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Phenolics compounds, flavonoids and antioxidant activity of chia seed extracts *(Salvia hispanica)* obtained by different extraction conditions

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Abstract

This study aimed to chemically characterize the chia seed *(Salvia hispanica)*, get chia seed extracts at different concentrations of ethanol and temperature using a factorial design 2^2 with triplicate at the central point and then analyze the extracts the content phenolics, flavonoids and antioxidant activity in vitro. The results showed that the chia seed has a high content of dietary fiber (374.4 g/kg), lipids (283.5 g/kg) and protein (231.7 g/kg). The same possess antioxidant activity, and the extract with a higher antioxidant activity was obtained by the method of stirring using temperatures of 60^0C and 80% ethanol concentrations. The content of phenolic compounds was found to be 2,639 g of gallic acid equivalent/kg of dry sample. The flavonoid content was 0.162 g equivalent of quercetin/kg dry sample. The IC_{50} found was of 3.841 mg/mL and FRAP 45.004 mmol trolox equivalent/ kg of dry sample.

Introduction

Lipid oxidation is a major concern in the food industry, because generate products that are undesirable both for the breakdown of lipids as well as for production of volatile compounds. These sensory changes promote the destruction of the essential components, causing a decrease of the nutritional value and the formation of toxic compounds during storage and food processing (Melo and Guerra, 2002). A good alternative to prevent the lipid oxidation in foods is to use natural antioxidants, which have better acceptance by consumers. Fruits, vegetables, cereals and spices are products that feature in its constitution compounds with antioxidant action, among which stand out phenolic compounds (Dimitrios, 2006).

Salvia hispanica or chia is native to the region extending from west central Mexico to northern Guatemala and has some compounds with potential antioxidant activity such as myricetin, quercetin, kaempferol and caffeic acid. It is known that the oxidation of chia seeds is minimal or absent, due to the presence of these compounds, having a great potential in the food industry (Ixtaina *et al*., 2011). Thus, this study aims to characterize the chia's seed, and evaluate the content of phenolic compounds, total flavonoids and antioxidant capacity in vitro of the extracts obtained by extraction in shaking using different temperatures and concentrations of ethanol.

Materials and Methods

Sample preparation

Chia seeds *(Salvia hispanica)* were purchased in commercial establishments in the city of Santa Maria - RS, Brazil, in January 2013. Initially, they have been dried in an oven with forced air (Marconi, MA-035/100, Piracicaba, Brazil) at 55^0C for 24 hours. Then, they have passed through an analytical grinding mill cooled to 4^0C (Quimis, model 298A21 Q, Diadema, Brazil) and then standardized in particle sizes of 60 Mesch (0.25 mm). After, they were placed in an amber bottle and stored in a freezer (-12^0C) until the time of analysis.

Characterization of the chia seeds

Moisture determinations (indirect gravimetric method at 105^0C), protein (Kjeldahl method), mineral residue (incineration method oven at 550^0C), dietary fiber (enzymatic method) and total lipid (Soxhlet) were performed according AOAC (2005). The carbohydrates were obtained by calculating the difference between the other fractions analyzed (AOAC, 2005), and the results were expressed on a wet basis.

Obtaining the chia seed extracts

To obtain the extracts of chia seeds was used a 2^2 Factorial design with three replicates at the

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Table 1. Factorial design 2^2 with triplicate at the central point (real and coded variables) used to obtain the chia seed extracts

Extract	Ethanol concentration (%)	Temperature (^{0}C)
	80	60
1	(+1)	(+1)
	50	60
2	(-1)	(+1)
	80	40
3	(+1)	(-1)
	50	40
4	(-1)	(-1)
	65	50
5	(0)	(0)
	65	50
6	(0)	(0)
	65	50
7	(0)	(0)

PC: Central point corresponds extractions 5, 6 e 7

Central Point, being the ethanol concentration and temperature independent variables, as shown in Table 1. The concentrations were defined based on the study described by Caudillo *et al.* (2008) with modifications. The milled chia seed were weighed and added solvent (hydroethanolic solution 50, 65 or 80%) in a 1:10 (1 g chia seed, and 10 ml solvent), to obtain the extract.

Later, each mixture was taken to a thermostated bath at a controlled temperature (40, 50 or 60^{0}C) (Solab, Model SL-152/10) and subjected to constant stirring (80 rpm) using a mechanical stirrer (Marconi MA-039, Piracicaba Brazil) for 60 minutes. Thereafter, the extracts were filtered through qualitative filter paper N°1 and centrifuged at 3000 rpm for 20 min. The extracts had their volumes completed with distilled water, packed and stored in amber bottles in a freezer (-12^{0}C) until analysis.

Determination of total phenolic compounds

The total phenolic content of the extracts was determined by the Folin-Ciocalteu colorimetric method, described by Singleton *et al.* (1999), with modifications. The extract samples, appropriately diluted, were mixed with the Folin-Ciocalteu reagent. After 6 minutes, the solution of 7.5% sodium carbonate (Na_2CO_3) was added and the tubes were kept in the dark at room temperature (approximately 23^{0}C) for 2 hours. After this time, the absorbance was determined at 765 nm in a spectrophotometer (Biospectro, SP-220, São Paulo, Brazil) and compared with a calibration curve of gallic acid Y=0,081x+0,0218, R^2 = 0,9967 (range 0 to 70 mg/L). The results were expressed as gram of gallic acid equivalent per kilogram of dry sample (g/kg GAE of dried sample).

Determination of total flavonoid

The total flavonoid content was determined using the colorimetric method described by Zhishen *et al*. (1999) with modifications. Here, 250 µL of the extract was mixed with 1250 µL of distilled water in a test tube, followed by addition of 75 µL of a solution of sodium nitrite ($NaNO_2$) 5%. After 5 minutes, 150 µL of a solution of aluminum trichloride ($AlCl_3$) at 10% was added and was left resting for further 5 minutes, and then added 500 µL of 1M NaOH and 775 µL distilled water, and the mixture was stirred . The absorbance was measured immediately at 510 nm using a spectrophotometer (Biospectro SP-220, São Paulo, Brazil) and compared with a quercetin calibration curve Y = 0,0082x + 0,0084, R^2 = 0,9961 (range 0 to 80 mg/L). The results were expressed as grams of quercetin equivalent per kilogram of dry sample (g/kg EQ dry sample).

Determination of antioxidant activity

Free radical sequestration capacity of 2,2-diphenyl-1-picryl-hydrazyl (DPPH)

The antioxidant activity of the extracts of chia seeds were determined through sequestration capacity of free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl) according to the methodology described by Brand-Williams *et al.* (1995). The technique consists of incubating for 30 minutes of 5 ml of an ethanol solution (80% v/v) of 0.1 mM DPPH with 5 mL of solutions containing increasing concentrations of chia extract (0.7, 1.5, 3.0, 6.25, 12.5, 25.0, 50.0 mg/mL).

The readings of the samples were performed in the spectrophotometer (Biospectro SP-220, São Paulo, Brazil) after incubation at a wavelength of 517 nm. The percentage of antioxidant activity (AA%) was calculated by the percentage of capture of DPPH radical, according to Equation 1.

$$AA\% = 100 - \left\{ \left[(Abs_{sample} - Abs_{blank}) \times 100 \right] / Abs_{DPPH} \right\} \quad (1)$$

Later, was calculated the concentration required to capture 50% of the free radical DPPH (IC_{50}) after evaluating the optimal concentration range.

Antioxidant power of iron reduction (FRAP)

In order to determine the FRAP of the extracts, the method described by Benzie and Strain (1996) was used. The FRAP reagent (Fe (III) solution -TPTZ) was obtained from the combination of 25 mL of 0.3 M acetate buffer, 2.5 ml of a solution TPTZ (tripiridiltriazina) 10 mM (3.12 g TPTZ in 1L of 40 mM HCl) and 2.5 ml of a 20 mM aqueous solution of ferric chloride.

In a test tube was added 200 µL of pre-diluted sample and 1.8 mL of the FRAP reagent and kept in a water bath at 37^{0}C for 30 min. Afterwards, the absorbance of the colored complex formed with Fe^{2+} and TPTZ was measured at 593 nm in a spectrophotometer (Biospectro SP 220, São Paulo, Brazil). FRAP reagent was used as a blank.

The compound trolox (range 0 to 25 µM) was used as standard for the calibration curve Y = 0,0265x + 0,002, R^2 = 0,9968, and the results were expressed in milimol the trolox equivalents per kilogram of dry sample (mmol/kg ET dry sample).

Statical analysis

In order to reduce the number of experiments (and to reduce the time and cost), 2^2 factorial design was used, with three repetitions at the central point. This allows the implementation of a statistical inference approach because it allows the calculation of waste and therefore the standard error and interval estimates (Rodrigues and Iemma, 2009).

The analyzes were performed in triplicate. The results were submitted to analysis of variance (ANOVA) and means compared using the Tukey test, with significance level of 95% ($p< 0.05$). The graphics and calculations of the effects were obtained using the software Statistica 9.0 (STATSOFT, INC).

Results and Discussion

Characterization of seeds of chia

The chemical composition of chia seed is shown in Table 2. The results show that chia seed is a good source of vegetable protein and lipids. These results are in agreement with Ayerza (1995) having a content of 250-390 g/kg lipids and 190-270 g/kg of protein in the chia seed. It is possible also note that the chia seed showed high content of dietary fiber, similar results to the study by Caudillo *et al.* (2008), where they were checked values from 369.7 to 399.4 g/kg of fiber food in chia seeds coming from the states of Jalisco and Sinaloa, Mexico. The high content of chia seed fibers can enhance satiety, regulate intestinal transit, reduce energy consumption and promote weight loss in users (Ayerza *et al*., 2002).

Chia seeds had low moisture content, which may contribute to greater chemical and microbiological stability thereof. With respect to ashes levels, were obtained 54.5 g/kg. These data differ from the results found by Sargi *et al.* (2013), which found 78.6 g/kg for moisture and 36.3 g/kg to ashes. According to Rodrigues (2005), the great variability in the physical and chemical characteristics of plant can be attributed to many factors, including the region where the plant was grown, climatic differences, fertility, soil pH and annual rainfall.

Table 2. Results of chemical composition of chia seeds *(Salvia hispanica)* on a wet basis (g/kg sample)

Fractions	(g/kg sample)
Moisture	32.7± 1.920
Ashes	54.5 ± 1.205
Protein	231.7 ± 2.890
Ether extract	283.5 ± 3.101
Dietary Fiber	374.4 ± 4.130
Carbohydrates	23.2±3.271

Means ± standard deviation of triplicate samples

Determination of total phenolic compounds

The content of phenolic compounds of chia seed extracts using different ethanol concentrations and temperatures are shown in Table 3.

According to Table 3 it can be observed that extracts 1, 3 and extracts the center point (5, 6, and 7) showed no statistical difference among themselves and among other groups ($p> 0.05$). However, the extract 1 showed the highest content of total phenolic content showing that major phenolic concentrations were obtained for extractions performed at high ethanol concentration and higher temperatures. On the other hand, the extract 2 showed statistical significance ($p <0.05$) than the others, and presented a lower value of phenolic compounds content.

Caudillo *et al.* (2008), analyzed the content of phenolic compounds on chia seed extracts obtained by stirring with ethanol at room temperature for 24 hours. In this study they found values of 8.8 g GAE/ kg of dry sample, evidencing the phenolic compounds present in chia seed would have a lower polarity and thus be more easily extracted in less polar solvents.

The effects of the variables (temperature and ethanol concentration) and their interactions on the extraction of phenolic compounds of chia seed are shown in Table 4. Significant effects ($p<0.05$) for the concentration of ethanol and interaction effects between ethanol concentration and temperature were observed. It was also observed that the interaction between ethanol concentration and temperature influenced positively in the extraction of phenolic compounds, namely the use of higher temperatures and higher concentrations of ethanol were more effective in the extraction of phenolic compounds of chia seed. Similar results were found by Spagolla *et al*. (2009), to quantify phenolic compounds from blueberry using different concentrations of ethanol and temperature of 70^{0}C. The total phenolic compounds were significantly higher when the

Table 3. Content of total phenolic, flavonoids, IC_{50} and FRAP of chia seed extracts

Extract	Condictions	Total phenolic (g/kg GAE)	Total flavonoid (g/kg EQ)	IC_{50} (mg/mL)	FRAP (mmol/kg ET)
1	60°C, ethanol 80%	2.639^{a}±0.584	0.162^{a}±0.003	3.841^{c}±0.118	45.004^{a}±4.012
2	60°C, ethanol 50%	0.860^{c}±0.239	0.055^{b}±0.014	8.237^{a}±0.430	18.005^{c}±3.101
3	40°C, ethanol 80%	2.187^{ab}±0.158	0.122^{a}±0.002	4.105^{bc}±0.202	35.036^{b}±3.004
4	40°C, ethanol 50%	1.775^{b}±0.077	0.110^{a}±0.027	4.943^{b}±0.324	28.024^{b}±2.064
5	50°C, ethanol 65%	2.264^{ab}±0.262	0.153^{a}±0.035	3.901^{c}±0.262	30.008^{b}±2.007
6	50°C, ethanol 65%	2.279^{ab}±0.233	0.159^{a}±0.008	4.365^{bc}±0.551	32.063^{b}±1.034
7	50°C, ethanol 65%	2.320^{ab}±0.061	0.142^{a}±0.013	5.110^{b}±0.494	34.023^{b}±2.009

Values expressed as mean ± standard deviation with different letters in the same column indicate significant difference ($p< 0.05$) by Tukey test.

Table 4. Calculation of the effects of variables (temperature and ethanol concentration) regarding to the content of flavonoids and phenolic compounds

	Phenolic compounds			Flavonoids		
	Efects	Standard Deviation	p	Efects	Standard Deviation	p
Means/Interaction	2.047	0.057	0.000*	0.123	0.007	0.000*
(1) Ethanol concentration (%)	1.095	0.152	0.001*	0.056	0.018	0.017*
(2) Temperature	-0.231	0.152	0.014	-0.010	0.018	0.574
1X2	0.684	0.152	0.001*	0.051	0.018	0.024*

1x2 = interaction between ethanol concentration and temperature

ethanol concentration ranging from 40 to 60%.

Determination of total flavonoid

The values found for total flavonoid content of chia seed extracts obtained by different ethanol concentrations and temperatures are shown in Table 3. According to Table 3 only extract 2 (50% ethanol) differed significantly from the others ($p <0.05$), with lower content of total flavonoids.

Buratto *et al.* (2011), when have analyzed the content of total flavonoids in Brazil nuts, they have obtained 0.34 g EQ/kg of dry sample. This value was higher than those found in chia seed extracts. In a study by Lin and Tang (2007), where it was evaluated the content of total flavonoids in vegetables, the values were 0.075 g EQ/kg for green peppers, 0.041 g EQ/kg for yellow pepper, values closer to those found for chia seed extracts, and 0.306 g EQ/kg for white onion.

In Table 4 it is possible to observe the effects of variables ethanol concentration and temperature on the response variable content of flavonoids. Significant effects of first order can be observed ($p <0.05$) in the concentration of ethanol, and the effect of interaction between ethanol concentration and temperature. The interaction between ethanol concentration and temperature positively affected the extraction of total flavonoids showing that, as the phenolic compounds, the use of higher temperatures and higher concentrations of ethanol were more effective in the extraction.

Determination of antioxidant activity

Free radical sequestration capacity of 2,2-diphenyl-1-picryl-hydrazyl (DPPH)

The results obtained in the determination of antioxidant activity in vitro by DPPH method of chia seed extracts obtained by different ethanol concentrations and temperatures are shown in Table 3. The extract 1, although not demonstrate statistical difference ($p> 0.05$) compared to extracts 3, 5 and 6, showed the higher antioxidant activity, with a lower IC_{50} value (3.841 mg/mL). Moreover, the extract 2 differed significantly from the others ($p <0.05$), with higher IC_{50} value (8.237mg/mL).

Table 5. Calculating the effects of the variables (ethanol concentration and temperature) for the IC_{50} response and FRAP

	IC_{50}			FRAP		
	Effects	Standard Deviation	p	Effects	Standard Deviation	p
Means/Interaction	4.863	0.089	0.000*	3.182	0.089	0.000*
(1) Ethanol concentration (%)	-2.730	0.219	0.000*	1.707	0.220	0.000*
(2) Temperature	1.746	0.219	0.000*	0.056	0.220	0.804
1X2	-1.902	0.219	0.000*	1.240	0.220	0.000*

1x2 = interaction between ethanol concentration and temperature

The *Ginkgo biloba* plant, considered with high antioxidant activity, showed an IC_{50} of 0.04072 mg /mL, in an experiment conducted by Mensor *et al*. (2001). Milani *et al.* (2011) studied the antioxidant activity of crude extract of persimmon. In this study, they found IC_{50} values of 0.2467 mg/mL. The chia seed extract showed low antioxidant activity when compared to the persimmon and the *Ginkgo biloba*, however, showed better antioxidant that mint extracts (Mentha arvensis), with IC_{50} 17.40 mg/mL (Morais *et al*., 2009).

In Table 5 it is possible to observe the effects of variables ethanol concentration and temperature on the IC_{50} response variable. First order significant effects can be observed ($p<0.05$) for ethanol concentration and temperature, and for interaction between ethanol concentration and temperature. It is observed that the interaction between ethanol concentration and temperature influenced inversely the IC_{50} value, that is, the higher ethanol concentrations and temperatures, the lower the IC_{50} value, the higher the antioxidant activity.

Similar effects were found in a study conducted by Caetano *et al.* (2009), which were used different concentrations for ethanol extraction residue acerola antioxidants. The results showed that the higher the concentration of ethanol used for extraction the greater the sequestration capacity of the free radical DPPH, therefore, the lower the IC_{50} values. The results were not temperature dependent, differing in this study, where the temperature positively influence the results.

It is observed a relationship between phenolics and flavonoids and the extract's ability to reduce free radical DPPH, as the extracts that showed higher values of these compounds had lower IC_{50}. This indicates that phenolic compounds and flavonoids are the mainly responsible for antioxidant activity of chia seed extracts.

Antioxidant power of iron reduction (FRAP)

The values found for FRAP (Antioxidant power of iron reduction) of chia seed extracts obtained by different ethanol concentrations and temperatures are shown in Table 3. Extracts 1 and 2 showed significant difference between them ($p<0.05$) and the others, and correspond to the maximum and minimum values (45.004 and 18.005 mmol ET/kg dry sample, respectively) found for FRAP of seed extracts of chia. The other extracts, however, showed no significant difference between them ($p> 0.05$).

A study conducted by Alothman *et al.* (2009) showed that the antioxidant power of reduction of iron (FRAP) of the extracts is dependent on the solvent used and its polarity. Thus, the extract 1, where higher concentrations of ethanol were used, showed better antioxidant properties, according to the FRAP method.

In Table 5 are the effects of variables ethanol concentration and temperature on FRAP response variable. First order significant effects were observed ($p<0.05$) in the concentration of ethanol, and interaction between ethanol concentration and temperature. It was observed that the interaction between ethanol concentration and temperature can affect positively FRAP variable.

The results are similar to the study conducted by Rockenbach *et al.* (2008), where they investigated the antioxidant power reduction iron (FRAP) on grape bagasse extracts of Tannat variety by using different concentrations of ethanol. The results of the study showed that the FRAP value increased as to increase the proportion of ethanol in the extraction, reaching maximum values of 592.4 mmol ET/kg dry sample when using 70% ethanol.

Conclusion

The results showed that chia seed has a high content of dietary fiber, lipids and protein, and

its extracts obtained by shaking have potential as an antioxidant. The highest levels of phenolic compounds, flavonoids and antioxidant activity were observed using the stirring method with a concentration of ethanol of 80% and temperature of 600C. There was a relationship between the content of phenolic compounds, flavonoids and antioxidant capacity in vitro, showing that these compounds are the main responsible for the antioxidant potential of chia seed extracts.

References

Alothman, M., Bhat, R. and Karim, A.A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 115(3): 785–788.

Association of Official Analytical Chemists. 2005. Official methods of analysis of the Association of Official Analytical Chemists.18th edn. Gaithersburg: AOAC.

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

Ayerza, R., Coates, W. and Lauria, M. 2002. Chia seed (*Salvia hispanica* L.) as na omega-3 fatty acid source for broilers: influence on fatty acid composition, cholesterol and fat content of white and dark meats, growth performance, and sensory characteristics. Poultry Science 81(6): 826-837.

Benzie, I.F.F and Strain, J.J. 1996. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. Analytical Biochemistry 239(1): 70–76.

Brand-Willians, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Science and Technology 28(1): 25-30

Buratto, A. P., Carpes, S. T., Vecchia, P., Schroll, E. M. and Appelt, P. 2011. Determinação da atividade antioxidante e antimicrobiana em castanha-do-pará *(Bertholletia excelsa)*. Revista Brasileira de Pesquisa em Alimentos 2(1): 60-65.

Caetano, A. C. S., Melo, E. A., Lima. V. L. G. and Araújo, C. R. 2009. Extração de antioxidantes de resíduos agroindustriais de acerola. Brazilian Journal Food Technology 12(2): 155-160.

Caudillo, E. R., Tecante, A., Valdivia, M. A. and Lopez, L. 2008. Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. Food Chemistry 107(2): 656-663.

Dimitrios, B. 2006. Sources of natural phenolic antioxidants. Trends in Food Science & Technology 17: 505-512.

Ixtaina, V. Y., Martínez, M. L., Spotorno, V., Mateo, C. M., Maestri, D. M. and Diehl, B. W. K. 2011. Characterization of chia seed oils obtained by pressing and solvent extraction. Journal of Food Composition Analysis 24(2): 166- 174.

Lin, J. Y. and Tang, C. Y. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry 101(1): 140–147.

Melo, E. A. and Guerra, N. B. 2002. Ação antioxidante de compostos fenólicos naturalmente presentes em alimentos. Boletim da Sociedade Brasileira de Ciência e Tecnologia de Alimentos, Campinas, 36(1): 1-11.

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C., Cintia, S., Coube, C. S. and Leitão, S. G. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 15(2): 127-130.

Milani, L. I. G., Terra, N. N., Fries, L. L. M., Rezer, A. A. S., Cichoski, A. J., Backes, A. M. and Parodia, C. G. 2012. In vitro antioxidant and antimicrobial properties of persimmon (*Diospyros kaki* L. cv. Rama Forte) extracts. Brazilian Journal of Food Technology 15(2): 118-124.

Morais, S., Cavalcanti, E. S. B., Costa, S. M. and Aguiar. L. A. 2009. Ação antioxidante de chás e condimentos de grande consumo no Brasil. Revista Brasileira de Farmacognosia 19(1): 315-320.

Rockenbach, I. I., Silva, G. L., Rodrigues, E., Kuskoski, E. M. and Fett, R. 2008. Influência do solvente no conteúdo total de polifenóis, antocianinas e atividade antioxidante de extratos de bagaço de uva *(Vitis vinifera)* variedades Tannat e Ancelota. Revista Ciência e Tecnologia de Alimentos 28: 238-244.

Rodrigues, M.I. and Iemma, A.F. 2005. Planejamento de Experimentos e Otimização de Processos. Campinas: Casa do Pão Editora.

Rodrigues, L. J. 2005. O pequi (*Caryocar brasiliense* Camb.): ciclo vital e agregação de valor pelo processamento mínimo. Lavras, Brasil, Universidade Federal de Lavras, Dissertação de Mestrado.

Sargi, S. C., Silva, B. C., Santos, H. M., Montanher, P. F., Boeing, J. S., Santos, O., Souza, N. E. and Visentainer, J. V. 2013. Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax, and perilla. Food Science and Technology 33(3): 144-158.

Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 299(1): 152-178.

Spagolla, L. C., Santos, M. M., Passos, L. M. L. and Aguiar, C .L. 2009. Extração alcoólica de fenólicos e flavonóides totais de mirtilo "Rabbiteye" *(Vaccinium ashei)* e sua atividade antioxidante. Revista de Ciências Farmacêuticas Básica e Aplicada 30(2): 187-191.

Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64(4): 555-559.