Studii şi Cercetări Ştiinţifice 2017, **18** (4), pp. 435 – 448
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

THE EFFECT OF SOXHLET AND ULTRASONIC-ASSISTED EXTRACTION ON ANTIOXIDANT COMPONENTS AND ANTIOXIDANT PROPERTIES OF SELECTED SOUTH ALGERIAN RED POTATOES CULTIVARS

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Received: May, 05, 2017
Accepted: December, 06, 2017

Abstract: The present work aimed to study the effect of two different commonly applied extraction techniques for the evaluation of total phenolic contents (TPC), total flavonoid contents (TFC) and total antioxidant capacity (TAC) of fresh south Algerian red potatoes cultivars (*Solanum tuberosum L*). These techniques are namely Soxhlet extraction (SE) and ultrasonic-assisted extraction combines with maceration (UAE-M) for 5 min, 2 hours and 24 hours. A 40 kHz probe was used for the sonication at 3 different amplitudes (30 %, 50 %, and 70 %) with a mixture of ethanol/water at ratios of 70:30 and 100 % v/v as solvent. TPC and TFC obtained using SE are respectively 13.94 mg gallic acid equivalents (GAE) / g extract and 11.32 mg rutin equivalents (RE) / g extract, the amount of these contents is increased to 22.29 mg GAE / g extract and 85.93 mg RE / g extract using UAE-M with 100 % ethanol at amplitude 30 % and 24 hours maceration time. HPLC analysis of samples extract shows four contents with different concentration. In addition, all the results demonstrate a statistically significant with ($p < 0.001$). It should be noted that the use of UAE-M proved to be faster and more efficient process when compared to Soxhlet extraction.

Keywords: *antioxidant capacity, Guemar, HPLC, maceration, red potatoes, ultrasonication, ultrasound amplitude*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is presently the fourth most important food crop in the world after maize, wheat and rice, with a production of 365 million tons [1]. Algeria is the 14th producer among the top 25 potato producing countries in the world with a total production of 5 million tons [1].
Potato is considered as an important source of polyphenolic and flavonoid compounds, most recent studies focused on the evaluation of its antioxidant activities and its phenolic and flavonoid contents [2 – 9]. However, very few studies dealt with the effect of extraction conditions of phenolic and flavonoid compounds from potato [10]. In food processing there are a several application of extraction techniques, traditional methods such as maceration and Soxhlet extraction are commonly used, also modern extraction methods such as microwaved-assisted extraction (MAE), ultrasound-assisted extraction (UAE) are used to obtain bioactive compounds from food and vegetables.
Ultrasound has been used in several operations in chemical engineering, such as waste-water treatment, drying, sonochemistry, and extraction [11 – 13]. In food and pharmaceutical sectors, ultrasound has been employed to extract bioactive compounds such as flavonoids [14], essential oils and alkaloids [15], polysaccharides [16], esters and steroids [17], and others substances [18, 19]. The advantages of ultrasound extraction lie in the possibility to operate with many samples in the same apparatus and also it requires short extraction times when compared with conventional solvent extraction [20].
In general terms, the extraction efficiency of target compound is usually a function of several variables process. Many research reports the influence of many factors on the extraction of phenolic compounds, the most important factors are solvent type, temperature, contact time and solvent-to-solid ratio [21]. These factors should always be optimized in order to produce good extraction yields in an economically advantageous process [20]. For solvent type, several solvent systems have been used to recover phenolic compounds from plant matrices. This work will focus on the use of ethanol and water and their combination, these substances being classified with the GRAS status (Generally Recognized as Safe) [22].
Herein we elected to compare Soxhlet extraction with ultrasound-assisted extraction combined with maceration on total phenolic contents, total flavonoid contents and on the total antioxidant capacity of selected fresh south Algerian red potatoes cultivars (*Solanum tuberosum* L.). The study aims to extract bioactive compounds with higher yield and less time using less solvent.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Coiocaltau's reagent (solution 2 M) was purchased from Sigma-Aldrich (Finland), gallic acid (99 %), ascorbic acid (99 %), rutin (97 %) were purchased from Alfa Aesar (Germany), ammonium molybdate 98 % was purchased from BioChem (Quebec, Canada). Hydrochloric acid was provided by Rathburn Chemical (Walkerburn,

Peebleshire, UK). Ethanol and di-sodium hydrogen phosphate were purchased from Fisher Scientific (Loughborough, UK) and all used as received.

Plant material

Red potatoes cultivars "Kondor" were collected in January 2015 from Guemar region located in the Wilaya of El Oued southeast Algeria were planted for three months before being used. Immediately after receiving, all samples of raw potatoes were peeled using a kitchen knife and prepared by simply cutting into 5 mm thick slices. 20 g of the slices of fresh peeled potatoes was extracted using 120 mL of a mixture of ethanol and water at ratio of 70:30 (v/v) as extraction solution. Subsequently all the samples taken after the treatment were filtered before analysis for removal of potato particles, then the filtrate was concentrated under reduced pressure at 60 °C by rotary evaporator and stored in a refrigerator.

Instruments

UV-Vis experiments were performed using a UV-1800 UV-VIS spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan).
High performance liquid chromatography (HPLC) analysis was conducted by using a LC-20AT (Shimadzu Scientific Instruments, Kyoto, Japan). System comprised a 2LC-10AT pump (A&B), a CTO-20A column oven, a SPD-20A UV-DAD detector, and CBM-20A interface.
Sonication with water bath type equipment (model WUC-D06H, Daihan Scientific, Korea) was used for ultrasound-assisted extraction.

Ultrasonic extraction

In this study the ultrasound assisted extraction procedure was used for the extraction, thus samples were further submitted to ultrasonication in bath (40 kHz) at three levels of power 30 %, 50 %, and 70 %, the extraction being performed at T = 33 ± 4 °C (this was the temperature reached by the extraction performed by UAE) [23]. The extracts were kept to macerate for 5 min, 2 h and 24 h, in dark at room temperature.

Soxhlet extraction

The sliced fresh potatoes (20 g) were continuously extracted with 120 mL using the appropriate solvent mixture for 3 h (5 cycles) at a maximum temperature of 70 °C in a Soxhlet apparatus.

Phytochemical investigation

Determination of TFC
TFC was determined as described by Zou *et al.* [24]. A mixture of 1 mL of extract, 2 mL of ultrapure water and 0.15 mL of aqueous 5 % $NaNO_2$ solution was prepared and left to react for 6 min. Then 0.15 mL of 10 % $AlCl_3$ solution was added and the resulting mixture was thoroughly stirred for 6 minutes, 2 mL of 4 % NaOH solution was

then added and left to stand for an additional 15 min. The absorbance of the obtained solution was then determined at 510 nm against a blank solution. Rutin was used as a standard for the measurement of TFC. Results were expressed in mg of rutin equivalents (RE) / g of extract.

Determination of TPC

TPC was determined with the Folin-Ciocalteu reagent according to a method described by Singelton and Rossi [25]. Briefly, 100 µL of suitably diluted extract was added to 0.5 mL of newly diluted 10-fold Folin-Ciocalteu reagent. Then 2 mL of 20 % aqueous Na_2CO_3 solution was added. The reaction mixture was set aside in the dark for 30 min, and then the absorbance was determined at 760 nm against a blank solution. Gallic acid was used as a reference and the results were presented as gallic acid equivalents (GAE) / g of extract.

Determination of TAC

The TAC of different samples was measured by the phosphomolybdenum method of Prieto *et al.* [26]. A portion of 100 µL of sample was added to 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were sealed and heated in water bath at 95 °C for 90 min. Then the samples were cooled to room temperature and the absorbance was measured at 695 nm. The antioxidant capacities were expressed as equivalent of ascorbic acid (mg ascorbic acid / g of extract).

HPLC analysis

Standard solutions

Quantitative determinations were carried out by external standard method. A stock solution of phenolic compounds was prepared by dissolving 10 mg of purified phenolic compound in a 50 mL volumetric bottle containing a sufficient volume of methanol (HPLC grade) to dissolve the phenolic compound, it was sonicated for about 10 min and then brought to volume with mobile phase. Daily working standard solutions of phenolic compounds were prepared by proper dilution of the one with the mobile phase: 10.0, 6.0, 4.0, 1.0 and 0.5 $\mu g \cdot mL^{-1}$. Any of these solutions (20 µL) was injected three times into the column, the peak area and retention times were recorded.

Chromatographic conditions

The phenolic compounds present in potatoes samples were analyzed by HPLC. An analytical column a Shim-pack VP-ODS C18 (4.6 mm × 250 mm, 5 µm), (Shimadzu Co., Japan) was used, the column was maintained at 30 °C. Elution was performed at a flow rate of 1 $mL \cdot min^{-1}$, using a mobile phase composed of a mixture of water and acetic acid at ratio of 98/2, v/v respectively (solvent A) and acetonitrile (solvent B) in gradient elution mode. The elution gradient was: 0 - 5 min, 5 % B; 10 min, 10 % B; 11 min, 20 % B; 20 min 20 % B; 30 min 40 % B; 40 min 50 % B; 50 min 80 % B. Chromatograms were acquired at 300 nm. Phenolic compound standards ascorbic acid, gallic acid, chlorogenic acid, vanillin, rutin were dissolved in extraction solvents and used for identification of phenolic acids presents in different extracts of potatoes. Peak identification in HPLC analysis was achieved by comparison of retention time and UV

spectra of reference standards. Quantification of individual phenolic compounds in the extracts was done using the peak area of reference compounds and reported as $\mu g \cdot mg^{-1}$ of extract.

Statistical analysis

All the manipulations were carried out in triplicate, and the results were presented as mean ± SD (standard deviation). Statistical analysis was performed using Excel 2007 and one way ANOVAs (Minitab version 14). In order to investigate the difference in levels of antioxidant capacities and phenolics between different varieties, the statistic significances at a 95 % confidence interval were achieved when $p < 0.001$.

RESULTS AND DISCUSSION

Phytochemical investigation

Determination of TFC

Table 1 and 2 show the amount of TFC obtained from the extract using UAE with (70:30 ethanol/water) and 100 % ethanol as solvent.

Table 1. *Total flavonoids content* obtained using ethanol/water (70:30)*

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	9.3230 ± 0.043	13.073 ± 0.010	8.5319 ± 0.003	6.9446 ± 0.019
2 h	11.7648 ± 0.007	9.4793 ± 0.007	12.0140 ± 0.004	
24 h	14.4272 ± 0.002	13.0321 ± 0.043	11.0244 ± 0.007	

*Total flavonoids content was expressed as mg rutin / g extract

Table 2. *Total flavonoids* content obtained using 100 % ethanol*

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	63.3336 ± 0.01	41.1461 ± 0.027	17.1184 ± 0.013	11.3242 ± 0.024
2 h	61.1119 ± 0.006	51.9105 ± 0.006	23.7503 ± 0.005	
24 h	85.9383 ± 0.008	69.1670 ± 0.049	58.2295 ± 0.018	

*Total flavonoids content was expressed as mg rutin / g extract

The UAE showed an advantage over SE, but a ratio of 100 % ethanol favored the extraction process resulting in increase of TFC for the two techniques. The amount of TFC obtained after 5 min of ultrasound treatment was higher compared to SE the three amplitude chosen by UAE increase the amount extracted from 17.1184 mg rutin / g extract at 70 % amplitude to 63.3336 mg rutin/g extract at 30 % of amplitude. Longer times of maceration following ultrasound extraction lead to the increase of the amount of TFC in the extract.

Determination of TPC

The concentration of TPC in the extracts, expressed as mg GAE / g sample depends on the solvent and the method of extraction, as shown in Tables 3 and 4.

Table 3. *Total phenolic content* obtained using ethanol/water (70:30)*

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	6.7781 ± 0.059	7.9936 ±0.067	5.1273 ± 0.032	6.7788 ± 0.017
2 h	9.3736 ±0.017	7.4522 ±0.094	6.9002 ±0.022	
24 h	10.3821 ± 0.007	8.1347 ± 0.018	7.2793 ± 0.007	

*Total phenolic content was expressed as mg gallic acid / g extract

The amount of phenolic compounds in the UAE extraction increases from amplitude 70 % (6.9426 mg gallic acid / g extract) to 30 % (17.5053 mg gallic acid / g extract). Also the maceration time after sonication of the extract have an effect on the level of TPC in samples, e.g. 24 hours of maceration led to the highest TPC with 22.2999 mg gallic acid / g extract, however using 5 minutes maceration time gave 17.5053 mg gallic acid / g extract.

Table 4. *Total phenolic content* content obtained using 100 % ethanol*

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	17.5053 ±0.053	14.4904 ± 0.003	6.9426 ± 0.016	13.9490 ± 0.023
2 h	15.2229 ± 0.065	14.4378 ± 0.016	9.2993 ± 0.029	
24 h	22.9299 ± 0.062	14.0127 ± 0.064	13.8694 ± 0.122	

*Total phenolic content was expressed as mg gallic acid / g extract

The best results were recorded using 100 % ethanol which showed advantage on ratio of 70:30 ethanol/water. The results obtained from SE increased from 6.77 (using 70:30 ethanol/water) to 13.94 (using 100 % ethanol), but are remarkably lower than those obtained by UAE.

Determination of TAC

The antioxidant capacity was evaluated using the phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes with a maximal absorption at 695 nm (Tables 5 and 6).

Table 5*. Total antioxidant capacity* obtained using ethanol/water (70:30)*

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	10.6776 ± 0.006	10.6838 ± 0.013	9.9551 ± 0.017	13.8983 ± 0.233
2 h	15.0973 ± 0.031	11.0793 ± 0.008	13.2861 ± 0.039	
24 h	15.3348 ± 0.003	9.7450 ± 0.025	15.2847 ± 0.015	

*Total antioxidant capacity was expressed as mg ascorbic acid / g extract

***Table 6**. Total antioxidant capacity* obtained using 100 % ethanol*

ULTRASOUND				SOXHLET
Maceration time	Amplitude			
	30 %	50 %	70 %	
5 min	36.3946 ± 0.022	20.2811 ± 0.079	11.3493 ± 0.014	23.1124 ± 0.029
2 h	28.4836 ± 0.044	20.8804 ± 0.015	19.5108 ± 0.060	
24 h	41.3346 ± 0.057	39.0802 ± 0.103	25.3192 ± 0.064	

*Total antioxidant capacity was expressed as mg ascorbic acid / g extract

The results show an increase of TAC at lower amplitude and higher maceration time from 10.6776 to 15.3348 mg ascorbic acid / g of extract for solvent ratio 70:30 (ethanol/water) and 36.3946 to 41.3346 mg ascorbic acid / g of extract for 100 % ethanol. It is clear that the TAC with UAE-M have an advantage over Soxhlet at two ratios of solvent.

Figures 1, 2 and 3 show the overall effect of maceration time (Figures 1a, 2a and 3a), amplitude (Figures 1b, 2b and 3b), method (Figures 1c, 2c and 3c) and solvent ratio (Figures 1d, 2d and 3d) on the TFC, TPC extracted from the potatoes and their TAC.

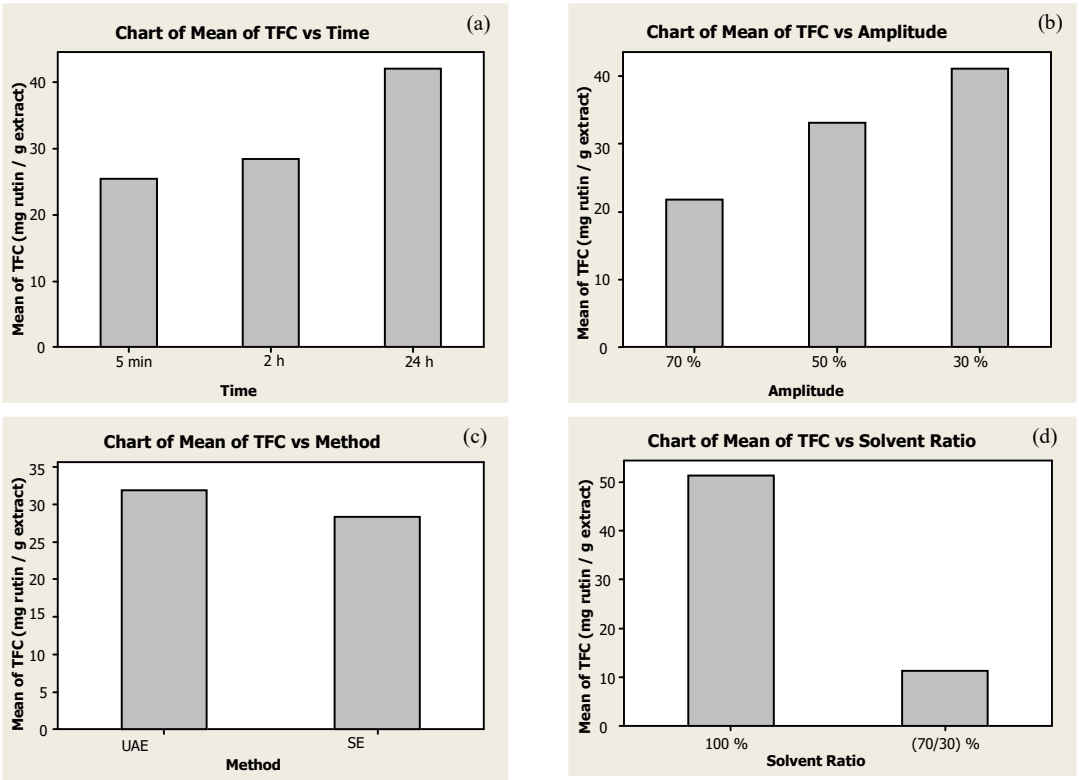

***Figure 1.** Effect of maceration time (a), amplitude (b), method (c) and solvent ratio (d) on the level of Total Flavonoids Content (TFC)*

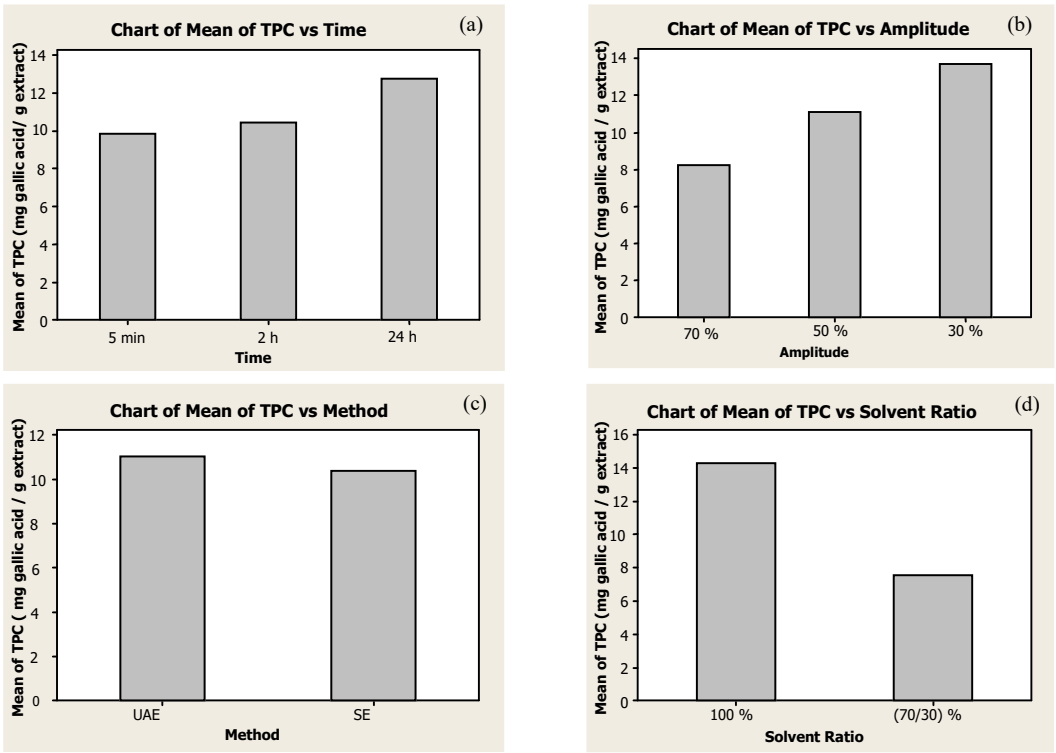

Figure 2. *Effect of maceration time (a), amplitude (b), method (c) and solvent ratio (d) on the level of Total Phenolic Content (TPC)*

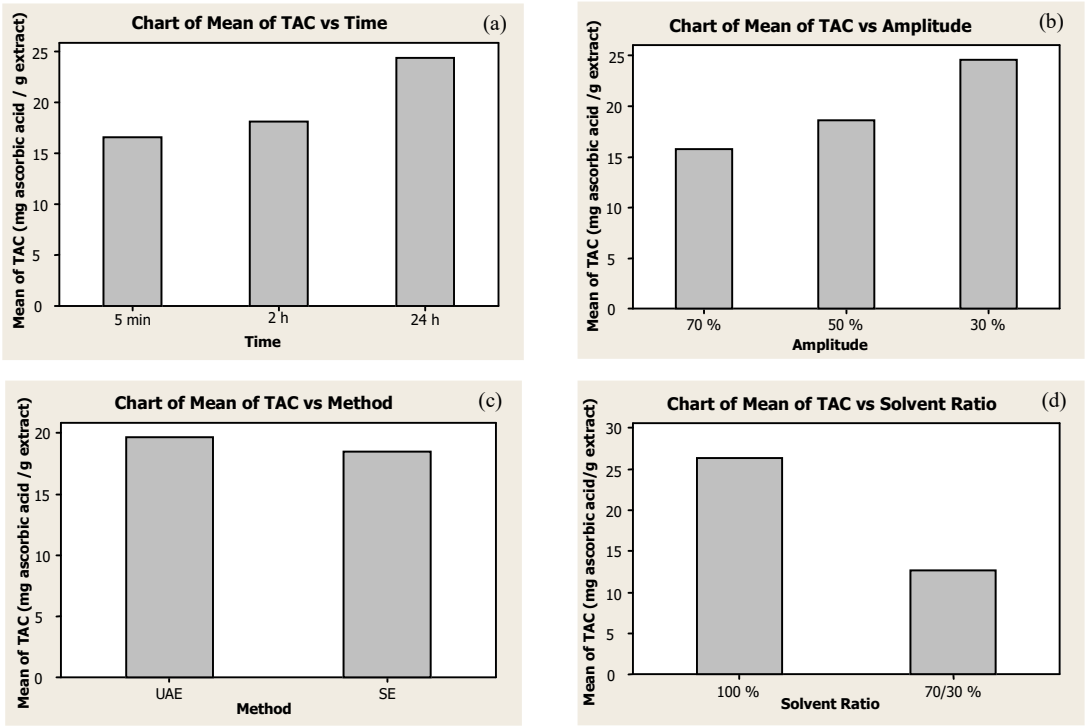

Figure 3. *Effect of maceration time (a), amplitude (b), method (c) and solvent ratio (d) on the level of Total Antioxidant Capacity (TAC)*

Table 7 shows relative between the amounts of TFC, TPC and TAC extracted using different conditions of extraction a further analysis of the variance of the main effects between the variables studied and their significance was performed using one way ANOVAs.

Table 7. *Analysis of variance for the main effects of factors studied*

	Factors	P value
TPC	Time of maceration	0.000
	Amplitude	0.000
	Method	0.000
	Solvent ratio	0.000
TFC	Time of maceration	0.000
	Amplitude	0.000
	Method	0.000
	Solvent ratio	0.001
TAC	Time of maceration	0.000
	Amplitude	0.000
	Method	0.000
	Solvent ratio	0.000

As it can be seen there is a statistically significant difference in the amounts of TFC, TPC and TAC using different maceration time ($p < 0.000$, Table 7) with the lowest amount obtained at 5 minutes maceration time. Furthermore a decrease of amplitude lead to an increase in TFC, TPC extracted and TAC as can be seen in Figures 1b, 2b and 3b, respectively. The effect of this factor has a significant statistically with ($p < 0.000$, Table 7). However the use of UAE-M extraction had an effect on the amounts of TFC, TPC extracted from potato and TAC (Figures 1c, 2c and 3c) with the lowest amount obtained by Soxhlet extraction. Table 7 shows a statistically significant with $p < 0.000$. It should be pointed out that the amount of TFC, TPC, and TAC increased with a higher ratio of ethanol with statistically significant ($p < 0.001$) (Table 7).

The amount extracted for both TPC and TFC is remarkably improved when using UAE-M over Soxhlet extraction. These results are in accordance with other data found in the literature which showed in an enhancement of the levels of phytochemicals extracted presented in different food matrixes after sonication [27 – 29]. However the UAE can bring a reduction in the size of vegetables and/or changes in the cell structure because of the ultrasonic cavitation, this structural disruption can provide a better contact between the solvents and cells allowing an increase in the content of the extracted compounds [30], additionally the increase of TPC and TFC depends on UAE conditions like amplitude, the decrease of this factor leads to higher amounts of TPC and TFC extract. The solvent ratios also show significant effect on the amount of TPC, TFC and TAC contents [31]. Finally, all these factors qualify the UAE-M to be a better technique than Soxhlet extraction. The most efficient method for the extraction of a high yield of bioactive compounds is to use UAE with 30 % of amplitude, ethanol as solvent and prior maceration of the sample for 24 hours.

HPLC analysis

All the standards were separated within 30 min and showed good resolution between analyte peaks. Figure 4 shows the chromatogram of mixed standard solution.

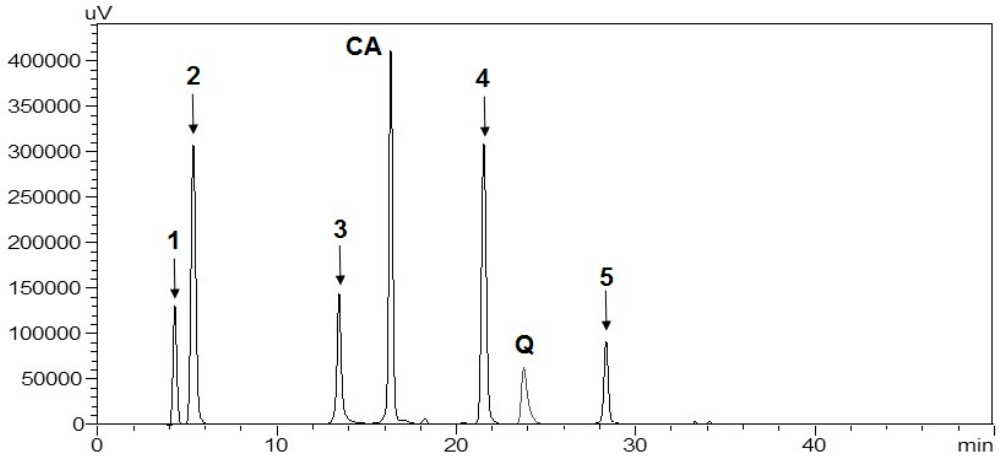

Figure 4. *Chromatograms of standard phenolic compounds: 1. ascorbic acid; 2.gallic acid; 3. chlorogenic acid; CA. caffeic acid; 4. vanillin; Q. quercetin; 5. rutin*

The calibration curve of the individual phenolic compound was based on these five concentrations of standard solutions. The peak area values were the average values of three replicate injections. The results of calibration equations and correlation coefficients are summarized in Table 8, and a good correlation was found between the peak area (y) and the concentrations (x) ($R^2 > 0.995$) for all the compounds in the range of concentration tested at 300 nm.

Table 8. *Calibration equations and regression coefficients for all standards*

Peak No	Standard	Retention Time [minutes]	Calibration equation	Regression coefficient (R^2)
1	Ascorbic acid	4.23	y = 2851.73x + 212	0.998
2	Gallic acid	5.29	y = 23616.36x - 723	0.996
3	Chlorogenic acid	13.39	y = 39775.06x - 188	0.999
4	Vanillin	21.46	y=80555.42x+321	0.995
5	Rutin	28.37	y=31189.46x+184	0.998

The HPLC analysis, based on the chromatographic profile of the extract of sliced fresh peeled potatoes of Kondor variety (Figures 5 - 8), shows that ascorbic acid ranges from 0.12 to 1.18 $\mu g \cdot mg^{-1}$, the gallic acid ranges from 0.81 to 1.37 $\mu g \cdot mg^{-1}$, the chlorogenic acid ranges from 0.27 to 1.53 $\mu g \cdot mg^{-1}$ for all the sample extract. However, the vanillin ranges from 0.004 to 0.18 $\mu g \cdot mg^{-1}$ for samples A, C and D. We note that the rutin (0.532 $\mu g \cdot mg^{-1}$) is only found in C sample. From the point of view of quantity, the A sample has the highest concentrations in ascorbic acid and chlorogenic acid.
The results show that all the samples contain at least three bioactive compounds using either UAE-M or SE as an extraction technique and two ratios for the ethanol as solvent.

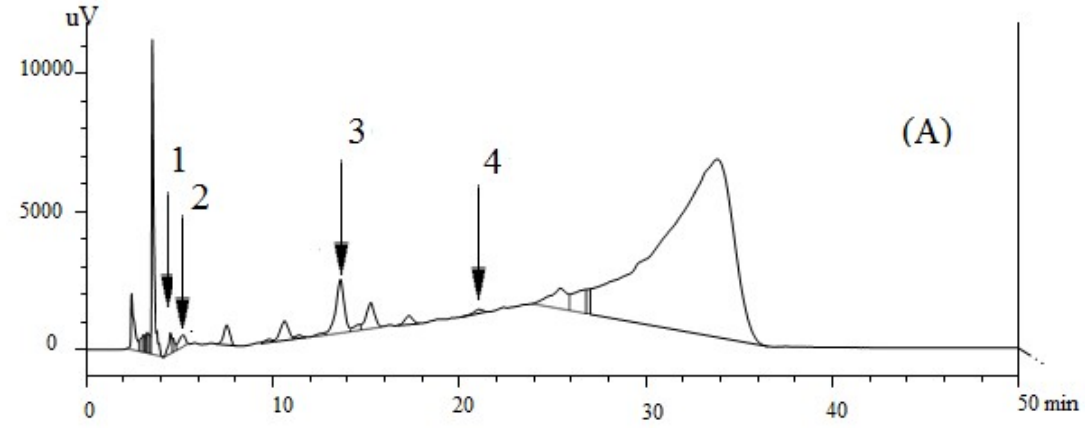

Figure 5. *Chromatograms of sample extract A:*
1. ascorbic acid; 2. gallic acid; 3. chlorogenic acid; 4. vanillin

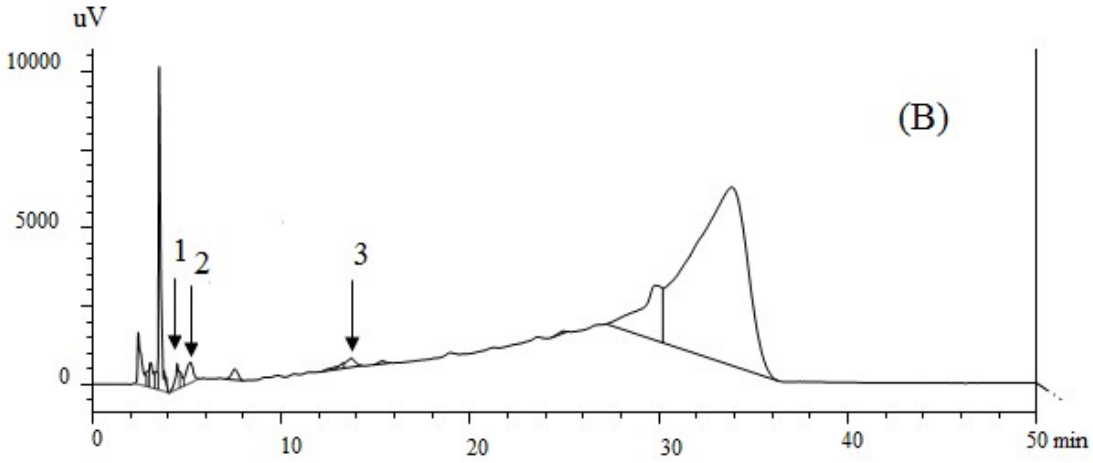

Figure 6. *Chromatograms of sample extract B:*
1. ascorbic acid; 2. gallic acid; 3. chlorogenic acid

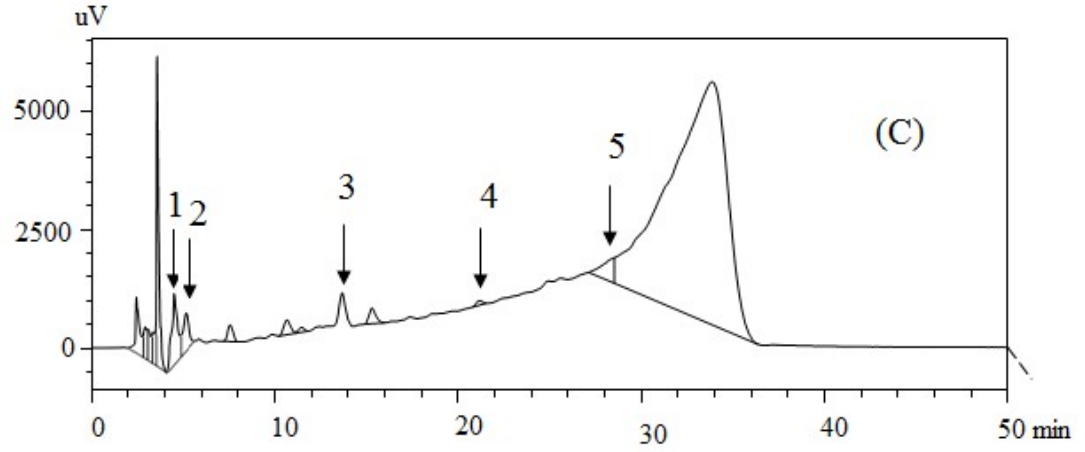

Figure 7. *Chromatograms of sample extract C:*
1. ascorbic acid; 2. gallic acid; 3. chlorogenic acid; 4. vanillin; 5. rutin

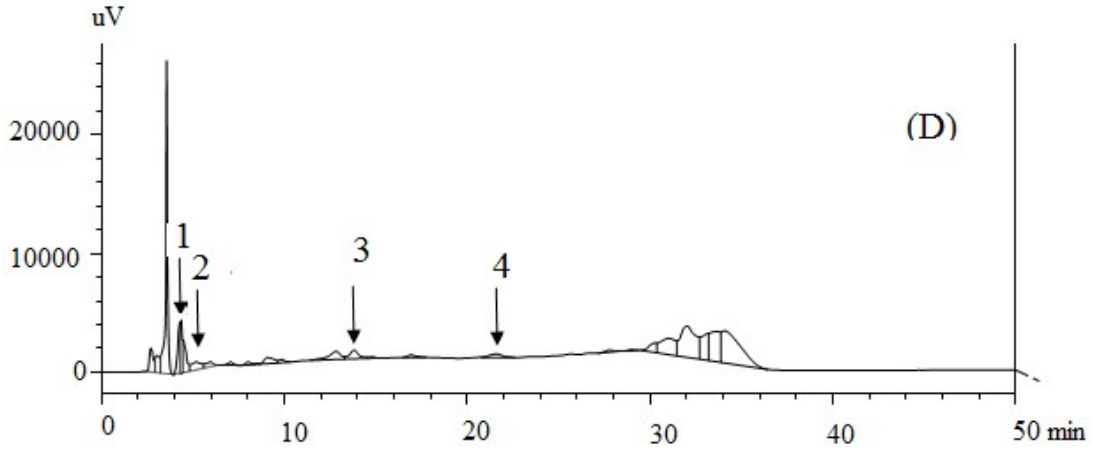

Figure 8. *Chromatograms of sample extract D:*
1. ascorbic acid; 2. gallic acid; 3. chlorogenic acid; 4. vanillin

From the chromatograms of four extract samples A, B, C and D, the quantities of bioactive compounds are shown in Table 8.

Table 9. *Constituents' content analyzed by HPLC*

Method	Ascorbic acid [µg·mg^{-1}]	Gallic acid [µg·mg^{-1}]	Chlorogenic acid [µg·mg^{-1}]	Vanillin [µg·mg^{-1}]	Rutin [µg·mg^{-1}]
A	1.1831	0.8108	1.5359	0.0702	-
C	0.4135	1.095	0.4768	0.0043	0.0095
D	0.3702	1.3752	0.6970	0.1869	
B	0.1288	1.0670	0.2768	-	-

A, C: sample extracted with UAE (30 % amplitude, 24 h maceration) and solvent ration [100 % ethanol, (70:30 ethanol/water)]
B, D: sample extracted with Soxhlet and solvent ration [100 % ethanol, (70:30 ethanol/water)]

Results showed that all the samples contain the three bioactive compounds (ascorbic acid, gallic acid, chlorogenic acid) using either UAE-M or SE methods but the A sample has the highest constituents of bioactive compounds.
The results obtained in this work demonstrate that the techniques used and the duration of maceration after sonication of the sample increases the amount of TFC, TPC, and TAC. The amplitude and the solvent ratio also affect the amount of TPC, TFC, and TAC. Finally, we can say that the UAE-M represents a better technique compared with SE.

CONCLUSION

In this study, two extraction techniques were used to evaluate the total phenolic contents, total flavonoids contents and total antioxidant capacity of roots extracts from fresh south Algerian red potatoes cultivars (*Solanum tuberosum* L.). These techniques are ultrasonic-assisted extraction combines with maceration and Soxhlet extraction. The obtained results indicated that the amount extracted of both total phenolic and flavonoids contents is remarkably improved when using UAE-M. The yield of

extraction also improved when maceration is used prior to ultrasonic-assisted extraction. The most efficient method for extraction of TPC and TFC is UAE-M with 30 % of amplitude, ethanol as solvent and maceration time of 24 hours. Finally, all obtained results are confirmed by HPLC analysis.

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