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Research Article

Composition, antioxidant activity and performance in canola oil of phenolic extract from wild rose hip with seed

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Abstract

In this study, the effectiveness of phenolic extracts from *Rosa woodsii* hip with seed (*Rosae pseudofructus cum fructibus*) in protecting vegetable oils against oxidative and thermal deterioration was assessed during accelerated storage of canola oil at 65^{o}C, Rancimat at 120^{o}C, and frying at 180^{o}C. At the end of the 7-day storage, formation of lipid hydroperoxides was 2.5 times higher in control canola oil compared to the fortified sample. Accumulation of polar components, polymerized triacylglycerols and other secondary degradation products during frying was reduced by up to 30% in the presence of the applied phenolic extracts. Similarly, the oxidative stability of canola oil significantly increased in the presence of extract as measured by Rancimat induction period (IP). HPLC-MS analysis indicated that quercetin, catechin and gallic acid were the major phenolic constituents of *Rosa woodsii* fruit extract, and are possibly the active antioxidant principle of the extract.

Keywords: Canola oil / Frying stability / Polyphenols / Natural antioxidants / *Rosa woodsii*

Practical applications

Oxidation of fats and oils or fatty fractions of foods is one of the major reasons for the deterioration of food. Therefore the search for highly effective antioxidants from natural sources to stabilize frying oils or food preparations is an ongoing story. The present paper demonstrates that phenolic extracts from *Rosa woodsii* Lindl (Rosaceae) can improve the frying and storage stability of edible oils. The findings can help food processors to improve quality and safety of food.

Introduction

Consumers' preference and intake of polyunsaturated fatty acids (PUFA) continue to increase in response to abounding evidence of their beneficial roles. However, because of their predisposition to oxidation, edible oils with high PUFA contents often exhibit poor stability resulting in rancidity. Besides the apparent economic losses, oxidative deterioration of oils/fats also results in the generation of free radicals and other toxic components, necessitating the need for effective fortification. To improve the shelf life of edible oils, antioxidants are often added, phenolic antioxidants being the most prominent mainly because of their high radical scavenging activity [1]. However, because of the harsh conditions employed and the presence of pro-oxidative components introduced from the food, most of the conventional antioxidant offers little or no protection to oils during frying [2]. The negative consumers' perception of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) further limits available choices for effective phenolic antioxidants [1].

Polyphenols are widely recognized as effective alternatives to synthetic antioxidants:

(i) they occur naturally in plant-based foods, creating special appeal to consumers; (ii) they present an array of interesting biological properties including antioxidant, antiviral, antifungal, antimicrobial, and anticarcinogenic; and (iii) depending on the number and position of the phenolic OH-group, they possess high radical scavenging and metal chelating potentials [3]. Hence, the recent trend in the search for natural antioxidants is the application of polyphenolic extracts from various parts of plants. Whereas there are several reports on the antioxidant activities of plant extracts and their polyphenolic components in vegetable oil under oxidative conditions ($< 120^{o}C$) [4 – 7], corresponding investigations under frying conditions, however, have not received much attention, and are usually limited to common spices and herbs [8 – 12]. However, underutilized wild edible fruits can also be an excellent source of antioxidative natural phenolic compounds.

Rosa woodsii Lindl (Rosaceae) is a shrub widely distributed in North America and has been used as medicine by the North American Aboriginal tribes. The fruits have been used in tea, jelly, jams, and mixed with dried salmon eggs to extend their shelf life [13]. Tannins and related polyphenols exhibiting potent inhibitory effect on HIV-1 reverse transcriptase have been reported from the leaf of Japanese *Rosa woodsii* [14]. Whereas the phenolic content and antioxidant activity of a number of Rosa species such as; *R. damascena*, *R. chinensis*, *R. canina*, *R. pimpinellifolia*, and *R. rugosa* have been reported [15 – 18], data on *R. woodsii*, however, has been very sparse. According to Yi et al. [13], the fruits of Canadian *R. woodsii* contained high phenolic content,

exhibiting significant antioxidant activity in a linoleic acid assay. Edible oil from *R. woodsii* is also deemed a valuable source of essential fatty acids, tocopherol and phytosterols [19]. The main aim of the present study was to evaluate the potential of phenolic extracts from *R. woodsii* in protecting polyunsaturated vegetable oils against thermo-oxidative deterioration.

Materials and Methods

Sample materials and chemicals

Fully ripened rose hip with seed (*Rosae pseudofructus cum fructibus*) from wildly grown *R. woodsii* were collected in the Oldman River Valley, Alberta, Canada (49° 42′ 0″ N, 112° 50′ 0″ W) during the months of August and October. Samples were randomly collected from several plants grown in three different locations. Refined, bleached and deodorized canola oil and frozen par-fried French fries in institutional pack were obtained from a local food store. All solvents were of HPLC grade (Merck, Germany). C18 SPE 1000-mg cartridges with 6 mL reservoir were obtained from J.T Baker (Deventer, Netherlands). Lipophilic Sephadex LH-20 was obtained from Sigma-Aldrich (Steinheim, Germany). The water used was either doubly distilled or of HPLC grades. All chemicals, including phenolic standards, were obtained from Sigma-Aldrich (Steinheim, Germany).

Extraction and partitioning of polyphenolic extracts

After de-stemming and removal of damaged fruits, the fresh fruits were air dried at ambient temperature and pulverized with a Grindomix GM 200 (Retsch, Haan, Germany) at 10,000 rpm for 30 sec. The ground samples (100 g) were homogenized with 1 L of extraction solvent (acetone/water/acetic acid; 70:29.5:0.5; v/v/v) for 2 min using a T 25 Ultra Turrax (IKA Labortechnik, Staufen, Germany) operating at 13,500 rpm followed by sonication at 50°C in an ultrasound bath for 1 h [20]. After filtration, the residue was re-extracted with fresh solvent following the same process. Acetone was removed from the combined filtrate under vacuum at 30°C using an RV 10C rotary evaporator (IKA Labortechnik, Staufen, Germany). Subsequently, the concentrated filtrate was successively extracted three times (150 mL, each) with hexane, ethyl acetate, and n-butanol. The hexane fraction was discarded while the ethyl acetate and n-butanol extracts were evaporated under vacuum at 40°C using a rotary evaporator, flushed with nitrogen and kept at -18^{O}C for further analyses.

Fractionation on C18 cartridge

The ethyl acetate and butanol extracts were further purified using a C18 SPE cartridge as follows: Extract (1 g) was dissolved in 50 mL methanol:water (70:30; v/v) and applied to a C18 SPE cartridge that had been preconditioned with 10 mL methanol. Eluates from parallel C18 cartridges were combined and evaporated *in vacuo* at 40^{O}C

Fractionation on Sephadex

The purified extracts obtained above were further fractionated on a Sephadex column as follows: Sephadex LH-20 powder (20 g) was swollen for 24 h in water and the suspension was poured into a glass column (20 × 350 mm). The extract (2 g) was suspended in water and applied unto the top of the column. The reservoir was filled with water and the flow rate was adjusted to about 1 mL/min. Subsequently, the extract was successively eluted with water (200 mL), 25% methanol (200 mL), 50% methanol (200 mL), 75% methanol (200 mL), 100% methanol (250 mL), and finally, methanol/acetone (1:1, v/v, 250 mL). Fractions were monitored by HPLC-PDA, pooled together based on the similarity of their HPLC chromatograms, and evaporated to dryness under vacuum at 40°C using a rotary evaporator. After the removal of residual solvent under a gentle stream of nitrogen, the residue was re- dissolved in 5 mL of methanol and kept at 4°C until used.

Separation and quantification of phenolic compounds by HPLC and HPLC-MS

High performance liquid chromatography was carried out using a LaChrom Elite® HPLC system equipped with an L-2130 Hitachi gradient pump and an L-2200 autosampler (Merck, Germany). The sample was separated at ambient temperature on a Lichrosphere 100 RP-18e column (5 µm; 250 × 4 mm; Merck, Germany) using a mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at a flow rate of 1 mL/min using the following gradient: 100% A at time 0 min; 95% A, 5% B (5 min); 65% A,
35% B (35 min); 45% A, 55% B (45 min); 20% A, 80% B (55 min); 20% A, 80% B (60 min);

100% A (63 min); 100% A (70 min). Injection volume was 10 µL and the analytes were detected at 280, 360, and 520 nm with an L-2455 PDA detector (Merck, Germany).
Phenolic compounds were identified with an ultrahigh resolution maXis impact q-TOF mass spectrometer (Bruker, Bremen, Germany) and by comparison of retention time and UV spectra with pure standards. The mass spectrometer was equipped with an ESI ion source operated in negative modes, acquiring MS and auto MS/MS data at 3 Hz acquisition speed and m/z 80 – 1300 scan range. MS source settings were as follows: dry gas temperatures, 180^{O}C; dry gas flow, 8 L/min; nebulizer gas pressure, 3 bars; and capillary voltage, 2500 V.

Molecular formula determination was carried out by combined evaluation of mass accuracy, isotopic patterns, adduct and fragment information using SmartFormula3D. TargetAnalysis software (Bruker, Germany) was used to screen extracts for phenolic compounds previously reported in the literature.

Determination of total phenolic content (TP)

The total phenolics (TP) in the extracts and fractions were determined using the method described by Singleton and Rossi [21] with some

modifications. In brief, 100 µL of Folin– Ciocalteu reagent, and 300 µL of Na_2CO_3 (20%) were added to 20 µL of appropriately diluted samples. Then the volume was completed to 2000 µL with distilled water. After a 30 min incubation period, absorbance was read at 765 nm. The concentration was calculated using gallic acid as standard, and the results were expressed as milligrams gallic acid equivalents (GAE) per gram extract.

DPPH radical scavenging assay

The DPPH assay was performed according to a method described by Nenadis and Tsimidou [22] with some modifications. Briefly, a methanolic DPPH solution (0.1 mM; 2960 µL) was added to 40 µL of different concentrations of extracts/fractions. The mixture was shaken vigorously and the decrease in absorbance was measured at 515 nm after 30 min of incubation in the dark. The blank solution contained the same amount of DPPH reagent and 40 µL of methanol and each test was performed in triplicate. The percentage of DPPH inhibition was calculated as follows:

$$\%\ DPPH = \left[\frac{A_c - A_s}{A_c}\right] \times 100$$

Where: A_c and A_s are the absorbance of the control and test samples, respectively. All standard deviations (SD) for DPPH tests were below 5.0%. BHT and α-tocopherol (200, 400, 800, and 1000 µg/g) were used as references. Calculated IC_{50} represents the concentration of antioxidant required to decrease the DPPH amount by 50%.

1.1 Antioxidant activity under storage and frying conditions

1.1.1 Addition of polyphenolic extracts to oil

A solution of the extract in methanol was added to canola oil oil in a flask to deliver the desired amount of phenolic content in gallic acid equivalent (GAE) per gram of oil. The solvent was evaporated under vacuum at 50°C using a rotary evaporator followed by sonication at 50°C in an ultrasound bath for 30 min. This procedure offers effective dissolution and dispersal of phenolic extracts in the oil. Residual solvent was removed by gentle stream of nitrogen.

Accelerated storage – Schaal oven test

The ability of the polyphenolic extracts to inhibit oxidative deterioration of oil during storage was determined using the Schaal oven test [23]. Canola oil (1.0

g), fortified with phenolic extracts at 200 μg GAE/g of oil, were introduced in the vials (2 ml, 12 × 32 mm). The uncapped vials were stored in darkness at 65°C for up to 7 days. Samples were examined at 24 h intervals by collecting individual vials at the particular period. The oxidative stability of the samples was evaluated by peroxide value (PV). The effectiveness of the new compounds
was compared with BHT (200 μg/g), as synthetic antioxidant. Experiments were set up in two repetitions for each tested antioxidant, and samples from each repetition were analysed in duplicate.

Thermal oxidation by Rancimat

Canola oils fortified with extracts at two different concentrations (200 and 500 μgGAE/g of oil) were submitted to thermo-oxidation under Rancimat conditions at 120 °C using a 743 Rancimat (Metrohm, Filderstadt, Germany) as previously reported [24]. In brief, 3.6 g oil was weighed into the reaction vessel, which was placed into the heating block kept at 120°C. The air flow was set at 20 L/h for all determinations. Volatile compounds released during the degradation process were collected in a receiving flask filled with 60 mL distilled water. The conductivity of this solution was measured and recorded. The software of the Rancimat automatically evaluated the resulting curves and calculated the induction period given in hours. BHT (200 μg/g) was used as the reference antioxidant.

Frying performance

Canola oil (100 g) fortified with phenolic compounds at 500 μgGAE/g of oil from the extract was weighed into 250 mL glass crystallizing dishes (9.5 cm diameter × 5.5 cm height; Schott Duran, Wertheim/Main, Germany). The oil was placed on a hot plate with a probe to control temperature at 180 ± 2°C and heated for 16 h with an hourly frying of 10 g of frozen French fries (approximately 4.5 × 1 × 1 cm) for 5 min. Oils were heated without replenishing and samples (2.0 g) were collected at the 4^{rd}, 8^{th}, 12^{th}, and 16^{th} hour and immediately frozen at - 18°C until analyzed. BHT (200 μg/g) was used as the reference antioxidant.

Quantitative analysis

Peroxide value (PV) was assessed according to procedure originally described by Hornero- Méndez et al. [25] as modified by Szterk et al. [26]. Briefly, 200 mg of oil was dissolved in 5 mL of hexane. Two hundred microliters of the solution was mixed with 5 mL of methanol/chloroform/HCl solution (1:1:0.012, v/v). Thereafter, 100 μL of $FeCl_2$ (0.4% water solution) and 100 μL of NH_4SCN (30% water solution) were added. The reaction was kept at room temperature for 5 min, and the absorbance was measured at 480 nm. A reaction blank containing all the reagents, except the sample, was used to zero the spectrophotometer readings.

Total polar components (TPC), dimerized and polymerized triacylglycerols (DPTG), and anisidine value (AnV) of frying oils were determined by Fourier-Transformed Near Infrared Spectroscopy (FT-NIR) following the DGF standard method C-VI 21 [27]. An MPA multipurpose FT-NIR analyser equipped with an OPUS LAB spectroscopy software interface (Bruker Optik GmbH, Ettlingen, Germany) was used for data acquisition and analysis.

Statistical analysis

Data are presented as means ± standard deviation (SD). Data were analyzed by single factor analyses of variance (ANOVA) using SPSS package (version 10.0). Statistically significant differences between means were determined by Duncan's multiple range tests for $P < 0.05$.

Results and discussions

Extraction, fractionation and phenolic compositions

Aqueous acetone has been reported to deliver extracts with higher phenolic content compare to aqueous methanol [20, 28], informing the choice of extraction solvent in the present study. According to d'Alessandro et al. [29], both the kinetic and yields of phenolic compounds considerably improved with ultrasonic assisted extraction. With the extraction protocol employed in the present study, the yields from the ethyl acetate and butanol extracts from *R. woodsii* fruits were 2.7 and 9.1%, respectively. Successive fractionation of the ethyl acetate extract (Rx) with Sephadex columns afforded a total of 7 fractions (coded, R6, R11, R14, R15, R16, R21, and R22) based on the similarity of phenolic compositions. Representative HPLC chromatograms are presented in Figure 1.

As shown in Figure 1, the major phenolic compounds of *Rosa woodsii* fruits extracts were gallic acid, catechin and quercetin. These phenolic compounds were also reported as predominant in the ethyl acetate extract of *R. damascena* [16]. Other phenolic components found in *R. woodsii* include: protocatechuic acid, B-type procyanidin, neochlorogenic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, quercetin, quercetin 3-β-D-glucoside (isoquercitrin), quercetin 3-D-galactoside (hyperoside), quercetin 3-*O*-α-L- arabinoside (guaijaverin), quercetin 3-*O*-α-L-rhamnoside (quercitrin), phloretin 2'-*O*-glucose (phloridzin), and myricetin (Table 1). However, unlike in *R. damascena* [16] and *R. chinensis* flowers [18], relatively lower content of quercetin glycosides were observed in *R. woodsii*

Based on HPLC data and total phenolic content (TP) analysis, the bulk of the phenolic compounds resided in the ethyl acetate extracts in spite of the higher extract yield recorded for butanol extraction. The TP for the purified ethyl acetate was 481 mg GAE/g (Figure 2), compared to 79 mg GAE/g for the corresponding butanol fraction, and the butanol fraction offered no unique phenolic mixtures. Thus, only the ethyl acetate extract was submitted for further antioxidant screening in our search for a natural phenolic mixture that can significantly improve the stability of vegetable oils during frying and storage.

Radical scavenging activity of phenolic extracts

The radical scavenging activity of the different phenolic fractions examined in this study is presented in Figure 2. All fractions exhibited excellent DPPH radical scavenging property, with activity significantly higher ($P < 0.05$) than tocopherol and BHT. The IC_{50} of the extracts ranges from 29 - 395 µg/mL as compared to BHT (704 µg/mL) and α-tocopherol (750 µg/mL), suggesting possible antioxidant potential in food. However, results from DPPH assay for potential antioxidative compounds are sometimes poorly correlated with performance in real food, principally because: (1) the nature and polarity of the radical encountered in food system is different from that of the DPPH radical; and (2) DPPH radical scavenging reaction occurs in an organic solvent, thereby excluding the effect of antioxidant's solubility and/or partitioning commonly observed in food substrates [30]. Hence, in the present study, the antioxidant activity of promising phenolic extracts/fractions were further examined in canola oil under storage and frying conditions.

Antioxidant activity in oil under storage conditions

The ability of the phenolic fractions from *R. woodsii* to protect fats/oils during ambient storage was evaluated in canola oil during accelerated storage at 65°C for 7 days. Formation of lipid hydroperoxides, the primary products of oxidative deterioration was monitored by PV (peroxide value). The increase in PV in the oil substrate over the storage period is depicted in Figure 3. All the tested extracts offered significant protection against oxidative deterioration of the oil. At the end of the storage period, the formation of lipid hydroperoxides in the oil was inhibited by up to 60% in the presence of the polyphenolic fractions. The purified ethyl acetate extract, Rx, and fraction R14 were particularly effective, with activity comparable to BHT, the synthetic antioxidant control. The comparable performance of R14 (containing predominantly quercetin) with the ethyl acetate extract, Rx, coupled with its superior activity over R6 (mainly gallic acid) and R11 (predominantly catechin) suggested quercetin as the active antioxidant principle of *R. woodsii* extract under the applied storage conditions. This may be related to the relatively better lipophilic nature and/or higher radical scavenging activity of quercetin, compared to gallic acid and catechin (Fig. 2). Quercetin has been shown to improve the oxidative stability of bulk canola and fish oils [31, 32].

Antioxidant activity under the Rancimat conditions

Although the oxidative conditions under the Rancimat are different from those during actual frying, the method can offer a fast assessment of the thermal stability and efficiency of prospective antioxidants under more challenging conditions than obtainable using the Schaal oven test. In contrast to the peroxide value, which provides a static measure for the assessment of fats and oils, the determination of oxidative stability by means of the Rancimat method is a dynamic measurement and the induction period (IP) measured by the Rancimat can offer an insight into the performance and thermo-oxidative stability of oils and the applied antioxidants [24]. The induction periods for control canola oil and the various fortified samples are presented in Figure 4. A concentration dependent activity was observed for the phenolic extracts. At the lower phenolic concentration (200 µg GAE/g of oil), the efficiency of the extracts was marginal, however at higher concentration (500 µg GAE/g of oil), a significant antioxidant activity was observed, with an increase of up

to 25% in the IP of fortified canola oil. Compared to BHT, the efficiency of the phenolic extracts was markedly better, especially at higher phenolic concentration. Unlike the results obtained under storage conditions, no significant difference was observed in the protective effect of Rx, R6, R11, and R14 (Fig. 4), suggesting a more complex interaction among the constituent phenolic compounds. It is also highly probable that the higher temperature available under Rancimat encouraged a better solubilisation of phenolic compounds in the oil, thus allowing even minor phenolic components in the extract to participate in the antioxidative process.

Performance during frying

The high temperature (>160°C), presence of water, oxygen, metals and other pro/antioxidative components from food materials often result in an array of chemical reactions that are difficult to simulate by accelerated tests such as Rancimat, necessitating the assessment of the phenolic extract under actual frying conditions. Based on availability and its performance in the storage and Rancimat tests (Figs. 3 and 4), the purified ethyl acetate extract (Rx) was chosen for the actual frying experiment. All the frying performance indices examined in the present study indicated that the ethyl acetate extract of *R. woodsii* (Rx) inhibited thermo-oxidative degradation of canola oil at a level significantly higher than BHT, the synthetic antioxidant control (Figs. 5 – 7).
As shown in Figure 5, the TPC increased significantly during the entire frying period, irrespective of oil samples, however, statistically significant differences ($P < 0.05$) were found among the samples. At the end of the 16 h of frying, the increase in TPC of the control canola oil was 43.6%, compared to 32.8% in samples fortified with the phenolic extract, Rx. Compared to BHT, the phenolic extract also showed a statistically significant ($P < 0.05$) superior performance during the entire frying period. Regression analysis indicated that the rate of TPC formation was 2.07, 2.70, and 2.67% per hour for samples fortified with phenolic extract, BHT, and the control canola oil, respectively. Thus, in contrast to its excellent performance during storage, BHT offered no protection under the frying condition employed in the present study (Fig. 5).

As a consequence of prolonged oxidation, oligomers of triacylglycerols are formed. Due to their high molecular weight, these components remain in the oil and can offer reliable indication of the extent of polymerization reaction occurring in the oil during frying. Similar to TPC results, phenolic extract from *R. woodsii* fruits exhibited significant anti- polymerization activity in the oil, with performance significantly higher than BHT (Fig. 6). At the end of the frying period, the formation of dimers and oligomers decreased by 31% in the presence of phenolic extracts.
Thermal decomposition of hydroperoxides during frying generates a number of secondary oxidation products, with carbonyl compounds being the most prominent. Anisidine value provides a reliable indication of the level of nonvolatile aldehydes in the oil, and by extension, the level of oxidative deterioration occurring in the frying oil. Figure 7 depicts the observed changes in anisidine value for canola oil with and without fortification during the frying operation. As expected, a significant increase in AnV was observed over the entire frying period, regardless of sample treatments. However, in agreement with other performance indices, canola oil supplemented with phenolic extract exhibited a significantly higher frying stability compared to canola oil control and the sample containing BHT, as assessed by the amount of carbonyl secondary oxidation products formed during the frying. At the end of frying, the AnV for canola oil control was 224 compared to 173 in the fortified sample, representing about 23% decrease in carbonyl compound formation as a consequent of the applied phenolic extract.
The poor performance of BHT during frying contrasts sharply with its excellent performance during storage, underscoring significant differences in the

nature and mechanisms of reaction occurring under the two conditions. Lipid oxidation under storage condition is primarily radical mediated; thus, the good radical scavenging activity of BHT coupled with its strong lipophilicity and small molecular size presumably enhanced its activity under the static storage condition. On the contrary, reactions during frying are dynamic and much more complex, involving both radical and non-radical reactions [2].

During deep-frying at elevated temperature with limited supply of oxygen and presence of food components, an acid-catalyzed, non-radical mechanism for the formation of C-C linked dimeric, polymeric or cyclic triacylglycerols predominates. Thus, synthetic antioxidants such as BHT, which operates almost exclusively by radical scavenging mechanism, tend to offer poor protection during frying [2, 33]. On the other hand, natural components, such as sterols and sesame oil's sesamolin, which can undergo acid-catalyzed decomposition reaction with activation energy lower than that of triacylglcerol dimerization, are effective polymerization inhibitor even though they exhibit negligible radical scavenging activity [2]. Furthermore, BHT is known to suffer evaporative loss and thermal inactivation/decomposition under frying condition and thus can only offer limited protection, which is usually restricted to the early stage of frying [34, 35]. On the contrary, the effectiveness of polyphenolic compounds appears to improve with temperature as indicated by a number of studies [36, 37]. Thus, beyond their excellent radical scavenging and metal chelating properties, the outstanding performance of *R. woodsii* phenolic extract under the current frying condition may be related to: (1) good thermal stability; (2) formation of decomposition products that can act as secondary antioxidants. For instance, thermal degradation of quercetin yielded protocatechuic acid as a major component [38], and acid-catalysed thermal decomposition of procyanidins can release (epi)catechin [39]; (3) synergistic interaction with endogenous antioxidants. For instance, quercetin and catechin have been reported to regenerate α-tocopherol from a homogenous solution of peroxidating methyl linoleate [40]; and (4) acid-catalysed release of aglycones from the glycosides.
Putting together, results from the present study showed that Canadian wild rose hip with seed is a rich source of natural antioxidative phenolic compounds. The natural phenolic mixture from this plant offered significant protection against deterioration of edible oils both under storage and frying conditions, presenting an excellent natural alternative to synthetic antioxidants commonly used in food processing industry. Further study is required on the impact of the extract on consumers' sensory perceptions of the oils and the foods prepared in them; suffice to say, however, that the addition of extracts did impact some colour, specific to each fruit, to the canola oil used for frying in the current study.

Acknowledgment

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The authors have declared no conflict of interest

Table 1: Identification of phenolic constituents of wild rose hip with seed (*Rosae pseudofructus cum fructibus*)

Peak	RT (min)	UV λ_{max} (nm)	$[M-H]^-$		MS^2	Identity
			Found	Expected		
A	9.5	225, 266	169.0143	169.0142	125.0241	Gallic acid
B	11.2	222, 260	153.1131	153.1125	109.0222	Protocatechuic acid
C	12.0	216, 226, 293	341.0868	341.0873	179.0349	Caffeoyl hexoside
D	13.2	218, 238, 325	353.0882	353.0878	191.0556	Neochlorogenic acid
E	16.1	215, 225, 278	289.0731	289.0718	245.0801, 203.0691	Catechin
F	17.1	218, 240, 325	353.0875	353.0878	191.0562, 179.0351	Chlorogenic acid
G	21.9	218, 237, 320	179.0353	179.0350	135.0448	Caffeic acid
H	24.9	234, 279	577.1369	577.1351	407.0763, 289.0720	B-type Procyanidin
I	25.4	227, 306	163.1494	163.1498	119.0455	*p*-Coumaric acid
J	26.1	218, 236, 314	193.0498	193.0500	149.0603	Ferulic acid
K	27.8	256, 353	609.1467	609.1461	301.0338	Rutin
L	28.3	255, 351	463.0881	463.0882	301.0334	Quercetin glucoside
M	29.0	254, 349	463.0876	463.0882	301.0335	Quercetin galactoside
N	30.5	254, 350	433.0779	433.0776	301.0324	Quercetin arabinoside
O	31.9	254, 349	447.0947	447.0933	301.0357	Quercetin rhamnoside
P	33.8	227, 283	435.1310	435.1297	273.0758, 167.0337	Phloridzin
Q	41.2	255, 369	301.0352	301.0354	178.9987, 151.1038	Quercetin
R	42.8	253, 371	317.0302	317.0297	178.9983	Myricetin

RT – retention time

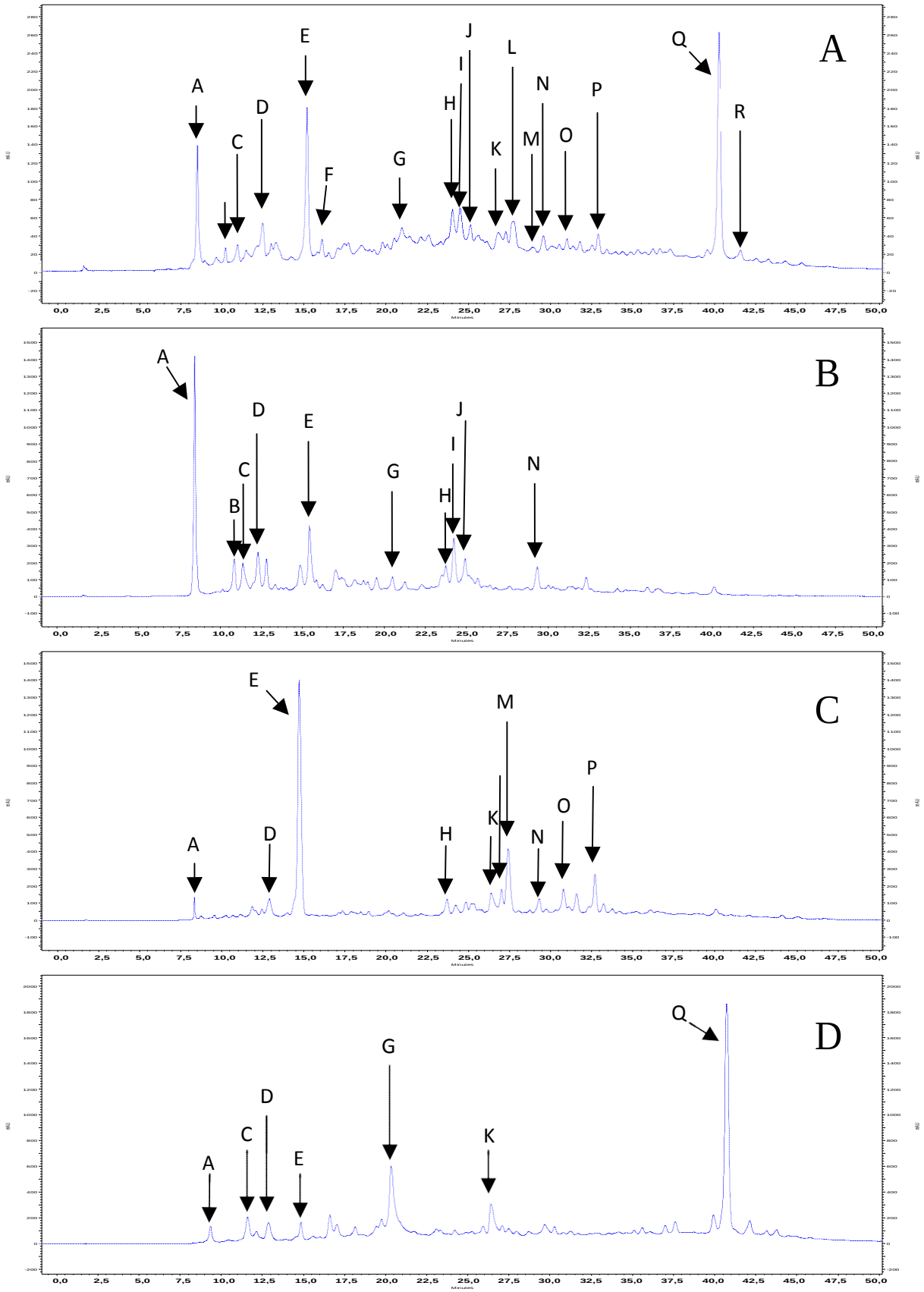

Figure 1: Representative chromatograms at 280nm of ethyl acetate extract and active fractions from *R. woodsii*. A = Rx (purified ethyl acetate extract); B = R6; C = R11; D = R14. R6, R11, R14 are coded fractions obtained from Rx using sephadex column. See text for conditions. See Table 1 for peak identifications.

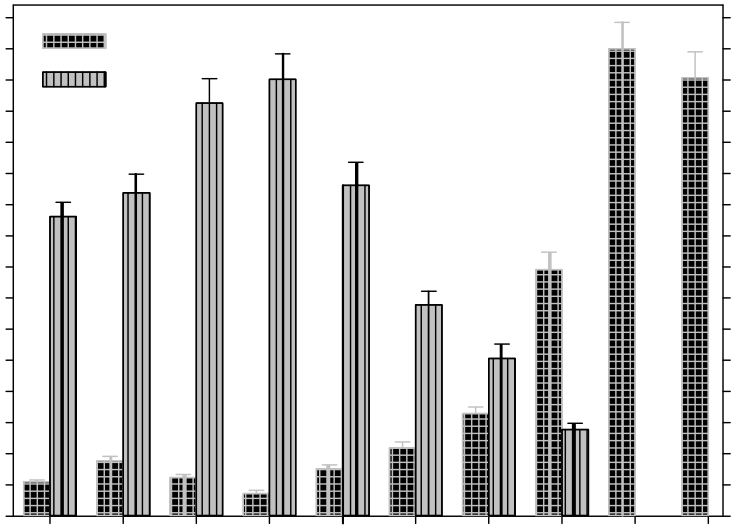

Figure 2: Total phenolics (TP) and DPPH radical scavenging activity of extract and phenolic fractions from R. woodsii fruits. Toc = α-tocopherol; BHT = butylated hydroxytoluene. R6, R11, R14, R15, R16, R21, and R22 are coded fractions obtained from Rx (purified ethyl acetate extract) using sephadex column. See text for conditions.

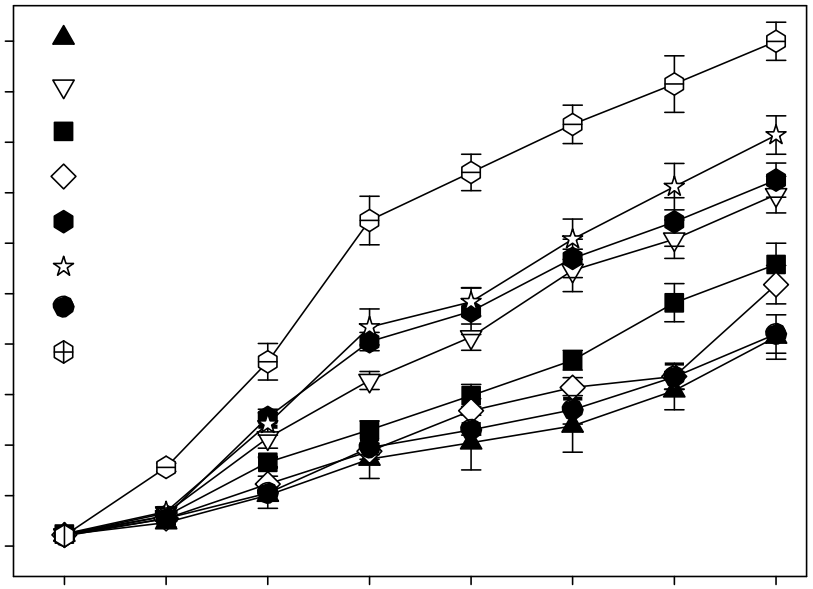

Figure 3: Changes in peroxide values during accelerated storage of canola oil fortified with phenolic extract/fractions from R. woodsii. Control = canola oil without exogenous antioxidant; BHT = butylated hydroxytoluene. R6, R11, R14, R15, and R16, are coded fractions obtained from Rx (purified ethyl acetate extract) using sephadex column. See text for conditions

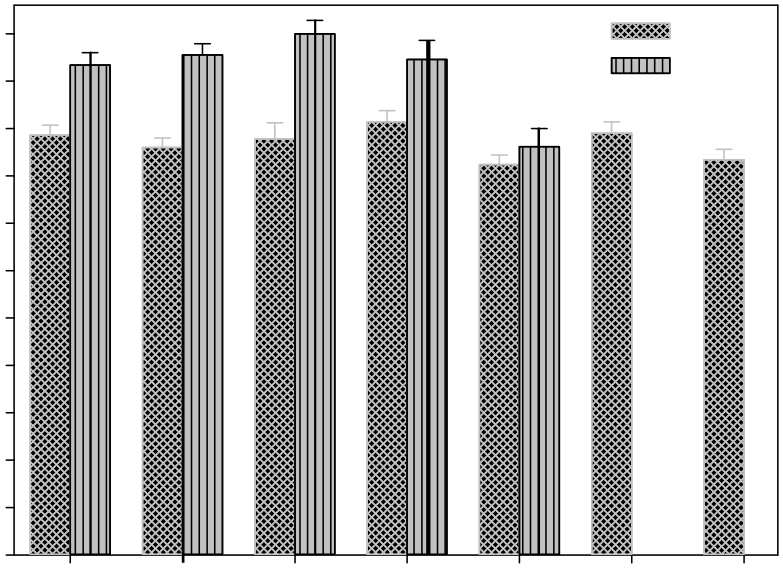

Figure 4: Oxidative stability under Rancimat condition of canola oil containing different phenolic fractions from R. woodsii fruits. Ctr = canola oil without exogenous antioxidant; BHT = butylated hydroxytoluene. R6, R11, R14, and R15 are coded fractions obtained from Rx (purified ethyl acetate extract) using sephadex column. See text for conditions

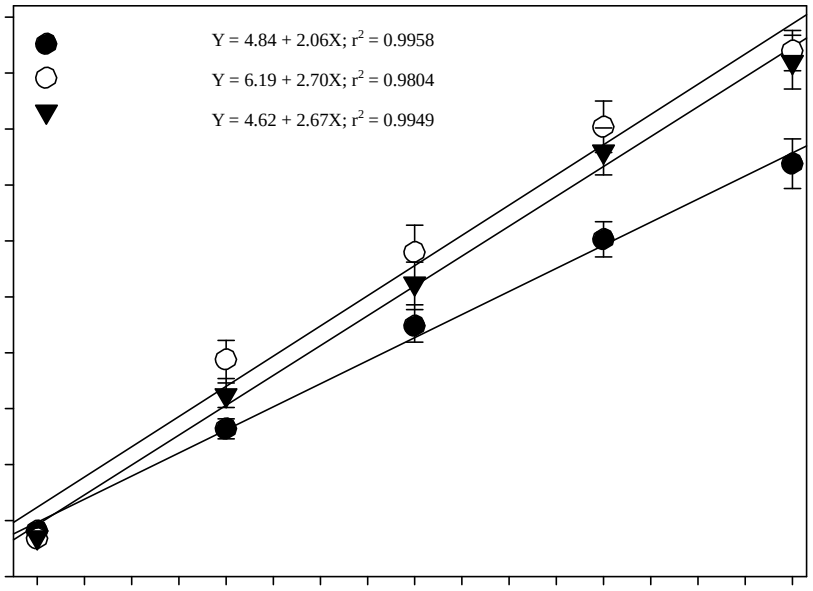

Figure 5: Formation of total polar components during frying in canola oil fortified with phenolic extract. Rx = purified ethyl acetate extract from R. woodsii fruits; Control = canola oil without exogenous antioxidant; BHT = butylated hydroxytoluene. See text for conditions

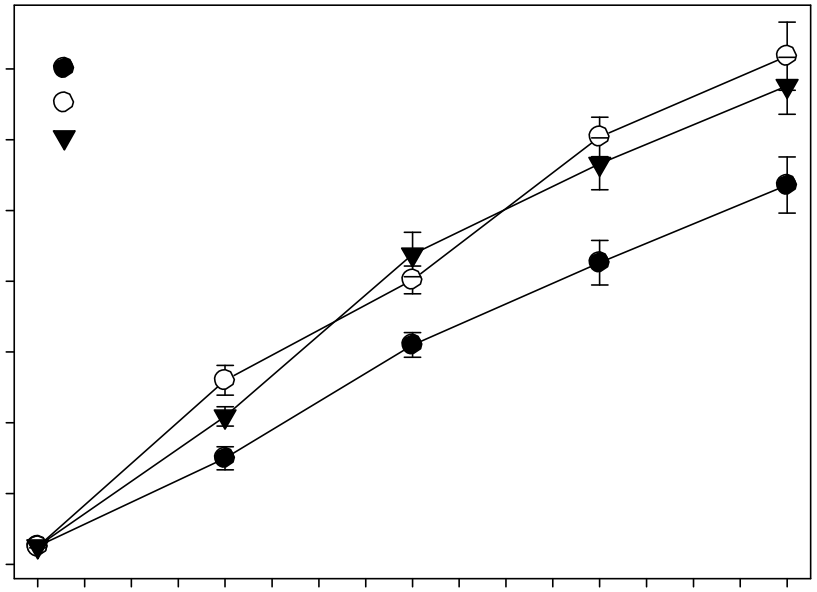

Figure 6: Formation of di- and polymerized triacylglycerides (polymers) during frying in canola oil fortified with phenolic extract. Rx = purified ethyl acetate extract from R. woodsii fruits; Control = canola oil without exogenous antioxidant; BHT = butylated hydroxytoluene. See text for conditions

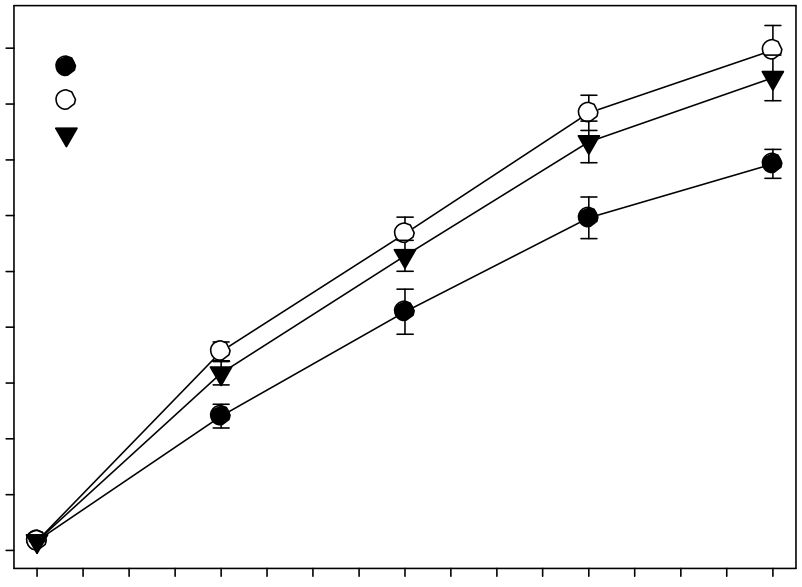

Figure 7: Formation of nonvolatile carbonyl components during frying in canola oil fortified with phenolic extract. Rx = purified ethyl acetate extract from R. woodsii fruits; Control = canola oil without exogenous antioxidant; BHT = butylatedhydroxytoluene. See text for conditions

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