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Nutritional evaluation of snacks obtained from chickpea and bovine lung blends

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Abstract

Chickpea snacks, containing 0, 5 and 10% of added bovine lung were obtained by extrusion at 130°C and 13% moisture content of the feed. These extruded products had improved contents of iron and protein compared to traditional ones, being able to provide up to 30% of the iron RDA in a 30g pack. Acceptability of these enriched snacks was assessed using a 9 point hedonic scale and was found high and comparable to commercial brands. Protein nutritional quality of these samples, measured by the NPR method, was similar and slightly lower than that of casein. The same behaviour was observed for the growth ratio of the experimental animals. Results indicate that it is possible to produce a highly acceptable snack of high nutritional quality that could be useful in feeding programs to counteract anaemia and malnutrition.

Keywords: Anaemia; Iron supplementation; Novel protein; Gram

1. Introduction

The food industry has been manufacturing products with additional acceptable nutritional attributes by means of several processes (Arêas, 1992; Cândido & Campos, 1995). Among the industrialization techniques, extrusion has been widely used because of its simplicity and low cost. This has contributed added value to by-products such as waste tissues and non-conventional legume grains. Generally, these foodstuffs have sensorial disadvantages but, after upgrading, they could be incorporated in human nutrition (Arêas, 1992; Bastos & Arêas, 1990; Bastos, Domenech & Arêas, 1991; Batistuti, Barros & Arêas, 1991; Kinsella, 1978; Smith, 1976).

Bovine lung is a raw material that, in spite of its great nutritional potential, has a strong aesthetic rejection, associated with its intrinsic low textural quality. It has been shown that its texture can be improved by extrusion (Arêas, 1992; Bastos & Arêas, 1990; Bastos et al., 1991; Campos & Arêas, 1993). Chickpea is another raw material with nutritional and agronomic advantages over other legumes and presents high potential as a functional ingredient for the food industry (Arêas, 1993; Attia, El-Tabey Shehata, Aman & Hamza, 1994; Avancini, Sales, Aguirre & Mantovani, 1992; Dodok, Modhir Abid Ali, Hazová, Halazová & Polacék, 1993). It has been shown that it could be textured to provide a highly acceptable snack product (Batistuti et al., 1991).

Although great progress has been made in texturization of lung by the extrusion process (Arêas, 1985, 1986a, b, 1992; Arêas & Lawrie, 1984; Bastos & Arêas, 1990; Bastos et al., 1991; Mittal & Lawrie, 1986), the achieved acceptability of these extruded products was far below the traditional commercial ones. Extruded chickpea, on the other hand, produced a snack comparable to commercial brands, with superior nutritional quality (Batistuti et al., 1991).

Thus, the use of bovine lung and chickpea mixtures could provide an acceptable ready-to-eat product that would contain high quality protein from chickpea and lung and iron and vitamins from the latter. The present work was performed to develop a snack product, with high nutritional quality in terms of protein and bioavailable iron, by use of this mixture.

2. Materials and methods

2.1. Materials

2.1.1. Snacks

Snacks were obtained by extrusion of mixtures containing chickpea and bovine lung as described by Batistuti

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et al. (1991) and flavoured with bacon for further evaluation. The flours were used as mixtures of 100:0, 95:5, and 90:10% of chickpea and bovine lung, respectively.

Chickpea (*Cicer arietinum* L.) var. Kabule, was bought from "Companhia de Entreposto e Armazéns Gerais — CEAGESP", São Paulo. The sample was prepared by grinding the beans in a knife mill (Mod. Termomatic, Marconi, Brazil) and by cool defatting with hexane.

Sadia S.A. (Paraná, Brazil) provided bovine lungs. They were excised from healthy animals (inspected by the Brazilian Agriculture Ministry Inspection Service), minced and frozen immediately after slaughter. The frozen lungs were lyophilized and milled to produce a powder that was defatted with chloroform.

The fat, in both samples, was extracted with 10 parts of solvent to 1 part of material by fast agitation through an electrical mixer for 30 min. This operation was performed with 30 l (hence 3 kg of dried lung or chickpea flours) solvent each time in a 100 l recipient. The residual solvent present in flour after extraction was evaporated in an air stream at ambient temperature.

2.1.2. Rat bioassay

The animal bioassay evaluated the protein nutritional value of chickpea and mixtures containing bovine lung and chickpea by weight gain on every other day and calculation of net protein ratio (NPR), that evaluated the quality of protein to maintain weight and promote growth. To compare the samples with the standard protein, casein, the relative NPR was calculated (Happich et al., 1984).

Male rats (*Rattus norvegicus*, var. albinos Rodentia mammalia), young (21–23 days), weighing 55 g, on average, were divided into six groups of eight animals. The animals were kept in individual cages that allowed collection of feces. These were collected every other day and their nitrogen contents analyzed by the micro-Kjeldahl method to calculate digestibility (AOAC, 1990).

Diets were prepared with 10% protein content, according to American Institute of Nutrition (AIN-93; Reeves, Nielsen, & Fahey, 1993). Casein was used as reference; the other groups were fed on cooked chickpea, extruded chickpea, extruded mixtures of 95:5 and 90:10% of chickpea and bovine lung, respectively, and an extra group was fed on a non-protein diet for the assessment of the endogenous nitrogen. The diets were offered ad libitum. Cooked chickpea diet was used without added oil since the whole flour was used and provided the necessary amount of lipids to the diet.

2.2. Methods

Proximate composition was carried out according to Instituto Adolfo Lutz (1985) and to AOAC (1990): dessication at 105°C, for moisture; calcination at 550°C, for ash; microkjeldhal for protein (N×6.25); defatting in Soxhlet apparatus with hexane for total lipids in chickpea and Folch method for residual lipids in bovine lungs (Bligh & Dyer, 1959). Carbohydrate was estimated by difference from 100.

Amino acids analysis was carried out on an auto analyser (Beckman, Mod 6300) by ion-exchange chromatography (Spackman, Stain & Moore, 1958). The samples were previously defatted and hydrolysed in HCl; methionine and cysteine were determined as methionine sulphone and cysteic acid, respectively, after quantitative oxidation with performic acid (Moore, 1963).

The samples were wet-ashed with concentrated nitric acid (HNO_3) and the solution was analysed for iron using plasma spectrometry (Angelucci & Mantovani, 1986; Baird ICP 2000).

Extrusion of samples was carried out in a laboratory single-screw extruder (Miotto Ltda — São Bernardo do Campo, São Paulo, Brazil), with L/D ratio 20:1, 20 mm barrel diameter. The conditions for extrusion were fixed at: 200 rpm screw rotation, 3.55:1 screw compression ratio, 5 mm die diameter, 30 rpm feed screw speed (70 g min^{-1}), 130°C central section temperature, 13% feed moisture. These conditions were reported to produce the highest acceptability for the production of pure chickpea snacks (Batistuti et al., 1991).

Expansion ratio was calculated as the ratio of extruded product diameter to the diameter of the die hole, both measured by a caliper rule. Values reported were averages of 30 measurements.

The force necessary to completely shear the extrudates was determined using an Instron apparatus (Mod. 1000 — Instron Corp.-USA) with a Warner Bratzler device. The crosshead speed was 100 mm min^{-1}, pressure transducer maximum capacity of 5 kg (50 N) and values reported were averages of 30 determinations.

Half of the snacks were flavoured with: 4% of bacon flavor; 22% hydrogenated vegetable fat; 0.9% of salt added to a final salt concentration of 2.5%.

The results were subjected to one-way analysis of variance ($P < 0.05$) using the Microcal Origin (version 6.0, Northampton, MA).

3. Results and discussion

The proximate compositions of the chickpea, bovine lung and extruded products are presented in Table 1. The composition of these materials would vary with production conditions, but they were in accordance with previously published reports (Arêas & Lawrie, 1984; Batistuti et al., 1991; Campos & Arêas, 1993; Romero-Baranzini, Yáñez-Farías & Barrón-Hoyos , 1995; Williams & Singh, 1987).

The protein content of chickpea is more than double that of wheat and is similar to that of fish, meat and eggs (Bhatty, Gilani & Nagra, 2000). Bovine lung is

Table 1
Proximate composition[a] (g/100 g) and iron content[a] (mg/100 g) of blends of chickpea and bovine lung before and after extrusion (dry basis)

	Moisture[e]	Protein	Ash	Lipids	Total carbohydrate[b]	Total iron[c] (mg/100 g)
Chickpea						
Non extruded	12.5±0.16	24.4±1.01	3.83±0.09	0.85± 0.08	71.0	5.10±0.02
Extruded	6.92±0.03	19.8±0.21	3.45±0.07	0.39± 0.04	76.4	5.19±0.07
Blends 95:5						
Non extruded	7.17±0.10	25.9±2.17	3.48±0.03	1.03±0.09	69.6	7.85±0.56
Extruded	7.20±0.37	23.0±0.60	3.54±0.08	0.39±0.06	73.1	7.18±0.16
Blends 90:10						
Non extruded	5.74±0.27	31.1±0.96	3.50±0.40	1.24±0.47	64.1	10.3±0.06
Extruded	6.67±0.05	26.6±0.55	3.63±0.02	0.32±0.05	69.5	9.18±0.23
Bovine lung						
Non extruded	12.17±0.26	94.1±1.04	5.80±0.08	1.07±0.06[d]	–	nd

[a] Average of three determinations±standard deviations.
[b] Obtained by difference.
[c] Determined by plasma spectrometry; nd, not determined.
[d] Bovine lung defatted by Folch method (Bligh & Dyer, 1959) has 4.05±0.04 of residual fat.
[e] Figures given are in wet basis.

higher in protein and ash content than chickpea. These compositional differences were reflected in the extruded blends of bovine lung and chickpea. Adding bovine lung to the mixture caused an increase in protein and ash and a reduction in carbohydrate contents of the extruded products.

The analysis of the iron in the above foodstuffs is also presented in Table 1. Total iron content was significantly higher ($P<0.05$) in extruded products that contain bovine lung when compared to pure chickpea extrudates. Assessment of the influence of the extrusion processing on iron bioavailability of bovine lung has been studied previously and the results showed an increment on total iron content and no influence on bioavailability of iron, irrespective of temperature and feed moisture conditions studied (Pinto, Colli & Arêas, 1997). In the present work, iron content was similar prior to and after extrusion, except for the blend containing 10% of bovine lung that had a lower iron content after extrusion ($P<0.05$).

The amount of iron observed in the extruded products, in the present work, allows the estimate that the daily consumption of a single 30 g package of snacks, produced by a 90:10 chickpea/lung mixture accounts for nearly 30% of the necessary iron for children between 7 and 10 years (OMS, 1998).

Essential amino acid analyses of chickpea, bovine lung and their extruded products are presented in Table 2 and were comparable to previous literature reports (Avancini et al., 1992; Campos & Arêas, 1993; Dhawan, Malhotra, Dahiya & Singh, 1991; Eggum, Hansen & Larsen, 1989; Khan, Akhtar, Ullah & Jaffery, 1995; Modgil & Mehta, 1993). Amino acid contents of the blends were also determined and values were close to the estimates for their composition. The sulphur amino acid contents of the chickpea differed considerably among different cultivars and within the same cultivars, depending on the region planted, ranging from 2.3 to 3.1 g/100 g of protein (Williams & Singh, 1987). Although chickpea proteins were found to be deficient in sulphur amino acids by some researchers (Avancini et al., 1992, Dhawan et al., 1991), this work showed sulphur amino acid contents (3.36 g/100 g protein) higher than provisional protein requirements (OMS, 1998) for children between 2 and 5 years but close to the range described in the literature. The careful quantitative oxidation of these residues by performic acid, carried out in the present work, allowed a more precise determination of these essential amino acids. The lysine content observed was higher than corn grits or wheat flour (Dodok et al., 1993) showing the superior nutritional quality of the snacks produced in the present work when compared to commercial brands made of corn or wheat.

Previous results have shown that foodstuffs with low moisture required higher energy inputs for extrusion and the high friction produced in the extruder resulted in a high degree of molecular fragmentation and irreversible association. These chemical transformations could promote loss in nutritional quality of protein (Bjork & Asp, 1983; Campos & Arêas, 1993; Cheftel, 1986). However, this seemed not to be the case in the present work where the drastic conditions adopted, to obtain the best snack, only slightly decreased the content of essential amino acids of the extruded products. Snacks from extruded blends containing 5 and 10% of bovine lung had a higher content of essential amino acids than pure chickpea and FAO/WHO provisional protein requirement (OMS, 1998) for children between 2 and 5 years.

The NPR values of proteins on all tested diets are presented in Table 3 and the results observed for weight

Table 2
Essential amino acid composition (g/100 g of protein) in raw materials and extruded products

Amino acid	Chickpea	Bovine lung	Blend 95:5[a]	Blend 90:10[a]	Extruded Chickpea[b]	Extruded bovine lung[c]	Extruded Blend 95:5[b]	Extruded Blend 90:10[b]	FAO/WHO (2–5 years)[d]
Threonine	3.81	4.21	3.87	3.93	3.59	3.89	3.22	3.36	3.40
Valine	4.96	6.40	5.20	5.39	4.68	4.96	4.84	5.07	3.50
Cystine	1.24	1.09	1.23	1.23	1.28	0.52	1.25	1.18	–
Methionine	2.12	2.38	2.16	2.20	2.31	1.60	2.58	2.40	–
Total Sulphur a.a.	3.36	3.47	3.39	3.43	3.59	2.11	3.83	3.58	2.50
Isoleucine	4.59	3.55	4.41	4.27	4.26	2.75	4.10	3.92	2.80
Leucine	7.93	8.69	8.06	8.15	8.10	7.85	8.32	8.34	6.60
Tyrosine	1.99	2.50	2.07	2.14	2.15	2.99	2.05	2.11	–
Phenylalanine	6.03	4.69	5.80	5.62	5.90	4.30	5.73	5.43	–
Total Aromatic a.a.	8.02	7.19	7.88	7.76	8.05	7.30	7.78	7.54	6.30
Histidine	2.77	2.75	2.77	2.76	3.19	nd	3.26	3.52	1.40
Lysine	7.07	6.94	7.05	7.03	6.63	5.81	6.90	6.70	5.80
Tryptophan	nd[e]	nd	nd	nd	nd	2.00	nd	nd	1.10

[a] Estimated from total amino acid content of chickpea and bovine lung.
[b] Extrusion conditions: 13% moisture, 130°C.
[c] From Campos and Arêas, 1993 (extrusion conditions: 18% moisture, 130°C).
[d] Provisional FAO/WHO/UNU protein (OMS, 1998).
[e] nd, not determined.

gain are shown in Fig. 1. The animals fed on diets having a dietary protein source from cooked chickpea, extruded chickpea, extruded blends of chickpea with 5 and 10% of bovine lung and casein, presented a linear weight gain, and those fed on a non-protein diet showed a loss of weight. No significant differences on weight gain among groups were observed, except for the extruded chickpea and the blend containing 10% of bovine lung (Student *t*-test) ($P<0.05$). Significant differences were observed between cooked chickpea and the other groups.

The NPR showed a significant difference between casein and experimental diets. No significant differences were observed between diets containing any of the extruded products. Cooked chickpea had a NPR similar to the extruded blend with 5% of bovine lung.

The effect of extrusion conditions on nutritional quality of extruded products is highly dependent on the moisture content and processing temperature. Protein solubility and nutritional value, generally reflect the degree of heat treatment received by the product (Gujska & Khan, 1990). The high energy inputs required for extruding at low moisture level can produce irreversible chemical modification of amino acids, resulting in changes in their composition, bioavailability or both, that affect nutritional quality (Campos & Arêas, 1993; Cheftel, 1986; Phillips, 1989). Racemization of some amino acids, in the drastic conditions of extrusion (Hayase, Kato & Fujimaki, 1975; Phillips, 1989), could also partially explain lower values for NPR in experimental diets when the content of amino acids is high in the extruded products, compared to FAO/WHO provisional protein (OMS, 1998).

Previous reports (Singh, 1985) showed that nutritive value of mixtures of chickpea and cereals (wheat, triticale and maize) were lower than those of the flours alone because of the lower protein quality of these cereals. The mixtures of the present work, have a nutritional quality comparable to casein, as lung protein also has a high nutritive value. These results indicate the high biological value of the tested proteins, suggesting that snacks containing chickpea and bovine lung could be incorporated into infant food as a high quality protein product with acceptable texture.

Digestibility was determined through protein content of faeces related to actual protein ingested. The protein content was corrected by the endogenous protein excreted by the rats in the aproteic diet. The results obtained are presented in Table 3 and showed similar digestibility (around 76%) for all samples containing chickpea alone or admixed with lung. Casein was totally digested, explaining why all nutritional indices calculated from the bioassays were higher for casein.

Table 3
NPR and digestibility of extrudates[a]

Groups	NPR[b]	NPRrel[c]	Digestibility[b]
Cooked chickpea	4.24±0.15b	90.6	76.5±11.7a
Extruded chickpea	3.87±0.26a	82.7	75.6±11.8a
Extruded blend 95:5	4.13±0.35a, b	88.3	76.9±10.3a
Extruded blend 90:10	3.90±0.32a	83.3	75.4±7.50a
Casein	4.68±0.19c	100	107±8.40b

[a] Average of six determinations±standard deviations.
[b] Different letters in the same column indicate significantly different values ($P<0.05$; Student test).
[c] Relative to casein.

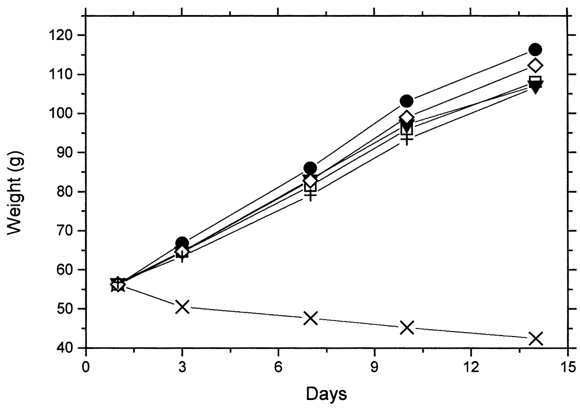

Fig.1. Weight gain of experimental animal groups fed on several diets. — × — non-protein; — ● — cooked chickpea; — □ — blend 95:5; — ◆ — blend 90:10; — ◇ — casein; — + — extruded chickpea.

The importance of texture and flavour to acceptability of food has long been acknowledged (Christensen, 1984; Suknark, Mcwatters & Phillips, 1998). The results for mechanical evaluation showed that shear strength increased and expansion ratio decreased with increase of bovine lung (Figs. 2 and 3).

The variance analysis for expansion performed on the data of Figs. 2 and 3, indicates the existence of significant linear effects of bovine lung addition ($P<0.0001$) and not significant flavor inclusion effect ($P>0.06$), showing that, as expected, the flavour process did not affect the expansion ratio. On the other hand, the addition of bovine lung reduced the expansion ratio of the snacks. This behaviour can been explained by the increase in the protein content of the feed (Faubion & Hoseney, 1982a, b; Gjuska & Khan, 1990)

Force necessary to completely shear the extruded samples demonstrated that the interaction between flavour and bovine lung was significant ($P<0.001$). Flavoured snacks showed more homogeneous results for shear strength (Fig. 2) where a linear effect of bovine lung addition can also be observed. Snacks without flavour presented some differences (test Tukey; $P<0.05$; Neter, Wasserman & Kutner, 1990): when these were prepared without bovine lung, the force necessary to completely shear was higher than the one with 5% of lung (Fig. 3), and did not show the expected linear behaviour.

Previous studies reported a decrease in expanded volumes and shear strengths of extruded products with high contents of starch, as protein content increased. This was especially observed when animal tissues were used, reducing the ability of the extrusion process to produce highly expanded snacks (Faubion & Hoseney, 1982a, b; Gjuska & Khan, 1990). Expansion ratio and shear strength, in our work, showed a tendency to this behaviour, although not significantly for non-flavoured snacks (Figs. 2 and 3).

The present results showed the high potential of snacks produced by chickpea and bovine lung mixtures

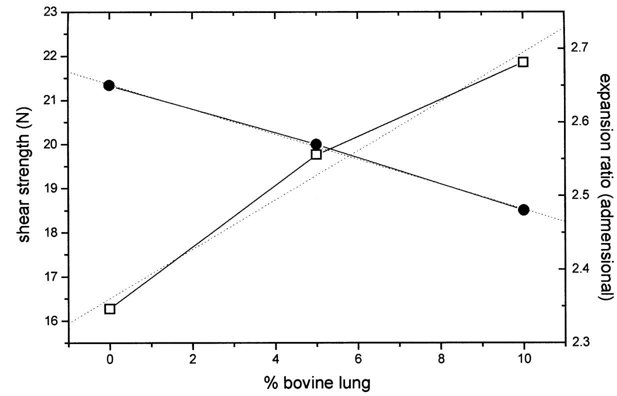

Fig. 2. Linear regression for expansion ratio and shear strength for flavoured extruded snacks of chickpea and bovine lung. Results are plotted against % of lung in the feed. — ● — expansion ratio; — □ — shear strength; - - - fitting for linear regression.

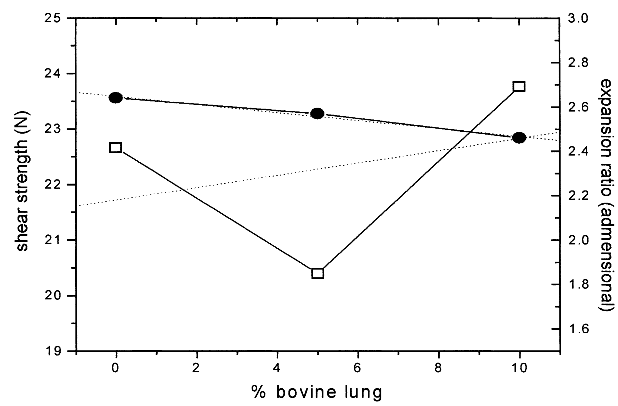

Fig. 3. Linear regression for expansion ratio and shear strength for non-flavoured extruded snacks of chickpea and bovine lung. Results are plotted against % of lung in the feed. — ● — expansion ratio; — □ — shear strength; - - - fitting for linear regression.

as nutritional supplements for infants. Some initial trials were performed and the snacks with bovine lung showed high acceptability, measured on a hedonic scale. More detailed tests were conducted and the results will be part of full report on sensory acceptability of these products. Furthermore, the higher contents of protein present (up to 24% in 90:10 chickpea/lung blends), together with the high iron content expected for these snacks when compared to commercial ones, make them attractive for nutritional programmes counteracting anaemia and malnutrition.

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