



An Assessment of Different Extraction Methods Antioxidant Activity And Physicochemical Property of Gamma Oryzanol :

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ABSTRACT:

A phytochemical with antioxidant properties and possible health advantages is oryzanol. In this work, our goal was to create a technique for extracting large amounts of γ -oryzanol from rice bran and confirming the substance's stability in several solvents. A central composite rotating design (DCCR - 24), which included 8 tests in axial circumstances and 4 center points for a total of 28 trials, was used to further refine the approach that produced the greatest γ -oryzanol content out of the five under examination. Temperature, duration, volume, and the hexane-to-isopropanol ratio were the variables under investigation. The following extraction parameters were used to produce a high concentration of oryzanol (13.98 mg.g⁻¹): 40° C, 40 min, and 75 mL of hexane:isopropanol (1:3). The method's limits of detection and quantification were 0.9 g g⁻¹ and 31 respectively.

Key Words: stability, phytochemistry, antioxidant, and spectrophotometer.

INTRODUCTION:

Although it can also be found in other paddy or rice milling byproducts, rice bran is the principal source of γ -oryzanol. Rice bran oil stands out among edible oils due to its high concentration of phytochemicals, such as oryzanol, lecithin, tocopherols, and tocotrienols, which are essential for both financial and nutritional reasons. While the rice bran oil is being refined, the bulk of these phytochemicals are eliminated as waste byproducts. Oryzanol is one such component that may be employed in medicinal, cosmetic, and nutraceutical products. Triterpene alcohols are alcohols made from triterpenes and contain ferulic acid sterol esters as their main component. It has a concentration of 1-2% in rice bran oil and serves as a natural antioxidant. In the article, it is described how to extract oryzanol.

Rice bran oil, which is essential for both economic and nutritional reasons and has a high concentration of phytochemicals such as oryzanol, lecithin, tocopherols, and tocotrienols, stands out among edible oils. However, during refinement, the majority of these phytochemicals are removed from the rice bran oil as unwanted byproducts. Oryzanol is one such ingredient that may be employed in the production of nutraceutical, pharmaceutical, and cosmetic goods. It is made up of ferulic acid sterol esters and triterpene alcohols, which are alcohols made from triterpenes. It is present in rice bran oil at concentrations of 1-2 percent and acts as a natural antioxidant. The many ways to extract -oryzanol from rice bran and the process by which rice bran becomes rice bran oil are both covered in the article.

The bran fraction (9, 12) refers to the portion of the bran and germ layers of the brown rice kernel that are removed during the polishing procedure to create white rice. Tocopherols, tocotrienols, g-oryzanol, and other beneficial nutraceutical components for health are also included (13–15). The second most prevalent constituent in milled rice, after carbohydrates (78% of the total), is protein (6-7%). Each grain of rice also contains additional healthy nutraceutical components such as phenolics, g-oryzanol, tocopherols, and tocotrienols (13–15). The second most prevalent ingredient in milled food is protein (6-7%) carbohydrates account up around 78% of the entire amount of rice. On the other hand, rice bran has a high protein content (11–15%) and is an excellent source of fiber (7–11%). Additionally, rice bran contains significantly more vitamins and minerals than the other rice milling fractions (16). Lipid content in rice bran is about 20%. Rice bran oil has attracted interest due to its unique fatty acid content and nutraceutical advantages (17). Bran has long been utilized as animal feed, but it is underestimated as a food source for humans (18, 19).

This study's objective was to evaluate the physicochemical properties, fatty acid compositions, and content of bioactive substances (anthocyanins, g-oryzanol, tocopherols, and phenolic compounds) in raw materials, especially in those varieties of rice bran that were designated as being of special grade.

Using statistical analysis, the samples were split into three main groups based on their nutritional characteristics, with group A having a high protein and fiber content and group B having a high fat and gross content. energy content and group C, which contains high amounts of functional, active substances like g-oryzanol but low levels of fat and energy. Only one sample of colored rice bran was found to contain anthocyanins.

Oleic, linoleic, and palmitic were the three free fatty acids that were most commonly discovered in rice bran samples. The findings of this study will support further research and uses of each rice bran component as a value-added component in the development of functional foods or other goods.

Animal tissues are shielded by antioxidants against aging and degenerative illnesses caused by free radicals. Numerous phytochemicals with antioxidant and other health benefits may be found in high concentrations in rice bran. Numerous research have focused on phytochemicals such as vitamin E, tocopherols (Ts), tocotrienols (T3s), and the -oryzanol fraction (CHOTIMAKORN et al., 2008; LOPES et al., 2012). According to LERMA-GARCA et al. (2009), the primary components of -oryzanol are trans-ferulic acid esters (trans-hydroxycinnamic acid) and phytosterols (sterols and triterpenic alcohols), which include cycloartenol, sitosterol, 24-methylenecycloartenol, and campesterol. Liquid-liquid phase extraction, solid-phase extraction, supercritical fluid extraction, and direct solvent extraction are the described extraction techniques for -oryzanol (XU; GODBER, 2000). It has been the most often utilized because it doesn't call for any special extraction equipment. These four techniques all include repeatedly extracting rice bran oil (RBO), typically using hexane. Despite the fact that hexane recovered more RBO, at ideal extraction circumstances, the most polar solvents—hexane:isopropanol (1:1 v/v)—extracted more -oryzanol from rice bran than hexane at high temperature (HU et al., 1996; XU; GODBER, 2000; SAENJUM et al., 2012). High performance liquid chromatography (HPLC) and ultraviolet spectrophotometry (UV) are the two methods used the most frequently to measure the amount of oryzanol (ROGERS et al., 1993). HPLC is accurate and effective,

however the high cost of the analysis is a drawback. On the other hand, the spectrophotometric method is straightforward, useful, and affordable, but the outcomes are not always accurate.

MATERIAL AND METHODS:

1. Rice bran Samples Standard, Reagents

Three paddy types (KDML 105, RJM, and HN) were purchased from a community enterprise in Thailand's Phichit Province. Between December 2014 and February 2015, they were collected. The paddy was dried in an oven until it had a moisture content of 13. To extract the bran, the dry paddy was ground and polished. The acquired rice bran was vacuum-packed in foil packets and kept at (20) pending usage. Initial oil content for rice bran for KDML 105 was 15.64, for RJM it was 16.84, and for HN it was 12.62. For the purpose of comparing the color of one popular brand of commercially refined RBO with the samples utilized in this investigation. The γ -oryzanol standard (purity > 98%) was bought from Wako Chemicals in the USA. every chemical utilized in Analytically pure reagents were utilized in every step of the experiment. Rio Grande do Sul state, Brazil's rice manufacturers contributed their surplus rice bran, which was frozen (at a temperature of -18 degrees Celsius) until it was needed.

2. Extraction of the rice bran oils:

2.2.1 Cold-press extraction(CPE)

Continuously fed into the hopper of a 2-HP screw press extractor was rice bran. The slits along the barrel's length were jammed with crude oil. The choke at the barrel's end simultaneously released the compacted rice bran.

2.2.2 Solvent extraction(SE)

The extraction procedure was carried out in a lab. The extraction was carried out for 3 hours with frequent stirring and the ratio of rice bran:hexane utilized was 1:3 (w/v). Under vacuum, filtering via filter paper No. 4 separated the bran from the extract. Extraction of supercritical carbon dioxide (SC-CO₂) 2.2.3 Using a SC-CO₂ extractor under the following conditions—60°C, 30 MPa of pressure, and 35 L/h of CO₂ flow—oil was extracted from rice bran. The extraction jar was filled with glass beads at the bottom and top before the sample of rice bran was put inside. To keep the temperature at the operational level, the cell was put in the heating chamber¹². Equation 1 was used to compute crude oil yields on a dry weight basis.:

$$\text{Yield (\%)} = \frac{\text{Weight of oil extracted(g)}}{\text{Weight of rice bran sample(g)}} \times 100$$

2.2.3 Extraction of γ -oryzanol used in CCRD

In a test tube, distilled water (50 mL) was used to suspend rice bran (10 g), and then ascorbic acid (2 g) was added. The mixture was then vortexed before being incubated for the amounts of time and at the temperatures listed in Table 2. The ratio of hexane:isopropanol (Table 2) was adjusted before being added, vortexing for 30 seconds, and centrifuging at 1320 g for 15 minutes. A separatory funnel was used to separate the organic layer. Hexane:isopropanol (10 mL) was used to extract the residue once again, and the procedure was repeated. At 70 ° C, a rotary evaporator was used to evaporate the mixed organic layers (XU; GODBER, 2000).

Table 1 – Extraction methods evaluated for γ -oryzanol extraction.Method Sample mass (g) Time (min) Solvent used for extraction Temperature of extraction ($^{\circ}$ C)

Method	Sample mass (g)	Time(min)	Solvent used for extraction	Temperature of extraction ($^{\circ}$ C)
1	60	20	Hexane	69
2	40	80	hexane:isopropanol (8:6)	40
3	40	120	chloroform:methanol (6:4)	Ambient
4	2	2	hexane	Ambient
5	20	40	hexane:isopropanol (2:2)	60

1: Zullaikah et al. (2009); 2: Zigoneanu et al. (2008); 3: Azrina et al. (2008); 4: Lilitchan et al. (2008); 5: Xu, Godber (2000).

Ambient temperature = $25 \pm 5^{\circ}$ C.

Table 2 – Values of the coded and real levels used in CCRD.

Factors	-2	-1	0	+1	+2
Temperature ($^{\circ}$ C)	20	30	40	50	60
Time (min)	20	30	40	50	60
Volume of hexane:isopropanol (mL)	10	30	50	70	90
Ratio of hexane:isopropanol	0.8:5.7	3:5	2:2	5:2	5.7:0.8

Ciênc. agrotec., Lavras, v. 36, n. 6, p. 665-673, nov./dez., 2012

2.2 Antioxidant property:

Determining antioxidant activity.

The bleaching of purple-colored methanol or ethanol solution of DPPH was used to test the capacity of polyphenol-rich extract to donate hydrogen atoms or electrons. The stable radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is used as a reagent in this spectrophotometric procedure 21, 22. To 2.5 ml of a 0.1 mM solution of DPPH, four ml of the aqueous extracts that had been dissolved in methanol or ethanol were added. After 30 minutes of room temperature incubation, the absorbance was measured at 515 nm against a blank. On table no. 6 lines 23 to 31, percentage inhibitions for both extracts were seen.

Formula :

$$I\% = (A_o - A_s / A_o) \times 100$$

Where, I = Percentage inhibition DPPH activity.

A_o = The absorbance of the standard solution.

A_s = The absorbance of the test compound.

2.3 Effect of bran color on the tocopherol and tocotrienol composition of rice:

According to multiple research (Finocchiaro et al. 2007, Yawadio et al. 2007, Huang and Ng 2011, Min et al. 2011, Gunaratne et al. 2013), and as shown in Table 5 for the endosperm, whole grain, and bran, differences in tocopherol and tocotrienol amounts in rice are not related to the bran color. However, in the husk, pigmented rice types have a total tocotrienol concentration that is 4.3 times greater (6.02 mg/kg) than

nonpigmented rice varieties. This is probably not because of the color of the bran, but rather because there have only been a few published research (around five) on the topic.

RESULTS:

3.1 Extraction of γ -oryzanol

The results of γ -oryzanol (mg g⁻¹) in the CCRD for the 4 variables under study (temperature, time, volume, and ratio of hexane:isopropanol) are shown in table (1&2). In this design, the γ -oryzanol yield varied from 1.50 to 13.98 mg g⁻¹. The best values were found in trials 5, 6, 7, 8, and 22, all of which showed high solvent volumes, at level +1 (70 mL) or +2 (90 mL), and proportion of hexane:isopropanol at level -1 (3:5) or 0 (2:2). An estimate of the main effect is obtained by evaluating the difference of process performance caused by a change from low (-1) to high (+1) levels of the corresponding factor (HAALAND, 1989). The measure of the process performance was the response of the γ -oryzanol content.

3.2 Antioxidant activity determination

The DPPH technique has been widely utilized to determine antioxidant activity from natural products. Gamma orygenol or any other plant that works as a free radical scavenger may be studied for their antioxidant activity using this approach since it is simple, reliable, and quick. In this procedure, the color changes from deep violet to yellow when DPPH interacts with an antioxidant molecule. The absorbance at 517 nm is then determined using a UV spectrophotometer (Table no. 3). The DPPH absorption inhibition in this investigation varied from 36.06 to 30.00%.

Table 3 – % Inhibition of Gamma Oryzanol

Concentration (µg/ml)	% Inhibition of ascorbic acid	% Inhibition of Gamma Oryzanol
25	93.53%	36.06%
50	93.81%	43.44%
75	94.79%	49.72%
100	94.87%	60.10%
125	99.16%	76.39%

Each result is the average of three measurements \pm SD.

3.3 Tocopherol and tocotrienol composition of rice:

Since they both consist of an amphiphilic 6-chromanol ring and a terpenoid side chain at position 2 of the ring, tocotrienols and tocopherols are jointly referred to as vitamin E or tocols. Tocopherols can be created by joining the chromanol head group to an unsaturated geranylgeranyl side chain, or to a saturated phytyl side chain, to create tocotrienols. After then, the head group can be methylated in a variety of ways to produce the four alternative forms (a, b, c, and d). A resonance-stabilized whole grain and husk are produced when the hydrogen atom from the free hydroxyl group on the chromanol ring, which is responsible for the antioxidant capabilities, is transferred to free radicals (Table 5). According to the rice grain fraction, α -tocopherol (8.12-105.2%) makes up the majority of the total tocol content, followed by γ -tocopherol (1.80-22.00%), δ -tocopherol (3.50-48.80%), and β -tocopherol (0.25-5.96). This ranking is similar to that inferred in most studies (Diack and Sask 1994; Yu et al. 2007; Heinemann et al. 2008; Imsanguan et al. 2008; Huang and Ng 2011; Min et al.

2011; Jeng et al. 2012; Zhang et al. 2012; Pascual et al. 2013); however, α -tocopherol was reported to be the major tocol in two Taiwanese rice varieties (Lin and Lai 2011).

Table 5. Contents of tocopherols and tocotrienols in Rice bran (mg/kg DW).

Color	α -Tocopherol	β -Tocopherol	γ -Tocopherol	Δ -Tocopherol	Total tocopherols
Nonpigmented	8.12–105.2 (n = 33)	1.80–22.00 (n = 12)	3.50–48.80 (n = 32)	0.25–5.96 (n = 21)	15.10–215.0 (n = 36)
Nonpigmented	47.11 - 22.61	7.50 - 8.4	20.74- 17.13	3.05 - 2.40	80.12 - 40.02
Pigmented	8.77–106.5 (n = 21)	0.75–6.02 (n = 9)	8.9 –55.18 (n = 18)	0.85–3.50 (n = 14)	25.30–180.1 (n = 18)
Pigmented	40.24 _ 20.47	4.24 - 2.12	25.40- 15.30	2.84 - 0.94	77.11 - 22.91

For each parameter, the first row values describe the minimum and maximum values (A–B) and the total number of studies from which data were extracted (n), whereas the second row values show the mean and SD. 2Pigmented rice refer to rice with black, purple, and red bran, whereas non pigmented rice refer to rice with brown bran.

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