

# Influence of Temperature on Gamma-Oryzanol Stability of Edible Rice Bran Oil during Heating

A. Srisaipet and M. Nuddagul

**Abstract**—Rice bran oil (RBO) presents a large amount of nutraceutical compounds such as gamma-oryzanol, a complex mixture of ferulate esters with sterol. It shows high ability to reduce cholesterol absorption and inhibition oxidation reaction. This research evaluates the stability of  $\gamma$ -oryzanol present in refined RBO by heating the oil under different temperatures. From the data, the concentration of  $\gamma$ -oryzanol are slightly changing at temperature of the oil higher than 120 °C for isopropanol, butanol and ethyl acetate as dissolve solvent. While hexane showed balanced of  $\gamma$ -oryzanol trend in all of studying temperature which is affected by the interference of the oil matrix. Thus isopropanol had been suggested for accuracy solvent for  $\gamma$ -oryzanol determination. Further, we found that that the acid value of RBO depends on temperature of heated oil and  $\gamma$ -oryzanol content.

**Index Terms**—Rice bran oil, gamma-oryzanol, organic solvent, heating stability.

## I. INTRODUCTION

Rice (*Oryza sativa*) is the most popular food crop in Asia and Thailand, due to it is main food for the population in this area. Rice bran, a by-product which is obtained from the rice milling processes, contains about 8-11 % (w/w) of the grain and compost of 16-22% of lipid. Rice bran can be used as feed or as a major source of rice bran oil (RBO) [1], [2].

Rice bran oil (RBO) is widely popular edible oil in east Asia countries such as China, Korea, Japan, Taiwan and Thailand. India and Thailand are the most important procedures of RBO and together sum over 225,000 metric tons of RBO per years [3]. The utilization of RBO has increased considerable in Western countries due to its well-known for wealth of phytochemical and it is very stable in at high temperature [4]. Gamma-oryzanol ( $\gamma$ -Oryzanol) derivatives in particular are found in only a very limited number of oils and it is the most important phytochemical components in RBO which is present at 13-20 times (w/w) greater content in rice bran than total tocopherols and tocotrienols [5].  $\gamma$ -Oryzanol is a mixture of whole group of ferulic acid ester of triterpene alcohols and phytosterol [6].  $\gamma$ -Oryzanol is mainly composed of four ester are cycloartanyl ferulate, 24-methylene cycloartanyl ferulate,  $\beta$ -sitosteryl ferulate and campesteryl ferulate [7]. It has been reported to lower blood cholesterol levels in humans and animals [8], [9], anti-inflammatory [10], anti-tumor activities [11], and

including antioxidant [7], [12], [13]. In addition to these putative health benefits, the antioxidants of RBO have a potential uses as additives to improve the storage stability of foods [14], [15].

Rice bran oil has a high nutritional value and it is very appealing as specialty oil in niche market. It is used as edible oil in many Thailand households. In a cooking process, the oil is heated at high temperature although the cooking time in households is shorter period than industrial process. However losses of phytochemicals in the oil can not be avoided.

The most commonly used technique for characterization is of  $\gamma$ -oryzanol in RBO is HPLC and the technique for determination  $\gamma$ -oryzanol content is UV spectrophotometry [16] because it is very simple, rapid and easy operating.

The objective of this study was to evaluate the stability of  $\gamma$ -oryzanol present in refined RBO when heated under different temperatures. The UV-spectrophotometry method is used to quantify the  $\gamma$ -oryzanol content in the RBO. Thereby, we simulate cooking condition in households which is shorter time and lower temperature than industrial process.

## II. MATERIALS AND METHOD

### A. Materials

The four brands (Chim, King, King (high oryzanol) and Alpha-1) of commercial rice bran oil were purchased from local supermarket. Gamma-oryzanol was supplied by the Vegetable Oil Refinery (Bangkok, Thailand). Butanol, Ethyl acetate, Isopropanol and hexane in analytical grade which purchase from LabScan (Bangkok, Thailand) were used as solvent for spectrophotometric technique. The bran was dried at 105 °C until the weight constant.

### B. Studying of Solvent Effect on the Accuracy of Gamma-Oryzanol Determination

Exactly 1.0 g dried bran was weight into screw cap test tube and added 4 ml of solvent (hexane, ethyl acetate, isopropanol and butanol). Extraction was done by mixing the substances for 30 minutes at room temperature (27 °C) and incubated overnight. Centrifugation was used for separate the bran from miscella for 5 minutes at 3,000 rpm. Supernatants was collected and recorded for  $\gamma$ -oryzanol absorption spectra. The four brand of commercial RBO were dissolved in solvent and the  $\gamma$ -oryzanol in the oil absorbance was done by using a U-100 UV-vis spectrophotometer (HITACHI, Japan). Quantification of  $\gamma$ -oryzanol in solvent was determined by standard curve.

### C. Stability of Gamma-Oryzanol in Heated Rice Bran Oil

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Rice bran oil was submitted to heating in oil bath at 80, 100, 120, 140, 160 and 180 °C for 20 minutes without air circulation and protection from light. The RBO samples were taken from the heating bath and were immediately cooled in ice bath to stop the reaction. The sample was dissolved with the solvent and the absorbance spectra of sample were detected at optimum wavelength.

#### D. Determination of Acid Value

Acid value (AV) of RBO was applied from AOCS Official method Method (AOCS, 1999). The oil sample was dissolved in isopropanol/ toluene mix (1:1), followed by addition of phenolphthalein indicator (1% in isopropanol) and neutralization with base. The sample is determined quantitatively by titration with 0.1 N potassium hydroxide. The AV values are express as mg KOH/ g of sample.

### III. RESULT AND DISCUSSION

#### A. Studying of Solvent Effect on the Accuracy of Gamma-Oryzanol Determination

Recently, the reputedly solvent for high oil extraction is hexane [17]. Therefore, the other solvent types were compared in accuracy determination of  $\gamma$ -oryzanol content. The all of extraction solvents are hexane, ethyl acetate, isopropanol and butanol. The maximum wavelengths ( $\lambda_{\max}$ ) and standard calibration of  $\gamma$ -oryzanol analysis in different were summarized in Table I which is closely to a previous reported [18]. The good linearly with correlation coefficients ( $R^2$ ) showed greater than 0.995, in the range studied.

TABLE I: MAXIMUM WAVELENGTHS ( $\lambda_{\max}$ ) AND STANDARD CALIBRATION FOR  $\gamma$ -ORYZANOL ANALYSIS IN DIFFERENT ORGANIC SOLVENT

Solvent	$\lambda_{\max}$ (nm)		Calibration curves	
	Ref.	Experimental	Regression Equation*	$R^2$
Hexane	314	315	$y = 0.0359x$	0.997
Ethyl	320	320	$y = 0.0386x$	0.999
acetate	326	327.5	$y = 0.0393x$	0.996
Isopropanol	326	328	$y = 0.0350x$	0.995
Butanol				

\*y = absorbance, x = concentration (ppm)

Rapid equilibrium extraction methods have been used to extracted oil from rice bran [19], [20]. Fig. 1 shows the concentrations of  $\gamma$ -oryzanol in the rice bran extracted by different organic solvent. The  $\gamma$ -oryzanol concentration range were found from 6,400 to 6,900 ppm which is nearly value in both of low polar (hexane and ethyl acetate) and high polar (isopropanol and butanol) solvent extracted. HU *et al.*, 1996 and Nanua *et al.*, 2000 [15], [21] reported the more polar solvents, isopropanol or isopropanol: hexane (1:1 v/v), extracted more vitamin E homologs or  $\gamma$ -oryzanol, respectively, from rice bran than hexane. Chen and Bergman 2005 [22] noted that the hydroxyl groups on the benzene ring of ferulate esters might be interacted with alcohol functional group of isopropanol, rendering more liberations of  $\gamma$ -oryzanol during extraction. Zigoneanu *et al.*, 2008 [23] proposed that no significant change in amount of  $\alpha$ -tocopherol dissolved in isopropanol and hexane during the processes of microwave and solvent extractions in heating

and evaporation at 40 °C. Proctor and Bowen, 1996 [19] suggested that isopropanol was used as the extraction solvent in comparison with hexane, the same amount of oil was extracted from rice bran. In addition, the polarity of butanol is very closely with isopropanol then its extractability was nearly isopropanol. These polar solvent are miscible, water on the rice bran surface can not retard the penetration of these solvents compared with polarity solvent. Thereby, Qiao *et al.*, 2005 [24] remarked that the different degree of coupled transport of isopropanol and butanol had affected to physicochemical properties of penetrants and membrane materials. For these reasons, these solvents include hexane, ethyl acetate, isopropanol and butanol will be used to study the  $\gamma$ -oryzanol concentration in commercial edible RBO.

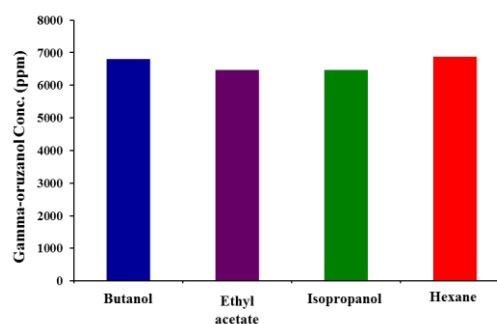


Fig. 1. The Gamma-oryzanol concentration in crude RBO solution which extracted by organic solvent.

#### B. Quantification of Gamma-Oryzanol in Commercial Rice Bran Oil

Due to the different  $\gamma$ -oryzanol content in each commercial RBO brand. The four brands (Chim, King, King (high oryzanol) and Alpha-1) of commercial RBO were selected to determine  $\gamma$ -oryzanol concentration in the oil. The  $\gamma$ -oryzanol content were label for higher than 2000, 4000 and 5,000 ppm for King, King (high oryzanol) and Alpha-1 brands, respectively and it was not labelled for Chim brand. The  $\gamma$ -oryzanol content analyzed by dissolving butanol, ethyl acetate, isopropanol and hexane at 27 °C is presented in Fig. 2. We observed that detection of  $\gamma$ -oryzanol in hexane show highest value in overall solvent in all RBO brand except the content in Alpha 1 brand which dissolved in butanol. However, the  $\gamma$ -oryzanol detection via dissolved in four solvent were in good agreement with the labelled value in all of RBO brand.

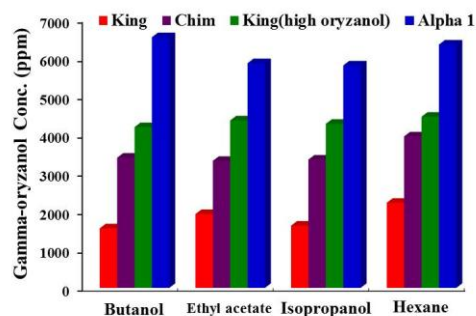


Fig. 2. The quantification of Gamma-oryzanol in commercial RBO analysis via different solvent.

Variation of  $\gamma$ -oryzanol concentration analysis with different temperature and solvent is exhibited in Fig. 3. When

isopropanol, butanol, ethyl acetate and hexane were used as dissolved solvent for determination  $\gamma$ -oryzanol content an increase temperature from 27 to 180 °C. The amount of  $\gamma$ -oryzanol did not vary significantly with temperature lower than 120 °C. For  $\gamma$ -oryzanol concentration, a significant change with temperature were higher than 120 °C. However, when hexane was used as dissolved solvent no significant change in the amount of  $\gamma$ -oryzanol on overall studying temperature.

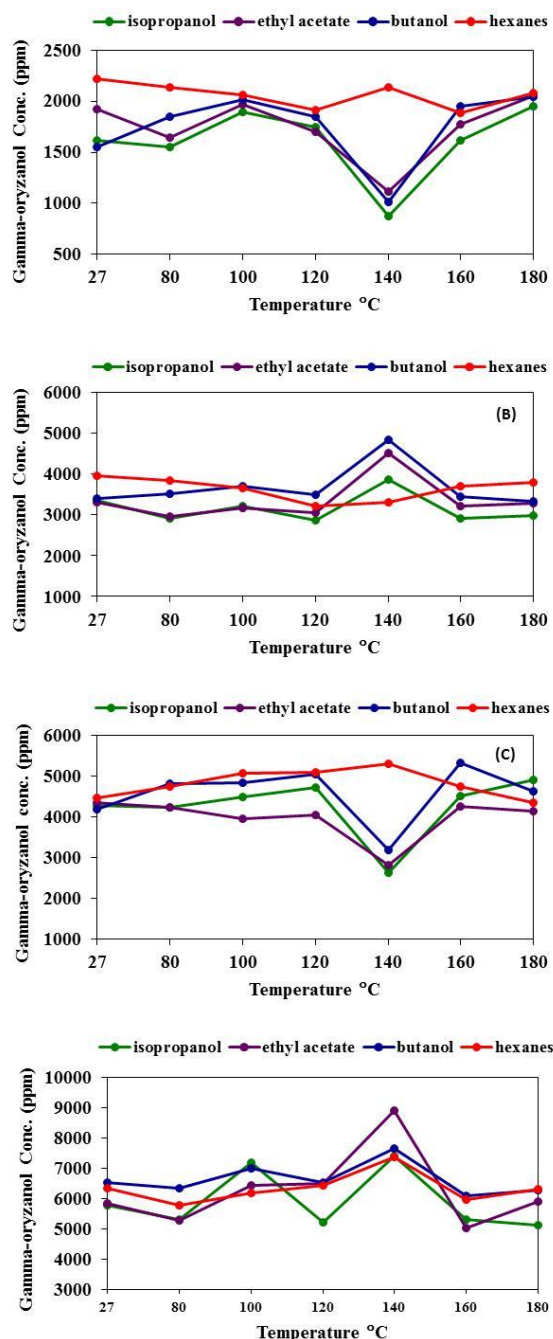


Fig. 2. The relationship between the Gamma-oryzanol concentrations (ppm) with variable temperature of edible commercial RBO in the different solvent analysis for the brands King (A) Chim (B) King (high oryzanol) (C) and Alpha 1 (D).

These results can be described by the research of Bucci *et al.*, 2003 [16]. They showed the data for using isopropanol and *n*-heptane to dilute the oil samples in determination of  $\gamma$ -oryzanol in RBO via spectrophotometric. Their results

indicated that the total concentration of  $\gamma$ -oryzanol is determined measuring the absorbance of an oil solution in *n*-heptane ( $\lambda_{\max} = 314$  nm) be not negligible and the results inaccurate because of the oil “matrix” is absorbed this wavelength. They suggested the using of isopropanol ( $\lambda_{\max} = 327$  nm) as solvent to improve the accuracy of the technique. Due to the fact that it is possible to get rid of the interference of the oil matrix. Kkuwijtjaru *et al.*, 2004 [25] reported that the degradation of  $\gamma$ -oryzanol in RBO could be found at the temperature higher than 120 °C described by a pseudo-first order kinetics model. Nystrom *et al.*, 2007 [26] found that the good heat stability of RBO has been attributed to its high content of steryl ferulates and tocopherols presents in the oil.

### C. Determination of Acid Value

Acid value (AV) is considered to be an indicator of oil quality. AV was found to increase with increasing temperature of the oil and depended on  $\gamma$ -oryzanol content in the oil (see Table II) because of ferulic acid in  $\gamma$ -oryzanol structure can react with KOH. It was due to the cleavage and oxidation of double bonds to form carbonyl compounds which oxidized to low molecular weight fatty acid during heating [27], [28]. Moreover, low molecular weight fatty acid in the oil may be lost through volatilization during heating [29].

TABLE II: ACID VALUE OF COMMERCIAL RICE BRAN OIL

RBO brands	$\gamma$ -oryzanol label (ppm)	Acid value (mg KOH/g of sample)	
		RT. (27°C)	Heated (180°C)
King	>2,000	0.22	0.29
Chim	No label	0.31	0.45
King(high oryzanol)	>4,000	0.53	0.58
Alpha 1	>5,000	0.60	1.03

## IV. CONCLUSION

Gamma-oryzanol concentration in edible commercial RBO was changed at high temperature than 120 °C due to the degradation of  $\gamma$ -oryzanol in the oil. It is presumed to a case of acid value increasing at high temperature from reacting of the ferulic acid in  $\gamma$ -oryzanol with KOH. For the determination of  $\gamma$ -oryzanol in RBO, isopropanol was recommended for accuracy solvent in determination  $\gamma$ -oryzanol.

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