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Article

Evaluation of Extraction Solvents and Hydrolysis Methods for Efficient Recovery of Ferulic Acid from Rice Bran

Phannipha Daisuk[1,a] **and Artiwan Shotipruk**[1,2,b*]

1 Bio-Circular-Green-economy Technology & Engineering Center, BCGeTEC, Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand
2 Research Unit on Sustainable Algal Cultivation and Applications, Faculty of Engineering, Chulalongkorn University, Bangkok 10330, Thailand
E-mail: [a]phanniphadaisuk@gmail.com, [b,*]artiwan.Sh@chula.ac.th (Corresponding author)

Abstract. This research aims to investigate various methods for the recovery of ferulic acid (FA), a valuable phenolic compound from rice bran (RB). The content of FA in RB was first determined, which was found predominantly in insoluble-bound form within the RB matrix (85.05%). The soluble forms exist in smaller amounts as esterified FA, known as γ-oryzanol (14.32%), and as free FA (0.64%). For soluble FA, Hansen solubility parameters consideration suggested that alcohols are suitable for extraction of free FA, while esters are more effective solvents for the esterified form. Experimental extraction of full-fat RB (containing approximately 25.85% crude oil) demonstrated that ethyl acetate (83.32% FA recovery) can effectively extract soluble forms of FA alongside the extracted oil, offering an alternative to conventional hexane extraction (69.86% FA recovery). While EtOH presented the highest recovery of free FA, the very low content of free FA in RB did not necessitate its recovery. On the other hand, the recovery of insoluble-bound FA was attractive, and pressurized hot water hydrolysis was demonstrated to yield as high as 64% recovery (180 °C, 90 min), which is considerably higher than that obtained with enzymatic hydrolysis (9.45% with 2 wt% enzyme loading at 40 °C, pH 5.6, for 24 hrs). This study promotes a sustainable and bio-circular economy through the efficient valorization of agricultural byproducts and the reduced reliance on harmful chemicals like hexane and strong alkali.

Keywords: Ferulic acid, enzyme hydrolysis, pressurized hot water hydrolysis, extraction.

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1. Introduction

Ferulic acid (4-hydroxy-3-methoxycinnamic acid, FA), a cinnamic acid derivative, is a ubiquitous phenolic compound found in various plant tissue [1, 2]. FA has gained considerable interest owing to its diverse biomedical effects, including antioxidant, anti-inflammatory, anti-microbial, anti-diabetic, and anti-aging properties, and its applications in the food and cosmetic industries expand rapidly in recent years [3–5].

Thailand, being a leading rice producer and exporter, produces approximately 7 million metric tons of paddy rice annually [6]. Large quantity of RB (constituting 9 wt% of the paddy rice) is thus being generated as an important byproduct of rice milling. While RB is mostly used as low-cost animal feed, a valuable source of edible oil, protein, and active ingredients is often overlooked. Consequently, this underutilization results in lost opportunities to extract high-value compounds like FA, which have significant industrial applications. Aligned with the country's policy to promote bio-circular economy, this study aims to investigate sustainable methods of recovering FA from RB.

Like in other plants, RB FA is present in three major forms: soluble-esterified from (FA that are esterified with triterpene alcohols or phytosterols, known as γ-oryzanol), soluble free FA, and insoluble-bound FA (FA that is linked with hemicellulose via esterified and lignin by etherified linkage [4, 7, 8]).

The esterified FA is generally extracted along with oil in a typical oil extraction process, where in a commercial scale, hexane is commonly used as an extraction solvent owing to its low cost. However, hexane is a volatile organic compound (VOC) that is toxic to human and the environment. As a result, a search for alternative solvents has recently become of the most important research activities.

For the recovery of the insoluble-bound FA, alkali hydrolysis has been shown to effectively liberate the FA by breaking down the FA-cellulose and FA-lignin linkages in various plant tissues [9]. This conventional approach generates large amount of wastewater, requiring neutralization for water treatment and releases other byproducts (e.g., furfural and hemicellulose) [8, 10], hindering downstream purification processes.

Alternative to alkali hydrolysis, enzymatic hydrolysis is a milder and greener approach to effectively and selectively cleave off FA that is bound to the rigid structure of cellulose and lignin of plant matrix [11]. Nevertheless, the process takes longer (20-50 hrs), and thus the incorporation of pretreatment using techniques such as autoclaving or ultrasonication is often required to help break down the plant cell complex structures and improve enzyme accessibility [7]. Other than enzymatic hydrolysis, pressurized hot water hydrolysis has been demonstrated to effectively release the bound FA from plant tissues within a much shorter period of time (3-70 min, depending on the conditions) [8, 12]. While much of the previous research has been conducted on recovering mostly bound FA (particularly from wheat or corn brans), there is a lack of comprehensive research on finding efficient methods to recover all different FA forms from RB, despite the abundance of FA in this agricultural byproduct, and is therefore the aim of this study. Firstly, the contents of different FA forms are quantified. Secondly, suitable solvents for extraction of soluble-esterified and free forms of FA were suggested, based on the consideration of Hansen solubility parameters (HSPs) which enables the prediction of relative solubility of the interested solutes in various solvents. Selected solvents having varying polarities were then experimentally tested for extraction of the two soluble FA forms. Lastly, the efficiency of enzymatic hydrolysis and pressurized hot water hydrolysis for extraction of the insoluble-bound form of FA from de-oiled RB were evaluated and compared.

2. Materials and Methods

2.1. Material and Chemicals

Parboiled RB was kindly provided by Thai Ruam Jai Vegetable Oil Co., Ltd. in Bangkok, Thailand. Extraction solvents, including ethyl acetate (EtOAc, 99.5%), iso-propyl alcohol (IPA, 99.8%), n-hexane (99%), methanol (MeOH, >99.8%), and acetonitrile (ACN, 99.9%), were obtained from Fisher Scientific UK Ltd. (Leicestershire, UK). Acetone (99.5%) and ethanol (EtOH, 99%) were supplied by ARC Chemicals (Istanbul, Turkey). Sodium hydroxide (NaOH, 99%) for alkali hydrolysis and hydrochloric acid (HCl, 37%) for pH adjustment were purchased from QRëC, New Zealand. Cellulase (35,000 U/g) and xylanase (200,000 U/g) were acquired from Megazyme South Ayrshire, Scotland. γ-Oryzanol (soluble-esterified FA, 97%) and free FA standards were obtained from Wako Chemicals, Japan.

2.2. Quantification of Different Forms of RB FA

To quantify the RB FA contents in various forms, sequential extractions and alkali hydrolysis were employed. Firstly, full-fat RB samples (2 g) were extracted repeatedly with hexane (40x3 mL, 60 min, 200 rpm at 30 °C) to obtain the soluble-esterified FA (γ-oryzanol). This step also provided a measure of total crude oil content (25.85%). Secondly, the remaining residue was extracted repeatedly with MeOH (40x3 mL, 60 min, 200 rpm at 30 °C) to obtain soluble-free FA. Finally, the residue from MeOH extraction was repeatedly extracted via alkali hydrolysis (20x3 mL of 2 molar NaOH, 3 hrs at 30 °C) to release the insoluble-bound FA. All extracts (9 samples) were analyzed using HPLC to determine the amounts of esterified and free FA. The total quantities of soluble-esterified, soluble-free, and insoluble-bound RB FA were calculated using Eq. (1)-(3).

$$\text{Total soluble esterified FA} = \frac{\text{Sum of esterified FA extracted by hexane}}{\text{Mass of starting RB sample}} \times \frac{164.18}{603.78} \quad (1)$$

$$\text{Total soluble free FA} = \frac{\text{Sum of free FA extracted by MeOH}}{\text{Mass of starting RB sample}} \quad (2)$$

$$\text{Total insoluble bound FA} = \frac{\text{Sum of free FA released by alkali hydrolysis}}{\text{Mass of starting RB sample}} \quad (3)$$

The constants 164.18 g/mol and 603.78 g/mol in Eq. (1) represent the molecular weights of free FA and γ-oryzanol, respectively. These values were used to convert the mass of γ-oryzanol to the equivalent mass of FA.

2.3. Screening of Solvents for Extraction Of Esterified and Free FA and Experimental Extraction

2.3.1. HSP evaluation and construction of solubility spheres

Initial screening for potential solvents for extracting soluble FA (esterified and free FA) was performed based on the consideration of HSPs, which are divided into three parameters: dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h). When the HSPs of a solvent are close to those of a solute, it is considered a good solvent. In such case, the R_a value, defined by Eq. (4) is small.

$$R_a = \sqrt{4(\delta_{d,A}-\delta_{d,B})^2+(\delta_{p,A}-\delta_{p,B})^2+(\delta_{h,A}-\delta_{h,B})^2} \quad (4)$$

To visualize the relative compatibility between a solute and a solvent, Hansen solubility spheres were constructed in a 3D space using MATLAB program. Here, the HSPs of the esterified and free FA, and the candidate solvents were first plotted. The center of each sphere represents the solute's HSPs, while its radius (R_0) defines the boundary between good and poor solvents. Solvents falling within each solute sphere are considered to have good solubility for the solute, while those outside it have poor solubility. The radius of each solute sphere, R_0, was determined based on experimental solubilities of the solute in several solvents having a wide range of polarities. The R_0 is typically assigned to be equal to the R_a value of the solvent having the largest R_a among all the good solvents for the particular solute.

In the case of esterified FA (γ-oryzanol), R_0 was assigned in our previous work, to be 8.25 $MPa^{0.5}$ (the R_a of acetone) [16]. To assign the R_0 value for free FA, experimental data on free FA solubilities in various solvents obtained from previous studies were considered, along with the HSPs of the solutes and the solvents (summarized in supplementary Table S1).

2.3.2. Experimental extraction of soluble esterified and free FA

To confirm the potential of the selected solvents for extracting soluble free and esterified FA, various selected solvents: hexane, EtOAc, acetone, IPA, EtOH, MeOH, and ACN were experimentally evaluated. Full-fat RB (2 g) was extracted with the tested solvent (20 mL) under stirring (200 rpm) at 30 °C for 60 min. The RB residue was separated from the extract by centrifugation (model U-320, BOECO Germany) at 4500 rpm for 10 min. The solvent was then evaporated using a vacuum oven (Binder, Germany) at 60 °C overnight, leaving the dried crude extract, which was then stored at 5 °C for subsequent quantification of free and esterified FA. The recovery (%) of free and esterified FA were calculated using the following Eq. (5) -(6).

$$\text{Recovery of free FA(\%)} = \frac{\text{Free FA recovered}\times 100}{\text{Total amount of free FA in RB}} \quad (5)$$

$$\text{Recovery of esterified FA(\%)} = \frac{\text{Esterified FA recovered}\times 100}{\text{Total amount of esterified FA in RB}} \quad (6)$$

2.4. Evaluation of Hydrolysis Methods to Release Insoluble-Bound FA

As an alternative to alkali hydrolysis, the efficiency of enzymatic hydrolysis and pressurized hot water in releasing insoluble-bound FA from de-oiled RB were experimentally evaluated and compared in terms of % recovery, defined by Eq. (7).

$$\text{Recovery of bound FA(\%)} = \frac{\text{Bound FA recovered}\times 100}{\text{Total amount of bound FA in RB}} \quad (7)$$

2.4.1. Enzymatic hydrolysis

De-oiled RB (2.5 g) was suspended in 50 mL of sodium acetate buffer (pH 5.6). To the suspension, an enzyme was added at 2% (w/w) loading, and the mixture was then placed in an incubation shaker operated at 130 rpm and 40 °C for 24 hrs. The enzymatic reaction was stopped with acetic acid, and the mixture was centrifuged (4500 rpm, 10 min). The supernatant (1 mL) was mixed with EtOH (1 mL) followed by filtration through a syringe filter (0.45 μm pore size) and the amount of released free FA was analyzed using HPLC. The effects of variables were investigated including enzyme types (cellulase, xylanase or the combination at 1:1 mass ratio) and the pretreatment methods (ultrasound and autoclave) on the percentage recovery of FA.

For ultrasound pretreatment, the suspension of de-oiled RB (2.5 g) in sodium acetate buffer (50 mL) was subjected to ultrasound irradiation (probe UP400St, 400 watts, 24 kHz) for 20 min at 50% amplitude. This process caused the temperature of the suspension to rise to 69 °C. After letting the suspension cool to room temperature, enzymatic hydrolysis was performed as described previously.

For autoclave pretreatment, de-oiled RB (2.5 g) was moistened with sodium acetate buffer (7 mL) and autoclaved at 121 °C for 15 min (total process time approximately 2 hrs). After autoclaving, 43 mL of sodium acetate buffer and 0.05 g of enzyme were added, and enzymatic hydrolysis proceeded as described above.

2.4.2. Pressurized hot water hydrolysis

The continuous flow pressurized hot water hydrolysis was employed following the procedure modified from Chainukool et al., (2014) [13]. De-oiled RB (1 g) was loaded into the extraction vessel (10 mL, Thar Design, USA), which was then installed in the oven (D63450, HARAEUS, Germany). Distilled water was continuously pumped (PU 980, JASCO, Japan) at a constant flow rate of 5 mL/min through a preheating inlet section within the oven and then through the extractor. The back-pressure regulator (AKICO, Japan) was adjusted to maintain a system pressure of 2 MPa. The hydrolysate was then collected at the outlet of the extractor every 10 min for a total of 90 min. Prior to HPLC analysis, 1 mL of hydrolysate was mixed with 1 mL of EtOH, and the mixture was then filtered through a syringe filter (0.45 μm pore size). The effect of temperature and time of pressurized hot water hydrolysis on the efficiency of liberating insoluble-bound FA was investigated.

2.5. Quantification of Free and Esterified FA

The contents of free and esterified FA in RB extracts and hydrolysis products were quantified using HPLC. Sample solutions (10 μL) were injected into reversed-phase Waters Sunfire C18 column (4.6 x 100 mm, particle size = 3.5 μm), connected to a UV/Vis detector (Waters Alliance e2695, USA) set at a wavelength of 325 nm. Isocratic elution was employed with a mobile phase mixture of ACN: MeOH: IPA (45:30:25 v/v/v) at a flow rate of 0.50 mL/min at 30 °C.

2.6. Statistical Analysis

In this study, all experimental data are presented as mean ± standard deviation (SD). Statistical significance of differences between groups was assessed using one-way ANOVA followed by Tukey's post-hoc test (IBM SPSS software). Significance was considered at $p < 0.05$.

3. Results and Discussion

3.1. Quantification of RB FA Contents in Different Forms

To comprehensively determine the distribution of FA within RB, a sequential extraction process involving a three-cycle hexane extraction, a three-cycle MeOH extraction, and three-cycle alkali hydrolysis was employed. As shown in Fig. 1, a large portion of esterified FA was extracted in the initial hexane cycle, while the subsequent hexane cycles yielded much smaller amounts. In total, hexane extraction yielded 0.91 mg of esterified FA of g dry weight RB. Free FA on the other hand was not detected in hexane extracts, but could be recovered with MeOH mostly in the 4th cycle, and in very small quantities in the 5th and 6th cycles, totaling 0.041 mg/g dry weight RB. This is a very small amount compared with those of the esterified FA and insoluble-bound FA. Insoluble-bound FA were primarily released during the 7th alkali hydrolysis cycle. Additional amounts of FA were released in the 8th and 9th cycles, giving the total content of insoluble-bound FA of 5.43 mg/g dry weight RB, which is similar to the amounts of 4.79-4.82 mg/g dry weight RB previously reported in literatures [14, 15]. The minor difference observed may be attributable to variations in rice variety, growing season, degree of milling, experimental setups, or analytical methods. The pie chart embedded in Fig. 1 shows the percentage distribution of the different FA forms within the RB sample, which indicates that the insoluble-bound form is dominant, accounting for 85.05%, followed by the esterified FA (14.32%), while free FA constitutes the smallest portion (0.64%).

3.2. Evaluation of Suitable Solvents for Extraction of Soluble Esterified and Free FA

3.2.1. Consideration of Hansen solubility spheres

To determine possible potential solvents for extraction of both soluble esterified and free FA, a preliminary evaluation based on the consideration of the HSPs of the solutes and several solvents were conducted. The Hansen solubility spheres for soluble-esterified and free FA were constructed, as depicted in Fig. 2. The esterified form, centered at δ_d =18.62 MPa$^{0.5}$, δ_p =6.49 MPa$^{0.5}$, δ_h =3.30 MPa$^{0.5}$ [16], exhibits a molecular interactions distinct from the free form, centered at δ_d =19.00 MPa$^{0.5}$, δ_p =8.89 MPa$^{0.5}$, δ_h =17.51 MPa$^{0.5}$ (determined by Group contribution method of Stefanis & Panayiotou, 2008 [17]). Due to its hydroxyl and carboxyl groups, the free form is dominated by hydrogen bonding interactions. The esterified FA, on the other hand, having non polar moieties from phytosterols or triterpene alcohols, it is primarily influenced by dispersion interactions. While the radius of the esterified FA was taken to be 8.25 MPa$^{0.5}$, as determined in our previous work [16], the radius of the free form was assigned in this study based on the experimental solubilities of free FA in various solvents, obtained from literature (supplementary Table S1). As seen from Table S1, free FA exhibited excellent solubility in solvents such as dimethyl sulfoxide (DMSO), glycols, alcohols, and esters (15.96-151.84 mg/mL) [18,19], but were poorly soluble in water, organochlorine solvents, and acetonitrile (0.75-2.43 mg/mL) [19–21]. The radius of the free FA sphere was thus assigned to be 14.40 MPa$^{0.5}$, corresponding to the R_a value of butyl acetate, the solvent with the largest R_a among those considered as good solvents.

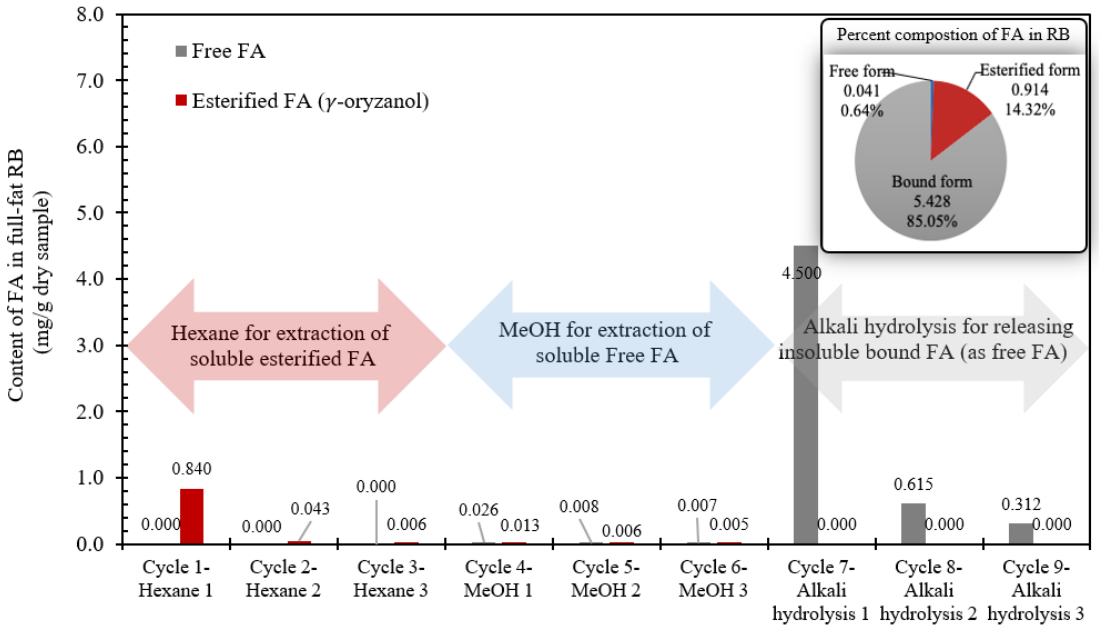

Fig. 1. The total FA contents of RB in different forms and those recovered after each of the 3-time repeated extractions.

By considering the HSP spheres in Fig. 2, potential solvents candidates for extraction of soluble FA can be suggested. Solvents like EtOAc, butyl acetate, and acetone, which fall within the overlapping region of both esterified and free FA spheres, are predicted to exhibit good solubility for both forms. Conversely, solvents located outside both spheres, including water, ACN, and hexane, are predicted to have poor solvency for both forms.

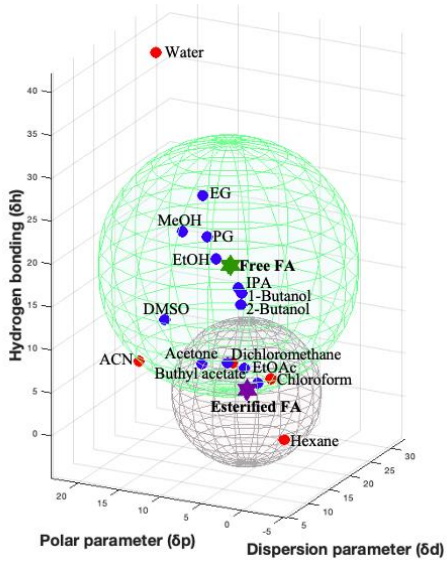

Fig. 2. Hansen solubility spheres of free and esterified FA.

On the other hand, solvents that exhibit good solubility for the free form but poor solubility for the esterified form include DMSO, alcohols, and glycols. Although the experimental solubility data agree with the Hansen prediction in most cases, it is importance to note that they do not agree in some cases. For instance, chloroform and dichloromethane, while falling within the overlapping region, exhibited high experimental solubility but the experimental solubility of free FA in these solvents are poor (1.45 and 2.43 mg/mL [21]). Nevertheless, HSP consideration allows initial screening/selection of candidate extraction solvents.

To validate the theoretical screening and assess the practical performance of some selected solvents, extraction experiments were conducted using full-fat RB sample. Acetone and EtOAc, expected to extract both soluble forms of FA, were selected for the experimental study. Additionally, MeOH, EtOH, and IPA, known for their high solubility of free FA, were included. For comparison, ACN and hexane, which are predicted to have poor solubility for both forms, were also tested.

The results depicted in Fig. 3 indicate that the highest total extract recovery was achieved with EtOAc and hexane, followed by acetone, IPA, EtOH, MeOH, and ACN. This trend aligns with expectations, as EtOAc and hexane are effective solvents for oils, the primary extractable component of RB. Conversely, highly polar solvents like MeOH and ACN exhibited the lowest total extract recovery.

3.2.2. Experimental extraction

Full-fat RB samples were extracted with selected solvents (hexane, EtOAc, acetone, IPA, EtOH, MeOH, and ACN) under identical conditions at room temperature (30 °C) for 60 min. Figure 3 illustrates the percentage recovery of total extract, esterified FA, and free FA extracted with various solvents.

For esterified FA, EtOAc yielded the highest recovery (87%), followed by acetone (80%). Hexane, EtOH, and IPA showed relatively high and comparable recoveries, ranging from 72% to 75%. MeOH and ACN demonstrated significantly lower recoveries of only 47% and 39%, respectively. These results generally correlate with Hansen solubility predictions, which indicated high

solubility of esterified FA in EtOAc and acetone, and low solubility in MeOH and ACN. However, the relatively high recoveries obtained with hexane, IPA, and EtOH, despite their predicted poor solvency for esterified FA, can be attributed to the co-extraction of esterified FA with oil (triglycerides), which are efficiently extracted by hexane and partially extracted by IPA and EtOH.

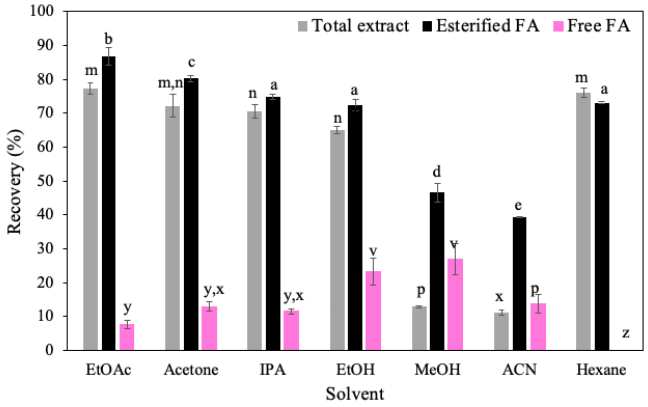

Fig. 3. Different solvents extraction of full-fat RB with 10 mL solvent to 1 g RB at 30 °C for 60 min.

Regarding free FA, MeOH yielded the highest recovery of 27%, followed by EtOH (23%). IPA, acetone, and ACN provided similar recoveries of 12-14%, while EtOAc extracted only 8%, and hexane could not extract any free FA. These experimental results also agree with Hansen solubility predictions, as MeOH, being the closest to the center of the free FA solubility sphere, exhibited the highest extraction efficiency, whereas solvents like EtOAc and acetone, positioned farther from the center, demonstrated lower extraction recoveries. It is worth noting that despite MeOH's predicted suitability for free FA extraction, the recovery was only 27%, which is lower than anticipated. This can be attributed to the presence of oil in the full-fat RB sample, which likely hindered the extraction of free FA. This hypothesis is supported by the significantly higher free FA recovery (64%) observed with MeOH extraction for de-oiled RB. However, as the free FA form is only present in a very low content in RB, their recovery is not economically viable.

Table 1. The recovery of total soluble FA (esterified and free form) from different solvents.

Solvent	Esterifed FA(mg/g RB)	Free FA (mg/g RB)	SUM FA (mg/g RB)	Overall FA recovery (%)
EtOAc	0.7926	0.0031	0.7957	83.32
Acetone	0.7327	0.0053	0.7379	77.27
IPA	0.6831	0.0047	0.6878	72.02
EtOH	0.6621	0.0094	0.6716	70.32
MeOH	0.4262	0.0109	0.4371	45.77
ACN	0.3589	0.0056	0.3645	38.17
Hexane	0.6672	0.0000	0.6672	69.86
Total	**0.9136**	**0.0406**	**0.9542**	**100**

Among all the solvents evaluated, EtOAc emerged as the superior solvent, achieving a significantly higher overall FA recovery (Table 1). This makes EtOAc a promising alternative to hexane for extracting total soluble FAs from full-fat RB, providing comparable oil extraction efficiency (77.37% vs 76.05%), but significantly outperforming hexane in recovering total soluble FAs (83.32% vs 69.86%). While the cost of EtOAc (USD 980-1702/ton) is comparable to hexane (USD 1030-1199/ton) [23], it is more favorable when considering safety, health and environmental criteria [24].

3.3. Comparison of Hydrolysis Methods for Releasing Bound FA

Alternative to alkali hydrolysis, two alternative methods: enzymatic hydrolysis and pressurized hot water hydrolysis, were evaluated for extraction of insoluble-bound FA.

3.3.1. Enzymatic hydrolysis

Figure 4 shows the effects of enzyme types: cellulase, xylanase, and a 1:1 mixture, on the recovery of insoluble-bound FA, with and without pretreatment either by autoclaving or ultrasonication. Without pretreatment, FA recoveries from RB enzymatic hydrolysis using either a single enzyme or the enzyme mixture were very low ranging from 4.41% to 5.44%. Xylanase and the enzyme mixture gave slightly (but significantly) higher recovery than that of cellulase. Without pretreatment, other studies on enzymatic hydrolysis of corn fiber and wheat bran showed relatively low FA recovery of 4-10%, depending on the type of enzyme used [2,14]. Regarding the enzyme type, the results in our and other studies [7,10] indicated that xylanase and cellulase were inferior to feruloyl esterase in extracting and recovery bound FA from plant materials. Although cellulase and xylanase break down the β-1-4 glycosidic bonds of cellulose and hemicellulose, unlike feruloyl esterase, they do not directly break down the ester or ether bond that link FA with lignin and arabionxylans within the plant matrix.

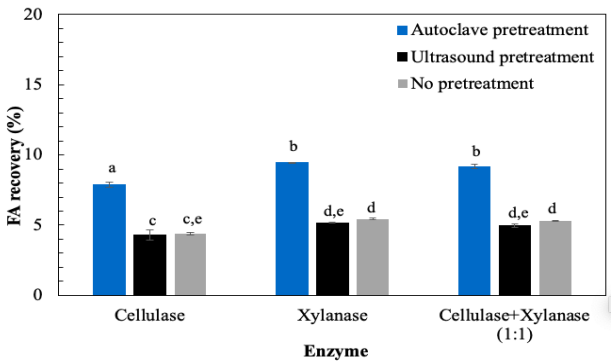

Fig. 4. Recovery of bound FA (%) by enzyme hydrolysis with and without autoclave or ultrasound pretreatment. Hydrolysis conditions: enzyme loading: 2%wt; incubation temperature: 40 °C; shaking: 130 rpm; incubation time: 24 hrs, and pH: 5.6.

Nevertheless, the high cost of feruloyl esterase led to a search for other alternatives or the exploration of assisted extraction techniques such as ultrasound and autoclave pretreatments. With ultrasound pretreatment, our results showed no improvement observed on the FA recovery, while on the other hand, the recovery could significantly be improved (approximately 2 times) with autoclave pretreatment. This is due to the high temperature (121 °C) and pressure (approximately 15-20 bar) during autoclave treatment, which can disrupt the structural integrity of the RB matrix, increasing enzyme accessibility to the substrate sites. Similar findings on the effectiveness of autoclave pretreatment have been reported by other studies. Valério et al. (2021) observed that pretreatment of corn fiber with water at elevated temperature of 140 °C at auto-generated pressure for 40 min prior to enzyme hydrolysis increased the recovery of bound FA from 7.83% to 28.94% [2]. The greater efficiency of autoclave over ultrasound pretreatment was also consistent with the findings reported by Al-Shwafy et al. (2023), on the recovery of FA from brewer's spent grain. The authors reported considerably higher % FA recovery when applying autoclave pretreatment prior to feruloyl esterase hydrolysis, compared with using ultrasound (43% vs 4%) [7].

3.3.2. Pressurized hot water hydrolysis

Alternative to enzymatic hydrolysis to extract bound FA from de-oiled RB, pressurized hot water hydrolysis was explored. The results in Fig. 5 illustrates the time profile percentage recovery of bound FA extracted by pressurized hot water hydrolysis in a semi-continuous flow system at various temperatures. The extraction profile can be divided into two distinct phases: from 0 to 20 min and from 50 to 90 min with transition occurring between 20 and 50 min.

The rapid extraction rate observed in the first phase was attributed to the hydrolysis of bound-FA molecules from the surface of the RB particles. The second phase, with a slower extraction rate, reflects the diffusion-controlled phase where the remaining bound FA molecules were extracted from the interior of the RB particles.

The highest overall recovery of bound FA was achieved at 180 °C, reaching 64% at 90 min, which is relatively high compared to that obtained with enzymatic hydrolysis (9.45%). At elevated temperature, water generates hydronium and hydroxide ions, which act as catalysts, promoting the breakdown of linkages of FA to lignin and/or arabinoxylans and thus the release of FA. At 100 °C, no bound FA was extracted, indicating that this temperature is insufficiently low to break the linkages of bound FA.

On the other hand, when the temperature was too high as in the case of 200 °C, low FA recovery decreased, which was likely due to the thermal degradation of FA molecules at such high temperature. In another study by Buranov and Mazza, (2009), the author also reported lower FA recovery from pressurized hot water extraction of corn bran at 220°C, than at 180 °C (13.3% vs. 16%) [8].

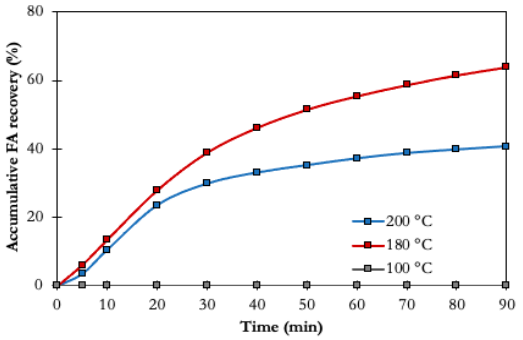

Fig. 5. Recovery of insoluble-bound FA by pressurized hot water hydrolysis extraction at flowrate of 5 mL/min and pressure 2 MPa.

Table 2. Summary of FA recoveries by pressurized hot water hydrolysis from different sources.

Raw materials	FA recovery (%)	Hydrolysis conditions	References
De-starched wheat bran (DWB)	13%	200 °C, 3.5 min, 1 g of DWB: 20 mL of water, batch system	[22]
Wheat bran	15%	160 °C, 1.5 MPa, 60 min, 2.6 g, 0.02 g of WB: mL of water, stirring speed 800 rpm, batch system	[12]
Corn bran	16%	180 °C, 5.2 MPa, 5 g flowrate 5 ml/min, 40 min	[8]
Flax shives	34%	180 °C, 5.2 MPa, 5 g flowrate 5 ml/min, 40 min	[8]
Wheat bran	25%	180 °C, 5.2 MPa, 5 g flowrate 5 ml/min, 40 min	[8]
Rice bran	64%	180 °C, 2 MPa, 1 g flowrate 5 ml/min, 90 min	This study

The decrease in the FA recovery at such high temperatures is supported by the study by Victoria Pazo-Cepeda (2020) which, based on thermogravimetric analysis (TGA), revealed that FA (free form) begins to decompose at 160 °C [12].

A summary of the literature on FA recovery from various sources using pressurized hot water hydrolysis is provided in Table 2. It is noted from the results of these different studies that, in addition to the type of raw materials and extraction conditions, extraction system for

the hydrolysis of bound FA could significantly influence the FA recovery. Flow-through systems such as that used in our study and Buranov and Mazza (2009) seemed to give higher FA recovery than the batch systems such as those used by Pazo-Cepeda et al. (2020) and Pazo-Cepeda et al. (2021) [12, 22]. Compared with the batch systems, the solute-solvent concentration gradient is better maintained in the flow-through systems, allowing the extraction to more rapidly takes place, which inturn help reducing thermal decomposition.

4. Conclusions

This study investigated the recovery of FA in various forms from RB. The majority of FA (85%) was found in the insoluble-bound form, with smaller amounts in esterified and free forms. Evaluation of HSPs revealed that esterified FA is preferentially soluble in low-polar solvents, while free FA is more soluble in alcohols (dominant of hydrogen bonding). Given the small quantity of free FA in RB, attempting to recover it would not be an economically viable option. For recovering the insoluble-bound FA, pressurized hot water hydrolysis demonstrated superior efficiency compared to enzymatic hydrolysis, achieving approximately 64% recovery at 180 °C, 2 MPa, 90 min. Future research could investigate the economic feasibility of scaling up pressurized hot water hydrolysis for industrial application, including detailed cost analysis of energy consumption, equipment investment, and downstream processing, environmental impact to determine its competitiveness against conventional method.

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Phannipha Daisuk was born in Trat Province, Thailand in 1988. is a Ph.D. student at the BioCircular-Green-economy Technology &Engineering Center (BCGeTEC), Department of Chemical Engineering, Chulalongkorn University, Thailand. She received a B.S. degree in Chemical Engineering Institute of Engineering, Suranaree University of Technology, Nakhonratchasima, Thailand in 2011. Her research interests lie in sustainable separation and purification technologies from bio derived resources.

Artiwan Shotipruk received her B.S. in Chemical Engineering from The Johns Hopkins University in 1995, her M.Eng. in Chemical Engineering from the University of Michigan, Ann Arbor in 1997, and her Ph.D. in Chemical Engineering from the University of Michigan, Ann Arbor in 2001. Her research focuses on the production of biofuels and bio-based chemicals using green technologies such as supercritical and subcritical fluid technology. Her research highlights include: Extraction and purification of natural active compounds from plant and microalgae for food and pharmaceuticals, biomass conversion for the production of fuels and high value chemicals, development of carbon-based catalysts for biorefineries, application of sub-and super-critical fluids for extraction and biomass conversion.

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Supplementary Data

Table S1. Literature data on experimental solubilities of free FA in various solvents and corresponding HSPs.

Solvent	Solubility (mg/mL)*	Temperature (°C)	References	δ_d ($MPa^{0.5}$)	δ_p ($MPa^{0.5}$)	δ_h ($MPa^{0.5}$)	R_a ($MPa^{0.5}$)
Organosulfur							
Dimethyl sulfoxide (DMSO)	151.84	298	[18]	18.4	16.4	10.2	10.55
Glycols							
Propylene glycol (PG)	74.41	298	[18]	16.8	10.4	21.3	6.01
Ethylene glycol (EG)	73.61	298	[18]	17	11	26	9.63
Alcohols							
MeOH	122.45	298	[18]	14.70	12.30	22.30	10.43
EtOH	81.70	298	[18]	15.80	8.80	19.40	6.68
IPA	50.44	298	[18]	15.80	6.10	16.40	7.07
1-Butanol	34.72	298	[18]	16	5.7	15.8	7.01
2-Butanol	35.95	298	[18]	15.8	5.7	14.5	7.76
Esters							
Methyl acetate	43.51	298.65	[19]	15.5	7.2	7.6	12.25
Ethyl acetate	28.43	302.55	[19]	15.8	5.3	7.2	12.66
Butyl acetate	**15.96**	**299.05**	[19]	**15.8**	**3.7**	**5.7**	**14.40**
Nitrile							
ACN	**17.21**	**298.2**	[20]	**15.3**	**18**	**6.1**	**16.37**
Organochlorine							
Dichloromethane	2.43	298.05	[19]	17.00	7.30	7.10	11.26
Chloroform	1.45	300.35	[19]	17.80	3.10	5.70	13.37
Water	0.75	298	[21]	15.5	16	42.3	26.73
Commonly used							
Acetone	-	-	-	15.50	10.40	7.00	12.72
Hexane	-	-	-	14.90	0.00	0.00	21.28
Solutes							
Free FA	-	-	This study	19.00	8.89	17.51	-
Esterified FA	-	-	[16]	18.62	6.49	3.30	-

* The solubilities of free FA, expressed in mg/ mL of solvent, were calculated from mole fraction (mole solute/ mole solvent) data reported in the literature.

It is noted that individual HSPs of the solvents in Table S1 and the soluble esterified FA have been reported in the literatures (Daisuk & Shotipruk, 2020; Hansen, 2007). The HSPs of free FA on the other hand was calculated using the Group Contribution method proposed by Stefanis and Panayiotou (2008)[19].