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Phenolic profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and inhibitory effects on α-glucosidase and pancreatic lipase

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ABSTRACT

Phenolic extracts from 20 Canadian lentil cultivars (*Lens culinaris*) were evaluated for total phenolic contents and composition, antioxidant activities (DPPH, FRAP, ORAC), and inhibitory properties against α-glucosidase and pancreatic lipase. Twenty one phenolic compounds were identified in the present study, with the majority being flavonoids, including kaempeferol glycosides, catechin/epicatechin glucosides and procyanidins. These phenolic compounds not only contributed significantly to the antioxidant activities, but they were also good inhibitors of α-glucosidase and lipase, two enzymes, respectively, associated with glucose and lipid digestion in the human intestine, thus contributing significantly to the control of blood glucose levels and obesity. More interestingly, it was the flavonols, not the flavanols, which showed the inhibitory activities against α-glucosidase and pancreatic lipase. Our result provides supporting information for developing lentil cultivars and functional foods with improved health benefits and suggests a potential role of lentil consumption in managing weight and control of blood glucose.

1. Introduction

Oxidative damage caused by free radicals is considered to be one of the aetiological factors associated with several human chronic diseases including cardiovascular diseases, neural disorders such as Alzheimer's and Parkinson's disease, diabetes and cancer (Xu, Yuan, & Chang, 2007). Natural phenolic compounds endogenous to food of plant origin have been well studied as antioxidants, which can delay or inhibit oxidative damage, thus preventing the onset of oxidative stress related diseases in the human body (Willett, 1994). Epidemiological and interventional studies have shown that the consumption of phenolic-rich foods is inversely associated with the prevalence of several chronic diseases (Kris-Etherton et al., 2002). Apart from antioxidant activity, phenolic compounds may also play a key role in the inhibition of α-glucosidase and lipase activities (He, Lv, & Yao, 2007; Zhang et al., 2010). The inhibition of α-glucosidase reduces intestinal glucose digestion and absorption, consequently controlling the post-prandial glycaemic response, which is key to the management of type 2 diabetes (Balasubramaniam et al., 2013). Studies have reported that phenolic compounds extracted from some beans are strong inhibitors of α-glucosidase and lipase (Sreerama, Takahashi, & Yamaki, 2012). Lipase is a key enzyme involved in triglyceride digestion. The inhibition of lipase is considered to be one of the more effective strategies for managing obesity (Xu, Han, Zheng, Lee, & Sung, 2005). Polyphenol constituents extracted from natural plants such as grape-seed and tea have been reported to inhibit the activity of lipase (Moreno et al., 2003; Nakai et al., 2005). Obesity is one of the most important pathogenic factors of type 2 diabetes. Nguyen, Nguyen, Lane, and Wang (2011) found that the prevalence of diabetes increases with increasing weight in the latest National Health and Nutrition Examination Survey (NHANES), suggesting that obesity control is an important intervention in an effort to reduce the incidence of diabetes.

Legumes are important basic staple foods for humans in many countries, providing not only ideal protein, carbohydrates (dietary

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fibre), minerals, and vitamins complementary to cereal-based diets, but also a wide range of phytochemicals including the phenolics with antioxidant and other bioactivities. Among legumes, lentils (*Lens culinaris*) have been gaining increasing attention for health benefits as human diet, and are considered to be an excellent source of dietary antioxidants largely due to their high level of bioactive phytochemicals (Zhang et al., 2014). An epidemiological study indicated that among several dietary flavonols and flavonol-rich foods, only the consumption of beans or lentil was associated with a lower incidence of breast cancer (Adebamowo et al., 2005). Lentils have one of the lowest glycaemic index (GI) among major staple foods (Jenkins, Wolever, Taylor, Barker, & Fielden, 1980), and many studies have shown the benefits of low GI foods in managing type 2 diabetes (Brand-Miller, Hayne, Petocz, & Colagiuri, 2003). Consumption of lentils also led to weight loss, which is recommended for all overweight and obese individuals who have diabetes or are at risk for diabetes by the American Diabetes Association (Papanikolaou & Fulgoni, 2008). On average, while the overall global pulse consumption is declining, the annual consumption of lentils is steadily increasing (Zou, Chang, Gu, & Qian, 2011). The consumption of lentils is limited in the western countries due to traditional eating custom, lack of consumer understanding, processing techniques and diversified food products. Developing diverse foods by incorporating lentil into the western diets has been highly recommended (Aguilera et al., 2010; Han & Baik, 2008).

Lentils belong to the genus *Lens* that includes many species and hybrids. This genetic diversity is likely to result in variability of their phytochemical composition and associated bioactive properties. While many studies have investigated the phenolic compounds and antioxidants in lentils (Boudjou, Dave Oomah, Zaidi, & Hosseinian, 2012; Dueñas, Sun, Hernández, Estrella, & Spranger, 2003), most of them have focused on traditional lentil cultivars, particularly those from the Mediterranean dry and arid regions with high legume consumption. Meanwhile, although the potential benefits of lentil consumption in diabetes management have been somewhat attributed to the resistant starch (García-Alonso, Goñi, & Saura-Calixto, 1998), contribution from other components such as phenolic compounds of lentils are less known. The effects of different lentil phenolics on α-glucosidase and lipase activities have not been studied. As such, in the present study, we have selected 20 most popular lentil cultivars grown in Canada to assess the inhibitory capability of phenolics against α-glucosidase and lipase activities. As these two enzymes are associated with glucose and lipid digestion in the human intestine and are critical in controlling blood glucose levels and obesity, results from this study will help further identifying and understanding fully the roles of dietary lentil phenolics in type 2 diabetes management. The antioxidant and enzyme inhibitory effects by the phenolics in different lentil cultivars will also provide supporting information for selecting lentil cultivars and for developing lentil-based functional foods with improved health benefits.

2. Materials and methods

2.1. Plant materials

The 20 lentil cultivars and breeding lines used for this study were the same as those used in our recent publication (Zhang et al., 2014). The 20 lentil cultivars are categorised into 2 groups based on their colours: 10 Red lentils: Blaze, Redcliff, Maxim, Rouleau, Redbow, Redberry, Impact, Imperial, Rosetown, and Dazil; 10 Green lentils: Imvincible, Greenland, Asterix, Imigreen, Impower, Improve, Sovereign, Milestone, Eston and Plato. The whole lentil samples were ground into fine powder, and stored for less than 6 months at −4 °C in sealed plastic bag prior to analysis.

2.2. Chemicals and reagents

All standard reference materials including *p*-hydroxybenzoic acid, syringic acid, *trans*-*p*-coumaric acid, epicatechin gallate, quercetin-3-xyloside, quercetin-3-glucoside, and kaempferol-3-glucoside, 1,3,5-tri(2-pyridyl)-2,4,6-triazine (TPTZ), L-ascorbic acid, gallic acid, Folin–Ciocalteu's phenol reagent, fluorescein, 2,2-diphenyl-1-picrylhydrazyl 107 (DPPH), Trolox and 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH), as well as the enzyme inhibition assay chemicals including rat intestinal acetone powder, pancreatic lipase, 4-methylumbelliferyl-α-D-glucoside (4-MUG) and 4-methylumbellifery oleate (4-MUO), acarbose, orlistat were purchased from Sigma (St. Louis, MO, USA). Sodium acetate, ferric chloride hexahydrate, monobasic sodium phosphate, dibasic sodium phosphate and HPLC-grade solvents, including methanol (MeOH), formic acid and hydrochloric acid (HCl) were purchased from Caledon Laboratories (Georgetown, ON, Canada).

2.3. Sample preparation

Phenolic compounds were extracted from lentils following a slightly modified version of the method described by Aguilera et al. (2010). Briefly, ca. 1 g lentil sample was accurately weighed and placed in a 50 mL screw-capped plastic tube and extracted with 20 mL of 70% MeOH containing 0.1% HCl (v/v) using a rotary shaker (Scientific Industries Inc., Bohemia, NY, USA) (at 400 rpm) overnight (ca. 15 h) at room temperature. The mixture was then centrifuged at 3000*g* for 10 min. The residue was re-extracted twice more, each with 10 mL of the same solvent and under the same conditions. The collected supernatants were brought to 40 mL with the extraction solvent, and filtered through a 0.45-μm PTFE membrane filter (VWR International, Mississauga, ON, Canada) in preparation for spectrophotometric, HPLC analyses and antioxidant assay.

2.4. Hydrolysis

The crude phenolic extract was hydrolysed with HCl (final concentration of 2 N) and heated at 85 °C for 1 h. Samples were allowed to cool down to room temperature and then centrifuged at 3000*g* for 5 min. The supernatant was filtered and subjected to HPLC analysis.

2.5. Purification of lentil extracts

To examine the enzyme inhibition activities, it was necessary to remove the interfering sugars from lentil extracts. The purification was conducted on 20cc (1 g) Oasis® HLB cartridges (Waters, Milford, MA, USA) employing a solid phase extraction (SPE) procedure. In brief, 40 mL of the supernatants collected above were concentrated to one quarter of its original volume on a rotary evaporator under reduced pressure at 50 °C. The SPE cartridge was activated with 20 mL pure methanol first and conditioned by 20 mL of 20% methanol. The concentrated extract (approximately 10 mL) was loaded onto the conditioned cartridge and sugars and other polar components were removed by washing with 10 mL of 5% methanol. The phenolic compounds were then eluted with 20 mL pure methanol and evaporated under nitrogen. The dried samples were reconstituted in water, and diluted to serial concentrations in preparation for enzyme inhibition assays.

2.6. Determination of total phenolic content (TPC)

The total phenolic content in crude extracts was determined by a colorimetric reaction using Folin & Ciocalteu's phenol reagent (Li, Deng, Wu et al., 2012). Briefly, 25 μL gallic acid standard or lentil

extract were mixed with 125 μL 0.2 M Folin–Ciocalteu reagent in a 96-well microplate and reacted for 10 min at room temperature. A 125 μL saturated sodium carbonate (Na_2CO_3) solution was then added and incubated for 30 min at room temperature before the absorbance of the reaction mixture was read at 765 nm using a visible–UV microplate kinetic reader (EL 340, Bio-Tek Instruments, Inc., Winooski, VT, USA). Calibration was achieved with an aqueous gallic acid solution (50–500 μg/mL). The total phenolic content (TPC) was expressed as mg gallic acid equivalent (GAE) per gram dry weight lentil (mg GAE/g DW) based on the calibration curve.

2.7. Determination of total flavonoids content (TFC)

The total flavonoid content of lentil extracts was determined according to a previously reported method with slight modification (Herald, Gadgil, & Tilley, 2012). In brief, 110 μL of $NaNO_2$ (0.066 M) was mixed with 25 μL of catechin standard (50–500 μg/mL) or lentil extracts in a 96-well microplate and allowed to react for 5 min at room temperature. Fifteen microlitres of 0.75 M aluminium chloride ($AlCl_3$) were as then added into each of the wells and incubated for 6 min at room temperature. The absorbance was read at 510 nm after the addition of 100 μL of 0.5 M NaOH solution. TFC was expressed as mg catechin equivalents (mg CAE)/g DW.

2.8. Determination of condensed tannin content (CTC)

Tannin content of the lentil extracts was determined by acidified vanillin assay (Downey & Hanlin, 2010). Briefly, 50 μL of lentil extracts or catechin standards (50–500 μg/mL) were mixed with 200 μL of vanillin reagent (containing 3% vanillin and 14% HCl in MeOH) in a 96-well microplate and allowed to react for 20 min at room temperature. The absorbance was recorded at 500 nm and the result was expressed as mg catechin equivalents (mg CAE)/g DW.

2.9. HPLC–DAD and HPLC–MS analyses of phenolic compounds

HPLC analysis was performed using an Agilent 1100 series HPLC system equipped with an auto-sampler, an inline degasser, a quaternary pump and a diode-array detector (DAD) and the ChemStation software. HPLC separation was performed on a Kinetex phenyl-hexyl 2.6 μm 100 × 4.60 mm column (Phenomenex Inc., Torrance, CA, USA). The mobile phase consisted of 5% formic acid in water (v/v) (solvent A) and 95% methanol/5% acetonitrile (v/v) (solvent B). Injection volume was 7 μL and flow rate was kept at 0.7 mL/min for a total run time of 60 min. The gradient solvent system used was as follows: 0–12 min, 0–20% B; 12–25 min, 20% B; 25–50 min, 20–80% B; 50–55 min, 80–100% B; 55–60 min, 100–0% B. Data were collected at 280 nm for phenolic acids and flavanols and 360 nm for flavonols. Phenolic compounds were identified by comparing retention time and UV absorption spectra with available external standards and confirmed by LC–MS. Compounds with no standard reference materials were tentatively identified by UV spectrum, MS data and by matching with published data. Quantification was performed with standard curves of external standards generated by plotting HPLC peak areas against the concentrations (0.5–200 μg/mL) ($r = 0.9999$). For compounds with no standards, quantification was based on calibration curves of similar compounds of the same phenolic subgroup. For instance, catechin glucoside, catechin gallate, procyanidins and related flavonoid derivatives were expressed as catechin equivalents; epicatechin glucoside and epicatechin gallate was expressed as epicatechin equivalen. Kaempferol glycosides and quercetin glycosides were quantified with the calibration curve of kaempferol and quercetin, respectively.

Mass spectra were obtained using a Dionex UHPLC UltiMate 3000 LC interfaced to an amaZon SL ion trap mass spectrometer (Bruker Daltonics, Billerica, MA, USA), following separation on a C18 column (Agilent Poroshell 120, 2.7 μm particle size, 150 × 4.6 mm, Santa Clara, CA, USA). The initial mobile phase condition was 2% acetonitrile in 0.1% formic acid. The gradient went to 98% acetonitrile in 0.1% formic acid in 30 min. The flow rate was maintained at 0.4 mL/min. The mass spectrometer electrospray capillary voltage was maintained at 4.5 kV and the drying temperature at 220 °C. The drying gas flow rate was set to 10 L/min with nebuliser pressure at 40 psi. Nitrogen was used as both nebulising and drying gas; helium was used as collision gas at 60 psi. For the monitoring of phenolic compounds, the mass-to-charge ratio was scanned across the m/z range 100–1500 in enhanced resolution negative-ion auto MS/MS mode. The smart parameter setting (SPS) was used to automatically optimise the trap drive level for precursor ions. The instrument was externally calibrated with the ESI (TuneMix, Agilent).

2.10. Antioxidant assays

2.10.1. DPPH assay

The antiradical activity of the lentil extracts was determined spectrophotometrically based on our previous reported method (Li, Deng, Liu, Loewen, & Tsao, 2013). Briefly, 200 μL of a methanolic solution of DPPH (350 μM) was mixed with 25 μL of lentil extract or a series of Trolox standard solutions (62.5–1000 μM) in a 96 well plate and let stand for 6 h at room temperature before the absorbance was recorded at 517 nm. The DPPH antioxidant activity was calculated as μmol Trolox equivalent per gram dry weight lentil (μmol TE/g DW).

2.10.2. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay followed a previously reported procedure (Li, Deng, Zhu et al., 2012). Briefly, 10 μL of L-ascorbic acid standard (62.5–1000 μM) or lentil extract were mixed with 300 μL of ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM FeCl3·6H2O at a ratio of 10:1:1 (v/v/v)) and allowed to react at room temperature for 2 h. The absorbance was read at 593 nm using the aforementioned microplate kinetic reader. The antioxidant activity was expressed as micromole ascorbic acid equivalents (AAE) per gram dry weight lentil (μmol AAE/g DW).

2.10.3. Oxygen radical absorption capacity (ORAC) assay

The ORAC assay was conducted according to existing protocols (Li, Deng, Zhu et al., 2012). Briefly, 25 μL of blank, Trolox standard or lentil extract (in triplicate) were mixed with 200 μL fluorescein solution (0.0868 nM) and incubated for 30 min at 37 °C, then added 25 μL AAPH, (153 mM). The fluorescence was measured every minute for about 120 min until it reached zero (excitation wavelength 485 nm, emission wavelength 528 nm) in a Bio-Tek Fluorescence Spectrophotometer equipped with an automatic thermostatic holder (PLX 800, Bio-Tek Instruments, Inc., Winooski, VT, USA). A calibration curve was constructed daily by plotting the calculated differences of area under the fluorescein decay curve between the blank and the sample for a series of standards of Trolox solutions (6.25–100 μM). The results were expressed as micromole Trolox equivalent (TE) per gram dry weight sample (μmol TE/g DW).

2.11. Enzyme inhibition assay

2.11.1. α-Glucosidase inhibition assay

4-Methylumbelliferyl-α-D-glucoside (4-MUG) was used as a fluorogenic substrate of α-glucosidase in this assay. Briefly, crude

enzyme from rat intestinal acetone powder was suspended in 0.1 M cold phosphate buffer (pH 6.9) at 25 mg/mL and hydrated for 1 h at 4 °C. After centrifugation at 10,000g, the supernatant was collected as working enzyme solution. Twenty five microlitres of sample (final concentrations: 10, 25 and 50 mg dried de-sugared extract/mL) prepared from de-sugared and lyophilised extract, or acarbose (positive control), or solvent blank were mixed with 75 μL of substrate (0.5 mM) in a well of a 96 well plate and incubated at 37 °C for 15 min while shaking. Then, 25 μL of the working enzyme solution were added into each well and allowed to incubate for 1 h at 37 °C. Finally, 125 μL of borate buffer (pH 9.8, 300 mM) were added to terminate the reaction. The fluorescence was measured at excitation wavelength of 360 nm and emission wavelength of 460 nm with the Bio-Tek Fluorescence Spectrophotometer (PLX 800, Bio-Tek Instruments, Inc., Winooski, VT, USA). The α-glucosidase inhibitory activity was expressed as IC_{50} which was calculated from the percent inhibition of the serial dilutions as mentioned above. IC_{50} is defined as the concentration of extract required to inhibit 50% of the enzyme activity, and expressed as milligram de-sugared extract per millilitre solvent (mg/mL).

2.11.2. Lipase inhibition assay

The inhibition of lipase activity was determined using a published method with modifications (Kawaguchi, Mizuno, Aida, & Uchino, 1997). Pancreatic lipase (type II, from porcine pancreas) and 4-MUO served as the reaction enzyme and fluorogenic substrate, respectively. In brief, the mixture of 225 μL of substrate (0.24 mM) and 25 μL sample solution of de-sugared extract, or orlistat (positive control), or solvent blank were incubated at 37 °C for 15 min, followed by addition of 25 μL of enzyme solution (0.55 mg/mL) in Tris–HCl buffer (0.1 M, pH 8.0) in each well. After reaction at 37 °C for 1 h, the fluorescence was measured at excitation wavelength of 360 nm and emission wavelength of 460 nm with the Bio-Tek Fluorescence Spectrophotometer. The lipase inhibitory activity was expressed in IC_{50} and similarly calculated as described for the α-glucosidase.

2.12. Statistical analysis

All assays or tests were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the means. Associations between enzyme activities and polyphenol concentrations were assessed by Pearson correlation. Differences were considered significant at $p < 0.05$. All statistical analyses were performed using Statistix for Windows version 9.0 (Analytical Software, Tallahassee, FL, USA).

3. Results and discussion

3.1. Total phenolic, flavonoid and condense tannin contents

Natural phenolic compounds widely distributed in food plants are considered to exert their beneficial health effects mainly through their antioxidant activities. Flavonoids and condensed tannins, the predominant phenolic compounds in legume seeds, are widely found in lentils, peas, soybeans and common beans, and as mentioned earlier, may also positively contribute to weight loss and type 2 diabetes risk reduction, in addition to the antioxidant activities.

TPC, TFC, and CTC of the 20 lentil cultivars are summarised in Table 1. TPC varied significantly among tested lentils, ranging from 4.56 to 8.34 mg GAE/g DW. Cultivar Asterix had the highest TPC at 8.34 mg GAE/g DW followed by Greenland and Redcliff at 7.62 and 7.02 mg GAE/g DW, respectively. Cultivars Asterix and Greenland also topped the list of TFC, containing 1.98 and 1.92 mg CE/g DW, respectively. CTC ranged from 3.00 to 7.80 mg CE/g DW, which accounted for 59.52–93.53% of the TPC, indicating condensed tannins are the main phenolic component in these lentils. The highest concentration (7.80 mg CE/g DW) of CTC was once again found in Asterix, followed by Greenland (5.88 mg CE/g DW) and Redcliff (5.82 mg CE/g DW). According to the Pearson's correlation coefficient, CTC was strongly correlated with the TPC ($R^2 = 0.8977$), and moderate correlations were found between TPC and TFC ($R^2 = 0.8076$) and between TFC and CTC ($R^2 = 0.7562$).

TPC and CTC of the 20 Canadian lentils in the present study were similar to those reported by others, whereas TFC was differed (Xu, Yuan, & Chang, 2007). The difference in TFC could be attributed to geographical location and genotype of the tested lentils. The TPC and CTC in lentils were significant higher than those reported for other legumes such as common beans, soybean and chickpea (Xu, Yuan, & Chang, 2007).

3.2. Identification and quantification of phenolic compounds

Fig. 1A shows typical HPLC chromatograms of the crude extract of lentil (Greenland) recorded at 280 and 360 nm, along with that of mixed standards. The retention time, UV spectra, the molecular and fragment ions of a total of 21 confirmed and tentatively identified phenolic compounds are presented in Table 2. *p*-Hydroxybenzoic acid, syringic acid, *trans*-*p*-coumaric acid, epicatechin gallate, quercetin-3-xyloside, quercetin-3-glucoside, and kaempferol-3-glucoside (peaks 2, 8, 10, 11, 15, 18 and 21, respectively) were positively identified by comparing their retention time and UV spectra with those of the corresponding commercial standards (Fig. 1A), and the results were confirmed by MS (Table 2).

Peak 1 showed a λ_{max} of 257 nm which was similar to that of *p*-hydroxybenzoic acid, and it exhibited a molecular ion $[M-H]^-$ at *m*/*z* 153 corresponding to dihydroxybenzoic acid. Peak 6 had a UV spectrum similar to that of sinapic acid, but it eluted from the column at a different retention time. While its molecular ion was not detected, a fragment ion $[F-H]^-$ at *m*/*z* 223 corresponded to a sinapic acid residue. This compound was tentatively identified as sinapic acid derivative. Peak 9 had a UV spectrum similar to that of *trans*-*p*-coumaric acid but with different retention time. The LC–MS showed a molecular ion $[M-H]^-$ at an *m*/*z* 279 and a fragment ion $[F-H]^-$ at *m*/*z* 163; the difference was loss of a malic acid molecule. Peak 9 was then tentatively identified as *trans*-*p*-coumaroyl malic acid, which was also reported by others in lentil (Aguilera et al., 2010).

Peaks 3, 5, 7, 16, and 17 all exhibited a λ_{max} of 279 nm and a UV spectrum characteristic of flavanol monomers (catechin or epicatechin) and procyanidin oligomers. LC–MS measurements showed that peaks 7, 16 and 17 had the same molecular ion $[M-H]^-$ at *m*/*z* 577 corresponding to a procyanidin dimer and a fragment ion $[F-H]^-$ at *m*/*z* 289 matching catechin or epicatechin; these compounds were tentatively identified as procyanidin dimer 1, 2 and 3, respectively. LC–MS data of peaks 3 and 5 showed the same molecular ion $[M-H]^-$ at *m*/*z* 451 and fragment ion $[F-H]^-$ at *m*/*z* 289, highly likely to be catechin or epicatechin glucoside. Based on the elution order of catechin and epicatechin on a reversed phase column, peaks 3 and 5 were tentatively identified as catechin glucoside and epicatechin glucoside, respectively. These two compounds have been detected in green lentils by Amarowicz et al. (2010).

Peak 4 presented a similar UV spectrum, but different retention time to that of epicatechin gallate which has been positively identified. It showed a negative molecular ion $[M-H]^-$ at *m*/*z* of 442 and a fragment ion $[F-H]^-$ at *m*/*z* of 289, the same as those of epicatechin gallate. This compound was then tentatively identified as catechin gallate, which has also been reported by Amarowicz et al.

Table 1
Total phenolic content, total flavonoid content and total condensed tannin content of 20 lentil cultivars.[A]

Cultivar		TPC[a] (mg GAE/g DW)	TFC[b] (mg CAE/g DW)	CTC[c] (mg CAE/g DW)
Red lentils	Blaze	5.04 ± 0.36 ab	0.60 ± 0.00 a	3.00 ± 0.12 a
	Redcliff	7.02 ± 0.48 ij	1.62 ± 0.12 e	5.82 ± 0.24 k
	Maxim	6.60 ± 0.42 ghi	1.50 ± 0.06 de	5.46 ± 0.30 ij
	Rouleau	6.30 ± 0.48 efgh	1.26 ± 0.18 c	4.62 ± 0.06 def
	Redbow	6.36 ± 0.30 fgh	1.26 ± 0.18 c	4.92 ± 0.42 fgh
	Redberry	6.00 ± 0.42 def	1.32 ± 0.12 cd	4.08 ± 0.36 bc
	Impact	6.48 ± 0.24 fghi	1.38 ± 0.06 cd	4.68 ± 0.24 def
	Imperial	6.72 ± 0.36 hi	1.50 ± 0.18 de	5.10 ± 0.18 ghi
	Rosetown	6.00 ± 0.30 defg	1.02 ± 0.12 b	4.50 ± 0.12 de
	Dazil	6.84 ± 0.24 hi	1.32 ± 0.12 cd	5.16 ± 0.18 hi
Green lentils	Imvincible	6.78 ± 0.30 hi	1.50 ± 0.18 de	5.58 ± 0.36 jk
	Greenland	7.62 ± 0.06 j	1.92 ± 0.12 f	5.88 ± 0.18 k
	Asterix	8.34 ± 0.54 k	1.98 ± 0.06 f	7.80 ± 0.12 i
	Imigreen	5.64 ± 0.24 bcd	0.84 ± 0.06 ab	4.38 ± 0.12 cd
	Impower	6.36 ± 0.48 fgh	1.32 ± 0.12 cd	4.74 ± 0.06 efg
	Improve	5.76 ± 0.36 cde	0.84 ± 0.18 ab	3.96 ± 0.18 b
	Sovereign	4.56 ± 0.24 a	0.66 ± 0.06 a	3.36 ± 0.06 a
	Milestone	6.12 ± 0.30 defg	1.26 ± 0.18 c	4.32 ± 0.24 cd
	Eston	6.54 ± 0.06 fghi	1.50 ± 0.18 de	4.92 ± 0.06 fgh
	Plato	5.34 ± 0.30 bc	1.38 ± 0.06 cd	4.08 ± 0.12 bc

[a,b,c]Total phenolic content, total flavonoid content, condensed tannin content, respectively.
[A] Values are mean ± SD, $n = 3$. Values followed by the different letter in the same column are significantly different ($p < 0.05$).

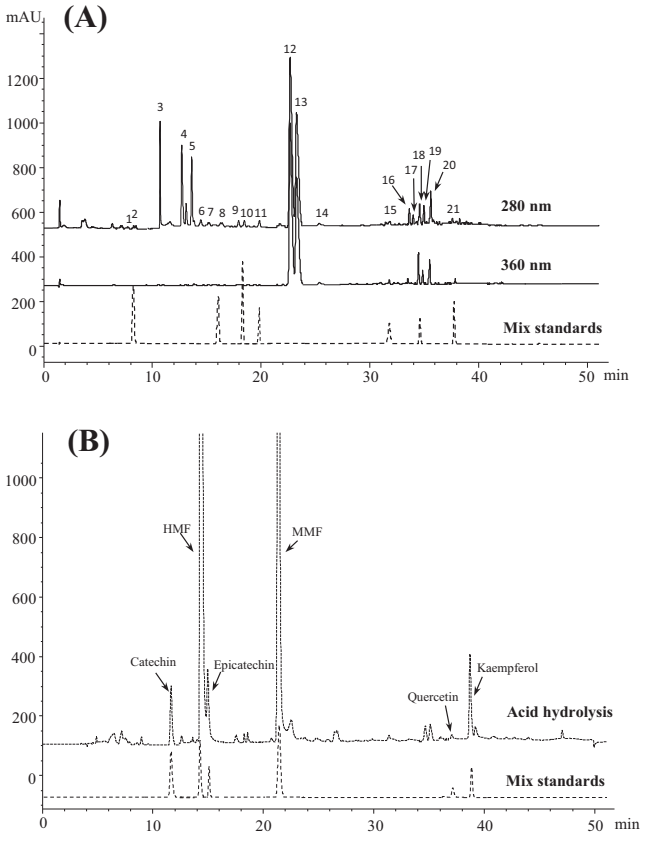

Fig. 1. HPLC chromatograms of phenolic extract of a typical lentil (Greenland) as detected at 280 nm and 360 nm, and a standard mixture at 280 nm (panel A); and acid hydrolysis mixture of the same extract as detected at 280 nm (panel B). Peaks without a number were not identified. 5-Hydroxymethyl-2-furan-2-carbaldehyde (HMF) and 5-methoxymethylfuran-2-carbaldehyde (MMF) were a sugar degradation product and its derivative.

(2010). Peaks 12, 13 and 14 all had similar UV spectrum to that of kaempferol. Peak 12 had a molecular ion $[M-H]^-$ of m/z 901, and two fragment ions $[F-H]^-$ at m/z 755 ($[M-H-146]^-$ from loss of deoxyhexose) and m/z 285 which corresponds to kaempferol aglycone after additional losses of two more molecules of hexose and another deoxyhexose. This compound was tentatively identified as kaempferol tetraglycoside which has been reported by Taylor, Fields, and Sutherland (2007). Peak 13 showed a molecular ion $[M-H]^-$ of m/z 755 and a fragment ion $[F-H]^-$ at m/z 285, and based on what was observed for peak 12, this compound was tentatively identified as kaempferol triglycoside, which has also been reported by Zou et al. (2011). Peak 14 showed a molecular ion $[M-H]^-$ of m/z 739 and a kaempferol aglycone fragment ion $[F-H]^-$ at m/z 285 (from losing a molecule of robinose and a rhamnose), and by comparing with the data reported by Aguilera et al. (2010), this peak was tentatively identified as kaempferol-3-robinoside-7-rhamnoside. Peaks 19 and 20 showed typical UV spectral pattern of flavonoids and were tentatively identified according to Zou et al. (2011).

While no further effort was made to confirm the exact position of the glycosyl bonds on the flavonoid core structure for the above tentatively identified glycosides, acid hydrolysis of the crude extract was conducted, and as shown in Fig. 1B, all glycosides (peak 3, 4, 5, 12, 13, 16, 17, 18 and 21) disappeared and 4 new peaks (monomeric catechin, epicatechin, kaempferol and quercetin) were produced. This result confirms the identification of the flavonoid aglycone moieties and endorses the intrinsic existence of their glycosides in lentils. **5**-Hydroxymethy-2-furfural (HMF) and 5-methoxymethylfuran-2-carbaldehyde (MMF) peaks in Fig. 1B are degradation products from the soluble sugar molecules of the extract or from those hydrolysed off the flavonoid glycosides (Chen et al., 2014).

Concentrations of the individual phenolic compounds are summarised in Table 3. Kaempferol glycosides dominated the phenolic profile of lentils, with the tetraglycoside and triglycoside having the highest concentrations ranging from 210.05 to 297.15 μg/g DW and 99.96 to 181.81 μg/g DW, respectively, followed by catechin glucoside, catechin gallate, epicatechin glucoside. *trans*-*p*-Coumaric acid and *p*-hydroxybenzoic acid were the main free phenolic acids found in all lentils, but in much lower amounts. These results suggest that the majority of extractable phenolics in tested lentils are flavonoid glycosides. The total phenolic indexes (TPI), sum of concentrations of all phenolics compounds detected in lentils, were between 594.63 and 952.55 μg/g DW, much lower

Table 2
Twenty one phenolic compounds identified by HPLC–DAD–MS in the lentil extracts.

Peak no.	Time (min)	Phenolics	λ_{max} (nm)	$[M-H]^-$ (m/z)	Fragment ions $[F-H]^-$ (m/z)	Identification
1	7.91	Dihydroxybenzoic acid	257	153		MS (Aguilera et al., 2010)
2	8.04	*p*-Hydroxybenzoic acid	223, 257	137		Rt, UV, MS
3	10.03	Catechin glucoside	245, 279	451	289	MS (Amarowicz et al., 2010)
4	11.71	Catechin gallate	277	442	289	MS (Amarowicz et al., 2010)
5	12.72	Epicatechin glucoside	279	451	289	MS (Amarowicz et al., 2010)
6	13.84	Sinapic derivative	246, 320		223	MS
7	14.75	Procyanidin dimer (1)	232, 279	577	289	MS
8	15.83	Syringic acid	277	197		Rt, UV, MS
9	17.50	*trans*-*p*-Coumaroyl malic acid	310	279	163	MS (Aguilera et al., 2010)
10	18.08	*trans*-*p*-Coumaric acid	310	163		Rt, UV, MS
11	19.92	Epicatechin gallate	277	442	289	Rt, UV, MS
12	22.75	Kaempferol tetraglycoside	266, 349	901	755, 285	MS (Taylor et al., 2007)
13	23.18	Kaempferol triglycoside	266, 349	755	285	MS (Zou et al., 2011)
14	25.51	Kaempferol-3-robinoside-7-rhamnoside	264, 353	739	285	MS (Aguilera et al., 2010)
15	31.82	Quercetin-3-xyloside	257, 355	433	301	Rt, UV, MS
16	33.38	Procyanidin dimer (2)	232, 279	577	289	MS
17	33.73	Procyanidin dimer (3)	232, 279	577	289	MS
18	34.33	Quercetin-3-glucoside	257, 355	462	285	Rt, UV, MS
19	34.75	Flavonoid derivative	270,318, 351 sh	523		MS (Zou et al., 2011)
20	35.38	Flavonoid derivative	270,318, 351 sh	523		MS (Zou et al., 2011)
213	37.40	Kaempferol-3-glucoside	266, 350	447	285	Rt, UV, MS

Rt, UV, MS: positively identified by matching retention time, UV/Vis and LC–MS/MS data; MS: tentatively identified by LC–MS/MS, UV/Vis and related references.

than the TPC (Table 1), which can be attributed to incomplete quantification of all peaks in the HPLC method, and to the potential interferences by other components in the TPC method. Similarly the total flavonol index (TFI) was also lower than the TFC, ranging from 351.82 to 528.42 μg/g DW. The lower TPI and TFI may also be caused by conjugation between the phenolic compounds with soluble components such as small peptides or oligosaccharides (Saulnier et al., 1999; Yokotsuka & Singleton, 1995).

3.3. Antioxidant activities and correlation with phenolics

The antioxidant activities varied widely and significantly among the hydrophilic extracts of the 20 tested lentils. As shown in Fig. 2A–C, the antioxidant activity as assessed by the DPPH assay ranged from 23.83 to 35.03 μmol TE/g DW, the FRAP value which is a measurement of the reducing power ranged from 18.75 to 34.52 AAE/g DW with the highest antioxidant activity being nearly double of the lowest. The ORAC values of the lentil extracts also varied from 105.06 to 168.03 μmol TE/g DW. The overall highest antioxidant activity was found in cultivar Asterix for all three antioxidant assays, which was consistent with the finding that this cultivar also had the highest TPC, TFC and CTC (Table 1).

Many previous studies have reported the contribution of total phenolic content to the antioxidant capacity of plant extracts (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009; Li, Deng, Wu et al., 2012). Attempts were made to analyse the correlation between the antioxidant activities (DPPH, FRAP and ORAC) and phenolic contents (TPC, TFC and CTC) using the Pearson's correlation coefficient (R^2). Strong correlations were observed between the TPC and TPI ($R^2 = 0.9074$), TPC and CTC ($R^2 = 0.8977$). From Table 1, it is clear that condensed tannins contribute significantly to the total phenolic content in lentils. The antioxidant activity as measured by the FRAP assay positively and strongly correlated with TPI, TPC, TFC and CTC, with correlation coefficients at $R^2 = 0.8548$, 0.9366, 0.8518, and 0.9317, respectively. These results suggest that phenolic compounds, especially condensed tannins, are major contributors to the reducing power of lentils. Antioxidant activities as measured by DPPH and ORAC methods showed moderate and low correlation with the phenolics, which were similarly observed in other foods (Li, Deng, Wu et al., 2012; Zou et al., 2011). This could be explained by the fact that phenolic compounds are not the only antioxidants in lentil extracts. The mechanisms and kinetics of the antioxidant action in different assays including the ORAC assay may inherently lack the correlation with phenolics.

As discussed above, the major phenolic compounds i.e. flavonols (including kaempferol and quercetin glycoside) and flavanols (including catechin/epicatechin-glucoside and procyanidin dimer) in the extracts may play the most significant role in the total antioxidant activities. To further corroborate this finding, seven available standards representing flavonols (kaempferol, kaempferol-glucoside, quercetin, and quercetin-arabinoside) and flavanols (catechin, epicatechin, procyanidin B1) were measured in the same antioxidant assays. Among the pure standards (20 μg/mL), quercetin exhibited the strongest antioxidant activities in all three assays having 367.62 μM TE, 645.98 μM AAE and 957.00 μM TE in the DPPH, FRAP and ORAC assay, respectively (Table 4). The antioxidant activity of kaempferol was significantly lower than that of quercetin, which can be well explained by the lesser hydroxyl group in the B-ring. Also important was the finding that the aglycones kaempferol and quercetin exhibited higher antioxidant activities than their respective glycosides, suggesting the glycosylation remarkably reduces the antioxidant activity of flavonols. Similar findings have also been reported by Baderschneider and Winterhalter (2001). In terms of the flavanols, monomers like catechin and epicatechin showed higher antioxidant activities than the dimer procyanidin B1 at the same concentration. The antioxidant activities of individual tested standards were significant lower than lentil extract after converting to the same equivalence, indicating that those phenolics determined by HPLC contribute to the antioxidant activities, but are not the only phenolic antioxidants in lentil extract. These results could also well-explain the difference between TPC and TPI. This could also be the result of a synergistic action among the different phenolic compounds.

3.4. Inhibitory activities on α-glucosidase and lipase

The phenolic-rich extracts from the different lentils were tested for their inhibitory ability on α-glucosidase and pancreatic lipase. α-Glucosidase inhibitors can interfere with enzymatic action in the brush-border of the small intestine, resulting in reduction of glucose absorption and post-prandial hyperglycaemia. This effect

Table 3
Concentrations (μg/g DW) of individual phenolics in 20 lentil cultivars ($n = 3$).[A].

Compounds	Red lentils									
	Blaze	Redcliff	Maxim	Rouleau	Redbow	Redberry	Impact	Imperial	Rosetown	Dazil
Dihydroxybenzoic acid	1.40 ± 0.21 a	2.99 ± 0.34 de	2.30 ± 0.19 c	1.49 ± 0.11 a	1.61 ± 0.28 ab	2.63 ± 0.32 cd	2.06 ± 0.19 bc	4.01 ± 0.42 e	1.94 ± 0.35 b	2.27 ± 0.16 c
p-Hydroxybenzoic acid	3.77 ± 0.42 c	5.26 ± 0.26 f	3.59 ± 0.33 b	4.48 ± 0.36 de	3.62 ± 0.14 bc	3.80 ± 0.18 c	4.22 ± 0.21 d	4.99 ± 0.36 ef	3.70 ± 0.26 c	5.80 ± 0.41 g
Catechin-glucoside	106.24 ± 5.14 bc	123.87 ± 3.28 de	128.07 ± 6.11 ef	121.10 ± 4.29 de	112.09 ± 3.56 c	101.97 ± 6.71 ab	118.47 ± 3.62 d	131.07 ± 4.57 f	120.12 ± 5.11 de	115.09 ± 4.98 cd
Catechin gallate	52.90 ± 2.14 b	70.79 ± 3.21 f	64.99 ± 2.91 de	63.69 ± 2.58 de	64.30 ± 1.95 de	59.25 ± 1.84 c	60.14 ± 3.11 cd	73.93 ± 2.34 f	61.85 ± 2.77 cd	72.09 ± 2.51 f
Epicatechin-glucoside	44.92 ± 1.99 a	72.63 ± 2.74 gh	53.79 ± 2.09 bc	61.71 ± 1.03 e	67.79 ± 3.12 f	55.98 ± 2.56 cd	58.98 ± 1.96 de	56.25 ± 2.47 cd	54.00 ± 1.85 c	82.80 ± 3.44 i
Sinapic derivative	0.48 ± 0.09 a	1.04 ± 0.14 d	0.71 ± 0.06 b	0.61 ± 0.07 b	0.62 ± 0.11 b	0.71 ± 0.09 b	0.86 ± 0.10 c	1.162 ± 0.07 d	0.60 ± 0.05 b	1.15 ± 0.12 d
Procyanidin dimer 1	4.90 ± 0.32 e	6.63 ± 0.47 f	3.25 ± 0.22 d	1.53 ± 0.21 a	1.68 ± 0.24 a	1.30 ± 0.18 a	4.83 ± 0.23 e	2.43 ± 0.54 bc	3.33 ± 0.28 d	7.45 ± 0.71 f
Syringic acid	2.20 ± 0.44 b	4.53 ± 0.51 de	2.90 ± 0.30 c	3.93 ± 0.19 d	1.83 ± 0.24 ab	1.83 ± 0.17 ab	2.80 ± 0.34 c	1.83 ± 0.15 ab	1.68 ± 0.24 ab	4.9 ± 0.36 ef
trans-p-Coumaroyl-malic acid	0.72 ± 0.05 ab	1.60 ± 0.14 e	1.51 ± 0.18 e	0.63 ± 0.04 a	1.08 ± 0.09 c	1.10 ± 0.13 c	1.16 ± 0.12 c	1.60 ± 0.06 e	0.83 ± 0.07 b	1.78 ± 0.15 e
trans-p-Coumaric acid	4.24 ± 0.58 a	8.93 ± 0.43 f	6.63 ± 0.18 d	5.11 ± 0.41 b	6.81 ± 0.27 d	5.48 ± 0.15 b	7.00 ± 0.19 d	7.69 ± 0.17 e	5.20 ± 0.54 b	11.19 ± 0.71 g
Epicatechin gallate	1.52 ± 0.07 cd	2.91 ± 0.12 f	2.73 ± 0.10 f	2.31 ± 0.18 e	1.64 ± 0.04 d	1.32 ± 0.12 bc	2.36 ± 0.21 e	2.34 ± 0.15 e	1.80 ± 0.06 d	2.68 ± 0.10 f
Kaempferol tetraglycoside	221.76 ± 5.32 ab	284.36 ± 7.58 fg	278.1 ± 3.79 f	268.71 ± 3.20 e	272.79 ± 4.57 ef	255.51 ± 3.06 d	280.68 ± 4.72 f	294.02 ± 3.38 g	254.56 ± 5.9 d	290.07 ± 2.43 g
Kaempferol triglycoside	107.3 ± 2.87 b	137.65 ± 3.05 g	133.71 ± 2.98 fg	123.91 ± 3.84 e	120.91 ± 2.91 de	115.33 ± 3.85 cd	151.81 ± 3.93 h	139.97 ± 4.89 g	115.06 ± 4.05 cd	125.54 ± 3.01 ef
Kaempferol-3-robinoside-7-rhamnoside	0.92 ± 0.08 a	1.51 ± 0.11 e	1.23 ± 0.08 c	0.92 ± 0.05 a	1.17 ± 0.10 bc	1.22 ± 0.08 c	1.32 ± 0.17 cd	1.40 ± 0.13 de	0.98 ± 0.05 a	1.58 ± 0.15 e
Quercetin xyloside	4.81 ± 0.21 a	8.73 ± 0.54 ef	7.60 ± 0.16 cd	8.86 ± 0.42 ef	4.58 ± 0.28 a	5.77 ± 0.81 b	8.32 ± 0.56 de	10.12 ± 0.59 fg	7.19 ± 0.36 c	9.85 ± 0.61 f
Procyanidin dimer 2	10.66 ± 0.76 a	26.08 ± 1.10 f	23.22 ± 1.15 e	18.98 ± 1.05 cd	18.16 ± 1.00 c	17.75 ± 0.92 c	20.62 ± 1.03 d	24.03 ± 1.20 ef	17.21 ± 1.05 c	24.72 ± 1.10 ef
Procyanidin dimer 3	13.66 ± 0.86 b	42.19 ± 2.15 g	27.99 ± 1.65 e	18.03 ± 0.92 c	21.99 ± 1.77 cd	14.07 ± 1.06 b	29.50 ± 1.68 ef	40.14 ± 2.11 g	14.21 ± 1.09 b	43.28 ± 2.18 g
Quercetin-3-glucoside	28.24 ± 1.85 ab	66.61 ± 1.91 g	41.16 ± 1.72 d	36.13 ± 1.11 c	32.59 ± 2.43 bc	31.64 ± 1.33 bc	46.88 ± 2.20 e	41.44 ± 2.13 d	28.51 ± 1.81 ab	45.52 ± 2.65 de
Flavonoid derivative	2.58 ± 0.47 cd	11.93 ± 0.96 i	10.53 ± 0.44 ij	5.43 ± 0.29 g	1.90 ± 0.15 bc	2.05 ± 0.18 c	6.48 ± 0.52 g	8.73 ± 0.31 h	2.28 ± 0.25 c	9.93 ± 0.82 i
Flavonoid derivative	4.38 ± 0.25 e	4.23 ± 0.38 e	3.03 ± 0.16 d	1.45 ± 0.14 a	2.80 ± 0.21 c	2.43 ± 0.09 bc	2.80 ± 0.20 c	7.15 ± 0.29 f	1.30 ± 0.17 a	8.05 ± 0.34 g
Kaempferol-3-glucoside	5.90 ± 0.18 cd	6.07 ± 0.13 cd	5.36 ± 0.22 b	4.40 ± 0.19 a	5.46 ± 0.25 b	5.66 ± 0.21 bc	5.77 ± 0.09 bc	7.43 ± 0.34 e	5.46 ± 0.14 b	7.84 ± 0.18 e
Total flavonol index (TFI)	368.93 ± 10.51 ab	504.93 ± 13.32 hi	467.16 ± 8.95 ef	442.93 ± 8.81 d	437.50 ± 10.54 d	415.13 ± 9.34 c	494.78 ± 11.67 gh	494.38 ± 11.46 gh	411.76 ± 12.31 c	480.40 ± 9.03 fg
Total phenolic index (TPI)	623.50 ± 24.30 ab	890.54 ± 29.55 h	802.40 ± 25.02 f	753.41 ± 20.68 de	745.42 ± 23.71 de	686.80 ± 24.04 cd	817.06 ± 25.38 fg	861.73 ± 26.67 gh	701.81 ± 26.45 d	873.58 ± 27.12 h
	Green lentils									
	Imvincible	Greenland	Asterix	Imigreen	Impower	Improve	Sovereign	Milestone	Eston	Plato
Dihydroxybenzoic acid	2.09 ± 0.36 bc	2.39 ± 0.27 c	3.56 ± 0.22 e	1.64 ± 0.31 ab	2.21 ± 0.38 c	1.64 ± 0.17 ab	1.55 ± 0.31 a	1.55 ± 0.18 a	2.33 ± 0.25 c	1.82 ± 0.13 b
p-Hydroxybenzoic acid	5.23 ± 0.44 f	5.11 ± 0.21 f	5.74 ± 0.51 g	3.23 ± 0.28 ab	3.50 ± 0.13 b	3.41 ± 0.24 b	3.29 ± 0.14 ab	3.41 ± 0.27 b	4.9 ± 0.34ef	2.93 ± 0.18 a
Catechin-glucoside	120.05 ± 5.36 de	127.55 ± 4.88 ef	130.1 ± 3.87 f	115.92 ± 3.11 cd	121.10 ± 4.52 de	99.27 ± 3.76 a	103.62 ± 4.09 ab	100.92 ± 4.01 a	108.87 ± 5.1 bc	107.22 ± 3.61 bc
Catechin gallate	61.51 ± 1.28 cd	71.88 ± 2.65 f	69.97 ± 2.60 ef	45.46 ± 1.54 ab	58.57 ± 1.93 c	50.65 ± 2.53 b	42.87 ± 2.10 a	68.88 ± 2.19 ef	66.97 ± 3.05 ef	49.15 ± 1.09 b

Epicatechin-glucoside	68.47 ± 3.17 f	83.55 ± 2.06 i	75.70 ± 2.40 h	50.93 ± 1.95 b	70.38 ± 1.62 fg	55.43 ± 3.18 cd	49.56 ± 1.52 b	51.47 ± 2.69 b	70.38 ± 3.19 fg	50.52 ± 1.21 b
Sinapic derivati	0.69 ± 0.08 b	0.67 ± 0.05 b	1.21 ± 0.08 d	0.60 ± 0.04 b	0.77 ± 0.08 c	0.79 ± 0.10 c	0.68 ± 0.08 b	0.99 ± 0.06 d	0.71 ± 0.05 b	0.61 ± 0.09 b
Procyanidin dimer 1	5.13 ± 0.19 e	8.88 ± 0.46 g	10.53 ± 1.40 h	1.68 ± 0.29 a	1.60 ± 0.11 a	2.13 ± 0.17 bc	3.78 ± 0.46 d	2.88 ± 0.55 c	2.88 ± 0.14 c	1.90 ± 0.33 ab
Syringic acid	2.13 ± 0.11 b	5.43 ± 0.47 f	9.18 ± 0.67 g	1.83 ± 0.19 ab	3.78 ± 0.44 d	2.35 ± 0.21 b	1.90 ± 0.27 ab	4.38 ± 0.30 de	4.15 ± 0.18 d	1.53 ± 0.29 a
trans-p-Coumaroyl-malic acid	0.85 ± 0.08 b	1.09 ± 0.05 c	1.38 ± 0.10 d	0.62 ± 0.07 a	1.30 ± 0.07 d	0.97 ± 0.05 c	0.75 ± 0.05 ab	0.85 ± 0.12 b	0.71 ± 0.05 ab	0.79 ± 0.06 b
trans-p-Coumaric acid	8.29 ± 0.42 ef	11.88 ± 0.50 g	12.94 ± 0.85 g	5.34 ± 0.24 b	8.24 ± 0.32 ef	6.72 ± 0.30 d	4.93 ± 0.15 ab	5.99 ± 0.21 c	7.64 ± 0.33 e	4.70 ± 0.18 ab
Epicatechin gallate	1.23 ± 0.14 ab	2.46 ± 0.09 ef	2.58 ± 0.13 ef	1.22 ± 0.08 ab	2.47 ± 0.15 ef	1.14 ± 0.05 a	1.37 ± 0.08 bc	2.36 ± 0.12 e	1.35 ± 0.17 bc	1.06 ± 0.06 a
Kaempferol tetraglycoside	296.34 ± 4.02 g	291.98 ± 5.88 g	297.15 ± 4.46 g	238.63 ± 7.63 c	268.84 ± 2.50 e	241.76 ± 4.32 c	210.05 ± 4.15 a	260.27 ± 5.32 de	270.34 ± 5.58 ef	224.89 ± 3.81 b
Kaempferol triglycoside	122.55 ± 2.91 e	130.30 ± 3.10 f	149.63 ± 4.83 h	107.98 ± 2.62 b	124.59 ± 1.74 e	110.84 ± 2.43 bc	99.96 ± 3.18 a	116.83 ± 2.64 cd	130.85 ± 2.81 f	111.93 ± 2.49 bc
Kaempferol-3-robinoside-7-rhamnoside	1.28 ± 0.14 c	1.45 ± 0.09 de	1.66 ± 0.12 f	1.06 ± 0.05 b	1.35 ± 0.11 cd	1.15 ± 0.10 b	1.19 ± 0.08 bc	1.19 ± 0.06 bc	1.38 ± 0.10 d	1.10 ± 0.05 b
Quercetin xyloside	8.11 ± 0.42 de	9.07 ± 0.26 f	12.84 ± 0.38 g	5.43 ± 0.31 b	5.60 ± 0.17 b	4.81 ± 0.22 a	5.73 ± 0.46 b	7.71 ± 0.51 cd	8.01 ± 0.71 cd	5.87 ± 0.31 b
Procyanidin dimer 2	34.55 ± 1.98 g	39.60 ± 2.56 g	48.33 ± 2.10 h	18.98 ± 1.01 cd	17.48 ± 0.95 c	18.57 ± 1.16 cd	12.57 ± 1.04 ab	19.94 ± 0.86 d	23.90 ± 1.74 e	13.93 ± 1.14 b
Procyanidin dimer 3	19.94 ± 1.09 c	44.38 ± 1.38 g	31.41 ± 0.90 f	14.21 ± 0.95 b	19.94 ± 0.75 c	14.21 ± 0.89 b	10.11 ± 0.85 a	23.22 ± 1.09 d	23.22 ± 1.01 d	11.47 ± 0.94 a
Quercetin-3-glucoside	49.47 ± 1.11 e	64.71 ± 3.38 fg	57.63 ± 3.98 f	27.96 ± 1.23 ab	33.68 ± 2.09 bc	27.15 ± 1.87 ab	30.55 ± 1.69 b	24.97 ± 1.05 a	34.90 ± 1.19 c	33.27 ± 2.11 bc
Flavonoid derivative	10.68 ± 0.63 ij	12.18 ± 1.02 j	14.65 ± 0.52 k	3.40 ± 0.26 e	1.30 ± 0.17 a	1.53 ± 0.21 ab	3.25 ± 0.09 e	6.10 ± 0.16 g	2.73 ± 0.23 d	4.15 ± 0.18 f
Flavonoid derivative	1.75 ± 0.09 ab	2.65 ± 0.10 bc	6.85 ± 0.28 f	1.83 ± 0.13 b	2.65 ± 0.17 bc	2.50 ± 0.15 bc	2.58 ± 0.25 bc	1.90 ± 0.11 b	1.98 ± 0.14 b	1.68 ± 0.14 ab
Kaempferol-3-glucoside	6.48 ± 0.33 d	7.60 ± 0.19 e	9.51 ± 0.64 f	4.42 ± 0.15 a	6.24 ± 0.24 cd	4.66 ± 0.12 a	4.34 ± 0.14 a	4.95 ± 0.31 ab	4.81 ± 0.11 a	5.11 ± 0.29 b
Total flavonol index (TFI)	484.23 ± 8.93 fg	505.11 ± 12.90 hi	528.42 ± 14.41 i	385.48 ± 11.99 b	440.30 ± 6.85 d	390.37 ± 9.06 b	351.82 ± 9.70 a	415.92 ± 9.89 c	450.29 ± 10.50 de	382.17 ± 9.06 b
Total phenolic index (TPI)	826.82 ± 24.35 fg	924.81 ± 29.65 hi	952.55 ± 31.04 i	652.37 ± 22.44 bc	755.59 ± 18.64 de	651.68 ± 22.23 bc	594.63 ± 21.18 a	710.76 ± 22.81 d	773.01 ± 26.47 ef	635.63 ± 18.69 b

[A] Values are mean ± SD, $n = 3$. Trace: determined with trace amounts. Values followed by the different letter in the same row are significantly different ($p < 0.05$).

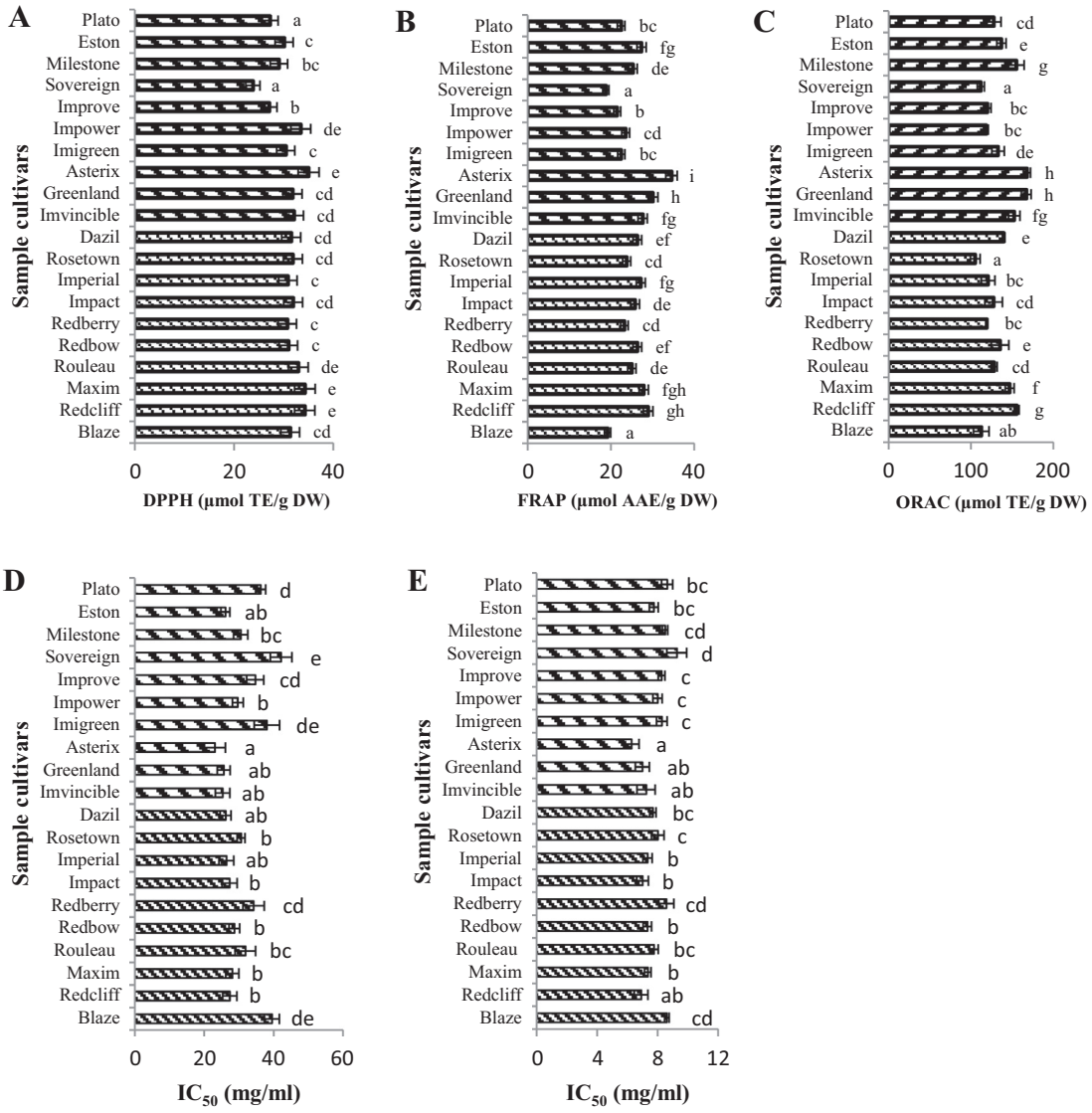

Fig. 2. Antioxidant activities and enzyme inhibitory activities of the hydrophilic extracts of 20 different lentil cultivars. (A) DPPH assay, values are expressed as μmol Trolox equivalent/g DW lentil (μmol TE/g DW); (B) FRAP assay, values are expressed as μmol ascorbic acid equivalent/g DW lentil (μmol AAE/g DW); (C) ORAC assay, values are expressed as μmol Trolox equivalent/g DW lentil (μmol TE/g DW); (D) IC_{50} of different lentil extracts on α-glucosidase and (E) lipase. IC_{50} is the concentration of the lentil extracts (de-sugared) that inhibits 50% of α-glucosidase or lipase activity. Values are means ± SD, $n = 3$. Values followed by the same letter in the same assay are not significantly different ($p < 0.05$).

Table 4
Antioxidant activities and enzyme inhibitory activities of pure standards.[A]

Compound	Antioxidant activity			Enzyme inhibition (IC_{50}) (μg/mL)	
	DPPH (μM TE)	FRAP (μM AAE)	ORAC (μM TE)	α-Glucosidase	Lipase
Catechin	276.50 ± 4.39 c	264.74 ± 3.30 c	764.72 ± 39.20 d	>200	>200
Epicatechin	282.40 ± 6.27 c	261.24 ± 10.42 c	726.92 ± 26.40 d	>200	>200
Procyanidin B1	137.32 ± 8.65 b	133.18 ± 7.26 a	376.80 ± 15.52 b	>200	>200
Kaempferol	148.07 ± 12.29 b	326.82 ± 5.90 d	482.56 ± 24.44 c	102.27 ± 2.06 b	33.02 ± 0.88 b
Kaempferol-glu	94.94 ± 3.12 a	197.60 ± 5.18 b	283.68 ± 6.52 a	100.63 ± 1.48 b	31.79 ± 1.42 b
Quercetin	367.62 ± 7.64 d	645.98 ± 15.45 f	957.00 ± 28.72 e	81.17 ± 1.03 a	22.54 ± 1.15 a
Quercetin-ara	288.85 ± 8.06 c	415.84 ± 9.80 e	516.52 ± 23.36 c	80.28 ± 1.21 a	20.81 ± 0.96 a

[A] Values are mean ± SD, $n = 3$. Values followed by the different letter in the same column are significantly different ($p < 0.05$). glu: glucose; ara, arabinoside.

is considered to be desirable in target for type 2 diabetes prevention and treatment. The inhibitory effect on α-glucosidase of the rat intestine varied widely and significantly among the hydrophilic extracts of the 20 tested lentils, and was dose-dependent. As shown in Fig. 2D, the IC_{50} values of the 20 extracts (de-sugared) ranged from 23.08 to 42.15 mg/mL. A low IC_{50} translates to a stronger α-glucosidase inhibition. The strongest inhibitory activity on α-glucosidase was found in cultivar Asterix, whereas cultivar

Sovereign possessed the weakest inhibitory activity. The inhibitory activities found in this study were similar with those reported for beans (Sreerama et al., 2012), but markedly higher than those of reported for other grains (Yao, Sang, Zhou, & Ren, 2009). The strongest correlation was observed between IC_{50} values for α-glucosidase and TFI ($R^2 = -0.8670$), followed by that between IC_{50} values for α-glucosidase and TPC ($R^2 = -0.8430$). These results suggest that phenolic compounds, especially flavonols e.g. kaempferol and quercetin glycosides, are the major contributors to the inhibitory activity on α-glucosidase. Activities of these flavonols have also been reported by Li et al. (2009).

Pancreatic lipase, a key enzyme responsible for triglyceride absorption in the small intestine, is secreted from the pancreas and hydrolyses triglyceride into glycerol and fatty acids. Suppression and delay of triglyceride digestion and absorption through inhibition of lipase is a key approach to the control of hyperlipidaemia and obesity. Similar to the effect on α-glucosidase, the hydrophilic extracts (de-sugared) of lentils inhibited the lipase activity (Fig. 2E). The IC_{50} values for pancreatic lipase inhibition also varied significantly from 6.26 to 9.26 mg/mL. Asterix again showed the highest lipase inhibitory activity, whereas the lowest activity was found in cultivar Blaze. The strongest correlation was also observed between the IC_{50} values and TFI ($R^2 = -0.8802$), indicating the most contribution to the lipase inhibitory activity may be from the flavonols. Sergent, Vanderstraeten, Winand, Beguin, and Schneider (2012) also reported that the phenolic compounds including quercetin and kaempferol exerted strong inhibitory activity on pancreatic lipase. It is also worth mentioning that the lentil extracts had much stronger inhibitory activity (lower IC_{50} values) toward lipase than α-glucosidase (Fig. 2). These results suggest that as an excellent source of pancreatic lipase inhibitors, lentils can be a useful dietary adjunct for the management of body weight and obesity.

Similar to the antioxidant activities, the same representative standard flavonols and flavanols were tested against the α-glucosidase and pancreatic lipase activities (Table 4). For flavonol aglycones, quercetin was a significantly stronger inhibitor than kaempferol against both α-glucosidase and pancreatic lipase, with IC_{50} at 81.17 and 22.54 μg/mL for quercetin, respectively, and 102.27 and 33.02 μg/mL for kaempferol, respectively (Table 4). Different from the antioxidant activities, flavonol glycosides of both kaempferol and quercetin showed higher inhibitory activity on both α-glucosidase and pancreatic lipase than their respective aglycones, although not statistically significance ($p > 0.05$). These results further indicate that the flavonols not only are strong antioxidants but major contributors to the inhibitory activity of lentil extract on the two important enzymes related to diabetes. Neither the catechins nor procyanidin B1 showed inhibitory activity against α-glucosidase or pancreatic lipase. Their IC_{50} values could not be calculated (Table 4). While the phenolics like the flavonols of lentils and the extracts containing them are strong inhibitors of α-glucosidase and pancreatic lipase, they are still significantly weaker inhibitors compared to acarbose and orlistat, two synthetic inhibitor drugs of the two enzymes used to manage postprandial blood glucose level and body weight. The IC_{50} values of acarbose and orlistat were 9.34 μg/mL and 1.14 ng/mL (data not shown) for α-glucosidase and pancreatic lipase, respectively, in the present study. Similar results were found by Ramdath, Padhi, Hawke, Sivaramalingam, and Tsao (2014) and Cha, Song, Kim, and Pan (2012).

In conclusion, in addition to the macronutrients, many micronutrients and phytochemicals in lentils may also be important contributors to human health. By determining the hydrophilic components of the most popular lentil cultivars grown in Canada, we demonstrated that lentils are an excellent source of phenolic compounds and condensed tannins. Phenolic compounds identified in the hydrophilic extract of lentils consisted of mainly flavanol and flavonol glycosides, as well as procyanidin oligomers. The FRAP antioxidant activity showed the strongest correlation with the TPC and TPI, whereas moderate and low correlations were found among the DPPH, ORAC values and various phytochemical contents. The present study also pointed to the flavonols, not the flavanols, as the most important contributors to the inhibitory activities of lentils against α-glucosidase and pancreatic lipase, and more interestingly, more so against the latter enzyme. Results obtained from this study may help to exploit the use of lentil as functional food and nutraceutical ingredients for promoting health, especially for preventing diabetes and obesity which affect millions of people worldwide.

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References

Adebamowo, C. A., Cho, E., Sampson, L., Katan, M. B., Spiegelman, D., Willett, W. C., et al. (2005). Dietary flavonols and flavonol-rich foods intake and the risk of breast cancer. *International Journal of Cancer, 114*(4), 628–633.

Aguilera, Y., Dueñas, M., Estrella, I., Hernández, T., Benitez, V., Esteban, R. M., et al. (2010). Evaluation of phenolic profile and antioxidant properties of Pardina lentil as affected by industrial dehydration. *Journal of Agricultural and Food Chemistry, 58*(18), 10101–10108.

Amarowicz, R., Estrella, I., Hernández, T., Robredo, S., Troszyńska, A., Kosińska, A., et al. (2010). Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). *Food Chemistry, 121*(3), 705–711.

Baderschneider, B., & Winterhalter, P. (2001). Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *Journal of Agricultural and Food Chemistry, 49*(6), 2788–2798.

Balasubramaniam, V., Mustar, S., Khalid, N. M., Rashed, A. A., Noh, M. F. M., Wilcox, M. D., et al. (2013). Inhibitory activities of three Malaysian edible seaweeds on lipase and α-amylase. *Journal of Applied Phycology, 25*(5), 1405–1412.

Boudjou, S., Dave Oomah, B., Zaidi, F., & Hosseinian, F. (2012). Phenolics content and antioxidant and anti-inflammatory activities of legume fractions. *Food Chemistry, 138*(2), 1543–1550.

Brand-Miller, J., Hayne, S., Petocz, P., & Colagiuri, S. (2003). Low-glycemic index diets in the management of diabetes a meta-analysis of randomized controlled trials. *Diabetes Care, 26*(8), 2261–2267.

Cha, K. H., Song, D.-G., Kim, S. M., & Pan, C.-H. (2012). Inhibition of gastrointestinal lipolysis by green tea, coffee, and gomchui (*Ligularia fischeri*) tea polyphenols during simulated digestion. *Journal of Agricultural and Food Chemistry, 60*(29), 7152–7157.

Chen, P. X., Tang, Y., Zhang, B., Liu, R., Marcone, M. F., Li, X., et al. (2014). 5-Hydroxymethy-2-furfural (HMF) and derivatives formed during acid hydrolysis of conjugated and bound phenolics in plant foods and the effects on phenolic content and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 62*(20), 4754–4761.

Downey, M. O., & Hanlin, R. (2010). Comparison of ethanol and acetone mixtures for extraction of condensed tannin from grape skin. *South African Journal for Enology & Viticulture, 31*(2), 154.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., & Merillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry, 57*(5), 1768–1774.

Dueñas, M., Sun, B., Hernández, T., Estrella, I., & Spranger, M. I. (2003). Proanthocyanidin composition in the seed coat of lentils (*Lens culinaris* L.). *Journal of Agricultural and Food Chemistry, 51*(27), 7999–8004.

García-Alonso, A., Goñi, I., & Saura-Calixto, F. (1998). Resistant starch and potential glycaemic index of raw and cooked legumes (lentils, chickpeas and beans). *Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 206*(4), 284–287.

Han, H., & Baik, B. K. (2008). Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (Glycine max), and their quantitative changes during processing. *International Journal of Food Science & Technology, 43*(11), 1971–1978.

He, Q., Lv, Y., & Yao, K. (2007). Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. *Food Chemistry, 101*(3), 1178–1182.

Herald, T. J., Gadgil, P., & Tilley, M. (2012). High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture, 92*(11), 2326–2331.

Jenkins, D., Wolever, T., Taylor, R. H., Barker, H. M., & Fielden, H. (1980). Exceptionally low blood glucose response to dried beans: Comparison with other carbohydrate foods. *British Medical Journal, 281*(6240), 578.

Kawaguchi, K., Mizuno, T., Aida, K., & Uchino, K. (1997). Hesperidin as an inhibitor of lipases from porcine pancreas and Pseudomonas. *Bioscience, Biotechnology, and Biochemistry, 61*(1), 102–104.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine, 113*(9), 71–88.

Li, H., Deng, Z., Liu, R., Loewen, S., & Tsao, R. (2013). Carotenoid compositions of coloured tomato cultivars and contribution to antioxidant activities and protection against H_2O_2-induced cell death in H9c2. *Food Chemistry, 136*(2), 878–888.

Li, H., Deng, Z., Wu, T., Liu, R., Loewen, S., & Tsao, R. (2012a). Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food Chemistry, 130*(4), 928–936.

Li, H., Deng, Z., Zhu, H., Hu, C., Liu, R., Young, J. C., et al. (2012b). Highly pigmented vegetables: Anthocyanin compositions and their role in antioxidant activities. *Food Research International, 46*(1), 250–259.

Li, H., Song, F., Xing, J., Tsao, R., Liu, Z., & Liu, S. (2009). Screening and structural characterization of alpha-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MS(n) and SORI-CID FTICR MS. *Journal of the American Society for Mass Spectrometry, 20*(8), 1496–1503.

Moreno, D. A., Ilic, N., Poulev, A., Brasaemle, D. L., Fried, S. K., & Raskin, I. (2003). Inhibitory effects of grape seed extract on lipases. *Nutrition, 19*(10), 876–879.

Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T., Shibata, H., et al. (2005). Inhibitory effects of oolong tea polyphenols on pancreatic lipase in vitro. *Journal of Agricultural and Food Chemistry, 53*(11), 4593–4598.

Nguyen, N. T., Nguyen, X.-M. T., Lane, J., & Wang, P. (2011). Relationship between obesity and diabetes in a US adult population: Findings from the National Health and Nutrition Examination Survey, 1999–2006. *Obesity Surgery, 21*(3), 351–355.

Papanikolaou, Y., & Fulgoni, V. L. III, (2008). Bean consumption is associated with greater nutrient intake, reduced systolic blood pressure, lower body weight, and a smaller waist circumference in adults: Results from the National Health and Nutrition Examination Survey 1999–2002. *Journal of the American College of Nutrition, 27*(5), 569–576.

Ramdath, D. D., Padhi, E., Hawke, A., Sivaramalingam, T., & Tsao, R. (2014). The glycemic index of pigmented potatoes is related to their polyphenol content. *Food & Function, 5*(5), 909–915.

Saulnier, L., Crépeau, M.-J., Lahaye, M., Thibault, J.-F., Garcia-Conesa, M. T., Kroon, P. A., et al. (1999). Isolation and structural determination of two 5, 5′-diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross-linked by oxidatively coupled ferulates. *Carbohydrate Research, 320*(1), 82–92.

Sergent, T., Vanderstraeten, J., Winand, J., Beguin, P., & Schneider, Y.-J. (2012). Phenolic compounds and plant extracts as potential natural anti-obesity substances. *Food Chemistry, 135*(1), 68–73.

Sreerama, Y. N., Takahashi, Y., & Yamaki, K. (2012). Phenolic antioxidants in some Vigna species of legumes and their distinct inhibitory effects on α-glucosidase and pancreatic lipase activities. *Journal of Food Science, 77*(9), C927–C933.

Taylor, W. G., Fields, P. G., & Sutherland, D. H. (2007). Fractionation of lentil seeds (*Lens culinaris* Medik.) for insecticidal and flavonol tetraglycoside components. *Journal of Agricultural and Food Chemistry, 55*(14), 5491–5498.

Willett, W. C. (1994). Diet and health: What should we eat? *Science, 264*(5158), 532–537.

Xu, B., Han, L. K., Zheng, Y. N., Lee, J. H., & Sung, C. K. (2005). In vitro inhibitory effect of triterpenoidal saponins from Platycodi Radix on pancreatic lipase. *Archives of Pharmacal Research, 28*(2), 180–185.

Xu, B., Yuan, S., & Chang, S. (2007). Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *Journal of Food Science, 72*(2), S167–S177.

Yao, Y., Sang, W., Zhou, M., & Ren, G. (2009). Antioxidant and α-glucosidase inhibitory activity of colored grains in China. *Journal of Agricultural and Food Chemistry, 58*(2), 770–774.

Yokotsuka, K., & Singleton, V. L. (1995). Interactive precipitation between phenolic fractions and peptides in wine-like model solutions: Turbidity, particle size, and residual content as influenced by pH, temperature and peptide concentration. *American Journal of Enology and Viticulture, 46*(3), 329–338.

Zhang, B., Deng, Z., Tang, Y., Chen, P., Liu, R., Ramdath, D. D., et al. (2014). Fatty acid, carotenoid and tocopherol compositions of 20 Canadian lentil cultivars and synergistic contribution to antioxidant activities. *Food Chemistry, 161*, 296–304.

Zhang, L., Li, J., Hogan, S., Chung, H., Welbaum, G. E., & Zhou, K. (2010). Inhibitory effect of raspberries on starch digestive enzyme and their antioxidant properties and phenolic composition. *Food Chemistry, 119*(2), 592–599.

Zou, Y., Chang, S. K., Gu, Y., & Qian, S. Y. (2011). Antioxidant activity and phenolic compositions of lentil (*Lens culinaris* var. Morton) extract and its fractions. *Journal of Agricultural and Food Chemistry, 59*(6), 2268–2276.