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Protein nutritional value of extrusion-cooking defatted lung flour

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The nutritional value of lung protein extruded at diverse conditions of feed moisture and process temperatures was determined by biological assay. No dependence of the nutritional quality of the proteins on process temperature was found in the extrusion of lung with 18% moisture content. Samples extruded at this low moisture (18%) presented a significant decrease in cysteine content and low biological value of the protein, although the decrease which has been observed in the chemical scores of total sulphur and other limiting amino acids was not enough to account for the observed decrease in biological value. Supplementation of this extruded protein with its limiting amino acids restored the initially observed nutritional value. Extrusion performed with 30% moisture lung flour protein resulted in a smaller loss of cysteine content after processing and bioassay comparable to non-processed samples or casein. Digestibilities of the lung samples decreased slightly after defatting and were not affected by their subsequent extrusion. In all lung samples digestibility was high, and thus could not explain the observed poor bioavailability of the protein extruded with 18% moisture content. Racemization could have occurred during the extrusion of this low moisture flour.

INTRODUCTION

Although extrusion-cooking has been largely used to upgrade the textural attributes of vegetable protein, little use has been made of this technology for waste tissues or low grade protein from animal origin (Harper, 1979, 1981; Kinsella, 1978). The great nutritional potential of these animal by-products has not yet been realized, mainly because aesthetic rejection is associated with their intrinsic lower textural quality, when compared to other animal products or to texturized vegetable protein. Recently, it has been shown that it is possible to upgrade by-products from the meat industry through their extrusion, provided that some steps, such as drying and defatting, are carried out on the raw materials (Arêas & Lawrie, 1984; Arêas, 1986*a*,*b*; Bastos & Arêas, 1990; Bastos *et al.*, 1991).

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The final extruded products obtained so far still lack all the desirable functional properties observed, for example, in texturized soya protein (Bastos & Arêas, 1990), but the great progress achieved to date makes it possible to forecast the use of extrusion texturization to upgrade and recover waste animal protein in the near future.

The composition of the raw materials used for the extrusion-cooking of these by-products is intrinsically different from that of soya or other vegetable protein sources. The former have, for example, negligible amounts of carbohydrates when compared to the latter (Arêas & Lawrie, 1984; Bastos *et al.*, 1991); and depending on the solvent used to defat the samples prior to extrusion, the lipid contents of lung flours can vary from 0·5 to 7%, the residual lipid being almost exclusively phospholipids (Arêas, 1985; Alcocer & Arêas, 1990). As little lipid oxidation occurs during extrusion (Harper, 1981) and the amount of carbohydrates is negligible, Maillard type reactions might be less important for the loss of protein nutritional value during

extrusion of these animal by-products than it is for soya and other vegetable sources (Noguchi *et al.*, 1982; Björck & Asp, 1984; Oates *et al.*, 1987). Also, iron is present in significant amounts in these waste tissues and could constitute another important nutrient to be considered since extrusion can result in a decrease in iron bioavailability after processing. An appraisal of the processing effects on the nutritional quality of these novel extruded products is thus necessary.

This work reports the effect of extrusion-cooking on the nutritional value of lung protein. The lung was dried, ethanol-defatted and extruded at diverse temperatures and moisture contents of the feed, which included the optimum conditions for texture formation (Bastos *et al.*, 1991). Amino acid composition by chemical analysis and bioassay were carried out for the protein before and after every step of processing.

MATERIALS AND METHODS

Materials

Raw bovine lungs were provided by Sadia-Frigobrás S.A. (Toledo, PR—Brazil) frozen in blocks of 20 kg in polyethylene bags. After thawing overnight at 5°C, they were minced twice in a homogenizer (Mod. D—Hobart Co., USA) through a perforated plaque with holes of 1 cm. The minced lung presented the following proximate analysis (see methods below): moisture 77·0%; lipids 4·2%; protein 18·0%; ash 1·0%. Part of this material was frozen at −30°C (during 10 h) and freeze-dried for 27 h at 36°C. This lyophilized product was then hammer-milled and used as a control for the subsequent processing and showed the following proximate analysis: moisture 3·5%; lipids 16·1%; protein 81·1%; ash 5·2%. To obtain the raw material for extrusion, another lot of the minced lung was spread in steel trays and dried in an circulating air oven (air temperature: 70°C) during approximately 8 h, being revolved occasionally. The oven-dried lung was then ground in a hammer mill and extracted with ethanol in a glass Soxhlet apparatus to produce the raw material to be further used for extrusion. Proximal analysis before defatting: moisture 14·0%; lipids 16·3%; protein 74·8%; ash 4·5%. After defatting: moisture 14·5%; lipids 0·44%; protein 86·1%; ash 3·0%. All the figures for proximal analysis are averages of three determinations which showed a coefficient of variability below 9%.

To obtain the desired moistures for extrusion, enough water was added, the samples were mixed thoroughly in a mixer (Mod. Planetária—Arno, São Paulo, SP, Brazil) and kept sealed in polyethylene bags at 5°C for 24 h.

Extrusion of the oven-dried and ethanol-defatted lung was carried out in a laboratory single-screw extruder (Miotto—São Paulo, SP, Brazil), 20 mm barrel diameter, *L/D* ratio 20 : 1, and the following conditions, which included those optimum for the extrusion of this material (Bastos & Arêas, 1990; Bastos *et al.*, 1991): feed moisture content: 18% and 30%; screw speed: 200 rev/min; die diameter: 3 mm; feed rate: 70 g/min; temperatures: 100, 130 and 160°C for the 18% moisture feed and 130°C for the 30% moisture feed.

All the protein sources (before and after extrusion) incorporated into the diets were sieved to 0·5 mm diameter.

Methods

Chemical analysis

Triplicate determinations of each component were carried out according to Instituto Adolfo Lutz (1985) and to AOAC (1980): desiccation to constant weight at 105°C for moisture, chloroform/methanol (2 : 1) extraction in Soxhlet extractors for lipids, micro-Kjeldahl for protein and calcination at 550°C to constant weight for ash. Iron was determined, after wet digestion of the samples with $HClO_4/HNO_3$, by atomic absorption in a spectrometer (Mod. 373—Perkin Elmer, USA) with a hollow cathode lamp at 248·3 nm, using $FeCl_3$ as standard. Amino acid analysis was carried out in duplicate on an auto analyser (Mod. Nicolas V—Honey Well, USA) on samples hydrolysed in HCl (6 N for 24 h) which were previously defatted with ethanol. Thus, no account of amino acid composition of the non-defatted samples could be obtained. Hydrolysis in LiOH (4 N for 24 h), for tryptophan determination was also carried out on the defatted sample, and methionine and cysteine were determined as methionine sulphone and cysteic acid, respectively, after quantitative oxidation with performic acid (Moore, 1963).

Animal experiments

The bioassay experiments were carried out in young rats (*Rattus norvegicus*, var. albinos Rodentia, mamalia), weighing 45 g on average, according to the 'Committee on Laboratory Animal Diets' (NRC, 1979). Each diet was tested on eight animals kept in individual metabolic cages. The diets were prepared with 10% protein content, 10% sucrose, 5% fibre, 3·5% salt mixture, 5·0% lipids (corn oil), 3·5% vitamin mixture, 0·2% choline bitartrate, corn starch to complete 100%, and offered *ad libitum* to the animals. Casein control diet was supplemented with 0·15% DL-methionine. The amount of lipid in the protein source was taken into consideration and discounted from the added oil. The salt mixtures for the lung samples were not supplemented with iron, since the amount originally present in all cases, as determined by atomic absorption spectrometry, was enough for the rat requirements. Casein was used as a reference and an extra group was fed on a non-protein diet for the assessment of the endogenous nitrogen. For NPU determination by carcass composition, the animals were fed during 14 days whereas for PER the feeding period was 28 days. Urine and faeces were collected separately in the metabolic cages, and their nitrogen contents analysed by the micro-Kjeldahl method (AOAC, 1980). Calculation of Digestibility Coefficient

(DC), Net Protein Utilization (NPU) and Biological Value (BV), were performed as follows:

$$DC = (N_I - N_F)/100 \qquad N_I = \text{Ingested nitrogen}$$
$$NPU = (N_R/N_I)100 \qquad N_R = \text{Retained nitrogen}$$
$$BV = (N_R/N_I)100 \qquad N_F = \text{Faecal nitrogen}$$

(AOAC, 1980; Bender & Doell, 1957; NRC, 1979; Pellet & Young, 1980; Saterlee, 1979).

RESULTS AND DISCUSSION

Figure 1 shows the weight gain of the animals fed on diets having as the only dietary protein sources: casein, extruded lung with 18% feed moisture and various processing temperatures, non-processed lung (lyophilized, oven-dried, defatted and non-defatted), and also a non-protein diet. Variance analysis of the results indicated no significant difference ($p < 0.05$) in weight gain of the animals or PER values calculated from these experiments among the various temperatures adopted for extrusion with lung flours of 18% feed moisture, and a significant decrease in weight gain or PER values when the extruded samples were compared to the casein control or non-extruded lung. No significant difference was observed in weight gain or PER value between the casein and non-extruded lung diets, indicating the high biological value of this latter protein source. These results also showed that the type of drying process (lyophilization or oven-drying with air stream) did not affect the nutritional quality of the protein, and that defatting did not alter, significantly, the nutritional behaviour of lung protein either.

Another set of experiments was then carried out, processing the ethanol-defatted lung by extrusion at 130°C with 18 and 30% feed moisture. These processed proteins were then fed to rats as before and Fig. 2 shows the observed weight gain of the animals. A diet with casein which served as a control and a group with lung protein of 18% moisture content processed at 130°C supplemented with its limiting amino acids, were also fed to rats in this experiment. It can be clearly seen from this figure that the increase in moisture content from 18 to 30%, for the extrusion of lung protein, prevented the loss of nutritional quality hitherto observed. Supplementation with the limiting amino acids restored the original nutritional performance of the processed protein at low moisture.

Amino acid analysis of the protein in the above diets is presented in Table 1. As the results obtained for the three extrusion temperatures tested on lung flour with 18% moisture content were similar, only the amino acid composition of the sample extruded at 130°C is displayed, for simplicity. The results show that extrusion of the lung at 18% moisture content produced a significant loss in cysteine content (>50% loss) and this was the major modification that occurred after processing. On the other hand, processing at 30% moisture caused little damage to this amino acid, its content being comparable to the original samples. However, chemical scores calculated, based on the provisional FAO/WHO (1985) protein, indicated that total sulphur amino acids

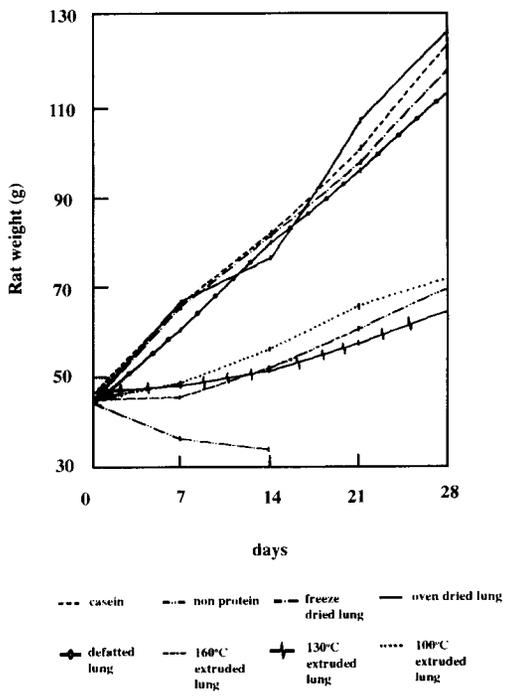

Fig. 1. Effect of processing on nutritional quality of lung protein. Weight gain of the animals fed on non-protein diet, casein and the following lung diets: freeze-dried, oven-dried, oven-dried and defatted, and extruded at various temperatures. Animals were kept in individual metabolic cages, with separate collection of faeces and urine. Weight determined every week. Results shown represent average of eight animals.

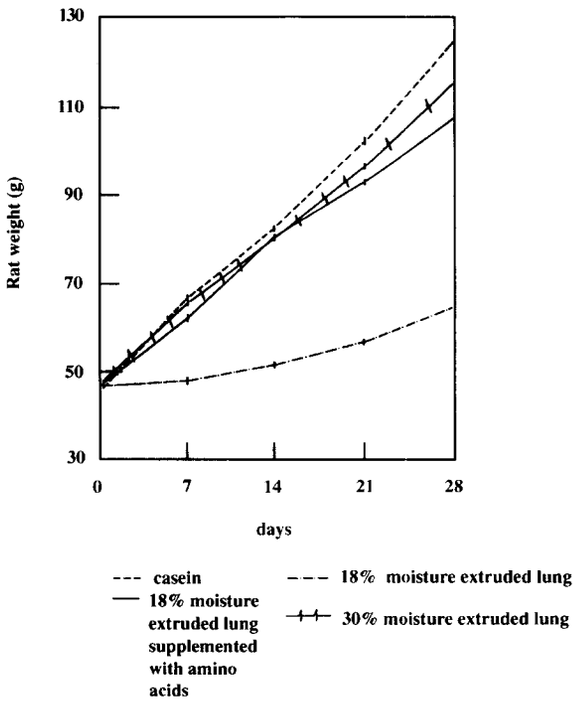

Fig. 2. Effect of moisture on nutritional quality of extruded lung protein. Weight gain of the animals fed on casein and the following lung diets: 18 and 30% moisture extruded lungs, 18% moisture extruded lung supplemented with amino acids. Animals were kept in individual metabolic cages, with separate collection of faeces and urine. Weight determined every week. Results shown represent average of eight animals.

Table 1. Essential amino acid content of lung protein after each stage of processing. (Lyophilized (control); oven-dried before and after extrusion at 130°C and diverse conditions of feed moisture, and extruded with 18% feed moisture supplemented with the limiting amino acids. Average of two determinations. Results expressed in mg of amino acid per g of total nitrogen. All samples were defatted with ethanol before hydrolysis)

Amino acid	Lung flour oven-dried ethanol-defatted and extruded at several conditions of moisture and temperature				
	Lyophilized	Oven-dried	18% moisture non-suppl. 130°C	18% moisture suppl. 130°C	30% moisture 130°C
Isoleucine	183	169	172	308	188
Leucine	548	480	491	491	471
Lysine	399	399	363	531	421
Methionine	119	84·0	100	—	104
Cystine	74·4	61·0	32·4	—	67·0
Total sulphur a.a.	193	145	132	306	172
Phenylalanine	289	280	269	269	291
Tyrosine	196	187	187	187	227
Total aromatic a.a.	485	467	456	456	518
Threonine	268	253	243	289	249
Tryptophan	119	97·8	125	125	89·0
Valine	270	349	310	366	336
Total	2 467	2 360	2 292	2 872	2 444
E/T	2·46	2·35	2·29	2·87	2·44

in the oven-dried lung and in the lung extruded with 18% moisture content were similar (Table 2). This could have occurred due to a combined effect of little loss of methionine in the latter sample and a slight decrease in this amino acid in the former. The literature has shown that moisture content of the extruder feed is an important aspect of loss in nutritional quality of protein after processing (Björck & Asp, 1984; Cheftel, 1986; Eggum *et al.*, 1986; Phillips, 1989) due to the high energy inputs required for extrusion under these conditions, the high friction resultant and, consequently, the high degree of molecular fragmentation and irreversible association which can occur. The chemical scores of all proteins tested and presented in Table 2, indicate overall better scores for the protein extruded with 30% moisture content. Primary limitants in the 18% moisture extruded lung were found to be sulphur amino acids with a chemical score of 60%. In spite of the close value observed for the oven-dried sample (63%), the nutritional value of this latter protein was much higher than the extruded protein, being comparable to the lyophilized lung and casein control ones. This can only be attributed to either the differences in the content of individual sulphur amino acids or other aspects which could be related to the bioavailability of the protein.

Table 3 presents the bioassay of the proteins on all tested diets. As the amino acid compositions among lung samples did not differ dramatically, a much lower digestibility would explain the low nutritional quality of the lung protein extruded at 18% moisture content, when compared to the original sample or to lung

Table 2. Chemical scores of lung protein (expressed as percentages) and limiting amino acids (compared to FAO/WHO protein), after each stage of processing. (Lyophilized (control); oven-dried before and after extrusion at 130°C and diverse conditions of feed moisture, and extruded with 18% feed moisture supplemented with the limiting amino acids. Average of two determinations. Results expressed in mg of amino acid per g of total nitrogen. All samples were defatted with ethanol before hydrolysis)

Amino acid	Lung flour oven-dried ethanol-defatted and extruded at several conditions of moisture and temperature				
	Lyophilized	Oven-dried	18% moisture non-suppl. 130°C	18% moisture suppl. 130°C	30% moisture 130°C
Isoleucine	73·3[b]	67·6[b]	68·8[b]	123	75·3[a]
Leucine	125	110	112	112	108
Lysine	116	116	106	155	122
Sulphur a.a.	88·4[b]	63·3[a]	60·5[a]	140	78·4[b]
Aromatic a.a.	129	125	122	122	138
Threonine	107	101	97·1	116	100
Tryptophan	190	157	199	199	143
Valine	86·4[b]	112	99·2	117	108

[a] Primary limitant.
[b] Secondary limitant.
[c] Tertiary limitant.

Table 3. Nutritional evaluation of lung protein after each stage of processing. (Lyophilized (control); oven-dried; oven-dried ethanol-defatted before and after extrusion at 130°C and diverse conditions of feed moisture, and extruded with 18% feed moisture supplemented with the limiting amino acids. PER, protein efficiency ratio; NPU, net protein utilization; DC, digestibility coefficient; BV, biological value)

Nutritional evaluation	Casein	Lung flour oven-dried ethanol-defatted and extruded at several conditions of moisture and temperature					
		Lyophilized	Oven-dried non-defatted	Oven-dried defatted	18% moisture non-suppl. 130°C	18% moisture suppl. 130°C	30% moisture 130°C
PER	2·2 ± 0·3	1·7 ± 0·2	1·7 ± 0·2	1·8 ± 0·3	0·7 ± 0·1	1·6 ± 0·4	1·8 ± 0·2
DC	93·3 ± 0·9	92·0 ± 2·1	91·0 ± 1·1	86·3 ± 1·6	87·0 ± 1·5	87·3 ± 2·0	89·6 ± 1·8
NPU	66·5 ± 4·4	53·5 ± 3·8	49·5 ± 3·8	58·4 ± 2·3	50·0 ± 4·4	55·3 ± 3·5	57·0 ± 3·9
BV	57·3 ± 3·5	45·7 ± 3·2	43·4 ± 2·6	53·0 ± 2·6	37·0 ± 3·1	48·0 ± 4·5	50·0 ± 3·5

DC $= (N_I - N_F)/100$ N_I = Ingested nitrogen
NPU $= (N_R/N_I)100$ N_R = Retained nitrogen
BV $= (N_R/N_I)100$ N_F = Faecal nitrogen

extruded at 30% moisture content. Digestibility of all samples, however, was high in all cases although a slight decrease which was observed after defatting and extrusion seemed not to have reduced digestibility any further. Some irreversible aggregation could have occurred in the defatting of these samples causing this decrease in digestibility coefficient. The solubility of lung protein after defatting declines (Alcocer & Arêas, 1990; Arêas & Mota, 1990) but this cannot be postulated to have affected the digestibility of protein because the extrusion of ethanol-defatted lung increases protein solubility, restoring its original levels (Bastos & Arêas, 1990; Bastos *et al.*, 1991). The possibility of racemization of some amino acids, occurring in the drastic conditions of extrusion employed in this work, as described in the literature for thermal processing (Bjarnason & Carpenter, 1970; Friedman *et al.*, 1981; Friedman & Masters, 1982; Hayase *et al.*, 1975; Phillips, 1989), cannot be disregarded, and could partially explain the observed behaviour. The more drastic the extrusion conditions (lower moisture of the flour) the more likely this could have been; and racemization cannot be detected either by protein solubility measurements or conventional chemical analysis of amino acids.

The effect of extrusion on the nutritional quality of the lung protein seemed to be highly dependent on the moisture content of the samples for processing, as reported in the literature for protein extrusion (Björk & Asp, 1984; Cheftel, 1986; Eggum *et al.*, 1986; Phillips, 1989). The high energy inputs required for proper processing at this low moisture level can produce irreversible chemical modification of amino acids resulting in changes either in their composition or bioavailability which are not detectable by ordinary chemical analysis, being evident only through biological assays. The optimum conditions found for lung processing require low moisture content (Bastos *et al.*, 1991) and this can considerably reduce its nutritional quality. Interactions of protein and lipids in these systems have been reported as an important factor for lung extrusion (Arêas & Lawrie, 1984; Arêas, 1986*a*,*b*; Bastos & Arêas, 1990), and high lipid content lung flours, which can be produced by defatting the dried lung with solvents of lower polarity, show good extrusion behaviour, being processable at higher moistures (Prudêncio-Ferreira, 1990; Bastos & Arêas, 1990; Arêas & Prudêncio, 1991). Optimization of extrusion conditions for better textural quality of these high lipid content flours and bioassay of the processed protein are thus required.

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