Effects of parboiling, storage and cooking on the levels of tocopherols, tocotrienols and γ-oryzanol in brown rice (Oryza sativa L.)

Cristina de Simone Iglesias Pascual a, Isabel Louro Massarett a, Fabiana Kawassaki a, Rosa Maria Cerdeira Barros a, José Alberto Noldin b, Ursula Maria Lanfer Marquez a,⁎

a Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 580, CEP 05508-900, São Paulo, SP, Brazil
b Epagri, Estação Experimental de Itajaí, CP 277, CEP 88301-970, Itajaí, SC, Brazil

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A B S T R A C T
Vitamin E and γ-oryzanol display a wide range of biological activities including hypocholesterolemic, anti-inflammatory and antioxidant activities. Although white rice is far more popular worldwide, consumption of brown rice is increasing, partially on account of the presence of bioactive compounds; however, the effects of parboiling, storage and cooking on them are not well-characterized. The effects of parboiling and a 6-month storage period on the contents of vitamin E and γ-oryzanol in three brown rice cultivars grown in different locations in Brazil were investigated. Also, their levels in branded non-parboiled and parboiled brown rice were monitored before and after cooking. Vitamin E homologues and γ-oryzanol were separated by RP-HPLC equipped with PDA and fluorescence detectors. The average levels of total tocols and γ-oryzanol in the raw brown rice cultivars studied were 25 and 188 mg/kg, respectively. Of the vitamin E homologues, γ-tocotrienol contributed with 74% of total tocots, followed by α-tocopherol, α-tocotrienol and γ-tocopherol in minor quantities. The combined processes, parboiling, storage and cooking, led to an approximate 90% reduction in tocots and only γ-tocotrienol was detectable after any of the processes. Parboiling followed by storage resulted in an approximate 40% loss of γ-oryzanol. Cooking had almost no further effect over γ-oryzanol levels in parboiled rice previously stored for 6 months.

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1. Introduction

Of all the bioactive lipophilic compounds found in the outer layers of brown rice kernels, vitamin E and γ-oryzanol are of greater scientific interest because of their potential health benefits. Vitamin E occurs in the form of eight stereoisomers, four tocopherols (α, β, γ, δ), and four tocotrienols (α, β, γ, δ), which differ in the saturation state of the isoprenoid side chain. The most abundant tocots found in indica rice are: γ-tocotrienol, α-tocopherol, α-tocotrienol and γ-tocopherol (Aguilar-Garcia, Gavino, Baragallo-Mosqueda, Hevia, & Gavino, 2007; Heinemann, Xu, Godber, & Lanfer-Marquez, 2008). In addition to their potential antioxidant activity, tocots have been reported to reduce lipid peroxidation, attenuate lipid-related risk factors (elevated LDL cholesterol levels, platelet aggregation), display anti-inflammatory properties and show anti-carcinogenic and cardiovascular protective effects. In fact, several novel beneficial structure-related effects of individual tocotocols and tocotrienols have been reported (Tiwari & Cummins, 2009).

Brown rice is also a unique source of γ-oryzanol, which is a mixture of at least 10 lipophilic phytosteroids (Xu & Godber, 1999). Several studies in animals and human beings have shown that this group of compounds displays antioxidant properties, total- and LDL-cholesterol-lowering and HDL-cholesterol-increasing effects (Gerhardt & Gallo, 1998; Rong, Ausman, & Nicolosi, 1997; Wilson, Idreis, Taylor, & Nicolosi, 2002). Other health benefits of γ-oryzanol, such as reduction of tumor incidence, inhibition of platelet aggregation and anti-inflammatory activity, have been observed (Lerma-Garcia, Herrero-Martinez, Simó-Alfonso, Mendonça, & Ramos-Ramos, 2009).

The factors affecting the accumulation of tocots and γ-oryzanol in brown rice have been investigated. Genetic and environmental factors, such as growing locations, harvesting and weather conditions, are suggested to influence their concentration in the grain (Aguilar-Garcia et al., 2007; Britz et al., 2007; Heinemann et al., 2008; Miller & Engel, 2006). Although studies on phytochemicals in brown rice have been widely carried out and have provided valuable information to guide the selection and the development of phytochemical-rich genotypes, little is known about the effects of processing and cooking on their actual contents in ready-to-eat foods. Worldwide, 170 million tons of rough rice is unofficially estimated to be parboiled every year, one-fifth of the average annual production (Amato & Elias, 2005). In fact, the production of parboiled rice is likely to increase, fueled not only by the technical advantages and nutritional benefits of the process, but also by its steadily increasing acceptance by consumers.

⁎ Corresponding author. Tel.: +55 11 3091 3684; fax: +55 11 3815 4410.
E-mail address: lanferum@usp.br (U.M.L. Marquez).

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Parboiling offers several advantages over other forms of processing such as higher yield, reduced stickiness of cooked rice, grain sterilization, enzyme inactivation, increased shelf life and migration of water-soluble vitamins and several minerals from the bran into the center of the grain (Amato & Elias, 2005; Heinemann, Fagundes, Pinto, Pentead, & Lanfer-Marquez, 2005). Rice parboiling is a hydrothermal treatment consisting of soaking (at a temperature above 58 °C), autoclaving (120 °C), causing total or partial starch gelatinization, and drying. Khatoon and Gopalakrishna (2004) found that vitamin E levels were greatly reduced after parboiling, whereas the γ-oryzanol content remained virtually unaltered. Finocchiaro et al. (2007), on the other hand, observed increased levels of tocotocols and an 8% loss of γ-oryzanol in cooked brown rice, compared with those of raw brown rice. In spite of the growing interest in rice parboiling little is known about the effects of thermal treatments as well as storage on phytochemicals in rice.

The aim of the current study was to assess the effects of parboiling, storage and cooking over the levels of total tocotocols, individual vitamin E homologues and γ-oryzanol in different brown rice cultivars.

2. Material and methods

2.1. Reagents

α-tocopherol, γ-tocopherol, α-tocotrienol and γ-tocotrienol were purchased from Sigma-Aldrich Co. Pure γ-oryzanol was kindly provided by Dr. Z. Xu and Dr. J. S. Godber (Department of Food Science, LSU Agricultural Center, Baton Rouge, LA, USA). All solvents used were analytical or HPLC grade.

2.2. Samples and sample preparation

Three cultivars (SCS 114 Andosam, SCSBRS Tio Taka, Epagri 109) of paddy indica rice (Oryza sativa L.) were grown in the experimental irrigated fields of the Institute of Agronomy at Epagri (Estação Experimental de Itajai, Brazil) and were harvested in 2008. The three varieties were then simultaneously grown on three different farms in each of three locations in the state of Santa Catarina, Brazil, totaling 27 brown rice samples: South Shore (29 °S 49°30’W), Upper Itajaí Valley (27°S 49°30’W) and North Shore (26°S 49°W). They differ in soil type, temperature and altitude (edaphoclimatic conditions). For example, South and North Shore are at an average altitude between 20 and 50 m, and the Upper Itajaí Valley is at 300 m. Immediately after harvest, 500 g of each rice sample was divided into two subsamples. One was parboiled and dehulled yielding parboiled brown rice and the other was dehulled to produce brown rice.

For chemical analysis, 200 g of each rice sample was cleaned up by removing immature and damaged grains and then stored at 5 °C until time of analysis. Grains were milled using an A10 Analytical Mill (Kinematica AG. Luzern, Switzerland) and then sieved through an 80-mesh sieve to obtain a homogeneous fine powder. The analyses were conducted within 8 h after milling. Moisture content of rice samples was determined by drying them to constant weight in an oven at 105 °C according to AOAC (1995). The moisture levels observed for all samples were lower than 13%.

Additionally, three samples of branded non-parboiled and three of parboiled brown rice were purchased from a local market in São Paulo and the vitamin E and γ-oryzanol contents in the samples were monitored before and after cooking.

2.3. Parboiling process

About 250 g of hulled rice was parboiled on a laboratory scale in the pilot plant of the Institute of Agronomy at Epagri (Estação Experimental de Itajai). Following drying, grains were dehulled in a proof mill (Suzuki, model MT) as follows: grains were soaked in distilled water (1:1.5 (w/v)) at 65 °C for 6 h, sterilized at 110 °C and 50.7 kPa for 7 min and then oven-dried at 95 °C for 24 h. After a 48-hour moisture equilibration period, grains were dehulled, packaged in polyethylene bags, and stored in a cold chamber at 5 °C until time of analysis.

2.4. Storage tests

The storage tests were performed as follows: nine samples were randomly chosen out of the 27 dehulled subsamples. Each of them was stored for 6 months at room temperature (25 ± 5 °C) in polyethylene pouches. Also nine corresponding parboiled subsamples were stored under the same conditions. The eighteen samples were then milled and analyzed for vitamin E and γ-oryzanol contents.

2.5. Cooking conditions

Thirty grams of each branded non-parboiled and parboiled brown rice sample was cooked for 30 min in a partially covered beaker containing 120 mL of distilled water to guarantee adequate texture and absorption of all water added. Cooked rice samples were freeze-dried, ground, sieved (80-mesh) and analyzed for vitamin E and γ-oryzanol contents.

2.6. Extraction of tocopherols, tocotrienols, and γ-oryzanol

Extraction was performed according to Aguilar-Garcia et al. (2007) with modifications to the sample size and the number of extractions. Briefly, 2 grams of rice flour (exact to 0.0001 g) in triplicate were mixed with 20 mL of HPLC-grade methanol in a capped test tube, vortexed for 2 min at 25 °C, centrifuged at 4500 g for 10 min and the supernatant was collected. The resulting residue was further extracted twice with 10 mL of methanol under the same conditions, and the combined supernatant fractions were concentrated under reduced pressure at 40 °C. The concentrates of non-parboiled and parboiled brown rice samples were then dissolved in 5 mL or 3 mL of HPLC-grade methanol, respectively. The extracts were filtered through a 0.22 μm membrane and 50 μL aliquots were transferred directly into a HPLC vial for the concomitant HPLC analysis of tocopherols, tocotrienols and γ-oryzanol.

2.7. HPLC analysis

Tocopherols, tocotrienols and γ-oryzanol were simultaneously separated by an analytical Shimadzu High Performance Liquid Chromatography (HPLC) system equipped with an LC-10ADVP pump, an SIL-10ADVP auto injector, an SPD-M10A VP photodiode array and a scanning fluorescence detector (Shimadzu, Kyoto, Japan) using the method described by Aguilar-Garcia et al. (2007). Chromatograms were recorded and processed using Shimadzu Class-VP V 6.14 software. Samples were injected onto a Syngeri Hydro-RP column (250 mm × 4.6 mm (i.d); Phenomenex, Torrance, CA) using an auto sampler. Elution was performed at 25 °C and at a flow rate of 1 mL/min. The initial composition of the mobile phase (45% acetonitrile, 45% methanol and 10% isopropanol) was held for 6 min, followed by a linear gradient to 25% acetonitrile, 70% methanol and 5% isopropanol in 10 min; the final composition was held for 12 min. Finally, the eluent composition was reset to its initial conditions and was held constant for 7 min to stabilize the baseline. Total runtime was 45 min including column equilibration.

Tocotrienol and tocopherol standards, monitored using the fluorescence detector at an excitation wavelength of 298 nm and an emission wavelength of 328 nm, were eluted at 4 well-defined peaks between 6 and 15 min, whereas the γ-oryzanol standard, monitored with PDA detection at 325 nm, was separated into 4 main peaks.
between 20 and 33 min (Fig. 1). On the chromatographic approach taken different number of individual compounds may be separated and probably each peak may contain one or more constituents. Under the experimental conditions employed, four peaks were separated but not individually identified and the quantification of total γ-oryzanol was based on the sum of the peak areas (Chen & Bergman, 2005; Xu & Godber, 1999).

### 2.8. Quantitative determination

Standard stock solutions of α-tocopherol (5.0 mg/mL), α-tocotrienol (2.5 mg/mL), γ-tocopherol (10.0 mg/mL) and γ-tocotrienol (5.0 mg/mL) were prepared using HPLC-grade hexane and stored at −20 °C away from light. The four vitamin E homologues were identified by comparing their retention times with those of published standards, and by spiking standards into samples (Britz et al., 2007; Chen & Bergman, 2005). Before use, 50–100 μL aliquots of each stock solution were diluted in HPLC-grade ethanol and each concentration was determined by UV absorption at their maximum absorbance wavelengths using the extinction coefficients (ε) in ethanol presented in Table 1. Concentrations were calculated by using Eq. (1).

Calibration was performed by using standard solutions of the vitamin E homologues and each concentration was determined by calculating the peak area and comparing it to the corresponding standard curve. Thus, further dilution of the extract and another injection were required for its quantification.

Although the separation of tocols was performed in a single run, the concentration of γ-tocopherol in rice exceeded the upper limit of the standard curve. Thus, further dilution of the extract and another injection were required for its quantification.

The calibration curve of γ-oryzanol was constructed by diluting its stock solution (1.0 mg/mL) in HPLC-grade methanol to yield final concentrations in the range of 30–150 μg/mL (seven points). The content of γ-oryzanol was calculated by adding up the areas of the four main peaks obtained and by comparing them to the combined areas of the standard.

### 2.9. Statistical analysis

Analyses were performed at least in triplicate and data were expressed as average ± standard deviation (SD) for all replicates. Statistical analysis was performed using STATISTICA 8.0 software at a significance level of 5% (p < 0.05) (Rodrigues & Lemma, 2005). Differences among growing locations and treatments (parboiling, storage and cooking) were evaluated using one-way ANOVA and the Tukey’s test.

### 3. Results and discussion

#### 3.1. Initial levels of tocols and γ-oryzanol in brown rice

The initial contents of individual tocotrienols (α-T3 and α-T) and tocopherols (α-T and γ-T), total tocols and γ-oryzanol in each sample of raw brown rice are shown in Table 2.

The average concentration of total tocols (24.9 mg/kg) (α-T3, γ-T3, α-T, and γ-T) ranged between 17.5 and 30.8 mg/kg rice (dry basis). No statistically significant difference in their levels was observed among growing locations or cultivars. A range of variation between 10.4 and 22.8 mg/kg was previously reported by our working group for 22 cultivars of indica brown rice grown in three Brazilian states (Rio Grande do Sul, Santa Catarina and Mato Grosso) and harvested in 2004 under different environmental and climatic conditions. At the same harvest (2004) japonica rice cultivars showed a higher content of total tocols (15.7–32.5 mg/kg) (Heinemann et al., 2008). On the other hand, in the current study the mean levels of tocols in indica varieties

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**Table 1**

<table>
<thead>
<tr>
<th>Substance</th>
<th>λ&lt;sub&gt;a&lt;/sub&gt; (nm)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>ε&lt;sub&gt;ethanol&lt;/sub&gt;&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Range of concentration of the standard curve (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-tocotrienol</td>
<td>292</td>
<td>3864</td>
<td>25–2000</td>
</tr>
<tr>
<td>γ-tocotrienol</td>
<td>296</td>
<td>3716</td>
<td>25–750</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>292</td>
<td>3264</td>
<td>25–2000</td>
</tr>
<tr>
<td>γ-tocopherol</td>
<td>298</td>
<td>3808</td>
<td>25–750</td>
</tr>
</tbody>
</table>

<sup>1</sup> Source: Adapted from Chen and Bergman (2005).
(24.9 mg/kg) are comparable to those noted for *japonica* cultivars (24.2 mg/kg). These results suggest that the genetic differences between *indica* and *japonica* rice subspecies may have been masked by agronomic and climatic conditions.

Britz et al. (2007) reported a wide variability in total tocols (38.48–90.92 mg/kg) in six different cultivars (*indica* (n = 2), *japonica* (n = 3) and *glaberrima* (n = 1)) grown in the United States and authors did not attribute variation to differences among subspecies. They also observed slight changes in the levels of tocols in five out of the 6 cultivars at higher temperature (4.5 °C above ambient, i.e., 35/27°C day/night). Finocchiaro et al. (2007) reported total vitamin E levels of 43.4 mg/kg in a single *japonica* cultivar grown in Italy.

Table 2 also shows the average contents of each vitamin E homologue in the brown rice cultivars studied. The levels of γ-tocotrienol, the most abundant tocol found in the samples, ranged between 13.6 and 24.0 mg/kg and averaged at 18.4 mg/kg, which corresponds to 74% of total tocols content (24.9 mg/kg). α-tocopherol, α-tocotrienol and γ-tocopherol, were present at lower concentrations representing 11%, 8%, and 7% of total tocols, respectively and no other vitamin E homologues were detected.

Likewise, γ-tocotrienol (46% of total tocols), followed by α-tocopherol (28%), was found to be the major component in 22 *indica* rice cultivars in a previous study carried out by our working group Heinemann et al. (2008). Similar observations were also reported for cultivars from Venezuela and some *japonica, indica* and *glaberrima* cultivars of different origins (Aguilar-García et al., 2007; Britz et al., 2007). However, α-tocopherol was reported to be the major compound in some rice varieties, especially of the *japonica* subspecies (Britz et al., 2007; Heinemann et al., 2008). Such differences in the proportion of individual tocols with respect to total levels may be attributed to genetic variability among subspecies. According to Bergman and Xu (2003) and Heinemann et al. (2008), the lack of correlation between the levels of α- and γ-homologues suggests that the biosynthesis of these tocols occurs through different metabolic pathways, which may explain why cultivars present specific homologues as major components.

The amounts of γ-oryzanol ranged between 151.0 and 228.1 mg/kg (at dry basis) and averaged at 187.9 mg/kg in raw rice samples (Table 2). No statistically significant difference was observed among cultivars or growing locations.

The average amounts of γ-oryzanol found in this study were statistically comparable to those previously reported for *indica* rice (190.1 mg/kg), but significantly lower than those for *japonica* rice (246.3 mg/kg) (Heinemann et al., 2008). Similarly, γ-oryzanol concentration in six rice cultivars of different subspecies was reported to range from 180 to 277 mg/kg and variation was not attributed to differences among subspecies (Britz et al., 2007). Miller and Engel (2006) investigated the levels of γ-oryzanol in 30 brown rice samples of various cultivars (unidentified ssp.), grown in different locations in Europe (Italy, France and Spain) and in different seasons (2000, 2001 and 2002). The authors observed that their levels ranged between 262.0 and 627.0 mg/kg and suggested that environmental factors rather than the stage of grain maturity account for the wide variability.

### 3.2. Effects of parboiling, storage and cooking on tocols and γ-oryzanol contents

The average content of tocotrienols (α-T3 and γ-T3), tocopherols (α-T and γ-T), and total tocols of all samples analyzed before and after the parboiling process are shown in Fig. 2. Parboiling affected mostly vitamin E, which presented an average loss of approximately 60% when compared to the initial level. Among the homologues, α-tocopherol was the most sensitive and parboiling caused a loss of 93% in respect to its original content, whereas γ-tocotrienol, the least influenced and the only detectable, displayed an approximate 60% reduction in its levels (final concentration —
The amounts of tocotrienols, tocopherols, and tocols in non-parboiled and parboiled brown rice during a 6-month period storage are presented in Fig. 3. Storage of brown rice at room temperature (25 ± 5 °C) caused a loss of 70% of tocols and remaining amounts were about 7.5 mg/kg. Storage of parboiled rice caused a small additional loss and residual amounts were only 3.7 mg/kg.

Three samples of branded non-parboiled brown rice, purchased from a local market in São Paulo, were also analyzed for vitamin E content. They displayed intermediate amounts of tocols (16.4 mg/kg) relative to our raw and 6-month-stored rice samples. On the other hand, branded parboiled rice showed concentrations (3.1 mg/kg) comparable to those of our parboiled samples, previously stored for 6 months. These results suggest that the content of vitamin E may vary depending on whether the rice is parboiled or not and how long it takes (including storage time at the manufacturer) before rice is finally consumed by the buyer. The levels of tocols in both parboiled and non-parboiled samples of branded rice were also analyzed after domestic cooking. The estimated combined effects of storage and cooking over their levels are also represented in Fig. 3. Cooking caused a further loss of tocols and the final amounts were irrelevant in both cooked non-parboiled and parboiled rice.

Khatoon and Gopalakrishna (2004) already reported high losses of tocols after parboiling a single Indian commercial brown rice sample. Very low amounts of tocopherols (α- and γ-) were found (2.18 mg/kg) while tocotrienols were not detected.

Changes in the amounts of tocols in non-parboiled and parboiled brown rice during a 6-month period storage are presented in Fig. 3. Storage of brown rice at room temperature (25 ± 5 °C) caused a loss of 70% of tocols and remaining amounts were about 7.5 mg/kg. Storage of parboiled rice caused a small additional loss and residual amounts were only 3.7 mg/kg.

In the present study, in parboiled rice, the accumulated losses caused by parboiling (20%) plus subsequent storage (18%) resulted in remaining amounts (118.6 mg/kg) corresponding to around 60% of γ-oryzanol originally existing in recently harvested rice, while in brown rice the remaining levels of γ-oryzanol after storage (150.5 mg/kg) were not less than 80% of the original contents.

The amounts of γ-oryzanol in the commercial rice samples were 133.0 mg/kg and 146.9 mg/kg for parboiled and non-parboiled rice, respectively. These amounts are within the same range of variation of those observed for the rice samples stored in the laboratory and confirm that γ-oryzanol is much more stable than vitamin E during parboiling and storage.

Cooking of brown or parboiled brown rice after storage did not cause additional relevant losses and so we concluded that the most important factors that cause γ-oryzanol losses are storage and parboiling.

4. Conclusions

The amounts of tocols and γ-oryzanol observed in indica raw brown rice of different cultivars grown in Brazil were little influenced by location or cultivar and variation was comparable to rice grown under different climatic, environmental conditions and year of harvest as previously reported by our working group.

In raw rice α-tocotrienol, the most abundant homologue, was responsible for ¾ of the total vitamin E content.

The combined treatments, parboiling, a 6-month period storage, and cooking, led to a significant loss in the concentrations of total tocols in brown rice and only little more than 10% of original α-tocotrienol content remained.
Parboiling followed by storage resulted in a loss of about 40% of γ-oryzanol contents, whereas cooking caused almost no change in their levels. Overall, vitamin E was far more affected than γ-oryzanol during the treatments.

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Appendix A. Equations

\[
C = \frac{A}{\varepsilon l},
\]

where

- \(A_{1cm}\) maximum absorbance of each substance
- \(\varepsilon\) extinction coefficient (mol/L)
- \(C\) molar concentration
- \(l\) 1 cm path length

References


