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Effect of Enzymatic Hydrolysis on the Antioxidant Properties of Alcoholic Extracts of Oilseed Cakes

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Summary

The aim of the present study is to compare changes in the total phenolic, flavonoid and reducing sugar content and antioxidant activity of alcoholic extracts of *Oenothera biennis*, *Borago officinalis* and *Nigella sativa* oilseed cakes before and after enzymatic hydrolysis. Extraction with ethanol and hydrolysis with different commercially available glycosidases: α-amylase, β-glucosidase, β-glucanase and their combinations in a ratio of 1:1:1 were investigated. Total phenolic, flavonoid and reducing sugar content, iron-chelating activity and antioxidant activity according to DPPH and ABTS tests were measured in non-hydrolysed extracts and compared with the results obtained for the extracts after the application of immobilised enzymes. As a result, the hydrolysed extracts had a higher phenolic and reducing sugar content as well as higher iron-chelating and antioxidant activities. Total phenolic content of *Oenothera biennis*, *Borago officinalis* and *Nigella sativa* oilseed cake extracts after enzymatic hydrolysis was higher in comparison with non-hydrolysed extracts, *i.e.* 2 times (for the enzyme combination), and 1.5 and 2 times (for β-glucanase) ($p<0.05$), respectively. The best results in increasing the flavonoid and sugar content as well as in iron-chelating activity were obtained after enzymatic hydrolysis of oilseed cake extracts by β-glucanase. Oilseed cake extracts after hydrolysis with an enzyme combination in a ratio of 1:1:1 had the highest increase in antioxidant activity.

Key words: Oenothera biennis, *Borago officinalis*, *Nigella sativa*, oilseed cake, polyphenols, enzymatic hydrolysis

Introduction

The oilseeds of *Oenothera biennis* (evening primrose or evening star), *Borago officinalis* (starflower) and *Nigella sativa* (black cumin or black seed) have a well-established status in pharmaceutical, medicinal, cosmetic and nutritional applications (*1–3*). They are mainly known as raw materials of plant origin possessing high biological activity, and are rich in triglycerides of fatty acids, especially γ-linolenic acid (8–14 % in *Oenothera biennis* and 17–25 % in *Borago officinalis* oilseeds) and linoleic acid (50–55 % in *Nigella sativa* and 70–75 % in *Oenothera biennis* oilseeds) (*4–7*). They are also found to be a rich source of unsaponifiables, which include antioxidant substances such as tocopherols, tocotrienols, carotenoids, flavonoids, phenolic acids and hydrolysable tannins, triterpenes and

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others (*8–12*). Some active compounds are still present in by-products left over from oil pressing. Thus, defatted seeds (oilseed cakes) seem to be a potential source of natural bioactive substances (*13–16*).

The secretion of active substances, especially of functional terpenes, flavonoids and phenolic acids, causes considerable difficulties on a technological scale due to the fact that some of these active compounds are present in a form associated with carbohydrates. Most of these compounds are present in glycoside and ester form, which is characterised by lower antioxidant activity and bioavailability as compared to the free aglycone form (*17,18*). However, it is possible to effectively improve the quality of raw material with the use of biotechnological methods, especially by enzymatic hydrolysis of the obtained extract. Enzymes from the class of hydrolases (EC 3), particularly the subclass of glycosidases (EC 3.2), allow to increase the efficiency of extraction of polyphenols from vegetable products and wastes as well as to enhance the efficiency of oil extraction from seeds (*19–27*).

The aim of the present study is to compare the antioxidant activity of oilseed cake extracts before and after enzymatic hydrolysis. For this purpose, extraction with ethanol and hydrolysis of the obtained extract with different commercially available glycosidases: α-amylase (EC 3.2.1.1) from *Aspergillus oryzae*, β-glucosidase (EC 3.2.1. 21) and β-glucanase (EC 3.2.1.6) from *Aspergillus niger*, as well as an enzyme combination in a ratio of 1:1:1 were investigated.

Materials and Methods

Plant material and the preparation of extracts

Oenothera biennis, *Borago officinalis* and *Nigella sativa* oilseed cakes were obtained from Efavit (Poznan, Poland). The oilseed cake samples were extracted with ethanol (50 %, by volume). The extraction was performed with a 10:1 solvent/raw material ratio (by volume per mass) in 100-mL flasks at a temperature of 50 °C for 24 h in a water bath shaker with a frequency of 200 cycles per min (Elpin-Plus, Lubawa, Poland). After 24 h, the extracts were hydrolysed by immobilised enzymes at 50 °C for 24 h on the water bath shaker with a frequency of 200 cycles per min (Elpin-Plus). The flasks with non-hydrolysed extracts (control) were also left on the water bath shaker for another 24 h. The obtained extracts (hydrolysed and non-hydrolyzed) were filtrated through Whatman filter paper (no. 1; GE Healthcare, Maidstone, UK). The solvents were evaporated under reduced pressure, using a rotary evaporator at 40 °C (Unipan 357; UNIPRO, Warsaw, Poland). Dry extract was dissolved in ethanol (50 %, by volume) (POCh S.A., Gliwice, Poland), collected and analysed. Total phenolic, total flavonoid and total reducing sugar contents of the extracts were investigated, and the free radical-scavenging activity was determined using DPPH and ABTS. Apart from that, the chelating activity was also measured.

Determination of enzyme activity and optimal reaction conditions for hydrolysis (pH, time and enzyme ratio)

The activity of α-amylase (EC 3.2.1.1) from *Aspergillus oryzae* (Sigma-Aldrich, St. Louis, MO, USA) was determined using the 3,5-dinitrosalicylic acid (DNS; Sigma-Aldrich) method described by Miller (*28*), with a slight modification. The soluble starch (Sigma-Aldrich) was used as a substrate for the determination of α-amylase activity, and maltose was used as the assay standard. The standard reaction mixture containing 40 mL of 1 % soluble starch solution in 0.1 mol/L of phosphate-citrate buffer (POCh) (pH=4.5, 5.0 or 5.5) was incubated with 8 mL of various ratios of α-amylase (1–5 %) in 0.1 mol/L of phosphate-citrate buffer (pH=4.5, 5.0 or 5.5) at 50 °C from 30 min to 48 h. The reaction was stopped by adding 1 mL of DNS solution. The DNS solution was prepared by mixing 10 g of 3,5-dinitrosalicylic acid with 200 mL of 2 M sodium hydroxide (POCh) and 300 g of potassium sodium tartrate (Sigma-Aldrich). The sample (2 mL) was mixed with DNS at the ratio of 1:1 (by volume) and incubated at 100 °C for 10 min. Next, the samples were cooled and transferred quantitatively to 10 mL of water. Absorbance was measured at a wavelength of 550 nm against a reagent sample containing a respective production medium instead of the exact sample. The concentration of maltose released during the starch hydrolysis was read out from a standard curve (previously prepared for maltose in the concentration range from 50 to 500 mg/L) (*29*).

Activities of β-glucosidase (EC 3.2.1.21) and β-glucanase (EC 3.2.1.6) from *Aspergillus niger* (Sigma-Aldrich) were also determined using the DNS method. Salicin (2-(hydroxymethyl)phenyl-β-D-glucopyranoside) (Sigma-Aldrich) was used as a substrate for the determination of the activity of β-glucosidase, β-glucanase and a combination of enzymes (in a ratio of 1:1:1). Glucose was used as the assay standard. The concentration of glucose (POCh) released during salicin hydrolysis was read out from a standard curve (previously prepared for glucose in the concentration range from 50 to 500 mg/L). The reported study involved the enzymes immobilised in sodium alginate.

Immobilisation of enzymes

Enzymes (1–5 %, by volume) in 0.1 mol/L of phosphate-citrate buffer (pH=4.5, 5.0 or 5.5) were mixed with a solution of 40 g/L of sodium alginate (Sigma-Aldrich) at 1:1 ratio (by volume). From the enzyme solution, beads of equal size were formed by direct instilling (using a syringe and a needle of 0.1 cm in diameter) into 0.2 mol/L of a solution of calcium chloride (POCh). The enzymes were left in the solution of calcium chloride for 20 min to harden, then they were separated from the solution and rinsed off with distilled water. The immobilised enzymes were transferred into flasks with oilseed cake extracts. Hydrolysis was conducted at 50 °C for 24 h in a water bath shaker with a frequency of 200 cycles per min (Elpin-Plus) (*30*).

Determination of total phenolic content

For the determination of total phenolic content (TPC), the method with Folin-Ciocalteu (POCh) reagent was used (*31*). The TPC was measured as follows: 0.5 mL of diluted extracts or standard solutions of gallic acid (25–500 mg/L; POCh) were added to a 50-mL volumetric flask containing 30 mL of distilled water, then 2.5 mL of 50 % (by mass per volume) Folin-Ciocalteu reagent were added to the mixture and shaken. After 5 min, 7.5 mL of 7 % sodium carbonate solution (by mass per volume) (POCh) were added with mixing and the solution was immediately filled up to 50 mL with distilled water. After incubation in a dark place for 2 h at room temperature, the absorbance of the solution was measured by the spectrophotometer Helios γ (Spectronic, Thermo Scientific, Cambridge, UK) at 765 nm. The results were calculated according to the calibration curve for gallic acid ($y=0.0009x$, $R^2=0.9993$, y=absorbance at 765 nm, x=concentration of gallic acid in mg/L). The TPC was expressed as mg of gallic acid equivalents (GAE) per g of extract dry mass.

Determination of total flavonoid content

The total flavonoid (TF) content assay was performed as previously described by Dragović-Uzelac *et al.* (*32*). A volume of 1 mL of diluted extracts or standard solution of quercetin (50–500 mg/L; POCh) was placed in a 10-mL volumetric flask, then 4 mL of distilled water and, after 5 min, 300 mL of sodium nitrate (1:20) and 3 mL of aluminium chloride (POCh) (1:10) were added. The mixture was shaken and 6 min later 2 mL of 1 mol/L of sodium hydroxide solution were added, again well shaken and centrifuged for 5 min at 2236×*g*. The supernatant was decanted and the absorbance was measured at 510 nm against the blank. The results were calculated according to the calibration curve for quercetin ($y=0.0003x$, $R^2=0.9973$). The content of TF was expressed as mg of quercetin equivalents (QE) per g of extract dry mass.

Determination of reducing sugar content

Reducing sugar content was determined using the DNS method described by Miller (*28*) with a slight modification. Glucose was used as the assay standard. The examined sample (2 mL) was mixed with DNS acid at the ratio of 1:1 (by volume) and incubated at 100 °C for 10 min. Next, the samples were cooled and transferred quantitatively to 10 mL of distilled water. Absorbance was measured at a wavelength of 550 nm against a reagent sample containing respective production medium instead of the exact sample. The results were calculated according to the calibration curve for glucose ($y=0.0009x+0.0144$, $R^2=0.9979$).

Determination of total antioxidant capacity by DPPH free radical assay method

The free radical-scavenging capacity of oilseed cake extracts was determined according to the previously reported procedure, using the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma-Aldrich) (*33*). The method was based on the reduction of stable DPPH nitrogen radicals in the presence of antioxidants. Briefly, 2 mL of oilseed cake extract at various concentrations were mixed with 2 mL of methanol (POCh) and 1 mL of 0.5 mM DPPH methanolic solution. The mixture was thoroughly mixed, kept in the dark for 20 min, and after that the absorbance was measured at 517 nm against the methanol as a blank. The DPPH activity was calculated as IC_{50} using a plot of the percentage of radical-scavenging activity against the extract concentration (in μg/mL) and determined as a concentration of the extract necessary to reduce DPPH radicals by 50 % (IC_{50}). Samples with a lower IC_{50} had a stronger antioxidant activity. Positive controls were quercetin and rutin (quercetin-3-*O*-rutinoside).

Determination of total antioxidant capacity by ABTS radical cation assay method

The radical-scavenging capacity of oilseed cake extracts was evaluated against 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS; Sigma-Aldrich) generated as previously reported (*34*). ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting 7 mmol/L of ABTS stock solution with 2.45 mM potassium persulphate (Sigma-Aldrich) in the dark at room temperature for 12–16 h before use. The next day, $ABTS^{\cdot+}$ solution (1 %, by volume) was diluted with 96 % ethanol (POCh) to an absorbance of 0.73±0.02 at 734 nm. After the addition of 0.16 mL of sample to 2 mL of diluted $ABTS^{\cdot+}$ solution, absorbance readings were taken after 1 min at 734 nm using the spectrophotometer Helios γ (Spectronic). Ethanol was used as a blank. The $ABTS^{\cdot+}$ activity was calculated as IC_{50} using a plot of percent radical-scavenging activity against extract concentration (in μg/mL) and determined as a concentration of the extract necessary to reduce $ABTS^{\cdot+}$ radicals by 50 % (IC_{50}). Samples with a lower IC_{50} had a stronger antioxidant activity. Positive controls were quercetin and rutin (quercetin-3-*O*-rutinoside).

Determination of chelating activity

The ferrous ion-chelating activity of the studied samples was estimated based on the decrease in the maximal absorbance of the iron(II)–ferrozine complex, according to previously reported method (*35*), with some modifications. Briefly, 1 mL of a solution of a test compound at various concentrations dissolved in ethanol was incubated with 0.5 mL of ferrous chloride tetrahydrate (1.0 mM; Sigma-Aldrich). The reaction was initiated by the addition of 1 mL of ferrozine (5.0 mM; Sigma-Aldrich), and then the total reaction volume was adjusted to 4 mL with ethanol. After the mixture had reached equilibrium (10 min), the absorbance was read at 562 nm. The control was prepared without the test compound. The chelating activity was plotted against the plant extract concentration (in μg/mL) to determine the concentration of the extract necessary for the chelation of 50 % (IC_{50}) of iron ions. Samples with a lower IC_{50} had a stronger chelating activity. Positive controls were quercetin and rutin (quercetin-3-*O*-rutinoside).

Statistical analysis

All results are presented as a mean values±standard deviations of at least six independent experiments (each performed in triplicate). Statistical analysis was performed

by using Student's *t*-test. A probability at $p<0.05$ was considered significant. Pearson's correlation coefficients (R^2) were calculated. IC_{50} values were obtained from dose-effect curves by linear regression.

Results and Discussion

In the experiment, hydrolases (α-amylase, β-glucosidase, β-glucanase and an enzyme combination in a ratio of 1:1:1) immobilised in sodium alginate showed the strongest ability to hydrolyse the substrates: water-soluble starch (for α-amylase) and salicin (for the β-glucosidase, β-glucanase and enzyme combination) during 48 h, pH=5.5, at a temperature of 50 °C (Table 1). The obtained results are comparable with literature data (*36*, *37*).

The next stage of the experiment was to determine the total phenolic, total flavonoid and reducing sugar content in the obtained extract. The results are shown in Fig. 1. A comparison of extracts revealed that *Oenothera biennis* contained the highest total phenolic content (in mg of GAE per g): 466.67 (Fig. 1a), followed by *Borago officinalis* with 374.07 and *Nigella sativa* with 196.96. The extract with the highest content of flavonoids (in mg of QE per g) and reducing sugars (in mg of glucose per g) was also *Oenothera biennis*: 211.11 (Fig. 1b) and 277.78

Table 1. Determination of optimal pH conditions for enzymes (α-amylase, β-glucosidase, β-glucanase and combination of enzymes during hydrolysis

Enzyme	γ(reducing sugars)/(mg/L)		
	pH=4.5	pH=5.0	pH=5.5
α-amylase	117.1±11.3	115.7±8.3	130.5±11.9
β-glucosidase	31.7±3.8	45.6±2.9	163.5±12.5
β-glucanase	44.6±2.1	103.7±10.6	127.5±11.5
combination of enzymes (1:1:1): α-amylase/β-glucosidase/β-glucanase	15.9±2.1	205.3±12.8	245.5±13.5

Hydrolysis conditions: time=48 h, temperature=50 °C. Starch was a substrate for α-amylase, maltose was a standard. Salicin was a substrate for β-glucosidase, β-glucanase and combination of enzymes (1:1:1), glucose was a standard. Data represent the mean values±standard deviation of three independent experiments, each performed in triplicate

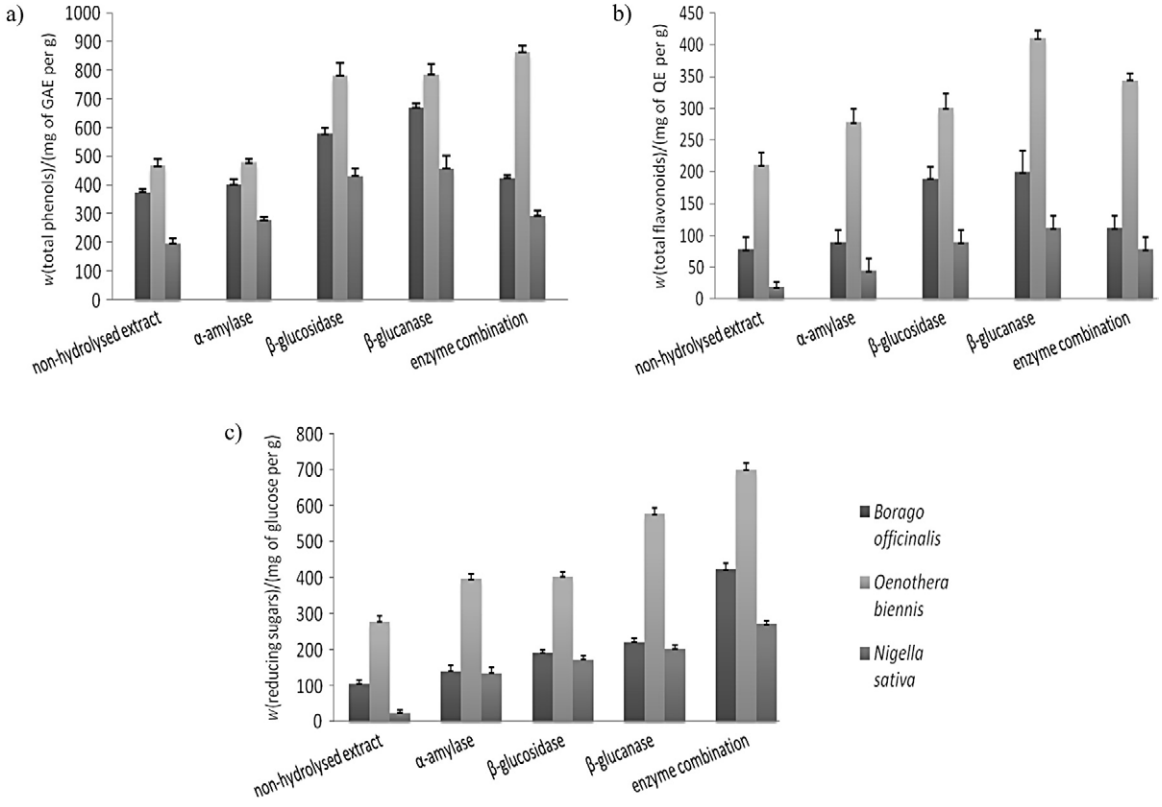

Fig. 1. Change in the: a) total phenolic, b) total flavonoid, and c) reducing sugar content of *Oenothera biennis*, *Borago officinalis* and *Nigella sativa* oilseed cake extracts before and after enzymatic hydrolysis by α-amylase, β-glucosidase, β-glucanase and a combination of enzymes. Data represent the mean values±standard deviation of six independent experiments, each performed in triplicate. Student's *t*-test: $p<0.05$

(Fig. 1c), followed by *Borago officinalis* with 77.78 and 105.56, and *Nigella sativa* with 19.25 and 24.07, respectively. The total phenolic content was at a similar level as that observed by earlier researchers: 495.38 and 653 mg/g in *Oenothera paradoxa* (*11,38*), 413 and 14.01 mg/g in *Borago officinalis* (*2,23*), and 78 mg/g in *Nigella sativa* (*39, 40*). The amount of polyphenols depends on the solvents and methods of determination that are used. Moreover, differences among the results are explained primarily by different sources of the biological material that is used, conditions of cultivation and also by different methods of sample preparation as well as extraction conditions in both experiments.

It was observed that enzymatic hydrolysis caused an increase in the total phenolic, flavonoid and reducing sugar content in all seed cake extracts in comparison with the non-hydrolysed extracts ($p<0.05$). Flavonoid and reducing sugar content after enzymatic hydrolysis was higher: 2 times with β-glucanase and the enzyme combination from *Oenothera biennis* ($p<0.05$), 2 times with β-glucanase and 4 times with enzyme combination from *Borago officinalis* ($p<0.05$), and 5 times with β-glucanase and enzyme combination from *Nigella sativa* ($p<0.05$), in comparison with the non-hydrolysed extracts, respectively (Figs. 1b and c). The total phenolic content in *Oenothera biennis*, *Borago officinalis* and *Nigella sativa* seed cake extracts after enzymatic hydrolysis was higher in comparison with the non-hydrolysed extracts: 2 times with the enzyme combination and 1.5 and 2 times with β-glucanase ($p<0.05$), respectively (Fig. 1a). This is primarily related to the biotransformation of polyphenols from their glycosidic to aglycone forms. The experiment showed that glycosidases, especially β-glucanase, as well as the enzyme combination in a ratio of 1:1:1 have a significant impact on the release of polyphenols from their glycosidic forms. The highest increase in the content of phenols, flavonoids and reducing sugars was observed in the *Oenothera biennis* extract hydrolysed by β-glucanase and the enzyme combination. The experiment showed that an enzyme combination in a ratio of 1:1:1 has a significant impact on the release of polyphenols from their glycosidic forms, which results in the release of reducing sugars. A higher sugar content is attributed to the increase in reducing sugar content after hydrolysis of the glycosidic form of polyphenols and to the hydrolysis of oligosaccharides or saponin glycosides from the extract (*41–43*).

Antioxidant properties of seed cake extracts before and after hydrolysis were evaluated by electron transfer (ET)-based assays (*44*) with synthetic DPPH radical and ABTS cation radical reaction (Fig. 2). The results indicate that after enzymatic hydrolysis of seed cake, the extract showed significantly higher antioxidant activity as compared to the non-hydrolysed extract ($p<0.05$). In both assays, the *Oenothera biennis* extract exhibited higher antioxidant activity (IC_{50} of 110 and 75 μg/mL for DPPH and IC_{50} of 100 and 65 μg/mL for ABTS assay) than *Borago officinalis* (IC_{50} of 210 and 125 μg/mL for DPPH and IC_{50} of 195 and 95 μg/mL for ABTS assay) and *Nigella sativa* extracts (IC_{50} of 500 and 350 μg/mL for DPPH and IC_{50} of 485 and 295 μg/mL for ABTS assay) before and after enzymatic hydrolysis with the enzyme combination. The results were compared with the results

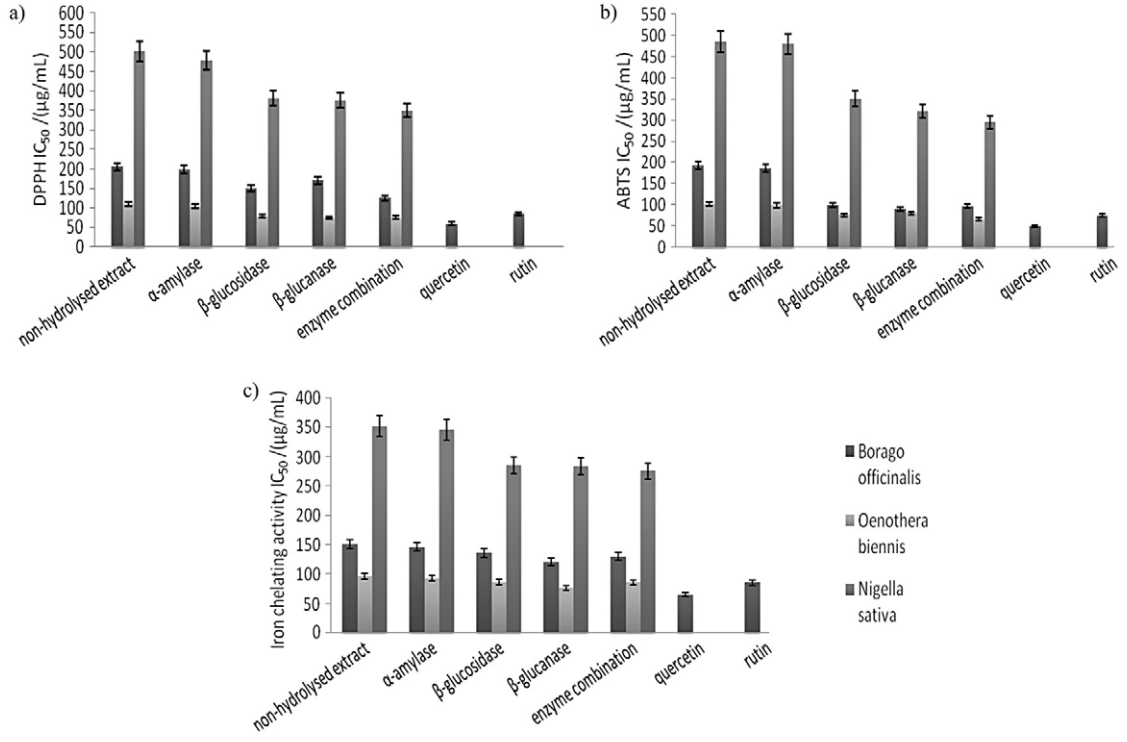

Fig. 2. Change of: a) DPPH radical-scavenging activity, b) ABTS radical-scavenging activity, and c) iron-chelating activity of *Oenothera biennis*, *Borago officinalis* and *Nigella sativa* oilseed cake extracts before and after enzymatic hydrolysis by α-amylase, β-glucosidase, β-glucanase and a combination of enzymes. Quercetin and rutin were used as positive control. Data represent the mean values±standard deviation of six independent experiments, each performed in triplicate. Student's *t*-test: $p<0.05$

of DPPH and ABTS assays for quercetin and rutin (quercetin-3-*O*-rutinoside). It was observed that quercetin (the aglycone form of flavonoid) had higher antioxidant activity (IC_{50}=65 μg/mL for DPPH and IC_{50}=50 μg/mL for ABTS) as compared to rutin (the glycosidic form of quercetin; IC_{50}=85 μg/mL for DPPH and 75 μg/mL for ABTS). Moreover, the antioxidant activity of *Oenothera biennis* seed cake extract after enzymatic hydrolysis with the enzyme combination (IC_{50}=75 μg/mL for DPPH assay and 65 μg/mL for ABTS assay) was at a comparable level to the antioxidant activity of quercetin.

The increase in the antioxidant activity was associated with the hydrolysis of glycoside molecules and also with the increase of free hydroxyl groups in the flavonoid ring. After enzymatic hydrolysis, the active ingredients may also dissolve more easily in the extraction medium. Previously, Laroze *et al*. (*45*) demonstrated that the use of commercial pectinases (Grindamyl™ CA 150; Danisco, Palo Alto, CA, USA, and Maxoliva®; DSM, Heerlen, The Netherlands) affects the increase of the total phenolic content and the antioxidant activity. Also, after hydrolysis of polyphenol glycosides by using β-glucosidase from *Rhizopus oligosporus* (*29,43*), it was observed that the phenolic content can increase by 66 % (from 3.0 to 5.0 mg/g) with the biotransformation of seed cake (5 g of aronia pomace and 5 g of evening primrose cake) (*25*).

In our experiment, the increase in the antioxidant activity was lower than the increase in total phenolic and flavonoid content after hydrolysis. However, it is worth mentioning that a significant correlation between polyphenolic and flavonoid content was observed (Table 2). Also, a high correlation was demonstrated between total phenolic content and antioxidant activity in the *Oenothera biennis* extract. Pearson's correlation coefficients (R^2) were 0.9523 and 0.9503 between the phenolic content and the DPPH and ABTS assays, respectively. Also, other authors found a positive correlation between the phenolic content and antioxidant activity in seed cake extracts and indicated that a high level of polyphenols results in high free radical-scavenging activity (*39,46*). A higher content of polyphenols and reducing sugars was obtained after the application of β-glucanase, while, when referring to antioxidant activity, better results were obtained by using β-glucosidase. This may be connected with the fact that the phenolics in the early stages of hydrolysis inhibit enzyme activity which, in turn, reduces antioxidant activity (*25*). The weakest effect on the increase of polyphenols and antioxidant activity was observed for α-amylase. This may be related to the inhibition of active ingredients found in the seed cake. Also, other authors observed a decrease in pancreatic α-amylase activity caused by the presence of phenolics in the fermented meal substrate (*47*). Furthermore, the higher content of reducing sugars and higher antioxidant activity but lower amount of phenolics and flavonoids may be connected with a competitive mechanism of enzyme inhibition and the restoration of glycosidic bonds (*48*).

Table 2. Pearson's correlation coefficients

	Polyphenols		
	Oenothera biennis	*Borago officinalis*	*Nigella sativa*
flavonoids	0.9752	0.9289	0.9374
reducing sugars	0.8285	0.8655	0.9471
DPPH scavenging activity	0.9523	0.8554	0.8545
ABTS scavenging activity	0.9503	0.8612	0.8799
iron-chelating activity	0.8887	0.9055	0.9380

Polyphenols scavenge free radicals and thus may reduce oxidative stress through several mechanisms that depend on their chemical structure. One of the mechanisms is the chelation of metal ions (iron or copper) (*49–51*). The chelating activity of seed cake extract using iron-(II)-ferrozine complex was expressed as the extract concentration (in μg/mL) that is necessary for the chelation of 50 % (IC_{50}) of iron ions (Fig. 2c). In our research the highest chelating activity was displayed by the *Oenothera biennis* extract: IC_{50}=95 and 65 μg/mL, followed by *Borago officinalis:* IC_{50}=150.50 and 110 μg/mL before and after enzymatic hydrolysis with β-glucanase, and the *Nigella sativa* extract*:* IC_{50}=351.50 and 290 μg/mL, before and after enzymatic hydrolysis with the enzyme combination, respectively. The results were compared with the IC_{50} values of chelating activity for quercetin and rutin. It was observed that quercetin (the aglycone form of flavonoid) had higher chelating activity (IC_{50}=65 μg/mL) than rutin (the glycosidic form of quercetin) (IC_{50}=85 μg/mL). Moreover, the chelating activity of *Oenothera biennis* oilseed cake extract after enzymatic hydrolysis with the enzyme combination (IC_{50}=65 μg/mL) was at a comparable level to the chelating activity of quercetin. Enzymatic hydrolysis of oilseed cake extracts increased with the chelating activity through the release of hydroxyl groups involved in the formation of the complex. The best results were observed for β-glucanase and the enzyme combination in a ratio of 1:1:1 (Fig. 2c). The strongest chelating activity of *Oenothera biennis* may be correlated with its high polyphenolic content, especially of low molecular mass phenolic antioxidants like (+)-catechin, (–)-epicatechin and gallic acid (*52–54*). The results obtained for *Borago officinalis* and *Oenothera biennis* oilseed cake extracts were higher than the values reported in the literature by Wettasinghe and Shahidi (*49*). In that study, extract from *Borago officinalis* oilseed cake at a concentration of 462 mM chelated 91 % of the iron ions, while the *Oenothera biennis* oilseed cake extract at a concentration of 506 mM chelated 100 % of the iron ions (*49*).

Conclusions

On the basis of the obtained results, it may be concluded that oilseed cake extracts from *Oenothera biennis*, *Borago officinalis* and *Nigella sativa* have a high polyphenolic content and thus display high antioxidant activity. Enzymatic hydrolysis can improve the quality of oilseed cake extracts by an increase in the phenolic and flavonoid content. It can also improve the antioxidant properties of oilseed cake extracts by biotransformation of polyphenolic glycosides to their aglycone form. This is accompanied by an increased reduction of sugar content. Thus, the obtained extracts may render better antioxidant activity than the non-hydrolysed extracts since

aglycones have free hydroxyl groups corresponding to the described phenomenon. Oilseed cake extracts after enzymatic hydrolysis may have numerous applications in food, pharmaceutical and cosmetic industries. However, the above observations require further research into the influence of enzymatic hydrolysis on oilseed cakes and into the biological properties (*e.g.* antimicrobial activity, enzyme inhibition) of products obtained by enzymatic hydrolysis.

References

1. M. Wettasinghe, F. Shahidi, R. Amarowicz, Identification and quantification of low molecular weight phenolic antioxidants in seeds of evening primrose (*Oenothera biennis* L.), *J. Agric. Food Chem. 50* (2002) 1267–1271.
2. M. Wettasinge, F. Shahidi, Antioxidant and free radical-scavenging properties of ethanolic extracts of deffated borage (*Borago officinalis* L.) seeds, *Food Chem. 67* (1999) 399–414.
3. M.F. Ramadan, L.W. Kroh, J.T. Mörsel, Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions, *J. Agric. Food Chem. 51* (2003) 6961–6969.
4. *Lipids, Lipophilic Components and Essential Oils from Plant Sources*, S.S. Azimova, A.I. Glushenkova, V.I. Vinogradova (Eds.), Springer, New York, NY, USA (2012).
5. A.K. Kiss, M. Derwinska, A. Dawidowska, M. Naruszewicz, Novel biological properties of *Oenothera paradoxa* defatted seed extracts: Effects on the metallopeptidase activity, *J. Agric. Food Chem. 56* (2008) 7845–7852.
6. B.H. Ali, G. Blunden, Pharmacological and toxicological properties of *Nigella sativa*, *Phytother. Res. 17* (2003) 299–305.
7. M.M. Engler, M.B. Engler, S.M. Paul, Effects of dietary borage oil rich in γ-linolenic acid on blood pressure and vascular reactivity, *Nutr. Res. 12* (1992) 519–528.
8. B. Matthaeus, Antioxidant activity of extracts obtained from residues of different oilseeds, *J. Agric. Food Chem. 50* (2002) 3444–3452.
9. M. Nogala-Kalucka, M. Rudzinska, R. Zadernowski, A. Siger, I. Krzyzostaniak, Phytochemical content and antioxidant properties of seeds of unconventional oil plants, *J. Am. Oil Chem. Soc. 87* (2010) 1481–1487.
10. N. Balasundram, K. Sundram, S. Samman, Phenolic compounds in plant and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses, *Food Chem. 99* (2006) 191–203.
11. W. Peschel, W. Dieckmann, M. Sonnenschein, A. Plescher, High antioxidant potential of pressing residues from evening primrose in comparison to other oilseed cakes and plant antioxidants, *Ind. Crops Prod. 25* (2007) 44–54.
12. P. Terpinc, B. Čeh, N. Poklar Ulrih, H. Abramovič, Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts, *Ind. Crops Prod. 39* (2012) 210– 217.
13. Y.Y. Soong, P.J. Barlow, Antioxidant activity and phenolic content of selected fruit seeds, *Food Chem. 88* (2004) 411–417.
14. C.A. Rice-Evans, N.J. Miller, G. Paganga, Antioxidant properties of phenolic compounds, *Trends Plant Sci. 2* (1997) 152–159.
15. H.K. Obied, M.S. Allen, D.R. Bedgood, P.D. Prenzler, K. Robards, R. Stockmann, Bioactivity and analysis of biophenols from olive mill waste, *J. Agric. Food Chem. 53* (2005) 823–837.
16. M. Wettasinghe, F. Shahidi, Evening primrose meal: A source of natural antioxidants and scavenger of hydrogen-derived free radicals, *J. Agric. Food Chem. 47* (1999) 1801–1812.
17. R. Zadernowski, M. Naczk, H. Nowak-Polakowska, Phenolic acids of borage (*Borago officinalis* L.) and evening primrose (*Oenothera biennis* L.), *J. Am. Oil Chem. Soc. 79* (2002) 335–338.
18. I. Merfort, V. Wray, H.H. Brakat, S.A.M. Hussein, M.A.M. Nawwar, G. Willuhn, Flavonol triglycosides from seeds of *Nigella sativa*, *Phytochem*istry, *46* (1997) 359–363.
19. H.V. Annegowda, C.W. Nee, M.N. Mordi, S. Ramanathan, S.M. Mansor, Evaluation of phenolic content and antioxidant property of hydrolysed extracts of *Terminalia catappa* L. leaf, *Asian J. Plant Sci. 9* (2010) 479–485.
20. K. Krygier, F. Sosulski, L. Hogge, Free, esterified and insoluble-bound phenolic acids. Extraction and purification procedure, *J. Agric. Food Chem. 30* (1982) 330–334.
21. H. Zhao, J. Dong, J. Lu, J. Chen, Y. Li, L. Shan *et al.*, Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.), *J. Agric. Food Chem. 54* (2006) 7277–7286.
22. P. Terpinc, T. Polak, N. Poklar Ulrih, H. Abramovič, Effect of heat treatment (*Camelina sativa*) seeds on the antioxidant potential of their extracts, *J. Agric. Food Chem. 59* (2011) 8639–8645.
23. C. Soto, J. Concha, M.E. Zuniga, Antioxidant content of oil and defatted meal obtained from borage seeds by an enzymatic-aided cold pressing process, *Process Biochem. 43* (2008) 696–699.
24. Z.G. Weinberg, B. Akiri, E. Potoyevski, J. Kanner, Enhancement of polyphenol recovery from rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*) by enzyme-assisted ensiling (ENLAC), *J. Agric. Food Chem. 147* (1999) 2959–2962.
25. H. Miszkiewicz, A. Jakubowski, Solid-state production of phenolic antioxidants from mixtures of aronia pomace and evening primrose waste, *Acta Sci. Pol. Biotechnol. 7* (2008) 13–25.
26. T. Zaki, I. Shahid, I. Maznah, Effects of hydrolytic conditions on recovery of antioxidants from methanolic extracts of *Nigella sativa* seeds, *J. Med. Plants Res. 5* (2011) 3152–3158.
27. Z. Zheng, K. Shetty, Solid-state bioconversion of phenolics from cranberry pomace and role of *Lentinus edodes* β-glucosidase, *J. Agric. Food Chem. 48* (2000) 895–900.
28. G. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem. 31* (1959) 426–428.
29. P. McCue, K. Shetty, Role of carbohydrate-cleaving enzymes in phenolic antioxidant mobilization from whole soybean fermented with *Rhizopus oligosporus*, *Food Biotechnol. 17* (2003) 27–37.
30. L. Stasiak-Rozanska, S. Blazejak, A. Ratz, Investigations into the optimization of parameters of glycerol biotransformation to dihydroxyacetone with the use of immobilized cells of *Gluconacetobacter xylinus*, *Pol. J. Food Nutr. Sci. 60* (2010) 273–280.
31. V.L. Singleton, J.A. Rossi Jr, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am. J. Enol. Vitic. 16* (1965) 144–158.
32. V. Dragović-Uzelac, Z. Savić, A. Brala, B. Levaj, D. Burasać Kovačević, A. Biško, Evaluation of phenolic content and antioxidant capacity of blueberry cultivars (*Vaccinium corymbosum* L.) grown in the northwest Croatia, *Food Technol. Biotechnol. 48* (2010) 214–221.
33. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci. Technol. 28* (1995) 25–30.

34. N. Pellegrini, D. Del Rio, B. Colombi, M. Bianchi, F. Brighenti, Application of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages, *J. Agric. Food Chem. 51* (2003) 260–264.

35. T.C.P. Dinis, V.M.C. Madeira, L.M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, *Arch. Biochem. Biophys. 315* (1994) 161–169.

36. I.Y. Volkov, N.A. Lunina, G.A. Velikodvorskaya, Prospects for the practical application of substrate-binding modules of glycosyl hydrolases, *Appl. Biochem. Microbiol. 40* (2004) 427–432.

37. D. Bajpai, V.K. Tyagi, Laundry detergents: An overview, *J. Oleo Sci. 56* (2007) 327–340.

38. A.K. Kiss, M. Naruszewicz, Polyphenolic compounds characterization and reactive nitrogen species scavenging capacity of *Oenothera paradoxa* defatted seed extracts, *Food Chem. 131* (2012) 485–492.

39. A.A. Mariod, R.M. Ibrahim, M. Ismail, N. Ismail, Antioxidant activity and phenolic content of phenolic rich fractions obtained from black cumin (*Nigella sativa*) seedcake, *Food Chem. 116* (2009) 306–312.

40. M.T.J. Johnson, A.A. Agrawal, J.L. Maron, J.P. Salminen, Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment, *J. Evol. Biol. 22* (2009) 1295–1307.

41. D. Amić, D. Davidović-Amić, D. Bešlo, V. Rastija, B. Lučić, N. Trinajstić, SAR and QSAR of the antioxidant activity of flavonoids, *Curr. Med. Chem. 14* (2007) 827–845.

42. D. Muth, P. Kachlicki, Methods of flavonoids' analysis in plant material, *Biotechnology, 85* (2009) 65–80.

43. Z. Zheng, K. Shetty, Solid-state production of beneficial fungi on apple processing wastes using glucosamine as the indicator of growth, *J. Agric. Food Chem. 46* (1998) 783–787.

44. A. Ratz-Lyko, J. Arct, K. Pytkowska, Methods for evaluation of cosmetic antioxidant capacity, *Skin Res. Technol. 18* (2012) 421–430.

45. L. Laroze, C. Soto, M.E. Zúñiga, Phenolic antioxidants extraction from raspberry wastes assisted by-enzymes, *Electron. J. Biotechnol. 13* (2010) 1–11.

46. D. Malencic, Z. Maksimovic, M. Popovic, J. Miladinovic, Polyphenol contents and antioxidant activity of soybean seed extracts, *Bioresour. Technol. 99* (2008) 6688–6691.

47. R.T.P. Correia, P. McCue, D.A. Vattem, M.M.A. Magãlhaes, G.R. Macêdo, K. Shetty, Amylase and *Helicobacter pylori* inhibition by phenolic extracts of pineapple wastes bioprocessed by *Rhizopus oligosporus*, *J. Food Biochem. 28* (2004) 419–434.

48. M. Brito-Arias: *Synthesis and Characterization of Glycosides*, Springer Science+Busines Media LLC, New York, NY, USA (2007).

49. M. Wettasinghe, F. Shahidi, Iron(II) chelation activity of extracts of borage and evening primrose meals, *Food Res. Int. 35* (2002) 65–71.

50. M. Ferrali, C. Signorini, B. Caciotti, L. Sugherini, L. Ciccoli, D. Giachetti, M. Comporti, Protection against oxidative damage of erythrocyte membrane by the flavonoid quercetin and its relation to iron chelating activity, *FEBS Let. 416* (1997) 123–129.

51. S.A.B.E. Van Acker, D.J. Van Den Berg, M.N.J.L. Tromp, D.H. Griffioen, W.P. Van Bennekom, W. J. F. Van Der Vijgh, A. Bast, Structural aspects of antioxidant activity of flavonoids, *Free Radic. Biol. Med. 20* (1996) 331–342.

52. M.S. Marzouk, F.A. Moharram, R.A. El Dib, S.M. El-Shenawy, A.F. Tawfike, Polyphenolic profile and bioactivity study of *Oenothera speciosa* Nutt. aerial parts, *Molecules, 14* (2009) 1456–1467.

53. H.D. Zinsmeister, I. Plitzko, H. Schels, Flavonols glycosides in South American species of *Oenothera* sect. Oenothera, *Phytochemistry, 16* (1977) 497–450.

54. A.K. Kiss, M. Derwinska, S. Granica, Quantitative analysis of biologically active polyphenols in evening primrose (*Oenothera paradoxa*) seeds aqueous extracts, *Pol. J. Food Nutr. Sci. 61* (2011) 109–113.