Chemical characterization of pan bread added of flaxseeds (*Linum usitatissimum*)

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RESUMO

A linhaça tem atraído a atenção dos consumidores devido ao seu alto teor nutricional. Nesta pesquisa, diferentes concentrações de linhaça marrom foram adicionadas à massa de pão para avaliar o perfil de ácidos graxos e composição química dos produtos. As concentrações ensaiadas foram de 0%, 3%, 6% e 9%. Os pães foram preparados em uma fábrica de panificação experimental e as análises foram realizadas no laboratório do Departamento de Agroindústria, Alimentos e Nutrição da Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo. Foram avaliados perfil de ácidos graxos, composição centesimal, valor calórico, minerais, fitatos, taninos, compostos fenólicos totais e digestibilidade in vitro de proteínas das 4 formulações. A quantidade de Ômega 3 aumentou significativamente em pães de acordo com a adição de linhaça. A presença natural de antioxidantes em toda a linhaça serviu para controlar a oxidação lipídica. Os minerais potássio, cálcio, enxofre, sódio, ferro, manganês e zinco, não apresentaram diferença estatística entre as formulações dos quatro pães. O processamento térmico foi eficiente para reduzir fitatos. O teor total de compostos fenólicos foi maior do que os taninos e fitatos. O processamento térmico melhorou a digestibilidade da proteína in vitro de pães. Portanto, conclui-se que a adição de sementes de linhaça em pães de forma, com o objetivo de melhorar a qualidade nutricional é viável.

Palavras-chave: Pão de forma, linhaça, Ômega 3, Ômega 6, fatores antinutricionais.

ABSTRACT

Flaxseed has attracted attention from consumers because of its high nutritional content. In this research, different concentrations of brown flaxseed were added to the pan bread dough to evaluate the fatty acids profile and chemical composition of products. The concentrations tested were 0%, 3%, 6% and 9%. The breads were prepared in an experimental baking plant and analyses were conducted in the laboratory of the Department of Agroindustry, Food and Nutrition of the Luiz de Queiroz Agriculture School - University of Sao Paulo, Brazil. Fatty acids profile, centesimal composition, caloric value, minerals, phytates, tannins, total phenolic compounds and in vitro protein digestibility of four products were evaluated. The amount of Omega 3 increased significantly in breads according to the addition of flaxseeds. The natural presence of antioxidants in the whole flaxseed served to control the lipids oxidation. Minerals such as potassium, calcium, sulphur, sodium, iron, manganese and zinc showed no statistical difference between the four bread formulations. The heat processing was efficient to reduce phytates. The total content of phenolic compounds was higher than tannins and phytates. The heat processing improved the in vitro protein digestibility of breads. Therefore, it could be concluded that the addition of flaxseeds in pan breads with the aim of improving the nutritional quality is feasible.

Keywords: Pan bread, Flaxseed, Omega 3, Omega 6, anti-nutritional factors

RESUMEN

La linaza ha atraído la atención de los consumidores debido a su alto contenido nutricional. En esta investigación, se añadieron diferentes concentraciones de linaza marrón a la masa de pan sartén para evaluar el perfil de ácidos grasos y la composición química de los productos. Las concentraciones ensayadas fueron de 0%, 3%, 6% y 9%. Los panes se prepararon en una planta experimental para hornear y los análisis se realizaron en el laboratorio del Departamento de Agroindustria, Alimentos y Nutrición de la Escuela de Agricultura de Queiroz Luiz - Universidad de Sao Paulo, Brasil. Se evaluaron los ácidos grasos perfil, composición centesimal, valor calorífico, minerales, fitatos, taninos, compuestos fenólicos totales y en la digestibilidad proteica in vitro de 4 productos. La cantidad de Omega 3 aumentó significativamente en los panes de acuerdo a la adición de semillas de lino. La presencia natural de antioxidantes en todo el linaza sirve para controlar la oxidación de lípidos. Minerales potasio, calcio, azufre, sodio, hierro, manganeso y zinc no mostraron diferencia estadística entre las 4 formulaciones de pan. El tratamiento térmico fue eficiente para reducir los fitatos. El contenido total de compuestos fenólicos fue mayor que los taninos y los fitatos. El tratamiento térmico mejora la digestibilidad de la proteína in vitro de panes. Por lo tanto, se puede concluir que la adición de semillas de lino en panes de molde con el objetivo de mejorar la calidad nutricional es factible.

Palavras Clave: Pan pan, linaza, Omega 3, Omega 6, factores antinutricionales.

1 INTRODUCTION

Due to the wide dissemination by the media, in general of the relationship between diet and health, the concern of the society with food has grown exponentially. A large quantity of new products that allegedly improve health has been daily presented by the food industry. Among the bioactive substances studied, fatty acids stand out, which is a group composed by polyunsaturated fatty acids, mainly Omega 3 and 6 found in fresh-water fish (salmon), vegetable oils, flaxseed, nuts and some types of plants. They are related to the prevention of cardiovascular diseases by reducing triglycerides and the blood cholesterol levels, increasing blood flow and reducing blood pressure. The linolenic acid is also involved in the hemoglobin formation, which transports oxygen throughout the blood. Flaxseeds can be used in the production of breads, cakes, biscuits and morning cereals. Its fibers collaborate in the release of toxins and fats as well as in the constipation treatment (MANDARINO; ROESSING; BENASSI, 2005).

White dough breads such as pan bread, for example, can be consumed, as long as in correct amounts, by virtually all people, because they complements the intake of carbohydrates, lipids and proteins that the body needs. Moreover, they are rich in sodium, calcium, phosphorus and potassium. The only restriction applies to celiac people, who have intolerance to bread made with wheat flour, rye and oat. Bread is an essential source of carbohydrates and is therefore an energy supplier of rapid metabolism. Bread is a universal food of daily use, part of breakfast, snacks or accompanying main meals (INMETRO, 2005).

Phytates are chemical compounds used by plants to store mineral phosphorus inside their cells. They are considered anti-nutritional factors, because they reduce the bioavailability of divalent minerals such as calcium, iron, magnesium, manganese, copper and zinc, mainly. However, from the decade of 1990 on, phytates have also been considered as potent antioxidant agents (preventing oxidation or aging of cells), thus playing important role in reducing the risk of many chronic and degenerative diseases such as some types of cancer and arthritis (EMBRAPA, 2004).

Tannins belong to the large group of polyphenols that have antioxidant function acting in the chelation of minerals such as copper and iron, making these elements unavailable for the Fenton reaction. Tannins interact with hydroxyl radical more than with metals in the formation of chelates, therefore, tannins have nutritional importance due to their antioxidant action. They can also give electron for highly reactive free radicals reducing the radical reactivity due to the displacement of the unpaired electron in the phenolic ring (CARBONARO; VIRGILI; CARNOVALE, 1996).

Due to the high nutritional content of flaxseeds, they were added at different concentrations as ingredient in the preparation of pan bread. The objective of this research was to assess the profile of fatty acids present in bread and analyze the chemical composition of products obtained taking into account the anti-nutritional factors.

2 LITERATURE REVIEW

The flaxseed seed (*Linum usitatissimum L.*) is a food item originating from linen that belongs to Linaceae family. Its name in Latin, linum usitatissimum, means “very useful” (MACIEL, 2006).

Linen is an herbaceous that varies from 40 to 80 centimeters of erect stems, with elongated and narrow leaves, its flowers are light-blue and its fruit is a spherical capsule with 10 seeds (HERBARIO, 2007).

The plant has a main stem from which several branches come and in these leaves sprout, the flowers and the spherical capsules containing two seeds in each one of the five compartments (COSKUNER, 2005).

Flaxseed is an oilseed, rich in protein, lipids and dietetic fibers (ALMEIDA, 2009). It has three components that present important pharmacological actions such as a-linolenic acid, soluble fibers and lignana, which are being assessed in clinical researches and studies related to breast cancer, prostate and colon, diabetes, lupus, bones loss, liver diseases, kidneys and cardiovascular, with favorable results regarding positive effects of the seed (CARRARA et al., 2009).

Phenolic acids are some of the substances part of the phenolic contents group. They are characterized for having a benzene ring, a carboxylic group and one or more hydroxyl and/or metoxyl groups in the molecule, providing antioxidant properties to the food and to the body, being, for that, indicated for cancer treatment and prevention, cardiovascular diseases and other diseases (FERGUSON ; HARRIS, 1999).

According to Portal Verde (2004) phenolic acids are phytochemicals abundant in flaxseed, and highlights: trans-ferrulico, trans-sinapic, trans-p-cumaric and trans-caffeic. The total of phenolic acids range from 7.9 mg/g to 10.3 mg/g in eight flaxseed varieties cultivated in Canada.

Flaxseed is rich in the essential fatty acid Omega-3, named alpha-linolenic acid (ALA) (HUSSAIN, 2006).

The alpha-linolenic fatty acid (Omega-3) is present in flaxseed approximately in 60%, which makes of this oilseed the major vegetal source of this essential fatty acid and its predominance is important in the prevention of heart diseases (MARTONI, 2004).

The fatty acid Omega-3 have several biological effects that make them useful in prevention and treatment of chronical diseases such as diabetes type II, liver diseases, rheumatoid arthritis, high blood pressure, coronary diseases, embolism and certain types of cancer (CONNOR, 2000).

Studies have shown that ingestion of 10 g of flaxseed per day causes hormone changes contributing to the decrease of cancer and diabetes risk, levels of total cholesterol and LDL, and favoring the decrease of antiplatelet aggregation (MACIEL, 2006).

The alpha-linolenic acid (ALA) may resist to cooking temperatures. One study performed with ground flaxseed addition to a bread flour mixture, the content ALA has kept practically the same after the cooking. It should be highlighted that, in one of the cases, the cooking time last for two hours with a temperature of 178ºC. ALA also remain stable during processing and cooking of spaguetti based on ground flaxseed (MANTHEY, 2002).

According to Novello and Pollonio (2011) flaxseed contains two substances that might interfere in the absorption of some nutrients, although in amounts that seem to be of little importance in human diet. The linatine inhibits the use of pyridoxine (B6 vitamin). Phytic acid forms insoluble complex with calcium, copper, iron, magnesium and zinc in intestine, reducing its absorption. There are no evidences that that may occur in any significant way in human beings.

3 MATERIALS AND METHODS

The breads were made in an experimental baking plant of the "Mario Dedini" SENAI school located at the city of Piracicaba, state of Sao Paulo, Brazil. The chemical, physical and sensorial analyses were conducted at the Bromatology Laboratory and at the Biochemistry and Experimental Analysis Laboratory from the Department of Agroindustry, Food and Nutrition of the *Luiz de Queiroz* Agriculture School (USP).

3.1 Preparation of breads

The ingredients used in the preparation of the pan bread are shown in Figure 1.

Ingredient	%	Weight g
Wheat flour	100	3000
Salt	2	60
Sugar	6	180
Enhancer	1	30
Butter	4	120
Biological Leaven	4	120
Milk Powder	3	90
Water	±60	1800

Fig. 1: Ingredients used in the formulation of control pan bread

The ingredients were separated and weighed in digital scale Filizola, model Pluris. Then, the dry ingredients were homogenized during two minutes into the spiral mixer Superfecta brand, model AE 15E. Soon after, the rest of the ingredients and 80% of the water were added. The remaining 20% of the water were used to determine the dough texture. Total dough homogenization was performed during four minutes. All these steps have been taken with the mixer in the 1^{st} speed. With equipment in the 2^{nd} speed, the dough was homogenized to the development of the gluten network until reaching the "elasticity point". Shortly after, the dough was removed from the mixer, divided into four equal parts and added of flaxseeds at concentrations 0%, 3%, 6% and 9% and each part, individually, was again homogenized in the mixer. Then, the doughs were brought to the table and covered with plastic for 15 minutes for fermentation. The doughs were placed in bread molds (10.5 cm of width x 10 cm of height x 20 cm of length) and taken to climatic baking chamber (85% of relative humidity and temperature from 36° to 39° C) for final fermentation for 60 minutes. Finally, the molds were brought to oven label Supremax, model Multi-213 at 180 º C for 30 minutes for baking.

3.2 Fatty acids determination

For the fatty acids determination, lipids were extracted from breads through method which consists of extracting oil in cold. Then, the esterefication of fatty acids in oil was conducted according to methodology proposed by Hartman and Lago (1973). After esterefication, the samples were sealed with parafilm and stored in freezer at - 20°C up to the time of reading, which was held in the gas chromatograph label Shimadzu model GC-14 B, equipped with a flame ionization detector, split-type injector, merged silica capillary column (50 m of length x 0.22 mm of internal diameter, Shimadzu-

Hicap, Australia). The chromatographic conditions were: column temperature of 180°C (isothermal); carrier gas, hydrogen at a flow rate of 1.05 mL / minute; detector and injector temperature of 250°C.

3.3 Centesimal Composition

The moisture content, crude protein, ether extract and ash analyses were carried out in accordance with procedures specified by AOAC (1995). The dietary fiber content was determined in accordance with methodology proposed by Asp, Johansson and Hallmer (1983). Carbohydrates were obtained through difference. The caloric value per 100 grams of each product was calculated through equation (1):

Caloric value = (grams of protein x 4) + (g of lipids × 9) + (g of carbohydrate x 4) (1)

3.4 Minerals determination

Mineral elements were determined in accordance with methodology proposed by Sarruge and Haag (1974). The reading of the samples absorbance was done in atomic absorption spectrometer, at wavelength of 248.3 nanometers.

3.5 Phytic acid determination

The total phytic acid content in samples was determined through method described by Grynspan and Cheryan (1989). The absorbance reading was held in spectrophotometer Beckman model DU 640 at wavelength of 500 nanometers. The phytic acid content was derived from the construction of the standard curve, with results were expressed in mg of phytic acid/g of sample.

3.6 Tannins determination

Tannins were analyzed according to methodology described by Price, Hagerman and Butler (1980). The reading was held on spectrophotometer Beckman model DU 640 at wavelength of 500 nanometers. The tannins concentration was obtained from a standard catechin curve, and the results were expressed in mg/g.

3.7 Total phenolic compounds determination

The determination of the total phenolic compounds concentration was carried out according to methodology described by Swain and Hillis (1959), through extraction with methanol, addition of the Folin-Denis reagent, saturation with sodium carbonate and subsequent absorbance reading at wavelength of 660 nanometers.

3.8 In vitro protein digestibility

To determine the protein digestibility, the method described by Akeson and Stahmann (1964) was used. The calculation of the *in vitro* digestibility percentage was conducted through equation (2).

$$\textit{In vitro}\ \text{digestibility} = [A-(B-C / A)] \times 100 \qquad (2)$$

where:

A = Amount of protein measured through Kjeldahl (%)
B = Average obtained from the digestibility (%)
C = White (%)

3.9 Statistical analysis

The statistical design was fully random. The Statistical Analysis System software was used. Analysis of variance was performed through the F test and the comparison of averages of different bread formulations was analyzed through the Tukey test ($p \leq 0.05$). (SAS, 1998).

4 RESULTS AND DISCUSSION

4.1 Fatty acids profile

The average percentages of the fatty acids profiles areas of pan breads added of 3%, 6% and 9% of flaxseeds and bread without the addition of flaxseeds (see Table 1).

Table 1: Average percentages of fatty acids found in fresh base bread with different flaxseed concentrations

Flaxseed Concentration (%)	Lauric Acid (12:0)	Myristic Acid (14:0)	Palmitic Acid (16:0)	Palmitoleic Acid (16:1)
0	1,12±0,29^{1a2}	2,69±0,67^{a}	22,18±3,42^{a}	0,85±0,04^{a}
3	0,54±0,08^{c}	1,70±0,26^{c}	18,44±1,54^{c}	0,65±0,11^{d}
6	0,73±0,38^{b}	2,07±0,79^{b}	19,24±2,89^{b}	0,71±0,03^{c}
9	0,48±0,17^{c}	1,62±0,64^{d}	16,35±2,42^{d}	0,79±0,13^{b}

Flaxseed Concentration (%)	Stearic Acid (18:0)	Oleic Acid (18:1)	Linoleic Acid (18:2)	Linolenic Acid (18:3)
0	6,22±0,53^{a}	22,07±2,34^{a}	22,54±1,46^{a}	1,84 ±0,32^{d}
3	6,17±0,29^{b}	18,16±1,78^{d}	21,96±0,38^{b}	7,86 ±0,49^{c}
6	5,63±0,56^{d}	21,37±1,24^{b}	20,27±0,72^{c}	13,61±1,42^{b}
9	5,66±0,21^{c}	20,03±2,34^{c}	20,06±0,26^{d}	16,57±1,51^{a}

[1] Average ± standard deviation
[2] Different letters in vertical indicate significant differences between treatments at significance level of 5%

According to data presented in Table 1, all treatments were different from each other in relation to the fatty acids profile. The control sample showed higher levels for all fatty acids assessed, except for linolenic acid. In relation to the linoleic acid (Omega 6), the control sample showed the greatest amount of linoleic acid but also showed the smallest amount of linolenic acid (Omega 3). The association between linoleic acid (Omega 6) and linolenic acid (Omega 3) was approximately 12:1 for the sample control; 3:1 for sample containing 3% of flaxseed; 1.5:1 for sample with 6 % of flaxseed and 1.2:1 for sample with 9% of flaxseed. In relation to the linolenic acid, its concentration increased significantly with the addition of flaxseeds in bread.

The World Health Organization (WHO) recommends ratios of 5:1 - 10:1 between linoleic acid (Omega 6) and linolenic acid (Omega 3) respectively in the diet (WHO, 1995), and the optimum balance is 5:1 between Omega 6 and Omega 3, respectively. It was observed that the increase on the flaxseed concentration in bread provided better balance in the Omega 6: Omega 3 ratio, and in the sample with the addition of 9% of flaxseed, this ratio was close to 1:1.

Among the fatty acids assessed, palmitic, oleic and linoleic acids presented the greatest results in the four bread samples, around 19.05%, 24.03% and 21.20% respectively. This composition contributes for the fragile stability of products and for the formation of undesirable compounds.

Gomez (2003) studied the stability of products added of flaxseeds for a period of 46 days using sample with zero days of storage as control. The sample that showed higher reduction of linoleic acid followed by an increase in the contents of palmitic, stearic and oleic acids was raw flaxseed meal stored at room temperature. On the other hand, cooked sample stored at a refrigeration temperature showed the smallest decrease in the content of that fatty acid.

According to the Brazilian Table of Food Composition (UNICAMP, 2006), the composition of fatty acids in 100 grams of traditional pan bread is: Tr for myristic acid, 0.39 g for palmitic acid, 0.01 g for palmitoleic acid; 0.08 g for stearic acid; 0.56 g for oleic acid; 0.69 g for linoleic acid and 0.04 g for linolenic acid. The composition of fatty acids established for pan bread with different flaxseed concentrations is according the literature.

4.2 Centesimal composition

The centesimal composition results found for pan breads with and without the addition of flaxseeds (see Table 2).

Table 2: Average centesimal composition of fresh base breads with different flaxseed concentrations

Flaxseed concentration (%)	Total moisture (%)	Ashes (%)	Protein (%)	Lipids (%)	Insoluble fiber (%)	Soluble fiber (%)	Carbohydrates (%)
0	18.64 ± 0.05^{1a2}	2.29 ± 0.04^{c}	9.65 ± 0.21^{bc}	3.34 ± 0.20^{c}	0.49 ± 0.08^{d}	0.40 ± 0.14^{d}	65.17^{b}
3	18.18 ± 0.28^{a}	2.34 ± 0.04^{cb}	9.42 ± 0.42^{c}	2.71 ± 0.45^{c}	1.15 ± 0.07^{c}	0.66 ± 0.11^{a}	65.52^{a}
6	15.93 ± 0.22^{b}	2.41 ± 0.01^{ab}	10.28 ± 0.30^{ab}	4.36 ± 0.23^{b}	1.32 ± 0.26^{b}	0.62 ± 0.23^{b}	65.08^{c}
9	14.61 ± 0.14^{c}	2.45 ± 0.01^{a}	10.60 ± 0.17^{a}	5.44 ± 0.38^{a}	2.30 ± 0.03^{a}	0.54 ± 0.08^{c}	64.05^{d}

[1] Average ± standard deviation
[2] Different letters in vertical indicate significant differences between treatments at significance level of 5%

Data in Table 2 show that the control sample and that containing 3% of flaxseed had the highest total moisture values. For ash, protein, lipids and insoluble fibers, the highest values were found for sample containing 9% of flaxseed. For soluble fiber and carbohydrates, the highest values were found for sample containing 3% of flaxseed.

The Brazilian Table of Food Composition (UNICAMP, 2006) establishes the following values for each 100 grams of traditional pan bread: 40.7% of moisture; 253 kcal, 12 grams of protein, 2.7 grams of lipids, 44.1 grams of carbohydrates, 2.5 grams of dietary fiber and 0.5 grams of ashes. These results are close to those found in the control sample, except for moisture and carbohydrates. It must take into consideration that the ingredients and the preparation process of breads are different, thus influencing directly the results.

In relation to labelling, the Ordinance No 27 of January 13th 1998 - Technical Regulation concerning the Supplementary Nutritional Information (BRAZIL, 1998) in respect to the content of dietary fiber, recommends that the product ready for consumption could be considered "SOURCE OF DIETARY FIBER" if it contains at least 3 grams of fibers/100g of product and could be considered "HIGH LEVELS OF DIETARY FIBER" if it contains at least 6 of fibers/100g. Pan bread added of 9% of flaxseed contains 2.84 grams of fiber/100g of product ready for consumption. Despite being very close to recommendations, it cannot be considered "SOURCE OF DIETARY FIBER". In a dry basis, bread with 9% of flaxseed presented 3.19 grams of fiber/100 g.

4.3 Caloric Value

The average caloric value of each product was determined per 100g of product and also per serving (see Table 3).

Table 3: Average caloric value per 100 grams of product in fresh basis, with different flaxseed concentrations

Flaxseed concentration (%)	Caloric value per 100g of product	Caloric value per serving/usual size
0	329.43[1c]	82.35[c] /1 slice of 25 grams
3	324.15[d]	81.03[d] /1 slice of 25 grams
6	340.68[b]	85.17[b] /1 slice of 25 grams
9	347.65[a]	86.91[a]/1 slice of 25 grams

[1]Different letters in vertical indicate significant differences between treatments at significance level of 5%

According to results presented in Table 3, it could be observed that sample containing 9% of flaxseed presented the highest caloric value per 100g of product per serving. The increase in caloric value of breads is proportional to the addition of flaxseeds, which is rich in the lipid fraction. These results for the caloric value per serving of product are close to the caloric value of breads available in market. According to the nutritional information displayed on the label of products, the traditional pan bread label Nutrella presents 71.42 Kcal per serving of 25 grams, while bread enriched with grains label Wickbold presents 66.5 kcal per slice of 25 grams (from grains added to the product, 3% are flaxseeds); bread Omega Vitta from Nutrella presents 72.5 kcal per slice of 25 g and contains in its formulation 1% of flaxseed flour and 1% of flaxseeds and marine oil.

4.4 Minerals

The average content of mineral assessed in the 4 bread samples is presented in Table 4.

Table 4: Average content of minerals (mg / g) assessed in samples of fresh base breads with different flaxseed concentrations

	0%	*3%*	*6%*	*9%*
Phosphorus	1.44±0.02[1b2]	1.53±0.04[b]	1.76±0.04[a]	1.82±0.04[a]
Potassium	2.49±0.25[a]	2.70±0.33[a]	2.85±0.34[a]	3.17±0.26[a]
Calcium	1.00±0.06[a]	0.88±0.02[a]	1.05±0.13[a]	0.98±0.09[a]
Magnesium	0.34±0.00[c]	0.43±0.00[bc]	0.47±0.05[b]	0.57±0.05[a]
Sulphur	0.88±0.00[ab]	0.87±0.03[b]	0.96±0.04[a]	0.93±0.02[ab]
Sodium	8.60±0.38[a]	8.26±0.12[a]	8.25±0.13[a]	8.89±0.74[a]
Iron	0.04±0.00[a]	0.09±0.03[a]	0.11±0.12[a]	0.05±0.01[a]
Manganese	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
Zinc	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]

[1] Average ± standard deviation

[2] Different letters in horizontal indicate significant differences between treatments at significance level of 5%.

The results presented in Table 4 show that samples containing 6% and 9% of flaxseed showed the highest phosphorus levels. In relation to magnesium, sample with 9% of flaxseed showed the highest level of this mineral. Sample containing 6% of flaxseed showed the highest sulphur level. Potassium, calcium, sodium, iron, manganese and zinc showed no statistical difference between the four samples. According to the Brazilian Table of Food Composition (UNICAMP, 2006) the composition of minerals in 100 grams of pan bread is: 156 mg calcium, 24 mg of magnesium, 0.51 mg of manganese, 105 mg of phosphorus, 5.7 mg of iron, 22 mg sodium, 65 mg of potassium, 0.06 mg of copper and, 1.3 mg of zinc. These data are similar to those found in this study (Table 2), except for sodium, potassium and manganese, which showed levels much higher than those presented in the Brazilian Table of Food Composition.

4.5 Anti-nutritional agents

Phytates, tannins and total phenolic compounds levels found in breads with flaxseeds and in bread without flaxseeds (see Table 5).

Table 5: Average content of phytates, tannins and total phenolic compounds determined in fresh base bread with different flaxseed concentrations

Flaxseed concentration (%)	Phytates mg/g	Tannins mg/g	Total phenolic compounds mg/g
0	1.85±0.04[1][d2]	0.957±0.00[c]	18.86±0.07[d]
3	2.50±0.11[c]	0.954±0.00[d]	22.53±0.2[c]
6	2.91±0.08[b]	0.984±0.00[b]	24.55±0.33[b]
9	3.78±0.24[a]	1.01±0.00[a]	27.20±0.16[a]

[1] Average ± standard deviation
[2] Different letters in vertical indicate significant differences between treatments at significance level of 5%

According to results presented in Table 5, the highest phytate level was found in sample containing 9% of flaxseed and the lowest level in the sample control. It seems that the level of phytate present in samples increased according to the addition of flaxseeds.

The phytic acid is present in many seeds as the main phosphate source, cereals and oily plants contain from 1 to 3% of phytic acid. Recently, Paik et al. (2004) created a food database containing phytates, and the group of cereals presented from 1.91 to 9.73 mg/g. The phytate content found in this study is within the range found by Paik et al. (2004). Therefore, it is observed that many foods of vegetal origin contain high phytate levels and in consequence may decrease the bioavailability of essential minerals. An alternative to reduce the phytic acid content would be the heat treatment or to add hexogen phytases or activated endogen phytases. The hydration of cereal grains, usually used before processing, can enable native phytases that degrade phytate into smaller size products. Phytate is also described in literature as an antioxidant with beneficial role in the lipid oxidation inhibition, in chronic processes such as heart disease and cancer and other purposes in the fields of pharmacy, biomedicine, chemistry and dentistry.

In relation to tannins, samples with 6% and 9% of flaxseeds were those that showed the highest contents of this element. Among the three compounds searched, tannins presented the lowest levels.

Tannins form complex with proteins, making them insoluble and inactivating enzymes. Moreover, they bind to other macromolecules such as starch, causing a reduction in the nutritional value of foods. Other harmful effects to diet are attributed to tannins such as undesirable color to food, damage to the intestinal mucosa and interference in the absorption of iron, glucose and vitamin B_{12} (GUZMÁN-MALDONADO; ACOSTA-GALEGO; PAREDES-LÓPEZ, 2000). However, other studies (GEHM et al., 1997) indicate that polyphenols (mainly flavonoids) from dark purple-red grapes have the ability to prevent the risk of cardiovascular diseases. Moreover, tannins seem to be important in some sectors of the food industry, as in the beer production, which contributes for the product stabilization through the reduction of its protein concentration. For the total phenolic compounds, sample with 9% and flaxseed showed the highest level and control sample showed the lowest level. It was observed that the total phenolic compounds level present in samples increased according to the addition of flaxseeds. Among the three compounds studied, the phenolic have shown the highest levels.

Velioglus et al. (1998) determined the total phenolic compounds in flaxseeds and found 5.09 mg / g of this substance. The values found in this study are in disagreement with literature, being higher than those.

Phenolic compounds are commonly found in edible and non-edible plants and have multiple biological effects, including antioxidant activity. In oilseeds, phenolic compounds occur as hydroxylated derivatives of benzoic and cinnamic acids, coumarins, flavonoids and lignans (OOMAH; KENASCHUK; MAZZA, 1995).

Flaxseed is being studied for its beneficial effects on health and it is considered a nutraceutical agent for being a natural source of phytochemicals. The demonstration of the clinical activity associated with the consumption of flaxseed has attracted interest in the study of this seed. Phenolic compounds, including flavonoids, tannic acid and ellagic acid are found in plants and have high antioxidant activity in several biological systems. Moreover, phenolic compounds of plants may withhold or delay the lipid oxidation onset, influencing not only the decomposition of hydroperoxides in food, but also in animal tissues (WETTASINGHE; SHAHIDI, 1999).

4.6 *In vitro* protein digestibility

The determination of the *in vitro* protein digestibility is one of the factors to be taken into consideration in the quality of protein. The results of the *in vitro* protein digestibility of bread samples with 3%, 6% and 9% of flaxseed and without addition of flaxseed (see Table 6).

Table 6: Average *in vitro* protein digestibility of fresh base breads with different flaxseed concentrations

Flaxseed concentration (%)	Protein digestibility (%)
0	73.46±0.59[1c2]
3	77.65±0.23[b]
6	77.28±0.31[b]
9	80.08±0.82[a]

[1] Average ± standard deviation
[2] Different letters in vertical indicate significant differences between treatments at significance level of 5%

It could be noted that the sample containing 9% of flaxseed presented better protein digestibility and the control sample presented the smallest digestibility percentage, samples containing 3% and 6% of flaxseed did not differ among themselves.

Berno et al. (2007) evaluated the protein digestibility of breads enriched with different whey protein concentrations (0, 5, 10 and 15%) and found the lowest digestibility for the standard sample and the samples characterized by the addition of whey protein in their formulation presented digestibility rates of more than 93%. This behavior was also observed (Table 6), namely, the standard sample presented lower digestibility rate and samples added of seed had increased digestibility percentage, although not exceeding 80% found in sample with 9% of flaxseed.

Carvalho et al. (2002) found that the heat treatment promoted an improvement on the protein digestibility due to the opening of the protein structure through its denaturation. However, heat treatment should be applied with discretion, because excessive heat treatment can cause decrease in the protein digestibility due to the formation of cross-links.

5. CONCLUSIONS

The concentration of linolenic acid (Omega 3) increased significantly in breads added of flaxseeds. The bread with 9% of flaxseed presented Omega 6: Omega 3 ratio around 1:1.

Among the fatty acids assessed, palmitic, oleic and linoleic acids were the main representative in the four bread samples that most contributed for the fragile stability of products and the formation of unwanted compounds; however, the presence of natural antioxidants in flaxseeds controlled the lipid oxidation.

None of the samples could be considered as "source of dietary fiber", since they did not contain 3g/100g of food ready for consumption.

Samples containing 6% and 9% of flaxseed showed the highest phosphorus levels and sample with 9% of flaxseed showed the highest magnesium level. Minerals potassium, calcium, sulphur, sodium, iron, manganese and zinc showed no statistical difference between the four bread formulations.

The phytate contents found in samples increased according to the addition of flaxseeds. Flaxseeds presented lower phytate levels after the baking process, indicating that the heat processing was efficient.

Samples with 6% and 9% of flaxseeds showed the highest tannin levels. But among the three compounds searched, tannins presented the lowest levels.

The total phenolic compounds content present in samples increased according to the addition of flaxseeds. Among the three compounds searched, the phenolic compounds showed the highest levels.

The heat processing improved the *in vitro* protein digestibility percentage, and sample with 9% of flaxseed presented the highest percentage.

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