Rape seeds as a source of feed and food protein

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Abstract: 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 were low glucosinolate and low erucic acid content. *Tetrachimena piriformis* was used for estimation of relative nutritive value of protein products. These values were compared with the same value for casein. Sufficiently high nutritive value (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 was lower that of soy protein isolates. We have concluded that protein products from rape seeds of modern selection are important source of feed and food proteins.

Key words: rape seed, rape protein, amino acids, *Tetrachimena piriformis,* nutritive value, functional properties

1 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 soy 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.

The presence of some undesirable components (glucosinolates, phytates, phenols, and crude fiber [1]) is a cause that rape meal is primarily used for livestock feeding or for some technic purpose. It was shown that canola protein could be used for producing of biodegradable materials such as films [2] thermal plastics [3], paper cover [4] and adhesives [5].

On the other hand rape seed and rape protein are considered to contain some substances that have technological or health benefits. Extracts obtained from rape seeds meal exhibit remarkable antioxidant activity, the extent of which depends on the cultivar [6]. The most significant phenolic compounds in rape seeds are sinapic acid derivatives. Mainly these phenolic compounds are thougth to be responsible for the antioxidant activity [7]. It was found that some

product of rape protein hydrolyses had antioxidant activity too [8,9]. Moreover rape proteins are supposed to protect development of overweight-metabolic syndrome-diabetes [10].

As well Brassicaceae plants contain glucosinolates which breakdown products are thought to inhibit carcinogenesis [11,12]. These products also have ability 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 [13].

The aim of our work was to study the relative nutritive value of seed protein products of winter and spring rape by biological methods. For this purpose we have used culture of *Tetrachimena piriformis* strain WH–14. Fatty acid composition, amino acid score and technological properties of protein products were also estimated.

2 Materials and Methods

2.1 Analysis of rape seeds

Rape (*Brassica napus)* seeds of winter (Artus, Lembke KG, Germany) and spring (Calibre, Lembke KG, Germany) varieties were used in our researches. Moisture content of seeds was determined using the gravimetric method. Fat content of seeds was measured according to Soxhlet's method. For this purpose 2 g of sample were extracted for 24 hrs using hexane as a solvent [AOAC, 1995]. Crude protein (Nx6.25) was determined by the Kjeldahl method according to AACC Method 46-12 (AACC, 1976). Glucosinolate content was measured as glucose released from glucosinolates in stoechiometric amounts under hydrolysis by the endogenous enzyme myrosinase using GLUCOTEST paper [14]. 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 during 2 min.

2.2 Determination of fatty acid composition

For the determination of fatty acid composition seed oil was extracted on the laboratory screw press. Fatty acid 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 γm film thickness (Agilent Technologies). The temperature of injector was 280 °C, detector — 290 °C. The column temperature was from 60 to 230 °C. The rate of carrier gas was 1.2 ml/min. Identification of the fatty acids was performed by comparison of the retention times with standards mixture of fatty acid methyl esters (37 Component FAME Mix, Supelco).

2.3 Determination of amino acid composition

The direct HCl hydrolysis was used to obtain hydsrolysates suitable for determination of all amino acid except cysteine and tryptophan. Hydrolysis was carried out in test tubes by adding of 1 ml HCl to dry sample, corresponding to 2 mg of protein. The mixture was frozen in a bath at - 80°C, evacuated and sealed. Than samples were exposed at 106 °C for 24 hr. in thermostat. After hydrolysis samples were cooled and HCl was removed from them by evacuating in 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 0.3 N litiy-citrate buffer, 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 micromole of the 17 commonly occurring amino acid was used to calculate the amount of amino acids in the samples.

2.4 Obtaining of protein isolates.

Protein was extracted from defatted rape seeds by sodium chloride solution (7 %, w/v pH 7.0) under constant stirring and temperature 50-55 °C during 40-50 min, meal:solution ratio was 1:10. After this insoluble residue was precipitated by centrifugation. The supernatant (protein extract) was used for isoelectric protein precipitation. After protein coagulation pellet was separated by centrifugation (3 000 x g). Protein pellet was collected and dried to 6-8 % fluidity.

2.5 Determination of toxicity and relative nutritive value of protein products

Tetrachimena piriformis (WH–14 strain) was used for determination of nutritive value and toxicity of protein products [15]. The presence of dead cells, changed shapes, characteristic of movement and growth depression of infusoria were the measure of toxicity. 50 mg of rape protein samples, 2 ml of 0.56 % sea salt solution (pH 7.0) and 0.04 ml of 3-days *Tetrachimena piriformis* culture were placed to the vials, mixed and incubated in thermostat at 25 °C during 24 and 72 hr. For better aeration the vials were periodically shaked during incubation. After incubation infusoria cells were fixed 5 % iodine solution in ethanol and analyzed under light microscope. Cell quantity was determined using 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 growned in the presense of a sample in comparison with the control.

2.6 Determination of Functional Properties

Water holding capacities (WHCs): 1 g of protein isolate was taken in 10 mL of distilled water and mixed vigorously for 2 min, The supernatants obtained after centrifugation at 3000 g for 20 min, were decanted and the weights of the sediments were determined, the WHCs values expressed as gram of water absorbed by 100 g of protein isolates.

Oil binding capacities (OBCs): 1 g (W) of protein isolate was taken into the reweighed

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 3000 g for 20 min, the supernatants were carefully poured immediately after the centrifugation and tubes with the sediments were weighted. The *OBCs* values expressed as gram of oil absorbed by 100 g of protein isolates.

Emulsifying capacity (EC): The samples (8.5 g) of each sample were mixed with 50 mL of distilled water for 2 min using a blender and vegetable oil was adding 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 % of oil relatively to protein products (v/w).

Foaming capacity (FC): 1 % protein isolate in deionized water was taken, pH was adjusted to 7.4 with 0.1N NaOH and 0.1N HCl. 100 mL of solution were blended for 3 min and poured into a 500 mL graduated cylinder. The volume of foam (V_f) and liquid (V_l) were immediately recorded and FC was calculated as % of obtained foam using the following equation:

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

2.7 Statistical analysis

Each samples were analyzed in triplicate, and the results were reported as mean ± SD. Differences were considered to be significant at validity α=0.95.

3 Results and Discussion

3.1. The physico-chemical properties

The physico-chemical properties of winter and spring rape seed varieties are presented in Table 1. There were not significant differences between seed of winter and spring rape with the exception of mass of 1000 seeds and glucosinolate content, they were higher in winter variety. Investigated seeds were low glucosinolate (Table 1) and low 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). The low linoleic (ω-6) content, high oleic and presence of linolenic (ω-3) acids determine the high nutritive value of rape oil. 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 [16]. On the other hand ω-3 to ω-6 ratio in tissue phospholipids in turn have been shown to affect multiple diseases ranging from cardiovascular [17] and psychiatric [18,1 9] disease to neurodevelopmental deficits [20]. At the same time modern diets are depleted in ω-3

fatty acids and abounded by ω-6 fatty acids, their ratio increased to 10 during previous century [21].

3.2. Amino acid composition of proteins

Content of the main amino acids of proteins isolated from rape seeds are shown in Table 3. The contents of majority of essential amino acids in rape proteins are higher than FAO/WHO scale. The exception is valin and isoleycin with 76-85 % content. The content of sulfur containing amino acid methionin and cystin was significantly higher in rape protein than in soy protein. According to our results there were not essential differences of amino acid composition between winter and spring varieties that were investigated. Our data are not completely agree with the publicated results [1]. The most prominent differences were in valin and isoleucine content. The content of valin was 73 and 77 % and isoleucine 76 and 81 % for spring and winter varieties relatively.

3.3. Toxicity and relative nutritive value of protein products

We did not detect any differences of shape, growth and reproduction of *Tetrachimena piriformis* between control samples and samples in the presence of rape cake, meal and protein isolates after 24 hr incubation (Fig. 1). This means that there was not any toxic influence of rape products on these cells. Relative nutritive values of rape protein products were in the range 81,1 to 95,94 % of nutritive value of casein that are sufficiently high for vegetable source of proteins. Relative nutritive values of spring rape cake were lowest, meal was higher and protein isolates the highest one. It is possible that heating of meal during hexane removing results in loss of some antinutritive substances in rape meal. On the other hand using of water solution for protein extraction accompanies removing of some substances to solution.

3.4. Functional properties of protein

Functional properties of protein isolates from rape meal were high (Fig. 2). Oil binding, emulsifying, foaming capacities exceeded the same properties of soy protein isolates considerably. It is worth to emphasize very high foaming capacity of rape proteins. The differences between two varieties were not very considerable. Only water holding capacities of rape protein isolates were lower relatively soy protein isolates, but according to specification for soy protein isolates this parameter can vary from 260 to 510 % and it depends very strong from technology of their obtaining.

4 Conclusions

Using of infusoria *Tetrachimena piriformis* it was shown that rape protein products had high relative nutritive values. These values were comparable with relative nutritive values of casein. Such as *Tetrachimena piriformis* has very short living cycle a lot of generation changed during our observation. We did not detect any negative influence of investigated samples on the

growth and development of *Tetrachimena piriformis.* We are concluding that rape protein products have not toxicity for living organism. On the other hand rape proteins have high biological value on the base on essential amino acid content and high functional properties. Taking into account these data and low glucosinolate content of rape seeds we suppose that these oilseeds are important source of food and feed proteins. But technology of food protein from rape seeds still requires development.

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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 oil.

Fatty acid	Spring rape oil	Winter rape oil
C 14:0	0.04	0.05
C 16:0	3.64	4,14
9c, C 16:1	0.17	0.18
C 18:0	1.88	1.57
9c, C18:1	66.74	62.84
9c, 12c, C 18:2	17.40	18.90
C20:0	0.68	0.58
9c, 12c, 15c, C 18:3	6.54	8.26
11c, C20:1	1.20	1.32
C20:2	1.20	1.63
C 22:0	0.33	0.28
13c, C 22:1	undetected	0.13
C 24:0	0.16	0.12

Table 3 Content of main essential amino acids in rape proteins

Amino acid	FAO/WHO protein, mg/100 mg of protein	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	6,0	**109,1**	6,5	**118,2**
methionine +cystine	3,5	2,1	5,3	**151,4**	5,5	**157,1**
valine	5,0	5,4	3,8	**76,0**	4,0	**80,0**
threonine	4,0	3,9	4,5	**112,5**	4,3	**107,5**
leucine	7,0	7,9	7,4	**105,7**	7,0	**100,0**
isoleucine	4,0	4,1	3,2	**80,0**	3,4	**85,0**
pheyilalanine + tyrosine	6,0	8,0	7,7	**128,3**	7,5	**125,0**

Table 4 Relative nutritive value of protein products

Samples	Cell quantity in 1 ml of medium	Relative nutritive value %
Casein	$(12,33\pm0,85)\cdot10^4$	100
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

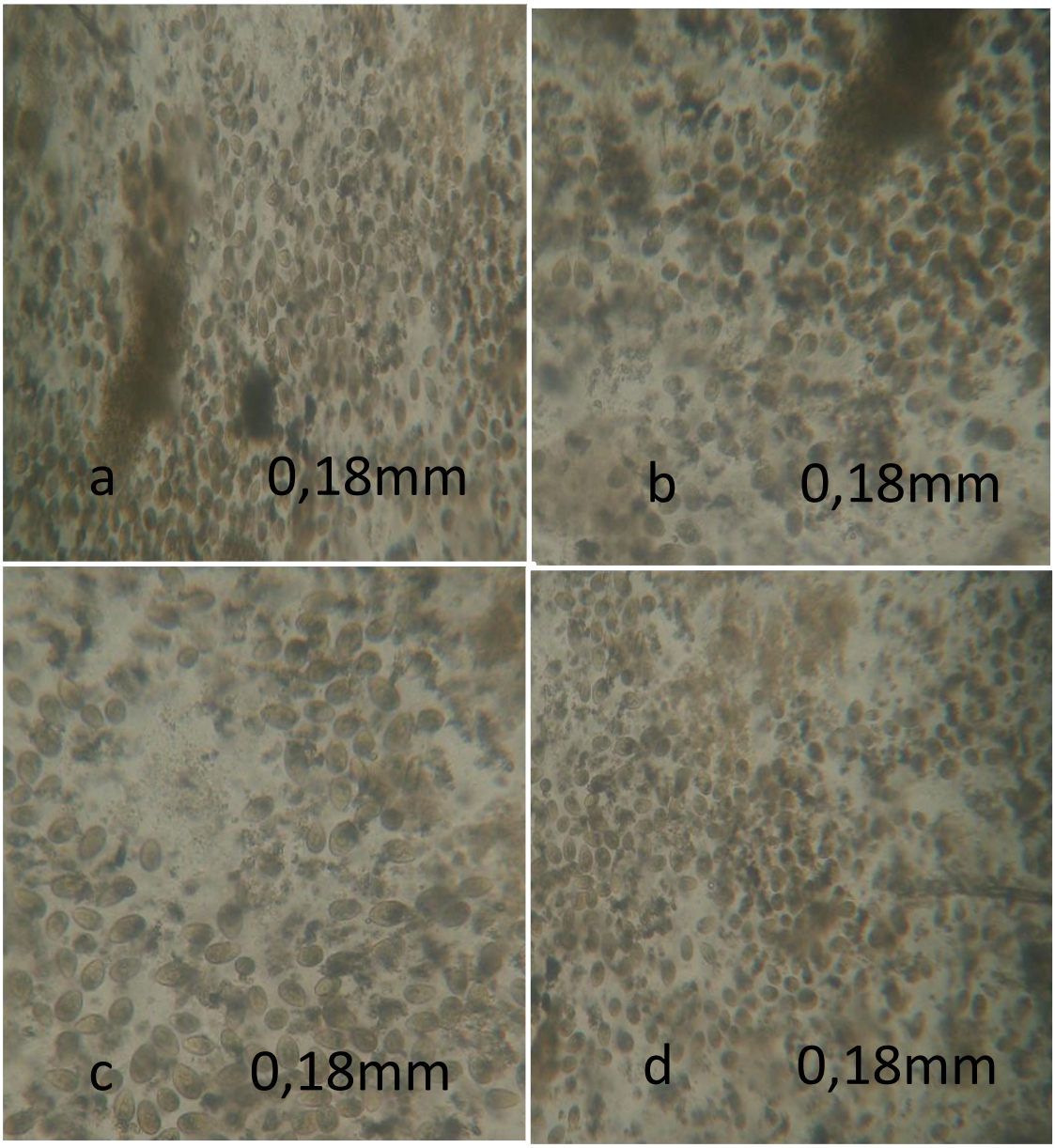

Fig. 1 Ligth micrographs showing the development of *Tetrachimena piriformis* after 24 hr incubation in the presence of casein (a), winter rape cake (b), winter rape meal (c), winter rape protein isolate (d).

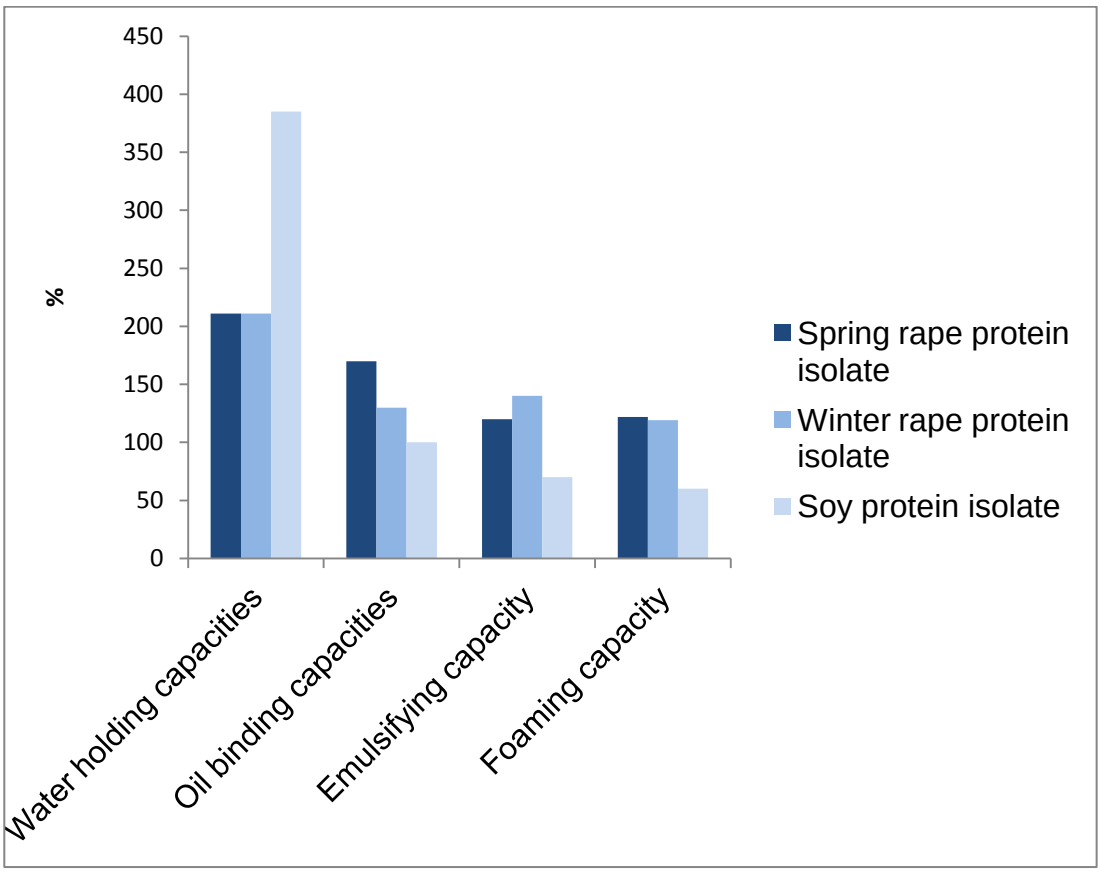

Fig 2. Functional properties of protein isolates from rape meal (data for soy protein isolate are given for comparison).