Comparative study on conventional, ultrasonication and microwave assisted extraction of γ -oryzanol from rice bran

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Revised: 26 December 2015 / Accepted: 6 January 2016 / Published online: 16 April 2016
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Abstract In present study, conventional, ultrasonic and microwave assisted extraction methods were compared with the aim of optimizing best fitting solvent and method, solvent concentration and digestion time for high yield of γ -oryzanol from rice bran. Petroleum ether, hexane and methanol were used to prepare extracts. Extraction yield were evaluated for giving high crude oil yield, total phenolic content (TPC) and γ -oryzanol content. Gas chromatography-mass spectrophotometry was used for the determination of γ -oryzanol concentration. The highest concentration of γ -oryzanol was detected in methanolic extracts of microwave treatment (85.0 ppm) followed by ultrasonication (82.0 ppm) and conventional extraction method (73.5 ppm). Concentration of γ -oryzanol present in the extracts was found to be directly proportional to the total phenolic content. A combination of 80 % methanolic concentration and 55 minutes digestion time of microwave

treatment yielded the best extraction method for TPC and thus γ -oryzanol (105 ppm).

Keywords γ -oryzanol · Ultrasonication · Microwave assisted extraction · Total phenolic content

Introduction

Today world's consumption of nutraceuticals in food is increasing regularly because they are fulfilling our nutritional aspects of food as well as disease resistance of the body. At the same time, many opportunities for the development of low cost novel techniques to produces dietary products have been created.

Highlights

1. The amount of solvent and processing time in γ -oryzanol extraction can be reduced by applying ultrasonication and microwave treatment.
2. In this study, methanolic extract of microwave treatment possessed maximum amount of γ -oryzanol as compared to conventional and ultrasonication.
3. Combination of 80 % methanolic concentration and 55 minutes microwave digestion resulted extraction of highest γ -oryzanol i.e. 105 ppm.

Electronic supplementary material The online version of this article (doi:10.1007/s13197-016-2175-2) contains supplementary material, which is available to authorized users.

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Rice (*Oryza sativa* L.) also known as the grain of life, is the major cereal crop and staple food for half of the world population. Rice bran is one of the most abundant byproduct from rice processing industry, which contains about 9.8 g/kg of biologically active γ -oryzanol, having antioxidant, antihypertensive, antimicrobial, cholesterol reducing, and antidiabetic nature (Bhatnagar et al. 2014; Gul et al. 2015; Kaur et al. 2015; Wang et al. 2015). γ -oryzanol is a mixture of ferulate (4-hydroxy-3-methoxycinnamic acid) esters of sterols (campesterol, stigmasterol and β -stigmasterol) and triterpene alcohols (cycloartenol, 24-methylenecycloartanol, cyclobranol) which are responsible for its biological functionality (Miller et al. 2003).

Numerous reports are available for the separation of nutraceuticals like γ -oryzanol from rice bran using traditional solvent extraction method (Heidtmann-Bemvenuti et al. 2012; Oliveira et al. 2012; Pascual et al. 2013; Bhatnagar et al. 2014; Ghasemzadeh et al. 2015; Wang et al. 2015). This method lacks efficiency in terms of time and solvent consumption. In addition to these demerits of solvent extraction system, it may lead to toxicity in food fortified and atmospheric pollution due to emission of waste organic solvents (Uquiche et al. 2008). To overcome these problems and to meet the increasing demand of nutraceuticals in foods and pharmaceuticals, different technique of extraction of plant derived phenolics compounds, were previously reported by the other studies including microwave (Dar and Sharma 2011) ultrasound-assisted methods (Ghasemzadeh et al. 2014a), supercritical fluid extraction methods (Tao et al. 2014) reflux (Ghasemzadeh and Jaafar 2014b) and soxhlet extractions (Bicchi et al. 2000). These techniques would be promising from an economical point of view, simple and efficient. These extraction methods need fewer amounts of organic solvents for extraction purpose and hence reduced emission of harsh solvent waste.

Microwave treatment and ultrasonication are the two most prominently used methods of extraction of γ -oryzanol as they are inexpensive, simple and efficient in terms of greatly rising γ -oryzanol yield, reducing amount of solvent and processing time (Pan et al. 2003; Holliday 2006; Thanonkaew et al. 2012). However, to date no information is available on comparative analysis of γ -oryzanol based extraction yield of conventional method, ultrasonication and microwave treatment. The objective of this study was to assess the γ -oryzanol extraction yield of traditional conventional method, ultrasonication and microwave treated solvent extracts from rice bran.

Material and methods

Materials

In the month of August, 10 kg of rice bran (from Pusa Basmati-4 variety of *Oryza sativa*) was procured in one slot

from Ricela Health Foods Limited (A.P Solvex) sangrur Punjab India. All the analytical grade chemicals used were procured from Himedia (Mumbai, India) and Sigma-Aldrich chemicals (Mumbai, India).

Oil content of rice bran

Oil content was analysed by AOAC (2000).

Experimental design

Rice bran was used as raw material for the extraction of γ -oryzanol in three different solvents (methanol, hexane, petroleum ether). Extraction was done by conventional solvent method, ultrasonication and microwave treatment at 38 °C for 60 min. Extractability of these three techniques was compared by accessing biomass or crude oil weight, total phenolic content and concentration of γ -oryzanol in chromatograms generated by gas chromatography-mass spectrometry (GC-MS). In this way, technique and better solvent for maximum extraction of γ -oryzanol, was optimized. Further, optimized technique was used for the optimization of solvent concentration and digestion time for maximum extraction of phenolic content and γ -oryzanol. Finally, the concentration of γ -oryzanol was accessed by GC-MS.

Pre-treatment of raw materials

Rice bran was sprayed with 30 mL/kg 0.1 N HCl (Hydrochloric acid) to arrest the lipolytic activity of lipase enzyme (naturally present in rice bran) and stored at 5 ± 1 °C. Treatment with HCl changes pH of medium of lipase activity, as its activity is reported at pH range of 7.5–8.0 (Aizono et al. 1971; Thanonkaew et al. 2012). Beside inactivation of enzymes, stabilization improves biomass extraction efficiency and reduces colour development.

Conventional solvent extraction of crude oil containing γ -oryzanol from rice bran

Petroleum ether, hexane and methanol were used for the conventional solvent extraction. Rice bran was mixed with different solvents in 1:3 (w/v) ratios. Each rice bran solution was then placed in shaking water bath at 38 °C for 60 min. The samples were centrifuged at 12,000 rpm and 4 °C for 30 min followed by filtration with whatmann filter paper 47. Solvents of filtered samples were evaporated by using vacuum rotary evaporator (Heidolph instruments GmbH & Co, Germany) at 60 °C. After that, samples were flashed with nitrogen gas by the help of nitrogen cylinder and stored at –20 °C under dark conditions for further analysis.

Ultrasonication assisted solvent extraction of crude oil from rice bran

A high intensity probe ultrasonic wave's generation system (GB-2500B ultrasound cleaner machine, China) equipped with auto-temperature controller, was used for the extraction purpose. Rice bran was added to petroleum ether, hexane and methanol in 1:3 ratios. All the rice bran solutions of different solvents were ultrasonicated at 24 kHz and 38 °C for 60 min. Rest of the procedure from centrifugation to nitrogen flushing and storage was same as above section. The semi-dried biomass or crude oil obtained was further processed for analysis.

Microwave assisted solvent extraction of crude oil from rice bran

Rice bran containing solvents (1:3 ratios) were weighed in pressure controlled teflon vessels. The microwave extraction system (Discover SP-D closed vessel microwave digestion, CEM Corporation, USA) equipped with auto-temperature controller was set to operate at 38 °C for 60 min using energy level of 800 W. After a ventilation period of 20 min each sample was cooled down, filtered by using whatmann 47 and further processed same as in ultrasound treatment (from centrifugation to nitrogen flushing and storage were same as conventional extraction system).

Yield determination

After vacuum evaporation of solvent extracts, polyphenolic biomass or crude oil was weighed for yield determination as well as analyzed for total phenolic content (TPC). TPC of crude oil was measured according to the method reported by (Thanonkaew et al. 2012) using Folin-Ciocalteu reagent with some modifications. A 0.2 mL crude oil solution (1.0 mg/mL DMSO) was added to 3 mL of 0.02 mg/mL Na_2CO_3 and mixed for 5 min. After adding 0.2 mL of Folin-Ciocalteu reagent, the final mixture was left for 30 min before reading the absorbance at 750 nm in UV-VIS spectrophotometer (Hach DR 5000, USA). All experiments were performed with three replicates. The total phenolic content was expressed as milligrams of ferulic acid equivalents per gram of crude oil (mg FAE/g crude oil), on the basis of calibration curve of ferulic acid.

Optimization of solvent concentration and digestion time in optimized technique

Different concentrations of optimized solvent as 50, 60, 70, 80, 90 and 100 % in 20 g of rice bran in the ratio 1:3 (w/v) were mixed thoroughly and then optimized technique for maximum extraction of TPC and γ -oryzanol, was performed. Rest of steps from centrifugation to nitrogen flushing and storage were same as conventional extraction system, to obtain oily semidried biomass. For digestion time optimization at optimized solvent concentration, digestion time was varied as 25, 35, 45, 55, 65, 70 and 75 min. Weight basis biomass yield was used to access maximum extractability. The concentration of γ -oryzanol was determined by GC-MS analysis.

Gas chromatography-mass spectrometric (GC-MS) analysis of oily semisolid biomass

GC-MS was done at Advance Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. GC-MS instrument (Shimadzu, Japan) was equipped with column Rtx-5 MS (60 m $\times$ 0.25 mm internal diameters with 0.25 μm film thickness), QP-quadrupole and an auto-sampler, was used for the analysis. γ -oryzanol in different samples was determined according to the method used by Kumar et al. (2010) with modifications. The oven temperature was programmed to reach 300 °C at the rate 10 °C per minute and held for 10 min for giving injection temperature 260 °C. Helium was used as a carrier gas, which flowed at a rate of 1.21 mL per minute. A 10 μL aliquot of sample (0.1 mg/mL w/v) was injected into the column at a split ratio 10:1. Gas chromatography was performed up to 35 min. Mass spectra were scanned in the range of m/z 40–950. Fat soluble components including γ -oryzanol, were identified by matching their recorded mass spectra with from library data (NIST version 08), which were provided by the software for GC-MS system. The MS was run for the duration of 40 min with interface temperature 280 °C and solvent cut time 3.50 min. Standard γ -oryzanol was used as authentic samples. The determination of γ -oryzanol was done by comparing with retention time of standard and was quantified as parts per million. The quantification was done with the help of standard curve equation of γ -oryzanol ($y = 5169500000x - 14,855,000$, $R^2 = 0.9984$), plotted as Peak area Vs γ -oryzanol concentration.

Table 1 Crude oil in mg/g of rice bran in different solvents and techniques

Solvents used	Conventional Extraction	Microwave Extraction	Ultrasonication Extraction
Petroleum ether	87.5 $\pm$ 0.02 ^{Aa}	169.1 $\pm$ 0.03 ^{Ac}	114.6 $\pm$ 0.01 ^{Ab}
Hexane	113 $\pm$ 0.12 ^{Ba}	191.6 $\pm$ 0.05 ^{Bc}	128.3 $\pm$ 0.03 ^{Bb}
Methanol	148 $\pm$ 0.07 ^{Ca}	203.6 $\pm$ 0.20 ^{Cc}	154.5 $\pm$ 0.04 ^{Cb}

Means $\pm$ SD in the same column with different capital letters superscripts indicating significant difference at $P \leq 0.05$. Means $\pm$ SD in the same row with different small letters superscripts indicating significant difference at $P \leq 0.05$

Fig. 1 Total phenolic content of different extracts. Each value is mean $\pm$ standard error of mean of triplicate samples and within the group bars with different letters are significantly different ($P < 0.05$)

Statistical analysis

Statistical significance was calculated by one-way and two way analysis of variance (ANOVA). Comparison between means was made by least significant difference (LSD) pair wise comparisons with help of Microsoft excel and systat software 6.0.1 version.

Results and discussion

Yield

Solubility of polyphenolic compounds depend on aqueous and non-aqueous medium. These are generally soluble in polar non-aqueous solvent (Hu et al. 1996; Proctor and Bowen 1996). Application of polar solvents on polyphenolic compound containing cells, lead to disruption of cell membrane and release of nutraceuticals into the solution (Oufnac et al. 2007). Methanol was able to extract out around 96.03 % of oil from rice bran as total oil content present in rice bran was 21.20 %. Table 1 and Fig. 1 represents yield of crude oil on weight and total phenolic content basis, respectively. Both yields followed the same trend such that:

Methanol extract > Hexane extract > Petroleum ether extract.

Methanol was proved to be better solvent for extraction of crude oil and polyphenolic compounds. Our study showed

resemblance with (Hu et al. 1996; Proctor and Bowen 1996) obtained, high yield of polyphenolic content in methanolic extracts of rice bran. They also concluded that the polar and non-polar nature of the solvent is an important criterion for extraction of polyphenolic compounds.

Our results showed that the amount of crude oil and total phenolic content of microwave treated methanolic extract was significantly higher than those of ultrasonication and conventional extracts i.e. 20.51 % and 0.889 mg FAE/g crude oil, respectively (Fig. 1). On the other hand, the total phenolic content of conventional and ultrasound treated petroleum ether and methanolic extracts was not significantly different at $P \leq 0.05$. This pattern may be due to the reason that, microwave homogeneously and rapidly delivers energy to the total volume of solvent and solid plant matrix, with subsequently leads to easy extraction of phenolic compound (Oufnac et al. 2007). Our results also matched with (Sun et al. 2006; Dar and Sharma 2011) obtained high TPC in rice bran microwave treated methanolic extracts as compared to simple conventional solvent extraction method. Thanonkaew et al. (2012) attained high yield of TPC in microwave treated rice bran extracts than conventional solvent method. Above trend is in line with the report of (Pan et al. 2003) demonstrated that better extraction efficiency of tea polyphenols and tea caffeine was obtained by microwave-assisted extraction as compared to ultrasonic, heat reflux and extraction at room temperature. Holliday (2006) also found great yield of phenolic content in microwave treated oat bran extracts than ultrasonication assisted and conventional solvent extracts. According to Toma et al. (2001) and Li et al. (2004) ultrasound treated plant solvent extracts diffuses the cell wall more faster than conventional extraction and causes cell rupture over a shorter period (especially methanolic extracts), up to certain extent showed similarity with our study. All the findings of our experiments were performed in triplicate and statistically justified at $P < 0.05$.

The possibility of presence of γ -oryzanol increases as the total phenolic content increases (Fig. 1). The concentration of γ -oryzanol in different extracts obtained by conventional, microwave and ultrasound treatment was estimated by GC-MS (Table 2). Chromatograms of methanolic extracts of different treatments are shown in Fig. 2. According to NIST08 library of mass spectra, γ -oryzanol shows retention time of 16.45 min in 40 min total time of scanning. The concentration

Table 2 Concentration of γ -oryzanol in different extracts (ppm)

Solvents used	Conventional Extraction	Microwave Extraction	Ultrasonication Extraction
Petroleum ether	59.352 $\pm$ 0.02 ^{Aa}	67.691 $\pm$ 0.03 ^{Ac}	58.369 $\pm$ 0.01 ^{Aa}
Hexane	62.010 $\pm$ 0.12 ^{Ba}	77.320 $\pm$ 0.05 ^{Bc}	67.365 $\pm$ 0.03 ^{Bb}
Methanol	73.512 $\pm$ 0.07 ^{Ca}	85.0 $\pm$ 0.20 ^{Cc}	82.032 $\pm$ 0.04 ^{Cb}

Means $\pm$ SD in the same column with different capital letters superscripts indicating significant difference at $P \leq 0.05$. Means $\pm$ SD in the same row with different small letters superscripts indicating significant difference at $P \leq 0.05$

Fig. 2 Chromatograms of **a** conventional method, **b** microwave and **c** ultrasound treated methanol extracts (Peak of oryzanol is indicated by encircled part of chromatogram)

Fig. 3 Oryzanol extraction optimisation on total phenolic content basis **a** Methanol concentration optimisation **b** Digestion time optimization

of γ -oryzanol was found to be directly proportional to phenolic content present in petroleum ether, hexane and methanolic extracts of different treatments, showed similarity with the

study (Thanonkaew et al. 2012). Highest amount of γ -oryzanol was present in microwave treated methanol extracts i.e. 85.0 ppm (Fig. 2b) followed by ultrasonication (82.032 ppm) (Fig. 2c) and conventional extraction method (73.512 ppm) (Fig. 2a) (Table 2). Our results were in agreement with the report of (Sun et al. 2006) obtained maximum amount of TPC and γ -oryzanol in microwave treated methanolic extracts of rice bran than hexane extracts.

Optimization of methanol concentration and digestion time

As shown in the earlier section, concentration of γ -oryzanol was directly proportional to the total phenolic content present in different solvent extracts. That is why; methanolic extract was selected for the optimization process of obtaining maximum yield of γ -oryzanol in relation to methanolic concentration and digestion time. Different methanolic concentrations (50–100 %) were tried for maximum extraction of total phenolic content under microwave treatment. It was found that from 50 % to 80 % methanolic concentration, total phenolic content was significantly ($P < 0.05$) increased and decreased thereafter from 80 to 100 % (Fig. 3a). This indicates that 80 % methanol concentration was more appropriate for yielding maximum total phenolic content. At 80 % methanol concentration, rice bran was digested from 25 to 75 min under microwave treatment. Maximum phenolic content was obtained at 55 min digestion time and on further increasing the digestion time, no significant increase in TPC was noticed (Fig. 3b). Therefore, microwave treatment of rice bran at 80 % methanolic concentration and 55 min digestion time was observed to give maximum TPC. The γ -oryzanol content of optimized condition was assessed by GC-MS and its concentration was

Fig. 4 Chromatogram of rice bran extract in 80 % methanol at 55 min digestion

noticed as 105 ppm (Fig. 4). Anwar et al. (2010) achieved high TPC in 80 % methanol as compared to 100 % methanol concentration in barley extracts, showed similarity with our results. Our findings are similar to (Kwon et al. 2003) obtained maximum yield of saponin (polyphenol) in microwave treated 80 % methanolic extract of ginseng plant as compared to simple solvent extraction method in 0.5 min.

Conclusions

Methanol was the most effective solvent for extraction of γ -oryzanol. Microwave treatment significantly boosted the γ -oryzanol extraction capability of methanol as compared to ultrasonication. In this way, use of solvent amount can be reduced by microwave application for γ -oryzanol extraction from rice bran.

Acknowledgment Present work was funded by director of Sant Longowal Institute of Engineering and Technology Longowal, Punjab-148106, India.

Compliance with ethical standards

Conflict of interest The authors have no competing interests to reveal.

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