Antioxidant properties of extracts from different oilseeds

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1 Introduction

The deterioration of lipids in food during storage and processing is an important reaction. Often lipid peroxidation is slowed down by synthetic antioxidants, but the interest in natural antioxidants increases. Consumers generally prefer these antioxidants for the stabilization of edible fats and oils against oxidative rancidity. In most cases tocopherols are used as natural antioxidants, but also extracts of rosemary, sage and other plant materials show good antioxidative effects /1, 2/.

During the oil pressing process great amounts of oil-cakes arise, which normally are used as fodder in animal nutrition but also as fuel in thermal power stations. These residues contain different bioactive substances, such as glucosinolates or phenolic compounds, with an important added value, which could be used as plant protection products or antioxidants.

The aim of the present work was the assessment of the antioxidant activity of extracts of oilseeds obtained with different solvents.

2 Material and Method

For the investigation seeds of rapeseed (*Brassica napus*), *Brassica verna*, *Brassica carinata*, *Lepidium campestre*, *Camelina sativa*, mustard (*Sinapis alba*), crambe (*Crambe abyssinica*) as well as sunflower (*Helianthus annuus*) were used.

Preparation of the extracts

15 g of each air-dried and defatted seed material were extracted three times with 200 ml of the appropriate solvent. The solvents used were water, 70 % acetone, 70 % methanol and a mixture of ethyl acetate and water (70:30) each. From the immiscible mixture of ethyl acetate/water only the extract obtained from the ethyl acetate phase was used for further investigations. First, the extraction was carried out by shaking the material over night with 200 ml solvent and afterwards by ultrasonic treatment of the material with 200 ml solvent for 45 min twice. The combined extracts were vacuum evaporated to remove the solvent at 40 °C and 30 Torr, weighted to determine the extraction yield and stored at –20 °C until use.

Determination of the Total Polyphenolic Compounds

About 0.1 g of the extract was dissolved in 25 ml 0.3 % HCl (in methanol/water (60/40)) and 100 µl of the resulting solution was added to 2 ml of 2 % Na_2CO_3. After 2 min 100 µl of 50 % Folin-Ciocalteau reagent was added and after further 30 min the absorbance was measured at 750 nm using a spectrophotometer. The concentration was calculated by using gallic acid as standard and the results were expressed as milligrams gallic acid equivalents (GAE) per gram extract /3/.

DPPH Radical Scavenging Method

An aliquot (0.5 ml) of the DPPH solution (about 50 mg/100 ml) was dissolved by 4.5 ml 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 at 515 nm was measured against a blank without extract using a spectrophotometer [4]. From a calibration curve with different amounts of DPPH the concentration of DPPH in the tube was calculated.

ß-Carotene Bleaching Method

40 mg of linoleic acid and 400 mg Tween 20 were transferred into a flask and 1 ml of a solution of ß-carotene (3.34 mg/ml) in chloroform were added. The chloroform was removed by rotary evaporation at 40 °C. Then

100 ml of distilled water were 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 the antioxidant solution was added and the absorbance was measured at 470 nm, immediately, against a blank, consisting of the emulsion without ß-carotene. The tubes were placed into a water bath at 40 °C and the absorbance was measured every 15 min up to 60 min /5/.

3 Results and Discussion

The amount of extractable compounds varied widely depending on the type of oilseed and the solvent used for the extraction. The results of testing different solvents for the extraction of phenolic compounds are given in figure 1. The data is presented in 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, which makes it easier to identify connections between the results. So it is clear that the extraction yields for methanol and acetone are very similar, while the yield for ethyl acetate is smaller. The highest amount of extractable compounds was found for water. Extracts of *Brassica carinata* extracted by water had the highest level and the lowest level was obtained by extraction of *Camelina sativa* with ethyl acetate. In general the amount of extractable substances decreases with decreasing polarity of the solvent in the order water, 70 % methanol, 70 % acetone and ethyl acetate.

One parameter for the characterisation of the extracts is the level of total phenolic compounds. The results of the colorimetric method for the determination of the total phenolic compounds, expressed as gallic acid equivalents, are shown on the next figure. The amount ranged from 2.1 to 19.4 percent, whereas most of the extracts show levels between 4 and 10 percent. The smallest level of total phenolic compounds was found for extracts from acetone. For this solvent the yields are similar for all residues, whereas the yields of the other solvents strongly vary depending on the type of oilseed.

Free radical scavenging is the accepted mechanism for an antioxidant to step in the lipid oxidation. A usual method to evaluate the scavenging activity of specific compounds on free radicals in a short time is the measurement of decolorisation of DPPH-donated protons at 520 nm. The anti-

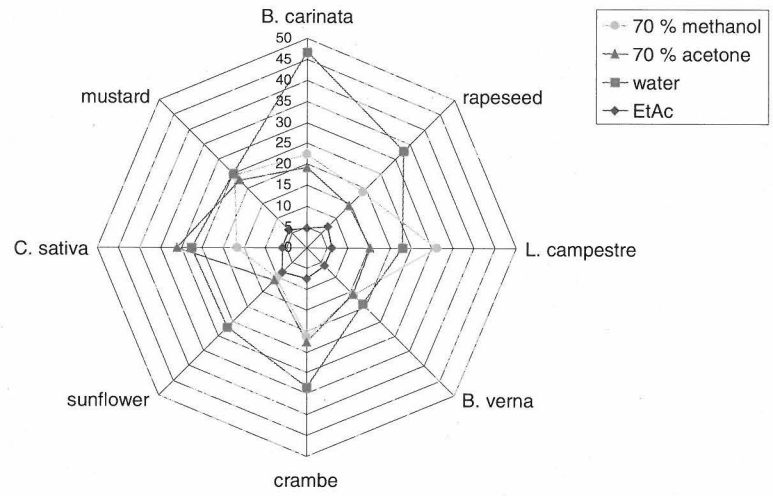

Figure 1: Amount of extractable substances [% of fat free residue]

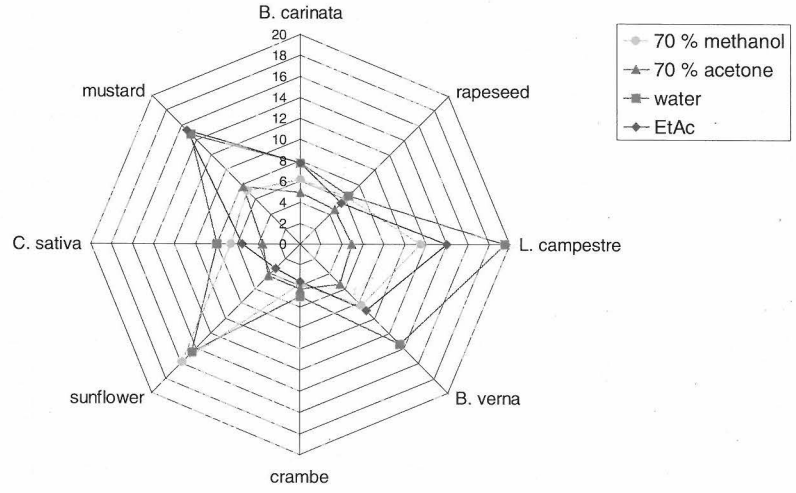

Figure 2: Content of total phenolic compounds (expressed as gallic acid equivalents)

oxidant reacts with a stable free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and as a consequence of its reduction the absorption at the characteristic wavelength of 520 nm disappears.

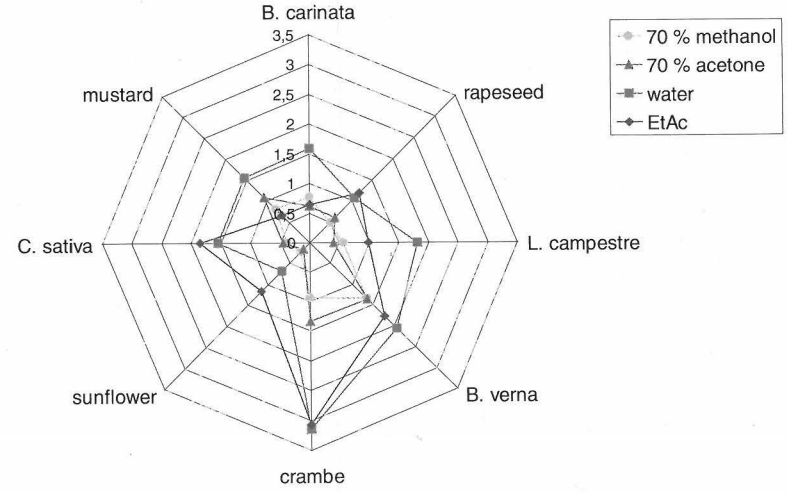

Figure 3: Effect of extracts on DPPH free radicals [expressed as mg extract allowing reduction of 50 % DPPH]

In Figure 3 the concentration of extract is given, which allowing the reduction of 50 % DPPH. It is obviously that the extracts of 70 % methanol and acetone, respectively, have the greatest antioxidant activity on the DPPH radicals. The effectiveness of the other extracts is a little smaller, the amount of extract necessary for a reduction of the initial concentration of DPPH by 50 % is higher. It should be noted that not for all seeds the antioxidant activities of the extracts obtained with the different solvents obey the order methanol > acetone > water > ethyl acetate.

In another model system ß-carotene undergoes a rapid discoloration in absence of an antioxidant. The free linoleic acid radical formed upon the abstraction of a hydrogen atom from one of its methylene groups attacks the ß-carotene molecules, causing loss of double bonds and therefore loss of its characteristic orange colour.

Figure 4 summarise the results of all extracts. The data is expressed in percentage of the initially available ß-carotene after an incubation of

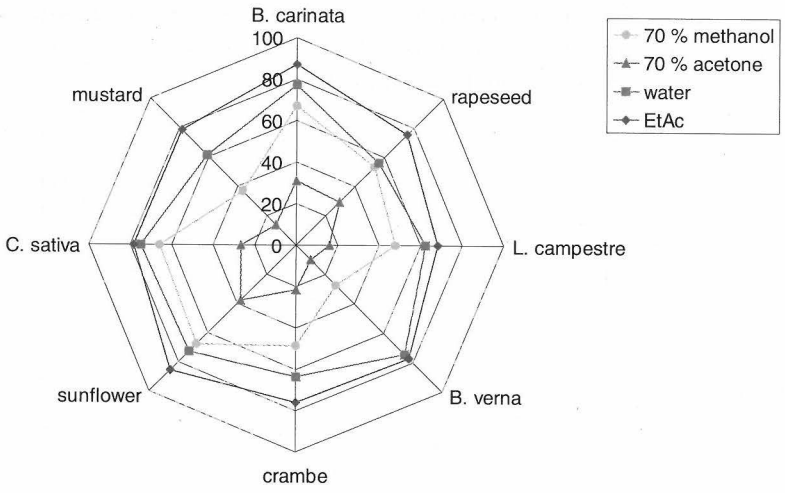

Figure 4: *Effect of extracts on a ß-carotene/linoleic acid system [% of ß-carotene after 60 min at 40 °C]*

60 min. at 40 °C. Under these circumstances the percentage of ß-carotene in a control sample without extract was 10 %. The highest impact is found with the addition of extracts obtained by extraction with ethyl acetate. After 60 min only about 20 percent of ß-carotene were decolourised. The extracts of acetone show the weakest antioxidant activity in the ß-carotene-linoleic acid-system. Apart from some exceptions, the antioxidant activity of the extracts from different oilseeds decreased in the order ethyl acetate extract > water extract > methanol extract > acetone extract.

In summary it may be said that the fat free residues of the investigated oilseeds contain considerable amounts of phenolic compounds. A further characterization of individual compounds is still necessary. All extracts show the ability to scavenge free radicals in the assays for evaluation of antioxidant activity. No correlation between the content of total phenolic compounds and the antioxidant activity was found.

4 Literature

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