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RAPID COMMUNICATION

Effect of processing on iron bioavailability of extruded bovine lung

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The iron bioavailability of extruded and non-extruded lung products was compared with that of ferrous sulfate by the haemoglobin regeneration method. Extrusion was performed in a laboratory extruder (L/D 20:1) at several processing temperatures and moisture contents of the feed (115–160°C; 16–30% moisture) with screw (4:1 compression ratio) speed fixed at 200 rpm. These conditions included the optimum observed for lung texturization. Bioavailability of iron was determined as efficiency of haemoglobin regeneration in rats fed bovine extruded lung-based diets, compared with non-extruded lung and casein standard diets. The results showed that iron bioavailability was high and comparable with ferrous sulfate standard, irrespective of the extrusion conditions adopted. These results showed that extruded lung can be used as a good iron source, even if processed in conditions deleterious for other nutrients. © 1997 Elsevier Science Ltd

INTRODUCTION

Extrusion cooking is being increasingly used by the food industry, particularly for the production of snacks and breakfast foods. This use has been recently extended to upgrade by-products from the meat industry, after drying and defatting of the raw material (Arêas & Lawrie, 1984; Arêas, 1986*a*,*b*; Bastos & Arêas, 1990; Bastos *et al.*, 1991). In previous work we have shown that protein is largely affected in nutritional terms when drastic conditions for extrusion, which are the ones for maximum texturization, are employed for lung texturization (Campos & Arêas, 1993). Another important nutrient in these waste tissues is iron, which is present in significant amounts and little information is, as yet, available about the extrusion impact on its nutritive properties.

The effect of extrusion on changes of the chemical composition, digestion and absorption of minerals is not perfectly established. Kevistö *et al.* (1986) have reported a reduction in mineral absorption from vegetable extruded products associated with a resistance of phytate digestion. However, Fairweather-Tait *et al.* (1987, 1989) observed in *in vivo* studies, using an extrinsic label, no effect of maize extrusion on iron absorption.

Lung, like most meats, has around 50% of total iron in the haeminic form. Animal tissues are known to have an enhancing effect on both haem and non-haem iron absorption. The mechanism behind this is not yet established but the interaction of iron with specific peptides produced during protein digestion may correspond with the iron absorption-enhancing properties of meat (Kapsokefalou & Miller, 1995).

The nutritional effects of extrusion cooking on iron bioavailability of soy and cereals have been reported in the literature (Mercier, 1993; Camire, *et al.*, 1990; Hazell & Johnson, 1989; Asp & Björck, 1989; Fairweather-Tait *et al.*, 1987, 1989; Ranger & Neale, 1984). Nevertheless, an appraisal of the processing effects on the nutritional quality of these novel extruded animal waste products is necessary.

The present work reports the effect of processing conditions on iron bioavailability of extruded lung. Lung tissue was dried, ethanol-defatted and extruded at various temperatures and feed moisture contents that included the optimum for the texturization of this material (Bastos *et al.*, 1991). Iron bioavailability was determined by the haemoglobin regeneration test in anaemic rats fed on experimental and control diets (Mahoney *et al.*, 1974).

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MATERIALS AND METHODS

Materials

Bovine lungs were provided frozen by Sadia-Frigobrás S.A. (Toledo, PR-Brazil). After thawing overnight at 5°C, they were minced in a homogenizer (MOD D-Hobart Co., USA) through a perforated plaque of 1 cm holes. Before extrusion, the sample was oven-dried in an air circulating oven (air temperature of 70°C) for 8 h and ethanol-defatted in a glass Soxhlet apparatus. To obtain the desired moistures for the extrusion feeding material, sufficient water was added, and the samples were mixed thoroughly in a mixer and maintained in polyethylene bags at 5°C for 24 h. Extrusion was carried out in a laboratory single-screw extruder (Miotto-São Paulo, SP, Brazil), 20 mm barrel diameter, L/D ratio 20:1.

Extrusion process variables for lung were established based on previous work by Bastos *et al.* (1991) and Bastos & Arêas (1990), in the range where extrusion of these materials was possible. A combination of barrel extrusion temperatures ranging from 115 to 160°C and feed moisture from 16 to 30% dry solid basis (d.s.b.) was employed.

Methods

Chemical analysis

Triplicate determinations of each component were carried out as follows: desiccation to constant weight at 105°C for moisture, ethyl ether extraction in Soxhlet extractors for lipids, micro-Kjeldahl for protein, and calcination at 550°C to constant weight for ash (AOAC, 1980; Instituto Adolfo Lutz, 1985). Iron was determined after wet digestion of the samples with $HClO_4/HNO_3$ (1:10), in an atomic absorption spectrometer with background correction (Mod. 373, Perkin Elmer, USA) employing a hollow cathode lamp at 248.3 mm, and $FeCl_3$ (Titrisol, Merck) as standard.

Animal experiments

Weaning male albino Wistar rats, weighing 58 g on average, were individually housed in stainless steel cages and maintained under controlled conditions of light and temperature. They received deionized/distilled water and were fed *ad libitum*. The bioassay experiments were carried out in accordance with the Committee on Laboratory Animal Diets (NRC, 1979).

The variables: weight of each animal (g), feed consumption (g) and blood haemoglobin (Hb) (g dl^{-1}), determined by cyanide haemoglobin method after tail bleed, according to Evelyn & Malloy (1938), were measured during the assay. The iron pool variation, which expresses available iron from ingested diets, or gain in haemoglobin iron (Miller, 1982) was calculated as: Pool Fe (mg) = 0.067 × weight of animal (g) × 0.035% × (Hb) (g dl^{-1}). The percentage of bioavailable iron (% Abs Fe) was then calculated by dividing the iron pool variation (mg) by total iron intake in the food (mg) multiplied by 100 (Mahoney *et al.*, 1974). A correction of iron absorption according to the variable amount of iron ingested in different groups was performed, as suggested by Miller (1982). We observed an exponential relationship between iron intake and percentage iron absorbed that was reproducible among different trails (Colli *et al.*, 1993). Thus, based on our experimental results of casein standard groups, with several iron concentrations, we derived the equation:

$$Y = e^{(4.1-0.0513X)}$$

where Y is the average percentage iron absorption of the casein standard groups of each experiment relative to the amount ingested (X). This equation was used to correct the percentage absorbed iron in all experimental groups according to the iron ingestion of each group.

Iron absorption of experimental groups was then related to that of casein standard group corrected to the amount ingested (% Cor Fe), and resulted in the relative biological value — RBV, according to the equation:

$$\text{RBV} = 100\ (\%\ \text{Abs Fe}/\%\ \text{Cor Fe}).$$

Anaemia was induced by feeding the animals immediately after weaning for a 3 week period with a semipurified, iron-free diet (AOAC, 1990). This low iron diet contained (in grams per kilogram of diet): corn starch 555.0, casein 200.0, sucrose 150.0, corn oil 50.0, gelatin 50.0, monosodium phosphate 20.0, calcium carbonate 20.0, potassium chloride 5.0, iodine added sodium chloride 5.0, vitamin mix 10.0, mineral mix (iron free) 2.7 and choline chloridrate 1.5.

During this period two control groups were investigated together with that of the depletion group to ascertain that no interference occurred in the animal growth. One of these groups contained the same depletion diet but with added ferrous sulfate and the other was a casein standard diet (NRC, 1979).

After 21 days, the rats of the depleted group presented body weights around 100 g and haemoglobin levels around 6.5 g dl^{-1}. They were then randomly assigned into eight homogeneous groups in respect of their iron pool (Hb concentration × body weight). The other two control groups were sacrificed. The iron bioavailability in this repletion phase was determined for groups feeding extruded repletion diets based on equal amounts of bovine lung in each condition of extrusion processing, and compared with that based on bovine defatted dry lung and that based on casein standard diet, (with $FeSO_4$). The amount of lung added was calculated to supply iron requirements for rats. This provided only 11% of protein, which was adjusted to 20% by adding 9% of pure and iron-free casein.

The test period for repletion spanned 13 days.

Data were analysed by one way variance analysis. Means were compared pairwise with the Tukey test for the equality of variances, and with the Brown–Forsythe and Sheffé test for the inequality (Neter *et al.*, 1985). The method of variance analysis with two fixed factors (two levels of temperature and three levels of moisture) was performed to verify the significance of the effect and interaction of factors on the model. This classic analysis assumes independent random errors with normal distribution and equal variance in every combination of temperature and moisture (Neter *et al.*, 1985).

RESULTS AND DISCUSSION

The iron contents of lung flours and their extruded products are shown in Table 1, where one can clearly see that the iron levels of extruded lung were significantly greater than the non-extruded ones. Several foods with iron contents from 400 to 600 mg g^{-1} are considered suitable as an iron source. The present results show that lung, be it extruded or not, can be a good source of dietary iron.

The extrusion process can result in an increase of the total iron content of the materials. This phenomenon has already been described by some researchers and must be taken into account as it could significantly affect the calculated iron bioavailability (Hazell & Johnson, 1989; Fairweather-Tait *et al.*, 1987, 1989; Maga & Sizer, 1978). In all cases the source of additional iron in the extruded samples was supplied by the hardened steel extruder.

Protein and iron concentration of the experimental diets and feed consumption, and protein and iron ingestion during the depletion period can be seen in Table 2. Feed consumption, and also animal growth, showed no significant difference among groups during the depletion period. However, the haemoglobin concentrations were distinct, being the same (around 12 g dl^{-1}) at the beginning and the end of the depletion period for the control groups and decreasing to 6.5 g dl^{-1} for the depletion groups. Figure 1 shows the growth curve and heamoglobin values for the depletion phase.

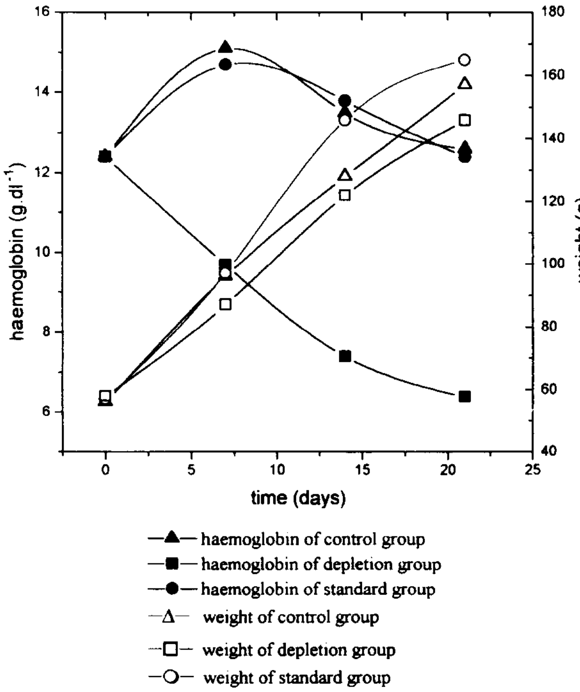

Fig. 1. Growth curves and haemoglobin values in the depletion phase of rats.

Table 1. Total iron content of bovine defatted dry lung bovine flours and their extruded products

Extrusion conditions Temperature/moisture	Fe μg g^{-1} (d.s.b)	C.V.
115°C/16%	750 ± 27	4
115°C/30%	685 ± 19	3
130°C/16%	691 ± 21	10
130°C/30%	672 ± 30	3
160°C/16%	747 ± 74	4
160°C/30%	667 ± 20	3
Non-extruded	582 ± 33	6

Table 2. Concentration of protein and iron from the feed and daily ingested iron and protein in the depletion phase

Groups	Depletion	Control	Standard
Protein (g%) (d.s.b.)	24 ± 0.4[a]	25 ± 1.7[a]	20 ± 1.7[b]
Iron (μg g^{-1}) (d.s.b)	16 ± 1.0	44 ± 2.1[c]	53 ± 2.0[c]
Ingested feed (g day^{-1})	11	14	13
Ingested protein (g day^{-1}	2.7	3.4	2.5
Ingested iron (μg day^{-1})	186	606	668

[a] 20% pure casein, 5% gelatin.
[b] 20% pure casein.
[c] $FeSO_4.7H_20$ sprayed from a concentrate solution.

We observed, in the present work, that $FeSO_4$ bioavailability of iron casein standard diet is higher than that of the lung diets. However, when corrected for iron ingestion, these values were reduced and resulted in relative biological values higher than 100% for the lung diets, after 13 days of the repletion experiment (Table 3). The efficiency of these diets in overcoming the anaemic state of the rats, measured by other parameters, can be seen in Table 3. Haemoglobin increased from the average of 6.5 g dl^{-1} in the anaemic rats to normal levels, around 12 g dl^{-1}. The iron pool, which was averaged approx. 2.2 mg at the beginning of the repletion experiment (non-anaemic rats: Pool HbFe approx. 4.2) increased to an average of 6.7 mg at the end, typical of normal rats of this age and weight. Relative biological value (RBV) presented values for extruded lung, significantly higher than the non-extruded sample and the casein standard group (Table 3) for any of the conditions of moisture and temperature tested. No interaction between the extrusion variables, temperature and moisture, was detected through variance analysis with two fixed factors ($P < 0.05$).

The results showed that bovine lung can be a good source of bioavailable iron and that extrusion, in any

Table 3. Effect of extrusion condition on iron bioavailability: haemoglobin repletion in anaemic rats in 13 days

Groups		Extrusion conditions						Non-extruded	Standard
		115°C, 16%	130°C, 16%	160°C, 16%	115°C, 30%	130°C, 30%	160°C, 30%		
Diet iron (μg g^{-1})		92 ± 3	90 ± 1	87 ± 3	89 ± 3	87 ± 3	90 ± 5	82 ± 8	53 ± 2
Ingested iron (mg)	t1	25 ± 2.3	24 ± 2.4	22 ± 2.8	23 ± 3.2	24 ± 3.4	25 ± 1.7	18 ± 1.8	14 ± 1.4
Body weight	t0	145 ± 11	141 ± 16	151 ± 13	147 ± 17	152 ± 12	139 ± 11	149 ± 11	142 ± 11
(g)	t1	247 ± 13	233 ± 19	238 ± 22	244 ± 28	256 ± 20	237 ± 17	218 ± 16	223 ± 21
Haemoglobin	t0	6.4 ± 0.7	6.6 ± 0.7	6.2 ± 0.5	6.6 ± 0.5	6.5 ± 0.8	6.7 ± 0.8	6.3 ± 0.7	6.7 ± 0.7
(g dl^{-1})	t1	13 ± 0.6	13 ± 0.5	13 ± 0.8	13 ± 0.9	13 ± 0.6	13 ± 0.5	12.5 ± 0.9	12 ± 1.1
HbFe pool	t0	2.2 ± 0.2^{a}	2.2 ± 1.0^{a}	2.2 ± 0.2^{a}	2.3 ± 0.4^{a}	2.3 ± 0.4^{a}	2.2 ± 0.4^{a}	2.2 ± 0.2^{a}	2.3 ± 0.2^{a}
(mg)	t1	7.9 ± 0.6	7.1 ± 0.7	7.6 ± 0.7	7.5 ± 0.9	8.1 ± 0.8	7.2 ± 0.6	6.5 ± 0.6	6.5 ± 1.0
%Abs Fe (bioavailable)	t1	23 ± 2.5^{a}	21 ± 2.1^{a}	25 ± 1.5^{a}	23 ± 2.7^{a}	24 ± 1.0^{a}	20 ± 0.8^{a}	23 ± 2.0^{a}	31 ± 5.7^{b}
% Cor Fe	t1	16 ± 0.7^{a}	18 ± 0.8^{a}	20 ± 1.1^{a}	19 ± 1.2^{a}	18 ± 1^{a}	17 ± 0.5^{a}	24 ± 1.0^{b}	—
RBV	t1	140 ± 17^{a}	118 ± 20^{ab}	132 ± 23^{a}	126 ± 17^{ab}	140 ± 25^{ab}	123 ± 17^{ab}	99 ± 16^{b}	—

In a row, means with the same superscript are not statistically different ($P < 0.05$). t0, beginning of repletion phase; t1, after 13 days; HbFe, haemoglobin iron pool; %Abs Fe, percentage of absorbed iron; % CorFe, percentage of iron ingested; RBV, relative biological value.

condition of temperature and feed moisture studied, did not affect this bioavailability. The results obtained in the present work suggested the possibility of using extruded bovine lung, not only as a protein source of high biological value (Campos & Arêas, 1993), but also as an iron source of high bioavailability.

The great progress achieved to date makes it possible to forecast the use of extrusion texturization to upgrade and recover waste animal protein/iron in the near future. It is necessary to determine the nutritional injury produced by processing and to focus on the mechanisms and conditions that could be responsible for it. Improvement in the equipment and process will help to optimize the technological and nutritional quality of extruded products. Optimization of extrusion processing has thus to take into account, not only acceptability parameters, but also nutritional performance of the final product.

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