# Phenolic and Polyphenolic Profiles of Defatted Camelina, Chia and Sophia Seeds and Their *In vitro* Antioxidant and Biological Activities

**By**

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**A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirement of the degree of master science**

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**August 2017**

**St. John's Newfoundland and Labrador Canada**

**Dedicated to My Respected Father and Mother**

# ABSTRACT

Phenolic compounds in oilseeds occur in the free, esterified and insoluble-bound forms. The phenolics in seeds act as natural antioxidants by preventing deteriorative oxidative processes in foods as well as oxidative stress and various disorders in the human body once consumed. The free, esterified and insoluble-bound phenolics were extracted from defatted camelina (*Camelina sativa*), chia (*Salvia hispanica*) and sophia (*Descurainia sophia*) seeds meals. All samples were evaluated for their total phenolic content (TPC), total flavonoid content (TFC), and total proanthocyanidin (PC) content as well as antioxidant activity of their various phenolic fractions. The TPC in camelina, chia and sophia defatted meal was 11.69 ± 0.44, 14.22 ± 0.44 and 22.40 ± 0.87 mg GAE per gram sample, respectively. The corresponding values for TFC were 6.81 ± 0.68, 8.45 ± 0.80 and 8.59 ± 0.13 mg CE per gram defatted meal, respectively. Meanwhile, the PC in camelina, chia and sophia meals was 3.73 ± 0.03, 0.08 ± 0.02 and 2.23 ± 0.06 mg CE per gram sample, respectively. Several *in vitro* free radical scavenging assays, namely 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, trolox equivalent antioxidant capacity (TEAC), hydroxyl radical scavenging capacity (HRSC), reducing power (RP), β-carotene/ linoleate model system and metal chelation activity were investigated for all fractions. In addition, inhibition activity against lipase, α-glucosidase, low density lipoprotein (LDL) oxidation and DNA strand scission induced by peroxyl and hydroxyl radicals for all fractions was examined in biological systems. High performance liquid chromatography (HPLC) and HPLC-tandem mass spectrometry (HPLC-$MS^n$) led to positive identification of 36 phenolic compounds belonging to simple phenols, phenolic acids and their derivatives, flavonoids and procyanidins in the three phenolic fractions of camelina, chia and sophia. Esterified fraction was the predominant form of phenolics compared to the free and insoluble bound forms of phenolics in both defatted

camelina and sophia seeds whereas the free phenolic fraction was the predominant form in defatted chia seed meal. Thus, camelina, chia and sophia seeds may serve as viable functional food ingredients with protective antioxidant potential but further research is required to confirm their cardiovascular diseases (CVD) preventive effects.

# Acknowledgement

I would like to thank my supervisor Professor F. Shahidi for his priceless professional and financial (through NSERC) support and personal inspiration throughout this project and for accepting me as graduate student in his research group which are deeply appreciated. I would also like to thank to my supervisory committee members the late Professor R. Hoover, as well as Dr. S. Debnath and Professor Julissa Roncal. I would also like to express my appreciation to the School of Graduate Studies at Memorial University of Newfoundland for financial support. Special thanks go to Linda Winsor and Celine Schneider at the Centre for Chemical Analysis, Research and Training (C-CART), Memorial University of Newfoundland for training me on the HPLC-DAD-MS/MS equipment and de Camargo and Dr. P. Ambigaipalan for HPLC-DAD-MS/MS analysis. My sincere thanks are also extended to all my friends and colleagues in Dr. Shahidi's research team for creating an enjoyable research environment and helping in each difficult situation. Finally, we want to give special thank to my parents and other family members for their encouragement during my master program.

# TABLES OF CONTENTS

# LISTS OF TABLES

**LISTS OF FIGURES**

# LISTS OF ABBREBIATIONS

| | |
|---|---|
| AAPH | 2,2'-azobis (2-aminopropane) dihydrochloride |
| DMSO | Dimethyl sulfoxide |
| DPPH | 1, 1-diphenyl-2-picrylhydrazyl |
| EDTA | Ethylenediaminetetraacetic acid |
| HPLC | High performance liquid chromatography |
| DAD | Diode array detector |
| LDL | Low density lipoprotein |
| PBS | Phosphate buffer solution |
| ROS | Reactive oxygen species |
| PUFA | Polyunsaturated fatty acids |
| TBA | Thiobarbituric acid |
| TE | Trolox equivalents |
| CVD | Cardiovascular disease |
| CD | Conjugated diene |
| HPLC-DAD-MS/MS | High performance liquid Chromatography-Diode Array Detector-tandem mass spectrometry |
| ABTS | 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonate) |
| GAE | Gallic acid equivalents |
| EPR | Electron paramagnetic resonance |

# Lists of Publications

1. Rahman, M. J., de Camargo, A. C., & Shahidi, F. (2018). Phenolic profiles and antioxidant activity of defatted camelina and sophia seeds, *Food Chemistry*, 240, 917-925.

2. Rahman, M. J., de Camargo, A. C., & Shahidi, F. (2017a). Phenolic and polyphenolic profiles of chia seeds and their in vitro biological activities. *Journal of Functional Foods*, *35*, 622-634.

3. Rahman, M. J., Ambigaipalan, P., & Shahidi, F. (2017b). Biological activities of camelina and sophia phenolics: Inhibition of LDL oxidation, DNA damage and pancreatic lipase and α-glucosidase activities, *Journal of Food Science*, Submitted.

# CHAPTER 1
# INTRODUCTION

Phenolic compounds occur widely in plants as secondary metabolites. Although the particular role of these secondary metabolites remains unclear, phenolic compounds are known to be important in the survival of a plant in its environment (Vuorela 2005; Puupponen-Pimiä et al. 2005). In general, phenolics are synthesized by plants during their normal growth in response to stress conditions such as infection, wounding, and UV radiation, among others (Naczk & Shahidi 2004). In addition to their role in plants, phenolics exhibit several bioactivities beneficial to humans. Many plant-derived foods, herbals and medicinal products are rich in phenolic compounds that can prevent, treat or cure diseases (Vuorela 2005; Scalbert 1993). In particular, phenolic compounds have been shown to exhibit protection against coronary heart disease and carcinogenesis (Albishi et al. 2013; Hertog et al. 1995). Epidemiological studies have shown that regular consumption of phenolic rich foods such as cereals, legumes and oilseeds as well as their products and by-products can protect against the risk of cardiovascular diseases, type 2 diabetes, gastrointestinal cancers, and a range of other disorders (Chandrasekera & Shahidi 2010; McKeown et al. 2002). Plant phenolics include simple phenols, phenolic acids (both hydroxybenzoic and hydroxycinnamic acid derivatives), flavonoids, isoflavonoids, stilbenes, hydrolysable and condensed tannins, lignans, and lignins (Naczk & Shahidi 2004; Dewick 2001). Phenolic compounds in oilseeds exist as free, soluble conjugates and insoluble-bound forms. The distribution of phenolic compounds is not equal in oilseeds, and a high proportion is found in the outer layers, namely the aleurone layer, testa, and pericarp, which form the main components in the bran fraction. Although insoluble-bound phenolics are not readily available for

absorption, they can be released under the low pH conditions of the gastrointestinal tract (Chandrasekera & Shahidi 2010; Liyana-Pathirana & Shahidi 2005) and upon colonic fermentation (Chandrasekera & Shahidi 2010; Kroon et al. 1997). Upon release, they can exert a localized effect on the gut lumen or could be absorbed into the bloodstream. Therefore, extraction and quantification of soluble and insoluble-bound phenolics and determination of their antioxidant activity in chemical and biological systems is of paramount importance to the understanding of the potential health benefits of oilseeds.

Camelina (*Camelina sativa)* is an ancient oilseed crop belonging to the Brassicaceae family. It is commonly known as gold of pleasure or false flax. It has been cultivated as a native oilseed crop in Northern Europe and Central Asia. In western Canada, camelina is a new oilseed crop that may have a promising future. Camelina oil is one of the most important edible oil sources in the world, with excellent nutritive value due to its abundance of essential fatty acids. The oil of camelina contains about 45% polyunsaturated fatty acids (PUFA), 35% monounsaturated fatty acids (MUFA), 10% saturated fatty acids (SFA), and up to 10% free fatty acids (FFA), as well as tocopherols, sterols, terpenes, and volatiles (Das et al. 2014). Camelina meal is the by-product of camelina deoiling process and commonly used for animal feed. Its amino acid content is ideal and it has a high content of fibre, several minerals, and vitamins. Defatted camelina meal consists of approximately 45% protein, 15% insoluble fiber, 10% soluble carbohydrates, 5% minerals, approximately 0.2% nucleic acids, and 4% or more of a mixture of phytochemical components (Aziza, Quezada, & Cherian 2010). Camelina contains more phenolic compounds than other oilseeds. The most significant of these are sinapic acid and its derivatives, most notable sinapine as is the case for canola seeds.

Chia (*Salvia hispanica)* is an annual oilseed plant that belongs to the Lamiaceae family. It is cultivated as a native plant in Southern Mexico and Northern Guatemala (Ayerza 1995), and has recently been marketed as a crop in South America (Ayerza & Coates 2011). In Canada, it is sold primarily as a health food commodity. In 2009, chia seeds received approval from the European Union as a novel food and can be used up to 5% in bread formulations (Segura-Campos et al. 2014; Commission of the European Communities 2009). Nowadays, chia is generally grown in Mexico, Guatemala, Bolivia, Argentina, Ecuador, and Australia (Segura-Campos et al. 2014; Guiotto 2013). It has been demonstrated that chia has great potential as a future crop plant (Segura-Campos et al. 2014; Guiotto 2013). Chia seeds contain a high amount of dietary fibre, protein, α-linolenic acid (C18:3 n-3, ALA), phenolic acids and vitamins (Rincón-Cervera et al. 2016; Valdivia-López & Tecante 2015; Porras-Loaiza et al. 2014). It contains a high oil content (25–32%), protein (18.5–22.3%), fibre (20.1–36.15%), and 59.9–63.2% α-linolenic acid as well as 18.9–20.1% of linoleic acid (Porras-Loaiza et al. 2014). Most of the species of the genus *Salvia* have homeopathic and horticultural importance as a source of many useful natural constituents, like polyphenols, such as chlorogenic and caffeic acids, myricetin, quercetin and kaempferol (Ixtaina et al. 2011; Reyes-Caudillo et al. 2008). Due to high diversity of secondary metabolites like phenolic compounds, *Salvia* plants possess excellent antioxidant capacity as well as antimicrobial activity and some are used against several pathological disturbances, such as atherosclerosis, brain dysfunction, and cancer (Cvetkovikj et al. 2013). Valenzuela et al. (2015) reported that chia oil intake provides good source of ALA, allows an important modification in the EPA content of erythrocytes in pregnant mothers and an increase of DHA in their milk.

Sophia (*Descurainia sophia*), commonly known as flaxweed, belongs to the Brassicaceae family, is found throughout Canada, and is well adapted to the climate of the Canadian Prairies where it is one of the most abundant weeds (HadiNezhad et al. 2015; Best 1977). Sophia has been used as a traditional medicine in many countries including China, India, and Iran (Khan et al. 2012). The seed is edible in the cooked or raw forms and contains 28 % protein, 33 % oil, and 4 % minerals (WHO 1997). The oil of sophia seed is good source of fatty acids which contain 69.91% polyunsaturated fatty acids (PUFAs), 21.79 % monounsaturatd fatty acids (MUFAs) and 8.30% saturated fatty acids (SFA) (HadiNezhad et al. 2015). Among PUFAs, the omega-3 fatty acids predominated (51.30%) (HadiNezhad et al. 2015). The seed is a rich source of bioactive compounds such as phenols, phenolic acids, flavonoids and flavonoid glycosides. Phenolic compounds such as *p*-hydroxybenzoic acid, isovanillic acid, *p*-hydroxybenzaldehyde, syringic acid, and 4-hydroxy-3, 5-dimethoxybenzaldehyde have been isolated from the whole seeds and meal of sophia (HadiNezhad et al. 2015; Sun 2005).

Although there have been studies on the free phenolics and their antioxidant activity in camelina meal (Terpinc et al. 2016; Terpinc et al. 2012); chia meal (Reyes- Caudillo et al. 2008; Marineli et al. 2014; Taga et al. 1984) and sophia meal (HadiNezhad et al. 2015; Sun 2005), there appears to be very little information available on the esterified and insoluble-bound phenolics in camelina (Terpinc et al. 2011) and none on the esterified and insoluble-bound phenolics in chia and sophia seeds. In the present study, the phenolic constituents of defatted camelina, chia and sophia seed meals were extracted by using an ultrasonic-assisted technique and alkaline hydrolysis and fractionated into their respective free, esterified (soluble), and insoluble-bound forms and the relative

proportions of various phenolic acids determined, both chemically and by using high-performance liquid chromatography-tandem mass spectrometry (HPLC-DAD-MS/MS). To the best of our knowledge, this is the first study that extensively examines all three forms of phenolics in defatted camelina, chia and sophia seed meals by both Folin Ciacalteu test and HPLC-DAD-MS/MS along with their contribution to the antioxidant and biological potential in several *in vitro* chemical systems.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Phenolics and polyphenolics

In the last few decades, phenolic compounds have gained increasing interest by researchers throughout the world. More research on phenolics and especially on polyphenolics is being done regularly because of their health benefits and due to their relatively large daily intake in food, including cereals, legumes, pulses, fruits, and vegetables that are responsible for many bioactivities. These compounds are potent antioxidants in food and biological systems and are involved in enzyme deactivation, apoptosis of certain cancerous cells, DNA repair, cell damage prevention, LDL oxidation inhibition and many other associated effects. Thus, they reduce the risk of development of several diseases due to their antioxidant power, among other factors in the human body. Figure 2.1 shows how research on phenolics has intensified since 1980 to 2016 (Source: Scopus, January 2017).

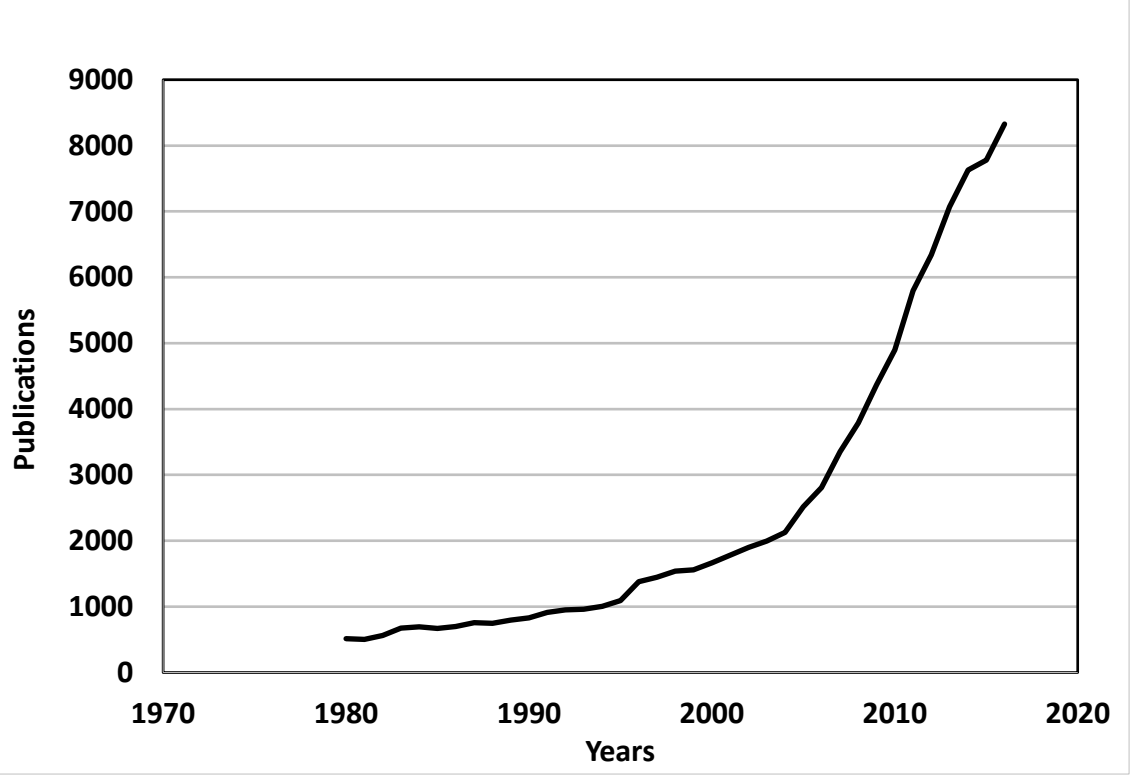



**Figure 2.1.** Number of publications with the keyword "phenolics" (Scopus, January 2017)

## 2.2 Occurrence of phenolics in plants

Plant metabolism and metabolites can be divided into primary and secondary. Generally, primary metabolism-originated compounds are mainly lipids, proteins, carbohydrates, and nucleic acids. These compounds are essential for plant to maintain its cell activity, among others (Giada 2013). On the other hand, secondary metabolism- originated substances such as phenolics, terpenoids, alkaloids and cyanogenic glycosides are produced from several biosynthetic pathways and play multiple functions in plant protection and human health (Giada 2013; Vickery & Vickery 1981). Among secondary metabolites, phenolic compounds are of the biggest and most widely distributed group of compounds in plants and are well studied for their antioxidant activity and other effects (Giada 2013; Scalbert & Williamson 2000).

Phenolics are synthesized by plants during normal development and are involved in response to stress conditions such as infection, wounding, and UV radiation, among others (Shahidi & Naczk 2003; Naczk & Shahidi 2004). In general, plant phenolics are derived from two aromatic amino acids, namely phenylalanine and tyrosine (Figure 2.2) through two metabolic pathways: the shikimic acid pathway, where, mainly, phenylpropanoids are formed and the acetic acid pathway in which the main products are the simple phenols (Shahidi & Naczk 2003; Naczk & Shahidi 2004; Giada 2013; Sánchez-Moreno 2002). Most plant phenolics are synthesized through the phenylpropanoid pathway (Giada 2013; Hollman 2001). The combination of both pathways leads to the formation of flavonoids, the most plentiful group of phenolic compounds in nature (Giada 2013; Sánchez-Moreno 2002). Additionally, condensation and polymerization processes lead to the formation of condensed tannins. Meanwhile, hydrolysable tannins are derivatives of gallic acid or hexahydroxydiphenic acid (Naczk & Shahidi 2004; Shahidi & Naczk 2003; Giada 2013; Stafford 1983).

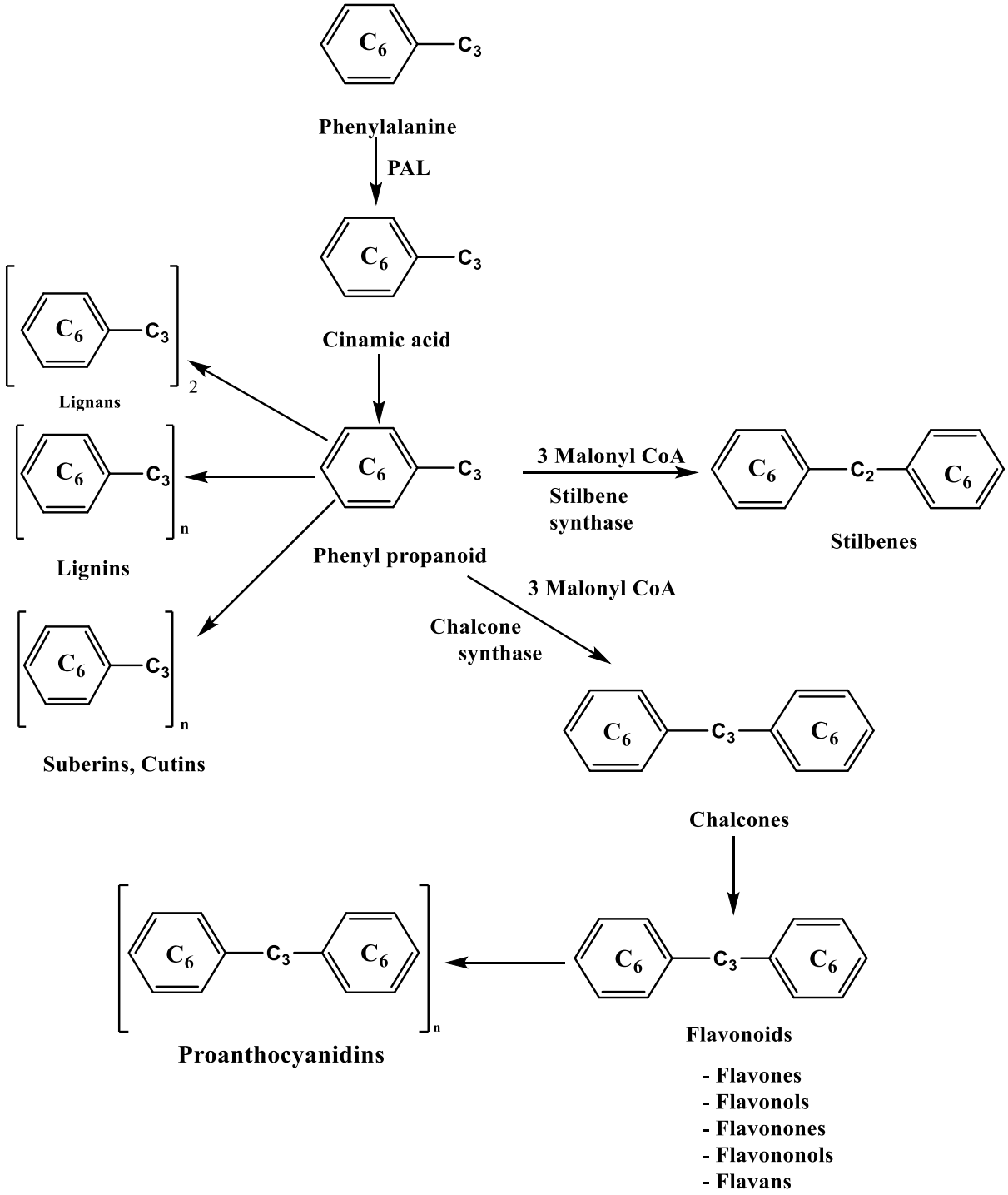



**Figure 2.2.** Synthesis of phenylpropanoids, stilbenes, lignans, lignins, flavonoids and tannins from phenylalanine through different enzymatic pathways. (Source: Naczk & Shahidi, 2004)

## 2.3 Classification and chemistry of phenolic compounds

Phenolic compounds are the major and most common group among the approximately 50,000 secondary plant metabolites (Grassmann et al. 2002). In plants, they are important constituents having several functions from overall fitness regulation to plant defence mechanism against insects, pathogens and extreme environmental conditions. As dietary phytochemicals for humans, phenolics exhibit a wide range of functional and biological activities. These activities depend on chemical structures of phenolic compounds. Phenolic compounds can be classified in different ways because they constitute many heterogeneous structures that range from simple molecules to highly polymerized compounds characterized by an aromatic ring with one or more hydroxyl groups. The aromatic ring (s) may also bear other functional substituents such as esters, methyl ethers and glycosides, and thus contributing to the great diversity of their structures. There are more than 8000 phenolic compounds identified in fruits, vegetables, seeds and related products. According to their distribution in nature, phenolic compounds in plants can be divided into two classes (Figure 2.3); simple phenolics which include various simple phenols, pyrocatechol, hydroquinone, and resorcinol, as well as aldehydes which are derived from benzoic acids that are components of essential oils, such as vanillin, and secondly complex phenolics which are divided into phenolic acids, such as hydroxybenzoic and cinnamic acid derivatives, flavonoids and their derivatives, coumarins, stilbenes, lignans and their polymerized counterparts, such as tannins and lignins.

As noted above, phenolics are generally classified into different groups. According to their location in the plant, phenolic compounds may also be classified as soluble

phenolics which include various simple phenols, flavonoids and tannins of low and medium molecular weight not bound to membrane compounds and insoluble-bound phenolics which are bound to cell wall polysaccharides and proteins to form insoluble-bound complexes. The soluble phenolic fraction includes both free and soluble conjugates, which are responsible for the *in vitro* antioxidant capacity of the extracts. On the other hand, phenolics in the insoluble-bound form are covalently bound to cell wall structural components (Acosta-Estrada et al. 2014; Wong 2006). They serve multiple functions in the cell wall by providing both physical and chemical barriers, protection against pathogen invasion and astringency that deters attack by insects and animals, antibacterial, antifungal and antioxidant functions (Acosta-Estrada et al. 2014; Liu 2007; Sancho et al. 2001). This classification is useful from the nutritional viewpoint, to the extent that their metabolic fate in the gastrointestinal tract and the physiological effects of each group will depend largely on their solubility characteristics. Insoluble-bound phenolic compounds are not digested, but may be partially fermented in the colon, and mostly or fully recovered in the feces, while a part of the soluble phenolics can cross the intestinal barrier and found in the blood, unchanged or as metabolites (Giada 2013; Sánchez-Moreno 2002). The antioxidant activity of food phenolic compounds is of nutritional interest, since it has been associated with the potentiation of the promotion of human health through prevention of several diseases (Lampe 1999).

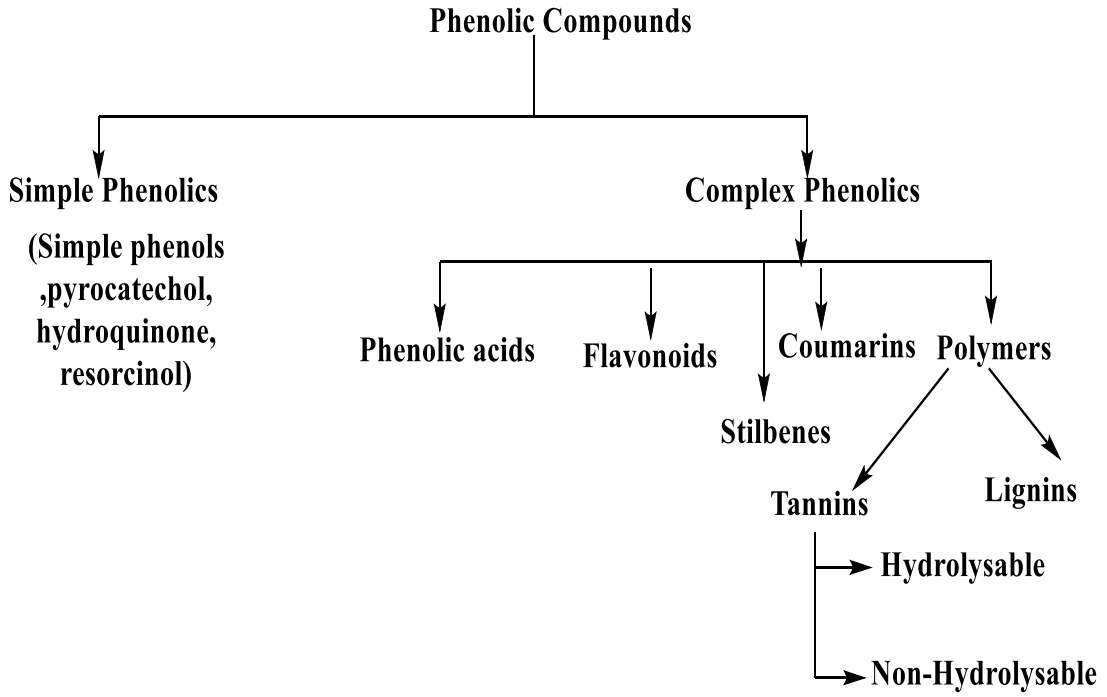



**Figure 2.3.** Classification of phenolic compound according to their distribution in plants

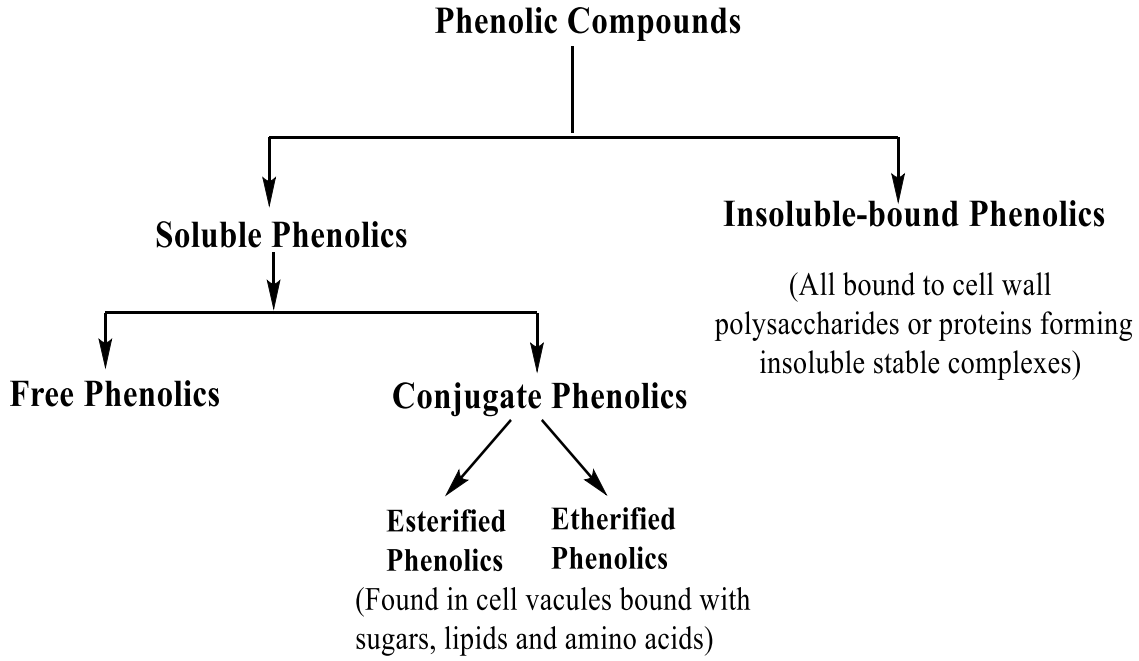
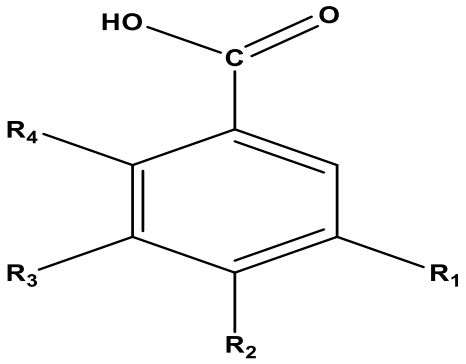



**Figure 2.4.** Classification of phenolic according to their location in plant foods

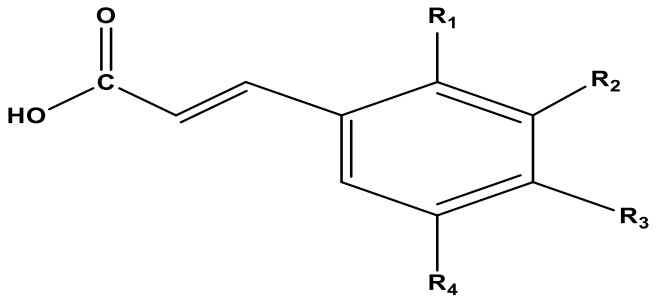
### 2.2.1 Phenolic acids and derivatives

Phenolic acids may constitute about one-third of the phenolic compounds in the human diet (Yang et al. 2001). Phenolic acids can be divided into two groups: benzoic acids and cinnamic acids and derivatives. Hydroxybenzoic acids have seven carbon atoms ($C_6$-$C_1$) and are the simplest phenolic acids found in nature. Cinnamic acids have nine carbon atoms ($C_6$-$C_3$). The general chemical formulas and names of the main benzoic and cinnamic acids are given in Figures 2.5 and 2.6, respectively. In the group of benzoic acids, most common phenolic acids are protocatechuic acid, vanillic acid, yringic acid, gentisic acid, salicylic acid, *p*-hydroxybenzoic acid and gallic acid (Sánchez-Moreno 2002). Among the cinnamic acids, *p*-coumaric, ferulic, caffeic and sinapic acid are most common in nature (Young et al. 2001). It has been documented that phenolic acids and their esters have high antioxidant activity, especially hydroxybenzoic and hydroxycinnamic acids and their derivatives such as chlorogenic acid, and although other characteristics also contribute to the antioxidant activity of phenolic acids and their esters, this activity is partly determined by the number of hydroxyl groups found in the molecules involved. In general, the hydroxylated cinnamic acids are more effective than their benzoic acids counterparts due to better radical scavenging activity arising from an additional resonance form possible for cimmanic acid derivatives (Shahidi & Naczk 1998; Sánchez-Moreno 2002).




1. Salicylic acid ($R_4$ = OH; $R_1$ = $R_2$ = $R_3$ = H)
2. Gallic Acid ($R_1$ = $R_2$ = $R_3$ = OH; $R_4$ = H)
3. Gentisic acid ($R_1$ = $R_3$ = OH; $R_2$ = $R_4$ = H)
4. *p*-Hydroxybenzoic acid ($R_2$ = OH; $R_1$ = $R_3$ = $R_4$ = H)
5. Vanillic acid ($R_1$ = $OCH_3$; $R_2$ = OH; $R_3$ = $R_4$ = H)
6. Protocatechuic acid ($R_1$ = $R_2$ = OH; $R_3$ = $R_4$ = H)
7. Syringic acid ($R_1$ = $R_3$ = $OCH_3$; $R_2$ = OH; $R_4$ = H)

**Figure 2.5.** The basic formula and names of the main benzoic acids (Source: Giada 2013)




1. *o*-Coumaric acid ($R_1$ = OH; $R_2$ = $R_3$ = $R_4$ = H)
2. *m*-Coumaric acid ($R_2$ = OH; $R_1$ = $R_3$ = $R_4$ = H)
3. *p*-Coumaric acid ($R_3$ = OH; $R_1$ = $R_2$ = $R_4$ = H)
4. Caffeic acid ($R_2$ = $R_3$ = OH; $R_1$ = $R_4$ = H)
5. Ferulic acid ($R_2$ = $OCH_3$; $R_3$ = OH; $R_1$ = $R_4$ = H)
6. Sinapic acid ($R_2$ = $R_4$ = $OCH_3$; $R_3$ = OH; $R_1$ = H)

**Figure 2. 6.** The basic formulas and names of the main cinnamic acids (Source: Giada 2013)

### 2.2.2 Flavonoids and derivatives

Flavonoids are important constituents of the human diet and are the most widely distributed and studied phenolic compounds in plant foods (Bravo 1998). They are most potent antioxidants from plants with excellent activity which is related to the presence of hydroxyl groups in positions 3' and 4' of the B ring, which confer high stability to the formed radical by participating in the displacement of the electron, and a double bond between carbons $C_2$ and $C_3$ of ring C together with the carbonyl group at the $C_4$ position, which makes the displacement of an electron possible from ring B. Additionally, free hydroxyl groups in position 3 of ring C and in position 5 of ring A, together with the carbonyl group in position 4, are also important for the antioxidant activity of these compounds (Sánchez-Moreno 2002). However, the effectiveness of flavonoids decreases with the substitution of hydroxyl groups with sugars, the glycosides so formed being less antioxidantive than their corresponding aglycones (Rice-Evans 1996).

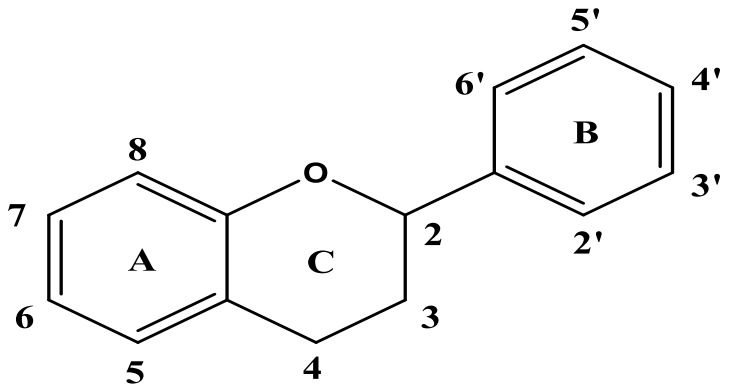



**Figure 2.7: Basic structure of flavonoids**

According to the degree of hydroxylation and the presence of a $C_2$-$C_3$ double bond in the heterocyclic pyrone ring, various flavonoids can be found in plants. Most common flavonoids are represented by flavonols, flavanols, flavones, isoflavones, flavan-3-ol

anthocyanidins or anthocyanins and flavanones which are structurally different according to the degree of hydrogenation and hydroxylation of the three ring systems involved with various functions in plants. Flavonoids also occur as sulphated and methylated derivatives, conjugated with monosaccharides and disaccharides and forming complexes with oligosaccharides, lipids, amines, carboxylic acids and organic acids, that constitute approximately 8000 compounds (Duthie et al. 2003). While certain classes of flavonoids (e.g. flavonones) are colourless, the others (e.g. anthocyanins) are always coloured, such as flower pigments and other plant parts (Harborne 1980). The basic chemical structures of the main classes of flavonoids are presented in Figure 2.8.

**Table 2.1**. Common sources of flavonoids and their derivatives

| Flavonoids | Flavonoids derivatives | Major sources |
|---|---|---|
| Flavonol | Quercetin, Rutin, Myricetin, Kaempferol | Tea, Red wine, Tomato, Apple, Cherry, and Onion |
| Flavanols | Catechin, Epicatechin, Gallocatechin | Tea and Apple |
| Flavones | Apigenin, Luteonin, Chrysin | Thyme and Parsley |
| Isoflavones | Genistein, Glycitein, | Soya bean and other legumes |
| Flavanones | Hesperidin, Narigenin | Grape fruit and Orange |
| Flavanonols | Taxifolin, Engeletin, Astilbin | White grape skin, Lemon and Sour orange |

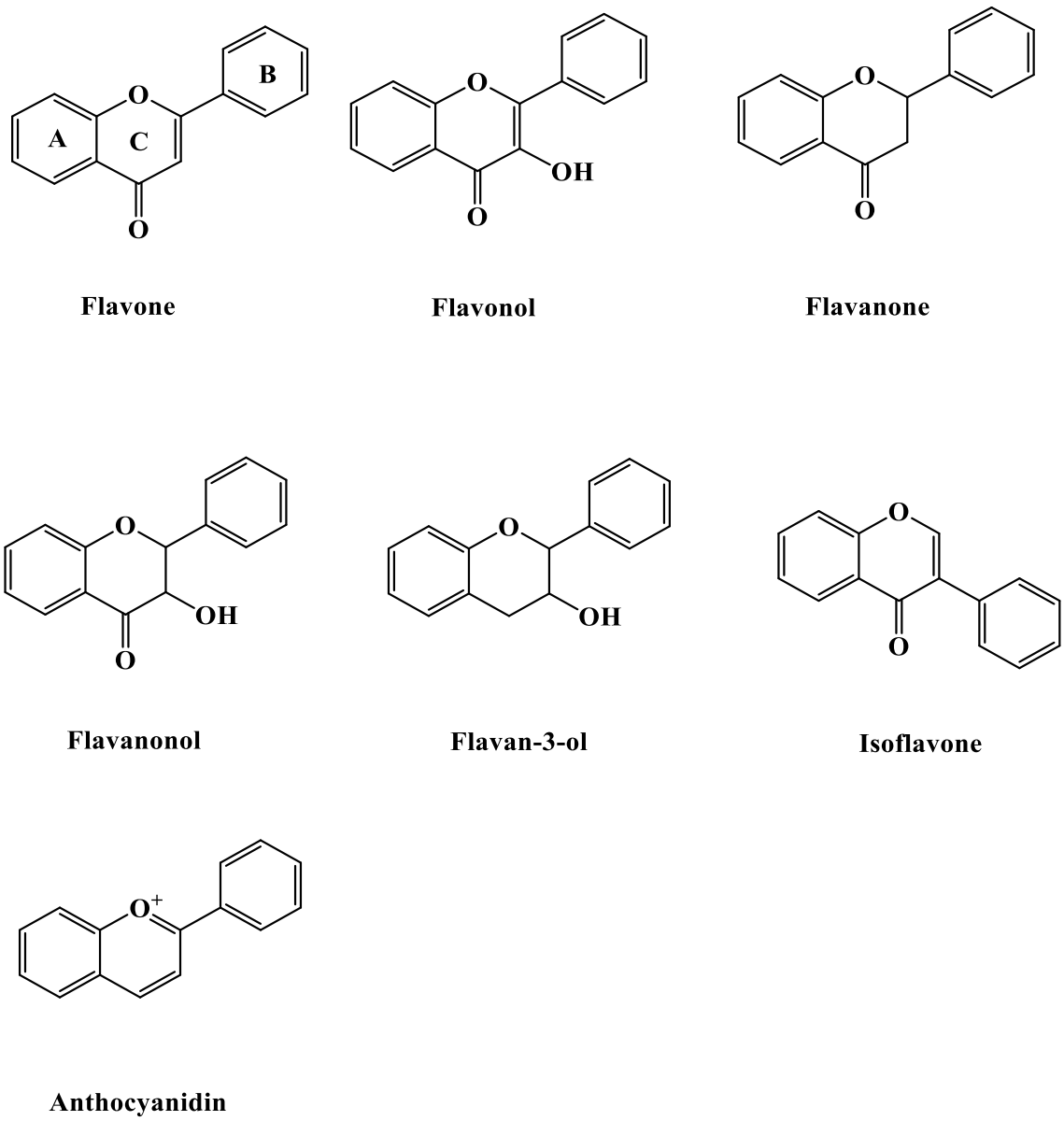



**Figure 2.8. Backbone chemical structures of the main classes of flavonoids.**

### 2.2.3 Tannins

Tannins are phenolic compounds with intermediate to high molecular weights (500-3000 Da) (Giada 2013; Sánchez-Moreno 2002) and classified into two major groups: hydrolysable tannins and non-hydrolysable or condensed tannins, also known as proanthocyanidins (Chung 1998). The hydrolysable tannins have a central glucose or a polyhydric alcohol partially or completely esterified with gallic acid or hexahydroxydiphenic acid, forming gallotannins and ellagitannins, respectively (Okuda et al. 1995). These metabolites are readily hydrolyzed with acids, bases or enzymes. However, they may also be oxidatively condensed to other galoyl and hexahydroxydiphenic molecules and form polymers of high molecular weight. The best known hydrolysable tannin is tannic acid, which is a gallotannin consisting of a pentagalloyl glucose molecule that can additionally be esterified with another five units of gallic acid (Bravo 1998). The condensed tannins are polymers of catechin and/or leucoanthocyanidin, not readily hydrolyzed by acid treatment, and constitute the main phenolic fraction responsible for the characteristics of astringency of foods (Giada 2013). Although the term condensed tannins is still widely used, the chemically more descriptive term "proanthocyanidins" has gained more acceptance. These substances are polymeric flavonoids. The proanthocyanidins most widely studied are based on flavan-3-ols (-)-epicatechin and (+)-catechin (Stafford 1983). The chemical structures of hydrolysable tannin and proanthocyanidins (nonhydrolyzable or condensed tannins) are shown in Figure 2.9.

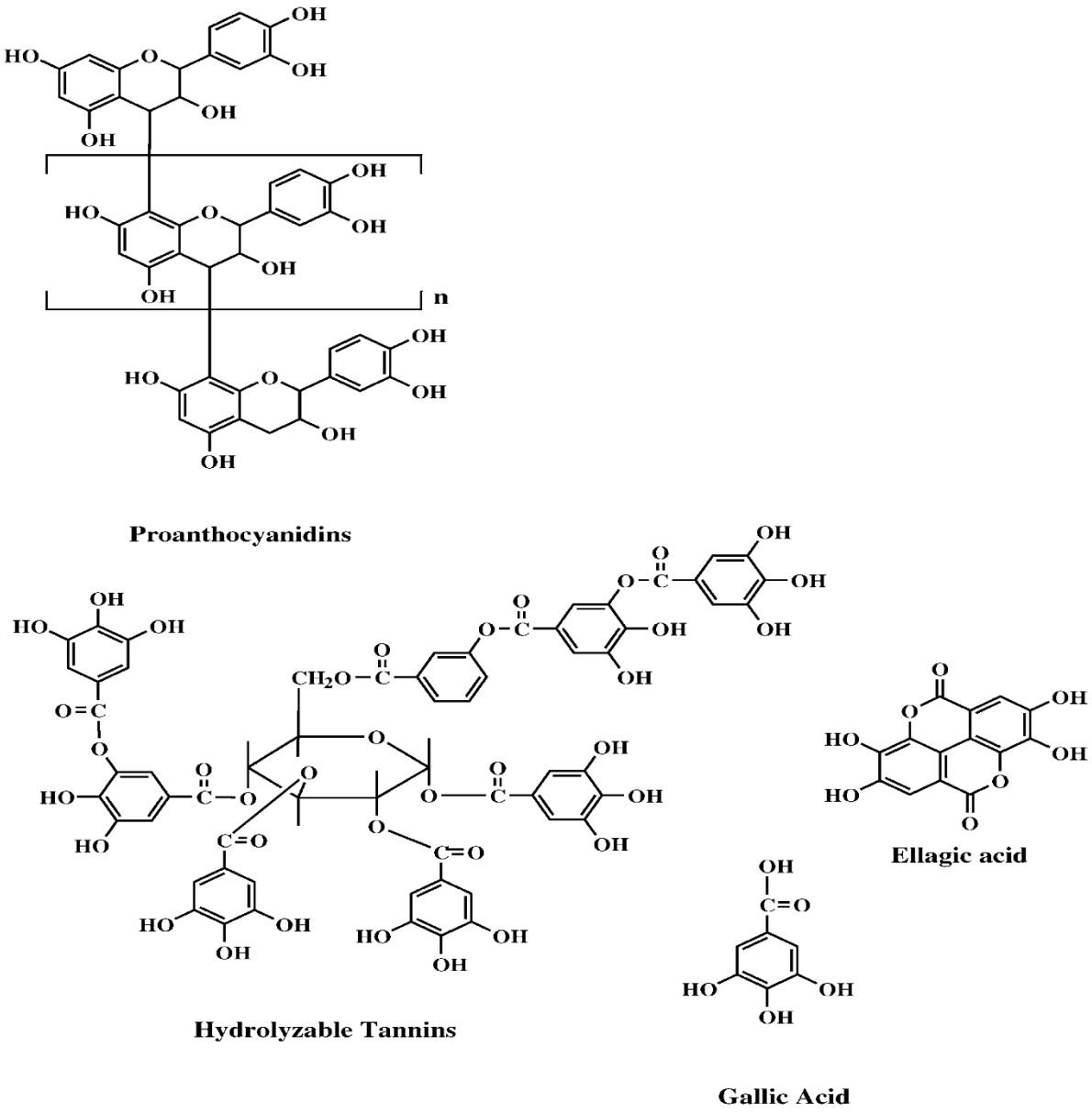



**Figure 2.9.** Chemical structures of hydrolysable tannin and proanthocyanidins (nonhydrolyzable or condensed tannins). (Source: Naczk & Shahidi, 2004)

## 2.4 Sources, extraction methods and analysis of phenolics

Phenolics are present abundantly in plant sources and their content may vary depending on the species and cultivar as well as environmental and agronomic conditions. The most common natural sources of phenolics and polyphenolics include fruits, vegetables, legumes, cereals, oilseeds, nuts, herbs and spices, among others. Fruits are rich sources of phenolic compounds and their antioxidant and biological activity *in vitro* systems has been well documented. Berries, grapes, apples, citrus, and pomegranates are among the common fruits available globally and serve as good sources of phenolics, especially flavonols (e.g. quercetin, kaempferol, myricetin and isorhamenetin), proanthocyanidins (e.g. procyanidins and prodelphinidins) and phenolic acids (mostly in esterified form, e.g. sinapic, gallic, ferulic, coumaric, caffeic and chlorogenic acids) (Zhong 2010). Stilbenes are predominant phenolics present in grape skin, leaves, seeds and stems as monomeric, oligomeric and polymeric forms. Resveratrol is the predominant stilbene found in grape skin as well as in wilting berries (Versari et al. 2001). Pomegranates are rich in hydrolysable tannins, particularly the gallagyl type tannins (e.g. punicalagin), its content is in the range of 150-190 mg/L juice (Gil et al. 2000).

Vegetables are a rich source of phenolics and polyphenols. The content and composition of phenolics in various groups of vegetables have been reviewed (Shahidi & Ambigaipalan 2015; Shahidi et al. 2010). Onions are a rich source of flavonoids of which quercetin is the most predominant one (Galdon et al. 2008). Roots (carrots, beets) and tubers (sweet potatoes, potatoes) are good sources of chlorogenic and caffeic acids while betalains contribute to the colour of beets. Green leafy vegetables such as lettuce, spinach and kale contain high levels of flavonoids at 0.80 - 2.241 mg/g fresh weight

(Howard et al. 2002). Phenolics are also found in flowers (broccoli and artichoke) and stems (asparagus) of vegetables at varying levels and compositions.

Cereals, legumes, oilseeds and nuts are recognized as good sources of phenolics with high amounts of phenolic acids and flavonoids that present in the aleurone layer of grains and seeds. In beans, a higher level of phenolics was detected in the hulls (6.7-27.0 mg catechin equivalents/g extracts) than in whole seeds (4.9-9.36 mg/g extracts) (Madhujith & Shahidi 2005). Major phenolic acids present in bean hulls include vanillic, caffeic, *p*-coumaric, ferulic and sinapic acids. These phenolic acids were also found in wheat bran at higher levels compared to its corresponding flour (Liyana-Pathirana & Shahidi 2007). Oilseeds are a potential source of phenolic acids and flavonoids. The major phenolic compounds present in oilseed are various phenolic acids, coumarins, flavonoids, tannins and lignins. In the family of brassica oilseeds, sinapic acid is the dominant phenolic acid.

Several extraction methods have been employed for the extraction of plant phenolics. The solvent extraction for phenolic compounds includes solid–liquid extraction (SLE), and liquid–liquid extraction, among others. Solvent extraction technique was mainly used in a laboratory scale (Kartsova & Alekseeva 2008). This technique has several drawbacks like use of a high volume of solvents, low selectivity, low extraction efficiency, long extraction time, solvent residue, and environmental pollution. Many novel extraction techniques have been developed and applied for the extraction of phenolic compounds without loss of their activity such as supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE). These techniques are

characterized by higher extraction yield, shorter extraction time, and final extract obtained in a solvent-free environment as a concentrate of biologically active compounds (Michalak & Chojnacka 2015; Kadam et al. 2013; Ibanez et al. 2012; Jeon et al. 2012). However, among all novel techniques, SFE method is preferred in the food and pharmaceutical industries because of minimal or no use of organic solvents, faster extraction rate and high yield without loss of activity of bioactive compounds (Michalak & Chojnacka 2015; Kadam et al. 2013; Ibanez et al. 2012). In addition to extraction from natural plant sources, some high-value phenolic compounds are also prepared by chemical or enzymatic synthesis and plant cell cultures as well as biosynthesis by microorganisms. Separation of phenolics may be necessary when one or more specific compounds are of interest in various plants/food materials and biological fluids (e.g. urine, plasma, blood serum, saliva). Techniques such as HPLC, LC-MS, LC-MS/MS and TLC, and electrophoresis such as capillary zone electrophoresis and micellar electrokinetic chromatography are among the main physicochemical methods for separation of phenolics (Zhong 2010; Kartsova & Alekseeva 2008).

## 2.5 Phenolic compounds as antioxidants

### 2.5.1 Lipid oxidation

Lipid oxidation is a major cause of food quality deterioration and also has negative effects in biological systems. The oxidation of foods may occur during harvesting and upon processing and storage. The oxidation process has several effects in foods such as development of off-odours and off-flavours, loss of essential fatty acids, fat soluble vitamins and other bioactives, and even formation of potentially toxic compounds (Zhong 2010; Shahidi 1994), thus decreasing shelf-life and nutirion of foods as well as

altering their texture and colour (Albishi 2012; Alamed et al. 2009). *In vivo* biological systems, oxidation has adverse cellular effects and may cause various diseases and health conditions including, atherosclerosis, inflammation, cancer and aging, among others (Kruidenier & Verspaget 2002; Floyd & Hensley 2002; Davies 2000; Dalton et al. 1999).

Lipid oxidation of foods has been well studied as it relates to nutritional and sensory quality of food and food products. Lipids are susceptible to oxidation because of their fatty acid composition, processing and storage conditions as well as presence of endogenous and exogenous antioxidants. Lipid oxidation is quite a complex process, which includes autoxidation, photooxidation, thermal and enzymatic oxidation (Shahidi 2000; Vercellotti et al. 1992). The unsaturated fatty acids lose a hydrogen atom and produce free radicals in the presence of initiators and the reaction can be catalyzed by light, heat, transition metal ions ($Cu^{2+}$, $Fe^{2+}$ etc.), haemoproteins, metalloproteins and cellular enzymes such as lipoxygenase. These lipid radicals subsequently react with oxygen and form peroxyl radicals, which act as the chain carriers of the rapidly progressing reaction by attacking new lipid molecules. This self propagating and self accelerating reaction may be repeated many times until no hydrogen source is available upon which radicals meet each other and the termination process, or the chain is interrupted by antioxidants or other means (Zhong 2010; de Man 1999).

Autoxidation is one of primary pathways that degrades lipids in food. It occurs via a free radical mechanism in which atmospheric oxygen is added to the unsaturated fatty acid chains of lipid molecules. The reaction can be catalyzed by various initiators as mentioned above. Autoxidation with the three aforementioned steps of initiation,

propagation and termination, leads to a series of complex chemical changes (Shahidi & Zhong 2005; Shahidi & Wanasundara 1992). A simplified scheme explaining the mechanism of autoxidation is given in Figure 2.10.

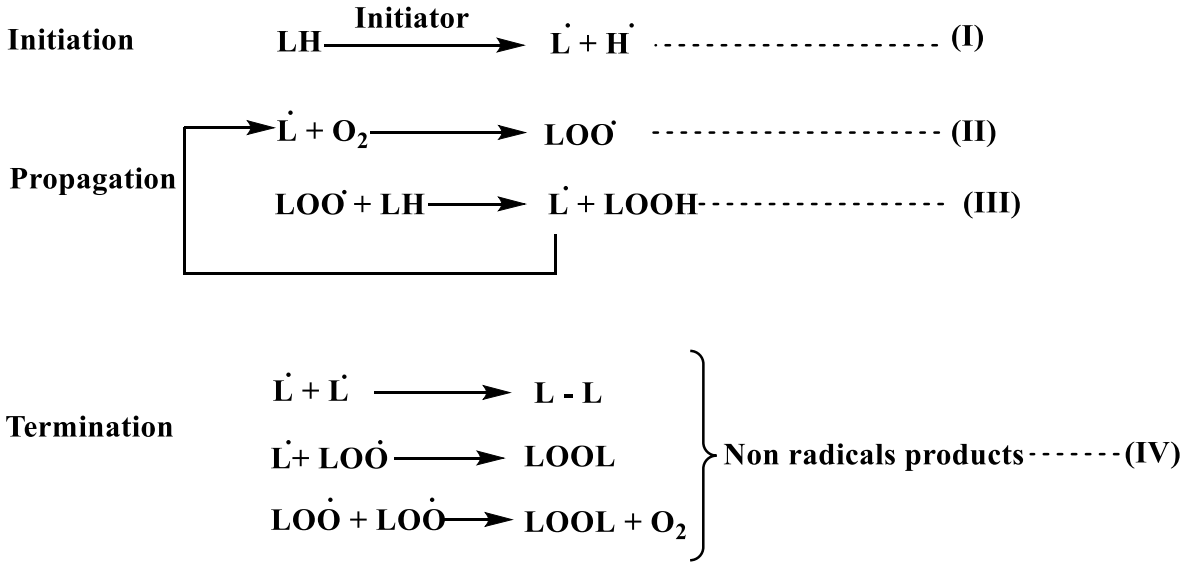



**Figure 2.10. Simple schematic pathways of lipid autoxidation reaction mechanism**

Oxidation in lipid-containing foods proceeds very slowly at the initial stage until crosses the induction period after which a sudden increase occurs. This initiation process (I) is quite complex and involves removing of a hydrogen atom from the lipid molecule (LH) to form a lipid radical ($L^{\cdot}$). Conjugated dienes and trienes are formed because of the rearrangement of the methylene interrupted double bonds in polyunsaturated fatty acids (PUFA). These conjugated dienes and trienes are good indicators of lipid oxidation (Shahidi & Zhong 2005). During propagation (II), the highly reactive alkyl radical of unsaturated fatty acids ($L^{\cdot}$) can react with atmospheric oxygen and form peroxyl radical ($LOO^{\cdot}$) or abstract a hydrogen atom from another lipid molecule (III) and form hydroperoxides (LOOH). These hydroperoxides are primary products of oxidation. Hydroperoxides are unstable and break down to a wide range of secondary oxidation

products, including aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids and epoxy compounds, among others, some of which have undesirable odours with very low threshold values. Meanwhile, alkoxyl ($LO^{\cdot}$), peroxyl ($LOO^{\cdot}$), hydroxyl ($^{\cdot}OH$) and new lipid radicals ($L^{\cdot}$) are generated from the decomposition of hydroperoxides, and further participate in the chain reaction of free radicals. In the termination stage of oxidation (IV), radicals neutralize each other through radical-radical coupling or radical-radical disproportionation to form stable non-radical products, including a variety of polymeric compounds (Zhong 2010; Erickson 2002).

### 2.5.2 Mechanism of antioxidant action of phenolic compounds

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Sang et al. 2002; Velioglu et al. 1997). Antioxidants have been used globally by food manufacturers for stabilizing food lipids. When added to foods, antioxidants reduce deteriorative processes and rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Sang et al. 2002; Jadhav et al. 1995). In the health-related areas antioxidants are used for health promotion due to their ability to protect the body against oxidative damage. They may be broadly classified based on their mode of action into primary antioxidants which break the chain reaction of oxidation by scavenging free radical intermediates, and secondary antioxidants, which prevent or retard oxidation by deactivation of oxidation initiators/accelerators or regeneration of primary antioxidants. Phenonic compounds and their derivatives can act as primary and, depending on their chemical structure, as secondary antioxidants due to their redox properties, which can play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides and other reactive

oxygen species (ROS), metal ion chelators, quenchers of secondary oxidation products, and inhibitors of prooxidative enzymes, among others (Shahidi & Zhong 2007; Sang et al. 2002; Osawa et al. 1995). Basically, the antioxidant action of phenolic compounds depends on the number and arrangement of the hydroxyl groups in the molecules of interest (Cao et al. 1997; Sang et al. 2002), among other factors. Phenolic compounds (AH) can donate hydrogen atoms to lipid radicals and produce lipid derivatives and antioxidant radicals (Reaction I), which are more stable and less readily available to promote autoxidation (Kiokias et al. 2008; Shahidi et al. 1992). The antioxidant free radical may further interfere with the chain-propagation reactions (Reactions II and III).

$$L^{\cdot}/LO^{\cdot}/LOO^{\cdot} + AH \longrightarrow A^{\cdot} + LH/LOH/LOOH \quad ----(I)$$

$$LO^{\cdot}/LOO^{\cdot} + A^{\cdot} \longrightarrow LOA/LOOA \quad ----------(II)$$

$$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot} \quad ----------(III)$$

**Figure 2.11. Antioxidant action of phenolic compounds**

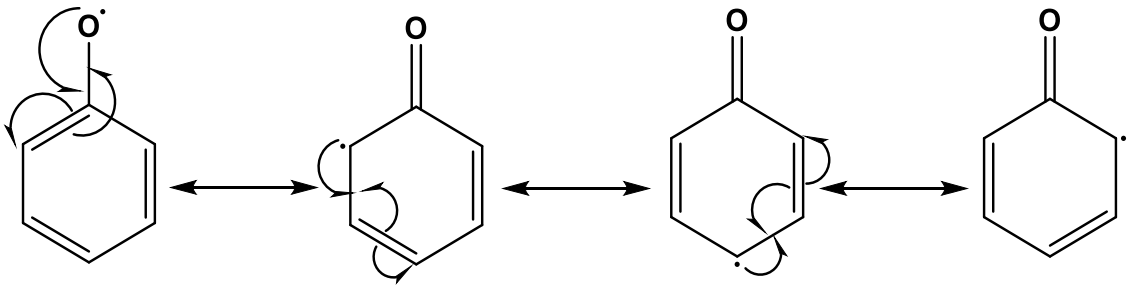



**Figure 2.12.** Resonance stabilization of phenoxyl radical

The resultant phenolic radicals are stabilized by delocalization of the unpaired electron around the phenol ring to form a stable resonance hybrid (Reische et al. 2002). These radicals have low reactivity and generally do not initiate the formation of new radicals, thus breaking the chain-reaction of free radical propagation (Nawar 1996). Moreover,

the phenolic radicals so formed can further scavenge free radicals by participating in the termination of oxidation. Therefore, phenolic antioxidants can trap two lipid radicals by donating a hydrogen atom to one radical and receiving an electron from another radical to form stable non-radical products (Young & Woodside 1999). Phenolic compounds may also act as secondary antioxidants that prevent or retard oxidation by suppressing the oxidation promoters, including metal ions, singlet oxygen, prooxidative enzymes and other oxidants. Phenolics, as reducing agents, can reduce lipid peroxides and related oxidants through redox reactions, and are also referred to as oxygen scavengers. Metal ions act as catalysts of oxidation reaction by producing free radicals through electron transfer (as shown below), but may be chelated by some polyphenols, hence being deactivated.

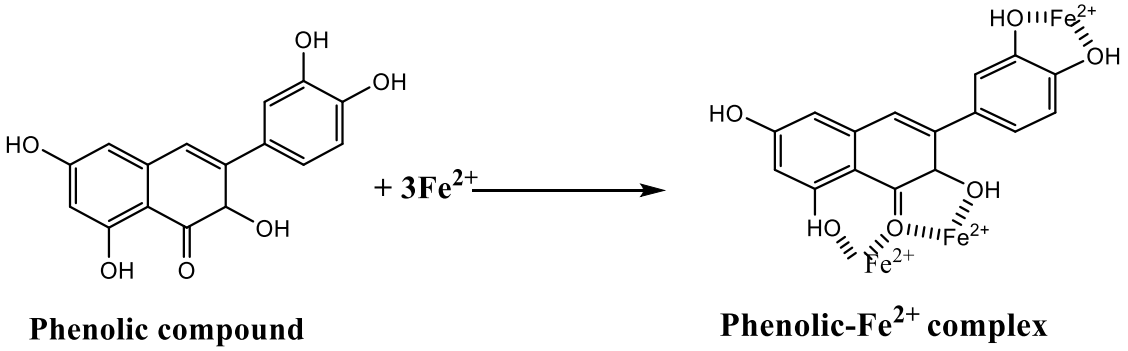



**Figure 2.13.** Metal chelation mechanism of phenolic compounds

### 2.6 Health benefits and bioavailability of phenolic compounds

Regular consumption of fruits, vegetables, legumes and various edible oilseeds may lower the risk of many diseases, including inflammation, cardiovascular disease (CVD), cancer, diabetes and neurodegenerative diseases. Many of the *in vitro* and *in vivo*

studies have shown that phenolics and polyphenolics possess antioxidant, anti-inflammatory, antiatherogenic, anticarcinogenic, antidiabetic, anti-allergic, antimicrobial and antiviral activities, among others. The mechanisms of these biological activities of phenolics and their related health effects have been reviewed (Zhong 2010; Aron & Kennedy 2008; Scalbert et al. 2005). Fruits, vegetables and various edible seeds are good sources of hydroxycinnamic acid conjugates and flavonoids. These phenolic compounds show a wide range of antioxidant activities *in vitro* (Shahidi & Ambigaipalan 2015; Rice-Evans et al. 1995) and are believed to exert protective effects against major diseases such as cancer and cardiovascular diseases (Shahidi & Ambigaipalan 2015; Boudet 2007). The health benefits of dietary phenolic compounds and flavonoids depend on the bioavailability of the individual compound during metabolism in the body. Increasing evidence shows that hydroxycinnamic acid derivatives and flavonoids can be absorbed into the human body in amounts that are, in principle, sufficient to exert antioxidant or other biological activities *in vivo* (Shahidi & Ambigaipalan 2015; Olthof et al. 2001; Scalbert & Williamson 2000). Dietary polyphenols are substrates for β-glucosidases, UDP-glucuronosyltransferase, or catechol-*O*-methyltransferase in the small intestine. Polyphenols taken from dietary sources are hydrolysed and degraded in the colon because of the activity of enzymes of the colonic microflora and show various bioactivities (Shahidi & Ambigaipalan 2015; Booth et al. 1957). Rechner et al. (2002) found that intact conjugated polyphenols are present at much lower levels than their degradation products due to the hydrolysis by colonic bacterial enzymes during metabolism in the liver. Grape anthocyanidins were found to be effective in preventing stomach mucosal injury induced by acidified ethanol, and their antiulcer property was thought to be due to both antioxidant activity and proteins binding ability (Saito et al.

1998). It has been reported that flavonoid intake from fruits and vegetables was inversely associated with all cause cancer risk and cancer of the alimentary and respiratory tract (Hertog et al. 1994). Quercetin was reported to show vasoactive and gastroprotective effects, as well as inhibition against heterocyclic amine (HCA)-induced mutagenesis (Alarcon 1994; Kahraman et al. 2003). Proanthocyanidin $A_2$ treatment effectively modulated expression of antioxidant enzymes and decreased UVB-induced skin tumours (Pan & Ho 2008). Isoflavones in soybean exhibit estrogenic activities and may protect against hormone-related cancer and cardiovascular diseases (Adlercreutz & Mazur 1997; Lichtenstein 1998). Recent research findings indicate that tea polyphenols can protect against different stages of carcinogenesis (Khan & Mukhtar 2010). EGCG (epigallocatechin-3-gallate), the main catechin in green tea, serves as a cancer chemopreventive agent (lungs, liver, gastrointestinal tract, skin and prostate cancer), as well as anti-obesity and cardiovascular protective compound (Khan & Mukhtar 2010; Klaus et al. 2005; Yang & Wang 1993). The antioxidant activity and beneficial health effects of EGCG as the main polyphenol of green tea was enhanced upon conjugation with docosahexaenoic acid (DHA) and the tetra ester so formed was able to arrest colon cancer effectively (Zhong, Chiou, Pan, Ho, & Shahidi 2012). Other bioactivities of phenolics include antiviral, anti-allergic, antidiabetic and analgesic properties, among others (Musci 1986; Nguyen et al. 1999; Hossain et al. 2008).

### 2.7 Phenolics and polyphenolics of camelina seeds

Camelina is an ancient oilseed crop. It has many vernacular names such as false flax and gold of pleasure (English), lendotter (German), and dorella (Italian) (Hrastar et al. 2009). It belongs to the cruciferae family (Brassicaceae), which includes mustard, canola,

rapeseed, crambe, broccoli, cabbage, cauliflower and several other vegetable and oilseed crops (Hrastar et al. 2009; Grady & Nleya 2010). It is a plant native to Northern Europe and Southeast Asia where it has been grown for at least 3,000 years. As an agricultural crop, camelina was grown in Europe and the former Soviet Union through World War II (Grady & Nleya 2010). Camelina is a new promising crop in Canada. It is widely cultivated in Canada and USA. In Montana (USA), camelina has been grown for the last several years on a commercial scale. The National Agricultural Statistics Service office reported 22,500 acres of camelina planted in 2007 and 12,200 acres in 2008 in Montana. Camelina is a cool-season crop. Plants are 2–3-feet tall at maturity. Seedpods are pear shaped and contain 8–10 seeds. The seeds are reddish-brown in colour and very small (less than 1/16 inch). Camelina is more resistant to seed shatter than canola (Grady & Nleya 2010).

The main product of camelina is its oil. The seeds of camelina contain around 30-40% oil on a dry weight basis. Usually, the oil is produced from seeds by crushing and warm pressing. The oil produced from the seeds is partly used as an edible oil, but most of it is used as a traditional home remedy, where it is thought to be useful for the treatment of stomach and duodenal ulcers, or applied topically for the treatment of burns, wounds and eye inflammations (Terpinc et al. 2012). The oil is a good source of essential and highly unsaturated fatty acids. It contains a high amount of oleic acid C18:1n-9 (15-20%), linoleic acid C18:2n-6 (15-20%), omega-3 (ω3) α-linolenic acid C18:3n-3 (30-40%), eicosenoic acid C20:1n-9 (15-20%), low content of erucic acid C22:1n-9 (about 3%), and high content of tocopherols (700 mg/kg) and phenolic compounds (128 mg/kg as chlorogenic acid), making it more stable toward oxidation than highly unsaturated linseed oil (Hrastar et al. 2009; Zubr & Matthäus 2002; Budin et al. 1995; Abramovič et

al. 2007). The high contents of ALA, tocopherols and other antioxidants make camelina oil nutritionally very attractive. During metabolism, α-linolenic acid is converted to some extent to the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) in the body (Kirkhus et al. 2013; Barceló-Coblijn & Murphy 2009). It has been reported that the intake of camelina oil compared to rapeseed oil gives significantly higher serum concentrations of ALA, EPA, and DHA, as well as a decrease in serum cholesterol in hypercholesterolaemic subjects (Kirkhus et al. 2013; Karvonen et al. 2002). The health benefits of EPA and DHA are well documented, including their protective effects on cardiovascular disease and autoimmune and mental disorders (Kirkhus et al. 2013; Calder 2006; McCann & Ames 2005; Mozaffarian 2008), but there is also a growing body of scientific data supporting the idea that 18:3 may exert beneficial effects by mechanisms other than simply acting as a precursor for EPA and DHA (Kirkhus et al. 2013; Boelsma 2001; Djoussé et al. 2005; Guizy et al. 2008; Nelson et al., 2007; Zatonski et al. 2008; Zhao et al. 2007). Camelina oil also contains phytosterols, which are known to have a cholesterol-lowering effect (Katan et al. 2003; Miettinen et al. 1995) and natural antioxidants such as tocopherols (vitamin E). Camelina oil is particularly rich in γ-tocopherol (Schwartz et al. 2008), making it very resistant to oxidation (Ehrensing & Guy 2008; Szterk et al. 2010). The consumption of camelina oil can help improving the general health of the population to desired levels (Waraich et al. 2013; Zubr 1997; Rokka et al. 2002; Lu and Kang 2008). Camelina oil is helpful in the regeneration of cells, skin elasticity and slenderness recovery (Waraich et al. 2013; Vollmann et al. 1996**).**

Camelina meal, obtained after oil extraction from the seeds typically contains 10–12% oil and 40% protein. It may be used to enhance the food quality of fish, meat, poultry, and

dairy products (Grady & Nleya 2010). The oilseed from *Camelina sativa* is of interest from an aquaculture perspective. Camelina meal is used as aquaculture feed. Hixson, Parrish and Anderson (2014) conducted a study on the use of camelina oil in the diet of farmed salmonids and Atlantic cod. They found significant omega-3 enrichment in fish tissue fatty acid profile including fish growth development. Camelina meal may also be used to produce omega-3 enriched meat, milk, and eggs. The US Food and Drug Administration (FDA) allows the use of camelina meal for up to 10% by weight of the total dietary ration fed to poultry broilers and has limited approval in Montana for up to 2% by weight of the total ratio fed to feed lot beef cattle and growing swine (Grady & Nleya 2010). However, the meal contains anti-nutritive compounds (glucosinolates) that can reduce livestock performance at high concentrations. Research has been conducted on the impact of higher levels of camelina on livestock performance and product quality (Grady & Nleya 2010).

The distribution of phenolics in plants at the tissue, cellular and subcellular levels is not uniform. The seeds of oil crops, particularly those with high contents of PUFA, provide an important source of antioxidants (Terpinc et al. 2012). The residue obtained after oil extraction from the seed is known as the cake or meal. This protein-rich by-product is currently used mainly for animal feed and as fertilizer. Recently oil cakes have become an attractive source to produce industrial enzymes, antibiotics, bio-pesticides, vitamins and other biochemicals (Ramachandran et al. 2007). Similarly, Matthäus (2002) reported that camelina cake contains a remarkable amount of bioactive substances such as glucosinolates, vitamins, and antioxidants.

Terpinc and Abramovič (2016) conducted a study on phenolic compounds, their occurrence and identification in the residues after pressing of the oil from camelina seeds of Slovenian origin, i.e. oilcake reported that almost all seed phenolics ended up in the oilcake. The major phenolic compounds were sinapine, 4-vinylphenol, 4-vinylguaiacol, 4-vinylsyringol, 4-vinylcatechol, ellagic acid, protocatechuic acid, 4-hydroxybenzoic acid, sinapic acid, salicylic acid, catechin, quercetin and quercetin glucoside. They also reported that the oilcake had high reducing power and radical scavenging activity. In the same study, heat treatment of seeds affected the amount of free, soluble and insoluble-bound phenolic compounds as well as antioxidant capacity of individual fractions. Terpinc et al. (2012) conducted a study on "The occurrence and characterisation of phenolic compounds in *Camelina sativa* seed, cake and oil ". They found that camelina seeds and its cake possess a similar phenolic profile which included ellagic acid, protocatechuic acid, *p*-hydroxybenzoic acid, sinapic acid, salicylic acid, catechin, rutin, quercetin and quercetin glucoside (Figures 2.13 & 2.14). Camelina cake showed higher reducing power and free radical scavenging activity, whereas camelina oil, with a relatively low phenolic content, exhibited a higher iron-chelating capacity and inhibitory effects against β-carotene discoloration in an emulsified system in the same study.

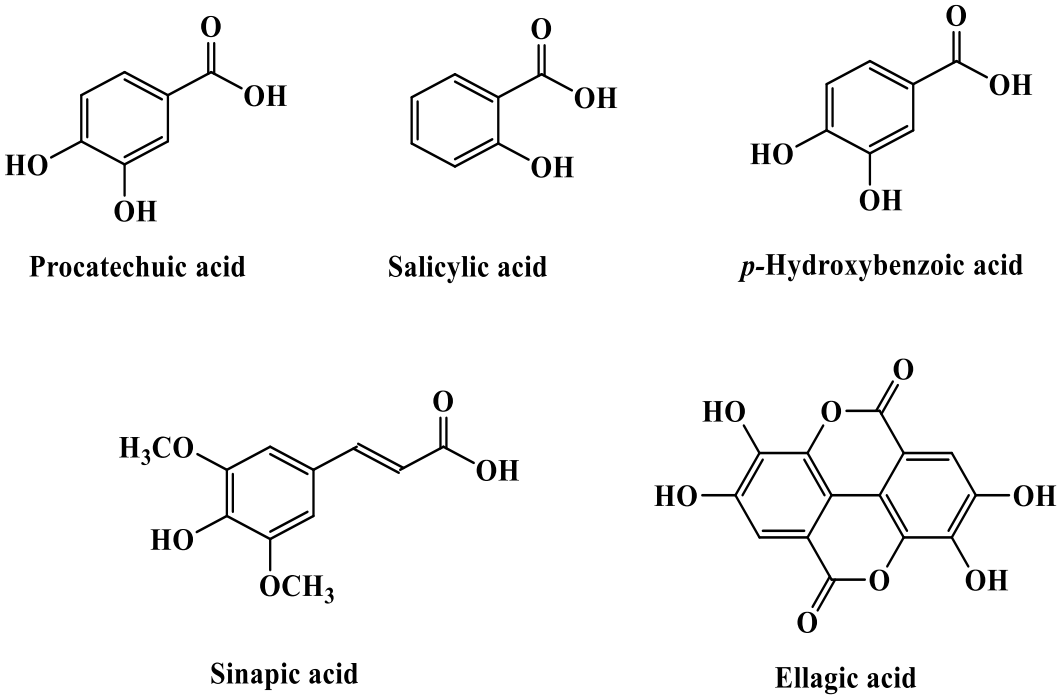



**Figure 2.14.** Chemical structures of Identified phenolic acids in camelina whole seeds and cake by LC- $MS^2$ (Name of compounds adopted from Terpinc et al. 2012)

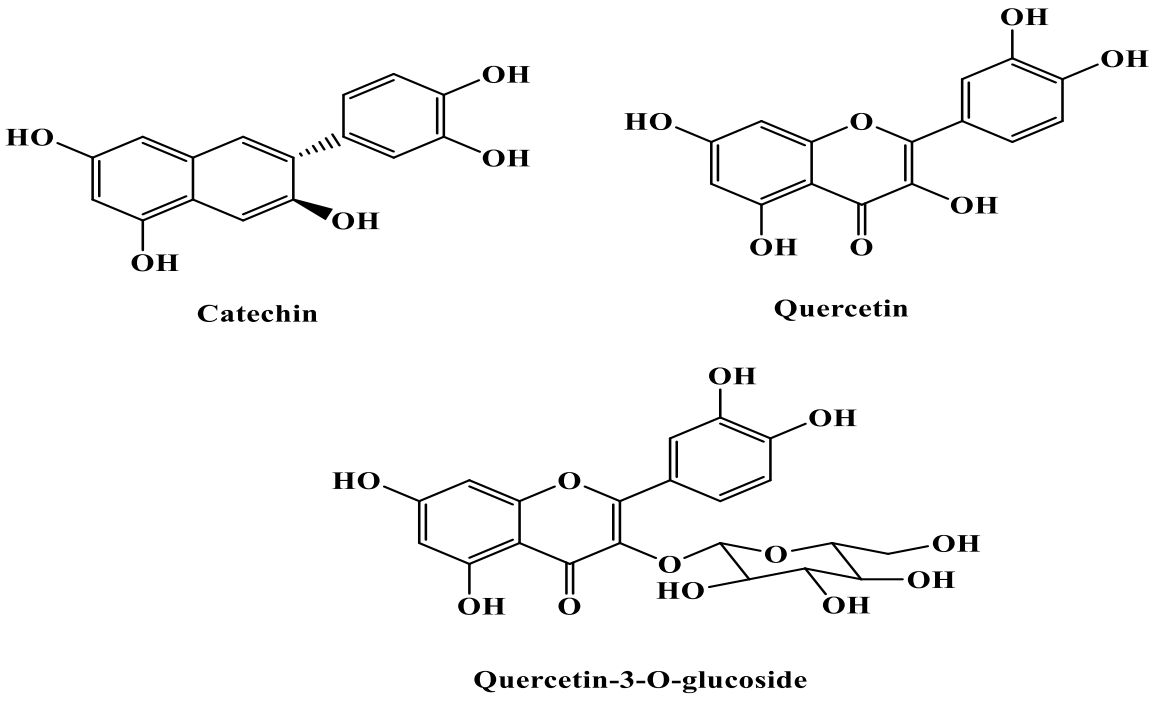



**Figure 2.15.** Chemical structures of flavonoids Identified in camelina whole seeds and cake by LC-$MS^2$ (Name of compounds adopted from Terpinc et al. 2012).

## 2.8 Phenolics and polyphenolics in sophia seeds

There is limited information on sophia seeds phenolics and polyphenolics as a potential source of bioactive compounds. The first study on phenolic analysis and their antioxidant activities in sophia seed was reported by HadiNezhad, Rowland and Hosseinian (2015). They extracted phenolics from whole sophia seed and deoiled meal by using a supercritical $CO_2$. More than 10 phenolic compounds were analysed by HPLC and sinapic acid was the dominant compound in both sophia whole seed and meal extracts. Sophia seed extracts showed a high level of antioxidant activity in the ORAC and β-carotene bleaching assays in the same study.

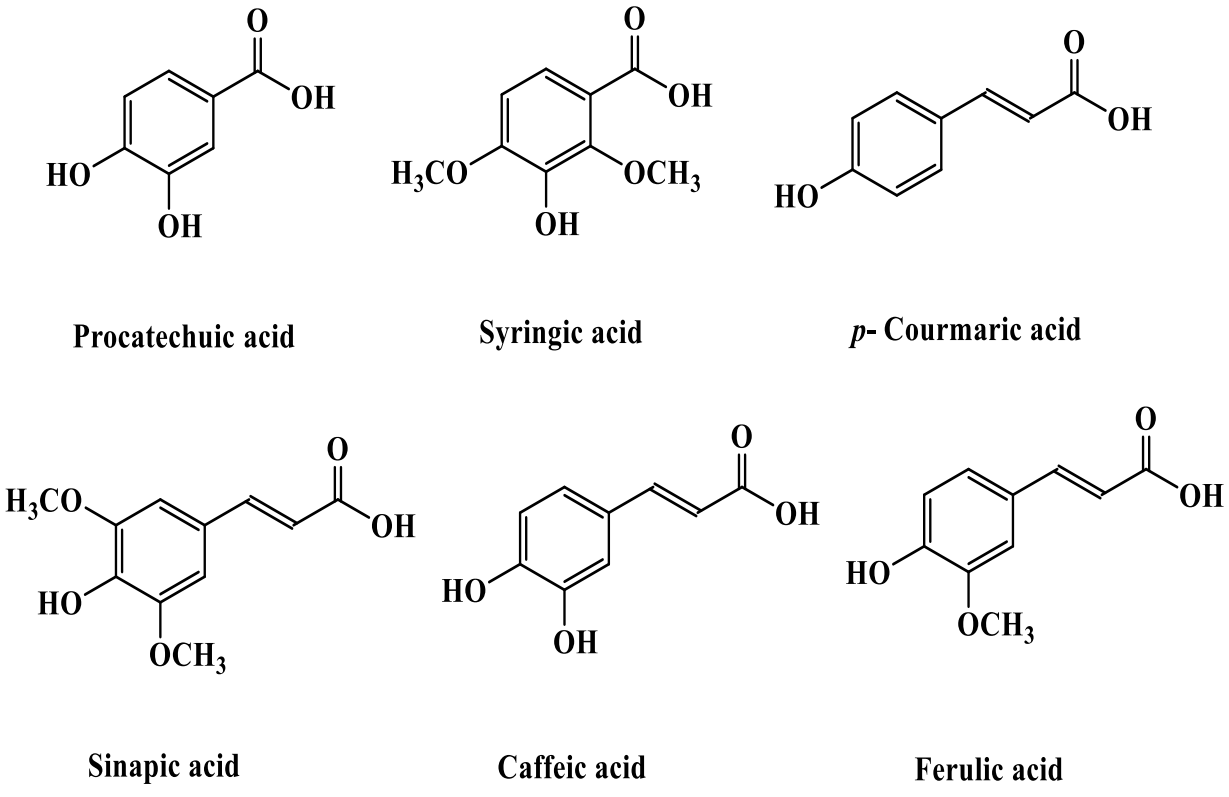



**Figure 2.16.** Chemical structures of phenolic acids identified in sophia whole seed by HPLC-PDA analysis (Name of compounds adopted from HadiNezhad, Rowland & Hosseinian 2015)

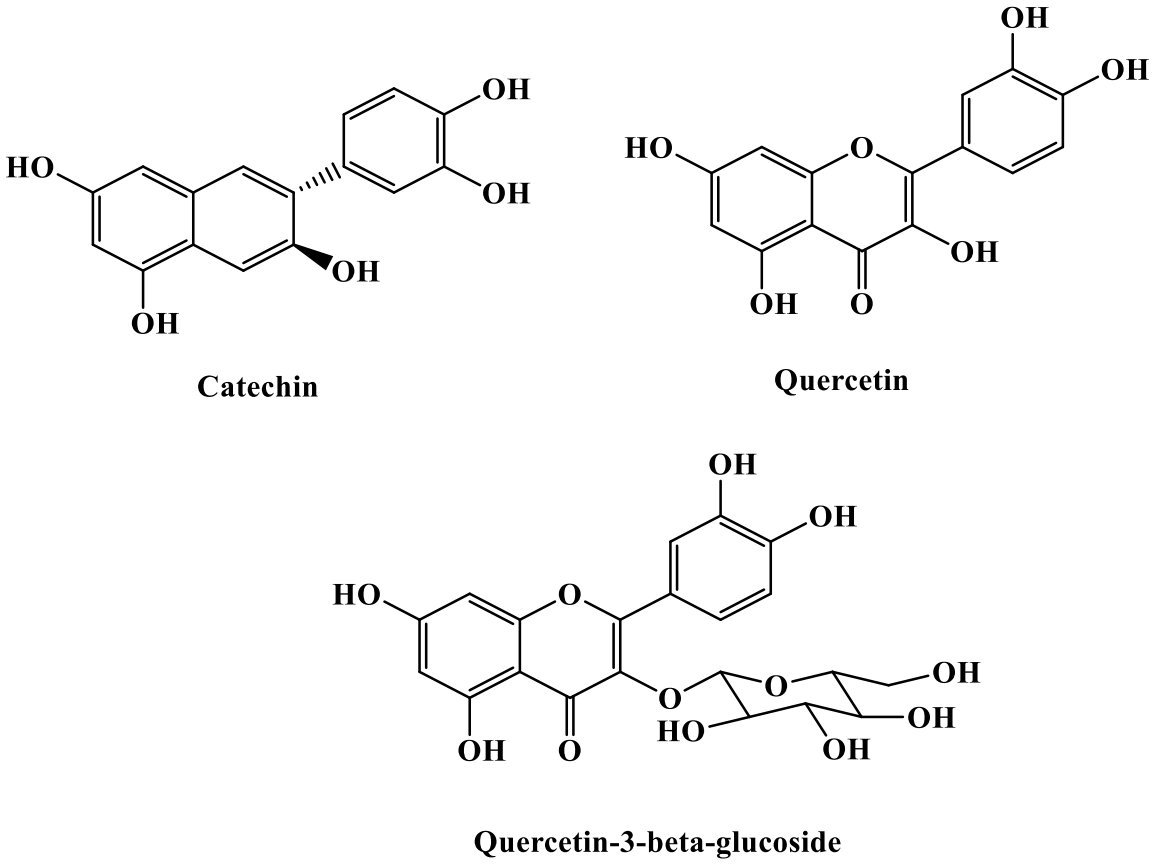



**Figure 2.17.** Chemical structures of flavonoids identified in sophia whole seed by HPLC-PDA analysis (Name of compounds adopted from HadiNezhad, Rowland & Hosseinian 2015)

## 2.9 Phenolics and polyphenolics in chia seeds

Many studies have been done on the phenolic profile of chia seeds and their potential antioxidant activity *in vitro*. While these studies were focussed on only crude phenolics of chia seeds, they still provide an overall idea on the phenolics present and their bioactivities. Reyes-Caudillo et al. 2008 reported that chia seeds contain 8.8 % of total phenolics on a dry weight basis. In the same study, the presence of caffeic acid, chlorogenic acid and quercetin was correlated with higher contents of phenolics in chia. Uribe et al. (2011) described that the chia seed is potentially a great source of

antioxidants and could have better health effects and used for preservation of lipid rich foods and food products. Ayerza and Coates (2001) identified and quantified chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol from chia seeds and evaluated their total antioxidant potential. Tepe et al. (2006) studied the antioxidant activity of ethanolic extract of chia seed and reported that polyphenols of chia seed inhibited free radical scavenging effect in a beta-carotene /linoleate model system. The free radical scavenging activity of chia seed was even greater than many natural sources of antioxidant such as those of *Moringa oleifera*, and sesame cake extract as described by Nadeem et al. (2013, 2014). Craig (2004) reported that polyphenols in chia seed protected it from oxidative deterioration. Reyes-Caudillo et al. (2008) also reported that chia seeds contain a wide range of phenolic compounds and their antioxidant potential was reviewed in the same study. Tepe et al. (2006) reported that phenolics of chia seed extract have potential antioxidant activity and their inhibition of lipid peroxidation was also reviewed in the same study. Quercetin, chlorogenic acid, and caffeic acid are believed to have anti-carcinogenic, antihypertensive, and neuron protective effects (Shahidi & Naczk 1995). Ayerza and Coates (2002) demonstrated that chia seed contained myricetin, quercetin, kaemferol, caffeic acid, flavonol glycosides and chlorogenic acid. Azeem et al. (2015a) found that 750 ppm chia seed extract significantly extended the shelf life of cottonseed oil at ambient temperatures.

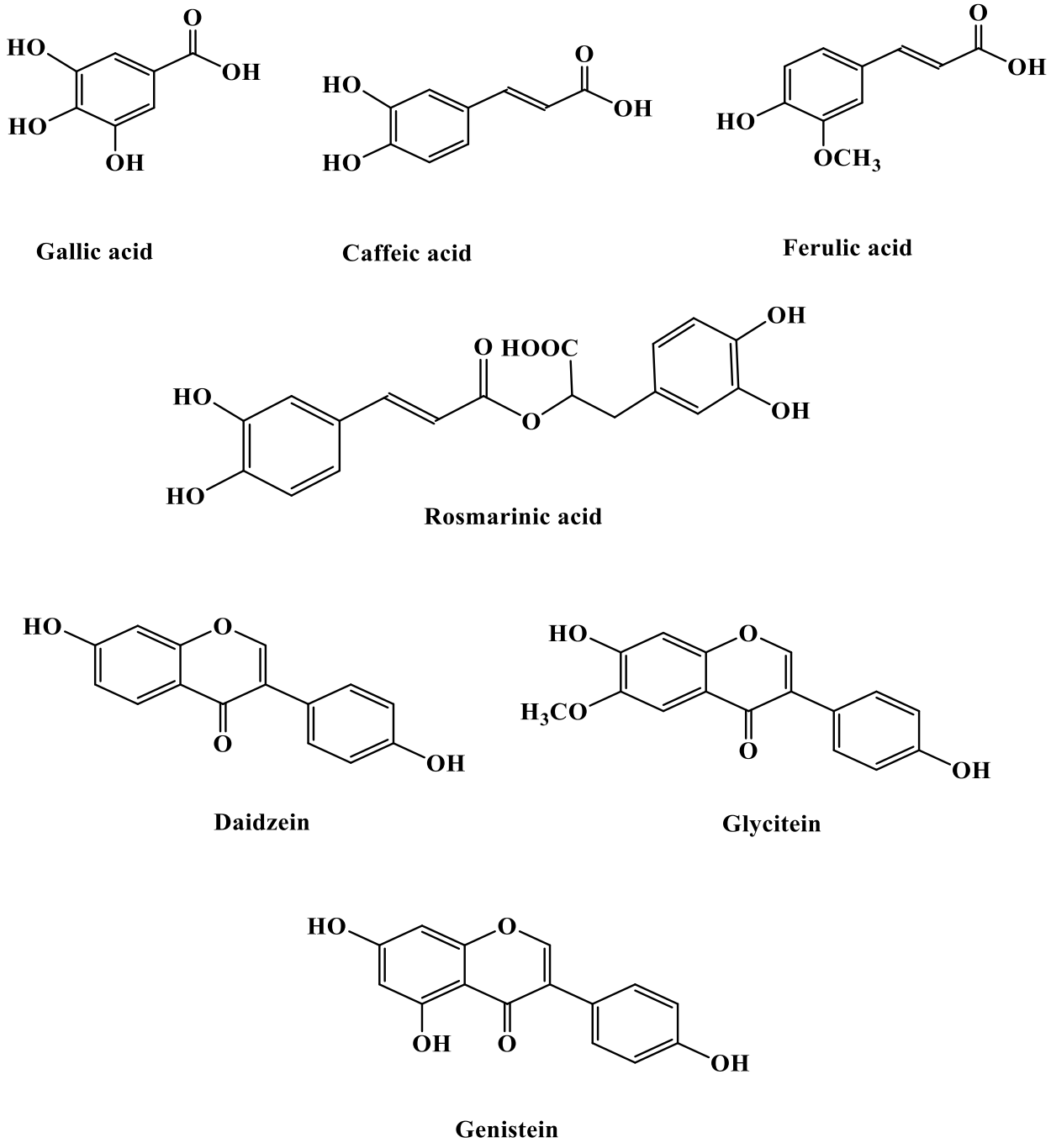



**Figure 2.18.** Chemical structures of phenolic acids and isoflavones identified in chia seeds by UHPLC analysis (Name of compounds adopted from Martínez-Cruz and Paredes-López 2014).

# CHAPTER 3
# MATERIALS AND METHODS

## 3.1 Sample collection and material procurement

The camelina, chia, and sophia seeds were used in this study. Camelina seeds were obtained via Professor C. Parrish, Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, NL, Canada. Chia seeds were bought from Costco wholesale, St. John's, NL, Canada. Sophia seed was a product of Daghdaghabad near the city of Hamedan in Iran and purchased from Tavazo store, Toronto, ON, Canada.

Standards of gallic acid, catechin, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonate) (ABTS), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), DPPH, trolox, ascorbic acid, and ethylenediaminetetraacetic acid trisodium salt ($Na_3EDTA$) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Organic solvents and reagents, namely diethyl ether, ethyl acetate, hexane, acetone, methanol, chloroform, formic acid, sodium chloride, mono- and dibasic potassium phosphates, hydrochloric acid, aluminum chloride, sodium nitrite, sodium hydroxide, potassium ferricyanide, ferric chloride, ferrous chloride, Folin-Ciocalteu's reagent, vanillin, trichloroacetic acid (TCA), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-disulphonic acid sodium salt (Ferrozine) and sodium carbonate were purchased from Fisher Scientific Ltd. (Ottawa, ON, Canada).

## 3.2 Sample preparation

All samples were ground using a coffee bean grinder (model CBG5 series, Black & Decker, Canada Inc., Brockville, ON, Canada) and passed through a 0.5 mm sieve to

obtain a fine powder and defatted by blending with hexane (1:5 w/v, 5 min, 3X) in a Waring blender (model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT, USA) at ambient temperature. Defatted samples were dried at $37^0$C and used immediately for extraction of phenolics.

**3.3 Extraction of phenolic compounds**

Free, esterified, and insoluble-bound phenolic compounds were extracted and fractionated according to Chandrasekara and Shahidi (2010) with some modifications. An ultrasonic-assisted extraction procedure was used for the extraction of soluble phenolic compounds. Defatted meal (510g) was mixed with 200-400 mL of 70% (v/v) acetone and then placed in an ultrasonic bath (300 Ultrasonik, Whittemore Enterprises, Inc., Rancho Cucamonga, CA, USA) and sonicated at the maximum power for 20 min at $30^0$ C. The resultant slurry was centrifuged for 5 min at 4000g IEC Centra MP4, International Equipment Co., Needham Heights, MA, USA) and the supernatant was collected and extraction wasrepeated two more times. After centrifugation, combined supernatants were evaporated under vacuum using a rotary evaporator at $40^0$C (Buchi, Flawil, Switzerland) to remove the organic solvents. Residues of whole oilseed samples were air-dried for 24 h and used to extract insoluble-bound phenolic compounds within a week. During all stages of extraction, extracts were protected from light by using aluminum foil.

**3.4 Extraction of free and esterified phenolic compounds**

After evaporation, the aqueous suspension of extract was adjusted to pH 2 with 6 M HCl, and free phenolics were then extracted five times with diethyl ether and ethyl acetate (1:1, v/v). The free phenolic extract was evaporated under vacuum using a rotary

evaporator at $40^0$C and dissolved in 5-10 mL of 80% methanol (HPLC grade). The esters remaining in the water phase were hydrolysed with 4 M NaOH for 4 h under a nitrogen atmosphere for the extraction of esterified phenolics. The liberated phenolics were then extracted from the hydrolysates five times with diethyl ether (1:1, v/v) and evaporated to dryness under vacuum and subsequently dissolved in 5-10 mL 80% methanol for comprehensive analysis of phenolics profile, determination of antioxidant and biological activities of camelina, chia and sophia seed meals.

**3.5 Extraction of insoluble-bound phenolic compounds**

The residue of the whole oilseed sample of camelina, chia and sophia obtained after extraction of soluble phenolics was hydrolyzed with 4M NaOH and stirred at room temperature for 4h under nitrogen. The resulting slurry was acidified to pH 2 with 6 M HCl and centrifuged as in the case of free phenolics. The liberated bound phenolic compounds were then extracted five times with diethyl ether and ethyl acetate (1:1, v/v), evaporated and then dissolved in methanol as described for esterified phenolics.

**3.6 Determination of total phenolic content (TPC)**

The total phenolic content (TPC) of each extract was determined according to Singleton and Rossi (1990). Briefly, 0.5mL of sample dissolved in methanol was taken in a centrifuge tube and Folin-Ciocalteu's reagent (0.5mL) was added to it. The contents were mixed thoroughly and 1 mL of saturated sodium carbonate was added to each tube for neutralization. Then, 8 mL of distilled water were added and vortexed thoroughly. Tubes were allowed to stand for 35 min at room temperature in the dark followed by centrifugation for 10 min at 4000g. The absorbance of the resultant blue colour supernatant was read at 725 nm (model HP 8452A diode array spectrophotometer,

Agilent Technologies, Palo Alto, CA, USA) using appropriate blanks for background subtraction. The content of total phenolic in each extract was determined and expressed as milligrams of gallic acid equivalents (mg GAE) per gram of defatted sample.

### 3.7 Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was determined using a colorimetric method explained by Kim, Jeong and Lee (2003) with slight modifications as described by Chandrasekara and Shahidi (2010). In 20 mL centrifuge tubes, 1mL of extract, dissolved in methanol, was mixed with 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ was added to it. The tubes were then allowed to stand for 5 min and subsequently 0.3 mL of 10% $AlCl_3$ was added to the reaction mixture and again allowed to stand for 1 min. Finally, 2 mL of 1 M NaOH and 2.4 mL of distilled water were added and mixed immediately. After centrifugation at 4000 g for 5 min, the tubes were kept in the dark at room temperature for 15 min. The absorbance was read at 510 nm against a blank prepared in a similar manner by replacing the extract with methanol. The TFC, calculated from a standard curve for catechin, was expressed as mg catechin equivalents (CE) per gram of defatted sample.

### 3.8 Determination of proanthocyanidin content (PC)

Total proanthocyanidin content of camelina, chia and sophia seeds was determined colorimetrically as explained by Price et al. (1978) with some modifications. The sample extract (0.2mL of it) in methanol was added to 1 mL of 0.5% vanillin-HCl reagent (0.5%, w/v vanillin in 4% concentrated HCl in methanol). The mixtures were then incubated for 20 min at room temperature and absorbance was read at 500 nm. A separate blank for each sample (4% HCl in methanol) was used; the content of proanthocyanidins was expressed as mg CE per gram of defatted seeds.

### 3.9 Identification of phenolic compounds by HPLC-DAD-ESI-$MS^n$ analysis

Phenolic profiles in the free (F), esterified (E), and insoluble-bound (B) fractions of defatted camelina, chia and sophia seed meals were identified and quantified by high performance liquid chromatography (HPLC) as described by Ambigaipalan et al. (2016) and de Camargo et al. (2014). The RP-HPLC analysis was carried out using an Agilent 1100 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump (G1311A), a degasser (G1379A), an ALS automatic sampler (G1329A), an ALS Therm (G1130B), a Colcom column compartment (G1316), a diode array detector (DAD, G1315B), and a system controller linked to a Chem Station Data handling system (Agilent Technologies, Palo Alto, CA, USA). Separations of phenolic compound were done with a SUPERLCOSILTM LC-18 column (4.6 * 250 mm * 5 µm, Merck, Darmstadt, Germany). The mobile phase consisted of 0.1% formic acid (eluent A) and 0.1% formic acid in acetonitrile (eluent B). The gradient solvent system used was as follows: 0 min, 100% A; 5 min, 90% A; 35 min, 85% A; 45 min, 60% A; held at 60% A from 45 - 50 min; subsequently mobile phase A was increased to 100% at 55 min, followed by column equilibration from 55 to 65 min. Injection volume was 50 µL and flow rate was adjusted to 0.5 mL/min for a total run time of 65 min. The detection of phenolic acids and flavonoids was performed at 280 nm. All samples were filtered through a 0.45 lm PTFE membrane syringe filter (Whatman Inc., Florham Park, NJ, USA) before injection.

HPLC-ESI-$MS^n$ analysis was performed as described above using an Agilent 1100 series capillary liquid chromatography mass selective detector (LC-MSD) ion trap mass spectrophotometer (Agilent Technologies) which was connected to the Agilent 1100 HPLC system via an electrospray ionization (ESI) in the negative mode for phenolic acids

and flavonoids. The data were achieved and analyzed with Agilent LC-MSD software (Agilent Technologies). The mass spectrometer was run in a scan range of m/z 50 to 2000, using smart parameter setting, drying nitrogen gas temperature of 350°C along with flow of 12 L/min, and nebulizer gas pressure of 70 psi. Limits of detection were in the range of 3 to 19 ng/g whereas the limits of quantification were in the range of 8 to 57 ng/g. Phenolic compounds were identified by comparing their retention times and UV absorption spectra with authentic standards and confirmed by LC-MS. Other compounds with no standard reference materials were tentatively identified using tandem mass spectrometry ($MS^n$) data, UV spectral data, and by matching with literature data. Quantification of phenolic compounds was done by DAD using standard curves of their authentic standards generated by plotting HPLC peak areas vs concentrations. For compounds with no standard reference materials, quantification was done based on standard curves of similar compounds of the same phenolic subgroup. The results of quantification of phenolic compounds were expressed as µg per gram defatted sample.

**3.10 Trolox equivalent antioxidant capacity (TEAC)**

The total antioxidant capacity of the tested oilseed extracts was measured according to the method described by van den Berg et al. (1999) with some modification. This assay is based on the scavenging of 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$). An $ABTS^{\bullet+}$solution was prepared in 100 mL phosphate buffer saline (0.1 M, pH 7.4, 0.15 M NaCl) (PBS) by mixing 2.5 mM AAPH with 2.5 mM ABTS stock solution (1:1, v/v). During heating at $60^0$ C for 20 min, the solution was protected from light by covering the container in a tin foil, and cooled to room temperature. Before mixing with

the extracts, $ABTS^{\bullet+}$solution was filtered using medium-porosity P5 filter papers (Fisher Scientific Co., Pittsburgh, PA, USA). Forty microlitres (40 µL) of the sample were mixed with 1960 µL of the $ABTS^{\bullet+}$ solution to determine the total antioxidant capacity and absorbance of the reaction mixture was read at 734 nm immediately at the point of mixing ($t_0$) and after 6 min ($t_6$). The decrease in absorbance at 734 nm after addition of both trolox and phenolic extract 6 min later was used for calculating TEAC values. The TEAC vales were determined using the equation below and and where ΔA is the reduction of absorbance and A is the absorbance at a given time. TEAC values were calculated as micromole trolox equivalents (TE) per gram of defatted sample.

$$\Delta A_{\text{Sample}} = [(A_{\text{Sample 0 min}} - A_{\text{Sample 6 min}}) - (A_{\text{Blank 0 min}} - A_{\text{Blank 6 min}})]$$

### 3.11 DPPH radical scavenging capacity (DRSC) using electron paramagnetic resonance (EPR)

DPPH radical scavenging capacity (DRSC) assay was carried following the method described by Madhujith and Shahidi (2006). Briefly, 1 mL of 0.3 mM solution of DPPH was mixed with 250 µL of appropriately diluted free, esterified and insoluble-bound phenolics extracts. Contents were mixed thoroughly and kept in the dark for 10 min at room temperature. The sample was subsequently passed through the sample cavity of a Bruker E- scan EPR spectrometer (Bruker E-scan, Bruker Biospin Co., Billercia, MA, USA) and the spectrum was recorded ($5.02 * 10^2$ receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100.000 G sweep width, 3495.258 G centre field, 5.12 ms time constant, 9.795 GHZ microwave frequency, 86.00 kHZ modulation frequency, 1.86 G modulation amplitude). DRSC of the extracts was calculated using the following equation.

DPPH radical scavenging capacity (%)

= [100 – (EPR signal intensity for the control - EPR signal intensity for the extract)] * 100

The DPPH scavenging activity of all extracts were expressed as micromoles TE/g defatted seed.

**3.12 Hydroxyl radical scavenging capacity (HRSC) by EPR**

The hydroxyl radical scavenging capacity (HRSC) was determined according to the method explained by Madhujith and Shahidi (2006) with slight modifications. Extracts of free, esterified and bound phenolic compounds of camelina, chia and sophia were dissolved in sodium phosphate buffer (PBS) (0.75M, pH 7.00) and diluted accurately. Briefly, 0.2 mL of each phenolic extracts was mixed with 0.2mL of $H_2O_2$ (10 mM), and 4mL of DMPO (17.6 mM). Then, 0.2mL of $FeSO_4$ (10mM) was added to the mixture to initiate the reaction. After 3 min, the mixtures were introduced into the sample cavity of the EPR spectrometer and the spectrum was recorded. Phosphate buffer saline (0.75M, pH 7.00) (PBS) was used as the control in place of the extract. Hydroxyl radical scavenging capacities of the extracts were calculated using the following equation.

Hydroxyl radical scavenging capacity (%) = [(EPR signal intensity for the control - EPR signal intensity for the sample)/EPR signal intensity for the control] *100.

The hydroxyl radical scavenging activity of the extracts was expressed as micromoles of micromoles of catechin equivalents (CE) per g of the defatted seed.

### 3.13 Reducing power (RP) assay

The reducing power was determined according to the method of Oyaiza (1986) with some modifications. In a centrifuge tube, 0.5 mL sample extract was mixed with 2.5 mL of a phosphate buffer solution (0.2M, pH 6.6) and subsequently to which 2.5 mL of potassium ferricyanide (1%, w/v) were added. The mixture was incubated at $50^0$C for 20 min, and then 2.5 mL of 10% TCA were added before centrifugation at 1750 g for 10 min. A 2.5 mL aliquot of the supernatant was taken in a test tube containing 2.5 mL of deionized water. After adding 0.5 mL of 0.1% (w/v) $FeCl_3$, the absorbance of the reaction mixture was read at 700 nm using a spectrophotometer. The standard curve was prepared using trolox. The reducing power (RP) of extracts were calculated using the following equation.

$$\text{Reducing Power (RP)} = [(\text{Absorbance}_{\text{Sample}} - \text{Absorbance}_{\text{Control}})/ \text{Absorbance}_{\text{Sample}}] * 100$$

The results were expressed as micromole of trolox equivalents (TE) per gram of defatted sample.

### 3.14 Ferrous ion chelating activity (FCA) assay

The ferrous ion chelating activity (FCA) of camelina, chia and sophia phenolic extracts was measured according to the method described by Dinis et al. (1994) with some modifications. Briefly, 0.2mL of phenolic extracts dissolved in PBS (0.75M, pH 7.00) was taken in a test tube and 0.025ml a solution of $FeCl_2$ (2mM) was added to each tube. The reaction was initiated by adding 0.1 ml of Ferrozine solution (5 mM), and the total volume was adjusted to 2 mL with distilled water. The mixture was left at room temperature for 10 min after shaking vigorously. The absorbance of the reaction mixture

was read at 562 nm and for the control, PBS was used instead of the extract. The inhibition percentage of Ferrozine-ferrous ion complex formation was calculated by the following equation.

Metal chelating effect (%) = [1- (absorbance of the sample / absorbance of the control)] * 100. The results were expressed as μmoles of EDTA equivalents per gram of defatted meal.

### 3.15 β -carotene-linoleate model system

A β-carotene–linoleate model system was used to evaluate the antioxidant activity of camelina, chia and sophia seeds meal in an oil-in-water emulsion (Amarowicz & Shahidi 1997). β- Carotene (10 mg) was dissolved in chloroform (10 mL), and an aliquot (1.2 mL) of it was transferred into a flask containing linoleic acid (40 mg) and Tween 40 (400 mg). A blank without β-carotene was also prepared (40 mg of linoleic acid + 400 mg of Tween 40). Chloroform was removed under a nitrogen stream; 100 mL of oxygenated distilled water were added to the flask, and the mixture was stirred vigorously for 30 min. Each 0.5 mL of camelina, chia or sophia meal phenolic extracts was dissolved in deionized water and mixed with 4.5 mL of the above emulsion. A control without sample and a mixture of blank (without β-carotene) was prepared for each sample. The absorbance at 470 nm was read immediately after the addition of the emulsion. The tubes were incubated in a shaking water bath at 50 °C, and the absorbance was read over a 105-min period at 15 min intervals. Antioxidant activity of the polyphenolics extract camelina, chia and sophia in protecting β-carotene/linoleic acid oxidation was calculated using the equation of Barros et al. (2007).

Antioxidant activity (%) = (β-carotene content after 105 min/ Initial β-carotene content) * 100

The corresponding $IC_{50}$ value, defined as the concentration of inhibitor required to inhibit 50% of the β-carotene bleaching was also calculated.

### 3.16 Inhibition of α-glucosidase activity assay

Alpha-glucosidase inhibitory activity of camelina, chia and sophia polyphenolic extracts was determined using the method of Eom et al. (2012) with slight modifications. An aliquot of the sample dissolved in methanol (10 µL) was mixed with 620 µL of potassium phosphate buffer (0.1 M, pH 6.8) in a 1.5 mL Eppendorf tube. α-Glucosidase solution (5 µL; 10U/mL) in PBS (0.1 M, pH 6.8) was added and incubated for 20 min at 37°C. After incubation, 10 µL a substrate *p*-nitrophenyl glucopyranoside (10 mM) were added to initiate the reaction and the reaction mixture was incubated at 37 °C for 30 min. After that, 650 µL of 1 M $Na_2CO_3$ were added to terminate the reaction and the quantity of released product (*p*-nitrophenol; yellow colour) was measured at 410 nm by using a UV visible spectrophotometer. Sample blanks without enzyme and a control without sample were also measured. α-Glucosidase inhibition was calculated using the equation below.

α-Glucosidase inhibition (%)= [(Abs $_{control}$ – Abs $_{sample}$)/ Abs $_{control}$] *100

The corresponding $IC_{50}$ value, defined as the concentration of inhibitor required to inhibit 50% of the α-glucosidase activity, was also calculated.

### 3.17 Inhibition of pancreatic lipase activity assay

Pancreatic lipase inhibition activity of camelina, chia and sophia phenolic extracts was determined as described by Marrelli et al. (2012). In this study, we used 5mg/mL concentration of lipase from porcine pancreas, dissolved in 1 M Tris–HCl buffer

(pH = 8.5). Phenolic extract of each of the camelina, chia and sophia (100 µL) was added in 4mL tris-HCl buffer solution (1M, pH 8.5). Then, 100 µL of pancreatic lipase solution were added and incubated for 25 min at 37 °C. A substrate solution of 4-nitrophenyl octanoate (10 mM) was prepared in dimethyl sulphoxide (DMSO) and diluted with ethanol (5 mM). To initiate the reaction, 100 µL of 4-nitrophenyl octanoate (5 mM) were added and incubated at 37 °C for 25 min. A control with methanol instead of sample and sample blanks without enzyme were prepared. The absorbance (yellow colour) was measured at 412 nm using a UV-visible spectrophotometer. The lipase inhibition was calculated using the equation below.

Lipase inhibition (%)= [(Abs $_{control}$ – Abs $_{sample}$)/ Abs $_{control}$] *100

The corresponding $IC_{50}$ value was also calculated for each sample extracts.

**3.18 Inhibition of cupric Ion-induced human low-density lipoprotein (LDL) peroxidation**

The inhibitory activity of phenolic extracts of camelina, chia and sophia meals on the cupric ion-induced human LDL oxidation was investigated using a method described by Ambigaipalan and Shahidi (2016). At first, 5 mg/mL LDL were dialyzed in 100 mL of freshly prepared PBS (10 mM, pH 7.4, 0.15 M NaCl) using a dialysis tube with the molecular weight cut off of 12−14 kDa (Fischer, Carle and Kammerer Scientific, Nepean, ON, Canada) at 4 °C under a nitrogen blanket in the dark for 12 h. After dialysis, diluted LDL cholesterol (0.04 mg LDL/mL) was mixed with the phenolics extracts of both samples (0.1 mg/mL). The reaction mixture was incubated at 37 °C for 15 min. After that, a copper sulphate solution of 0.1 mL (100 µM) was added to initiate the oxidation reaction. Subsequently, the samples were incubated at 37 °C for 22 h. The formation of conjugated dienes (CD) was measured using a diode array spectrophotometer (Agilent,

Palo Alto, CA, USA) at 234 nm. A positive control was excluding phenolic extracts. The appropriate blank for each sample was prepared by replacing LDL cholesterol and $CuSO_4$ with distilled water for background correction.

**3.19 Supercoiled plasmid DNA strand scission inhibition assay**

The inhibition activity of camelina, chia and sophia phenolic extracts on supercoiled strand DNA scission induced by hydroxyl and peroxyl radicals was evaluated as stated by Hiramoto et al. (1996) and Liyanapathirana and Shahidi (2006) with slight modifications. Supercoiled plasmid DNA (pBR 322 from *Escherichia coli* RRI) (50 µg/mL) was dissolved in PBS (0.5 M, pH 7.4). Phenolics extracts of camelina, chia and sophia meals at various concentration (0.25-1.5 mg/mL) were prepared in PBS. In a 0.5 mL Eppendorf tube, 2 µL each of a solution of supercoiled plasmid DNA, PBS, phenolic extract, $H_2O_2$ (1 mM), and FeSO4 (0.5 mM) were added to determine the inhibitory activity of test material on DNA strand scission induced by hydroxyl radical. A control with DNA alone and a blank devoid of phenolic extracts were included with each set. The mixture was incubated at 37 $^0$C for 1 h in the dark. After incubation, loading dye (5µL) consisting of 0.25% bromophenol blue, 0.25% xylene cyanol, and 50% glycerol in distilled water were added to the reaction mixture.

The inhibitory effect of camelina, chia and sophia phenolic extract against DNA oxidation induced by peroxyl radical was also investigated in another set of studies. In this assay, AAPH in PBS (17.25 mM) was mixed with DNA and the extracts to a final volume of 10µL. The mixture was incubated at 37 $^0$C for 1 h in the dark. A control with DNA alone and a blank devoid of phenolic extracts were prepared with each set. The samples were electrophoresed using a 0.7% (w/v) agarose gel prepared in Tris-acetic acid EDTA (TAE)

buffer (40 mM Tris acetate, 1mM EDTA, pH 8.5). SYBR safe (100 µL/L of TAE buffer) was added as a gel stain. Submarine gel electrophoresis was run at 80 V for 1.5 h using a model B1A horizontal mini gel electrophoresis system (Owl Separation Systems Inc., Portsmonth, NH, USA) and a model 500 V power supply (WMR International Inc., West Chester, PA, USA) at room temperature in TAE buffer. The bands were visualized under trans illumination of UV light using an Alpha Imager gel documentation system (Cell Biosciences, Santa Clara, CA, USA). The images were analyzed using Chemilmager 4400 software (Cell Biosciences) to quantify DNA scission. The inhibitory effect of the seeds extracts was calculated using the retention of the normalized supercoiled DNA as given below.

DNA retention % = (intensity of supercoiled DNA with the oxidative radical and extract/ intensity of supercoiled DNA in control) *100.

The concentration of extracts that retain 50% ($EC_{50}$) of supercoiled DNA was also calculated.

**3.20 Statistical analysis**

All tests were done in triplicate from three individuals extracts, and mean values and standard deviations were reported. One-way ANOVA was used, and the mean separations were analysed by Tukey's HSD test ($p < 0.05$) using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

# CHAPER 4

# RESULTS AND DISCUSSION

## 4.1 Total phenolic content (TPC)

The total phenolic content (TPC) of soluble (free and esterified) and insoluble-bound fractions of defatted camelina, chia and sophia seed meals were determined using Folin-Ciocalteu assay. This is the most common spectroscopic method to measure the total amounts of phenolics. In this assay, under alkaline conditions, phenolic groups are deprotonated and form phenolate ions due to reduction of the phosphotungstic-phosphomolybdic complex in the Folin Ciocalteu reagent producing a blue colour. The soluble phenolic fraction includes both free and soluble ester conjugates, which are responsible for the *in vitro* antioxidant capacity of the extracts. Free and esterified phenolics may be absorbed in the small intestine and gastrointestinal track and could be highly metabolized or rapidly eliminated (Manach 2004). After metabolism, they can be found in the blood and other target organs as metabolites with biological activities. On the other hand, phenolics in the insoluble-bound form are covalently bound to cell wall structural components such as cellulose, hemicellulose (e.g. arabinoxylans), lignin, pectin and rod-shaped structural proteins (Acosta-Estrada et al. 2014; Wong 2006). Insoluble bound phenolic compounds are not digested in the small intestine, but passed into the colon where they are hydrolyzed by intestinal enzymes and colonic microflora before being absorbed and render various bioactivities like soluble phenolics (Manach 2004). In addition to health benefits, they have multiple functions in the cell wall by providing both physical and chemical barriers, protection against pathogen invasion and astringency that deters attack by insects and animals, as well as rendering antibacterial,

antifungal and antioxidant functions (Acosta-Estrada et al. 2014; Liu 2007; Sancho et al. 2001).

The total phenolic content (TPC) of soluble (free and esterified) and insoluble-bound phenolic extracts of camelina, chia and sophia meals are summarized in Table 4.1. The total phenolic content (TPC) in camelina, chia and sophia defatted meal was 11.69 ± 0.44, 14.22 ± 0.44 and 22.40 ± 0.87 mg GAE per gram sample, respectively.

**Table 4. 1.** Total phenolic content (TPC), flavonoids content (TFC) and proanthocyanidins content (PC) of the defatted camelina, chia and sophia meals.

| Samples | Free phenolic | Esterified Phenolic | Insoluble-Bound | Total |
|---|---|---|---|---|
| Total phenolics content (mg GAE/ g defatted sample) | | | | |
| Camelina | 4.07 ± 0.45Bd | 6.80 ± 0.30Ac | 0.82 ± 0.13Cf | 12.96 ± 0.87C’ |
| Chia | 8.69 ± 0.53Ab | 0.95 ± 0.07Cf | 4.59 ± 0.16Bd | 14.22 ± 0.44B’ |
| Sophia | 4.14 ± 0.36Bd | 15.85 ± 0.15Aa | 2.49 ± 0.08Ce | 22.40 ± 0.42A’ |
| Total flavonoids content (mg CE/ g defatted sample) | | | | |
| Camelina | 2.26 ± 0.28Bc | 4.32 ± 0.47Aa | 0.22 ± 0.00Ce | 6.8 ± 0.68B’ |
| Chia | 4.86 ± 0.8A4a | 0.23 ± 0.01Ce | 3.35 ± 0.03 Bb | 8.45 ± 0.80A’ |
| Sophia | 2.53 ± 0.18Bc | 4.95 ± 0.16Aa | 1.10 ± 0.19 Cd | 8.59 ± 0.13A’ |
| Total proanthocyanidins (Condensed tannin) content (mg CE / g defatted sample) | | | | |
| Camelina | 3.13 ± 0.01Aa | 0.28 ± 0.01B c | 0.32 ± 0.01Bc | 3.73 ± 0.03A’ |
| Chia | 0.05 ± 0.56A d | 0.00 ± 0.00 b | 0.02 ± 0.01 Ad | 0.08 ± 0.02C’ |
| Sophia | 1.77 ± 0.02Ab | 0.28 ± 0.04Bc | 0.17 ± 0.00C | 2.23 ± 0.06B’ |

Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different upper case letters within a row and different lower case letters among all fractions are significantly different ($p < 0.05$) but same letters are not significantly different ($p > 0.05$). GAE, Gallic acids equivalents and CE, Catechin equivalents.

Sophia seed extract showed significantly ($p < 0.05$) higher total phenolics content 2.24 % (22.40 ± 0.87 GAE per gram sample) compared to camelina meal 1.14% and chia seed meal 1.42 % (11.69 ± 0.44 and 14.22 ± 0.44 mg GAE per gram sample, respectively). The

TPC of free, esterified, and insoluble-bound fractions of camelina, chia and Sophia was in the range of 0.82 ± 0.13 to 15.85 ± 0.18 mg GAE per gram defatted sample (Table 4.1). Sophia's esterified fraction showed significantly ($p< 0.05$) higher phenolic content (15.85 ± 0.18 mg GAE per gram sample) among all fractions of three samples tested whereas the lowest phenolic content was found in the insoluble-bound fraction of camelina (0.82 ± 0.13 mg GAE per gram /g sample). No significant difference was observed between chia esterified and camelina insoluble-bound phenolics contents 0.95 ± 0.07 and 0.82 ± 0.13 mg GAE per gram sample, respectively). In case of free form of phenolics, chia had the highest free phenolics (8.69 ± 0.53 mg GAE per gram sample), whereas camelina and sophia showed practically the same amount of free phenolics (4.07 ± 0.45 and 4.14 ± 0.36 mg GAE per gram sample, respectively). For the insoluble-bound form of phenolics, chia showed the highest content (2.50 ± 0.08 mg GAE per gram sample) compared to camelina (0.82± 0.13 mg GAE per gram sample). Esterified fraction was the predominant form of phenolics compared to the free and insoluble-bound forms in both defatted camelina and sophia seeds whereas the free phenolic fraction was the predominant form in defatted chia seed meal. The variation in phenolic content among defatted seeds of camelina, chia and sophia may be attributed to the variety, growing location, storage and harvesting period that were not considered in this study. According to Shahidi (1992), total phenolic acids content in various cultivars of rapeseed/ canola meals was in the range of 13-18 mg per gram, on a dry and defatted weight basis. The phenolic acid content of free, esterified and insoluble-bound fractions in mustard meal was 10.81, 15.38 and 0.228 mg per gram sample, respectively (Shahidi & Naczk 1990). In agreement with these published data, the total, free, esterified and insoluble-bound phenolics extracts of camelina, chia and sophia seeds varied with respect to their different

phenolics fractions. The results of this study clearly showed that the distribution of TPC among free, soluble esters and insoluble-bound phenolic extracts may be different depending on the variety as shown for different seed meals (Shahidi & Naczk 1990; Krygier et al. 1982; Naczk & Shahidi 1989). Furthermore, the Folin-Ciocalteu assay is non-specific and results may be affected by the presence of co-existing simple carbohydrates and/or amino acids in the crude extracts which could interfere with determinations of TPC, leading to discrepancies of the results obtained in the present work (Chandrasekara & Shahidi 2010; Zielinski & Kozlowska 2000).

**4.2 Total flavonoid content (TFC)**

Flavonoids are the most studied group of polyphenols in plant foods with well documented antioxidant activity. In this study, total flavonoid content (TFC) was determined using the chelating power of flavonoids with aluminum (III). Flavonoids form a pink-coloured complex with aluminum (III) through the 4-keto and neighboring hydroxyl group.

The TFC of camelina, chia and sophia meals is shown in Table 4.1; the respective values are 6.81 ± 0.68, 8.45 ± 0.80 and 8.59 ± 0.13 mg CE per gram defatted meal. Both chia and sophia seeds showed higher flavonoids content compared to camelina seeds and no significant difference ($p > 0.05$) was found for the flavonoids contents between chia and sophia phenolics extract (Table 4.1). The TFC of free, esterified, and insoluble-bound fractions of camelina, chia and sophia seeds varied from 0.22 ± 0.0 to 4.95 ± 0.16 mg per gram defatted meals. Esterified fraction of camelina and sophia showed a higher flavonoids content (4.32 ± 0.47 and 4.95 ± 0.16 mg per gram sample, respectively) among all fractions of three types of seeds tested. This might be due to their high

phenolic content that we found in the same study. No significant ($p > 0.05$) difference existed in the free flavonoids of chia and esterified flavonoids contents of camelina and sophia meals whereas the content of insoluble-bound flavonoids was significantly different at $p < 0.05$. The flavonoids content in camelina, chia and sophia defatted meals were significantly different ($p < 0.05$). In this study, camelina and sophia meals had a higher flavonoid content in esterified fraction compared to their free and insoluble-bound flavonoids while chia meal showed higher flavonoids in the free fraction. The variation of flavonoid contents among different fractions of camelina, chia and sophia meals might be due to the same reasons as total phenolic content mentioned above in the same study. This is the first study on TFC of free, conjugate esterified and insoluble-bound phenolics of camelina, chia and sophia seed meals as determined calorimetrically, hence comparison with published data is not possible.

**4.3 Total proanthocyanidins (PC; condensed tannin) content**

Proanthocyanidins are polymeric polyphenols consisting of flavan-3-ol units. They are biologically active and may adversely affect the nutritional value and biological availability of proteins and minerals if present in high quantities in plant food and food products (Chandrasekara & Shahidi 2010). Vanillin reagent (0.5%) was used in this study to assay the tannin content (Price, Van Scoyoc, & Butler 1978). This method is specific for flavanols and dihydrochalcones, which have a single bond at the 2, 3 position and free meta-oriented hydroxyl groups (Naczk & Shahidi 1989). Vanillin may react with condensed tannins and its monomeric components. The sensitivity of this reaction depends on the type of solvent employed. Absolute methanol was used for carrying out

the vanillin-tannin reaction because in this solvent the vanillin reagent is less sensitive to the monomeric units (Naczk & Shahidi 1989).

The proanthocyadinins content (PC) of the three seed meals is shown in Table 4.1. In this study, total proanthocyanidin content (TPC) in camelina, chia and sophia meals was 3.73 ± 0.03, 0.08 ± 0.02 and 2.23 ± 0.06 mg CE per gram sample, respectively. We found that camelina seed meal showed significantly higher (3.73 ± 0.03 mg CE per gram sample) PC compared to chia and sophia seed meals (0.08 ± 0.02 and 2.23 ± 0.06 mg CE per gram sample, respectively). Proanthocyanidins predominated in the free phenolic fraction of both camelina and sophia meals (Table 4.1). This might be because both seeds belong to the same family. Camelina free phenolic extracts showed significantly higher (3.73 ± 0.03 mg CE per gram sample) proanthocyanidin content compared to others phenolic extracts of camelina, chia and sophia. Low amounts of TPC were detected in both insoluble-bound fraction of all seed meals tested (Table 4.1). In this study, chia seed meal showed a low amount of proanthocyanidins content compared to camelina and sophia seeds meals while no proanthocyanidins was found in chia esterified fraction, possibly due to a low amount of phenolics that was observed in this work. The condensed tannin content in various canola varieties ranged from 6.82 to 7.72 mg per gram of oil-free meal, expressed as catechin equivalents (Naczk & Shahidi 1989). Consistent with these published data, we found low amounts of proanthocyanidins among camelina, chia and sophia seed meals which indicates that proanthocyanidin contents varied because of their family of origin and genotype, among others.

### 4.4 Identification and quantification of phenolic compounds by HPLC-DAD-ESI-$MS^n$

Gallic, trans-sinapic, and chlorogenic acids, as well as protocatechuic acid pentoside, catechin, and epicatechin were detected in camelina and sophia but not in chia. *Cis*-*p*-coumaric, *cis*-caffeic, hydroxycoumaric acid, *cis*- and *trans*-ferulic acids as well as genistein were specific to chia, whereas carboxyprotocatechuic acid and isorhamnetin-pentoside were found only in sophia. Chlorogenic acid and dihydrodihydroxyisorhamnetin were detected only in their free form, whereas gallic acid was found only in the fraction containing phenolics released from their insoluble-bound form.

#### 4.4.1 Phenolic acids and their derivatives

Nineteen phenolic acids were tentatively or positively identified in the present study from the different phenolic extracts of camelina, chia and sophia defatted seed meals (Table 4.2). Compound 1, which showed $[M - H]^-$ at m/z 137, gave a product ion at 93 in $MS^2$ due to the loss of a hydrogen and a carboxyl group $[M - H - CO_2]^-$, therefore being tentatively identified as *p*-hydroxybenzoic acid (Chandrasekara & Shahidi, 2011). Compounds 2 (protocatechuic acid), 5 (gallic acid), and 17 (ellagic acid) were positively identified using authentic standards. The literature has reported that cis isomers usually elute before their trans counterparts during the chromatographic separation (Ma, Kosińska-Cagnazzo, Kerr, Amarowicz, Swanson, & Pegg 2014); therefore, compounds 3 and 4 were tentatively identified as *cis*- and *trans*-*p*-coumaric acids due to their identical deprotonated ion at m/z 163 and a signal at 119 in $MS^2$, which is characteristic of *p*-coumaric acid (de Camargo, Regitano-d'Arce, Gallo, & Shahidi 2015). Accordingly, compounds 6 and 7 were also tentatively identified as *cis*- and *trans*-caffeic acids because both showed m/z signals at 179 in MS and at 135 in $MS^2$, therefore matching

with the characteristic loss of carboxyl group [M - H - $CO_2$]$^-$, as previously reported for caffeic acid (de Camargo, Regitano-d'Arce, Gallo, & Shahidi, 2015). Betés-Saura et al. (1996) also demonstrated that cis-caffeic acid eluted before its trans isomer using a similar solvent system in HPLC, thus supporting our identification. Compound 8 gave deprotonated ion at m/z 181 and at m/z 137, 93 in $MS^2$, the latter ones matching with the identity of *p*-hydroxybenzoic acid (Chandrasekara & Shahidi 2011). Because of the loss of carboxyl group [M-H-$CO_2$]$^-$, compound 8 was tentatively identified as carboxy *p*-hydroxybenzoic acid. Compound 9 showed the same deprotonated ion of compound 8 at m/z 181; however, it gave m/z signals at 163 and 119, which are typical of *p*-coumaric acid as confirmed with an authentic standard. Compound 9 was tentatively identified as hydroxycoumaric acid due to the loss of water [M-H-$H_2O$]$^-$. Compounds 10 and 11 showed the same molecular ion [M-H]$^-$ at m/z 193 and at m/z 135 in $MS^2$ which matches with the fragmentation pattern of ferulic acid (Chandrasekara & Shahidi, 2011). Betés-Saura et al. (1996) reported that *cis*-ferulic acid eluted before *trans*-ferulic acid, thus compounds 10 and 11 were tentatively identified as c*is*- and *trans*-ferulic acids, respectively, in the present study. Compound 12 was tentatively identified as hydroxycaffeic acid (Ambigaipalan, de Camargo, & Shahidi 2016; de Camargo, Regitano-d'Arce, Biasoto, & Shahidi,2014) due to its molecular ion [M-H]$^-$ at m/z 197, followed by loss of water [M-H-$H_2O$]$^-$, which gave m/z at 179 followed by 135 in $MS^2$. The latter ones were compared with an authentic standard of caffeic acid. Compound 13 showed deprotonated ion at m/z 197 and m/z signals at 153 and 109 in $MS^2$, the second two are characteristic of protocatechuic acid, as confirmed with an authentic standard. Therefore, due to loss of carboxyl group [M-H-$CO_2$]$^-$, compound 13 was tentatively identified as carboxyprotocatechuic acid. Compounds 14 and 15 gave [M – H]$^-$ at m/z

223, which is typical of sinapic acid. Accordingly, they were tentatively identified as *cis*- and *trans*-sinapic acids, respectively. Furthermore, several studies (Ambigaipalan, de Camargo, & Shahidi, 2016; Betés-Saura, Andrés-Lacueva, & Lamuela-Raventós 1996; Ma, Kosińska-Cagnazzo, Kerr, Amarowicz, Swanson, & Pegg 2014) have demonstrated that usually *cis* isomers elute first during the chromatographic separation, which supports the present identification. Compound 16 showed deprotonated ion at 285 and gave product ions at 153 and 109. The last two matched with an authentic standard of protocatechuic acid; however, due to its loss of pentose loss of pentose $[M - H - 132]^-$, compound 16 was tentatively identified as protocatechuic acid pentoside. Compound 18, with a deprotonated ion at 353 showed loss of hexose $[M - H -162]^-$ and gave a product ion at 191 which matches with the fragmentation pattern in previous studies (Chandrasekara & Shahidi 2011), thus being tentatively identified as chlorogenic acid. Rosmarinic acid (compound 19) was tentatively identified due to its deprotonated molecular ion at m/z 359, followed by loss of hexosyl group $[M - H - 162]^-$, and two molecules of water $[M - H - 162 - H_2O - H_2O]^-$, thus giving m/z signals at 197, 179, and 161, respectively, which matches with the literature data (Justesen 2000).

**Table 4.2.** Identification of phenolic compounds in camelina, chia and sophia meals by HPLC-MS/MS

| C# | Phenolic acids | MW | [MH]$^-$ (m/z) | MS$^2$ products ions | Camelina | | | Chia | | | Sophia | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | F | E | B | F | E | B | F | E | B |
| 1 | *p*-hydroxybenzoic acid$^+$ | 138 | 137 | 93 | * | | * | * | | | * | | |
| 2 | Protocatechuic acid$^+$ | 154 | 153 | 109 | * | | * | * | | | * | | * |
| 3 | *Cis*-*p*-coumaric acid | 164 | 163 | 119 | | | | * | | | | | |
| 4 | *Trans*-*p*-coumaric acid | 164 | 163 | 119 | * | | | * | * | | * | | |
| 5 | Gallic acid$^+$ | 170 | 169 | 125 | | | * | | | | | | * |
| 6 | *Cis*-caffeic acid | 180 | 179 | 135 | | | | * | * | | | | |
| 7 | *Trans*-caffeic acid | 180 | 179 | 136 | * | * | * | * | * | * | * | | |
| 8 | Carboxy *p*-hydroxybenzoic acid | 182 | 181 | 137, 93 | | | * | * | | | | | * |
| 9 | Hydroxycoumaric acid | 182 | 181 | 163, 119 | | | | | * | | | | |
| 10 | *Cis*-ferulic acid | 194 | 193 | 135 | | | | * | | | | | |
| 11 | *Trans*-ferulic acid | 194 | 193 | 135 | | | | * | * | * | | | |
| 12 | *Cis*-hydroxycaffeic acid | 198 | 197 | 179, 135 | | | * | | | * | | | * |
| 13 | Carboxyl protocatechuic acid | 198 | 197 | 153, 109 | | | | | | | * | | |
| 14 | *Cis*-sinapic acid | 224 | 223 | 179 | | * | | * | * | | * | * | |
| 15 | *Trans*-sinapic acid | 224 | 223 | 179 | * | * | * | | | | * | * | * |
| 16 | Protocatechuic acid pentoside | 286 | 285 | 153, 109 | * | * | * | | | | * | | * |
| 17 | Ellagic acid$^+$ | 302 | 301 | 283, 257 | * | * | * | | * | * | * | * | * |
| 18 | Chlorogenic acid | 354 | 353 | 191 | * | | | | | | * | | |
| 19 | Rosmarinic acid | 360 | 359 | 197,179, 161 | | * | * | * | * | | * | * | |
| | **Flavonoids & Procyanidins** | | | | | | | | | | | | |
| 20 | Genistein | 270 | 269 | 133 | | | | | | * | | | |
| 21 | Glycitein | 284 | 283 | 165 | | | | | | | | | * |
| 22 | Catechin$^+$ | 290 | 289 | 245, 205, 179 | | | * | | | | * | | * |
| 23 | Epicatechin$^+$ | 290 | 289 | 245, 205, 179 | * | | * | | | | * | | * |
| 24 | Quercetin$^+$ | 302 | 301 | 179, 151, 107 | * | | | | * | | * | | |
| 25 | Myricetin | 318 | 317 | 151, 179 | * | * | * | | | | * | * | * |
| 26 | Apigenin | 270 | 269 | 241, 225, 183 | | | | | | | | | |
| 27 | Dihydrodihydroxyisorhamnetin | 354 | 353 | 315 | * | | | * | | | * | | |
| 28 | Daidzein | 416 | 415 | 253 | * | | | * | * | * | | | |
| 29 | Kaempferol-hexoside | 448 | 447 | 285 | | | | * | * | * | * | | * |
| 30 | Isorhamnetin-pentoside | 448 | 447 | 315 | | | | | | | * | | * |
| 31 | Quercetin-hexoside | 464 | 463 | 301, 179, 151 | * | * | * | * | | | * | * | * |
| 32 | Rutin | 610 | 609 | 301 | | * | * | | * | | * | | * |
| 33 | Procyanidin dimer A type | 576 | 575 | 285, 407, 449 | | | | | | | * | | * |
| 34 | Procyanidin dimer B1 | 578 | 577 | 289, 425, 451 | | | | * | | | | | |
| 35 | Procyanidin dimer B2 | 578 | 577 | 289, 407, 425 | * | | | | | | * | | |
| 36 | Procyanidin dimer B3 | 578 | 577 | 289, 425, 452 | | | | | * | | | * | * |

Abbreviations are: MW, molecular weight; [M-H]$^-$ is deprotonated molecular; C#, compound number; F, free; E, esterified; and IB, insoluble-bound. * Indicates the presence of the compound in the fraction. $^+$ Identified with authentic standard

**Table 4.3.** Quantification of phenolic compounds in camelina, chia and sophia defatted meal (μg per gram sample) by HPLC-MS/MS.

| | **Free Phenolics** | | | | |
|---|---|---|---|---|---|
| No. | Compounds | MW | Camelina | Chia | Sophia |
| 1 | *p*-Hydroxybenzoic acid | 138 | 13.78 ± 0.78 | tr | 43.82 ± 2.97 |
| 2 | Protocatechuic acid | 154 | 19.34 ± 0.32 | 13.01 ± 0.96 | 19.09 ± 2.64 |
| 3 | *Cis*-*p*-coumaric acid | 164 | nd | 4.02 ± 0.60 | nd |
| 4 | *Trans*-*p*-coumaric acid | 164 | 10.41 ± 0.01 | 12.42 ± 1.32 | 4.82 ± 0.63 |
| 5 | Gallic acid | 170 | nd | nd | nd |
| 6 | *Cis*-caffeic acid | 180 | nd | tr | nd |
| 7 | *Trans*-caffeic acid | 180 | 10.81 ± 1.92 | 10.16 ± 0.76 | 6.08 ± 0.48 |
| 8 | Carboxyl *p*-hydroxybenzoic acid | 180 | nd | tr | nd |
| 9 | Hydroxycoumaric acid | 182 | nd | tr | nd |
| 10 | *Cis*- ferulic acid | 194 | nd | 32.97 ± 4.38 | nd |
| 11 | *Trans*-ferulic acid | 194 | nd | 54.67 ± 0.81 | nd |
| 12 | *Cis*-hydroxycaffeic acid | 198 | nd | nd | tr |
| 13 | Carboxyl protocatechuic acid | 198 | nd | nd | 8.96 ± 0.13 |
| 14 | *Cis*-sinapic acid | 224 | nd | tr | tr |
| 15 | *Trans*-sinapic acid | 224 | 619.8 ± 4.13 | nd | 396.7 ± 7.6 |
| 16 | Protocatechuic acid pentoside | 286 | tr | nd | nd |
| 17 | Ellagic acid | 302 | tr | nd | nd |
| 18 | Chlorogenic acid | 354 | 17.02 ± 1.67 | nd | tr |
| 19 | Rosmarinic acid | 360 | nd | 738.2 ± 17.9 | tr |
| | Total free phenolic acid | | 691.16 | 865.4 | 479.47 |
| | Flavonoids & Procyanidins | | | | |
| 20 | Genistein | 270 | nd | nd | nd |
| 21 | Glycitein | 284 | nd | nd | nd |
| 22 | Catechin | 290 | nd | nd | 45.06 ± 3.88 |
| 23 | Epicatechin | 290 | nd | tr | nd |
| 24 | Quercetin | 302 | 56.32 ± 7.43 | nd | tr |
| 25 | Myricetin | 318 | 22.64 ± 6.48 | 10.07 ± 1.39 | 12.80 ± 1.32 |
| 26 | Apigenin | 270 | nd | nd | nd |
| 27 | Dihydrodihydroxyisorhamnetin | 354 | 21.73 ± 1.69 | 36.02 ± 3.5 | tr |
| 28 | Daidzein | 416 | 477.2 ± 71.1 | 734.1 ± 16.2 | 734.1 ± 16.2 |
| 29 | Kaempferol-hexoside | 448 | nd | tr | 1889.2 ± 16.39 |
| 30 | Isorhamnetin-pentoside | 448 | nd | nd | 413.2 ± 4.27 |
| 31 | Quercetin-hexoside | 464 | 89.28 ± 0.34 | tr | 283.28 ± 8.97 |
| 32 | Rutin | 610 | 277.5 ± 0.77 | nd | nd |
| | Total free flavonoids | | 944.67 | 780.19 | 3377.64 |
| 33 | Procyanidin dimer A type | 576 | nd | nd | 28.52 ± 2.05 |
| 34 | Procyanidin dimer B1 | 578 | nd | 22.67 ± 2.50 | nd |
| 35 | Procyanidin dimer B2 | 578 | nd | nd | nd |
| 36 | Procyanidin dimer B3 | 578 | nd | nd | nd |
| | Total free procyanidins | | - | 22.67 | 28.52 |
| | **Total phenolics content** | | **1686.59** | **1668.26** | **4013.39** |

Data represent the mean values for each sample ± standard deviations (n = 3).

**Table 4. 3 continued…**

| No. | Esterified phenolics Compounds | MW | Camelina | Chia | Sophia |
|---|---|---|---|---|---|
| Phenolic acids | | | | | |
| 1 | *p*-Hydroxybenzoic acid | 138 | nd | nd | tr |
| 2 | Protocatechuic acid | 154 | nd | nd | nd |
| 3 | *Cis*-*p*-coumaric acid | 164 | | nd | |
| 4 | *Trans*-*p*-coumaric acid | 164 | tr | 6.01 ± 0.01 | nd |
| 5 | Gallic acid | 170 | nd | nd | tr |
| 6 | *Cis*-caffeic acid | 180 | nd | 178.6 ± 17.8 | nd |
| 7 | *Trans*-caffeic acid | 180 | 16.36 ± 0.32 | 42.4 ± 1.65 | nd |
| 8 | Carboxyl p-hydroxybenzoic acid | 180 | nd | nd | 9.79 ± 0.43 |
| 9 | Hydroxycoumaric acid | 182 | nd | tr | nd |
| 10 | *Cis*-ferulic acid | 194 | nd | nd | nd |
| 11 | *Trans*-ferulic acid | 194 | nd | tr | nd |
| 12 | *Cis*-hydroxycaffeic acid | 198 | tr | nd | nd |
| 13 | Carboxyl protocatechuic acid | 198 | nd | nd | 8.96 ± 0.13 |
| 14 | *Cis*-sinapic acid | 224 | 13.98 ± 0.55 | tr | 22.61 ± 0.30 |
| 15 | *Trans*-sinapic acid | 224 | 1899 ± 10.16 | nd | 481.1 ± 4.82 |
| 16 | Protocatechuic acid pentoside | 286 | tr | nd | nd |
| 17 | Ellagic acid | 302 | 25.21 ± 0.51 | tr | tr |
| 18 | Chlorogenic acid | 354 | nd | nd | nd |
| 19 | Rosmarinic acid | 360 | tr | 31.03 ± 2.98 | tr |
| Total esterified phenolic | | | 1954.55 | 258.04 | 522.46 |
| Flavonoids | | | | | |
| 20 | Genistein | 270 | nd | nd | nd |
| 21 | Glycitein | 284 | nd | nd | 91.98 ± 12.66 |
| 22 | Catechin | 290 | nd | nd | tr |
| 23 | Epicatechin | 290 | tr | nd | nd |
| 24 | Quercetin | 302 | tr | 309.5 ± 12.51 | tr |
| 25 | Myricetin | 318 | 20.66 ± 1.03 | 18.24 ± 0.77 | tr |
| 26 | Apigenin | 270 | nd | 76.51 ± 1.75 | nd |
| 27 | Dihydrodihydroxyisorhamnetin | 354 | nd | nd | tr |
| 28 | Daidzein | 416 | tr | 110.46 ± 10.56 | tr |
| 29 | Kaempferol-hexoside | 448 | tr | tr | nd |
| 30 | Isorhamnetin-pentoside | 448 | nd | nd | tr |
| 31 | Quercetin-hexoside | 464 | 99.79 ± 2.94 | nd | 91.05 ± 2.39 |
| 32 | Rutin | 610 | tr | 83.38 ± 1.84 | nd |
| Total esterified flavonoids | | | 120.45 | 598.09 | 183.03 |
| Proanthocyanidins | | | | | |
| 33 | Procyadinin dimer A type | 576 | nd | nd | tr |
| 34 | Procyadinin dimer B1 | 578 | nd | nd | tr |
| 35 | Procyadinin dimer B2 | 578 | tr | nd | nd |
| 36 | Procyadinin dimer B3 | 578 | 6.95 ± 0.21 | 23.94 ± 5.7 | nd |
| | Total esterified proanthocyanidins | | 6.95 | 23.94 | - |
| | **Total esterified phenolic** | | **2081.95** | **880.07** | **705.49** |

**Table 4.3 continued…**

| Insoluble bound phenolics | | | | | |
|---|---|---|---|---|---|
| No. | Compounds | MW | Camelina | Chia | Sophia |
| Phenolic acids | | | | | |
| 1 | *p*-Hydroxybenzoic acid | 138 | 16.60 ± 0.47 | nd | nd |
| 2 | Protocatechuic acid | 154 | 31.11 ± 1.51 | nd | 17.24 ± 1.55 |
| 3 | *Cis*-*p*-coumaric acid | 164 | nd | nd | nd |
| 4 | *Trans*-*p*-coumaric acid | 164 | nd | nd | nd |
| 5 | Gallic acid | 170 | 8.86 ± 0.09 | nd | 4.79 ± 0.05 |
| 6 | *Cis*-caffeic acid | 180 | nd | nd | nd |
| 7 | *Trans*-caffeic acid | 180 | 7.19 ± 0.22 | 72.02 ± 0.71 | nd |
| 8 | Carboxyl p-hydroxybenzoic acid | 180 | tr | nd | nd |
| 9 | Hydroxycoumaric acid | 182 | nd | nd | nd |
| 10 | *Cis*- ferulic acid | 194 | nd | nd | nd |
| 11 | *Trans*- ferulic acid | 194 | nd | 69.70 ± 0.21 | nd |
| 12 | *Cis*-hydroxycaffeic acid | 198 | 5.08 ± 0.01 | 67.44 ± 0.49 | 2.87 ± 0.03 |
| 13 | Carboxyl protocatechuic acid | 198 | tr | nd | nd |
| 14 | *Cis*-sinapic acid | 224 | nd | nd | nd |
| 15 | *Trans*-sinapic acid | 224 | 172.02 ± 8.72 | nd | 70.48 ± 3.4 |
| 16 | Protocatechuic acid pentoside | 286 | nd | nd | nd |
| 17 | Ellagic acid | 302 | 3.54 ± 0.46 | tr | tr |
| 18 | Chlorogenic acid | 354 | nd | nd | nd |
| 19 | Rosmarinic acid | 360 | tr | nd | 31.03 ± 2.98 |
| Total insoluble bound phenolic | | | 244.4 | 209.16 | 126.41 |
| Flavonoids | | | | | |
| 20 | Genistein | 270 | nd | 607.8 ± 6.64 | nd |
| 21 | Glycitein | 284 | | nd | |
| 22 | Catechin | 290 | 12.49 ± 0.22 | nd | 8.78 ± 0.25 |
| 23 | Epicatechin | 290 | nd | nd | nd |
| 24 | Quercetin | 302 | nd | nd | tr |
| 25 | Myricetin | 318 | 5.45 ± 0.66 | | 3.01 ± 0.40 |
| 26 | Apeginin | 270 | nd | 152.5 ± 1.93 | nd |
| 27 | Dihydrodihydroxyisorhamnetin | 354 | nd | nd | nd |
| 28 | Daidzin | 416 | tr | tr | nd |
| 29 | Kaempferol-hexoside | 448 | nd | 76.18 ± 0.10 | tr |
| 30 | Isorhamnetin-pentoside | 448 | nd | nd | tr |
| 31 | Quercetin-hexoside | 464 | 48.49 ± 6.47 | nd | 21.54 ± 0.94 |
| 32 | Rutin | 610 | tr | nd | 21.54 ± 0.64 |
| Total insoluble bound flavonoids | | | 66.43 | 335.54 | 54.87 |
| Proanthocyanidins | | | | | |
| 33 | Procyadinin dimer A type | 576 | nd | 3.10 ± 0.30 | 6.22 ± 0.004 |
| 34 | Procyadinin dimer B1 | 578 | nd | nd | tr |
| 35 | Procyadinin dimer B2 | 578 | tr | nd | nd |
| 36 | Procyadinin dimer B3 | 578 | 5.29 ± 1.84 | 30.49 ± 2.12 | nd |
| Insoluble bound proanthocyanidins | | | 5.29 | 33.59 | 6.22 |
| **Total insoluble phenolic content** | | | **316.12** | **578.29** | **187.45** |

The quantification of phenolic acids of camelina, chia and sophia defatted meals is summarized in Table 4.3 as determined by HPLC-DAD-MS/MS. Both camelina and sophia esterified fractions contained higher amounts of phenolic acids (1954.55 and 522.46 µg per gram sample, respectively) compared to the free and insoluble-bound fractions while the free fraction of chia seed meal showed higher amounts of phenolic acids (865.45 µg per gram sample) compared to the esterified (258.04 µg per gram sample) and the insoluble-bound fractions (209.16 µg per gram sample), respectively. Sinapic acid was the major phenolic acid found in the free and esterified fractions of both camelina and sophia seeds with concentrations of 2532.78 and 900.41 µg per gram sample, respectively. Rosmarinic acid was the major phenolic acid found in the free and esterified fractions with concentrations of 738.2 ± 17.9 and 31.03 ± 2.98 µg per gram sample, respectively, while total contribution (free, esterified & bound) of caffeic acid and ferulic acid was 303.13 and 157.34 µg per gram sample, respectively, as both *cis* and *trans* forms. Martinez-Cruz and Paredes-Lopez (2014) also identified and quantified rosmarinic acid as the major phenolic compound in chia seed with a concentration of 926 µg per gram sample which is higher compared to our findings; the differences may possibly be due to differences in genetic and environmental factors. Rosmarinic acid has been reported to have many biological activities such as inflammatory, antiviral, antibacterial, antimutagen and astringent (Martinez-Cruz & Paredes-Lopez 2014; Tepe 2008). Caffeic acid was the second major phenolic acid quantified in chia meal with a higher content of 303.13 µg per gram sample than that reported by Martinez-Cruz and Paredes-Lopez (2014) for Mexican chia seeds (27.4 µg per gram seed). Caffeic acid is a good free radical scavenger and enzyme inhibitor, and it may also show binding activity with specific receptors (Martinez-Cruz & Paredes-Lopez 2014; Son & Lewis 2002). Olthof,

Hollman, and Katan (2001) reported that caffeic acid inhibits low density lipoprotein (LDL) oxidation *in vitro* and thus might protect against cardiovascular diseases. A small amount of gallic acid was found in the insoluble-bound fraction of the both camelina and sophia seed samples at 8.86 and 4.79 µg per gram sample, respectively, but gallic acid was not identified in chia. Moreover, camelina showed a higher total phenolic acid content (2890.11 µg per gram sample) than chia and sophia seed meal (1332.65 and 1128.34 µg per gram sample, respectively).

### 4.4.2 Flavonoids and procyanidins

Eleven (11) flavonoids and four (4) procyanidins were positively or tentatively identified

Freo camelina, chia and sophia seed meals (Table 4.2). Genistein (m/z 269 & 133), which was detected only in the fraction containing phenolics released from their insoluble-bound form of chia, was tentatively identified as compound 20 (Orcic et al. 2014). Compounds 22, 23, and 24 were positively identified by comparing their retention times and fragmentation patterns with those of authentic standards. Compounds 25 and 26 were tentatively identified as myricetin according to the published data and comparing their retention time, fragments ions and UV spectral data (Chandrasekara & Shahidi 2011). Compound 27 was tentatively identified as dihydrodihydroxyisorhamnetin due to its deprotonated molecular ion $[M - H]^-$ at m/z 353 and an $MS^2$ product ion at m/z 315, the latter one reflecting the loss of two atoms of hydrogen and one molecule of water $[M- H - H - H - H_2O]$. Compound 28 was tentatively identified as daidzin, an isoflavone in the glucoside form, because it gave $[M - H]^-$ at m/z 415 and an $MS^2$ signal at m/z 253 (Chen, Zhao, Plummer, Tang, & Games 2005), which is consistent with the loss of hexose $[M - H - 162]^-$, thus releasing the aglycone daidzein. Kaempferol-hexoside

(compound 29) with the deprotonated molecular ion at m/z 447, showed loss of hexose and gave an $MS^2$ product ion at 285, which matches with the molecular weight of kaempferol, therefore being tentatively identified as such (de Camargo, Regitano-d'Arce, Biasoto, & Shahidi 2014). Similar to kaempferol-hexoside, compound 30 showed deprotonated molecular ion at m/z 447; however, it gave $MS^2$ signal at 315 (de Camargo, Regitano-d'Arce, Gallo, & Shahidi 2015), which matches the molecular weight of isorhamnetin, therefore allowing its tentatively identification as isorhamnetin-pentoside. Compound 31, which was tentatively identified as quercetin-hexoside (de Camargo, Regitano-d'Arce, Biasoto, & Shahidi 2014), exhibited a deprotonated molecular ion at m/z 463, followed by loss of hexose which gave m/z at 301 in $MS^2$. Other fragment ions (m/z at 179 and at 151 in $MS^2$) were confirmed by using a quercetin standard. Compound 32 was tentatively identified as rutin according to the fragmentation pattern reported in the literature (Gavrilova, Kajdžanoska, Gjamovski, & Stefova 2011). Procyanidin dimer A type (compound 33) was indentified temporaily with published data matched up products ions/ ions fragments reported by Ambigaipalan, de Camargo, & Shahidi (2016). Compounds 34, 35, and 36 were identified positively or tentatively as procyanidin dimers B1, B2, and B3, respectively. Compound 34 produced a molecular ion $[M - H]^-$ at m/z 562 with its $MS^2$ fragment ions at m/z 289, 425, 451 which matched with published data (Chandrasekara, & Shahidi 2011; Ambigaipalan, de Camargo, & Shahidi 2016). Compound 35 was identified as procyanidin dimer B2 that had a deprotonated ion at m/z 575 and fragmentation in $MS^2$ produced ions at m/z 289 (typical deprotonated (epi) catechin) and m/z 407, 425, and 451, which is consistent with previously published data (Chandrasekara, & Shahidi 2011; Ambigaipalan, de Camargo, & Shahidi 2016). Procyanidin dimer B2 consists of epicatechin-(4β-8)-epicatechin.

Similarly, compound 36 was identified as procyanidin dimer B3 based on its molecular ion $[M-H]^-$ at 577 and $MS^2$ fragments at m/z 289, 425, and 451 (Chandrasekara, & Shahidi 2011; Ambigaipalan, de Camargo, & Shahidi 2016).

Kaempferol-hexoside was the major flavonoid (1889.2 ± 16.39 µg per gram sample) in sophia seeds and found in the free phenolics fraction followed by daidzein (734.1 ± 16.2 µg per gram sample), isorhamnetin-pentoside (413.2 ± 4.27 µg per gram sample), quercetin-hexoside (283.28 ± 8.97 µg per gram sample), catechin (45.06 ± 3.88 µg per gram sample), myricetin (12.80 ± 1.32 µg per gram sample), all of which were found in the same fraction of sophia seed. On the other hand, daidzein (477.2 ± 71.1 µg per gram sample) and rutin (277.5 ± 0.77 µg per gram sample) were found in the free phenolics fraction of camelina seed as a major flavonoid followed by quercetin (56.32 ± 7.43 µg per gram sample), quercetin-hexoside (89.28 ± 0.34 µg per gram sample) and myricetin (22.64 ± 6.48 µg per gram sample). Quercetin-hexoside and myricetin were found in the free, esterified and insoluble-bound fractions of camelina seed (Table 4.3) where their corresponding total contributions were 237.56 and 48.75 µg per gram sample, respectively. Daidzein was the major flavonoid found in the free (734.1 ± 16.2 µg per gram sample) and esterified (110.46 ± 10.56 µg per gram sample) fractions of defatted chia seeds. Martinez-Cruz and Paredes-Lopez (2014) also quantified daidzein in chia seed with a concentration of 6.6 µg per gram sample which is lower than our finding, possibly because of differences in genotype and agronomic conditions. Quercetin (309.5 ± 12.51 µg per gram sample) and rutin (83.38 ± 1.84 µg per gram sample) were found in the esterified fraction but only trace amounts of quercetin were found in the insoluble-bound fraction. Genistein and quercetin-hexoside were detected only in the insoluble-

bound phenolic fraction of chia at concentrations of 91.98 ± 12.66 and 91.05 ± 2.39 µg per gram sample, respectively. Myricetin and apigenin were identified for the first time in chia; myricetin was present in the free, esterified, and insoluble-bound fractions (10.07 ± 1.39, 18.24 ± 0.77and 2.07 ± 0.49 µg per gram sample, respectively) and apigenin was found in the esterified and insoluble-bound fractions only at concentrations of 76.51 ± 1.75 & 152.51 ± 1.93 µg per gram sample, respectively. Flavonoids, especially isoflavones, are well known as anti-carcinogenic agents and they also have numerous applications in the prevention of inflammation, cardiovascular diseases and many other disorders (Martinez-Cruz & Paredes-Lopez 2014; Vacek et al. 2008). Quantification of flavonoids by HPLC-DAD-MS/MS (Table 4.3) revealed that free phenolics (as flavonoids) fraction of both camelina and sophia existed in higher amounts compared to esterified and insoluble-bound fractions while chia seed revealed that free phenolics (as flavonoids) fraction was present in higher amounts (780.19 µg per gram sample) compared to esterified and insoluble-bound fractions (598.09 & 335.54 µg per gram sample, respectively).

Proanthocyanin dimer B1 and B2 ware found in the free phenolics fraction of both camelina and sophia seeds at 50.76 ± 4.64 and 127.76 ± 3.23 µg per gram sample, respectively, whereas proanthocyanin dimer B3 was also present in the insoluble-bound fraction of camelina seed (5.29 ± 1.84 µg per gram sample). For chia seed meal, procyanidin dimer B1 was indentified only in the free fraction (22.67 ± 2.50 µg per gram sample) whereas procyanidin dimer B3 was found in both esterified and insoluble-bound fractions at 23.94 ± 5.7and 30.49 ± 2.12 µg per gram sample, respectively and procyanidin dimer B2 exited in trace amounts in the insoluble-bound fraction.

Proanthocyanin dimer A type is another proanthocyanidins which was identified and quantified by HPLC-DAD-MS/MS at 28.52 ± 2.05 and 6.22 ± 0.004 µg per gram sample, respectively, in the esterified and insoluble-bound fractions of both camelina and sophia meal whereas Procyanidin dimer A type was found (3.10 ± 0.30 µg per gram sample) only in the insoluble-bound fraction of chia. This is the first comprehensive study for identification and quantification of procyanidins in chia meal. Therefore, no information is available on quantification of procyanidins of camelina, chia and sophia meals to compare our results with it. The sum of the concentrations of all phenolic compounds detected in camelina, chia and sophia meals by HPLC-DAD-MS/MS was much lower than the TPC determined by Folin-Ciocalteu method (Table 4.1), which shows possible interference of other non-phenolic compounds in the latter method, and to a lesser extent, because of incomplete quantification of all compounds in the HPLC-DAD-MS/MS method. In addition, total flavonoid content by HPLC-DAD-MS/MS was lower than that by the $AlCl_3$ method, possibly due to conjugation of flavonoids with soluble components such as small peptides or oligosaccharides (Zhang et al. 2015).

## 4.5 *In vitro* antioxidant and biological activities of defatted camelina, chia and sophia meals

Antioxidant activity is the most extensively investigated chemical property of phenolic and polyphenolic compounds. Phenolic compounds exhibit antioxidant activity because of their redox potential through transfer of a hydrogen atom or an electron, acting as a reducing agent, and possibly also by chelation of metal ions and inhibition of the activity of oxidases. In this study, we determined ABTS, DPPH, and hydroxyl radical scavenging, reducing power (RP), metal ion chelation and inhibition of bleaching of β-carotene in an oil-in-water emulsion system of the test samples in order to investigate the primary and

secondary oxidation inhibition ability of the phenolic extracts of camelina, chia and sophia seed meals. Several *in vitro* biological assays such inhibition of pancreatic lipase, α-glucosidase, LDL oxidation and DNA strand scission were also investigated.

### 4.5.1 Trolox equivalent antioxidant capacity (TEAC)

The TEAC assay is widely used to assess the antioxidant capacity of different biological matrices. The ability of antioxidant compounds to reduce the ABTS radical cation to its non- radical form is compared with that of trolox, which is a water-soluble analogue of alpha-tocopherol. In this study, the TEAC test is performed in an aqueous buffer; thus, only water-soluble compounds are measured. In the modified TEAC assay (van den Berg et al. 1999) that was used in this study, ABTS radical cations with a characteristic blue-green colour were pre-generated by heating ABTS with the thermolabile azo compound AAPH before addition of the extracts. As documented in the literature (van den Berg et al. 1999), some compounds show a biphasic reaction pattern that includes fast and slow reactions in the TEAC assay. Therefore, TEAC values depend on the time point used to read the absorbance. In the present study, TEAC at 6 min was chosen as it includes more of the slow reaction as many antioxidants also demonstrate a slow reaction as well with ABTS radical (Madhujith & Shahidi 2006; Liyana-Pathirana & Shahidi 2007).

The trolox equivalent antioxidant capacity (TEAC) of defatted camelina, chia and sophia seed meals as µmole trolox equivalents/g sample is shown in Table 4.4; the total values were 178.38 ± 0.80, 138.17 ± 4.98 and 344.87 ± 7.5 µmole TE per gram defatted sample, respectively. Sophia seed meal showed a significantly ($p< 0.05$) higher total TEAC value (344.87 ± 7.5 µmole TE per gram sample) than camelina (178.38 ± 0.80 µmole TE per gram sample) and chia (138.17 ± 4.98 µmole TE per gram sample), respectively. In this

study, all seed meals had significantly ($p$ < 0.05) different ABTS radical scavenging capacity among their three phenolic fractions. The TEAC value of free, esterified, and insoluble-bound fractions of camelina, chia and sophia were in the range of 14.11 ± 2.51 to 261.29 ± 6.04 µmole TE per gram defatted meals. The esterified extract of sophia showed the highest TEAC value (261.29 ± 6.04 µmole TE per gram sample) compared to the other fractions between three varieties tested. In the case of insoluble-bound phenolic extracts, chia showed a higher TEAC values (58.35 ± 1.89 µmole TE per gram sample) compared to camelina (14.11 ± 2.51 µmole TE per gram sample) and sophia (39.54 ± 1.6 µmole TE per gram sample). The TEAC value of camelina and sophia esterified phenolics was significantly ($p$< 0.05) higher than those of free and insoluble-bound phenolics in both seeds whereas chia meals had a higher TEAC value in its free phenolic fraction (Table 4.4). This may be due to the high phenolic content found in Folin-Ciocalteu test for sophia esterified phenolic fraction. There was no significance difference ($p$ > 0.05) famong camelina free and sophia free and esterified phenolics. No previus study on the ABTS radical scavenging activity of camelina and sophia seed meals has been carried out, therefore direct comparison with the literature data is not possible. However, in case of chia seed meal, our ABTS result was higher (138.17 ± 4.98 µmole TE per gram sample) tahn that of the Mexican chia (84.51 ± 6.38 µmole TE per gram) but lower than Brazilian chia (255.88 ± 16.94 µmole TE per gram), as reported by Dick et al. (2015). Vázquez-Ovando et al. (2009) also reported the ABTS radical scavenging capacity of Mexican chia as 488.88 µmole TE per gram, which is much higher than our finding. This variation may be attributed to differences in genotype, extraction procedure used and other agronomic conditions of chia seed tested. The trolox equivalent antioxidant capacity (TEAC) and total phenolics content (TPC) of different

fractions of camelina, chia and sophia were highly correlated ($r^2$ = 0.91; $p$<0.01). This suggests that different phenolics fractions of defatted camelina, chia and sophia meals might have excellent free radical scavenging power.

### 4.5.2 DPPH radical scavenging capacity (DRSC) by EPR

DPPH radical is a synthetic organic radical with a deep purple colour that is widely used to evaluate free radical scavenging properties of foods and plant extracts as well as purified phenolic compounds. DPPH is a more stable radical compared to the highly reactive and transient hydroxyl and peroxyl radicals that are responsible for lipid peroxidation and tissue injury in biological systems (Chandrasekara & Shahidi 2010). The DRSC assay is based on the ability of antioxidants to donate a hydrogen atom or an electron to the DPPH radical to convert it to the non-radical form, which occurs slowly (Chandrasekara & Shahidi 2010; Moon & Shibamoto 2009). In the present study EPR spectroscopy was used to determine signal intensity of DPPH radical left following reaction with phenolic extracts of camelina, chia and sophia meals in the test system (Figure 4.1).

Table 4.4 shows DPPH radical scavenging capacity (DRSC) of defatted seeds of camelina, chia and sophia which as 27.82 ± 1.44, 35.64 ± 2.44 and 71.05 ± 5.61 µmole TE per gram defatted sample, respectively. Sophia seed meal was highly effective in DPPH radical scavenging compared to chia and camelina seed meals. The total DRSC of free, esterified and insoluble-bound phenolics fractions of defatted meals of camelina, chia and sophia ranged from 1.28 ± 0.31 to 55.93 ± 6.04 µmole TE per gram sample. Sophia meal esterified fraction had the highest efficacy (55.93 ± 6.04 µmole TE per gram sample) in DPPH radical scavenging compared to all fractions tested for camelina, chia and sophia

seed meals. Similar results were found for ABTS radical scavenging and reducing power assays, possibly due to the high phenolic content of sophia meal esterified fraction and the high content of phenolics, especially tannins and flavonoids. Chia free fraction showed a higher DPPH radical inhibition effect compared to its esterified and insoluble-bound fraction while esterified phenolic fraction in both camelina and sophia seeds showed significantly ($p<0.05$) higher DPPH radical scavenging activity (23.93 ± 2.00 and 55.22 ± 6.04 µmole TE per gram sample, respectively) compared to their free and insoluble-bound counterparts. There was no significant difference in free and insoluble-bound phenolics of camelina and sophia meals (Table 4.4). No information is available on DPPH radical scavenging activity of camelina and sophia seed meals to compare our results with published data, however, chia seed meal showed much lower total DPPH radical scavenging activity (27.7 ± 1.14 per gram sample) than DPPH results for Brazilian chia seeds (436.61 ± 9.67 µmole per gram seed) reported by de Silva Marineli et al. (2014) that might be attributed to genotype and other environmental factors. The DRSC and total phenolic content (TPC) was highly correlated ($R^2$= 0.97), indicating that camelina, chia and sophia phenolics may play a role in quenching free radicals in both food and biological systems.

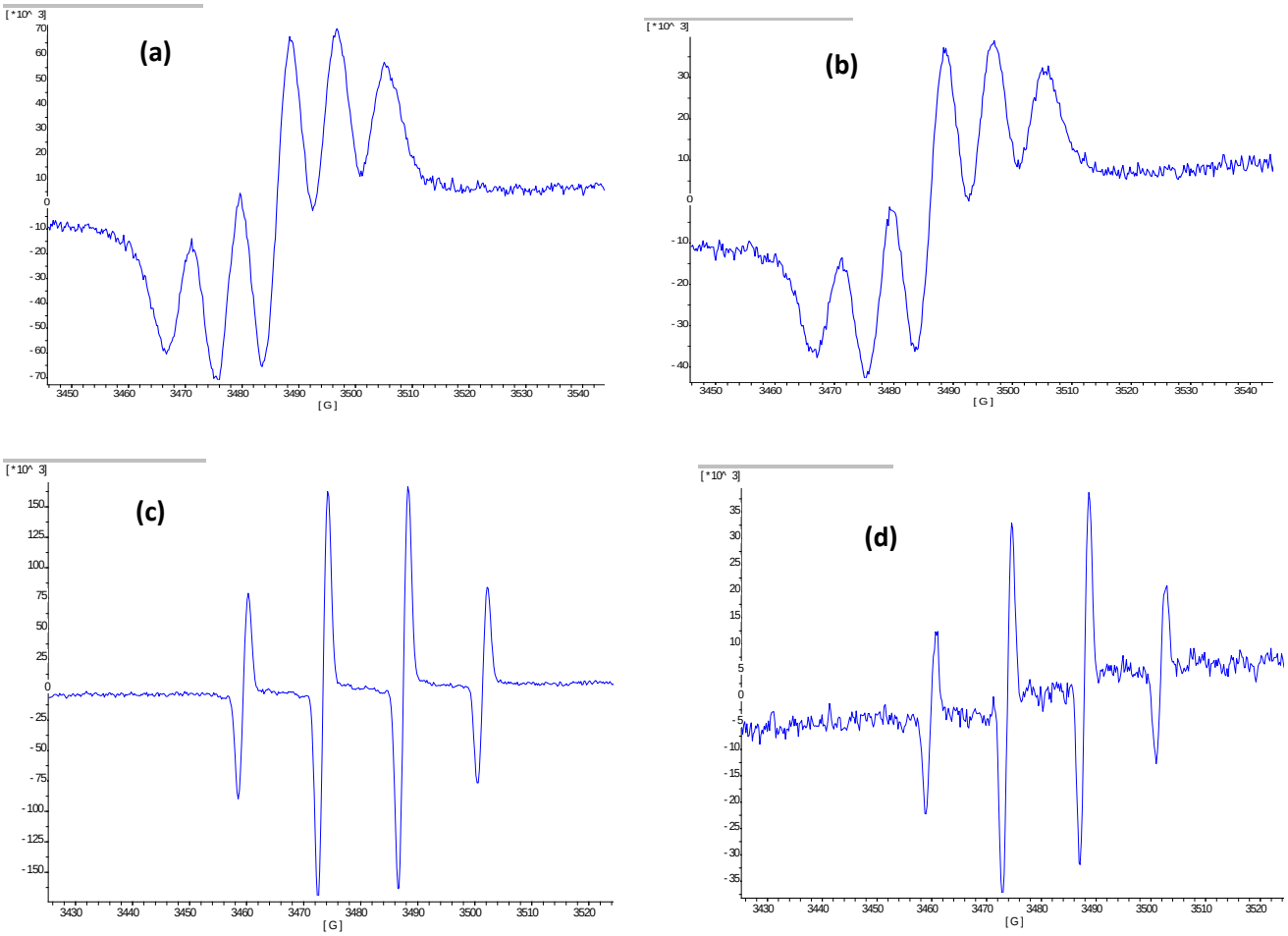



**Figure 4.1.** The EPR signal intensity of DPPH alone (a) and DMPO-OH radical adduct alone. The EPR signal intensity of DPPH (b) and DMPO-OH radical adduct (d) was significantly reduced in the presence of sophia esterified phenolic extract.

**Table 4.4.** Antioxidant activity of defatted camelina, chia and sophia samples reflected in their reducing power and free radical scavenging activity by different methods

| Samples | Free phenolic | Esterified Phenolic | Insoluble-Bound | Total |
|---|---|---|---|---|
| Trolox equivalent antioxidant capacity (µmole TE/ g defatted sample) | | | | |
| Camelina | 44.55 ± 2.89Be | 119.72 ± 1.01Ab | 14.11 ± 2.51Cf | 178.38 ± 0.80B' |
| Chia | 73.40 ± 0.96Ac | 6.42 ± 3.04Cf | 58.35 ± 1.89Bd | 138.17 ± 4.98C |
| Sophia | 44.04 ± 1.77 Be | 261.29 ± 6.04 Aa | 39.54 ± 1.6Be | 344.87 ± 7.50A' |
| Reducing power (µmole TE/ g defatted sample) | | | | |
| Camelina | 39.51 ± 0.70Bd | 59.40 ± 1.62Ac | 6.44 ± 0.10Ci | 105.35 ± 1.8C' |
| Chia | 73.06 ± 0.64Ab | 10.17 ± 0.19Ch | 37.19 ± 0.13Bf | 120.42 ± 0.46B' |
| Sophia | 49.74 ± 0.39Bd | 81.30 ± 0.82Aa | 26.05 ± 0.66 Cg | 157.10 ± 0.85A' |
| DPPH scavenging activity (µmole TE/ g defatted sample) | | | | |
| Camelina | 7.93 ± 0.29Bc | 23.93 ± 2.00Ab | 3.78 ± 0.93Cc | 35.64 ± 2.44B' |
| Chia | 16.56 ± 0.75Ab | 1.28 ± 0.31Cf | 9.98 ± 0.50Bc | 27.82 ± 1.44C' |
| Sophia | 8.33 ± 0.17Bc | 55.22 ± 6.04Aa | 7.50 ± 0.74Bc | 71.05 ± 5.61 A' |
| Hydroxyl radical scavenging activity (µmole CE/ g defatted sample) | | | | |
| Camelina | 4.84 ± 1.18Bc | 21.93 ± 3.85Ab | 2.21 ± 0.68Bc | 28.98 ± 4.28B' |
| Chia | 23.29 ± 6.18Ab | 21.73 ± 1.96Ab | 17.90 ± 1.37Ab | 62.92 ± 8.96A |
| Sophia | 22.46 ± 1.47Bb | 39.26 ± 1.50Aa | 5.36 ± 0.97Cc | 67.09 ± 3.32A' |
| Metal chelation activity (µmole EDTA/ g defatted sample) | | | | |
| Camelina | 12.92 ± 0.04 Ab | 10.88 ± 0.41 Bc | 13.87 ± 0.83Aab | 37.02 ± 4.28 |
| Chia | 6.29 ± 0.33Be | 14.28 ± 0.15Aa | 2.50 ± 0.11C f | 23.08 ± 0.47 |
| Sophia | 7.92 ± 0.56 Bd | 9.76 ± 0.21 Ac | 6.91 ± 0.07 Cde | 24.61 ± 0.59 |

Data represent the mean values for each sample ± standard deviations (n = 3. Means followed by the different upper case letters within a row and different lower case letters among all fractions are significantly different *($p < 0.05$)* but same letters indicate are not significantly different ($p > 0.05$). TE, Trolox equivalents and CE, Catechin equivalents, EDTA, Ethylenediaminetetraacetic acid.

### 4.5.3 Hydroxyl radical scavenging capacity (HRSC)

Hydroxyl radical (•OH) is an extremely reactive oxygen-centered radical, which can be generated in the body and may attack all biological molecules such as DNA, proteins, and polyunsaturated fatty acids (PUFA) in membranes, among others (Chandrasekara & Shahidi 2010). In addition, its significant role as an initiator of lipid peroxidation is well documented. In this study, hydroxyl radicals were produced through the Fenton reaction, in which ferrous sulphate reacts with $H_2O_2$ to generate hydroxyl radicals. As the

resulting hydroxyl radicals are short-lived, a spin trap, 5, 5-dimethyl-1-pyrrolidine-*N*-oxide (DMPO) is used to make them stable for detection by EPR spectrometry as DMPO-OH radical adducts. Table 4.4 shows the hydroxyl radical scavenging capacity (HRSC) of defatted meals of camelina, chia and sophia. Sophia meal showed a higher hydroxyl radical inhibition activity compared to camelina and chia seed meals. In the present study, the hydroxyl radical scavenging capacity (HRSC) of free, esterified and insolube-bound phenolic extracts of defatted seeds of camelina, chia and sophia ranged from 2.21± 0.68 to 39.26± 1.50 µmole CE per gram defatted sample (Table 4.4). Esterified extracts of sophia showed the highest ($p$<0.05) HRSC value (39.26 ±1 .50 µmole CE per gram defatted sample) compared to other phenolics extracts of camelina, chia and sophia tested. For camelina and sophia, esterified fraction showed a higher hydroxyl radical scavenging activity compared to the free and insoluble-bound fractions whereas chia had a higher hydroxyl radical activity in the free phenolic fraction. This is the first comprehensive study on hydroxyl radical scavenging activity for camelina, chia and sophia seed meals, hence the results cannot be compared with the literature. HRSC and total phenolics content (TPC) of different fractions of camelina, chia and sophia were poorly correlated ($r^2$ = 0.45, $p$<0.01). This variation might be because hydroxyl radicals are very reactive and their scavenging efficacy depends on the chemical structure and the type of the phenolics involved. These results suggest that different phenolic fractions of defatted camelina, chia and sophia meals might have excellent hydroxyl radical scavenging power in peroxidation that attacks all biological molecules such as DNA, proteins, and polyunsaturated fatty acids (PUFA) in membranes, among others.

### 4.5.4 Reducing Power (RP)

The reducing power (RP) of free and esterified and insoluble-bound phenolic extracts of camelina, chia and sophia mmeals was evaluated by the potassium ferricyanide method. In this method, phenolic compounds react with potassium ferricyanide($Fe^{3+}$) to form potassium ferrocyanide ($Fe^{2+}$), which then reacts with ferric chloride to form ferrous ion complex that has an absorption maximum at 700 nm (Alshikh, de Camargo, & Shahidi 2015; Jayanthi & Lalitha 2011). Phenolic compounds with reducing power (RP) showed that they could donate electrons thus reducing oxidized intermediates produced during lipid peroxidation processes, acting as antioxidants. In this assay, the colour of the tested sample turns into various shades of green from yellow colour based on the reducing power of the phenolic extract (Alshikh, de Camargo, & Shahidi 2015; Jayanthi & Lalitha 2011). The absorbance increases with increasing phenolic contents in the extracts which shows a stronger reducing capacity.

Table 4.4 summarizes the RP of defatted camelina, chia and sophia meals which varied from 6.44 ± 0.10 to 81.30 ± 0.82 µmole TE/g sample for all fractions. However, the total RP of camelina, chia and sophia meals was 105.35 ± 1.8; 120.42 ± 0.46 and 157.10 ± 0.85 µmole TE per gram sample, respectively. Sophia seed meal had a significantly ($p < 0.05$) higher RP value compared to camelina and chia seed meal (Table 4.4). While comparing RP among all fractions of three seeds tested, the esterified extract of sophia showed the highest ($p < 0.05$) RP value (81.30 ± 0.82 µmole TE per gram sample). The same results were also observed in DPPH radical scavenging assay in this same study, possibly due to high phenolic content that we found in the Folin-Ciacalteu's test in our study. The reducing power in the insoluble-bound phenolic fraction of defatted seeds of

sophia was 26.05 ± 0.66 µmole TE per gram sample which is 4 times higher than that of defatted camelina meal (6.44 ± 0.10 µmole TE per gram sample) and 6 times lower than that of chia seed meal (37.19 ± 0.13 µmole TE per gram sample). The reducing power (RP) among camelina, chia and sophia was significantly ($p < 0.05$) different. The variation in reducing power of different phenolics fractions in camelina, chia and sophia defatted meals is dictated by their phenolic content and the type of phenolics present. The total reducing power (RP) of chia seed meal (120.42 ± 0.46 µmole TE per gram sample) was much lower than the ferric reducing antioxidant power (FRAP; 405.71 µmole TE per gram) of Brazilian chia seed reported by de Silva Marineli et al. (2014) and the difference might be due to different genotype and other environmental factors; other results cannot be compared with the literature as there are no previous studies on this topic. RP was highly correlated with TPC ($R^2$= 0.83), indicating that the phenolic extract of camelina, chia and sophia might serve as good reducing agents and as a viable source of natural antioxidants that could be used directly in selected foods or food formulations.

**4.5.5 Ferrous ion chelating activity**

Transition metal ions such $Fe^{2+}$ and $Cu^{2+}$ catalyse peroxidation in foods and biological systems. In the body, ferrous ions may produce hydroxyl radicals via Fenton's reaction which leads to the destruction of biomolecules, thus causing disease conditions and aging. Chelating agents such as phenolics and polyphenolics can prevent oxidation by binding metal ions and serve as effective secondary antioxidants (Chandrasekara & Shahidi 2010). In our study, we used the ferrozine method for determination of metal chelation activity of camelina, chia and sophia polyphenolics extracts. In this assay, phenolic compounds and Ferrozine compete for the $Fe^{2+}$ ion and form a coordinate

complex and stop transfer of electrons. Thus, phenolic compounds with cleating ability reduce the intensity of the purple colour of the complex.

Table 4.4 shows the metal chelating activity of the phenolic extracts of camelina, chia and sophia meals. Total ferrous ion chelating activity of camelina, chia and sophia phenolic extracts was 37.51 ± 0.74; 23.08 ± 0.47 and 24.61 ± 0.59 µmole EDTA Eq per gram sample, respectively. Camelina meal showed a higher metal chelation activity (37.51 ± 0.74 µmole EDTA Eq per gram sample) while no significant difference existed in ferrous ion chelation between chia and sophia meals. In case of camelina seed, the insoluble-bound phenolic fraction showed significantly ($p< 0.05$) higher metal chelation activity (13.87 ± 0.83 µmole EDTA Eq per gram sample) compared to the free and esterified fractions. On the other hand, sophia's esterified phenolics showed significantly higher metal chelation activity (9.7 ± 0.21 µmole EDTA Eq per gram sample) compared to the free and insoluble-bound phenolics. Esterified phenolic fraction of both chia and sophia as well as insoluble-bound phenolics of camelina which showed high chelating activity were reported to contain flavan-3-ol monomers as well as dimmers such as procyanidin B1 and B2 which could be responsible for their high ferrous ion chelating activity (Chandrasekara & Shahidi 2010). Comparison of these results with the literature is not possible as no prior work has been done with such details earlier. No correlation existed between TPC and metal chelation activity indicating that metal chelation activity did not depend on the quantity of phenolics present, but to the specific type of phenolic compounds and the number of hydroxyl groups and their arrangement in the molecule. Thus, these results show that camelina, chia and sophia meal extracts may serve as good sources of food ingredients with secondary antioxidant activity.

### 4.5.6 β-carotene bleaching assay

In the β-carotene /linoleate model system, free radicals are formed by abstracting a hydrogen atom from the active bis-allylic methylene group of linoleic acid in C-11 between the two double bonds. Free radicals attack β-carotene, causing the molecule to lose its conjugation, resulting in the loss of the characteristic yellow-orange colour of the molecule. Phenolic compounds protect β-carotene from bleaching by protecting linoleate from oxidation (Chandrasekara & Shahidi 2010). Thus, in this study the antioxidant activity of the oilseed extracts was investigated in the beta-carotene/linoleate emulsion system, which is more relevant to foods and biological systems.

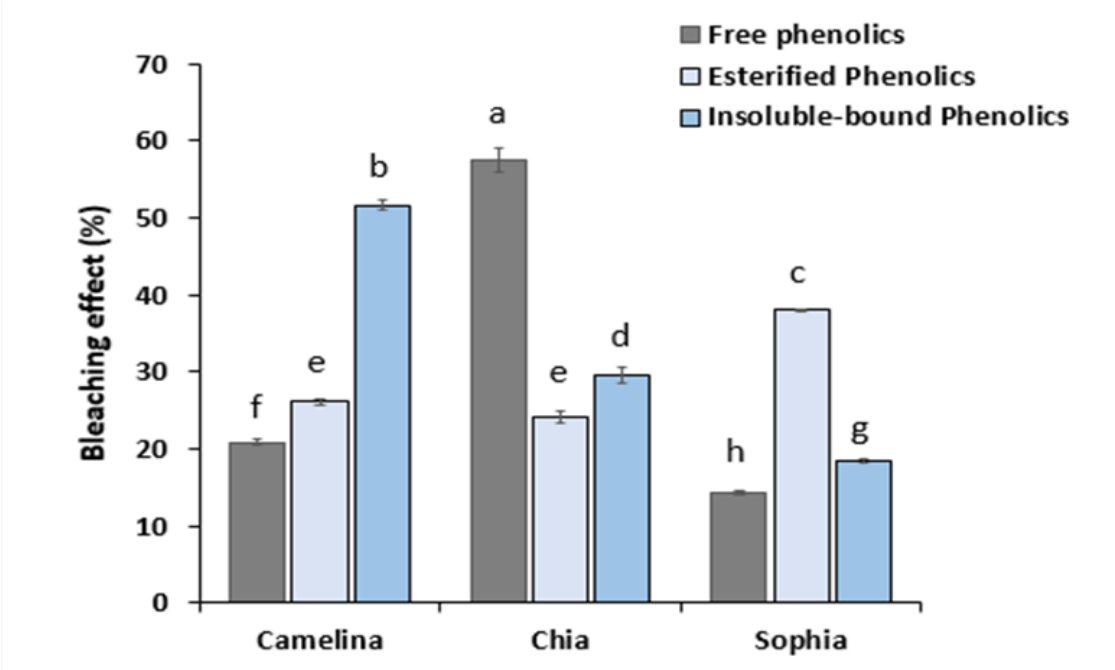



**Figure 4.2.** Inhibition of bleaching of β-carotene in beta-carotene/linoleate model system by camelina, chia and sophia seeds phenolic extracts. Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different lower case letters among all fractions are significantly different *(p* < 0.05) but same letters indicate are not significantly different (*p* > 0.05).

The inhibition of bleaching of β-carotene in oil-in-water emulsion system was screened at 0.5mg/ mL extract of camelina, chia and sophia phenolics as shown in Figure 4.2 and Table 4.5. The percentage inhibition of bleaching of β-carotene by camelina, chia and sophia seed extracts was significantly varied from 14.38 ± 0.21 to 57.53 ± 1.51% in different fractions whereas the $IC_{50}$ values ranged from 0.43 ± 0.01 to 1.73 ± 0.02 mg per mL of extract. A low $IC_{50}$ indicates a higher inhibition activity. In this work, free seed extract of chia showed a significantly higher β-carotene bleaching inhibition (57.53 ± 1.51%) with the lowest $IC_{50}$ value at 0.43 ± 0.01 mg/ mL of extract. On the other hand, the lowest β-carotene bleaching inhibition (14.38 ± 0.21 %) of the free phenolic extract of sophia seed had the highest $IC_{50}$ of 1.73 ± 0.02 mg/mL. For comelina and chia seeds, the free phenolic extracts were more effective against β-carotene bleaching compared to other fractions while esterified sophia seed extract was more effective in β-carotene oxidation. This is the first comprehensive study on β-carotene bleaching of camelina seed meal, hence the results cannot be compared with the literature. However, HadiNezhad, Rowland and Hosseinian (2014) reported that sophia whole seed phenolic extract was effective against β-carotene bleaching with an $IC_{50}$ of 1.0 ± 0.1 mg/mL extract while that for sophia meal had $IC_{50}$ of 1.6 ± 0.2 mg/mL extract in the same study. Reyes- Caudillo et al. (2008) reported that chia seed collected from two different places showed β-carotene bleaching inhibition 79.3 ± 0.13 and 73.5 ± 0.19% respectively, which is almost the same as that for the free phenolic fraction but lower compared to the total β-carotene bleaching inhibition of chia seed meal, again due to genotype and agronomic conditions of the chia seed tested. However, camelina, chia and sophia seeds showed higher inhibition activity against β-carotene bleaching in β-carotene/linoleate system which reflects many foods and biological systems.

### 4.5.7 Inhibition of α-glucosidase activity

α-Glucosidase is a key enzyme in the digestive tract. It is a membrane-bound enzyme located on the epithelium of the small intestine. α- Glucosidase breaks down dietary carbohydrates into glucose. If the pancreas does not produce a sufficient amount of insulin, or if the body does not properly use the insulin it makes, glucose (sugar) builds up in the blood instead of being used for energy, thus may cause type 2 diabetes. In addition, glucosidases are important enzymes in the processing of glycoprotein and glycolipids and are involved in the formation of essential glycoproteins required in viral assembly, secretion, and infection (Gruters et al. 1987). Phenolic and polyphenolic compounds with specific structural features may bind enzymes by complex formation through hydrogen bonding or other mechanisms. Therefore, we evaluated the effect of phenolic extracts of camelina, chia and sophia meals on the inhibition of α-glucosidase to examine their possible antidiabetic effects and enhancement of the body's host immune system.

The inhibitory effect of camelina, chia and sophia phenolic extract on α-glucosidase in *in vitro* assay is shown in Table 4.5 and Figure 4.3. In this assay, we used the original concentration of phenolic extracts (9.3 -33.1 mg/ mL). The inhibitory effects of camelina, chia and sophia meals on α-glucosidase enzyme was significantly ($p < 0.05$) different among all phenolics fractions (Table 4.3) and varied from 0.47 ± 0.29 to 63.86 ± 1.25%. The free phenolics extract of sophia showed a significantly higher (63.86 ± 1.25%) inhibitory effect against α-glucosidase whereas the lowest inhibition activity (0.47 ± 0.29 %) was found for the insoluble-bound fraction of camelina meal. Free phenolics showed a higher α-glucosidase inhibitory activity in all three seeds compared to other tested phenolic fractions in this study. However, sophia seed extract was more effective

compared to camelina and chia seeds. The concentration of the phenolic extract of camelina and sophia meals required for 50 % α-glucosidase inhibition ($IC_{50}$) was also calculated and found to range from 25.92 ± 0.51 to 1281.39 ± 767.40 mg/ mL of extract. As low $IC_{50}$ explains higher α-glucosidase inhibition activity, the highest inhibitory activity on α-glucosidase was found in sophia free phenolics at the lowest $IC_{50}$ value of 25.92 ± 0.51 mg/mL of extract. The highest $IC_{50}$ value (1281.39 ± 767.40 mg/mL of extract) was found in the insoluble-bound phenolics of camelina meals, indicating its lowest inhibitory activity on α-glucosidase. A direct comparison of these results with the literature is not possible as no prior data exists for detailed determinations.

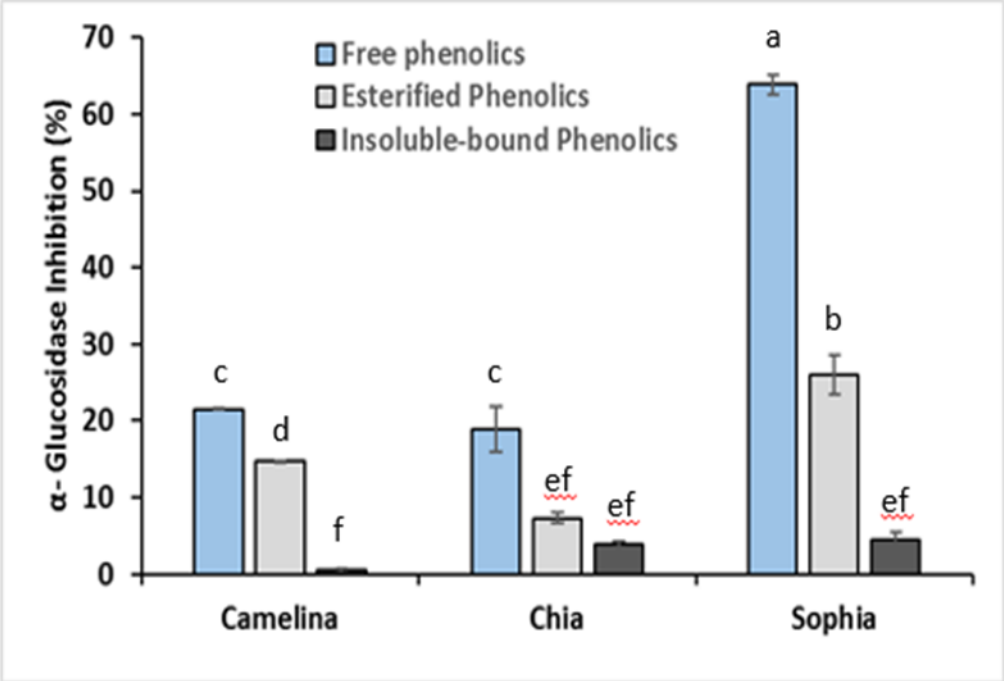



**Figure 4.3.** α-Glucosidase inhibition activity of camelina, chia and sophia meals. Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different lower case letters among all fractions are significantly different ($p < 0.05$) but same letters indicate are not significantly different ($p > 0.05$).

**Table 4.5.** Effect of camelina, chia and sophia seeds phenolic extracts on inhibition of bleaching of β-carotene in a water-in-oil model system, pancreatic lipase, α-glucosidase, LDL oxidation and DNA strand scission induced by hydroxyl and peroxyl radicals ($IC_{50}$ mg/mL extract).

| Samples | Free | Esterified | Insoluble-Bound |
|---|---|---|---|
| Antioxidant activity in β-carotene/linoleate model system | | | |
| Camelina | 1.19 ± 0.01c | 0.95 ± 0.01d | 0.48 ± 0.00f |
| Chia | 0.43 ± 0.01 | 1.032 ± 0.03 | 0.84 ± 0.02 |
| Sophia | 1.73 ± 0.02a | 0.65 ± 0.00e | 1.35 ± 0.01b |
| α-Glucosidase inhibition activity | | | |
| Camelina | 10.89 ± 0.13c | 6.93 ± 0.01e | 4.15 ± 0.04f |
| Chia | 56.54 ± 9.4 | 127.49 ± 12.78 | 192.54 ± 19.54 |
| Sophia | 16.87 ± 0.11a | 9.47 ± 0.18d | 12.23 ± 0.02b |
| Pancreatic lipase inhibition activity | | | |
| Camelina | 10.89 ± 0.13c | 6.93 ± 0.01e | 4.15 ± 0.04f |
| Chia | 10.05 ± 0.09 | 16.58 ± 0.22 | 17.10 ± 0.08 |
| Sophia | 16.87 ± 0.11a | 9.47 ± 0.18d | 12.23 ± 0.02b |
| Low density lipoprotein (LDL) oxidation inhibition | | | |
| Camelina | 0.06 ± 0.01a | 0.02 ± 0.00b | 0.02 ± 0.01b |
| Chia | 0.03 ± 0.01 | 0.02 ± 0.0 | 0.07 ± 0.01 |
| Sophia | 0.07 ± 0.03a | 0.02 ± 0.00b | 0.03 ± 00ab |
| DNA strand scission inhibition induced by hydroxyl radical | | | |
| Camelina | 12.19 ± 0.36c | 6.05 ± 0.10d | 5.06 ± 0 .01e |
| Chia | 13.70 ± 1.59 | 20.95 ± 0.76 | 25.74 ± 3.7 |
| Sophia | 22.01 ± 0.25a | 4.09 ± 0.10f | 15.74 ± 0.15b |
| DNA strand scission inhibition induced by peroxyl radical | | | |
| Camelina | 5.50 ± 0.03b | 2.68 ± 0.00d | 2.42 ± 0.05e |
| Chia | 2.75 ± 0.01 | 7.74 ± 0.01 | 5.26 ± 0.03 |
| Sophia | 8.35 ± 0.03a | 1.14 ± 0.00f | 5.40 ± 0.01c |

Data represent the mean values for each sample ± standard deviations (n = 3. Means followed by the different lower case letters among all fractions are significantly different ($p < 0.05$) but same letters indicate are not significantly different ($p > 0.05$).

### 4.5.8 Inhibition of pancreatic lipase activity

Pancreatic lipase is an important enzyme in gastrointestinal tract. It is secreted from the pancreas and hydrolyses triacylglycerols to glycerol and fatty acids in the small intestine that may cause obesity. Thus, inhibition of pancreatic lipase provides a good strategy in weight management and may help controlling hyperlipidaemia and obesity.

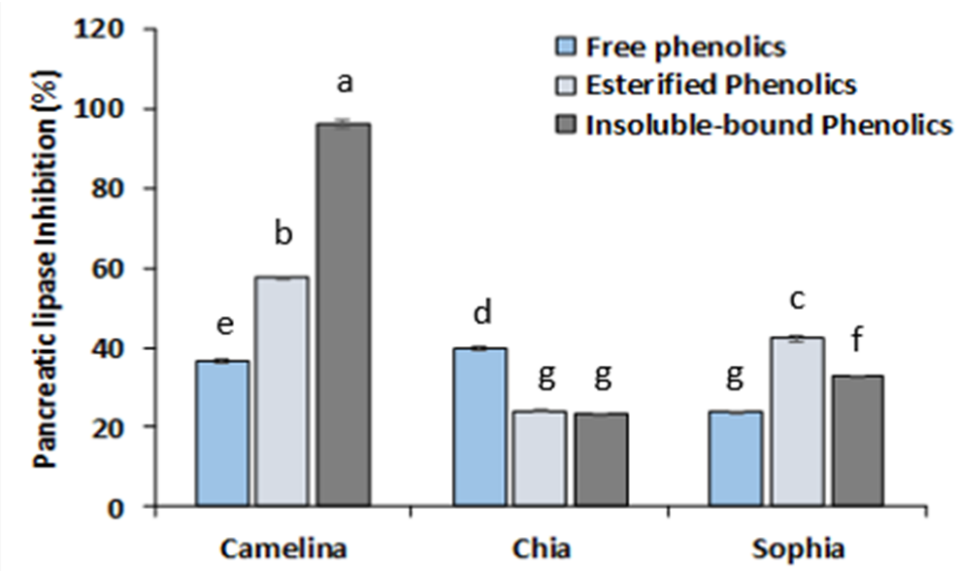



**Figure 4.4.** Pancreatic lipase inhibition activity of camelina, chia and sophia meals. Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different lower case letters among all fractions are significantly different *(p* < 0.05) but same letters indicate are not significantly different (*p* > 0.05).

Camelina, chia and sophia seed extracts showed high inhibition against pancreatic lipase activity which was screened at 6 mg/mL of extract as shown in Figure 4.4 and Table 4.5. Camelina, chia and sophia seed extracts showed a significantly ($p$<0.05) wide range of pancreatic lipase inhibition activity which varied from 23.39 ± 0.11 to 96.19 ± 1.08% while the corresponding $IC_{50}$ values of the extracts of camelina and sophia defatted meals ranged from 4.15 ± 0.04 to 16.87 ± 0.11 mg/mL of extract. Insoluble-bound phenolics extract of camelina showed the highest inhibitory effect on pancreatic lipase with lowest $IC_{50}$ value of 4.15 ± 0.04 mg/mL, whereas the lowest inhibition activity (23.39 ± 0.11 %) was found for the insoluble-bound fraction of chia meal with highest $IC_{50}$ value at 17.10 ± 0.08 mg/mL of extract. In case of chia seed, the free phenolic fraction showed a high lipase inhibitory activity compared to other fractions whereas esterified fraction of sophia and the insoluble-bound phenolic fraction of camelina was

more effective for pancreatic lipase inhibition. Based on the results shown here it is evident that besides the content of phenolics, their composition/ structural features and possibly other factors may also play a role in the enzyme inhibition activity of each type of phenolics involved. Since this is the first study on pancreatic lipase inhibition activity of camelina, chia and sophia seed meals, a comparison with the literature is not possible.

### 4.5.9 Low-density lipoprotein (LDL) oxidation inhibition

The oxidized low-density lipoprotein (Ox-LDL) plays a role in atherogenesis and coronary heart disease in humans (Ambigaipalan, de Camargo & Shahidi 2016; Jialal & Devaraj, 1996; Witztum & Steinberg 1991), and LDL oxidation may proceed via different pathways. The development of atheromatous plaques occurs due to uptake of oxidized LDL, through scavenger receptors followed by cholesterol accumulation and foam cell formation (Ambigaipalan, de Camargo & Shahidi 2016; Chisolm & Steinberg 2000). In this study, we examined the oxidative susceptibility of LDL catalyzed by $Cu^{2+}$ in an *in vitro* system by monitoring conjugated dienes formation after 12 h of incubation at $37^0$C. The conjugated dienes are formed when a hydrogen atom abstract from a PUFA followed by molecular rearrangement. The formation of conjugated dienes indicates the initiation phase of LDL oxidation.

The LDL oxidation inhibition effect of camelina, chia and sophia seed meals in a $Cu^{2+}$ catalyzed *in vitro* system was screened using 0.02 mg/ mL of phenolic extract as summarized in Figure 4.5 and Table 4.5. The LDL oxidation inhibition activity of camelina, chia and sophia phenolic extracts ranged from 13.99 ± 3.09 to 50.09 ± 2.80%.

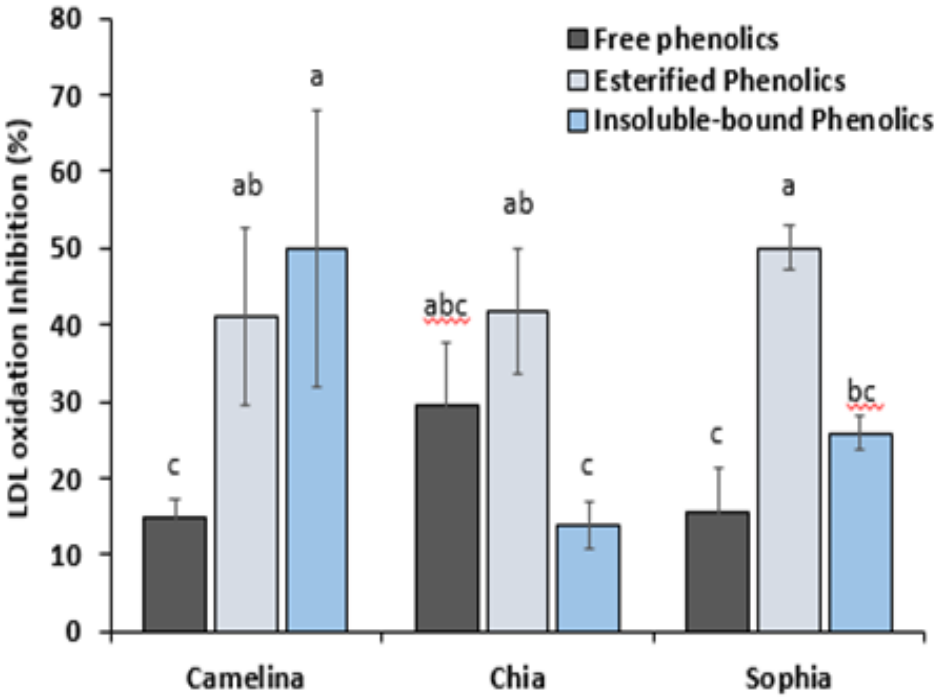



**Figure 4.5.** LDL oxidation inhibition effects of camelina, chia and sophia meals. Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different lower case letters among all fractions are significantly different *(p* < 0.05) but same letters indicate are not significantly different (*p* > 0.05).

Sophia esterified phenolic fraction showed the highest inhibition effect (50.09 ± 2.80%) on LDL oxidation compared to other fractions of all three seeds tested. There was no significant difference (*p* > 0.16) for the inhibition of LDL oxidation among esterified fractions of all three seeds and the free phenolic fraction of chia seed meal. Similar results were also found at *p* > 0. 30 for the esterified phenolics fraction in both camelina and sophia as well as for the free and insoluble-bound phenolics of chia and sophia seed, respectively. The concentration of the phenolic extracts of camelina and sophia meals required for 50 % LDL oxidation inhibition ($IC_{50}$) was also calculated and found to range from 0.02 ± 0.01 to 0.07 ± 0.03 mg/ mL of extract. No significance difference (*p* > 0. 14)

existed at the $IC_{50}$ value of free phenolics fractions in all three seeds as well as the insoluble-bound phenolics of chia and sophia seeds. A comparision of our findings with the literature is not possible since no prior work has been conducted.

### 4.5.10 Supercoiled plasmid DNA strand scission assay

Oxidative stress in cells is caused by ROS such as hydroxyl and peroxyl radicals. Because of oxidative stress, DNA damage may occur which is the first step in many pathological conditions such as mutagenesis and carcinogenesis, among others. DNA damage is a free radical mediated process that may occur at both the phosphate backbone and the nucleotide bases of DNA molecules. A wide variety of modifications may be observed in DNA molecules due its damage by ROS, including strand scission, sister chromatid exchange, DNA-DNA and DNA-protein cross-links as well as base modification (Chandrasekara & Shahidi 2011; Valko et al. 2004; Davies 1995). Hydroxyl radical is generated through Fenton reaction in the presence of transition metal under physiological conditions from hydrogen peroxide (product of superoxide anion dismutation). Hydroxyl radical can abstract a hydrogen atom from the deoxyribose moiety as well as pyrimidine and purine bases of DNA, thus producing single strands (Chandrasekara & Shahidi 2011; Stolc, Valko, Valko, & Lombardi 1996). Double strand breaks, which occur near to each other on both strands, may be due to the multiple hydroxyl radical attacks and could lead to lethal damage of the cells (Chandrasekara & Shahidi 2011; Ward 1985). Peroxyl radicals have also been shown to be involved in DNA strand breakage and base modification (Rodriguez et al. 1999). In this work, free radical induced DNA oxidation inhibition was investigated for camelina, chia and sophia seed meals extracts at a concentration of 8.0 and 4.0 mg/mL, and the retention of supercoiled

DNA and $IC_{50}$ values were also calculated. When oxidation occurs in DNA by peroxyl or hydroxyl radical, the supercoiled form of DNA may be converted to a nicked open circular form and a linear form (Ambigaipalan, de Camargo, & Shahidi 2016). Supercoiled DNA moves faster through an agarose gel network compared to the linear form of DNA. Thus, we observed two rows of DNA such as nicked (N) and supercoiled (S) (Figure 2). Areas under these bands were used to calculate percentage inhibition of supercoiled DNA oxidation and also $IC_{50}$ values.

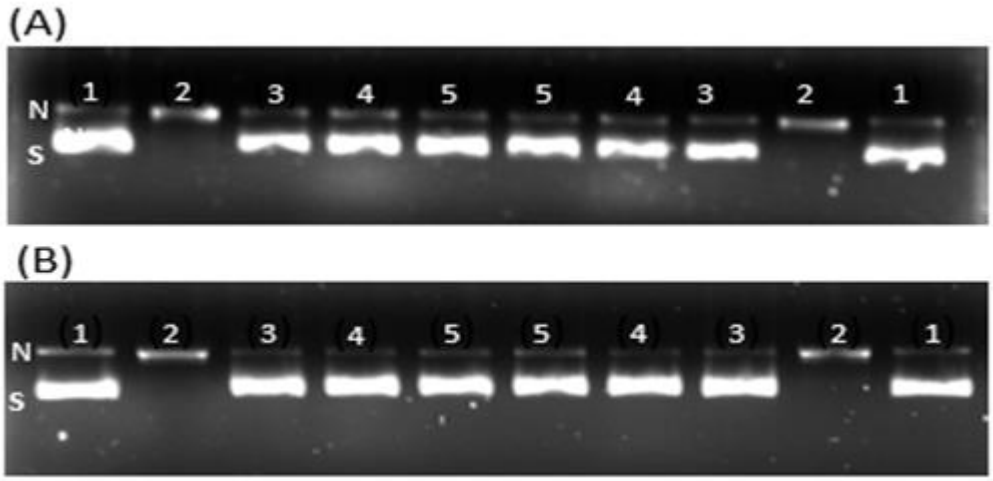



**Figure 4.6.** Agarose gel electrophoresis of inhibition of supercoiled DNA strand scission induced by hydroxyl radical (A) and peroxyl radical in the presence of camelina phenolic extracts (duplicates). Lanes: (1) blank; (2) control; (3) camelina free; (4) camelina esterified and (5) camelina insoluble bound phenolic extract. N, nicked DNA; S, supercoiled DNA.

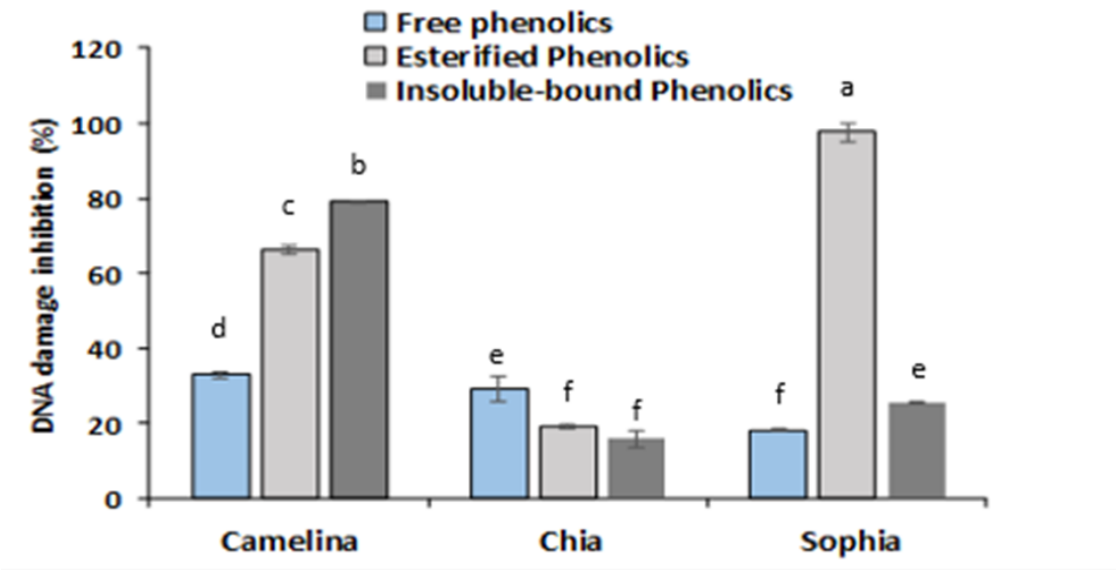



**Figure 4.7.** Hydroxyl radical induced DNA damage inhibition of camelina, chia and sophia meals. Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different lower case letters among all fractions are significantly different *(p* < 0.05) but same letters indicate are not significantly different (*p* > 0.05).

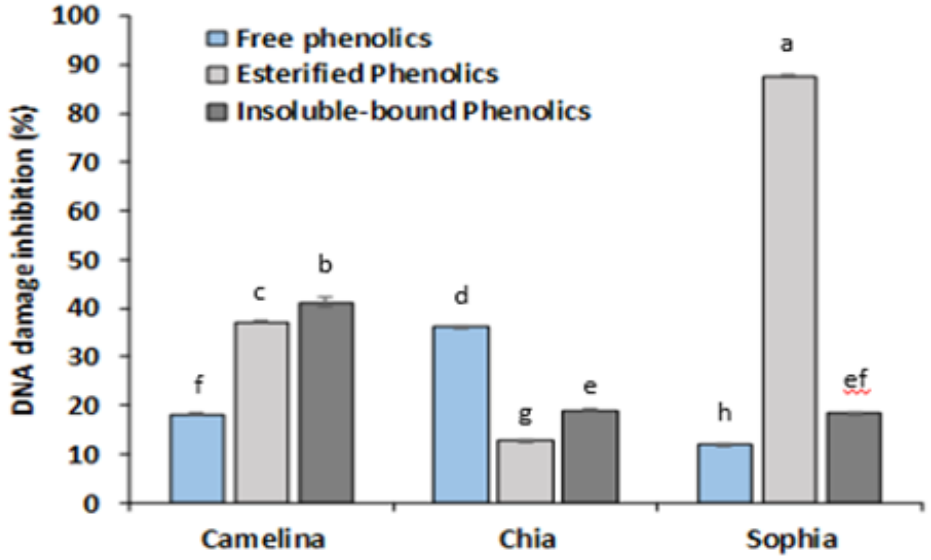



**Figure 4.8:** Peroxyl radical induced DNA damage inhibition of camelina, chia and sophia meals. Data represent the mean values for each sample ± standard deviations (n = 3). Means followed by the different lower case letters among all fractions are significantly different *(p* < 0.05) but same letters indicate are not significantly different (*p* > 0.05).

Figures 4.7 and 4.8 as well as Table 4.5 summarize the inhibition effect of camelina, chia and sophia seed meals against DNA strand scission induced by both peroxyl and hydroxyl radicals which varied significantly ($p$ <1.00) from 15.7 ± 2.28 to 97.61 ± 2.40 % and from 11.97 ± 0.04 to 87.61 ± 0.08 %, respectively. The insoluble-bound phenolics fraction of camelina seed meal showed significantly ($p$ < 0.05) higher inhibition effect (78.93 ± 0.21%) against hydroxyl radical induced DNA strand scission with the lowest $IC_{50}$ value of 4.09 ± 0.10 mg/mL of extract while esterified sophia phenolic extract showed the highest inhibition activity against peroxyl radical induced DNA strand scission. The lowest inhibition activity was found for insoluble-bound phenolic fraction of chia against hydroxyl and peroxyl radicals during DNA damage. A similar result was found in the inhibition of cupric ion-induced LDL oxidation in our study.

# CHAPTER 5

# SUMMARY, CONCLUSION AND FUTURE DIRECTIONS

The phenolic and polyphenolic profiles of camelina (*Camelina sativa*), chia (*Salvia hispanica*) and sophia (*Descurainia sophia*) seed meals were evaluated and compared. Defatted camelina, chia and sophia seeds meals were rich sources of various phenolic compounds and displayed higher antioxidant and biological activities in several *in vitro* systems. Sophia seed meal showed significantly higher phenolics content compared to the camelina and chia. Esterified phenolics were predominant in both camelina and chia seed meals, whereas free phenolics predominated in chia seed. Phenolic content in free, esterified and insoluble fraction of camelina, chia and sophia defatted meals was significantly different. Sinapic acid was the predominant phenolic compound in both camelina and sophia, while chlorogenic acid was a major phenolic compound in chia seed meal in the HPLC-DAD-MS/MS analysis. Daidzein was the predominant flavonoid in chia meal whereas quercetin-hexoside was predominant in both camelina and sophia meals. Free radical scavenging activity and reducing power of sophia seed meal was better than those of camelina and chia. On the other hand, camelina seed meal showed a higher metal chelation activity compared to sophia and chia meals, indicating that it may act as a better secondary antioxidant. Camelina, chia and sophia defatted meal not only showed significant free radical scavenging and antioxidant activity, but also inhibited pancreatic lipase and α-glucosidase activities. These enzymes are found in the digestive tract associated with lipid and carbohydrate digestion, respectively, and control obesity and blood glucose level in the human body. The findings of this work provide supporting information that chia meal may inhibit human LDL oxidation and DNA nicking

caused by free radicals and may also exert potential for prevention of CVD once consumed. Thus, seed meals of camelina, chia and sophia may serve as nutritional supplement and bioactive phenolics based on our HPLC-DAD-MS/MS data and *in vitro* antioxidant and biological assays. However, *in vivo* confirmation is still needed. In addition, investigation on the absorption and metabolism of camelina, chia and sophia meals phenolics need be carried out in *in vivo* systems. Besides *in vivo* studies, the application of the camelina, chia and sophia meals as antioxidant preservatives in food processing and preservation may provide means for full and better utilization of the resources. The effects of genotypes, growing conditions, harvesting time and storage on the phenolic and polyphenolic profiles of defatted camelina, chia and sophia seeds and their antioxidant and biological activities in both *in vitro* and *in vivo* deserve attention in further studies.

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