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ORIGINAL

Optimization of ethanol extraction of antioxidative phenolic compounds from torrefied oak wood (*Quercus serrata*) using response surface methodology

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Abstract A torrefaction treatment process followed by ethanol extraction was applied for extracting antioxidant components from oak wood. Response surface methodology (RSM) was applied to optimize the ethanol extraction conditions of antioxidant compounds from torrefied oak wood (severity factor $Ro = 4.23$). Response values were assessed such as total polyphenol content, total flavonoid content, and DPPH radical scavenging activity. Optimal extraction conditions were found as follows: ethanol concentration 69.15 %, extraction temperature 71.60 °C and processing time 70.15 min for total polyphenol content; ethanol concentration 66.93 %, extraction temperature 69.52 °C and time 66.09 min for total flavonoids content; ethanol concentration 68.18 %, extraction temperature 51.77 °C and time 74.22 min for DPPH radical scavenging activity. The experimental values agreed with those predicted within a 95 % confidence interval indicating the suitability of RSM in optimizing the ethanol extraction of antioxidant compounds from the torrefied oak wood. However, no significant correlation was found between antioxidant activity (DPPH), neither with total polyphenol content nor with total flavonoid content.

Introduction

Synthetic antioxidants have widely been used as butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and propyl gallate (Devi et al. 2007). However, many researchers have reported adverse effects of synthetic antioxidants, such as toxicity and carcinogenicity (Velioglu et al. 1998).

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There is a growing interest to replace such compounds with naturally occurring antioxidants.

Phenolic compounds of plants such as flavonoids, lignans, tannins, and various terpene-related compounds are potent antioxidants (Jaina et al. 2015; Ghasemi et al. 2009; Pokorny 2007). Nowadays, the interest is mainly focused on the development of phenolic compounds which are abundant and low-cost agricultural, forest, or industrial wastes (El-Shourbagy and El-Zahar 2014; Wanyo et al. 2014; Hamid et al. 2012).

However, many antioxidative phenolic compounds in plants are most frequently present in a covalently bound form with an insoluble polymer (Niwa and Miyachi 1986). Therefore, it is necessary to find an effective processing method to release the compounds. Several methods such as heat treatment (steam explosion and torrefaction), far-infrared (FIR) radiation, and enzymatic treatment have been studied to liberate and activate low molecular weight natural antioxidants from various plants (Gong et al. 2012; Ahajji et al. 2009; Lee et al. 2003; Duh et al. 2001).

Heat treatment of wood is a thermochemical pretreatment at temperatures between 200 and 300 °C (Chen and Kuo 2011). Antioxidative phenolic compounds can be present in the raw material or be produced during the heat treatment process. Heat treatment of wood primarily targets the selective breakdown of hemicelluloses. Cell wall-linked phenolic compounds are also solubilized during this type of treatment (Sivonen et al. 2002; Ahajji et al. 2009). A variety of compounds appears in the liquors obtained by heat treatment, including sugar oligomers, monomeric sugars, sugar degradation products (furfural and hydroxymethylfurfural), organic acids (citric and malic acids originating from the cells of the biomass, formic and levulinic acids from sugar degradation products, acetic acid from acetyl groups), and extractives and phenolics (Palmqvist and Hägerdal 2000). Preliminary studies have revealed that heat treatment of wood increased the phenol content, the concentration of stable phenoxyl-free radicals, and the antioxidant activities. The concentration of some phenolic compounds, such as syringyl and guaiacyl compounds increased after torrefaction (Shoulaifar et al. 2014; Ahajji et al. 2009). Ahajji et al. (2009) applied torrefaction (temperature of 210, 235, and 250 °C during 1 h) to beech and sprucewood, which allowed a significant increase of antioxidant activity. Castro et al. (2008) showed that the steam-explosion pretreatment improves the production of inhibitors such as syringaldehyde, vanillin, 4-formyl-benzoic acid methyl ester and desaspidinol. Among heat treatment methods, torrefaction is being used in the pellet production process. However, little information is available about the extraction of antioxidative phenolic compounds from torrefied wood.

Extraction is widely used as a process of separation to obtain antioxidative phenolic compounds from the plant. However, different plants may require different extraction conditions to achieve maximum recovery of antioxidative phenolic compounds (Chirinos et al. 2007). Optimization of extraction can be achieved by empirical or statistical methods, which are essential for economical commercial application of the antioxidative phenolic compounds extraction process. Factors influencing the extraction yield and the quality of the extracted antioxidative

compound were found as type of extraction solvent, solvent concentration, plant solvent ratio, agitation, extraction time and temperature (Nobre et al. 2005).

Response surface methodology (RSM) is an effective optimization tool for investigating many factors and their interactions influencing the response with fewer experimental trials (Tan et al. 2009). Accordingly, RSM has been increasingly utilized to optimize extraction conditions for antioxidative phenolic compounds over the past few years (Vázquez et al. 2012; Sun et al. 2011; Silva et al. 2007).

According to the literature review, no optimal extraction conditions were developed for antioxidative phenolic compound extraction from torrefied oak wood using a mixture of ethanol and water as an extraction solvent. Therefore, development of optimal extraction conditions using RSM for extraction of torrefied oak wood was considered to be important in order to maximize recovery of antioxidative phenolic compounds. The aim of this study was to investigate the effects of factors such as ethanol concentration, extraction temperature, and extraction time on the extraction of antioxidative phenolic compounds (total polyphenol content and total flavonoid content) and the DPPH radical scavenging activity of extracts from torrefied oak wood.

Materials and methods

Material

Oak wood (*Quercus serrata*) was supplied by the Korea Forest Research Institute. The dimensions of the oak wood chips were smaller than 30 × 50 × 30 mm^3, and dust (particle sizes <1 mm) was removed prior to torrefaction. An external dryer was used to dry the chips to <15 % moisture prior to torrefaction.

Torrefaction

Torrefaction of the oak wood chips was achieved using a laboratory scale reactor. A prescribed amount of oak wood chips (1 kg) was weighed and placed in the center of the reactor. The oak wood chips were torrefied at different lengths of time (5–10 min) and at a range of temperatures (200–240 °C). The severity of the torrefaction [Eq. (1)] was designated by a single factor, the severity factor *Ro*, which combines the effects of time (*t*, min) and temperature (*T*, °C) (Heitz et al. 1987).

$$Ro = [t \times \exp[(T - 100)/14.75]] \quad (1)$$

After torrefaction, the material was ground and sieved to a maximum particle size of 60 mesh. The torrefied material was stored in a desiccator at room temperature until chemical analysis.

Extraction procedure of torrefied oak wood

The phenolic compounds were isolated from the extracts following previously described methods, with certain modifications (Gong et al. 2012).

Extractions of torrefied oak wood were performed in glass bottles using distilled water or ethanol as the solvent. In general, an amount of 3 g of torrefied oak wood was placed in a glass bottle with 60 mL of solvent. The bottle was then placed in a shaking water bath at a speed of 100 rpm (1.7 s^{-1}). Upon completion of extraction, the extract was filtered through a paper filter. The filtrates were collected, placed in glass centrifuge tubes, and stored at −20 °C until use. Each extraction was carried out in triplicate.

Determination of total polyphenol content

The total polyphenol content was determined by the Folin–Ciocalteu method according to the reported procedure (Li et al. 2008). In detail, extracts (1 mL) with proper dilution were mixed with 1 mL of Folin–Ciocalteu reagent (10 times dilution) and allowed to react for 3 min at 30 °C in the dark. Then, an amount of 0.8 mL of saturated Na_2CO_3 solution was added, and the mixture was allowed to stand for 1 h. The absorbance of the reaction mixture at 765 nm was measured by using a spectrophotometer (HITACHI U-3000, Tokyo, Japan). A calibration curve of gallic acid was prepared, and the total polyphenol content was standardized against gallic acid and expressed as mg GAE equivalent per gram of dry torrefied wood.

Determination of total flavonoid content

The total flavonoid content was determined by aluminum chloride colorimetric assay (Ghasemi et al. 2009). Briefly, 0.5 mL of extract was mixed with 1.5 mL of methanol, 0.1 mL of 10 % $AlCl_3{\cdot}H_2O$, 0.1 mL of 1 M KCH_3CO_2, and 2.8 mL of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture at 510 nm was measured by using a spectrophotometer (HITACHI U-3000, Tokyo, Japan). The total flavonoid content was calculated as quercetin from a calibration curve. A calibration curve of quercetin was prepared, and the total flavonoid content was standardized against quercetin and expressed as mg QE equivalent per gram of dry torrefied wood.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity was calculated based on the change of absorbance due to the decrease in 2,2-diphenyl-1-picrylhydrazyl in relation to the control value (Ghasemi et al. 2009). Considering the color of the extract, an ethanol solution (1 mL) was used as a color blank instead of 0.5 mM DPPH in ethanol (1 mL). As the control, water or ethanol (1 mL) was added instead of the extract. In the following, a 0.1 mM ethanolic DPPH solution was prepared. The initial

absorbance of the DPPH in ethanol was measured at 517 nm, and it did not change throughout the period of the assay. An aliquot (0.1 mL) of each extract (with appropriate dilution if necessary) was mixed with 3.0 mL of ethanolic DPPH solution and incubated for 30 min at 30 °C in the dark. Then, the absorbance at 517 nm was measured. The DPPH radical scavenging activity (%) was calculated by the following equation:

$$\text{DPPH } (\%) = \left(1 - A_{\text{sample}}/A_{\text{control}}\right) \times 100 \tag{2}$$

where A_{sample} is the absorbance in the presence of the sample, and A_{control} is the absorbance in the absence of the sample.

Experimental design of RSM

Response surface methodology (RSM) was used to optimize process conditions for ethanol extraction of phenolic compounds from torrefied oak wood. RSM was applied to the extraction process to determine maximum yields of total polyphenol content, total flavonoids content, and DPPH radical scavenging activity by adjusting the levels of the process variables such as ethanol concentration (60–80 %, X_1), extraction temperature (60–80 °C, X_2), and extraction time (60–80 min, X_3). Box–Behnken design was applied to optimize the extraction conditions (Table 1). Seventeen experiments were performed in random order to minimize the effects of unexpected variability in the observed responses due to extraneous factors. Response surfaces, represented in Figs. 4, 5, and 6, were created using the RSREG (SAS: statistical analysis system, SAS Institute, USA). The experimental data were fitted to a second-order polynomial model [Eq. (3)], and the regression coefficients were obtained by multiple linear regression

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{2} \sum_{j=2}^{3} \beta_{ij} X_i X_j \tag{3}$$

where X_1, X_2, and X_3 are the independent variables affecting the responses of Y; β_0, β_i ($i = 1, 2, 3$), β_{ii} ($i = 1, 2, 3$), and β_{ij} ($i = 1, 2, 3$) are the regression coefficients for the intercept, linear, quadratic, and cross-product terms, respectively.

Statistical Analysis

The received data were assessed by analysis of variance to determine the extraction condition effects. Statistical analysis was carried out according to the results from the ANOVA test by comparing the data mean to the Duncan's test. Duncan's multiple comparison range test was applied to determine significant differences between the means.

Table 1 Box–Behnken design for the RSM

Run	Independent variables			Dependent variables		
	X_1	X_2	X_3	Total polyphenol content, Y_1 (mg GAE/g dry torrefied wood)	Total flavonoids content, Y_2 (mg QE/g dry torrefied wood)	DPPH radical scavenging activity, Y_3 (%)
1	1	0	−1	37.2	9.7	85.2
2	0	0	0	44.5	12.8	88.8
3	1	0	1	38.0	10.8	86.1
4	−1	1	0	40.2	11.3	84.8
5	0	0	0	45.0	12.9	88.1
6	0	−1	−1	38.2	12.3	84.4
7	−1	0	−1	39.2	12.0	86.4
8	1	−1	0	37.7	10.1	86.9
9	−1	0	1	38.3	11.1	82.2
10	0	−1	1	36.8	10.7	88.3
11	1	1	0	37.5	10.4	87.2
12	0	1	1	40.6	11.0	86.3
13	0	0	0	44.3	12.4	88.9
14	0	0	0	45.0	12.7	88.8
15	−1	−1	0	36.8	11.2	88.1
16	0	1	−1	39.0	11.8	86.6
17	0	0	0	44.8	12.8	88.9

Independent variables	Levels		
	−1	0	1
X_1, ethanol concentration (%)	60	70	80
X_2, extraction temperature (°C)	60	70	80
X_3, extraction time (min)	60	70	80

Results and discussion

Selection of torrefaction condition and extraction solvent

A solvent system was selected for the purpose of extraction such as preparation or analysis, the nature of the components of interest, the physicochemical properties of the matrix, the availability of reagents, and equipment, cost, and safety concerns. Ethanol and water are considered as preferable solvents regarding safety, human health and handling (Westcott and Muir 1998). In addition, absolute ethanol is known as the most cost-effective solvent for commercial applications (Yu et al. 2002). Therefore, water and ethanol were selected as the extraction solvents in the present study.

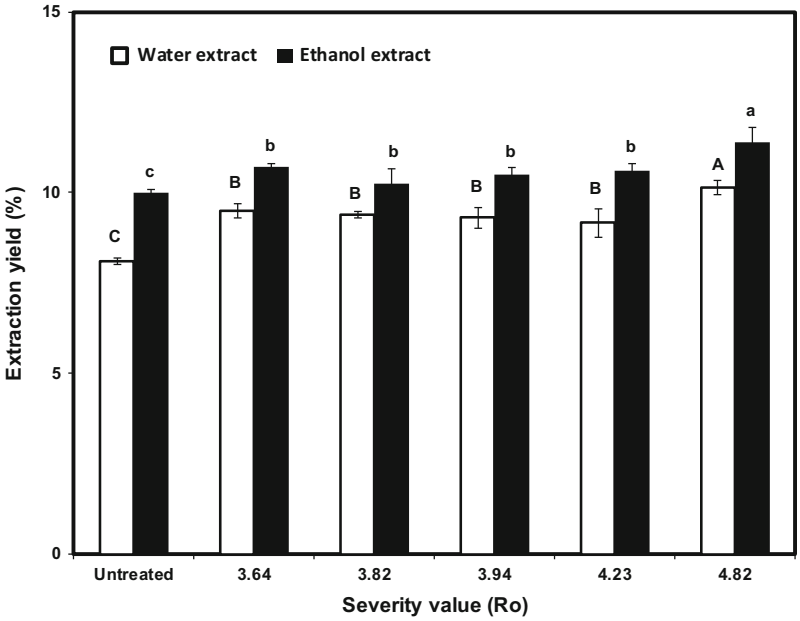


Fig. 1 Effect of torrefaction and extraction solvent on extraction yield. The data are expressed as the mean ± SD ($n = 3$). The significance is expressed in *uppercase letters* (water extract) and in *lowercase letters* (ethanol extract)

The extraction yields of torrefied oak wood in water and ethanol were determined as shown in Fig. 1. The oak wood (untreated) extracted with water and ethanol gave extract yields of 8.1 and 10.0 %, respectively. Torrefaction resulted in a significant increase in the extraction yields for the water and ethanol solvents. The extraction yield in water extract increased from 8.1 % (untreated oak wood) to 10.3 % for the severity (*R*o) 4.82 torrefied oak wood. Similarly, the extraction yield in ethanol extract increased from 10.0 % (untreated oak wood) to 11.4 % at the severity level *R*o = 4.82 (torrefied oak wood). In general, the total amount of extractable material increased after torrefaction at a temperature level of 240 °C. The increase in extracts following torrefaction might be due to degradation of some high molecular weight components and changing these components from non-soluble to soluble ones in the solvents. After torrefaction, the components have changed; wood compounds were either degraded from high molar mass compounds, i.e., hemicelluloses and lignin, or formed by polymerization/cross-linking of low molar mass compounds (Shoulaifar et al. 2014). A variety of antioxidant compounds was obtained by torrefaction, including sugar degradation products (furfural and hydroxymethylfurfural), organic acids (citric and malic acids originating from the cells of the biomass, formic and levulinic acids from sugar degradation products, acetic acid from acetyl groups), extractives and phenolics (Garrote et al. 2004). Ethanol compared to water was more effective in terms of extract yield. Overall, the results showed that ethanol (organic solvent) is an effective solvent for enhancing extract yields in torrefied oak wood.

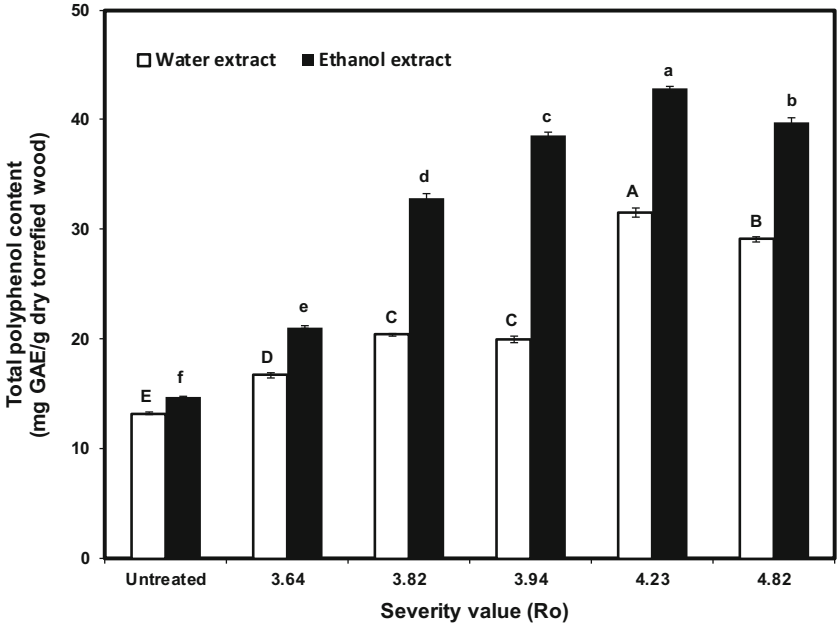
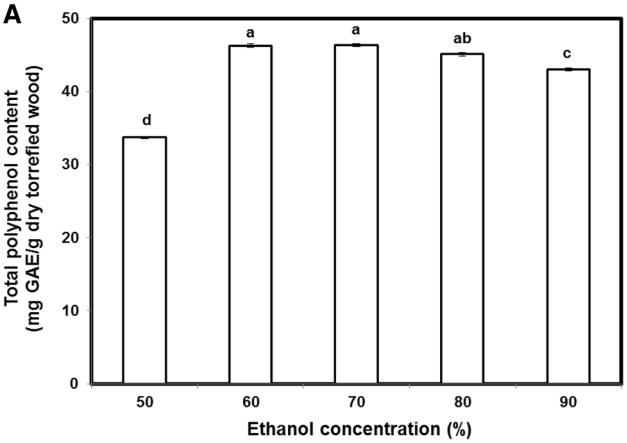


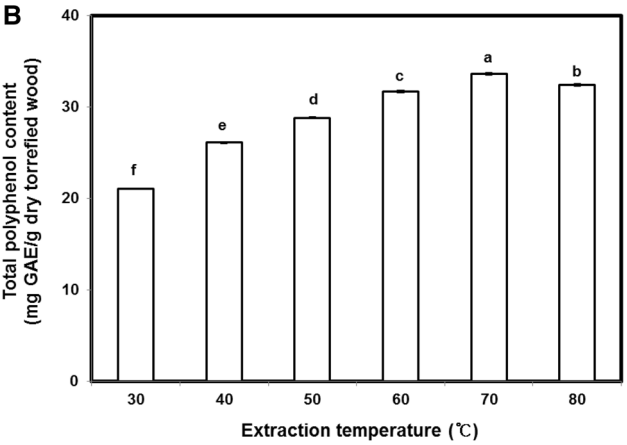
Fig. 2 Effect of torrefaction and extraction solvent on total polyphenol content. The data are expressed as the mean ± SD ($n = 3$). The significance is expressed in *uppercase letters* (water extract) and in *lowercase letters* (ethanol extract)

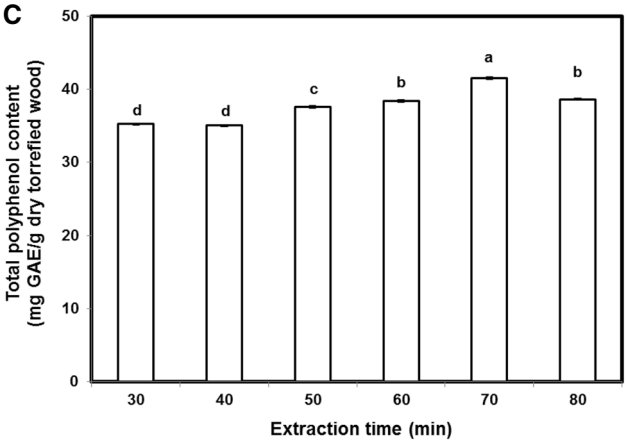
Fig. 3 Effects of ethanol concentration with a 60 min extraction at 60 °C, (**A**) extraction temperature with an extraction time of 60 min and ethanol concentration 70 % (**B**) and time with ethanol concentration 70 % at 70 °C, (**C**) on the total polyphenol of extracts from torrefied oak wood [severity value (*R*o) 4.23]. The data are expressed as the mean ± SD ($n = 3$) ▶

Considering the effect of thermal treatment, previous studies have reported that thermal treatments of lignocellulosic biomass generally result in incremental increase of antioxidant capacity and phenolic content (Shoulaifar et al. 2014; Garrote et al. 2004). To obtain phenolic compounds from lignocellulosic biomass, effective thermal treatment was necessary. Therefore, the effect of torrefaction condition on the total phenolic content of the torrefied oak wood extract was considered as important.

Figure 2 shows the influence of torrefaction conditions in the range of severity (*R*o) 3.64–4.82 on the total polyphenol content of the torrefied oak wood extract. Both water extract and ethanol extract showed a maximum total polyphenol content at a severity level (*R*o) of 4.23 which increased from 13.2 to 31.5 and from 14.7 to 42.8 mg GAE/g dry torrefied wood, respectively. A similar increase in phenolic compounds after torrefaction was reported by Ahajji et al. (2009) and Shoulaifar et al. (2014). The value of phenolic compound at 250 °C was approximately two times higher than the value obtained for untreated beech wood (Ahajji et al. 2009). Similarly, it has been reported that the amount of phenolic compounds in birchwood


A
Total polyphenol content
(mg GAE/g dry torrefied wood)
50
40
30
20
10
0
a
a
ab
c
d
50
60
70
80
90
Ethanol concentration (%)


B
Total polyphenol content
(mg GAE/g dry torrefied wood)
40
30
20
10
0
a
b
c
d
e
f
30
40
50
60
70
80
Extraction temperature (℃)


C
Total polyphenol content
(mg GAE/g dry torrefied wood)
50
40
30
20
10
0
a
b
c
d
d
b
30
40
50
60
70
80
Extraction time (min)

increased gradually with increasing torrefaction temperature from 240 to 255 °C. However, the amount of phenolic compounds decreased at a temperature of 270 °C (Shoulaifar et al. 2014). In addition, Fig. 2 also shows that the total polyphenol content significantly decreased with increasing temperature. Many different extraction solvents have been utilized to extract phenolic compounds from thermally treated lignocellulosic biomass. Garrote et al. (2004) reported that a selective recovery of the phenolic compounds from thermally treated lignocellulosic biomass can be achieved by extraction with solvents such as ethyl acetate or diethyl ether. The extraction of phenolic compounds from a sample is directly related to the compatibility of the compounds with the solvent system according to the ''like dissolves like'' principle.

Selection of experimental ranges for RSM

In a preliminary study, the effects of factors such as ethanol concentration, extraction temperature and time on the extraction of total polyphenol content were investigated to determine the appropriate experimental levels for RSM design. To investigate the effect of ethanol concentration on the extraction of total polyphenol content, a range from 50 to 90 % of ethanol concentration was used for the investigation. As shown in Fig. 3A, the extraction amount of total polyphenol content was greatly influenced by the concentration of ethanol. The total polyphenol content of the extract initially increased with increasing ethanol concentration until reaching a maximum (46.4 mg GAE/g dry torrefied wood) at 70 %, after which the total polyphenol content decreased. The reason might be related to the polarity of ethanol and the solubility of polyphenol in torrefied wood. Ethanol is a low polar solvent, whereas water is a strong polar solvent, and they can be blended with each other in any proportion. With the addition of water to ethanol, the polarity of the complex solvent will increase continuously (Zhang et al. 2007). Therefore, 70 % ethanol was selected as the center point for further RSM experiments.

In the present study, the effect of temperature on the extraction of total polyphenol content was investigated. The statistical analysis showed that significant differences existed among the tested temperatures ($p < 0.05$). As shown in Fig. 3B, the total polyphenol content of the extract increased with increasing temperature up to 70 °C, resulting in a maximum total polyphenol content (33.6 mg GAE/g dry torrefied wood) at 70 °C. This finding can easily be explained considering that the increase in temperature positively affects the polyphenol solubility in the extraction of the total polyphenol content. However, further increases in temperature resulted in a decrease in the total polyphenol content. The results may be explained by the fact that an increase in extraction temperature may be effective for the release of the antioxidant compound from the lignocellulosic biomass. Further, temperatures higher than the optimized level may cause the degradation of some phenolic compounds (Shoulaifar et al. 2014).

Extraction time is also an important factor for extraction of polyphenols from lignocellulosic materials. It is associated with the final concentration of total polyphenol content, the efficiency of extraction, and the energy cost. The effect of extraction time on total polyphenol content was investigated as the final step in a

series of preliminary experiments. It was found that the total polyphenol content in the extract increased when extraction time was increased from 30 min to 70 min (Fig. 3C). Above 70 min, the total polyphenol content decreased, most likely due to the decomposition of phenolic compounds during the prolonged extraction time (Makris et al. 2007). Thus, the RSM center point of extraction time was determined at 70 min. As a result, experimental levels for RSM were selected as ethanol concentration of 60–80 %, extraction temperature of 60–80 °C, and extraction time of 60–80 min.

Optimum ethanol extraction conditions by RSM

In a preliminary study, ethanol extraction was found to be effective in extracting phenolic compounds from torrefied oak wood. The ethanol extraction of phenolic compounds from torrefied oak wood was optimized through the RSM approach. Seventeen experiments were employed to optimize the parameters, including ethanol concentration, extraction temperature, and extraction time, aiming for the highest total polyphenol content, also for total flavonoid content and antioxidant activity evaluated by DPPH assay.

The experimental conditions and corresponding responses of the dependent variables (total polyphenol content, total flavonoid content, and DPPH radical scavenging activity) are listed in Table 1.

Table 2 Regression coefficients and predicted second-order polynomial models for total polyphenol content, total flavonoid content and DPPH radial scavenging activity

Model parameter	Total polyphenol content		Total flavonoid content		DPPH radical scavenging activity	
	Regression coefficient	p value	Regression coefficient	p value	Regression coefficient	p value
Intercept	−452.412500	0.0001**	−68.725000	0.0191	−52.787500	0.4191
Linear		0.0001**		0.0162		0.5108
X_1	5.618750	<.0001***	1.321500	0.0053*	0.888750	0.0292
X_2	4.875000	0.0001**	0.821500	0.0425	0.437500	0.2057
X_3	3.676250	0.0001**	0.266500	0.4483	2.698750	0.0184
Quadratic		0.0001**		0.0005**		0.0076*
X_1^2	−0.038125	<.0001***	−0.012600	0.0003**	−0.0168750	0.0142
X_2^2	−0.033375	<.0001***	−0.007100	0.0076*	−0.0002625	0.6293
X_3^2	−0.032125	0.0001**	−0.005600	0.0223	−0.0020375	0.0058*
Interaction		0.0001**		0.1416		0.0554
X_1X_2	−0.009000	<.0001***	0.000500	0.8066	0.009000	0.1355
X_1X_3	0.004250	0.0041*	0.005000	0.0385	0.012750	0.0482
X_2X_3	0.007500	0.0001**	0.002000	0.3430	−0.010500	0.0898

* Significant ($p < 0.01$)

** Significant ($p < 0.001$)

*** Significant ($p < 0.0001$)

Table 3 Fitted quadratic model in terms of coded variables for Y_1, Y_2, and Y_3 responses

Response	Second-order polynomial equation	R^2	Lack of fit
Total polyphenol content	$Y_1 = -452.412500 + 5.618750X_1 + 4.875000X_2 + 3.676250X_3 - 0.038125X_1^2 - 0.009000X_1X_2 - 0.033375X_2^2 + 0.004250X_1X_3 + 0.007500X_2X_3 - 0.032125X_3^2$	0.9984	1.81^{NS}
Total flavonoid content	$Y_2 = -68.725000 + 1.321500X_1 + 0.821500X_2 + 0.266500X_3 - 0.12600X_1^2 + 0.000500X_1X_2 - 0.007100X_2^2 + 0.005000X_1X_3 + 0.002000X_2X_3 - 0.005600X_3^2$	0.9349	8.42^{NS}
DPPH radical scavenging activity	$Y_3 = -52.787500 + 0.888750X_1 + 0.437500X_2 + 2.698750X_3 - 0.0168750X_1^2 + 0.009000X_1X_2 - 0.0002625X_2^2 + 0.012750X_1X_3 - 0.010500X_2X_3 - 0.0020375X_3^2$	0.8602	21.78^{NS}

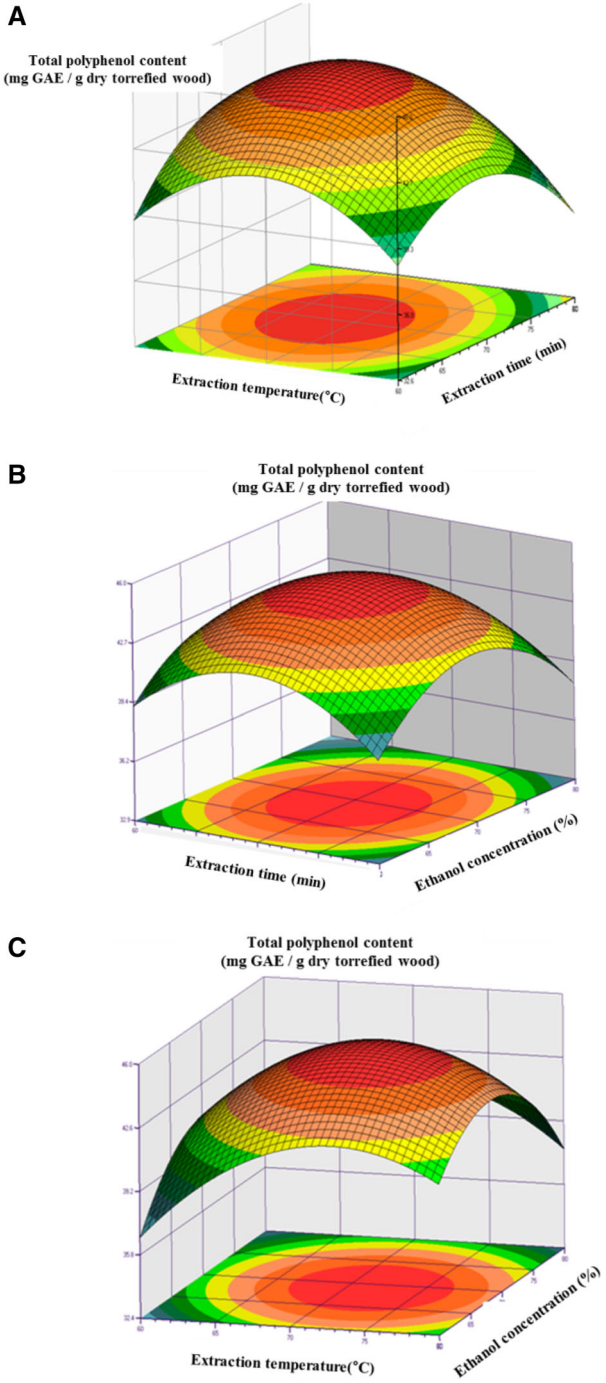
NS Not significant ($p > 0.05$)

Fig. 4 Response surface plots showing the effects of extraction temperature (°C) and time (min) while ethanol concentration was kept at 69.15 % (**a**), ethanol concentration (%) and extraction time (min) while extraction temperature was kept at 71.60 °C (**b**), ethanol concentration (%) and extraction temperature (°C) while extraction time was kept at 70.15 min (**c**), on the total polyphenol (mg GAE/g dry torrefied wood) of extracts with ethanol as solvent ▶

The experimental data for total polyphenol, total flavonoids, and DPPH radical scavenging activity were fitted to a response surface quadratic model, and the acquired equation was tested for adequacy of fit to the data. The regression coefficients and the response surface were used to study the impacts of variables on the extraction of total polyphenol, total flavonoids, and DPPH radical scavenging activity. Regression coefficients of the predicted second-order polynomial models for total polyphenol, total flavonoids, and DPPH radical scavenging activity are shown in Table 2.

In the total polyphenol, the linear ($p < 0.0001$), quadratic ($p < 0.0001$), and interaction ($p < 0.0001$) effects of ethanol concentration, extraction temperature, and extraction time were highly significant, which showed the existence of an optimal value within the experimental area. This suggested that the change in either factor will significantly influence the total polyphenol, as shown in Table 2. However, in the total flavonoids and DPPH radical scavenging activity, only the quadratic ($p < 0.001$) effect of ethanol concentration, extraction temperature, and extraction time was significant. A model was considered significant if its p value (also known as the "Prob > F" value) is lower than 0.01, indicating only a 1 % chance that a "Model F value" could occur because of noise. The "p value" values were also used to evaluate the significance of the effects of each linear, quadratic and interaction term on the response. The results of analysis of variance (ANOVA) showed a significant result for the model.

The results of fitting the second-order polynomial model to the data are presented in Table 3. The following quadratic equation describes the predicted model in the terms of coded values. The R^2 values were 0.9984, 0.9349, and 0.8602 for total


A
Total polyphenol content
(mg GAE / g dry torrefied wood)
Extraction temperature(°C)
Extraction time (min)
B
Total polyphenol content
(mg GAE / g dry torrefied wood)
Extraction time (min)
Ethanol concentration (%)
C
Total polyphenol content
(mg GAE / g dry torrefied wood)
Extraction temperature(°C)
Ethanol concentration (%)

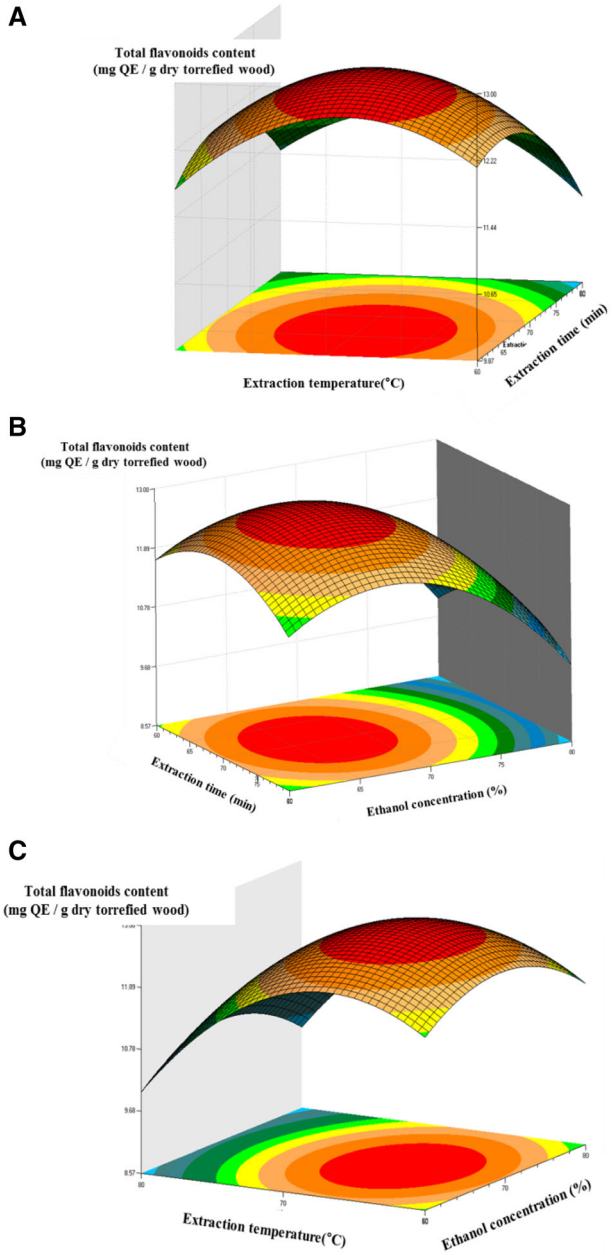


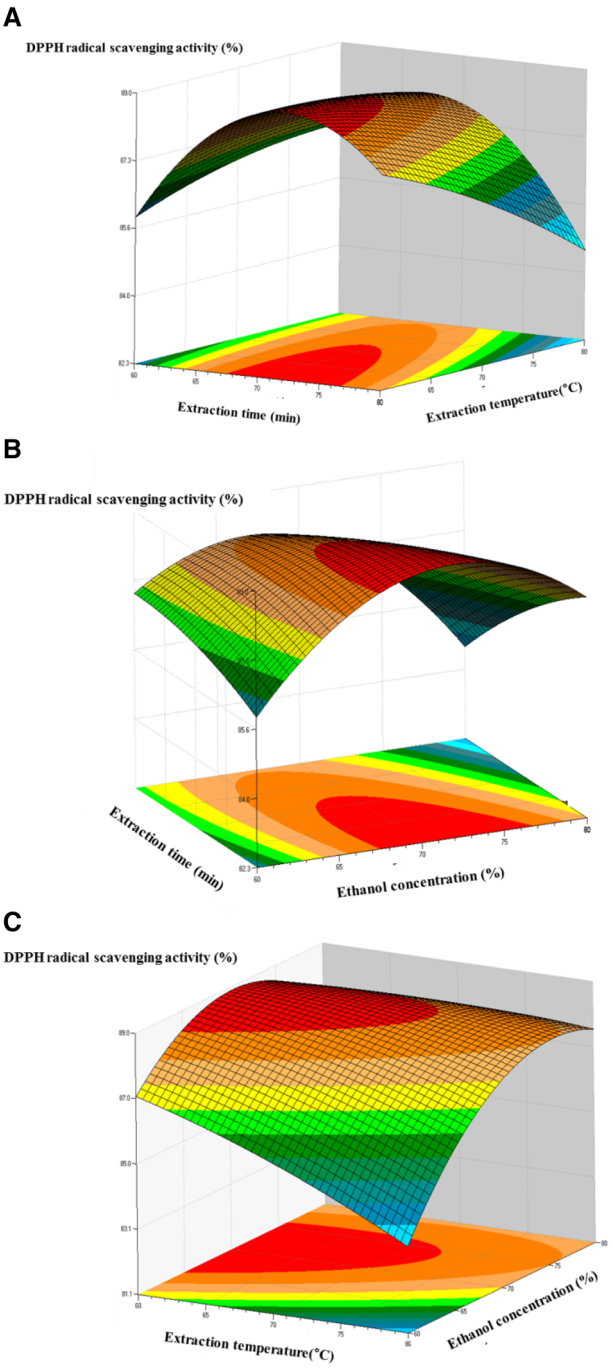
Fig. 5 Response surface plots showing the effects of extraction temperature (°C) and time (min) while ethanol concentration was kept at 66.93 % (**a**), ethanol concentration (%) and extraction time (min) while extraction temperature was kept at 69.52 °C (**b**), ethanol concentration (%) and extraction temperature (°C) while extraction time was kept at 66.09 min (**c**) on the total flavonoids (mg QE/g dry torrefied wood) of extracts with ethanol as solvent

polyphenol, total flavonoids, and DPPH radical scavenging activity, respectively, which means that the most variation (>86 %) of the total polyphenol, total flavonoids, and DPPH radical scavenging activity could be predicted by the models, while only 1, 7, and 14 % variation, respectively, could not be explained by the models. The high coefficients of determination (R^2) illustrated that the model was well adapted to the response (an R^2 value >0.75 indicates a suitable model) (Ven et al. 2002). The lack of fit of the model indicated whether the estimated response surface represents the actual shape of the surface. The lack of fit was not significant ($p > 0.05$) in the models (Halim et al. 2009), meaning that the model was well fit.

The three-dimensional response surface plot simulated by RSREG is the graphical representation of the regression equation. Figures 4, 5, and 6 plot graphically the regression equation for total polyphenol, total flavonoids, and DPPH radical scavenging activity, respectively.

Table 4 presents the optimum conditions for total polyphenol, total flavonoids, and DPPH radical scavenging activity, and their predicted and observed values. The optimization conditions of ethanol extraction for total polyphenol, total flavonoids, and DPPH radical scavenging activity from torrefied wood were 69.15 %, 71.60 °C, and 70.15 min, 66.93 %, 69.52 °C, and 66.09 min, and 68.18 %, 51.77 °C, and 74.22 min, respectively. Upon comparison of the optimal ethanol extraction conditions for total polyphenol, total flavonoids, and DPPH radical scavenging activity, it was found that they were different. Good correlation was found between total polyphenol and total flavonoids (correlation coefficient = 0.82), but no correlation was found between total polyphenol and antioxidant activity (correlation coefficient = 0.55) or total flavonoids and antioxidant activity (correlation coefficient = 0.42, data not shown. This lack of relationship is in agreement with other reports (Ghasemi et al. 2009; Ebrahimzadeh et al. 2014). It is known that only flavonoids with a certain structure, and particularly the hydroxyl position in the molecule, can act as proton donators and show radical scavenging activity (Hou et al. 2003; Mensor et al. 2001). Furthermore, the extracts from thermally treated lignocellulosic biomass are very complex mixtures of many different compounds with distinct activities (Shoulaifar et al. 2014; Gong et al. 2012; Castro et al. 2008; Palmqvist and Hägerdal 2000).

Under these optimal conditions, the model predicted a maximum response of total polyphenol, total flavonoids, and DPPH radical scavenging activity of 45.3 mg GAE/g dry torrefied wood, 12.9 mg QE/g dry torrefied wood, and 89.0 %, respectively. Mean values of 44.8 mg GAE/g dry torrefied wood, 12.3 mg QE/g dry torrefied wood, and 87.8 % of total polyphenol, total flavonoids, and DPPH radical scavenging activity were acquired from real experiments. From Table 4, it can be observed that the difference between the predicted results and the experimental values under the optimal extraction conditions for total polyphenol, total flavonoids, and DPPH radical scavenging activity was small (<10 %). Overall, a close relationship between the experimental values and the predicted values indicates that a satisfactory model was developed. This demonstrates that the response model was adequate to reflect the expected optimization.


A
DPPH radical scavenging activity (%)
Extraction time (min)
Extraction temperature(°C)
B
DPPH radical scavenging activity (%)
Extraction time (min)
Ethanol concentration (%)
C
DPPH radical scavenging activity (%)
Extraction temperature(°C)
Ethanol concentration (%)

◀**Fig. 6** Response surface plots showing the effects of extraction temperature (°C) and time (min) while ethanol concentration was kept at 68.18 % (**a**), ethanol concentration (%) and extraction time (min) while extraction temperature was kept at 51.47 °C (**b**), ethanol concentration (%) and extraction temperature (°C) while extraction time was kept at 74.22 min (**c**) on the DPPH radial scavenging activity (%) of extracts with ethanol as solvent

Table 4 Comparison between the predicted value and observed value for the response variables, ethanol concentration, extraction temperature, and extraction time

Response	Predicted value	Observed value[a]	Difference (%)
Total polyphenol content (mg GAE/g dry torrefied wood)[b]	45.3	44.8 ± 0.3	0.4
Total flavonoid content (mg QE/g dry torrefied wood)[c]	12.9	12.3 ± 0.1	2.4
DPPH radical scavenging activity (%)[d]	89.0	87.8 ± 0.5	1.8

[a] Means of triplicate determination

[b] Optimum conditions: 69.15 %, 71.60 °C, 70.15 min

[c] Optimum conditions: 66.93 %, 69.52 °C, 66.09 min

[d] Optimum conditions: 68.18 %, 51.77 °C, 74.22 min

Conclusion

The experimental design approach succeeded in optimizing the extraction conditions of antioxidant compounds from torrefied oak wood. The conditions for ethanol extraction of torrefied [severity (*R*o) 4.23] oak wood were optimized by using a Box–Behnken design and RSM. The optimal conditions were found to be as follows: (a) total polyphenol content: 69.15 % ethanol concentration, 71.60 °C, 70.15 min; (b) total flavonoid content: 66.93 % ethanol concentration, 69.52 °C, 66.09 min; and (c) DPPH radical scavenging activity: 68.18 % ethanol concentration, 51.77 °C, 74.22 min. Under these conditions, the predicted value of total polyphenol content (45.3 mg GAE/g dry torrefied wood), total flavonoid content (12.9 mg QE/g dry torrefied wood), and DPPH radical scavenging activity (89 %) was close to the observed values (44.8 mg GAE/g dry torrefied wood, 12.3 mg QE/g dry torrefied wood, 87.8 %, respectively). The three variables investigated in the present study could be ranked as follows in terms of influence on the extraction performance: ethanol concentration > extraction temperature > extraction time. Further, no significant correlation was found between antioxidant activity (DPPH) either with total polyphenol content or with the total flavonoid content, suggesting that the structure–function of the phenolic compound has a greater influence on the antioxidant activity than the total polyphenol content and total flavonoid content.

For prospective research, a study should be carried out to determine the main components in the high phenolic content in ethanol extract from torrefied oak wood. Furthermore, isolation and purification of the extracted phenolic compounds from torrefied oak wood to use as nutritional supplements, foods, or cosmetic additives should be investigated.

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