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Effect of proteolytic modification and methionine enrichment on the nutritional value of soya albumins for rats

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Proteolytic modification, particularly when combined with methionine enrichment, significantly reduced the lectin content and modified the trypsin inhibitor activity of a soya albumin preparation. During both peptic hydrolysis and enzymatic peptide modification, the amount of soya bean agglutinin was reduced. This reduction was verified by the decrease in the intensity of protein staining and immunoblots of the peptic hydrolysate and of the product produced by enzymatic peptide modification, and confirmed by haemagglutination activity measurements and ELISA technique. The functional activity of Kunitz trypsin inhibitor was also partially abolished whereas that of the Bowman-Birk inhibitor was not significantly altered by the enzymatic modifications. In line with the reduction in some of the antinutrients, the nutritional performance of rats fed a diet containing this enzymatically modified product was significantly improved in comparison with that of the control animals fed the original soya albumin but was poorer than the lactalbumin-fed controls. Enzymatic peptide modification of soya albumin fractions by transpeptidation and covalent methionine enrichment is suitable to improve their nutritional value partly by compensating for their methionine deficiency and partly by modifying the structure of the soya antinutrients. Changes in the structure by proteolytic hydrolysis and synthesis of peptide chains can lead to alteration in protein conformation, resulting in modified biological activity and increased nutritional value. (J. Nutr. Biochem. 7:481–487, 1996.)

Keywords: enzymatic modification; methionine enrichment; soya albumin; nutritional value; lectin content; trypsin inhibitor activity

Introduction

Soya meal is extensively used in animal nutrition and the use of soya products in human food is also increasing. Unfortunately, as soya beans contain a number of antinutritive components, mainly the lectin (soya bean agglutinin, SBA) and trypsin inhibitors (Kunitz-type trypsin inhibitor, KTI and Bowman-Birk-type protease inhibitor, BBI), the efficiency of the nutritional utilization of diets containing soya bean is below of that expected on the basis of chemical composition, thus limiting the wider and more general use of soya products[1,2] particularly those rich in antinutrients such as soya whey.

By recognition and specific binding to the glycosyl side chains of receptors of endogenous or exogenous growth factors, lectins can alter the metabolism of brush border epithelium,[3] modify glycosylation,[4] interfere with food digestion and absorption, and change the microbial ecology of the gut.[5] Exposure to high concentrations of these lectins can lead to intestinal damage and malabsorption of dietary components, and the severity of the antinutritional effects are in most instances correlated with the agglutinating activity of the lectins.[6] Recognition of the importance of interaction between dietary lectins, gut receptors, and bacteria

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Received February 9, 1996; accepted May 30, 1996.

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0955-2863/96/$15.00
PII S0955-2863(96)00093-9

has helped to explain the differences between toxic and non-toxic lectins and to work out effective strategies for improving the quality and safety of food.[7]

Recent observations have indicated that some of the harmful effects of dietary lectins on metabolism and health are due to their powerful modulation of the body's hormone balance and that these are also mediated and reinforced by immune responses to the lectins.[8,9] As these physiological responses make significant contributions to the efficiency of the overall utilization of soya products, it is of great importance to understand how these reactions are involved in the mechanism of the antinutritional effects of the dietary soya bean lectin.

The antinutritional effects of trypsin inhibitors are thought to be due to their interference with digestion in the gastrointestinal tract. Although the effects of trypsin inhibitors on the small intestine differ from those of lectins,[10] both these types of antinutrients are powerful inducers of the growth of the pancreas.[11] Indeed, continuous overstimulation of the pancreas in both animals and man may be one of the most deleterious effects of diets containing these antinutrients, particularly, in the long-term.

To reduce the antinutritional effects, presently all soya products have to go through an expensive heat treatment procedure or other processing to reduce the concentration of antinutrients. However, these can also lead to losses of essential amino acids and the production of toxic by-products. An understanding of the biochemical and gastroenterological causes of the poor nutritional value of soya proteins could lead to developing cost-efficient methods of detoxification procedures and bring considerable economical benefits particularly if with these methods the use of soya products of little nutritional and economic value such as soya whey could be increased.

Protein modification for human use has ancient roots.[12] Amongst the biochemical/biotechnological procedures, proteolysis and proteolytic modification reactions are of special importance for optimizing food proteins with respect to nutritional quality and functional and biological activities.[13,14,15,16] The goal of enzymatic modification is to improve the nutritional quality of proteins mainly from plants and to formulate more nutritious diets to fit present day healthy lifestyles. Plant proteins are usually deficient in one or more essential amino acids. In legumes this is methionine, whose deficiency is one of the main factors limiting utilization. As methionine is involved in many reactions potentially beneficial for health,[17] when the sulphur-containing amino acid content is suboptimal, this limits not only the nutritional value of legume proteins but may also jeopardize food safety.

Protein conformation can be substantially altered by enzymatic peptide modification (EPM) catalyzed by proteases.[18] Moreover, this same process can also be used for amino acid-enrichment of the peptide chains of these proteins.[19,15,20] The aim of the present work was therefore, to develop an EPM method to improve the nutritional value of soya bean and at the same time to overcome the methionine deficiency of its proteins. It was indeed shown in feeding trials with rats that the nutritional value of soya proteins is significantly improved by the EPM modification method described in the present paper.

Methods and Materials

Chemicals

All chemicals used were the highest quality purchased from Sigma Chemical Co. (Poole, Dorset, UK). Soya beans (*Glycine max*) were purchased from Real Foods (Edinburgh, UK). A soya albumin fraction was prepared as previously described.[21] Briefly, seed meal was defatted by extraction with petroleum ether (sample:solvent, 1:20, w/v) at room temperature overnight, and air-dried. The defatted meal was extracted by stirring with 0.2 M citrate/phosphate buffer pH 7.0 (sample to solvent ratio 1:10; w/v) overnight at +1°C, centrifuged at 50,000 × g for 20 min, the supernatant decanted and its content recovered by freeze-drying.

Enzymatic hydrolysis and EPM

Proteolytic treatment of soya bean albumin (SBALB) was carried out with pepsin (substrate: enzyme, 100:1, w/w, Serva) in solution at pH 2.0 for 2 hr with continuous stirring, after which the solution was freeze-dried. The peptic hydrolysate of soya bean albumin fraction (SBALB-AH) was dissolved in water to give a solution of 20%, w/v, adjusted to pH 4.0 with dilute NaOH and used as the substrate of the enzymatic peptide modification process in the presence of L-methionine ethyl ester at a ratio of 0.4:1 to the protein (w/w). The reaction mixture was incubated with pepsin (substrate:enzyme, 100:1, w/w) for 16 hr at 37°C without stirring, dialyzed against distilled water at 5°C for 24 hr to remove the excess of free methionine and methionine ethyl ester and freeze-dried (soya bean albumin fraction after enzymatic peptide modification: SBALB-EPM).

Haemagglutination assay

For the estimation of the haemagglutinating activity (HU) soya preparations were extracted with phosphate-buffered saline, pH 7.0 and the solutions were serially diluted and mixed with trypsin-treated rat blood cells.[22,23] One unit of HU was defined as the amount of material in the last dilution giving 50% agglutination of the cells. The specific activity was given as HU kg^{-1} of material.

Trypsin inhibitor activity

The protease inhibitor activity of KTI and BBI was determined as previously described.[24] Commercial trypsin (Sigma UK, type III from bovine pancreas) or chymotrypsin (Sigma UK, type 1-S from bovine pancreas), in which the true enzyme content was estimated by active site titration using p-nitrophenyl-p'-guanidinobenzoate and N-trans-cinnamoyl imidazole respectively,[25,26] was added in excess to the extracts and the mixtures were left to react at 0°C. The level of uninhibited trypsin activity in the reaction mixture was assessed using N α-benzoyl-DL-arginine-p-nitroanilide as substrate[27] and the level of uninhibited chymotrypsin was evaluated using glutaryl-phenylalanine-p-nitroanilide as substrate.[28] Trypsin and chymotrypsin inhibitory activities were expressed as g enzyme inhibited per kg meal and the protease inhibitor content of the test diets was calculated on the basis of the level of meal inclusion in the diet.

SDS-PAGE electrophoresis

This was carried out in thin layer gels (135 mm × 165 mm × 0.7 mm) by a method adapted from Laemmli.[29] The total acrylamide content of the running gel was 17.6% with 0.45% cross-linkage and that of the stacking gel was 3.95% with 1.42% cross-linkage. Samples (10 $mgxml^{-1}$) were incubated at 100°C for 2 min in Tris-glycine buffer, pH 8.3 containing 3% SDS and 0.1% (v/v)

β-mercaptoethanol. After electrophoresis the gels were stained with 0.5% Coomassie Brilliant Blue R (Sigma, UK).

PAGE in non-dissociating media

This was carried out the same way as the SDS-PAGE, but the samples contained no SDS or β-mercaptoethanol and were not boiled. The running buffers were also without SDS or β-mercaptoethanol.

Transblotting

After SDS-PAGE, the gels were transblotted and reacted with the appropriate antibodies as described previously.[10]

ELISA

An indirect, competitive ELISA method was used as before.[10]

Animal handling and experimental protocols

Male Hooded-Lister spf (specified pathogen-free) rats of the Rowett colony, weaned at 19 days were fed on a stock diet until they were 30 days old, weighing 80 ± 1 g. The rats were kept singly in metabolic cages throughout the experiment and pre-fed on a semi-synthetic, good quality diet containing lactalbumin (100 g kg^{-1} diet) control diet (6 g rat^{-1} day^{-1}) (30) for 3 days. Two groups of rats (5 animals per group) were then changed to diets in which half of the lactalbumin protein was replaced with either SBALB or with SBALB-EPM into which methionine was incorporated (*Table 1*). The peptic hydrolysis product, SBALB-AH, was not tested in animal experiments. Throughout the experiment the rats were pair-fed and their food intake was restricted to that of the SBALB group, which contained approximately 26.8 g KTI, 5.6 g BBI and 3.3 g lectin per kg of diet. In the test diet the inclusion of SBA-EPM was the same as in the SBALB diet but because of the modification, the effective antinutrient concentration was less; 20.7 g KTI, 5.5 g BBI and 2.3 g lectin. The third group was fed the control lactalbumin (LA) diet throughout the experiment. All diets were fully supplemented with amino acids, vitamins, and minerals to target requirements to provide optimal growth.[31] Feeding was for 10 days and water was given *ad libitum*. The rats were weighed daily. In the morning of day 11, all rats were given 1.5 of their appropriate diets and killed by halothane overdose 2 hr later. Stomach, jejunum (20 cm piece; 5 to 25 cm from pylorus), the remaining parts of the small intestine, caecum, colon, pancreas, and liver were removed, washed with ice-cold saline, blotted dry, and weighed. A section of the small intestine (2 cm; 5 cm from pylorus), together with representative sections of the pancreas, stomach, colon, and caecum were cut, weighed, and fixed in 4% phosphate-buffered (pH 7) formalin for histological examination. The remainder of the tissues was freeze-dried to constant weight and used for chemical analyses.

Table 1 Composition of experimental diets

	Diet (g/kg)		
	Control	Soya bean albumin	MET-enriched soya bean albumin
Soya albumin	—	118	—
MET-enriched soya albumin	—	—	112
Lactalbumin	120	—	—
Corn starch	380	382	388
Potato starch	100	100	100
Glucose	150	150	150
Corn oil	150	150	150
Vitamins[1]	50	50	50
Minerals[1]	50	50	50
Silicic acid	0.40	0.40	0.40
L-leucine	—	2.34	2.34
L-isoleucine	—	2.58	2.58
L-valine	—	2.58	2.58
L-tryptophan	—	0.16	0.16
L-methionine	—	1.22	—

[1]The vitamin mixture contained 200 mg thiamin, 200 mg pyridoxine, 200 mg riboflavin, 200 mg p-amino benzoic acid, 600 mg nicotinic acid, 400 mg calcium pantothenate, 100 mg folic acid, 100 mg biotin, 8,000 mg inositol, 240 mg retinol, 50 mg cholecalciferol, 1,200 mg all-rac-a-tocopherol, 2 mg menadione, 500 mg cyanocobalamin, and 16 g choline chloride made up to 1 kg with corn starch.[23]

[2]The mineral mixture comprised 400 mg $CuSO_4.7H_2O$, 5 g $FeSO_4.7H_2O$, 4 g $MnSO_4.4H_2O$, 3.6 g $ZnSO_4.7H_2O$, 40 mg KI, 40 mg KIO_3, 120 mg NaF, 10 mg NH_4VO_3, 80 mg $NiCl_2.6H_2O$, 120 mg $SnCl_4.5H_2O$, 6 mg $NaSeO_3$, 0.96 g $CrK(SO_4)_2.12H_2O$ [chrome alum], 410 g $CaCO_3$, 314 g KH_2PO_4, 22 g KCl, 102 g $MgSO_4.7H_2O$, and 142 g Na_2HPO_4 in approximately 1 kg.[23]

Chemical analysis

Tissues were extracted in the presence of an internal standard of 1,7-diamino heptane with 10% (w/v) perchloric acid (15 mg tissue per ml) for 30 min at 0°C, centrifuged and individual polyamines in the supernatant were measured by HPLC.[32]

The protein content of the residue insoluble in perchloric acid was determined by a modified Lowry method[33] after solubilization in 0.3 mol L^{-1} NaOH. RNA[34] and DNA[35] concentrations were estimated as described previously.[7]

Statistical analysis

The results were subjected to one-way analysis of variance (ANOVA) using the Minitab computer program (Penn State University, State College, USA). When $P < 0.05$, the significance between groups was estimated by Student's *t*-test.

Results

Effect of EPM on soya antinutrients

Evaluation of the SDS-PAGE patterns after Coomassie-blue staining (*Figure 1*) and immunoblotting (*Figure 2*) of different amounts of SBA, SBALB, SBALB-AH, and SBALB-EPM showed that during the peptic hydrolysis, the zone corresponding to SBA was reduced and decreased further after modification by EPM. Furthermore, the SBALB preparation contained 4.2×10^7 haemagglutination units/100 g, whereas in SBALB-EPM this was significantly less, 3.0×10^5. Similarly, SBALB was shown by ELISA to contain 3.3 g SBA but in the EPM product this was reduced to 2.3 g/100 g.

There were also changes in total trypsin inhibitor activity of the modified soya albumins as shown by electrophoretic separation followed by reacting the gels with trypsin (*Figure 3*). Thus, the intensity of staining of the KTI zone was significantly reduced (*Figure 3*) even though the inhibitor activity of BBI was essentially not different after the enzymatic modifications. This was also confirmed by quantitative enzyme assays. Thus, the SBALB preparation inhibited 345 mg trypsin/g material; this was reduced to 265 mg in

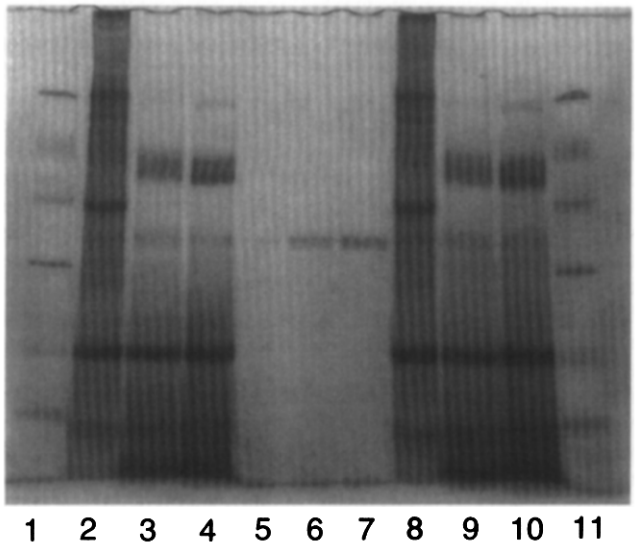

Figure 1 SDS PAGE of proteins of soya albumin and enzymatically modified soya albumin samples. The lanes contained the following samples: 1. low molecular weight standard (LMW, Pharmacia); 2. SBALB (50 mg); 3. SBALB-AH (140 mg); 4. SBALB-EPM (140 mg); 5. SBA (0.5 mg); 6. SBA (2.0 mg); 7. SBA (3.0 mg); 8. SBALB (70 mg); 9. SBALB-AH (180 mg); 10. SBALB-EPM (180 mg), and 11. LMW. Staining was by Coomassie-blue.

SBALB-AH and further decreased to 219 mg trypsin/g in the EPM product. In contrast, the corresponding values for chymotrypsin inhibition showed less changes; the values were 173, 188, and 133 mg chymotrypsin inhibited/g of SBALB, SBALB-AH, and SBALB-EPM, respectively. Based on ELISA assays with the respective polyclonal antibodies of the two inhibitors, SBALB contained 26.8 g KTI and 5.6 g BBI but these values were reduced by the EPM modification to 20.7 g KTI and 5.5 g BBI per 100 g material.

Effects of EPM on nutritional performance

The final body weight of rats fed diets containing unmodified soya albumin for 10 days was significantly less than that of pair-fed control animals in spite of both groups having similar starting body weight and the same food intake (but a slightly higher N intake with SBALB). In contrast, the weight gain of rats fed diets in which the soya albumin was replaced by an equal weight of the EPM product was considerably higher (*Table 2*). Whereas the fecal output on the SBALB diet increased significantly, with the EPM-protein it did not differ from that of the controls. Similarly, the fecal N loss found with SBALB diet was also significantly reduced by the EPM treatment. In comparison with control values the volume of urine in rats fed SBALB diet was greatly increased. However, although there was a further significant increase in urine output with the SBALB-EPM diet, the N and urea N contents of the urine samples from these rats were significantly less than those obtained from rats fed SBALB diet (*Table 2*). As a result, the N balance with the modified protein was significantly improved when compared with the untreated soya albumin although it did not reach the values of the control animals (*Table 2*).

Although the dry body weight and its N content were significantly reduced in rats fed diets containing unmodified soya albumins in comparison with control animals, after EPM treatment the extent of the reduction in these values was significantly less (*Table 3*). In contrast, the total body lipid content was not different on the three different diets.

The weight and chemical composition of the small intestine of rats fed SBALB diet significantly increased but this was partly reversed by the EPM modification (*Tables 3 & 4*). In contrast, the modest increase in pancreatic weight in rats fed SBALB diet was significantly further increased with the EPM product. The dry weight of spleen, liver, and heart of rats fed SBALB diet was considerably less than that of the control animals and this was not changed by the EPM treatment. However, the loss of gastrocnemius muscle mass of rats fed SBALB-diet was further significantly increased in rats fed diets containing SBALB-EPM (*Table 3*). In contrast the significant reduction in the thymus weight on SBALB diet was completely reversed by the EPM treatment; in fact the thymus weight became significantly larger than that of the control rats (*Table 3*).

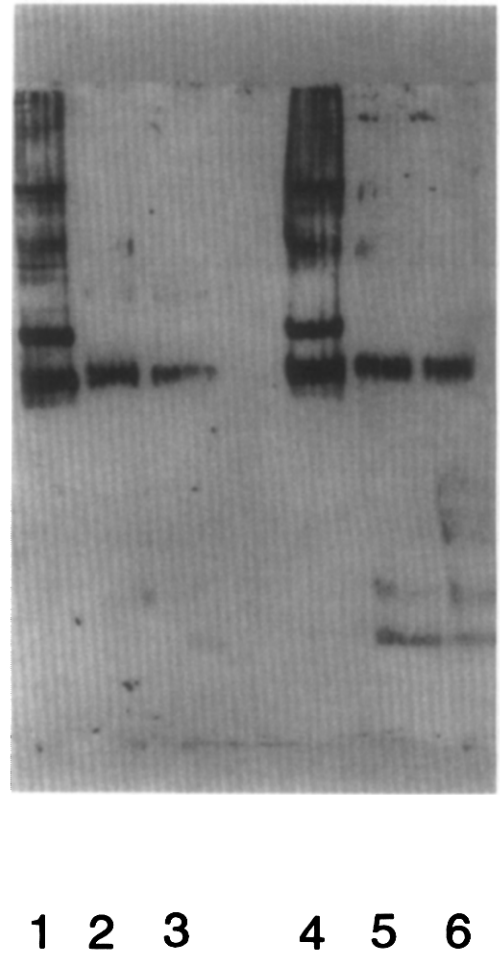

Figure 2 Immunoblotting of different soya proteins. The lanes contained the following materials: 1. SBALB (140 mg); 2. SBALB-AH (140 mg); 3. SBALB-EPM (140 mg); 4. SBALB (180 mg); 5. SBALB-AH (180 mg) and 6. SBALB-EPM (180 mg). After electrophoretic transfer, the blots were reacted with anti-SBA rabbit IgG antibody followed by peroxidase-antiperoxidase reaction, and finally the colors were developed with 4-chloronaphthol-H_2O_2 reagent.

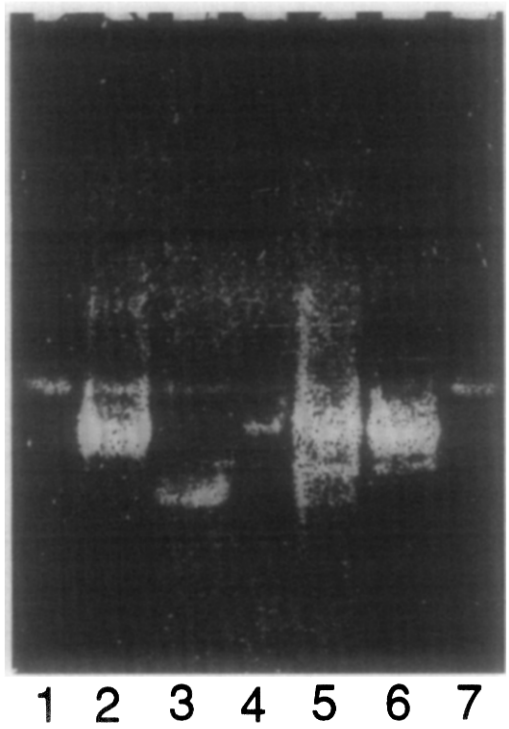

Figure 3 Reaction of trypsin inhibitors and enzymatically modified soy albumin samples with trypsin after native PAGE. Samples run were: 1. Kunitz trypsin inhibitor (KTI); 2. soya albumin; 3. proteolytic hydrolysate of soya albumin; 4. Bowman-Birk inhibitor (BBI); 5. peptic hydrolysate of soya albumin; 6. peptic EPM of soya albumin; 7. KTI. The bands were visualized by exposure to trypsin and its substrate N-acetyl-DL-phenylalanine β-naphthyl ester followed by negative staining with tetrazotized o'-dianisidine-$ZnCl_2$ complex.

Discussion

An enzymatic peptide modification method was developed with the aim to increase covalently bound methionine content and decrease the amounts of antinutrients in soya protein preparations. A major objective of this work was to improve the nutritional value of soya albumin proteins and to reverse, at least in part, the previously well-demonstrated negative effects of antinutrients, the lectin, and the two trypsin inhibitors contained in soya albumin preparations.[10]

In the first step of this modification the protein was subjected to peptic hydrolysis. In the second step, transpeptidation and covalent methionine incorporation into the protein chains occurred in the presence of pepsin, as catalyst. From a wide range of proteolytic enzymes tested pepsin proved to be the most effective catalyst bringing the covalently attached methionine content of SBALB-EPM to target requirements (*Table 1*).

During peptic hydrolysis the amount of SBA in SBALB-AH was reduced, judged from the decrease in the intensity of protein staining and immunoblots of the SBA bands after SDS-PAGE, with further decreases in the amount of SBA

Table 2 Nutritional performance of rats fed diets containing unmodified soya albumin, an EPM-modified soya albumin preparation or control diet for 10 days

	Diet			
	LA (control)	SBALB	SBALB-EPM	SD
Food (g)	60.0	60.0	60.0	0
Intake N (mg)	990[a]	1050[b]	1080[c]	0
Initial BW (g)	83.4[a]	84.2[a]	83.4[a]	1.1
Final BW (g)	94.4[a]	79.3[b]	83.5[c]	1.5
Change (g)	+11.0[a]	−4.9[b]	+0.1[c]	1.7
Feces (mg)	3127[a]	4994[b]	3743[a]	361
Fecal N (mg)	146[a]	433[b]	290[c]	25
Urine (mL)	22.5[a]	30.5[b]	36.5[c]	2.0
Urine N (mg)	155[a]	484[b]	392[c]	40
Urea N (mg)	101[a]	391[b]	299[c]	42
N Balance (mg)	+689[a]	+133[b]	+398[c]	55

Values represent means of five rats per treatment.
Different superscripts refer to significant differences between treatments ($P < 0.05$), based on Students *t*-test.

Table 3 Changes in dry body weight and composition and organ weight of rats kept fed control or soya protein diets for 10 days

	Diet		
	LA (control)	SBALB	SBALB-EPM
Dry body weight (g)	27.9 ± 0.7[a]	22.7 ± 1.1[b]	24.4 ± 0.6[c]
lipid (g)	5.2 ± 0.5[a]	4.9 ± 0.3[a]	4.7 ± 0.2[a]
N (g)	3.1 ± 0.1[a]	2.5 ± 0.1[b]	2.7 ± 0.1[c]
Dry organ weights (mg)			
Small intestine	604 ± 22[a]	767 ± 24[b]	690 ± 60[b]
Pancreas	138 ± 8[a]	150 ± 12[ab]	167 ± 11[b]
Spleen	63 ± 5[a]	45 ± 4[b]	48 ± 4[b]
Liver	1000 ± 51[a]	907 ± 154[ab]	890 ± 41[b]
Thymus	49 ± 3[a]	36 ± 7[b]	73 ± 14[c]
Heart	91 ± 5[a]	79 ± 3[b]	81 ± 5[b]
Gastrocnemius muscle	214 ± 8[a]	179 ± 7[b]	151 ± 14[c]

Values represent means of five rats per treatment.
Different superscripts refer to significant differences between treatments ($P < 0.05$), based on Students *t*-test.

Table 4 Chemical composition of small intestine and pancreas of rats fed control or soya protein diets for 10 days

	Diet		
Tissue	LA (control)	SBALB	SBALB-EPM
Small intestine			
Protein (mg)	427 ± 25[a]	501 ± 13[b]	463 ± 34[ab]
RNA (mg)	495 ± 97[a]	1290 ± 189[b]	948 ± 198[c]
DNA (mg)	28 ± 1[a]	34 ± 1[b]	29 ± 4[a]
Putrescine (nmol)	836 ± 100[a]	1122 ± 152[b]	1198 ± 232[b]
Spermidine (nmol)	1744 ± 113[a]	2159 ± 72[b]	1892 ± 75[a]
Spermine (nmol)	2025 ± 165[ab]	2082 ± 38[a]	1944 ± 74[ab]
Pancreas			
Protein (mg)	22 ± 3[a]	18 ± 1[b]	22 ± 4[ab]
RNA (mg)	4.6 ± 1.4[a]	2.5 ± 1.0[ab]	2.4 ± 0.7[b]
DNA (mg)	0.09 ± 0.01[a]	0.07 ± 0.01[b]	0.10 ± 0.01[a]
Putrescine (nmol)	30 ± 16[a]	93 ± 45[b]	50 ± 26[ab]
Spermidine (nmol)	5470 ± 1030[a]	7614 ± 2889[a]	6766 ± 1837[a]
Spermine (nmol)	1355 ± 358[a]	1554 ± 98[a]	1967 ± 599[a]

Values represent mean of five rats per treatment.
Different superscripts refer to significant differences between treatments ($P < 0.05$).

during EPM modification (*Figures 1 & 2*). This reduction was confirmed both by haemagglutination activity measurements and ELISA.

The functional activity of KTI was also partially abolished, whereas that of the BBI was not significantly altered by EPM treatment (*Figure 3*). These results were also confirmed in quantitative protease inhibition assays. Furthermore, ELISA data showed that about one quarter of the original KTI content of the SBALB preparation was eliminated by EPM but that there were no changes in BBI content.

In line with the reduction in some of the antinutrients, the nutritional value of the enzymatically modified EPM product was significantly improved. Thus, probably due to the high antinutrient content of SBALB preparations,[23,10] rats fed diets based on soya albumin proteins lost weight during the 10-day experimental period (*Table 2*) even though that the methionine deficiency of these proteins was compensated for by supplementing the diet with methionine to target requirements (*Table 1*). In contrast, when rats were fed diets containing SBALB-EPM, they maintained their initial body weight or even improved it slightly. This was reflected in the N balance values, which also showed significant improvement with the EPM product. The fecal output with the modified protein was also reduced significantly, nearly back to values measured in controls.

Changes in the weight of organs of rats fed diets containing SBALB-EPM were also in line with the reduced antinutrient content of the modified product. Thus, the weight increase of rat small intestine induced by the high SBA content of SBALB preparations was significantly reduced in rats fed SBALB-EPM diet (*Table 3*). Changes in the protein, RNA, DNA, and polyamine contents of the small intestine of rats fed the different soya diets were well correlated with the weight changes of this tissue (*Table 4*). The effectiveness of the EPM treatment became even more obvious from the observation that, on a proportional body weight basis, the small bowel in these rats did not differ from that of control rats that had been fed a good quality protein (lactalbumin).

Significant pancreatic enlargement was induced in rats fed diets containing SBALB, which is a rich source of both the agglutinin and trypsin inhibitors (*Table 3*). The increase in the spermidine content of the tissue appeared to support this increased metabolic activity although the difference between the pancreas from control and SBALB-fed rats did not reach full significance (*Table 4*). Interestingly, the pancreatic enlargement was not reduced but rather increased by incorporating the EPM product in the rat diet even though that the amounts of both SBA and KTI were significantly less after the enzymic modification and methionine incorporation. However, as pancreatic protein synthesis has a high methionine requirement, the results might indicate that covalently linked methionine in soya proteins was possibly more readily available to satisfy this high demand of the hypertrophic growth even when the amounts of the trophic stimulants were substantially reduced as in the EPM product.

In conclusion, enzymatic peptide modification of soya albumins by transpeptidation and methionine enrichments is suitable to improve the nutritional values of soya proteins, partly by compensating for their methionine deficiency and partly by modifying the structure of the soya antinutrients, the most important of which was that SBA and KTI activities were significantly decreased. This reduction in the activities of these two antinutrient may possibly be the result of modification by proteolysis and resynthesis of peptide sequences and the decrease in the number of peptide-bonds in soya proteins, and/or the incorporation of a hydrophobic amino acid (i.e., methionine) into the terminals of the protein chains. Either of these, or the combination of the two events, can lead to alterations in protein conformation resulting in modified biological activity and increased nutritional value.

Acknowledgments

The skillful assistance of Ms. Betty Zarai and Ms. Aniko Bekes in the preparation of EPM product is gratefully acknowledged. The help and contribution of Mrs. Kissne, Eva Valentine, and Mr. Istvan Makk are appreciated. This work is part of the COST 98 programme. The financial support of OMFB (Hungary) and SOAFD (RRI) is greatly appreciated.

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