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MICROSTRUCTURE OF EXTRUDED MIXTURES OF CEREALS AND OIL SEED PROCESSING RESIDUES

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Abstract

The utilization of valuable by-products of seed processing residues as coextrusion materials was investigated. By mixing sunflower, pumpkin, corn or rice germ presscake with cereals (wheat, corn, rice), the good protein quality of the former group might improve the biological value of the resulting coextrudates. The microstructure of such coextruded products was analysed with reference to their chemical compositions, nutritional characteristics and functional properties. As seed processing residue was increased, the microstructure of the products became more compact and uniform and the air/solids ratio decreased considerably. The results showed that the highest acceptable concentration of the additive was not more than 20-40 % for the applied high temperature short-time procedure.

Introduction

Extrusion technologies are being used increasingly in the processing of cereal-based foods, snacks and related products. Usage has grown rapidly because of the development of technologies capable of producing a wide range of products economically. High-temperature, short-time (HTST) procedures are versatile and continuous. The resulting products are sterile and very digestible (Seib 1976).

Because mechanical and heat treatment can or has the potential to disorganize the original structure of raw materials (Björck et al 1984), the physicochemical character, ultrastructure and texture of the main components of the starting materials (starch, proteins and lipids) can change significantly during extrusion (Seib 1976, Williams 1977).

The modifications of starch due to extrusion were detailed by several workers (Mercier and Feillet 1975, Faubion and Hosney 1982a, Owusu-Ansah et al. 1983, Goodman and Rao 1984, Villareal and Juliano 1987). Some of changes expected to occur in proteins during extrusion have been reviewed as well (Faubion and Hosney 1982b). Heat-sensitive components (vitamins, fatty acids, etc.) are effectively preserved by short term thermal processing while the relative amount of dietary fiber slightly increases under the extreme conditions of temperature and pressure (Varo et al. 1983, Björck et al. 1984).

Successful coextrusion of maize protein with soy produced a uniquely textured product (Neuman et al 1984, Aguilera and Kosikowski 1978). Koeppe et al (1987) coextruded amaranth seeds and maize gluten meal to produce nutritionally complemented products. Similar work has been reported by Peri et al. (1983) concerning the production of a corn germ-milk coextruded snack product.

In spite of these successes, the use of HTST extrusion cooking for the texturization of certain oilseed proteins or mixtures of plant materials has not been investigated satisfactorily. The objective of the present work was to study the effect of extruding seed processing residues (sunflower seed, pumpkin, maize germ, rice germ) with cereals (wheat, maize, rice). The changes in chemical composition, microstructure, functional properties (expansion, water- and oil-absorption capacity, emulsion activity index) and nutritional

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quality of the extruded products were investigated as a function of concentration of the seed processing residues. This study aimed at the utilization of these valuable industrial by-products (seed processing residues) as food ingredients in human nutrition.

Materials and Methods

Materials

Oilseed press cakes, sunflower seed, pumpkin seed, maize germ, and rice germ were obtained from the Herbaria Co., Budapest. The fresh cold pressed (350 atm) by-products were ground by Cyclotec 1093 (Tecator, Sweden) sample mill to an average particle size <0.32 mm and were used immediately for extrusion. The commercially available cereal meals (hard wheat, maize, rice) were obtained from Hungarian Grain Trust (Budapest).

Extrusion

The seed processing residues and cereal components were mixed, pairwise in a cylindrical powder mixer of 0, 20, 40 or 60% (w/w) seed processing residues. An industrial extruder (GMV01, GMV Székesfehérvár, Hungary) with a 35 mm diameter single screw (1/d ratio=4/1, pitch 46mm) was used to extrude the mix. The extrusion parameters were as follows: 155 °C barrel temperature (heated by an electrical induction system), 100 rpm screw speed and six 4 mm restriction dies. The total residence time of the product in the extruder was about 8 sec. The products were cooled by air and ground into powder for analysis.

Proximate analysis

Raw materials and extrudates were analysed by AACC (1983) and AOAC (1980) methods: Crude protein (Kjeldahl; AACC method 46-10), moisture (AACC method 44-19), ash (AACC method 08-01), crude fiber (AOAC method 14020) and lipid (AOAC method 14018).

Functional properties

Bulk density was estimated by measuring the weight of 10 cm³ unextruded (ground) and 1000 cm³ extruded material. Graduated cylinders containing the extrudates were tapped 30 times to allow uniform compacting of the material. The mean of nine replicates was reported. The water absorption capacity (WAC) was determined by a modified capillary absorption method at ambient temperature according to Enslin (1933). Samples of 50 mg were placed on the surface of a glass filter and the amount of absorbed water was determined from the changes of the water level in the capillary. The WAC value was expressed as g water absorbed/g sample. The results represent the mean of three replicate measurements.

Oil absorption capacity (OAC) was measured by modified method described by Lin et al. (1974). A 0.5 g sample was mixed (1 min) with 3 cm³ sunflower oil in a plastic centrifuge tube and allowed to stand 20 min at ambient temperature. The slurry was centrifuged at 4000 rpm (1800 g) for 25 minutes. The tubes were inverted on filter paper for 10 minutes and then reweighed. The measured data were expressed as g absorbed oil/g sample. Samples were measured in triplicate.

Both the emulsifying activity index (EAI) and emulsion stability index (ESI) were deter-

mined by the method of Pearce and Kinsella (1978). Samples of 0.6 g were homogenized in 12 cm³ 0.1 M phosphate buffer, pH 7.4, with 4 cm³ sunflower oil at 15000 rpm (6000 g) for 1 min. Samples of 100 μ l were collected after different incubation times (time range 0.5-60.5 min) and diluted one hundred times with 0.01 M phosphate buffer containing 0.05% sodium dodecylsulphate. The absorption of the samples was measured at 500 nm, and EAI was calculated according to Pearce and Kinsella (1978).

Electron microscopy

The extrudates were cut and glued with carbon paint. The specimens were coated with gold (thickness 50 nm, vacuum 10⁻⁵ torr). During evaporation the sample holder was cooled by water to prevent samples from heating.

The extrudates were examined in a JEOL JSM 840 SEM using the secondary electron images. In order to reduce charging and beam damage, low accelerating voltages (5-10 kV) and currents (10⁻¹¹-10⁻¹⁰ A) were used.

Nutritional evaluation

Amino acid analyses were performed using a Mikrotechna AAA 881 amino acid analyser equipped with an automatic sample applicator. Duplicate determinations were conducted on duplicate samples.

In vitro biological value of proteins were calculated from amino acid data according to Morup and Olesen (1976) using a computer program.

Results and Discussion

Chemical composition

The raw materials used (Table 1) allowed us to vary the chemical composition of the coextrudates over a wide range. By altering the amount of macrocomponents present (protein, starch, lipid), parameters of nutritional importance could be changed accordingly. The nutritional properties of the products were determined by their amino acid composition and by the amount and ratio of essential amino acids (Table 2).

Table 1. Chemical composition of raw materials^a

Meal	Crude	Lipid	Mois-	Ash	Crude
	protein		ture		fiber
	%	%	%	%	%
Wheat	11.1	0.33	6.0	0.44	0.90
Maize	9.9	0.93	6.2	0.80	0.90
Rice	8.6	0.11	2.7	0.72	0.70
Sunflower seed	46.9	8.78	6.3	7.10	9.00
Pumpkin seed	55.3	8.75	7.1	8.10	9.20
Maize germ	17.8	14.05	5.6	3.41	12.90
Rice germ	20.3	13.73	7.3	8.23	13.90

a - triplicate determinations of each sample

Table 2. Amino Acid composition of raw materials (mg amino acid/g sample)

Amino acid	Wheat	Maize	Rice	Sun-flower seed	Pump-kin seed	Maize germ	Rice germ
ASP	5.41	7.52	9.32	48.05	41.97	15.82	13.99
THR	4.08	5.12	5.46	24.12	19.22	8.28	5.46
SER	4.56	4.38	3.48	16.59	23.30	7.46	5.73
GLU	34.97	20.98	13.10	90.84	79.91	24.73	23.31
PRO	12.55	4.33	2.40	24.10	19.53	5.86	8.14
GLY	4.37	3.31	3.55	24.88	34.30	11.66	10.49
ALA	3.48	7.46	4.17	18.63	26.22	9.87	9.40
CYS	0.87	0.81	0.98	6.76	2.90	1.26	7.76
VAL	3.02	3.80	2.81	16.59	27.25	7.50	6.55
MET	1.08	1.59	1.59	7.45	14.88	2.82	1.93
ILE	3.08	2.43	2.10	14.58	15.07	5.05	4.83
LEU	5.85	12.32	5.81	23.68	41.65	10.79	8.43
TYR	4.63	5.92	4.98	19.48	18.52	8.69	7.83
PHE	5.32	6.74	4.34	23.58	27.79	13.69	7.28
LYS	2.91	3.30	4.32	20.36	29.95	13.69	14.61
HIS	2.83	4.04	2.25	14.44	17.61	11.23	7.52
TRP	0.06	0.05	0.13	1.86	0.16	0.55	3.32
ARG	4.43	4.88	7.07	45.02	98.44	24.33	20.01

The lipid and crude protein determination of the products confirmed that during the extrusion process no loss of protein or lipid occurred. Based on a given raw material composition, any required product can be realized. However, sensitive amino acids did change during extrusion which resulted in a decreased nutritive value of the products (see below).

Functional properties

While the bulk density (BD) of the products decreased due to extrusion in all cases (Table 3), there were large differences in BD depending on the cereal components used. This could be due to different behaviors by the various starches (Mercier and Feillet 1975) and proteins (Faubion and Hosney 1982b) tested. In all cases, the BD values were considerably influenced by the addition of the seed processing residues. The changes of BD as a function of additive level were less pronounced in mixtures containing wheat. Using maize and rice as cereal components, considerable changes were observed. In the mixtures of maize + rice germ 20%, rice + sunflower seed 20%, and rice + rice germ 20%, the BD values of the products were lowest and most comparable to that of extruded pure wheat.

The water and oil absorption capacities of the pure cereals generally increase upon extrusion (Lawton et al. 1985). The values obtained for extruded maize and rice (Tables 4 and 5) did not differ significantly in either parameter while wheat showed lower WAC and OAC values compared to extruded maize and rice. On comparing the WAC of pure extruded cereals to that of coextrudates, WAC values were found to decrease with increasing the amount of the oilseed component (Table 4). Low concentrations of seed processing residues (20%) resulted in 10-30% decreases in the WAC of extrudates. WAC was least affected when maize germ residue was used as the

Table 3. Bulk density of raw materials and their extrudates prepared from pure cereals or mixtures of cereals and seed processing residues^d

Additive	Wheat		Maize kg/dm ³		Rice	
	raw	extr. ^a	raw	extr. ^a	raw	extr. ^a
None	0.715	0.152	0.633	0.073	0.710	0.064
Sun-flower seed						
20%	0.704	0.346	0.631	0.375	0.674	0.184
40%	0.642	0.352	0.624	0.419 ^b	0.634	0.305 ^b
60%	0.640	0.317	0.620	0.404	0.586	0.329 ^b
Pumpkin seed						
20%	0.705	0.373	0.640	0.293	0.704	0.246
40%	0.649	0.371	0.634	0.395 ^b	0.668	0.261
60%	0.615	0.299 ^c	0.607	0.375 ^b	0.608	0.249
Maize germ						
20%	0.638	0.337	0.638	0.364	0.661	0.237
40%	0.625	0.387 ^b	0.612	0.409 ^b	0.609	0.376 ^b
60%	0.586	0.418 ^b	0.574	0.413 ^b	0.580	0.397 ^b
Rice germ						
20%	0.677	0.412	0.629	0.207	0.709	0.117
40%	0.652	0.433	0.617	0.397 ^b	0.663	0.198 ^b
60%	0.625	0.412	0.587	0.425 ^b	0.617	0.296 ^c

a - extruded samples

b - significantly different from the 20% mixtures of the same additive, $p < 0.05$

c - significantly different from the 20% and 40% mixtures of the same additive, $p < 0.05$ (Student's t-test)

d - three determinations on three replicate samples

seed processing residue component. Higher substitutions resulted in more drastic lowering of the WAC, e.g. 65% decrease in wheat; pumpkin seed (40:60).

The oil absorption capacity of coextrudates was decreased to 40-60% of control (0% processing residue) values at the level of 20% seed processing residue in the extrudate (Table 5.). The same trends were observed for all types of cereal components used.

The extrudates and coextrudates showed relatively low (1-2 g/g) EAI and stable ESI indices. The EAI and ESI values obtained did not differ significantly as a function of seed processing residues added.

Microstructure

Figure 1 (a-c) shows that the microstructures of the extruded cereals differed significantly. However, each pure cereal showed rather uniform extrudate structure, in the sense that the holes or compartments had similar shapes and the cell walls were rather typical for the particular

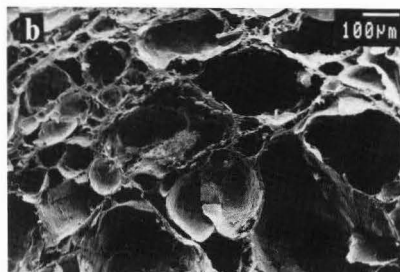


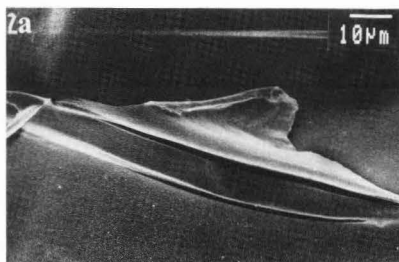
Figure 1. Low magnification scanning electron micrographs of extrudates of a) rice; b) maize; c) wheat.

Figure 2. Scanning electron micrographs of a) extruded rice (further magnification of a similar area indicated by the arrows in Fig. 1a); b) coextrusion of rice with 20% rice germ residue; c) coextruded rice with 60% rice germ residue.

cereal. Fig. 1a shows the flaky sheet type matrix being characteristic of extruded rice whereas extruded maize (Fig. 1b) had a loose structure with uniform cell sizes. The location of large and small cells was random. The largest compartments were about 1 mm in diameter. The loose microstructure of these two cereal products explains their low bulk density. Extruded wheat (Fig. 1c) had fibrous surfaces and contained irregularly shaped cells. The different coextrudates showed a significantly different microstructure at low and high magnification. The structure of each particular coextrudate varied within the sample. Only typical changes due to the addition of seed processing residue are presented in Figures 2 to 4.

The effect of increasing processing residue content on extrudate microstructure is shown in Figs. 2a-c. The addition of 20% rice germ disrupted the flaky structure of extruded rice with the appearance of small granules (Fig. 2b). With increasing amounts of the additive, a compact, granular structure (Fig. 2c) was observed at higher magnifications. Upon the addition of seed processing residues, the ratio of dispersed air was reduced (bulk density increased) and the structure became more compact and the cell walls showed less variability within the sample.

Effects similar to the above mentioned were observed for coextrudates of processing residues and maize. Adding rice germ, pumpkin, maize germ and sunflower seed processing residues at 60% concentration caused the original microstructure



Microstructure of coextrudates

Table 4. Water absorption capacity of extrudates from cereals and from mixtures of cereals and seed processing residues

Additive	Wheat Maize Rice g water/g material			
	None	4.3 ^c	4.6 ^c	4.5 ^c
Sunflower seed	20%	4.0	3.9	3.0
	40%	2.9 ^a	2.1 ^a	2.7 ^a
	60%	1.7 ^b	2.2 ^a	2.5 ^b
Pumpkin seed	20%	2.8	3.9	4.3
	40%	2.1 ^a	2.6 ^a	3.3 ^a
	60%	1.5 ^b	1.5 ^b	2.3 ^b
Maize germ	20%	3.4	3.5	4.3
	40%	3.4	3.2 ^a	4.0 ^a
	60%	2.7 ^a	3.3 ^a	3.5 ^b
Rice germ	20%	2.4	3.9	3.8
	40%	1.9 ^a	2.2 ^a	3.5 ^a
	60%	2.0 ^a	2.2 ^a	3.0 ^b

- a - significantly different from the 20% mixtures of the same additive, $p < 0.05$
 b - significantly different from the 20% and 40% mixtures of the same additive, $p < 0.05$
 c - values of pure extruded product significantly ($p < 0.05$) different from values of coextrudates with the same cereal

Table 5. Oil absorption capacity of extrudates from cereals and from mixtures of cereals and seed processing residues

Additive	Wheat Maize Rice g oil/g material			
	None	2.1 ^c	2.8 ^c	2.8 ^c
Sunflower seed	20%	1.3	1.2	1.5
	40%	1.1 ^a	1.1	1.0 ^a
	60%	1.1 ^a	0.8 ^a	1.1 ^a
Pumpkin seed	20%	1.5	1.6	2.4
	40%	1.2 ^a	1.1 ^a	1.3 ^a
	60%	1.2 ^a	1.2 ^a	1.2 ^a
Maize germ	20%	1.2	1.1	1.0
	40%	1.0 ^a	0.9	0.9
	60%	0.9 ^a	1.0	0.9
Rice germ	20%	1.1	1.5	1.6
	40%	0.8 ^a	0.9 ^a	1.1 ^a
	60%	0.9 ^a	0.9 ^a	0.9 ^b

- a - significantly different from the 20% mixtures of the same additive, $p < 0.05$
 b - significantly different from the 20% and 40% mixtures of the same additive, $p < 0.05$
 c - values of pure extruded product significantly ($p < 0.05$) different from values of coextrudates with the same cereal

to disappear. The coextrudates had compact structure containing intact starch granules 5-15 μ m and in some areas gelatinized starch was observed (Fig. 3a-e).

On adding seed processing residues to wheat, the original lamellar structure (observed at higher magnifications) completely disappeared (Fig. 4a-e). With 20% seed processing residue addition some fibrous-lamellar structure at the surface of compartments could still be observed (Fig. 4b-c). Increasing the amount of the additive to 60%, a rippled structure with randomly distributed granules of different size was observed in some areas of the sample. These granules, appearing only with the addition of 60% residues seem to be intact starch particles with a maximum diameter of 20 μ m.

Nutritional evaluation

Protein nutritional quality indices (Morup and Olesen 1976) of extrudates prepared from cereals and from mixtures of cereals and seed processing residues were calculated from the samples' amino acid compositions (Table 6). The relatively low biological values of cereals were increased by the addition of seed processing residues. The biological value of extrudates made with rice germ and sunflower seed increased considerably. The identity of the cereal component played a role as well. All three cereals are well complemented with rice germ at concentrations of 20-40%. Sunflower seed improved the biological value of extrudates at all seed processing residue concentrations. Wheat and maize improved only when supplemented with large amounts of pumpkin seed. The nutritional quality of these coextrudates was not significantly improved by the addition of maize germ.

There were significant ($p < 0.05$) differences (Table 6.) between measured and calculated biological values in extruded products. These results suggest that the destruction of limiting amino acids (tryptophan, cysteine and methionine) during extrusion has to be taken into consideration.

Conclusions

Functional properties and microstructural evaluations suggested that HST extrusion is an applicable technology to process various cereals containing seed processing residues as coextrudates.

The bulk density of the coextrudates increases with an increase in the amount of seed processing residues in the mixture. A pronounced decrease was observed in the WAC and OAC of the coextrudates. Because of the above unfavorable tendencies, the amount of seed processing residues in the products is limited (max. 20-40%). In contrast, an advantageous increase in nutritional value was observed by addition of seed processing residues.

The extrudates of pure cereals showed cellular microstructures of different character but became more compact when the processing residues were added. Increasing the seed processing residue content reduced the proportion of air in the products which paralleled a shift in the ratios of gelatinized/intact starch.

The recommended cereals and seed processing residues to form coextrudates of acceptable quality are shown by our results to be composed of

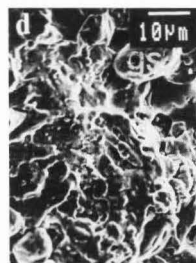
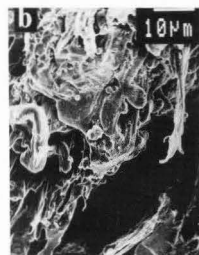
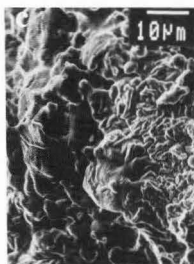
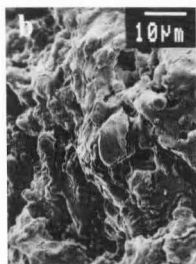


Figure 3. Scanning electron micrographs of a) extruded maize and coextrusions of maize with seed processing residues of b) 60% rice germ; c) 60% pumpkin; d) 60% maize germ; e) 60% sunflower.
(s) starch, (gs) gelatinized starch.

Figure 4. Scanning electron micrographs of a) extruded wheat and coextrusions of wheat with different amounts of seed processing residues; b) 20% rice germ; c) 20% pumpkin; d) 60% rice germ; e) 60% pumpkin.
(s) starch, (gs) gelatinized starch.

Table 6. *In vitro* biological value of proteins in extrudates calculated by Morup and Olesen (1976) index

Additive	Wheat		Maize		Rice	
	calcu- lated ^a	mea- sured ^b	calcu- lated	mea- sured	calcu- lated	mea- sured
None	35.8	33.6	29.8	28.0	69.1	62.9
Rice germ	20% 97.6 40% 97.3 60% 82.5	- - 78.5	86.2 106.4 89.9	- - 84.5	90.4 86.6 76.6	- - 70.7
Pump- kin seed	20% 53.3 40% 59.3 60% 60.4	- - 56.5	47.2 55.3 59.4	- - 64.6	68.4 66.1 63.3	- - 48.6
Maize germ	20% 47.4 40% 50.6 60% 50.2	- - 46.3	42.2 49.9 50.5	- - 56.9	62.2 57.5 54.1	- - 50.2
Sun- flower seed	20% 61.7 40% 71.0 60% 76.0	- - 69.6	56.1 67.3 73.9	- - 68.7	77.5 79.5 80.3	- - 72.7

a - calculated values represent the theoretical predictable biological values

b - measured values were calculated by amino acid composition of extrudates

maize or rice as cereal and sunflower seed or rice germ as seed processing residue component.

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Discussion with Reviewers

D.J. Gallant: Cytochemistry would helpfully resolve the question "how the oil seed interacts with the other components of cereals and oil seed residues". Did you try to study it either under the light or transmission electron microscope?
Authors: Specific staining is planned for cytochemical evaluation of coextrudates to reveal the interaction of macrocomponents during extrusion. Light microscopy results will be presented in a separate paper.

W.J. Wolf: Sunflower seeds contain chlorogenic acid and other phenolic acids. Does the presence of these compounds cause problems of undesirable colors in the coextrudates?

Authors: The colour of the products was basically determined by the colour of the cereal component obtained after hydrothermic treatment. The white or yellow colour of the cereal components turned into clear brown or green due to addition of sunflower seed and pumpkin, respectively. The colours were stable during storage. For these reasons the chlorogenic acid and phenolic components of the seed processing residues were not measured.

W.J. Wolf: The oilseed press cakes contain appreciable amounts of lipids (Table I). Do these residual lipids cause stability problems in the coextruded materials?

Authors: The organoleptic properties of the coextrudates are certainly influenced by the characteristics of the lipids. For this reason coextrusion was done from freshly pressed oilseed residues. The oxidation of the sensitive components will have to be prevented by adequate packaging and by adding antioxidants. Storage experiments are planned to investigate the stability of the products.

W.J. Wolf: For a food product to be successful, it must have organoleptic properties that are acceptable to consumers. Have you evaluated any of the extruded materials for their organoleptic characteristics, i.e., flavour and texture?

Authors: Preliminary experiments were carried out to analyze the organoleptic properties of the coextrudates. The flavours were present.

J.M. Fabuon: What are the small granules (about 1 μ m) shown in Fig. 3b-e?

Authors: The 1-2 μ m size granules are believed to be protein bodies according to previous works of Pagani et al. (1986).

J.M. Fabuon: Why does the addition of the seed processing residues inhibit or prevent starch gelatinization?

Authors: The elevated seed processing residue content (higher amount of lipids) presumably prevented the water migration that paralleled the reduced heat conduction. These unfavourable temperature conditions and the decreased amount of available water resulted in reduced gelatinization.

P.D. Richards: The main body of the work relates the microstructure to the functional and nutritional aspects. The authors should explain why the nutritional and related data are essential for this work?

Authors: The purpose of the coextrusion is to obtain products with increased nutritive value. Measuring the change of these parameters to evaluate the products is essential.

P.D. Richards: Have the authors made any investigations of biodigestibility *in vivo* to determine the nutritional value of the products?

Authors: Parallel *in vivo* rat trials and *in vitro* measurements for determination of the biological value and the true digestibility of proteins for extruded products were performed. The correlations between *in vivo* and *in vitro* data were determined for both parameters and will be reported elsewhere.

Additional Reference

Pagani MA, Gallant DJ, Bouchet B, Resmini P. (1986) Ultrastructure of cooked spaghetti. Food Microstructure 5: 111-129.