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Effect of phospholipid on protein structure and solubility in the extrusion of lung proteins

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Bovine lung flours with residual phospholipid contents of 6·7% and 0·5% were extruded in a single-screw extruder at various temperatures and feed moistures. Disulphide bonds, followed by hydrophobic and electrostatic interactions, were the major interactions responsible for stabilization of extrudate structure. The high lipid content flour (6·7% phospholipid) presented a decrease in protein solubility after extrusion, whereas the low lipid flour (0·5% phospholipid) showed the opposite behaviour. Amino acid composition was not affected by the diverse processing conditions. Infrared spectra showed the presence of β-sheet antiparallel structures in the extrudates even in the most drastic conditions of processing employed. Protein solubility in buffer with reducing agents plus sodium dodecyl sulphate of both extrudates was lower in the internal core than in the edges; such behaviour was not observed in a control of soya. Optical microscopy of transverse sections of lung extrudates showed a tendency of collagen to locate on their external parts. These results can be explained by the recently proposed 'suspension model' for biopolymer extrusion.

INTRODUCTION

The mechanisms of texturization in protein extrusion are still controversial. New peptide bonds formed in the drastic conditions of processing have been claimed to be the major interaction for the stabilization of product structure (Burgess & Stanley, 1976; Simonsky & Stanley, 1982; Aguilera & Stanley, 1986; Stanley, 1986, 1989). However, this conclusion was contested by reports of disulphide linkages, followed by hydrophobic and electrostatic interactions, as the main forces accountable for the structure of extrudates (Hager, 1984; Jeunik & Cheftel, 1979; Martinez-Serra & Villota, 1992; Rhee *et al.*, 1981; Sheard *et al.*, 1984, 1986). Another controversial point in the literature about protein extrusion is the role of lipids in the process. The presence of lipids in the feed is believed to impair extrusion. Lipid was found to interact with protein because of extrusion processing (Izzo & Ho, 1989; Guzman *et al.*, 1992; Ho & Izzo, 1992) in a similar way to what is observed for starch (Mercier *et al.*, 1979). Also, lipid–protein interactions, which occur in the feed material, were reported to benefit extrusion performance and final extrudate structure (Arêas & Lawrie, 1984; Arêas, 1986*a*,*b*). The way lipids affect protein extrusion is not yet clear. A better understanding of the mechanisms involved in protein extrusion and the effect of lipid in this process is essential for extending its use to a series of protein sources not yet used.

Recently, a 'suspension model' for the melt flowing into the extruder was suggested to explain conflicting results both for protein interaction mechanisms and for the role of lipids in biopolymer extrusion (Mitchell & Arêas, 1992). According to this model, a second insoluble phase is present suspended in the melt during extrusion. The amount of this phase and the ability of the macromolecules present in the soluble phase to form the final three-dimensional network would thus determine the practicability of extrusion of a given feed material. All interactions in the process could then either facilitate flow of the macromolecules or form aggregates that can contribute to the final three-dimensional network of the extrudate and to the amount of the second phase during processing. The present work investigates the effect of lipid on the extrusion texturization mechanism and the applicability of the 'suspension model' for lung flours with two distinct lipid contents. The flours were produced by defatting dried lung with chloroform (which resulted in 6·7% of residual phospholipid) and ethanol (0·5% residual

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phospholipid). Protein was solubilized before and after extrusion by reagents of known mode of action on the protein, allowing the mechanisms responsible for protein insolubilization to be assessed (Hager, 1984). Determination of amino acid composition was carried out before and after all the extrusion procedures adopted. Infrared spectroscopy was also used to verify which protein structural features occurred and how they were affected by the extrusion process.

MATERIALS AND METHODS

Materials

Lung was provided by Sadia, Frigobrás S.A. (Toledo, PR, Brazil) frozen in blocks of 20 kg in polyethylene bags. After thawing overnight at 5°C, it was minced twice in a Hobart homogenizer (Mod. D—Hobart Co., USA) through perforated holes of 1 cm. It was then spread in steel trays and dried in an oven with a 70°C air stream during approximately 8 h, being revolved occasionally. The dried lung was then ground in a hammer mill and extracted with ethanol or chloroform to give two distinct types of flour with the following proximal composition: ethanol defatted lung—14·5% moisture, 94·2% protein (d.b.) and 0·5% lipids (d.b.); chloroform defatted lung—11·5% moisture, 89·2% protein (d.b.) and 6·7% lipids (d.b.) (see methods below).

Soya protein isolate (Proteimax-90, LG (low gel), from Sanbra SA (Brazil)) was granulated to avoid feed problems. It presented the following proximate composition: moisture 3·7%, protein 83·6% (d.b.) and lipids 1·5% (d.b.). To obtain the desired moistures for extrusion, enough water was added and the samples kept sealed in polyethylene bags at 5°C for 24 h.

For the determination of solubility of, separately, the external part and the core, the extrudates were scratched regularly over a steel file until their diameters were reduced to half the original value, maintaining their cylindrical shapes. The removed material was considered the external part of the extrudates, the remaining core being the central part. For the solubility assays all the samples were ground and sieved, the material used being classified between 0·25 and 0·5 mm diameter.

Methods for proximal composition

Conventional methods (desiccation to constant weight at 105°C for moisture; micro-Kjeldhal method (AOAC, 1980) for protein; chloroform/methanol 2:1 extraction in Soxhlet apparatus for lipids) were employed for the determination of the proximal composition of the samples.

Extrusion

Extrusion of the lung flours and a 30% moisture soya isolate which served as a control was carried out in a small laboratory single-screw extruder with an *L/D* ratio of 20:1 (Miotto Ltd, São Paulo, Brazil). The following conditions were adopted: screw of compression ratio 4:1; screw speed 200 rev/min; die diameter 3 mm; feed rate 70 g min^{-1}. Independent heat elements provided control over three distinct zones of the barrel. The temperature of the central zone was used as the independent variable for processing. Temperatures of the first zone and at the die were set 10°C lower than the central zone ones to give the ideal temperature profile for soya and lung extrusion in this equipment (Barros *et al.*, 1987; Bastos *et al.*, 1991). The soya control was extruded at 140°C.

Solubility in several buffer systems

Protein solubility of the original and extruded samples was determined in 0·035 M phosphate buffers pH 7·6 with and without the following reagents, alone or combined: 8 M urea, 0·1 M sodium sulphite, 0·1 M acrylonitrile, 0·1 M 2-mercaptoethanol, 1·5% sodium dodecyl sulphate. Total protein was determined in the powder samples by the micro-Kjeldhal method (AOAC, 1980). Each sample (30 mg) (after being classified to 0·25–0·5 mm) was homogenized with 15 ml of the solvent in a Turratec homogenizer (Mod. TE102, Tecnal, Brazil) at medium speed for 3 min. The samples were then centrifuged for 15 min at 20 000*g* (centrifuge Mod. RC2-B, Sorvall, USA) and the supernatant filtered. Soluble protein was determined in the supernatant by the method of Lowry *et al.* (1951), modified by Besandoun & Weinstein (1976). The amounts of protein solubilized by the various solvent systems employed were used to calculate the percentages of the protein in five states (Hager, 1984):

State 1: buffer-soluble protein;
State 2: protein insoluble in buffer due mainly to non-covalent interactions;
State 3: protein insoluble in simple buffers due to disulphide interactions;
State 4: protein insoluble in simple buffers due to a combination of disulphide and non-covalent interactions;
State 5: indeterminate, that is calculated by difference.

Sulphhydryl and disulphide analysis

Sulphhydryl and disulphide were determined in the samples by the Ellman's reactive, DTNB (5,5'-dithiobis-2-nitrobenzoic acid), according to Beveridge *et al.* (1974), with some modifications. The amount of CNT chromophore after DTNB reaction was determined at 412 nm, using the extinction coefficient of $1·36 \times 10^4$ M^{-1} cm^{-1}. Sulphhydryl groups were determined in 0·09 M tris-glycine buffer pH 8·0, 4 mM in EDTA, before and after addition of 8 M urea and 1·5% SDS. Parallel determinations of sulphhydryl groups were carried out after addition of 2-mercaptoethanol to reduce disulphide bridges, precipitation of the protein

Table 1. Ethanol defatted lung (0·5% residual phospholipid) extrudates. Soluble and insoluble protein at pH 7·6 before and after extrusion at diverse conditions due to non-covalent forces, disulphide bonds, and combination of disulphide and non-covalent forces (according to classification presented in the experimental section) (%)

State of protein	Solubility type	Control	Ethanol defatted lung flour extruded at					
			16% moisture			30% moisture		
			115°C	135°C	160°C	115°C	135°C	160°C
1	Protein soluble in simple buffer	2·55	4·75	6·71	6·53	3·15	4·66	4·90
2	Protein insoluble by non-covalent forces (%)	20·85	20·10	14·86	10·89	23·04	17·72	14·16
3	Protein insoluble by disulphide covalent forces (%)	26·73	18·04	3·03	0·57	17·89	11·66	8·86
4	Protein insoluble due to combination of disulphide and non-covalent interactions (%)	Trace	Trace	4·70	11·41	Trace	Trace	6·55
5	Indeterminate (%)	49·87	57·11	70·60	70·60	55·92	65·96	65·53

through 16% trichloroacetic acid (TCA) and resuspension of the precipitated protein in the buffer added of 8 M urea and 0·5% SDS. The latter determination was used to calculate the amount of disulphide bridges present on the protein.

Amino acid analysis

Amino acid analysis was carried out in an auto analyser (Mod. Honey Well (Nicholas V, USA)), after 6 N HCl hydrolysis at 110°C for 22 h. Cysteic acid and methionine sulphone were analysed after oxidation of the samples with performic acid.

Infrared spectroscopy

Infrared spectra in the region 650–4000 cm^{-1} were obtained in original and extruded samples after their extensive desiccation under vacuum, mixing with KBr and tablet formation at moderate compression. The equipment employed was a Perkin-Elmer spectrometer (Mod. 710 A) calibrated with polystyrene at 1601 cm^{-1}.

RESULTS AND DISCUSSION

Tables 1 and 2 present the percentage of soluble and insoluble protein after solubilization experiments in several buffer systems and classified in five states, as described in the experimental section. Tables 3 and 4 show the results for sulphhydryl and disulphide contents determined in these samples.

As a general behaviour, the low moisture samples exhibited, after extrusion, higher amounts of protein insoluble in buffer with a combination of all reagents used (SDS, urea and 2-mercaptoethanol) and smaller contents of disulphide bonds than the higher moisture ones. The lipid content of the initial feed determined the type of interaction between the proteins after extrusion. When the residual lipid content was low (0·5% of residual lipid), initial protein solubility in buffer alone was low and tended to increase after extrusion (Tables 1 and 2) as described before (Bastos & Arêas, 1990). Insoluble protein due to non-covalent forces and disulphide bridges in this sample decreased after processing. This disruption of disulphide linkages, depending on process temperature, was accompanied by loss of cysteine as observed in Table 3. The amount of indeterminate protein in this sample (State 5) increased after extrusion. These results could be explained by intensive breakdown of the protein followed by irreversible aggregation. The low lipid flours were a case limit for extrusion, as the torque of the extruder electric motor rose to high levels during processing. The opposite behaviour was observed in the high residual lipid flour (6·7% of phospholipid). The solubility of this sample in buffer alone was initially high and decreased after extrusion. The insoluble protein due to disulphide and non-covalent interactions remained practically constant. They were, together with

Table 2. Chloroform defatted lung (6·7% residual phospholipid) extrudates. Soluble and insoluble protein at pH 7·6 before and after extrusion at diverse conditions, due to non-covalent forces, disulphide bonds, and combination of disulphide and non-covalent forces (according to classification presented in the experimental section) (%)

State of protein	Solubility type	Control	Chloroform defatted lung flour extruded at	
			16% moisture	30% moisture
			135°C	135°C
1	Protein soluble in simple buffer	11·59	4·27	1·25
2	Protein insoluble by non-covalent forces (%)	24·19	24·07	38·49
3	Protein insoluble by disulphide covalent forces (%)	24·72	21·53	36·21
4	Protein insoluble due to combination of disulphide and non-covalent interactions (%)	Trace	Trace	Trace
5	Indeterminate (%)	39·50	50·13	24·85

Table 3. Free and total sulphhydryl and disulphide bridges in lung protein flour with 0·5% residual phospholipid (ethanol–defatted) before and after diverse extrusion conditions[a]

Determination	Control	Ethanol defatted lung flour extruded at					
		16% moisture			30% moisture		
		115°C	135°C	160°C	115°C	135°C	160°C
Free SH (without denaturing agents)	13·7	24·7	22·0	22·6	15·9	15·3	19·2
Total SH (with denaturing agents)	14·4	26·3	25·6	29·0	18·9	21·8	21·0
Disulphide	289	218	150	131	266	215	200

[a] Results expressed in moles 10^{-10} mg^{-1} protein.

non-covalent interactions, the major interactions found in these extrudates. Similar results are observed for soya (Jeunik & Cheftel, 1979; Hager, 1984; Sheard *et al.*, 1984, 1986), except that lung extrudates present higher contents of State 5 protein (insoluble protein due to indeterminate types of interaction). Torque during processing did not rise to high levels as in the case of the low lipid flour. Table 4 indicates that, in the extrusion of this flour, free sulphhydryl content decreased and most of the cysteine was conserved, being involved in disulphide bridges. The amino acid composition of the original raw materials before and after being submitted to the diverse extrusion conditions (Table 5) confirmed these results and showed only minor changes and no obvious relationship of any amino acid with the diverse processing conditions.

The remarkable similarities observed in protein–protein interaction after extrusion of lung protein (6·7% of residual lipid, which are almost exclusively phospholipid derivatives—Alcocer & Arêas, 1990), and the ones reported for soya protein (Jeunik & Cheftel, 1979; Hager, 1984; Sheard, 1984, 1986; Areâs & Prudêncio, 1991), indicate that the presence of more lipids in the lung flours favoured interactions between cysteine residues. The generally accepted view of the lipid role in extrusion is that its presence in the feed causes slippage at the barrel, thereby impairing or even preventing successful processing (Harper, 1979, 1981; Kinsella, 1978). The presence of lipids in the feed generally reduces expansion of extruded products and their overall textural quality. However, in the present case, these interactions benefited extrusion, improving product quality. In this case no physical slippage at the barrel surface occurred. A smoother flux at or near molecular level was obtained, probably due to the interactions between lipids and proteins, facilitating flow. This caused less aggregation of protein inside the extruder and less energy input, compared to the low lipid flour, resulting in better overall quality of extrudates.

Rehydration of lung extrudates in buffer with all reagents (urea, SDS and 2-mercaptoethanol) showed no homogeneity. The samples did not dissolve completely as observed for a soya extrudate control. Instead, and in spite of their overall lower resistance compared to the soya ones when retorted at 120°C in water, their structure failed to collapse. An apparent harder core of insoluble material remained. This odd behaviour can be explained by the 'suspension model' for biopolymer extrusion (Mitchell & Arêas, 1992), which considers that the melt was constituted of a continuous phase where insoluble particulate materials were present. This second phase would impair the flow of the material through the extruder, especially when its volume fraction approaches 0·64 (value for the closing pack of spheres). Near this value, insoluble particles flowing in a tube would tend to locate at its centre. This would produce the observed hard core. The existence of this second phase was checked by measuring protein solubility (in buffer with all cleaving reagents) in the core and in the external part of the extrudates (Table 6). The significant lower protein solubility observed in the core indicates that the second phase, as expected, migrated to the centre in the last stages of extrusion where a homogeneous flow was produced at the die end.

The viability for lung extrusion, in spite of the high

Table 4. Free and total sulphhydryl and disulphide bridges in lung protein flour with 6·7% residual phospholipid (chloroform defatted) before and after diverse extrusion conditions[a]

Determination	Control	Chloroform defatted lung flour extruded at	
		16% moisture	30% moisture
		135°C	135°C
Free SH (without denaturing agents)	23·8	17·6	13·6
Total SH (with denaturing agents)	51·8	36·7	15·1
Disulphide	325	208	314

[a] Results expressed in moles 10^{-10} mg^{-1} protein.

Table 5. Ethanol defatted (0·5% residual phospholipids) and chloroform defatted (6·7% residual phospholipids) proteins. Amino acid composition before and after extrusion of 16 and 30% moisture flours at 135°C. Average of two determinations. Results expressed in moles of amino acid per milligram of protein $\times 10^{-8}$

Amino acid	Ethanol defatted lung flour			Chloroform defatted lung flour		
	Control	16% moisture	30% moisture	Control	16% moisture	30% moisture
Lysine	27·4	28·0	26·6	23·7	24·2	23·0
Histidine	9·7	11·9	9·4	8·5	9·6	8·0
Arginine	26·0	29·5	26·1	21·5	24·4	20·7
Aspartic acid	36·3	43·6	43·2	34·6	39·0	33·6
Threonine	20·0	22·4	20·6	16·3	20·0	17·1
Serine	24·6	27·4	26·5	23·8	25·6	20·6
Glutamic acid	46·1	57·5	51·6	41·8	50·4	41·8
Proline	31·5	38·9	36·3	27·9	34·8	30·9
Glycine	71·5	88·3	78·8	69·1	80·9	62·4
Alanine	46·6	60·8	55·0	46·3	53·0	41·5
Half cystine[a]	6·4	5·4	5·6	7·2	6·4	6·6
Valine	26·6	27·9	18·2	25·2	24·5	23·6
Methionine[b]	5·1	6·5	6·2	5·7	5·6	5·4
Isoleucine	14·7	15·3	12·1	13·7	12·4	12·2
Leucine	35·6	40·7	31·3	36·4	33·2	30·5
Tyrosine	11·3	12·3	10·0	12·0	11·4	9·9
Phenylalanine	16·4	17·8	14·6	17·1	16·3	14·8

[a] Determined as cysteic acid.
[b] Determined as methionine sulphone.

content of insoluble protein, could be attributed to the presence of collagen that, once solubilized in extrusion conditions, would provide the continuous phase for a coherent melt to be formed. According to the process described above, this collagen, being soluble, would tend to locate at the external part of the extrudate. Optical microscopy of a transverse section of a rehydrated extrudate of lung (0·5% residual phospholipid and 16% moisture content obtained at 135°C), which was specifically stained such that collagen appears as a blue colour and all other proteins as yellow, is presented in Fig. 1. It can be seen that collagen was located mainly in the external parts of the extrudates (dark blue) although some collagen was found near the centre but in a lower concentration (pale blue).

Infrared spectra were obtained for lung flours of 0·5 and 6·7% of residual phospholipid content before and after extrusion in the diverse conditions adopted in the present work and the results are displayed in Figs 2 and 3. Recent reports on infrared spectroscopy applied to extruded soya protein claimed that the differences observed in some amide vibration modes after soya extrusion could be attributed to the increase in the content of peptide bonds (Aguilera & Stanley, 1986; Stanley, 1989). Four characteristic vibration modes are known for the peptide bond in the region 600–1800 cm^{-1} both for infrared and Raman spectroscopy. They are: amide I, II, III and IV (a rare vibration mode at 630 or 780 cm^{-1}), the amide I (around 1650 cm^{-1}) and III (1230–1300 cm^{-1}) modes being the ones which provide the most relevant structural information for protein studies (Myiazawa, 1960; Myiazawa & Blout, 1961; Parker, 1983; Krimm & Bandekar, 1986). In the spectra obtained (Figs 2 and 3)

Table 6. Protein solubility radially across the extrudates in phosphate buffer pH 7·6, containing 8 M urea, 0·1 M sodium sulphite and 0·1 M acrylonitrile

Samples and extrusion conditions	Solubility (%)	
	External part of extrudate	Internal part of extrudate
Lung (0·5% phospholipid) 16% moisture extruded at 135°C	32·44[a]	25·65[b]
Lung (0·5% phospholipid) 30% moisture extruded at 135°C	38·47[a]	29·85[b]
Lung (6·7% phospholipid) 16% moisture extruded at 135°C	44·62[a]	32·31[b]
Lung (6·7% phospholipid) 30% moisture extruded at 135°C	54·47[a]	46·97[b]
Soya isolate 30% moisture extruded at 140°C	89·39[a]	92·64[a]

Different superscript letters in the same line indicate significant differences between the two values ($p < 0·05$).

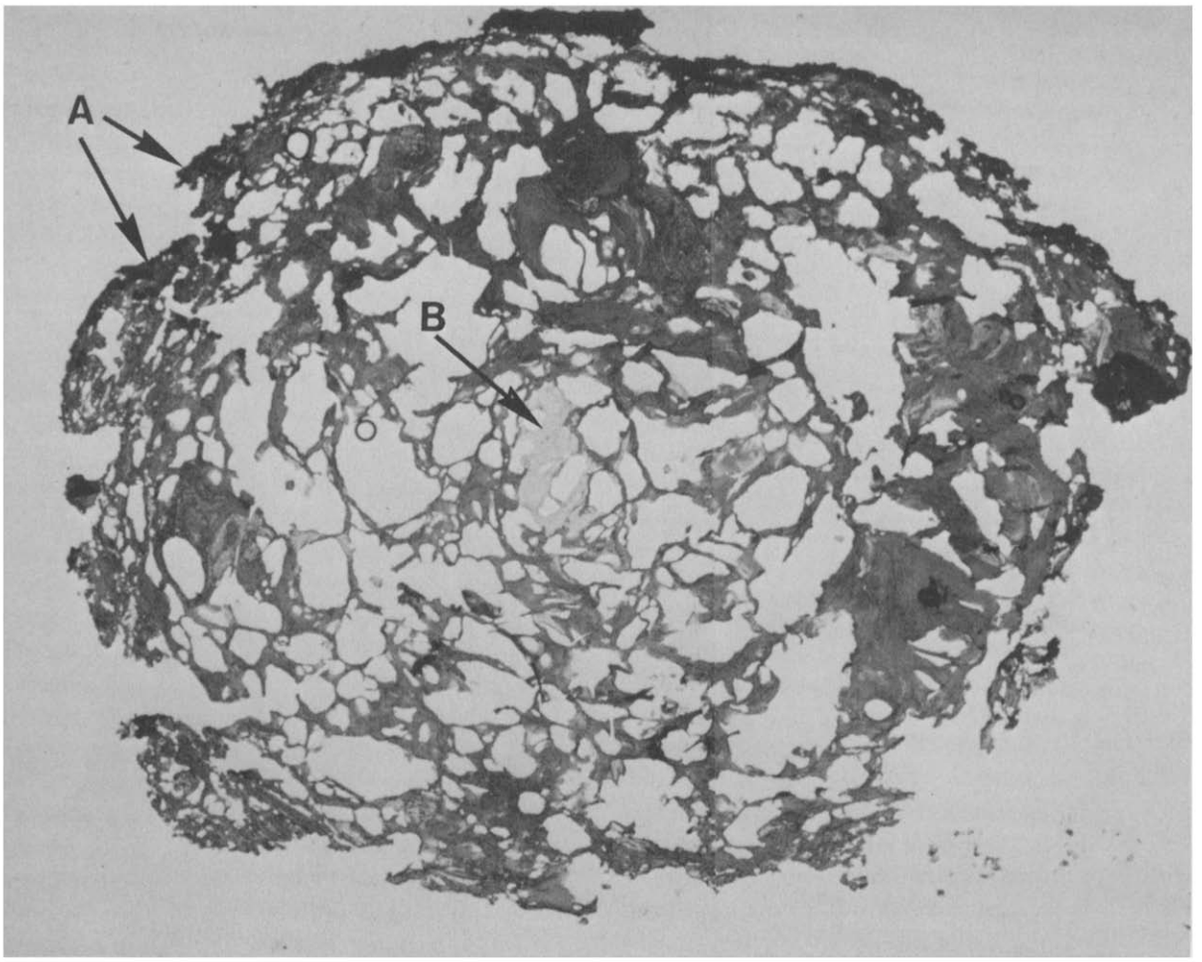

Fig. 1. Optical microscopy of a transverse section (8 mm thick, obtained from the frozen sample) of rehydrated extrudate from lung flour with 0·5% residual phospholipid content and 16% moisture, extruded at 135°C. Staining with combined dyes shows collagen in a blue colour and other proteins in yellow. The arrows indicate the preferential location of collagen at the external part of the extrudate (A) (dark area) with less collagen in the central part (B) (pale area). Magnification 12·5×.

the vibration modes for amide I, observed at 1650 cm^{-1}, and amide II, observed around 1550 cm^{-1}, were unequivocally assigned as two distinctive vibration modes for the peptide bond. The other amide modes could not be assigned from the spectra. It is very difficult with this technique to quantify the amounts of peptide bonds present in the samples based on the intensity of the several amide vibration modes. The use of FTIR can improve resolution and allow more information to be obtained, but quantitative information of this type is not yet reported in the literature. The peak at 1685 cm^{-1} with a shoulder at 1630 cm^{-1}, consistently observed before and after any extrusion condition had been used, can be assigned to anti-parallel β-sheet structures. This interpretation is supported by the literature of infrared spectroscopy related to protein conformation (Myiazawa & Blout, 1961; Krimm & Bandekar, 1980; Parker, 1983; Arêas *et al.*, 1989). This demonstrated that β-sheet structures are present in the extrudates, whichever extrusion conditions are employed. Despite the high energy inputs and high shear employed in extrusion cooking and, consequently, the high degree of denaturation and loss of secondary structure expected in the protein, the results obtained in the present work indicate that, even under these drastic conditions, the process favours proteins undergoing juxtaposition at the molecular level. Protein interactions at the end of the barrel stabilize the extrudate three-dimensional structure including anti-parallel β-sheet conformations. The presence of substantial amounts of tertiary and secondary structures has already been observed in thermal gelation of globular proteins (Clark & Lee-Tuffnell, 1986). This indicates that, for gelation, soya protein does not require that the molecules are completely unfolded to the three-dimensional structure of the gel or that these structures are reformed after or because of the gelation process. The present results indicate that, in the extrusion of lung protein, the same probably occurs. It is not yet possible to predict the contribution of each intra- and intermolecular interaction involved in the formation of these β-sheet structures produced upon extrusion, and in solubilization studies these interactions would be disrupted by urea (Robinson & Jencks, 1965) and consequently would be accommodated in State 2 of the classification presented in the experimental section.

The present results clearly indicate that protein–protein interactions in the extrusion of lung proteins

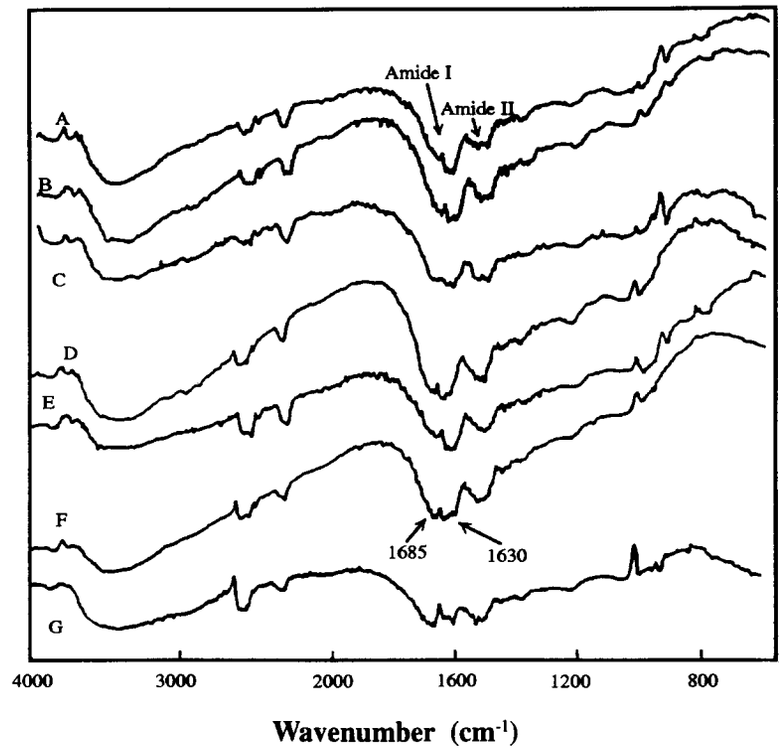

Fig. 2. Transmittance infrared spectra of lung protein defatted with ethanol (0·5% residual phospholipid) (A) and its products; (B) extruded at 115°C/16% moisture; (C) extruded at 115°C/30% moisture; (D) extruded at 135°C/16% moisture; (E) extruded at 135°C/30% moisture; (F) extruded at 160°C/16% moisture; (G) extruded at 160°C/30% moisture.

were dependent on the initial amount of lipids in the feed. Low lipid content flours produced extrudates with high proportions of indeterminate types of interaction (probably irreversible aggregates made up by stable covalent interactions) and less disulphide bridges and non-covalent interactions. Extrudate structures, both in the high and low lipid flours, were stabilized by disulphide and non-covalent interactions. No evidence of increase in the amount of peptide bonds was found by infrared spectroscopy. Anti-parallel β-sheet structures were detected by this technique in all extrudates, irrespective of the extrusion conditions adopted. The internal core of lung extrudates presented higher proportions of insoluble protein (in buffer with urea, SDS and 2-mercaptoethanol) than their external parts. Collagen was found to locate preferentially in the external part of the extrudates. These results agree with the proposed 'suspension model' for biopolymer extrusion.

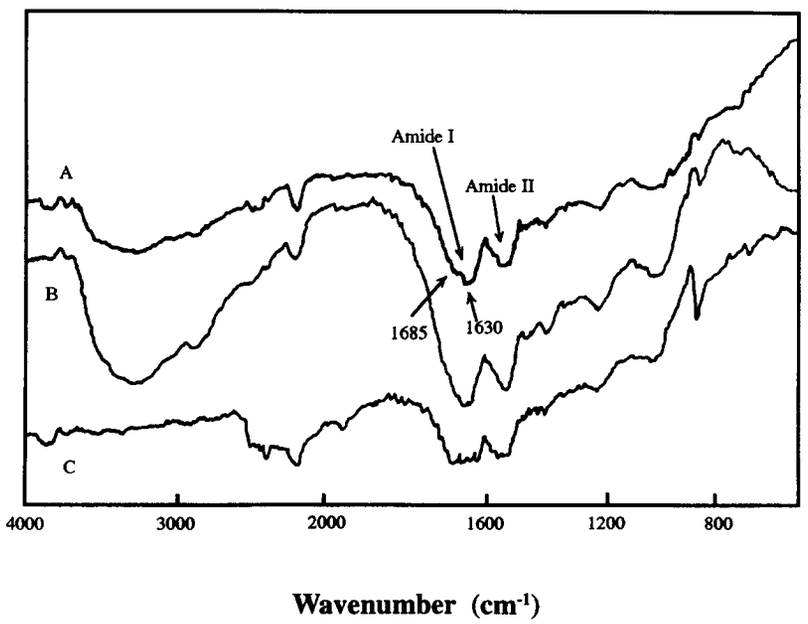

Fig. 3. Transmittance infrared spectra of lung protein defatted with chloroform (6·7% residual phospholipid) (A) and its products; (B) extruded at 135°C/16% moisture; (C) extruded at 135°C/30% moisture.

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REFERENCES

Aguilera, J. M. & Stanley, D. W. (1986). Extrusion processing as applied to proteinaceous materials. In *Food Engineering and Process Applications*, Vol. 2, ed. M. Maguer & P. Jelen. Elsevier Applied Science Publishers, London, pp. 131–41.

Alcocer, M. J. C. & Arêas, J. A. G. (1990). Lipid composition and hydration characteristics of lung protein isolates defatted by several solvents. *J. Food Sci.*, **55**, 19–22.

AOAC (1980). *Official Methods of Analysis*, 13th edn. Association of Official Analytical Chemists, Washington, DC, 505 pp.

Arêas, E. P. G., Laure, C. J., Gabilan, N., Araújo, P. S. & Kawano, Y. (1989). Raman and infrared studies on the conformation of porcine pancreatic and *Crotalus durissus terrificus* phospholipases A_2. *Biochim. Biophys. Acta*, **997**, 15–21.

Arêas, J. A. G. (1986*a*). Hydrophobic and electrostatic interactions on hydration characteristics of defatted offal protein isolates. *J. Food Sci.*, **51**, 880–2.

Arêas, J. A. G. (1986*b*). Effect of lipid–protein interactions on extrusion of protein isolates. *J. Food Sci.*, **51**, 1311–13.

Arêas, J. A. G. & Lawrie, R. A. (1984). Effect of lipid–protein interactions on extrusion of offal protein isolates. *Meat Sci.*, **11**, 275–99.

Arêas, J. A. G. & Prudêncio, S. H. (1991). Protein–protein interactions in the extrusion of vegetable and animal protein. *Proc. I Int. Brazilian Congress on Proteins*, ed. B. Oliveira & W. C. Sgarbieri, Editora da Universidade Estadual de Campinas, Brazil, pp. 533–46.

Barros, R. M. C., Silva, R. S. F., Borsato, D. & Arêas, J. A. G. (1987). Otimização das condições de extrusão de farelo de soja pelo emprego da metodologia de superfície de resposta. *Bol. Centro Pesquisa Proc. Alim.*, **5**, 61–70.

Bastos, D. H. M. & Arêas, J. A. G. (1990). Lung proteins: effect of defatting with solvents and extrusion cooking on some functional properties. *Meat Sci.*, **28**, 223–35.

Bastos, D. H. M., Domenech, C. H. & Arêas, J. A. G. (1991). Optimization of extrusion cooking of lung proteins by response surface methodology. *Int. J. Food Sci. Technol.*, **26**, 403–8.

Besandoun, A. & Weinstein, D. (1976). Assay of proteins in the presence of interfering materials. *Anal. Biochem.*, **70**, 141–51.

Beveridge, T., Toma, S. J. & Nakai, S. (1974). Determination of SH- and SS-groups in some food protein using Ellman's reagent. *J. Food Sci.*, **39**, 49–51.

Burgess, G. D. & Stanley, D. W. (1976). A possible mechanism for thermal texturization of soy-bean protein. *Can. Inst. Food Sci. Technol. J.*, **9**, 228–31.

Clark, A. H. & Lee-Tuffnell, C. D. (1986). Gelation of globular proteins. In *Functional Properties of Macromolecules*, ed. J. R. Mitchell and D. A. Ledward. Elsevier Applied Science, London, pp. 203–72.

Guzman, L. B., Lee, T. C. & Chichester, C. O. (1992). Lipid binding during extrusion cooking. In *Extrusion Cooking Science and Technology*, ed. J. L. Kokini, C. T. Ho & M. V. Karwe. Marcel Dekker, New York, pp. 427–36.

Hager, D. F. (1984). Effects of extrusion upon soy concentrate solubility. *J. Agric. Food Chem.*, **32**, 293–6.

Harper, J. M. (1979). Food extrusion. *Crit. Rev. Food Sci. Nutr.*, **11**, 155–215.

Harper, J. M. (1981). *Extrusion of Foods*, Vols 1 and 2. CRC Press, Boca Raton, FL.

Harper, J. M. (1986). Extrusion texturization of foods. *Food Technol.*, **40**, 70–6.

Ho, C. T. & Izzo, M. T. (1992). Lipid–protein and lipid–carbohydrate interactions during extrusion. In *Food Extrusion Science and Technology*, ed. J. L. Kokini, C. T. Ho & M. V. Karwe. Marcel Dekker, New York, pp. 415–26.

Izzo, M. T. & Ho, C. T. (1989). Protein–lipid interaction during single screw extrusion of zein and corn oil. *Cereal Chem.*, **66**, 47–51.

Jeunik, J. & Cheftel, J. C. (1979). Chemical and physicochemical changes in field bean and soybean proteins texturized by extrusion. *J. Food Sci.*, **44**, 1322–5.

Kinsella, J. E. (1978). Texturized proteins: fabrication, flavoring and nutrition. *Crit. Rev. Food Sci. Nutr.*, **10**, 147–207.

Kokini, J. L., Ho, C. T. & Karwe, M. V. (eds) (1992). *Food Extrusion Science and Technology*. Marcel Dekker, New York, 734 pp.

Krimm, S. & Bandekar, J. (1980). Vibrational analysis of peptides, polypeptides, and proteins. V. Normal vibrations of β-turns. *Biopolymers*, **19**, 1–29.

Krimm, S. & Bandekar, J. (1986). Vibrational spectroscopy and conformation of peptides, polypeptides and proteins. *Adv. Prot. Chem.*, **38**, 181–364.

Lowry, O. H., Rosebrough, N. J., Lewisfarr, A. & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Martinez-Serra, M. D. & Villota, R. (1992). Reactivity, functionality, and extrusion performance of native and chemically modified whey proteins. In *Extrusion Cooking Science and Technology*, ed. J. L. Kokini, C. T. Ho & M. V. Karwe. Marcel Dekker, New York, pp. 387–414.

Mercier, C., Charbonniere, R., Gallant, D. & Gulbt, A. (1979). Structural modification of various starch by extrusion cooking with twin-screw French extruder. In *Polysaccharides in Food*, ed. J. M. V. Blanshard & J. R. Mitchell. Butterworths, London, pp. 153–70.

Mitchell, J. R. & Arêas, J. A. G. (1992). Structural changes in biopolymers during extrusion. In *Food Extrusion Science and Technology*, ed. J. L. Kokini, C. T. Ho & M. V. Karwe. Marcel Dekker, New York, pp. 345–60.

Myiazawa, T. (1960). Perturbation treatment of the characteristic vibrations of polypeptide chains in various configurations. *J. Chem. Phys.*, **32**, 1647–51.

Myiazawa, T. & Blout, E. R. (1961). The infrared spectra of polypeptides in various conformations: Amide I and amide II. *J. Am. Chem. Soc.*, **83**, 712–19.

Parker, F. S. (1983). *Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry*. Plenum Press, New York, 550 pp.

Rhee, K. C., Kuo, C. K. & Lusas, E. W. (1981). Texturization. In *Protein Functionality in Foods*, ed. J. P. Cherry. *ACS Symp. Series*, **147**, 51–88.

Robinson, D. R. & Jencks, W. P. (1965). The effect of compounds of urea-guanidinium class on the activity coefficient of acetyltetraglycine ethyl ester and related compounds. *J. Am. Chem. Soc.*, **87**, 2462–70.

Sheard, P. R., Ledward, D. A. & Mitchell, J. R. (1984). Role of carbohydrates in soya extrusion. *J. Food Technol.*, **19**, 475–83.

Sheard, P. R., Mitchell, J. R. & Ledward, D. A. (1986). The extrusion behavior of different soya isolates and the effects of particle size. *J. Food Technol.*, **21**, 627–41.

Simonsky, R. W. & Stanley, D. W. (1982). Texture–structure relationships in textured soy protein. V: the influence of pH and protein acylation on extrusion texturization. *Can. Inst. Food Sci. Technol.*, **15**, 294–301.

Stanley, D. W. (1986). Chemical and structural determinants of texture of fabricated foods. *Food Technol.*, **40**, 65–8.

Stanley, D. W. (1989). Protein reactions during extrusion cooking. In *Extrusion Cooking*, ed. C. Mercier, P. Linko & J. M. Harper. American Association of Cereal Chemists, St Paul, MN, pp. 321–41.