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Phytochemicals of lentil (*Lens culinaris*) and their antioxidant and anti-inflammatory effects

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Abstract

Lentils contain a plethora of bioactive phytochemicals such as extractable and insoluble-bound phenolics, carotenoids, tocopherols, saponins, phytic acid, and phytosterols, which have been increasingly attributed to the health benefits of lentil consumption in the diet. The concentration and stability of these phytochemicals in lentils may be affected by several processing parameters including different thermal processing, exogenous enzyme treatment and germination. Consumption of lentils has been associated with the risk reduction of many diseases due to the potential antioxidant activity and anti-inflammatory potential of phytochemicals in lentils. This mini review is intended to provide most current information on the phytochemical composition of lentils, and the potential antioxidant and anti-inflammatory properties of these compounds.

Keywords: Lentils; Phytochemicals; Phenolics; Carotenoids; Antioxidants; Anti-inflammatory.

1. Introduction

Legumes, commonly referred to as "pulses", are one of the most extensively cultivated crops throughout the world and are typically marketed as dry products (Schneider, 2002). They are consumed as a basic staple food in many countries, providing ideal protein, carbohydrates (including dietary fibers), fatty acids, minerals, and vitamins complementary to cereal-based diets (Gumienna et al., 2009; Marathe et al., 2011). Pulses are defined by the Food and Agriculture Organization (FAO) of the United Nations as grain legumes harvested only for their seeds (Mudryj et al., 2012). FAO lists eleven primary pulses including lentils, and excludes leguminous seeds used primarily for oil extraction,such as soybeans and groundnuts, or those consumed in immature form as vegetables, such as green beans and green peas (Dahl et al., 2012). Lentils (*Lens culinaris*) have been gaining increasing attention for their nutritive value as human diet. It is sometimes called "poor man's meat", which originated in ancient Europe (Bhatty, 1988). For these reasons, lentils have long been recognised as an inexpensive, excellent alternative to animal proteins, and are considered as a potential whole food source for people affected by micronutrient malnutrition (Thavarajah et al., 2009). Lentil crop was first introduced into southern Manitoba and Saskatchewan in Canada during the grain surplus years of the early 1970's due to favorable price compared with depressed cereal prices (Bhatty, 1988). Canada is by far the world's largest lentil exporter to the global marketplace, selling to over 100 countries each year, and produces about 25% of the total world output (Thavarajah et al., 2009). The most commonly grown lentil cultivars are the large green "Laird" cultivar and the red lentil (http://www.pulsecanada.com/food-health/what-is-a-pulse/lentil).

In recent years, many studies have shown potential health benefits of pulses, including lentils, beyond satisfying basic nutrient requirements for humans (Rochfort and Panozzo, 2007). Epidemiological and interventional studies suggest that pulse consumption is inversely associated with the incidence of several chronic diseases, such as coronary heart disease, type II diabetes mellitus, cardiovascular diseases, cancer and aging (Amarowicz and Pegg,

2008; Villegas et al., 2008). Some of these purported benefits of consuming a pulse-based diet have been attributed to their high content of phytochemicals that exert antioxidant and anti-inflammatory activity *in vitro* and *in vivo* (Amarowicz et al., 2004; Xu and Chang, 2012). However, the consumption of pulses like lentils is limited in western countries, with only about one in eight people consuming pulses on a daily basis (Mudryj et al., 2012), due to traditional eating customs, lack of consumer education and understanding on the nutritional values, processing techniques and available diversified food products. Despite this, incorporation of pulses into western diets has been highly recommended for consumers to receive maximum health benefits (Aguilera et al., 2010). In order to heighten public awareness of the nutritional benefits of pulses as part of sustainable food production aimed towards food security and nutrition, FAO has declared 2016 the International Year of Pulses (IYP) (http://www.fao.org/pulses-2016/en/).

Macronutrients such as carbohydrates and proteins are the main components of lentils, and current research suggests protein hydrolysates and peptides may be responsible for some of the observed health benefits, and dietary fibres and their colonic fermentation products i.e. short chain fatty acids (SCFA) may be a contributing factor to gut and colon health. Micronutrients such as phenolics have also shown strong antioxidant and anti-inflammatory effects. However, despite these findings, there is no consensus on the exact bioactive component(s) in lentils that contribute to the health benefits. There is a need for a comprehensive overview on the possible bioactive components in lentils. Inflammation, especially low grade inflammation, is the root cuase of oxidative stress induced chronic diseases. Food components with antioxidant and anti-inflammatory activities therefore are naturally targets of the health benefits of lentils. The present contribution intends to provide a review of the recent advances in the phytochemicals of lentils and their antioxidant and anti-inflammatory effects and how they might contribute to reduction in human health risks.

2. Phytochemicals in lentils

In general, phytochemicals could be broadly defined as all plant derived chemicals including macronutrients such as carbohydrates, lipids and proteins. However, in the present contribution, the term "phytochemicals" refers to those small non-essential bioactive compounds or secondary metabolites that occur naturally in plants. These phytochemicals such as flavonoids and carotenoids are usually responsible for the color and other organoleptic properties of food, but also have other significant biological activities. Lentils have been reported to contain an array of different phytochemicals such as phenolics, condensed tannins, carotenoids, tocopherols, saponins, phytic acid, and phytosterols, making them the major sources for phytochemicals in the diet. Table 1 summarizes the different phytochemicals reported for lentils.

2.1. Extractable phenolics

Polyphenolic compounds are perhaps the most diverse group of phytochemicals that are known for their various biological properties. To date, more than 8,000 polyphenolic substances have been identified. These polyphenols can be classified into sub-groups such as phenolic acids and flavonoids according to their molecular structures. Phenolic acids are simple phenolics that can be further divided into hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives. Flavonoids are the most important phenolic subclass, characterized by a C_6–C_3–C_6 backbone structure. Flavonoids can be further classified into different sub-groups of flavan-3-ols, flavonols, flavones, flavanones, anthocyanidins and isoflavones, and oligomers such as proanthocyanidins. The phenolic compounds in plant are conventionally extracted by ethyl or methyl alcohols, acetone or their aqueous mixtures, which is only efficient in extracting soluable phenolics., leaving behind other phenolics that exist in the bound form. For this reason, the majority of the reported measurement of phenolics in food samples is confined to the soluble or extractable phenolic fraction.

Lentils are a significant dietary source of these extractable phenolics. The major phenolic compounds found in lentils include sub-classes of phenolic acids, flavan-3-ols, condensed tannins (proanthocyanidins), anthocyanidins, flavonols, stilbenes, flavones, and flavanones. Lentil was reported to have the highest total phenolic content (TPC) of 7.53 mg gallic acid equivalents (GAE)/g dry weight (DW) among 8 different types and varieties of pulses, as well as 2.21 mg catechin equivalents (CE)/g DW of total flavonoids content (TFC) (BJ Xu & Chang, 2007). Lentils also contain 1.5–2.6 mg/g of total phenolic acids (Xu and Chang, 2010). Amarowicz et al. (2009, 2010) identified 24 phenolic compounds including phenolic acids and flavonoids in the extractable fraction of red lentil and 20 in green lentil. They found that *p*-hydroxybenzoic acid, *trans*-*p*-coumaric acid, *trans*-ferulic acid and sinapic acid were major phenolic acids in red lentil, whereas *trans*-*p*-coumaric acid and *trans*-ferulic acid were mainly present in green lentil. The content of individual flavonoids in lentils ranged from 0.27 to 289 µg/g DW. Flavonols, flavan-3-ols and condensed tannins (proanthocyanidins), such as kaempeferol glycoside, quercetin diglycoside, catechin, epicatechin, prodelphinidin dimer and digallate procyanidin, were identified as the predominant flavonoids of lentils in the study reported by Zou et al. (2011). Alshikh et al. (2015) further fractionated the crude phenolic extract of lentils and found that majority of the extractable phenolics were in esterified form (2.32–21.54 mg GAE/g) rather than in free form (1.37–5.53 mg GAE/g). Catechin, epicatechin and procyanidins B were predominant flavonoids in both free and esterified fractions of all tested lentils. They also identified and quantified methyl vanillate and prodelphinidin dimer A in lentils for the first time. Others have found that monomeric flavan-3-ols (catechin and epictechin), as well as limited amount of flavone (luteolin) were present in 11 selected lentil cultivars (Xu and Chang, 2010). Our recent study revealed that flavonols such as kaempeferol glycoside, and flavan-3-ols mainly catechin and epicatechin glycosides were the predominant phenolics in the aqueous methanolic extract of 20 lentil cultivars, and it was these compounds that contributed most to the antioxidant capacity of lentils (Zhang et al., 2015). The varied flavonoid compositions in lentils among published data might be attributed to the different genotype, growing environment, extraction condition and chromatography system. Anthocyanins are pigmented flavonoids found in dark colored lentil varieties, and there is very limited information available in the literature. Early studies showed that lentil seeds contained a compound that resembled a diglycosyl derivative of delphinidin (D'Arcy and Jay, 1978). Takeoka et al. (2005) first isolated a major anthocyanin from the acidified methanolic extract of Beluga black lentils, and identified as delphinidin 3-O-(2-O-β-D-glucopyranosyl-α-L-arabinopyranoside).

The distribution of phenolic compounds differs greatly in the cotyledon and the seed coat of lentils. Although the lentil seed coat accounts for only 8 to 11% percent of the whole seed weight, it provides significant contribution to the overall benefits of lentils (Duenas et al., 2006). Mirali et al. (2014) reported that phenolic compounds in lentil seed coat were more abundant and diverse than in the cotyledon. The seed coat was found to contain a large amount

Table 1. Phytochemical studies related to lentils

Phytochemicals	Samples	Extract solvent	Content	Concentration	Processing effects	References
Extractable Phenolics	Red Chief lentil from Spokane	Acidic 70% acetone	TPC	7.53 mg GAE/g DW		Xu and Chang, 2007;
	Eleven lentils from North Dakota	70% acetone with 0.5% acetic acid	Benzoic acid derivates Cinnamic acid derivates Total phenolic acid Flavan-3-ol Flavone Total flavonoids	169.7–248.1 μg/g 1315.6–2381.7 μg/g 1543.7–2551.4 μg/g 266.9–4946.7 μg/g 18.22–77.13 μg/g 3524.1–6870.8 μg/g		Xu and Chang, 2010;
	Green and Red lentil from Poland	80% acetone	Phenolic acids Flavan-3-ol monomeric Proanthoccyanidins Flavonol	0.06–73.46 μg/g 6.65–289 μg/g 5.3–154.8 μg/g 0.27–287.84 μg/g		Amarouwica et al. 2010; Amarouwica et al. 2009;
	Lentil from Spokane (Morton cultivar)	70% acetone with 0.5% acetic acid (Crude extract)	TPC	70.0 mg GAE/g Extract		Zou et al., 2011;
		Eluted with 80% MeOH	TPC	377.2 mg GAE/g Extract		
	Lentils from Saskatoon, Canada	Supernatants were extracted with diethyl ether and ethyl acetate (Free phenolic fraction)	TPC Hydroxybenzoic acids and derivatives Hydroxycinnamic acids and derivatives Flavonoids and derivatives	1.37–5.94 mg GAE/g defatted sample 0–0.81 μg/g 0.63–10.4 μg/g 150–270 μg/g		Alshikh, et al., 2015;
		Aqueous phase was hydrolyzed by alkali (Esterified phenolic fraction)	TPC Hydroxybenzoic acids and derivatives Hydroxycinnamic acids and derivatives Flavonoids and derivatives	2.32–15.4 μg/g 0–7.13 μg/g 2.86–15.8 μg/g 109–486 μg/g		
	Green and red lentils from Canada	70% MeOH with 0.1% HCl	TPC Total flavonol index (TFI) Total Phenolic index (TPI)	4.56–8.34 mg/g DW 351.82–528.42 μg/g 594.63–952.55 μg/g	Domestic cooking decreased the flavanols, and increased the flavonols	Zhang et al., 2015; Zhang et al., 2014b;
	Two lentil (*Lens culinaris*) cv Pardina and Crimson	30% dimethylformamide	TPC	11.8–12.0 mg GAE/g	TPC decreased 80% by decortication, 16–41% by cooking, 22–42% by soaking	Han and Baik, 2008;
	Lentil cv. CDC Richlea	Acidic 70% acetone	TPC	around 7.8 mg GAE/g	All thermal processing decreased total phenolics by around 60%	Xu and Chang, 2009;

Table 1. Phytochemical studies related to lentils - *(continued)*

Phytochemicals	Samples	Extract solvent	Content	Concentration	Processing effects	References
	Lentil cv. Pardina	80% methanol with HCl	Hydroxybenzoic and hydroxycinnamic compounds Flavonols and flavones Stilbenes Proanthocyanidins	0.73–10.02 µg/g 0.33–6.20 µg/g 0.93 µg/g 0.29–31.50 µg/g	All enzymatic treatments decreased hydroxycinnamic and proanthocyanidins content, treatment of tannase increased quercetin 3-O-rutinoside and luteolin content	Dueñas et al., 2007;
	Lentils (*Lens culinaris* L., var. Castellana)	80% methanol with HCl	Phenolic acids Flavan-3-ol	13.3–342 µg/g 0.1–0.5 µg/g	Soaking decreased all of the lentil phenolics, whereas germination resulted in an overall increase of phenolics	López-Amorós et al., 2006;
	Lentil var. Tina from Poland	70% acetonee with 1% HCl	TPC Phenolic acids Flavonoids	18.98 mg/g FW 0.78–44.46 µg/g FW 0.25–393.79 µg/g FW	p-hydroxybenzoic, benzoic and caffeic acids was significant increased on days 3 and 4 after germination	Świeca et al., 2012;
Insoluble-Bound Phenolics	Lentils from Saskatoon, Canada	Residue was hydrolysed with 4 M NaOH	TPC Hydroxybenzoic acids and derivatives Hydroxycinnamic acids and derivatives Flavonoids and derivatives	1.21–9.68 mg GAE/g defatted sample 0.20–1.87 µg/g 0.07–4.74 µg/g 73.1–458 µg/g		Alshikh, et al,, 2015;
	Lentils from Canada	Residue was hydrolyzed with 2 M NaOH	TPC Phenolic acids Total phenolic index (TPI-B)	0.11–0.29 mg GAE/g 1.09–71.04 µg/g 115.72–217.25 µg/g	Domestic cooking negatively affected the release of bound phenolics in lentil	Zhang et al., 2014b;
	CDC Richlea lentils	Residue was hydrolysed with 4 M NaOH	TPC	4.78 mg GAE/g	Gemination significant increased the phenolic content	Yeo and Shahidi, 2015
Carotenoids and Tocopherols	Green and white lentils from France	Hexane:acetonee (1:1, v/v)	Carotenes Neoxanthin Lutein	0.020–0.028 mg/100 g edible portion 0.042 mg/100 g edible portion 1.061–1.196 mg/100 g edible portion		Biehler et al., 2012;
	Green and red lentils from Canada	Hexane/isopropanol (3:2, v/v)	all-trans-lutein all-trans-Zeaxanthin Total carotenoids idex α-Tocopherol γ-Tocopherol δ-Tocopherol Total tocopherol	3.07–14.31 µg/g 0.25–2.09 µg/g 4.64–19.63 µg/g 0.16–0.90 µg/g 36.32–63.54 µg/g 0.33–1.25 µg/g 37.38–64.38 µg/g	Domestic cooking increased release of tocopherols and carotenoids	Zhang et al., 2014; Zhang et al., 2014b;

Table 1. Phytochemical studies related to lentils - *(continued)*

Phytochemicals	Samples	Extract solvent	Content	Concentration	Processing effects	References
	Lentil from Ireland local store	Hexane/diethyl-ether (1:1, v/v)	α-Tocopherol β+γ-Tocopherol	1.6 mg/100 g 4.5 mg/100 g		Ryan et al., 2007;
	Lentils cv Agueda, cv Almar, cv Paula, and cv Alcor. From Spain	Pure methanol	α-Tocopherol β-Tocopherol γ-Tocopherol δ-Tocopherol	3.84–8,69 µg/g 1.94–3.81 µg/g 91.11–104.68 µg/g 2.01–2.74 µg/g	Germination and cooking decreased β-, γ- and δ-Tocopherol, increased α-Tocopherol	Fernandez-Orozco et al., 2003;
Saponins	Lentils (Lens esculenta var. Magda 20 and Lyda) from Spain	70% ethanol with 0.01% EDTA and 1-butanol	soyasaponinVI	703–1139 mg/ kg DW	Soaking did not modify the saponin content, cooking degraded soyasaponin VI into soyasaponin I	Ruiz, Price et al., 1996;
	Lentils from Spain	Methnol	Total saponin content	654–1269 mg/ kg DW		Ruiz et al., 1997;
	Lentils from Italian	70% ethanol	soyasaponin I soyasaponin βg (VI)	28–407 mg/kg DW 110–1242 mg/ kg DW		Sagratini et al., 2009;
	Lentils (Lens esculenta var. Magda 20) from Spain	Methnol	Soyasapogenol B	0.34 mg/g DW	Germination signifaicant increased saponin content at day 6	Ayet et al., 1997;
Phytic acid	Lentils (Lens esculenta var. Magda 20) from Spain	0.5 M HCl	IP4 IP5 IP6 Total Inositol phosphates content	0.09 mg/g 0.72 mg/g 4.91 mg/g 5.67 mg/g	Germination signifaicant decreased phytic acid	Ayet et al., 1997;
	Lentils (*Lens esculenta* var. Angela)	Acetic acid/ sodium hydroxide 0.1 N, pH 5.5	IP3 IP4 IP5 IP6	0.27 g/kg 0.48 g/kg 0.75 g/kg 2.69 g/kg	commercial phytase decreased IP4, IP5 and IP6, whereas did not affect IP3	Frias et al., 2003;
Phytosterols	Lentil from Ireland local store	Hexane/diethyl-ether (1:1, v/v)	β-Sitosterol Campesterol Stigmasterol	123.4 mg/100 g 15.0 mg/100 g 20.0 mg/100 g		Ryan et al., 2007;
	Lentils from Greece (cooked)	Hexane extract after hot saponification	β-Sitosterol Campesterol Stigmasterol	15.4–24.2 mg/100 g 2.60–2.63 mg/100 g 2.18–2.58 mg/100 g		Kalogeropoulos et al., 2010;

of monomeric flavan-3-ols, proanthocyanidin oligomers and polymers, as well as small amounts of glycosides of flavonols such as quercetin, myricetin, luteolin, and apigenin. Phenolic acids, on the other hand, such as hydroxybenzoic and hydroxycinnamic acids, in both free and bound forms, were mainly present in the cotyledon of lentils (Dueñas et al., 2002). Interestingly, a stilbene trans-resveratrol-5-glucoside was identified for the first time in the seed coat of lentils (Dueñas *et al.*, 2002). The same research group further reported the structural compositions of proanthocyanidins, the major group of polyphenols present in the seed coat of lentils (Dueñas et al., 2003). The proanthocyanidins in the seed coat of lentil mainly included monomeric, oligomeric, and polymeric flavan-3-ols. The major monomeric flavan-3-ol was (+)-catechin-3-glucose, with lesser amounts of (+)-catechin and (−)-epicatechin aglycones. Various dimer, trimer, and tetramers constituted of catechin, gallocatechin, and catechin gallate units were identified in the oligomeric fraction, and several procyanidins and prodelphinidins from pentamers to nonamers in the polymeric fraction. Approximately 65−75% of proanthocyanins in the seed coat of lentils are polymers with 7–9 mDP (mean degree of polymerization), and 20−30% are oligomers with an mDP of 4−5. Proanthocyanins are strong antioxidants and have been reported to have many health beneficial effects, thus it is highly recommended that lentils are consumed in-whole.

2.2. Insoluble/Bound phenolics

Apart from most studied extractable phenolics, there still exist easily neglected insoluble or bound phenolics that are commonly associated with cell wall materials. The lack of assessing bound phenolics leads to underestimation of the total phenolic content and the overall actual health benefits of lentils or other pulses. In general, organic solvents such as ethanol, methanol and acetonee are employed to directly extract the soluble phenolics (extractable phenolics) in plants, whereas insoluble-bound phenolics can only be released upon alkali, acid, or enzymatic treatment of samples before extraction (Andreasen et al., 2001; Bartolome and Gómez-Cordovés, 1999; Zupfer et al., 1998). The insoluble-bound phenolics are considered to contribute additional health benefits because they may avoid degradation under upper gastrointestinal digestion conditions, and are absorbed into teh circulation system or epithelial cells after being released by intestinal microflora fermentation (Andreasen et al., 2001). However, unlike other plants such as cereals, fruits and vegetables, insoluble-bound phenolics of pulses, particularly lentils, and their potential contribution to health benefits have not been well studied. Han and Baik (2008) found that bound phytochemicals contributed more to total antioxidant activity in lentils than free (extractable) phytochemicals. The TPC of insoluble-bound phenolics in lentils ranged from 0.18 to 17.5 mg GAE/g, whereas TFC ranged from 0.03 to 4.13 mg CE/g (Alshikh et al., 2015; Zhang et al., 2014b). Alshikh et al. (2015) identified 15 compounds including hydroxybenzoic acids, hydroxycinnamic acids, flavonoids and their derivatives in the insoluble-bound fraction of lentils. Catechin, catechin-3-glucoside and procyanidin dimer Bweree the predominant phenolic compounds identified in the insoluble-bound fractions of 6 tested lentil cultivars. In our previous study, five phenolic compounds including gallic acid, protocatechuic acid, catechin, epicatechin and 3-hydroxycinnamic acid were identified in the insoluble-bound fractions of lentils (Zhang et al., 2014b).

2.3. Carotenoids and tocopherols

Carotenoids and tocopherols are well-known lipophilic antioxidants that are synthesized in whole or in part from the plastid isoprenoids (DellaPenna and Pogson, 2006). Carotenoids comprise a large isoprenoid family and most are C40 tetra-terpenoids derived from phytoene. It provides plants with distinctive red, orange, and yellow colours. Tocopherols are a group of nutrients that constitutes vitamin E that are essential to health of all mammals. Lentils are a good source of both carotenoids and tocopherols. Biehler et al. (2012) reported presence of lutein, carotenes and neoxanthin in lentils, among which lutein (1.061–1.196 mg/100 g edible portion) was the major carotenoid. Our recent study examined the carotenoid and tocopherol compositions in 20 lentil cultivars (10 green and 10 red) (Zhang et al., 2014a). All-*trans*-lutein accounted for 64–78% of the total carotenoid content (TCC) in lentils, followed by all-*trans*-zeaxanthin, which constitutes 5–13% of TCC. The total lutein and zeaxanthin contents (total of all-*trans* and *cis*-isomers) in lentils ranged from 4.32–17.29 μg/g DW to 0.32–2.73 μg/g DW, respectively. Total tocopherols of lentils were from 37–64 μg/g DW, predominantly γ-tocopherol (96–98% of the tocopherol content), followed by δ- and α-tocopherols. Similar results were repored by Ryan et al. (2007) who did not separate all tocopherol isomers. All α-, β-, γ- and δ-tocopherol isomers were reported in lentils in another study (Fernandez-Orozco et al., 2003). When consumed daily, these carotenoids and tocopherols of lentils can provide substantial nutritional and health benefits.

2.4. Saponins

Saponins are a diverse group of compounds characterized by a carbohydrate moiety attached to a steroid or triterpenoid aglycone in their structures. These phytochemicals have long been considered undesirable due to their toxicity and haemolytic activity. Saponins in pulses such as lentils however are attracting considerable interest in recent years due to their ability to lower plasma cholesterol levels in human and suppress cancer growth. Pulses including lentils are considered among the best sources of saponins. Total saponin content of lentils has been reported to be in the range of 654–1269 mg/kg DW, and the main saponins were soyasaponin I and VI in Spanish lentils (Ruiz et al., 1996; Ruiz et al., 1997). Sagratini et al. (2009) tested 32 Italian lentils, and found soyasaponin I and soyasaponin βg (also known as VI) to be 28–407 mg/kg and 110–1242 mg/kg.

2.5. Phytic acid

Phytic acid (or inositol hexaphosphate, IP6) is a saturated cyclic acid, and is considered as the major source of phosphorus in many plant tissues or organs, especially in seeds. The catabolites of phytic acid are called lower inositol polyphosphates including inositol tri- (IP3) , tetra- (IP4) and penta-phosphate (IP5). Phytic acid has been proposed to serve a vital role in protecting the seeds against the deleterious effects of oxygen and iron (Graf and Eaton, 1990). Lentils have been reported to contain 86% of IP6 (4.91 mg/g), 13% of IP5 (0.72 mg/g) and trace amount of IP4 (0.09 mg/g) (Ayet et al., 1997).

2.6. Phytosterols

Phytosterols, primarily β-sitosterol, campesterol, and stigmasterol, are integral natural components of plant cell membranes. They are proposed to have a wide range of biological effects including anti-inflammatory, anti-oxidative, anti-carcinogenic activities

and cholesterol-lowering ability (Berger et al., 2004). Studies have shown that phytosterols can inhibit the intestinal absorption of cholesterol, thus lowering total plasma cholesterol and low-density lipoprotein (LDL) levels (de Jong et al., 2003). Pulses including lentils are one of the important dietary sources of phytosterols, along with vegetable oils, nuts and cereal grains. β-Sitosterol was found to be the most prevalent phytosterol in lentils, of which the concentration was 123.4 mg/100 g, followed by 20.0 mg/100 g of stigmasterol and 15.0 mg/100 g of campesterol (Ryan et al., 2007). In another study, Kalogeropoulos et al. found that the predominant β-sitosterol ranged from 15.4 to 24.2 mg/100 g in cooked lentils, whereas the contents of stigmasterol and campesterol ranged from 2.60–2.63 to 2.18–2.58 mg/100 g, respectively (Kalogeropoulos et al., 2010).

3. Processing effects on phytochemicals in lentils

Lentils are usually processed before consumption. Processing not only improves the flavour and palatability of lentils, but also significantly affects their phytochemical content and profiles, as well as the antioxidant capacities. The stability of phytochemicals during processing has been a major concern. Han et al. reported that the total antioxidant activity and total phenolic content (TPC) of lentils was reduced by ca. 80, 16–41 and 22–42% by decortication, cooking and soaking, respectively (Han and Baik, 2008). Interestingly, they observed a loss of 94.8% in the total antioxidant activity of bound phenolics of lentils by decortication, indicating that most of the bound phytochemicals are distributed in the seed coat, whereas the total antioxidant activity of free phenolics increased by 10–36% in lentils after cooking probably due to the release of the conjugated phenolics during cooking. Xu et al. (2009) compared the influences of four thermal processing methods (conventional boiling, conventional steaming, pressure boiling, and pressure steaming) on phytochemical profiles, antioxidant capacities, and antiproliferation properties of commonly consumed cool-season food legumes including lentils. All thermal processing resulted in significant ($P < 0.05$) reductions in total phenolic, procyanidin, total saponin, phytic acid, chemical antioxidant capacities (ferric reducing antioxidant power, FRAP; and peroxyl radical scavenging capacity, ORAC), and cellular antioxidant activity (CAA), as well as anti-proliferation capacities of lentils, as compared to raw lentils. Conventional boiling, pressure boiling and pressure steaming caused significant ($p<0.05$) decreases in gallic, chlorogenic, sinapic, *p*-coumaric acid, subtotal, and total phenolic acids, and significant increases in 2,3,4-trihydroxybenzoic acid, whereas conventional steaming did not cause significant changes in the chlorogenic and sinapic acid, subtotal cinnamic acid and total phenolic acid. Different thermal processing methods present significant differences. Their results indicated that steaming was a better cooking method than boiling in retaining antioxidants and phenolic components, whereas boiling was effective in reducing saponin and phytic acid contents (Xu and Chang, 2009). Our recent study found that cooking favours the release of carotenoids, tocopherols and flavonols (kaempferol glycosides) but leads to losses of flavanols (monomeric and condensed tannin) (Zhang et al., 2014b). However, a significantly reduction in tocopherols including α-, β-, γ- and δ-tocopherols resulted from cooking was observed by Fernandez-Orozco et al. (2003). Elhardallou and Walker (1994) determined a loss of 60.5% in phytic acid in lentils during autoclaved cooking. Ruiz et al. (1996) observed that soaking did not modify the saponin content or composition of lentils regardless of the pH of the soaking solution. The native soyasaponin VI in lentils, however, was found to be partially degraded into soyasaponin I during cooking, and an overall loss of 15–31% in total saponin content was found for lentils.

Exogenous enzyme treatment and germination could also have significant effects on the phytochemicals in lentils. Dueñas et al. investigated the effect of the enzymes tannase, α-galactosidase, phytase and viscozyme on the phenolic composition of lentils, and found that all exogenous enzyme treatment changed phenolic composition of lentil flours, particularly those of the hydroxycinnamic compounds and proanthocyanidins that are significantly decreased after the enzymatic treatments, whereas quercetin 3-O-rutinoside and luteolin increased and reached the highest concentration by treatment of tannase. *Trans*-resveratrol was only observed in the lentils treated by the tannase and phytase, and gallic acid was formed by the action of phytase, α-galactosidase and tannase. The treatments of viscozyme, α-galactosidase or tannase increased the antioxidant capacity as compared to raw lentils, and the quercetin 3-O-rutinoside was evident to be the main compound affecting antioxidant activity (Dueñas et al., 2007). After addition of commercial phytase to lentil flour, a reduction of 85–91, 57–69 and 6–27% in IP6, IP5 and IP4, respectively, was observed, whilst did not significantly change the content of IP3 (Frias et al., 2003). In general, the phenolic content in lentils tend to steadily decline during germination due to leaching into the soaking water, binding with other organic substances such as carbohydrates or proteins, and the activation of endogenous enzymes such as hydroxylases and polyphenoloxydases. López-Amorós et al. (2006) reported a general decrease in all of the lentil phenolics including hydroxybenzoic acids, hydroxycinnamic acids, (+)-catechin and procyanidin oligomers after soaking when compared with raw seeds, thereafter germination resulted in an overall increase of phenolics in lentils, with the exception of protocatechuic acid where no increase took place. It is worth noting that some of hydroxycinnamic compounds such as *transp*-coumaric acid and *trans*-ferulic acid , which had decreased or disappeared after soaking, were detected and increased from the beginning of the germination period. The hydroxycinnamic compounds are the constituents of plant cell walls, in various bonds and esterified forms, linked to arabinoxylans and lignin. The changes observed in these hydroxycinnamic compounds during germination therefore could be well explained by the action of the endogenous esterases. Yeo et al. (2015) even proposed a new indicator, the ratio of insoluble bound phenolics (IBPs) to soluble phenolics (SPs), to monitor changes in the antioxidant activity of lentils during germination. They observed an increase of TPC in both SPs (from 3.35 to 4.25 mg GAE/g of defatted weight (DW)) and IBPs (from 4.78 to 6.45 mg GAE/g of DW) of lentils during germination. Total flavonoids contents (TFC) of SPs decreased from 2.49 to 1.96 mg CE/g of DW during the 4 days of germination probably due to the degradation of flavonoids by oxidants such as ROS produced in the mitochondria, whereas that of IBPs increased from 2.98 to 3.85 CE mg/g (Yeo and Shahidi, 2015). Cevallos-Casals and Cisneros-Zevallos (2010) found that TPC of SPs from green lentils was increased significantly during 7 days germination. The condition of germination and illumination was found to have varied effects on the phenolic components of lentils and their biological activity. Phenolics were stimulated by cultivation under continuous light, and the content of *p*-hydroxybenzoic, benzoic and caffeic acids was significant increased on days 3 and 4 after germination (Świeca et al., 2012). Additionally, the contents of β-, γ- and δ-tocopherols were reported to decrease by 33.6–42.5, 29.3–55.4 and 23.7–47.1%, respectively, in germinated lentil seeds of all cultivars, but the contents of α-tocopherol increased by 1.6–48.9% in germinated seeds when compared to that of the raw lentils of all cultivars being investigated (Fernan-

dez-Orozco et al., 2003). Ayet et al. (1997) observed that germinated lentil seeds at day 6 contained highest levels of soyasapogenol B, whereas total phytic acid amounts were greatly reduced after 6 days germination.

4. Antioxidant effects

Given the fact that *in situ* analysis of the antioxidant activity *in vivo* is currently impossible, the antioxidant properties of phytochemicals in plant was mostly evaluated by chemical-based assays, such as Trolox equivalent antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), FRAP, ORAC, total radical-trapping antioxidant parameter (TRAP), inhibition of photochemiluminescence (PCL), inhibition of oxidation of human low-density lipoprotein cholesterol (LDL) and DNA, and iron(II) chelation activity. These antioxidant assay methods are mainly based on two mechanisms, the hydrogen atom transfer (HAT) and the single electron transfer (SET). In the methods of ORAC and PCL, lentil phytochemicals may act as free radical scavengers by donating a hydrogen atom, while assays that determine the ability to inhibit LDL and DNA oxidation by lentil phytochemicals, the antioxidant properties may be based on both hydrogen donation and metal chelation. Commonly used methods such as TEAC and FRAP are considered to detect the ability of lentil phytochemicals to transfer single electron (SET) to reduce any compound including metals, carbonyls and free radicals, whereas DPPH method is considered to follow both HAT and SET system. Several secondary constituents in lentil, mainly phenolics, appeared to serve as powerful antioxidants by preventing against oxidative and free radical mediated reactions. Most of researches on the phenolic profiles of lentils hitherto overviewed use these chemical-based methods to determine the antioxidant activities. These methods may give different equivalent numbers. The total antioxidant activity determined by the ABTS (2,2′-azinobis-3-ethyl-benzthiazoline-6-sulfonic acid) assay was highest in lentils at around 14 µmol Trolox equivalent antioxidant capacity (TEAC)/g among 5 tested legumes, and insoluble-bound phenolics contributed 82–85% of total antioxidant activity in lentils (Han and Baik, 2008). These findings were also confirmed by Pellegrini et al. who observed that lentils had the highest total antioxidant capacity measured by FRAP and TRAP among tested pulses, but came second to broad beans by TEAC. Similarly, Xu et al. reported that lentils had the highest DPPH and ORAC activity in comparison with green pea, yellow pea and chickpea (Xu and Chang, 2008). Alshikh et al. (2015) found that all fractions including free, esterified and insoluble-bound from selected lentils showed varied reducing power and scavenging activity against DPPH, hydroxyl radicals and ABTS radical cation. Their potential bioactivity was further confirmed through inhibition of cupric ion induced human LDL peroxidation and peroxyl radical induced DNA strand breakage. According to our recent studies, the DPPH, FRAP and ORAC values of phenolics in lentils were in the range of 23.83–35.03 µmol TE/g DW, 18.75–34.52 AAE/g DW and 105.06–168.03 µmol TE/g DW, respectively, whilst the antioxidant activities of lentil hydrophobic fraction containing carotenoids and tocopherols were determined as 3.61–4.48 µmol TE/g DW and 2.73–6.23 µmol TE/g DW in the DPPH and PCL assay, respectively (Zhang et al., 2014a; Zhang et al., 2015).

CAA has recently been developed for the evaluation of antioxidant activity to overcome the lack of biological and physiological relevance of the chemical-based assays,. This method measures the ability of antioxidants to prevent the formation of fluorescent dichlorofluorescein (DCF) by 2, 20-azobis (2-amidinopropane) dihydrochloride (ABAP)-generated peroxyl radicals in live cell lines. CAA is considered more biologically relevant than the chemistry-based assays due to accounting of the absorption, metabolism and location of antioxidants inside a living cell. Lentil was reported to exhibit the greatest CAA with the lowest IC_{50} value (0.67 mg/mL), followed by yellow pea and green pea, whereas raw chickpea did not exhibit dose-dependent CAA. Pressurized steaming reduced CAA of lentil with increased IC_{50} value of 1.88 mg/mL (Xu and Chang, 2009). The same authors determined CAA of 11 selected lentil cultivars with the IC_{50} values ranging from 0.30 to 1.41 mg/mL, and revealed that the CAA results significantly correlated with chemical antioxidant assay ORAC (Xu and Chang, 2010).

5. Anti-inflammatory effects

Inflammation is known as a basic defense mechanism of responses to infection, burn, toxic chemicals, allergens or other noxious stimuli. However, excessive or persistent inflammation may lead to tissue destruction and many chronic diseases. There are two scenarios when the inflammatory response itself damages host tissue and causes organ dysfunction. One being an extremely acute or subacute inflammatory response that occurs when there is severe attacks from pathogens (sepsis) or debris from damaged host cells, and the other is pathologic inflammation or low grade inflammation, which when regulation of its pathways is disrupted, triggers chronic diseases such as atherosclerosis, type 2 diabetes and Alzheimer's disease (Tabas and Glass, 2013). As inflammatory reactions often include the formation of tissue-damaging oxidation products, i.e. increased oxidative stress, compounds with high antioxidant activity may inhibit inflammation. Interestingly, lentils have long been used by ancient treatment remedies to treat some inflammatory symptoms, such as skin infections by its water paste and the treatment of burns, after being roasted, milled and applied directly to affected areas (Sezik et al., 2001; Teklehaymanot et al., 2007). Additionally, regular consumption of pulse foods, particularly lentils, have been evidenced by many researches to reduce the incidence of developing chronic inflammatory disease including type 2 diabetes, cardiovascular diseases (CVD) and cancers (Adebamowo et al., 2005; Anderson et al., 2007; Rizkalla et al., 2003). Phenolic-rich lentils have the potential to reduce blood pressure due to its angiotensin I-converting enzyme (ACE) inhibitor activity (Boye et al., 2010; Hanson et al., 2014). The recent study observed that bioactive compounds such as legumin, vicilin and convicilin in lentil present higher ACE-inhibitory and cardioprotective activity (Garcia-Mora et al., 2014). In a hypertensive animal model, lentils administration can reduced the total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and pathological manifestations of cardio-morphometric analysis (Lukito et al., 2001). The specific bioactive components that exert these protective benefits on inflammatory-related diseases still need to be further identified and the mechanisms explored. Cyclooxygenases (COX) and lipoxygenase (LOX) are two major metabolic routes controlling eicosanoid biosynthesis. COX-1 and COX-2 regulate inflammatory responses differentially, thus different types of inhibitors are required for the anti-inflammatory effects. COX-2 inhibitors increase inflammation risk whereas COX-1 inhibitors reduce it. COX-2 is a key enzyme catalyzing the production of prostaglandins (PG) in response to inflammatory stimuli (Surh, 2002). The inhibition of prostaglandin E2 (PGE2) and nitric oxide (NO) production has been considered a potential therapy for different inflammatory disorders. While the nuclear transcription

factor (NF-κB) is the target of the intracellular signaling pathways responsible for induction of COX-2 expression, it can be a positive regulator of COX-2 in diverse cell types (Surh et al., 2001). Many studies reported that plant-derived phenolics and flavonoids exhibit excellent anti-inflammatory activity by regulating the levels of various inflammatory cytokines or mediators including IL-1, IL-6, IL-10, TNF-α, NF-κB, NO, iNOS, LOX, COX-1 and COX-2 (Wu et al., 2011). Boudjou et al. (2013) found that aqueous ethanol (80%) extract of lentil hulls exhibited high anti-inflammatory activities preferentially inhibiting 15-LOX (IC_{50}, 55 μg/ml), with moderate COX-1 (IC_{50}, 66 μg/ml) and weak COX-2 (IC_{50}, 119 μg/ml) inhibitory effects on the COX pathway. Current research on the anti-inflammatory activity of lentil is limited to the *in vitro* studies of phenolics fraction. We have recently reported that phenolics of lentils showed dose-dependent anti-inflammatory activity against pro-inflammatory cytokines COX-2, IL-1β and IL-6 in TNF-α-induced inflammation in Caco-2 cells. The antioxidant and anti-inflammatory activities were positively correlated with the total and individual phenolic contents (Zhang et al. 2017). Concisely, these studies suggest that the dietary consumption of polyphenolic-rich lentils should be on a regular basis, having the potential to reduce the risk of inflammatory-related chronic diseases. More *in vitro* and *in vivo* studies are needed to investigate the anti-inflammatory mechanisms of different phytochemicals in lentils.

6. Interaction with microbiome

The human intestinal tract is home to more than 100 trillion microorganisms. Gut microbes are believed to be involved in major physiological activities, such as protecting gut epithelial cells from pathogens invasion, stimulating the immune system, increasing nutrient availability, stimulating bowel motility. After consumption, foods containing phenolics may undergo a serial of enzyme reactions, following alteration of physiochemical properties in the digestive tract, including the mouth, stomach, small intestinal and large intestine (colon). Those free Phenolic compounds are released from the food matrix in the stomach and small intestinal (gastrointestinal tract) by enzymes and acidic or base conditions, whereas majority of insoluble-bound phenolics survive in gastrointestinal tract digestion and transfer into the colon (large intestine), and release single phenolics or metabolits by the activity of digestive enzymes or colon microbiota. It was reported that only 5–10% of free phenolics can be absorbed in the small intestine, while the remaining 90%–95% degrade and move directly to the colon (Scalbert, A. & Williamson, G. 2000). Therefore, the interaction between the insoluble-bound phenolics and microbiome might play a critical role in the potential health benefits of lentils. The metabolism and absorption mechanism of insoluble-bound phenolics of lentils in colon after microbiota fermentation has not yet been well studied, which is worth investigating in future research.

7. Summary

The above review on the phytochemical composition, as well as their antioxidant and anti-inflammatory activity, indeed showed that lentils contain a plethora of health promoting bioactives in addition to the macronutrients such as starch and protein. Many factors can affect the health benefits of lentils, as the content and composition of phenolics, carotenoids, tocopherols and other nutrients hitherto mentioned can vary significantly in different cultivars, and by different processing methods. Lentils have been traditionally consumed as whole seeds, however, processing of lentil into different fractions, i.e. flour, protein and starch, has been in steady sharp growth in recent years due to increased consumer needs in alternative plant protein (other than soy protein) and specialty foods such as gluten-free foods. Lentil processing generates significant amount of seed coats which are currently of no or low value, yet studies have shown that these are the major source of dietary fiber and antioxidant and anti-inflammatory phenolics, in both free and insoluble-bound forms. The high content of bound phenolics, and the *in vitro* antioxidant and anti-inflammatory effects of lentil hulls warrant further studies *in vivo*. In addition, potential synergistic effects may exist among different classes of phytochemicals. Researches on the potential health benefits of lentil bioactives have not paid much attention to the bioaccessibility, bioavailability *in vivo*. In order to be absorbed and to reach the target cells or tissues at a certain level for these compounds, they have to survive the gastrointestinal tract, and be uptaken and transported. This requires a multidisciplinary in approach in future studies.

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