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Phenolic extracts from *Sorbus aucuparia* (L.) and *Malus baccata* (L.) berries: Antioxidant activity and performance in rapeseed oil during frying and storage

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Abstract

In the present study, phenolic extracts and fractions from Canadian rowanberry (*Sorbus aucuparia*) and crabapple (*Malus baccata*) were screened for antioxidant activity using DPPH radical scavenging activity, and b-carotene bleaching assays. Furthermore, rapeseed oil was supplemented with extracts/fractions and performance was assessed during accelerated storage at 65 °C, under Rancimat at 120 °C, and during frying at 180 °C. A number of phenolic fractions showed significantly higher radical scavenging and antioxidant activity in the oil than the synthetic antioxidant, butylated hydroxytoluene (BHT). At the end of the 7-day storage, the peroxide value was reduced by up to 42% in the presence of extracts. The extent of thermooxidative degradation was significantly lower in oils fortified with the fruit extracts, with fractions from *Sorbus* species being more effective. Results from the present study suggested that polyphenolic extracts from these fruits can offer effective alternative to synthetic antioxidants during frying and storage of vegetable oils.

Keywords: Polyphenols Rowanberry Crabapple Oxidative stability, Natural antioxidants

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1. Introduction

Due to their inherent chemical compositions, vegetable oils undergo oxidative deterioration during frying and storage, resulting in the generation of free radicals as well as lipid degradation products and finally in quality deterioration. Additionally some of the degradation products are suspected to be toxic, such as acrolein or 4-hydroxy-nonenal. The stability of the oil is a measure of its resistance to several of the destructive reactions, especially oxidation, occurring during processing and storage. In general, oils that are more unsaturated oxidize more readily than less unsaturated one, prompting several modifications of the fatty acid composition of conventional oils to obtain the so called high-oleic oils (Matthäus, 2006). However, because of the current consumers' preference for polyunsaturated oils, their protection against oxidative deterioration without compromising the level of essential fatty acids is of paramount importance. One such measure is the application of antioxidants.

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and *tert*-butyl hydroquinone (TBHQ) are often added to processed oils to retard oxidative degradation during storage and frying (Warner, 2004). Although BHT and BHA are effective antioxidants at ambient and accelerated storage temperatures (Nenadis, Zafiropoulou, & Tsimidou, 2003), they offer relatively little protection during frying of food (Gertz, 2004). They are known to evaporate and thus do not remain in the frying oil long enough to provide protection against thermooxidative degradation (Augustin & Berry, 1983). Aside from the poor protection, especially under frying conditions, use of common synthetic antioxidants has also been limited due to their perceived detrimental effect on human health (Gertz, 2004). Consequently, there is a growing interest in the search for effective natural antioxidants.

A 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 °C), corresponding investigations under frying conditions, however, have received lesser attention, and are mostly limited to common spices and herbs (Al-Bandak & Oreopoulou, 2011; Aranha & Jorge, 2012; Che Man & Jaswir, 2000; Houhoula, Oreopoulou, & Tzia, 2004; Kalantzakis & Blekas, 2006; Karoui, Dhifi, Jemia, & Marzouk, 2011). However, ornamental plants with edible fruits can present a renewable source for natural polyphenolic antioxidative mixture.

Rowanberry (*Sorbus aucuparia*) and crabapple (*Malus baccata*) are popular ornamental plants widely grown in gardens and parks in Europe and North America. The fruits of rowanberry are used in the treatment of intestinal obstructions, chronic diarrhoea, various liver and gallbladder diseases. Rowanberry has been traditionally used for jelly and jams because of their nutritive and medicinal properties (Hukkanen, Pölönen, Kärenlampi, & Kokko, 2006). A number of polyphenolic compounds including quercetin, isoquercetin, hyperin, rutin, catechin, epicatechin, and chlorogenic acid have been reported in rowanberry (Kylli et al., 2010; Olszewska, Presler, & Michel, 2012; Termentzi, Kefalas, & Kokkalou, 2008).

Although the phenolic profile and antioxidant activity of Malus genus such as apples has been widely reported, relatively little is known about crabapples. A strong antiproliferative activity of crabapple juice toward human leukemic HL-60 cells was reported by Yoshizawa, Sakurai, Kawaii, Soejima, and Murofushi (2004). According to Wei et al. (2009), the leaf extracts of Siberian *M. baccata* had much stronger inhibitory abilities on fatty acid synthase of chicken liver than that from green tea. To the best of our knowledge, no data is available on the potential of polyphenolic extracts from rowanberry and crabapple to inhibit oxidative degradation of vegetable oils during frying and storage, necessitating the present study.

2. Materials and methods

2.1. Sample materials and chemicals

Fully ripened rowanberry and crabapples were collected between September and October, 2012 from the respective ornamental plants in different parts of Lethbridge, Alberta, Canada ($49°42^{0}0^{00}$N, $112°50^{0}0^{00}$W). Rapeseed 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 LH20 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).

2.2. 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 s. The ground samples (100 g) were homogenised 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 30 min. 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 defatted with hexane (3 × 500 mL) and successively extracted with ethyl acetate and *n*-butanol (3 × 500 mL each). The hexane extract was discarded while the ethyl acetate and *n*-butanol extracts were evaporated under vacuum at 30 °C, flushed with nitrogen and kept at -18 °C for further analysis.

2.3. Fractionation on C18 cartridge

The ethyl acetate and butanol extracts were further partitioned into the 50% and 100% methanol soluble fractions using a C18 SPE cartridge as follows: Extract (1 g) was dissolved in 50 mL methanol:water (50:50; v/v) and applied to a C18 SPE cartridge that had been preconditioned with 10 mL methanol. After collecting the 50% methanol soluble fraction, the column was washed with 20 mL 100% methanol to obtain the 100% methanol soluble fraction. Respective eluates from separate C18 SPE cartridges were combined and evaporated under vacuum at 30 °C using a rotary evaporator.

2.4. Fractionation on Sephadex

In order to obtain fractions with varying mixture of phenolic compounds, the 50% methanol fractions obtained by C18 partitioning 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 onto 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 (200 mL), and finally, methanol/acetone (1:1, v/v, 200 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 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.

2.5. Separation and identification 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 both positive and negative modes, acquiring MS full scan and auto MS/MS data at 2.5 Hz acquisition speed and *m/z* 80–1300 scan range. MS source settings were as follows: dry gas temperature, 200 °C; dry gas flow, 8 L/min; nebulizer gas pressure 2 bars; capillary voltage 4500 V; broadband collision-induced dissociation (bbCID), 25 eV; in-source CID, 60 eV. Molecular formula determination was carried out by combined evaluation of mass accuracy, isotopic patterns, adduct and fragment information using SmartFormula3D (Bruker, Bremen, Germany).

2.6. 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 (1965) 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]L 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. Each test was performed in triplicates.

2.7. DPPH radical scavenging assay

The DPPH assay was performed according to a method described by Nenadis and Tsimidou (2002) with some modifications. Briefly, a methanolic DPPH solution (0.1 mM; 2960]L) was added to 40]L 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]L of methanol and each test was performed in triplicate. The percentage of DPPH inhibition was calculated as follows:

$$\%\text{DPPH} = \frac{A_c - A_s}{A_c} \times 100$$

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 a-tocopherol were used as references. Calculated IC_{50} represents the concentration of antioxidant required to decrease the DPPH amount by 50%.

2.8. b-Carotene bleaching assay

Determination of the antioxidant activity using a b-carotene/linoleic acid system was as described by Miraliakbari and Shahidi (2008) with some modifications. In brief, 40 mg of linoleic acid and 400 mg of Tween 20 were transferred into a flask, and 1 mL of a solution of b-carotene (2 mg/mL) in chloroform was added. Chloroform was removed by rotary evaporation at 40 °C. Then 100 mL of distilled water was added slowly to the residue and the solution was vigorously agitated to form a stable emulsion. An aliquot of 4.8 mL of this emulsion was transferred into a test tube containing 0.2 mL of sample or methanol, and the absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without b-carotene. The tubes were then incubated at 50 °C in the dark and the absorbance was read after 2 h. BHT and a-tocopherol at 45]g/g concentration were used as reference antioxidants. Each test was performed in triplicate.

The antioxidant activity (AA) was calculated in terms of percentage inhibition relative to the control using the following equation:

$$\text{AA}(\%) = 1 - \frac{A_{s0} - A_{s2}}{A_{c0} - A_{c2}} \times 100$$

where A_{s0} and A_{s2} are the absorbance of the test samples measured at 0 and 2 h, respectively, while A_{c0} and A_{c2} denote the absorbance of the control (b-carotene-containing emulsion and methanol instead of sample), measured at 0 and 2 h, respectively.

2.9. Antioxidant activity under storage and frying conditions

2.9.1. Addition of polyphenolic extracts to oil

A solution of the extract in methanol was added to rapeseed 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 polyphenolic extracts in the oil. Residual solvent was removed by gentle stream of nitrogen.

2.9.2. 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. Rapeseed oil (1.0 g), fortified with phenolic extracts at 200]g 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, as synthetic antioxidant. Experiments were set up in two repetitions for each tested antioxidant, and samples from each repetition were analysed in triplicate.

2.9.3. Thermal oxidation by Rancimat

Rapeseed oils fortified with extracts at two different concentrations (200 and 500]gGAE/g of oil) were submitted to thermo-oxidation under Rancimat conditions using a 743 Rancimat (Metrohm, Filderstadt, Germany). 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. BHT was used as the reference antioxidant. Experiments were set up in two repetitions for each tested antioxidant.

2.9.4. Frying performance

Rapeseed oil (100 g) fortified with phenolic compounds at 500]gGAE/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 in a hot plate with a probe to control temperature at 180 ± 2 °C and heated for 8 h per day for 2 days 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 (3.5 g) were collected at the end of each frying day and immediately frozen at -18 °C until analysed. Experiments were set up in two repetition for each tested antioxidant and samples for each repetition were analysed in duplicate ($n = 4$).

2.10. Quantitative analysis

2.10.1. Determination of peroxide value (PV)

PV was assessed according to procedure described by Arkadiusz, Roszko, Sosińska, Derewiaka, and Lewicki (2010). 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]L of $FeCl_2$ (0.4% water solution) and 100]L of NH_4SCN (30% water solution) were added. The reaction was run at room temperature for 5 min, and the absorbance measured at 480 nm using all reagents for the blank sample.

2.10.2. Determination of polar materials, anisidine value and iodine value

The total polar components (TPC), dimerized and polymerized triacylglycerols (DPTG), anisidine value (AnV) and iodine value (IV) of frying oils were determined by Fourier-Transformed Near Infrared Spectroscopy (FT-NIR) following the DGF standard method C-VI 21 (DGF, 2013). 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.

2.11. Statistical analysis

Data are presented as means ± standard deviation (SD). Data were analysed 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 $P < 0.05$.

3. Results and discussions

3.1. Extraction, fractionation and total phenolic contents of fractions

The extraction and fractionation protocol employed in the current study is depicted in supplementary Fig. 1S. According to Kähkönen, Hopia, and Heinonen (2001), aqueous acetone was a better extraction solvent for apple, offering a significantly higher phenolic content than aqueous methanol. It is also well known that ultrasound assistance considerably improves both the kinetic and yields of phenolic compounds from vegetal sources (d'Alessandro, Kriaa, Nikov, & Dimitrov, 2012). With the extraction protocol employed in the present study, 2.6 and 5.8 g of ethyl acetate and butanol soluble fractions, respectively, were obtained from 100 g of rowanberry. These values were in agreement with previous data by Olszewska et al. (2012). The corresponding ethyl acetate and butanol fractions from crabapple were 1.7 and 6.4 g, respectively. Successive C18 and Sephadex fractionation of extracts yielded 8 and 9 fractions for rowanberry and crabapple, respectively, based on the similarity of HPLC phenolic profiles. Representative chromatograms are presented in Fig. 1 and Fig. 2, and as supplementary data in Figs. S2 and S3 for rowanberry and crabapple, respectively.

Although the amount of extract was higher in butanol fraction than the corresponding ethyl acetate fraction, the ethyl acetate fractions were significantly richer in phenolic compounds than the butanol fractions. The TP for C18 SPE purified ethyl acetate extract for rowanberry (RW_x) and crabapple (CA_x) were 103 and 183 mg GAE/g, respectively. The respective TP for the C18 SPE purified butanol extracts (RW_b, CA_b) were 42 and 61 mg GAE/g. A similar trend was reported by Olszewska and Michel (2012) for *Sorbus aria* ethyl acetate and butanol extracts. Depending on the purity of the fraction and the nature of the phenolic compounds, phenolic contents up to 809 mgGAE/g were estimated in the various fractions employed for subsequent antioxidant evaluation (Table 1). Activities of fractions were successively screened by a number of established tests in search for a natural polyphenolic mixture that can significantly improve the stability of vegetable oils during high temperature processing and storage.

3.2. Radical scavenging activity of phenolic extracts

The radical scavenging activity of the different phenolic fractions examined in this study is presented in Fig. 3. The IC_{50} of the extracts ranges from 30–3713 lg/mL and 7–3438 lg/mL for rowanberry and crabapple, respectively. Under the same DPPH assay experimental conditions, the IC_{50} for BHT and a-tocopherol were 704 and 750 lg/mL, respectively. The radical scavenging activity of crabapple ethyl acetate extract was significantly higher than the corresponding extract from rowanberry, which is not surprising considering the higher TP value for the crabapple extract (Hukkanen et al., 2006). However, contrary to the present study, Hallmann, Orpel, and Rembialkowska (2011) reported a higher TP in Polish rowanberry compared to the corresponding apple fruits. The observed difference may be due to the differences in geographical locations and the part of fruits examined. Whereas in the present study, whole fruits were utilised, Hallmann et al. (2011) only analysed the flesh after removing the seeds and peel.

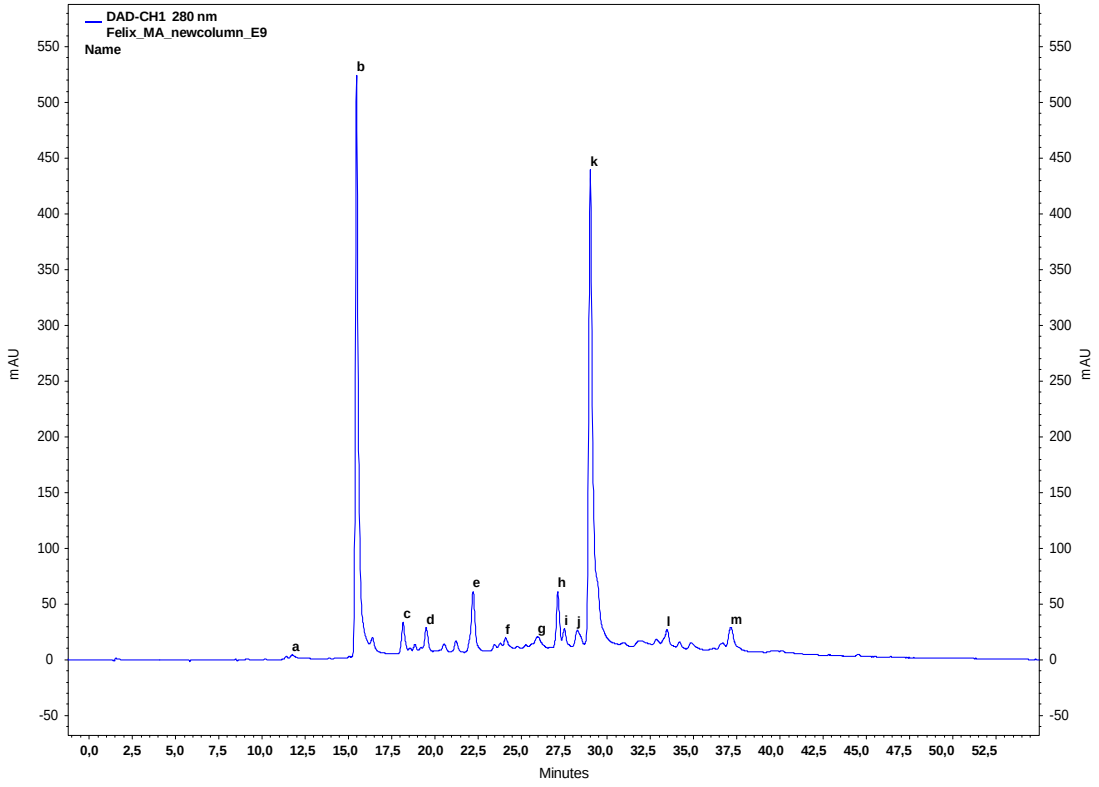

Figure 1. HPLC chromatogram at 280 nm of an active phenolic fraction, RW_3, from rowanberry fruit extract. Peaks: a = neochlorogenic acid; b = chlorogenic acid; c = epicatechin; d = caffeic acid; e = feruloylquinic acid; f = ferulic acid; g = rutin; h = Quercetin-3-O-glucoside; i = Quercetin malonylglucoside; j = Kaempferol-3-O-glucoside; k = Quercetin-3-O-rhamnoside; l = phoridzin; m = Quercetin. For conditions, see text.

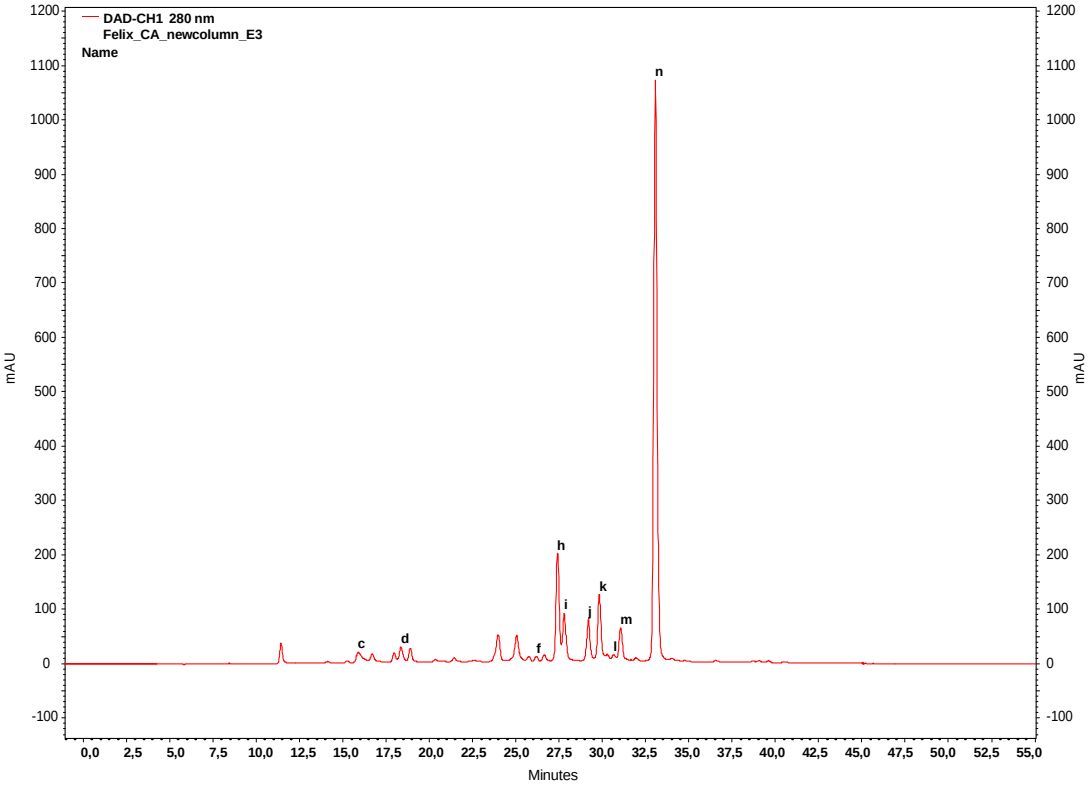

Figure 2. HPLC chromatogram at 280 nm of an active phenolic fraction, CA_6, from crabapple fruit extract. Peaks: a = neochlorogenic acid; b = catechin; c = chlorogenic acid; d = epicatechin; e = caffeic acid; f = ferulic acid; g = rutin; h = Quercetin-3-O-glucoside; i = Quercetin malonylglucoside; j = Kaempferol-3-O-glucoside; k = Quercetin-3-O-rhamnoside; l = Quercetin glycoside; m = phloretin-2^0-xyloside; n = phloridzin; o = phloretin. See supplementary Fig. S2 for missing letters; For conditions, see text; For abbreviation see Fig. S1.

Table 1
Total phenolic contents, antioxidant acitivity and performance of selected fractions during frying in rapeseed oil.

Fractions	TP (mgGAE/g)	AA* (%)	Frying							
			DTPC (%)		DDPTG (%)		DAnV		IV (110.7)**	
			8 h	16 h	8 h	16 h	8 h	16 h	8 h	16 h
RWx	103 ± 7^{a}	78.2 ± 1.9^{a}	–	–	–	–	–	–	–	–
RW1	229 ± 12^{b}	73.5 ± 2.5^{b}	–	–	–	–	–	–	–	–
RW2	865 ± 5^{c}	73.7 ± 2.0^{b}	–	–	–	–	–	–	–	–
RW4	237 ± 21^{b}	74.4 ± 1.8^{b}		–	–	–	–	–	–	–
RW5	$209 \pm 15^{b,e}$	73.9 ± 2.4^{b}	–	–	–	–	–	–	–	–
RW3	809 ± 13^{c}	86.2 ± 1.8^{c}	20.4 ± 1.5^{a}	35.8 ± 2.2^{a}	14.5 ± 1.0^{a}	24.8 ± 1.5^{a}	102.0 ± 4.7^{a}	176.8 ± 8.8^{a}	101.4 ± 1.2^{a}	92.5 ± 0.7^{a}
Control[d]	–	–	28.9 ± 1.3^{b}	42.4 ± 2.4^{b}	17.9 ± 0.5^{b}	29.8 ± 1.6^{b}	133.1 ± 4.1^{b}	204.4 ± 9.2^{b}	98.7 ± 1.2^{b}	89.1 ± 1.0^{b}
BHT	–	92.2 ± 3.1^{d}	24.3 ± 2.1^{c}	40.7 ± 1.8^{b}	15.7 ± 0.7^{c}	28.5 ± 1.1^{b}	122.3 ± 4.9^{c}	201.2 ± 6.1^{b}	$99.9 \pm 1.3^{a,b}$	89.9 ± 0.8^{b}
CA6	751 ± 16^{d}	78.9 ± 1.7^{a}	21.7 ± 1.7^{ac}	35.5 ± 2.1^{a}	13.5 ± 0.9^{a}	24.7 ± 1.3^{c}	100.2 ± 5.8^{a}	182.8 ± 10.4^{a}	102.1 ± 1.4^{a}	92.9 ± 1.3^{a}
CA3	$204 \pm 18^{b,e,i}$	69.1 ± 3.4^{e}	–	–	–	–	–	–	–	–
CA7	$153 \pm 11^{g,h}$	69.7 ± 2.3^{e}	–	–	–	–	–	–	–	–
CA2	138 ± 15^{g}	78.1 ± 2.1^{a}	–	–	–	–	–	–	–	–
CA1	164 ± 8^{h}	70.6 ± 1.8^{e}	–	–	–	–	–	–	–	–
CAx	183 ± 9^{i}	65.2 ± 2.7^{f}	–	–	–	–	–	–	–	–
Toc	–	82.8 ± 2.8^{c}	–	–	–	–	–	–	–	–

TP = total phenolics; DTPC = change in total polar compounds during frying; DDPTG = change in dimeric and polymeric triacyglycerides during frying; DAnV = change in anisidine value during frying. Values bearing the same superscripts within the same column are not significantly different at $p < 0.05$. [d]Rapeseed oil without added antioxidant.
* AA = antioxidant activity.
** IV (110.7) = iodine value of fresh rapeseed oil.

Although DPPH assay is an excellent method to evaluate the radical scavenging activity of potential antioxidative compounds, the results are sometimes poorly correlated with performance in real food, principally because; (i) the nature and polarity of the radicals encountered in food system is different from that of the DPPH radical; and (ii) the reaction is carried out in an organic solvent and the impact of antioxidant partitioning is not evaluated (Decker, Warner, Richards, & Shahidi, 2005). In the b-carotene bleaching assay, the nature of the radical is somewhat similar to those occurring in vegetable oil and the partitioning of applied antioxidant is also taken into consideration. Thus, in the presence study, the chain breaking activity of the phenolic extracts was

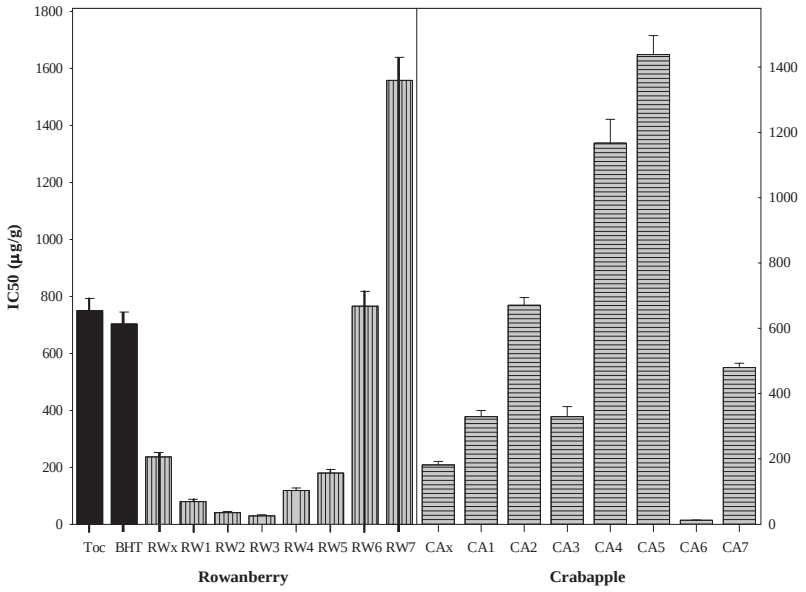

Figure 3. DPPH radical scavenging activity of various polyphenolic fractions from rowanberry (RW) and crabapple (CA). IC50 is the concentration of extract required to decrease DPPH amount by 50%. See text for details.

assessed by the b-carotene assay. However, fractions (RW_6, RW_7, CA_4, and CA_5) with DPPH radical scavenging activity weaker than that of a-tocopherol (IC_{50} 750 lg/g) were excluded from further study with the b-carotene assay. Extracts were added at concentration that delivered phenolic compounds of approximately 50 lg GAE/mL. As shown in Table 1, all the tested extracts showed good antioxidant activity with fraction RW_3 (AA = 86%) from rowanberry offering comparable activity with BHT (AA = 92%), the synthetic antioxidant control. Kylli et al. (2010) also reported an excellent antioxidant activity of rowanberry extract in an oil-in-water emulsion (10% o/w) as assessed by the formation of conjugated dienes. Whereas the DPPH radical scavenging activity of the purified ethyl acetate extract (CA_x) from crabapple was higher than the corresponding extract from rowanberry (RW_x), a reversal of potency was observed under the b-carotene bleaching assay, presumably due to differential solubility of the phenolic constituents of the two extracts in this assay.

3.3. Antioxidant activity in oil under storage conditions

The ability of the promising fractions from rowanberry and crabapple to protect vegetable oils during ambient storage was assessed using the widely accepted Schaal oven test at 65 °C for up to 7 days. Lipid hydroperoxides, the primary oxidation products, are the precursors of several secondary oxidation products, such as the aldehydic compounds, responsible for rancidity in edible oils. Thus, their formation dramatically impacts oil shelf lives and consumer acceptance. The increase in peroxide value (PV) of the rapeseed oil substrate over the storage period is depicted in Fig. 4. All the tested extracts offered significant protection against oxidative deterioration of the rapeseed oil. Formation of hydroperoxides in the oil was inhibited by up to 42% and 30% in the presence of fractions from rowanberry and crabapple, respectively (Fig. 4). Fractions RW_1, RW_3 and RW_4 offered the best protection among the rowanberry extracts while CA_x, CA_3, CA_6 were the most promising fractions from crabapple. At equivalent phenolic content (200 lg/g), and under the conditions employed in the present study, none of the fractions showed comparable efficiency as BHT. The reduced antioxidant activity compared with BHT may be due to the influence of some pro-oxidant impurities in the phenolic fractions.

3.4. Antioxidant activity under the Rancimat conditions

Although the oxidative conditions under the Rancimat are different from those during actual frying, the method can provide a fast assessment of the thermal stability and efficiency of an antioxidative compound under a harsher condition than 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 the oxidative stability by means of the Rancimat method is a dynamic measurement. Thus, the induction period (IP) measured by the Rancimat can be a useful "screening" method for frying oils (Matthäus, 2006). The induction period (IP) for the rapeseed oils with or without exogenous phenolic compounds at 200 and 500 lg GAE/g from rowanberry and crabapples is presented in Fig. 5. The phenolic extracts exhibited a concentration dependent increase in activity, with efficiency markedly better than BHT at the higher concentration. The poor efficiency of the fractions at 200 lg/g is presumably related to the thermal degradation of the phenolic constituents under the harsher Rancimat condition, compared to the Schaal oven test. However, at 500 lg GAE/g of oil, RW_1, RW_3, RW_4, and the purified rowanberry ethyl acetate extract, RW_x significantly extended the IP of the rapeseed oil. Fractions CA_3, CA_6, and CA_x from crabapple also exhibited strong antioxidant activity under the Rancimat conditions (Fig. 5).

3.5. Performance during frying

Based on their consistence in all the screening tests, the performance of fractions RW_3 from rowanberry and CA_6 from crabapple was further evaluated during a 2-day actual frying of French fries in rapeseed oil. Concentration of 500 lg GAE/g of oil was chosen as the minimum concentration based on the results obtained under the Rancimat condition (Fig. 5).

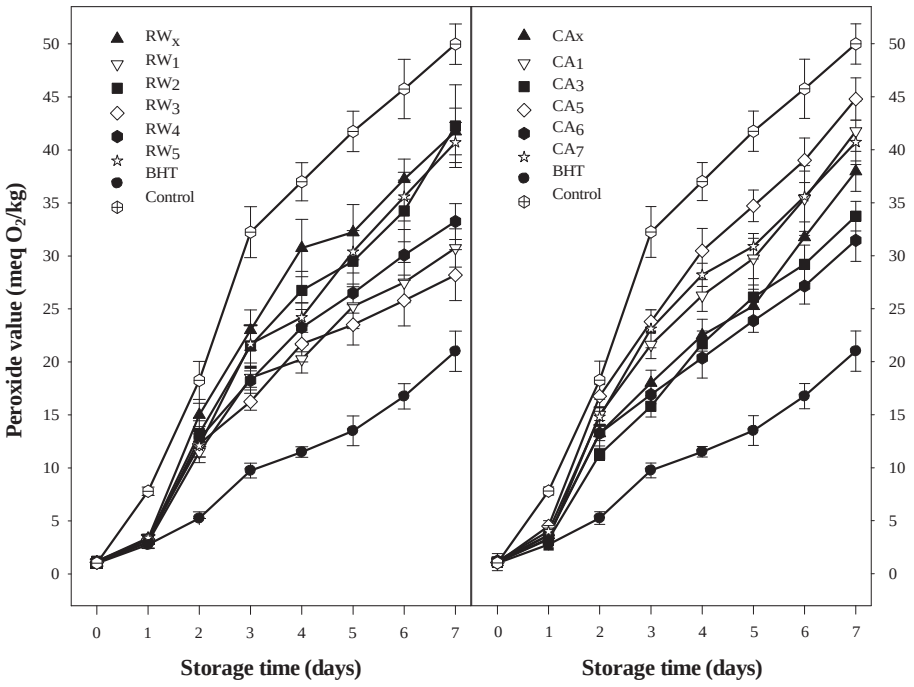

Figure 4. Changes in peroxide values during accelerated storage of rapeseed oil fortified with different polyphenolic fractions from rowanberry (RW) and crabapple (CA). BHT – butylated hydroxytoluene; control – refined, bleached and deodorized (RBD) rapeseed oil without extract/fraction. See text for details.

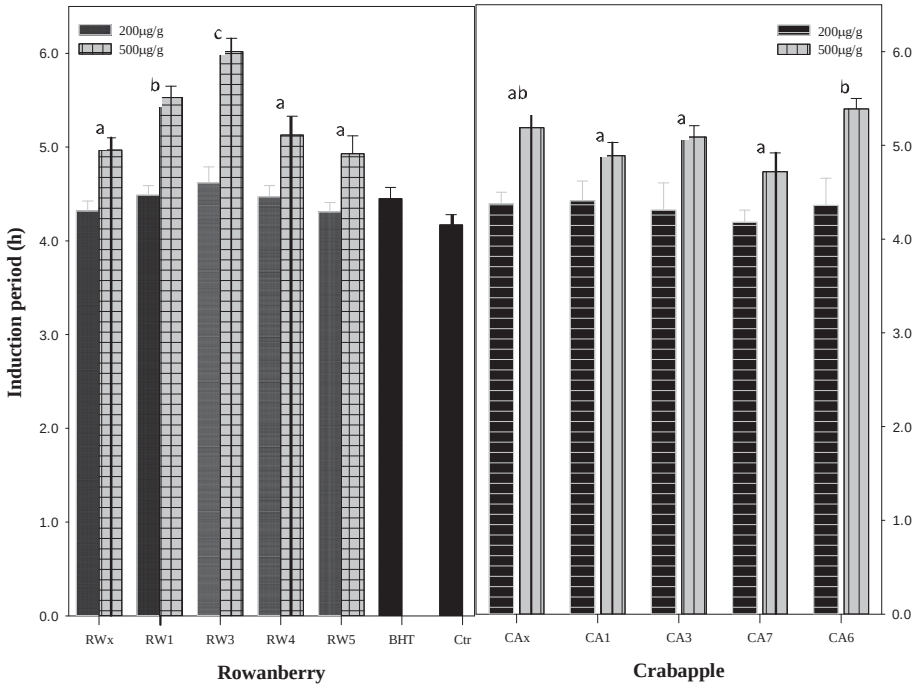

Figure 5. Oxidative stability of rapeseed oil fortified with different polyphenolic fractions from rowanberry (RW) and crabapple (CA) as measured by Rancimat induction period. BHT – butylated hydroxytoluene; Ctr – refined, bleached and deodorized (RBD) rapeseed oil without extract/fraction. Differences between fractions bearing the same letter are not statistically significant at 500]g/g applied concentration. See text for details.

3.5.1. Total polar components (TPC)

Because the components measured are non-volatile and are representative of the major reactions occurring during frying, TPC is one of the most reliable parameters for assessing the frying stability of fats/oils. As shown in Table 1, the TPC increased significantly during the entire frying period, irrespective of oil samples, however, statistically significant differences ($P < 0.05$) were found among the treatments. At the end of the 8 h of frying, the TPC of the rapeseed oil control was 29% and 25% higher than the amounts found in samples fortified with RW_3 and CA_6, respectively. At the end of the 8 h of frying, the TPC for RW_3 and CA_6 fortified rapeseed oils were below the 25% discard level defined in many European countries; the corresponding value for the rapeseed oil control, however, was 28.9%, a 4-percent point

higher than the discard level. Compared to BHT, the polyphenolic fractions, RW_3 and CA_6 also showed a significant superior performance under the frying protocol used in the present study, with the effect being more significant at the higher frying time (Table 1).

It is interesting that the performance of BHT during frying (Table 1) is at variance with its excellent antioxidant activity observed during storage (Fig. 4), possibly due to differences in the nature and mechanism of the chemical and physical reactions occurring under both conditions (Gertz, 2004). Lipid oxidation under storage condition is principally 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, with the non-radical acid-catalysed mechanism for the formation of C–C linked dimeric, polymeric or cyclic triacylglycerols becoming significant (Gertz, 2004). Since TPC measures the totality of thermooxidative degradation products, contrary to PV, the inefficiency of BHT as an antioxidant under frying condition becomes apparent.

3.5.2. Dimerized and polymerized triacylglycerols (DPTG)

High molecular weight degradation products, mainly dimers and oligomers of acylglycerols are formed during frying and their quantification is a very reliable parameter for frying fats/oils thermo-oxidative stability. In the present study, the formation of DPTG in rapeseed oil was considerably reduced in the presence of the applied polyphenolic fractions, indicating a strong anti-polymerization activity (Table 1). Whereas, no significant difference ($P > 0.05$) was observed in the performance of RW_3 and CA_6 as measured by the accumulation of DTPG, both polyphenolic fractions did exhibit a significantly higher protection than BHT at the end of the 2-day frying period. Compared to the control rapeseed oil without exogenous antioxidant, BHT showed a significant protection only during the first day of frying, but the difference at the end of the second day of frying was marginal (Table 1). The failure of BHT under prolonged frying operation is well known; presumably due to evaporative losses or thermal inactivation (Augustin & Berry, 1983). On the contrary, natural polyphenolic compounds are much less volatile, and their effectiveness appears to improve with temperature (Elhamirad & Zamanipoor, 2012). This may be due to improved solubility of the polyphenolic constituents in response to increase in temperature and heat-induced formation of secondary antioxidants with different or complementary antioxidant activity. Further, unlike BHT which operates almost exclusively by radical scavenging activity, the anti-polymerization activity of polyphenolics, in addition to the metal-chelating activity of their aglycones, may include acid-catalysed hydrolysis of the glycosides. This competing acid-catalysed reaction of antioxidants has been suggested as the reason for the effectiveness of natural components, such as sterols and sesamolin, despite their negligible radical scavenging activity (Gertz, 2004).

3.5.3. Anisidine and Iodine values (AnV, IV)

Thermal decomposition of lipid hydroperoxides during frying generates a number of secondary oxidation products, with carbonyl compounds being the most prominent. Although some of the aldehydes produced are volatile and lost by evaporation during frying, a significant amount remains and is assessed by AnV. Changes in AnV of rapeseed oil with and without fortification are presented in Table 1. As expected, a significant increase in AnV was observed over the entire frying period, regardless of sample treatments. In agreement with TPC and DPTG results, rapeseed oil supplemented with RW_3 and CA_6 exhibited a significantly higher frying stability compared to the unfortified rapeseed oil control, as assessed by the amount of non-volatile carbonyl secondary oxidation products formed during the frying (Table 1).

Data from IV paralleled those from AnV, showing consistent decrease over the course of the frying period regardless of sample treatments (Table 1). The IV decreased from 110.7 in the fresh oil to 92.4, 92.9, 89.9, and 87.4 in samples fortified with RW_3, CA_6, BHT, and the control rapeseed oil, respectively, at the end of the 2-day frying. IV is a measure of unsaturation in the oil and the observed decrease was a consequence of thermo-oxidative loss of unsaturated fatty acid. Thus, the significantly higher IV in the fortified oil compared to the control rapeseed oil is a testimony to the antioxidant activity of the polyphenolic fractions during frying.

3.6. Phenolic constituents of fractions

Based on their performance, the phenolic profiles of fractions RW_3 and CA_6 were examined by HPLC–MS and the results are contained in Figs. 1 and 2, respectively. In agreement with several previous studies (Hukkanen et al., 2006; Kylli et al., 2010; Olszewska et al., 2012), chlorogenic acid and its isomer, neochlorogenic acid were the major phenolic compounds in rowanberry, representing more than 85% of the total phenolic compounds (Fig. 1SA). Indeed, according to Hukkanen et al. (2006), the amount of chlorogenic acid in rowanberry is comparable to the amount present in coffee. The fact that no significant differences were observed in the frying performance of rapeseed samples fortified with RW_x (P80% chlorogenic acid; Fig. 1SA), RW_2 (>70% neochlorogenic acid; Fig. 1SB) and RW_5 (ca. 50% each chlorogenic isomer; Fig. 1SC) suggested a common antioxidant mechanism and a lack of synergy between the chlorogenic acid isomers. Similarly, the additional presence of feruloylquinic acid in RW_4 (Fig. 1SD) did not result in any significant improvement in performance under Rancimat (Fig. 5). On the contrary, the presence of quercetin-3-O-rhamnoside (Q-Rh) in RW_3 (Fig. 1SF) markedly increased the performance of this fraction, presumably due to the high reducing and radical scavenging activity of this flavonoid compared to the hydroxycinnamic acid derivatives (Aaby, Hvattum, & Skrede, 2004). The comparatively poor thermal stability of Q-Rh is possibly offset by the relatively better thermal stability of chlorogenic acid in the fraction. This may explain the better performance of RW_3 under Rancimat, compared to RW_1 lacking the chlorogenic acid component (Fig. 1SE). According to Van der Sluis, Dekker, and van Boekel (2005), the loss of Q-Rh at 100 °C in the presence of oxygen was at least 4 times higher than that of chlorogenic acid.

Representative chromatograms for crabapple fractions are presented in supplementary Fig. 2S. Although significant amount of chlorogenic acid was also found in crabapple, its major phenolic compound was phloridzin (Fig. 2SA), representing up to 25% of the total phenolic compounds. Wang, Wang, and Li (2013) also reported phloridzin as the major phenolic compound in crabapple from China. The higher radical scavenging and the relatively better lipophilicity of phloridzin probably accounted for the higher performance of CA_6 compared to other fractions where chlorogenic acid predominates (Figs. 2SB–D).

4. Conclusion

The radical scavenging, antioxidant and antipolymerization activity of polyphenolic extracts from seeds of two widely available ornamental plants were evaluated. Results from the present study indicated that the ethyl acetate extracts and a number of purified fractions from rowanberry (*S. aucuparia* L.) and crabapple (*M. baccata* L.) possess considerable potential as natural antioxidants for polyunsaturated vegetable oils both during frying and storage. All the frying performance indices evaluated in the present study

(total polar components, oligomerized triacyglycerols, anisidine value and iodine value) clearly showed that polyphenolic fractions from these fruits offered significantly better protection to rapeseed oil than BHT, the synthetic antioxidant positive control. A possible influence of lipophilicity and thermal stability of constituent polyphenolic compounds on the frying performance of fractions was observed, thus, further studies on the fate of polyphenolic compounds during frying are underway. A study on the effect of applied polyphenolic extracts on the sensory attributes of fats/oils and the prepared food is also warranted.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.02.139.

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