Effect of processing on the antinutritive factors and nutritive value of rapeseed products

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The antinutritive factors and nutritive value of rapeseed products were evaluated. The reductions in antinutritional factors by heat processing ranged from 47 to 94% for glucosinolates, from 9 to 43% for phytic acid and from 41 to 67% for tannic acid after toasting and autoclaving treatments. Isolation of proteins eliminated 72-95% of glucosinolates, 74-92% of phytic acid and 100% of tannic acid for protein concentrate and isolate. All processes destroyed trypsin inhibitor activity completely. Rapeseed products have a balanced amino acid composition and their proteins are relatively high in sulphur-containing amino acids and lysine. The chemical score and limiting amino acids of rapeseed products varied considerably, depending on the type of process. Heat treatment and isolation of proteins improved the in-vitro digestibility of rapeseed proteins. The essential amino acid index of rapeseed products ranged from 66 to 81%. Rapeseed meal has high levels of riboflavin, niacin and pyridoxine. Riboflavin and niacin are more stable to the heat process than thiamine, pyridoxine and pantothenic acid. Many of these vitamins are lost during isolation of proteins. The meals are rich in Ca, K, P and Mg. Some minerals increased and others decreased during the processing.

INTRODUCTION

Rapeseed is one of the world's major oilseed crops. After extraction, the oil rapeseed meal contains about 40% protein. Its proteins are rich in lysine and consist of adequate quantities of sulphur-containing amino acids, which are the limiting amino acids in most cereal and oilseed proteins. Sarwar et al. (1984) compiled the results of a study of protein quality by six collaborating laboratories. The results indicated that the quality of rapeseed protein was similar to casein and superior to proteins from other plant sources such as soybean, pea and wheat. The utilization of rapeseed protein has been limited by the presence of many antinutritional factors such as glucosinolates, phytic acid, phenolics and hull. There are a number of procedures for removing the undesirable glucosinolates and their hydrolysis products. A recent procedure describes hydrothermal treatment of the intact seeds by heating in a solution of sodium sulphate at 100°C (Mothadi-Nia et al., 1986). Extraction of the ground seeds with a two-phase solvent system has been reported, one phase consisting of a 10% solution of ammonia in methanol or in methanol containing 5% water and the second phase consisting of hexane (Diosady et al., 1985). Soaking of intact seeds

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under mild citric acid or ammonium carbamate conditions at room temperature has also been done after which the defatted meal was treated with alcohol/ ammonia mixture (Schwenke *et al.*, 1990). Phytic acid lowers the bioavailability of minerals (Nolan & Duffin, 1987) and inhibits proteolytic enzymes (Serraino *et al.*, 1985).

The nutritive value of rapeseed meal is greatly improved by heat treatment which inactivates the native thioglucosidase enzyme in the seed or meal (Josefsson, 1975a). Phytic acid was lowered significantly by common methods of domestic processing and cooking, including soaking, cooking, autoclaving and sprouting of the legume grains (Duhan *et al.*, 1989).

The objective of this study was to evaluate the effect of dry and wet heat treatment, as well as isolation of proteins, on the antinutritive factors and nutritive value of rapeseed products.

MATERIALS AND METHODS

Materials

Rapeseed meals

Three cultivars of double zero rapeseed are grown in Hungary; our sample was a mixture of these cultivars: Lindore, Santana and Tandem. Defatted raw rapeseed



meal and toasted rapeseed meal (110° C for 1 h) were obtained from the Research Institute of Vegetable Oil and Detergent Industry, Budapest. Autoclaved meals (121° C for 1 h and $1\frac{1}{2}$ h) were prepared from raw meal.

Rapeseed protein isolate

(a) Extraction of protein. The meal was extracted with NaOH (pH 12·1) at room temperature. The meal-to-solvent ratio was 1:20 (w/v) and the mixture was shaken for 1 h. Insoluble material was removed by centrifuging at 5000 rpm for 15 min.

(b) Precipitation of protein. To the clear supernatant was added sodium hexametaphosphate (0.08 g/g protein) as a precipitation aid, according to the method of Gillberg and Törnell (1976), as well as 0.1% sodium metabisulphite followed by precipitation of proteins at pH 3.2 using 1 M HCl. The precipitate was soaked in 0.2% sodium metabisulphite for 1 h followed by washing with distilled water.

The precipitate was dried with acetone and vacuum oven at 30°C for 4 h.

Rapeseed protein concentrate

The method of Liu *et al.* (1982) modified by Thompson *et al.* (1982) was used with slight modifications.

All rapeseed products were ground to pass through a 400 μ m sieve.

Analytical methods

Crude protein $(N \times 6.25)$ content was assayed by the Kjeldahl procedure using the automatic Kjel-Foss equipment (Model 16210 made in Denmark). The total glucosinolates were determined according to Möller *et al.* (1986) based on the colour reaction with a palladium reagent. The calibration was performed using sinigrin as a standard.

Phytic acid was determined by the method of Makower (1970). Tannic acid was determined photometrically according to the method of Hagerman and Butler (1978).

Trypsin inhibitor activity was assayed according to the method of Kakade *et al.* (1974), with the modification described by Petres and Kárpáti (1981). Activity was calculated as trypsin inhibitor units (TIU) per mg protein.

Total carbohydrate was determined by Schoorl's method after a hydrolysis for 3 h with 2.5% HCl. For determining the oligosaccharides, the TLC method of Tanaka *et al.* (1975) was applied.

Determination of amino acid composition was carried out using a Mikrotechna AAA 881 automatic amino acid analyser according to the method described by Moore and Stein (1963). Hydrolyses of the proteins were performed in the presence of 6 M HCl at 110°C for 24 h in nitrogen atmosphere. Sulphur-containing amino acids were determined after performic acid oxidation. Tryptophan was chemically determined by the method of Miller (1967).

The in-vitro true digestibility (TD) and apparent digestibility (AD) were determined as described by Salgó *et al.* (1984) by measuring the decrease in pH during digestion with trypsin and pancreatin. The in-vitro protein digestibility (IVPD) was determined according to the procedure of Akeson and Stahmann (1964).

Essential amino acid index (EAAI) was calculated according to Oser (1959) using the amino acid composition of freeze-dried whole egg protein.

Chemical scores of amino acids were calculated using the FAO/WHO (1973) reference pattern.

Phosphorus was assayed photometrically using the phosphorus molybdate complex. Sodium, potassium and calcium were determined after nitric acid digestion (Lindner & Dworschák, 1966) by flame photometer (type Flamon, Hungary). Micro elements (Cu, Zn, Fe, Mn, Mg) were determined after dry-ashing by a Perkin-Elmer Model 403 atomic absorption spectrophotometer.

Energy values were calculated from the protein, carbohydrate and fat contents.

Vitamins of the B-group were determined by microbiological methods as described by György and Pearson (1967).

RESULTS AND DISCUSSION

Table 1 shows that the raw rapeseed meal contains high and low levels of total glucosinolates and phytic acid, respectively, compared with those reported by Tzeng et al. (1988); this difference might be due to interspecies variation. On the other hand, phytic acid content is similar to those noted by BinZhou et al. (1990) and Schwenke et al. (1990). Glucosinolates, phytic acid and tannic acid concentrations markedly decreased with the increase in the heat treatment, while trypsin inhibitor activity was destroyed completely by heat processing. Autoclaving caused more effective reduction in concentration of antinutritional factors than toasting. Autoclaving might have a deesterification effect on phytic acid. Josefsson (1975b) reported that there was a drastic reduction in glucosinolate content when the rapeseed had been heat-treated for 1 to 2 h at 115 or 120°C. Duhan et al. (1989) found that the autoclaving lowered phytic acid by 20-26% in chickpea and

 Table 1. Effect of processing on the antinutritional factors of rapeseed products (calculated on dry weight basis)

Sample	Gluco- sinolates ^a (µmol/g)	Phytic acid ^b (%)	Tannic acid ^b (%)	Trypsin inhibitor ^b (unit/mg protein)	
Raw meal	35.41	3.27	0.90	1.74	
Toasted meal	18.67	2.98	0.53	0.0	
Autoclaved meal, 1 h	13.60	2.84	0.42	0.0	
Autoclaved meal, 11/2 h	2.28	1.88	0.30	0.0	
Protein concentrate	9.83	0.86	0.00	0.0	
Protein isolate	1.85	0.27	0.00	0.0	

⁴ Mean of three determinations.

^b Mean of two determinations.

Sample	Total carbohydrate	Fructose	Sucrose	Raffinose	Stachyose
	(%)	(%)	(%)	(%)	(%)
Raw meal	18.72	0.0	4.74	Traces	0.95
Toasted meal	17.89	0.0	4.42	Traces	0.88
Autoclaved meal, 1 h	18.95	0.09	1.70	Traces	0.83
Autoclaved meal, 1 ¹ / ₂ h	17.12	0.54	1.16	Traces	0.39
Protein concentrate	1.11	0.0	0.0	0.0	0.0
Protein isolate	1.61	0.0	0.0	0.0	0.0

Table 2. Effect of processing on the total carbohydrate, mono- and oligosaccharide contents of rapeseed product (calculated on dry weight basis)

by 35-40% in black gram grains. Tannins in seeds are mostly located in the seed coat, and it is possible that the loss of tannins during processing could be due to (a) a reduced extractability, (b) a change in chemical reactivity or (c) an actual removal (Barroga et al., 1985). The decrease in tannic acid content observed in toasting treatment may be apparent and due to a change in solubility and polymerization, while in autoclaved treatments, protein concentrate and isolate is attributed to an actual decrease. On the other hand, isolation of the protein reduced glucosinolate content by almost 95%. Sosulski and Dabrowski (1984) found that preparation of protein isolate by an alkaline method reduced 96% of flour glucosinolate content. Isolated proteins had a low phytic acid content as compared to those reported by Tzeng et al. (1988). Furthermore, the isolation reduced the phytic acid content in proteins by almost 92%, whereas BinZhou et al. (1990) reported that preparation of protein isolate by three-stage countercurrent extraction at alkaline pH reduced (by one-third) the phytic acid content of defatted rapeseed meal.

Both protein concentrate and isolate are free from tannic acid and trypsin inhibitor. Liener and Kakade (1980) reported that protease inhibitors had been removed during precipitation and purification of protein isolate.

Table 2 shows that raw meal has a low content of oligosaccharides causing flatulence as compared to other oilseeds such as soybean, and pumpkin seed. The amount of flatulence factors and sucrose gradually decreased by increasing heat treatment while the concentration of fructose increased. This increase could be due to the decomposition of sucrose during heat treatment. Rackis *et al.* (1970) reported that toasted soybean meal still retains a part of its flatus-producing activity, although cooking in water causes a significant reduction in flatulence-related oligosaccharides (Ku *et al.*, 1976).

Rapeseed concentrate and isolate are free from flatulence factors. Anderson *et al.* (1979) reported that protein isolate and polysaccharide of high molecular weight are free from flatulence factors.

Amino acids	Raw meal	Toasted meal	Autoclaved meal 1 h	Autoclaved meal 1 ¹ / ₂ h	RPC ^a	RPI ^b	FAO/ WHO (1973)
Isoleucine	3.73	2.48	3.41	4.15	4.10	2.78	4.00
Leucine	6.65	6 ⋅90	7.44	7.88	6.81	8.25	7.00
Lysine	8.88	9.72	7.13	6.17	10.8	7.32	5.50
Cystine	1.97	0.71	1.70	2.06	1.87	3.06	
Methionine	1.84	1.75	2.15	1.53	1.50	1.50	
Total sulphur containing	3.81	2.46	3.85	3.59	3.37	4.56	3.50
Tyrosine	2.67	2.92	3.55	3.12	1.80	2.72	_
Phenylalanine	3.89	3.58	4.43	4.07	3.33	3.76	_
Total aromatics	6.56	6.50	7.98	7.19	5.13	7.48	6.00
Threonine	5.50	6.07	5.20	3.59	4.37	3.42	4.00
Tryptophan	1.28	0.54	1.27	0.88	1.62	2.76	1.00
Valine	4.40	3.97	4.61	5.03	3.96	3.60	5.00
Total essential amino acids	40.8	38.6	40 ·9	38.5	4 0·1	39.2	36.0
Histidine	4.18	3.84	4.45	3.87	4.08	5.08	
Arginine	6.63	6.64	7.50	6.90	5.76	7.20	
Aspartic acid	8.72	11.6	7.26	11.9	9.34	7.24	_
Glutamic acid	18.3	19.0	19.0	20.5	21.6	18·7	—
Serine	3.95	2.68	3.53	4.09	2.74	5.54	
Proline	7.47	6.42	5.64	4.87	7.73	6.58	_
Glycine	5.53	6.11	6.39	5.53	4.86	5.87	
Alanine	4.45	5.03	5.37	4.10	3.80	4.65	_
Total non-essential amino acids	59.2	61.4	59 ·1	61.5	59·9	60.8	

Table 3. Amino acid composition of rapeseed products (g/16 g N)

^a RPC: Rapeseed protein concentrate.

^b RPI: Rapeseed protein isolate.

Table 4. Effect of processing on the nutritive value of rapeseed proteins									
Sample	Crude ^a protein (%)	IVPD ^b (%)	TD ^c (%)	AD ^d (%)	EAAI ^e (%)	CS ^f (%)	First limiting amino acid	Second limiting amino acid	
Raw meal	42.9	78·8	80.4	67.4	80.2	77	Val	Ile	
Toasted meal	4 3·2	82.5	91·1	77.5	65.9	43	Try	Ile	
Autoclaved meal, 1 h	42.1	91·4	96-1	82.2	81.4	76	Ile	Val	
Autoclaved meal, 1 ¹ / ₂ h	4 2·1	80.3	87.5	74.2	76.5	78	Try	Thr	
Protein concentrate	89.4	83.5	82.1	69·0	75.8	71	Val	Tyr+Phe	
Protein isolate	92.2	89.6	90.4	76.8	77·9	65	Ile+Val	Thr	

Table 4. Effect of processing on the nutritive value of rapeseed proteins

^a On dry weight basis.

^b In-vitro protein digestibility.

^c True digestibility.

^d Apparent digestibility.

^e Essential amino acid index.

^fChemical score.

Table 3 indicates that rapeseed meal protein has the highest levels of lysine and sulphur-containing amino acids when compared with other oilseed meals. Our results agree well with those reported by Horváth and Senkálszky-Ákos (1990) and Rutkowski (1971), who reported that the amino acid composition of rapeseed meal is very similar to that of meals of other valuable oil plants and is characterized by a relatively high methionine, cysteine and lysine content. The toasted treatment resulted in an increase in the lysine content by almost 10%. On the other hand, lysine concentration gradually decreased by increasing the time of autoclaving. Mostafa et al. (1988) reported that lysine and total protein were protected by the moisture present during a 30 min dry heating at 140°C. The unavailable lysine content of the autoclaved (at 120°C for 1 h) oilseed flours was significantly higher than in the nonheated flours (Rooney et al. 1972). These results differ from those of Yadav and Liener (1978) on navy beans; they noted that the lysine content of protein had not been affected either by autoclaving for 15 min at 121°C or by roasting. The toasting has an adverse effect on the level of cystine, isoleucine and tryptophan, contrary to the autoclaving which had little effect. Rapeseed protein isolate was much higher in sulphur-containing amino acids and tryptophan than rapeseed meal, while rapeseed concentrate had a greater proportion of lysine than meal and isolate.

In the comparison of the amino acid composition of rapeseed meal and isolate to the FAO/WHO (1973)

pattern, it can be seen that rapeseed meal and isolate have the highest contents of all essential amino acids with the exceptions of isoleucine and valine, and leucine for meal and threonine for isolate.

Table 4 shows that the total protein contents were about 42%, 89% and 90% for meal, concentrate and isolate respectively. The changes in protein contents resulting from the heat treatments were quite small. The IVPDs were 78.8%, 83.5% and 89.6% for meal, protein concentrate and isolate respectively. This result is in agreement with those reported by Horváth and Senkálszky-Ákos (1990) for rapeseed meal. The in-vitro digestibility of heated meals and isolated proteins was improved due to the heat treatments, of the destruction of the trypsin inhibitor or inactivation of tannic acid. Dhingra and Kapoor (1985) noted that the low in-vitro digestibility of mango seed protein could be due to the presence of tannins which bind proteins and form undigestible complexes. It is interesting to note that the IVPD and TD of 1¹/₂ h autoclaved meal was lower than that of the meal autoclaved for 1 h. These results agree well with those reported by Yadav and Liener (1978) on navy beans. EAAI of raw rapeseed meal was 80.2%, which is similar to that reported by Sosulski and Sarwar (1973) for turnip rapeseed meal. It is higher than those of rapeseed, peanut, cottonseed, sunflower and soybean meals (Rutkowski, 1971). The value of EAAI is considerably lower than the value noted by Schwenke et al. (1990) for rapeseed meal. This difference may be due to interspecies variation. The toasting

Table 5. Effect of processing on the B-group vitamins of rapeseed products (dry weight basis)

Sample	Thiamine (µg/100 g)	Riboflavin (µg/100 g)	Niacin (µg/100 g)	Pyridoxine $(\mu g/100 g)$	Pantothenic acid (µg/100 g)
Raw meal	818	330	8 068	1 864	479
Toasted meal	573	324	7 791	1 641	328
Autoclaved meal, 1 h	438	320	7 749	1 432	424
Autoclaved meal, 11/2 h	159	310	7 498	1 246	270
Protein concentrate	63	103	1 039	75	8
Protein isolate	77	62	368	635	40

Sample	Na	Ca	К	P (all	Cu l in mg/10	Zn 0g)	Fe	Mn	Mg	Energy (kJ/100 g)
Raw meal	43.6	497	1 836	1 478	0.53	9.04	19.6	7.74	436	1 196
Toasted meal	44 ·8	539	1 876	1 572	0.69	9.41	19.0	7.61	381	1 236
Autoclaved meal, 1 h	50.6	624	1 809	1 573	1.59	8.12	20.0	7.23	334	1 237
Autoclaved meal, 1 ¹ / ₂ h	57·0	647	1 365	1 574	1.66	7.75	21.0	7.20	310	1 175
Protein concentrate	1 111	584	195	3 909	0.04	2.65	11.1	3.54	420	1 689
Protein isolate	277	294	500	2 534	0.05	7.96	6.6	1.11	84.0	1 756

Table 6. Effect of processing on the minerals and energy of rapeseed products (dry weight basis)

process lowered the EAAI from 80.2% to 65.9%; this lowering is attributed to the reduction of isoleucine, cystine and tryptophan concentrations during the toasting process. There was a slight improvement in EAAI by the autoclaving process for 1 h, while prolongation of the autoclaving process decreased the EAAI. Protein concentrate and isolate had lower EAAIs than rapeseed meal. The chemical score of raw meal (77%) implied a high nutritive value, and valine is the first limiting amino acid. The chemical score and limiting amino acids of rapeseed products varied considerably, depending on the types of process.

Table 5 shows that raw rapeseed meal contains high levels of riboflavin, niacin and pyridoxine as compared to soybean meal. Our results are in agreement with those reported by Rutkowski (1971) and Aherne and Lewis (1978). However, the contents of thiamine and pantothenic acid are rather low. Heat treatments were destructive of thiamine, pyridoxine and pantothenic acid. The reductions in their concentrations ranged from 33 to 80% (autoclaved for $1\frac{1}{2}$ h). DeRitter (1982) noted that the losses of thiamine and pantothenic acid are increased as temperature and time increase. Tannenbaum et al. (1985) found that during canning of vegetables, losses of vitamin B_6 range from about 60 to 80%. Among the heat treatments, autoclaving for 1 h caused more pronounced reductions in thiamine and pyridoxine than toasting. However, it is interesting to note that toasting resulted in a more effective reduction of pantothenic acid than autoclaving for 1 h. On the other hand, both riboflavin and niacin were stable to the heat process. Tannenbaum et al. (1985) reported that riboflavin is stable in food under most processing or cooking conditions. Also, they showed no loss in niacin content during milk processing and meat roasting. The concentrations of vitamins of the B-group drastically decreased during the preparation of protein concentrate and isolate; this reduction may be attributed to the dissolving and precipitating during their preparation.

Table 6 shows that rapeseed meal is rich in Ca, K, P and Mg. These results agree well with those reported by Aherne and Lewis (1978). The mineral contents of rapeseed meal are relatively high as compared to those of groundnut meal but similar to those reported for soybean and sunflower meals (Rutkowski, 1971). There was a small change in the mineral content by the heat treatments, with the exception of Cu which increased by 30-213%. The increase in Cu content in processed meals may be due to contamination during processing. Tannenbaum *et al.* (1985) found that the mineral content may increase during the heat process, as is the case for Ca in blanching spinach. Protein concentrate and isolate have much lower contents of all minerals except sodium and phosphorus. The high sodium and phosphorus content is attributed to the extraction of the meal with sodium hexametaphosphate during the preparation of the concentrate or its addition during the preparation of the isolate or the addition of sodium metabisulphite during the preparation of both proteins. An intake of 100 g of the meal or both concentrate and isolate is capable of supplying about 10% and 14% of energy requirement for the adult male i.e. 12 000 kJ/day (NRC, 1980).

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