Studies on the Nutritional Quality of Rapeseed Protein Isolates

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Abstract

Protein efficiency ratio (PER), net protein utilization (NPU), nitrogen digestibility (ND), organ weights, and amino acid composition were investigated for rapeseed protein isolates (RPI) prepared by alkaline countercurrent extraction and isoelectric precipitation of defatted rapeseed meal (DRM).

The PER values for 3 kinds of RPI obtained at pI of 6.7, 5.6 and 5.0 were 2.8, 3.1 and 2.9, and for DRM 2.5 while the NPU values for these RPI appeared to be 68, 73 and 71 %, and for DRM 56 %, respectively. Mean ND (85 %), food intake (87), and weight gain (37.6) for RPI were significantly different from those of DRM (71 %, 77, and 28, respectively). There was no signicant difference in the PER among three RPI and casein (3.0), nor in the NPU among those proteins and casein (74 %).

These data attributed to the favorable amino acid patterns of proteins isolated which contained balanced essential amino acids in proportions which meet the requirements of human adult(FAO/WHO, 1973).

Introduction

Rapeseeds are grown as an oilseed crop in many countries of the world. The rapeseed contains about 40 % oil and 25 % protein. The defatted rapeseed meal is characterized by relatively high content of lysine[1]. However, the use of such meal in food for human is not allowed due to the presence of the goitrogenic compounds, *L*-5-vinylthiooxazolidone and isothiocyanate[2]. In the previous work, a process has been developed for the isolation of proteins, free of such toxic substances, from defatted rapeseed meal which was extracted with a cold alkaline solution at a pH of 11[3]. The results suggested that the protein isolated have desirable functional properties and its potential utilization in food systems.

As a continued work, the biological test on protein quality of the rapeseed protein isolates in animals was studied. Data were determined for protein efficiency ratio, net protein utilization, nitrogen digestibility, organ weights, and amino acid composition.

Materials and Methods

Materials

Rapeseed of a winter variety, *Brassica napus*, was purchased from a local breeder using standard cultural practices of Jeju city in June. The rapeseeds were ground with a mill and dehulled by wind sifting. Oils in the dehulled rapeseed fraction were extracted with cold hexane in the ratio of 1 to 20 (rapeseed : cold hexane) by shaking thoroughly in a flask without heating, and finally reextracted with petroleum ether. Residual solvent was removed from the defatted meal in an air stream at room temperature, and the dried meal was disintergrated in a hammer mill equipped with a 0.5 *mm* screen.

Three samples, A, B and C, of protein isolates were prepared from defatted rapeseed meal by countercurrent extraction and isoelectric precipitation methods of Yang *et al.*[3]. The protein isolated resulted in a recovery of 92.2 % of the meal nitrogen, and showed three distinct isoelectric points (RPI A, pI 6.7 ; RPI B, pI 5.6 ; RPI C, pI 5.0). Detailed technical and documentary processes were described elsewhere[3].

Chemical composition of defatted rapeseed meal (DRM) and rapeseed protein isolates (RPI)

The chemical composition of DRM and RPI is shown in Table 1. A semi-micro Kjeldahl procedure was used for nitrogen determination[4], and protein was calculated as total N×5.5[5]. Oil, fiber, ash, and nonprotein nitrogen were determined in duplicate according to the A.O.A.C. methods[6]. *L*-5-vinylthiooxazolidone and isothiocyanate were analyzed after enzymic hydrolysis of glucosinolate according to the method of Appelqvist *et al.*[7]. The amino acid composition was analyzed by the method of Benson and Patterson[8]. The sample was hydrolyzed with 6 *N* HCl for 24 hr at 110°C. Cysteine was determined as cysteic acid after oxidation by performic acid. Tryptophan was analyzed according to the method of Inglis *et al.*[9].

Protein efficiency ratio (PER)

PER values were determined by the method of Osborne *et al.*[10]. Weanling albino male rats of the Sprague-Dawley strain, about 21 day old weighing average 45~55 *g*, were used. Thirty rats were divided into four (tests) and casein (control) groups. Each group consisted of six rats. Four groups were as follows : RPI A, RPI B, RPI C, and DRM. All groups in each experiment had the same average initial body weight. The rats were caged individually with a diet containing 10 % protein level and allowed to a diet and water *ad libitum* for 4 weeks. Room temperature was 24~25°C, humidity 50 %, and lightning consisted of 12 hr light/12 hr darkness. All rats were fed a stock laboratory diet for 2 days prior to being placed on the experimental diets. Body weight and food intake were measured twice weekly. The content of the basal diet was supplemented with a 4 % salt mixture[11], 5 % corn oil, 2 % vitamin mixture[11], 0.1 % choline solution and enough corn starch to adjust to 100. ANRC(Animal Nutritional Reference Council) reference casein (Shefield Chemical Co., Union, NJ) was the standard.

Net protein utilization (NPU)

NPU values were determined by the procedure of Bender and Doell[13]. Male albino rats, 21 day-old, were fed the experimental diets for 10 days. Ten rats were used in each dietary groups. A protein-free diet and diets containing 10 % protein level were made at expense of corn starch. The protein level of the experimental diets was calculated from the total amino acid content of the protein hydrolyzate. An otherwise adequate but protein-free basal diet contained the following ingredients in percent : corn starch 86.9, corn oil 5, salt mixture 4, vitamin mixture 2, non-nutritive fiber 2, choline solution 0.1. Sixty rats were divided into 6 groups (RPI A, RPI B, RPI C, DRM, protein-free, and casein). Casein was used as the standard (control). Each composition of the salt and vitamin mixtures was according to Harper[13]. Other experimental conditions were followed by the same procedure as described in PER experiments.

At the termination of the experiment, The rats were sacrificed by the injection of 15 *mg* of sodium pentobarbital in 0.5 *ml* of water. After autoclaving the carcasses in individual mason jars for 4 hr, each carcass was ground with a Waring blender and

representative duplicate samples were taken for the determination of nitrogen content by macro-Kjeldahl procedure[14].

During the 10 days feeding experiment, feces of each rat were collected in a 125 *ml* Erlenmeyer flask containing 50 *ml* of 20 % HCl. After autoclaving for 2 hr and cooling to room temperature, the fecal sample was pressed through a sieve and made to 250 *ml* with water. Duplicate 10 *ml* aliquots were used for the nitrogen determination.

Analysis of variance was used to test the differences among the nutritional values of the experimental diets. Duncan's new multiple range test[15] was used to determine the differences among treatment means.

Nitrogen digestibility (ND)

ND values were analyzed by measuring fecal nitrogen excretion after feeding a nitrogen-free diet, as described by the method of Saunder and Kohler[16].

Organ weights

At the termination of measuring NPU, thyroid and liver were removed. Fresh weights of the thyroid and liver were recorded and the tissues were stored at −20°C. A two-way analysis of variance appropriate to the random block design was carried out for all the variables according to the method of Cochron and Snedecor[17].

Results and Discussion

Chemical analysis

As shown in Table 1, protein contents of defatted rapeseed meal (DRM) and its protein isolates were 38.77, 99.7, 99.8, and 99.5 %, respectively. On the other hand, oxazolidinethione and isothiocyanate contents in DRM were 0.46 and 0.22 *mg*, whereas 3 RPI samples showed no contents of such toxic factors. There was no significant difference among the protein contents of 3 RPIs. DRM contained the greatest amount of nitrogen-free extract (42.35 %), whereas RPIs the lowest. It is worth noting that all 3 RPIs were low in fiber, an advantage for human on low fiber diets.

The amino acid profiles in Table 2 are typical of those for RPI, which contained very well amino

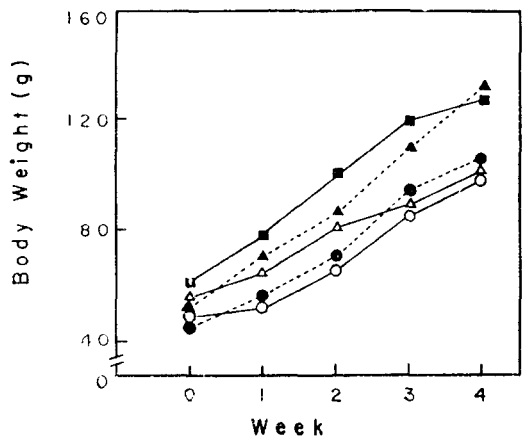

Fig. 1. Body weight gain during the 4-week protein efficiency test on the 10 % protein diets
△—△: RPI A (pI 6.7), ▲···▲: RPI B (pI 5.6), ■—■: RPI C (pI 5.0), ○—○: DRM, ●···●: Casein

acid balance as compared with amino acid requirements of human adult (FAO/WHO, 1973). The amounts of 10 kinds of amino acids were higher in RPI B obtained at pI 5.6 than in RPI A obtained at pI 6.7 or in RPI C obtained at pI 5.0.

Protein efficiency ratio

Table 3 shows the results of food intake, protein intake, weight gain, and PER. The PER values revealed that RPI B obtained at pI of 5.6 had better protein quality than RPI C (pI 5.0) and RPI A (pI 6.7), while DRM had a lower value than 3 RPIs. The analysis of variance showed that the PER values were significant for 3 RPIs prepared from DRM. Lower PER in the case of RPI A (pI 6.7) obtained from DRM may be due to a comparably less amount of essential amino acids present than those of RPI B (pI 5.6) and RPI C (pI 5.0).

Growth rate in different periods was uniform in the test rats fed an amount of diets of each RPI (Fig. 1), and the weight gains of the test rats fed each RPI were higher than those of rats fed DRM diet. The gain in weight for the group fed RPI B (pI 5.6) was significantly higher than that of any other groups. The results agreed well with those obtained El Nocklashy *et al.*[19] for a RPI obtained at pH 6.0 prepared from *Brassica napus*, *Erglu*,

Table 1. Chemical composition of defatted rapessed meal and protein isolates (dry weight basis)

Component	*Brassica napus*, variety			
	Defatted rapeseed meal	Protein isolates		
		A	B	C
Protein, %	38.77	99.7	99.8	99.5
Oil, %	3.02	0.0	0.0	0.0
Ash, %	8.25	0.2	0.1	0.2
Fiber, %	4.16	0.0	0.0	0.0
Nitrogen-free extract, %	42.35	0.4	0.2	0.3
Oxazolidinethione, *mg/g*	0.46	0.0	0.0	0.0
Isothiocyanate, *mg/g*	0.22	0.0	0.0	0.0

A : RPI obtained through isoelectric precipitation at pI of 6.7
B : RPI obtained through isoelectric precipitation at pI of 5.6
C : RPI obtained through isoelectric precipitation at pI of 5.0

Table 2. Essential amino acid content of defatted rapeseed meal, protein isolates, FAO/WHO pattern, and requirements of humans (*g*/100*g* protein)

Essential amino acid	*Brassica napus*, variety				FAO/WHO[b]		
	Defatted rapeseed meal	RPI			Amino acid (%)	Requirements	
		A[a]	B[a]	C[a]		Child (%)	Adult (%)
Lysine[c]	6.24	3.79[c]	5.5	4.49	5.5	7.5	2.2
Tryptophan	0.88	0.82	1.04	0.93	1.0	0.5	0.6
Valine	4.00	4.01	5.01	4.10	5.0	4.1	1.8
Cystine	1.64	1.72	2.42	2.18	—	—	—
Methionine	1.81	1.82	1.86	1.80	—	—	—
Total sulfur amino acid	3.45	3.54	4.28	3.98	3.5	3.4	2.4
Isoleucine	4.00	3.19[c]	3.66[c]	3.39[c]	4.0	3.7	1.8
Leucine	7.45	6.25	7.52	6.10	7.0	3.4	2.4
Phenylalanine	3.21	3.28	3.49	3.44	—	—	—
Tyrosine	2.38	1.55	3.00	2.42	—	—	—
Aromatic amino acid	5.59	4.83	6.49	5.86	6.0	3.4	2.5
Threonine	3.53	3.92	4.88	4.31	4.0	4.4	1.3

[a] Yang *et al.* (1978) [b] FAO/WHO (1973)[18] [c] Limiting amino acid

measured in chicks.

Net protein utilization, nitrogen digestibility and organ weights

The growth data of young rats fed for 10 days on an otherwise but protein-free diet or the same diet supplemented from each RPI or DRM or casein are summarized in Table 4. The nitrogen digestibility and the NPU of the diet containing 10 % RPI B (pI 5.6) were higher than those of RPI C (pI 5.0).

The nitrogen digestibility and the NPU of the diet containing 10 % casein were significantly ($p < 0.01$) higher than comparable values of the diet containing 10 % RPI B (pI 5.6). The results indicated, however, that the growth rate with fed RPI B was lower in initial stages, but it was better than casein in the longer run.

The fecal nitrogen value of the group fed DRM diet was remarkably higher than that of the rat fed diet containing the comparable level of casein. Although the protein content of all diets was same, the DRM diet had non-nutritive crude fiber (4.16 %)

Table 3. PER values of rapeseed protein isolates, defatted rapeseed meal and casein

Protein	Initial body weight(*g*)	Food intake per animal (*g*/28 days)	Protein intake per animal (*g*/28 days)	Mean Wt. gain per animal (*g*/28 days)	PER
RPI A*	45.2±0.03[a]	233[b]	23.3±0.15[a]	65.3±2.3[a]	2.8±0.00[a]
RPI B	50.4±0.23	285	28.5±0.46	86.8±4.2	3.1±0.04
RPI C	54.2±0.54	289	28.9±0.53	85.4±2.1	2.9±0.07
DRM	50.5±0.63	230	23.0±0.44	60.2±0.5	2.5±0.08
Casein	45.1±0.27	238	23.8±0.65	66.1±7.9	3.0±0.02

[a] Standard error. [b] Average value of one animal; 6 animals in each group. * See footnote in Table 1. Results expressed at PER adjusted to 3.0 for standard casein

Table 4. Net protein utilization, nitrogen digestibility and organ weight of rapeseed protein isolates, defatted rapessed meal and casein standard

Protein[a]	Food intake (*g*)	Weight gain (*g*)	Fecal nitrogen (*g*)	Nitrogen[b] dig. (%)	NPU[c] (%)	Thyroid* (*mg*)	Liver* (*mg*)
PPI A	83	32	225	80	68	2.2	0.5
RPI B	92	45	230	89	73	2.5	0.7
RPI C	87	36	226	86	71	2.3	0.6
DRM	77	28	324	71	56	3.1	1.0
Protein-free	40	−10	73	—	—	2.0	0.2
Casein (10 %)	90	38	227	90	74	2.2	0.4

[a] Ten rats per diet group
[b] Nitrogen distibility=N intake-(fecal N-fecal N of protein-free group)/N intake×100
[c] Net protein utilization=(Carcass N-carcass N of protein-free group)/N intake×100
* Dry weight

and nitrogen-free extract (42.35 %).

The weights of the thyroid and liver of rats fed DRM diet or each RPI diet were significantly affected by the test diets, but on equal body weight basis, the DRM diet group had slightly higher in the longer run. Eklund *et al.*[20] reported no abnormal symptom of any enlargement of thyroids in young male and female rats by feeding of rapeseed protein concentrate (RPC) diets for 12 weeks. A similar observation was reported by Anderson *et al.*[21] in pregnant rats fed rapeseed flour. Thus, by plant breeding and by the processing involved in the preparation of rapeseed protein concentrates, the anti-thyroid substances have been reduced to very low levels. The liver weights for all of the RPI groups were higher than those for casein-fed rat. On equal body weight basis, however, these differences were eliminated. In their 12-week studies, just as they did not observe any difference in body weight gains.

Although the amino acid patterns of all of the RPI were adequate and comparable, the poor performances of the defatted rapeseed meal are certainly due to the presence of rather large amounts of isothiocyanate, oxazolidienthione, and glucosinolate.

All samples tested contained essential amino acids in proportions which meet the requirements of human adult. The good food intake, the excellent weight gain, the high protein efficiency ratio, the good nitrogen digestibility and adequate net protein utilization observed with rapessed protein isolates from the one variety of *Brassica napus*, can be attributed to the favorable amino acid patterns derived from them.

In summary, the results indicated that the nutritional quality of rapeseed protein isolates is relative to casein but may be improved by the addition of some amino acids. It can be expected that the protein isolates will contribute to filling the protein gap of the world's population. According to FAO/WHO for human requirements, lysine, methionine,

and isoleucine are the limiting amino acid in rapeseed proteins, and found in proportions even higher than those of human requirements. It would serve as an excellent protein supplement to cereal grains and also may be used as the source of protein.

요 약

역류 추출 및 등전 침전에 의한 평지씨 단백질의 분리(한국 식품 과학 회지, **10**(2), 162 (1978)에 이어 그들의 영양가(단백질 효율, 정미 단백질 이용율, 질소 소화율, 성장율, 아미노산 조성 및 기관 중량)를 실험하였으며 다음과 같은 결과를 얻었다. 각 등전점(pI, 6.7, 5.6, 5.0)에서 분리한 3가지 단백질의 PER는 각각 2.8, 3.1 및 2.9였고 평지씨 박(탈지박)의 PER는 2.5로 낮았으며 한편 3가지 단백질의 NPU(정미 단백질 효율)은 각각 68, 73 및 71%였고 탈지 평지씨 박은 56%로 제일 낮았다. 분리된 3가지 단백질의 평균 질소 화율은 85%, 사료 섭취량은 87 g 그리고 체중 증가량은 37.6 g였으며 이에 반하여 탈지 평지씨박의 그것들은 각각 71%, 77 g 및 28 g 등으로 현저한 차이를 보였다. PER의 값에 관한 분리된 3가지 단백질 간에는 큰 차가 없었고 이들의 NPU 값과 카제인의 NPU 값은 서로 비견할 수 있었으며 특히 등전점 5.6에서 분리한 단백질은 카제인의 PER 값이나 NPU 값에 비해 약간 높은 값을 보였으며 아미노산 조성에서도 필수 아미노산이 고루 함유된 우수한 단백질로 나타났다. 또한 갑상선 및 간장의 무게 증가는 나타나지 않았으며 성장저해 인자가 전연 없었다. 분리된 3가지 단백질 중의 아미노산은 FAO/WHO의 성인 요구량에 비해 평형을 나타냈거나 상회하였다.

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