JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Effect of Heat Treatment of Camelina (*Camelina sativa*) Seeds on the Antioxidant Potential of Their Extracts

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ABSTRACT: The effect of different heat treatments of camelina (*Camelina sativa*) seeds on the phenolic profile and antioxidant activity of their hydrolyzed extracts was investigated. The results showed that total phenol contents increased in thermally treated seeds. Heat treatment affected also the quantities of individual phenolic compounds in extracts. Phenolics in unheated camelina seeds existed in bound rather than in free form. A temperature of 160 °C was required for release of insoluble bound phenolics, whereas lower temperatures were found to be optimal to liberate those present as soluble conjugates. The best reducing power and alkyl peroxyl radical scavenging activity in the emulsion was expressed by phenolics which were bound to the cell wall, whereas the best iron chelators and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavengers were found to be those present in free form. The heat treatment of seeds up to 120 °C increased the reducing power and $DPPH^{\bullet}$ radical scavenging ability of extracts, but negatively affected iron chelating ability and their activity in an emulsion against alkyl peroxyl radicals.

KEYWORDS: heated camelina seeds, alkaline hydrolysis, free, soluble conjugate, insoluble bound phenolics, flavonoids, antioxidant activity

INTRODUCTION

The extraction of phenolics from various oilseeds requires the application of thermal and mechanical pretreatment to induce rupture of the oil-containing cells, to improve the crushing capacity, and consequently to make these compounds more extractable.[1,2] As reported,[3] thermal processing disrupts the cell membranes and cell walls, resulting in cleavage of covalently bound phenolic compounds and their release from bound forms. In addition, high temperatures also inactivate a number of naturally occurring enzymes present in the seed that would otherwise have negative effects on the stability of phenolic compounds.[4] Besides formation of the desired flavor, texture, and color, thermal treatment is also critical for the nutritional value of roasted seeds. Their antioxidant capacity may be strongly affected through the degradation of some heat-labile bioactive substances or by formation of new compounds having antioxidant activity, mainly attributed to Maillard reaction products.[5]

Phenolic acids exist in oilseeds in free, soluble conjugate, and insoluble bound forms. Having both carboxylic acid and hydroxyl groups in their structure, phenolic acids may form both ester and ether linkages. That is, some of the phenols are associated with cell wall materials, especially complex carbohydrates such as lignin and arabinoxylan. These are designated as insoluble bound phenolics.[6] Others are present in cytoplasmic vacuoles linked to various carbohydrates or organic acids or with one another and are regarded as soluble conjugates. They can be esterified with alcohols, other phenolic acids, phenols, and alkaloids.[7,8] Bound phenolics may be released by alkali, acid, or enzymatic hydrolysis prior to extraction.[7−9] Although acidic and enzymatic hydrolyses are more representative of human digestion, alkali hydrolysis is more generally used. The latter liberated nearly twice the amount of phenolics as acid hydrolysis, as evaluated by the Folin−Ciocalteu (FC) assay.[10] However, according to HPLC analysis, some phenolic acids are more labile under alkali conditions, whereas others prefer acid hydrolysis.[11] Moreover, enzymatic conditions do not allow classification of phenolics into free, esterified, and insoluble-bound forms.[7] Like the other phenolic compounds, flavonoids also exist in the bound form rather than in the free form.[12,13]

In recent years, there has been growing interest in studying phenolic compounds from oilseeds, one of the latter being camelina (*Camelina sativa*), also known as false flax or gold of pleasure. Camelina belongs to the Cruciferae family. To our knowledge, the phenolic antioxidants of camelina seeds have been tested in their free form only. In our previous study[14] we reported that besides sinapine and sinapic acid, other compounds such as flavonoids, ellagic acid, hydroxybenzoic and hydroxycinnamic acids, and their 4-vinyl derivatives contribute to the antioxidant activity of camelina seeds.

The objectives of the present study were to investigate the effect of different heating conditions on the yield and phenolic profile of camelina seed extracts considering its free, soluble, and insoluble bound fractions using alkaline hydrolysis. To evaluate the antioxidant properties of these extracts, four antioxidant assays utilizing various reaction mechanisms were used. Heat treatment is a part of the processing of these oilseeds, and this study was performed to determine the optimal heating condition to produce seeds with enriched antioxidant potential and improved physicochemical value.

MATERIALS AND METHODS

Sample Material. Seeds from the camelina plant (*C. sativa* (L.) Crantz) grown in the year 2009 were obtained from a local farm in the Koroška region, Slovenia.

Received: April 21, 2011
Revised: July 4, 2011
Accepted: July 11, 2011
Published: July 11, 2011

ACS Publications © 2011 American Chemical Society

dx.doi.org/10.1021/jf2016072 | *J. Agric. Food Chem.* 2011, 59, 8639–8645

Chemicals. All chemicals and reagents used for the present work were of analytical quality and purchased from Merck (Darmstadt, Germany), Sigma-Aldrich GmbH (Steinheim, Germany), Kemika (Zagreb, Croatia), Riedel-de-Haën (Seelze, Germany), and Carlo Erba (Milano, Italy). For preparation of solutions ultrapure water (Milli-Q, Millipore) was used.

Synthesis of 4-Vinyl Derivatives from Hydroxycinnamic Acids. 4-Vinyl derivatives of hydroxycinnamic acids (4-vinylphenol (4-VP), 4-vinylguaiacol (4-VG), 4-vinylcatechol (4-VC), and 4-vinylsyringol (4-VS)) were prepared by thermal decarboxylation of *p*-coumaric, ferulic, caffeic, and sinapic acids, respectively, and confirmed by MS and NMR as explained in our recent publication.[15]

Isolation and Conformation of Sinapine. Sinapine (3,5-dimethoxy-4-hydroxycinnamoylcholine) was isolated from kale (*Brassica oleracea*) according to the procedure described in our previous investigation.[14]

Heat Treatment of Camelina Seeds. Seeds were heated in an electrical oven (model VO 500, Memmert, Scwabach, Germany) at three different temperatures: 80, 120, and 160 °C for 30 min. Afterward, the seeds were cooled in air for an hour to room temperature. Unheated seeds were used as control.

Sample Preparation. Seeds were ground in a seed mill and twice defatted with hexane (1:4, w/v) on a Vibromix 314 EVT shaker (Tehnica Železniki) for 1 h at room temperature. After filtration under vacuum, the defatted residue was air-dried for 1 h to remove residual hexane and used for solvent extraction of phenolic compounds. In parallel, an aliquot of defatted seed was used for water content determination (by oven-drying at 105 °C to constant weight in triplicate).

Free Phenolic Compounds. Extraction (in triplicate) was carried out with 80% (v/v) methanol using a defatted seeds-to-solvent ratio of 1:5 (w/v). After 1 h of shaking at room temperature, the solid was recovered by filtration and subjected to an additional extraction step under the same conditions. The solvent in the combined extract was evaporated, and the dry methanolic extract from 5 g of defatted seeds (in triplicate) was redissolved in 4 mL of water acidified with HCl (pH 2) and extracted with ethyl ether (4 mL), three times for 5 min. The phenolics that partitioned in the combined ether fractions represented free phenolic compounds. The ether was evaporated at 30 °C, and the residue was reconstituted in methanol and filtered through a 0.45 μm membrane filter.

Soluble Conjugates. The acidified water phase (in triplicate), from which in the previous step the ether fraction had been removed, was neutralized to pH 7 with 2 M NaOH and dried using a vacuum-evaporator. The dry residue was redissolved in 4 mL of 2 M NaOH and stirred on a Vibromix 314 EVT shaker (Tehnica Železniki) at room temperature. After 4 h, hydrolysis was interrupted by acidification to pH 2 using 6 M HCl. Extraction with ethyl ether (4 mL, 5 min) was performed three times to obtain the soluble conjugate fraction. The ether extracts were combined, evaporated, reconstituted in methanol, and filtered.

Bound Phenolic Compounds. To the solid residue from the methanol extraction (2.5 g) was added 100 mL of 2 M NaOH (in triplicate) and shaken for 4 h on a Vibromix 314 EVT shaker (Tehnica Železniki) at room temperature. After alkaline hydrolysis, the solution was acidified to pH 2 with 6 M HCl. The liberated phenolic compounds were then extracted three times with ethyl ether. The ethyl ether layers were pooled, evaporated, reconstituted in methanol, and filtered to obtain the insoluble-bound phenolic fraction.

The whole extraction procedure giving fractions of free phenolic compounds, soluble conjugates, and bound phenolics was performed according to a modified method described by Kim et al.[11] All phenolic fractions were stored at 4 °C until analysis.

Determination of Total Phenolic (TP) Content. The total phenolic content was measured at 765 nm ($A_{765\ nm}$) on a model 8453 Hewlett-Packard UV−visible spectrophotometer (Hewlett-Packard, Waldbronn, Germany) with a 1 cm cell according to the Folin−Ciocalteu method,[16] with slight modification as explained previously.[14] Results were expressed in milligrams of chlorogenic acid (CA) per 1 g of dry matter of defatted seeds.

Determination of Total Flavonoid (TF) Content. The total flavonoid content was determined using the aluminum chloride colorimetric assay.[17] Results were expressed in milligrams of rutin per 1 g of dry matter of defatted seeds.

Determination of Flavone and Flavonol (FF) Content. Another aluminum chloride colorimetric assay[18] was used to determine the flavone and flavonol content; it is based on the formation of a complex between the aluminum ion Al(III) and the carbonyl and hydroxyl groups of the tested compounds. Results were expressed in milligrams of quercetin per 1 g of dry matter of defatted seeds.

Determination of Flavonone and Dihydroflavonol (FD) Content. To quantify flavones and dihydroflavonols, the 2,4-dinitrophenylhydrazine (2,4-DNP) method was applied.[18] This assay is based on the interaction between flavanones/dihydroflavanols and 2,4-DNP in an acidic environment to form colored phenylhydrazone. Results were expressed in milligrams of naringenin per 1 g of drymatter of defatted seeds.

Characterization of Phenolic Compounds in Camelina Extracts Using HPLC and MS. Identification and quantification of phenolics were performed on the basis of their UV−vis spectra and MS spectra and by chromatographic comparison with standards as fully described in our previous investigation.[14] The contents of compounds identified were calculated by their regression equations from the curves of standards and expressed as micrograms of compound per 1 g of dry matter of defatted camelina seeds. The repeatability of the analysis was ±2%.

Antioxidant Activity Assays. The reducing power was evaluated (in triplicate) as the absorbance at 740 nm according to a modified[14] method of Juntachote et al.[19] and expressed as the coefficient of reducing power C_R.

The DPPH$^\bullet$ radical scavenging activity was determined (in triplicate) according to the method of Brand-Williams et al.[20] as described previously.[14] The capability of extracts to scavenge the DPPH$^\bullet$ radical was expressed as the corresponding coefficient: $C_{DPPH\bullet} = (1 - A_{s\ 517\ nm}/A_{c\ 517\ nm}) \times 100\%$, where $A_{s\ 517\ nm}$ is the absorbance of the sample solution after 30 min and $A_{c\ 517\ nm}$ is the absorbance of the control solution with no antioxidant added.

The antioxidant activity of extracts in an aqueous emulsion system of linoleic acid was determined (in triplicate) according to a slightly modified[14] β-carotene bleaching (BCB) test of Moure et al.[21] The final concentrations of the tested fractions in the emulsion were 20 ppm. The coefficient of antioxidant activity (C_{AA}) was expressed as percentage inhibition relative to the control after 60 min of incubation: $C_{AA} = [1 - (A_{s\ 470\ nm\ (t=0)} - A_{s\ 470\ nm\ (t=60)})/(A_{c\ 470\ nm\ (t=0)} - A_{c\ 470\ nm\ (t=60)})] \times 100\%$, where $A_{s\ 470\ nm\ (t=0)}$ and $A_{c\ 470\ nm\ (t=0)}$ are the absorbances measured at zero time of incubation for the test sample and control, respectively, and $A_{s\ 470\ nm\ (t=60)}$ and $A_{c\ 470\ nm\ (t=60)}$ are the absorbances measured in the test sample and control, respectively, after incubation for 60 min.

The chelating activity of extracts for the ferrous ion Fe^{2+} was measured (in triplicate) according to the method of Decker et al.,[22] with some modifications.[14] The ability of the extract to chelate ferrous ion (C_{CA}) was calculated as $C_{CA} = (1 - A_{s\ 562\ nm}/A_{c\ 562\ nm}) \times 100\%$, where $A_{c\ 562\ nm}$ is the absorbance of the control and $A_{s\ 562\ nm}$ is the absorbance in the presence of sample.

Statistical Analysis. All analyses were performed in triplicate, and results are presented as the mean ± standard deviation. Statistical assessment was carried out with the program system SPSS for Windows (version 18). The results of antioxidant activity assays were analyzed using one-way analysis of variance (ANOVA). Differences were considered to be significant at the $P < 0.05$ level. Comparison of treatment means was based on Duncan's multiple-range test. The Pearson

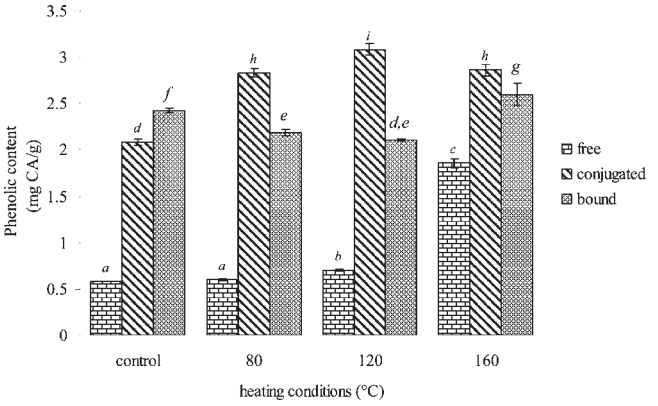

Figure 1. Total phenolic contents in free, soluble conjugate, and insoluble bound fractions of camelina seeds as affected by different heating conditions. Values are the mean ± standard deviation (n = 3). Values with different letters are significantly different (P < 0.05).

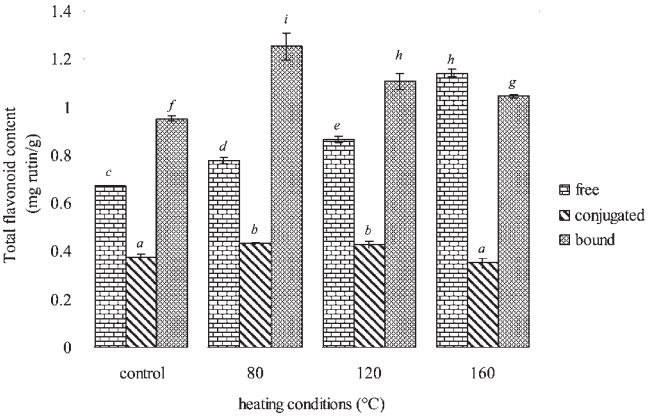

Figure 2. Total flavonoid contents in free, soluble conjugate, and insoluble bound fractions of camelina seeds as affected by different heating conditions. Values are the mean ± standard deviation (n = 3). Values with different letters are significantly different (P < 0.05).

correlation test was employed to determine the correlation coefficients among means.

RESULTS AND DISCUSSION

The contents of free, conjugated, and insoluble-bound phenolics as determined by the FC method from camelina seeds heated under different conditions are shown on Figure 1. In thermally untreated seeds the corresponding values amounted to 0.58 ± 0.01, 2.08 ± 0.03, and 2.42 ± 0.02 mg CA/g, respectively. Regardless of the heating temperature, free phenolics were the least represented, indicating that the greater part of camelina phenolics were not simply extractable by aqueous methanol, but released by the alkali treatment of the samples. It has to be stressed that the total phenolic content of camelina seeds amounting to 15.4 ± 0.9 mg CA/g, as determined in our previous investigation,[14] notably exceeded the values obtained in the present investigation. The shorter extraction time, smaller defatted seeds-to-solvent ratio, and absence of an extraction step using ultrasound lowered the yield of total phenolics in the present investigation, whereas the 4 h hydrolysis also partly destroyed the most sensitive phenolics. Furthermore, intermediate evaporation of the extracts, their reconstruction, and additional extraction with ethyl ether contributed to the lower yield.

As seen in Figure 1, lower heating temperatures did not significantly influence the content of free phenolics, whereas additional increase of temperature resulted in higher amounts of free TP. Heat treatment of the seeds also significantly affected the amount of soluble conjugate fraction, which in heated camelina seeds dominated over the phenolics bound on cell wall material. The results of the present investigation revealed that treatment at 160 °C was required for release insoluble bound phenolics, whereas lower temperatures were found to be more appropriate to liberate those present as soluble conjugates.

With regard to the total of flavonoid compounds, their contents in free, conjugated, and bound fractions of thermally untreated camelina seeds were 0.67 ± 0.01, 0.37 ± 0.01, and 0.95 ± 0.01 mg rutin/g, respectively (Figure 2). The results presented in Figure 3 show that in unheated camelina seeds the amounts of free, conjugated, and bound fractions of FF were 0.103 ± 0.001, 0.0071 ± 0.0001, and 0.040 ± 0.001 mg quercetin/g, respectively. As demonstrated in Figure 4, the content of free, soluble conjugate, and insoluble bound

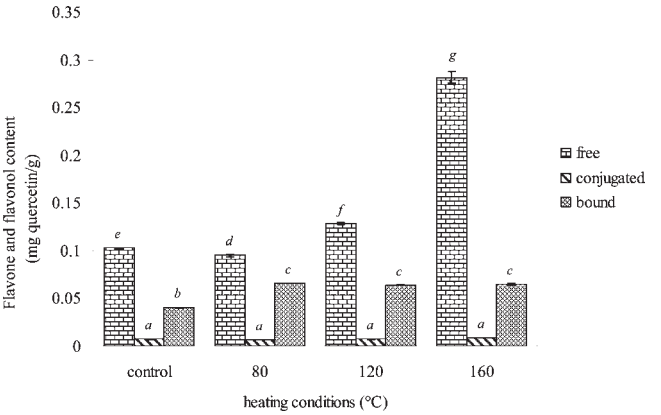

Figure 3. Flavone and flavonol contents in free, soluble conjugate, and insoluble bound fractions of camelina seeds as affected by different heating conditions. Values are the mean ± standard deviation (n = 3). Values with different letters are significantly different (P < 0.05).

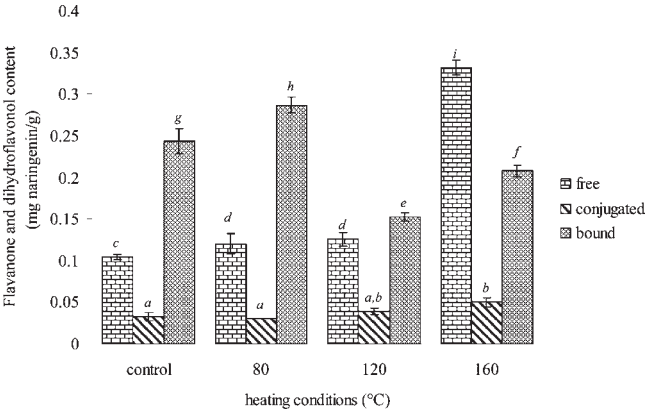

Figure 4. Flavone and dihydroflavonol contents in free, soluble conjugate, and insoluble bound fractions of camelina seeds as affected by different heating conditions. Values are the mean ± standard deviation (n = 3). Values with different letters are significantly different (P < 0.05).

flavones and dihydroflavonols in thermally untreated camelina seeds amounting to 0.104 ± 0.003, 0.033 ± 0.004, and

dx.doi.org/10.1021/jf2016072 |J. Agric. Food Chem. 2011, 59, 8639–8645

Table 1. Phenolic Compounds Identified in Different Fractions of Camelina Seed As Affected by Different Heat Treatments[a]

	peak											
	1	2	3	4	5	6	7	8	9	10	11	12
	protocatechuic acid	catechin	sinapine	ellagic acid	sinapic acid	rutin	4-VC	salicylic acid	quercetin	4-VP	4-VS	4-VG
control-F[b]	0.34	0.001	87.11	0.009	0.008	31.02	3.26	1.26	0.029	nd	2.92	0.40
control-C	nd	0.004	0.63	0.011	2.798	0.09	3.74	0.0	0.010	0.27	0.67	3.33
control-B	12.19	0.026	2.24	0.023	0.079	0.32	7.39	2.06	0.010	0.83	6.50	1.01
80 °C-F	0.65	0.002	83.26	0.003	0.009	26.57	3.20	1.75	0.029	nd	3.64	0.56
80 °C-C	nd	0.005	0.64	0.035	2.912	0.21	4.47	0.27	0.010	0.29	0.50	2.58
80 °C-B	30.43	0.025	2.53	0.018	0.930	0.23	6.97	1.96	0.010	0.93	8.28	0.88
120 °C-F	1.21	0.002	119.27	0.0016	0.006	31.61	8.56	2.29	0.054	nd	20.06	1.64
120 °C-C	nd	0.011	0.82	0.022	2.986	0.18	4.75	0.58	0.012	0.30	0.43	2.45
120 °C-B	31.71	0.033	2.42	0.003	0.966	0.32	7.18	2.61	0.016	1.05	7.66	0.42
160 °C-F	3.36	0.004	147.83	0.0017	0.003	38.19	24.43	9.24	0.118	nd	52.49	0.39
160 °C-C	nd	0.018	2.32	0.034	3.071	0.13	4.64	1.24	0.018	0.44	1.96	2.05
160 °C-B	54.23	0.019	2.64	0.026	0.814	0.31	2.73	1.34	0.042	0.76	3.10	0.50

[a] The values presented are expressed as μg/g dry weight of defatted camelina seeds; nd, not determined. [b] F, free; C, soluble conjugates; B, insoluble bound phenolics.

0.244 ± 0.015 mg naringenin/g, respectively, was strongly affected by different heating conditions.

Because TF were expressed as rutin equivalent, whereas their subgroups were given as quercetin for FF and naringenin equivalent for FD content, we cannot expect that the sum of their amounts will completely coincide with the values of TF. Moreover, sometimes hydrolysis conditions that are optimal for the release of some bound phenolics are inappropriate for others present in the same plant, resulting in degradation and, consequently, in the loss of these compounds.[13,23] In addition, different forms of phenolic compounds (free, esterified, and insoluble bound) are not equally sensitive to heat treatment. Despite that, we can observe similar trends. In the case of TF and FD, the content of the insoluble bound fraction predominated over that of the free one. The latter finding is in accordance with Huang et al.,[12] who reported that flavonoids, like other phenolic compounds, existed in bound rather than in free form. However, in the case of FF the opposite was found. Soluble conjugates were the least representative and the most constant regardless of the heating condition. Heating positively affected only the amount of free flavonoids, indicating that the flavonoids found in camelina extracts were liberated by heat treatment. The highest amounts of free TF, FF, and FD contents were found in seeds heated at 160 °C, whereas lower temperatures were optimal for insoluble bound FF and FD, just like for TF, giving the highest yield at 80 °C. In accordance with the lower optimal temperature found for conjugate and bound fractions of TF, FF, and FD in comparison to total phenolics, we could suggest that these flavonoid fractions are more thermally labile than the conjugate and bound fractions of other phenolics present in camelina seeds.

Qualitative and quantitative analyses of phenolic compounds were performed using HPLC-UV-MS and the results are summarized in Table 1. Peak 1 (t_R = 2.12 min, λ_{max} = 260 nm, and $[M - H]^-$ = 153 m/z) was identified as protocatechuic acid, which existed mostly in insoluble bound form and did not form any soluble esters. Its content increased with increasing temperature. Peak 2 (t_R = 3.07 min, λ_{max} = 280 nm, and $[M - H]^-$ = 289 m/z) was identified as catechin, which existed mostly in insoluble bound form. Its content in all forms increased up to 120 °C, followed at 160 °C by a noticeable decrease of the insoluble bound fraction, indicating that the latter is partly transformed into the two other forms. Peak 3 (t_R = 4.64 min, λ_{max} = 328 nm, and $[M + H]^+$ = 311 m/z) was characterized as sinapine. It was almost completely present in free form. For all three fractions it was found that the highest heating temperature yielded the highest content. Peak 4 (t_R = 7.92 min, λ_{max} = 356 nm, and $[M - H]^-$ = 301 m/z) was identified as ellagic acid. In untreated seeds the largest fraction of ellagic acid occurred in insoluble bound form. In heated seeds the soluble conjugate fraction predominated. Peak 5 (t_R = 8.85 min, λ_{max} = 322 nm, and $[M - H]^-$ = 223 m/z) was identified as sinapic acid, which in camelina seed mainly formed soluble conjugates. Its content increased with increasing temperature. Peak 6 (t_R = 9.10 min, λ_{max} = 354 nm, and $[M - H]^-$ = 609 m/z) was characterized as rutin. Almost 99% of all rutin existed in free form, regardless of heat treatment. The highest content was achieved at 160 °C. Peak 7 (t_R = 10.35 min, λ_{max} = 260 nm, and $[M - H]^-$ = 135 m/z) was identified as a decarboxylation product of caffeic acid, 4-VC. In untreated seeds the amount of the bound form prevailed over that of free and soluble conjugate fractions. In heated seeds the amount of bound form decreased, whereas the free 4-VC increased, and in samples treated at 160 °C free 4-VC was dominant. Peak 8 (t_R = 11.06 min, λ_{max} = 304 nm, and $[M - H]^-$ = 137 m/z) was identified as salicylic acid. Salicylic acid existed mostly in insoluble bound form, whereas in seeds heated at 160 °C this fraction decreased and the free fraction predominated. Peak 9 (t_R = 12.59 min, λ_{max} = 358 nm, and $[M - H]^-$ = 301 m/z) was identified as quercetin, which mostly occurred in free form. With increased heating temperature the amounts of all forms of quercetin increased. Peak 10 (t_R = 13.38 min, λ_{max} = 260 nm, and $[M - H]^-$ = 119 m/z) was identified as the decarboxylation product of *p*-coumaric acid, 4-VP. Interestingly, there was no free 4-VP found in any of the tested samples. In our previous investigation[14] we succeeded in identifying this compound. As already mentioned, the total phenolic content determined in our recent publication[14] was much higher than in the present study. A possible explanation might be that the amount of 4-VP was under the detection limit. The insoluble bound

fraction of 4-VP was more abundant than the corresponding soluble esters. Peak 11 (t_R = 13.53 min, λ_{max} = 260 nm, and $[M-H]^-$ = 179 m/z) was characterized as the decarboxylation product of sinapic acid, 4-VS. Among the 4-vinyl derivatives of the hydroxycinnamic acids, 4-VS was by far the most abundant in camelina seeds. Similarly as for 4-VC and 4-VP, the insoluble bound fraction predominated in nonheated seeds and decreased in seeds heated at 160 °C. In the same seeds free 4-VS was far more abundant, indicating that it was liberated by heat. Peak 12 (t_R = 13.84 min, λ_{max} = 260 nm, and $[M-H]^-$ = 149 m/z) was the least polar phenolic compound identified in the tested extracts. 4-VG is the decarboxylation product of ferulic acid, which in camelina seeds, regardless of the heating temperature, mainly existed in esterified form. Besides soluble esters, heating also negatively affects the insoluble bound fraction. On the other hand, free 4-VG increased with increasing temperature up to 120 °C, followed by a noticeable decrease at 160 °C. This suggests that 4-VG is more heat sensitive than other 4-vinyl derivatives.

The increased amount of 4-vinyl derivatives in heated seeds was expected because these compounds are formed by thermal decarboxylation of hydroxycinnamic acids, but this is in contrast to results obtained in a previous investigation,[14] in which a decrease of these compounds in cake (heated seeds) was observed. It has to be stressed that in the present investigation thermal treatment was performed on the whole seeds, but in the previous one[14] the seeds were crushed before heating. The loss of these highly volatile compounds during heating might be prevented to a greater extent when the seed is in its whole form. Thus, the physical form of the seed might be of great influence.

Literature data concerning the effect of different heating conditions on the content of individual phenolic compounds (those found in camelina seeds) content are scarce. However, as can be seen in Table 1, sinapic acid occurred in camelina seeds mostly in esterified form, probably with choline, forming sinapine, the major phenolic ester in crucifer seeds,[24] whereas sinapine and 4-VS, another sinapic acid derivative, occurred mainly in free form. As previously reported,[25] heating of the seeds led to an increase of the 4-VS content; the optimum temperature for its formation in rapeseed was determined to be 160 °C. The latter is in agreement with our results, where this temperature highly increased the amount of free 4-VS.

The effect of heating on the antioxidant capacity of the free, esterified, and insoluble bound phenolic fractions from camelina was evaluated by four chemical assays: the reducing power assay, the DPPH$^\bullet$ assay, chelating ability, and the β-carotene bleaching method.

Overall, from Table 2 it can be concluded that regardless of whether the seeds were thermally treated or not, at a concentration of 0.004 mg/mL the most effective reducing power was found for phenolics which were bound to the cell wall, whereas the weakest reductants were found to be those present as soluble conjugates. Heating the seeds at 120 °C coincided with increased reducing power of free camelina phenolics, whereas stronger heating conditions remarkably negatively affected the reducing capacity of this fraction. A similar trend was observed for the insoluble bound fraction. Heat treatment of any kind played a negative role in the reducing power of soluble conjugates.

As already mentioned, 120 °C seems to be the optimal temperature to obtain compounds having representative reducing activity, on the other hand, simultaneously for the least marked heat degradation of the naturally occurring reductants.

Table 2. Coefficients of Reducing Power (C_R), DPPH$^\bullet$ Radical Scavenging Activity ($C_{DPPH\cdot}$), Antioxidant Activity in Emulsion (C_{AA}), and Chelating Ability (C_{CA}) of Different Phenolic Fractions of Camelina Seed As Affected by Different Heat Treatments[a]

sample	C_R	$C_{DPPH\cdot}$ (%)	C_{AA} (%)	C_{CA} (%)
control-F[b]	0.578 ± 0.010 e	86.9 ± 0.1 g	95 ± 3 ef	49 ± 3 f
control-C	0.535 ± 0.008 d	86.3 ± 0.8 g	78 ± 4 c	na
control-B	0.628 ± 0.002 g	60.1 ± 0.4 c	98 ± 1 f	7.9 ± 0.7 b
80 °C-F	0.584 ± 0.008 ef	89.1 ± 0.1 h	89 ± 2 de	44 ± 1 e
80 °C-C	0.413 ± 0.004 ab	73.6 ± 1.0 d	72 ± 5 bc	na
80 °C-B	0.680 ± 0.010 h	51.8 ± 0.8 a	96 ± 2 f	8.0 ± 0.6 b
120 °C-F	0.647 ± 0.012 g	90.6 ± 0.4 i	85 ± 6 d	37 ± 3 d
120 °C-C	0.426 ± 0.003 b	77.7 ± 1.3 f	64 ± 6 a	na
120 °C-B	0.708 ± 0.017 i	55.5 ± 0.6 b	99 ± 1 f	2.2 ± 0.3 a
160 °C-F	0.454 ± 0.009 c	75.3 ± 0.8 e	75 ± 1 bc	15.2 ± 0.9 c
160 °C-C	0.400 ± 0.017 a	73.3 ± 1.6 d	70 ± 4 b	na
160 °C-B	0.602 ± 0.023 f	51.7 ± 0.2 a	97 ± 2 f	2.7 ± 0.1 a

[a] Values are the mean ± standard deviation (n = 3). Values with different letters in the same column are significantly different ($P < 0.05$); na, no activity. [b] F, free; C, soluble conjugates; B, insoluble bound.

Table 3. Correlations among the Coefficient of Reducing Power (C_R), DPPH$^\bullet$ Radical Scavenging Activity ($C_{DPPH\cdot}$), Antioxidant Activity in Emulsion (C_{AA}), Chelating Ability (C_{CA}), and Individual Phenolic Compounds

	C_R	$C_{DPPH\cdot}$	C_{AA}	C_{CA}
protocatechuic acid	0.56	−0.84	0.64	−0.32
catechin	0.44	−0.88	0.46	−0.56
sinapine	0.02	0.56	−0.02	0.75
ellagic acid	−0.60	−0.26	−0.47	−0.54
sinapic acid	−0.64	0.02	−0.70	−0.70
rutin	0.03	0.60	0.03	0.83
4-VC	−0.13	−0.02	−0.16	0.00
salicylic acid	−0.05	0.02	−0.06	0.07
quercetin	−0.13	0.18	−0.11	0.29
4-VP	0.48	−0.92	0.50	−0.61
4-VS	−0.05	0.07	−0.09	0.16
4-VG	−0.54	0.35	−0.70	−0.46

That is, as observed by Duh et al.,[26] the reducing power of barley decreased at higher heating temperatures. Moreover, our results are in accordance with those of Kim et al.,[2] who found that increased heat treatment of grape seeds increased their reducing power, but only to a certain level, as too elevated temperatures or prolonged heating times considerably lowered their reducing effectiveness. With regard to Table 3, where correlation studies between the phenolic profile and antioxidant activity of the tested extracts are presented, we suggest that protocatechuic acid, catechin, and 4-VP are the main contributors to the reducing power of camelina seeds.

The results summarized in Table 2 show that the best DPPH$^\bullet$ scavengers at a concentration of 0.02 mg/mL were free phenolics. $C_{DPPH\cdot}$ of the free fraction increased from 86.9% for the control to 90.6% for seeds treated at 120 °C, whereas higher temperatures significantly decreased this activity. Heating of any kind negatively affected the $C_{DPPH\cdot}$ values for soluble conjugate

and insoluble bound fractions. The observation that heating significantly affected not only reductants but also the reactivity of radical scavengers toward the stable DPPH$^{\bullet}$ radical is partly in accordance with recent studies,[26,27] in which a strong decrease of antioxidant activity was recorded during heat treatment. On the other hand, Jeon et al.[3] reported that heat treatment significantly increased the radical scavenging activity, whereas other investigators[2] found that their effectiveness increased with temperature but decreased for samples heated above 200 °C. Therefore, it can be concluded that radical scavengers from different plant materials possess varying heat stability. Regression analysis (Table 3) showed that rutin and sinapine contribute most to the free radical scavenging activity of camelina seeds.

Data on the antioxidant activity of camelina fractions in an emulsion are summarized in Table 2. Insoluble bound phenolics were the most effective as scavengers of the alkyl peroxyl radical at a concentration of 20 ppm. Heating had no significant influence on their C_{AA}. The antioxidant activity of free and soluble conjugate fractions was reduced when compared to the unheated seeds. Depending on the degree of heating the behavior of C_{AA} can probably be attributed to the loss of polyphenolic compounds and to successive formation of other antioxidant compounds such as Maillard reaction products or pyrolysis products when more severe heating conditions are applied.[28] Durmaz et al.[29] reported a loss of antioxidant activity during the advanced phases of apricot kernel heating, partly as a result of the formation of intermediates, which might act as lipid oxidation stimulator agents.

Similarly as observed for the reducing power assay, the insoluble bound phenolics were the most effective as scavengers of the alkyl peroxyl radical, whereas soluble esters displayed the weakest action. This is interesting, because these two methods differ not only in their mechanism but also in the homogeneity of the reaction medium. Moreover, according to the Table 3, the phenolic compounds that correlate well with the reducing power also demonstrate the best activity against alkyl peroxyl radicals in the emulsion, namely, protocatechuic acid, catechin, and 4-VP.

Completely different results were obtained for the chelating ability of camelina extracts, for which, interestingly, the best iron chelators were the free phenolics. The chelating ability of this fraction was statistically significantly negatively affected by temperature. The effectiveness of the insoluble bound fraction under the same tested concentration of 0.12 mg/mL was considerably lower and notably affected by heat treatment. The soluble conjugate of unheated and heated seeds expressed no chelating ability at all.

It seems that chelators are extremely sensitive to heat treatment, especially in the free fraction. Higher temperatures also resulted in a lower chelating effect for ferrous ions in the study performed by Duh et al.[26] Regression analysis (Table 3) showed that rutin and sinapine contribute most to the chelating ability of camelina seeds.

In conclusion, a number of phenolic compounds were identified in all thermally treated and nonheated camelina extracts. Both the phenolic profile and overall antioxidant properties of camelina seeds were significantly affected by the heating conditions. We observed that heat treatment caused partial degradation of some phenolic compounds and increased the concentration of others. The increase in individual phenolic content was primarily attributed to the increased release of phenolic acids and flavonoids from the cell structural materials. The different trend for free, soluble esters, and insoluble bound phenolics can be explained by their different heat stabilities, and also by transformation of one form to another. The appropriate heat treatment for camelina seeds is determined by the desired antioxidant properties and provides some important information about further exploitation of this oilseed crop. Because heating of the seeds is unavoidable in terms of the formation of the desired flavor, texture, color, and nutritional value, treatment at 80 °C would be recommended as the least harmful with regard to their chelating ability and antiradical action in emulsified systems and at 120 °C for optimal reducing power and free radical scavenging activity.

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Funding Sources

We thank the Slovenian Research Agency for financial support through Research Program P4-0121.

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