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Elucidation of antioxidant compounds recovery capacity from "Cẩm" purple rice bran by different sustainable extraction techniques

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ABSTRACT

"Cẩm" rice is a pigmented rice with the purple color that was well known as local cultivar in Tien Giang province (Vietnam). The bran of this rice is usually not properly used; however, it contains rich antioxidants that could be utilized for demanding healthy and nutritional foods. This study is aimed to study the different green and sustainable extraction methods for rice bran's antioxidants, including microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), ultrasound followed by microwave (U+MAE), microwave followed by ultrasound (M+UAE), and conventional method as the control. The results showed that high extraction efficiency was found when Cẩm rice bran was extracted with the aid of sustainable extraction techniques such as sonication or microwave. The study also reported that dual synergistic-assisted extraction presented a higher content of bioactive compounds and their antioxidant activities compared with single-assisted extraction. In addition, U+MAE was shown to have a higher capacity to extract and maintain antioxidant activities than M+UAE. This study provides the fundamental information for further application and optimization process of extract of antioxidant compounds from Cẩm rice bran, as source of antioxidant and nutraceutical ingredient.

1. INTRODUCTION

Rice is widely acknowledged as a prominent staple food on a global scale. A wide variety of rice cultivars were annually cultivated and harvested in the nation of Vietnam. According to Van Tai *et al.* [1] reported that the rice producing industry in Vietnam yields a substantial amount of waste, which has promise for conversion into value-added commodities. Rice bran is recognized as a byproduct that is produced as a result of the rice growing process. Huang and Lai [2] conducted a study which provided evidence that rice bran possesses a substantial quantity of various nutrients and antioxidant compounds. The quality of rice is often related to the presence of high levels of bioactive compounds and nutrients [3,4]. According to the findings of Ngo *et al.* [5], there is a possibility that these bioactive compounds exhibit the capacity to modulation the digestion behavior and exhibit properties that are beneficial in managing diabetes and demonstrated that rice bran is rich in essential vitamins and minerals. The rice cultivar referred to as "Cẩm" exhibits a unique purple outer layer. It was considered as "new rice varieties" which was adapted and recognized by the Ministry of Agriculture and Rural Development in Vietnam in 2012 (No. 387/QĐ-CTT). Historically, it has been conventionally employed in gastronomy and has moreover functioned as a fundamental constituent in the manufacturing of many commodities, encompassing instant rice, sprouted rice flour, and bread suitable for those with gluten intolerance [6]. Nevertheless, once the milling and polishing procedures were concluded, the rice bran known as "Cẩm" was also discarded, indicating that its utilization was not efficiently executed [7,8]. In a recent study conducted by Loan *et al.* [7], it was demonstrated that the rice bran known as "Cẩm" exhibits substantial potential for the creation of value-added products. The high protein content and presence of other necessary components are responsible for this feature. Furthermore, a distinct investigation carried out by Loan *et al.* [8] revealed that these specific rice cultivars showcase a significant level of anthocyanins, with a precise measurement of 46.3 mg/100 g. The optimization of the extraction process has been documented [8]; however, additional research is required to assess its applicability in the context of culinary applications.

MAE and UAE have emerged as "green and sustainable" methodologies that have garnered increased interest within both academic and industry domains in recent times [9,10]. The phenomenon of MAE process is characterized by the rotational movement of solvent molecules induced

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by the presence of an electric field, leading to the dissipation of energy through friction [11]. The aforementioned phenomenon involves the conversion of electromagnetic energy into thermal energy, resulting in an elevation of the temperature of the solvent [11,12]. The elevation of solvent temperature leads to the evaporation of water, resulting in an augmentation of pressure within plant cells. The application of high pressure and temperature induces the disruption of plant cell walls, resulting in the formation of a multitude of microchannels [12]. This phenomenon facilitates the extraction of bioactive compounds from the plant matrix into solvents, leading to a higher quantity of recovered bioactive molecules. The extent of thermal conversion of electromagnetic energy relies on both the strength of the electromagnetic field and the dielectric properties of solvents [13,14]. The ultrasound-assisted extraction (UAE) has demonstrated enhanced extraction efficiency in comparison to traditional extraction methods by the utilization of the acoustic cavitation effect within the extraction solutions [13,15]. When rarefaction cycles cause the repulsion of molecules in the medium, resulting in the formation of cavitation bubbles, the subsequent explosion of these bubbles induces shearing forces and turbulent effects on the surface of the material, leading to the disruption of the cell wall. The permeability of cell walls, which have been disrupted or broken down, facilitates the ingress of solvents into the material, hence increasing the extraction efficiency of bioactive chemicals [15]. The UAE can be considered a proponent of green emerging technology due to its notable extraction capacity, reduced energy consumption, decreased time requirements, and minimized solvent usage [15,16]. The combination of UAE and MAE has been employed to augment the retrieval of bioactive components from botanical sources. This includes the extraction of phenolic compounds from butterfly pea flower [17] and the refinement of antioxidant phenolics from banana peel [18]. The synergistic extraction method is a recently developed extraction process that offers advantages in terms of speed, efficiency, and cost-effectiveness. The combination of ultrasonic and microwave technologies capitalizes on the microwave's ability to penetrate and heat materials, as well as the ultrasonic capacity for cavitation [19]. However, it is limit study on rice bran using these techniques, especially it has not been elucidated on purple rice bran from Vietnam. Thus, there is a need to compare the extraction capacity of single extraction or combination with assistance of microwave or ultrasound. The study could provide fundamental information for further optimization or application of extract from "Cẩm" rice bran, which might lead to enhance the economic value of this material.

2. MATERIALS AND METHODS

2.1. Materials

Cẩm rice bran [Figure 1] was collected after milling and polishing process at local company in Tien Giang province (Vietnam). The defatted rice bran was milled to pass 80 mesh sieve for further extraction [20]. The powder was kept in a dark airtight bag at 4°C until the extraction was conducted.

2.2. Extraction Process

2.2.1. MAE process

The extraction solvent employed in this study is a food-grade ethanol solution with a concentration of 60% [18]. The extraction method was conducted using an electric microwave oven manufactured by Electrolux in Korea. The extraction process was conducted using a solid-liquid ratio of 1:10 (weight/volume) and a microwave power of 500 W for a duration of 5 min.

Figure 1: Cẩm rice bran.

2.2.2. UAE process

The extraction process was conducted using an ultrasonic bath (M2800H-J, 110 W, Japan). The bran was weighed according to design and the solvent was added in the same ratio as the MAE process. The extraction process was conducted with a period of 30 min, a temperature of 70°C, and a frequency of 60 Hz [21].

2.2.3. M+UAE and U+MAE processes

The same conditions were used with dual-assisted extraction microwave following ultrasound-assisted extraction (M+UAE) and ultrasonication combined with microwave-assisted extract (U+MAE). After completing each extraction process, sample was immediately transferred to next process.

The conventional extraction was conducted with the temperature of 60°C for 30 min at continuous shaking water bath, considering as control sample.

The crude extracts were promptly subjected to centrifugation at a speed of 10,000 revolutions per minute for a duration of 1 minute. Subsequently, they were filtered through filter paper using a vacuum apparatus (V-700, Büchi, Switzerland) immediately following the extraction process. The gathered samples were placed in glass vials and thereafter stored at a temperature of 4° Celsius until they were ready for further analysis. The crude liquid extract underwent vacuum evaporation and subsequent drying to ascertain the overall extraction yield. The findings were presented in terms of the proportion of total extractable solids per 100 g of dry sample and expressed as a weight-to-weight percentage (% w/w).

2.3. Analysis of Total Phenolic Content (TPC)

The determination of TPC was conducted using the Folin-Ciocalteu test, as outlined by Wanyo *et al.* [22]. In this experiment, a volume of 300 µL of rice bran extract was subjected to a reaction with 2.25 mL of a Folin-Ciocalteu reagent solution with a concentration of 10%. The resulting mixture was then left undisturbed at room temperature for a duration of 5 min. Subsequently, 2.25 mL of a sodium carbonate solution with a concentration of 60 g/L was introduced into the aforementioned combination. The absorbance of the reaction was measured at a wavelength of 725 nm after being kept at room temperature for a duration of 90 min. The standard curve of gallic acid was employed to determine the TPC of the extract.

2.4. Analysis of Total Anthocyanin Content

The total anthocyanin content was followed the differential pH method. Using different buffer at pH = 4.5 and pH = 1 for analyzing the absorbance of reaction. Briefly, to establish the appropriate dilution factor, it is necessary to dissolve the sample in a KCl buffer with a pH of 1 until an absorbance of <1.2 at a wavelength of 510 nm is attained. The measurement of the absorbance of the aqueous solution was conducted at wavelengths of 510 nm and 700 nm to establish the zero point. The cyanidin-3-glucoside exhibited a maximum wavelength of 510 nm, whereas the remaining sediments in the sample were accounted for and corrected at 700 nm. The sample exhibited complete transparency, as indicated by a zero absorbance reading at a wavelength of 700 nm. Every individual sample was solubilized in a buffer solution using a pre-established dilution factor (df). Following dilution with KCl buffer (pH 1) and Na-acetate buffer (pH 4.5), the sample (0.5 mL) with buffer (3.5 mL) underwent an incubation period of 15 minutes before measurement. The measurement of absorbance for each solution was conducted at wavelengths of 510 nm and 700 nm, with distilled water serving as the reference blank. The calculation and process of analysis were clearly mentioned in study of Thuy *et al.* [23].

2.5. Determination of Antioxidant Activity

The antioxidant activity of purple rice bran extract was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay and the 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity (DPPH) assay. The FRAP assay method employed in this study was based on the protocol outlined by Tabaraki and Nateghi [24]. A total volume of 50 μL of extract was utilized for the reaction, which involved mixing it with 1.5 mL of the FRAP reagent. The absorbance value of the combination at a wavelength of 593 nm was measured using the Shimadzu UV18000 UV-Vis spectrophotometer (manufactured in Japan) following a 4-min incubation period. Three duplicates were utilized to establish a standard curve for the $FeSO_4$ solution, which was subsequently employed to determine the FRAP value of the treatment. The findings were quantified in terms of micromoles of Fe(II) per gram of rice bran on a dry weight basis.

3. RESULTS AND DISCUSSION

3.1. Yield of Extraction

Microwave and sonication process have different mechanism effect on the yield of extraction of antioxidant compounds from purple rice bran. Figure 2 shows that applying the aid of these processes could enhance remarkably the recovery capacity compare with conventional method (CE). The dissipation factor in microwave assisted extraction is indicative of the extent to which microwave energy is converted into thermal energy. A higher dissipation factor signifies a greater capability for conversion [25]. The alteration of material structure was seen as a result of the microwave effect, which has been found to potentially enhance the extraction yield [18,26]. The degradation of bioactive compounds during microwave-assisted extraction (MAE) might be related to the liberation of excessive heat generated by microwaves, which acts as the activation energy for various degradation mechanisms [27]. Moreover, the application of sonication facilitated the rupture of cells and the generation of turbulence, leading to a swift increase in the concentration of antioxidants in the solvent. Consequently, the equilibrium concentration achieved in the UAE procedure was higher compared to the MAE approach [28] as similar as the result of this study. Moreover, the synergistic extraction showed higher recovery

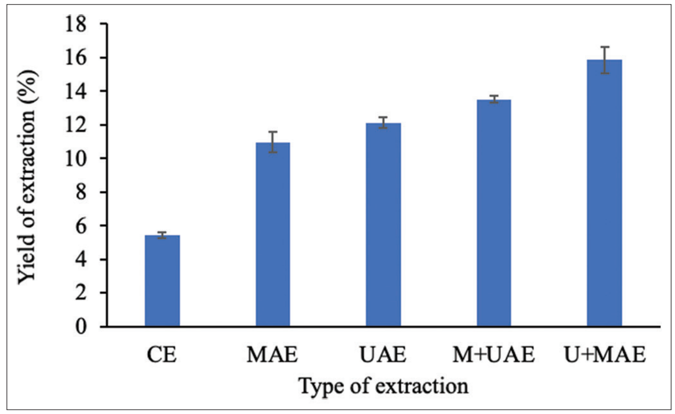

Figure 2: Effect of green extraction method on the yield of extraction of purple rice bran. CE is conventional extraction; MAE is microwave-assisted extraction; UAE is ultrasound-assisted extraction; M+UAE is microwave-ultrasound assisted extraction; U+MAE is ultrasound-microwave-assisted extraction.

yield compared with the single assisted extraction. The combination of advantages in the penetrating heating effect of microwave and cavitation of ultrasonic led to greatly increased in efficiency extraction [19]. However, ultrasonic-microwave assisted synergistic extraction presented a slightly higher capacity than M-UAE. This method also commonly used in extraction the bioactive compounds from various sources as presented in study of Li *et al.* [19].

3.2. Content of Antioxidant Compounds

The extraction was operated with assistance of microwave and sonication process, even single assisted or synergic extraction, showing the higher capacity for extracting the bioactive compounds from Cẩm rice bran [Table 1]. MAE has been found to improve extraction efficiency in comparison to conventional extraction methods. This is attributed to the interaction between microwaves and polar molecules that present in the extraction media, resulting in the generation of heat and a rise in internal pressure within the solid material [14,17,29]. The primary mechanisms involved in UAE are cavitation and the mechanical mixing effect, which contribute to enhanced extraction efficiency and a reduction in extraction time. These mechanisms contribute to enhanced extraction efficiency and a reduction in extraction time [13,17,18]. Furthermore, the utilization of ultrasound in chemical processes circumvents the thermal degradation of heat-sensitive substances due to its non-thermal nature [13].

When MAE was used, recovery capacity of TPC and TAC of conventional extraction time was increased by 34.29% and 31.51%, respectively. According to Bayramoglu *et al.* [30], the application of microwaves to solid media results in an elevation in internal pressure, leading to improved extraction efficiency. Consequently, MAE enables the leaching of phenolic compounds in a shorter duration compared to conventional extraction methods. However, the extraction yield of TPC and TAC of UAE was higher than CE by 40.95% and 73.45%, respectively. It was also higher than MAE method. It is might due to ultrasound has been found to offer a more rapid and intensified mixing effect, resulting in the reduction of external resistance and the enhancement of mass transfer. Consequently, raising the power level of ultrasound has been observed to improve extraction efficiency, as supported by studies conducted by Vinatoru *et al.* [13]. Mixing takes place at the

Table 1: Effect of green extraction method on the total phenolic and total anthocyanin content of extract from purple rice bran.

Method	TPC (mgGAE/100 g)	TAC (µg/100 g)
CE	43.07±0.54	24.75±0.52
MAE	57.85±0.45	32.55±0.46
UAE	60.71±0.55	42.94±0.51
M+UAE	64.38±0.94	42.74±0.62
U+MAE	67.86±0.51	45.45±1.00

Data are expressed as mean±standard deviation, TPC: Total phenolic content, TAC: Total anthocyanin content.

Table 2: Effect of green extraction method on antioxidant activities of extract from purple rice bran.

Method	DPPH (%)	FRAP (µmol Fe (II)/g)
CE	54.09±0.46	35.26±0.46
MAE	68.96±0.53	49.52±0.48
UAE	73.22±0.47	55.17±0.27
M+UAE	82.12±0.57	57.04±0.48
U+MAE	86.53±0.89	60.56±1.00

Data are expressed as mean±standard deviation.

solid-liquid interface by the utilization of ultrasound. Consequently, the thickness of the boundary layer diminishes. Besides, the heat increasing using UAE was lower than MAE, therefore, it could reduce the rate of bioactive compound degradation.

Dual-assisted extraction also showed the higher yield of antioxidant compounds than the single-assisted extraction. It also showed that lowering the extraction yield was found when microwave process was done before ultrasonication, especially it was not increasing yield of anthocyanin. The findings suggest that the extraction process has the potential to disrupt the structure of plant tissue, hence increasing the reactivity of the extraction site. This enhanced reactivity is achieved through the combined use of ultrasonic cavitation and microwave irradiation, as demonstrated by Arasi *et al.* [31]. The observed pattern can be attributed to the effects of sonic cavitation on the surface of plant cells. Previous studies have demonstrated that an appropriate level of ultrasonic exposure can effectively augment the mechanisms of sonoporation and erosion. These mechanisms, in turn, play a crucial role in promoting the diffusion of phenolics and flavonoids into the extractant. This increased diffusion encourages greater interaction between the compounds and the solvent, ultimately leading to an improved extraction yield [32]. Nevertheless, the prolonged duration resulted in an excessive exposure of the plant matrix to microwave radiation, which may potentially result in the thermal destruction of flavonoid compounds [33,34]. Furthermore, the use of this amalgamation of technologies was employed for the purpose of degrading phenolic compounds. The synergistic action of sono-generated radicals, in tandem with the expeditious thermal impact of microwaves, exhibits a noteworthy efficacy in the degradation process of polar molecules.

3.3. Antioxidant Activities

The statistical analysis revealed significant variations in the antioxidant activity of extracts obtained by various techniques [Table 2].

Both FRAP and DPPH assay showed the similar results, the antioxidant activities followed the order: U+MAE > M+UAE > UAE > MAE > CE. It is related to the content of antioxidant compounds in the extract. Although the slightly lower of TAC in the extract using M+UAE comparing with U+MAE, the antioxidant activities still higher due to higher TPC content. The content of antioxidant compounds and their activities is positive correlated in many previous studies [17,18,28,29,35]. It is also presented that in spite of TAC could create the color for bran and the main component, other antioxidant compounds still significantly contributed on the antioxidative activity [36,37]. The obtained results may suggest the significant impacts of various extraction forces on attaining substantial yields of distinct chemicals. The distinct influences exerted on the yields of phenolic compounds suggest the necessity of individually targeting these components in forthcoming biorefinery processes.

4. CONCLUSIONS

The present study aimed to explore the efficacy of microwave and ultrasonic aided extraction methods in extracting antioxidant capacity from Cẩm rice bran. The utilization of microwave and ultrasonic powers resulted in a substantial enhancement of the recuperation of antioxidant capacity in the extracted samples. The UAE exhibited a higher extraction yield in terms of antioxidant capacity when compared to both MAE and conventional extraction methods. More interestingly, the syneresis-assisted extraction was significantly enhanced the antioxidants recovery, especially U+MAE process showed highest efficiency. This study could be the fundamental information for further optimization and application of this material, improving the usage as well as economic value.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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