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**Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils**
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**Abstract**
Vegetable oils are the richest dietary sources of vitamin E. Vitamin E determination levels in foods are of great importance to adjust the ingestion of nutrients by the population. The purpose of this paper is to determine the concentration of alpha-tocopherol and gamma-tocopherol in vegetable oils and compare the alpha-tocopherol value to the nutritional requirement of vitamin E. The analysis was performed using High Performance Liquid Chromatography. The values expressed as mg/kg for alpha and gamma-tocopherol were, respectively, 120.3±4.2 and 122.0±7.9 in canola oil; 432.3±86.6 and 92.3±9.5 in sunflower oil; 173.0±82.3 and 259.7±43.8 in corn oil; 71.3±6.4 and 273.3±11.1 in soybean oil. A significant difference was encountered between the alpha-tocopherol concentrations in vegetable oils. Similar results were found for gamma-tocopherol, except for corn and soybean oils. It was concluded that the soybean oil was not considered a source of vitamin E. The canola and corn oils were considered sources, and the sunflower oil was considered an excellent source.

**Keywords:** vitamin E; vegetable oils; nutritional recommendations.

1 Introduction

Vitamin E is the most potent lipid-soluble antioxidant in human plasma. Dietary components with antioxidant activity of vitamin E include α-, β-, γ- and δ-tocopherols and α-, β-, γ- and δ-tocotrienols. These compounds containing a chromanol ring attached to a saturated phytol (tocopherols) unsaturated (tocotrienols) and vary as to the number of methyl groups on the chromanol ring (Traber, 2012). Among the tocopherol homologs, the alpha-tocopherol has the highest biological potency (Ortíz et al., 2006).

Digestion of tocopherols and tocotrienols is similar to lipids, so that the emulsification and incorporation into mixed micelles takes place. Then, these compounds are absorbed by the enterocytes and transported primarily in the proximal intestine. In these cells, the tocopherols are incorporated into chylomicrons and transported by the lymphatic system (Nagy et al., 2013).

Os chylomicrons reach the blood circulation, where its triglycerides are hydrolyzed by the lipoprotein lipase (Asakura, 2006). Tocophersols reach the liver via the remnant chylomicrons and alpha-tocopherol is preferably incorporated into VLDLs by having greater affinity for the transport protein alpha-tocopherol (α-tocopherol transport protein or α-TTP). Therefore, alpha-tocopherol is the isomer that is in higher concentrations in plasma and tissues (Fu et al., 2014).

Several reactive oxygen / nitrogen species induce oxidative stress. The removal of these compounds is the main function of antioxidants, vitamin E being the most important and abundant fat-soluble antioxidant in vivo. The peroxyl radicals are removed by vitamin E in order to disrupt and inhibit lipid peroxidation reactions (Niki, 2014). The alpha-tocopherol presents anti-inflammatory activity and modulates the expression of proteins involved in cholesterol metabolism (Wallert et al., 2014).

At the post-translational level, the alpha-tocopherol modulates proteins, such as kinase, lipooxygenase and phospholipase A2. At the transcriptional level, this compound is capable to modulate the transcription of the genes involved in the cell signaling and regulation of the cell cycle. The alpha-tocopherol also has the function of cell proliferation inhibition, platelet aggregation and monocytes adhesion (Azzi et al., 2004; Zingg & Azzi, 2004).

Gamma-tocopherol is regarded as the most potent free-radical remover NO(X) among the isomers of vitamin E. In addition, studies show that gamma-tocopherol has strong anti-inflammatory activity and is related to inhibition of carcinogenesis (Ju et al., 2010).

The vitamin E deficiency has been reported primarily in preterm infants, patients with severe malnutrition, genetic disorders of lipid metabolism and chronic gastrointestinal diseases. The main symptoms of this vitamin deficiency is hemolytic anemia and neurological symptoms (ataxia, peripheral neuropathy, myopathy, pigmentary retinopathy) (Péter et al., 2013).

Gamma-tocopherol is the most abundant tocopherol in the American diet; however, only alpha-tocopherol is related to the daily requirement of vitamin E, which makes this vitamin one of the most difficult nutrients obtained from the diet. Only 8% of men and 2% of women in the United States reach the estimated average requirement (Traber, 2006). A study performed with 2344 Brazilian adults verified a low daily ingestion of alpha-tocopherol, which average was lower...
than 50% of the recommended ingestion for the population mentioned (Pinheiro et al., 2011).

The richest dietary sources of vitamin E are vegetable oils. Other important sources of this vitamin are whole grains and nuts (Traber, 2012).

Triacylglycerols are the predominant components of vegetable fats and oils. Minor compounds include: mono- and diglycerides, free fatty acids, sterols, tocopherols, among others. Tocopherols play an important role for inhibiting the lipid oxidation in vegetable oils. The result of the autoxidation of fats and oils is the development of objectionable flavors and odors characteristic of the condition known as “oxidative rancidity” (Asif, 2011).

The determination of the minor compounds of vegetable oils, among them the tocopherol group, is essential for analytical evaluation of the quality, origin, extraction method, refining and possible adulteration that might occur in this product (Cert et al., 2000).

The control of vitamin E levels in foods is also of great importance to ensure that the daily ingestion is adequate, as this is an essential factor for good health (Sánchez-Pérez et al., 2000). In order to adjust the ingestion of nutrients by the population it is important to know the main food-sources, as well as the most consumed foods (Batista et al., 2007). There is little information concerning the concentration of vitamin E in foods sold in Brazil and the main tables of food composition do not have data concerning vitamin E content, or only show data of total tocopherol, without specifying the concentration of each isomer in the food.

The purposes of this paper was to determine the concentration of alpha- and gamma-tocopherol of vegetable oils acquired in large supermarkets of the city of Natal/RN and the relation of the values found for alpha-tocopherol to the nutritional requirement of vitamin E for adults.

2 Materials and methods

2.1 Samples

Four types of vegetable oils were analyzed: canola, sunflower, corn and soybean. Out of each type of oil, 3 main brands were analyzed. Out of each brand, 2 different batches were acquired and 2 bottles were acquired of each batch. This methodology was elaborated based on the sampling plan of industrialized products of the Brazilian Table of Food Composition (Universidade Estadual de Campinas, 2011). Moreover vegetable oils with labels that did not inform that it had the addition of tocopherols to the product were also selected.

Vegetable oils which manufacture had occurred until one month before the date of analysis. The selection of oils was performed at large supermarkets of Natal/RN and these products were transported to the Biochemistry Laboratory of Foods and Nutrition, located in the Department of Biochemistry of the Federal University of Rio Grande do Norte (RN), Brazil.

2.2 Dilution of the samples

For sample preparation, the dilution of 40 μL of certain types of vegetable oil in 960 μL of dichloromethane was performed (UV/HPLC - Vetec, Rio de Janeiro, Brazil) and shaken for 1 minute. The dichloromethane was chosen for the dilution because it allows a good solubility of oils and is also miscible with the solvent used in the mobile phase (methanol).

2.3 Determination of alpha- and gamma-tocopherol using High Performance Liquid Chromatography

After the dilution of the vegetable oil, direct injection of the sample was performed for the determination of the alpha and gamma-tocopherol through High Performance Liquid Chromatography (HPLC). For this purpose, 20 μL was applied in the Shimadzu chromatograph, using AD LC-10 Shimadzu pump, connected to a SPD-10A Shimadzu UV-VIS Detector and Chromatopac C-R6A Shimadzu Integrator to a LC Perkin Elmer CLC-ODS column (M) 4.6 mm × 25 cm. The mobile phase was methanol at 100%, in an isocratic system with 1.0 mL/min flow and wavelength of 292 nm.

The chromatographic method using detection by ultraviolet or fluorescence was standardized according to IUPAC and AOCS standards (Cert et al., 2000). The HPLC use, in a reverse phase, is not capable to separate beta- and gamma-tocopherol. However, for edible vegetable oils, this was presented as a minimum error of quantification, since, in accordance with the literature, the oils do not contain beta-tocopherol or contain relatively small amounts (0-28.0 mg/kg depending on the analyzed oil) in comparison to the gamma-tocopherol (Gliszczynska-Swiglo & Sikorska, 2004).

The identification and quantification of the alpha-tocopherol and gamma-tocopherol in the samples had been established by comparison with the time of retention and the area of the respective standards (Figure 1). The concentration of the standards was confirmed by the specific extinguishing coefficient (ε 1%, 1 cm = 75.8 to 292 nm for alpha-tocopherol and ε 1%, 1 cm = 91.4 to 298 nm for gamma-tocopherol), both in absolute ethanol (Merck, São Paulo, Brazil) (Nierenberg & Nann, 1992; Podda et al., 1996). The analytical procedure of sample preparation and of the standards was performed under a temperature of about 25 °C and in pipes packed with paper aluminum.

The linearity of the method was verified through the calibration curves for the alpha-tocopherol and the gamma-tocopherol. These were constructed by a linear regression (concentrations of standards × standards area) and a value of r² = 0.9992 and r² = 0.9999 were obtained for the alpha-tocopherol and the gamma-tocopherol, respectively. The limits of detection and quantification of alpha-tocopherol had been based on the linearity of the standard curve, obtaining the values of 13 ng/20μL and 26 ng/20μL, respectively. The limits of detection and quantification of gamma-tocopherol had been based on the linearity of the standard curve, obtaining the values of 5.75 ng/20μL and 11.50 ng/20μL, respectively. The precision of the method was verified through repetition of the test, and the variation coefficient was lower than 4%.
3.1 Concentration of tocopherols in vegetable oils

Regarding the quantitative analysis, the following average concentrations of alpha-tocopherol referring to each type of oil were found: (120.3±4.2) mg/kg of canola oil; (432.3±86.6) mg/kg of sunflower oil; (173.0±82.3) mg/kg of corn oil and (71.3±6.4) mg/kg of soybean oil.

In relation to gamma-tocopherol concentrations the following results were found: 122.0±7.9 mg/kg of canola oil; 92.3±9.5 mg/kg of sunflower oil; 259.7±43.8 mg/kg of corn oil and 273.3±11.1 mg/kg of soybean oil.

After statistical analysis, a significant difference between the alpha-tocopherol concentrations was verified in the oils evaluated. After comparison between the gamma-tocopherol concentrations in the analyzed products, it was noted that there is a statistically significant difference between the types of oils, except among corn and soybean oils (p = 0.286). Considering that, it is noted that the lipid portion of grains or seeds of which the vegetable oils are extracted has well distinct concentrations of tocopherols.

3.2 Concentration of tocopherol by vegetable oils brands

The concentrations of alpha- and gamma-tocopherol in vegetable oils related to the analyzed brand are shown in Tables 1 and 2.

It was verified that there isn’t a significant difference between the alpha-tocopherol concentrations in the three analyzed brands of canola oils as well as of the soybean oils (Table 1). A similar result was verified for the gamma-tocopherol concentrations in the analyzed products, it was noted that there is a statistically significant difference between the types of oils, except among corn and soybean oils (p = 0.286). Considering that, it is noted that the lipid portion of grains or seeds of which the vegetable oils are extracted has well distinct concentrations of tocopherols.

3.3 Tocopherol distribution in vegetable oils

It was observed that, among the analyzed products, the richest in alpha-tocopherol are the sunflower and corn oils. Moreover, the gamma-tocopherol has a higher concentration in soybean and corn oils. It was also observed that the
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3.4 Vegetable oils as food source of vitamin E

Considering the amount of alpha-tocopherol in the portion of oils recommended (Figure 3), it was found that a portion of canola, sunflower, corn and soybean oil are equivalent, respectively, to 6.4%; 23.1%; 9.2% and 3.8% of the nutritional requirement of vitamin E. Thus, the soybean oil was not considered as a food-source, the canola and corn oils were classified as an excellent source of vitamin E.

4 Discussion

Vitamin E is the main fat-soluble vitamin in vegetable oils, which contain concentrations of total tocopherols in the range of 200-1000 mg/kg. These compounds are generally derived from the extraction of oil seeds (Chen et al., 2011).

The concentration of alpha-tocopherol found in the canola oil was lower than the value displayed on the food table composition of the United States Department of Agriculture (2008) (175.0 mg/kg). It was also lower than the values found in Canada (274.0 mg/kg), Chile (247.0 mg/kg) and Brazil (184.0 mg/kg; 269.0 mg/kg) (Ahmed et al., 2005; Romero et al., 2007; Guinazi et al., 2009; Branco, 2009). On the other hand, the concentration of alpha-tocopherol obtained in this study was higher than the value found in a study conducted in Costa Rica (69.0 mg/kg) (Monge-Rojas & Campos, 2011). In relation to the gamma-tocopherol concentration, it was verified that the values found are lower than the value found in Canada (237.0 mg/kg) (Ahmed et al., 2005), in Brazil (442.0 mg/kg;
501.0 mg/kg) (Guinazzi et al., 2009; Branco, 2009), in Chile (348.0 mg/kg) (Romero et al., 2007) and in the American food table (273.0 mg/kg) (United States Department of Agriculture, 2008) and is in accordance with the results obtained in Costa Rica (100.0 mg/kg) (Monge-Rojas & Campos, 2011).

In relation to the sunflower oil, it was observed that the alpha-tocopherol concentration was similar to the concentration found on the food table composition of the United States Department of Agriculture (2008) and in Lechner’s paper et al. (1999) (411.0 mg/kg and 425.0 mg/kg, respectively). Moreover, it is higher than the result found by Ortiz et al. (2006) (359.0 mg/kg) and by Monge-Rojas & Campos (2011) (150.0 mg/kg). However, the concentration found was lower than the concentration found by Barrera-Arellano et al. (2002), Franke et al. (2007), Branco (2009), Gliszczyńska-Świglo et al. (2007), Kamal-Eldin (2006) and Schwartz et al. (2008), that were 575.0 mg/kg; 541.8 mg/kg; 666.0 mg/kg; 575.0 mg/kg; 671.0 mg/kg and 590.0 mg/kg, respectively. In relation to the gamma-tocopherol concentration, it was noted that the value found in our paper was higher than the value found in the literature (Ortíz et al., 2006; Branco, 2009; Monge-Rojas & Campos, 2011; Lechner et al., 1999; Barrera-Arellano et al., 2002; Franke et al., 2007; Gliszczyńska-Świglo et al., 2007; Kamal-Eldin, 2006; Schwartz et al., 2008).

It was verified that the concentration of alpha-tocopherol in the corn oil analyzed had similar value to the value found in the food table composition of the United States Department of Agriculture (2008) (143.0 mg/kg) and to the value found in studies performed in Brazil (169.0 mg/kg) (Lima & Gonçalves, 1997) and in Austria (180.0 mg/kg) (Schwart et al., 2008). However, it was lower than the value found in Egypt (417.0 mg/kg) (Ramadan & Wahdan, 2012), in the United States (222.1 mg/kg) (Franke et al., 2007), Brasil (240.0 mg/kg) (Branco, 2009), Poland (207.0 mg/kg) (Gliszczyńska-Świglo et al., 2007), Sweden (222.0 mg/kg) (Kamal-Eldin, 2006) and Spain (204.0 mg/kg) (Sánchez-Pérez et al., 2000). However, the concentration of alpha-tocopherol was higher than the value obtained in Costa Rica (69 mg/kg) (Monge-Rojas & Campos, 2011). Regarding the concentration of gamma-tocopherol found in the corn oil, it was verified that this is lower than the concentration found in the literature and similar to that obtained by Monge-Rojas and Campos (2011) (220 mg/kg) (Sánchez-Pérez et al., 2000; Branco, 2009; Franke et al., 2007; Gliszczyńska-Świglo et al., 2007; Kamal-Eldin, 2006; Schwartz et al., 2008; Lima & Gonçalves, 1997; Ramadan & Wahdan, 2012).

The concentration of alpha-tocopherol in the soybean oil analyzed is similar to the concentration in the work performed in Canada (92.8 mg/kg) (Ahmed et al., 2005) and found in the food table composition of the United States Department of Agriculture (2008) (81.8 mg/kg). However, it is lower than the concentration found in Spain (149.0 mg/kg) (Barrera-Arellano et al., 2002), Poland (152.0 mg/kg) (Gliszczyńska-Świglo et al., 2007), Brazil (Guinazzi et al., 2009; Lima & Gonçalves, 1997; Branco, 2009; Carvalho et al., 2008) (121.0 mg/kg, 134.0 mg/kg, 181.0 mg/kg, 180.1 mg/kg) and Sweden (116.0 mg/kg) (Kamal-Eldin, 2006). Moreover, this concentration obtained appeared higher than that in a study conducted in Costa Rica (25.0 mg/kg) (Monge-Rojas & Campos, 2011). The concentration of gamma-tocopherol in the soybean oil was lower than the values found in the literature and higher to that result found in Costa Rica (210.0 mg/kg) (Ahmed et al., 2005; Guinazzi et al., 2009; Branco, 2009; Monge-Rojas & Campos, 2011; Barrera-Arellano et al., 2002; Gliszczyńska-Świglo et al., 2007; Kamal-Eldin, 2006; Lima & Gonçalves, 1997; Carvalho et al., 2008).

The tocopherols are one of the main groups regarding the portion of the minor compounds in vegetable oils. Oils originated from the same species can have variable content and composition of these minor compounds, due to climatic and agronomic conditions; fruit quality or seed origin; oil extraction system and refining procedure (Cert et al., 2000).

Traditionally, the purpose of the vegetable oils refining is to eliminate all the impurities in crude oils in order to get a vegetable oil of good quality (Medina-Juárez et al., 2000). The volatility of tocopherols is less than free fatty acids and greater than the triacylglycerols. Thus, during the refining process, tocopherols are partially lost during deodorization (Chen et al., 2011). A study of sunflower oil showed that the concentration of tocopherols and isomers, individually analyzed are reduced during refining of vegetable oils (Naz et al., 2011).

Considering the high potential of anti-oxidant of tocopherols in vegetable oils and the nutritional contribution of vitamin E, it is evident that the establishment of the ideal operational conditions is important during the refining process in order to reduce the loss of these tocopherols (Medina-Juárez et al., 2000).

The variability in the vegetable oils composition by intrinsic or external factors can be related to the concentrations of alpha and gamma-tocopherol found in our work, which are conflicting with the literature. The food industries must verify the origin of the grain or seed and select raw material of higher quality for production of vegetable oils. Moreover, the conditions of culture, storage and processing of the seeds or grains must be proper and constantly evaluated and monitored for the preservation of the organoleptic and nutritional quality of the final product.

Considering the differences between the several brands, the hypothesis is that the factors mentioned by Cert et al. (2000) and by Medina-Juárez et al. (2000) can have influenced the alpha- and gamma-tocopherol concentration in the sunflower and corn oils analyzed.

The richest in alpha-tocopherol are the sunflower and corn oils. This information regarding the sunflower oil is in accordance with the statement that this compound is found in higher concentrations in wheat germ oils, olive oil and sunflower oil (Traber, 2006; Gliszczyńska-Świglo et al., 2007; Smolarek & Suh, 2011; Velasco et al., 2010).

The gamma-tocopherol has a higher concentration in soybean and corn oils and similar information was related by Traber (2006) and by Gliszczyńska-Świglo et al. (2007). The tocopherol ratio varies according to the oil type, as well as by other factors, for example, climate and origin of the culture (Ortíz et al., 2006).
Some studies reported the inadequate ingestion of alphatocopherol (Traber, 2006; Pinheiro et al., 2011). Considering this, it is important to verify the relation between the alpha-tocopherol concentration in the oils analyzed and the nutritional requirement of vitamin E. According to the Dietary Reference Intakes (DRI), the daily recommendation of vitamin E for healthy individuals from 14 years is of 15 mg of alpha-tocopherol (Institute of Medicine, 2000). This recommendation is based on studies of vitamin deficiency induced in humans and in trials of hemolysis in vitro caused by hydrogen peroxide (Ramalho et al., 2006).

Despite the gamma-tocopherol predominance in the detriment of alpha-tocopherol in soybean oil, this product is the main contributor for the ingestion of vitamin E for the population (Guinazi, 2004). Considering the findings of this paper, the consumption of oils with higher concentrations of alpha-tocopherol (sunflower and corn) needs to be stimulated in order to improve the ingestion of this vitamin.

5 Conclusions

It is concluded that the sunflower and corn oils have the highest concentrations of alpha-tocopherol, whereas the vegetable oils with higher concentrations of gamma-tocopherol were the soybean and corn oils. Moreover, the soybean oil was not considered as a food source of vitamin E, the canola and corn oils had been classified as a food source and the sunflower oil was considered as an excellent source of this vitamin.

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References


Lechner, M., Reiter, B., & Lorbeer, E. (1999). Determination of tocopherols and sterols in vegetable oils by solid-phase extraction and subsequent capillary gas chromatographic analysis. Journal of...


