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Extruded flaxseed meal enhances the nutritional quality of cereal-based products

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Abstract

Human consumption of flaxseed is increasing due to its health benefit properties and extrusion processes can enhance its nutritional quality. Extruded flaxseed meal (EFM) obtained in a pilot plant was characterized and incorporated in flour mixes and cereal-based bars to demonstrate its nutritious usefulness. Amino acid content was not affected by extrusion and, despite lysine was the limitating amino acid, the chemical score (CS) was 83%. Thiamin and riboflavin decreased slightly as consequence of extrusion, phytic acid did not change and trypsin inhibitor activity was undetectable. Proximate composition and nutritional quality determined by biological and chemical indexes were compared among EFM, flour mixes (FM) and cereal bars (CB). They presented high protein levels (26%, 20% and 17%, respectively), good biological value (BV) (80, 79 and 65, respectively), acceptable true protein digestibility (TD) (73, 79 and 78, respectively), and high dietary fiber (33, 20.5 and 18%, respectively). The ratio of ω6:ω3 for CB was within the WHO/FAO recommendations. These results open a new venue for the usefulsess of nutritious/healthy extruded flaxseed flours into ready-to-eat cereal-based products with improved nutritional quality.

Keywords

Flaxseed, extrusion, cereal bars, nutritional value.

Abbreviations

BV	Biological Value
CB	Cereal bars
CS	Chemical Score
EFM	Extruded flaxseed meal
FM	Flour mixes
NPR	net protein ratio
NPU	Net protein utilization
PDCAAS	Protein digestibility corrected amino acid score
IP5	Inositol penta-phosphate
IP6	Phytic acid or inositol hexa-phosphate
RNPR	Relative net protein ratio
TD	True digestibility
TIA	Trypsin inhibitor activity

Introduction

Flaxseed (*Linum usitatissimum*), also named linseed, is an important oilseed crop that is traditionally incorporated as feed component to improve the body performance and health parameters of animals [1]. Flaxseed meal has been found to be of outstanding interest for human consumption as a rich source of α-linolenic acid, lignan content, phenolic compounds and also biactive peptides; the potential health benefits of these components have a direct link to many chronic diseases such as cardiovascular disorders, obesity and hormone-dependent cancers [2-4]. The consumption of different byproducts of flaxseed meal (grain, milled or oil), has been associated with the levels of α-linolenic acid in blood, oxidative prevention and regulation of glucose metabolism [5-6]. Therefore, the demand for human food and livestock markets is increasing due to the unique properties of this ancient crop.

Flaxseed meal is also a source of high-quality protein with favourable ratio of essential amino acids to human nutrition, being Lys the limiting amino acid making flaxseed suitable to complement cereal and legume proteins [2]. Therefore, flaxseed products are nutritive and healthy ingredients that can be incorporated into traditional cereal based matrices as bread and pasta [7], and in ready-to-eat snack foods characterized for their high consumer acceptability [8].

Extrusion cooking is an economical on-going food processing technology widely used for the production of textured foods. The advantages against other heating systems include versatility, high production capacity, low operating costs and shorter cooking times. Extrusion cooking not only improves the nutrients digestibility but also their bioavailability by the inactivation of heat labile non-nutritive factors [9], and it can induce positive or negative effects on the bioactive compounds depending on the extrusion process conditions [10]. Therefore, the inclusion of extruded products in enriched foods is of increasing concern. The fortification of puffed, a corn meal-based product, with extruded flaxseed meal has been marketed as a ready-to-eat functional breakfast with potential health benefits [11]; however, the nutritional quality of foods enriched with extruded flaxseed meal products has not been yet established.

Argentina has been one of the major flaxseed producers in the past, but nowadays the cultivation represents only the 3% of the world production. A preliminary study performed in our laboratory with extruded flaxseed fortified products showed good organoleptic properties. The extrusion of flaxseed could be an excellent opportunity to encourage its production and, once established its nutritional quality, guarantee its incorporation in derived food products. Therefore, the present study was aimed to characterize the composition of extruded flaxseed meal compared with raw flaxseed and to evaluate the nutritional quality of two fortified cereal-based food products by biological and chemical indexes.

Materials and Methods

Raw seeds. Brown flaxseed (*Linum usitatissimum* var. *panambi*) cultivated in Gualeguay, province of Entre Rios (Argentina) was provided by Cereal and Oilseeds Centre from National Institute of Industrial Technology (INTI, Argentina).

Extruded flaxseed meal (EFM). Whole flaxseeds were extruded at 95-100ºC according to Frias et al. [9]. Then, extruded flaxseeds were submitted to fat extraction using a screw press, milled and dried in a thermopneumatic transport system at 150 ºC for 1-2 seconds and, consequently, a final meal product with a moisture content of ~7% was obtained.

Flour mixes (FM). It was obtained by mixing whole wheat flour (50%), partially defatted soymeal (21%), EFM (9%), wheat bran (15%) and wheat germ (5%). All the ingredients, except EFM, were previously submitted to a heating process at 110-130ºC for 5 min.

Cereal bars (CB). Mixture of dry ingredients (64% of extruded pea, 27% of FM, 5.4% of dehydrated whole milk and 3.6% of sunflour seeds) was combined with agglutinant (54.5% of honey, 36.5% of whisk egg white and 9% of soybean oil), in a proportion of 0.56:0.44 (w/v) and dried at 105ºC for 30 min.

Chemical analysis

Nitrogen content, determined by AOAC 984.13 [12], was multiplied by 6.25 to obtain protein content. Moisture content, ash content, fat content and dietary fibre were determined according to AOAC [13]. Percentage of carbohydrates was estimated by difference [100 – (% proteins + % fat + % water + % fibre + % ash] and energy value was calculated by the Atwater general factors system [14]. Fatty acids were quantified by GC-flame ionization [15]. Amino acids, thiamine, riboflavin, phytic acid and TIA were also determined [9].

Protein quality evaluation by biological assays. Protein quality assessment was established by biological value (BV), net protein utilization (NPU) and true protein digestibility (TD), net protein ratio (NPR) and relative net protein ratio (RNPR) [9]. For these items, three experimental diets were studied: Extruded flaxseed meal (EFM), flour mixes (FM) and cereal bars (CB). Other two diets were simultaneously used as controls: one free protein diet and one protein reference diet constituted with casein supplemented with 2% methionine. With the exception of the free protein diet, all the diets were adjusted to 10% protein and the provided diets were the only source of protein. The influence of different diets on metabolic utilization of nitrogen was studied in 4 weeks old (recently weaned) Wistar albino rats with an initial body weight of 55±5g fed for 10 days. A total of 60 rats were divided into 5 groups of 12 animals for each diet. Diet composition, food intake, body weight, change in body weight, nitrogen intake, and faecal nitrogen excretion were determined in all rats and the experimental conditions were those described previously [9]. Throughout the experimental period, all animals had free access to water and diets were consumed *ad libitum*.

Protein quality evaluation by chemical indexes. The comparison of essential amino acid content in each sample versus its content in the recommended protein reference for 3-10 year old children was calculated [9, 16]. The amino acid with the lowest percentage is called limiting amino acid and this percentage is considered the chemical score (CS). The protein digestibility corrected amino acid score (PDCAAS) was calculated multiplying CS by TD [9, 17].

Statistical analysis

Extrusion experiments were performed in duplicated. Flour mixes and cereal bars were elaborated several times in order to carry out analytical analysis and biological studies. Analytical data were expressed as the mean ± standard deviation of two independent determinations of each replicate. Data were subjected to multifactor analysis of variance (ANOVA) using the least-squared difference test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, MD, USA). Biological data were expressed as the mean ± SD of 12 independent determinations.

Results and Discussion

In order to study the influence of extrusion on flaxseed, the content of some nutritive compounds such as amino acids and vitamins in raw seed and extruded products were compared, as well as some non-nutritive factors including phytic acid and trypsin inhibitor activity (TIA) (Table 1). Among non-essential amino acid Glu was present in the largest amount in raw flaxseed, followed by Asp and Arg, then Gly and Ser and, finally, Ala and Pro, and no significant differences ($P \leq 0.05$) with extruded flaxseeds were found. Regarding essential amino acids, Leu was the predominant one, followed by Val, Thr, Lys, Ile, and then Met+Cys, His, Tyr. Trp was the amino acid present in the lowest amount and extrusion did not cause significant ($P \leq 0.05$) differences. Lys was present in noticeable amount (3.6-3.8 g/100g protein) and values are in the range found in the literature for the flaxseed flours [2]. Results confirm the stability of amino acids to extrusion conditions providing proteic products of relevant nutritional value.

Extruded flaxseed meal provides thiamin and riboflavin vitamins, although in a 49 and 32% less amount than the untreated flaxseed (Table 1), as recently highlighted in different extruded cereals [18]. Extrusion cooking did not significantly (P≤0.05) modify the content of phytic acid (IP6) and pentainositol phosphate (IP5) (Table 1), values within the range reported for other flaxseed varieties [19]. TIA was not detectable in raw and extruded flaxseed meal suggesting the suitability of incorporating flaxseed meal into different derived foods without protein availability detriment.

Table 2 shows the proteic assessment of raw and extruded flaxseed meal by chemical indexes. The percentage of amino acids provided by raw and extruded flaxseeds vs. requirements of children between 3 and 10 years old [16] was quite high for most of the EAA, with exception of Lys that showed a CS of 80% for raw flaxseed vs. 83% after extrusion. In this sense, this product could be combined reasonably well to complement the protein quality of cereal and legume mixtures. Therefore, characterization of extruded flaxseed meal was carried out.

Moisture content reached levels of 6.5%, as stated during its preparation. Protein content was rather high (26.4%), as well as fat content (25%), dietary fiber (33%), whilst the content of carbohydrates was rather low (6%) and energy value of 353 Kcal/100g (Table 3).

The fatty acid content and composition of EFM product is collected in Table 4. Saturated fatty acids were present in rather lower amount (11.7%) than monounsaturated (23.5%), and the predominant fraction was represented by polyunsaturated fatty acids (64.4%). This profile indicates that the composition of fatty acids reported in raw flaxseed is almost kept after processing (EFM) with levels of linolenic acid ranging 50% of total fatty acids, that makes EFM an attractive ingredient for fortify cereal-combined flours.

Animal studies carried out with EFM diet allowed the evaluation of the protein quality and the results are presented in Table 5. EFM presented a good protein quality: Biological value (BV) was reasonably fair (80) whilst true digestibility (TD) was rather low (73) possibly due to the high dietary fibre. TD was used to calculate the protein digestibility corrected amino acid score (PDCAAS), and a value of 61% was obtained (Table 2). This value matched well to that obtained for net protein utilization (NPU) in the animal studies (58%, Table 5), exhibiting similar results between those obtained by biological methods vs. chemical methods corrected by the TD. These results show that extrusion process carried out in the described conditions conduct to the protein denaturalization, improving its bioavailability and, hence, its notorious nutritional quality.

EFM is presented here as a proteic feasible option to follow WHO guidelines [16] and it can be used to supplement the nutritional quality of other plant food products. With this aim, two cereal-based products supplemented with EFM were elaborated: mixture of cereal flours to facilitate its usefulness in small bakery industries (FM: flour mixes) and a ready-to-eat snack product (CB: cereal bars).

FM presented a high content of proteins (20.3%) whilst fat content was rather low (6%) (Table 3), and within values recommended for bakery products. Carbohydrates represented a 43%, mainly due to the presence of pea and cereal flours in the mixture. Dietary fiber content was also high (20.5%) and energy value of only 3 Kcal/g. These levels of dietary fiber could make FM qualified as a highlighted dietary fiber contributor, since food with levels above 6% can be claimed "high fibre food", according to Argentinean legislation [20]. Regarding the ω6:ω3 ratio, the proportion 18:2 increased compared to 18:3 mainly due to the linoleic acid provided by wheat germ (Table 4). In spite of this, 18:3 predominated in flour mixes showing an unbalanced ω6:ω3 ratio (1.5:1), which it is far from the WHO/FAO recommendations (5:1 - 10:1) [21]. Regarding the protein quality, biological value was maintained (79 for FM vs. 80 for EFM) (Table 5), which indicates complementarity of essential amino acids. Similarly, true digestibility in the FM also increased (from 73 in EFM to 79 in FM) possibly due to the lower dietary fiber content.

Flour mixes (FM) was incorporated to cereal bars based on extruded peas in order to obtain an enriched ready-to-eat snack product of acceptable protein quality. Consumers have the perception that cereal bars are convenient and healthy food for breakfast, aimed at children population and, even, as a portable food to consume at convenient time. In addition, its consumption has been quadriplicated worldwide during last decade. Recent studies carried out in Argentina have shown scare ingredient diversity in their formulations, low protein content presence of trans fatty acids due to the inclusion of hydrogenated vegetable oils and high sugar content due to the presence of corn syrup as agglutinant . Our results show that protein content of cereal bars was rather high (17.6%; Table 3) compared with current commercial products present in the market analyzed in our laboratory (5-6%); fat content reached values aimed in the formulation (9%); carbohydrates were at a level of 52.6%; they can still be considered “high fiber foods” (11.3%) making them very attractive by its low calorie content (3.6 Kcal/g). Cereal bars reached a ratio ω6:ω3 of 6.8:1, value within the WHO/FAO recommendations [21]. However, since these unsaturated fatty acids are easily oxidized, this aspect will limit the shelf life of these products; otherwise antioxidant should be added [15].

Conclusions

The cereal bars enriched with extruded flaxseed meal provide an acceptable nutritional quality, with enhanced quality and quantity of proteins, dietary fiber and ω6:ω3 ratio. Particularly, it is of a great interest to formulate this type of products aimed at short-age children, due to the international concern on the poor nutritional quality of snacks and related products highly consumed by this population group. This goal is fully applicable to cereal bars as novel ready-to-eat food in which the WHO/FAO guidelines are aimed at contributing to a “healthy diet”.

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Table 1. Non-essential amino acids, essential amino acids, thiamin, riboflavin and inositol phosphate content of raw and extruded flaxseed meal.

	Raw flaxseed	Extruded flaxseed meal
Non essential amino acids (g/100g protein)		
Asp	9.92 ± 0.08[a]	10.48 ± 0.02[a]
Glu	19.46 ± 0.19[a]	19.19 ± 0.11[a]
Ser	5.00 ± 0.09[a]	5.47 ± 0.05[a]
Gly	5.90 ± 0.07[a]	6.18 ± 0.03[a]
Arg	9.42 ± 0.20[a]	9.39 ± 0.10[a]
Ala	3.77 ± 0.09[a]	4.08 ± 0.01[a]
Pro	3.73 ± 0.04[a]	3.87 ± 0.07[a]
Essential amino acids (g/100g protein)		
His	2.43 ± 0.09[a]	2.50 ± 0.05[a]
Val	4.84 ± 0.10[a]	4.80 ± 0.02[a]
Met	1.66 ± 0.08[a]	1.52 ± 0.01[a]
Cys	0.95 ± 0.04[a]	1.12 ± 0.04[a]
Ile	3.85 ± 0.12[a]	3.98 ± 0.06[a]
Leu	5.72 ± 0.10[a]	6.08 ± 0.13[a]
Phe	4.75 ± 0.04[a]	4.88 ± 0.09[a]
Tyr	2.34 ± 0.04[a]	2.63 ± 0.11[a]
Lys	3.83 ± 0.06[a]	3.66 ± 0.09[a]
Thr	4.14 ± 0.05[a]	4.35 ± 0.04[a]
Trp	1.50 ± 0.03[a]	1.55 ± 0.02[a]
Vitamins (μg/100 g d.w.)		
Thiamin	97.23±5.23[a]	49.51±2.64[b]
Riboflavin	75.83±5.24[a]	51.91±2.80[b]
Inositol phosphates (g/100 g d.w.)		
IP_6	0.55±0.02[a]	0.55±0.02[a]
IP_5	0.05±0.01[a]	0.05±0.01[a]

IP_6, inositol hexa-phosphate; IP_5, inositol penta-phosphate.

Different superscripts in the same row indicate significant difference (P≤0.05).

Table 2. Protein evaluation of raw and extruded flaxseed meal by chemical indexes.

Essential Amino acids	Requirements children 3-10 years old*	% amino acid raw flaxseed/ requirements	% amino acid extruded flaxseed meal / requirements
His	1.6	152	154
Val	4.0	121	120
Met+Cys	2.4	109	133
Ile	3.1	124	139
Leu	6.1	94	103
Phe+Tyr	4.1	173	199
Lys	4.8	80	83
Thr	2.5	166	186
Trp	0.7	227	3.42
Chemical score		80	83
Limiting amino acid		Lys	Lys
PDCAAS**		-	61

*FAO (2007) g/100g protein
**PDCAAS = Protein Digestibility Corrected Amino Acid Score.

Tabla 3. Proximate composition and energy of extruded flaxseed meal, flour mixes and cereal bars

	Extruded flaxseed meal	Flour mixes	Cereal bars
Moisture	6.49±0.00	7.77±0.04	7.12±0.33
Ash (g/100 g dw)	3.23±0.00	3.26±0.06	2.19±0.01
Proteins (g/100g dw)	26.35±0.05	20.25±0.05	17.65±0.05
Fat (g/100 g dw)	24.56±0.15	5.70±0.20	9.00±0.20
Total Dietary Fibre (g/100 g dw)	32.95±1.05	20.50±0.80	11.35±0.15
Carbohydrates (g/ 100 g dw)	6.18	42.5	52.6
Energy (Kcal/100 g dw)	353	302	362

Table 4. Fatty acids of extruded flaxseed meal, flour mixes and cereal bars

Fatty Acids (g/100g fat)	Extruded Flaxseed Meal	Flour Mixes	Cereal Bars
C 14:0 (myristic)	0.1	0.1	0.1
C 16:0 (palmitic)	6.7	10.3	9.7
C 16:1 (palmitoleic)	0.2	0.1	0.1
C 18:0 (stearic)	4.8	3.9	4.0
C 18:1 (oleic)	22.3	20.8	21.3
C 18:1 (cis-octadecenoic)	0.9	1.2	1.3
C 18:2 (linoleic)	14.3	37.6	54.4
C 18:3 t (trans-octadecatrienoic)	0.2	0.1	-
C 18:3 (linolenic)	49.9	24.9	8.0
C 20:0 (araquidic)	0.2	0.2	0.3
C 20:1 (eicosenoic)	0.1	0.3	0.2
C 22:0 (behenic)	0.2	0.2	0.4
C 24:0 (lignoceric)	0.1	0.2	0.1
∑ saturated	11.7	14.5	14.1
∑ monounsaturated	23.5	22.4	22.9
∑ polyunsaturated	64.4	62.6	62.4
ω6:ω3	0.3:1	1.5:1	6.8:1

Table 5. Evaluation *in vivo* of protein of extruded flaxseed meal, flour mixes and cereal bars

	Casein	Extruded flaxseed meal	Flour mixes	Cereal bars
NPU	83.6±5.4^{a}	58.4±6.4^{bc}	61.9±6.4^{b}	50.9±6.4^{c}
TD	95.6	73	79	78
BV	87.9±5.8^{a}	80.0±8.7^{a}	79.4±5.9^{a}	65.2±8.3^{b}
NPR	5.50±0.25^{a}	3.22±0.27^{b}	3.80±0.31^{c}	3.35±0.37^{bc}
RNPR	100.0±4.6^{a}	58.6±4.8^{b}	69.1±5.6^{c}	60.9±6.8^{bc}

Different superscripts in the same row indicate significant difference ($P \leq 0.05$).
NPU: Net protein utilization; TD: True digestibility; BV: Biological value;
NPR: Net protein ratio; RNPR: Relative net protein ratio.