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Article *in* Journal of Applied Research on Medicinal and Aromatic Plants · February 2021

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Journal of Applied Research on Medicinal and Aromatic Plants 22 (2021) 100301

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Optimization of microwave extraction method of phenolic compounds from red onion using response surface methodology and inhibition of lipoprotein low-density oxidation

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ARTICLE INFO

Keywords:
Red onion
Microwave extraction
Lipoprotein low density
Phenolic compounds
Antioxidant activity

ABSTRACT

In the present study, we have optimized by surface response methodology (BBD model) phenolic compounds recovery using microwave-assisted extraction (MAE) as an innovative technique. The extraction was carried out under different experimental conditions: ethanol/water (x_1, 30–80 %), time (x_2, 30–180 s), microwave power (x_3, 500–900 W) and solvent to sample ratio (x_4, 20:1 40:1, v/w). Total phenolics content (TPC) and antioxidant activities (AA) of onion extract were investigated on 2,2−9-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$)$^{\bullet}$, *1,1-diphenyl-2-picrylhydrazyl radical* ($DPPH^{\bullet}$), oxygen radical absorbance capacity (ORAC) tests, and on biological model oxidation, human low-density lipoprotein (LDL). The maximum predicted TPC under the optimal MAE conditions (70 % ethanol), 700 W microwave power, 65 s extraction time and 25 mL g^{-1} solvent to solid ratio), was 10.90 ± 0.88 mg GAE g^{-1} DW. The AA of MAE or conventional extraction (CE) extract showed similar activities to neutralize DPPH, ABTS or AAPH derived radicals. The modified Gompertz model has been successfully used to describe CDs production kinetic in LDL Cu^{2+}-mediated oxidation. This allowed the estimation of the lag time (*Tlag*) and the oxidation propagation rate (R_p) with precision. LDL oxidation assay showed that red onion extract increased significantly *Tlag* and giving an increased protection ratio at higher concentration (2.95 μmol L^{-1}) of 180 % and 172 % for MAE and CE methods, respectively. However, R_p and CDs production remained constant. Results showed that microwave used for extraction does not affect the composition and antioxidant properties of onion sample. Red onion may be an important source of bioactive compounds and could promote health consumers.

1. Introduction

The reactive oxygen species (ROS) plays an important role in disease development. Recently, the interest and necessity of antioxidative nutraceuticals for prevention and promotion of health have been largely reported (Lee et al., 2004). These radical species (ROS) may attack protein, lipid and DNA causing various physiological disorders such as atherosclerosis, vasospasms, cancers, trauma, stroke, asthma, retinal damage and liver injury… (Lee et al., 2004).

The colour of fruits and vegetables represent a lot about their nutritional value. It is attributed to bioactive substances like minerals, vitamins, antioxidants and phytochemicals. Fruits and vegetables have low calorific value, but play a considerable role in the human diet as a main source of bio-active compounds (Patel et al., 2019). For this reason, they are gaining interest and popularity, and are used as antioxidants, nutraceuticals and medicines to treat several diseases (Patel et al., 2019).

Onion (*Allium cepa* L.), one of the very important agricultural economic crops, is largely planted around the global as vegetables with around 640 million tons of outputs (Mallor et al., 2014). Onions is rich in

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https://doi.org/10.1016/j.jarmap.2021.100301
Received 8 April 2020; Received in revised form 23 November 2020; Accepted 18 February 2021
Available online 25 February 2021

antioxidants and specially flavonoids, with protective effects against different chronic and/or degenerative pathologies (Pérez-Gregorio et al., 2011), and is reported to be the richest source of flavonoids in the human diet (Škerget et al., 2009). The major classes of flavonoids in onions fruits are anthocyanins (cyaniding glycosides) and flavonols (quercetin glycosides) and these phytochemical compounds are effective and stronger scavengers of various free radicals (Benkeblia, 2005). In addition, onions can be considered as a good source also of natural additives to retard deterioration of foods (Navas et al., 2006). In fact, flavonoids from onion, due to their antimicrobial and antioxidant properties, may be used as additives to increase food shelf-life by inhibiting oxidative deterioration and microbial spoiling (Naz et al., 2008; Ramos et al., 2006). Generally, red cultivars possess the highest phenolics and flavonoids and exhibit the highest antioxidant activities among cultivars (Škerget et al., 2009).

However, the significant loss of flavonoids during storage and processing of nutraceutical products and flavonoid-rich food commodities has been observed (Zafrilla et al., 2001). Hence, food scientists are trying to enhance the presence of flavonoids in foods by exploiting new natural sources with development and optimization of extraction methods for efficient recovery and maximum phenolic compounds yield (Routray and Orsat, 2012).

Nowadays, in the most of the conventional extraction techniques like maceration or supercritical fluid extraction (SFE) used to recover phytochemicals compounds was supplemented with « assisted » extraction methods where an additional physical phenomenon is applied to intensify the process and increase the yield (Renard, 2018). Since 2005, microwave (MAE) and ultrasound-assisted extraction (UAE) were the most studied applications according to the scientific publication trends (Mandal et al., 2007). Microwave processing modifies physical properties of the biological tissues by increasing the pressure builds up inside the treated biomaterial which improves the porosity of biological material and thus, offers better penetration of extracting solvent (Kratchanova et al., 2004; Yeoh et al., 2008).Vian et al. (2009); (Zill e et al., 2011) have optimized the extraction of flavonoids from various varieties of onion using solvent-free microwave hydro diffusion and gravity (MHG) technique and reported that MHG is the preferable method compared to the conventional method, as the samples showed the highest antioxidant capacities, and offers advantages like cleaner feature (no solvent used) and shorter extraction time (23 min). Jin et al. (2011) optimized the extraction of quercetin from onion skin using conventional solvent extraction (CSE), MAE and UAE. The MAE was the best productive method but only two parameters (ethanol concentration and time) were optimized. Kumar et al. (2014) studied the extraction by MAE of quercetin from solid onion (*Allium cepa L.*). Indeed, Yan et al. (2015) have evaluated the extraction of flavonoids from onion by hot water, ethanol and ultrasonic extraction method and their determination by ultraviolet–visible spectroscopy.

In our knowledge, there is no report in the literature on the optimization of MAE (with solvent) of phenolic compounds from red onion variety. For this, the present work consists to study the optimization of microwave extraction method of phenolic compounds from the onion bulb using response surface methodology (RSM). The different operating factors were studied and the range for each factor is fixed from a previous preliminary study (data not shown), as ethanol/water (x_1, 30–80 %), time (x_2, 30–180 s), microwave power (x_3, 500–900 W) and solvent to sample ratio (x_4, 20:1−40:1, v/w). Also, a comparative study between MAE and the conventional method was carried out in the respect to TPC and antioxidant activities evaluated by chemical tests such as DPPH, ABTS and ORAC assays, and by a biological test such as inhibition of low-density lipoprotein oxidation induced by copper (Cu^{++}). The kinetic of conjugated dienes (CDs) production from LDL oxidation was plotted by a modified Gombertz model.

2. Material and methods

2.1. Chemicals and standards

Butylatedhydroxytoluene (BHT), and 2 N Folin-Ciocalteu reagent were purchased from Sigma Aldrich Chemical Co. (Saint Quentin Fallavier, France). Gallic acid and 2',7'-dichloro-fluorescein were obtained from Merck (Darmstadt, Germany). Ethanol (EtOH) used for preparing standard solutions was from Prolabo (Paris, France). 2.2'-Azobis-2-amidinopropane hydrochloride (AAPH) was from Biovalley (Conches, France).

2.2. Plant material preparation

Red onion samples were from Bejaia region (Algeria). They were cut manually and dried in a ventilated oven at 40 °C for 4 days. Then, they were grounded using an electric grinder. The powder obtained is subsequently sieved by an electric sifter until to obtain a fine powder ($\leq$ 125 μm), which is stored in a closed bottle and protected from light.

2.3. Extraction procedure

2.3.1. Microwave-assisted extraction (MAE) method

The extraction procedure was previously described in our previous study (Dairi et al., 2014). A domestic microwave oven (NN-S674MF, LG, Japan, 32 L, 1000 W; variable in 100 W increments, 2.45 GHz), with cavity dimensions of 22.5 × 37.5 × 38.6 cm was used to extract onion phenolic compounds. The extraction vessels are made from Pyrex and have a capacity of 150 mL. The vessel was placed in the middle of the oven and connected to the conderser to condense the vapors generated during extraction of the sample and the mixture was exposed to microwave radiation. The temperature inside the vessel doesn't exceed 85 °C. Then, the extraction was carried out under different experimental conditions: Ethanol/water (x_1, 30–80 %), time (x_2, 30–180 s), microwave power (x_3, 500–900 W) and solvent to sample ratio (x_4, 20:1 40:1, v/w) (Table 1). The range and conditions of microwave-assisted

Table 1
Box–Behnken design with the observed responses and predicted values for yield of total phenolics content (TPC) of dried onion fruits using microwave-assisted extraction.

X_1-Ethanol/ water (%)	X_2-Time (s)	X_3-Power (W)	X_4-Liquid-solid (mL/g)	Y-TPC (mg GAE g^{-1} DW)
30	105	700	40	9.68
55	180	500	30	10.09
55	105	500	20	8.40
55	30	700	40	10.20
55	180	700	20	10.97
30	105	500	30	8.13
80	30	700	30	9.89
55	30	500	30	9.19
80	105	700	40	8.77
80	180	700	30	12.39
55	180	700	40	9.63
80	105	700	20	11.26
30	105	900	30	7.67
55	105	500	40	8.55
55	105	700	30	10.03
55	30	900	30	10.69
55	105	900	20	9.00
55	105	700	30	10.69
30	180	700	30	8.92
80	105	900	30	9.42
30	30	700	30	11.95
30	105	700	20	8.54
55	105	700	30	10.43
55	30	700	20	10.30
55	105	900	40	8.55
55	180	900	30	8.41
80	105	500	30	8.62

extraction (MAE) were accomplished according to a preliminary study that was carried out to determine the appropriate range for each extraction variable. After MAE treatment, the sample was filtered with a sintered glass at 0.45 μm using a vacuum pump and stored at 4 °C until further use.

2.3.2. Conventional extraction (CE) method

The extraction method used for dried onion samples was as reported in a previously published protocol (Dairi et al., 2014): 20 mL of solvent EtOH/water (50/50; v/v) was added to 1 g of dried onion in a round bottom flask. The mixture was stirred carefully for 60 min with magnetic agitation at room temperature. After that, the process was the same as for MAE procedure.

2.4. Optimisation procedure

According to the single-factor experimental results (data not shown), influencing factors on the response were confirmed, and then a Box–Behnken design (BBD) with response surface methodology (RSM) was conducted to design the experimental work, and to identify the relationship between the response and variables as well as to determine conditions that could optimise the extraction process. In this investigation, four factors are chosen and coded as follow: X_1: ethanol concentration (30–80 %), X_2: extraction time (30–180 s), X_3: microwave power (500–900 W) and X_4: solvent-solid ratio (20–40 mL/ g) (Table 1). Then, a second-order polynomial model which indicated 27 experiments to be required for this procedure (Table 1). The general equation of the second-degree polynomial equation is:

$$Y = B_0 + \sum_{i=1}^{k} Bixi + \sum_{i=1}^{k} Biix^2 + \sum_{i>j}^{k} Bijxixj + E \quad (1)$$

Where Y represents the response function (in this case the TPC yield); B_0 is a constant coefficient; *Bi, Bii* and *Bij* are the coefficients of the linear, quadratic and interactive terms, respectively, and *xi* and *xj* represent the coded independent variables. According to the variance analysis, the regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between independent and dependent variable and to deduce the optimum conditions, the regression coefficients were used to generate 3D surface plots from the fitted polynomial equation. The factor levels were coded as −1 (low), 0 (central point or middle) and 1 (high), respectively. The variables were coded according to the following equation:

$$x_i = \frac{(X_i - X_0)}{\Delta X} \quad (2)$$

Where x_i is the (dimensionless) coded value of the variable X_i; X_0 is the value of X at the center point and ΔX is the step change.

2.5. Measurement of antioxidant activities

2.5.1. Chemical tests

2.5.1.1. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging assay. The DPPH assay was performed according to the method developed by Dudonne et al. (2009). 3 mL of DPPH• solution (60 μmol L^{-1}) were mixed with 100 μL of sample extracts or of standard such as BHA or α-tocopherol at various concentrations. The mixtures were incubated for 20 min at 37 °C in a water bath, and the absorbance was recorded at 515 nm.

The DPPH• inhibition is expressed as percentage and was calculated according to the following equation: % loss of DPPH •= [(AC (DPPH•) – AA (sample)/AC (DPPH•)] x 100, where AC (DPPH•) is the control absorbance at time =0 min; and AA (sample) is the absorbance of DPPH• in the presence of antioxidant at time =20 min. The antioxidant activity of each test sample and standards was expressed as the concentration required to inhibit 50 % DPPH• radicals (IC_{50} expressed as mg L^{-1}) and calculated from the log-dose inhibition curve.

2.5.1.2. 2,2-9-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+}$)• radical cation decolorization assay. $ABTS^{+}$ •scavenging activity was determined according to a method of Re et al. (1999). Before use, the ABTS+ •solution was diluted until an absorbance of 0.700 ± 0.020 at 734 nm with ethanol. Then, 1 mL of $ABTS^{+}$ •solution was mixed to 1 mL of onion extract or standard ethanolic solutions (BHA or α-tocopherol) at different concentrations. The results were expressed as the reduction percentage of absorbance for each sample concentration expressed as mg GAE L^{-1} for onion extract or as mg L^{-1} for pure standards. The IC_{50} (concentration providing 50 % inhibition) was calculated from the log-dose inhibition curve.

2.5.1.3. Oxygen radical absorbance capacity (ORAC). ORAC values were measured with fluorescence spectrometer (Victor^{2}Wallac–Perkin-Elmer, USA) by inhibition of 2′,7′dichlorofluorescein (DCF) consumption as reported by Ishimoto et al. (2011) with slight modifications as mentioned in our previous work (Dairi et al., 2014). The analyzer was programmed to record the fluorescence of DCF every minute after addition of AAPH. Briefly, 50 μL test samples or 50 μL Trolox solutions (0–20 μmol L^{-1}), 100 μL DCF solution (50 nmol L^{-1}), and 100 μL AAPH solution (20 mmol L^{-1}) were added to the wells of a 96-well plate. Then, the fluorescence of DCF was recorded every for 90 min at 485 nm-excitation and 535 nm-emission wavelengths. ORAC levels were expressed as mol of Trolox equivalent (TE) per mol of antioxidant (pure compounds) or per mol of GAE for onion extracts. For this, a calibration curve was established between the time needed to obtain 50 %-fluorescence decay versus the Trolox concentrations.

2.5.2. Biological test

LDL isolation and evaluation of onion effect on LDL oxidation mediated by Cu^{2+} ions

The procedure is the same that was reported in our previous work (Dairi et al., 2014). LDL was isolated from fresh plasma of healthy human subjects, obtained from the "French Blood Establishment", in accordance with its ethical rules. Their oxidizability was monitored at 234 nm for 5 μmol L^{-1} Cu^{2+} oxidation as previously indicated (Monde et al., 2011). Briefly, isolated LDL was diluted to 1 μmol apoB L^{-1}, added with the various onion extract concentrations to be tested, and then 10-fold diluted in oxygenated PBS at pH 7.4. The cumulative conjugated dienes (*CDs*) production at any time *t* was noted $CDs^{(t)}$, and it was plotted against time. The modified Gompertz model was used to describe CDs production kinetic:

$$CDs^{(t)} = CD_{\max}\ exp\left[- exp\left(\frac{R_p\ e}{CD_{max}}\ (Tlag - t) + 1\right)\right] \quad (3)$$

Where $CD_{\max}$ is the maximal cumulative *CDs* production (CD-mol apoB-mol^{-1}), R_p the cumulative *CDs* production rate (CD-mol apoB-mol^{-1} min^{-1}) and *Tlag* the lag time (min).

To evaluate the antioxidant abilities of the extracts, we used the notion of specific antioxidant activity (SAA), which was calculated as the slope of the linear relationship obtained between relative lag time (r*Tlag*), and concentrations of the different tested compounds (Monde et al., 2011). Relative *Tlag* was defined as [*Tlag*+/*Tlag*−] × 100, with + and – denoting LDL with and without antioxidants, and *Tlag* was defined as the time corresponding to the end of the 1 st kinetic phase during which absorbance (A) do not or only slowly increase. An increased protection ratio was calculated using the following formula: protection ratio (%) = [(*Tlag*+ – *Tlag*−)/*Tlag*−] ×100, with + and − denoting LDL with and without tested antioxidants. Finally, the *CD*max value was calculated as A_{max}/ε, where A_{max} corresponded to maximal oxidized product accumulation and ε is the specific absorption coefficient of *CDs*,

and was then expressed as mol CD_{max} mol^{-1} apoB min^{-1}. An inhibitory ratio of the rate of oxidation propagation (R_p) was finally calculated using the following formula:

$$\text{Inhibitory ratio } (\%) = \left[R_p^- - R_p^+ \Big/ R_p^-\right] \times 100 \quad (4)$$

2.6. Parameter estimation and statistical methods

The modified Gompertz model parameters were estimated using nonlinear regression with a curve fitting toolbox (MATLAB 6.5, The Math-Works Inc., Natick, MA, USA). The root mean squared error (*RMSE*) between all experimental $Y^{(t)}$ and predicted data, adjusted coefficients of determination ($R^2_{adjusted}$) and confidence intervals (calculated with 95 % of probability) were used as goodness-of-fit indicators for the estimated parameters. Analyses were carried out three times or more and results were reported as mean values ± standard deviation (SD). Data were compared on the basis of the mean values. Differences among means of variety groups were tested using a Tukey-Kramer HSD (Software JMP version 7.0) with a significance level of 0.05.

3. Results and discussion

3.1. Optimization of microwave-assisted extraction (MAE)

Based on the preliminary study (data not shown), the ranges of each independent variable (ethanol concentration X_1, extraction time X_2, extraction power X_3, and liquid-solid ratio X_4) influencing the TPC yield were chosen. Then, a Box-Behenken design model analyzed randomly the experimental data obtained from the 27 trials. Table 1 showed that the TPC ranged from 8.13 to 12.39 mg g^{-1} DW, which means that the studied factors have an influence on the extraction yield of these bioactive compounds.

The analyze of variance and the statistical parameters shown in Table 2 indicate that the model is adequate to predict the extraction yield of antioxidants by microwave procedure from onion samples. At a 95 % confidence level, a model was considered significant, if the *p*-value is < 0.05. In fact, *p*-value probability of the regression model were <0.05 which indicates a high significance of the model. Determination coefficient (R^2), adjusted determination coefficient (R^2 adj), predicted determination coefficient (R^2 pre) and variation coefficient (CV, %) were determinated to verify the goodness of the proposed models with the experimental data. The statistical analysis showed that the determination coefficient R^2 is 0.96 which means that only 4% of the total variations are not explained by the model. Indeed, the value of $R^2_{adjusted}$ (0.92) is high, $R^2_{predicted}$ 0.81 is in reasonable agreement with $R^2_{adjusted}$ and that confirm that the model is highly significant. CV value (3,45 %) and root mean square error (*RMSE* = 0.33) were very low, which indicate that the experimental and predicted values are very close and thus, there is a better precision and reliability of the experimental data. The proposed model presented also an adequate precision value of 17.652 (more than 4), which corresponds to an adequate signal.

Two statistical parameters, *p*-value and F-value, are determined to evaluate the significance of each coefficient as shown in Table 2. Smaller *p*-value and greater F-value mean that the studied variables are significant on the considered response. The proposed model has a *p*-value < 0.0001, which means that it is significant and can be used to optimize the different extraction variables.

Table 2 shows that the independant variables have a significatif effects on TPC yield. Onion TPC recovery was affected more significantly by ethanol concentration X_1 ($p < 0.0001$), followed by ratio X_4 ($p = 0.02$).But all the other linear terms (time X_2 and power X_3) were insignificant ($p > 0.05$). In addition, various interactions between factors were observed such as X_1X_2 ($p < 0.0001$), X_1X_4 ($p = 0.0001$ and) X_2X_3 ($p = 0.0005$). This means that each factor affect one another. Finally, the quadratic terms presented a significant effect on the response for all the independant variables : X_3^2 ($p < 0.0001$), X_2^2 ($p = 0.0016$), X_4^2 ($p = 0.0040$) and X_1^2 ($p = 0.0374$). The mathematical modeling of the extraction of TPC as a function of the different parameters is represented by the following Eq. (5) which shows the relation between the microwave extraction variables and the TPC as response (Y) excluding non-significant terms:

$$Y(\text{TPC}) = 10.38 + 0.45x_1 - 0.26x_4 + 1.38x_1x_2 - 0.91x_1x_4 - 7.9x_2x_3 - 0.34x_1^2 + 0.58x_2^2 - 1.4x_3^2 - 0.51x_4^2 \quad (5)$$

To visualize the mutual interaction between variables on TPC yield a three-dimensional response surface were plotted as shown in Fig. 1.

Fig. 1A shows the response surface plotted from the interaction between solvent concentration (X_1) and the irradiation time (X_2). From the result, an increase in the TPC content has been observed with the increase in the ethanol concentration from 45 % to 65 % and the irradiation time from approximately 55–80 s. The recovery of TPC principally depends on the ethanol concentration as its quadratic, linear and interaction (with time and liquid-to-solid) effects were highly significant ($p < 0.001$). This finding was in agreement with our previous conclusions on extraction of natural phenolic compounds from *Citrus limon* peels (Dahmoune et al., 2013), *Pistacia lentiscus* leaves (Dahmoune et al., 2014) and *Myrtus communis* leaves (Dahmoune et al., 2015). Other literature studies reported also the same conclusions about ethanol concentration on TPC recovery from plant materials, such as apple

Table 2
Analysis of variance (ANOVA) for the experimental results obtained by using microwave assisted extraction for red onion phenolic compounds recovery.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	35.73	14	2.55	23.01	< 0.0001	significant
X_1-ethanol	2.48	1	2.48	22.40	0.0005	
X_2-Time	0.27	1	0.27	2.46	0.1427	
X_3-Power	0.048	1	0.048	0.43	0.5225	
X_4-Ratio	0.80	1	0.80	7.17	0.0201	
X_1X_2	7.65	1	7.65	68.92	< 0.0001	
X_1X_3	0.40	1	0.40	3.58	0.0829	
X_1X_4	3.29	1	3.29	29.70	0.0001	
X_2X_3	2.53	1	2.53	22.79	0.0005	
X_2X_4	0.38	1	0.38	3.47	0.0873	
X_3X_4	0.090	1	0.090	0.81	0.3854	
X_1^2	0.61	1	0.61	5.48	0.0374	
X_2^2	1.83	1	1.83	16.52	0.0016	
X_3^2	10.49	1	10.49	94.57	< 0.0001	
X_4^2	1.39	1	1.39	12.57	0.0040	
Residual	1.33	12	0.11			
Lack of Fit	1.11	10	0.11	1.00	0.5967	not significant
Pure Error	0.22	2	0.11			
Cor Total	37.06	26				

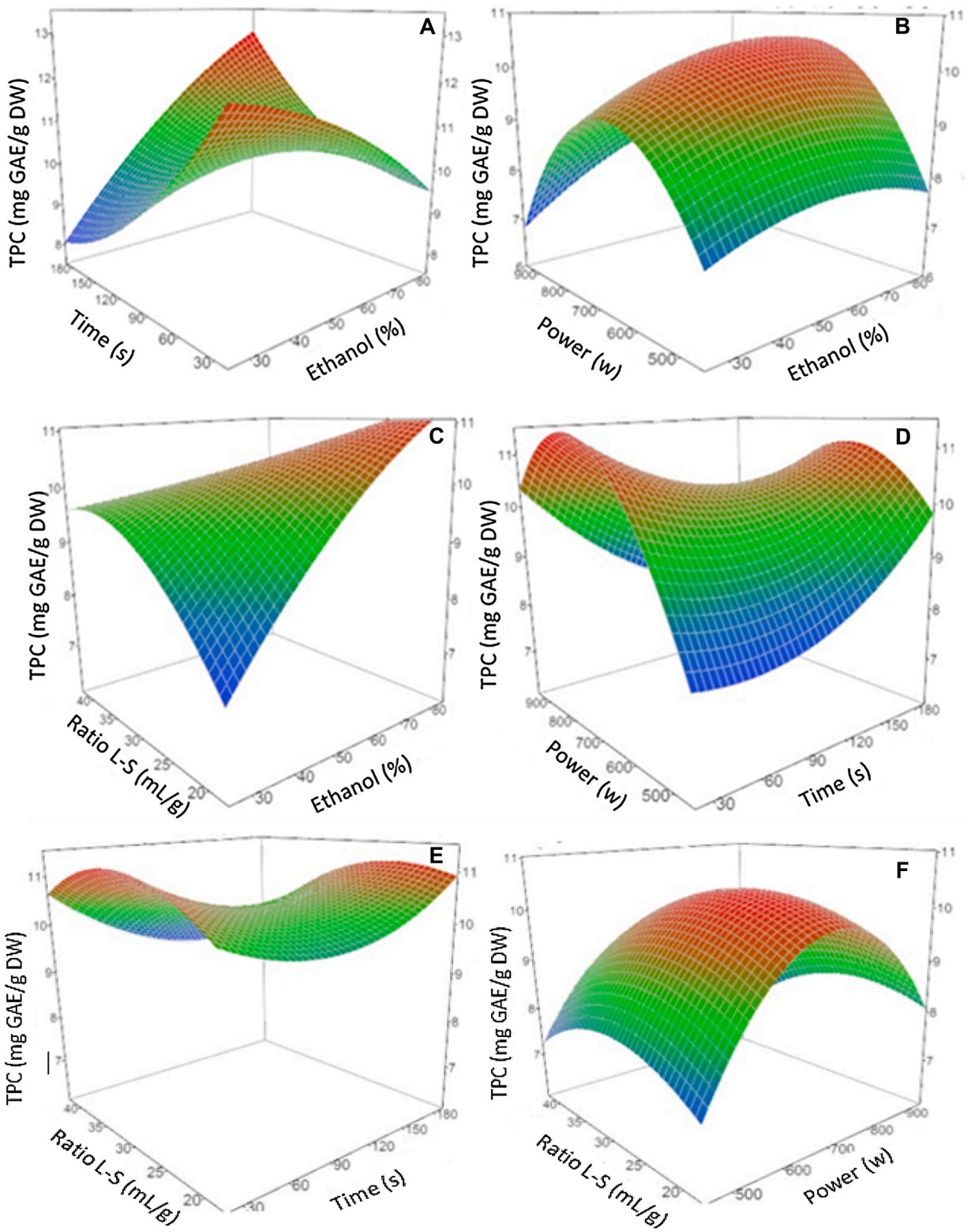

Fig. 1. Response surface analysis for the total phenolics yield from onion with microwave assisted extraction with respect to ethanol and irradiation time (A); ethanol and power (B); ethanol and liquid-solid ratio (C); irradiation time and microwave power (D); irradiation time and liquid-solid ratio (E); microwave power and liquid-solid ratio (F).

pomace (Bai et al., 2010) and green tea leaves (Pan et al., 2003). In the other hand, the tendency has been reversed when the irradiation time and solvent concentration were higher than 85 s and 70 %, respectively, and thus, a remarkable decrease in the TPC yield is noted which is already demonstrated in the preliminary study that a longer exposition time by microwave causes degradation of the thermolabile constituents. Fig. 1B shows the interaction effect between ethanol concentration and liquid/solid ratio. It is observed that TPC content increased with the increase in the solvent concentration from 45 % to 80 % and the liquid-solid ratio from approximately 20–35 mL / g. Moreover, a

stability in the TPC content is observed when the liquid-solid ratio is within the range of 35–40 mL / g. This suggested that higher ratio could reduce the microwave extraction efficiency. The Fig. 1C shows the effect of extraction time and microwave power on TPC recovery from onion sample. The increase in microwave power and extraction time increased phenolic compound content up to a value approximately of 10.50 mg g^{-1} DW. However, a long extraction time with a strong microwave power gave a low content. This is probably due to the compounds degradation by microwave heating.

According to the proposed model, the optimal conditions to carry out for onion phenolic compounds recovery under microwave extraction are: ethanol concentration 70 %, microwave power 700 W, irradiation time 65 s and liquid-solid ratio of 25 mL/g. Under these conditions, the model predicted a maximum response of 10,56 ± 0,47 mg g^{-1} DW. In order to validate again the established model, three additional experiments were carried out, and onion TPC content presented a value of 10,90 ± 0,88 mg GAE/ g DW which is very closed and significantly not different with the predicted one. This suggest that the model had a high suitability to optimize microwave extraction conditions for onion compounds recovery. Since the microwave irradiations are not totally absorbed, by the extraction solvent, we have calculate the true energy and power absorbed under these optimized conditions, and the results give values of 1,716 W mL^{-1} and 111, 54 J s^{-1} for absorbed power density (APD) and absorbed energy density (AED) respectively.

3.2. Microwave-assisted extraction vs conventional extraction

3.2.1. Total phenolic compounds content

The total phenolics content (TPC) and antioxidant activities (DPPH, ABTS and ORAC tests) of red onion sample and standards are presented in Table 3. Both methods, MAE and CE, presented the same TPC level, 10.90 ± 0.88 and 10.82 ± 0.11 mg GAE g^{-1} DW for MAE and CE extracts, respectively. This result is in agreement with several studies showing that microwave extraction method could constitute an alternative method for phenolic compounds recovering from plants (Dairi et al., 2015). In the work of Zill e et al. (2011), the solvent-free microwave hydro diffusion and gravity (MHG) technique is considered as the preferred extraction procedure in comparison to the conventional technique for onion phenolic compounds recovery, and this is because microwaves induced vacuoles and cell wall disruptions, thus promoting the effectiveness of this method.

3.2.2. Effect of extraction methods on antioxidant activities

There is evidence of discrepancies in antioxidant activities of natural compounds when evaluated in different model systems. For this, in the present study, the antioxidant activities of onion extracts were measured using several assays, including DPPH, ABTS and ORAC assays and Cu^{++} mediated-LDL oxidation in vitro model. These popular methods are based on the ability and capacity of phenols to transfer H-atoms or electrons to radicals including peroxyl radicals (ORAC, inhibition of lipid peroxidation) and metal ions (Antolovich et al., 2002).

Table 3
Total phenolics content and antioxidant activities of red onion extracts.

	TPC (mg GAE g^{-1} DW)	DPPH (IC_{50})	ABTS (IC_{50})	ORAC (mol TE mol^{-1} antioxidant)
Onion MAE	10.90 ± 0.88[a]	328.36 ± 17.66 [a]	2.96 ± 0.14[b]	1.20 ± 0.12[a]
Onion CE	10.82 ± 0.11[a]	343.58 ± 7.73 [a]	3.65 ± 0.12[a]	0.95 ± 0.09[ab]
BHA	–	95.81 ± 1.39 [c]	0.89 ± 0.07[d]	0.72 ± 0.14[b]
α-tocopherol	–	167.29 ± 7.07 [b]	1.49 ± 0.11[c]	–

The values were expressed as the mean of three measurements (n = 3) ± standard deviation (SD). Means followed by different letters in the same column are significantly different ($p < 0.05$).

3.2.2.1. Chemical assays. The model of scavenging DPPH free radicals is widely used to evaluate the antioxidant ability of plant extract. The DPPH scavenging activity of onion extracts was expressed as IC_{50}. Lower IC_{50} value indicated higher antioxidant activity. The both onion extracts obtained by MAE or CE, exhibited the same scavenger ability towards DPPH radicals ($p < 0.05$), and this is in agreement with phenols content, and which confirms previous findings, that phenols are mainly responsible for the radical scavenging activity of extracts (Škerget et al., 2009). However, MAE or CE onion extracts exhibited an antioxidant activity 3.42 or 3.58 time lower than BHA, respectively, and 1.96 or 2.05 time lower than α-tocopherol, respectively. Thus suggesting that antioxidant complex mixture such as plant extract showed less efficacy than pure antioxidant when the evaluation assay used revealed only one mechanism of action, and this is confirmed by several studies (Dairi et al., 2015).

In the ABTS assay, which allowed to determine the electron-donating capacity of antioxidant compounds, showed that onion extract obtained by MAE and CE method may reduce ABTS radicals with a dosedependent manner (with a slight advantage for MAE extract) and giving an IC_{50} value of 2.96 ± 0.14 mg GAE L^{-1} and 3.65 ± 0.12 mg GAE L^{-1}, respectively. This result is in agreement with the work of Lu et al. (2011) who studied the antioxidant activities of onion. The comparison of onion antioxidant ability to standards showed similar result as observed with DPPH assay. In fact, MAE or CE onion extracts exhibited a reducing ability 3.32 or 4.10 time lower than BHA, respectively, and 1.98 or 2.45 time lower than α-tocopherol, respectively.

In the ORAC test, the antioxidants act as inhibitor of AAPH-induced oxidation of fluorescein by reducing the peroxyl radicals (ROO·) derived from AAPH decomposition, and this assay could be considered as a preferred method because of its biological relevance to the in vivo antioxidant efficacy (Oueslati et al., 2012). Table 3 showed that onion extracts obtained by MAE and CE methods showed similar results with an ORAC value of 1.20 ± 0.12 and 0.95 ± 0.09 mol TE mol^{-1} GAE, respectively. Our ORAC values for onion extracts were comparable to those described by Zill e et al. (2011) (1.36 mmol TE $mmol^{-1}$ GAE or 1.09 mmol TE $mmol^{-1}$ GAE for solvent free microwave hydrodiffusion and gravity MHG or CE extract). It was observed also that onion extracts were more effective than BHA with an ORAC value of 0.72 ± 0.14 mol TE mol^{-1}. This result did not correlate with ABTS or DPPH result. This supposes that in ORAC assay, the antioxidants present in the onion extracts could neutralise as well as AAPH-derived radicals and/or regenerate oxidized fluorescein. Indeed, onion extract is rich in quercetin derivatives as reported previously by several studies (Beesk et al., 2010; Lombard et al., 2002) and it is well established that molecules with higher number of hydroxyl group were more potent antioxidant in AAPH-derived radicals neutralisation (Cao et al., 1997).

3.2.2.2. Inhibition of copper-induced LDL oxidation by onion extract. Oxidation of low-density lipoprotein (LDL) has been strongly suggested as a key factor in the pathogenesis of atherosclerosis. Thus, the inclusion of some anti-oxidant compounds in daily dietary foodstuff may prevent the LDL from oxidation and may decrease both the development and the progression of atherosclerosis (Ahmadvand et al., 2011). In this present study, we evaluate the inhibitory effects of *Allium cepa* (onion) extracts on LDL oxidation induced by $CuSO_4$ in vitro at different physiological concentrations (0–2.95 μmol GAE L^{-1}). The kinetics of lipid oxidation was monitored at 234 nm and which correspond to CD formation. Fig. 2 illustrates the good fit obtained between experimental cumulative CDs production data and the predicted curves using the optimized kinetic parameters reported in Table 4. The $R^2_{adjusted}$ values and the relative uncertainty (mean value of estimated parameters/confidence interval) for all tested concentrations of polyphenol extracts were calculated to be > 0.99 and < 10 %, respectively. The RMSE value was in average 9.1

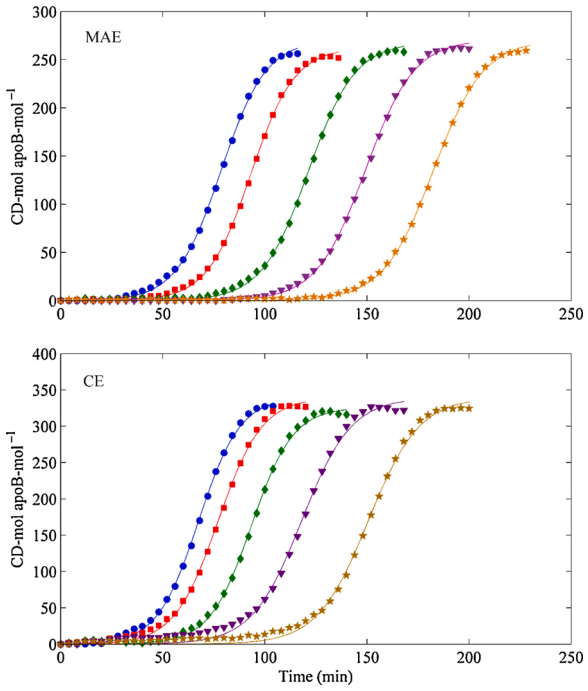

Fig. 2. Experimental (symbols) and simulated (solid lines) CDs production kinetic by LDL Cu2+-mediated oxidation in the presence of polyphenol extracts of onion obtained by MAE or CE methods (● 0, ■ 0.74, ◆ 1.48, ▼ 2.21 and ★ 2.95 μmol GAE L^{-1}).

(mol apoB mol^{-1}) for all trials, indicated that the predictions describe the experimental data satisfactorily. This suggests that the kinetics of CDs production by LDL Cu^{2+}-mediated oxidation in the presence of onion extract for MAE or CE methods were well represented by the modified Gompertz model. To our knowledge, this model has never been used before to describe CDs production kinetic by LDL Cu^{2+}-mediated oxidation.

As shown in Fig. 2, the presence of onion extracts in the media increased, in a concentration-dependent manner, the lag phase of conjugated diene (CD) formation. Thus, suggesting that onion extract protect LDL from oxidation induced by copper as previously shown for others plant extracts (Carbonneau et al., 2014; Dairi et al., 2014; Monde et al., 2011). As reviewed, red onions are very rich in flavonols particularly quercetin or quercetin derivatives (quercetin 4′-glucoside and quercetin 3,4′-diglucoside) in all onion cultivars, and also the anthocyanins of red onions (approximately 10 % of the total flavonoid content) are mainly cyanidin glucosides acylated with malonic acid or non-acylated. In this regard, it is worthwhile to mention that onions are considered among the major sources of antioxidant compounds (Slimestad et al., 2007; Tedesco et al., 2015). This result was in agreement with the work of Ahmadvand et al. (2011) where it was shown that extract of *Allium cepa* was able to inhibit LDL oxidation and increase their resistance against in vitro oxidation. Indeed, the work of Carbonneau et al. (2014) studied LDL oxidation under the same experimental conditions used in this present work showed that quercetin present the strongest activity in delaying LDL oxidation compared to other flavonoids, and this is due to its chemical structure with the presence of the two adjacent 3′, 4′ OH groups at ring B or 3−OH (when ring C contains a double bond between C2–C3 and the carbonyl group at position 4) (Vaya et al., 2003).

To better understand the action mechanism of onion extracts on oxidation kinetic, we have determined their effect on different characteristic kinetic parameters (Table 4). As shown in Table 4, onion extract increased significantly *Tlag* at different concentrations tested giving an increased protection ratio at higher concentration (2.95 µmol L^{-}1) of 180 % and 172 % for MAE and CE methods, respectively. However, the oxidation propagation rate (R_p) presented a slight decrease at the higher concentration but remain significantly not different compared to the oxidation kinetic of the control. the R_p decreased significantly ($p < 0.05$) and giving an inhibitory ratio of 10.93 and 15.66 % for MAE and CE, respectively, which is not in agreement with our last wok (Dairi et al., 2014) where it was observed a decrease of R_p when *Myrtus communis* extract was used (at the same concentration). This observation confirmed the relationship between plant extract composition and antioxidant activity. Indeed, the formation of oxidation products evaluated as CD_{max} remained constant for all tested concentrations. SAAs of onion extracts were evaluated under the Cu^{2+}-mediated oxidation system, and results were expressed as µmol of GAE L^{-1} (Fig. 3). Higher SAA value, indicated higher antioxidant activity. As shown in the Fig. 3 onion MAE extract showed higher antioxidant activity than onion CE extract one giving SAA values of 67.47 ± 2,66 and 57.81 ± 4.71 µmol of GAE L^{-1} respectively, and which is in agreement with ORAC results. However, onion extract SAA values were lower 3,21 and 3,75 times than quercetin, respectively. The lowest SAA value was observed for α-tocopherol which lacking in the catechol structure. In our previous studies, it was observed that plant extract was more or less effective than pure compounds and this is depending on their chemical structure and plant extract composition (Carbonneau et al., 2014; Dairi et al., 2014).

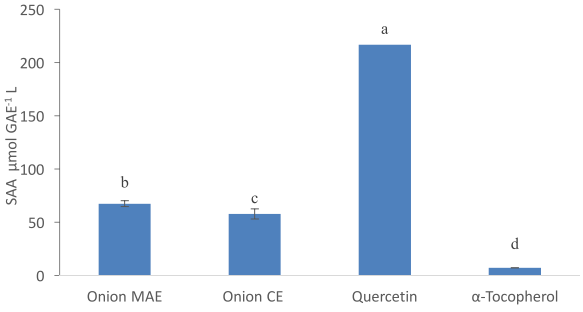

Fig. 3. SAA values of onion extracts and quercetin and tocopherol standards. Results are expressed as means of 3 experiments ± SD. Different letters indicate that samples are significantly different ($p < 0.05$).

Table 4
Effect of polyphenol extracts of onion obtained by MAE or CE methods on the estimated kinetic parameters of LDL oxidation mediated by Cu^{2+} ions. Mean values ± 95 % confidence interval.

Concentration (µmol GAE L^{-1})	*Tlag* (min)		Rp (CD-mol apoB-mol^{-1} min^{-1})		CD_{max} (CD-mol apoB-mol^{-1})		R^2 adjusted		*RMSE*	
	MAE	CE	MAE	CE	MAE	CE	MAE	CE	MAE	CE
0	55.9 ± 1.9^{e}	45.9 ± 2.0^{e}	6.4 ± 0.6^{a}	8.3 ± 0.8^{a}	261.5 ± 5.5 ^{a}	345.2 ± 11.9 ^{a}	0.994	0.994	8.5	10.8
0.74	70.9 ± 1.7^{d}	54.5 ± 2.1^{d}	6.3 ± 0.5^{a}	8.0 ± 0.8^{a}	259.1 ± 4.6 ^{a}	341.6 ± 10.1 ^{a}	0.995	0.994	7.3	11.2
1.48	97.5 ± 1.7^{c}	71.7 ± 1.84^{c}	6.0 ± 0.5^{a}	8.0 ± 0.7^{a}	264.8 ± 4.3 ^{a}	331.0 ± 8.5 ^{a}	0.996	0.995	7.2	9.6
2.21	124.3 ± 1.6^{b}	92.8 ± 2.5^{b}	5.9 ± 0.4^{a}	7.2 ± 0.7^{a}	268.2 ± 4.1 ^{a}	340.6 ± 11.6 ^{a}	0.997	0.992	6.7	12.7
2.95	156.8 ± 1.6^{a}	124.7 ± 2.1^{a}	5.7 ± 0.4^{a}	7.0 ± 0.6^{a}	265.6 ± 4.0 ^{a}	341.3 ± 10.1 ^{a}	0.997	0.993	6.4	10.6

RMSE: Root Mean Squared Error between experimental and estimated CDs. Means followed by different letters in the same column are significantly different ($p <$ 0.05).

Transition metal ions may induce lipid peroxidation via a complex series of reactions, being dependent on the oxidizable lipids, concentrations of antioxidants, and preformed hydroperoxides (Pinchuk and Lichtenberg, 2014). As reviewed by Pinchuk and Lichtenberg (2002), if an antioxidant prolongs the lag and reduces the maximal rate of copper-induced peroxidation, the first conclusion is that the mechanism implies is either binding of copper ions or blocking of copper-binding sites of the lipoprotein. On the other hand, it was shown in several studies that addition of antioxidants in LDL oxidation system, delay vitamin E consumption which let suppose that the added antioxidant may also act as a regenerating agent of vitamin E (Monde et al., 2011). These remarks concluded that in a complex system, the antioxidant may act by various ways to exert their effects, contrary when chemical tests were used.

4. Conclusion

Our study showed that microwave-assisted extraction may be an alternative method to recover phenolic compounds from *Allium cepa* (onion) and in a short period compared to the conventional one. This procedure may be used to recover valuable products from onion and thus, their application in food, pharmaceutic and cosmetic industries. The chemical tests revealed that MAE onion extract present similar antioxidant activities and, in some case, better antioxidant competence like observed in ABTS, DPPH and ORAC assay. Modified Gompertz model was used to describe CDs production kinetic by LDL Cu^{2+}-mediated oxidation; it has been successfully fitted. This fitting allowed us to estimate the lag time (*Tlag*) and the oxidation propagation rate (R_p) with precision. MAE onion extract present higher SAA value than CE onion extract, and which means that MAE onion extract prevents Cu^{2+} induced-LDL oxidation by delaying *Tlag* phase more effectively than the extract obtained by the conventional extraction. However, the R_p and CDs max production remained constant. These remarks showed that microwave used for extraction do not affect the composition and/or antioxidant properties of onion sample and the optimized parameters could be used to recover effectively onion phenolic compounds.

Declaration of Competing Interest

The authors report no declarations of interest.

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