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Optimisation of ultrasound assisted extraction of rice bran proteins: effects on antioxidant and antiproliferative properties

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RESEARCH ARTICLE

Abstract

In this study, it was aimed to increase added value of rice bran which is a by-product of paddy processing. Optimisation of ultrasound assisted extraction produced the highest rice bran protein yield (39.85%) with the parameters as solid/liquid ratio of 0.43, power of 48.25% amplitude, and ultrasound application time of 29.89 min. Based on the optimised solid/liquid ratio and time, antioxidant and antiproliferative properties of protein isolates treated with different level of ultrasound power were determined. Total antioxidant capacity (TEAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, total phenolic content (TPC), metal chelating activity and the effects on HepG2 cytotoxicity of rice bran protein isolates were evaluated. The highest values of TEAC (17.73%), DPPH (63.61%), TPC (297.43 mg GAE/mg), and metal chelating activity (55.34%) were obtained from samples treated with 100% ultrasound power. Th results of the study showed that the ultrasound power had significant effect ($P<0.05$) on TPC, metal chelating activity, and DPPH scavenging activity of rice bran proteins.

Keywords: rice bran, ultrasound, optimisation, antioxidant, antiproliferative

1. Introduction

The food processing industry produces a large amount of waste and by-products which may result in potential environmental problems. Therefore, their valorisation requires special attention and in this context, evaluation of by-products of cereal milling industry is an important issue that needs to be investigated. For this purpose, optimisation of ultrasound assisted extraction was performed to release proteins from low added value rice bran and antioxidant and antiproliferative properties of rice bran proteins.

Rice bran is the most important waste in paddy processing. Rice grain consists approximately 10% bran and the bran contains 11.3-14.9% protein together with 34.0-62.0% starch, 15.0-19.7% oil and nutraceuticals (Mustafa and Ertan, 2008).

Extractions of functional compounds of food by ultrasound assistance have become emerging subject (Chen *et al.*, 1998; Hu *et al.*, 2013; Saunders, 1990; Wu *et al.*, 2001). Ultrasound which is comparatively cheap and easy technique (Hu *et al.*, 2013) may help to extract intracellular components by disintegrating the cell wall structure through cavitation and mechanical interactions.

Ultrasound assisted extraction is also being considered as promising technique for the extraction of proteins from various food matrix. Applying ultrasound assisted extraction on rice bran has been reported to produce protein with high efficiency (Tang *et al.*, 2002). Studies also showed that protein hydrolysate which was obtained from rice bran has antioxidant activity (Adebiyi *et al.*, 2008) such as antioxidant activity against peroxidation (Chanput *et al.*, 2009).

In this research, rice bran was used for protein extraction by ultrasound. Extraction procedures were optimised by central composite design (CCD) of response surface methodology (RSM). Protein extracts were produced at optimum ratio, time and four different ultrasound powers. The effect of ultrasound power on antioxidant (2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, total phenolic compound, total antioxidant activity, metal

chelating activity) and antiproliferative (cytotoxicity on HepG2) properties were evaluated.

2. Materials and methods

Materials

Rice bran was obtained from Çeltiksan Food and Agricultural Products Ltd. (İpsala, Edirne, Turkey). Sodium phosphate dibasic was purchased from Merck (Darmstadt, Germany), DPPH, ferrozin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and other chemicals were purchased from Sigma (St. Louis, MO, USA).

Methods

Moisture, protein and lipid content

Moisture content was determined by calculating the weight loss. Samples were weighed to be 10 g per tarred petri dishes and the calculation was made based on the weight loss at 105 °C for 4 h (IUPAC, 1987). Protein content of rice bran was determined using a nitrogen analyzer (CHNS-932; Leco, St. Joseph, MI, USA) by Dumas method (AOAC, 2005). Soxhlet extraction with hexane was used for lipid analysis (AOCS, 1997).

Preparation of rice bran sample

Defatting

Samples were heated with 1/5 (w/v) hexane at 45 °C for 4 h at 220 rpm and samples were centrifuged (Hettich, Tuttlingen, Germany) for 10 min and dried 1 day in the fume-cupboard (Faema MPN, Changzhou, China). Particles were downsized and sieved through 250 micron sieve. The samples were stored until analysis at 4 °C (Carbone and Mencarelli, 2015).

Ultrasound application

Samples were weighed in the specified amounts (10, 30, 43 and 50%) in 250 ml beakers and 100 ml distilled water were added. Temperature control was achieved by covering the beaker with iced water. Ultrasound device (UP400S; Hielscher, Teltow, Germany), 400 W, 24 kHz and with titanium probe (H22D, 22 mm) maximum amplitude of 120 µm was used. The probe was placed up to 4 cm inside the sample then ultrasound was applied. After ultrasound treatment samples were centrifuged at 4,200 rpm for 10 min and filtered (0.42 µ, syringe filter) and then stored at -18 °C.

Optimisation

Response surface methodology (RSM) using CCD (Design Expert, Trial Version 7; Stat-Ease Inc., Minneapolis, MN, USA) was employed to determine the optimum levels of the three variables (A, solid/liquid ratio; B, ultrasound power; C, time) and three levels (-1, 0, +1) were used to evaluate the optimum combinations regarding the response, protein yield. All the ranges for the parameters were selected based on the literature and the preliminary experimental work. The range of independent variables and their levels were depicted in Table 1.

The variation of protein yield related to the three variables A, B and C were evaluated using a quadratic polynomial model given by the following equation:

$$Y= \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2$$

where Y is response variable, β_0 is the fixed response at central point, β_1, β_{11} and β_{12} are the linear, quadratic and interaction coefficients, respectively. A, B and C are the independent variables.

Protein content

Bradford dye solution was prepared with 50 ml of coomassie brillant blue, 50 ml of 95% ethanol and 50 ml of 85% phosphoric acid. Samples (40 µl) that was diluted according to pre-trials and Bradford solution (200 µl) were placed in microplate and absorbans were measured at 620 nm by a microreader (Multiscan FC; Thermo, Istanbul, Turkey). Standard curve was formed with bovine serum albumin at various concentrations (0.2, 0.5, 0.6 and 0.8 mg/ml) and protein contents were determined by the equation obtained from the curve (Sánchez-Moreno *et al.*, 1998).

Ultrasound application after optimisation

Designated value of solid/liquid ratio (43%) and time (30 min) were used and four different amplitude (Table 2).

Table 1. Coded and uncoded levels of the independent variables.

Independent variables	Code units	Coded levels		
		-1	0	+1
Solid/liquid ratio (%)	A	10	30	50
Ultrasonic power (amplitude, %)	B	20	60	100
Time (min)	C	10	20	30

Table 2. Different point that was evaluated of antioxidative and antiproliferative properties.

Sample	Solid/liquid ratio (%)	Time (min)	Power (amplitude, %)
Control	43	30	0.00
1	43	30	20.00
2	43	30	48.25
3	43	30	65.91
4	43	30	100.00

Preparing protein isolate

Rice bran protein isolates were prepared by the alkali extraction method (Adebiyi *et al.*, 2008; Dinis *et al.*, 1994). The solution mixture was adjusted to pH 10 with 0.5 M NaOH, stirred for 2 h at 30 °C, and centrifuged at 4,100 rpm for 15 min. Then, the supernatant was adjusted to pH 4.5 with 2 M HCl, maintained at 4 °C for 60 min for protein precipitation, and then centrifuged at 4,100 rpm for 15 min. The residue was washed with five volumes of distilled water and neutralised with 0.5 M NaOH. The neutralised protein solution was lyophilised and stored at -20 °C.

Bioactive properties

Total phenolic content

Phenolic compounds of the extracts were determined by Folin-Ciocalteu colorimetric method with some modification (Sagdic *et al.*, 2013). Total phenolics were expressed as gallic acid equivalents (mg GAE/ml extract). Folin-Ciocalteu reagent was diluted with distilled water. 7% $NaCO_3$ was prepared with distilled water to adjust pH 10. 400 µl samples, 2 ml diluted Folin-Ciocalteu reagent and 1.6 ml $NaCO_3$ was added in tube and incubated 1 h then absorbances were read at 765 nm by spectrophotometer (UV1700 Pharmaspec; Shimadzu, Kyoto, Japan). The same procedure was applied for the gallic acid prepared (0-1 mg/ml). The absorbance was measured and linear calibration curve was plotted. The equation of calibration curve equation y = 0.8689x + 0.0024 was used for calculation.

Trolox equivalent antioxidant capacity

7 mM ABTS was weighed and dissolved with distilled water in 50 ml volumetric flask. Subsequently, 5 ml 12.25 mM potassium persulfate solution was added and volumetric flask volume was made up to 50 ml with distilled water. This solution was kept in the dark 12 h. Phosphate buffer was prepared at pH 7.4 with sodium monobasic, dibasic sodium and sodium chloride. This buffer was used for diluting the sample and ABTS solution. 20 µl of sample and 2 ml of ABTS was added in spectrophotometer tube. The absorbance of the mixture was measured after incubation in the dark for 6 min at 734 nm in a spectrometer (Wattanasiritham *et al.*, 2016). The percentage inhibition was calculated using following equation:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{absorbance sample}}{\text{absorbance ABTS}}\right) \times 100$$

Standard of trolox was drawn for 2, 1.5, 1 and 0.5 mM trolox absorbance. Trolox equivalent amount of the samples was calculated from the equation. The equation of calibration curve was y = 8.668x – 1.010.

DPPH radical scavenging activity

The scavenging activity of rice bran protein isolate for the radical 2,2-diphenyl-1 picrylhydrazyl (DPPH) was measured as described by Sánchez-Moreno *et al.* (1998). 0.1 mM DPPH reagent was prepared with ethanol. Sample (100 µl) was prepared in different concentrations and 3,900 µl DPPH reagent added the absorbance determined by a spectrometer (UV1700 Pharmaspec; Shimadzu) at 517 nm after 30 min incubation. Radical scavenging activity was expressed as percentage inhibition using following equation (Sánchez-Moreno *et al.*, 1998).

Percentage inhibition of DPPH by the sample:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{absorbance sample}}{\text{absorbance of control}}\right) \times 100$$

Metal chelating activity

Metal chelating activity was measured according to Dinis *et al.* (1994). 400 µl extracted protein and 50 µl 2 mM $FeCl_2$ was incubated 30 min then 200 µl 5 mM ferrozine solution and 3,350 µl ethanol were added in the incubation medium and after 30 min the absorbance was measured at 562 nm by spectrometer (UV1700, Pharmaspec, Shimadzu). The calculation was carried out by metal chelating activity (%):

$$\text{Metal chelating activity \%} = \left(1 - \frac{\text{absorbance sample}}{\text{absorbance of control}}\right) \times 100$$

Cytotoxic on HepG2 cell line

Culturing the cells

Dulbecco's modified eagle's medium (DMEM) was used by adding 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin-streptomycin. The HepG2 cell line was reproduction in incubator containing 37 °C, 95% moisture 5% CO_2 by DMEM medium.

Determination of cell viability

DMEM medium was used by adding 10% FBS, 1% L-glutamine, 1% penicillin-streptomycin. The HepG2 cell line was reproduction in incubator containing 37 °C, 95% moisture 5% CO_2 by DMEM medium. Trypan blue dye test was used for determination of cell viability (Louis and Siegel, 2011).

Statistical analysis

The measurements were replicated 3 times. Statistical analysis was performed using the software SAS (Version 8.2; SAS Institute Inc., Cary, NC, USA). The analysis of variance (Bermúdez-Aguirre D) and Tukey tests were used for the checking of the statistical significance with confidence level of 95.0%. The ANOVA produced parameters, lack of fit, coefficient of determination (R^2) and F-test, were employed to evaluate the model adequacy. The model was fitted by multiple linear regressions and response surface plots were obtained for three responses.

3. Results and discussion

Moisture, lipid and protein content

The moisture, lipid and protein contents of rice bran samples were found as 10.6, 15.5 and 10.6%, respectively. The chemical composition of cereals varies depending on the growing conditions, irrigation, fertilisation, etc. In a study, moisture, oil and protein content of rice bran has been reported as 12.28, 14.12 and 13.29%, respectively (Bagchi *et al.*, 2016).

Optimisation of extraction by central composite design

Total number of 20 designed runs of experimental points, values of the independent process variables (A, B and C) and measured values of the response are presented in Table 3.

The extraction was performed with water. The maximum protein content was found 39.45%. Optimum extraction point was 0.43 solid/liquid ratio, 48.25% amplitude and 30 min. It was reported that optimum tryptophan extraction conditions from rice were 30% amplitude power, 5 min, 30 °C and 8% methanol (Setyaningsih *et al.*, 2017). The

Table 3. Central composite design of three variables with their observed response.

Design point	Block	Independent variables			Response
		Solid /liquid ratio (%)	Power (amplitude, %)	Time (min)	Protein content (%)
1	1	0.10	100.00	30.00	8.26
2	1	0.10	60.00	20.00	12.16
3	1	0.30	60.00	30.00	30.03
4	1	0.50	20.00	10.00	31.55
5	1	0.50	100.00	10.00	31.26
6	1	0.30	60.00	20.00	36.29
7	1	0.10	20.00	10.00	10.07
8	1	0.10	20.00	30.00	13.19
9	1	0.30	60.00	20.00	29.42
10	1	0.30	60.00	20.00	34.20
11	1	0.10	100.00	10.00	7.94
12	1	0.30	60.00	20.00	34.69
13	2	0.30	20.00	20.00	31.52
14	2	0.50	60.00	20.00	36.55
15	2	0.30	100.00	20.00	30.82
16	2	0.30	60.00	10.00	38.25
17	2	0.50	100.00	30.00	35.83
18	2	0.50	20.00	30.00	39.45
19	2	0.30	60.00	20.00	31.27
20	2	0.30	60.00	20.00	35.00

results of ANOVA showed that while solid/liquid ratio had significant effect on extraction yield, the effect of amplitude and time were insignificant (P>0.05). A study on ultrasound assisted amylase degradation reported the highest rice protein extraction rate by applying ultrasound frequency of 20/35 kHz (Yang *et al.*, 2018). The model was important and lack of fit was insignificant (Table 4). The ANOVA shows the relationship among the ultrasound extraction and significant independent variables with a R^2=0.957. The sample variation of 95.7% for the protein extraction from rice bran by ultrasound was attributed to the independent variables, and only 4.3% of the total variation could not be explained by the model. The P-value was found smaller than 0.0001 and lack of fit value was insignificant (P=0.2616) and therefore the model is valid (Table 4).

The variation in protein yield a function of the process variables were depicted as contour plots (Figure 1). Figure 1A and 1B shows that when the solid/liquid ratio was selected at optimum point, the response increases but the effects of the power as amplitude and time parameters were insignificant (P>0.05).

Antioxidant properties

Total phenolic content

Total phenolic content (TPC) of samples is presented in Table 5. It seems that ultrasound power (as percentage of amplitude) at fixed solid/liquid ratio (0.43 rice bran protein isolate/distilled water) and time (30 min) cause statistically significant (P<0.05) alterations in TPC of the rice bran proteins. TPC of the sample that was applied 100% amplitude ultrasound power was similar to sample that was applied 65.91% amplitude ultrasound power but higher and different from control and 20 and 48.25% amplitude ultrasound power applied samples. Rice bran has been known to have antioxidant activity due to its phenolic components (Mariod *et al.*, 2010; Arab *et al.*, 2011; Wang *et al.*, 1999). It was shown that TPC of rice bran was higher compared to husk and endosperm fractions (Butsat and Siriamornpun, 2010). Studies report that higher phenolic components can be extracted from wheat (Hromádková *et al.*, 2008) and rice (Farahmandfar *et al.*, 2015) bran samples after ultrasound treatment.

Table 4. Statistical result of central composite design.

Source[1]	Sum of squares	df[2]	Mean square	*F*-value	*P*-value (Prob > *F*)
Model	2,120.833	9	235.6481	24.81597242	<0.0001
A-A	1,513.733	1	1,513.733	159.4103849	<0.0001
B-B	13.60366	1	13.60366	1.432593433	0.2589
C-C	5.884965	1	5.884965	0.619742313	0.4494
AB	1.245644	1	1.245644	0.13117803	0.7248
AC	10.19317	1	10.19317	1.073436465	0.3246
BC	4.699936	1	4.699936	0.494947631	0.4978
A^2	238.5036	1	238.5036	25.11667936	0.0005
B^2	17.08568	1	17.08568	1.799283368	0.2095
C^2	0.620298	1	0.620298	0.065323234	0.8035
Residual	94.95826	10	9.495826		
Lack of fit	61.40679	5	12.28136	1.830226407	0.2616
Pure error	33.55147	5	6.710294		
Corr. total	2,215.792	19			
R^2	**0.957**				
Adj R^2	0.919				
Pred. R^2	0.765				
Adeq. precision	14.103				

[1] A = solid/liquid ratio; B = power; C = time.
[2] df = degree of freedom.

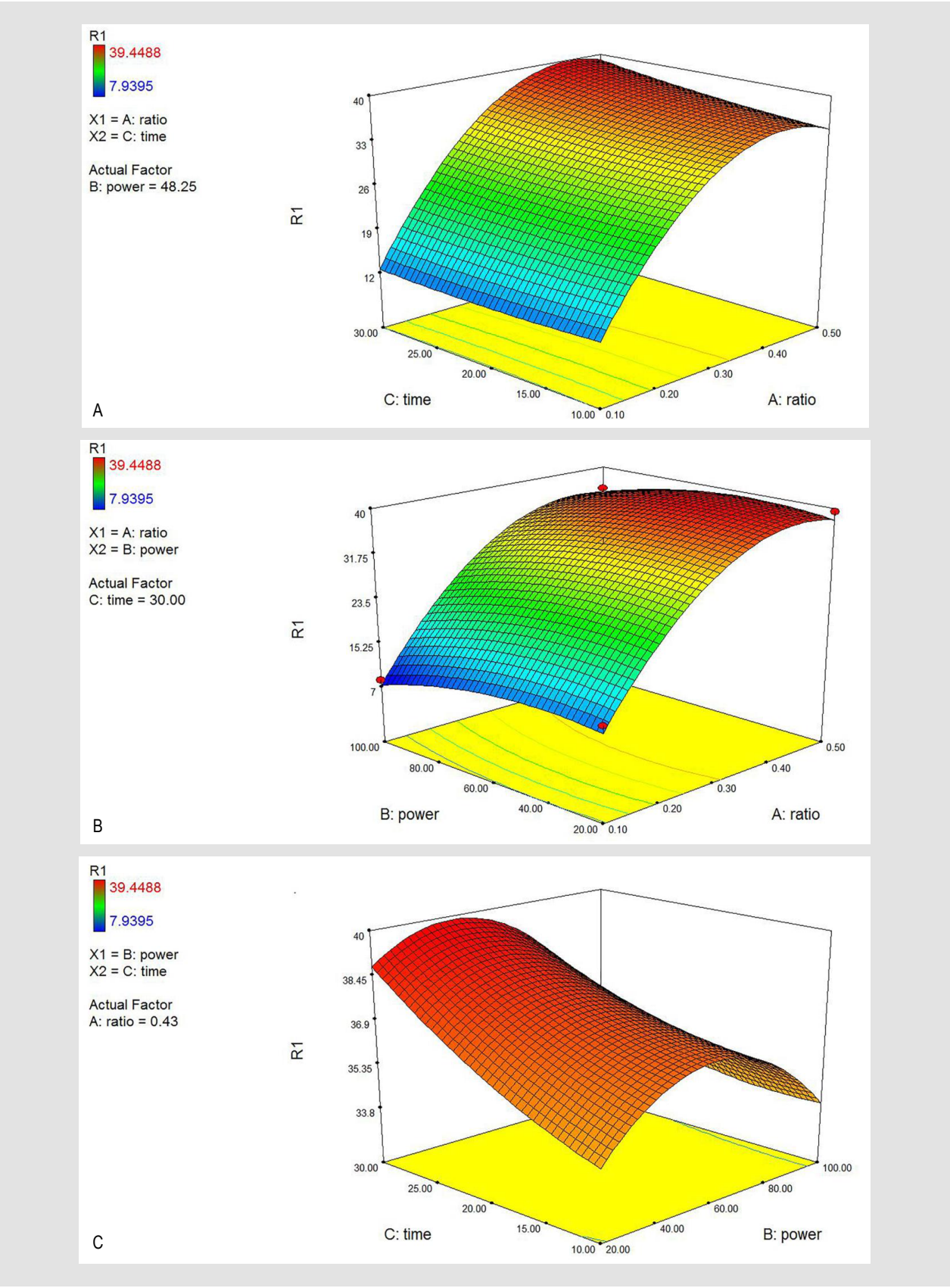

Figure 1. Response surface plots (3D) of protein extraction as a function of significant interaction between factors: (A) ratio and time; (B) ratio and power; (C) time and power.

Table 5. Total phenolic content (TPC) and antioxidant activity of samples and living cell.[1,2]

Ultrasound power (amplitude, %)	TPC (mg GAE/g sample)	TEAC (µg trolox/ mg sample)	DPPH inhibition (%)	DPPH IC_{50}[2]	Metal chelating activity (%) (1 mg/ml)	Metal chelating IC_{50}	Living cell (%)	Living cell IC_{50}
Control	286.30±0.27^{b}	16.47±1.27^{a}	54.41±0.56^{b}	0.037±0.001^{b}	34.17±1.37^{c}	1.40±0.02^{a}	18.43±0.74^{c}	1.71±0.12^{a}
20	272.49±0.82^{c}	15.14±0.37^{a}	55.66±1.38^{b}	0.036±0.001^{b}	40.73±1.69^{b}	1.16±0.03^{b}	16.14±0.65^{d}	1.81±0.14^{a}
48.25	283.43±3.26^{b}	15.16±0.72^{a}	59.46±3.04^{ab}	0.033±0.003^{a}	46.65±0.56^{b}	0.96±0.02^{c}	24.62±4.00c^{b}	2.20±0.05^{a}
65.91	299.34±3.52^{a}	16.23±0.28^{a}	61.86±2.17^{a}	0.032±0.001^{a}	45.82±2.43^{b}	1.01±0.06^{c}	32.35±1.39^{b}	1.87±0.11^{a}
100	297.43±1.90^{a}	17.73±1.49^{a}	63.61±0.15^{a}	0.032±0.001^{a}	55.34±0.67^{a}	0.58±0.01^{d}	45.3±1.21^{a}	3.00±0.30^{b}

[1] Mean values ± standard deviation. Different letters following the values in the columns indicate significant (P<0.05) differences.
[2] DPPH = 2,2-diphenyl-1-picrylhydrazyl; GAE = gallic acid equivalents; IC_{50} = 50% inhibition concentration; TEAC = trolox equivalent antioxidant capacity.

DPPH radical scavenging activity

The 100% ultrasound power applied sample had highest and similar DPPH scavenging activity value as compared with 65.91 and 48.25% amplitude ultrasound power applied samples, respectively. When the ultrasound power was increased, DPPH scavenging activity of samples was also increased. Similarly, a study evaluating the ultrasound assisted extraction of antioxidant components from rice reported that when the power was increased, percentage scavenging activity also increase (Zhang *et al.*, 2003). The amount of antioxidant sample that is necessary to reduce 50% the concentration of DPPH (IC_{50}) is generally used for the assessment of antioxidant capacity (Inglett *et al.*, 2011). In this study, the IC_{50} value of 100% amplitude ultrasound power applied sample was found as 0.032 g/ml. The DPPH scavenging activity of rice bran (Arab *et al.*, 2011; Butsat and Siriamornpun, 2010; Chotimarkorn *et al.*, 2008; Shao *et al.*, 2014) has previously been reported and attributed to its sulfhydryl groups of amino acids (Elias *et al.*, 2008).

Trolox equivalent antioxidant capacity

Free radicals have been considered important for the oxidation reaction which may lead to damage at cell and cause diseases such as cancer and heart disease (Rai *et al.*, 2006). Antioxidants especially natural ones play an important role for human health by preventing the formation of oxidation (Saleem *et al.*, 2005). It was reported that antioxidant compounds of rice bran may protect the cells against oxidative stress (Xu *et al.*, 2001). The results current study shows that ultrasound power (% amplitude) had no effect on trolox equivalent antioxidant capacity (TEAC) (P>0.05). The highest value (17.73 µg trolox/mg sample) was obtained from the 100% amplitude ultrasonund power applied sample (Table 5). The ABTS radical scavenging activity of rice bran extracts has been reported as 33.61-59.85 (Zigoneanu *et al.*, 2008) or 8.67-14.25 µmol trolox equivalent/g sample (Premakumara *et al.*, 2013).

Metal chelating activity

The metal chelating activity and IC_{50} values were presented in Table 5. Statistical analysis showed that samples were not different from each other and control (P>0.05) means the effect of ultrasound power on metal chelating activity is insignificant. The highest metal chelating activity was measured as 55.34% for the 100% amplitude power applied sample and IC_{50} value was calculated as 0.58 mg/ml. Metals such as iron and copper have been known to have accelerating effect on oxidation reaction (Xie *et al.*, 2008). The metal chelating activity of rice bran has been attributed to γ-oryzanol (Zhang *et al.*, 2003) or bioactive protein and peptides which bind metal ions via free amino or hydroxyl groups (Liu *et al.*, 2010). In a study on ultrasound assisted extraction of antioxidant components from rice bran, metal chelating activity was reported as 57.2% (Zhang *et al.*, 2003) which is comparable to the metal chelating activity value of present study. When the ultrasound power was increased, metal chelating activity also increased similar to TPC, DPPH radical scavenging activity and trolox equivalent antioxidant capacity. It has been known that TPC shows identical tendency to antioxidant properties (Shoemaker *et al.*, 2005; Velioglu *et al.*, 1998).

Antiproliferative activity

Antiproliferative activity of rice bran protein isolate was evaluated by measuring the cytotoxic effect on HepG2 (liver cancer cell) line. HepG2 cell line generally used for the determination of toxic effect of food components (Liu *et al.*, 2010). Minimum sample concentration for the toxic effect on HepG2 was determined as 1 mg protein isolate/ml medium through pre-trials. The effect of rice bran protein concentration was found significant (P<0.05) on cell toxicity. The higher concentration resulted in the higher toxic effect. The effect of ultrasound power as indicated by percentage amplitude on cytotoxicity was significant (P<0.05). The increase in ultrasound power resulted in a decrease in

cytotoxic effect. The highest toxic effect (83.96%) was determined for 20% amplitude ultrasonic power applied sample (Table 5). A study on toxic effect of rice bran proteins on colon cancer cell line (Caco-2), human breast cancer cell line (MCF-7) and liver cancer cell line reported that the application of 600-700 µg/ml rice bran protein produce toxic effect of 84% on Caco-2, 80% on MCF-7 and 84% on HepG2 (Kannan *et al.*, 2009). Cytotoxic effect on different cell lines for bioactive compounds (Forster *et al.*, 2013), enzyme assisted extracts (Revilla *et al.*, 2013; Santa-María *et al.*, 2013), various solvent extracts (Leardkamolkarn *et al.*, 2011) and antioxidant extracts (Lee, 2005) from rice bran have been reported. In addition γ-oryzanol (Kim *et al.*, 2012; Yasukawa *et al.*, 1998) and tocopherol and tocotrienol (Kawamura *et al.*, 1993; Packer *et al.*, 2001) of rice bran have been shown to have anticancer activity.

4. Conclusions

In this study RSM was successfully used to determine optimal levels of experimental parameters for the ultrasound assisted protein extraction from rice bran. Three extraction variables (solid/liquid ratio, ultrasonic power and extraction time) were optimised for obtainment the highest extraction yield for protein. The optimum points for the three responses were estimated as: solid/liquid ratio, 0.43; extraction time, 30 min; ultrasound power, 48.25% amplitude. It was seen that ultrasound has effect on extraction yield. After optimisation, antioxidant and antiproliferative properties of samples was evaluated at optimum solid/liquid ratio, time and four different ultrasound powers. It was seen that ultrasound power has an effect on antiproliferative property as assessed cytotoxicity on HepG2 cell line and antioxidant properties of rice bran protein extracts. It has been proved that ultrasound assisted extraction can be applicable to rice bran protein giving rise to higher yields. In addition, the extracts optimally produced from rice bran are likely to be potential health beneficial ingredients for the formulation of functional foods. Therefore, the results of this study may provide data on the investigations aimed at to improve the added value of rice bran, a by-product of paddy processing.

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