PAPER

EXTRACTION AND PURIFICATION OF FERULIC ACID AS AN ANTIOXIDANT FROM SUGAR BEET PULP BY ALKALINE HYDROLYSIS

A. AARABI[1], M. HONARVAR[1]*, M. MIZANI[1], H. FAGHIHIAN[2] and A. GERAMI[3]
[1]Department of Food Science and Technology, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Chemistry, Shahreza Branch, Islamic Azad University, Shahreza, Iran
[3]Faculty of Management and Accounting, Qazvin Branch, Islamic Azad University, Qazvin, Iran
*Corresponding author: m.honarvar@srbiau.ac.ir

ABSTRACT

Extraction of ferulic acid from sugar beet pulp was carried out using alkaline hydrolysis (NaOH) method and the effects of parameters on extraction were assessed. HPLC method and FT-IR spectrum of precipitate in purification method performed to proved that the isolated compound was ferulic acid. Finally the antioxidant activity of isolated ferulic acid was evaluated by $ABTS^{+\cdot}$ method. Time, temperature and NaOH concentration were the most significant factors influencing ferulic acid extracton and severe alkali concentration has a negative dissociation effect on the ferulic acid content for all temperature/time conditions. The optimal conditions for extraction time, temperature and NaOH concentration were (12 hours, 41°C, and 2 M) respectively. Antioxidant capacity for isolated and pure ferulic acid was 0.39±0.01 and 0.55±0.01 respectively. Its showed that sugar beet pulp is potent source of ferulic acid that can be extracted and use as an antioxidant.

Keywords: antioxidant activity, ferulic acid, HPLC, response surface method, sugar beet pulp

1. INTRODUCTION

Extraction of major phenolic compounds from agricultural crop residues is important for the development of value-added products from renewable by-products. One of the functional compounds that may be extracted from agricultural biomass is ferulic acid (FA), which is the most abundant hydroxycinnamic acid found in plant cell walls that are covalently linked to polysaccharides and lignin (TILAY *et al*., 2008). This phenolic compound is widely used in food, pharmaceutical and cosmetic industries because of different technologically benefitial functions as an antioxidant, anti-microbial and cross-linking agent (GRAF, 1992, MICARD *et al*., 1999; OOSTERVELD *et al*., 2001; ZHAO *et al*., 2008) and also because of its therapeutic effects against cancer, diabetes, cardiovascular diseases (GHATAK *et al*., 2010, DI DOMENICO *et al*., 2009). Ferulic acid has been commercially produced from γ-oryzanol in rice bran oil because of easier process. However, the main part of this valuable phenolic acid is presented in plant cell walls cross linked with polysaccharides which may be released by enzymatic or chemical processes (OU *et al*., 2007).
One of these potential sources is sugar beet pulp (SBP), a main by- product of sugar beet industries. It is a valuable by- product, but at present it is only used as animal feed. JANKOVSKA *et al*. (2001) set-up a method to determine the ferulic acid content of sugar beet pulp by high-pressure liquid chromatography. A few researches have been previously accomplished to extract ferulic acid from sugar beet pulp by alkaline and enzymatic methods (COUTEAU *et al*, 1997; KROON *et al*, 1996; JANKOVSKA *et al*., 2005; DONKOH *et al*., 2012), However, optimization of this process is necessary to understand the interactions between different parameters during extraction, and to minimize the negative effects of chemical processes. The main objective of this research was to compare total phenolic contents of methanol and alkaline sugar beet pulp extracts and optimize alkaline hydrolysis of sugar beet pulp for ferulic acid extraction through response surface methodology via central composite design. Finally antioxidant capacity of isolated ferulic acid from sugar beet pulp extract was evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Sugar beet pulp (SBP) was provided by Isfahan Sugar Factory (Isfahan, Iran). *Trans*-ferulic acid as external standard, ABTS (2,2'-Azino-Bis(3- ethylbenzthiazoline-6-Sulphonic acid)) and trolox was purchaced from Aldrich Chemical Co. (Milwaukes, W1, USA), Sodium Hydroxide, ethanol, methanol, KBr, Gallic acid, ethyl acetate, potassium persulfate and Folin- Ciocalteu reagent were obtained from Merck Chemical Company.

2.2. Instrumentation

Mill type laboratory (Panasonic MX -J120-P made in Japan). HPLC (Agilent Technologies), equipped with a Zorbax C_{18} column (length 150 mm×4.6mm dpi. 5 μm particle size, 300 Å pore size, Agilent Technologies 1200 series, USA) and coupled online with a UV/Vis Agilent Technologies detector. Perkin Elmer spectrum 65 FT-IR spectrometer (USA). Perkin Elmer spectrophotometer, PTFE syring–driven filters (0.22 μm pore size) were provided by Biofil (Germany). Rotary evaporator (Heildolph co., Germany).

2.3. Sample preparation

Sugar beet pulp (SBP) was soaked in water for 3 hours to extract sugar residues and then it was dried in vacuum oven at 40°C for 12 h and ground in a laboratory mill (Panasonic MX -J120-P made in Japan). The powdered sample was passed through a sieve with mesh size 1 mm was taken for further investigations.

3. PREPARATION OF PLANT EXTRACTS

3.1. Extraction with methanol

Methanol was the most commonly used extraction solvent in the assay of phenolic compounds herbs in literatures (DAR *et al*., 2011). In this study, 5 g SBP was mixed in methanol solution (99% v /v) and the extraction of phenolic compounds was performed by reflux for 6 hours at 60°C temperatures. Then, the pH of metalonic extracts was adjusted to 2.0, with HCl 6M for lignin precipitation. The mixture was filtered off, and subsequently the filtrate was centrifuged at 9000 rpm for 2 min. The supernatant was filtered and, evaporated to remove excess methanol followed by using a rotary evaporator (Heildolph Co., Germany) under reduced pressure at 40°C.

3.2. Extraction with sodium hydroxide

In this method, 5 g SBP was placed in an Erlenmeyer flask attached to a condenser, and mixed with a 100 mL NaOH (1M) solution. It was heated up to 60°C for 6 h and then cooled down to 20° C. Once the extraction process was completed, pH was reduced to 2.0, so that the hemicellulose would precipitate. The final mixtures were filtered off, and subsequently 150mL ethyl acetate was added to the filtrates in a 250mL baffled Erlenmeyer flask and was shaken in magnet stirrer (300 rpm) at room temperature for15 min to carry out a liquid-liquid extraction (MAX *et al*., 2009). The supernatant was vacuum evaporated to remove excess solvent. Then the concentrated extract, which contained the phenolic compounds was analyzed for total phenolic compounds.

3.3. Determination of total phenolics content

The total phenolics content of extracts was determined in accordance with a protocol described by TURKMEN and VELIOGLU (2005)[15]. 1 mL aliquot of each sample was mixed with 5 ml of Folin-Ciocalteu reagent (10% in distilled water) in a test tube. After 5 min, 4 ml of sodium carbonate (7.5% in distilled water) were added to each tube, the test tubes were cap-screwed and vortexed. Mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. The absorbance was measured at 765 nm with a UV-vis spectrophotometer. Gallic acid was used as standard and the analyses were done in triplicate. The results were expressed as mg gallic acid (GAE)/g sugar beet pulp extract.

3.4. Experimental design

Response surface methodology (RSM) was used to determine optimum conditions for extraction of ferulic acid from sugar beet pulp. The experiments were designed according to the central composite design (CCD), the most widely used form of RSM. Three factors including time (x_1), temperature (x_2) and sodium hydroxide concentration (x_3) were chosen, and ferulic acid concentration (y) were determined using optimization method (Table 1).

Table 1: Independent variables and their coded and actual values.

Parameters	Symbols	Coded levels of variables				
		-1.6817(-α)	-1	0	1	1.6817(+α)
Time (hour)	X_1	2	4.0	7	9.9	12
Temperature(°C)	X_2	30	36.0	45	53.9	60
Concentration of NaOH(Molar)	X_3	0.5	0.8	1.25	1.69	2

Each factor was studied at five different levels (-1.68, -1, 0, 1, 1.68). All variables were taken at a central coded value considered as zero. Equation (1) represents the three-variable mathematical model:

$$y= \beta_0+\beta_1x_1+\beta_2x_2+\beta_3x_3+\beta_{12}x_1x_2+\beta_{13}x_1x_3+\beta_{23}x_2x_3+\beta_{11}x_1^2+\beta_{22}x_2^2+\beta_{33}x_3^2 \quad \text{(Eq.1)}$$

where *y* is ferulic acid concentration, β_0 is the intercept term, β_1, β_2 and β_3 are the linear terms, β_{11}, β_{22} and β_{33} are the quadratic terms and β_{12}, β_{13} and β_{23} are the interaction terms between three independent variables. The design contains a total of 20 experimental trials.

3.5. Determination of ferulic acid concentration

The prepared methanolic extracts were passed through 0.22 μm PTFE filters and 10 μl of the filterates were injected into a HPLC system, Agilent Technologies, equipped with a Zorbax C_{18} column (length 150 mm×4.6mm dpi. 5 μm particle size, 300 Å pore size, Agilent Technologies 1200 series, USA) and coupled online with a UV/Vis Agilent Technologies detector. The flow rate was 1.0 mL/min and the oven temperature was 35°C. The mobile phase consisted of methanol and water (1% HAc)(65:35, v/v) and the detector was set at 320 nm. All quantitative analyses were made by the external standard method using ferulic acid as an analytical standard.

3.6. Conformity tests to declare the mathematical model validity

Conformity tests were carried out with the same experimental conditions to examine the accuracy of the mathematical model correlations. The error percentage was then calculated according to equation 2 (Madadi *et al*., 2012):

$$Error(\%) = (actual\ values - predicted\ values)/(actual\ values) \quad \text{(Eq.2)}$$

3.7. Ferulic acid purification

Ethanol 95% was added to the brownish extracts to obtain a final concentration of 30% ferulic acid. Then, the murky solution was centrifuged at 11,000 g for 20 min (BURANOV *et al*., 2009). The supernatant was vacuum evaporated to purify ferulic acid. For further purification of the ferulic acid, it was dissolved in a 6 ml anhydrous ethanol, resulting in a less intense murky solution, and again centrifuged for 20 min at 11,000 g. The supernatant was vacuum evaporated and precipitate was analyzed using Fourier transform infra-red (FT-IR) and the methanolic solution of precipitate was injected to high performance liquid chromatography (HPLC) (BURANOV *et al*., 2009).

3.8. FT-IR spectroscopy

Isolated ferulic acid spectra was recorded on a FT-IR spectrometer in the range of 400-4000 cm^{-1} using a KBr disc containing 1% finely ground samples (KUNST *et al*., 2003).

3.9. Measurement of antioxidant capacity of isolated ferulic acid

Antioxidant capacity of isolated ferulic acid from sugar beet pulp and pure ferulic acid were performed immediately by ABTS method. The method used was the ABTS_+ (radical cation) decolourisation assay (Alanon *et al*., 2011). This assay was based on the ability of different substance to scavange ABTS cation radical (ABTS•+(2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)). The radical cation was prepared by mixing 7mM of ABTS stock solution with 2.45mM potassium persulfate(1/1,v/v) and leaving the mixture for 4-16h until the reaction was complete and the absorbance was stable. The ABTS•+ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734nm for measurement. The photometric assay was conducted on 0.9 ml of ABTS•+ solution and 0.1 ml of tested sample and mixed for 45 sec; measurements were tacken immediately at 734nm after 15min. The antioxidant activity of the tested sample was calculated by determining the decrease in absorbance by using the following equation:

$$E= ((Ac-At)/A)\times 100$$

Where At and Ac are the respective absorbance of tested sample and ABTS•+ was expressed as μmol. Trolox chosen as standard antioxidant and the standard reference curve was constructed by plotting % inhibition value against Trolox concentration between 10 and 600 μM. Antioxidant activity measurement, expressed as Trolox equivalent antioxidant capacity (TEAC). Each sample was measured in triplicate. Mean and standard deviation (n = 3) were calculated.

3.10. Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's test was carried out to test for differences between extractants (methanol and alkaline hydrolysis) in the statistical program SPSS ver. 15.0. Significance of differences was defined at the 5% level ($p < 0.05$). The experimental design and statistical analysis were performed using MiniTab software (version 16).

4. RESULTS AND DISCUSSION

4.1 Total phenolic content

Significant differences were found in total phenolic contents between two extracts. alkaline extracts contained higher amounts of polyphenols than methanolic extract. The total phenolics content of methanolic and alkaline extract were 121.45 ± 1.32 and 758.638 ± 3.27 mg GAE/100 g db, respectively. The results showed that alkaline treatment led to retained higher phenolics, which might be due to an alkaline hydrolysis breaks the ester bond linking phenolic acids to the cell wall and thus is an effective way to release phenolic compounds from polysaccharides. It is clear that chemical processes are more efficient to extract phenolic compounds by hydrolyzing the covalent esteric bonds.

In structure of sugar beet pulp cell wall, phenoic compounds such as ferulic and cumaric acids were etherified to lignin and arabinoxylans and forms an alkali-labile cross-link between these two cell wall polymers (TORRE *et al*., 2008). Such relatively higher content of alkali-labile cross-linkages within the lignin network or between lignin and polysaccharides might explain the fast and easy solubilisation of both phenolic acids by alkaline treatments (NOOR HASYIERAH *et al*., 2011). In other researches, alkaline treatments are commonly used to extract bound phenolic acids and other related compounds from cereal grains, and alkaline hydrolysis totally releases the bound phenolics in a short time at high alkali concentration and temperature (OUFNAC *et al*., 2007). Phenolic acids such as benzoic and cinnamic acids could not be effectively extracted with pure organic solvents, so mixtures of alcohol-water or alcohol-alkali are recommended. Our results are in agreement with the published results (STALIKAS, 2007; OUFNAC *et al*., 2007).

4.2. Identification of ferulic acid

The intense peak at Rt= 3.39 min in the chromatogram of alkaline extracts revealed the presence of ferulic acid in the sugar beet pulp extract. (Fig. 1 a and b).

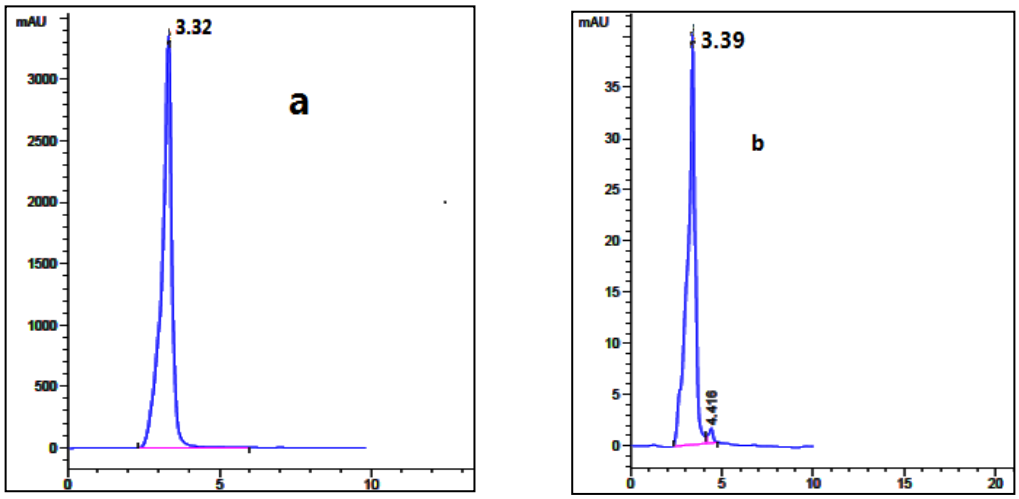

Figure 1: HPLC chromatogram of ferulic acid in C18 reverse phase chromatography at 320 nm.
a) standard, b) alkali treated sugar beet pulp extract (peak of ferulic acid = 3.39 min).

Concentration of ferulic acid were determined according to calibration curve. The concentration of FA in the alkaline hydrolysates obtained in different extraction conditions designed by RSM are presented in Table 2.
Based on the RSM method, result of experimental analysis was presented in Table 3. In these results, those effects with calculated P-values less than 0.05 would be significant in the studied range of parameters.

Table 2: Experimental extraction conditions and ferulic acid concentration.

Run Order	Time (h) X_1	T(°C) X_2	NaOH (M) X_3	Concentration of ferulic acid (g/100g)
1	7.0	45.0	1.25	0.800
2	7.0	45.0	1.25	0.789
3	9.9	53.9	1.69	1.000
4	4.0	53.9	0.80	0.342
5	7.0	45.0	0.50	0.230
6	4.0	53.9	1.69	0.600
7	7.0	45.0	1.25	0.700
8	2.0	45.0	1.25	0.341
9	7.0	60.0	1.25	0.800
10	7.0	45.0	2.00	1.000
11	12.0	45.0	1.25	0.946
12	4.0	36.0	0.80	0.241
13	9.9	53.9	0.80	0.754
14	7.0	45.0	1.25	0.633
15	7.0	45.0	1.25	0.700
16	7.0	45.0	1.25	0.620
17	4.0	36.0	1.69	0.565
18	7.0	30.0	1.25	0.476
19	9.9	36.0	0.80	0.415
20	9.9	36.0	1.69	1.100

Based on the results shown in Table 3, the extraction of ferulic acid was significantly affected by time (x_1), temperature (x_2), NaOH concentration (x_3) and the coupling terms between x_2 and x_3, while the interaction between terms x_1x^2 and x_1x_3, and the second order effect of term x_1,x_2 and x_3 on FA concentration were insignificant ($P > 0.05$).
Therefore, after eliminating the statistically insignificant values, the final model is given in Eq. (3) as follows:

$$y = -2.10380 + 0.035\, x_1 + 0.051 x_2 + 1.355\, x_3 - 0.0158 x_2 x_3 \qquad \text{(Eq.3)}$$

The positive and negative signs in front of the terms indicate the synergistic and antagonistic effect of each term on the model response, i.e. FA concentration. The coefficient of determination (R^2) for empirical equation from Eq. (3) was 0.952.

Table 3: ANOVA analysis for Central Composite Design.

Source	DF	Seq SS	Adj SS	Adj MS	F	P	
Linear	3	1.11115	1.11115	0.370385	62.14	0.000	
A -Time	1	0.47184	0.47184	0.471844	79.16	0.000	Sign ≤0.05
B-Temp	1	0.06196	0.06196	0.061963	10.40	0.009	Sign ≤0.05
C-Concentration	1	0.57735	0.57735	0.577348	96.86	0.000	Sign ≤0.05
time*time	1	0.00444	0.00733	0.007332	1.23	0.293	
temp*temp	1	0.00658	0.00865	0.008651	1.45	0.256	
con*con	1	0.01535	0.01535	0.015347	2.57	0.140	
Interaction							
time*temp	1	0.00133	0.00133	0.001326	0.22	0.647	
time*con	1	0.01523	0.01523	0.015225	2.55	0.141	
temp*con	1	0.03188	0.03188	0.031878	5.35	0.043	Sign ≤0.05
Lack-of-Fit	5	0.03109	0.03109	0.006218	1.09	0.463	
Pure Error	5	0.02852	0.02852	0.005703			

R^2 = 95.21 & α= 0.05 - Significant parameters (P-value < 0.05).

In order to determine the effect of the three independent variables on extraction yield, extraction parameter graph and response surface were generated as a function of two variables, while the third one was held constant at its middle level. The optimum region was determined in terms of maximum concentration of ferulic acid (Fig. 2).
To assess the quality of the model and to measure how well the suggested model fit the experimental data, the parameters, F-value, lack of fit and R^2 were used (Montgomery, 2005). In our research F-values were 82.85 and lack of fit of the model was not significant, which implied that the models were significant. The determination coefficients (R^2>0.95) were obtained from ANOVA of the quadratic regression models, indicating that less than 4.8% of the total variations was not explained by the suggested model.

4.3. Effect of the each process parameter on ferulic acid extraction

4.3.1. Temperature

As shown in Fig. 3, increasing the extraction reaction temperature may cause higher concentration of ferulic acid released, while the other two factors (i.e. time & akali concentration) were constant. It is well known that sugar beet root contains significant quantities of ferulic acid which is etherified to lignin and /or arabinoxylans and through alkali-labile cross-linkages (SCALBERT, 1986). Therefore, cleavage of these bonds at the same time may be possible at higher temperatures. An increase in temperature from 30 to 60°C and time from 2 to 12 hr resulted in an increment of FA from 0.3 to 0.9g/100g when alkali concentration was fixed at the middle level (Fig. 2a).

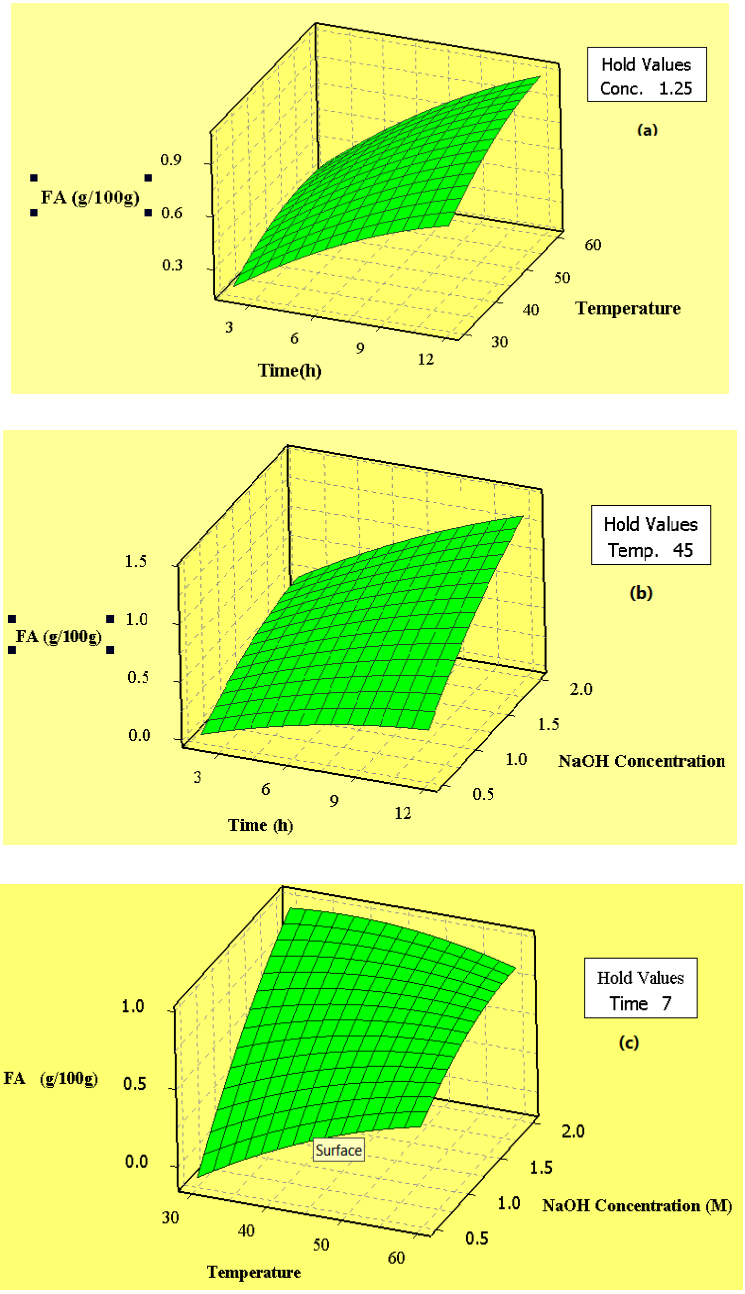

Figure 2: 3D response surface plot of the interactions. (a) time and concentration when temperature was fixed at 45°C, (b) temperature and time when concentration was fixed at 1.25 M, (c) concentration and temperature when time was fixed at 7 h

XU investigations (2005) showed that an increase in alkaline treatment temperature had an important effect on the release of ester-linked p-coumaric and ferulic acids from the cell walls of various cereal straws.

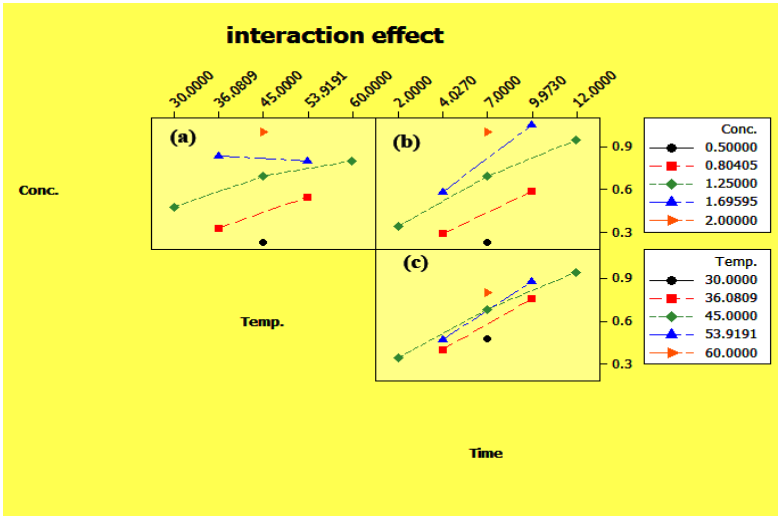

Figure 3: Interactions between extraction parameters NaOH concentration and temperature(a), NaOH concentration and time(b), time and temperature (c).

4.3.2. NaOH concentration

As presented in Fig. 2b, the ferulic acid concentration significantly increased with NaOH concentration. This is because raising the alkaline treatments may dissolve lignin by cleavage of ester linkages in lignin–polysaccharide complexes, which lead to the release and solubilization phenolic acids. Different alkaline compounds such as NaOH, $Ca(OH)_2$, NH_4OH, and H_2O_2 have been previously utilized as catalysts for hydrolysis treatments (RODRIGUEZ-VAZQUE *et al*., 1994). In the present study, NaOH was selected because of its more selective action, compared to the other agents, in releasing phenolic compounds such as ferulic acids (TORRE *et al*., 2008). It may be obvious that using a too mild alkaline condition may not act effectively for ferulic acid extraction.These results have also been confirmed in previous studies (MUSSATTO *et al*., 2007; TORRE *et al*., 2008).
The ffect of time in alkaline hydrolysis on the extracted FA concentration is shown in Fig. 2b. By increasing reaction at constant temperature, alkali has more time to release ferulic acid. A positive interaction between the time and NaOH concentration and the time and temperature process on the extracted ferulic acid has been observed (Figs. 3 b and c), However for higher concentrations of NaOH (1.69M), the slope of graph was increased as compared to the low concentrations (0.8 M). At higher concentration of NaOH, accelerated solubilisation of ferulic acid has been observed. It may be emphasized that ferulic acid solubilization exhibited a time- dependent behaviour and reached a maximum concentration after 12 h of hydrolysis with 2.0N NaOH (Fig. 3 b).
But the main point that should be considered is that severe alkali concentration has a negative dissociation effect on the FA for all temperature / time conditions (Fig. 2c and Figs. 3 a and b).
According to the obtained results, the main key factors affecting the releasing rate of phenolic compounds are alkali concentration and hydrolysis duration. The same result has previously been reported for the effect of these factors on the yield of extraction from the other sources such as paddy straw, sugar cane baggas and agricultural wastes (NOOR HASYIERAH *et al*., 2011; XU *et al*., 2005; TILAY *et al*., 2008).

According to the results in Table 3, interaction between temperature and NaOH concentration (x_2x_3) had a statistically significant effect on the extraction yield while the others (i.e: x_1x_3 and x_1x_2) were insignificant. Increasing the temperature from 36°C to 53°C at a constant time (7 h) and low concentration of alkali (0.8 M) improved the ferulic acid extraction (from 0.32 to 0.54 g/100g FA), but at the same temperature range, using high concentration of NaOH caused a reduction of FA concentration (Fig. 3a). This is because of the opposite effect of temperature and concentration of NaOH on FA extraction, which is shown in Eq. (3) by negative sign of the term, x_2x_3.

A clear decrease in ferulic acid concentration took place after the threshold was exceeded and confirmed this negative interaction, so that when temperature was increased from 45 to 60 °C and alkali concentration was raised from 0.5 to 2.0M, an oxidative degradation might happen (Fig. 2c). No significant degradation was detected at the lowest alkali level (0.5M). It has also been previously shown that ferulic acid, in its monomeric form, is more resistant to oxidation than its dimeric form during alkaline hydrolysis (BAUER *et al*., 2012).

Finally, it may be emphasized that the amount of ferulic acid obtained through CCD in the present research was 1.29 g/100 g, which is higher than the maximum amount reported by ZHAO (2008) in sugar beet pulp (0.800 g/100 g); and this improvement may be mainly due to the optimum condition selected for the extraction process.

Normal probability plot of residuals produced an approximately straight line which indicated a normal distribution of residuals (the deviation between predicted and actual values) and confirmed the accuracy of the model (NOOR HASYIERAH *et al.,* 2011).

4.4. Validating the optimal conditions

The optimum conditions, as suggested by the software, were 41°C, 2 M NaOH, 12 h corresponding to 1.33 g/100 g (predicted value). For verification of these conditions, four replicates were carried out to determine the highest experimental value of ferulic acid. The highest ferulic acid production obtained at these conditions was 1.29 g/100 g.

The results of the conformity test on the basis of determining the error percentage demonstrated that process optimization in CCD reliably predicted the FA with acceptable accuracy(3.1%).

4.5. FT-IR spectra and validation of precipitate

In the purification stage, the pericipiate that obtained from alkaline extract of 5 g sugar beet pulp was analysed by FT-IR and HPLC method. The FT-IR spectrum of the precipitate was compared with the spectrum of the pure ferulic acid to confirm it (Fig. 4).

The FT-IR spectrum of sample clearly showed the existence of main functional groups in the ferulic acid structure, and the strong and broad band at 3,331.08cm $^{-1}$ is characteristic of the OH group in phenolic compound. A part from this, C-H stretching of the aromatic ring was at the 2925.82 cm $^{-1}$ band. The band at 1,649.02 cm^{-1} corresponds to that of the carbonyl group (C = O). Stretching band at 1,328.87 cm $^{-1}$ is characteristic of C-H vibration on the methyl group, While the vibration for C = C on the aromatic ring is at 692.77 cm^{-1} band (ROBERT, 1998).

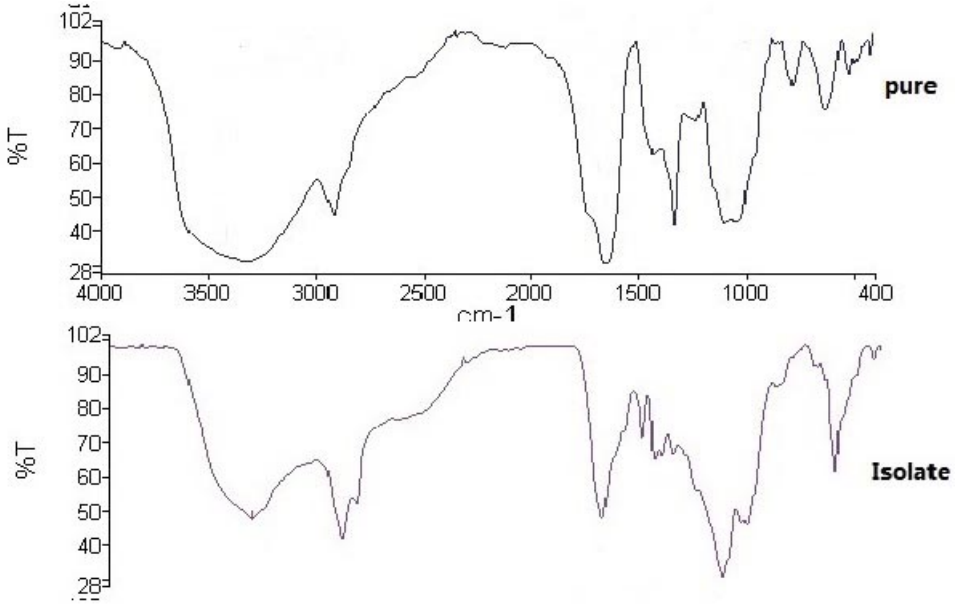

Figure 4: FT-IR spectra of ferulic acid (isolate and pure).

The precipitate was then analyzed using high performance liquid chromatography (HPLC) to reaffirm the results as characterized by the IR analysis. 0.15 mg of precipitate was collected and dissolved in 10 ml of methanol solution acting as the solvent before the analysis. Finally, the 10 μl of concentrated sample was injected into a HPLC machine. The validation was performed with sample of precipitate based on the relative retention times (RRTs) and relative peak areas (RPAs). The percent of recovery was 64.88% and precision was assessed by analyzing three replicate samples and the relative standard deviation (RSD) was below 2.02% and 4.47% for RRTs and RPAs, respectively.

4.6. Measurement of antioxidant capacity of isolated ferulic acid

Antioxidant capacity results expressed as μmol of Trolox equivalents per milligram of samples. The ABTS cation radical (ABTS•+) (Pisoschi and Negulescu2011) which absorbs at 734 nm (giving a bluish-green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2'-azino-bis(3- ethylbenzthiazoline-6-sulphonic acid)). In the presence of Trolox (or of another hydrogen donating antioxidant), the nitrogen atom quenches the hydrogen atom, yielding the solution decolorization. Antioxidant capacity for isolated and pure ferulic acid was 0.39±0.01 and 0.55±0.01 respectively. Significant differences were found at a significance level of $p < 0.05$ between isolated and pure samples in the antioxidant capacity values which indicates that precipitates need more purification stage to made pure. Result showed that sugar beet pulp is potent source of ferulic acid that can be extracted and use as an antioxidant.

5. CONCLUSIONS

The present study demonstrated that alkaline treatment led to release higher phenolic compounds than methanolic method and the results showed that temperature, time and NaOH concentration had significant effects on the ferulic acid solubilization in alkaline media for extraction. The coefficient of determinations (R^2) for predicted ferulic acid

content showed good correlation with the experimental data at 95% confidence level. The amount of extracted ferulic acid at optimized conditions obtained from the model (i.e: 12 h, 41°C and 2 M) was 1.29 g/100 g. The FT-IR spectrum of isolated sediment clearly showed the existence of main functional groups in the ferulic acid structure. Significant differences were found at a significance level of $p < 0.05$ between isolated and pure ferulic acid in the antioxidant capacity values which indicates that precipitates need more purification stage to made pure. In conclusion suggest that sugar beet pulp is potent source of ferulic acid that can be extracted and use as an antioxidant.

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