Analytical Methods

Optimisation of an ultrasound-assisted extraction method for the simultaneous determination of phenolics in rice grains

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Abstract

Fifteen phenolic compounds were determined in rice grains by ultrasound assisted extraction and ultra-performance liquid chromatography with photodiode array detection. Primarily, an UAE method has been developed and validated for the extraction of phenolics from rice grains. For the optimization, a Box–Behnken Design based on six factors including extraction temperature (10–70 °C), solvent composition (0–50% methanol in water), cycle (0.2–0.7 s^{-1}), ultrasound amplitude (30–70%), and solvent to sample ratio (2.5:1 to 5:1) was employed. Multi-response optimization (MRO) was performed to develop simultaneous extraction method of 15 phenolics. The optimal UAE conditions were: extraction temperature 45 °C, time 25 min, cycle 0.4 s^{-1}, ultrasound amplitude 47%, solvent composition 80% methanol in water at pH 4.25, and sample to solvent ratio 1:5. Linearity, LODs, LOQs, precision and recovery were determined. In addition, the method was successfully applied to analyse a number of rice samples with different varieties.

Keywords: Ultrasound-Assisted extraction; Phenolics; UPLC-PDA; Rice grains

1 Introduction

Rice (*Oryza sativa*) is an essential world-wide cereal that provides calories for over half of the world's population (OECD-FAO, 2011). It becomes more important attributable to exclusive compounds with proven benefits for human health and these include phenolics (Deng et al., 2013). These facts trigger numerous of researches into phenolics thus extensively disclose the composition of these compounds in the grain (Chi, Lee, Kim, Kim, & Chung, 2007; Walter & Marchesan, 2011; Zhou, Robards, Helliwell, & Blanchard, 2004).

Phenolic compounds naturally present in rice grains including phenolic acids and phenolic aldehydes (Huang & Ng, 2012; Qiu, Liu, & Beta, 2010). The most common compounds of phenolic acids in rice grains are derived from hydroxycinnamic acid (C_6−C_3; an aromatic ring linked to a three-carbon chain) and hydroxybenzoic acid (C_6−C_1; an aromatic ring linked to a carbon atom) (Goufo & Trindade, 2014). Besides the aforementioned two major forms of phenolic compounds, aldehyde analogues are also described as phenolic compounds.

Given their role as powerful antioxidants, phenolic compounds persist to be fascinating to investigate thus far. Positively, it is crucial to come up further researches with a reliable method for their analysis. However, the development of an analytical extraction method of multi-analytes from a complex matrix imparts a number of difficulties due to feasible rapid interaction of targeted analytes with other components in the matrix. Ultrasound-Assisted Extraction (UAE) emerge a solution to this problem as for an advanced technology that can hasten mass transfer thus increase the extraction kinetics (Wu, Lin, & Chau, 2001). Furthermore, UAE has formerly been proved to be effective in extracting phenolic compounds from a different part of rice seed other than the grain, viz., rice bran (Tabaraki & Nateghi, 2011). On top of that, it has been also successfully utilised in the sample preparation for the determination of total phenolic compounds in rice grains by Cooklin (Chooklin, 2013, 2014). Henceforth, extraction method based on UAE for the individual

phenolic compounds in rice grain is promising to achieve.

There are another advanced extraction techniques: microwave-assisted extraction (MAE) and pressurized liquid extraction (PLE) for example, however, UAE is a cheaper technique, also it is simpler to use (L. Wang & Weller, 2006). Additionally, UAE is not limited by solvent, it means solvent must not be transparent for microwave for example. Therefore, UAE is used for a wide range or compounds, including phenolics in complex biological matrices (Ghafoor, 2009; Golmohamadi, Möller, Powers, & Nindo, 2013; Tao, Wu, Zhang, & Sun, 2014).

The UAE is a useful extraction technique for compound in solid foods because of the cavitation effect (Pringet, Fabiano-Tixier, & Chemat, 2013; Vinatoru, 2001). Cavitation is related to the power of ultrasounds applied to the sample. Nevertheless, there are some other factors that are important regarding the extraction efficiency, including the temperature of the extraction, solvent composition, the frequency of ultrasounds, and the cycle used. The aforementioned factors can influence the efficiency of UAE thus need to be optimized to extract the analytes of interest.

There are several chemometric tools used for optimization of extraction processes. For example, Box-Behnken designs (BBD) have been applied, first to evaluate the effects of working variables in the efficiency of the extraction by UAE, later the optimization can be developed, for example using response surface methodology (RSM) (Widiastuti Setyaningsih, Duros, Palma, & Barroso, 2016; Tabaraki & Nateghi, 2011). Given that the optimization process includes several responses, it is strongly suggested to optimize all the response simultaneously because of the resulted solutions equal to the variables by study would be gathered (Candioti, De Zan, Cámara, & Goicoechea, 2014). When this occurs, the overall solution must be considered in an optimal region, directing to a specific degree of compliance with the proposed criteria for each factor of the system. Subsequently, multiresponse optimization (MRO) can be reached using a desirability function to find a compromise solution (Islam, Alam, & Hannan, 2012). Therefore, the aim of this study was to optimize the UAE method utilising BBD in conjunction with MRO next to desirability function for a simultaneous extraction of individual phenolic compounds in rice grains. Afterwards, the optimised method was applied to determine phenolic compounds in rice extracts as a part of method validation.

2 Materials and methods

2.1 Chemicals and reagents

HPLC-grade methanol, ethyl acetate, and acetic acid were obtained from MercK (Darmstadt, Germany). Caffeic acid (CAF), *p*-Coumaric acid (*p*-COU), syringic acid (SYR), chlorogenic acid (CHL), isovanillic acid (IVA), and quercetin (QUE) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Isoferulic acid (IFA) was purchased from Extrasynthese (Genay, France). Ferulic acid (FER), *p*-hydroxybenzoic acid (*p*-HBA), sinapic acid (SIN), *p*-hydroxybenzaldehyde (*p*-HB), protocatechuic aldehyde (PRA), protocatechuic acid (PRO), vanillin (VAN) and vanillic acid (VAA) were purchased from Fluka (Buchs, Switzerland). The phenolic standard solutions were prepared in aqueous methanol 50:50 (v/v) and stored in a freezer at −32 °C. Water was purified using a Milli-Q purification system (Billerica, MA, USA).

2.2 Rice samples

Rice samples were obtained from an Asian market in Seville, Spain. They were grounded in an Ultraturrax homogenizer (IKA® T25 Digital, Germany) right before the extraction. The final extraction method was applied to four different rice varieties including white, brown, black, and red-pigmented rice.

2.3 Ultrasound-assisted extraction (UAE)

UAE was performed using a 24 kHz and 200 W UP200S ultrasonic device (Hielscher Ultrasonics GmbH, Teltow, Germany) with a diameter probe of 7 mm. The UAE temperature was controlled by a water bath (Frigiterm, J.P. Selecta, Barcelona, Spain) to operate from –10 °C to 100 °C. The rice grains were accurately weighed (2 g). A defined solvent was added into the extraction vessel with sample-to-solvent ratio according to the experiences in the experimental design. After applying the ultrasounds, the liquid extract was separated from the solid material using a centrifuge (J. P. Selecta, Barcelona, Spain) at 4 °C for 5 min operating at 1144.8×g. Fresh solvent was used to wash the solid material, then collecting with the extract and adjusted to the initial volume. A 0.22 µm nylon filter was used to remove any impurity before the injection in the chromatographic system.

2.4 Determination of phenolic compounds

A UPLC (ACQUITY UPLC® H-Class) system with Photodiode Array Detector (PDA) Waters Corporation, Milford, MA, USA) was used for the chromatographic analyses. A fixed wavelength at 280 nm was used for peak integrations during the optimization. Peaks identities were confirmed using the chromatographic and spectroscopic properties of commercial standards, additionally spiking procedures were used for confirmation.

A reverse phase (100 mm length; 2.1 mm I.D.; RP 18 CORTECS UPLC® silica-based solid-core particle; 1.6 µm particle size from Waters) was used for the chromatographic analyses. A binary solvent system was used as mobile phase. Solvent A was 0.01% acetic acid in water and solvent B was 2% acetic acid in acetonitrile. The 4.0 min gradient program was as follows (%B): 0–3 min, 6.4–50%; 3–4 min, 50–100%. The flow rate was set at 0.6 mL min^{-1}.

2.5 Design of experiment

The Box Behnken design (BBD) was used for the evaluation of the effects by the extraction variables on the recovery of phenolic compounds. Six independent factors including solvent (x_1), temperature (x_2), amplitude (x_3), cycle (x_4), pH (x_5), and sample-to-solvent ratio (x_6), each in 3 levels, was studied. The design domain of the levels of independent factors are listed in Table 1. The full BBD consisted of 54 extractions that were performed in random order (Table 2).

Table 1 The levels of independent factors.

Factors	–1	0	+1	Unit
Solvent Composition (x_1)	0	25	50	% methanol in water
Temperature (x_2)	10	30	50	°C
Amplitude (x_3)	30	40	70	%
Cycle (x_4)	2	4.5	7	s^{-1}
pH (x_5)	3	5	7	–
Sample-to-solvent ratio (x_6)	1:2.5	1:3.75	1:5	mg mL^{-1}

Table 2 Box–Behnken design for six factors with their observed responses.

DOE	UAE factors						Responses (Relative value to maximum response, %)								
	x_1	x_2	x_3	x_4	x_5	x_6	PRO	*p*-HBA	PARA	VAA	IVA	VAN	*p*-COU	FER	SIN
1	0	0	−1	0	−1	−1	24.87	38.35	63.38	47.27	42.21	44.29	37.84	56.89	24.68
2	0	0	1	0	−1	−1	20.43	33.85	57.53	41.97	31.39	44.61	35.02	64.10	20.10
3	0	0	−1	0	1	−1	18.07	33.35	54.15	27.64	20.15	44.67	37.45	66.06	23.60
4	0	0	1	0	1	−1	17.81	35.03	53.89	19.74	16.01	44.36	36.66	58.82	25.78
5	0	0	−1	0	−1	1	54.30	52.86	81.26	43.77	27.60	73.74	65.44	91.81	37.08
6	0	0	1	0	−1	1	36.35	49.93	77.67	50.53	38.52	68.84	59.50	79.76	28.27
7	0	0	−1	0	1	1	50.85	54.62	77.93	26.78	32.92	83.51	65.15	84.51	33.10
8	0	0	1	0	1	1	34.66	56.79	74.98	45.89	42.76	75.88	58.06	91.86	38.85
9	0	−1	0	−1	−1	0	35.04	38.72	18.10	63.70	17.44	83.05	65.95	63.76	29.90
10	0	1	0	−1	−1	0	21.12	48.55	59.42	40.46	100.00	60.64	68.51	67.29	40.82
11	0	−1	0	1	−1	0	40.58	44.72	23.58	65.59	22.61	93.00	73.07	71.70	28.86
12	0	1	0	1	−1	0	22.33	57.10	67.50	57.35	41.75	71.65	78.39	77.90	53.53
13	0	−1	0	−1	1	0	40.73	44.54	48.66	55.08	28.50	86.34	66.34	63.86	32.51
14	0	1	0	−1	1	0	26.77	48.33	64.37	49.64	49.26	54.79	51.30	55.59	41.82
15	0	−1	0	1	1	0	44.67	48.38	54.04	61.28	30.18	83.17	68.07	66.09	27.58
16	0	1	0	1	1	0	25.10	48.29	63.01	56.54	59.86	65.46	65.00	67.27	54.54
17	−1	0	−1	−1	0	0	79.50	82.46	90.79	81.39	58.95	43.89	68.65	75.17	45.76
18	1	0	−1	−1	0	0	15.50	22.92	43.84	14.07	15.35	55.76	66.44	88.90	10.42
19	−1	0	1	−1	0	0	76.75	81.71	90.08	84.23	66.59	43.75	51.00	70.26	45.83

20	1	0	1	−1	0	0	17.37	23.61	47.39	17.63	17.54	83.44	72.00	11.57	38.33
21	−1	0	−1	1	0	0	72.53	76.73	100.00	77.23	63.79	47.48	53.94	73.83	59.92
22	1	0	−1	1	0	0	17.03	23.57	51.19	15.43	17.06	76.72	73.41	94.22	11.04
23	−1	0	1	1	0	0	65.37	64.81	86.76	64.17	54.91	44.25	54.36	66.48	56.44
24	1	0	1	1	0	0	18.51	29.11	53.18	19.90	15.75	81.02	74.25	88.46	22.11
25	0	−1	−1	0	0	−1	17.00	37.72	47.15	49.49	37.67	65.82	48.54	48.30	22.23
26	0	1	−1	0	0	−1	18.91	38.87	45.41	51.12	47.64	36.06	43.54	48.35	31.06
27	0	−1	1	0	0	−1	16.41	38.14	45.90	48.36	38.10	59.19	39.00	42.29	18.49
28	0	1	1	0	0	−1	19.35	38.10	40.92	39.40	49.48	35.21	41.48	46.73	34.53
29	0	−1	−1	0	0	1	37.48	48.34	55.51	62.59	26.31	78.33	74.84	70.14	32.08
30	0	1	−1	0	0	1	58.64	58.34	77.59	59.28	46.53	89.54	81.13	80.92	62.60
31	0	−1	1	0	0	1	50.94	54.07	50.36	59.91	45.15	91.93	69.41	66.61	38.83
32	0	1	1	0	0	1	31.71	63.99	75.37	66.27	53.14	84.98	93.97	87.49	66.48
33	−1	−1	0	0	−1	0	67.98	76.12	50.97	82.52	60.97	56.24	62.68	62.45	38.27
34	1	−1	0	0	−1	0	12.71	19.14	28.78	18.42	9.68	72.75	75.61	77.27	20.99
35	−1	1	0	0	−1	0	36.85	46.78	17.42	48.24	59.20	26.32	53.58	48.66	50.76
36	1	1	0	0	−1	0	12.69	31.67	43.05	26.25	22.65	89.61	75.78	87.09	25.71
37	−1	−1	0	0	1	0	72.43	78.34	53.28	89.42	64.58	56.37	65.98	65.38	42.80
38	1	−1	0	0	1	0	10.32	24.99	23.94	12.80	13.39	63.59	74.82	76.60	18.06
39	−1	1	0	0	1	0	28.55	51.88	41.00	64.77	65.17	28.02	62.12	58.13	58.25
40	1	1	0	0	1	0	12.53	32.14	43.53	26.41	26.58	88.74	79.33	89.33	26.34
41	−1	0	0	−1	0	−1	55.33	63.31	70.59	63.26	55.19	35.18	37.58	50.76	48.36
42	1	0	0	−1	0	−1	21.24	29.06	73.10	15.54	13.21	85.03	54.14	68.11	30.86
43	−1	0	0	1	0	−1	55.37	47.71	43.17	75.45	37.92	13.24	44.99	48.83	15.36
44	1	0	0	1	0	−1	23.41	32.78	76.73	30.98	18.46	70.57	60.89	67.60	81.19
45	−1	0	0	−1	0	1	100.00	100.00	70.80	93.51	71.72	47.22	88.02	79.81	90.12
46	1	0	0	−1	0	1	16.04	25.50	53.44	20.61	20.39	95.26	100.00	92.07	100.00
47	−1	0	0	1	0	1	90.22	86.88	92.35	100.00	76.99	49.36	80.89	95.70	41.07
48	1	0	0	1	0	1	16.13	29.02	59.14	21.18	29.25	100.00	98.45	100.00	94.99
49	0	0	0	0	0	0	44.93	55.70	91.73	49.50	20.56	74.54	68.22	82.68	92.58
50	0	0	0	0	0	0	38.25	53.16	81.81	55.38	19.44	65.58	57.39	72.36	60.30
51	0	0	0	0	0	0	39.31	52.42	82.86	52.10	17.49	70.73	58.24	75.43	64.06
52	0	0	0	0	0	0	36.26	52.37	81.72	50.95	21.84	64.15	61.59	70.33	60.53
53	0	0	0	0	0	0	36.26	54.05	79.70	46.21	29.46	49.31	76.25	79.92	81.56

54	0	0	0	0	0	0	39.01	53.95	79.44	66.89	36.93	55.73	65.70	60.94	62.79

Resulting data from the chromatographic analyses of the extracts, were evaluated to developed a mathematical model for the response to fit a second order polynomial function as shown in Fig. 1.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij x_i x_j} + \varepsilon \quad (1)$$

x_1, x_2, … , x_k are the UAE factors that influence the extraction efficiency; y; β_0, β_{ii} ($i = 1, 2,\ldots, k$), β_{ij} ($i = 1, 2, \ldots, k$; $j = 1, 2,\ldots, k$) are unknown parameters; ε is a random error.

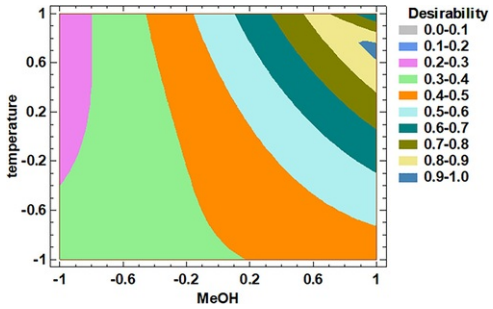


Fig. 1 2-D contour plot of the interaction between extraction temperature and solvent composition.

The least square method was used to estimate the β coefficients whilst only second-order interactions are considered.

2.6 Statistical analysis

In order to find the optimum UAE providing the highest recovery a multiresponse optimisation was accomplished using a desirability function provided by STATGRAPHICS Centurion XVI (Statpoint Technologies, Inc., USA). Gnumeric 1.12.17 was utilised to analyse the experimental data generated from single factor experiments. The Analysis of Variance (ANOVA, $p = 0.05$) was used to determine the significance of differences between the means. In the event that ANOVA suggest a significant difference, Least Significant Difference (LSD, $p = 0.05$) test was used to check the differences among the means.

3 Results and discussion

3.1 Performance of the chromatographic method

The level of individual phenolics were the responses used for the optimization, therefore first the chromatographic method was validated for the phenolic compounds based on the ISO 17025 and ICH Guideline Q2 (R1) (ICH, 2005; ISO, 2005). The analytical properties, as shown in Table S1 in the Supplementary Material, were checked.

Linear ranges were obtained for 1 to 15 mg L^{-1} and 10 to 100 mg L^{-1} with the coefficient of determination (R^2) both higher than 0.997. Limit of detection (LOD) and limit of quantification (LOQ) were estimated using the slope and standard deviation of the regression line. It was observed that IVA has the lowest LOD and LOQ (0.635 and 1.925 mg L^{-1} respectively) while SYR has the highest limits (1.113 and 3.373 mg L^{-1} respectively). This demonstrates an effective performance of the chromatographic method.

Repeatability (intra-day precision) was evaluated by analyzing nine replicates of the same samples within a day whilst inter-day precision was assessed by analysing three replicates in three consecutive days. These two levels of precision were estimated using coefficient of variance (CV) for the data of retention time and peak area. According to the AOAC manual for the Peer-Verified Methods program, the acceptable CV limit was ±10% (AOAC, 2012). The method provided a high precision for both intra- and inter-day precision because their CV values were <10%.

3.2 Stability study of phenolics

The stability of phenolic compounds under UAE conditions was performed prior to the development of UAE method. Extraction temperature was the most important factor affecting the stability of phenolic compounds. The level of phenolic compounds in standard solutions was checked before and after applying UAE temperatures of 10 and 70 °C for 20 min. These preliminary temperatures were set respect to the lowest and highest temperatures that could produce adequate precision by the system. A binary solvent of methanol:water was chosen as this normally used for the extraction of phenolics from plants (Alberti et al., 2014; González-Montelongo, Lobo, & González, 2010; Sarkis, Michel, Tessaro, & Marczak, 2014). Extractions were performed using medium setting of ultrasound amplitude (50%) and cycle (0.5 s^{-1}). The extraction temperature was controlled by circulating a constant temperature liquid that transferred heat to water which maintain the temperature of the extraction vessel. The recovery of phenolic compounds attained after being exposed to

different UAE temperatures are listed in Table S2 in the Supplementary Material.

For each set of UAE experiment, the results were compared with references of the same standard solution without UAE checking at before (initial reference) and after (last reference) the experiment. This comparison was used to assess the level of phenolics remained in the standard solution (hereafter referred to as recovery) after the ultrasound exposure. The level of phenolic in both references (initial and ultimate) were not significantly different, therefore, no degradation for any untreated compound during the set of experiment.

Every studied phenolics was stable in a low temperature of UAE (10 °C). Most of the compounds attain higher than 95% recovery while no >8.5% degradation takes place during the experiments. Since the temperature increases, some phenolics were found to have less stability presenting recovery lower than 90% including *p*-COU (89.8%) and QUE (87.7%). It is, therefore, a lower temperature than 70 °C was set as the maximum level on the design domain, viz., 50 °C.

3.3 The UAE method development

Taking into consideration the stability of phenolics, the working temperature for the UAE has been set. The subsequent study was to evaluate the factors that were likely to affect the recovery of phenolics from the rice samples by means of an experimental design. BBD was created to assess the effects on the recoveries of six factors related to UAE conditions. The studied factors were the solvent composition (0–50% methanol in water, x_1), extraction temperature (10–50 °C, x_2), ultrasound amplitude (30–70%, x_3), cycle (0.2–0.7 s^{-1}, x_4), pH of the solvent (3–7, x_5), and sample-to-solvent ratio (1:2.5–1:5, x_6). Due to the differences of unit and range of each factor, the values was normalized to a range of –1 to +1 thus a more even response could be obtained (Baş & Boyacı, 2007). Relative values to the maximum chromatographic signal (%) of peak areas were used representing the responses of individual phenolics and these are presented in Table S1.

A BBD with 54 experiments including 6 centre points was accomplished thus a mathematical model for the resulting data was subsequently estimated. The total response for the optimizations were the recovery of nine individual phenolic compounds that were selected as producing sufficiency signals on the response to DOE: p-HBA, PRO, IVA, PRA, VAA, VAN, FER, p-COU, and SIN.

3.4 Prediction capability of the regression model

The RSM results were first calculated based on the experimental design generated by BBD to produce a regression model for each response. The acquired mathematical model was used to estimate a predictive equation for the individual phenolic compounds in rice samples considering the significant factors. Only the significant factors ($p < 0.05$) were considered for the optimization to keep the number of factors for an optimized design low thus avoid a high variability. The equation for the model obtained for PRO is as it follows:

$$y = 0.39 - 0.25x_1 + 0.11x_6 - 0.11x_1x_6 + 0.10x_1x_2 - 0.05x_2 - 0.06x_2x_2 - 0.05x_5x_5 \quad \textbf{(2)}$$

where y is the level of PRO in the sample and x_i are the studied factors (x_1, solvent composition; x_2, extraction temperature; and x_5, pH; x_6, sample-to-solvent ratio).

ANOVA checks the significance and the adequacy of the model ($p > 0.05$) whilst the R^2 statistics shows how the model describes the variability of the responses. The errors of the predicted value calculated by ANOVA indicated that the residual standard deviations were ranging from 0.05434 (*p*-HBA) to 0.17916 (SIN) while the mean absolute error ranging from 0.02632 (*p*-HBA) to 0.08680 (SIN). The average variation for the estimated results by the RSM was 4.14%. The model prepared for *p*-COU was the best fit with variation ranged from 0.003 to 9.86% whilst the highest variation was found for the model of SIN (0.13–31.96%). The high variability (>20%) for SIN extraction was occur only in one experiment in low value of recovery (0.24 μg g^{-1}). Also of interest is the R^2 statistic, the values were ranged from 68.1% (SIN) to 95.51% (p-HBA). Hence, the regression model can be utilized to predict the responses in the optimization process.

The developed model suggest the effect of the studied factors on the response over the BBD domain as the response surface equation created by STATGRAPHICS Centurion XVI for each response was plotted. Afterwards, MRO was used to simultaneously optimise the nine responses by constructing the desirability function based on the RSM for each optimized response. The optimization defined by MRO was to achieve the maximum recovery (100%). To reach the optimum UAE conditions, the desirability function was plotted as a 2-D contour plot of the interaction between extraction temperature and solvent composition, which showed the optimum point of the simultaneous multi-response optimization (Fig. 1).

3.5 Response optimization

Prior to MRO, the main effects and interactions of studied factors were analyzed. The most influential factors were solvent composition (x_1), UAE temperature (x_2) and sample-to-solvent ratio (x_6) that were acknowledged in Eq. (2). Additionally, there was a significantly strong interaction between the solvent composition and extraction temperature ($p < 0.05$). This suggests that the solvent and temperature had divergent influence on the amount of extracted phenolic compounds. However, the influence was subjected to the type of individual phenolic compounds. The interaction effect between solvent and temperature was higher for more polar compounds due to have higher interaction with water content in the solvent.

Using the MRO results, the factors of solvent composition (x_1) and extraction temperature (x_2) were plotted (Fig. 1). A high extraction yield (94.06%) was achieved within the design domain at coordinates + 1.0000 for solvent composition (x_1, 50% methanol in water) and + 0.7166 for extraction temperature (x_2, 45 °C). The complete MRO suggestions for the rest studied variables were - 0.15953 for ultrasound amplitude (x_3, 47%), - 0.06543 for cycle (x_4, 0.4 s^{-1}), - 0.3945 for pH (x_5, 4.25), and + 0.9999 for solvent to sample ratio (x_6, 1:5).

The optimum point for the solvent composition was in the corner of the design domain (+ 1.0000). Henceforth, it was then needed to check values over the highest studied level (50% methanol in water).

3.6 Further optimization on solvent composition

The extraction yields obtained with extraction solvents of different compositions are shown in Fig. 2. It was found that the highest extraction yield was reached when applying the solvent composition of 80% methanol in water. Consequently, the optimum extraction solvent was set for 80% methanol in water.

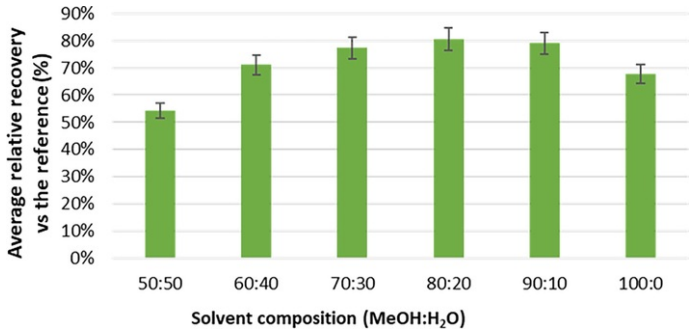


Fig. 2 Recoveries for phenolics using different solvents.

3.7 Kinetic study

Certain sample matrices can retain analytes within pores or other structures. In rice grain matrix, phenolic compounds are either soluble in the vacuole or bound to the cell wall (Shibuya, 1984). Increasing the extraction time permits the cavitation effects of ultrasound to increase the permeability of plant tissues to release the analytes from cell then diffuse into the extraction solvent. Therefore, the extraction kinetics should always be explored to produce a complete recovery.

Using different extraction times and the best extraction conditions, the extraction kinetics was studied. The resulting data of this specific study are presented in Fig. 3. It reveals that the recoveries for the total phenolic compounds by UAE increased until a plateau starting at 25 min. Henceforth, 25 min was assign as the optimized extraction time.

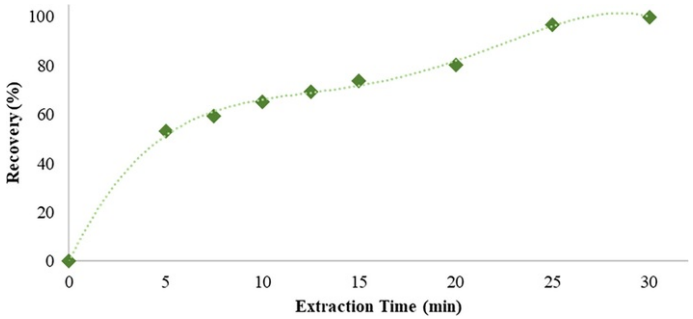


Fig. 3 Amount of extracted compounds in different extraction time.

The optimized extraction time reported by recent studies on the UAE for phenolic compounds is ranging between 6 and 60 min depend on the sample matrices (Carrera, Ruiz-Rodríguez, Palma, & Barroso, 2012; Corbin et al., 2015; Ince, Şahin, & Şümnü, 2013; Luo et al., 2015; Jin Wang et al., 2012; Jing Wang, Sun, Cao, Tian, & Li, 2008; X. Wang et al., 2013). A longer extraction time is expected when extracting the compounds from more complex matrices. In rice sample, phenolic compounds are distributed in different concentration and composition throughout the grain which composed of fibrous layers and protein bodies (O., 1993). Additionally, as the rice cell is solid and compact, the extraction time needed to recover the phenolic compounds from rice (25 min) is relatively longer than semi-solid or aqueous matrices i.e. grape (6 min).

3.8 Method validation

The absolute responses of the rice samples with and with-out analytes spiked before the UAE process were compared to define the UAE recovery (%R). The standard solution of phenolic compounds used for spiking was also measured. The spiked and non-spiked rice samples were extracted and phenolic targeted compounds were quantified on the resulting extracts. The extraction recoveries developed by UAE for the studied compounds were listed in Table S3 in the Supplementary Material. As it can be seen that the

recoveries ranging from 80.51% (*p*-HBA) to 95.99% (PRA) were obtained. These values of the recovery were within the acceptable range refers to AOAC guidelines (80–110%) thus indicated high accuracy of the developed method (AOAC, 2012). As the previous kinetic study clearly stated that no higher recovery is produced using longer extraction times and no degradation was observer neither, it must be assumed that no full recoveries for spiked compounds must be related to some interactions with the matrix, which cannot be overcome using the extraction conditions.

Two levels of precision, viz. repeatability and intermediate precision, was considered to evaluate the precision of the UAE method. Both levels were assessed according to ICH guidelines. The precision, indicated as CV, of the UAE method was ranged from 0.15% (*p*-HB) to 1.05% (VAN) for repeatability ($n = 9$) and 1.21% (PRO) to 8.39% (VAN) for intermediate precision ($n = 3 \times 3$). The developed UAE method has been proved as a highly precise extraction method because the CV values for both repeatability and intermediate precision were below the acceptable limit (±10% refer to AOAC).

3.9 Real sample application

In order to check its applicability, the proposed UAE method was applied to extract real rice samples. Five different rice samples *were* extracted using the optimized conditions of the developed and validated UAE. The results obtained from the real sample application (Table 3) revealed that the contents of phenolics are noticeably diverse among rice samples, i.e., polished (white), whole-grain (brown) and pigmented grain. This variation in the studied compounds is tangible considering the chemical and physical properties of the different types of rice varieties in which demonstrates the usefulness of the proposed method on the grains assessment.

Table 3 The level of phenolic compounds in the studied rice grain samples.

Phenolics (μg g^{-1})	Rice Varieties				
	Red	Black	Brown	Brown (Sushi)	White (Sushi)
PRO	48.41 ± 0.51	127.21 ± 0.08	2.00 ± 0.01	2.72 ± 1.06	2.40 ± 0.97
PRA	13.39 ± 0.11	2.82 ± 0.20	1.73 ± 0.32	1.19 ± 0.04	1.04 ± 0.27
p-HBA	1.41 ± 0.02	0.96 ± 0.24	1.52 ± 0.07	TR	TR
CAF	7.20 ± 0.30	ND	7.20 ± 0.06	3.45 ± 0.28	0.94 ± 0.65
CHL	3.98 ± 0.12	ND	2.33 ± 0.01	2.13 ± 1.03	1.05 ± 0.25
VAA	6.88 ± 0.06	27.79 ± 0.20	3.43 ± 0.03	1.97 ± 0.56	1.52 ± 0.21
p-HB	0.93 ± 0.01	ND	TR	TR	ND
IVA	3.36 ± 0.04	ND	1.97 ± 0.03	2.73 ± 0.31	1.13 ± 0.06
SYR	0.47 ± 0.16	ND	TR	TR	ND
p-COU	5.45 ± 0.95	3.48 ± 0.70	4.11 ± 0.09	1.51 ± 1.48	0.49 ± 0.30
VAN	4.73 ± 0.63	6.62 ± 0.44	2.51 ± 0.03	1.98 ± 0.15	0.92 ± 0.22
FER	12.16 ± 0.13	2.25 ± 0.05	22.68 ± 038	10.66 ± 0.19	4.02 ± 2.86
SIN	0.64 ± 0.01	ND	1.24 ± 0.03	TR	TR
QUE	TR	118.12 ± 0.58	TR	TR	TR
IFA	1.91 ± 0.01	ND	1.80 ± 0.01	2.03 ± 0.02	TR
SUM	110.93 ± 2.6	289.30 ± 0.12	52.52 ± 1.02	28.33 ± 0.04	13.49 ± 3.38

Note. ND, not detected due to less than LOD.

TR, trace due to the concentration less than LOQ but higher than LOD.

The results indicate that the pigmented rice group has higher value for the total extracted phenolic compounds (110.9 ± 2.6 μg g^{-1} and 289.3 ± 0.1 μg g^{-1} for black and red-pigmented rice respectively) compare to the non-pigmented rice samples (52.5 ± 1.0 μg g^{-1}, 28.3 ± 0.1 μg g^{-1} and 13.5 ± 3.4 μg g^{-1} for brown, brown (sushi) and white (sushi) rice samples). This fact is relevant with result reported by Tian, Nakamura, and Kayahara (2004), who revealed that higher levels of phenolic compounds are obtained from grains with a darker

colour than those that are white or pale yellow.

The second largest amount of phenolic compound was also found in the brown rice group. Nonetheless, the similarity in rice products, which was whole-grain rice, does not essentially mean that the grains own similar level of phenolic taking into account the different physical chemical characteristic of the grains. Sushi differ from regular rice for its sticky texture of the cooked grains due to have lower amylose content whilst possess higher amount in amylopectin. The result showed that the regular brown rice has higher concentration of total phenolics ($52.5 \pm 1.0\ \mu g\ g^{-1}$) than sushi brown rice ($28.3 \pm 0.1\ \mu g\ g^{-1}$). This finding corresponds with the results reported by Chi et al. (2007), who determined the phenolic compounds in Korean rice varieties and mentioned that the regular rice (Ilpumbye) has a higher level of phenolics than the glutinous type (Hwasunchalbyo).

The real sample application of the proposed UAE was also confirmed that the level of phenolic compounds in sushi brown or un-polished rice ($52.5 \pm 1.0\ \mu g\ g^{-1}$) were higher than the white or polished rice of the same variety ($13.5 \pm 3.4\ \mu g\ g^{-1}$). These results are matched with previously reported data (W. Setyaningsih, Saputro, Palma, & Barroso, 2016) that showed polishing process could reduce the concentration of phenolics in the grain since these compounds are mainly located in the external layers of the grain (Vichapong, Sookserm, Srijesdaruk, Swatsitang, & Srijaranai, 2010; Walter & Marchesan, 2011).

4 Conclusions

The Box–Behnken design in conjunction with the multi-response optimisation permitted the optimization of the UAE conditions for individual phenolic compounds extraction from rice grains. The optimum UAE conditions included the use of 80% methanol in water for 25 min at 45 °C with an amplitude 47%, cycle $0.4\ s^{-1}$, pH 4.25 and sample-to-solvent ratio of 1:5. The developed method presented acceptable values for linearity and precision (RSD < 9.0%). Henceforth, the proposed UAE method is an effective technique for the determination of individual phenolic compounds in rice samples.

Uncited references

Wang et al. (n.d.).

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Conflict of interest.

Authors declare no conflict of interest

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.02.107.

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Appendix A. Supplementary data

The following are the Supplementary data to this article:

Multimedia Component 1

Supplementary data 1

Multimedia Component 2

Supplementary data 2

Highlights

- Ultrasound-assisted extraction allows for fast extraction phenolics from rice grains.
- Phenolics are stable under the ultrasound-assisted extraction conditions.
- Fifteen phenolics can be recovered from rice grains by ultrasound-assisted extraction.

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