JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds

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Residues of the oil-extracting process of oilseeds contain bioactive substances such as phenolic compounds, which could be used as natural antioxidants for the protection of fats and oils against oxidative deterioration. Thus, the extraction of such constituents from residual material can be considered to contribute to the added value of these residues, which could justify their isolation. In the present work the fat-free residues of eight different oilseeds whose oils are usable for nutritional applications, and also as renewable resources, were extracted with 70% methanol, 70% acetone, water, and ethyl acetate/water, respectively. The resulting extracts were investigated regarding their content of total phenolic compounds by the Folin−Ciocalteau assay, sinapine, flavanoids, and the UV-absorption spectra. Further, the antioxidant activity of the extracts was characterized by the DPPH method, the β-carotene−linoleic acid assay, and ESR investigations. The fat-free residues of the different oilseeds contained considerable amounts of extractable substances. The yields decreased with decreasing polarity of the solvent in the order water, 70% methanol, 70% acetone, and ethyl acetate. The ratio of total phenolic compounds to the extractable compounds ranged from 3 to 19%. There was no significant correlation between the amount of total extractable compounds and the total phenolic compounds ($p < 0.001$). All extracts showed remarkable antioxidant activities determined with the different methods. The effects depended strongly on the solvent used for the extraction as well as on the extracted residue. A correlation between the methods used for the characterization of the antioxidant activity and the composition of the residues could not be shown.

KEYWORDS: Antioxidant activity; β-carotene bleaching; DPPH; electron spin resonance; extraction; oilseeds; phenolic compounds

INTRODUCTION

The autoxidation of lipids, as well as the enzymatic oxidation, during storage and processing is the major reaction in fats, oils, and fat-containing foods responsible for the deterioration in food quality. It affects the color, flavor, texture, and especially the nutritive value of the foods (*1*).

Antioxidants are able to prevent the radical chain reactions of oxidation by cutting in the initiation and propagation step, which leads to the termination of the reaction and a delay in degradation reactions. During the late 1940s the ability of phenolic compounds to inhibit lipid oxidation was discovered. This led to the application of synthetic antioxidants in food industry. However, the synthetic antioxidants such as *tert*-butyl hydroxyanisole (BHA) or *tert*-butyl methylphenol (BHT) have some critical disadvantages, because they are highly volatile, unstable at elevated temperatures, restricted by legislative rules (*2*−*4*), and, above all, they are suspected to have some toxic properties. Therefore, consumers generally prefer natural antioxidants for the stabilization of edible fats and oils against oxidative rancidity. Tocopherols are the most widely applied antioxidants of this group (*5*). However, since several years ago, flavonoids have also come under investigation for use as natural antioxidants in food preservation (*6*−*8*).

The flavonoids occur ubiquitously in food plants, because they are important in plants for normal growth development and defense against infection and injury. The ability of such compounds to act as antioxidants is based on the fact that they are able to form delocalized unpaired electrons, stabilizing the formed phenoxyl radical after reaction with lipid radicals (*9*). These properties allow the molecule to act as reducing agents, hydrogen donator, and singlet oxygen quencher. The molecules have also a metal celation potential (*10*).

Many different plant materials have recently become a major interest of scientific research as a result of naturally occurring antioxidants. Extracts from rosemary or sage, other herbs, spices, cereal crops, barks, and roots belong to the well-investigated sources for natural compounds with antioxidant activity (*7*, *11*−*14*). Such compounds are tocopherols, phospholipids, amino acids, peptides, and phenolic compounds (*5*, *15*).

Oilseeds also contain different substances with antioxidant activity (*14*, *16*, *17*). These are phenolic compounds such as

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10.1021/jf011440s CCC: $22.00
Published on Web 05/03/2002

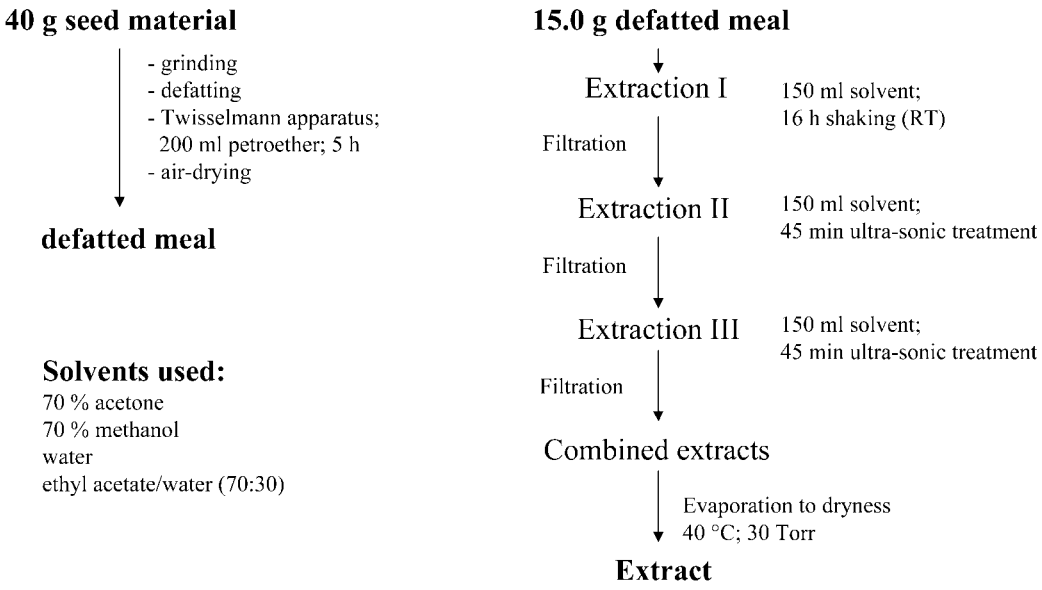

Figure 1. Extraction method.

hydroxylated derivatives of benzoic and cinnamic acid, coumarins, flavonoid compounds, or lignins (*18*). Although most of the tocopherols and parts of the phospholipids are removed during the oil-extracting process, parts of the phenolic compounds remain in the fat-free residue. These byproducts of the oil-extracting process are normally used as fodder in animal nutrition or as fuel in thermal power stations. From an economical point of view, the residues could be attractive sources for different bioactive substances such as glucosinolates or phenolic compounds, which are retained in the residues in significant amounts. The extraction of these constituents from residual material can be considered to contribute to the added value of these residues, which could justify their isolation.

Very few data on the antioxidant activity of extracts obtained from the residues of the oil-extracting process are available. Thus, the aim of this work was to evaluate the antioxidant activity of such extracts. For the extraction four different solvents were used: 70% acetone, 70% methanol, water, and ethyl acetate/water (70:30). Acetone and methanol and mixtures of these solvents with different proportions of water were described in the literature frequently (*19*−*22*) for the extraction of phenolic compounds. Further on, ethyl acetate is often used as extraction solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols (*23*, *24*). By addition of water the amount of phenolic compounds as well as the selectivity of this solvent can be increased (*25*, *26*). Especially di- and oligomer proanthocyanidins with additional hydroxyl groups are not soluble in the ethyl acetate phase and remain in the water phase.

MATERIALS AND METHODS

Materials. For the investigation seeds of rapeseed (*Brassica napus*), *Brassica verna*, *Brassica carinata*, *Lepidium campestre*, *Camelina sativa*, mustard (*Sinapis alba*), crambe (*Crambe abyssinica*), and sunflower (*Helianthus annuus*) were used. Seeds of sunflower and rapeseed are important sources for the production of edible oils, but the oil of these seeds is also used for industrial applications. The residues find use in the nutrition of animals. However, the other seeds used in this investigation are known as renewable resources for the production of oil for industrial applications in the oleochemical industry. The residues of these oilseeds are not suitable for the application in animal nutrition, because of the amount and the composition of bioactive compounds. So it is useful to think about the utilization of these residues of the oil-extracting process.

Preparation of the Extracts. The extracts were obtained according to the diagram in **Figure 1**. Approximately 40 g of seed material was crushed and defatted by a Twisselman apparatus with 200 mL of petroleum benzene. The fat-free residue was air-dried, and from 15.0 g of this material the antioxidant components were isolated by the use of four different solvents. The solvents used were water, 70% acetone, 70% methanol, and ethyl acetate/water (70:30), and each was used separately. From the immiscible mixture of ethyl acetate/water, only the extract obtained from the ethyl acetate phase was used for further investigations. Each residue was extracted three times with 200 mL of the appropriate solvent. First, the extraction was carried out overnight by shaking, and then it was repeated two times with 200 mL of solvent by ultrasonic treatment for 45 min. The combined extracts were vacuum evaporated to remove the solvent at 40 °C and 4×10^3 Pa, then weighed to determine the extraction yield, and stored at −20 °C until use.

Determination of the Total Polyphenolic Compounds. The amount of total polyphenolic compounds was measured by a method described by Taga et al. (*27*). In brief, about 150 mg of the extract was dissolved in 10 mL of methanol, and 2 mL of this solution was filled up with 0.3% HCl to 5 mL. A 100-μL aliquot of the resulting solution was added to 2 mL of 2% Na_2CO_3 and after 2 min 100 μL of Folin−Ciocalteau reagent (Merck, Darmstadt, Germany) (diluted with methanol 1:1) was added. After a further 30 min the absorbance was measured at 750 nm using a spectrophotometer. The concentration was calculated using gallic acid as standard, and the results were expressed as milligrams gallic acid equivalents (GAE) per gram extract.

Determination of Flavanoids. The determination of flavanoids was performed according to a colorimetric assay of Delcour and de Varebeke (*28*). About 150 mg of the extract was diluted in 10 mL of methanol, and 1 mL of this solution was pipetted into a test tube. A total of 5 mL of the chromogen reagent (1.000 g 4-dimethylaminocinnamaldehyde (Fluka, Buchs, Germany) dissolved in a cooled mixture of 250 mL of concentrated HCl and 750 mL of methanol, completed to 1 L with methanol) was added to the extract solution, and after 10 min the absorbance was read at 640 nm against a blank with water instead of extract solution. The chromogen reagent was stable for one week in the dark. A calibration curve was prepared with (+)-catechin, and the results were expressed as (+)-catechin equivalents.

Determination of Sinapine. About 80 mg of each extract was dissolved in 25 mL of methanol/water (1:1). The HPLC method for sinapine was performed according to a modified method of Clausen et al. (*29*) under isocratic conditions as described by Clausen et al. (*30*). A 1-mL aliquot of the solution was further diluted 1:10 and then injected onto a LiChrospher 60 RP-select B column (5 μm) 125 × 4 mm (Merck,

Darmstadt, Germany) used with a flow rate of 1.0 mL/min without further purification (*31*). The mobile phase used consisted of 0.01 M sodium heptanesulfonic acid, 0.01 M sodium dihydrogenphosphate, and 0.01 M dibutylamine in acetonitrile/water (2:8), adjusted to pH 2.0. The UV-detector was set at 325 nm. Calibration and evaluation of the method were done with sinapine thiocyanate isolated from a rapeseed sample (*32*).

Determination of Antioxidant Activity with the DPPH Radical Scavenging Method. For determination of the antioxidant activity of different extracts, the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma, Steinheim, Germany) radical was used. Determination of scavenging stable DPPH free radicals was a very fast method to evaluate the antioxidant activity of the extracts in a very short time. With this method it was possible to determine the antiradical power of an antioxidant by measurement of the decrease in the absorbance of DPPH at 515 nm. As a result of a color change from purple to yellow the absorbance decreased when the DPPH radical was scavenged by an antioxidant through donation of hydrogen to form a stable DPPH-H molecule. In the radical form this molecule had an absorbance at 515 nm which disappeared by acceptance of an electron or hydrogen radical from an antioxidant compound to became a stable diamagnetic molecule. The method described by Hatano et al. (*33*) was used with some modifications. For each extract different concentrations were tested. An aliquot (0.5 mL) of the DPPH solution (about 50 mg/100 mL) was diluted in 4.5 mL of methanol, and 0.1 mL of a methanolic solution of the extract was added. The mixture was shaken vigorously and allowed to stand for 45 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (without extract) with a spectrophotometer. From a calibration curve obtained with different amounts of extract, the ED_{50} was calculated. The ED_{50} was that concentration of an antioxidant which was required to quench 50% of the initial DPPH radicals under the experimental conditions given.

Determination of Antioxidant Activity with the β-Carotene Bleaching Method. In this method a model system made of β-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. The free linoleic acid radical formed upon the abstraction of a hydrogen atom from one of its methylene groups attacked the β-carotene molecules, which lost the double bonds and, therefore, its characteristic orange color. The rate of bleaching of the β-carotene solution was determined by measurement of the difference between the initial reading in spectral absorbance at 470 nm at time 0 min and after 60 min.

Determination of the antioxidant activity using a β-carotene/linoleic acid system was carried out according to the method described by Taga et al. (*34*). 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 β-carotene (3.34 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. To an aliquot of 5 mL of this emulsion, 0.2 mL of an antioxidant solution was added, and the absorbance was measured at 470 nm, immediately, against a blank consisting of the emulsion without β-carotene. The tubes were placed in a water bath at 40 °C and the absorbance was measured every 15 min up to 60 min.

Evaluation of Free Radical Scavenging Activities by Electron Spin Resonance (ESR). Direct detection of free radicals is not possible because of their short life and high reactivity. The use of a spin-trapping technique combined with electron spin resonance spectrometry still allowed the determination of the identity of free radicals. The principle of this method was that a compound was added to a sample which reacted with the free radicals to form radical-adducts that were very much more stable and longer-lived than the original radical species. This technique is the only analytical method which is able to determine free radicals directly (*35*). Such unpaired electrons may have a spin of either $+^1/_2$ or $-^1/_2$. They act as small magnets in an electromagnetic field, so that they can be detected in the ESR.

In a system consisting of hydrogen peroxide, sodium hydroxide, and dimethyl sulfoxide, superoxide anions, hydroxyl radicals, and methyl radicals were formed. Thus, this system can be used for the characterization of the radical scavenging properties of phenolic compounds. The determination was carried out according to the method described by Yamaguchi et al. (*36*). Aliquots of 50 μL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Deisenhofen, Germany), 50 μL of 25 mM NaOH, and 50 μL of the sample solution (aqueous) were mixed in a little tube. Then 5 μL of 5,5-dimethyl-1-pyrrolin-*N*-oxide (DMPO) (Sigma-Aldrich, Deisenhofen, Germany) and 50 μL of 30% hydrogen peroxide were added. The mixture was put into the flat quartz cell, and after 10 min was set into the ESR apparatus and the scanning was begun.

The conditions of the ESR apparatus (EMS 104 EPR analyzer (Bruker Analytik GmbH, Rheinstetten, Germany)) were as follows: power, 9.95; sweep width, 100; modulation [G], 2.54; sweep time [s], 21.0; filter time constant [ms], 20.5; amplification, 20; number of sweeps, 30; receiver offset, 0; receiver phase, 0; and magnetic field offset, 0.

RESULTS AND DISCUSSION

Amount of Extractable Compounds vs Extractable Phenolic Compounds. In general the amount of extractable substances decreased with decreasing polarity of the solvent in the order water, 70% methanol, 70% acetone, and ethyl acetate. The results of testing different solvents for the extraction of phenolic compounds are given in **Table 1**. It was evident that the different oilseeds contained noticeable amounts of extractable compounds. Additionally, it was obvious that the different solvents used for the extraction of the fat-free residues had different capabilities to extract substances from these residues. There was a dependence on the sort of oilseed extracted, as well as the solvent used for the extraction.

As the results presented in **Table 1** show, the extraction with water was most effective. With this solvent, from most of the fat-free residues of the oilseeds, the highest amounts of compounds were extracted. So the extraction of the fat-free residue of *Brassica carinata* with water resulted in the highest amount of total extractable compounds. In that case nearly half of the content of the residue could be found in the water extract (466.7 mg/g residue). The extraction abilities of acetone and methanol were very similar to one another, whereas the extraction yield with ethyl acetate was only small in comparison with that of the other solvents. The lowest level was obtained by extraction of *Brassica carinata* with ethyl acetate. With this solvent only 47.3 mg/g extract could be removed from the fat-free residue of the seeds. The reason was that the use of ethyl acetate as extraction solvent improved the selectivity of the extraction process. Small proanthocyanidins are well soluble in ethyl acetate, whereas especially di- and oligomer proanthocyanidins remain in the water phase. Because of this selectivity of the extraction solvent, the extraction yield of ethyl acetate was much smaller than those for the other solvents.

The amount of total phenolic compounds in the extracts obtained with the different solvents was determined by the Folin−Ciocalteau assay. The results of this colorimetric method, expressed as gallic acid equivalents are shown in **Table 1**. Expressed as absolute values, the extracts contained between 2.6 mg and 44.7 mg of phenolic compounds per gram of extractable compounds. These amounts were comparable with results described in the literature for other extracts of plant products (*14*, *15*, *37*−*39*). Both the plant material and the extraction solvents influenced the amount of total phenolic compounds.

The results of the total phenolic compounds given in **Table 1** showed that crambe, extracted with different solvents, contained the lowest amounts (3.6−16.6 mg/g) of total phenolic compounds in the extractable compounds of the fat-free residues. For rapeseed (4.0−21.2 mg/g) and *C. sativa* (3.2−21.8 mg/g) the extraction yields were also very small. The highest contents

Table 1. Total Extractable Compounds (EC), Total Phenolic Compounds (PC) (measured by Folin–Ciocalteau assay), Flavanoids (measured by 4-dimethylaminocinnamaldehyde reagent), and Sinapine of Different Extracts Obtained from Fat-Free Residues of Oilseeds (results are given in mg/g extract)

	EC (mg/g)	PC (mg/g)	PC/EC (%)	flavanoids (mg/g)	sinapine (mg/g)
			70% methanol extract		
B. carinata	223.0	13.6	6.1	7.49	99.9
rapeseed	187.3	11.8	6.3	7.96	94.6
L. campestre	311.0	35.6	11.5	128.80	0.5
B. verna	159.0	13.1	8.2	11.47	100
crambe	214.0	8.1	3.8	9.36	9.6
sunflower	102.0	16.1	15.8	12.03	0.0
C. sativa	166.7	11.1	6.6	142.79	56.5
mustard	245.0	17.6	7.2	0.97	122.1
			70% acetone extract		
B. carinata	192.7	9.4	4.9	9.32	129.7
rapeseed	143.3	6.6	4.6	5.20	85.6
L. campestre	150.7	7.4	4.9	72.49	1.8
B. verna	156.7	8.5	5.4	18.61	100
crambe	224.0	9.6	4.3	5.69	11.5
sunflower	110.0	4.7	4.3	11.23	0.0
C. sativa	309.3	11.1	3.6	36.15	47.5
mustard	228.0	17.6	7.7	1.21	85.5
			water extract		
B. carinata	466.7	36.0	7.7	2.04	17.5
rapeseed	326.0	21.3	6.5	0.74	11.7
L. campestre	229.3	44.7	19.5	3.54	0.7
B. verna	188.7	25.2	13.4	5.26	57.0
crambe	332.7	16.6	5.0	0.64	2.6
sunflower	267.3	38.8	14.5	1.45	0.0
C. sativa	275.3	21.8	7.9	11.77	9.6
mustard	247.3	36.5	14.8	0.07	32.5
			ethyl acetate extract		
B. carinata	47.3	3.6	7.7	8.25	37.9
rapeseed	72.0	4.0	5.5	4.19	39.4
L. campestre	59.3	8.3	13.9	47.92	0.0
B. verna	60.0	5.4	9.0	19.83	0.0
crambe	74.0	2.6	3.6	9.30	13.7
sunflower	80.7	2.7	3.3	4.49	0.0
C. sativa	58.0	3.2	5.5	59.75	0.0
mustard	60.7	9.2	15.2	2.18	60.9

of total phenolic compounds were found for *L. campestre* (7.4−44.7 mg/g) and mustard (9.2−36.5 mg/g).

The dependence of the total phenolic compounds on the extraction solvent depicts that the smallest absolute levels were found for extracts obtained with ethyl acetate (2.6−9.2 mg/g). The highest content of total phenolic compounds was found in extracts obtained with water (21.3−44.7 mg/g), and here, *L. campestre* contains nearly 45 mg/g. Methanol also showed high extraction yields (8.1−35.6 mg/g), whereas the contents obtained with acetone were much smaller (4.7−17.5 mg/g). Again, it was obvious that the selectivity of the extraction with ethyl acetate resulted in lower yields of the interesting compounds.

The ratio of total phenolic compounds to the total extractable compounds ranged from 3 to 19% (**Table 1**). This meant that in all cases between 81 and 97% of the extractable compounds were other than phenolic compounds. The highest part of total phenolic compounds in the total extractable compounds was found for extracts obtained with water (5.0−19.5%), whereas 70% acetone showed the lowest share of phenolic compounds in the extracts measured with the Folin−Ciocalteau method. For this solvent the part of phenolic compounds in the extractable compounds ranged from 3.6 to 7.7%. Although the extraction yield of total extractable compounds with ethyl acetate was very small, the part of phenolic compounds ranged from 3.3 to 15.2%.

A comparison of the results obtained from the investigation of the total extractable compounds and the total phenolic compounds expressed as GAE showed that there was no significant correlation between these two characteristic features ($p < 0.001$). The method for the determination of the total phenolic compounds was based on the reaction with the Folin−Ciocalteau reagent; this reaction was not specific for phenols only, because other reducing compounds such as sugars or ascorbic acid (*40*) can interfere. The increasing polarity of the solvent seemed to extract increasing amounts of soluble solids, but not inescapable phenolic compounds or flavanoids from the residues. This meant that a high value of extractable compounds did not necessarily correspond to a high value of phenolic compounds.

Further Characterization of the Phenolic Compounds. For a further specification of the phenolic compounds, the content of flavanoids as well as sinapine was determined. Additionally the ultraviolet−visible absorption spectrum of the extracts was picked up.

The content of flavanoids was determined by a colorimetric assay based upon the reaction of the A rings with the chromogen *p*-dimethylaminocinnamaldehyde. Similar to the determination of the total phenolic compounds with the Folin−Ciocalteau assay, the reaction was specific for components with a single bond at the 2,3 position and meta-oriented di- or tri-hydroxy substituted benzene rings. Thus, the results of both methods should be comparable, but as presented in **Table 1**, there were great differences in the results obtained with the two methods. Especially, the extracts from *L. campestre* and *C. sativa*,

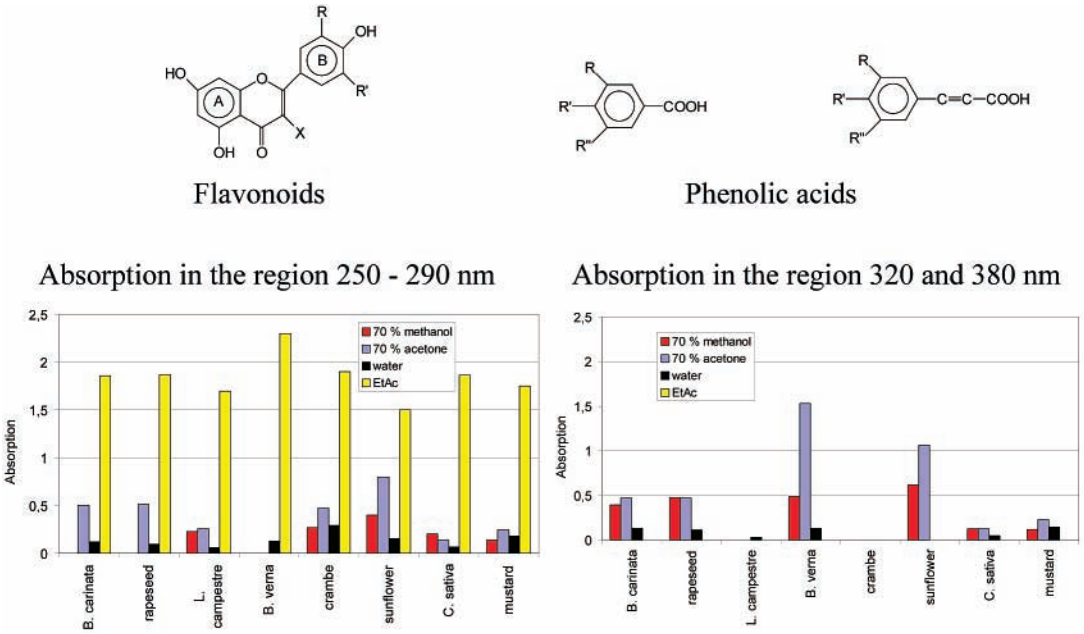

Figure 2. Absorption of extracts obtained from different oilseeds.

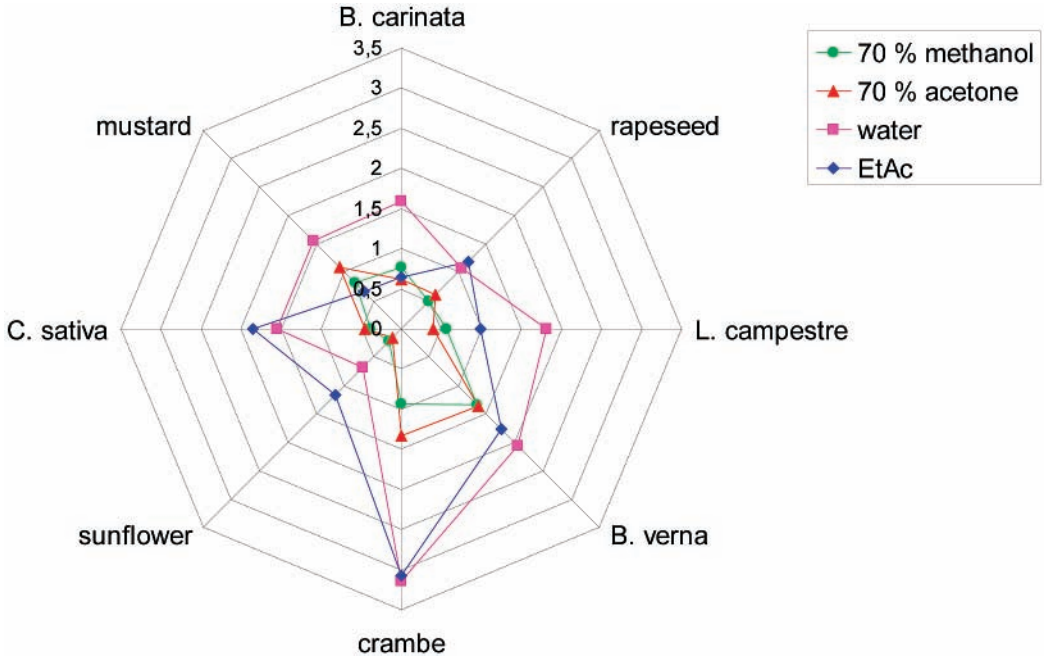

Figure 3. Effect of extracts on DPPH free radicals [expressed as mg extract allowing reduction of 50% DPPH] (The antiradical power of the extracts is expressed as concentration of extract which allows the reduction of 50% DPPH. The data are presented in the form of a net and each category in the graph has its own axis which starts in the center. All values of the same series are connected by lines.).

obtained with different solvents, showed considerable amounts of flavanoids as measured with the chromogen. For the other extracts, the amounts of flavanoids measured with this method were smaller than the amounts obtained by the Folin−Ciocalteau assay. Only in extracts obtained with ethyl acetate were higher contents of flavanoids found. Other authors also found a poor correlation between the results obtained with the Folin−Ciocalteau assay and the method with *p*-dimethylaminocinnamaldehyde (*28*), because substances other than flavanoids react with the chromogen reagent and influence the results.

Sinapic acid is the predominant phenolic acid in rapeseed, as well as in seeds of other members of the family Brassicaceae (*41*, *42*). This phenolic acid occurs in free, esterified, glycosidic, or insoluble-bound forms, and the most important phenolic ester in this family is sinapine, the choline ester of sinapic acid (*41*). **Table 1** showed that the seeds belonging to the family Brassicaceae contain significant amounts of sinapine. In *B. carinata*, another aromatic choline ester similar to sinapine was detected. As expected, no sinapine was found in extracts of sunflower seeds. The amounts in *L. campestre* and crambe were very small. Water and ethyl acetate were noticeably less suitable for the extraction of sinapine.

Ultraviolet−visible absorption spectroscopy is one of the most useful techniques available for structure analysis of phenolic compounds. Phenolic compounds exhibit two major absorption bands in the ultraviolet/visible region: a first band in the range between 320 and 380 nm and a second band is in the 250 to 285 nm range. The first band represents the A ring absorption, whereas the second band is a result of the B ring absorption. As can be seen from **Figure 2** all extracts showed a more or

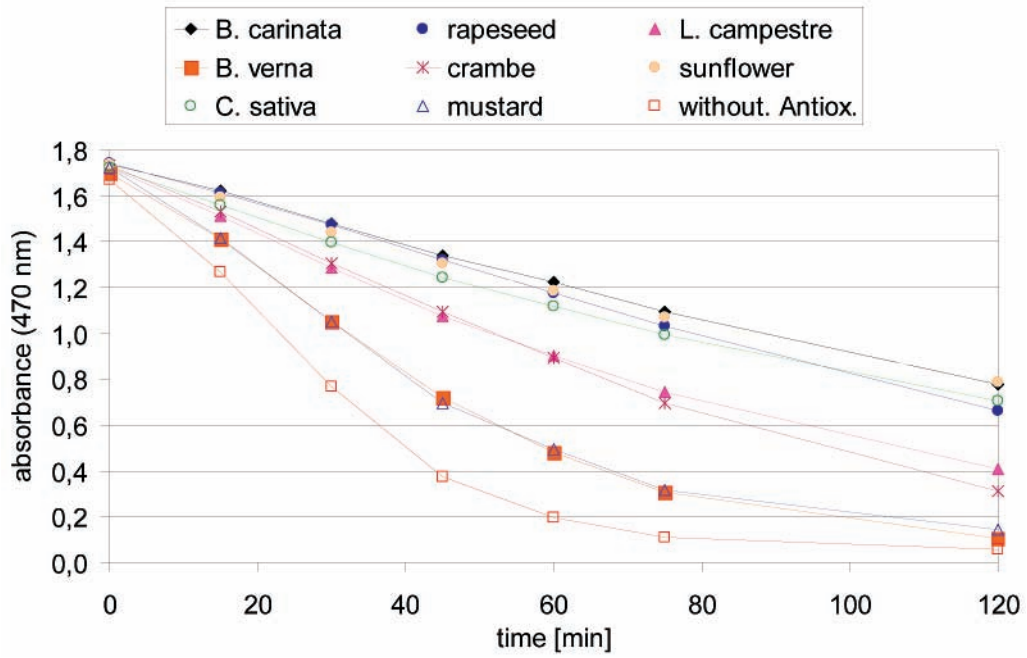

Figure 4. Co-oxidation of β-carotene in a β-carotene/linoleic acid system at 40 °C in the presence of different methanolic extracts.

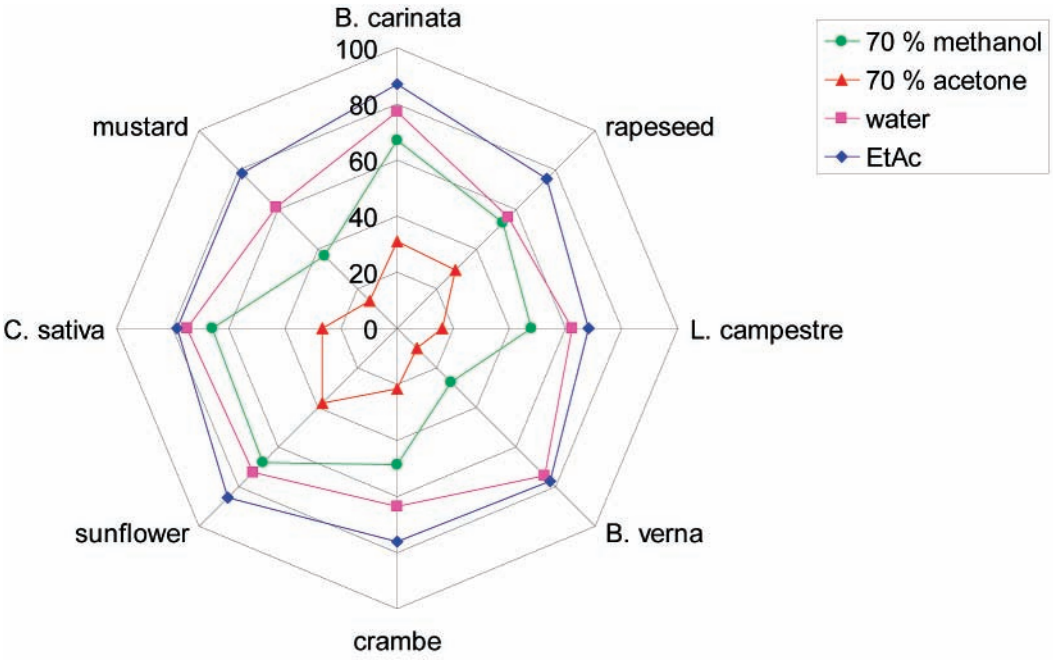

Figure 5. Effect of the extracts on a β-carotene/linoleic acid system [% of β-carotene after 60 min at 40 °C].

less strong absorption in the range of the B ring absorption, whereas the absorption in the range of the A ring was remarkably smaller. Extracts obtained from ethyl acetate showed no absorption in this region because the phenolic acids were removed with the aqueous phase from the solvent mixture ethyl acetate/water. Ethyl acetate was more effective in the extraction of flavonoids, as the high absorption in the region between 250 and 290 nm showed. The absorption of the other extracts in the range between 250 and 285 nm indicated that they contained only small amounts of flavonoids. In fat-free residues of crambe and *L. campestre*, respectively, extracted with the different solvents, no or only very small amounts of phenolic acids were detected by UV absorption.

Antioxidant Properties of the Extracts. Three different methods have been used for the determination of the antioxidant properties of the extracts isolated from the residues of different oilseeds: DPPH free radical scavenging, inhibition of β-carotene co-oxidation in a linoleate model system, and electron spin resonance spectrometry in an H_2O_2/NaOH/DMSO system.

Figure 3 shows the effect of the different extracts on the DPPH free radicals. It was obvious that the extracts of 70% methanol and 70% acetone had the greatest antioxidant activity on the DPPH radicals. The effectiveness of the extracts obtained with the other solvents was a little smaller, i.e., the amount of extract necessary for a reduction of the initial concentration of DPPH by 50% was higher. It was conspicuous that the antioxidant activity of the extracts obtained with the different solvents did not obey the order ethyl acetate > water > acetone > methanol for all seeds, but most samples followed this order. Considering the antioxidant effect of the different extracts of the residues it was obvious that the extracts obtained from sunflower residues by extraction with 70% methanol and 70%

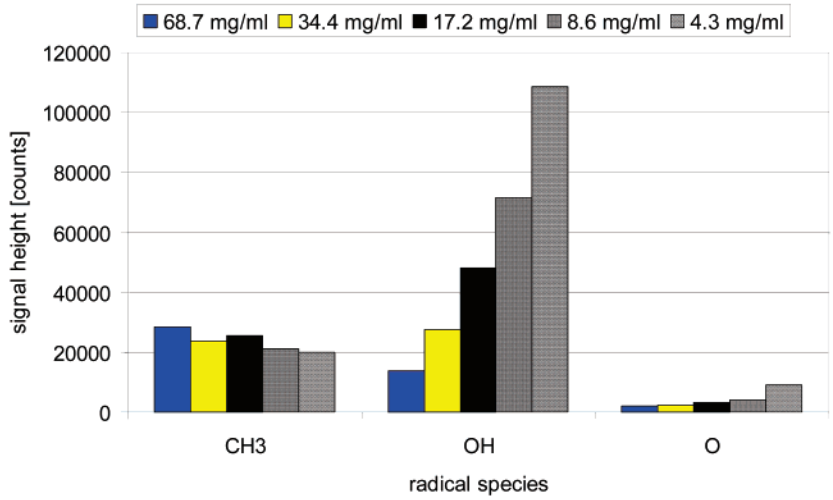

Figure 6. Radical scavenging properties of a methanolic extract from *Brassica carinata* characterized by ESR.

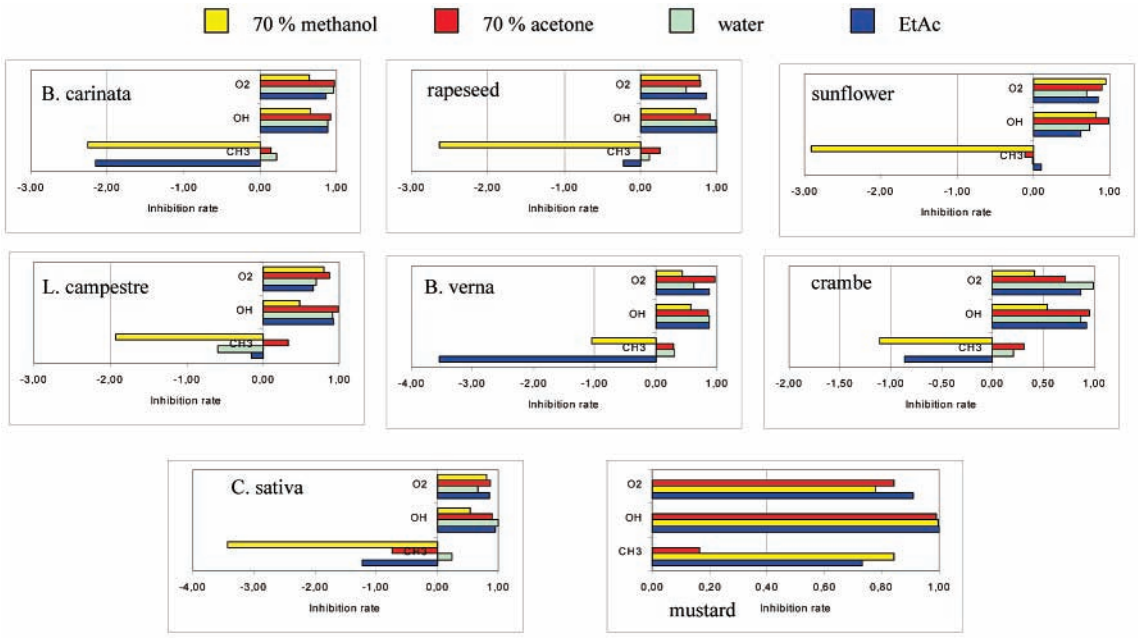

Figure 7. Radical scavenging properties of extracts from different oilseeds characterized by ESR.

acetone showed the strongest effect on the DPPH radicals. In that case only about 0.2 mg of extract was necessary to reduce the DPPH radicals by 50%. Also having strong effects on the DPPH radicals were extracts from *B. carinata*, rapeseed, *C. sativa*, and *L. campestre*, extracted by the above-mentioned solvents. By contrast, more than 3.0 mg of extract from crambe residues, extracted by ethyl acetate or water was necessary to get the same result. In summary, extracts of sunflower residues were by far the most suitable for stabilization of the DPPH radicals. From this extract only between 0.15 and 1.16 mg extract, depending on the extraction solvent, was necessary to reduce the DPPH radicals by 50%. Most ineffective were the extracts obtained from *B. verna* and crambe. From these residues the highest amounts of extracts were necessary to reduce the DPPH radicals.

Figure 4 indicates the decrease in absorbance of β-carotene in the presence of different methanolic extracts in the coupled system of β-carotene and linoleic acid. It was obvious that the control sample, without addition of extract solution, oxidized most rapidly. The methanolic extracts were markedly effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β-carotene in comparison with the control. Most effective are the methanolic extracts of *B. carinata*, sunflower, *C. sativa*, and rapeseed, whereas the effects of extracts from *B. verna* and mustard were not so strong.

In **Figure 5** the results of all extracts obtained from the β-carotene/linoleic acid assay were summarized. The data are expressed in percentage of the initial available β-carotene after an incubation of 60 min at 40 °C. Under these circumstances the percentage of β-carotene in a control sample without extract was 10%. The highest impact resulted from addition of extracts obtained by extraction with ethyl acetate. After 60 min only about 20% of β-carotene were decolorized. The extracts of acetone showed the weakest antioxidant activity in the β-carotene−linoleic acid system. Apart from some exceptions the antioxidant activity of the extracts from residues of different oilseeds decreased in the order ethyl acetate extract > water extract > methanol extract > acetone extract. In all, extracts obtained from *B. carinata* and sunflower with different solvents were most effective in inhibiting the oxidation of β-carotene. From these residues all extracts showed the strongest effect on the β-carotene−linoleic acid system, whereas extracts obtained from *L. campestre*, mustard, and *B. verna* had only a relatively small antioxidant effect.

Table 2. Correlation Factors between Parameters Describing the Amount of Extractable and Phenolic Compounds and Methods Describing the Antioxidant Activity of the Extracts

	DPPH*	β-carotene bleaching	ESR[c]
EC[a]	0.0102	0.0422	0.0224
PC[b]	0.0117	0.0092	0.0079
sinapine	0.0539	0.1901	0.1027
flavanoids[d]	0.0751	0.004	0.055

[a] EC, extractable compounds. [b] PC, phenolic compounds (measured by Folin–Ciocalteau assay). [c] ESR, electron spin resonance spectroscopy. [d] As measured by 4-dimethylaminocinnamaldehyde reagent.

Figure 6 shows the effect of a methanolic extract obtained from *B. carinata*, desolved in water, and added to a reaction system consisting of H_2O_2/NaOH/DMSO, at five different concentrations. The influence on the signal height of the three free radical adducts generated from combined components H_2O_2/NaOH/DMSO was measured by ESR and is presented in the figure. While the hydroxyl radical was suppressed with increasing concentration of extract added, the methyl radical showed the contrary effect: with increasing concentration of extract the signal height increased. This led to the assumption that the addition of extract resulted in the formation of methyl radicals. The concentration of superoxide anions was reduced, but the effect was not very marked.

Results of the ESR measurements of all extracts are summarized in **Figure 7**. The extracts were added in a concentration of 3% to the above-mentioned system and the inhibition rate was given as a ratio between signal height caused by the extract and signal height of a blind sample without extract. It was obvious that all extracts were able to scavenge superoxide anions and hydroxyl radicals in a remarkable range. In the literature it was described that hydroxyl radicals were the major active oxygen species causing lipid oxidation (*43*). In contrast to the antioxidant effect of the extracts on superoxide anions or hydroxyl radicals, the addition of some extracts led to the formation of methyl radicals in the system, expressed as negative inhibition rate. In these extracts some substances were able to form methyl radicals.

Looking at the correlation between the amount of phenolic compounds of the extracts obtained with different solvents from the fat-free residues, and measured with different methods, as well as the antioxidant activities of these extracts, it was obvious that no correlation can be given. **Table 2** summarizes the correlation coefficients. This result was similar to those reported in other publications in the literature (*37*), where no correlation was found for the antioxidant activity of berry and fruit wines and liquors and the phenolic compounds in these beverages. It is also similar to the work of Kähkönen et al., who investigated different plant extracts and found no significant correlation between the total phenolic content and the antioxidant activity measured as oxidation of methyl-linoleate (*39*). As an explanation one can give the work of Singleton (*44*), who described for wine the poor response of anthocyanins for the Folin−Ciocalteau assay in comparison with that of gallic acid or catechin. The main reason for the poor correlation between the amount of phenolic compounds and the antioxidant activity was probably the fact that the method determined the sum of phenolic compounds, whereas the individual phenolic compounds had very different responses on the Folin−Ciocalteau reagent and made different contributions to the antioxidant activity. For this reason, it could be that the amount of total phenolic compounds did not correspond to the antioxidant activity of the phenolic substances of the extracts. Additionally, it was described in the literature that substances such as sugars or ascorbic acid present in the extracts influence the results of methods intended to register the antioxidant activity (*40*). However, there were also other investigations which described a correlation between the DPPH method and the amount of phenolic compounds; in particular the content of flavan-3-ols showed a high correlation with the antioxidant activity (*45*). The different findings regarding the correlation between phenolic compounds and antioxidant activity described in the literature and also confirmed in the present paper indicate the diversity of the great group of phenolic compounds and their different responses to different methods for the determination of the antioxidant activity. The sum parameters measured in this work to characterize the extractable compounds of the residues can give only an indication about the presence of phenolic compounds. For a deeper explanation of the effects of the extracts on the methods for the determination of the antioxidant activity it would be necessary to characterize the individual phenolic compounds.

CONCLUSIONS

The fat-free residues of different oilseeds, whose oil is usable for edible applications, and also as a renewable resource, contain considerable amounts of phenolic compounds. The extracts obtained from these residues have remarkable antioxidant activity, the extent of which depends on the type of residue and the solvent used for the extraction. The investigation showed that the amount of extractable compounds, as well as the content of total phenolic compounds, gave no concrete indication of the real antioxidant activity of the extracts. There is no correlation between the amount of phenolic compounds and the methods describing the antioxidant activity of the extracts.

More work should be done to characterize individual phenolic compounds of the extracts of oilseeds in order to assign certain antioxidant effects to individual compounds of the resulting extracts.

ACKNOWLEDGMENT

I thank Dr. C. Wiezorek from the Chemischen Landes und Staatlichen Veterinäruntersuchungsamt Münster for making it possible to carry out the ESR measurements in that institute.

LITERATURE CITED

(1) Shahidi, F.; Wanasundara, P. K. J. P. D. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67−103.
(2) Branen, A. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J. Am. Oil Chem. Soc.* **1975**, *52*, 59−63.
(3) Linderschmidt, R.; Trylka, A.; Goad, M.; Witschi, H. The effects of dietry butylated hydroxytoulene on liver and colon tumor development in mice. *Toxicology* **1986**, *387*, 151−160.
(4) Tappel, A. Antioxidants protection against peroxidation. *Inform* **1995**, *6*, 780−783.
(5) Kamal-Eldin, A.; Appelqvist, L.-A. The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids* **1996**, *31*, 674−701.
(6) Pratt, D. E.; Hudson, B. J. F. Natural antioxidants not exploited commercially. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London, 1990; pp 171−192.
(7) Löliger, J. The use of antioxidants in foods. In *Free Radicals and Food Additives*; Aruoma, O. I., Halliwell, B., Eds.; Taylor & Francis: London, 1991; pp 121−150.
(8) Halliwell, B.; Aeschbach; R.; Löliger, J.; Aruoma, O. I. The Characterization of Antioxidants. *Food Chem. Toxicol.* **1995**, *33*, 601−617.

(9) Gordon, M. H. The Mechanism of Antioxidant Action in vitro. In *Food Antioxidants;* Hudson, B. J. F., Ed.; Elsevier Applied Science: London, 1990; pp 1−18.
(10) Rice-Evans, C.-A.; Miller, N. J.; Bolwell, P. G.; Gramley, P. M.; Pridham, J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* **1995**, *22*, 375−383.
(11) Nakatani, N. Antioxidative compounds from spices. *Nippon Nogeikagaku Kaishi* **1987**, *62*, 170−173.
(12) Plumb, G. W.; Chambres, S. J.; Lambert, N.; Bartolome, B.; Heaney, R. K.; Wanigatunga, S.; Arumoma, O. I.; Halliwell, B.; Williamson, G. Antioxidant aciton of fruit, herb, and spice extracts. *J. Food Lipids* **1996**, *3*, 171−188.
(13) Ramarathnam, N.; Osawa, T.; Ochi, H.; Kawakishi, S. The contribution of plant food antioxidants to human health. *Trends Food Sci. Technol.* **1995**, *6*, 75−82.
(14) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.
(15) Tsaliki, E.; Lagouri, V.; Doxastakis, G. Evaluation of the antioxidant activity of lupin seed flour and derivatives (*Lupinus albus* ssp. Graecus). *Food Chem.* **1999**, *65*, 71−75.
(16) Yen, G.-C.; Duh, P.-D.; Tsai, C.-L. Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food Chem.* **1993**, *41*, 67−70.
(17) Duh, P.-D.; Yeh, D.-B.; Yen, G.-C. Extraction and identification of an antioxidative component from peanut hulls. *J. Am. Oil Chem. Soc.* **1992**, *69*, 814−818.
(18) Oomah, B. D.; Kenaschuk, E. O.; Mazza, G. Phenolic acids in flaxseed. *J. Agric. Food Chem.* **1995**, *43*, 2016−2019.
(19) Naczk, M.; Shahidi, F. Phenolic Constituents of Rapeseed. In *Plant Polyphenols: Synthesis, Properties, Significance*; Hemingway, R. W.; Laks, P. E., Eds.; Plenum Press: New York, 1992; pp 895−910.
(20) Leung, J.; Fenton, T. W.; Mueller, M. M.; Clandinin, D. R. Condensed Tannins of Rapeseed Meal. *J. Food Sci.* **1979**, *44*, 1313−1316.
(21) Gupta, R. K.; Haslam, E. Vegetable tannins - structure and biosynthesis. In *Polyphenols in Cereals and Legumes*; Hulse, J. H., Ed.; IDRC: Ottawa, ON, 1980; pp 15−24.
(22) Naczk, M.; Shahidi, F.; Sullivan, A. Recovery of rapeseed tannins by various solvent systems. *Food Chem.* **1992**, *45*, 51−54.
(23) Shen, Z.; Haslam, E.; Falshaw, E.; Begley, M. Procyanidins and polyphenols of *Larix gmelini* bark. *Phytochemistry* **1986**, *25*, 2629.
(24) Scholz, E.; Rimpler, H. Proanthocyanidins from *Krameria triandra* root. *Planta Med.* **1989**, *55*, 379.
(25) Jende-Strid, B.; Moller, B. L. Analysis of proanthocyanidins in wild-type and mutant barley (*Hordeum vulgare* L.). *Carlsberg Res. Commun.* **1981**, *46*, 53.
(26) Pekic, B.; Kovac, V.; Alonso, E.; Revilla, E. Study of the extraction of proanthocyanidins from grape seeds. *Food Chem.* **1998**, *61*, 201−206.
(27) Taga, M. S.; Miller, E. E.; Pratt, D. E. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* **1984**, *61*, 928−931.
(28) Delcour, J. A.; de Varebeke, D. J. A new Colourimetric Assay for Flavanoids in Pilsner Beers. *J. Inst. Brew.* **1985**, *91*, 37−40.
(29) Clausen, S.; Olsen, O.; Sorensen, H. Separation of aromatic choline esters by high performance liquid chromatography. *J. Chromatogr.* **1983**, *260*, 193−199.
(30) Clausen, S.; Larsen, L. M.; Plöger, A. Aromatic Choline Esters in Rapeseed. Sorensen, H., Ed.; Martinus Nijhoff/Dr. W. Junk Publishers: 1985; vol. 11, pp 61−71.
(31) Matthäus, B.; Zubr, J. Variability of specific components in *Camelina sativa* oilseed cakes. *Ind. Crops Prod.* **2000**, *12*, 9−18.
(32) Kerber, E.; Buchloh, G. The sinapine content of Seeds of Crucifers. *Angew. Botanik* **1980**, *54*, 47−54.
(33) Hatano, T.; Kagawa, H.; Yasuhara, T.; Okuda, T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.* **1988**, *36*, 1090−2097.
(34) Taga, M.. S.; Miller, E. E.; Pratt, D. E. Chia Seeds as a Source of Natural Lipid Antioxidants. *J. Am. Oil Chem. Soc.* **1984**, *61*, 928−931.
(35) Holley, A. E.; Cheeseman, K. H. Measuring free radical reactions in vivo. *Br. Med. Bull.* **1993**, *49*, 494−505.
(36) Yamaguchi, F.; Yoshimura, Y.; Nakazawa, H.; Ariga, T. Free Radical Scavenging Activity of Grape Seed Extract and Antioxidants by Electron Spin Resonance Spectrometry in an H_2O_2/NaOH/DMSO System. *J. Agric. Food Chem.* **1999**, *47*, 2544−2548.
(37) Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. Antioxidant Activity of Berry and Fruit Wines and Liquors. *J. Agric. Food Chem.* **1998**, *46*, 25−31.
(38) Yen, G.-Ch.; Hsieh, Ch.-L. Antioxidant Activity of Extracts from Du-zuhong (*Eucommia ulmoides*) toward Various Lipid Peroxidation Models in Vitro. *J. Agric. Food Chem.* **1998**, *46*, 3952−3957.
(39) Kähkönen, M. P.; Hopia A. I.; Vuorela, H. J.; Rauha, J.-P.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.
(40) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(41) Bouchereau, A.; Hamelin, J.; Lamour, I.; Renard, M.; Larher, F. Distribution of Sinapine and Related Compounds in Seeds of Brassica and Allied Genera. *Phytochemistry* **1991**, *30*, 1873−1881.
(42) Matthäus, B. Antinutritive Compounds in Different Oilseeds. *Fett/Lipid* **1997**, *99*, 170−174.
(43) Yen, G.-Ch.; Wu, J.-Y. Antioxidant and radical scavenging properties of extracts from *Ganoderma tsugae. Food Chem.* **1999**, *65*, 375−379.
(44) Singleton, V. L. Analytical fractionation of the phenolic substances of grapes and wine and some practical uses of such analyses. In *Chemistry of Winemaking*; Webb, A. D., Ed.; American Chemical Society: Washington, DC, 1974.
(45) Goupy, P.; Hugues, M.; Boivin, P.; Amiot, M. J. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. *J. Sci. Food Agric.* **1999**, *79*, 1625−1634.

Received for review October 31, 2001. Revised manuscript received March 21, 2002. Accepted March 21, 2002.

JF011440S