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Chemical composition, functional properties, and bioactivities of rapeseed protein isolates

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Abstract

In order to utilize rapeseed protein from oil industry waste for food applications, rapeseeds were pretreated to remove the oil using hexane. Two protein isolates were prepared, one by precipitation at controlled pH and the other by ultrafiltration. The precipitated and ultrafiltered protein isolates, respectively, contained 70.8% and 98.7% protein. The ultrafiltered protein isolate had a better emulsification capacity than had whole egg. The ultrafiltered protein isolate had a protein solubility of 52.5–97.2% in the range pH 3–9, whilst the maximum protein solubility of the precipitated protein isolate was 26.4% in the pH range 7–9. There were no significant differences between the precipitated and ultrafiltered protein isolates regarding their angiotensin converting enzyme inhibition are their bile acid-binding capacity. Their bile acid-binding capacity and angiotensin converting enzyme inhibition capacities were lower than of those de-oiled soybean. They showed stronger DPPH radical-scavenging activity than did de-oiled soybean.

Keywords: *Brassica napus*; Protein isolate; Functional properties; ACE inhibition; Bile acid-binding; DPPH radical-scavenging activity

1. Introduction

Rapeseed is one of the important oilseed crops. After oil extraction, a protein-rich meal results and this is usually used for animal feeds or fertilizers. The protein meal has a well-balanced amino acid composition (Pastuszewska, Jablecki, Swiech, Buraczewska, & Ochtabinska, 2000). However, it also contains glucosinolates, phytates and phenolics which are problematic/toxic for food use. Since those toxic compounds have significantly lower molecular weights than have rapeseed proteins, precipitation at controlled pH or separation by ultrafiltration could potentially be used to separate the proteins from those toxic compounds. In this study, we have tried to prepare protein isolate from de-oiled rapeseed by precipitation and by ultrafiltration. These protein isolates should be useful for food applications, for changing the emulsifying capacity and for improving the texture of the products. In addition, isolation of the proteins may have health benefits, such as angiotensin I converting enzyme (ACE, dipeptidyl-carboxypeptidase) inhibition, bile acid-binding and free radical-scavenging activities.

ACE is an important enzyme in the control of blood pressure. Inhibition of ACE is beneficial for patients suffering from hypertension. Since it is recommended to use foods to inhibit ACE, many studies have investigated ACE inhibitory fractions in food. It is well documented that the hydrolyzed protein fraction effectively inhibits ACE. Up until now, hydrolyzed protein from cheese whey (Abubakar, Saito, Kitazawa, Kawai, & Itoh, 1998), casein (Kohmura et al., 1989), soy sauce (Kinoshita, Yamakoshi, & Ikeuchi, 1993), soybean (Okamoto, Hanagata, Kawamura, & Yanagida, 1995), tuna (Kohama et al., 1988) and

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bonito (Matsumura, Fujii, Takeda, Sugita, & Shimizu, 1993) have been reported to show ACE inhibition. Marczak et al. (2003) commented on the antihypertensive peptides from rapeseeds. However, they focussed on isolating the ACE inhibitory fraction for pharmaceutical use, not for food applications. In the present study, we have investigated the ACE inhibition of rapeseed protein isolates from a food processing point of view.

Bile acids, synthesized in the liver from cholesterol, help the emulsification and absorption of fats by micelle formation (Hall, Kok, & Javitt, 1988). We have reported the bile acid-binding capacity of lupin protein isolates and demonstrated that they had a bile acid-binding capacity similar to the cholesterol-reducing agent, cholestyramine (Yoshie-Stark & Wäsche, 2004). Bile acid-binding promotes the consumption of cholesterol in the liver and hence information about the bile acid-binding capacity of rapeseed protein isolates is important.

Radicals, such as superoxide, hydrogen peroxide and hydroxyl, are physiological metabolites that commonly exist throughout the body. It is well known that radicals cause disease and therefore it is advantageous to remove radicals to reduce the risk of disease. Matthäus (2002) reported the radical-scavenging activity of certain fractions from industrial oilseed residues. His approach was to extract the antioxidant fraction but there was no report about the radical-scavenging activity of protein isolates from de-oiled rapeseeds.

Proteins are digested in the gastrointestinal tract. We therefore evaluated the ACE inhibition, bile acid-binding capacity and radical-scavenging activities of de-oiled rapeseed (rapeseed meal), rapeseed protein isolates and their hydrolyzates.

As our study planned to utilize protein isolates for food applications, we analyzed the functional properties of rapeseed protein isolates and investigated the health aspects of rapeseed protein (mentioned above) using *in vitro* techniques.

2. Materials and methods

2.1. Materials

Rapeseeds (*Brassica napus* var. Express) were obtained from NPZ Hans-Georg Lembke KG, Germany. Kernels and hulls were separated in a dry mill and the kernels were flaked with a roll mill. The rapeseed flakes were de-oiled using hexane and these flakes were used for the protein isolate. The flakes were milled using a Retsch ZM-100 mill (Düsseldorf, Germany) to a powder (<0.1 mm).

The rapeseed proteins were extracted from the de-oiled flakes. The extraction procedure is shown in Fig. 1. As we have previously reported (Wäsche & Schönweiz, 2003), the total protein was extracted at slightly alkaline pH and a protein fraction was precipitated at slightly acidic pH. From the results of a preliminary test, as mentioned in the report of Wäsche and Schönweiz (2003), the conditions of mixing (solid:liquid = 2:10), pasteurization, and spray-drying were fixed, and the resulting protein powder was microbiologically safe for use in food processing. Ultrafiltered protein isolate was recovered from the acid supernatant by cross-flow ultrafiltration (Polysulfon, Asahi Chemical, Japan) with a molecular weight cut-off at 10 kDa.

Pepsin, pancreatin, ACE from rabbit lung, bile acid analysis kit and DPPH radicals were purchased from Sigma (St. Louis, MO, USA). All the other reagents used for the experiments were of analytical grade.

2.2. Proximate analysis

The chemical composition (dry matter, nitrogen content, ash content and oil content) of the de-oiled rapeseed flakes and the processed protein samples were analyzed in accordance with the official method (Anon., 1995).

2.3. Functional properties

The functional properties of the de-oiled rapeseed flakes and the protein isolates were determined using standardized methods.

The protein solubility was analyzed, following the method of Morr et al. (1985). The nitrogen solubility index (NSI) was determined in accordance with the official AOCS (1998) or AACC (2000) methods. The protein solubility curve was obtained by mixing protein samples (1 g) in 50 ml of 100 mM sodium chloride solution at a set pH and at ambient temperature for 60 min. The non-dissolved fraction was separated by centrifugation at 20,000*g* for 15 min. The protein remaining in solution was determined by nitrogen analysis, and then multiplying the recorded nitrogen value by 6.25. The protein solubility is given as the percentage of dissolved protein compared to the protein content of the starting sample.

To analyze emulsifying capacity, the protein solution (1%, w/w) was stirred at constant temperature (20 °C) in a 1 l laboratory reactor (IKA) with a stirrer and a Ultra-Turrax emulsifying system (IKA-Werke GmbH & Co. KG, Staufen, Germany). The oil was automatically added by a titration system (Metrohm GmbH & Co. KG, Herisau, Switzerland). The conductivity was continuously measured and used as parameter for the determination of the inversion point of the emulsion. The amount of oil which was added up to the inversion point of the emulsion was used to calculate the emulsifying capacity [ml oil/g protein].

For the emulsion stability test, emulsions [1:10:10 (w/v/v)] were prepared in a 1 l laboratory reactor (IKA) with a stirrer and an emulsifying system as mentioned above. The homogenization was done at 11,000 rpm for 5 min. After homogenization, the emulsion was poured into centrifuge tubes. The tubes were heated at 80 °C for 30 min and then stored at 5 °C for 12 h. After storing, the samples were centrifuged at 4500*g* for 10 min at 20 °C. The stability

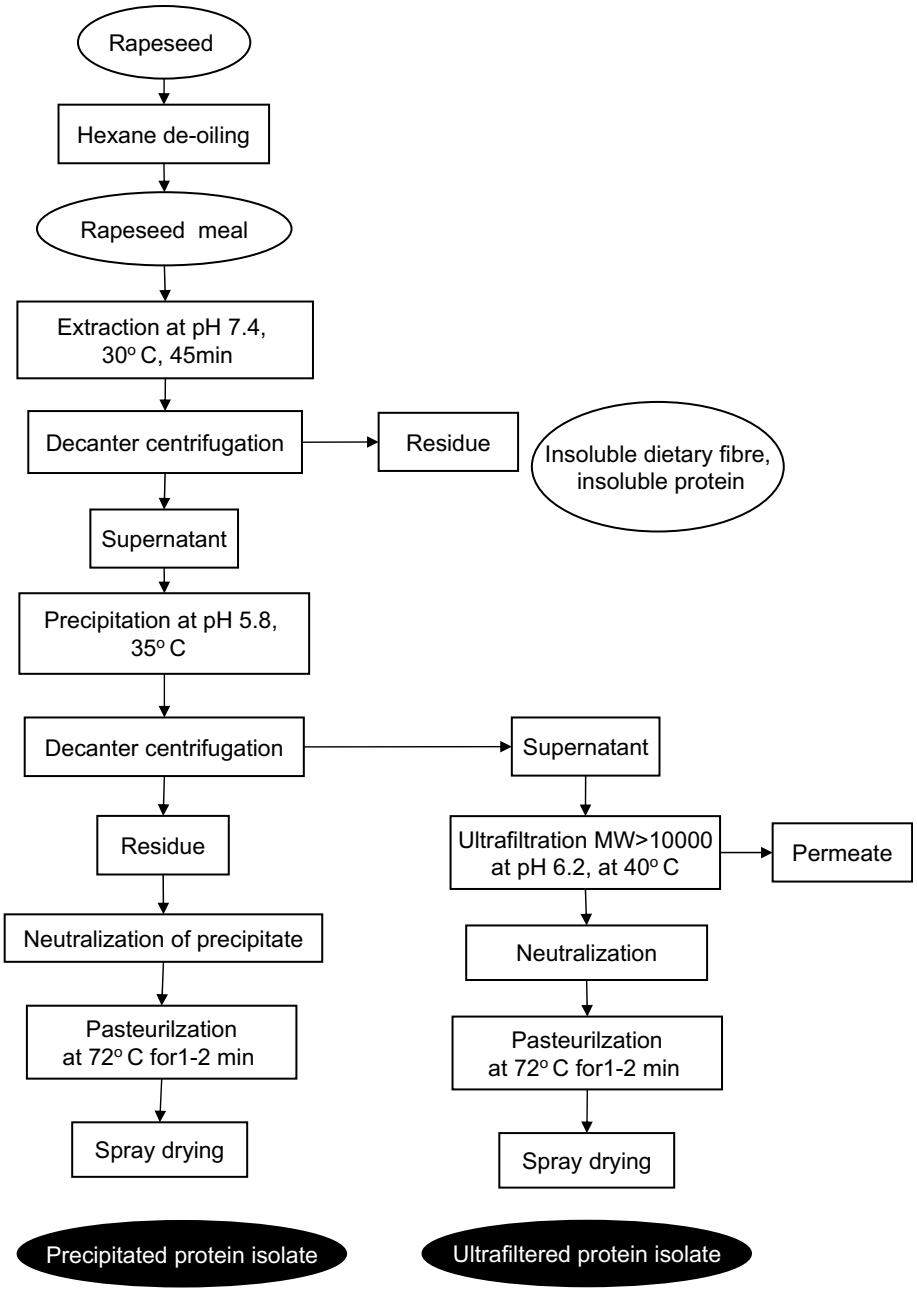

Fig. 1. Protein extraction process from rapeseed meal.

of the emulsion after centrifugation was calculated as follows:

$$\text{Emulsification stability } (\%) = (\text{volume of emulsified layer})/(\text{total volume}) \times 100$$

2.4. Protein hydrolysis for ACE inhibitory assay, bile acid-binding assay and radical-scavenger studies

Protein hydrolysis was performed by following the methods of Pihlanto-Leppälä, Rokka, and Korhonen (1998) Mullally, Meisel, and FitzGerald (1997) with some modification as mentioned in our report (Yoshie-Stark & Wäsche, 2004). The degree of hydrolysis was analyzed following the method of Nielsen, Petersen, and Dambmann (2001).

2.5. ACE inhibitory activity

The ACE inhibitory activity was measured spectrophotometrically using hippuryl-L-histidyl-leucine (Hip-His-Leu) as the substrate, according to the method of Cushman and Cheung (1971) and Hernández-Ledesma et al. (2003) with minor modification, as mentioned in our report (Yoshie-Stark, Bez, Wada, & Wäsche, 2004). Captopril, which is reported to have strong ACE inhibition, was also used for this study.

2.6. Bile acid-binding assay

The *in vitro* bile acid-binding assay was carried out by the method of Yoshie-Stark and Wäsche (2004). Briefly, de-oiled rapeseed powder or freeze-dried hydrolyzed samples were mixed with 1.5 mM sodium cholate solution in 100 mM sodium phosphate buffer (pH 7.0) at a concentration of 1 mg/ml and incubated at 37 °C for 2 h. Samples were centrifuged and the supernatants were collected and analyzed for bile acid using a spectrophotometer (530 nm, Sigma bile acid analysis kit 450). Cholestyramine resin, a bile acid-binding and cholesterol lowering drug, was also evaluated for its bile acid-binding ability.

2.7. DPPH radical-scavenger study

Samples were prepared by the method of Duh, Tu, and Yen (1999). The method, used to determine the DPPH radical-scavenging activity, was carried out by the protocol described in the report of Yoshie-Stark et al. (2004). From a calibration curve obtained with different dilutions of the extract, the median effective dose (ED_{50}) was calculated. The ED_{50} is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the given experimental conditions. Trolox, which is known to be a strong DPPH radical-scavenger, was also tested.

2.8. Statistical analysis

The results are presented as mean values ± SD ($n = 3$–8). ANOVA was used to calculate significant differences.

3. Results and discussion

3.1. Chemical composition

The chemical compositions of raw rapeseed, de-oiled rapeseed (rapeseed meal), precipitated protein isolate and ultrafiltered protein isolate are shown in Table 1. After the protein isolation process shown in Fig. 1, rapeseed meal protein (as 100%) was fractionated to precipitated protein isolate (37%) and ultrafiltered protein isolate (32%). The extraction at pH 7.4 did not dissolve all proteins in rapeseed meal; 22% of protein became a pellet with insoluble dietary fibre. When rapeseeds are harvested, they contain 19.0% of protein and 54.2% of fat. After de-oiling, the rapeseed had a fat content of 6.4%. When we produced lupin protein isolate, the de-oiled lupins had less than 2% fat (Wäsche, Müller, & Knauf, 2001). This is much lower than that of rapeseed meal. After processing, the precipitated protein isolate and ultrafiltered protein isolate, respectively, contained 70.8% and 98.7% of protein and 8.2% and 1.2% of fat. The ultrafiltered protein isolate had a higher percentage of protein than had the precipitated protein isolate. This shows that the protein isolation method produces different protein fractions, one including fibres and the other not. Thus, factors such as functional properties, important for apply food processing and bioactivity, related to health claims, were expected to be different, depending on the fraction.

3.2. Functional properties

Fig. 2 shows the protein solubilities of the precipitated protein isolate and ultrafiltered protein isolate as a function of pH. Precipitated protein isolate was not solubilized at pH 3 and 4 but was solubilized at pH 5–9 by 21.3–26.4%. Ultrafiltered protein isolate showed good solubility. It had a solubility greater than 90% at pH 5–9. The protein solubilities of ultrafiltered protein isolate were 52.5% and 60.4% at pH 3 and 4, respectively. Ultrafiltered protein isolate contained a higher percentage of protein than did precipitated protein isolate; however, the protein solubility was greater than the solubility of the precipitated protein isolate at all tested pH values. When we tested the solubility of lupin protein isolates in an earlier study, their maximum solubility was 72% (data not shown).

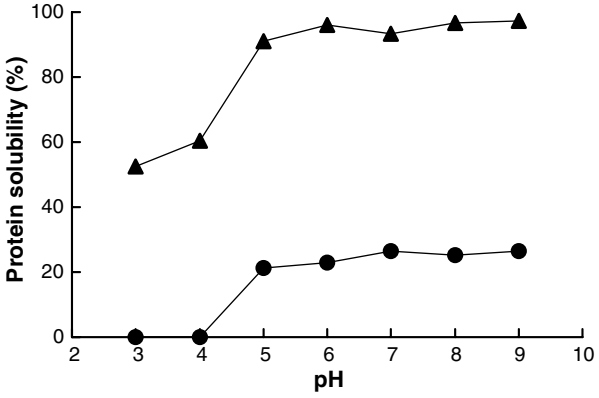

Fig. 2. Protein solubility as a function of pH for rapeseed protein isolates: precipitated protein isolate (•) and ultrafiltered protein isolate (▲).

Table 1
Chemical composition of de-oiled rapeseed, rapeseed protein isolates and de-oiled soybean

	Dry matter (%)	Protein (%, DM)	Ash (%, DM)	Fat (%, DM)	Fibre (%, DM)
Rapeseed (raw)	92.99	19.0	3.60	54.2	23.2
Rapeseed (hexane de-oiled)	92.20	48.2	7.90	6.37	37.5
Precipitated protein isolate	91.01	70.8	10.8	8.23	10.2
Ultrafiltered protein isolate	92.25	98.7	3.05	1.18	–
Hexane de-oiled soybean	90.00[a]	61.0[a]	6.00[a]	2.00[a]	6.00

[a] Data from Yoshie-Stark and Wäsche (2004).

Commercial soy protein showed a minimum solubility of 52% at pH 4.0 and a maximum solubility of 62% at pH 10.0. Commercial sesame protein showed a minimum solubility of 8% at pH 6.0 and a maximum solubility of 75% at pH 2.0 (López, Flores, Gálvez, Quirasco, & Farrés, 2003). Compared to those values, ultrafiltered rapeseed protein isolate showed a higher solubility at all pH values. Egg white ovalbumin is known to have a minimum solubility at pH 4.5 (iso-electric point). By contrast, ultrafiltered rapeseed protein isolate showed 60.4% solubility at pH 4 and 91.1% solubility at pH 5. This indicates that ultrafiltered rapeseed protein may be useful as a replacement for egg white, at various pH levels, for allergic people. There are some reports concerning plant protein isolates for food applications, but many of these show lower solubility at pH 4–5 (Achouri, Zhang, & Shiying, 1998; Molina Ortiz & Wagner, 2002). Low solubility decreases the potential use of the protein in food applications. Highly soluble protein such as ultrafiltered rapeseed protein isolate is more advantageous.

The nitrogen re-solubility at pH 7, emulsification capacity and emulsification stability are shown in Table 2. Precipitated protein isolate had 26.4% nitrogen re-solubility, whilst ultrafiltered protein isolate had 93.3% at pH 7. Ultrafiltered protein isolate showed significantly higher N re-solubility at pH 7. Compared to the nitrogen re-solubility of protein isolates from lupin (19.2–33.8%, El-Adawy, Rahma, El-Bedawey, & Gafar, 2001; Lqari, Vioque, Pedroche, & Millán, 2002), ultrafiltered rapeseed protein showed higher N re-solubility (93.3%). Precipitated and ultrafiltered protein isolates have emulsification capacity values of 400 and 693 ml oil/g protein, respectively. There are many reports relating to the emulsification capacity of plant proteins from lupin (164–169 ml oil/g protein) (El-Adawy et al., 2001), mung bean (245 ml oil/g protein, El-Adaway, 2000), soy (107 ml oil/g protein, Gao, Nguyen, & Utioh, 2001), pea (69–76 ml oil/g protein, Gao et al., 2001) and sesame (130 ml oil/g protein, Khalid, Babiker, & EL Tinay, 2003). In our study, both protein isolates from rapeseed showed emulsification capacities higher than the values given in these reports. When we determined the functional properties of whole egg and egg white (shown in Table 2) for comparison purposes, the emulsification capacity and emulsification stability of ultrafiltered protein isolate were found to be greater than for whole egg and smaller than for egg white. Taking all of the functional properties into consideration, the ultrafiltered protein isolate is considered to have the better functional properties for food applications.

Table 2
Functional properties of rapeseed protein isolates and eggs

Sample	N re-solubility at pH 7 (%)	Emulsification capacity (EC) (ml/g)	Emulsification stability (%)
Precipitated protein isolate	26.4	400	88.5
Ultrafiltered protein isolate	93.3	693	96.0
Egg, whole	65.0[a]	495[a]	89.0[a]
Egg, white	100[a]	800[a]	100[a]

[a] Data from Yoshie-Stark et al., 2004.

3.3. ACE inhibition

In this study, results are expressed as percent ACE inhibition by 150 μg samples (final concentration in the reaction tube was 1 mg/ml). From our preliminary studies, the concentration of captopril was fixed at 1.5 ng (0.01 μg/ml) in our experiments.

As shown in Fig. 3, ACE was inhibited by de-oiled rapeseed and its hydrolyzate to a degree of 21.6–23.1%, by precipitated protein isolate and its hydrolyzate to a degree of 17.4–26.3% and by ultrafiltered protein isolate and its hydrolyzate to a degree of 14.7–24.6%. It was also inhibited by soybean and its hydrolyzate to a degree of 1.1–25.0% and by 0.01 μg/ml captopril to a degree of 48.0%. The inhibition capacity increased with the progress of the hydrolysis, as reported by others (Yust et al., 2003; Vermeirssen, Van Camp, & Verstraete, 2002), but there was no significant difference in ACE inhibition upon hydrolysis.

Rapeseed protein isolates had a degree of hydrolysis (DH, see Table 3) of 3.42–9.05% and de-oiled soybean had a DH of 3.34–5.66%. The highest ACE inhibition (26.3%) was shown by pepsin–pancreatin digested precipitated protein isolate with a DH of 9.05%, whilst pepsin–pancreatin-digested ultrafiltered protein isolate had a DH of 7.23%, corresponding to 24.6% of ACE inhibition. Compared to de-oiled soybean, all the rapeseed samples showed higher ACE inhibition. We expected stronger ACE inhibition by ultrafiltered protein isolate because of the high protein solubility and higher DH value. However, the

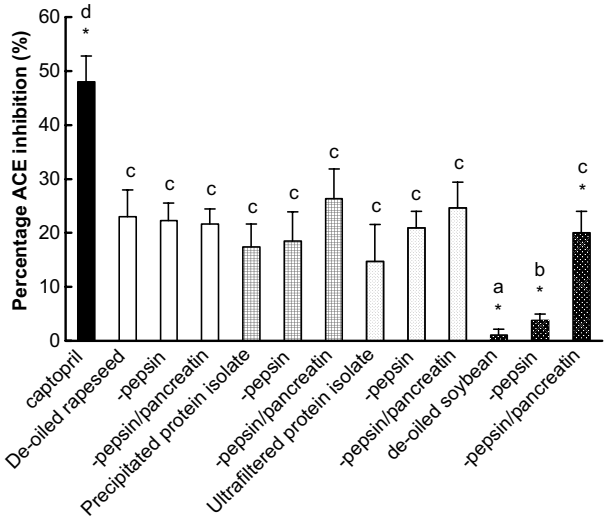

Fig. 3. ACE inhibition by de-oiled rapeseed, rapeseed protein isolates, de-oiled soybean and their hydrolyzates. *Data from Yoshie-Stark et al. (2004). Different letters indicate significant differences ($p < 0.05$).

Table 3
Degree of hydrolysis (%)

	Pepsin	Pepsin–pancreatin
Rapeseed (hexane de-oiled)	11.9	17.3
Precipitated protein isolate	4.55	9.05
Ultrafiltered protein isolate	3.42	7.23
Hexane de-oiled soybean	3.34[a]	5.66[a]

[a] Data from Yoshie-Stark and Wäsche (2004).

precipitated protein isolate showed the greater ACE inhibition. A more active peptide for ACE inhibition may be present in the precipitated protein isolate. Precipitated protein isolate might therefore be a good source of extractable bioactive compounds for reducing blood pressure.

We tested 4–5 more concentration points for IC_{50} (50% ACE inhibition) values and found the IC_{50} values of captopril, soybean pepsin/pancreatin hydrolyzate (DH 5.66%), de-oiled rapeseed pepsin–pancreatin hydrolyzate (DH 17.3%), precipitated protein isolate pepsin/pancreatin hydrolyzate (DH 9.05%) and ultrafiltered protein isolate pepsin/pancreatin hydrolyzate (DH 7.23%) to be 0.004 μg/ml, 0.37 mg/ml, 0.51 mg/ml, 0.37 mg/ml and 0.47 mg/ml, respectively. There were no significant differences between the rapeseed samples and soybean. Marczak et al. (2003) reported that rapeseed protein digest, using pepsin (IC_{50} 0.16 mg/ml), showed stronger ACE inhibition than did a digest using pepsin–pancreatin (IC_{50} 0.70 mg/ml). Compared to their results, our pepsin–pancreatin hydrolyzate from precipitated protein isolate (IC_{50} 0.37 mg/ml) showed stronger ACE inhibition. This protein isolate may be useful as a good source of natural ACE inhibitor. Wu and Ding (2002) reported the IC_{50} values of enzymatic hydrolyzate of soy protein at 0.34 mg/ml. The ACE inhibition by soybean hydrolyzate showed quite similar activity. Captopril was reported to show a variety of IC_{50} values under various experimental conditions, e.g. 0.00035 μg/ml (Vermeirssen et al., 2002) and 0.085–0.092 μg/ml (Watanabe, Mazumder, Nagai, Tsuji, & Terabe, 2003). Bovine skin gelatin hydrolyzate, legumin hydrolyzate, whey digest and pea protein digest were, respectively, reported to have IC_{50} values of 0.76 mg/ml (Kim, Byun, Park, & Shahidi, 2001), 0.18 mg/ml (Yust et al., 2003), 0.90 mg/ml (Vermeirssen et al., 2002) and 1.36 mg/ml (Vermeirssen et al., 2002). Compared to these reported values, our experimental IC_{50} for captopril showed a similar value and rapeseed samples showed ACE inhibition activity similar to soybean hydrolyzate. Considering the small degree of hydrolysis, de-oiled rapeseed and protein isolates might also be powerful ACE inhibitors.

3.4. Bile acid-binding

As shown in Fig. 4 (top), sodium cholate was bound by cholestyramine, de-oiled rapeseed, precipitated protein isolate, ultrafiltered protein isolate, de-oiled soybean and their hydrolyzates. Sodium cholate was bound by de-oiled rapeseed and its hydrolyzate to a degree of 11.6–16.0%, by precipitated protein isolate and its hydrolyzate to a degree of 5.77–12.7% and by ultrafiltered protein isolate and its hydrolyzate to a degree of 7.38–11.1%. It was also bound by cholestyramine to a degree of 53.1% and by soybean and its hydrolyzate to a degree of 11.9–18.5%. As shown in Fig. 4 (bottom), sodium deoxycholate was bound by cholestyramine, de-oiled rapeseed, precipitated protein isolate, ultrafiltered protein isolate, de-oiled soybean and their hydrolyzates. Sodium cholate was bound by de-oiled rapeseed and its hydrolyzate to a degree of 13.0–30.7%, by precipitated protein isolate and its hydrolyzate to a degree of 10.0–12.6% and by ultrafiltered protein isolate and its hydrolyzate to a degree of 5.81–22.8%. Sodium deoxycholate was bound by cholestyramine to a degree of 58.2% and by soybean and its hydrolyzate to a degree of 12.6–15.0%. The degree of hydrolysis increased following the digestion process. However, neither the sodium cholates or the

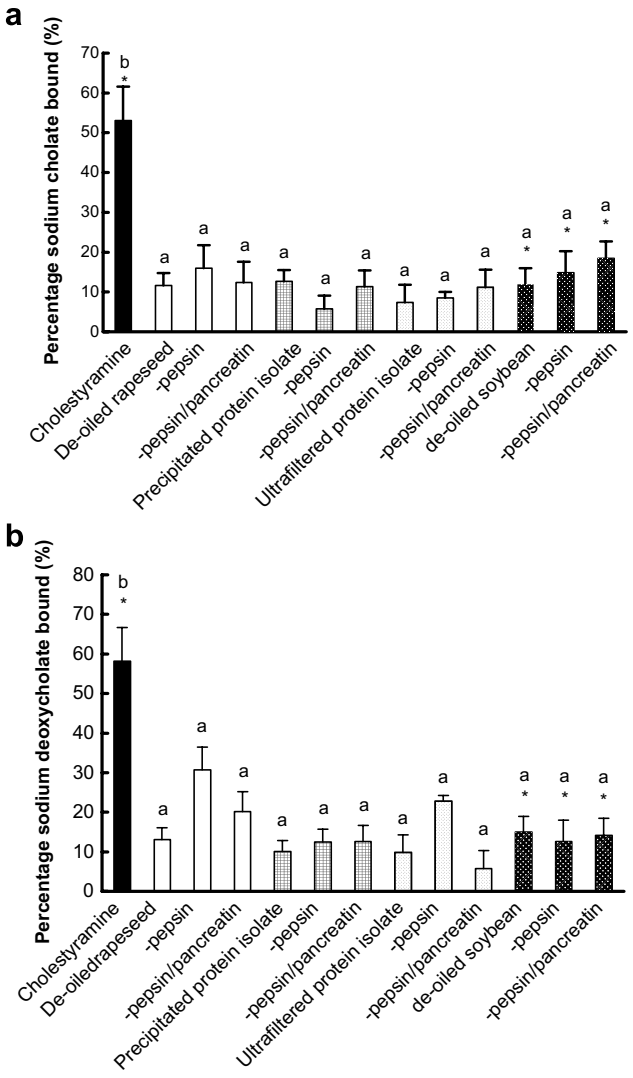

Fig. 4. Bile acid-binding by de-oiled rapeseed, rapeseed protein isolates, de-oiled soybean and their hydrolyzates. Top: Sodium cholate binding and bottom: sodium deoxycholate binding. *Data from Yoshie-Stark and Wäsche (2004). Different letters indicate significant differences ($p < 0.05$).

sodium deoxycholate-binding capacity were significantly affected by hydrolysis. There was no significant difference between any tested samples, except for cholestyramine.

We tested the bile acid-binding at a final bile acid concentration of 1.5 mM because of the concentration of bile acid in the body (1.5–7 mM). Camire, Zhao, and Violette (1993) and Camire and Dougherty (2003) tested cholate binding at a cholate concentration of 12.5 mM, and they reported cholate binding of 75% by cholestyramine, 15–20% by three types of raisin and 10% by wheat bran. In our study, de-oiled rapeseed and precipitated protein isolates showed 11–16% and 5.8–13% sodium cholate binding, and 13–31% and 10–13% sodium deoxycholate binding. As de-oiled rapeseed contained approximately 35% of fibre, these similar values to the sodium cholate binding by wheat bran may be mainly due to the effect of fibre, not due to the protein in the sample. However, ultrafiltered protein isolate without fibre still showed bile acid binding after pepsin–pancreatin digestion. This indicates that some large molecular weight protein fraction binds bile acid in our gastrointestinal tract. As deoxycholate is a secondary bile acid which is produced by bacteria in the intestine, it is advantageous to bind and remove this type of secondary bile acid from the body. However, none of the tested samples showed specific binding to deoxycholate.

3.5. DPPH radical-scavenging activity

The DPPH radical-scavenging activities of de-oiled rapeseed, rapeseed protein isolates, de-oiled soybean and their hydrolyzates are shown in Fig. 5. As this study is the first attempt at testing the radical-scavenging activity of rapeseed proteins, hot water-soluble fractions were evaluated, as mentioned in the described method. For these fractions, de-oiled soybean and hydrolyzate showed ED_{50} at 29.3–31.7 mg/ml, whilst de-oiled rapeseed, rapeseed protein isolates and their hydrolyzates showed ED_{50} at 2.30–5.36 mg/ml. Under our experimental condition, trolox showed ED_{50} at 0.148 mg/ml. All of the rapeseed samples tested showed much stronger DPPH radical-scavenging activity than did soybean samples but the radical-scavenging effect was not comparable to trolox. Ultrafiltered rapeseed protein isolate and hydrolyzate had significantly higher ED_{50} (5.13–5.37 mg/ml) than had de-oiled rapeseed, precipitated rapeseed protein isolate and their hydrolyzates (2.30–3.02 mg/ml). This shows that precipitated rapeseed protein isolate has a greater radical-scavenging capacity than has ultrafiltered protein isolate. When we analyzed the protein concentration and total phenolic compound contents of tested extracts, there was no clear correlation between the ED_{50} value and the phenolic content or protein concentration (data not shown). Although some reports have mentioned a correlation between ED_{50} and total phenol content (Matthäus, 2002), our samples did not show this tendency, due to the crude protein isolate from pilot plant scale production. The degree of hydrolysis did not affect the DPPH radical-scavenging activity in this test. Further research should test organic soluble fractions and more DH varieties in order to clarify the factors which affect the radical-scavenging activity.

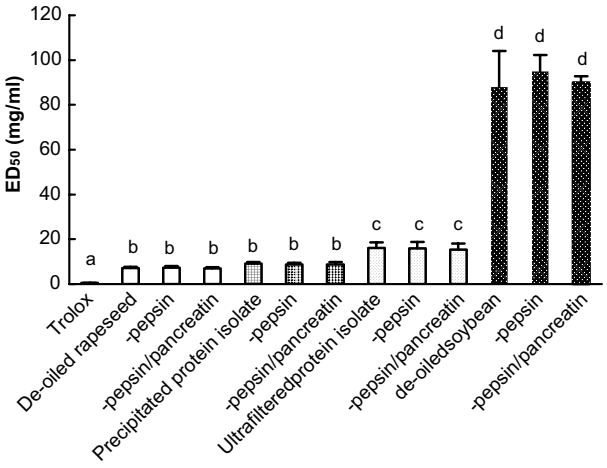

Fig. 5. DPPH radical-scavenging by the water-soluble fraction of de-oiled rapeseed, rapeseed protein isolates, de-oiled soybean and their hydrolyzates. * Data from Yoshie-Stark et al., 2004. Different letters indicate significant differences ($p < 0.05$).

4. Conclusions

Two types of rapeseed protein isolates have been shown to have interesting functional properties. Ultrafiltered protein isolate had good solubility, emulsification capacity and emulsification stability. These results indicate the potential of rapeseed proteins for replacing egg protein in food production. The precipitated protein isolate showed stronger ACE inhibition than did the ultrafiltered protein isolate. In the case of the bile acid-binding capacity, the precipitated protein isolate showed higher binding, presumably because of the higher concentration of fibre. The DPPH radical-scavenging capacity of the precipitated rapeseed protein isolate was higher than that of the ultrafiltered protein isolate. All told, the ultrafiltered protein isolate had the better functional properties, whilst the precipitated protein isolate gave better results in bioactive tests. These results provide a useful indication of the functional properties and health aspects of rapeseed protein as a potential replacement for animal proteins.

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