Industrial Crops and Products 77 (2015) 123–132

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Optimization of the recovery of phenolic compounds from Algerian grape by-products

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ARTICLE INFO

Article history:
Received 28 April 2015
Received in revised form 17 August 2015
Accepted 18 August 2015

Keywords:
Grape by-products
Phenolics
Extraction methods
Optimization
Response surface methodology

ABSTRACT

Grape seeds and skin are by-products of wine making or juice making operations which are considered as a good source of bioactive compounds. In this study, the total phenolic content (TPC) from *Vitis vinifera* L. cv. *Ahmar Bou-Amar* seeds and skin was optimized by conventional solvent extraction (CSE) and microwave-assisted extraction (MAE) using response surface methodology (RSM), then a comparative study was carried out. The optimal conditions for seeds extracted by CSE were: 74.33% v/v of ethanol concentration, 65.23 min extraction time, 0.1 g/70.86 ml solid/liquid ratio, and for those extracted by MAE were: 59.88 s irradiation time and 373.15 W microwave power. The extract obtained under these conditions showed a TPC of 96.56 ± 1.29 mg GAE/g and 73.15 ± 0.20 mg GAE/g DW for CSE and MAE, respectively. Concerning skin, the optimal conditions for CSE were: 51.46% v/v of acetone concentration, 89.80 min extraction time, 0.1 g/32.25 ml solid/liquid ratio and for MAE were: 113.74 s irradiation time and 384.44 W microwave power. The extract obtained under these conditions showed a TPC of 39.57 ± 0.23 mg GAE/g and 54.84 ± 0.41 mg GAE/g DW for CSE and MAE, respectively. The TPC of seeds extract obtained with MAE was 24% lower than that of the CSE extract; also, the antioxidant activity of CSE extract is better than that of MAE extract. While, the TPC of skin extract obtained with MAE was 28% higher than that of the CSE extract and the antioxidant capacity was significantly higher than that of the CSE extract. The results indicate that the extracts of cv. *Ahmar Bou-Amar* seeds and skin contain a high quantity of polyphenols; therefore, they can be considered as a good source of natural antioxidants.

1. Introduction

Grapes (*Vitis vinifera* L.), are the world's largest fruit crop (Ghafoor et al., 2010; Yang et al., 2009), with a total production of approximately 75.1 million tons in 2013 (O.I.V., 2015). Although, a high part of grapes is made into wine, another part is dried into raisins, a significant part is consumed as table grapes (Nelson, 1979). The cv. *Ahmar Bou-Amar* grape is originated in Algeria, it is widespread in the Kabily region with a low commercial value, and is generally consumed as a fresh fruit.

Vitis vinifera L. contains large amounts of phytochemicals such as phenolic compounds (Yang et al., 2009) which offer health benefits via their important antioxidant activity (Andjelkovic et al., 2013). The grape polyphenolic compounds are found essentially in seeds and skins with approximately 75% (Ghafoor et al., 2010); these levels are influenced by the grape variety and environmental factors (Cadot et al., 2008; Cheynier et al., 1998; Katalinic et al., 2010).

Grape seeds and skins which are a low-value by-products of wine making or juice making operations are considered to be a cheap and a good source of the high-quality phenolic compounds (Bucić-Kojić et al., 2007; Spigno et al., 2007), so they can be exploited as natural antioxidant agents to neutralize free radicals in biological systems (Bucić-Kojić et al., 2007; Ghafoor et al., 2010).

Extraction is a critical step in the isolation of active compounds from plant material (Afoakwah et al., 2012; Dragović-Uzelac et al., 2012). Different extraction methods have been investigated in this regard; conventional extraction methods are generally based on choosing the accurate solvent in order to enhance the solubility of active compounds (Dragović-Uzelac et al., 2012; Ravalji et al.,

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http://dx.doi.org/10.1016/j.indcrop.2015.08.039
0926-6690/

2012). These methods necessitate longer extraction time which produces a thermal deterioration for the majority of the active compounds (Wong-Paz et al., 2014). Recently, environmental friendly techniques are being interested to develop the "Green Chemistry" concept such as ultrasound-assisted extraction (Bimakr et al., 2013) and microwave-assisted extraction (MAE) (Afoakwah et al., 2012; Rafiee et al., 2011; Wong-Paz et al., 2014; Zheng et al., 2011).

Microwave-assisted extraction is considered as a novel extraction method that combines microwave and conventional solvent extraction (Lopez-Avlia and Young, 1994). It is a technique used to extract active compounds from a range of raw materials using the energy of microwave radiation to heighten the temperature of the solvents successfully and rapidly (Afoakwah et al., 2012; Zheng et al., 2011). MAE is also able to decrease jointly the solvent consumption and extraction time in comparison to conventional techniques. Moreover, it has the possibility to improve extraction quality (Hithamani and Ramalakshmi, 2013; Ravalji et al., 2012; Wong-Paz et al., 2014).

Considering the variability of the structures of phenolic compounds present in different parts of grape (Rivera-Dominguez et al., 2010), it is important to develop an optimal extraction method for each matrix. As the extraction methods are affected by different parameters as well as the interaction of all these factors, the use of an optimization modeling is necessary to facilitate the determination of the optimum extraction conditions. Response surface methodology (RSM) is a mathematical technique which is able to generate statistical models and take into consideration the possible interrelationships between the different test parameters while reducing the number of essays and allowing for considerable reduction of operation cost and time (Dahmoune et al., 2014).

Many authors investigated Conventional Solvent Extraction procedure (CSE) of phenolic compounds and their properties from grape seeds and skins and from other plant materials (Bordiga et al., 2011; Katalinic et al., 2010; Pinelo et al., 2005a). However, limited information has been published on the use of microwave for the extraction of total phenolic compounds from grape seeds and skins. Moreover, there is a little published information about optimization modeling and comparison of CSE and MAE extraction process from grape by-products. Finally, to the best of our knowledge, no literature report exists on the extraction of polyphenols from grape cv. *Ahmar Bou-Amar*. Therefore, there is a need to develop an optimal method for the extraction of phenolic compounds from the by-products of this variety. Thus, the objectives of the current study are to: (1) optimize the phenolic compounds extraction from seeds and skin of grape *Vitis vinifera* cv. *Ahmar Bou-Amar* by CSE and MAE, studying the effects of different parameters on the extraction efficiency, using Box-Behnken Design (BBD) and Central Composite Design (CCD) of RSM. (2) Compare the optimized CSE with MAE process based on the total phenolic content (TPC) and the antioxidant activity.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteau's phenol reagent, sodium carbonate (Na_2Co_3), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4) and all solvents used were obtained from Prolabo (made in CE). Potassium persulfate ($K_2S_2O_8$), trifluoroacetic Acid ($C_2HF_30_2$) and ABTS: 2,2-azinobis-3-ethylbenzothiazoline-b6-sulfonic acid ($C1_8H_{18}NaO_6S_4$) were purchased from Sigma Aldrich (Germany). Potassium ferricyanide ($C_6N_6FeK_3$), ferric chloride ($FeCl_3{\cdot}6H_2O$), trichloroacetic acid, gallic acid and quercetin from Biochem-chemopharma (UK).

2.2. Biological material

The fruit samples of *Vitis vinifera* cv. *Ahmar Bou-Amar* were collected in the area of Amizour (Bejaia, Algeria). Grape seeds and skin were manually separated from the pulp and dried at 40 °C in a hot air oven. The dried samples were milled with an electrical grinder, the powder was sifted by a standard sieve and just the portion with particle size < 500 μm was used.

2.3. Effect of solvent nature

In a first stage, the effect of solvent nature was studied: four solvents were used: 50% (v/v) methanol, 50% (v/v) ethanol, 50% (v/v) acetone and water. The extraction was carried out with a CSE by fixing extraction time (30 min) and sample/solvent ratio (100 mg/10 ml) at ambient temperature, after that, each sample was centrifuged at 10 000 rpm, then filtered through Whatman $N^0$1 paper by Büchner funnel. The supernatant was used in order to determine total phenolic compounds (TPC).

2.4. Preparation of extracts according to the experimental design

2.4.1. CSE process

Samples were extracted using the best solvent selected in the first step. In order to predict optimal extraction conditions and to determine the best combinations of three independent parameters for CSE, namely: solvent concentration, extraction time and solid/liquid ratio, a 3^3 factorial experimental design through Box–Behnken with RSM was used on the recovery of total phenolic compounds. The different levels for each independent parameter were chosen from a series of preliminary experiments without using mathematical models. The levels of independent variables were reported in Table 1.

50 mg of seeds and 100 mg of skin powders were extracted with different volumes of appropriate solvent, different extraction times and solid/liquid ratio as showed in Table 2. After extraction, each sample was centrifuged at 10,000 rpm, then filtered through Whatman No1 paper by Büchner funnel. The extracts obtained under the optimum conditions by RSM, were concentrated under vacuum using a rotary evaporator and recuperated in 55% ethanol acidified with 0.005% of trifluoroacetic acid (TFA) and stored at 0 °C.

2.4.2. MAE process

Using the best solvent, solvent concentration and sample/solvent ratio fixed at optimum conditions of CSE process, the samples were extracted with MAE, the independent parameters of extraction time and microwave power were analyzed using a central composite design to assess their effects and interactions on the extraction of phenolics compounds. A RSM was used to optimize the extraction process. The different levels for each independent parameter were chosen from a series of preliminary experiments without using mathematical models. The levels of independent variables were reported in Table 1.

The microwave apparatus used in this study is a domestic one (NN-S674MF, Samsung, Malaysia), it was modified in order to condensate the vapors generated during extraction process giving a stable extract volume. Concerning the extraction, 50 mg of seeds and 100 mg of skin powders were placed in a 250 ml volumetric flask containing the extraction solvent. The samples were extracted at different irradiation times and microwave powers (Table 2). After extraction, each sample was centrifuged at 10,000 rpm, then filtered through Whatman No1 paper by Büchner funnel. The extracts obtained under the optimum conditions by RSM, were concentrated under vacuum using a rotary evaporator and recuperated in 55% ethanol acidified with 0.005% of trifluoroacetic acid (TFA) and stored at 0 °C.

Table 1
Levels of different independent variables in RSM for the optimization of extracts by CSE and MAE.

Extraction process	Independent variables	Levels					
		Seeds			Skin		
		(-1)	(0)	(+1)	(-1)	(0)	(+1)
CSE	X_1-solvent concentration	50	65	80	40	55	70
	(% v/v)	30	60	90	60	90	120
	X_2-time (min)	1	1.75	2.5	2.5	3.75	5
	X_3–ratio (mg/ml)						
MAE	X_1-Power (W)	200	400	600	200	400	600
	X_2-time (S)	30	75	120	60	120	180

Table 2
Preparation conditons of extracts according to the experimental design with RMS by CSE and MAE.

Essay	CSE Factors			Essay	MAE factors	
	X_1-solvent concentration (% v/v)	X_2-time (min)	X_3–ratio (mg/ml)		X_1- time (s)	X_2-power (W)
1	−1	+1	0	1	0	0
2	+1	0	−1	2	0	0
3	−1	0	−1	3	−1	0
4	0	−1	−1	4	91	−1
5	0	0	0	5	+1	−1
6	−1	0	+1	6	0	+1
7	0	+1	+1	7	−1	+1
8	+1	−1	0	8	0	0
9	+1	0	+1	9	0	−1
10	0	−1	+1	10	+1	0
11	0	+1	−1	11	+1	+1
12	−1	−1	0			
13	+1	+1	0			
14	0	0	0			
15	0	0	0			

To obtain the optimum operating conditions and to explain the performance of the method, a second-order model was used according to the following equation:

$$Y = \beta o + S\beta i Xi + S\beta ii Xi^2 + S\beta ij XiXj \quad (1)$$

Where Y is the predicted response (TPC mg GAE/g DW), βo, βi, βii, and βij are regression coefficients for intercept, linear, quadratic and interaction terms, respectively and Xi and Xj are the actual levels of the independent variables.

2.5. Analysis of total phenolic compounds

Total phenolic content was measured according to the method described by Velioglu et al. (1998). 200 μl of the extracts were mixed with 1500 μl of Folin–Ciocalteau reagent (diluted tenfold). After 3 min, 1500 μl of 6% sodium carbonate were added. The test tube contents were mixed and preserved for 1 h at room temperature in the dark. Then, the absorbance was measured at 765 nm versus a blank prepared without extract. Gallic acid was used as a standard and the results were expressed as milligram gallic acid equivalents per gram of dry weight (GAE/g of DW).

2.6. Evaluation of the antioxidant activity

2.6.1. $ABTS^+$ method

The $ABTS^+$ radical scavenging activity of extracts was determined according to the method of Awika et al. (2003). The $ABTS^+$ radical was prepared by combining a volume of 8 mM $ABTS^+$ with the same volume of 3 mM potassium persulfate and incubating for 16 h in the dark at room temperature. The working solution was obtained by diluting with 50% methanol to an absorbance of 0.70 ± 0.02 at 734 nm. The results were expressed as Quercetin mg equivalents per gram of dry weight (mg QE/g DW). The percentage inhibition was calculated according to the following formula:

$$\%\text{Inhibition} = [(A\text{control} - A\text{sample})/\text{control}] \times 100 \quad (2)$$

Where A is the absorbance of the control or of the sample.

2.6.2. Ferric reducing power (FRP)

The reducing power of the extracts was evaluated using the method described by Oyaizu (1986); 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% potassium ferricyanide were added to 1 ml of the sample. The mixture was incubated at 50 °C for 20 min. After that, 1 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 1700 g. Then 0.2 ml of 0.1% ferric chloride was added to the mixture of 1 ml of supernatant and 1 ml of distilled water. The absorbance was measured at 700 nm. Quercetin was used as a standard and reducing power was expressed as quercetin mg equivalents per gram of dry weight (mg QE/g DW).

2.7. Statistical analysis

All the essays were repeated tree times and the results were expressed as means ± standard deviation (SD). Statistical analysis was done with JMP software (Version 7.0, SAS) to find the response surfaces and contour plots of the response model. Data were analyzed by analysis of variance (ANOVA) and the fitting of the mathematical models was determined by evaluating the lack of fit and coefficient of determination (R^2). While the effect of type solvent on the TPC in the first stage and the results of comparison between CSE and MAE (ABTS, FRP and TPC) were statistically assessed by ANOVA using STATISTICA 5.5 to find significant differences among the different results. The statistical significance of each result was determined at 5% probability level ($p < 0.05$).

3. Results and discussion

3.1. Effects of the solvent on the total phenolic content

Preliminary experiments were conducted to determine optimum extraction solvent for each matrix. The efficiency of ethanol, methanol, acetone and water on the extraction of the total phenolic content from grape seeds and skin, was compared. Significant differences ($p < 0.05$) solvent extraction for skin samples as shown in Table 3, the higher content of TPC was detected in acetone extract (27.90 ± 0.39 mg GAE/g DW) followed by the ethanol, methanol and water extracts. While for seeds, the higher content of TPC was detected in ethanol extract (86.51 ± 5.36 mg GAE/g DW) followed by the methanol, water then acetone extracts. Therefore, in the following study, acetone was chosen to be the best solvent extraction for skin matrix and ethanol was chosen to be the best solvent extraction for seeds. These results are in agreement with the previous published results. Indeed, aqueous ethanol was used by several authors for the extraction of phenolic compounds from grape, grape seeds, skins and/or pomace (Bucić-Kojić et al., 2007; Carrera et al., 2012; Katalinic et al., 2010; Pourali et al., 2014; Spigno et al., 2007) and Yilmaz and Toledo (2006) have used aqueous acetone. Kennedy et al. (2002) extracted the phenolic compounds from grape skins with 66% aqueous acetone. Cadot et al. (2011, 2006) used different solvents for skins and seeds: acetone/methanol/H_2O/TFA (50:15:35:0.05) for skins and acetone/H_20 (60:40) for seeds.

3.2. Optimization of the CSE conditions

The experimental design and corresponding responses for the obtaining of grape cv. *Ahmar Bou-Amar* seeds and skin extracts are presented in Table 4. Model presented the total of 15 experiments. Tree replications at the central points were included in order to evaluate experimental error measurement (Goupy and Creighton, 2006).

Following the results obtained on different solvents extraction, seeds and skins were extracted with the selected appropriate solvent (ethanol and acetone for seeds and skins respectively). The influence of the variables: solvent concentrations, solid/liquid ratio, and extraction time on the extraction of TPC was investigated through a 3^3 factorial experimental design with response surface methodology (RSM) and Box–Behnken design. The experimental and predicted values of TPC at various experimental conditions are given in Table 4

As shown, TPC of seeds ranged from 87.99 to 95.97 mg GAE/g DW, while the best result was obtained when solvent concentration, extracting time and solid/solvent ratio were 65% (v/v), 60 min and 1.75 (m/v), respectively. On the other hand, TPC for skin range from 33.19 to 39.14 mg GAE /g DW, where the central point presents the best extraction conditions. The obtained results were higher than those obtained by Casazza et al. (2010). Indeed, these authors have found values of 55.98 ± 0.58 and 5.60 ± 0.20 mg GAE/g in ethanolic extracts from grape seeds and skin, respectively. Bucić-Kojić et al. (2007) found the concentration ranged from 14.72 mg GAE/g DW to 66.81 mg GAE/g DW in 50% ethanolic extract of grape seeds. Krishnaswamy et al. (2012), have obtained a value of 13.5 ± 0.48 mg GAE/g DW in 30% ethanolic extract from grape seeds.

The second order polynomial equation was generated to describe the empirical relationship between the TPC of seeds (Y_1) and skin (Y_2) and operational conditions (solvent concentration, solid/extraction time and solid/liquid ratio) in terms of coded values: the models were simplified by elimination of statistically insignificant terms.

$$Y_1 = 95.65 + 0.46X_1 + 0.35X_2 - 1.78X_3 - 0.87X_2X_3 - 0.54{X_1}^2 - 2.68{X_2}^2 - 2.41{X_3}^2 \quad (3)$$

$$Y_2 = 38.85 - 0.43X_1 - 0.55X_2 - 1.21X_3 - 0.87X_2X_3 - 1.07{X_1}^2 - 1.71{X_2}^2 - 1.19{X_3}^2 \quad (4)$$

The results of the analysis of variance (ANOVA) for the adequacy of the selected mathematical models are reported in Table 5. The significance of every factor and their interactions are verified by p-values, where the values less than 0.05 indicate significance and less than 0.001 indicate high significance.

very low p-values ($p < 0.0001$ and $p = 0.0022$ for seeds and skin, respectively) indicated that each generated model was statistically significant and suggests that the CSE of grape seeds and skin could be well described with those appropriate models. The values of R-squared are close to 1 for each model ($R^2 = 0.9924$ and 0.9722 for seeds and skin, respectively), which is very high and indicates a good correlation between the experimental and the predicted values, and indicated that each model could explain 99.24% and 97.22% of the variation in the TPC in grape seeds and skin, respectively. Also, the value of F-value for the lack of fit, of both models, was not significant ($p > 0.05$), thus confirming the validity of the model. All results indicated that both models could work well for the prediction of TPC extraction from grape seeds and skin.

The regression analysis of the data showed that all the three parameters (solvent concentration (X_1), solid/liquid ratio (X_2) and extraction time (X_3)) have a significant effect ($p < 0.05$) on both seeds and skin extracts. Meanwhile, solid/liquid ratio has the dominant effect on TPC, followed by solvent concentration and extraction time. However, interactions X_1X_2 and X_1X_3 did not exhibit any significant effect in any cases. Whereas, the effects of X_2X_3, ${X_1}^2$, ${X_2}^2$ and ${X_3}^2$ on the TPC of seeds and skin extract were significant ($p < 0.05$).

The Eq. (3) shows that ethanol concentration and extraction time have significant positive linear effects on TPC grape seeds extract, whereas, solid/liquid ratio and their quadratic interaction have significant negative effects. Concerning skin, Eq. (4), indicates that all parameters and their quadratic interaction have significant negative effects on TPC. The influence of the three parameters on TPC of grape seeds and skin is depicted in Fig. 1. Results indicate that, yield of TPC increased with increasing ethanol concentration up to 74% and then it remained significantly constant. While the TPC in the grape skin extracts increased with decreasing of acetone concentration, so the maximum TPC was obtained with minimal value of acetone concentration. Similar results have been obtained by Chew et al., (2011); the 40% ethanol extract of *Centella asiatica* contained more phenolic compounds than 60%, 80% and 100% ethanol extracts. Also, Al-Farsi and Lee (2008) reported that 50% acetone was the most efficient solvent for phenolic extraction from date seeds.

From Fig. 1A to F, it can be concluded that the TPC, independently of solvent concentration and solid/liquid ratio, increased rapidly for grape seeds and slightly for skin extracts, respectively, with the increase of the extraction time from 30 to 60 and from 60 to 90 min for grape seeds and skin extracts, respectively; then slightly decreased from 65 to 90 and rapidly from 91 to 120 min for grape seeds and skin extracts, respectively. In general, the highest value of TPC was achieved at an extraction time of 65 and 90 min for seeds and skin extracts, respectively. But, after this point, the TPC was decreased. Similar results were reported in the literature (Spigno et al., 2007; Wissam et al., 2012; Yang et al., 2013). Pileno et al. (2005b) showed that the TPC of grape extracts decrease with the increase of the extraction time. This phenomenon

Table 3
Effect of solvent nature on the extraction of TPC from seeds and skin.

Solvents	TPC of seeds extracts (mg GAE/g DW)	TPC of skin extracts (mg GAE/g DW)
Water	57.56 ± 0.23^{b}	07.58 ± 0.83^{d}
Ethanol 50%	86.51 ± 5.36^{a}	20.61 ± 1.64^{b}
Methanol 50%	69.18 ± 1.41^{b}	13.09 ± 0.79^{c}
Acetone 50%	38.92 ± 0.68^{c}	27.90 ± 0.39^{a}

Results are reported as means ± S.D. Different letters in the same column refer to means statistically different according to ANOVA and Tukey's test.

Table 4
Box–Behnken design with the observed responses and predicted values of TPC referred to DW of seeds and skin using CSE.

Essay	Factors			Reponses			
	X_1- solvent concentration (% v/v)	X_2-time (min)	X_3– ratio (mg/ml)	Seeds TPC mg GAE /g DW		Skin TPC mg GAE /g DW	
				Experimental	predicted	Experimental	predicted
1	−1	+1	0	92.06 ± 0.84	92.00	36.06 ± 0.70	36.26
2	+1	0	−1	95.13 ± 1.63	95.31	36.89 ± 1.05	37.21
3	−1	0	−1	93.61 ± 1.36	93.64	38.11 ± 0.37	38.37
4	0	−1	−1	91.38 ± 1.29	91.13	36.95 ± 1.70	36.83
5	0	0	0	95.74 ± 1.09	95.65	39.14 ± 0.36	38.85
6	−1	0	+1	91.01 ± 0.90	90.83	35.98 ± 1.17	35.66
7	0	+1	+1	87.99 ± 0.54	88.24	33.19 ± 0.37	33.31
8	+1	−1	0	92.16 ± 0.78	92.22	36.68 ± 0.55	36.48
9	+1	0	+1	91.01 ± 0.38	90.98	35.32 ± 0.31	35.07
10	0	−1	+1	89.33 ± 1.10	89.30	35.70 ± 0.69	36.16
11	0	+1	−1	93.54 ± 1.48	93.57	37.94 ± 1.38	37.48
12	−1	−1	0	91.71 ± 0.48	91.93	36.88 ± 1.13	36.74
13	+1	+1	0	93.76 ± 0.82	93.54	34.63 ± 1.02	34.77
14	0	0	0	95.97 ± 0.67	95.65	38.83 ± 1.84	38.85
15	0	0	0	95.24 ± 0.78	95.65	38.58 ± 0.66	38.85

Table 5
Analysis of variance (ANOVA) of the quadratic model obtained with CSE. df. degrees of freedom.

Source	Seeds				Skin			
	Df	Sum of squares	*F*-value	*P*-value Prob > *F*	Df	Sum of squares	*F*-value	P-value Prob > *F*
Model	9	76.9125	72.9768	<0.0001	9	37.0018	19.4359	0.0022
X_1 –Solvent	1	1.6718	14.2766	0.0129	1	1.5287	7.2272	0.0433
X_2- Time	1	0.9583	8.1834	0.0353	1	2.4088	11.3874	0.0197
X_3-Ratio	1	25.5628	218.2930	2.56815e-5	1	11.7540	55.5664	0.0006
X_1X_2	1	0.3906	3.3360	0.1273	1	0.3791	1.7926	0.2382
X_1X_3	1	0.5779	4.9356	0.0769	1	0.0788	0.3727	0.5681
X_2X_3	1	3.0648	26.1724	0.0037	1	3.0548	14.4414	0.0126
X_1^2	1	1.1126	9.5012	0.0273	1	4.2876	20.2693	0.0063
X_2^2	1	26.5220	226.4838	2.34629e-5	1	10.7707	50.9180	0.0008
X_3^2	1	21.4407	183.0925	3.94951e-5	1	5.2694	24.9108	0.0041
Residual	5	0.5855	0.7301	0.6221	5	1.0576	3.8692	0.2122
Lack of fit	3	0.3060			3	0.9022		
Pure error	2	0.2794			2	0.1554		
Cor total	14	77.4980			14	38.0595		
		$R^2 = 0.9924$				$R^2 = 0.9722$		

could be explained by Fick's second law of diffusion, when the solvent saturates the extracted compound, the concentration gradient becomes null after a particular duration and the phenomenon stops (Rodrigues et al., 2008; Yang et al., 2013). However, it was found that augmentation of the extraction time prolonged exposure to oxygen and light (Wissam et al., 2012), thus, increase the chances for formation of free radicals which can be scavenged by phenolic compounds (Naczk and Shahidi, 2004). Also plant cells contain enzymes capable for altering the phenolic compounds, in particular polyphenol oxidase via reactions enzymatic browning (Tomãs-Barberãn and Espin, 2001), the longer extraction time may favor those reactions (Garcia-Salas et al., 2010). Hence, an excessive extraction time was not useful to extract more phenolic antioxidants (Chaalal et al., 2012).

With regard to solid/liquid ratio, when this parameter decreases from 2.5 to 1 for seeds and from 5 to 2.5 for skin an increase on TPC was observed. Our results agree with those reported by Chaalal et al. (2012), who confirmed an increase of TPC when the solid/liquid ratio was decreased from 0.08 (0.8 g/10 ml) to 0.02 (0.2 g/10 ml). One of the probable explanation for this phenomenon is that regularly usage of larger volume of solvent could obtain larger amount of phenolic compounds (Ince et al., 2014); according to mass transfer principles, the driving force during mass transfer is the concentration gradient between the sample and the solvent, which is greatest when a small sample/solvent ratio is used (Pinelo et al., 2005b).

The obtained results show that the optimum extraction conditions for the recovery of the TPC using CSE from seeds (1) are: 74.33% v/v of ethanol concentration, 65.23 min extraction time and 0.1 g/70.86 ml solid/liquid ratio with a predicted yield of 96.23 mg

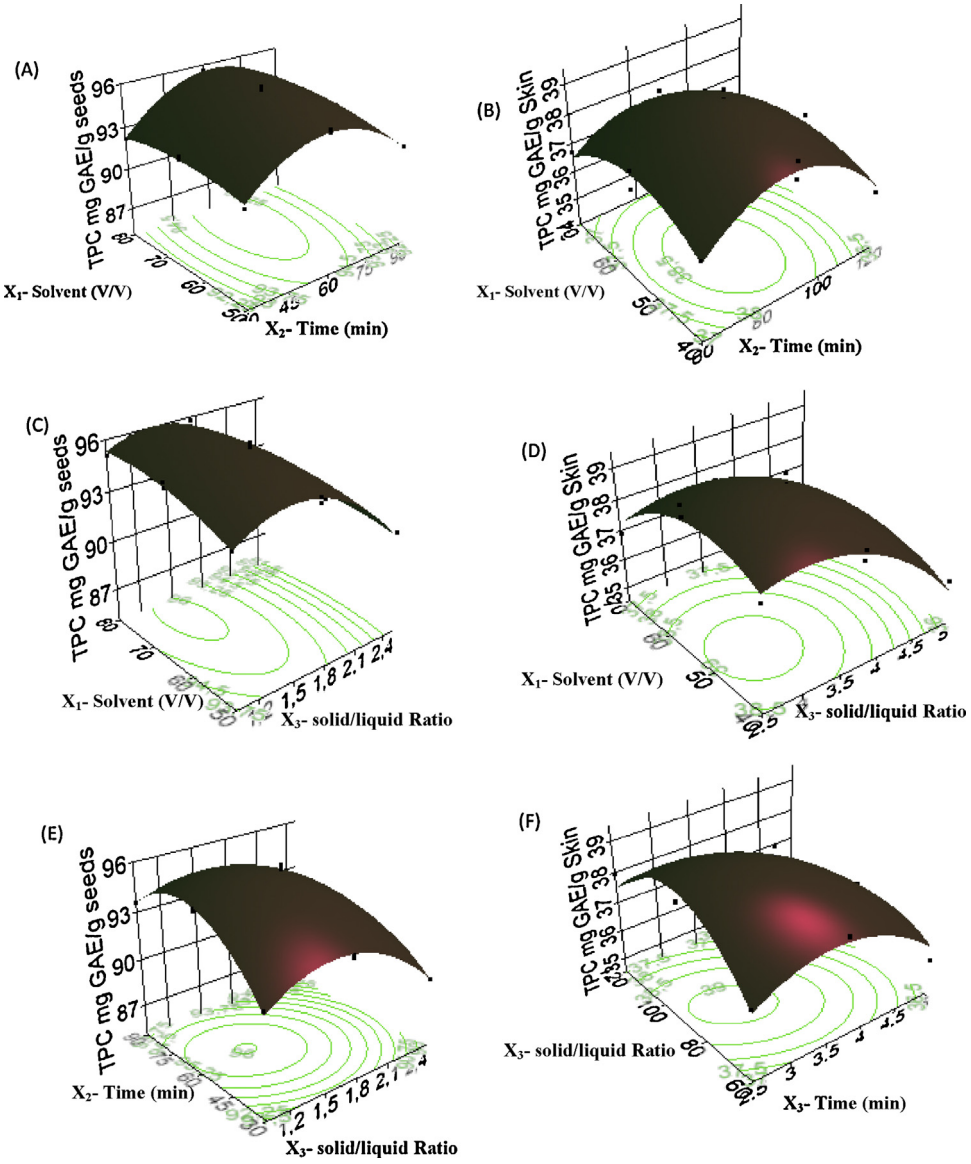

Fig. 1. Three-dimensional presentation of the developed response surface model for the TPC from grape seeds and skin with CSE with respect to extraction time and solvent percentage (A: seeds; B: skin); solid/liquid ratio and solvent percentage (C: seeds; D: skin); extraction time with solid/liquid ratio (E: seeds; F: skin).

GAE/g. A verification experiment at the optimum conditions was performed and the practical recovery of 96.56 ± 1.29 mg GAE/g was obtained. While those from the skin (2) are: at 51.46% v/v of acetone concentration, 89.80 min extraction time and 0.1 g/32.25 ml solid/liquid ratio with a predicted yield of 39.22 mg GAE/g. A verification experiment at the optimum conditions was performed and the practical recovery of 39.57 ± 0.23 mg GAE/g was obtained.

3.3. Optimization of the MAE conditions

RSM based on a Central Composite Design (CCD), through a 2^3 factorial experimental design, to optimize the Microwave-Assisted Extraction (MAE) of total phenol content from seeds and skin, was investigated. The experimental values of TPC extracts at various experimental conditions are presented in Table 6. In this new design, only the irradiation time and microwave power were studied. The solvent concentration and solid/liquid ratio were fixed at optimum that was found in CSE study. Each factor, containing three levels, was chosen from a series of preliminary experiments without using mathematical models. The model presented the total of 11 experiments. Three replications at the central points were included in order to evaluate experimental error measurement (Goupy and Creighton, 2006). The obtained results showed that the maximum TPC of the grape seeds and skin extracts were 72.92 and 55.82 mg GAE/g DW, respectively.

Eq. (5) and (6), show the relationship between irradiation time and microwave power for the extraction of total phenolic compounds of grape seeds (Y_1) and skin (Y_2), respectively.

$$Y_1 = 72.33 - 3.47X_1 - 4.98X_2 - 6.88X_1X_2 - 3.51{X_1}^2 - 9.22{X_2}^2 \quad (5)$$

$$Y_2 = 54.31 - 0.82X_1 - 0.72X_2 - 2.30X_1X_2 - 3.08{X_1}^2 - 3.12{X_2}^2 \quad (6)$$

The results of ANOVA, goodness of fit and the adequacy of the models are showed in Table 7. The ANOVA response surface quadratic regression model showed that the models were highly significant for both matrixes ($p < 0.001$) with highly F-value (129.22 and 69.87 for seeds and skin, respectively), at the same time, a high proportion of variability was explained by the RSM models for TPC as indicated by R^2 ($R^2 = 0.99$ and 0.98 for seeds and skin, respectively) therefore, the models were adequate and explained most of the variability for each matrix. Also, the lack-of fit statistics

Table 6
Central Composite Design with the observed responses and predicted values of TPC referred to DW of seeds and skin using MAE.

Essay	Factors		Reponses			
	X_1–time(s)	X_2–power (W)	Seeds TPC mg GAE/g DW		Skin TPC mg GAE/g DW	
			Experimental	predicted	Experimental	predicted
1	0	0	71.83 ± 0.79	72.32	54.92 ± 0.84	55.06
2	0	0	72.44 ± 0.46	72.32	55.82 ± 0.82	55.06
3	−1	0	72.00 ± 0.60	72.29	52.60 ± 0.98	52.79
4	−1	−1	60.68 ± 0.95	61.17	47.76 ± 0.34	47.78
5	1	−1	67.30 ± 1.08	67.97	51.28 ± 1.02	51.21
6	0	1	56.74 ± 1.23	58.12	50.58 ± 0.72	51.09
7	−1	1	65.74 ± 0.73	64.97	51.51 ± 0.64	51.3
8	0	0	72.92 ± 0.79	72.32	55.01 ± 0.52	55.06
9	0	−1	69.23 ± 1.00	68.08	52.48 ± 0.57	52.53
10	1	0	65.39 ± 1.19	65.33	50.90 ± 0.69	51.27
11	1	1	44.86 ± 1.90	44.25	45.12 ± 0.61	44.82

Table 7
Analysis of variance (ANOVA) of the quadratic model obtained with MAE. df. degrees of freedom.

Source	Seeds				Skin			
	df	Sum of squares	*F*-value	P-value Prob > *F*	df	Sum of squares	*F*-value	P-value Prob > *F*
Model	5	723.1177	129.2295	2.78383e-5	5	94.8697	69.9606	0.0001
X_1–Time	1	207.6950	72.5813	0.0004	1	4.0520	14.9408	0.0118
X_2-Power	1	480.6025	148.7268	8.61256e-5	1	3.1520	11.6220	0.0190
X_1X_2	1	496.4884	189.1988	<0.0001	1	21.1829	78.1057	0.0003
X_1^2	1	85.2034	31.2173	0.0032	1	24.1343	88.9880	0.0002
X_2^2	1	539.8073	215.4265	3.49442e-5	1	24.6201	90.7793	0.0002
Residual	5	5.5956	5.5803	0.1557	5	1.3560	1.4520	0.4326
Lack of fit	3	4.9984			3	0.9293		
Pure error	2	0.49984			2	0.4266		
Cor total	10	728.7133			10	96.2257		
		R^2 = 0.9923				R^2 = 0.9859		

(p = 0.1557 and 0.4326 for seeds and skin, respectively), which was used to test the adequacy of the model was not significant. Thus, it can be concluded that the models were well fitted and could be used to predict the TPC from grape seeds and skin extracts with MAE.

As shown in Table 7, ANOVA results indicated that effect of irradiation time (X_1) and microwave power (X_2) on TPC of seeds and skin extracts is highly significant ($p < 0.0001$) such as their interaction and their quadratic terms. According to Eq. (5) and (6) the negative coefficients for X_1 and X_2 indicate linear effects that may decrease the responses (TPC). The relationship between TPC and dependent variables is graphically presented in three dimensional response surface plots (Fig. 2). From those figures, it can be concluded that the TPC slightly increases, at a constant irradiation time, with an increase of microwave power from 200 to 380 and 410 W for seeds and skin, respectively, then rapidly decreases from 460 and 480 to 600 W for seeds and skin, respectively.

It was also observed that the increase in the irradiation time at lower microwave power, leads to the increase of the response. At lower microwave power, the impact of extraction time has a positive effect on the TPC up to the extraction time of 84 s and 146 s for seeds and skin extracts respectively, and then it has a negative impact. It is very interesting to note that the TPC (1) decreases with the increasing of the microwave power for longer extraction times and (2) increases at low microwave power with long extraction time and high power with short exposure. There is a strong interaction between these two parameters, which is based on the function shape. These results conclude that the most efficient mean to extract phenols from grape seeds and skins by MAE is the application of a moderate microwave power for a short exposure. Therefore, the longer extraction time with higher microwave power can affect the quality of the phenolic compounds. Afoakwah et al. (2012) reported that, the extraction process of MAE is widely influenced by time exposure to heat. Thus, the quantity of target compounds extracted can be increased with an increase in the extraction time, but there is an associated risk of deterioration of thermo labile ones. The results obtained can be explained by the fact that the increase of the microwave power in short extraction time leads to the increase of the content of phenolic compounds by increasing the solubility of the target analytes, the diffusion rate, and the mass transfer (Afoakwah et al., 2012; Dahmoune et al., 2013; Garcia-Salas et al., 2010). However, it was noted that increasing the power up to a certain values may promote possible concurrent decomposition of phenolic compounds (Afoakwah et al., 2012; Muthuselvi et al., 2012). Additionally, high power may encourage solvent loss through evaporation (Garcia-Salas et al., 2010; Thabit et al., 2014).

Optimal conditions for seeds extract are: irradiation time of 58.63 s and microwave power of 373.15 W with a predicted TPC yield of 73.29 mg GAE/g DW. For skin extract: irradiation time of 113.74 s and microwave power 384.44 W with a predicted TPC yield of 54.38 mg GAE/g DW. The validity of those predicted optimal values of TPC for each matrix extracts, was also experimentally confirmed: 73.15 ± 0.20 and 54.84 ± 0.41 mg GAE/g DW for seeds and skin, respectively, very close to the values predicted by the models.

3.4. Comparison between CSE and MAE

Table 8 shows the concentrations of phenolic compounds and the antioxidant activity (ABTS radical scavenging activity and reducing power) of the extracts obtained under the best conditions. The results indicate that TPC of seeds extract obtained with MAE was significantly lower (73.15 ± 0.20 mg/g) than that obtained with CSE (96.56 ± 1.29 mg/g). The same observation is

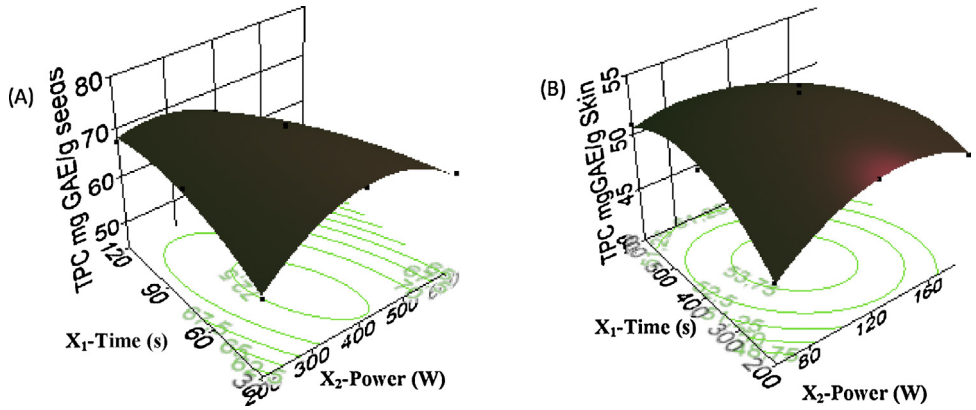

Fig. 2. Response surface model for the TPC from grape seeds and skins with MAE to irradiation time and microwave power (A: seeds; B: skin).

Table 8
Total phenolic content and antioxidant activity of different extracts.

Matrixes	TPC mg GAE/g DW		ABTS mg QE/g DW		FRP mg QE/g DW	
	CSE	MAE	CSE	MAE	CSE	MAE
Seeds	96.56 ± 1.29^{a}	73.15 ± 0.20^{b}	182.24 ± 5.44^{a}	134.62 ± 1.99^{b}	181.90 ± 1.09^{a}	122.94 ± 1.10^{b}
Skin	39.57 ± 0.23^{b}	54.84 ± 0.41^{a}	104.94 ± 0.54^{b}	108.36 ± 1.14^{a}	90.98 ± 0.94^{b}	93.52 ± 1.11^{a}

Results are reported as means ± S.D. Different letters in the same line and in two consecutive columns refer to means statistically different according to ANOVA and Tukey's test.

noted for the antioxidant activity, indeed the ABTS and the FRP values (182.24 ± 5.44 and 181.90 ± 1.09 mg EQ/g DW, respectively) of extract obtained by CSE were significantly higher than those obtained by MAE (134.62 ± 1.99 and 122.94 ± 1.10 mg EQ/g DW, respectively).

The lower TPC of seeds extracts obtained with microwave extraction as compared to conventional extraction can due to their heat sensibility. The effect of temperature cannot be generalized since it strongly depends on typology of compounds (Spigno et al., 2007).

The heat treatment causes irreversible changes in tannins structure, indeed, Padmaja (1989), has reported the decrease of tannin contents in cassava leaves dried at 60 °C. Several studies showed that extraction of tannins and proantocyanidins, which are the major phenolic compounds in grape seeds (Bordiga et al., 2011; Cadot et al., 2006; Shi et al., 2003), decrease with the increase of temperature (Makkar and Becker, 1996; Padmaja, 1989; Spigno et al., 2007). Liazid et al., (2007) also showed that the microwave radiation can easily degrade unstable phenolics due to their structural properties. Phenolic compounds with higher number of hydroxyl substituent in their aromatic rings are unstable and can easily be degraded under high temperature conditions. The degradation of phenolic compounds can generate a decline on the antioxidant activity, which can explain the lower antioxidant activity of extracts obtained by MAE in comparison to those obtained by CSE method. Indeed, several authors found a significant correlation between TPC and antioxidant activity (Slusarczyk et al., 2009; Velioglu et al., 1998).

In the other hand, TPC of skin extracts obtained by different methods showed significant difference (Table 6). The TPC obtained by MAE method (54.84 ± 0.41) was significantly ($p < 0.05$) higher than that obtained by CSE method (39.57 ± 0.23 mg/g). The same observation is noted for the antioxidant activity. Indeed the ABTS and the FRP values (104.94 ± 0.54 and 90.98 ± 0.94 mg EQ/g DW, respectively) of extract obtained by CSE were lower than those obtained by MAE (108.36 ± 1.14 and 93.52 ± 1.11 mg EQ/g DW, respectively).

For extracting phenolic compounds, Hithamani and Ramalakshmi, (2013) and Rafiee et al. (2011) have shown that MAE was more effective than the conventional extraction methods with reducing in extraction time. The reduction of the extraction time was due to the heating mechanism of the microwaves (Rafiee et al., 2011; Yang et al., 2013). An enhance in temperature adds to the efficacy of the process, indeed heat (1) decreases the viscosity of the solvent, which makes easy its passage through the solid matrix so the efficiency of the extraction. In the other hand, it (2) increases the permeability of cell walls which promotes the solubility and the diffusion coefficients of the biological active compounds to be extracted (Afoakwah et al., 2012; Muthuselvi et al., 2012; Wissam et al., 2012); As a result, microwave in comparison to conventional extraction can extract polyphenols in shorter times (Bayramoglu et al., 2008).

The shorter extraction time in microwave processing can reduce the degradation of biological active compounds (Ince et al., 2014; Ravalji et al., 2012), which explains the result of the antioxidant activity of grape skin extracts. In fact, the lower activity of CSE extract could be a result of the extension in the extraction time; the long exposure of the extracts to unfavorable conditions such as light, together with oxygen and temperature, is the most important factor that facilitates degradation reactions (Garcia-Salas et al., 2010).

4. Conclusion

The aim of this study was, on the one hand, to obtain a better survey into optimization of extraction conditions of phenolic compounds from Algerian grape cv. *Ahmar Bou-Amar* with two extraction methods (CSE and MAE) using RSM, and on the other hand to compare those extraction methods based on the maximum TPC and antioxidant activity measured with ABTS and FRP.

The results indicated that the mathematical models used in the present investigation work well for the prediction of TPC extraction from grape cv. *Ahmar Bou-Amar* seeds and skin. Thus, the two studied extraction methods were successfully optimized by BBD and CCD of RSM. On the other hand, the TPC of seeds extract obtained

with MAE was 24% lower than that of the CSE extract; also, the antioxidant activity of CSE extract was better than that of MAE extract. While, the TPC of skin extract obtained with MAE was 28% higher than that of the CSE extract and the antioxidant capacity was significantly higher than that of the CSE extract. Therefore, (1) it can be concluded that, MAE is a good alternative for the extraction of phenolic compounds from grape skin of the studied variety, since it provides a high quantity and a high antioxidant quality compared to CSE by drastically reducing the time of the process (98%). (2) In order to maximize recovery of TPC from grape seeds of the studied variety, it is better to realize the extraction with CSE method with longer extraction time compared to MAE, but it is noteworthy to point that, MAE permits to extract in only 58.63 s about 76% of TPC which were extracted by CSE during 65.23 min.

Thus, from the point of view of industrial application, MAE can be recommended for extracting antioxidant compounds from grape cv. *Ahmar Bou-Amar* byproducts with a reduction in extraction time of about 98% compared to CSE. Additionally, for their high contents of phenolic compounds in comparison to others varieties, the extracts of grape cv. *Ahmar Bou-Amar* seeds and skin can be considered as a good source of natural antioxidants and may be exploited as natural antioxidant agents for health food as substituted antioxidant to the synthetic antioxidants with great commercial interest in the food and phyto-pharmaceutical market.

Acknowledgements

We would like to thank all members of UVV laboratory at the INRA of Angers (France) for their precious help, especially Gérard Barbeau, Marie-Hélène Bouvet and Anne Mège. As well as a special thank to Mrs Wahiba Azegagh for her providing English language help.

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