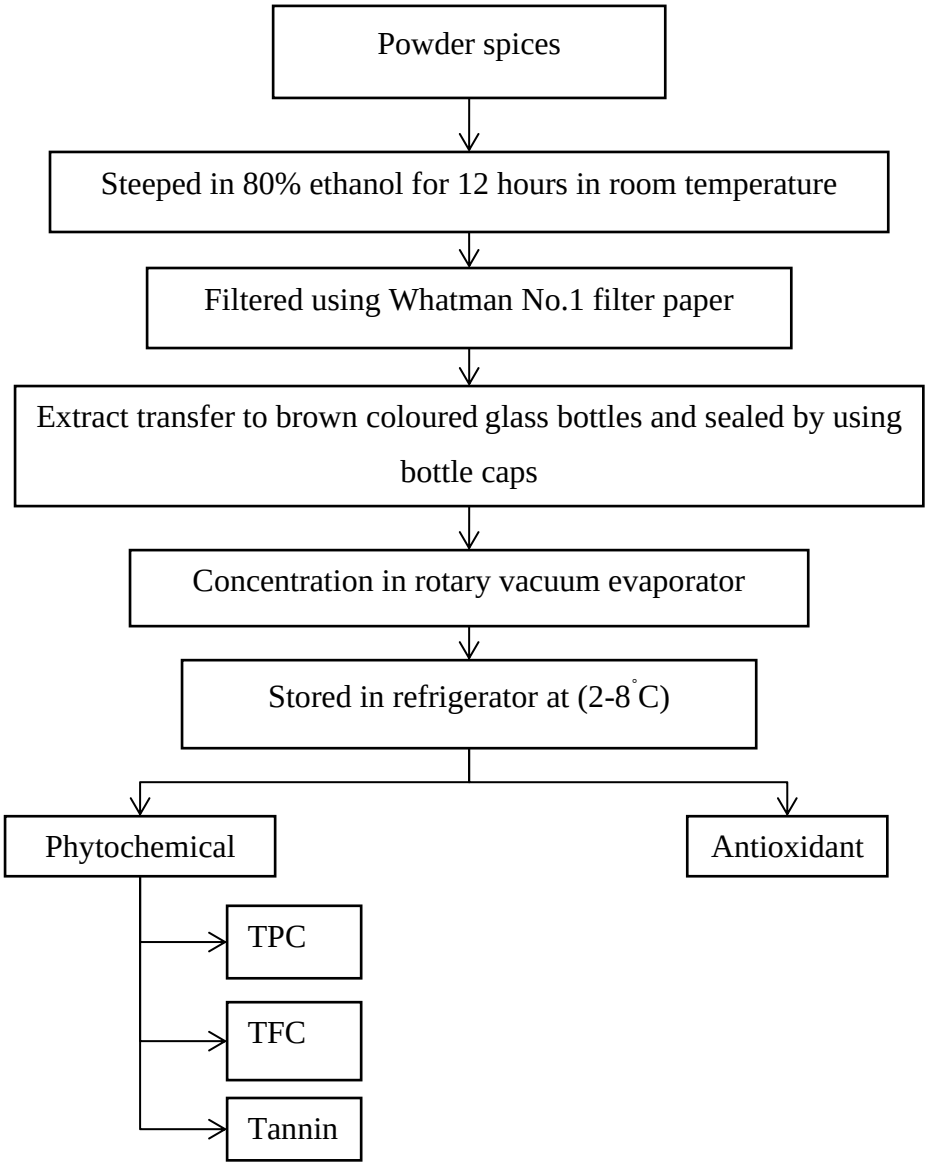

Fig 3.2 Flow diagram of extract preparation

3.3 Experimental procedure

3.3.1 Proximate analysis

3.3.1.1 Determination of moisture content

Moisture content involves refluxing the food with an immiscible solvent with a higher boiling point and lower specific gravity than water. toluene, heptane or xylene. The refluxed water settles while the solvent floats in a volumetric measuring tube. Distilled volatile oils remain mixed with the solvent and are not measured. The device used for this moisture determination method is called the Dean and Stark device (Ranganna, 1986). This result was expressed as a percentage.

$$\text{Moisture content (\%)} = \frac{\text{volume of water (ml)}}{\text{wt. of sample taken}} \times 100$$

3.3.1.2 Determination of crude protein

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

$$\text{Nitrogen (\%)} = \frac{\text{(sample titre-blank titre)} \times \text{Noramality of HCL} \times 14 \times 100}{\text{Wt of sample} \times 100}$$

$$\text{\% of crude protein} = \text{Nitrogen} \times 6.25$$

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 (Ranganna, 1986). The calculated data were presented as g/100 g on dry basis.

$$\text{\% of total ash} = \frac{\text{(wt of ash)}}{\text{wt of sample taken}} \times 100$$

3.3.1.4 Determination of crude fat

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

$$\text{\%crude fat} = \frac{\text{(wt of ether soluble materials)} \times 100}{\text{wt of sample}}$$

3.3.1.5 Determination of crude fibre

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 given in (Ranganna, 1986). The calculated data were presented as g/100 g on dry basis.

$$\%\text{of crude fibre} = \frac{\text{loss in wt noted}}{\text{wt of sample taken}} \times 100$$

3.3.1.6 Determination of carbohydrate

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

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

3.3.2 Ultimate analysis

3.3.2.1 Determination of iron

Iron in the sample was 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 thiocynate to form a red ferric thiocynate which was measured calorimetrically at 480 nm (Rangana, 1986).

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

3.3.2.2 Determination of calcium

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

$$\text{Calcium } \left(\frac{\text{mg}}{100}\right) = \frac{\text{titre} \times 0.2 \times \text{Total volume of ash solution} \times 100}{\text{volume taken for estimation} \times \text{wt of sample taken for ashing}}$$

3.3.2.3 Determination of ascorbic acid

The dichlorophenol dye, which was blue in alkaline solution and red in acid solution, was reduced by ascorbic acid to a colourless form. Result was presented in mg of ascorbic acid per 100mg (Nonogaki *et al.*, 2010).

$$\text{Vitamin C}\left(\frac{\text{mg}}{100}\right)=\left(\frac{\text{Titer}\times\text{dyefactor}\times\text{volume made up}\times 100}{\text{aliquot of extract taken(ml)}\times\text{wt of sample(g)}}\right.$$

3.3.3 Procedure for extraction

Plant materials were extracted as per (W. Ahmad *et al.*, 2014a) with slight modification.10 g of powdered spices were steeped in 80% ethanol (100 ml) for 12 h at room temperature. They were then filtered using Whatman No.1 filter paper. Finally, extracts were transferred to brown coloured glass bottles, sealed by using bottle caps and stored at 4 ± 2ºC until analysis. The extract concentration was determined by evaporating in rotary vacuum evaporator.

3.3.4 Phytochemicals Quantitative Analysis

3.3.4.1 Total phenolic content

TPC was determined using the Folin–Ciocalteu method (Singleton *et al.*, 1999) with slight modifications. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin- ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na2CO3 aqueous solution. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at wavelength = 765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GAE/g of extract).

3.3.4.2 Total flavonoid 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 pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate (NaNO3) was mixed and stand for5 minutes. 0.2 ml of 5% Aluminum Chloride (AlCl3) was pipetted out, mixed in the tube and allowed to stand for 5 minutes. This followed 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 510nm against a reagent blank. The test result was correlated with standard curve of Quercetin (20, 0, 60, 80, 100µg/ml) and the total flavonoid content is expressed as mg QE/g of dry weight.

3.3.4.3 Total tannin content

The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35% Na2CO3solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and100µg/ml) were prepared in 29the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of TAE /g of extract (Miean and Mohamed, 2001; Ribarova *et al.*, 2005; R. Singh *et al.*, 1970).

3.3.4.4 Free Radical Scavenging Activity Using (DPPH)

Extract(100µL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5mL). The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/ VI'S spectrometer) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula (Hatano *et al.*, 1988).

$$\%\text{of inhibition} = \frac{\left(A_{control} - A_{sample}\right)}{A_{control}} \times 100$$

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control).

I%=percentage of inhibition

The radical scavenging activities of the extracts are expressed in terms of their IC_{50} values.The data were presented as mean values ± standard deviation (n 3). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (H. Zhao *et al.*, 2008).

3.3.5 Statistical Analysis

Analysis was carried out in triplicate. Data on analysis of TPC, TFC, DPPH radical scavenging activity, and tannin, in different frying time of fenugreek seeds and cumin seeds using ethanol solvent was tabulated for comparison and using Microsoft Excel 2021. Data

were statistically processed by GeneStat version 12.1.0.3338 for analysis of variance (ANOVA). Means of data were compared by post hoc method at 5 % level of significance.

Part IV

Result and Discussion

Fenugreek seeds and cumin seeds were purchased from the local market of Dharan, Nepal. The sample were winnowed, handpicked and deep fried at different time interval (15, 30, 45, 60) sec at constant temperature (220℃). The fried sample were grinded to fine powder and extracted by using ethanol solvent. These extracts were used for further quantitative analysis of total phenolic content, total flavonoid content, tannin and DPPH free radical scavenging activity.

4.1 Proximate composition

Proximate analysis provides inexpensive yet valuable information, particularly from a nutritional and biochemical perspective. The dried samples were analyzed for the contents of moisture, protein, fiber, ash and fat according to the (Ranganna,1986), the carbohydrates content was calculated by subtracting the previous components from 100.The experiment were made in triplicates, and then the means were calculated.

The results are typically expressed in percentages and the term "crude" is often used as a modifier due to the general nature of the tests employed for the determination of various constituents such as crude protein, crude fat, and crude fiber. Therefore, proximate constituents represent only a category of compounds present in biological materials (Acharya and Karki, 2008).

4.1.1 Proximate composition of raw fenugreek seeds

Table 4.1 Proximate composition of raw fenugreek seeds

Parameters	**Values(% dry basis)**
Crude protein	24.81±0.27
Crude fat	4.07±0.16
Ash content	3.26±0.05
Crude fiber	15±0.22
Carbohydrate	52.86±0.24
Moisture content	10.76±0.24

The values presented are the mean ± sd of three determinations, and all values are expressed on a dry basis.

Table 4.1 shows the proximate composition of fenugreek seeds, which indicates that the protein, fat, ash, crude fiber, carbohydrate and moisture content of the fenugreek seeds are 24.8%, 4.07%, 3.26%, 15%,52.86% and 10.76%respectively, on a dry weight basis. Comparing these results with those of DFTQC (2017), it can be seen that all of the proximate parameters (26.2%, 5.8%, 3%, 7.2% ,44.1% and 13.7% respectively) are in agreement with that report.

The result was also in accordance with (Mahmood and Yahya, 2017) who showed the protein, fat, crude fiber, ash and carbohydrate content of fenugreek seeds was (28.3-28.45)%, (6.9-7.15)%, 17%, (3.082-3.56)% and (45.2-46)% respectively. However, the composition of the spices may vary according to the genus, species, growing conditions, and many other factors (Fried *et al.*, 2008). Therefore, this composition may be agreeable.

4.1.2 Proximate composition of raw cumin seeds

Table 4.2 Proximate composition of raw cumin seeds

Parameters	**Values (%dry basis)**
Crude protein	16.04 ± 0.15
Crude fat	13.97 ± 0.11
Ash content	6.24 ± 0.08
Crude fiber	10.22± 0.23
Carbohydrate	53.53 ± 0.60
Moisture content	9.36±1.26

The values presented are the mean ± sd of three determinations, and all values are expressed on a dry basis.

Table 4.2 shows the proximate composition of cumin seeds, which indicates that the protein, fat, ash, crude fiber, carbohydrate and moisture content of the cumin seeds are 16.04%, 13.97%, 6.246%, 10.227%, 35.74% and 9.36% respectively, on a dry weight basis. Comparing these results with those of DFTQC (2017), it can be seen that all of the proximate parameters (18.7%, 15%, 5.8%, 12%, 36.6% and 11.9% respectively) are in agreement with that report. The result was also in accordance with (Patil *et al.*, 2017) who showed the protein, fat, crude fiber, ash and carbohydrate content of cumin seeds was 12%, 15%, 11%, 8% and 33% respectively

However, the composition of the spices may vary according to the genus, species, growing conditions, and many other factors (Fried *et al.*, 2008). Therefore, this composition may be agreeable.

4.2 Mineral composition

4.2.1 Mineral composition of fenugreek seeds

Table 4.3 Mineral composition of fenugreek seeds

Minerals	**Values (mg/100g)**
Iron	5.076 ± 0.11
Calcium	134.9 ± 0.10

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

Table 4.3 shows the mineral composition of fenugreek seeds according to which iron and calcium content of the fenugreek seeds are 5.076 and 134.9mg per 100g dry basis respectively on dry weight basis. Comparing these results with the results DFTQC (2017) it is seen that parameters (6.5 and 160 respectively) are nearly agree with that report. However, the composition of grain may vary according to genus, species, growing conditions and many more factors. So, this composition may be agreeable.

4.2.2 Mineral composition of cumin seeds

Table 4.4 Mineral composition of cumin seeds

Minerals	**Values (mg/100g)**
Iron	11.45 ± 0.12
Calcium	1097 ± 3.6

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

Table 4.4 shows the mineral composition of cumin seeds according to which iron and calcium content of the cumin seeds are 11.45 and 1097mg per 100g dry basis respectively on dry weight basis. Comparing these results with the results DFTQC (2017) it is seen that parameters (11.7 and 1080 respectively) are nearly agree with that report. However, the composition of grain may vary according to genus, species, growing conditions and many more factors. So, this composition may be agreeable.

4.3 Ascorbic acid content of cumin seeds

The mean value of ascorbic acid in cumin seeds was found to be 4.58±0.31 mg/100g.Comparing this result with the results DFTQC (2017) it is seen that the ascorbic acid content (3mg/100) is nearly agree with that report.

4.4 Effect of frying time on tannin content of spices

Tannins are a group of polyphenolic compounds that are found in many plant-based foods, including spices. They are responsible for the astringent taste and are known to have various health benefits, such as antioxidant and anti-inflammatory properties (Khanbabaee and Van Ree, 2001).

Deep frying resulted in a significant reduction in tannin content compared to raw seeds. However, the extent of the reduction varied depending on the frying temperature and duration. At higher frying temperatures and longer durations, a greater reduction in tannin content was observed (Sharara, 2017).The spices were deep fried in different time interval(15, 30, 45 and 60) second. The change in tannin content of the spices were analyzed in different frying time interval as well as in raw sample. The reduction in tannin content of spices after being fried in oil is demonstrated in below.

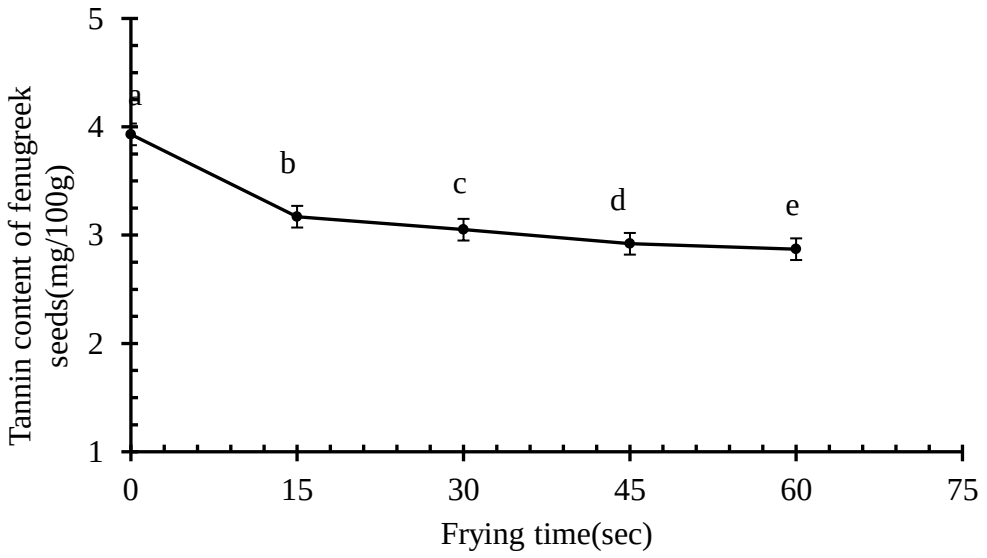

Fig 4.1 Effect of frying time on tannin content of fenugreek seed

The mean value of tannin content in raw fenugreek seed was found to be 3.963 ± 0.1mg/100 g on the basis of dry matter. Tannin content was reduced on the progressive

interval of frying. The mean value of progressive interval of frying was 3.172 ± 0.22 mg/100 g, 3.038 ± 0.8mg/100 g, 2.914 ± 0.52 mg/100 g, and 2.87 ± 0.12mg/100 g on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between tannin content of fenugreek seed in the different interval of frying ($p < 0.05$).

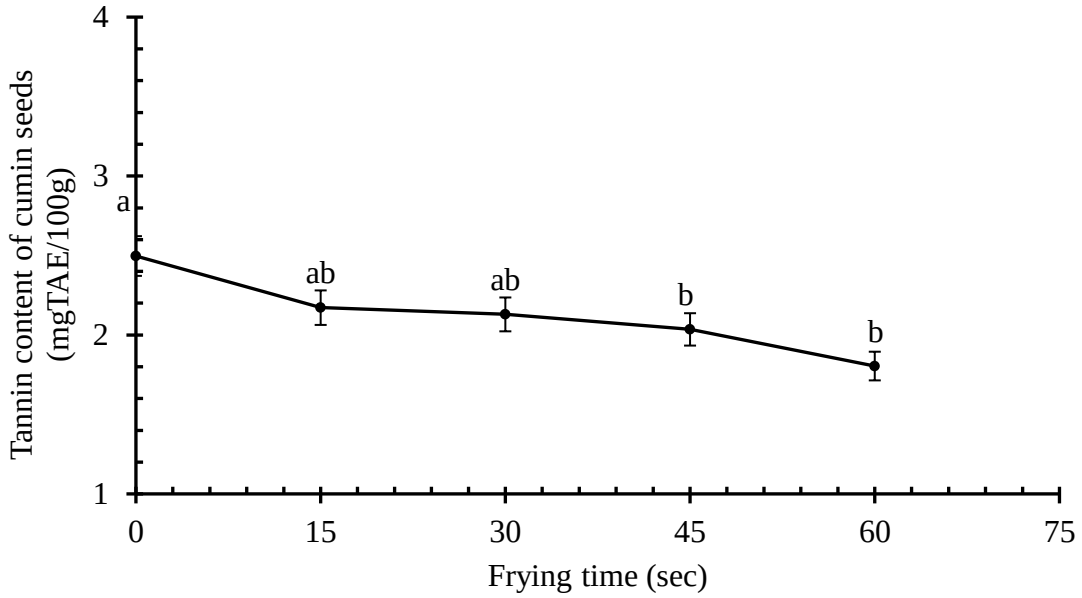

Fig 4.2 Effect of frying interval on tannin content of cumin seeds

The mean value of tannin content in raw cumin seed was found to be 2.497 ± 0.45mg/100 g on the basis of dry matter. Tannin content was reduced on the progressive interval of frying. The mean value of progressive interval of frying was 2.172 ± 0.06 mg/100 g, 2.129 ± 0.55mg/100 g, 2.035 ± 0.62 mg/100 g, and 1.804 ± 0.18 mg/100 g on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between tannin content in the raw and other frying interval ($p < 0.05$) while there was no significant difference between 60 and 45 sec frying interval and 15 and 30 sec frying intervals.

It is important to note that while deep frying can cause a reduction in tannin content, it can also have other effects on the nutrient content of spices. For example, deep frying can lead to the formation of potentially harmful compounds such as acrylamide and trans fats.

Our results showed substantial decrease in the total tannin content in the both the spices i.e., fenugreek and cumin processed by different frying interval and are in agreement with the earlier studies. Our results are well in agreement with (Nawaz *et al.*, 2018) who reported decrease in tannin content of ginger around (60-77%) after thermal treatment. The decrease in phytochemical content in response to an increase in the treatment time in both cases may be attributed to the thermal degradation of the heat-sensitive bioactive phytochemical compounds present in spices.

4.5 Effect of frying time on total phenol content of spices

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.*, 2006).The spices were deep fried in different time interval (15, 30, 45 and 60) second. The change in total phenol content of the spices were analyzed in different frying time interval as well as in raw sample. The reduction in total phenol content of spices after being fried in oil is demonstrated in below fig.

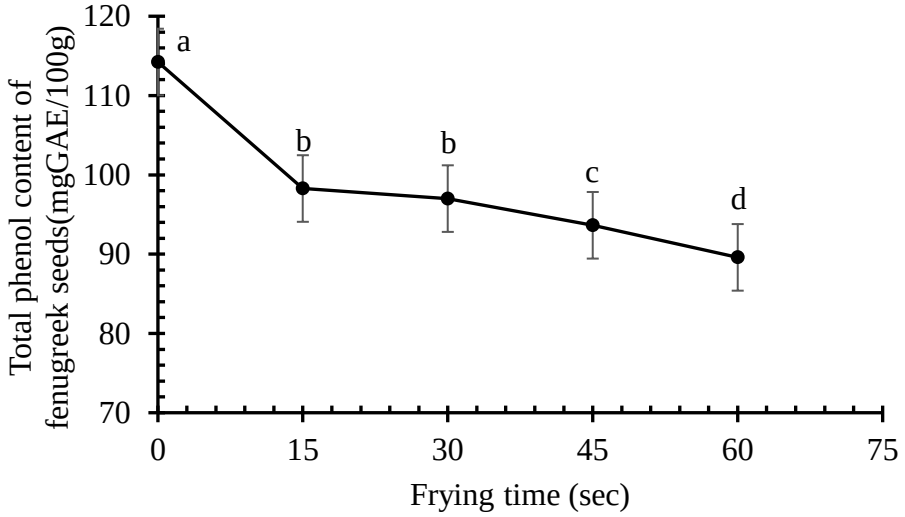

Fig 4.3 Effect of frying time on total phenol content of fenugreek seeds

The mean value of total phenol content in raw fenugreek seed was found to be 114.23 ± 1.19 mg/100 g on the basis of dry matter. Total phenol content was reduced on the progressive interval of frying. The mean value of progressive interval of frying was 98.27 ± 0.91 mg/100 g, 96.97 ± 0.97 mg/100 g, 93.64 ± 1.46 mg/100 g, and 89.59 ± 1.39 mg/100 g

on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between tannin content in raw, 45 and 60 sec of frying ($p < 0.05$) while there was no significance different in total phenol content in 15 and 30 sec of time interval.

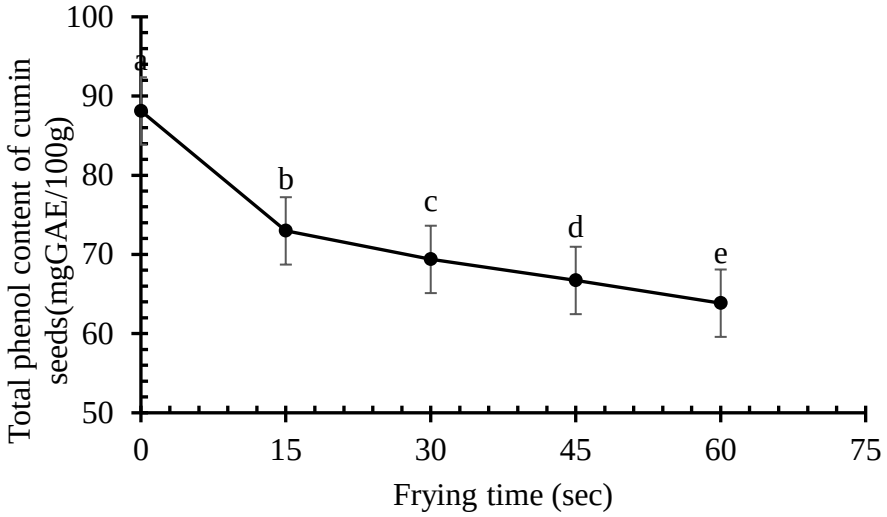

Fig 4.4 Effect of frying time on total phenol content of cumin seeds

The mean value of total phenol content in raw cumin seed was found to be 88.12 ± 1.35 mg/100 g on the basis of dry matter. Total content was reduced on the progressive interval of frying. The mean value of progressive interval of frying was 72.97 ± 1.31 mg/100 g, 69.37 ± 1.24 mg/100 g, 66.72 ± 1.17 mg/100 g, and 63.84 ± 1.22 mg/100 g on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between total phenol content of cumin seeds in raw and different interval of frying ($p < 0.05$).

Our results showed substantial decrease in the total phenolic content in the both the spices i.e., fenugreek and cumin processed by different frying interval and are in agreement with the earlier studies. Our results are well in agreement with (Alide *et al.*, 2020), who reported decrease in total phenol content of garlic by 57.4-59.1%, 60.4-62.16%, 67.69-72.76% and 68.03-75% on different cooking interval (15, 30, 45 and 60) min respectively. Similar results for total phenolic content (30% of losses) in fried tomatoes is reported by (Sahlin *et al.*, 2004). Regarding to the flavonoids and the phenolics of the oil, (Andrikopoulos *et al.*, 2002), reported losses in these compounds that increase with frying sessions. Thus, the losses can

be of 20% in the first cycle and arise up to 80% in the cycle eight. Temperature affects both flavonoid and phenolic content of foodstuff and also the polyphenol compounds present in the oil used for frying.

In addition, the increase or decrease in the phytochemical content of spices depends on the type of cooking that was used to determine the effect of temperature and sometimes the type of spices, duration of cooking and the type of oil used (Turkmen *et al.*, 2005).

4.6 Effect of frying time on total flavonoid content of spices

Flavonoids are a group of naturally occurring compounds that are widely distributed in plants and are responsible for many of their colors. (Erdman Jr *et al.*, 2007). The spices were deep fried in different time interval (15, 30, 45 and 60) second. The change in total flavonoid content of the spices were analyzed in different frying time interval as well as in raw sample. The reduction in total flavonoid content of spices after being fried in oil is demonstrated in below figure.

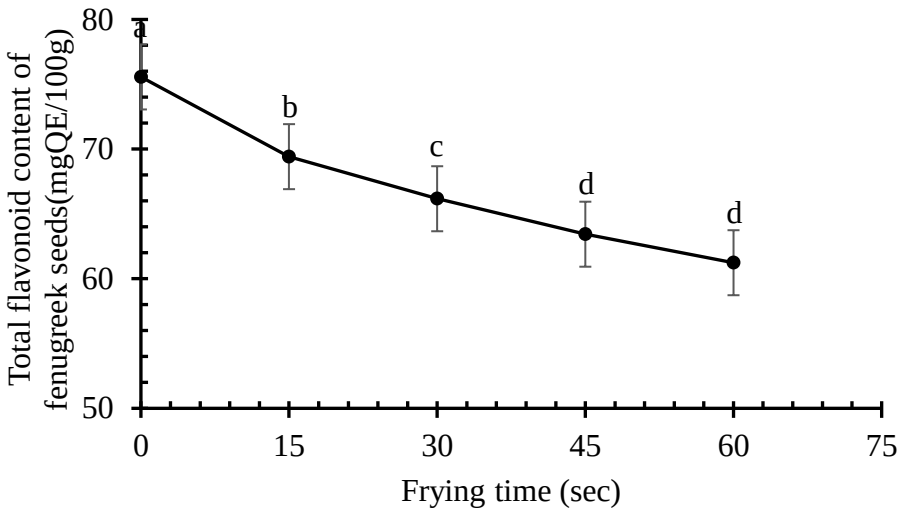

Fig 4.5 Effect of frying time on total flavonoid content of fenugreek seeds

The mean value of total flavonoid content in raw fenugreek seed was found to be 75.57 ± 0.64 mg/100 g on the basis of dry matter. Total flavonoid content was reduced on the progressive interval of frying. The mean value of progressive interval of frying was 69.42 ± 0.8 mg/100 g, 66.17 ± 0.74 mg/100 g, 63.43 ± 1.11 mg/100 g, and 61.23 ± 2.23 mg/100 g on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between

total flavonoid content in raw, 15 and 30 sec of frying ($p < 0.05$) while there was no significance difference in total flavonoid content in 45 and 60 sec of frying time interval.

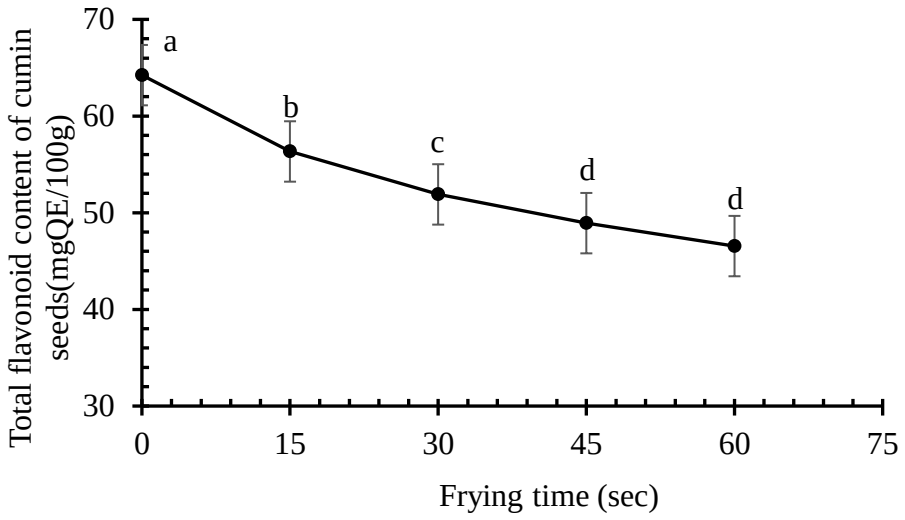

Fig 4.6 Effect of frying time on total flavonoid content of cumin seeds

The mean value of total flavonoid content in raw cumin seed was found to be 64.234 ± 1.01 mg/100 g on the basis of dry matter. Total flavonoid content was reduced on the progressive interval of frying. The mean value of progressive interval of frying was 56.34 ± 1.32 mg/100 g, 51.89 ± 1.9 mg/100 g, 48.92 ± 1.45 mg/100 g, and 46.55 ± 1.25 mg/100 g on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between total flavonoid content in raw, 15 and 30 sec of frying interval ($p < 0.05$) while there was no significant difference between 45 and 60 sec of frying interval.

Our results showed substantial decrease in the total flavonoid content in the both the spices i.e., fenugreek and cumin processed by different frying interval and are in agreement with the earlier studies. Our results are well in agreement with (Crozier *et al.*, 1997), who reported losses of conjugated quercetin of 21% and 35% in fried onions and tomatoes, respectively.

The result was accordance to (Abacan *et al.*, 2017), the value of total flavonoid content of garlic gradually decline by 50.5-52.45%, 61.8-74.45%, 71.9-74, 9% and 72.26-76% on different cooking interval 15, 30, 45 and 60 min respectively. However, the cooking time

considered (≤5 min) was too short to significantly affect the TPC, TFC and antioxidant activity of spices.

4.7 Effect of frying time on antioxidant activity of spices

Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins (Yashin *et al.*, 2017).

The spices were deep fried in different time interval (15, 30, 45 and 60) second. The change in DPPH antioxidant activity (IC_{50}) of the spices were analyzed in different frying time interval as well as in raw sample. The reduction in DPPH antioxidant activity of spices after being fried in oil is demonstrated in below figure. Lower IC_{50} values indicate higher antioxidant activity.

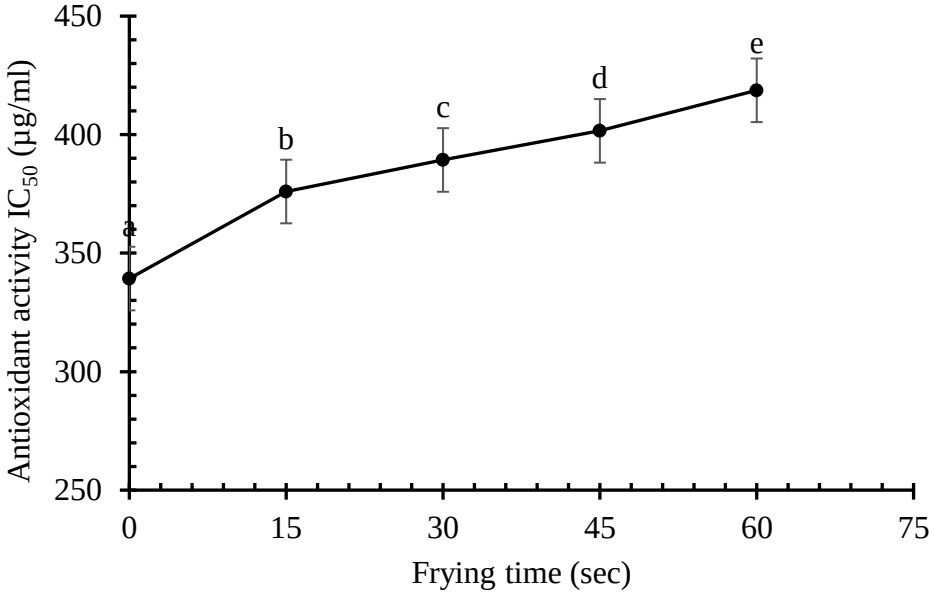

Fig 4.7 Effect of frying time on antioxidant activity of fenugreek seeds

The mean value of IC_{50} value of raw fenugreek seed was found to be 339.3 ± 1.57 µg/ml on the basis of dry matter. IC_{50} was increased on the progressive interval of frying. The mean value of progressive interval of frying was 376 ± 1.58 µg/ml, 389.3 ± 0.85 µg/ml, 401.6 ± 0.64 µg/ml, and 418.7 ± 1.76 µg/ml on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between IC_{50} value between raw and different frying interval ($p<0.05$).

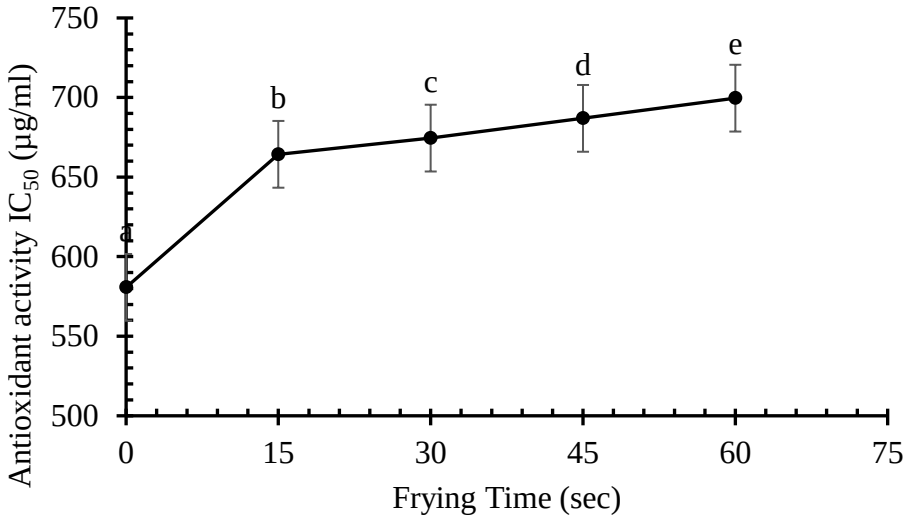

Fig 4.8 Effect of frying time on antioxidant activity of cumin seeds

The mean value of IC_{50} value of raw cumin seed was found to be 580.7 ± 0.53 µg/ml on the basis of dry matter. IC_{50} was increased on the progressive interval of frying. The mean value of progressive interval of frying was 664.3 ± 2.15 µg/ml, 674.5 ± 2.5 µg/ml, 686.9 ± 2.34µg/ml and 699.6 ± 3.10 µg/ml on the basis of dry matter on different frying interval (15, 30, 45 and 60) sec respectively. The analysis of variance (Appendix B) showed that there was significant difference between IC_{50} value between raw and different frying interval ($p<0.05$).

Our results showed substantial decrease in antioxidant activity in the both the spices i.e., fenugreek and cumin processed by different frying interval and are in agreement with the earlier studies. Our study are in agreement with (Chuah *et al.*, 2008), who reported significant reduction (23-36%) in DPPH radical scavenging activities for colored pepper after thermal treatment for 5 to 30 minutes. Since the spices were heated at high temperature it may result in significant decrease ($p<0.05$) of radical scavenging activity (Bordoloi *et al.*, 2017).

(Sultana *et al.*, 2008) reported that when spices are submitted to cooking processes, such as pressure-cooking, microwaving, baking, grilling, deep frying, variations appear in their antioxidant activity or scavenger capacity. These variations depend on the spice themselves (bioactive structures), the cooking method, the bioavailability of phenolics temperature, the localization of the structures in the vegetables, cutting, chopping, stability of the structure to

heat (Pedraza-Chaverrí *et al.*, 2006), the synergic activity of the structures, and on the reaction systems assayed (for example, β-carotene is an efficient singlet oxygen quencher but is not a hydrogen donor) (Yamaguchi *et al.*, 2001).

Part V

Conclusion and recommendation

5.1 Conclusion

On the basis of this study following conclusions were drawn.

- Spices offer various health benefits, including antioxidant properties, anti-inflammatory effects, and the enhancement of flavor and aroma in culinary dishes.
- Tannin content in raw fenugreek seeds was found 3.96±0.02mg/100g and reduced by 18.5-20%, 21.5-24%, 31% and 33-35% and in raw cumin seeds was 2.497±0.45 and reduced by 12-15%, 16-17.5%, 19.5% and 20-21% in different frying interval (15, 30, 45, 60) sec respectively.
- Total phenol content in raw fenugreek seed was 114±1.19 mg/100g and was reduced by 12.5%,18.5%,20% and 21.6% and in raw cumin seeds was 88.12±1.35mg/100g and reduced by 15-16%,19.2%,21.5% and 22-24.5% in different frying time (15,30,45,60) sec respectively.
- Total flavonoid content in raw fenugreek was 75.57±0.64mg/100g and reduced by 9%, 11.5%, 13-14.5% and 18% and in raw cumin seeds was 64.23±1.01mg/100gand reduced by 8-12%, 13.5-15%, 17% and 19.5% in different frying interval (15, 30, 45, 60) sec respectively.
- The DPPH radical scavenging activity (IC_{50}) was found maximum in raw fenugreek seeds i.e..,339.3±1.57µg/ml and minimum in fried cumin seeds(60sec) i.e..,699.6±3.10µg/ml. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.
- Frying of fenugreek and cumin seeds, which are commonly used in cooking, results in a significant reduction in their antioxidant potential as well as their tannin, total phenol, and total flavonoid content.
- The reduction of antioxidants in fried fenugreek and cumin seeds suggests that they may provide less health benefits when consumed in this form.

5.2 Recommendation

The following recommendation can be drawn from conclusion.

- Research can be done by varying other factors like temperature, and other spices too.
- Frying time should be kept as low as possible.

- To maximize the health benefits of fenugreek and cumin seeds, it is recommended to consume them in their raw or lightly processed form, such as adding them to salads or using them as a seasoning in recipes, rather than frying or cooking them for extended periods.

Part VI

Summary

Frying of spices refers to the technique of heating whole or ground spices in hot oil or ghee until they release their aromas and flavors. This process helps to enhance the overall flavor of a dish and is commonly used in Indian, Middle Eastern, and Southeast Asian cuisine. The frying of spices is typically done at the beginning of cooking a dish, or added towards the end to enhance the flavor of a finished dish. It is important to be careful not to burn the spices while frying, as this can result in a bitter taste and unpleasant aroma.

Changes in food and oil depends on the characteristics of the food, oil type, surface/volume ratio of the oil, rate of air incorporation of into the oil, temperature, heating process, length of immersion and the kind of material the frying container is made of. Additionally, the longer the oil is used, greater is the induction of adverse reactions. Extended exposure of oil to high temperatures and atmospheric air can generate highly oxidized, potentially toxic products.

For this study the spices (fenugreek and cumin seeds) were purchased from the local market of Dharan. They were winnowed, handpicked. They were fried in oil at constant temperature (220°C) and for different time interval (15, 30, 45 and 60 sec) and were milled to powder. The prepare sample was taken for further analysis of phytochemical and antioxidant activity.

Tannin content was decreased from 3.96 to 3.17, 3.04, 2.92 and 2.87 mg tannic acid/100g in fenugreek seeds and from 2.51 to 2.17, 2.129, 2.035 and 1.80 mg tannic acid/100g in cumin seeds in different interval of time (15, 30, 45 and 60 sec).

Total phenol content was decreased from 114.22 to 98.2, 97.003, 93.6 and 89.5 mg GAE/100g in fenugreek seeds and from 88.12 to 72.97, 69.37, 66.71 and 63.84 mgGAE/100g in cumin seeds in different interval of time (15, 30, 45 and 60 sec).

Total flavonoid was decreased from 75.5 to 69.4, 66.17, 63.4 and 61.23 mgQE/100g in fenugreek seeds and from 64.234 to 56.35, 51.89, 48.92 and 46.54 mgQE/100g in cumin seeds in different interval of time (15, 30, 45 and 60 sec).

The DPPH radical scavenging activity (IC_{50}) was increased from 339.27 to 375.99, 389.3, 401.38 and 418.705 µg/ml in fenugreek seeds and from 580.67 to 650.9, 674.5, 686.8 and 699.6 µg/ml in cumin seeds in different time interval (15, 30, 45 and 60 sec). Lower IC_{50} values indicate higher antioxidant activity.

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Appendices

Appendix A

1. Standard curve of gallic acid for total phenol content

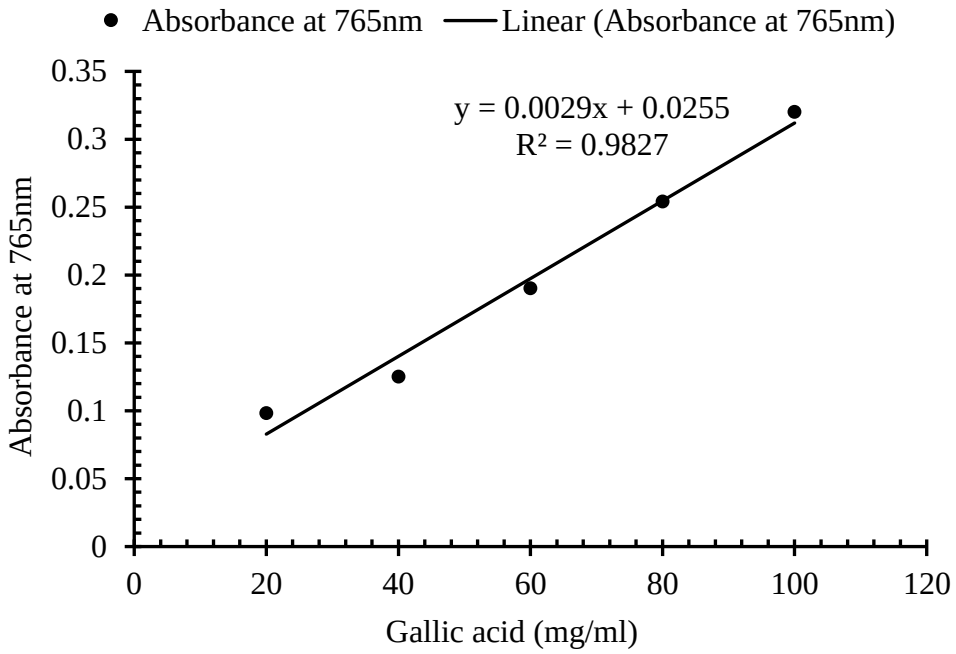

Fig. A.1 Standard curve of gallic acid for total phenol content

2. Standard curve of tannic acid for tannin content

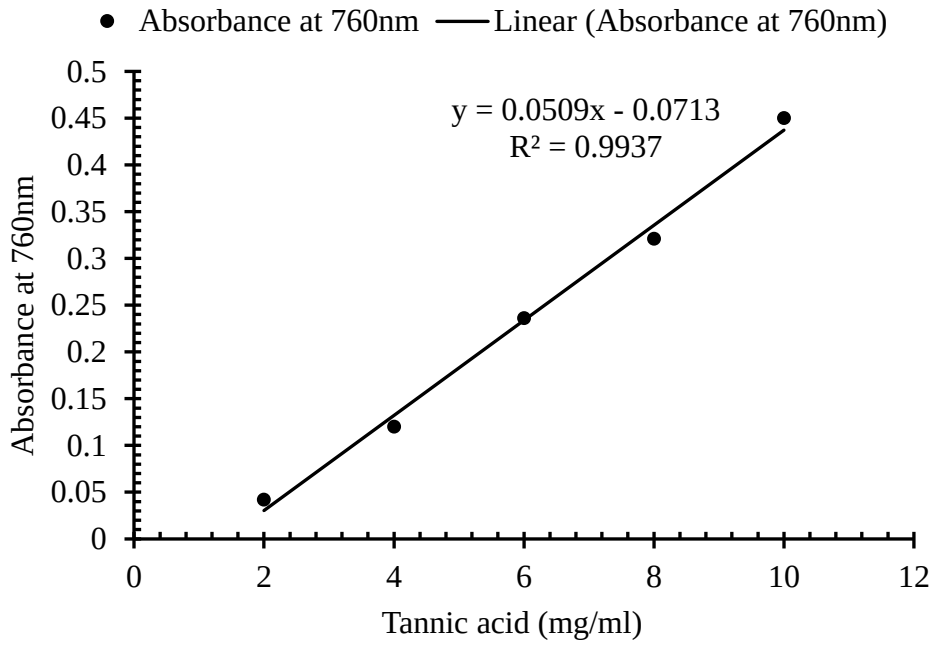

Fig. A.2 Standard curve of tannic acid for tannin content

3. Calibration Curves of Quercetin for flavonoid

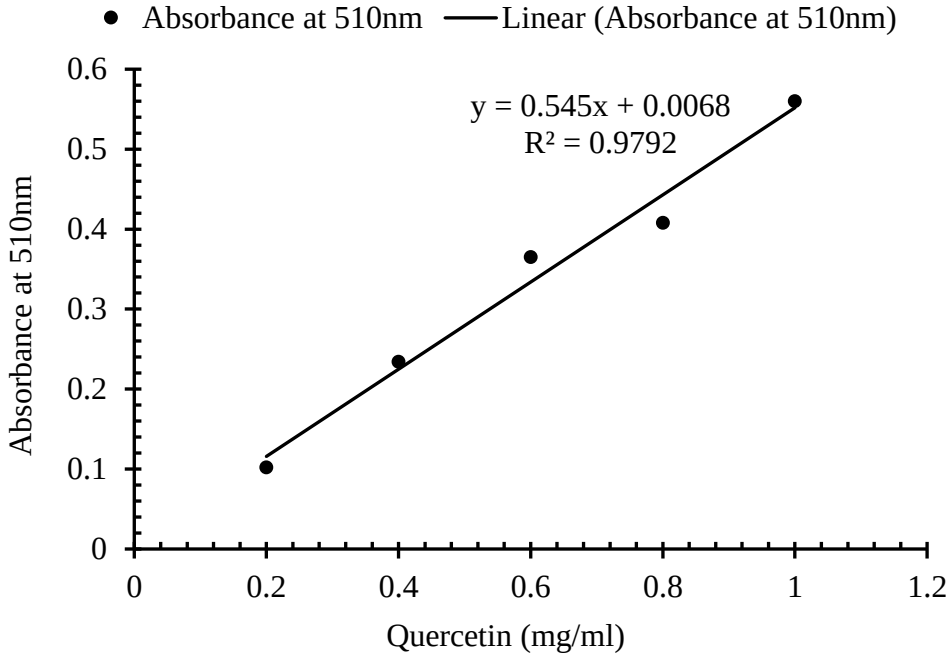

Fig. A.3 Calibration Curves of Quercetin for flavonoid

Appendix B

Table B.1 ANOVA for tannin content of fenugreek seeds

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	2.396638	0.599159	1224.61	<.001
Residual	10	0.004893	0.000489		
Total	14	2.40153			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.2 LSD of means of tannin content of fenugreek seeds

Treatment	Mean	Column A	l.s.d	d.f.
F60*	2.87 ± 0.12	E	0.04024	10
F45*	2.91 ± 0.52	D		
F30*	3.03 ± 0.80	C		
F15*	3.17 ± 0.22	B		
Raw Fenu*	3.963 ± 0.10	A		

(* = Significantly different)

Table B.3 ANOVA for total phenol content of fenugreek seeds

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	1058.109	264.527	181.63	<.001
Residual	10	14.564	1.456		
Total	14	1072.674			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.4 LSD of means of total phenol content of fenugreek seed

Treatment	Mean	Column A	l.s.d	d.f.
F60*	89.59 ± 1.39	D	2.196	10
F45*	93.64 ± 1.46	C		
F30	96.97 ± 0.97	B		
F15	98.27 ± 0.91	B		
Raw fenu*	114.23 ± 1.19	A		

(* = Significantly different)

Table B.5 ANOVA for total flavonoid content of fenugreek seed

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	377.263	94.316	60.07	<.001
Residual	10	15.700	1.570		
Total	14	392.962			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.6 LSD of means of total flavonoid content of fenugreek seed

Treatment	Mean	Column A	l.s.d	d.f.
F60	61.23 ± 2.23	D	2.280	10
F45	63.43 ± 1.11	D		
F30*	66.17 ± 0.74	C		
F15*	69.42 ± 0.80	B		
Raw fenu*	75.57 ± 0.64	A		

(* = Significantly different)

Table B.7 ANOVA for antioxidant activity of fenugreek seeds

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	10805.165	2701.291	1462.17	<.001
Residual	10	18.475	1.847		
Total	14	10823.640			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.8 LSD for mean of antioxidant activity of fenugreek seeds

Treatment	Mean	Column A	l.s.d	d.f.
F60*	418.7 ± 1.76	E	2.473	10
F45*	401.6 ± 0.64	D		
F30*	389.3 ± 0.85	C		
F15*	376 ± 1.58	B		
Raw fenu*	339.3 ± 1.57	A		

(* = Significantly different)

Table B.9 ANOVA for tannin content of cumin seed

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	0.75369	0.18842	3.79	0.04
Residual	10	0.49766	0.04977		
Total	14	1.25135			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.10 LSD for mean of tannin content of cumin seed

Treatment	Mean	Column A	l.s.d	d.f.
C60	1.804 ± 0.18	B	0.4058	10
C45	2.035 ± 0.62	B		
C30	2.129 ± 0.55	AB		
C15	2.172 ± 0.06	AB		
Raw Cumin*	2.497 ± 0.45	A		

(* = Significantly different)

Table B.11 ANOVA for total phenol content of cumin seed

Source of variation	Degree of freedom	Sum of squares	Mean squares	Variance ratio	F probability ratio
Treatment	4	1085.862	271.465	169.66	<.001
Residual	10	16.001	1.6		
Total	14	1101.862			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.12 LSD of mean of total phenol content of cumin seed

Treatment	Mean	Column A	l.s.d	d.f.
C60*	63.84 ± 1.22	E	2.301	10
C45*	66.72 ± 1.17	D		
C30*	69.37 ± 1.24	C		
C15*	72.97 ± 1.31	B		
Raw Cumin*	88.12 ± 1.35	A		

(* = Significantly different)

Table B.13 ANOVA for total flavonoid content of cumin seed

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	585.335	146.334	72.23	<.001
Residual	10	20.258	2.026		
Total	14	605.593			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.14 LSD for mean of total flavonoid content of cumin seed

Treatment	Mean	Column A	l.s.d	d.f.
C60	46.55 ± 1.25	D	2.589	10
C45	48.92 ± 1.45	D		
C30*	51.89 ± 1.90	C		
C15*	56.34 ± 1.32	B		
Raw Cumin*	64.23 ± 1.01	A		

(* = Significantly different)

Table B.15 ANOVA for antioxidant activity of cumin seed

Source of variation	Degree of freedom	Sum of square	Mean of square	Variance ratio	F probability ratio
Treatment	4	26420.053	6605.053	1255.26	<.001
Residual	10	52.619	5.262		
Total	14	26472.672			

Since $p<0.05$, there is a significant difference between the samples in different frying interval so LSD testing is necessary.

Table B.16 LSD for mean of antioxidant activity of cumin seed

Treatment	Mean	Column A	l.s.d	d.f.
C60*	699.6 ± 3.10	E	4.173	10
C45*	686.9 ± 2.34	D		
C30*	674.5 ± 2.50	C		
C15*	664.3 ± 2.15	B		
Raw Cumin*	580.7 ± 0.53	A		

(* = Significantly different)

Appendix C

Table C.1 List of chemicals used

Chemical	Supplier /manufacturer	Other specification
Sodium hydroxide (NaOH)	Thermo Fisher Scientific India Pvt.Ltd	Pellets ,AR grade,98%
Hydrochloric acid (HCL)	Thermo Fisher Scientific India Pvt.Ltd	36%,LR grade
Boric acid	Merek (India) Limited	amorphous
Sulphuric acid (H_2SO_4)	Thermo Fisher Scientific India Pvt.Ltd	97%,LR grade
Potassium permanganate	Avantor performance materials limited	99%,Assay
Potassium thiocyanate	Thermo Fisher Scientific India Pvt.Ltd	97%,Assay
Tannin acid	Avarice laboratories Pvt.ltd	Analytical reagent
Nitric acid	Fisher Scientific India Pvt.Ltd	68-75%,Assay
Sulphuric acid	Fisher Scientific India Pvt.Ltd	97%,Assay
DPPH	Hi Media Laboratories Pvt.Ltd	
Hydrochloric acid	Fisher Scientific India Pvt.Ltd	35-37%,Assay
Quercetin	Avarice laboratories Pvt.ltd	Analytical reagent
Gallic acid	Avarice laboratories Pvt.ltd	Analytical reagent
L-ascorbic acid	S.D.fine chemicals Ltd	99%,Assay

Table C.2 List of equipment used

Physical apparatus	**Specification**
Electric balance	Phoneix instruments ,India
Spectrophotometer	Labtronics,India
Soxhlet apparatus	Y.P.scientific glass work
Hot air oven	Victolab India
Incubator	Y.P.scientific glass work
Muffle furnance	Accumax ,India
Cabinet dryer	AIset YDL-2000
Colorimeter	Jenway Ltd.,UK
Centrifuge	Y.P.scientific glass work
Heating mantle	Y.P.scientific glass work
Rotary vacuum eveporator	OEM manufacturer ,India

List of plates

P1 Reading absorbance on spectrophotometer

P2 Using rotary vacuum evaporator for extraction

P3 Lab work on progress