ABSOLUTE QUANTIFICATION OF FATTY ACIDS IN CHIA SEEDS PRODUCED IN BRAZIL

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Chia is a good source of fibre, protein and antioxidants, and is also rich in polyunsaturated fatty acids, particularly α-linolenic acid (18:3 n-3). This fact makes it increasingly interesting for studies of its composition and nutritional quality, because it may be useful for inclusion in diets or in novel food formulations. The objective of this study was to determine the chemical and fatty acid composition and the indices of nutritional quality of the lipid fraction of chia seed produced in Brazil. In respect to chemical composition, an average grade of 8.14% moisture, 20.11% protein, 27.72% lipid and 3.94% ash was detected. Sixteen fatty acids were quantified in the chia, highlighting the prevalence of polyunsaturated fatty acids, mainly 18:3 n-3. All indices of nutritional quality of lipid fraction assessed (Hypocholesterolaemic/ hypercholesterolaemic FA ratio, atherogenic index and thrombogenic index) indicated that chia has a high lipid fraction quality and its inclusion in diet or as an ingredient in food products can be an ally in the reduction of cardiovascular problems.

KEY WORDS: FATTY ACID; ATHEROGENIC INDEX; THROMBOGENIC INDEX; HYPOCHOLESTEROLAEMIC/ HYPERCHOLESTEROLAEMIC FA RATIO.

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1 INTRODUCTION

Chia (*Salvia hispanica* L.) is an annual herbaceous plant, native to the mountainous areas of western and central Mexico (Bueno et al., 2010). These seeds were used as food by the pre-Columbian civilizations of Central America and the indigenous peoples of the southwest of the continent for hundreds of years. According to Ayerza (1995) the use of chia as a food source for the Mayan people was a sign of energy, vitality and support. The intake of these seeds is observed in countries such as Mexico, Colombia and Guatemala (Ayersa and Coates, 2009).

Chia is considered a significant source of lipids, carbohydrates, proteins, minerals and fibre (Bushway et al*.,* 1981). Tosco (2004) reported that chia seeds have a high content of antioxidants and contain more omega-3 than flaxseed. The presence of α-linolenic acid (18:3 n-3, ALA) highlights the use of chia as a functional food and for its use in the formulation of products such as cereal bars, yoghurts and breads. Rupflin (2011) developed a functional cereal bar based on amaranth, honey, chia and cashew nuts, and obtained a highly nutritious product.

According to Uribe et al. (2011) the lipid fraction of chia consists of a high content of polyunsaturated fatty acids: α-linolenic acid (18:3 n-3, ALA) and linoleic acid (18:2 n-6, LA). Omega-3 fatty acids (FA) have positive effects on health and an adequate intake of them, together with n-6 FAs, may reduce the risk of many diseases such as hypertension, diabetes, arthritis, and other inflammatory disorders and cancers (Simopoulos, 2011).

The polyunsaturated fatty acids (PUFA) participate in lipoprotein metabolism and eicosanoid synthesis which makes them of particular interest in relation to the prevention and treatment of various cardiovascular diseases. The administration of PUFA rich oils or their concentrates in humans has shown beneficial effects on immune function by inhibiting the proliferation of lymphocytes and antibody production of pro-inflammatory cytokines. Other positive effects resulting from the intake of PUFA have been observed in certain inflammatory and allergic processes such as atopic eczema, psoriasis, asthma and rheumatoid arthritis. Positive effects have also been observed for premenstrual syndrome, diabetes, and certain types of cancer (Jadhav et al*.*, 1996; Jakobsen et al*.*, 2008; Gogus & Smith, 2010).

The aims of this study were to determine the chemical composition of chia, to quantify its fatty acid content by gas chromatography and to determine the nutritional quality of its lipid fraction.

2 MATERIAL AND METHODS

2.1 SAMPLING

Chia samples were obtained from three different producers in the state of Rio Grande do Sul, Brazil, between the months of January to June, 2011. The seeds were ground, packed into polyethylene bags and stored at -18°C until further analyses could be performed. The analyses were carried out in quadruplicate and the results were expressed as the mean, and standard deviation.

2.2 DETERMINATION OF CHEMICAL COMPOSITION

Moisture, protein (N x 6.25) and ash content were determined according to Cunnif (1998) procedures. Carbohydrate content was determined as the remaining percent weight according to the formula: [100 – (moisture + ash + crude fat + crude protein)]. Total lipids were extracted according to the method of Bligh & Dyer (1959) using chloroform, methanol and water in the ratio (2:2:1) as solvent. The lipid content was obtained by gravimetric analysis.

2.3 FATTY ACID (FA) COMPOSITION

The FA composition consisted of the conversion of lipids into FA methyl esters (FAME), using the methylation method described in Hartman and Lago (1973), modified by Maia and Rodriguez-Amaya (1993). The FAME were separated via gas chromatography (Finnigan, model 9001, USA), which had a flame ionization detector and a fused silica capillary column (SGE BPX70; 60 m x 0.25 mm x 0.25 mm in 100% bonded cyanopropyl). The rate of flow of the gases used was 1.2 mL min^{-1} of H_2 as the carrier gas, 30 mL min^{-1} for N_2 as the auxiliary gas and 35 and 350 mL min^{-1}, respectively, for H_2 and synthetic air to the flame. The injector and detector temperature was 250 °C and 255 °C, respectively. The column was programmed at 185 °C for 10 min, followed by a ramp of 4 °C min^{-1} to 240°C for 1 min. The total time for each test was 24 min and 45 s. Injections of 1 µL for each solution containing the FAME were carried out in triplicate.

The areas corresponding to the peaks of the chromatograms were obtained by integration with software Clarity Lite (DataApex, 2.7.03.498, Czech Republic). FAME were identified by comparison of retention times of the sample constituents with Sigma FAME standards and by spiking.

The fatty acids in mg g^{-1} total lipids were quantified in relation to the internal standard, methyl tricosanoate (23:0), purchased from Sigma, and calculated according to the method of Joseph and Ackman (1992), using Equation 1:

$$C\ (mg\ g^{-1}) = (A_x \,.\, M_{23:0\,.}\, T_{RF}) / (A_{23:0} \,.\, M_A \,.\, F_{CT}) \qquad (1)$$

where:
C: concentration of fatty acid X;
A_X: area of FAMEs;
$A_{23:0}$: internal standard area;
$M_{23:0}$: internal standard mass added to the sample (mg);
M_A: sample mass (g);
TRF: theoretical response factor of FAMEs;
FCT: conversion factor to express the results in mg of fatty acids g^{-1} of total lipids (TL).
FA levels in the samples were analysed by ANOVA and the Tukey test ($p<0.05$).

2.4 INDICES OF NUTRITIONAL QUALITY OF LIPID FRACTION

A better approach for the nutritional evaluation of fat is the utilization of indices based on the functional effects of FA, e.g., indices of nutritional quality of lipids. These indices are known as the hypocholesterolaemic/ hypercholesterolaemic FA ratio (HH), computed according to the knowledge of the effects of individual FA on cholesterol metabolism (Dietschy, 1998); the atherogenic (AI) and thrombogenic indices (TI), indicate that C12:0, C14:0 and C16:0 are atherogenic and that C14:0, C16:0 and C18:0 are thrombogenic (Ulbricht & Southgate, 1991).

The nutritional quality of the lipid fraction from chia seeds was evaluated from the FA composition data. Equations 2, 3 and 4 were used to determine the AI, TI and HH ratios.

$$AI = [(C12:0+(4xC14:0)+C16:0)]/ (\Sigma MUFA+\Sigma n\text{-}6+\Sigma n\text{-}3) \qquad (2)$$

$$TI = (C14:0+C16:0+C18:0)/ [(0.5x\Sigma MUFA)+(0.5x\Sigma n\text{-}6+(3x\Sigma n\text{-}3)+(\Sigma n\text{-}3/\Sigma n\text{-}6)] \qquad (3)$$

$$HH = (C18:1cis\text{-}9+C18:2n\text{-}6+C20:4n\text{-}6+C18:3n\text{-}3+C20:5n\text{-}3+C22:5n\text{-}3+C22:6n\text{-}3)/ (C14:0+16:0) \qquad (4)$$

These indices were analysed by ANOVA and the Tukey test ($p<0.05$).

3 RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION

Table 1 shows the averages of results obtained from the chemical composition of the chia seed samples.

The moisture content found (14.8%) was higher than that cited by Ixtaina et al*.* (2008) and Segura-Campos et al*.* (2013), which was 7.00% and 6.32%, respectively. However, this value is higher than indicated for preservation and storage of grain, which ranged between 11.00 and 13.00% (Bala, 1997). It is known that the higher the total lipid content of the grain, the lower the moisture content should be to prevent damage to seeds in the storage step.

TABLE 1 – CHEMICAL COMPOSITION OF THE CHIA SEED SAMPLES.

Analysed parameters	**Composition (g 100 g^{-1})**
Moisture	8.14 ± 0.76
Ash	3.94 ± 0.45
Crude protein	20.11 ± 1.16
Total fat	27.72 ± 2.86
Carbohydrates	40.39 ± 1.60

Values are the mean of four replicates **± standard deviation**

Segura-Campos et al*.* (2013), found an ash content of 4.32% in chia, whereas in this study a content of 3.94% was observed. This content is related to functional and nutritional properties in food, and is dependent on climatic conditions and soils where the produce was grown, as well as the method of determination.

The seeds studied showed a 20.11% crude protein content, which was similar to the range of values found by Weber et al*.* (1991) and Porras-Loaiza et al*.* (2014). This value is close to the crude protein content of golden flaxseed (23.06%) and brown flaxseed (21.94%) (Tarpila et al*.,* 2005) but higher than that found in amaranth (14.80%) and cereals such as oat (15.30%), wheat and maize (14.00%), barley (9.20%) and rice (8.50%). Chia comprises all essential amino acids, and is considered as being an excellent source of bioactive compounds (Segura-Campos et al*.,* 2013).

Total lipids were extracted by the cold method, which is considered fast, accurate, precise, simple, low-cost, and also reduces exposure of the fatty acids to the oxidation process which is accelerated by heating (Pérez-Palacios et al*.,* 2008). The content of total lipids was 27.72%, which is consistent with data found in the literature (25–38%) (Ayerza, 1995). Porras-Loaiza et al. (2014) also found a fat content ranging from 21.5–32.7% in chia seed grown in Mexico. The yield of total lipids is directly related to the method used, as demonstrated in the work by Ixtaina et al. (2008) where the percentages found for the seeds studied ranged from 26.70 to 33.60% and from 20.30 to 24.80%, in the extraction with n-hexane solvent and pressure, respectively. The use of only non-polar solvent, such as n-hexane, can lead to greater extraction of total lipids (Tanamati et al., 2005).

The carbohydrate content (40.39%) was higher than reported by Martinez et al. (2012) (33.40%), but very close to the content of 41.80% reported by Tosco (1994).

According to Regulation (EC) No 258/97 of the European Parliament and of the Council (2013), the composition of chia seeds should be comprised of 4.00 to 9.00% moisture, 20.00 to 22.00% crude protein, 30.00 to 35.00% lipid and 4.00 to 6.00% ash.

In general, the results obtained about the chemical composition of the chia seeds studied are consistent with the literature. Small differences can be explained due to genetic diversity, climatic conditions, soil type and management during cultivation of the samples.

3.2 FATTY ACID (FA) COMPOSITION

Sixteen fatty acids were quantified in the lipid fraction of chia seeds, as shown in Table 2.

The content of saturated fatty acids (SFA) was 11.12%, and the main acids are palmitic (16:0) and stearic (18:0), with 68.04 mg g^{-1} and 27.39 mg g^{-1} total lipids, respectively.

The amount of monounsaturated fatty acids (MUFA) was 7.40%, highlighting oleic acid (18:1 n-9), with a concentration of 56.16 mg g^{-1}.

The polyunsaturated fatty acids (PUFA) are the most abundant in chia seed (81.48%). The α-linolenic (18:3 n-3) showed a concentration three times higher than linoleic acid (18:2 n-6). Similar results were obtained by Martínez et al*.* (2012) who found 82.50% of PUFA in the lipid fraction of chia, which was composed of 22.00% linoleic acid (LA) and 60.50% α-linolenic acid (ALA).

TABLE 2 – CONCENTRATION (MG G^{-1} TOTAL LIPIDS) OF FATTY ACIDS IN CHIA SEEDS.

Fatty acid	**Concentration (mg g^{-1})**
C14:0	0.50 ± 0.01
C16:0	68.04 ± 1.42
C16:1 n-9	0.70 ± 0.01
C16:1 n-7	1.59 ± 0.01
C18:0	27.39 ± 0.04
C18:1 n-9	56.16 ± 0.01
C18:1 n-7	7.52 ± 0.07
C18:2 n-6	174.13 ± 0.07
C18:3 n-6	2.34 ± 0.15
C18:3 n-3	551.02 ± 5.90
C20:0	2.88 ± 0.04
C20:1 n-9	0.28 ± 0.01
C20:2 n-6	1.07 ± 0.01
C20:3 n-3	0.39 ± 0.01
C20:5 n-3	1.01 ± 0.01
C24:0	0.78 ± 0.01
Parameters	
ΣSFA	99.59 ± 1.42
ΣMUFA	66.25 ± 0.07
ΣPUFA	729.96 ± 5.90
Σn-6	177.54 ± 0.17
Σn-3	552.42 ± 5.90

Values are the mean of four replicates **± standard deviation.** SFA: Saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Linoleic acid (LA) and α-linolenic acid (ALA) are both essential fatty acids that cannot be synthesized by humans and must be supplied with diet (Aranceta & Pérez-Rodrigo, 2012). Essential FAs act as precursors for the synthesis of more highly unsaturated and longer-chain omega-3 and omega-6 fatty acids. Omega-3 and n-6 fatty acids are essential components of cell-membrane phospholipids and they have several other functional roles (Tapiero et al., 2002). Due to the excessive use of vegetable oil, rich in LA, in the human food chain and also due to reduced intake of fish or plants (rich in ALA), the ratio between n-6/ n-3 is now unbalanced in most western diets (Blasbalg et al., 2011). Thus, chia, due to its fatty acid composition, is an excellent ingredient for use in diet or in the formulation of new food products, contributing to increases of the intake of 18:3 n-3.

The high content of ALA in chia seeds makes it a potential functional food (Almeida et al., 2009). The recommended daily intake of this fatty acid is approximately 1.60 g/day for an adult (Health Canada, 2012). Consumption of 9.50 g of chia seeds supplies the daily requirement of this fatty acid.

3.3 INDICES OF NUTRITIONAL QUALITY OF LIPID FRACTION

Through the fatty acid composition analysis, it was possible for the nutritional quality of the lipid fraction of chia to be determined, as shown in Table 3. The concentration of lauric acid (C12:0), arachidonic acid (C20:4 n-6), docosapentaenoic acid (C22:5 n-3) and docosahexaenoic acid (C22:6 n-3) was considered as zero for calculations of AI and HH, because they were not identified in the total lipids of chia.

TABLE 3 – INDICES OF THE NUTRITIONAL QUALITY OF THE LIPID FRACTION IN CHIA SEEDS.

PUFA/SFA	n-6/n-3	HH	AI	TI
7.33	0.32	11.41	0.09	0.054

HH: Hypocholesterolaemic/hypercholesterolaemic FA ratio - (C18:1n-9+C18:2n-6+C20:4n-6+-C18:3n-3+C20:5n-3+C22:5n-3+C22:6n-3) / (C14:0+C16:0). **AI:** Atherogenicity index - [(C12:0 + 4xC14:0+C16:0)] / ΣAGMI+ Σn-6+Σn-3 **TI:** Thrombogenicity index - (C14:0 + C16:0 +C18:0) / [(0,5xΣAGMI)+(0,5xΣn-6)+(3xΣn-3)+(Σn-3/n-6)].

The PUFA/SFA and n-6/n-3 ratios of diet or food are important indices for human health. According to current nutritional recommendations, the PUFA/SFA ratio in human diets should be above 0.45 (Wood et al., 2004) and, within the PUFA, a ratio of 1:1 to 2:1 n-6/n-3 fatty acids should be the target ratio for health (Simopoulos, 2011). Lower ratios of PUFA/ SFA in the diet as a whole may increase the incidence of cardiovascular disease (WHO, 2003). The results for the PUFA/SFA and n-6/n-3 ratios were 7.33 and 0.32, respectively. These indices evidence the high quality of the lipid fraction of chia seeds.

The indices of atherogenicity (AI) and thrombogenicity (TI) indicate the potential for stimulating platelet aggregation. The smaller the values of AI and TI are, the larger the amounts of anti-atherogenic fatty acids present in a certain oil/fat and therefore, the greater the potential for preventing the development of coronary heart disease. According to Tonial et al. (2011), there are no recommended values for AI and TI. It is understood that lower values of these indices indicate a healthier ratio (Fuchs et al., 2013). The HH index considers specific effects of fatty acids on cholesterol metabolism, and high HH values are desired from a nutritional standpoint (Turan et al., 2007).

The most hypercholesterolaemic saturated fatty acids are myristic (14:0), palmitic (16:0) and lauric acids (12:0), in decreasing order of activity. Stearic acid (18:0), although saturated, does not seem to have an effect on blood lipoproteins (Mahan & Escott-Stump, 2005). The concentrations of these acids in chia seeds were 0.50 mg g^{-1} (14:0) and 68.04 mg g^{-1} (16:0), respectively. Lauric acid was not quantified in the samples.

The HH ratio in chia seeds was 11.41, much higher values than those reported by Ramos Filho (2008) for fish from the Brazilian Pantanal (1.49–1.84).

The atherogenicity (AI) and thrombogenicity (TI) indices were 0.09 and 0.054, respectively. These indices suggest that the inclusion of chia in the human diet could prevent the development of coronary heart diseases.

4 CONCLUSION

Chia seeds had high protein and lipid content, of which the lipids consisted mainly of ALA. The PUFA/ SFA, n-6/n-3 and HH ratios and the values of atherogenicity (AI) and thrombogenicity (TI) indices show chia to be nutritionally favourable. These results confirm that chia can be employed directly in the diet, as well as in the formulation of new products, contributing to the improvement of the nutritional quality index of the lipid fraction of the population's diet.

SEMENTES DE CHIA (*SALVIA HISPANICA* L.): COMPOSIÇÃO EM ÁCIDOS GRAXOS, PROXIMAL E QUALIDADE NUTRICIONAL DE SUA FRAÇÃO LIPÍDICA.

RESUMO

Chia é uma boa fonte de fibras, proteínas, antioxidantes, sendo também rica em ácidos graxos poli-insaturados, particularmente o ácido linolênico (18:3 n-3). Essa é a razão pelo interesse crescente no estudo da composição química e qualidade nutricional dessa semente, que pode ser incluída na dieta ou utilizada como ingrediente em novas formulações. O objetivo deste estudo foi determinar a composição química e em ácidos graxos de amostras de chia comercializadas no Brasil e avaliar os índices de qualidade nutricional de sua fração lipídica. Com relação à composição encontraram-se valores médios de umidade de 8,14%, 20,11% de proteínas, 27,72% de lipídios totais e 3,94% de cinzas. Dezesseis ácidos graxos foram quantificados, destacando a prevalência de ácidos graxos poli-insaturados, principalmente 18:3 n-3. Todos os índices de qualidade nutricional analisados (Razão entre ácidos graxos hipocolesterolêmicos/ hipercolesterolêmicos, índice de aterogenicidade e índice de trombogenicidade) indicam que a chia é um alimento com excelente qualidade lipídica e sua inclusão na dieta ou como um ingrediente em formulações alimentares pode ser uma aliada na redução de problemas cardiovasculares.

PALAVRAS-CHAVE: ÁCIDO GRAXO; ÍNDICE ATEROGÊNICO; ÍNDICE TROMBOGÊNICO; RELAÇÃO FA HIPOCOLESTEROLÉMICA / HIPERCOLESTEROLÊMICA.

REFERENCES

1 ALMEIDA, K.C.L.; BOAVENTURA, G.T.; GUZMAN-SILVA, M.A. A linhaça (*Linum usitatissimum*) como fonte de ácido α-linolênico na formação da bainha de mielina. **Revista de Nutrição**, v.22, n.5, p.747-754, 2009.

2 ARANCETA, J.; PÉREZ-RODRIGO, C. Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. **British Journal of Nutrition**, v.107, p.S8–S22, 2012.

3 AYERZA, R. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. **Journal of the American Oil Chemists' Society**, v.72, n.9, p.1079–1081, 1995.

4 AYERZA, R.; COATES W. Influence of environment on growing period and yield, protein, oil and α-linolenic content of three chia (*Salvia hispanica* L.) selections. **Industrial Crops and Products**, v.30, n.2, p.321–324, 2009.

5 BALA, B.K. **Drying and storage of cereal grains**. India: Oxford & IBH Publishing Co., 1997

6 BLASBALG, T.L.; HIBBELN, J.R.; RAMSDEN, C.E.; MAJCHRZAK, S.F.; RAWLINGS, R.R. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. **American Journal of Clinical Nutrition**, v.93, n.5, p.950–962, 2011.

7 BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology,** v.37, n.8, p.911-917, 1959.

8 BUENO, M.; DI SAPIO, O.; BAROLO, M.; BUSILACCHI, H.; QUIROGA, M.; SEVERIN, C. Análisis de la calidad de los frutos de *Salvia hispanica* L. (Lamiaceae) comercializados en la ciudad de Rosario (Santa Fe, Argentina). **Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas**, v.9, n.3, p.221-227, 2010.

9 BUSHWAY, A.A.; BELYA, P.R.; BUSHWAY, R.J. Chia seed as a source of oil, polysaccharide and protein. **Journal of Food Science,** v.46, n.5, p.1349 – 1350, 1981.

10 CAPITANI, M.I.; SPOTORNO, V.; NOLASCO, S.M.; TOMÁS, M.C. Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. **LWT - Food Science and Technology**, v.45, n.1, p.94–102, 2012.

11 COMMISSION DECISION 2013/50/EU of 22 January 2013 authorizing an extension of use of Chia (*Salvia hispanica*) seed as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. **Official Journal of the European Union.** Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:021:0034:0035:EN:PDF

12 Acessed on 25 March 2014.

13 CUNNIF, P.A. **Official Methods of Analysis of AOAC International** (6th Ed.) Arlington: Association of Official Analytical Chemists, 1998.

14 DIETSCHY, J.M. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentration. **Journal of Nutrition**, v.128, n. , p.444S–448S, 1998.

15 FUCHS, R.H.B.; RIBEIRO, R.P.; MATSUSHITA, M.; TANAMATI, A.A.C.; BONA, E.; SOUZA, A.H.P. Enhancement of the nutritional status of Nile tilapia (*Oreochromis niloticus*) croquettes by adding flaxseed flour. **LWT - Food Science and Technology**, v.54, n.2, p.440-446, 2013.

16 GOGUS, U.; SMITH, C. Review article. n-3 Omega fatty acids: a review of current knowledge**. International Journal of Food Science & Technology**, v.45, n.3, p.417–436, 2010.

17 HARTMAN, L.; LAGO, R.C.A. Rapid preparation of fatty acid methyl from lipids. **Laboratory Practice**, v.22, n.4, p.474-476, 1973

18 HEALTH CANADA (2012). **Do Canadian adults meet their nutrient requirements through food intake alone**. Available at: http://www.hc-sc.gc.ca/fn-an/alt_formats/pdf/surveill/nutrition/commun/art-nutr-adult-eng.pdf Accessed on: 14 May 2014.

19 IXTAINA, V.Y.; NOLASCO, S.M.; TOMÁS, M.C. Physical properties of chia (*Salvia hispanica* L.) seeds. **Industrial Crops and Products**, v.28, n.3, p.286-293, 2008.

20 JADHAV, S.J.; NIMBALKAR, S.S.; KULKARNI, A.D.; MADHAVI, D.L. **Food antioxidants:** technological, toxicological, and health perspectives. New York: Marcel Dekker, 1996.

21 JAKOBSEN, M.U.; OVERVAD, K.; DYERBERG, J.; HEITMANN, B.L. Intake of ruminant trans fatty acids and risk of coronary heart disease. **International Journal of Epidemiology**, v.37, n.1, p.173-182, 2008.

22 JOSEPH, J.D.; ACKMAN, R.G. Capillary column gas chromatography method for analysis of encapsulated fish oil and fish oil ethyl esters: collaborative study. **Journal of AOAC International,** v.75, n.5, p.488–506, 1992.

23 MAHAN, L.K.; ESCOTT-STUMP, S.K**. Alimentos, Nutrição & Dietoterapia**. São Paulo: Roca, 2005.

24 MAIA, E.L.; RODRIGUEZ-AMAYA, D.B. Avaliação de um método simples e econômico para a metilação de ácidos graxos com lipídios de diversas espécies de peixes. **Revista do Instituto Adolfo Lutz**, v.53, n. 1/2, p.27-35, 1993.

25 MARTÍNEZ, M.L.; MARÍN, M.A.; SALGADO, F.C.M.; REVOL, J.; MARÍA, C.P. Chia (*Salvia hispanica* L.) oil extraction: Study of processing parameters. **LWT - Food Science and Technology**, v.47, n.1, p.78-82, 2012.

26 PÉREZ-PALACIOS, T.; RUIZ, J.; MARTÍN, D.; MURIEL, E. Analytical methods comparison of different methods for total lipid quantification in meat and meat products**. Food Chemistry**, v.110, n.4, p.1025–1029, 2008.

27 PORRAS-LOAIZA, P.; JIMÉNEZ-MUNGUÍA, M.T.; SOSA-MORALES, M.E.; PALOU, E.; LÓPEZ-MALO, A. (2014). Physical properties, chemical characterization and fatty acid composition of Mexican chia (*Salvia hispanica* L.) seeds. **International Journal of Food Science & Technology,** v.49, n.2, p.571–577, 2014.

28 RAMOS FILHO, M.M.; RAMOS, M.I.; HIANE, E.M.T. Perfil lipídico de quatro espécies de peixes da região pantaneira de Mato Grosso do Sul. **Ciência e Tecnologia de Alimentos,** v.28, n.2, p.361-365, 2008.

29 RUPFLIN, D.I.A. Caracterización de la semilla del chan (*Salvia hispánica L.*) y diseño de un producto funcional que la contiene como ingrediente. **Revista de la Universidad del Valle de Guatemala, v.**23, p.43-49, 2011.

30 SALAZAR-VEGA, M.I.; ROSADO-RUBIO, G.; CHEL-GUERRERO, L.A.; BETANCUR-ANCONA, D.A.; CASTELLANOS-RUELAS, A.F. Composición en ácido graso alfa linolénico (w-3) en huevo y carne de aves empleando chia (*Salvia hispanica* L.) en el alimento. **INCI**, v.34, n.3, p.209-213, 2009.

31 SANTOS-SILVA, J.; BESSA, R.J.B.; SANTOS-SILVA, F. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. **Livestock Production Science**, v.77, n. 2-3, p.187-194, 2002.

32 SEGURA-CAMPOS, M.R.; SALAZAR-VEJA, I.M.; CHEL-GUERRERO, L.A.; BETANCUR-ANCONA, D.A. Biological

potential of chia (Salvia hispanica L.) protein hydrolysates and their incorporation into functional foods. **LWT - Food Science and Technology**, v.50, n.2, p.723-731, 2013.

33 SIMOPOULOS, A.P. Importance of the omega-6/omega-3 balance in health and disease: evolutionary aspects of diet. **World Review of Nutrition and Dietetics**, v.102, p.10-21, 2011.

34 TANAMATI, A.; OLIVEIRA, C.O.; VISENTAINER, J.V.; MAKOTO, M..; SOUZA, N.E. Comparative Study of Total Lipids in Beef Using Chlorinated Solvent and Low-Toxicity Solvent Methods. **Journal of the American Oil Chemists' Society**, v.82, n.6, p.393-397, 2005.

35 TONIAL, I.B.; OLIVEIRA, D.F.; BRAVO, C.E.C.; SOUZA, N.E.; MATSUSHITA, M.; VISENTAINER, J.V. Caracterização físico-química e perfil lipídico do salmão (*Salmo salar* L.). **Alimentos e Nutrição**, v.21, n.1, p.93-98, 2010.

36 TOSCO, G. Os benefícios da Chia em humanos e animais. **Revista Atualidades Ornitológicas** n.119, p.7, 1994.

37 TURAN, H.; SÖNMEZ, G.; KAYA, Y. Fatty acid profile and proximate composition of the thornback ray (*Raja clavata* L. 1758) from the Sinop coast in the Black Sea. **Journal of Fisheries Sciences**, v.1, n.2, p.97-103, 2007.

38 ULBRICHT, T.L.V.; SOUTHGATE, D.A.T. Coronary heart disease: seven dietary factors. **Lancet**, v.338, n.8773, p.985-992, 1991.

39 URIBE, J.A.R.; PEREZ, J.I.N.; KAUIL, H.C.; RUBIO, G.R.; ALCOCER, C.G. Extraction of oil from chia seeds with supercritical CO_2. **The Journal of Supercritical Fluids**, v.56, n.2, p.174-178, 2011.

40 WEBER, C.W.; GENTRY, H.S.; KOHLHEPP, E.A; MCCROHAN, P.R. The nutritional and chemical evaluation of chia seeds. **Ecology of Food and Nutrition**, v.26, n.2, p.119-125, 1991.

41 WOOD, J.D.; RICHARDSON, R.I.; NUTE, G.R.; FISHER, A.V.; CAMPO, M.M.; KASAPIDOU, E., SHEARD, P.R.; ENSER, M. Effects of fatty acids on meat quality: a review. **Meat Science**, v.66, n.1, p.21-32, 2004.

42 TAPIERO, H.; BA, G.N.; COUVREUR, P.; TEW, K.D. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. **Biomedicine & Pharmacotherapy**, v.56, n.5, p.215–222, 2002.

43 TARPILA, A.; WENNBERG, T.; TARPILA, S. Flaxseed as a functional food. **Current Topics in Nutraceutical Research**, v.3, n.3, p.167-188, 2005.