Polish Journal of Food and Nutrition Sciences

DE GRUYTER OPEN

Pol. J. Food Nutr. Sci., 2014, Vol. 64, No. 2, pp. 109–114
DOI: 10.2478/pjfns-2013-0007
http://journal.pan.olsztyn.pl

Original article
Section: Food Technology

Rape Seeds as a Source of Feed and Food Proteins

Tamara Nosenko[1], Tetyana Kot[1], Volodymyr Kichshenko[2]*

[1]Department of Fat Technology, National University of Food Technologies, Kyiv, 01601 Ukraine
[2]State Enterprise "Ukrmetrteststandard", Kyiv, Ukraine

Key words: rape seeds, proteins, amino acids, toxicity, nutritive value, functional properties

Amino acid content of proteins, fatty acid composition of oil, glucosinolate content, nutritive value of protein products and functional properties of protein isolates from rape seeds of spring and winter varieties of modern selection were studied in this work. Investigated rape samples had low glucosinolate and low erucic acid content. *Tetrachimena piriformis* was used for estimation of relative toxicity of protein products. These values were compared with the same value of casein. Sufficiently high nutritive values (90.1–95.9%) of winter rape cake, both samples of rape meal and protein isolates were detected. Rape seed protein isolates had high oil binding, emulsifying and foaming capacities. At the same time, water holding capacity of rape seed protein isolates was lower than that of soy protein isolates. We have concluded that protein products from rape seeds of modern selection are important sources of feed and food proteins.

INTRODUCTION

Supplying of food and feed by high biological value protein is still actual at the present time. Oil seeds are considered as a source of food and feed protein. Traditionally, the source of plant protein is soybean seeds. At the same time, protein content of other oil seeds is high too and their biological value is sufficient enough. Particularly, rape seeds with low or zero erucic acid content are an important source of edible oils but their proteins are still underestimated [Hu & Diosady, 2013].

The presence of some undesirable components like glucosinolates, phytates, phenols, and crude fiber make that rape meal is primarily used for livestock feeding or for some technical purpose [Hu & Diosady, 2013]. It was shown that canola protein could be used to produce biodegradable materials such as films [Jang *et al.,* 2011], thermal plastics [Manamperi *et al.*, 2010], paper cover [Palomino *et al.*, 2012] and adhesives [Li *et al*., 2012].

On the other hand, rape seeds and rape protein are considered to contain some substances that have technological or health benefits. Extracts obtained from rape seeds meal display remarkable antioxidant activity, that depends on the cultivar [Amarowicz *et al.*, 2003]. The most significant phenolic compounds in rape seeds are sinapic acid derivatives: mainly these phenolic compounds are thought to be responsible for the antioxidant activity [Thiyam *et al.*, 2006]. It was found that some products of rape protein hydrolyses had antioxidant activity too [Zhang *et al.*, 2009; Thiyam-Holländer & Schwarz, 2013]. Moreover rape proteins are supposed to prevent development of overweight-metabolic syndrome-diabetes [Mariotti *et al.*, 2008].

But at the same time there are published data which prove that breakdown products of glucosinolates from Brassicaceae plants possess the ability to inhibit carcinogenesis [Hayes *et al.*, 2008; Traka & Mithen, 2009]; these products also have capacity to induce antioxidant, detoxification and cytoprotective genes through activation of Nrf2 (NF-E2 related factor 2) and inhibit the pro-inflammatory reactions by repression of NF-jB (nuclear factor-jB). Certain isothiocyanates can block the activation of several carcinogens to their ultimate carcinogenic forms. Indoles can affect apoptosis in breast and prostate cancer cells [Bonnesen *et al.*, 2001].

The aim of this work was to study amino acid score, functional properties of protein isolates and relative toxicity of protein products from winter and spring rape seeds by biological assays with test organisms *Tetrachimena piriformis* strain WH–14, as their reactions on the chemical and biological influence are considered to appropriate the high animal responses [Mykytyuk, 1987]. In addition, these infusoria have a very high metabolic level allowing rapid response to the nutritive medium composition.

Fatty acid composition of oil was also estimated. The seeds of winter and spring varieties were chosen for our investigation in order to compare their properties. The winter rape is very popular in Ukraine as well as in other countries because of good crop capacity, but at the same time this variety has low frost resistance and its crops are often destroyed by frost.

* Corresponding Author: E-mail: tnosenko@yahoo.com (Prof. T. Nosenko)

MATERIALS AND METHODS

Analysis of rape seeds

Rape (*Brassica napus)* seeds of winter (*Artus*, Lembke KG, Germany) and spring (*Calibre*, Lembke KG, Germany) cultivars were analyzed. Moisture content of seeds was determined using the gravimetric method. Fat content of seeds was measured according to Soxhlet method. For this purpose, 2 g of sample were extracted for 24 h using hexane as a solvent. Crude protein (N × 6.25) was determined with Kjeldahl method according to AOAC Method [1984]. Glucosinolate content was measured as glucose released from glucosinolates in stoichiometric amounts under hydrolysis by the endogenous enzyme myrosinase using GLUCOTEST paper according to Interstate Standard [1987]. For glucosinolate hydrolysis, 0.5 g of crushed seeds were mixed with 5 mL of distilled water and incubated in the presence of activated carbon for 2 min.

Determination of amino acid composition of protein isolates

The direct acid hydrolysis of protein isolates was used to obtain hydrolysates suitable for determination of all amino acids except cysteine and tryptophan. Hydrolysis was carried out in test tubes by adding 1 mL of HCl to dry sample, corresponding to 2 mg of protein. The mixture was frozen in a bath at -80°C, the tubes were vacuumized, sealed and incubated at 106°C for 24 h in a thermostat. After hydrolysis, the samples were cooled and HCl was removed by evacuating in a dessicator containing NaOH pellet. After drying of samples, 4 mL of deionized water was added and drying procedure was repeated. Dry samples were dissolved in citrate buffers (0.3 mol/L, pH 2.2) and used for amino acid analyses.

Amino acid analyzer T 339 (Czech Republic) was used for amino acid content analysis. Standard amino acid mixture containing 0.5 μmol/L of the 17 commonly occurring amino acids was used to calculate the amount of amino acids in the samples.

Protein isolation

Proteins were extracted from defatted rape seeds (rape meals) by sodium chloride solution (70 g/L, pH 7.0) under constant stirring, at temperature of 50–55°C for 40–50 min, meal: solution ratio was 1:10 (w:v). Afterwards, the insoluble residue was precipitated by centrifugation. The supernatant (protein extract) was used for isoelectric protein precipitation at pH 4.8. After protein coagulation, pellet was separated by centrifugation (3,000 × g), protein pellet was collected and dried to 6–8% fluidity.

Determination of toxicity and relative nutritive value of protein products

Tetrachimena piriformis (WH–14 strain) cultures were used for the determination of relative nutritive value and toxicity of the protein products [Mykytyuk, 1987]. The dead cells, changed shapes, characteristic of movement and growth depression of infusoria were measures of toxicity. For analyses, 50 mg of rape protein samples (rape seeds cakes, rape seeds meals and rape seeds protein isolates), 2 mL of sea salt solution (5.6 mg/mL, pH 7.0) and 0.04 mL of 3-day *Tetrachimena piriformis* cultures were placed in vials, mixed and incubated in a thermostat at 25°C for 24 and 72 h. For better aeration the vials were periodically shaken during incubation. After incubation, infusoria cells were fixed in iodine solution in ethanol (50 g/kg) and analyzed under light microscope. Cell number was determined using a counting chamber. The control samples contained casein instead of rape protein products. Relative nutritive values of investigated samples were represented as a number of cells grown per sample, compared with the control.

Determination of protein functional properties

The water holding capacities (WHC) of the extracted seed proteins were measured as described by Ashraf *et al.* [2012] by taking 1 g of protein and resuspending it in 10 mL of distilled water and mixing vigorously for 2 min. The supernatants obtained after centrifugation at 3,000 × g for 20 min, were decanted and the weights of the sediments were determined. The WHC values were expressed as gram of water absorbed per 100 g of protein extracted.

The oil binding capacities (OBCs) of the extracted seed proteins were measured using the method of Ashraf *et al.* [2012] taking 1 g of protein that was deposited and reweighed in 50 mL centrifuge tubes and thoroughly mixed for 3 min with 10 mL of vegetable oil. Samples were allowed to stand for 30 min and the mixtures were centrifuged at 3,000 × g for 20 min, the supernatants were carefully poured immediately after the centrifugation and tubes with the sediments were weighed. The OBC values were expressed as gram of oil absorbed per 100 g of protein isolates.

The emulsifying capacity (EC) of the extracted seed proteins was determined according to Karki *et al.* [2009] by taking 8.5 g of each sample and mixing it with 50 mL of distilled water for 2 min using a blender and adding vegetable oil slowly with continuous blending. The process was stopped after every 2 min to check for emulsion breakage. The maximum volumes of oil that was emulsified were measured and emulsifying capacity was determined as the volume of oil relative to 1 g of protein isolates.

The foaming capacity (FC) of the extracted seed proteins was determined according to Makri *et al.* [2005] by taking 1% of the protein extracted and resuspending it in deionized water, with pH adjusted to 7.4 with 0.1N NaOH and 0.1N HCl. The solution (100 mL) was blended for 3 mins and poured into a 500 mL graduated cylinder. The volumes of foam (V_f) and liquid (V_l) were immediately recorded and FC was calculated using the following equation:

$$FC = \frac{V_f}{V_l} \times 100$$

Determination of fatty acid composition of oil

For determination of fatty acid composition, seed oil was extracted by screw press. For preparation of fatty acid methyl esters 100 mg of oil were dissolved in 2 mL solution (0.5 g/L) of butylated hydroxytoluene (BHT) in heptane. Then 100 μL of sodium in methanol solution (46 mg/mL) were added, the solution has been mixed for 2 min and kept for 15 min. From 1 to 2 g of sodium hydrosulfate were added. The sam-

ples were filtrated through the anhydrous sodium sulfate and then 2 mL of BHT heptane solution were added. Solution obtained was filtrated one more time through 0.45 μm membrane cellulose filter and the filter was washed with 1 mL of the same solvent. The two filtrates were combined and the solution was used for analysis.

Fatty acid methyl ester composition was determined by gas-liquid chromatography of fatty acid methyl esters. They were analyzed on Hewlett Packard gas chromatograph model HP 6890 with capillary column HP-88 (88%-cyanopropyl aryl-polysiloxane, 100m x 0.25 mm x 0.25 μm film thickness, Agilent Technologies). The temperature of injector was 280°C, and of detector was 290°C. The temperature program of heating rate from 60 to 260°C was as follows:

- holding at 60°C – 4 min,
- heating from 60 to 150°C at 4°C/min, holding at 150°C – 10 min,
- heating from 150 to 180°C at 3°C/min, holding at 180°C – 5 min,
- heating from 180 to 190°C at 3°C/min, holding at 190°C – 2 min,
- heating from 190 to 230°C at 3°C/min, holding at 230°C – 2 min,
- heating from 230 to 260°C at 4°C/min, holding at 260°C – 2 min.

The rate of gas carrier flow was 1.2 mL/min, sample volume was 1.0 μL. Identification of the fatty acids was performed by comparison of their retention times with standard mixture of fatty acid methyl esters (37 Component FAME Mix, SUPELCO).

Statistical analysis

Samples were analyzed in triplicate. Statistical analysis was performed using Microsoft Excel 2007 (Microsoft, City of Redmond, USA). The results were reported as mean±SD. Differences were considered to be significant at validity of α=0.95.

RESULTS AND DISCUSSION

The physico-chemical properties of rape seeds varieties

The physico-chemical properties of winter and spring rape seed cultivars are presented in Table 1. There were no significant differences between the seed varieties with the exception of mass of 1000 seeds and glucosinolate content; they were higher in winter cultivar. The investigated seeds were low in glucosinolate (Table 1) and low in erucic acid (Table 2). The main fatty acids were oleic (62.8–66.7%), linoleic (17.4–18.9%) and linolenic (6.5–8.3%). Because of dietary intake of ω-3 and ω-6 fatty acids determinates the proportions of bioactive 20- and 22-carbon ω-3 and ω-6 highly unsaturated fatty acids in tissue phospholipids [Lands *et al.,* 1992] the low linoleic (ω-6) content, high oleic acid content and the presence of linolenic (ω-3) acids determine the high biological value of rape oil. On the other hand, ω-3 to ω-6 ratio in tissue phospholipids has been shown to affect multiple diseases ranging from cardiovascular [Harris, 2008] and psychiatric [Samieri *et al.*, 2008; Hibbeln, 2009] diseases to neurodevelopmental deficits [Hibbeln & Davis, 2009].

TABLE 1. The physico-chemical properties of rape seeds.

Properties	Spring rape seeds	Winter rape seeds
Moisture (%)	6.4±0.3	4.2±0.2
Oil content (%)	43.7±0.4	43.6±0.5
Protein content (%)	25.9±0.3	23.7±0.2
Glucosinolate content (%)	0.6±0.1	0.8±0.1
Mass of 1000 seeds (g)	4.2±0.1	5.3±0.1

TABLE 2. Basic fatty acid content (% of total fatty acids) in rape seed oils.

Fatty acid	Spring rape oil	Winter rape oil
C 14:0, myristic	0.04±0.001	0.05±0.001
C 16:0, palmitic	3.64±0.04	4.14±0.05
9c, C16:1, palmitoleic	0.17±0.01	0.18±0.02
C18:0, stearic	1.88±0.04	1.57±0.03
9c, C18:1, oleic	66.74±0.23	62.84±0.18
9c, 12c, C18:2, linoleic	17.40±0.16	18.90±0.19
C20:0, arachidic	0.68±0.01	0.58±0.02
9c,12c,15c, C18:3, α-linolenic	6.54±0.01	8.26±0.06
11c, C20:1, 11-eicosenoic	1.20±0.05	1.32±0.04
C20:2, 11,14-eicosadienoic	1.20±0.06	1.63±0.03
C 22:0, behenic	0.33±0.02	0.28±0.01
13c, C 22:1, erucic	undetected	0.13±0.01
C 24:0, lignoceric	0.16±0.02	0.12±0.01

At the same time, modern diets are depleted in ω-3 fatty acids and abounded in ω-6 fatty acids, their ratio decreased to 1:10 in the previous century [Blasbalg *et al.*, 2011].

Amino acid composition of protein isolates from rape seeds

Contents of the main amino acids of proteins extracted from rape seeds are shown in Table 3. The contents of the majority of essential amino acids namely lysine, sum of sulfur containing methionine and cystine, threonine, leucine and sum of phenylalanine and tyrosine in rape proteins are higher than FAO/WHO scale [1985]. The exception is valine with the score of 76 and 80% for spring and winter rape, respectively, and isoleucine with 80 and 85% for spring and winter rape, respectively. The scores of sulfur containing amino acids: methionine and cystine were 151.4 and 157.1% for spring and winter rape, respectively. They were significantly higher than these of amino acids scores in soybean seed proteins. The content of lysine, sum of methionine and cystine, valine and isoleucine was higher in proteins from winter cultivar seeds and proteins from spring cultivar seeds containing more threonine, leucine and sum of phenyalanine and tyrosine. Our data do not completely agree with the published results [Hu & Diosady, 2013]. The greatest differences were in valine and isoleucine contents, notably score of valine was 73 and 77% and that of isoleucine was 76 and 81% for spring

TABLE 3. Content of main essential amino acids in soy and rape protein isolates relative to FAO/WHO scale protein.

Amino acid	FAO/WHO protein (mg/100 mg of protein) [1985]	Soy protein (mg/100 mg of protein)	Spring rape protein isolate		Winter rape protein isolate	
			mg/100 mg of protein	% to FAO/ WHO protein	mg/100 mg of protein	% to FAO/ WHO protein
Lysine	5.5	6.1±0.18	6.0±0.17	109.1	6.5±0.20	118.2
Methionine+cystine	3.5	2.1±0.06	5.3±0.16	151.4	5.5±0.17	157.1
Valine	5.0	5.4±0.16	3.8±0.11	76.0	4.0±0.12	80.0
Threonine	4.0	3.9±0.12	4.5±0.14	112.5	4.3±0.13	107.5
Leucine	7.0	7.9±0.24	7.4±0.22	105.7	7.0±0.21	100.0
Isoleucine	4.0	4.1±0.12	3.2±0.10	80.0	3.4±0.10	85.0
Phenylalanine + tyrosine	6.0	8.0±0.23	7.7±0.23	128.3	7.5±0.23	125.0

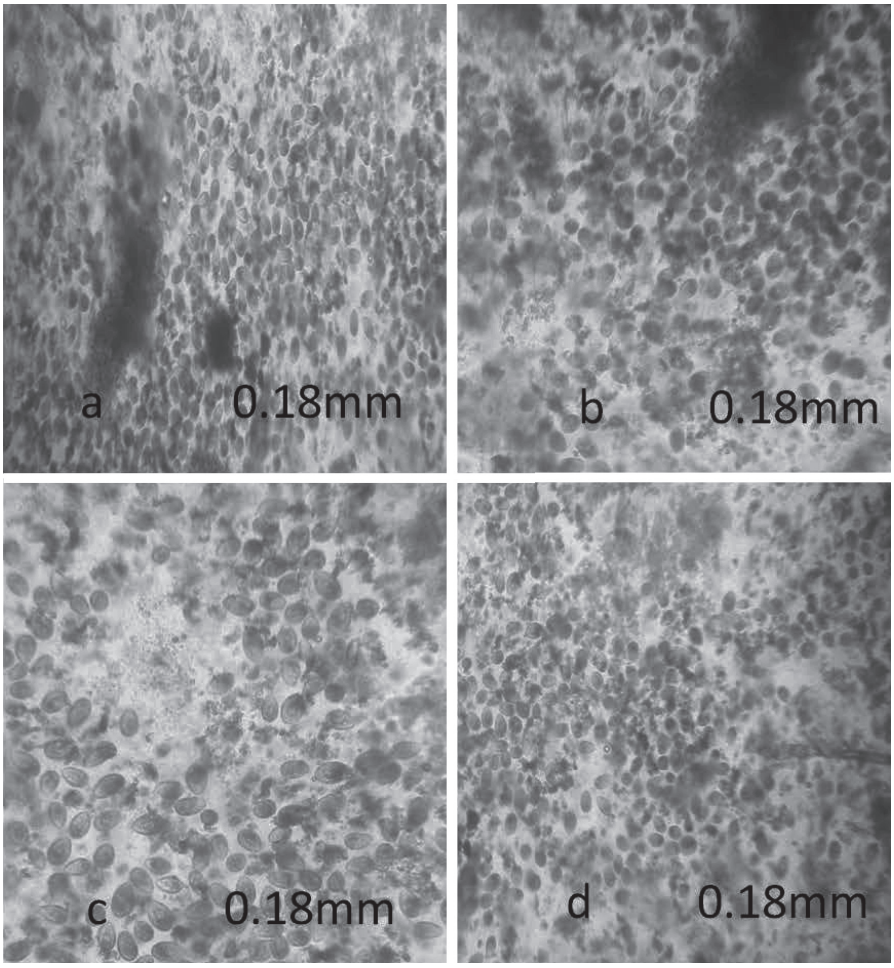

FIGURE 1. Light micrographs showing the development of *Tetrachimena piriformis* after 24 h incubation in the presence of casein (a), winter rape cake (b), winter rape meal (c), winter rape protein isolate (d).

and winter cultivars, respectively. The differences could be attributed to different rape cultivars.

It is known that amino acid composition can influence the functional properties of proteins based on predominance of hydrophobic or hydrophilic amino acids.

Toxicity and relative nutritive value of protein products

Light micrographs of *Tetrachimena piriformis* culture after 24 h incubation in the presence of casein (as a control), winter rape cake, winter rape meal and winter rape protein isolates are shown in Figure 1. No differences were detected in the shape, growth and reproduction of *Tetrachimena piriformis* between control sample and samples in the presence of winter rape cake, meal and protein isolates. The same results were obtained in the presence of spring rape cake, meal and protein isolates (data are not shown). Based on these results we can suggest that protein-containing products from rape seeds have no toxic influence on the cells of this test-organism.

Results of estimation of relative nutritive values of rape protein products are presented in Table 4. The relative nutritive values of rape protein products were in the range from 81.1 to 95.94% of nutritive value of casein that are sufficiently high for

TABLE 4. Relative nutritive value of protein products.

Samples	Cell count in 1 mL of medium	Relative nutritive value (%)
Casein	$(12.33\pm0.85)\cdot10^4$	100.00
Spring rape cake	$(10.00\pm0.63)\cdot10^4$	81.10
Winter rape cake	$(11.10\pm0.29)\cdot10^4$	90.02
Spring rape meal	$(11.28\pm0.89)\cdot10^4$	91.48
Winter rape meal	$(11.23\pm1.02)\cdot10^4$	91.82
Spring rape protein isolate	$(11.52\pm2.89)\cdot10^4$	93.43
Winter rape protein isolate	$(11.83\pm1.59)\cdot10^4$	95.94

vegetable source of proteins. According to our results, the protein isolates have the highest relative nutritive values that are apparently due to the removal of some antinutritive substances during their processing. The protein isolates from winter cultivar have a higher relative nutritive value than these from spring cultivar. The relative nutritive value of cake from spring rape seeds was the lowest and equaled 81.1% and relative nutritive values of seed meals of both cultivars were very close and reached 91.48 and 91.82% for spring and winter rape, respectively.

Functional properties of seed protein isolates

Besides biological value the functional properties of proteins are very important for their use in food systems. Water holding, oil binding, emulsifying and foaming capacities of protein isolates from rape meals were compared with the same capacities of soy protein isolates (Figure 2). Water holding capacities of protein isolates from both rape cultivars were high, namely, about 210% for both cultivars but they were substantially lower comparing with soy isolates. Poor water binding of rape proteins compared with soy proteins is possibly due to their low hydrophilic properties determined by proportion of hydrophilic and hydrophobic amino acids. Particularly, rape proteins are abounded by such hydrophobic amino acids as methionine and cystine. On the other hand, functional properties related to hydrophobicity of proteins, namely, oil binding, emulsifying and foaming capacities of rape protein isolates considerably exceeded the same properties of soybean protein isolates. Oil binding capacity of protein isolates from spring rape meal was about 30% higher than that of winter rape but protein isolates from winter rape had slight higher emulsifying capacity and foaming capacities were almost equal for both cultivars. Noteworthy are very high foaming capacities of rape proteins, they were twice higher compared with soy proteins.

CONCLUSIONS

Using infusoria *Tetrachimena piriformis* it was shown that rape protein products had not shown toxicity to living organisms. Their relative biological values were comparable with relative value of casein. Such as *Tetrachimena piriformis* has

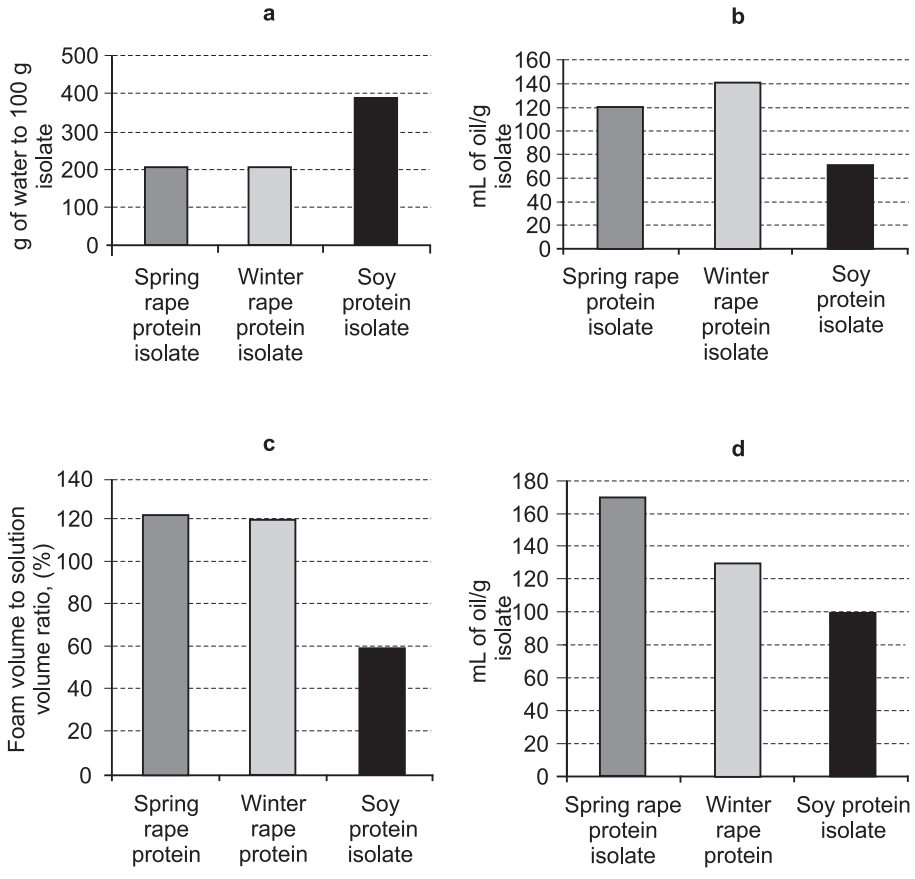

FIGURE 2. Functional properties of protein isolates from rape meal (data for soy protein isolates are given for comparison), a- water holding capacities, b- emulsifying capacity, c- foaming capacity, d- oil binding capacities.

a very short living cycle we could detect there was no negative influence of the investigated rape samples on the reproduction, growth and development of *Tetrachimena piriformis*.

Rape protein isolates have a high nutritive value on the basis of essential amino acid content and high functional properties. Taking into account these data and low glucosinolate content of rape seeds we suppose that these oil seeds are important sources of food and feed proteins. But technology of food protein isolates from rape seeds still requires development in order to improve their organoleptic properties.

ACKNOWLEDGEMENTS

Authors are thankful to Dr. V. Dzhmil (Department of Veterinary and Sanitary Expertise of Agrarian University) for his valuable support in analysis of toxicity and nutritive value of rape products.

This work was supported by grant number 0112U001084 from the Ministry of Education and Science of Ukraine.

REFERENCES

1. Amarowicz R., Raab B., Shahidi F., Antioxidant activity of phenolic fractions of rapeseed. J. Food Lipids, 2003, 10, 51–62.
2. Ashraf S., Saeed S.M.G., Sayeed S.A., Ali R., Impact of microwave treatment on the functionality of cereals and legumes. Int. J. Agric. Biol., 2012, 14, 365–370.
3. Blasbalg T.L., Hibbeln J.R., Ramsden Ch.E., Majchrzak S.F., Rawlings R.R., Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am. J. Clin. Nutr., 2011, 93, 950–962.
4. Bonnesen C., Eggleston I.M., Hayes J.D., Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. Cancer Res., 2001, 61, 6120–6130.
5. FAO/WHO/UNU. Energy and protein requirements. Reports of a joint FAO/WHO/UNU expert consultation. World Health Organization Technical Report Series 724. WHO. Geneva, Switzerland, 1985.
6. Harris W.S., The omega-3 index as a risk factor for coronary heart disease. Am. J. Clin. Nutr., 2008, 87 1997S–2002S.
7. Hayes J.D., Kelleher M.O., Eggleston I.M., The cancer hemopreventive actions of phytochemicals derived from glucosinolates. Eur. J. Nutr., 2008, 47 (Suppl 2), 73–88.
8. Hibbeln J.R., Davis J.M., Considerations regarding neuropsychiatric nutritional requirements for intakes of omega-3 highly unsaturated fatty acids. Prostaglandins Leukot. Essent. Fatty Acids, 2009, 81, 179–186.
9. Hibbeln J.R., Depression, suicide and deficiencies of omega-3 essential fatty acids in modern diets. World Rev. Nutr. Diet, 2009, 99, 17–30.
10. Hu L., Diosady L.L., Processing of canola proteins: A review. 2013, *in*: Canola and rapeseed: Production, Processing, Food Quality and Nutrition, (eds. Usha Thiyam-Holländer, N.A. Michael Eskin, Bertrand Matthäus), CRC Press Taylor and Francis Group, Boca Raton, USA, pp.59–78.
11. Interstate Standard GOST 9824–87. Rape and colza seeds. Varietal and sowing characteristics. Specification. 1987, Standard publisher, Moscow, 6 p. (in Russian).
12. Jang S.-A., Lim G.-O. Song K.B., Preparation and mechanical properties of edible rapeseed protein films. J. Food Sci., 2011, 76, 218–223.
13. Karki B., Lamsal B.P., Grewell D., Pometto III A.L., Leeuwen J., Khanal S.K., Jung S., Functional properties of soy protein isolates produced from ultrasonicated defatted soy flakes. J. Am. Oil Chem. Soc., 2009, 86, 1021–1028.
14. Lands W.E., Libelt B., Morris A., Kramer N.C., Prewitt T.E., Bowen P., Schmeisser D., Davidson M.H., Burns J.H., Maintenance of lower proportions of (n-6) eicosanoid precursors in phospholipids of human plasma in response to added dietary (n-3) fatty acids. Biochim. Biophys. Acta, 1992, 1180, 147–162.
15. Li N., Qi G., Sun X.S., Stamm M.J., Wang D., Physicochemical properties and adhesion performance of canola protein modified with sodium bisulfite. J. Am. Oil Chem. Soc., 2012, 89, 897–908.
16. Makri E., Papalamprou E., Doxastakis G., Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, pea & broad bean) in admixture with polysaccharides. Food Hydrocoll., 2005, 19, 583–594.
17. Manamperi W.A.R., Chang S.K.C., Ulven C.A., Pryor S.W., Plastics from an improved canola protein isolate: preparation and properties. J. Am. Oil Chem. Soc., 2010, 87, 909–915.
18. Mariotti F., Hermier D., Sarrat C., Magné J., Fénart E., Evrard J., Tomé D., Huneau J.F., Rapeseed protein inhibits the initiation of insulin resistance by a high-saturated fat, high-sucrose diet in rats. Br. J. Nutr., 2008, 100, 984–991.
19. Mykytyuk P.V., Micromethod of toxic and biological estimation of fish and hydrobionts, Kyiv, 1987, p. 23 (in Russian).
20. Official Methods of Analysis, 14th edn., Association of Official Analytical Chemists, Washington, D.C., 1984.
21. Palomino J., Tressel R.P., Pudel F., Special prepared rapeseed protein for paper board coating. 2012, *in:* Book of Abstract 10th Euro Fed Lipid Congress: Fats, Oils and lipids: from science and Technology to Health, 23–26 September, Cracow, Poland, p. 24.
22. Samieri C., Féart C., Letenneur L., Dartigues J.F., Pérès K., Auriacombe S., Peuchant E., Delcourt C., Barberger-Gateau P., Low plasma eicosapentaenoic acid and depressive symptomatology are independent predictors of dementia risk. Am. J. Clin. Nutr., 2008, 88, 714–721.
23. Thiyam U., Stöckmann H., Felde T.Z., Schwarz K., Antioxidative effect of the main sinapic acid derivatives from rapeseed and mustard oil by-products. Eur. J. Lipid Sci. Technol., 2006, 108, 239–248.
24. Thiyam-Holländer U., Schwarz K., Rapeseed and canola phenolics: antioxidant attributes and efficacy. 2013, *in:* Canola and rapeseed: Production, Processing, Food Quality and Nutrition, (eds. Usha Thiyam-Holländer, N.A. Michael Eskin, Bertrand Matthäus), CRC Press Taylor and Francis Group, Boca Raton, USA, pp. 277–298.
25. Traka M., Mithen R., Glucosinolates, isothiocyanates and human health. Phytochem. Rev., 2009, 8, 269–282.
26. Zhang S. B., Wang Z., Xu S. Y., Gao X. F., Purification and characterization of a radical scavenging peptide from rapeseed protein hydrolysates. J.Am.Oil.Chem.Soc., 2009, 86, 959–966.

Submitted: 11 November 2013. Revised: 13 February and 27 February 2014. Accepted: 3 March 2014. Published on-line: 23 April 2014.