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Biochemical Characterization of Tunisian *Cichorium Intybus* L. Roots and Optimization of Ultrasonic Inulin Extraction

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Abstract: In this study Cichorium intybus L. roots were tested for its chemical composition, antioxidant activity, and phenolic profile. Optimization of ultrasonic inulin extraction using response surface methodology (RSM) was further investigated. Chicory roots were found to have high value of total carbohydrates (70.43%), soluble fiber (66.93), Neutral detergent fiber (NDF) (33.07%), potassium (380 mg/100g), calcium (540 mg/100g) and sodium (140 mg/100g). Chicory roots exhibit a high content of flavonoids, polyphenols, and tannins. Antioxidant activity measurement reveals the capacity of Chicory roots to scavenge diphenylpicrylhydrazyl (DPPH) radicals. Phenolic acids profile shows the abundance of vanillic acid (19.64%) followed by protocatechuic acid (15.67%). The effect of three independent variables namely extraction time, the ratio of water to raw material and temperature on inulin extraction was studied. Optimum deciding responses were Inulin content, Total Soluble Solids (TSS) content and Water produced inulin yield. The optimal ultrasonic extraction conditions were: extraction time 87 min, liquid to solid ratio 38 (ml/g) and ultrasonic temperature 61˚C. Under these conditions, the inulin content, TSS content and produced inulin yield were 35.92%, 24.72%, and 32.53%, respectively. The produced inulin was characterized by the Fourier infrared transformation (FTIR) and observed by means of scanning electron microscopy (SEM).

Keywords: Ultrasonic extraction, Cichorium intybus L., produced inulin, antioxidant activity, phenolic profile.

Introduction

Cichorium intybus L. (chicory) belongs to Asteraceae family and widely distributed in Asia and in Europe [1]. In Tunisia, the production of chicory is very limited in terms of cultivated area and that we consume in general only the leaves. The rest including roots are intended for animal feeding. Chicory is a plant with tuberous roots that store inulin, a fructan polysaccharide, composed of high fructose content (about 94%) and a terminal glucose molecule. In the human organism, inulin act in a similar way than dietary fibers, contributing to the improvement of the gastro-intestinal system conditions. The fresh chicory plant contains about 68% inulin, 14% sucrose, 5% cellulose, 6% protein, 4% ash, and 3% other compounds, while dried chicory contains approximately 98% inulin and 2% other compounds [2]. The chicory roots are a source of many bioactive compounds such as fructans, polyphenolic acids (5-caffeoylquinic–chlorogenic, dicaffeoylquinic, chicoric acid), polyphenol glycosides, including derivates of quercetin, apigenin, luteolin and sesquiterpenes [3]. The conventional technology used to produce inulin involves the following steps: liquid extraction, filtration, evaporation and spray drying. The energetic costs of this process are very high due to the concentration and drying stages. Ultrasonic extraction has been widely used to isolate bioactive substances from different parts of plants [4]. Using ultrasound is a highly efficient tool for the fast extraction of active compounds. In fact, ultrasound treatment could disrupt tissues of the cell plant

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materials and enhanced the mass transfer of the solvents into the materials and the soluble constituents into the solvents. The efficiency of the extraction process is influenced by many conditions such as power, frequency, temperature and time of sonication. In this context, the aims of this work are to characterize the Tunisian Cichorium intybus L. roots and to optimize the ultrasonic extraction of inulin. Therefore a response surface methodology (RSM) was designed to optimize a mathematical model representing the relationship between the response (Inulin content, TSS content and produced inulin yield) and some parameters of the ultrasonic extraction, such as extraction time, ratio of water to raw material and extraction temperature.

Materials and methods

Preparation of plant materials

The chicory was harvested in the spring, during February 2014, before flowering stage. The chicory roots were provided by farmers in Hammam Ejdid - Hammamet located in the north-eastern region of Tunisia. The highest inulin concentration occurs in chicory roots during the above-mentioned harvesting period [5]. After flowering, the inulin concentration in roots decreases, due to its conversion into fructose, which will be absorbed by the plant later. After harvesting, the fresh chicory roots were washed several times with water jet to remove remaining soil and other impurities. In sequence, deteriorated parts were removed and roots sliced into pieces of 3 to 5 mm in thickness and dried in an oven at 60 ◦C for 48 h until a constant weight was obtained [6]. The dried material was then ground in a high-speed disintegrator to obtain a powder and passed through a 40-mesh sieve with a particle diameter of less than 0.5 mm [7]. The chicory roots powder was then stored at –10 °C in sealed bags for future use. Dried and grounded chicory roots were used as the starting material.

Chemical analysis

Moisture, protein, ash and fat contents of each sample were determined according to the AOAC nos 934.01, 940.26, 920.152 and 930.09, respectively [8]. Total carbohydrates were determined by the phenolsulphoric acid method of Dubois et al. [9] Reducing sugars were estimated by 3,5-dinitrosalicylic acid (DNS)[10]. Both measurements were determined using glucose (Sigma) as standard. The total soluble solids were measured using a hand Abbe´refractometer and expressed as the degree Brix (ºBrix). The measurements were taken at 20º C. The contents of hemicellulose, cellulose, neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined in the chicory root powder by the Van Soest detergent method using Fibertec system 2010 (Foods, Sweden)[11]. To remove carbon, the chicory root powder was incinerated in a muffle furnace at 550°C for 16 h [12]. Obtained ash was dissolved in HNO3 [13] and the mineral constituents were determined using an atomic absorption spectrophotometer (Hitachi Z-6100, Japan). All analytical determinations were performed at least in triplicate.

Phytochemical composition of chicory roots extract

Polyphenol extraction

2.5 g of chicory roots powder were extracted by magnetic stirring with 25 mL of pure methanol (80%) for 30 min at room temperature (25º C)[14]. The extract was then kept for 24 h at 4°C, filtered through a Whatman No.4 filter paper, evaporated under vacuum to dryness and stored at 4ºC until further analysis. Obtained extract will serve for the quantification of polyphenols components and the evaluation of antioxidant activities.

Determination of total phenolics contents

The total phenolics content was determined by the Folin–Ciocalteu spectrophotometric method (UV–VIS) [15]. 1 ml of the methanol extract was mixed with 1 ml of Foline–Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 10 ml of 7% sodium carbonate solution (w/v) were added, and the final volume was made up to 25 ml with distilled water. After 1 h of reaction at ambient temperature, the absorbance at 720 nm was measured by a Spectrophotometer. Measurements were calibrated to a standard curve of prepared gallic acid solution. Total phenolic content of chicory root powder was expressed as mg gallic acid equivalents per gram of dry weight matter (mg GAE/g DM). All samples were analyzed in three replications.

Determination of total flavonoid content

For the determination of total flavonoid contents, 250 µl of the methanolic extract was mixed with 75 µl NaNO2 (5%) [16]. After 6 min, we added 150 µl of 10% aluminum chloride and 5 min later, 500 µl of NaOH (1 M) was added to the mixture. Finally, the mixture was adjusted to 2.5 ml with distilled water. The absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content of chicory roots was expressed as mg catechin equivalents (CE) per gram of dry weight matter (mg CE/g DM). The calibration curve range was 50–500 mg ml−1. All samples were analyzed in three replications.

Determination of condensed tannins

In presence of concentrated H2SO4, condensed tannins were transformed by the reaction with vanillin to anthocyanidols [17]. 50 µl of the methanolic extract appropriately dilute was mixed with 3 ml of 4% methanol vanillin solution and 1.5 ml of H2SO4.

The absorbance was measured at 500 nm after 15 min. Condensed tannin contents of chicory roots were expressed as mg catechin equivalents (CE) per gram of dry weight through the calibration curve with catechin. The calibration curve range was 50–600 mg ml−1. All samples were analyzed in three replications

DPPH radical scavenging activity

The DPPH• scavenging activity was estimated according to Hatano et al. [18]. Briefly, 2 ml of methanolic extracts at different concentrations ranging from 1 to 200 µg ml−1 were added to 0.5 ml of a 0.2 mmol−1 DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark, and then the absorbance was measured at 517 nm. For each dilution of the extract, the DPPH• scavenging activity was calculated using the following equation:

DPPH scavenging effect (%) = $((A_0 – A_1) /A_0)\times100$

Where A0 is the absorbance of the control at 30 min, and A1 is the absorbance of the sample at 30 min. The antiradical activity was finally expressed as IC50 (µg ml-1). A lower IC50 value corresponds to a higher antioxidant activity of the plant extract [19]. All samples were analyzed in three replications.

Hydrolysis and identification of phenolic compounds using RP-HPLC

Chicory roots powder was hydrolysed according to the method of Proestos et al [20], slightly modified. 40 ml of methanol containing BHT (1 g l^{-1}) were added to 0.5 g of a dried chicory root sample. Then 10 ml of 6 M HCl were added. The mixture was stirred carefully and then sonicated for 15 min and refluxed in a water bath at 90 ◦C for 2 h. For the determination of the phenolic compound acids, the obtained extract was injected to RP-HPLC (Agilent Technologies 1100 series liquid chromatograph coupled with a UV–Vis multi-wavelength detector). The separation was carried out on a 250 × 4.6-mm, 4-m Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 ml/min. The gradient program was as follows: 15% A/85% B 0–12 min, 40%A/60% B 12–14 min, 60%A/40%B 14–18 min, 80%A/20% B 18–20 min, 90%A/10%B 20–24 min, 100% A 24–28 min [21]. The injection volume was 20µl, and peaks were monitored at 280 nm and 330nm. Samples were filtered through a 0.45µm membrane filter before injection. Peaks were identified according to their retention times compared with those of standards.

Ultrasonic extraction of inulin powder

Chicory roots powder was extracted in an ultrasonic cleaner (BRANSON 3210 ,130 watt, 70% amplitude and a frequency of 20 kHz , Institute Pasteur , Tunisia.) with distilled water, using selected extraction time (range from 30 to 90 min), ultrasonic temperature (range from 60 to 80°C), solvent to raw material (range from 20 to 40 ml/g). The temperature of the bath was controlled with a constant temperature water-bath. After filtration, inulin extract samples were collected, concentrated to 20°Brix by a rotary evaporator at about 40°C [22] and analyzed for total carbohydrates, reducing sugar and total soluble solids content (°Brix). The inulin content in each sample was measured with the difference between total carbohydrates and reducing sugars [23]. Then, inulin extract solutions were lyophilized (72 h at -49 ◦C and $62*10^{-3}$ mbar), collected and weighed to get produced inulin powder. The produced inulin powder yield was determined by the following formula:

Produced inulin powder yield (%) = (produced inulin powder weight (g)/chicory root powder weight (g)) × 100

Determination of inulin quality by FT-IR and SEM analysis

Produced inulin powder, at optimal ultrasonic extraction conditions, and commercial inulin (Sigma–Aldrich) were investigated for FT-IR and SEM analysis. Fourier transform infrared spectra (FT-IR) of inulin samples were recorded on a Nicolet MAGNA-IR 560 spectrometer. Inulin samples were dispersed in a matrix of KBr at room temperature, the mixture was pressed into pellets. The range of spectra was from 400 to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1}. The microstructures of inulin samples were observed by scanning electron microscopy (SEM). Samples were sprinkled onto carbon double-sided tape on SEM stubs and covered with gold using a sputter coater to make the specimen conductive. Then, these gold-coated specimens were analyzed in a Microtrac Semtrac Mini (Nikkiso, Tokyo, Japan) scanning electron microscope.

Experimental design

When many factors affect any desired response, it can be an exhausting task to optimize a process. Therefore, response surface methodology (RSM) was employed to optimize multiple variables to predict the best performance conditions with a minimum number of experiments [24]. In the present study, a statistical experimental design based on central composite design (CCD) (for the experiments) and RSM (for identifying the optimal levels) [25] were planned to optimize the ultrasonic extraction of inulin and to analyze the effect of each variable, their interactions, and second-order terms. Table1shows maximum (coded as 1), minimum (coded as −1) and central (coded as 0) levels for each independent variable in the CCD: ultrasonic extraction time (X_1), ratio of water to raw material (X_2) and ultrasonic temperature (X_3). The experimental responses inulin content (Y_1),

Total Soluble Solids (TSS) content (Y_2) and produced inulin yield (Y_3) for different selected levels of variables are shown in Table 2 for 19 runs.

Table 1. Independent variables and factors levels in the response surface design.

Variables	factors Levels		
	-1	0	1
Extraction time (min)	30	60	90
Ratio of water to raw material (ml/g)	20	30	40
Temperature (˚C)	60	70	80

Table 2. Experimental conditions of the central composite design and the corresponding experimental responses.

Assay	X_1 ultrasonic extraction time	X_2 ratio of water to raw material	X_3 ultrasonic temperature	Y_1 Inulin content (%)	Y_2 TSS content (%)	Y_3 Produced inulin yield
1	-1	-1	-1	28.70	23.64	17.215
2	1	-1	-1	21.50	27.13	21.983
3	-1	1	-1	34.64	24.60	23.140
4	1	1	-1	30.93	25.90	27.956
5	-1	-1	1	30.01	29.26	27.088
6	1	-1	1	23.95	31.58	24.506
7	-1	1	1	27.69	20.32	25.273
8	1	1	1	25.12	20.56	22.740
9	-1	0	0	45.15	21.39	32.470
10	1	0	0	40.26	23.26	33.587
11	0	-1	0	27.39	26.35	37.905
12	0	1	0	30.95	21.32	39.984
13	0	0	-1	48.84	23.16	40.519
14	0	0	1	49.80	22.27	42.847
15	0	0	0	47.79	21.88	49.158
16	0	0	0	47.82	21.78	48.154
17	0	0	0	47.93	21.58	47.191
18	0	0	0	47.69	21.57	49.214
19	0	0	0	47.88	21.44	48.885

In this work, the relationship between the inulin content, TSS content, produced inulin yield and the three selected quantitative variables was approximated by the following second order polynomial equation:

$$Y = b_0 + b_1 * X_1 + b_2 * X_2 + b_3 * X_3 + b_{11} * (X_1)^2 + b_{22} * (X_2)^2 + b_{33} * (X_3)^2 + b_{12} * (X_1*X_2) + b_{13} *X_{13}+b_{23} *X_{23} + e$$

Where (Y) is the calculated response function, X_1, X_2 and X_3 are the levels of the independent variables, b_0 is the intercept term, b_1, b_2 and b_3 are the linear coefficients, b_{11}, b_{22} and b_{33} are the quadratic coefficients, b_{12}, b_{13} and b_{23} are the interaction coefficients and e is the global error. The coefficients were determined by multiple linear regressions. The Nemrod-w software package was used for the regression analysis of the experimental data obtained. The Fit quality of the polynomial model equation was determined by calculating the coefficient R^2. Significance level was given as *** P< 0.001, **P< 0.01, *P< 0.05. All differences with a p-value superior to 0.05 were not considered significant. For CCD validation, optimum conditions were fixed on the basis of the data obtained from experimental design.

Statistical analysis

The results of the chemical and phytochemical composition of chicory roots powder were statistically analyzed. Analytical values were determined, using three independent determinations. The values of the different parameters were expressed as mean (n=3) ± standard deviation (x ± SD), and reported on a dry matter basis.

Results and Discussion

Chemical Composition of chicory root

The chemical composition of *Cichorium intybus* L. roots is presented in table 3. Results reveal that chicory roots contain a high level of moisture (87.57%) and total carbohydrate (70.43%) contents. However, low-fat (3.01%), protein (5.17%) and ash (8.96) contents were found. The obtained results were similar to the values recorded by Monti et al. [26] who found that crude protein and ash ranged from 8.56 to 15.73% and 9.58 to 13.75%, respectively. On the other hand, the chicory roots were characterized

by its high concentration of soluble fiber (66.93%), NDF (33.07%), ADF (18.04%), lignin (16.91%) and hemicelluloses (15.03%). The Total soluble sugars in chicory roots used in our study are about 12.33%. These results are in accordance with those obtained with chicory roots from Egypt as determined by Mona et al.[27]. Indeed, insoluble fiber content and total soluble sugars were found to be 30.73% and 11.06%, respectively. As it's known that the mainly soluble fibers present in chicory roots were fructans, their presence confirms the choice of using chicory roots for inulin extraction.

Table 3. Chemical composition of *Cichorium intybus* L. roots (g/100 g DM).

Components	Values
Moisture content	87.57±0.05
Protein	5.17±0.17
Fat	3.01±0.5
Ash	8.96±0.07
Total carbohydrates [a]	70.43±0.05
Total soluble sugars	12.33±0.04
NDF	33.07±1.54
ADF	18.04±0.86
Lignin	16.91±0.61
Hemicellulose	15.03±0.68
Cellulose	1.13±0.61
Soluble fiber	66.93±0.01

[a] Total Carboyhydrates = 100- moisture- fat- protein- ash,
NDF: neutral detergent fiber, ADF: acid detergent fiber

Mineral compositions (calcium (Ca), potassium (K), sodium (Na), iron (Fe), zinc (Zn), manganese (Mn), lead (Pb), copper (Cu), Cobalt (Co) and chromium (Cr) of chicory roots were summarized in table 4. In the present study, a predominance of Ca (540 mg/100 g DM), K (380 mg/100 g DM) and Na (140 mg/100 g DM) was observed. Potassium is an essential mineral micronutrient in human nutrition. It is the major cation inside animal cells, and it is thus important in maintaining fluid and electrolyte balance in the body [28]. Na, Ca and K are in large quantity compared with those of Mona et al.[27]. The heavy metal composition (iron, zinc, manganese, lead, copper, cobalt and chromium) revealed that lead (0.79 mg/100 g DM) was the most abundant element in the chicory roots followed by Iron (0.74 mg/100 g DM). Trace element plays a crucial role in the medicinal value of a plant, in human health and to cure diseases. The obtained results are different from the values reported by Kaneez et al. [29]. The high content of minerals in chicory roots was attributed to its deep tap root which can absorb minerals that are inaccessible to shallow rooted plant species. Hence, the chemical composition of *Cichorium intybus* L. roots (high amount of carbohydrates, soluble fibers, and minerals) could be justified by the search for value-added use of by-products of this plant to improve food quality.

Table 4. Mineral composition of *Cichorium intybus* L. roots(mg/100g DM).

Minerals	Values
Cr	0.06±0.02
Co	0.09±0.01
Cu	0.07±0.01
Mn	0.26±0.01
Zn	0.17±0.1
Pb	0.79±0.01
Fe	0.74±0.01
Na	140±0.00
K	380±0.00
Ca	540±0.00

Phytochemical composition of chicory roots

The phytochemical composition of *Cichorium intybus* L. roots showed the presence of polyphenols, tannins and flavonoids (Table 5). The Tannins content of *Cichorium intybus* L. roots is about 305.1 mg/100g DM. The obtained Tannins content are lower than those found by Shad et al.[30] in chicory roots (1510 mg/100g DM). While tannins content in *Cichorium intybus* L. root was found to be higher than the range (41-166 mg/100g DM) reported by Kumari et al. [31] in leafy vegetables. This difference can be explained by geographical variables (climate and soil composition), plant origin and extraction procedures. The Total flavonoids content of chicory roots is about 67.75 mg/100g DM. The content of polyphenols was about 341.25 mg EAG/100g DM.

The ability of *Cichorium intybus* L. roots to scavenge DPPH$^{\bullet}$ free radicals was measured (table 5). The IC_{50} value was found to be 54µg/ml and the level of antiradical efficiency was 18.52. Our finding is similar to that of Shad et al. [30] who showed that chicory roots are good free radical scavengers due to its high DPPH radical inhibition. *Cichorium intybus* L. has been found to have an important medicinal role in the defending system against endogenous free radicals.

Table 5. Total flavonoids, total phenolics, Condensed tannins contents and DPPH antioxidant activity of *Cichorium intybus* L.roots extract.

Parameters	Total phenolic[v]	Total Flavonoids[y]	Condensed tannins [y]	DPPH	
				IC_{50} [w]	AE [x]
Chicoryroots	341.25±5.8	67.75±43.16	305.1±51.62	54±0.001	18.52±0.01

[v]mg GAE/100 g DM);
[w] The inhibitory concentration (µg/ml): amount of antioxidant needed to decrease the initial DPPH concentration by 50%; [x]Antiradical efficiency: $1/IC_{50}$; [y]mgCatechin equivalents (CE)/100 g DM.

The phenolic acids composition of chicory roots is presented in Table 6. Results revealed that vanillic acid is the major phenolic compound (6.51 mg/g of DM), followed by protocatechuic acid (5.2 mg/g DM), Quercetin acid (4.95 mg/g DM) and cafeic acid (4.88 mg/g DM). Additionally, we have also detected chicoric, 3.4-dihydroxybenzoic, gallic, chlorogenic, trans cinnamic, 3,5-dicaffeolquinic, quercetin-3-glucuronide acids.

Table 6. Content (mg/g DM) of phenolic acids in *Cichorium intybus* L. roots extract.

Phenolic compounds	Content (mg/g DM)	%
Gallic acid	1.68	5.08
3.4-dihydroxybenzoic acid	3.67	11.07
Quercetin	4.95	14.94
Trans cinnamic acid	0.70	2.13
Quercetin-3-o-glucuronide	0.06	0.19
3,5-dicaffeolquinic acid	0.08	0.27
Protocatechuic acid	5.19	15.67
Cafeic acid	4.88	14.72
Vanillic acid	6.51	19.64
Chlorogenic acid	1.59	4.80
Chicoric acid	3.81	11.49
Total Phenolic Compounds	33.15	100

Results are in agreement with Innocenti et al.[32] who reported the presence of the same acids in four widely distributed cultivars of chicory: Catalogna, Witloof, and two commercial red varieties. Phenolic acids appeared to be mainly responsible for the strong antioxidant activities. Thus, our data support conclusions of others who attributed antioxidant activities to the presence of phenolic compounds in chicory roots [33,34].

Validation of the Model

The regression coefficients and the analysis of variance (ANOVA) indicate the high significance of the model (Table7). For statistical models, the aim for the determination of the coefficient R^2 is the prediction of future outcomes on the basis of other related information. In this study, responses Y_1, Y_2, and Y_3, yielded R^2 values of 0.99, 0.99 and 0.98, respectively. The high values of R^2 showed the good agreement between the experimental results and the theoretical values predicted by this model[35] (R^2 Pred Y_1 = 0.97; R^2 Pred Y_2 = 0.98; R^2 Pred Y_3 = 0.95 for Y_1, Y_2, and Y_3, respectively). Values of the adjusted determination coefficient were also very high to advocate for a high significance of the model[36] (R^2Adj Y_1 = 0.99; R^2Adj Y_2 =0.99; R^2AdjY_3 = 0.97) (Table 8).The significance of each coefficient was determined by P-values which were listed in Table 7. The analysis of the optimization study indicated that the coefficients b_3 (independent variable X_3) is not influential on the response Y_2 and b_1 (independent variable X_1), b_2 (independent variable X_2) and b_{12} (interaction variable X_{12}) are not influential on the response Y_3, since the value of significance for these coefficients is higher than 5%.

By means of Multi Linear Regression method [37] a quadratic regression equation was developed based on statistical experimental design [38]. The significance of all regression coefficients was checked by means of Student's test. The final equations for the optimization of ultrasonic extraction of inulin parameters derived from the application of the method (after eliminating non-significant terms) are given below:

$$Y_1= 47.449-2.442X_1+ 1.778X_2-0.804X_3 -4.279 X_1^2-17.808X_2^2+ 2.340X_3^2+0.872X_{12}+0.285 X_{13}-2.461X_{23}+e$$

$$Y_2= 21.683 +0.921X_1-2.527X_2+0.600X_1^2 +2.111X_2^2+0.990X_3^2 -0.533X_{12}-0.278X_{13} - 2.461X_{23}+e$$

$$Y_3 = 47.3456 + 1.1641X_3 - 12.8485X_1^2 - 6.9325X_2^2 - 4.1940X_3^2 - 1.8374X_{13} - 1.9349X_{23} + e$$

Table 7. Regression coefficients of the central composite design.

	Y_1 (Inulin content)		Y_2 (TSS content)		Y_3 (Produced inulin yield)	
Term	**Coefficient**	***P*-value**	**Coefficient**	***P*-value**	**Coefficient**	***P*-value**
b_0	47.449	***	21.683	***	47.345	***
b_1	-2.442	***	0.921	***	0.558	30.7%
b_2	1.778	***	-2.527	***	1.039	7.2%
b_3	-0.804	***	-0.044	59.5%	1.164	*
b_{11}	-4.279	***	0.600	**	-12.848	***
b_{22}	-17.808	***	2.111	***	-6.932	***
b_{33}	2.340	***	0.990	***	-4.194	**
b_{12}	0.872	***	-0.533	***	0.012	98.1%
b_{13}	0.285	**	-0.278	*	-1.837	*
b_{23}	-2.065	***	-2.461	***	-1.934	**

*** P< 0.001, **P< 0.01, *P< 0.05

Table 8. Regression statistics.

	Y_1 (Inulin content)	Y_2 (TSS content)	Y_3 (Produced inulin yield)
R-squared (R^2)	0.997	0.997	0.989
Adjusted R -squared (R^2Adj)	0.993	0.994	0.978
Predicted R-squared (R^2pred)	0.978	0.980	0.958
Predicted Residual Sum of Squares (PRESS)	40.928	3.429	89.525
Number of degrees of freedom	4	9	9

Interpretation of the Response Surface Model

The graphical representation for each response was developed as a simultaneous function of the three independent variables according to their significance to the response. The examination of the 3D surface plots and 2D contours for inulin content, TSS content and produced inulin yield as a function of levels of the independent variables (ultrasonic extraction time, the ratio of water to raw material and ultrasonic temperature) are shown in Figures 1 to 3. These plots are useful in studying the effects of the variation of different factors in the domain studied and consequently, in determining the optimal experimental conditions.

Inulin content

Yield is one of the major concerns for ultrasonic inulin extraction as it is closely related to the production cost and efficiency. Results revealed that the inulin content of different extraction treatments varied from 21.5% to 49.80% based on ultrasound extraction conditions. The effects of different independent variables on the inulin content were studied and the results are listed in Figure 1a–c.

As shown in Figure 1a, when the ultrasonic temperature (X_3) was fixed at 70° C, the inulin content increased with the increase of ultrasonic extraction time (X_1) when the ratio water to raw material (X_2) is between 25 (ml/g) and 35(ml/g). Figure 1bshowed the effects of ultrasonic extraction time (X_1) and ultrasonic temperature (X_3) on inulin content. The interactions between the ratio water to raw material (X_2) and extraction temperature (X_3), when ultrasonic extraction time (X_1) was fixed at 60 min, were displayed in Figure 1c. It can be noted that the ultrasonic temperature (b_3<0) has a negative effect on the inulin content, in the studied values range. As it can be seen from Figure 1b, the temperature of 61°C was the one that presented better inulin content, independently of the used ratio water to raw material and extraction time. After this temperature, the inulin content started to reduce with increasing extraction time. This result is in agreement with the observed by Toneli et al.[39] indicating an increase in mass production rate with increase in inlet air temperature during the inulin spray drying. Indeed, these results can be explained as it is the ultrasonic use which could improve mass transfer and the solvent's ease of access to the cell material of the fiber. The effects of cavitation, including macro-turbulence generated by the implosion of cavitation bubbles, and micro-jets caused by cavitation on the product surface facilitated the diffusing of all-trans b-carotene extracts of ultrasonic extraction to make the osmotic pressure between the inside and the outside of the cell different, so that the extraction of ultrasonic extraction quickly arrives at extraction equilibrium.

On the other hand, the increase of the ultrasonic time could induce by ultrasound waves, the

expansion and collapse of cavities near the cell wall as well as the turbulent vibration on the solvent–solid interfaces led to a cell disruption and speeded up both the release and diffusion of inulin into water. Thus extraction yield was significantly improved. An excessively lengthening ultrasonic times, will also induce the degradation of inulin, and then the inulin content decreased. Therefore, longer time of ultrasonic extraction was unnecessary after the maximum extraction yield was achievement.

A positive effect (b_2>0) of the ratio of water to raw material onthe inulin content was observed. In fact, the inulin content increased remarkably with the increased ratio of water to raw material from 30 to 40 ml/g. A larger ratio of water to raw material indicates greater concentration difference between the interior plant cells and the exterior solvent, and the diffusion of inulin occurred more quickly. At the same time, more inulin which could be dissolved in water, further improvement in the inulin extraction yield. For an efficient extraction, the solvent used for inulin extraction must be able to solvate the target compounds, while leaving the sample matrix intact. Therefore, water was the best solvent for ultrasonic inulin extraction.

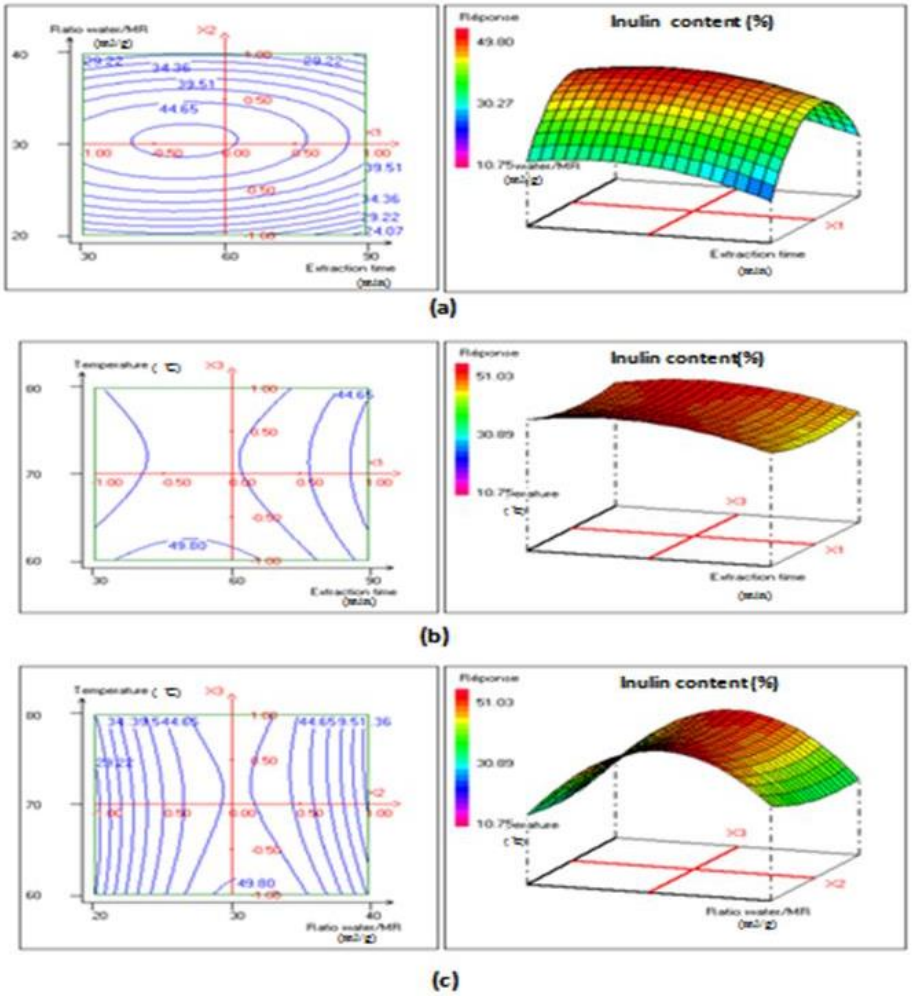

Figure 1. Three-dimensional response surface and contour plots for the effect of **(a)** ultrasonic extraction time and ratio of water to raw material at constant temperature (70 ºC), **(b)** ultrasonic extraction time and temperature at constant ratio of water to raw material (30ml/g) and **(c)** ratio of water to raw material and temperature at constant ultrasonic extraction time (60 min) on inulin content. Data are presented as means ±SE of three replicated determinations.

Total Soluble Solids (TSS) content

Figure 2a–c shows the influence of independent variables on the TSS content of chicory roots extract. The examination of the isoresponse contours and three-dimensional plots showed that the higher TSS content values were obtained at the optimal ratio of water to raw material and an extraction temperature of 61ºC.The rate of TSS content was not affected by ultrasonic temperature changes. Figure 2c showed the effect of extraction time and ratio of water to raw material at fixed temperature (70° C) on the TSS content. It can be seen also that the ratio of water to raw material (b_2=-2.52 <0) has a negative strong effect on the TSS content. At 70° C, the maximum TSS content (between 23.39% and 25.44%) was

reached at a ratio of water to raw material between 20 ml/g and 30 ml/g. On the basis of above result, it is clear that the TSS content of chicory roots extract increases as the ultrasonic extraction time increases and the ratio of water to raw material decrease to 30 ml/g.

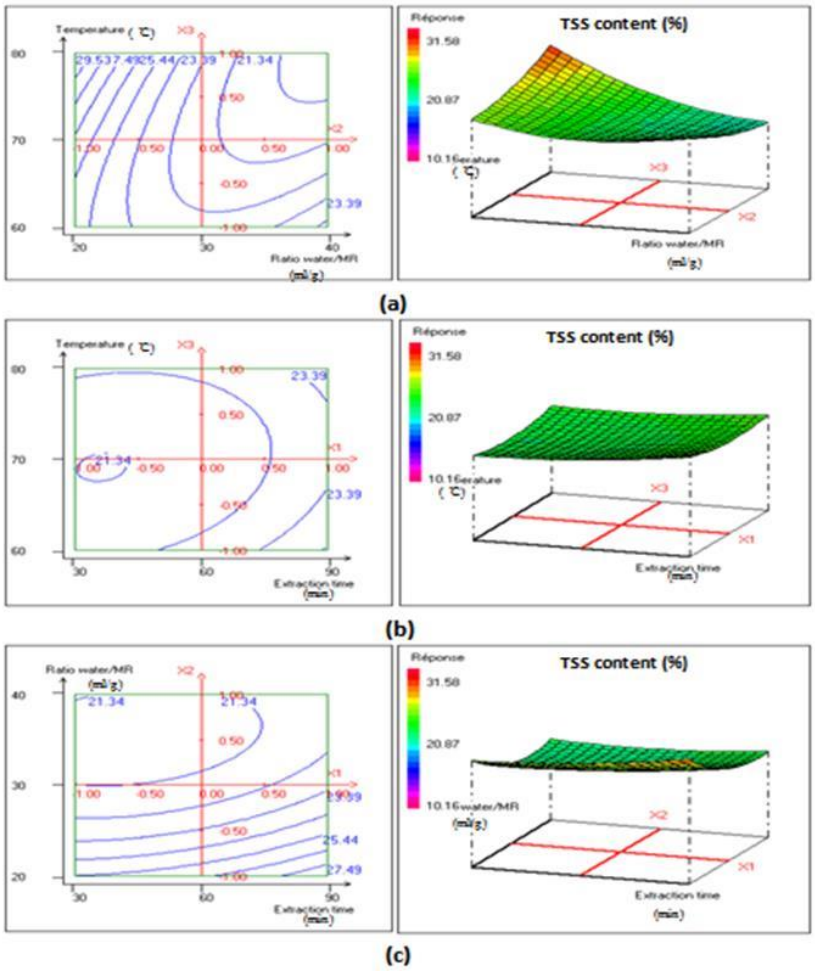

Figure 2. Three-dimensional response surface and contour plots for the effect of **(a)** ratio of water to raw material and temperature at constant ultrasonic extraction time (60 min), **(b)** ultrasonic extraction time and temperature at constant ratio of water to raw material (30ml/g) and **(c)**) ultrasonic extraction time and ratio of water to raw material at constant temperature (70 ºC) on TSS content of chicory roots. Data are presented as means ±SE of three replicated determinations**.**

Produced inulin yield

The produced inulin yield was not affected by ultrasonic extraction time, the ratio of water to raw material and their interaction changes. As shown in Figure 3a-c, when the ultrasonic temperature (X_3) was fixed at 70°C, the produced inulin yield increased as the ultrasonic extraction time (X_1) and the ratio of water to raw material increased. The produced inulin yield was positively (b_3= 1.16) correlated with the ultrasonic extraction temperature when the temperature was higher than 60°C.

In this context Wasan.[40], Winarti et al.[41] and Gaafar et al.[42] reported that the solubility of inulin was correlated to extraction temperature, the solubility of inulin at 10°C is about 6% whereas at 90°C it is about 35 %. The interaction between ultrasonic extraction time (X_1) and ultrasonic temperature (X_3), when the ratio of water to raw material (X_2) was fixed at 30 ml/g, was displayed in Figure 3b.The interaction between the ratio of water to raw material (X_2) and ultrasonic temperature (X_3), when the ultrasonic extraction time (X_1) was fixed at 60 min, was displayed in Figure 3c.

The produced inulin yield rise (>31.5%) when the ratio of water to raw material drop between 30 and 40 ml/g, the ultrasonic extraction time is between 60 and 90 min and the ultrasonic temperature is between 60 and 65°C. According to Milani et al.[43], the inulin extraction yield increased significantly when the amount of water used for the extraction was increased. This is because more liquid was able to increase the driving force of inulin out of the powder and then release to the medium.

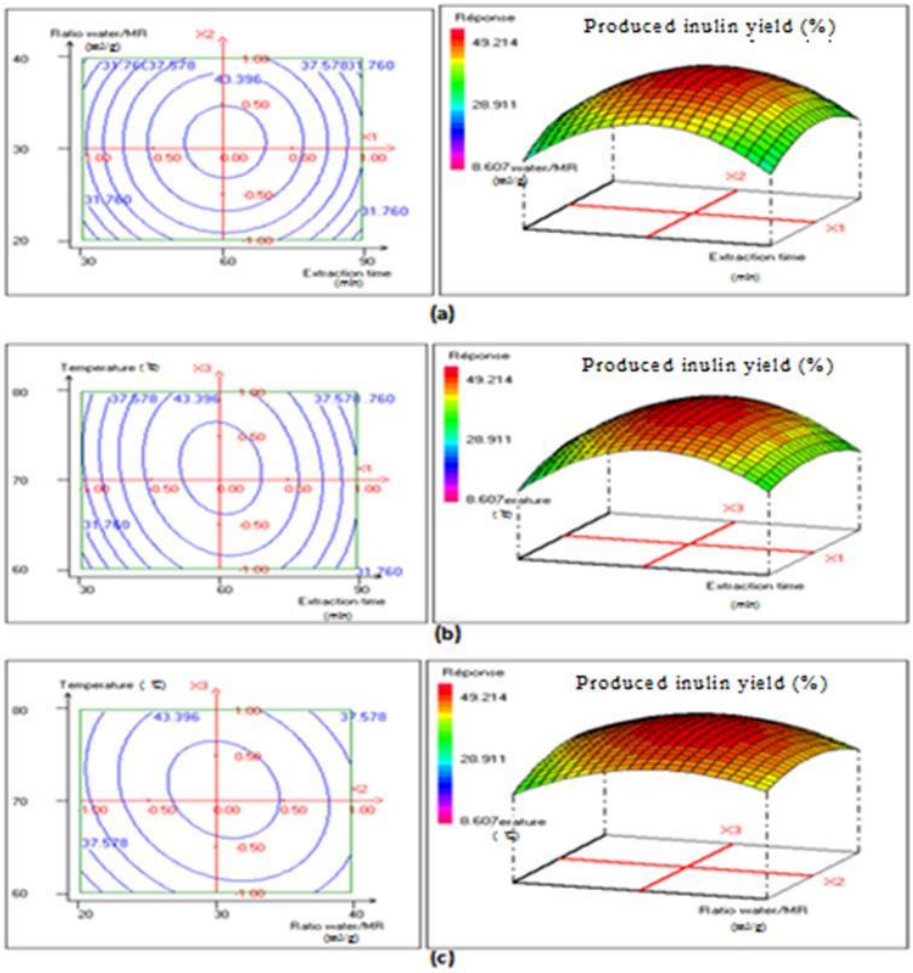

Figure 3. Three-dimensional response surface and contour plots for the effect of **(a)** ultrasonic extraction time and ratio of water to raw material at constant temperature (70 ºC), **(b)** ultrasonic extraction time and temperature at constant ratio of water to raw material (30ml/g) and **(c)** ratio of water to raw material and temperature at constant ultrasonic extraction time (60 min) on produced inulin yield of chicory roots. Data are presented as means ±SE of three replicated determinations.

The desirability

Optimization of ultrasonic parameters for the extraction of inulin from chicory roots was carried out to obtain desired criteria for each response. The aim of desirability function was to optimize different combinations of ultrasonic parameters such as ultrasonic extraction time, the ratio of water to raw material and ultrasonic temperature.

In this study, maximum global desirability was 91.48% (Table 9). Among all optimum points, the best desirability value 98.12% for inulin content (78.20% for TSS content and 98.12 % for produced inulin yield, respectively) was found at ultrasonic extraction time of 87 min, the ratio of water to raw material of 38 ml/g and ultrasonic temperature of 61 ˚ C. Under these optimal conditions, the best inulin content, TSS content and produced inulin yield were 35.92%, 24.72% and 32.53%, respectively.

Table 9. Desirability (d) values in optimal conditions.

	Optimal conditions	
	X_1 :87 min / X_2 : 38 **ml/g** / **X_3 :** 61 **ºC**	
	Value	**di (%)**
Inulin content (Y_1)	35.92	98.12
TSS content (Y_2)	24.72	78.20
Produced inulin yield (Y_3)	32.53	98.12
Total desirability		91.48

Structures of the produced inulin analyzed by FT-IR and SEM

The IR spectra of produced and commercial inulin powders showed that they have similar profiles (Figure 4). Results show the presence of a large band with a maximal absorption at about 3367 cm^{-1} which indicate the stretch vibrations of hydroxyl groups of inulin (OH-valent poly-saccharide), while the peak at 2931 cm^{-1} was the stretching vibration CH of CH_2. Our finding is similar to that of Wu and Lee [44].The observed peak at 1647 cm^{-1} spectra suggests the presence of water in the produced inulin (Figure 4.b). Bands in the 1500 cm^{-1} and 1200 cm^{-1} area were assigned to the stretching vibration of CH deformation. The bands between 1120cm^{-1} and 1020cm^{-1} corresponding to the stretching vibrations of COC and COH in the glucose molecule. Absorption band in the region 950-1250 cm^{-1} with the peak at 1033 cm^{-1} reveals the presence of OH groups of glucose in inulin powders. While the presence of fructose with β-configuration glycosidic bonds in produced and commercial inulin was indicated at absorption with maxima at 600 cm^{-1} -800 cm^{-1} and 900 cm^{-1}.

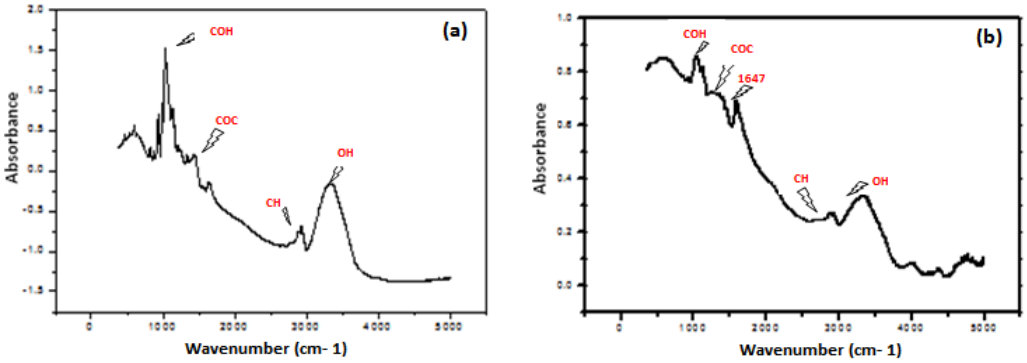

Figure 4. IR absorption spectra of inulin powders; (a) commercial inulin powder, (b) produced inulin powder from chicory roots.

Representative micrographs of inulin powders were illustrated in Figure 5. Clear differences were observed in the microstructures of commercial and produced inulin powders. Spheres for commercial inulin (Figure 5b) appear with a little and smooth surface, while those for produced inulin (Figure 5a) show a large porous surface. The large particle size of produced inulin was mainly because of the presence of water in inulin powder, this observation was in agreement with results found with FTIR analysis (peak at 1647 cm^{-1} spectra shows the presence of water). The higher water content of produced inulin shows the appearance of agglomerated inulin particle.

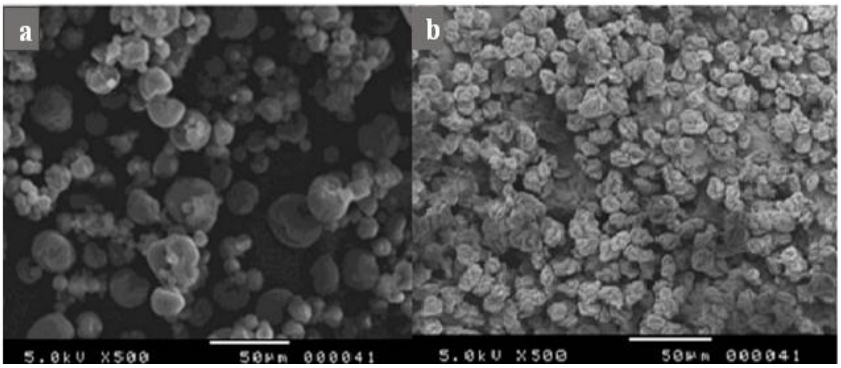

Figure 5. Scanning electron microscopy at 500× magnification of inulin powders; (a) produced inulin powder from chicory roots, (b) commercial inulin powder.

Conclusion

The present study provides data for supporting the use of *Cichorium intybus* L. roots extracts as a source of natural antioxidant and phenolic compounds. On the other hand, ultrasonic extraction has been considered as a potential alternative to traditional solid-liquid extraction for the extraction of inulin powder from chicory roots for medicine and functional foods use. The results obtained for the optimization of the ultrasonic extraction of inulin powder from chicory roots varied according to the response considered. All the tested variables presented statistically significant effects for a confidence level of 95%.This difference leads to the conclusion that the nature of the extracted inulin powder varies with modifications of the experimental conditions. The conditions were optimized using RSM and the optimum values for ultrasonic extraction time, the ratio of water to raw material and ultrasonic temperature were found to be 87 min, 38 ml/g and 61°C.

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