Kinetics of Tocopherol Degradation During the Storage of Wheat Germ Oil

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Tocopherol degradation in wheat germ oil was studied during storage at different temperatures. Two types of wheat germ were used: heat-treated germ (HT) and raw germ (RG). The oil was extracted (Soxhlet, *n*-hexane) and stored at 25 and 47 °C (82 days) and at -20 and 5 °C (600 days). Tocopherols were determined by HPLC. Total tocopherol, α-tocopherol, and β-tocopherol decreased following first-order kinetics. At 25 and 47 °C, the values of the degradation rate constants for total tocopherol were 5.00×10^{-3} days^{-1} (RG), 3.00×10^{-3} days^{-1} (HT), and 5.37×10^{-2} days^{-1} (RG), 2.57×10^{-2} days^{-1} (HT), respectively, indicating an increase in tocopherol degradation rate with increasing temperature. The reaction rates of total tocopherol were similarly affected at 5 and −20 °C, with lower rate constants (4×10^{-4} and 2×10^{-4} days^{-1}, respectively). α-tocopherol degraded faster than β-tocopherol, presenting higher rate constants. Low temperatures favour the preservation of tocopherols.

Keywords: wheat germ, oil, storage, kinetics, tocopherols

INTRODUCTION

Wheat germ constitutes about 2–3 % of the whole wheat kernel, and 10 % of the germ is oil.[1] The germ is removed from the endosperm during wheat milling and is mainly used for fodder and oil production. Wheat germ oil has important health benefits, such as lowering blood cholesterol levels, improving physical strength, and possibly reducing the effects of aging, due to its high concentration of nutrients. It is rich in tocopherols (vitamin E), phytosterols, policosanols, thiamine, riboflavin, and niacin.[2]

Of all the vegetable oils, wheat germ oil has the highest tocopherol content (over 2000 mg/kg), consisting mainly of α-tocopherol (over 0.46 g/g, 46 mass %), with β-tocopherol and γ-tocopherol in lower proportions, and low concentrations of δ-tocopherol.[3,4,5] The most important chemical property of tocopherols is their antioxidant activity. The main physiological function of α-tocopherol and other tocols is to delay the progression of a variety of degenerative diseases.[6] Vitamin E is only synthesized by higher plants and cyanobacteria, and it is essential for humans to get it from their diet. The phytosterols present in wheat germ oil include sitosterol and campesterol.[1,2] This oil is also valued for its fatty acid content, which includes linoleic (0.55 g/g, 55 mass %), palmitic (0.16 g/g, 16 mass %), oleic (0.14 g/g, 14 mass %), and linolenic (0.7 g/g, 7 mass %) acids.[7]

Despite its important nutritional value, wheat germ has the disadvantage of having a short shelf life due to the presence of unsaturated fatty acids and hydrolytic and oxidative enzymes, such as lipase and lipoxygenase. The oxidation of the fatty acids and vitamins in the wheat germ can be prevented and its shelf life extended by heat-induced enzyme inactivation or by removing the oil fraction.[8] Heating is an important part of many food processing operations. Many desirable changes, as well as undesirable reactions, occur in vegetable oils when they are heated at an elevated temperature. However, during heating, vegetable oils are very susceptible to quality changes, caused by chemical instability, that are dependent on both chemical composition and environmental factors. Lipid oxidation is one of the major deleterious reactions during heating that markedly affects the quality of vegetable oils.[9]

The degradation of lipid quality due to oxidation is of great economic and nutritional importance for the food industry due to the loss of essential fatty acids and fat-soluble vitamins. Lipid peroxidation also produces free radicals, which are associated with health issues such as carcinogenesis. Although antioxidants cannot hinder the self-oxidation of lipids or revert the formation of peroxides, they can delay the process by removing the fatty acid radicals.[10]

Oil storage results in considerable destruction of tocopherols, depending on the oil, time, temperature, light, and concentration of antioxidants. For example, safflower oil loses 45 % of its tocopherol content after storage at 10 °C for 6 months and 70 % of tocopherols after storage at 37 °C for 3 months.[11] Player et al.[12] and Abramovic et al.[13] studied the degradation of α-, γ-, and δ-tocopherol in soybean and *Camelina sativa* oils, respectively, during storage in the dark at 50 °C. They found that total tocopherol content in oil decreased during storage, where α-tocopherol completely disappeared after 16 and 27 days, respectively. Koski et al.[14] found that α-tocopherol in canola oil was completely consumed within 7–11 days of storage in the dark at 60 °C.

Even though tocopherols are stable during freezing, Bunnell was one of the first to conclude that low storage temperatures do not prevent the oxidation of tocopherols, and substantial losses can occur over time.[11] For temperatures between 6–18 °C, a

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lower α-tocopherol degradation rate was observed in virgin olive oil.[15]

On the other hand, when Chung[16] studied the oxidative degradation kinetics of tocopherols as a function of time and temperature in solutions of α-, γ-, and δ-tocopherol in glycerol heated to 100–250 °C for 5–60 min, he determined that the reaction followed first-order kinetics. Lavelli et al.[17] observed a decrease in α-tocopherol following pseudo first-order kinetics in extra virgin olive oil stored at 40 °C and 25 °C for 8 months.

Given the importance of tocopherols in disease prevention and for extending the shelf life of food products, their preservation during storage becomes a priority.[18] The aim of the present work was to study the effects of temperature and storage time for wheat germ oil on tocopherol concentration by analyzing the degradation kinetics of tocopherols.

MATERIALS AND METHODS

Samples

The wheat germ was provided by Molino Cañuelas SACIFI (Buenos Aires Province, Argentina). Two types of wheat germ were studied for comparison: raw germ (RG) and heat-treated germ (HT). The treatment of the wheat germ was performed by the provider using a turbo-dryer with steam at 1400 kPa pressure and temperature at 160 °C for 90 sec, feed intake = 35 °C and output = 60 °C. Samples of 50 kg of each type of wheat germ were prepared. These were stored in plastic containers at 5 °C until further use.

The wheat germ samples were characterized for: moisture (AOCS Ba 2a-38[19]), oil content (IUPAC 1.122[20]), nitrogen content (AOCS Ba 4a-38[19]), crude fibre content (AOCS Ba 6-84[19]), and ash (AOCS Ba 5a-49[19]). Protein content was calculated as nitrogen content × 6.25. Carbohydrate content was estimated as equal to nitrogen-free extract (NFE), calculated using Equation (1).

$$NFE = 100 - (oil + protein + crude\ fibre + ash) \quad (1)$$

The wheat germ oils were characterized for free fatty acid content (free acidity expressed as the percentage of oleic acid, IUPAC 2.201[20]), peroxide value (IUPAC 2.501[20] and AOCS Cd 8-53[19]), fatty acid composition (Izquierdo[21]), and tocopherol concentration (AOCS Ce 8-89[19]).

Sample preparation

Wheat germ oils (RG, HT) were extracted with *n*-hexane in a Soxhlet apparatus (Buenos Aires, Argentina) by ten thermal cycles at 80 °C, using 150 mL of solvent and 50 g of germ, following the modified IUPAC Standard Method (IUPAC[20]). This technique was used for all the cases, and is valid for the conditions used. This method is widely employed and very accessible for extraction of cereal and oleaginous oils. In this work, extraction time was 8 h. The solvent was removed using a rotary vacuum evaporator at 40 °C (Büchi, Waterbath B-480, Switzerland). The recovered oil was kept in a caramel-coloured bottle and the residual hexane was removed under a nitrogen stream.

The oil samples were then stored at 25 and 47 °C for 82 days and at -20 and 5 °C for 600 days in 1.5 mL plastic vials on sealed trays to protect them from light. During that time, tocopherol content of the oil was analyzed, and for the oil stored at higher temperatures (25 and 47 °C), the acid composition was also tested following the standard methods mentioned above. For each analysis three oil samples were used.

Tocopherol Concentration

Tocopherol concentration in the wheat germ oil was measured through high-performance liquid chromatography (HPLC) in normal phase and isocratic mode, according to standard methods IUPAC 2.432[20] and AOCS Ce 8-89.[19] A series 1050 Hewlett Packard machine (Waldbronn, Germany) with a fluorescence detector (Agilent, 1100 Series, $\lambda_{excitation}$: 292 nm, and $\lambda_{emission}$: 330 nm), a Hichrom column, Lichrosorb Si 60, 250 × 4.6 mm inner diameter, and 5 μm particle size (Merck, Darmstadt, Germany) was used. A solution of isopropanol 0.005 L/L (0.5 % v/v) in hexane was used as the mobile phase, with a flow rate of 1.5 mL/min. The peaks in the chromatogram were identified by taking into account the retention times of the different tocopherol isomers. The isomers were quantified using the external standard method with α-tocopherol as a reference (Sigma T3251, 95 % purity; AOCS Ce 8-89 standard method), and the results were expressed as μg tocopherol/g oil.

Degradation Kinetics of Tocopherols

The degradation kinetics of tocopherols were analyzed by considering first-order kinetics, taking into account previous works by Chung.[16]

The values of the reaction rate constants (k_{exp}) for the different oils were calculated using the integrated rate equation for first-order reactions (Equation 2):

$$\ln Ct = \ln C_o - k_{\exp} \times t \quad (2)$$

where Ct is the tocopherol concentration at different storage times (t), and C_o is the initial tocopherol concentration. Plotting ln Ct versus t, the value of the reaction rate constant (k_{exp}) can be determined from the slope of the curve for each working temperature.

The analysis of the influence of temperature on the reaction rate, which is observed in the increase in the rate constant (k_{exp}) with increasing temperature, is represented using the Arrhenius equation (Equation 3):

$$\ln k_{\exp} = \ln A - Ea/RT \quad (3)$$

where k_{exp} is the experimental rate constant corresponding to the thermal degradation of tocopherols at the absolute temperature T (K), Ea is the activation energy (energy per unit amount of substance), A is the Arrhenius pre-exponential factor (identical units to k_{exp}), and R is the ideal gas constant (8.3144 J/ mol K). The values of the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were obtained using the Eyring equation (Equation 4):

$$\ln\left(\frac{k_{\exp}}{T}\right) = \frac{\Delta H^{\ddagger}}{RT} + \frac{\Delta S^{\ddagger}}{R} + \ln\left(\frac{R}{Na\ h}\right) \quad (4)$$

where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the enthalpy and entropy of activation, respectively; T is the temperature in Kelvin (K) at which each rate constant k_{exp} was determined; R is the gas constant (8.3144 J/ mol K); N_a is the Avogadro constant (6.02×10^{23} molecules/mol), and h is Planck's constant (6.6252×10^{-34} J sec). Based on the graphic representation of ln (k_{exp}/T) as a function of $1000/T$, the corresponding activation parameters can be determined.

Statistical Analysis of the Data

An analysis of variance (ANOVA) was performed using Infostat 6.0 software.[22] When significant differences were observed among means of the studied variables ($p < 0.05$), they were compared using Tukey's test.

RESULTS AND DISCUSSION

Characterization of Wheat Germ and Wheat Germ Oil

The proximate compositions of raw (RG) and heat-treated (HT) wheat germs and the characteristics of the oils of both germs are shown in Table 1.

The samples of RG and HT wheat germ presented oil, protein, and crude fibre contents close to the ranges reported in the literature.[3,23,24]

Both oils exhibited high free acidity, indicating the action of the lipases present in the wheat germ and/or an incomplete deactivation of those enzymes. Both samples also presented similar fatty acids composition, and no influence of the heat treatment could be detected on that content.

Tocopherol Content

The initial concentration of total tocopherols was 3440 ± 28.6 μg/g of oil in the RG oil and 3545 ± 33.6 μg/g of oil in the HT oil, with α-tocopherol being the most abundant isomer (0.642 and 0.656 g/g, 64.2 and 65.6 mass %, respectively), followed by β-tocopherol (0.333 and 0.328 g/g, 33.3 and 32.8 mass %), and small amounts of γ-tocopherol (0.025 and 0.016 g/g, 2.5 and 1.6 mass %). Statistical analysis of the data showed that the HT wheat germ oil presented significantly higher concentrations of total tocopherols ($p = 0.0023$), α-tocopherol ($p = 0.0033$), and β-tocopherol ($p = 0.0081$). The higher tocopherol concentration in HT wheat germ oil can be attributed to a dilution effect, because a higher oil percentage was extracted from the RG (Table 1). The lower total tocopherol concentration in the RG oil is also in agreement with the higher peroxide index detected in that sample, because it has less protection against oxidation.

Oil Storage

Effects of oil storage on tocopherols

A statistical analysis (ANOVA) was performed to study the variation of tocopherols in the oil of each type of wheat germ independently. Each type of germ (RG and HT) was analyzed at temperatures of 47 and 25 °C considering five storage times (0, 9, 23, 49, and 82 days) (Table 2), and at 5 and −20 °C for another set of three storage times (0, 300, and 600 days) (Table 3). For both groups of temperatures analyzed, the variables of time and temperature significantly affected ($p < 0.0001$) the total tocopherol concentration for both wheat germ oil samples. A significant time × temperature interaction ($p < 0.0001$) was also detected. Total tocopherol concentration decreased significantly with storage time for all the studied temperatures, and in general the decrease was higher with increasing temperature. The effect of temperature was more pronounced on the RG oil (with lower tocopherol content), and the tocopherol concentration decreased significantly during storage at 47 °C (98 % in 82 days) compared with 25 °C (36.2 % in 82 days). Losses of 85.9 and 29.4 % of tocopherols were observed for the HT wheat germ oils at 82 days of storage at 47 and 25 °C, respectively (Table 2). These losses were greater than those observed for the oils stored at 5 and −20 °C (21.5 and 9.1 % for RG samples, 19.7 and 11.3 % for the HT samples, respectively) (Table 3). A similar pattern was reported by Bauernfeind and Desai[11] and Okogeri and Tasioula-Margar[15] when respectively studying the storage of safflower and virgin olive oil. Regarding α-tocopherol (the most abundant tocol), in general it exhibited similar behaviour to the total tocopherols. The

Table 1. Proximate composition of RG and HT wheat germ (g/100 g db) and initial physicochemical characteristics of RG and HT oils

	Wheat germ	
Component	RG	HT
Moisture	14.00 ± 0,01	7.65 ± 0.11
Oil	10.3 ± 0.05	8.4 ± 0.05
Ash	4.00 ± 0.37	4.32 ± 0.13
Crude fibre	1.19 ± 0.01	1.75 ± 0.01
Protein*	32.68 ± 0.11	32.25 ± 0.26
NFE	51.83 ± 0.52	53.28 ± 0.19
	RG oil	HT oil
Free fatty acids (% oleic acid)	8.2 ± 0.15	8.9 ± 0.03
Peroxide value (mequiv peroxide/kg)	1.9 ± 0.12	1.2 ± 0.19
Fatty acids[1]		
Palmitic acid (16:0)	18.5	18.2
Stearic acid (18:0)	0.5	0.5
Oleic acid (18:1)	13.8	14
Linoleic acid (18:2)	60.8	60.5
α-Linolenic (18:3)	6.4	6.8

Results are expressed as (x ± d), x being the average, and d, the standard deviation (n = 3).
*Factor: 6.25; NFE: nitrogen-free extract.
[1]Expressed as fatty acids % total methyl esters.

Table 2. Total, α- and β- tocopherols concentration (μg tocopherol/g oil) for RG and HT oils stored at 47 °C and 25 °C for different periods of time. Values followed by different letters, for each wheat germ oil, indicate significant differences. (Tukey's test: $p \leq 0.05$, n = 3)

	Tocopherols concentration (μg/g)			
Time (days)	RG		HT	
Totals	47 °C	25 °C	47 °C	25 °C
0	3440 j	3440 j	3545 k	3545 k
9	2450 e	3190 gh	3395 ghi	3345 fgh
23	1691 c	3130 g	2976 d	3336 fg
49	415 b	2903 f	1293 b	3206 e
82	53 a	2195 d	499 a	2739 c
α-toc				
0	2210 i	2210 i	2325 i	2325 i
9	1444 e	2053 h	2158 f	2280 h
23	121 c	1814 g	1600 c	2163 gf
49	30 ab	1600 f	374 b	1990 e
82	6 a	1205 d	35 a	1690 d
β-toc				
0	1146 i	1146 i	1164 ghi	1164 ghi
9	1036 ef	1088 gh	1102 ef	1144 fgh
23	800 c	1050 efg	900 c	1114 fg
49	90 ab	1000 de	490 b	1059 de
82	37 a	960 d	300 a	1027 d

Table 3. Total α- and β- tocopherols concentration (μg tocopherol/g oil) for RG and HT oils stored at 5 °C and −20 °C for different periods of time. Values followed by different letters, for each wheat germ oil, indicate significant differences. (Tukey's test: $p \leq 0.05$, $n = 3$)

	Tocopherols concentration (μg/g)			
Time (days)	RG		HT	
Totals	5 °C	−20 °C	5 °C	−20 °C
0	3440 e	3440 e	3545 d	3545 d
300	3025 b	3234 d	3279 c	3269 c
600	2701 a	3126 c	2848 a	3145 b
α-toc				
0	2210 e	2210 e	2299 e	2299 e
300	1954 bc	2010 d	2044 cd	2005 c
600	1500 a	1953 b	1741 a	1946 b
β-toc				
0	1146 e	1146 e	1164 cde	1164 cde
300	1090 bcd	1080 abc	1130 bc	1135 bcd
600	1051 ab	1012 a	1090 ab	1065 a

gradual decrease in α-tocopherol concentration over storage time at 47 and 25 °C for the oils of both samples (RG and HT) is presented in Figures 1a and 1b, respectively. Gutiérrez and Fernández[25] reported that virgin olive oil stored at 2 °C and in darkness maintained unaltered α-tocopherol contents. However, in oils stored at 30 °C with illumination, the α-tocopherol level fell 97.3 % after 180 days. In contrast, Goffman and Möllers[10] reported that total tocopherol contents remained unaltered at 5 °C over 24 weeks of storage, and at 40 °C tocopherols were completely degraded in canola oil.

Although a small effect of storage on β-tocopherol concentration was detected at the lower temperatures (25, 5, and −20 °C), this decrease was not statistically significant. In contrast, β-tocopherol concentration decreased progressively over time at 47 °C, with a loss of 70 % for the HT oil and approximately 90 % for the RG oil. γ-tocopherol decreased significantly only during storage at 47 °C (losses of 70 and 80 % for HT and RG wheat germ oils, respectively; data not shown).

Degradation kinetics of tocopherols

The results obtained show that the degradation of total tocopherols and of α- and β-tocopherol in the temperature range studied (−20–47 °C) followed first-order kinetics, a model similar to that reported by Chung[16] to study the degradation kinetics of the different isomers of α-tocopherol in a glycerol solution at 100–250 °C, and to that reported by Lavelli et al.[17] for α-tocopherol in olive oil stored at 40 and 25 °C. Figures 2 and 3 show the plots of residual α-tocopherol content versus time of storage at 47 °C for RG and HT oils, respectively.

The rate constants and correlation coefficients for total tocopherols in the RG and HT oils determined at each temperature are presented in Table 4. The calculated values of k_{exp} ranged from 0.0001 day^{-1} (at −20 °C) to 0.0537 day^{-1} (at 47 °C), and the values were similar for both oils at each temperature studied. The higher rate constant values at higher temperatures (47 and 25 °C) indicated a higher degradation rate of tocopherols with increasing temperature.

On the other hand, in both oils the degradation rate constants obtained for α-tocopherol (Table 4) were higher than those for β-tocopherol (Table 4), suggesting faster α-tocopherol degradation compared with β-tocopherol.

When Lavelli et al.[17] studied the effect of storage at 40 °C on the content of α-tocopherol in extra virgin olive oils, they determined rate constants of decreasing tocopherols in the $0.77–1 \times 10^{-3}$ day^{-1} range. Widicus et al.[26] reported rate constants in the $3.23–15.94 \times 10^{-3}$ day^{-1} range for the degradation of α-tocopherol in a model food system containing no fat, stored isothermally at 20, 30, or 37 °C. Hidalgo et al.[18] determined rate constants in the $0.04–9.50 \times 10^{-3}$ day^{-1} range for the degradation of α-tocopherol in flours of different varieties of wheat stored between −20–38 °C. These results, together with those obtained in the present work,

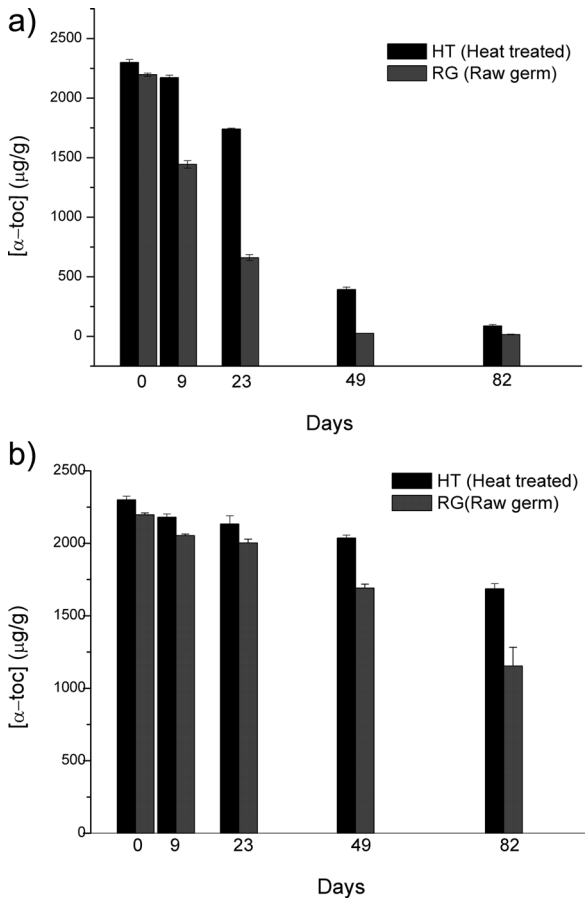

Figure 1. Concentration of α-tocopherol (μg tocopherol/g oil) of wheat germ oils stored for different periods of time (a) at 47 °C; (b) at 25 °C. RG: raw wheat germ; HT: heat-treated wheat germ.

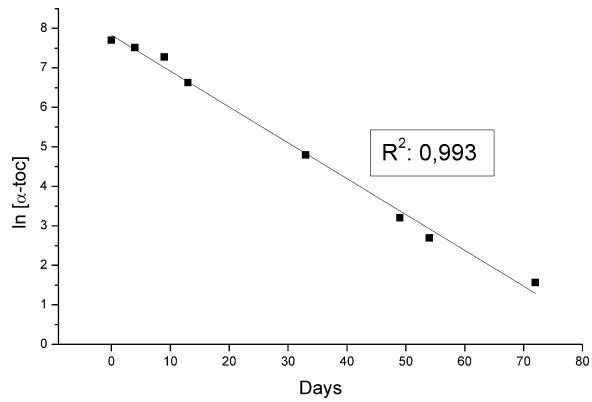

Figure 2. Plot of residual α-tocopherol (μg tocopherol/g oil) in RG wheat germ oil stored at 47 °C for different storage time periods.

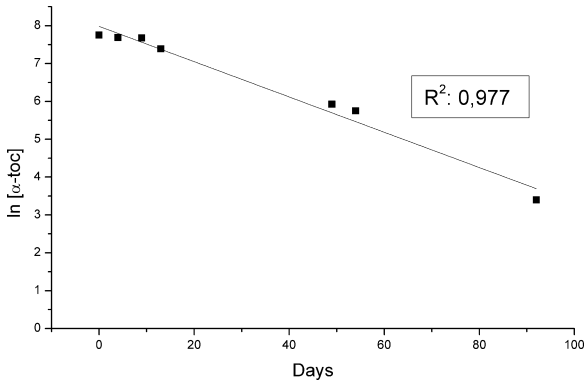

Figure 3. Plot of residual α-tocopherol (μg tocopherol/g oil) in HT wheat germ oil stored at 47 °C for different storage time periods.

show an important effect of temperature on the degradation rate of α-tocopherol.

The thermodynamic parameters for α-and β-tocopherol are presented in Table 5. The activation energies (E_a) and enthalpies indicate that the degradation mechanisms were similar for the tocopherols in both oils. These results are in agreement with those reported by Widicus et al.,[26] who showed that the experimental activation energies for the degradation of α-tocopherol are in the 37.03–54.60 kJ mol^{-1} range, and those reported by Labuza[27] for the natural loss of α-tocopherol in seaweed meal (41–43.9 kJ mol^{-1}).

According to these results, the fact that ΔH is positive for the thermal degradation reaction shows that this process is endothermic. In addition, $\Delta S < 0$ indicates that there is a decrease in the entropy change.

A higher activation energy implies that a smaller temperature change is needed to induce a certain change in the rate of degradation.[9]

CONCLUSIONS

The degradation reaction of tocopherols during storage of wheat germ oil between -20–47 °C follows first-order kinetics, with an increase in the degradation rate with increasing temperature. The storage temperature with the highest effect on tocopherol degradation was 47 °C, whereas when the wheat germ oil was stored at 5 and −20 °C, the highest amounts of total tocopherols and of each isomer were preserved. The highest rate constants corresponded to α-tocopherol, indicating a lower stability than β-tocopherol. No effects of the stabilization process of wheat germ on the degradation kinetics of tocopherols during oil storage were detected. The study of the changes of tocopherols in wheat germ oil is a contribution to improving storage conditions and treatments in order to preserve the quality and nutritional value of the oil.

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NOMENCLATURE

RG Raw wheat germ
HT Heat-treated wheat germ

Table 4. Reaction rate constants and correlation coefficients for total α- and β-tocopherols degradation in RG and HT oils

Temperature (°C)	Rate constant (k_{exp})(days^{-1})	R^2	Rate constant (k_{exp})(days^{-1})	R^2
Totals	RG		HT	
47	0.0537	0.990	0.0257	0.992
25	0.005	0.992	0.003	0.996
5	0.0004	0.998	0.0004	0.980
−20	0.0002	0.986	0.0001	0.978
α-				
47	0.0907	0.992	0.0466	0.980
25	0.0073	0.986	0.0036	0.990
5	0.0006	0.958	0.0005	0.992
−20	0.0002	0.914	0.0003	0.880
β-				
47	0.0463	0.990	0.0165	0.974
25	0.0026	0.976	0.0016	0.988
5	0.0001	0.988	0.0001	0.974
−20	0.000 02	0.986	0.000 03	0.986

Table 5. Activation energies and thermodynamic properties for the thermal degradation of α- and β-tocopherol in RG and HT oils

Tocopherol	Oil	*Ea* (kJ/mol)	ln *A*	$\Delta H^{\ddagger}$ (kJ/mol)	$\Delta S^{\ddagger}$ (J/mol K)
α-tocopherol	RG	61.7	20.23	59.4	−84.44
	HT	50.03	15.03	47.73	−127.66
β-tocopherol	RG	78.75	25.99	76.45	−36.78
	HT	64.37	19.65	62.11	−89.49

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