

Degradation of Tocopherols in Rice Bran Oil Submitted to Heating at Different Temperatures

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Abstract

The objective of this study has been to evaluate the stability of α -, ($\gamma+\beta$)-, and δ -tocopherols in rice bran oil chemically refined submitted to heating in a heater without air circulation and shielded from light, at temperatures of 100°C and 180°C. The collection of samples took place after 48, 96, 144, 192, 240, 336, and 432 h of heating and were stored in amber-colored flasks and frozen (-18°C). The analyses of tocopherols took place in accordance with the method by Chen and Bergman (2005), with slight modifications, utilizing a system of high efficiency system of liquid chromatography. It was observed that the α -tocopherol is present at higher concentration in rice bran oil (328.4 mg/kg), followed by ($\gamma+\beta$)-tocopherol (99.1 mg/kg), and δ -tocopherol (7.7 mg/kg). The α -tocopherol in rice bran oil submitted to 100°C showed a reduction of 28.65% at the end of 432 h of heating whereas when submitted to 180°C temperature; its reduction was of 100% at the end of 240 h of heating. The contents of ($\gamma+\beta$)- and δ -tocopherol in rice bran oil at the end of 432 h of heating at 100°C was of 79.9 and 6.4 mg/100 g, respectively.

Introduction

Edible oils constitute, from a nutritional point of view, an excellent source of energy and an important vehicle of liposoluble vitamins in the human organism, in addition to being a source of essential fatty acids. However, oils are highly unstable at high temperatures and, as a result, subject to drastic changes in their quality and in their functional and nutritional properties when submitted to heating. These thermo-oxidating alterations are associated with a variety of illnesses such as cardiovascular problems, cancer, obesity, and multiple sclerosis (1,2).

Rice bran oil is extensively consumed as an edible oil in several Asian countries including Japan, China, Korea, India, Thailand, and Pakistan. India and Thailand are the main producers of rice bran oil and together sum over 225,000 tons of oil per year (3).

The utilization of this oil has increased considerably in Western countries due to its nutraceutical potential, the most

important components of which include tocopherols (Figure 1) and the *γ*-orizanol fraction (4–9).

Tocopherols are a composite of elements that exert biological activities (11,12) and assist in the stabilization of vegetable oils. This group includes the α -, β -, γ -, and δ -tocopherols, which differ among themselves by the localization of the methyl groups in the aromatic ring (13–17).

Tocopherols are the most abundant natural antioxidants found in vegetables, being recognized as excellent biological antioxidants, which protect cellular membranes and increase stability in vegetable oils and animal fat (18, 19). This compound inhibits lipid peroxidation by acting as captors of lipid peroxy radicals, preventing these radicals from reacting with the lateral chains of adjacent fatty acids or with membrane proteins. The OH group of the α -tocopherol donates its hydrogen atom to the peroxy radical (LOO-), forming an α -tocopheryl and a hydroperoxide, thus interrupting the chain reaction of the lipid peroxidation (20).

The α -tocopherol is found in practically all vegetable oils and presents the greatest biological activity among tocopherols. Its biological activity doubles that of the β - and γ - and is 100 times greater than that of the δ -tocopherol (17,21). However, the thermal stability of the α -tocopherol was lower than the others. Lampi et al. (10) also found that α -tocopherol at high concentrations in rapeseed oil, when heated in the presence of oxygen in the dark during 16 days at 40°C, presented lower stability than γ -tocopherol. Research carried out by Player et al. (22) evaluating the stability of the α -, γ -, and δ -tocopherols during the oxidation of soy bean oil at 50°C demonstrated that the α -tocopherol is degraded faster than the γ - and δ -tocopherols, when the α -tocopherol was completely destroyed within 16 days while the γ - and δ -tocopherols remained after heating for 24 days.

The objective of this study was to evaluate the stability of toco-

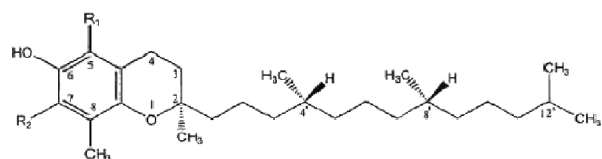


Figure 1. Structure of tocopherols (27).

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pherols present in refined rice oil when submitted to continuous heating under different temperatures.

Materials and Methods

Reagents and samples

Analytical-grade isopropanol, acetonitrile (Vetec, Rio de Janeiro, Brazil) and methanol (Synth, Diadema, Brazil) were used. Standards of α -tocopherol (99%, Merck, Darmstadt, Germany), γ -tocopherol (> 96%, Sigma), and δ -tocopherol (> 90%, Sigma) were also used.

Sample of rice bran oil were obtained from Irgovel (Indústria Riograndense de Óleos Vegetais Ltda., Pelotas, Brazil). The samples were collected directly from the chemical refining process of this company.

Instrumentation

A high-performance liquid chromatography (HPLC) system (Shimadzu) was utilized and was equipped with a solvents delivery module LC-10Atvp degasifier FCV-10Alvp, reodyne pump DGU-14a, control system SCL-10Aup, column oven CTO-10Asvp, and auto-sampler SIL-10AF. Separation was carried out in a reverse-phase analytical column, Shim-PAK-CLC-ODS (4.6 mm \times 150 mm \times 5 μ m). A fluorescence detector was utilized, with excitation of 290 nm and emission at 330 nm. Data were acquired and processed utilizing software Class-VP.

Methods

Rice bran oil was submitted to heating in a heater without air circulation and shielded from light. Two five-liter beakers were utilized, and three liters of oil were placed in each. One of the beakers was previously heated to 180°C \pm 2 and the other to 100°C \pm 2 and afterwards placed inside the heater at the corresponding temperatures.

In each experiment, samples were collected at pre-determined time intervals and stored in amber-colored flasks, which were frozen at -18°C up to the moment of the analyses. The experiment was carried out in duplicate.

Sample collection took place every 48 h for 432 h. The procedures for the determination of tocopherols were adapted from Chen and Bergman (23). Portions of ca. 250 mg oil were weighted and diluted with 5 mL of a 7:3 (v/v) isopropanol-acetonitrile mixture. To remove suspended solids, the mixtures were centrifuged at 9000 rpm (7245 g) for 6 min (NT-800 micro centrifuge, Nova Técnica, Piracicaba, Brazil), and the sample was transferred to a 1.5-mL vial. Aliquots of 20 to 40 μ L were injected in the LC.

The HPLC separation was performed at 25°C with a constant flow rate of 1 mL/min. The initial and final mobile phases were a 50:40:10 (A) and a 30:65:5 (B) acetonitrile-methanol-isopropanol mixtures (v/v/v), respectively. Isocratic elution of phase A for 5 min was followed by a linear gradient for 10 min to phase B, followed by 5 min of isocratic elution with phase B, and then returned to phase A in 5 min. Standards of α -, γ -, and δ -toco-

pherols were used to construct external calibration curves. The analyses were done in triplicate.

Statistical analysis

Analysis of variance (ANOVA) and comparison of averages by the Tukey's test were carried out using the program STATISTICA v. 6.0 (12). A 5% significance level was used in all cases.

Results and Discussion

It was observed that the α -tocopherol exists in higher initial concentration in rice bran oil followed by the (γ + β)- and δ -tocopherol, respectively of 328.4 mg/kg, 99.1 mg/kg, and 7.7 mg/kg (Table I, zero time). Pestana et al. (24) found for rice bran oil contents of α -tocopherol, (γ + β)- and δ -tocopherol of 215.4, 77.4, and 3.8 mg/kg respectively.

The α -tocopherol showed lower stability during heating, its content in rice bran oil submitted to 100°C showed a gradual reduction up to 432 h of heating, totalling a loss during this period of 28.65%. In rice bran oil submitted to 180°C, it was observed that the α -tocopherol showed degradation above 90% at the end of 144 h of heating, reaching degradation of 100% at the end of 240 h of heating (Table I).

The content of (γ + β)-tocopherol in rice bran oil submitted to 100°C showed degradation of 19.37% in the period of 432 h of heating, finalizing with 79.9 mg/kg. Rice bran oil submitted to 180°C showed a rapid reduction of (γ + β)-tocopherol, reaching a degradation percentage of 96.06% in 240 h of heating. (γ + β)-Tocopherol was reduced by more than 98.59% during heating at 180°C at the end of the heating period (432 h).

The content of δ -tocopherol in rice bran oil submitted to 100°C showed reduction of 0% and 10.39% for the periods of 48 h and 240 h respectively, reaching a degree of degradation of 16.88% in the period of 432 h. The δ -tocopherol present in rice bran oil submitted to heating at 180°C showed a gradual degradation in all the exposition period of the oil to heating, showing a reduction of 3.90% and 63.64% respectively, for the periods of 48 h and 240 h of heating. At the end of the period of 432 h, it showed degradation of 96.10%.

Table I. Contents of α -, (γ + β)-, and δ -Tocopherol (mg/Kg) in Rice Bran Oil Submitted to Temperatures*

Time (h)	100°C			180°C		
	α -T	(γ + β)-T	δ -T	α -T	(γ + β)-T	δ -T
0	328.4 a	99.1 a	7.7 a	328.4 a	99.1 a	7.7 a
48	321.7 b	88.4 b	7.7 a	217.5 b	56.8 b	7.4 a
96	316.6 c	86.4 b	7.5 b	134.4 c	50.5 c	7.5 a
144	315.1 d	85.9 c	7.3 b	32.7 d	17.6 d	6.6 b
192	286.6 e	82.7 c	7.2 b	2.0 e	8.7 e	4.2 c
240	268.8 f	81.3 d	6.9 c	0	3.9 f	2.8 d
336	257.7 g	81.0 d	6.9 c	0	2.0 ef	0.8 e
432	234.3 h	79.9 e	6.4 d	0	1.4 gf	0.3 fe

* Tests were run in an Oven Without Air Circulation and Shielded From Light. Values followed by the same low-cap letters in the same column do not differ among themselves by 5% in significance by the Tukey test ($p > 0.05$).

When comparing the behaviour to heating of the α -, ($\gamma+\beta$)-, and δ -tocopherols identified in rice oil (Figures 2A and 2B), it was observed that the α -tocopherol was the compound which showed a more rapid degradation at both heating temperatures. However, the 180°C temperature differed from the 100°C by influencing on the greater degradation speed of the α -, ($\gamma+\beta$)-, and δ -tocopherol.

The content of ($\gamma+\beta$)- and δ -tocopherol showed only a slight decrease during the heating period at 100°C, well below the decrease of the α -tocopherol. However, both presented a sharply higher decrease at the 180°C temperature, which demonstrates the greater stability of the α -, ($\gamma+\beta$)-, and δ -tocopherols in relationship to the α -tocopherol during oil heating at temperatures lower than 180°C.

The degree of degradation of tocopherols found in this study for rice oil at 100°C and 180°C temperatures showed the order: α - > ($\gamma+\beta$)- > δ -tocopherol, indicating that α -tocopherol was less stable. We are in agreement with the results found by Player et al. (22) and Lampi and Kamal-Eldin (25). In accordance with Steel et al. (26), the degradation curve of tocopherols demonstrates that the α - and ($\gamma+\beta$)-tocopherols were also destroyed more rapidly than the β - and δ -tocopherols present in soy bean oil submitted to 180°C.

Zambiazzi (27) describes that rice bran oil at a temperature of 65°C showed greater stability than sunflower, canola, and soy-bean oils. The same author tells us that the α -tocopherol present in rice bran oil degraded more rapidly than the ($\gamma+\beta$)- and δ -tocopherol.

The greater stability shown by the δ -tocopherol may be due to its lack of capacity to donate its phenolic hydrogen to the free radicals. According to Kalucka et al. (28), the lower antioxidant effect of the δ -tocopherol may be associated to its greater stability when compared with the α -tocopherol, which is oxidated

more rapidly into tocopheryl radical, which can participate in chain reactions that result in the acceleration of oxidation.

According to Kalucka et al. (28), the δ -tocopherol would also be more stable because it would not participate so easily as the α -tocopherol, in secondary reactions with hydroperoxides to form more radicals at higher temperatures.

Kim and Lee (29) and Player et al. (22) also emphasized that antioxidants have a capacity to donate their hydrogen from phenolic groupings to free radicals, and the α -tocopherol, for having a high antioxidant activity in vegetable oils, has low stability at high temperatures.

The total content of tocopherols in rice bran oil submitted to heating at 100°C showed a gradual reduction in the entire heating period, reaching losses of 13.49% and of 26.08%, respectively, for the periods of 192 h and 432 h (Figure 3).

In accordance with the results, it has been observed that the higher rates of degradation in the total content of tocopherols were observed in oil heated at 180°C, which showed the greatest losses at the period of 144 h of heating (86.9%). The tocopherol content of the rice bran oil heated at 100°C showed higher degradation after 144 h of heating and, even then, the degradation content remained lower than the one presented during the first 144 h when heated at 180°C.

Ko et al. (30) observed that short periods of heating in rice bran oil and rice bran, both in microwave and electric ovens utilizing temperatures of 170°C, 180°C, and 190°C caused total degradation of vitamin E. According to Nyström et al. (8), the good stability of rice oil has been attributed to the high content of estheryl pherulate (present in the fraction of the γ -orizanol) and to the contents of tocopherols present in the oil.

Conclusion

At temperatures of 100°C and of 180°C, it was verified that degradation of tocopherols occurred and that it increased considerably with the increase in temperature. With this, it was observed that rice bran oil submitted at 180°C temperature suffered greater loss of tocopherols, resulting in the increasing order of degradation of α -, ($\gamma+\beta$)-, and δ -tocopherols.

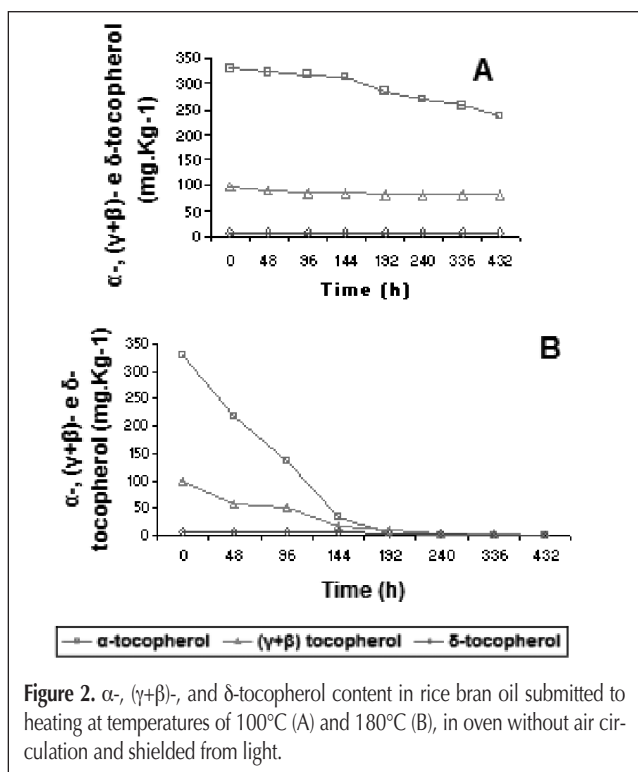


Figure 2. α -, ($\gamma+\beta$)-, and δ -tocopherol content in rice bran oil submitted to heating at temperatures of 100°C (A) and 180°C (B), in oven without air circulation and shielded from light.

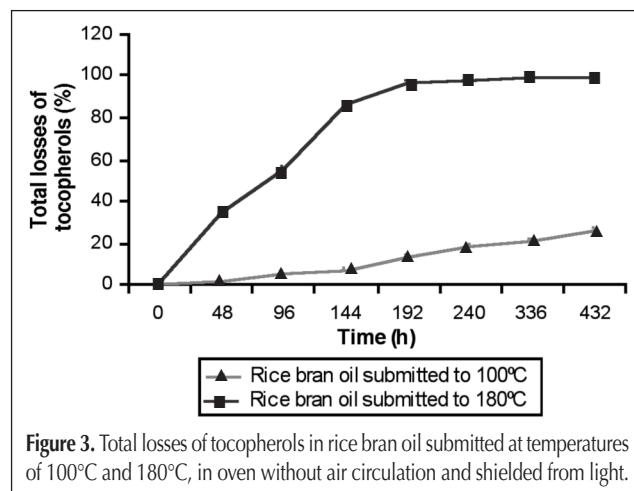


Figure 3. Total losses of tocopherols in rice bran oil submitted at temperatures of 100°C and 180°C, in oven without air circulation and shielded from light.

References

- V.C. Ramalho and N. Jorge. Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos. *Química Nova*. **29(4)**: 755–760 (2006).
- A.D.I. Morgharbel and R.J.S. Freitas. Influência do aquecimento nos meios de fritura sobre o grau de acidez e índice de iodo. *Brasil Alimentos*. **20**: 35–37 (2003).
- V.V. Hoed, G. Depaemelaere, J.V. Ayala, P. Santiwattana, R. Verhé, and W.D. Greyt. Influence of chemical refining on the major and minor components of rice bran oil. *J. Amer. Oil Chem. Soc.* **83(4)**: 315–321 (2006).
- J. Kim, J. Godber, J. King, and W. Priyawitkul. Inhibition of cholesterol autoxidation by the nonsaponifiable fraction in rice bran in an aqueous model system. *J. Amer. Oil Chem. Soc.* **78**: 685–689 (2001).
- K. Wamer, W.E. Neff and F.J. Eller. Enhancing quality and oxidative stability of aged fried food with tocopherol. *J. Food Chem.* **51**: 623–627 (2003).
- T.Y. Ha, S. Han, S.R. Kim, I.H. Kim, H.Y. Lee, and H.K. Kim. Bioactive components in rice bran oil improve lipid profiles in rats fed a high-cholesterol diet. *Nutrition Research*. **25**: 597–606 (2005).
- C.E.C. Rodrigues, M.M. Onoyama, and A.J.A. Meirelles. Optimization of the rice bran oil deacidification process by liquid-liquid extraction. *J. Food Engineering*. **73**: 370–378 (2005).
- L. Nyström, T. Achrenius, A.M. Lampi, R.A. Moreau and V. Piironen. A comparison of the antioxidant properties of steryl ferulates with tocopherol at high temperatures. *Food Chem.* **46(2)**: 1–8 (2006).
- C. Balachandran, P.N. Mayamol, S. Thomas, D. Sukumar, and A.Sundaresan. An ecofriendly approach to process rice bran for high quality rice bran oil using supercritical carbon dioxide for nutraceutical applications. *Bioresource Technol.* **99**: 2905–2912 (2008).
- A.M. Lampi, L. Kataja, A.K. Eldin, P. Vieno. Antioxidant activities of α - and γ -tocopherols in the oxidation of rapeseed oil triacylglycerols. *JOACS*. **76(6)**: 749–755 (1999).
- H. Yoshida, S. Takagi, H. Lenaga, and C. Tsuchiya. Regional Distribution of Tocopherols and Fatty Acids Within Soybean Seeds. *J. Amer. Oil Chem. Soc.* **75(7)**: 767–774 (1998).
- S.L. Abidi. Tocol-Derived Minor Constituents in Selected Plant Seed Oils. *J. Amer. Oil Chem. Soc.* **80(4)**: 327–333 (2003).
- P.A. Morrissey and J.A. Sheehy. Optimal nutrition: vitamin E. *Proceedings of the Nutrition Society*. **58**: 459–468 (1999).
- R.B. Flohé. Bioactivity of vitamin E. *Nutrition Research reviews*. **19**: 174–86 (2006).
- W.I.W. Rosli, A.S. Babji, A. Aminah, S.P. Foo, and A.B.D.Malik. Vitamin e contents of processed meats blended with palm oils. *J. Food Lipids*. **13**: 186–98 (2006).
- S.C. Cunha, J.S. Amaral, J.O. Fernandes, and M.B.P.P. Oliveira. Quantification of tocopherols and tocotrienols in portuguese olive oils using HPLC with three different detection systems. *J. Agric. Food Chem.* **54**: 3351–3356 (2006).
- S.M. Nolasco, L.A.N. Aguirrezábal, and G.H. Crapstec. Tocopherol oil concentration in field-grown sunflower is accounted for by oil weight per seed. *J. Amer. Oil Chem. Soc.* **81(11)**: 1045–1051 (2004).
- M.L. Takur and V.S. Srivasta. Vitamin E metabolism and its application. *Nutrition Res.* **16(10)**: 1767–1809 (1996).
- B.F. Haumann. Antioxidants: firme seeking products they can label as natural. *Inform.* **12**: 1002–1013 (1990).
- N.A. Porter, S.E. Caldwell and K.A. Mills. Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*. **30**: 4 (1995).
- N. Romero, P. Robert, L. Masson, J. Ortiz, K. Gonzalez, K. Tapia and C.Dobarganes. Effect of α -tocopherol, α -tocotrienol and Rosa mosqueta shell extract on the performance of antioxidant-stripped canola oil (*Brassica sp.*) at high temperature. *Food Chem.* **104**: 383–389 (2007).
- M.E. Player, H.J. Kim, H.O. Lee and D. B Min. Stability of α -, γ - or δ -tocopherol during soybean oil oxidation. *J. Food Sci.* **71(8)**: 456–460 (2006).
- M.H. Chen and C.J. Bergman. A rapid procedure for analysing rice bran tocopherol, tocotrienol and g-oryzanol contents. *J. Food Comp. Anal.* **18**: 139–151 (2005).
- V.R. Pestana, R.C. Zambiasi, C.R.B. Mendonça, M.H.Bruscatto, M.J. Lermagarcia, and G. Ramis-Ramos. Quality changes and tocopherols and c-orizanol concentrations in rice bran oil during the refining process. *J. Amer. Oil Chem. Soc.* **85(11)**: 1013–1019 (2008).
- A.M. Lampi and A. Kamal-Eldin. Effect of α - and γ -tocopherols on thermal polymerization of purified high-oleic sunflower triacylglycerols. *J. Amer. Oil Chem. Soc.* **75(12)**: 1699–1703 (1998).
- C.J. Steel, M.C. Dobarganes, and D. Barrera-Arellano. The influence of natural tocopherols during thermal oxidation of refined and partially hydrogenated soybean oils. *Grasas y Aceites*. **56**: 46–52 (2005).
- R. Zambiasi. The role of endogenous lipid components on vegetable oil stability. Manitoba/Canadá. Tese de Doutorado, Food and Nutritional Sciences Interdepartmental Program, University of Manitoba. p. 304 (1997).
- M.N. Kalucka, J. Korczak, I. Elmadfa, and K.H. Wagner. Effect of α - and δ -tocopherol on the oxidative stability of a mixed hydrogenated fat under frying conditions. *Eur. Food Res. Technol.* **221**: 291–297 (2005).
- H.J. Kim, O.H. Lee, and D.B. Mim. Effects and prooxidant mechanisms of oxidized α -tocopherol on the oxidative stability of soybean oil. *J. Food Sci.* **72(4)**: 223–230 (2007).
- S.N. Ko, C.J. Kim, C.T. Kim, H. Kim, S.H. Chung, S.M. Lee, H.H. Yoon, and I.H. Kim. Changes of vitamin E content in rice bran with different heat treatment. *European J. Lipid Sci. Technol.* **105(5)**: 225–228 (2003).

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