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A Method for Ferulic Acid Production from Rice Bran Oil Soapstock Using a Homogenous System

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Featured Application: The homogenous base-catalyzed hydrolysis method can be applied for producing ferulic acid from rice bran oil soapstock.

Abstract: Ferulic acid (FA) is widely used as an antioxidant, e.g., as a Ultraviolet (UV) protectant in cosmetics and in various medical applications. It has been produced by the hydrolysis of γ -oryzanol found in rice bran oil soapstock. In this study, the base-catalyzed, homogenous hydrolysis of γ -oryzanol was conducted using various ratios of potassium hydroxide (KOH) to γ -oryzanol, initial concentrations of γ -oryzanol in the reaction mixture, and ratios of ethanol (EtOH) (as cosolvent)/ethyl acetate (EtOAc) (γ -oryzanol solution). Acceleration of the reaction using a planar type of ultrasound sonicator (78 and 130 kHz) at different reaction temperatures was explored. By using a heating method, the 80% yield of FA was attained at 75 °C in 4 h under homogeneous conditions (initial concentration of γ -oryzanol 12 mg/mL, the KOH/ γ -oryzanol ratio (*wt/wt*) 10/1, and EtOH/EtOAc ratio (*v/v*) 5/1). With the assistance of 78 and 130 kHz irradiation, the yields reached 90%. The heating method was applied for the γ -oryzanol-containing extract prepared from rice bran oil soapstock. From soapstock, the 74.3% yield of FA was obtained, but 20% of the *trans*-FA in the reaction mixture was transformed into *cis*-form within one month.

Keywords: ferulic acid; γ -oryzanol; soapstock; homogeneous reaction; ultrasound-assisted hydrolysis; *cis-trans* isomerization

1. Introduction

Ferulic acid (FA) is an antioxidant widely used in the cosmetic industry applied in terms of Ultraviolet (UV) protection and to protect against various inflammatory diseases [1]. It also reduces the cholesterol levels in serum and liver, protects against coronary disease, and acts as antimicrobial and anti-inflammatory agents [2]. On the other hand, FA has been investigated for blocking the formation of nitrosamine from nitrites both in vitro and in vivo in humans and mice [3]. Besides, FA

also helps to prevent e.g., cardiovascular disease, diabetes, Alzheimer's, colon cancer and reduces blood cholesterol [2,4–9].

FA is an ubiquitous phenolic compound in plant tissues such as citrus fruits, bananas, eggplant, cabbage, beetroot, broccoli, coffee [1,10], corn bran, rice bran, flax shives, wheat [11], paddy straw [12], *Radix Angelicae sinensi* [13], and *Ligusticum chuanxiong* [14]. In cereal, FA is located in the bran. It also exists in ester forms such as steryl ferulates (γ -oryzanol), which was firstly recognized in 1954 in rice bran oil (RBO). γ -Oryzanol is a mixture of ferulate compounds, of which the four main ferulates are cycloartenyl ferulate, 24-methylene-cycloartenyl ferulate, campesteryl ferulate and β -sitosteryl ferulate. Rice bran is unique source of γ -oryzanol [15]. In rice refining and processing, 96% of the γ -oryzanol contained in rice bran has been discarded as the by-products [16].

The preparation of FA from RBO soapstock has received much attention, particularly in rice-producing countries such as India, China, Japan, Thailand, and the United States. One of patents on the FA manufacturing process has been filed by the TSUNO company (Japan) [17]. The process for FA production was as follows: alkaline hydrolysis of crude γ -oryzanol (purity 80%, 10 g), base catalyst of NaOH (5 g), water (20 mL), and solvent of isopropanol (20 g). Using the method, a molar yield of FA of 86% was obtained at 90 °C in 8 h. There have been many reports of the use of ultrasound irradiation to accelerate reactions and extraction [18–24]. However, no reports were found regarding the effect of ultrasound on hydrolysis of γ -oryzanol.

Asian countries have the biggest capacity for rice production in the world. In Vietnam, the total amount of rice bran produced is about three million tons per year, but the potential inherent value of rice bran has not been fully exploited. In 2003, the first Vietnamese factory for RBO production with a capacity of 500 tons per day [25] was constructed in Can Tho City. The production of FA from γ -oryzanol contained in RBO soapstock is a promising manufacturing.

In this study, we examined the FA production from γ -oryzanol standard using alkaline catalyst of potassium hydroxide (KOH) with homogeneous solvent (water/EtOH/EtOAc) system, and by assistance of ultrasonic irradiation. In order to obtain high yield of FA, the experiments were conducted by varying the parameters as follows: the initial concentrations of γ -oryzanol, the ratios of KOH to γ -oryzanol (w/w), the ratios of solvent EtOH to EtOAc (v/v), ultrasound frequencies, reaction temperatures, and the duration of reaction. The method developed was applied to hydrolysis of the γ -oryzanol-containing extracts (Ory-extract) from RBO soapstock. The *trans-cis* isomerization of ferulic acid in the mixture after reaction was explored during one-month storage.

2. Materials and Methods

2.1. Chemicals and Equipments

The purities of *trans*-FA, γ -oryzanol, and ethyl ferulate standards (Sigma Aldrich, Saint Louis, MO, USA) were all over 99%. Another FA standard (Wako, Osaka, Japan) consists of *cis*- and *trans*-isomer was also purchased for identification. Inorganic compounds (potassium hydroxide, acetic acid, sulfuric acid, and phosphoric acid) and solvents (EtOH, EtOAc, ethyl ether, acetone, tetrahydrofuran, and isopropanol) were analytical grade, and purchased from Merck (Darmstadt, Germany). Acetonitrile (ACN), methanol (MeOH), and water used as mobile phases were high performance liquid chromatography (HPLC) grade, and purchased from Merck. The soapstock used was provided from Wilmar Argo Vietnam Co., Ltd. (Can Tho city, Vietnam).

2.2. Extraction of the γ -oryzanol from Soapstock

The Ory-extract from the RBO soapstock was prepared as follows. A 20 mL of EtOAc was added to 20 g of soapstock in a 100 mL Erlenmeyer flask with screw stopper. The flask was placed in the ultrasonic water bath (Model: S300H, Elmasonic, 37 kHz, Singen, Germany) at room temperature, and irradiated for 10 min. The mixture was allowed to stand until the residue was completely settled at the bottom. The upper liquid phase was filtered, and the filtrate was put into a 50 mL volumetric flask.

An additional 20 mL of solvent was added to the residue. The extraction was simultaneously repeated by two times, and the filtrates were subsequently transferred to the volumetric flask.

2.3. Homogeneous Hydrolysis of γ -oryzanol

2.3.1. Selection of Solvents

The solubility of γ -oryzanol in various kinds of solvents (MeOH, EtOH, EtOAc, ethyl ether, acetone, ACN, tetrahydrofuran, isopropanol, and water) was examined. A 9.6 g of γ -oryzanol was put into 100 mL of a solvent in an Erlenmeyer flask with a screwed cap. After closing, the flask was shaken vigorously, and left for a while at room temperature. The solvents in which the solubility of γ -oryzanol is more than 96 mg/mL were EtOAc, ethyl ether, acetone, and tetrahydrofuran. Among these solvents, EtOAc is the most suitable because it has the highest boiling point of 77.1 °C. EtOAc was used for extraction of γ -oryzanol from soapstock and preparation of standard solution, while EtOH was chosen as a cosolvent to homogenize the γ -oryzanol/EtOAc solution with aqueous alkaline solution.

It has been reported that the hydrolysis and transesterification reaction proceeds rapidly under homogeneous conditions consisting of water [26]. The experiment of γ -oryzanol hydrolysis was conducted under the homogeneous binary solvents systems consisting of EtOAc and aqueous KOH solution dissolved in a cosolvent of EtOH.

2.3.2. Hydrolysis Process by Heating Method

The γ -oryzanol solutions of concentration 48, 80, and 96 mg/mL were prepared by dissolving 4.8, 8.0, and 9.6 g, respectively, of γ -oryzanol in 100 mL of EtOAc. The aqueous KOH solutions of concentration 240, 384, 400, 480 mg/mL were prepared by dissolving 12, 19.2, 20, 24 g, respectively, of KOH in an aqueous solution (50 mL).

The effects of γ -oryzanol concentrations on the yield of FA were explored when the ratio of KOH/ γ -oryzanol was 10/1 (*wt/wt*). γ -oryzanol solutions of various concentrations (48, 80, and 96 mg/mL, 16 mL) were added to EtOH (80 mL) in a reaction glass vessel as shown in Figure 1a. The vessel has two necks and flat base bottom (inner diameter of 6.5 cm, 21.5 cm in height, and thickness of 2 mm) [27]. The vessel was placed in a water bath controlled at 75 °C using a SDT-04P thermal controller (Sansyo, Osaka, Japan), and held for more than 5 min. Aqueous KOH solutions of various concentrations (240, 400, and 480 mg/mL, 32 mL) were added to the vessel to prepare solutions with different initial concentrations of γ -oryzanol in the mixture (6, 10 and 12 mg/mL). After being dispersed uniformly, the vessel was held in the water bath without stirring and without acceleration by ultrasonic irradiation (hereinafter called the heating method).

In order to investigate the effect of ratio of KOH/ γ -oryzanol (KO ratio) on the FA yield, the γ -oryzanol concentration in the reaction mixture was 12 mg/mL, and the concentration of KOH solution was varied as follows. A γ -oryzanol solution (96 mg/mL, 16 mL) was added to ethanol (80 mL) in a reaction vessel. After mixing, the vessel was placed in a water bath controlled at the temperature of 75 °C and held for more than 5 min. Aqueous KOH solutions of concentrations (240, 384, and 480 mg/mL, 32 mL), corresponding to KO ratios of 5/1, 8/1, and 10/1, were added.

In addition, the effect of the cosolvent ratio was examined by varying the ratio of EtOH/EtOAc (*v/v*) (EH/EA ratio) from 2/1 to 9/1 while maintaining the KO ratio 10/1 and initial concentration of γ -oryzanol 12 mg/mL.

For determination of the reaction products, a 100- μ L aliquot of mixture was taken out and mixed with concentrated acetic acid (100 μ L) to terminate the reaction every 30 min. The mixed solution was diluted to 1.5 mL with ethanol and an aliquot (10 μ L) was subjected to HPLC analysis. The reaction was monitored for 4 h.

2.3.3. Ultrasound-Assisted Hydrolysis

As described in Section 2.3.2, the homogeneous reaction mixture prepared in the vessel was placed on the surface of the transducer of the sonicator, and irradiated with frequencies of 78 and 130 kHz (50W) at the temperatures of 60 and 75 °C for 4 h in Figure 1a. The total volume of reaction mixture was 128 mL, and the depth of solution in the reaction vessel was round 4 cm. The ultrasound sonicator used was a planar-type QUAVA Mini QR-001 in Figure 1b (Kaijo Cooperation, Tokyo, Japan). A 100 μ L of reaction mixture was taken out in every 30 min, and submitted for HPLC analysis described in Section 2.3.2.

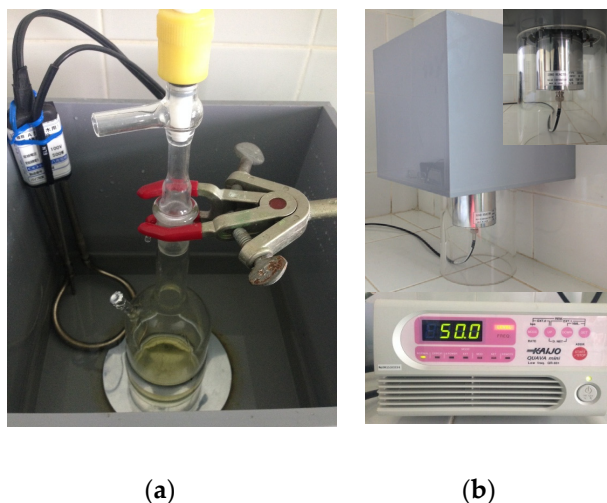


Figure 1. The reaction vessel placed on the transducer in a temperature controlled water bath (a) and the planar type of transducer and its controller (b).

2.4. Homogenous Hydrolysis of Ory-Extracts and Their Storage

The hydrolysis of Ory-extract was examined using heating method with same conditions of the authentic γ -oryzanol as follows: the initial γ -oryzanol concentration in reaction mixture of 12 mg/mL, the KO ratio of 10/1 (*wt/wt*), EH/EA ratio of 5/1 (*v/v*), and reaction temperature at 75 °C for 4 h.

After reaction, the product solution was hold at the temperature of 28 ± 3 °C for 1 month in the laboratory avoiding exposure of direct sunlight.

2.5. Quantitative Analysis Method of γ -oryzanol, FA, and Ethyl Ferulate

The HPLC system (GL-7480, GL Science Inc., Tokyo, Japan) equipped with a photo diode array detector (PDA) (GL-7452, GL Science Inc., Tokyo, Japan) was used. The analytical column was a reversed-phase Inertsil ODS-3 C₁₈ (4.6 mm x 250 mm, 5 μ m). The mobile phases were an aqueous phosphoric acid solution (0.283 M) (A) and a mixed solution of ACN and MeOH (*v/v* 1:1) (B). The analytical conditions were as follows: a gradient from 30:70 (A:B) to 100:0 for 15 min, and held for 25 min, then a gradient to 70:30 for 5 min, and then, 30:70 for 5 min. The eluent flow rate was 0.8 mL/min. The UV absorbance was monitored at 325 nm. The calibration curves of *trans*-FA, EF, and γ -oryzanol showed the good linearity, and their coefficient of determination (R^2) was more than 0.9990. The calibration curve of γ -oryzanol was estimated by the sum of the areas of four main components versus the corresponding concentrations (Figure 2). The obtained values were the averages of triplicate measurements.

The *cis*-FA concentration was estimated based on the *trans*-FA calibration curve. The percentage of *trans*-isomer presented in the mixture of two isomers was calculated as follows:

$$Pt = \frac{St}{St + Sc} \times 100\% \quad (1)$$

where Pt is the percentage of *trans*-FA in the mixture, St and Sc are the concentrations of *trans*- and *cis*-FA, respectively.

3. Results and Discussion

3.1. HPLC Chromatography

The HPLC chromatograms (UV 325 nm) of authentic γ -oryzanol and its hydrolysis products were shown in Figure 2. The peaks eluted in the range of 33–45 min were corresponding to the four ferulate components of γ -oryzanol. The peaks at retention time of 31.5, 36.0, 38.0, 44.5 min were assigned to cycloartenyl ferulate (Or_1), 24-methylene cycloartenyl ferulate (Or_2), campesteryl ferulate (Or_3), and sitosteryl ferulate (Or_4), respectively [28]. The peaks appeared at retention times of 9.1, 9.3 min were assigned to the *trans*- and *cis*-FA by comparison with their standards. As hydrolysis proceeded, ethyl ferulate appeared at retention time of 14.4 min as the reaction intermediate.

The FA produced by hydrolysis was the *trans*-form as shown in Figure 2a. The result indicated that the four ferulate components of γ -oryzanol are configured in the *trans*-form.

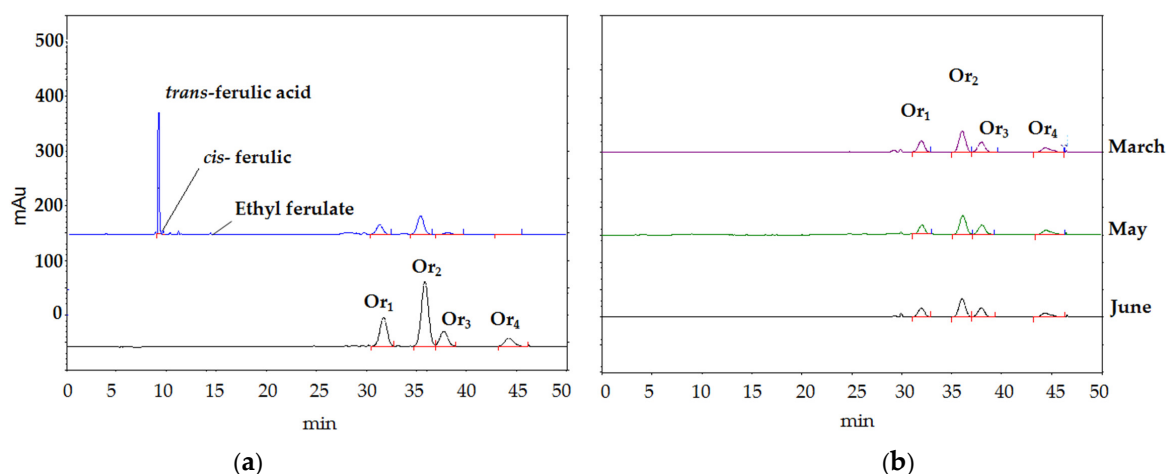


Figure 2. The High performance liquid chromatography (HPLC) chromatograms of (a) authentic γ -oryzanol before (lower line) and after hydrolysis (upper line); and (b) the Ory-extracts from the rice bran oil (RBO) soapstock samples obtained in March, May, and June of 2016.

The Ory-extracts were prepared from the Vietnamese RBO soapstock samples collected in March, May, and June of 2016. The HPLC chromatograms of the Ory-extracts are shown in Figure 2b. The four peaks of ferulates appeared in the range of 31.5–44.5 min were as same as those of an authentic γ -oryzanol as shown in Figure 2a. However, no peaks of FA were detected. The results indicated that no FA exists in free form, but in the ester form such as γ -oryzanol and/or conjugated form with bio matrix in RBO soapstock. The content of γ -oryzanol in the Vietnamese RBO soapstock was $3.1 \pm 0.3\%$ (*wt/wt*). It was reported that the soap stock obtained by alkali refining of the rice bran oil contained 1.3–3.1% γ -oryzanol [29]. After three extractions with the EtOAc solvent, Ory-extract could effectively be prepared for the following experiments.

3.2. Hydrolysis of γ -oryzanol with Homogeneous Systems

3.2.1. Effect of Initial Concentration of γ -oryzanol and KO Ratio

The yields of FA were investigated by varying γ -oryzanol concentrations and KO ratios in homogeneous systems consisting of EtOH/EtOAc and aqueous KOH solution. The experiments were conducted by varying the concentrations of γ -oryzanol from 6 to 12 mg/mL, while the KO ratio was 10/1. The reaction was monitored at 75 °C for 4 h. The FA yields were shown in Figure 3a. The yields of FA were in the range of 50%–80%, and increased with increasing initial concentration of γ -oryzanol. When the concentration of γ -oryzanol was more than 12 mg/mL at 60 °C, the precipitates appeared and the system became heterogeneous, and as a result, the yield couldn't be increased. With the initial concentration of 12 mg/mL and KO ratio of 10/1 (corresponding to molar ratio of 107/1), the FA yield reached 80%.

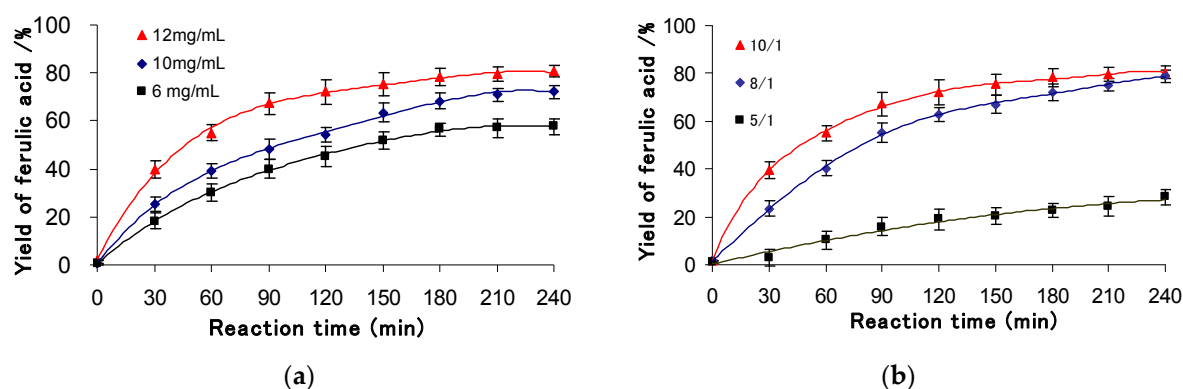


Figure 3. Effect of initial concentration of γ -oryzanol (a) and KOH/ γ -oryzanol (*wt/wt*) ratio on yield of ferulic acid (FA) (b)

When the KO ratio was greater than 10/1 and the concentration of γ -oryzanol was 12 mg/mL, the precipitates occurred under the reaction temperature as high as 75 °C. Therefore, the experiments were conducted by the KO ratios of 5/1, 8/1, and 10/1, respectively. The results were shown in Figure 3b. The yields of FA obtained were in the range of 28%–80%. The FA yield increased with the increase of KO ratio. When the KO ratio was greater than 8/1, the FA yield reached 80% as the maximum yield. These results indicated that the yield of FA depended on KO ratio. By the homogeneous system, the 80% yield of FA could be attained at the temperature of 75 °C in 4h. The temperature was lower and the reaction time was half in comparison with those reported by Taniguchi et al. [17].

3.2.2. Effect of the Cosolvent Ratio

The effect of cosolvent ratios (EH/EA) on yields of FA were examined with the following conditions: the initial concentration of γ -oryzanol 12 mg/mL, KO ratio 10/1, reaction temperature 75 °C, and 4 h. The EH/EA ratio was varied from 2/1 to 9/1, however the precipitate appeared with the ratio of 2/1. The results were shown in Figure 4. The FA yield reached the maximum of 80% at the ratio of 5/1. When the ratio increased to more than 6.5/1, the yields gradually decreased. It could be caused by the solubility of γ -oryzanol in EtOH as it decreased with the increase of EtOH. The EH/EA ratio of 5/1 was chosen for the following experiments.

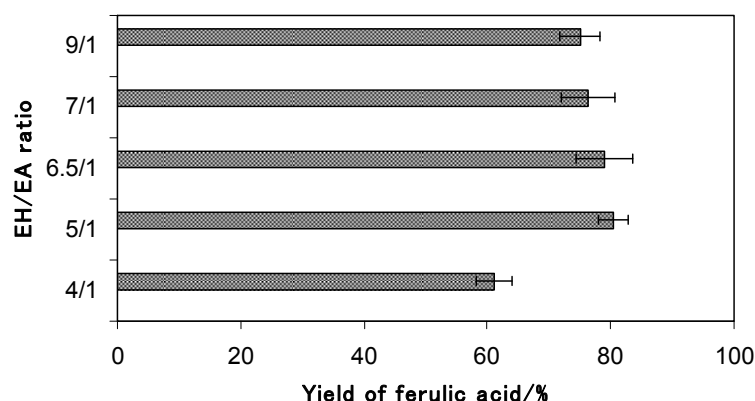


Figure 4. Effect of ethanol/ethyl acetate (*v/v*) (EH/EA) ratio on yield of FA.

3.2.3. Effect of Temperature and Ultrasound Irradiation

The effects of temperature and ultrasound irradiation on the yields were investigated by varying the temperatures of 60 and 75 °C, and the irradiation frequencies of 78 and 130 kHz (50W), respectively. The results were shown in Figure 5. The yield of FA was 45% at 60 °C, and 80% at 75 °C using the heating method. However, the yields increased to 65% at 60 °C and 90% at 75 °C with assistances of the ultrasonic irradiations (78 and 130 kHz). No significant effects on the FA yield were observed by the difference of those two frequencies. These results indicated that the hydrolysis of γ -oryzanol can be accelerated not only by the temperature, but also with agitation of ultrasonic irradiation, although it has been reported that the effect of ultrasound irradiations becomes less intense with increasing temperature [30]. Using the heating method with the homogeneous system, the maximum yield of FA was attained to be 80%, and with assistance of ultrasonic irradiation, the yield reached 90% at 75 °C in 4 h.

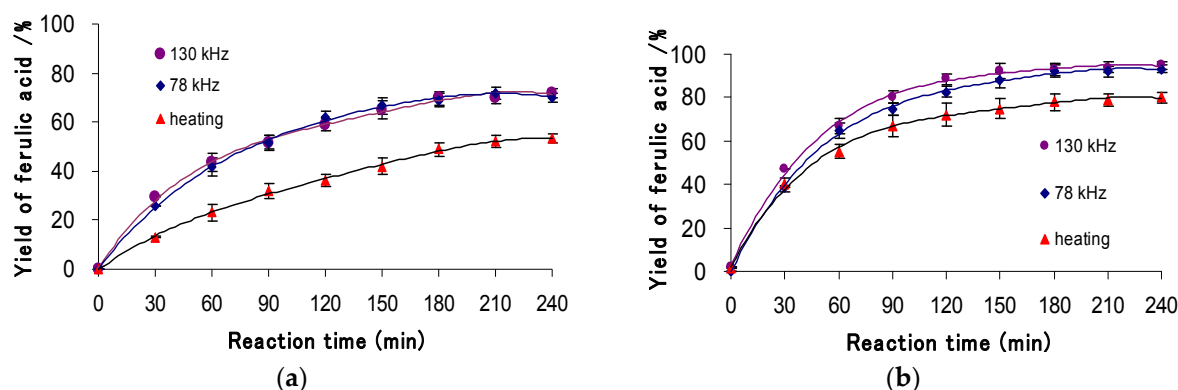


Figure 5. Effect of ultrasonic frequency (kHz) on yield of FA (a) at temperature of 60 °C; and (b) at temperature of 75 °C.

3.3. Hydrolysis of Ory-Extract from RBO Soapstock

The heating method was applied to hydrolysis of Ory-extract contained 96 mg/mL of γ -oryzanol. A 30 mL of Ory-extract solution in EtOAc was submitted for hydrolysis. The reaction conditions were described in Section 2.4. The yield of FA obtained was 74.3%, and was comparable with that of the authentic γ -oryzanol, but slightly lower than the value estimated from the results described in Section 3.2. The lower yield obtained might be explained by the consumption of basic catalyst amount of KOH by ingredients extracted together with γ -oryzanol from soapstock.

3.4. The Cis-Trans Isomerization of FA during Storage

The isomerization of FA from *trans*- to *cis*- form in alkaline solution was monitored for one month as it has been known that double bond present in the side chain of FA is subjected to *cis-trans* isomerization [31]. The isomerization process immediately occurred after reaction, with the percentage of *trans*-isomer at 97.6%. After one week, this percentage was 97.4%, and one month later decreased to 80% (Figure 6).

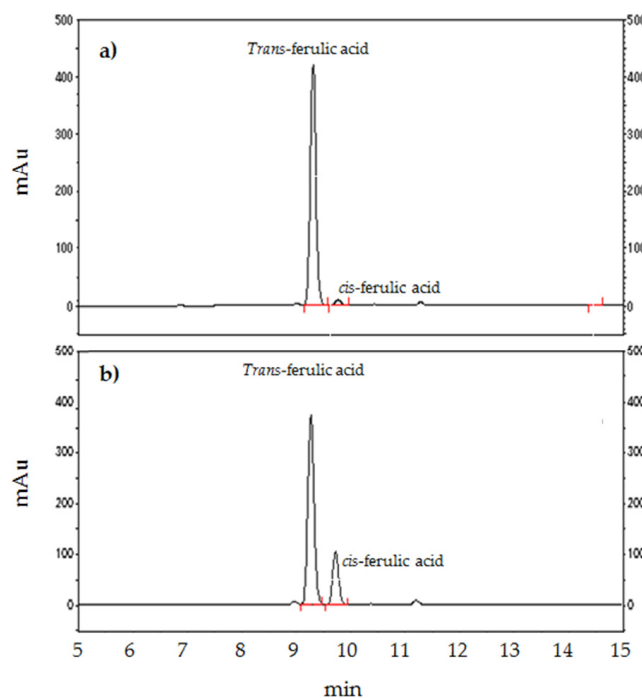


Figure 6. HPLC chromatogram of ferulic acids (a) one week after hydrolysis; (b) holding after one month.

Sun et al. reported that the isomerization by hydrogen peroxide/UV light was strongly pH dependent [32]. Under exposure to UV light, *trans*-FA was gradually converted to *cis*-form, resulting in up to 30%–40% of *cis*-FA [33]. On the contrary, the standard solution of *trans*-FA was stable when stored in the refrigerator, and hence, it is recommended that purification of *trans*-FA is conducted as early as possible after reaction.

4. Conclusions

The alkaline hydrolysis of γ -oryzanol was investigated using a homogeneous reaction system. Using the authentic sample of γ -oryzanol, the various reaction parameters of hydrolysis were examined in order to obtain the maximum yield of FA. The parameters obtained in this experiment were as follows: the ratio of cosolvent EtOH/EtOAc (*v/v*) 5/1, the initial γ -oryzanol concentration 12 mg/mL, and the KOH/ γ -oryzanol ratio (*wt/wt*) 10/1. An 80% yield of FA was obtained at 75 °C in 4 h using a conventional heating method, while the FA yield reached more than 90% with the assistance of an ultrasound irradiation of 78 (or 130) kHz (50W). By applying the heating method for hydrolysis of the Ory-extract from the RBO soapstock, the FA yield was 74.3%—the value of which was lower than in comparison with an authentic sample. However, in alkaline solution, 20% of *trans*-FA was converted into *cis*-form within one month.

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Conflicts of Interest: The authors declare no conflict of interest.

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