Degradation of Gamma-oryzanol in Rice Bran Oil during Heating : An Analysis Using Derivative UV-spectrophotometry

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Abstract

Gamma-oryzanol, a group of ferulic acid esters of phytosterols and triterpene alcohols, has been reported to exhibit antioxidant activity and other health beneficial properties. In this study, the degradation of gamma-oryzanol in rice bran oil during the heat treatment was investigated. The quantitative analysis of the gamma-oryzanol in rice bran oil was performed by second order derivative UV-spectrophotometry. The degradation kinetics was described by the first-order reaction model and degradation rate constants were 0.0089, 0.0315 and 0.0763 at 120, 150 and 200°C, respectively. Temperature dependence of the rate constant obeyed Arrhenius relationship, and activation energy was calculated to be 40.76 kJ/mol.

Keywords : Gamma-oryzanol; Rice bran oil; Derivative UV- spectrophotometry; Degradation kinetics.

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Introduction

Phytochemicals have been received much interests in recent years because of their potential in preventing or curing some of human chronic diseases, e.g. cancers, coronary heart disease (CHD), diabetes etc. Thai people are now encouraged to consume unpolished rice instead of polished rice because unpolished rice is a good source of vitamin B, vitamin E, and other phytochemicals. One of the phytochemicals that found at high concentration in rice is gamma-oryzanol which is a group of ferulic acid esters of phytosterols and triterpene alcohols (Cheruvanky, 2003) (Figure 1).

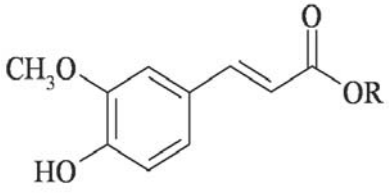

Fig. 1 Chemical structure of gamma-oryzanol. R represents phytosterols or triterpene alcohols.

Gamma-oryzanol exists mainly in bran layers, and therefore it is also found in extracted rice bran oil. Gamma-oryzanol acts as an efficient natural antioxidant in the oil. Several studies have demonstrated the potential of gamma-oryzanol in lowering blood cholesterol (Cicero and Gaddi, 2001; Kim et al., 2001; Seetharamaiah and Chandrasekhara, 1988; Seetharamaiah and Chandrasekhara, 1989; Xu et al., 2001).

To receive the health benefits from gamma-oryzanol in rice bran oil, the compound must be stable against several severe conditions during the food preparation processes, but gamma-oryzanol which acts as antioxidant can be lost during the thermal oxidation. Data of degradation of gamma-oryzanol in rice bran oil is not available; therefore, the purpose of this study was to gain the results of

degradation kinetic of gamma-oryzanol in rice bran oil during the heating conditions which simulate the process such as deep-fat frying.

Materials and method

Materials

Commercial rice bran oil was purchased from local market. Gamma-oryzanol was received as a gift from Tsuno Rice Fine Chemical Co., Ltd (98.5% purit y, Wakayama, Japan). Isopropanol (J.T. Baker, USA) was used as a solvent for UV-spectrophotometric analysis.

Degradation study

Rice bran oil (2 mL) was added to glass test tubes and then the tubes were heated in a silicone oil bath which was pre-heated at 120, 150, or 200°C. At the time interval, three tubes of the rice bran oil were randomly taken from the heating bath and were immediately cooled in an ice-water bath to stop the degradation reaction. The samples were taken at proper time intervals until 50 to 75% of gamma-oryzanol was lost.

Determination of gamma-oryzanol

In this study, we used the derivative UV-spectrosphotometry method described by Bucci et al. (2003) to quantify the gamma-oryzanol content in the rice bran oil. The rice bran oil sample was dissolved with isopropanol. Then the absorbance spectra of the sample were measured at the wavelength from 280 to 400 nm in a 1-cm length glass cuvette using UV/Visible spectrophotometer (JASCO V-530, JASCO Corporation, Japan). The software Spectra Manager for Windows (Version 1.24.00 Build 1, JASCO Corporation, Japan) was used for both controlling the spectrophotometer and for differentiation calculation. The spectra were measured at scan speed of 100 nm/min and the data were recorded at 1 nm interval. Each sample was scanned five times and the average of those five spectra was used for next calculation steps.

To reduce the distortion of the spectra, smoothing operation using Savitzkey-Golay method (Talsky, 1994) with 25 points interval was carried out. Then the second order derivatives (^{2}D) of the spectra were calculated using the data interval (Ä•) of 10 nm.

Kinetics model

Degradation kinetics of many compounds in food at constant temperature follows the first-order kinetics model (Taoukis et al., 1997) which can be expressed as

$$-\frac{dC}{dt} = kC \qquad (1)$$

where C is the concentration of the compound, t is time and k is reaction rate constant. Integration of eq 1 gives

$$\mathrm{In}C = \mathrm{In}C_0 - kt \qquad (2)$$

where C_0 is initial concentration of the compound. By plotting the logarithm of concentration of the compound (ln C) over time (t) during degradation process, we can obtain the reaction rate from the slope of the simple linear regression line. From eq 2 the time required for the compound to be degraded to half of its initial concentration (half-life) is calculated from

$$t_{1/2} = \frac{\mathrm{In}2}{k} \qquad (3)$$

Results and discussion

Derivative UV-spectrophotometry

All absorbance spectra of the standard gamma-oryzanol in isopropanol at different concentrations (from 5.09 to 30.54 mg/L) showed the maxima at 327 nm (Figure 2), which were comparable with that reported by Bucci et al. (2003). The second order differentiation of the spectra was performed and the derivative spectra (^{2}D) (multiplied by 10^4) are shown in Figure 3. The peak-peak method (Talsky, 1994) was used to evaluate the derivative spectra.

The distance from a maximum (~ 360 nm) to a minimum (~ 330 nm) was correlated with the concentration of gamma-oryzanol. The linear relation between the peak-peak distances and the concentrations (X) was obtained, and the regression equation was

$$^2D = 0.8770X - 0.5296 \quad (4)$$

The coefficient of determination, R^2, was found to be 0.9991.

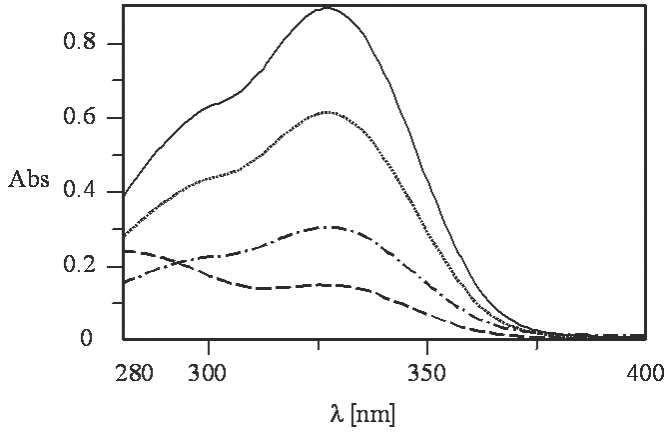

Fig. 2 Absorbance (Abs) spectra of standard gamma-oryzanol in isopropanol at the wavelengths from 280 to 400 nm. The concentrations of standard solution were 5.09 mg/L (– – –), 10.18 mg/L (– · – · –), 20.36 mg/L (·····) and 30.54 mg/L (——).

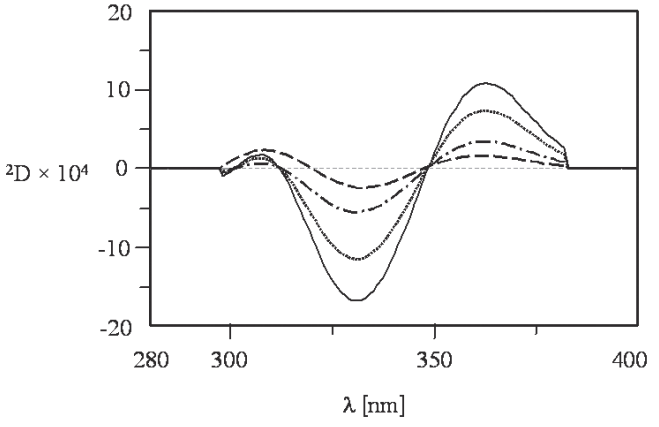

Fig. 3 Second order derivatives ($^2D \times 10^4$) of the absorbance spectra of standard gamma-oryzanol in isopropanol at the wavelengths from 280 to 400 nm. The concentrations of standard solution were 5.09 mg/L (– – –), 10.18 mg/L (– · – ·), 20.36 mg/L (·····) and 30.54 mg/L (——).

Limit of detection (LOD) of the method was calculated from the standard curve of gamma-oryzanol (eq 4), and the LOD was defined as the gamma-oryzanol concentration giving the ^2D equal to blank signal (y-axis intercept) plus three residual standard error ($s_{y/x}$) from the regression analysis (Miller and Hawthorne, 2000). Therefore, the value of ^2D at the limit of detection was found to be -0.5296 + 3(0.3684), that is 0.5756. Use the regression equation then yielded the detection limit of 0.32 mg/g oil.

The absorbance spectra and the ^2D spectra of the rice bran oil (unheated) are showed in Figure 4. The initial concentration of gamma-oryzanol in rice bran oil used in this study was calculated to be 2.61±0.20 mg/g oil. This value was in the same range with that reported in literature (Seetharamaiah and Prabhakar, 1986). The ^2D spectra of heated oil showed the maxima and minima at the same wavelength for every heating condition studied, that is the oil matrix did not change the absorbance pattern. Therefore, the method used in this study should be able to provide reliable concentration values.

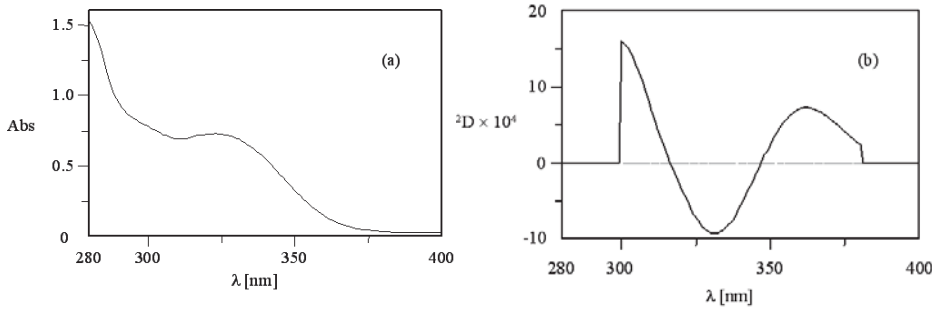

Fig. 4 Absorbance spectra (a) and the second derivative spectra (b) of the rice bran oil in isopropanol at the wavelengths from 280 to 400 nm.

Degradation kinetics of gamma-oryzanol

The concentration of gamma-oryzanol in rice bran oil decreased as the heating time increased for all temperature studied. The results suggested that first-order kinetics model could be applied to approximately describe the degradation reaction of the gamma-oryzanol in rice bran oil. The temperature effects on degradation rate are shown in Figure 5 and in Table 1. Increasing the heating temperature from 120°C to 200°C resulted in increased rate of degradation of gamma-oryzanol and decreased half-life of the compound about 10 times. The main reaction that caused the degradation of gamma-oryzanol could be the oxidation of the compound by oxidation products of oil which formed during the thermal treatment. Loss of antioxidants (á-tocopherol, hydroxytyrosol derivatives and tyrosol derivatives) due to thermal oxidation of olive oil was reported at temperature 60 and 100°C (Nissiotis and Tasioula-Margari, 2002) and 180°C (Brenes et al., 2002).

Table 1 Kinetic constant of degradation of gamma-oryzanol (k), determination coefficient (r^2) and standard error (SE) using eq 2 and $t_{1/2}$ using eq 3.

Temperature (°C)	k (h^{-1})	r^2	SE	$t_{1/2}$ (h)
120	0.0089	0.7747	0.0011	77.88
150	0.0315	0.8362	0.0028	22.00
200	0.0763	0.7089	0.0122	9.08

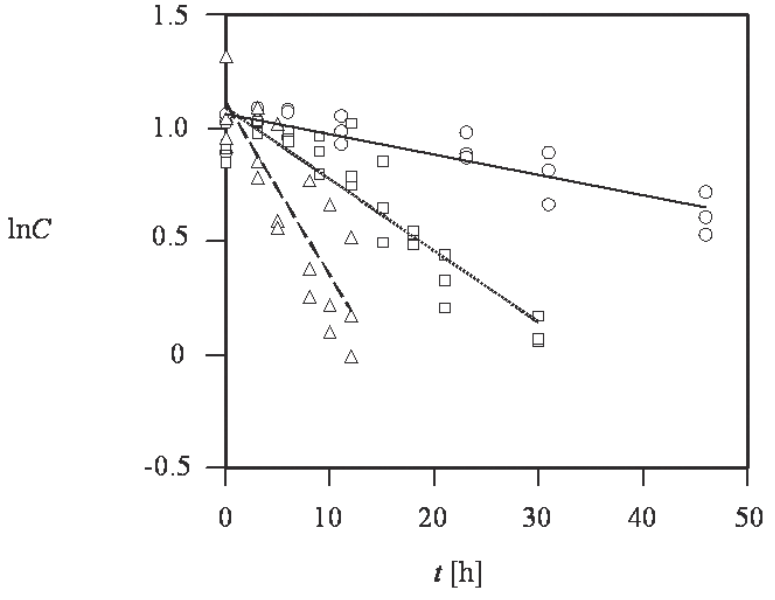

Fig. 5 Degradation of gamma-oryzanol in rice bran oil during the heating process at 120°C (O), 150°C (□) and 200°C (Δ).

Plotting the logarithm of rate constants of the reaction (ln k) against reciprocal of the absolute temperature ($10^3/T$) (Figure 6) showed that the temperature dependence of degradation rate constant obeyed Arrhenius relationship :

$$\text{In } k = \text{In } k_0 - E_a / RT \qquad (5)$$

where k is the reaction rate constant (h^{-1}); k_0 is the pre-exponential constant (h^{-1}); E_a is the activation energy (kJ/mol); R is the universal gas constant (8.314 kJ/mol K) and T is the absolute temperature (K). Using eq 5, we calculated the activation energy (E_a) from the slope of the linear regression line in Figure 6 to be 40.76 kJ/mol.

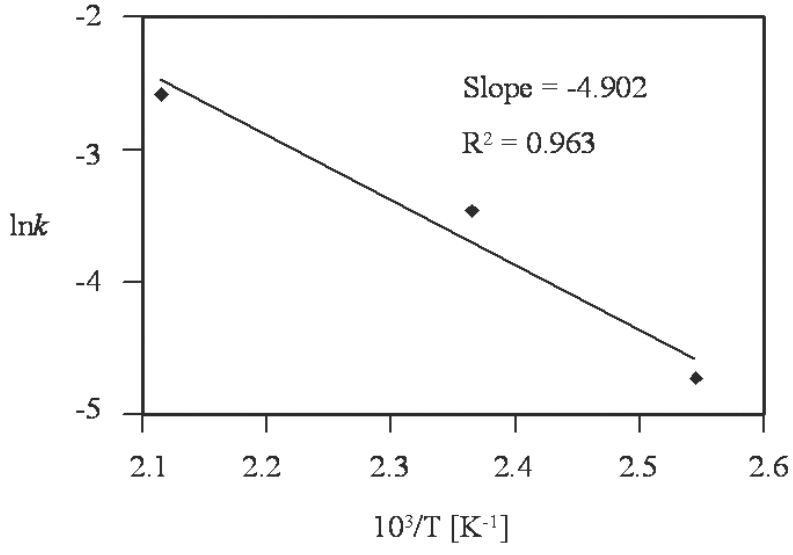

Fig. 6 Arrhenious plot for degradation of gamma-oryzanol in rice bran oil.

Conclusion

We found that degradation of gamma-oryzanol in rice bran oil obeyed the first-order kinetic. The rate constant and activation energy of the reaction at different temperatures may be used to predict retained amount of the compound during the high temperature processing such as frying. Because of limitation of the analytical method used in this study, further studies should be conducted with more efficient analytical methods such as high pressure liquid chromatography which is able to monitor change of each gamma-oryzanol compounds.

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