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Optimization of Phenolic compounds Extraction from Olive Leaves using Experimental Design Methodology

I. Sifaoui[1*], N. Chammem[2], M. Abderrabba [1], M. Mejri[1]
[1] *Laboratoire de matériaux- molécules et applications, IPEST, Tunisia*
[2]*Laboratoire d'Ecologie et de Technologie Microbienne, INSAT, Tunisia*

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**Corresponding Author. E-mail: ines.sifaoui@hotmail.com; Tel: (+21671740048) ; Fax : (+21671746551)*

Abstract
Olive products, such as oils, olives and leaves infusions, have been used in the past to treat many health problems. This study aims to develop a methodology for maximizing the efficiency of extraction of polyphenols from olive leaves. For this purpose, two experimental design approaches (a central composite design (CCD) and a mixture design) have been used. The optimal extraction parameters obtained from the CCD were: an incubating temperature of 58 °C, an extraction time of 54 min, a pH of 8, an agitation speed of 246 rpm, and a liquid-to-solid ratio of 77:1. Under the over mentioned conditions, the phenolic compounds content has increased two fold.

Keywords: Olive leaves; extraction; experimental design; Antioxidant compounds.

1. Introduction
Reactive oxygen species (ROS) are considered as the major molecules contributing to the induction and/or amplification of a large number of human pathologies such as cancer, chronic inflammation, Alzheimer's, Parkinson's and heart diseases. Many studies have associated the low incidence of cardiovascular diseases and some cancers in the Mediterranean area with the diet which is rich in fruits, vegetables, wine, and olive oil containing high levels of phytochemicals and particularly phenolic compounds (1).
Phenolic compounds, which are secondary metabolites from plants, have been linked to biological activity and health benefits in animals and humans. Such compounds play important roles as antioxidant, antimicrobial, antiviral, and antitumoral agents, just to name a few (2). These compounds have the ability to protect the cells from the ROS damage by scavenging harmful ROS that are produced in excess of those normally required for various metabolic reactions. Flavonoïds represent the major class of secondary plant phenolic compounds having a positive effect on human health, and they have been associated to anti-ischaemic, antiplatelet, anti-inflammatory, and antimicrobial activities (3).
Olive trees, widely cultivated in the Mediterranean countries, and their fruits have been extensively used in culinary food and as a natural remedy in traditional medicine (4). Olive Leaf Extract (OLE) has also been known as an effective remedy for fever and other diseases such as malaria (5-8). More recently, pharmaceutical and food industries started to use several olive products considering their richness in bioactive molecules, (phenolic, triterpenic acids, and flavonoids) (9-12). In these industries, phenolic compounds are generally extracted from plant materials or chemically synthesized. Several methods have been used to recover bioactive compounds from natural sources, including solid/liquid extraction with organic solvents, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluids extraction, and high-pressure processes (13). Among the various methods, the solid/liquid extraction method is still commonly used for phenolics extraction from plant material (14). However, in addition to selecting the appropriate method, the extraction yield depends on several parameters such as the temperature, the duration of extraction, the particle size, and the solvent to herb ratio. Therefore, these parameters should be taken into consideration in order to attain optimal extraction yields.
The aim of the present work was to apply the response surface methodology (RSM) approach to optimize the extraction conditions of phenolic (TP) and flavonoid (TF) compounds from olive leaves, considering the

following variable extraction parameters: temperature, time of extraction, liquid-to-solid ratio, pH, and agitation speed.

2. Materials and Methods

2.1. Samples

Olive leaves from the Swebea Elgia olive tree variety collected during the 2010-2011 during the ripening fruit season, with a maturing index between 3 and 4, were used to optimize the experimental conditions for the polyphenols extraction. Air-dried olive leaves were ground to a fine powder using a mill.

2.2. Extraction protocol

2.2.1. Selection of relevant variables and experimental ranges

As an initial step, liquid-to-solid ratio, temperature, and time of contact were investigated in order to determine the appropriate experimental ranges to be considered during the optimization process. The impact of the liquid-to-solid ratio on the content of phenolic (TP) and flavonoid (TF) compounds in the olive leaves extract was conducted using the following four ratios: 10:1, 20:1, 40:1, and 80:1 (v/w). The extraction was made with water at 50 ºC and an extraction period of 30 min. Additionally, to investigate the impact of temperature on the TP and the TF content, a liquid-to-solid ratio of 20:1 and a contact time of 30 min were used with temperatures ranging from 25 ºC to 65ºC. Finally, a kinetic study was performed to select the time necessary to reach an asymptotic region during the extraction procedure. The axial (30ºC and 60ºC) and central (45 ºC) temperatures, previously chosen for the experimental design, were used in the kinetic study.

2.2.2. Experimental design

The phenolic compounds extraction from olive leaves was optimized through the RSM approach. A central composite design (CCD) was employed in this regard. Incubator temperature (X_1), extraction time (X_2), pH (X_3), agitation (X_4), and liquid-to-solid ratio (X_5) were chosen as independent variables. The range and center point values of the independent variables presented in Table 1 were based on the results of preliminary experiments.

Table 1: Experimental domain of Central Composite Design (CCD)

Xj	**Factor levels**		
	-1	0	1
Temperature (°C)	30	45	60
Time (min)	1	30	59
pH	4	7	10
Agitation (rpm)	80	280	480
Ratio	20	50	80

Each variable was coded at three levels: -1, 0, and 1. The coded and uncoded (actual) levels of the independent variables are also presented in Table 1. Contents of phenolic and flavonoid compounds were selected as the responses for the combination of the independent variables and are presented in Table 2.

Once the experiments were performed, the response variable (extraction yield) was fitted with a second-order polynomial regression model in order to correlate the response variable to the independent operating variables. The general form of the second-order polynomial equation is as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k-1} \beta_{ii} X_i^2 + \sum_{i}^{k} \sum_{j} \beta_{ij} X_{ij} + \xi$$

where Y is the response (dependent variable); β_0 is a constant coefficient; βi, βii, and βij are the coefficients for the linear, quadratic, and interaction effect; Xi and Xj are the factors (independent variables); and ξ is the error.

According to the analysis of variance, the regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculations to generate dimensional and contour maps of the responses from the regression models.

Table 2: CCD with the observed responses and predicted values for Total Phenol (TP mg EAG extract), Total Flavonoid (TF mg ER g^{-1} extract)

N° Exp	T (°C)	Time (min)	pH	Agitation (rpm)	Ratio (v:w)	TP	TF
1	30	1	4	80	80	30.50	20.92
2	60	1	4	80	20	36.87	20.80
3	30	59	4	80	20	44.94	22.40
4	60	59	4	80	80	50.60	27.44
5	30	1	10	80	20	30.50	19.98
6	60	1	10	80	80	36.57	23.52
7	30	59	10	80	80	46.96	28.18
8	60	59	10	80	20	50.60	26.73
9	30	1	4	480	20	35.36	18.86
10	60	1	4	480	80	37.92	23.18
11	30	59	4	480	80	42.29	25.85
12	60	59	4	480	20	56.61	26.91
13	30	1	10	480	80	30.01	23.41
14	60	1	10	480	20	37.41	21.38
15	30	59	10	480	20	53.71	25.69
16	60	59	10	480	80	56.29	31.93
17	30	30	7	280	50	53.06	26.57
18	60	30	7	280	50	58.06	29.65
19	45	1	7	280	50	37.09	22.35
20	45	59	7	280	50	54.19	27.89
21	45	30	4	280	50	47.04	27.00
22	45	30	10	280	50	47.32	27.88
23	45	30	7	80	50	52.28	27.02
24	45	30	7	480	50	55.93	28.10
25	45	30	7	280	20	49.99	26.98
26	45	30	7	280	80	51.14	30.24
27	45	30	7	280	50	51.34	28.09
28	45	30	7	280	50	52.46	28.19
29	45	30	7	280	50	52.28	28.33
30	45	30	7	280	50	52.03	28.49

Statistical analysis

Nemrodw software package, France, was used to analyze the experimental data. *P* values lower than 0.05 were considered to be statistically significant. SigmaPlot software was used to study the correlation between the antioxidant activity and different components of the extracts.

2.3. Determination of total phenolic content

Total phenolic content of olive leaves extracts were determined by the Folin–Ciocalteu procedure (15). 0.5 ml of diluted extract (dilution of 1:50 in extraction solvent), 2.5 ml of Folin–Ciocalteu reagent (Sigma-aldrich), diluted 10 times with distilled water, and 2 ml of aqueous sodium carbonate solution Na_2CO_3 ($75g.l^{-1}$) were added after 3 minutes. Samples were vortexed and incubated at 50 °C for 5 min and then cooled. The absorbance of the samples was measured immediately against the blank solution at 760 nm using a Uvi Light *XS 2* spectrophotometer (Secomam, France). Results were expressed as mean values (milligrams of equivalent gallic acid per gram of dry weight) against a calibration curve of gallic acid (0-200µg ml^{-1}). Samples were analyzed in triplicates.

2.4. Determination of total flavonoids content

Estimation of the total flavonoids content in crude extracts was performed according to the procedures described by Jia et al. and modified by Dewanto et al. (16,17). In summary, 0.25 ml of the olive leaf extract (at a dilution

of 1:10 in water) or the rutin standard solution was mixed with 1.25 ml of distilled water in a test tube followed by addition of 75 µl of a 5% $NaNO_2$ solution. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for 5 min before 0.5 ml of 1 M NaOH was added. The mixture was brought to 2.5 ml by adding distilled water and mixed with a vortex. The absorbance was measured immediately against the blank solution at 510 nm. Results were expressed as mean values (milligrams of equivalent rutin per gram of dry weight) against a calibration curve of rutin (0-200µg ml^{-1}). Samples were analyzed in triplicates.

3. Results and discussion

3.1. Optimization of extraction conditions

At the beginning of this study, the liquid: solid ratio, temperature, and time of extraction were studied in order to determine the appropriate experimental ranges to be considered during the optimization process.

3.1.1. Liquid: solid ratio

The impact of the liquid to solid ratio (v/w) on the extraction yield of phenolic and flavonoid compounds from olive leaves was studied using four ratios, 10:1, 20:1, 40:1, and 80:1; over a 30 min extraction period using water at 50 ºC. The results of the one-way analysis of variance showed that there was a significant difference among the studied ratios. The yield of the phenolic compound increases according to the mass transfer principles. Indeed, when higher liquid to solid ratio were used, the molecular diffusion between both phases was enhanced. Although, the volume of the solvent used must be optimize to provide high recoveries with minimum solvent consumption. According to the results obtained, liquid-to-solid ratios of (20:1, 50:1, and 80:1; v:m) were chosen as different levels for the variable liquid to solid ratio of the CCD.

3.1.2. Effect of temperature

The impact of temperature on the TP and TF content was investigated at temperatures ranging from 25 ºC to 60 ºC. According to figure 1 the increase in temperature improves the yields due to enhanced solvent extraction, both as a result of higher diffusion coefficients and greater solubility of compounds. These findings are in agreement with the results of Al-Farsi et al. [18].

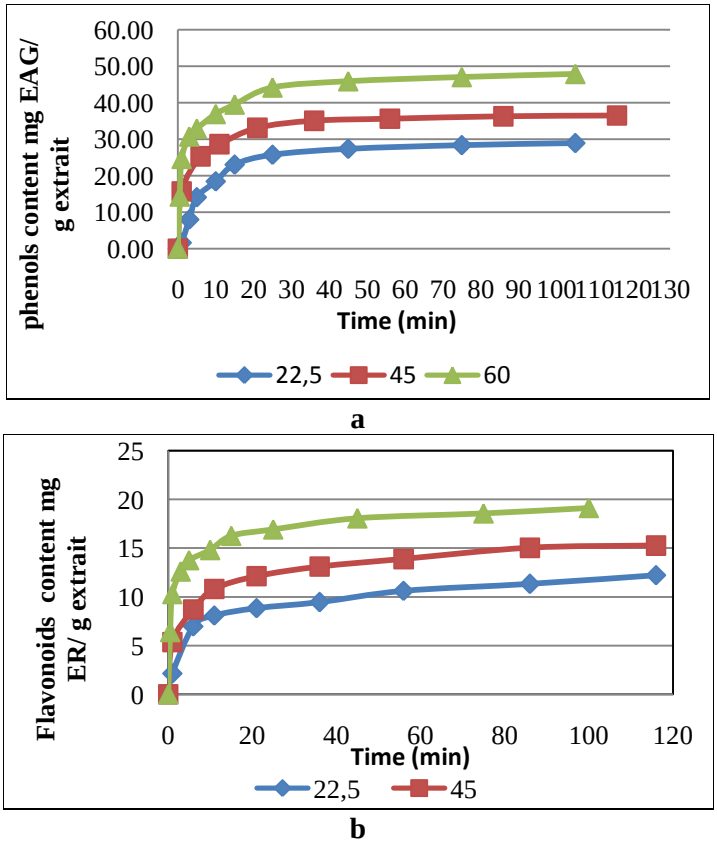


Figure 1: Evolution of Phenols (a) and flavonoids (b) contents as function of temperature and time.

3.1.3. Kinetics of extraction

The kinetics of phenolic and flavonoid compounds extraction was studied in order to determine the optimal extraction rate and to allow the choice of an appropriate experimental range to be included in the RSM for the time variable. The liquid to solid ratio was fixed at 24:1 (v:m) using water. According to Fig. 1, an asymptotic region was reached after 50 min of extraction for the three temperatures studied. Furthermore, prolonging the extraction period rendered the extraction procedure uneconomical, and with no significant increases in the amount of extracted phenols and flavonoids compounds. Nonlinear regressions were applied to the data using an exponential model:

C (mg EAG/g dry extract) = a*(1-exp(-b*t))+c*(1-exp(-d*t))

The exponential model has been found to adequately represent the experimental data for all the three temperatures and the range of time used, with a good determination coefficient ($R^2 \geq 0.99$) as shown in Table 3.

Table 3: Exponential models for the kinetics of extraction of polyphenols from olive leaves

Temperature (°C)	C (mg EAG/g dry extract) = a*(1-exp(-b*t))+c*(1-exp(-d*t))				
	a	b	c	d	R^2
22.5	26,0432	0,1290	17,8420	0,0018	0,9977
45	19,6912	1,1157	16,8186	0,1550	0,9997
60	27,3467	0,6480	20,2330	0,0380	0,9991

To assess the effect of temperature and time of contact on TF and TP content, temperatures of 30, 45, and 60 °C were used for three different times: 1, 30, and 59 min.

3.1.4. Optimization of extraction by RSM

The extraction of phenolic compounds from olive leaves was further optimized through the RSM approach. A central composite design (CCD) was employed for this purpose. Incubator temperature (X_1), extraction time (X_2), pH (X_3), agitation speed (X_4), and liquid-to-solid ratio (X_5) were chosen as independent extraction parameters (variables). The range and center point values of the three independent variables, presented in Table 1 were based on the results of preliminary experiments. TP and TF content were selected as the responses for the combination of the independent variables as shown in Table 2.

The design, observed responses, and the predicted values of 30 runs using RSM design are presented in Table 2. Results obtained show that the experimental model fitted well the theoretical one. In addition, it was observed that the TP ranged from 30.01 mg of equivalent Gallic acid per g of dry weight to 58.06 mg of equivalent Gallic acid per g of dry weight. The maximum level was found under the following experimental conditions: a temperature of 60 °C, an extraction time of 30 min, a pH of 7, an agitation speed of 280 rpm and a ratio of 50:1. A moderate range of TF was also found (18.06– 31.93 mg/g) and the maximum point (31.93mg/g) was obtained when the following conditions were used: a temperature of 60 °C, an extraction time of 59 min, a pH of 10, an agitation speed of 480 rpm and a ratio of 80:1. Since the found optimal extraction conditions were not the same for TP and TF, an optimum process should be investigated in order to obtain higher extracted amounts of both compounds.

a- Total phenolic content

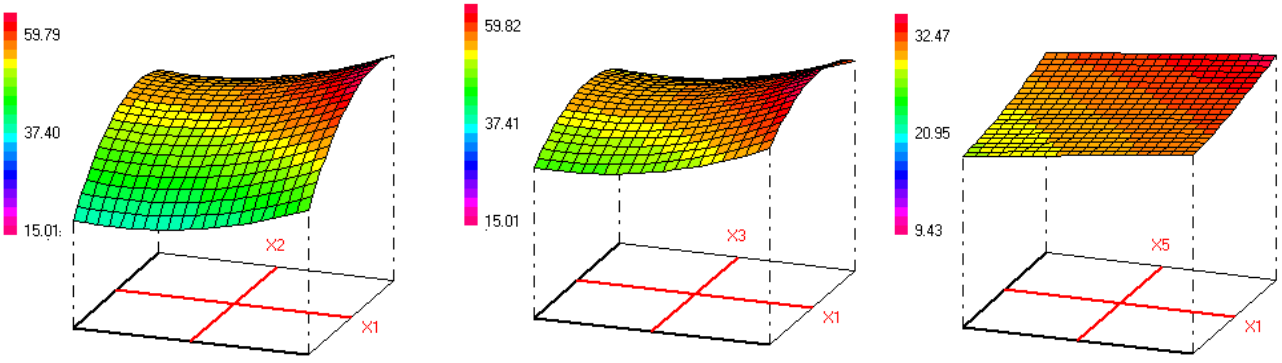
Linear term of extraction time (X_2), incubator temperature (X_1), agitation (X_4), liquid-to-solid ratio (X_5), and quadratic term of temperature, time and pH (X^2_1,X^2_2,X^2_3) showed the largest effect ($p < 0.0001$) on phenolic content of the extracts. It was followed by the linear and quadratic terms of liquid-to- solid ratio and interaction term of incubator temperature and ratio (X_{15}). Linear term of pH, and the other interaction terms of X_{12}, X_{14}, X_{34}, X_{25} were not significant ($p > 0.05$).

b- Total flavonoids content

Linear term of incubator temperature (X_1), extraction time (X_2), pH (X_3), agitation (X_4), ratio (X_5) and quadratic term of time (X^2_2) showed the largest effect ($p < 0.001$) on flavonoids content. It was followed by the quadratic terms pH, quadratic term of agitation, interaction term of incubator temperature and time (X_{12}), interaction term of incubator temperature and pH(X_{13}), interaction term of time and pH(X_{23}), interaction term of time and agitation (X_{24}) and interaction term of pH and ratio (X_{35}). Quadratic term of temperature, ratio and the other interaction terms of X_{14}, $X_{34,}$ $X_{15,}$ X_{25} and X_{45} were not significant ($p > 0.05$).

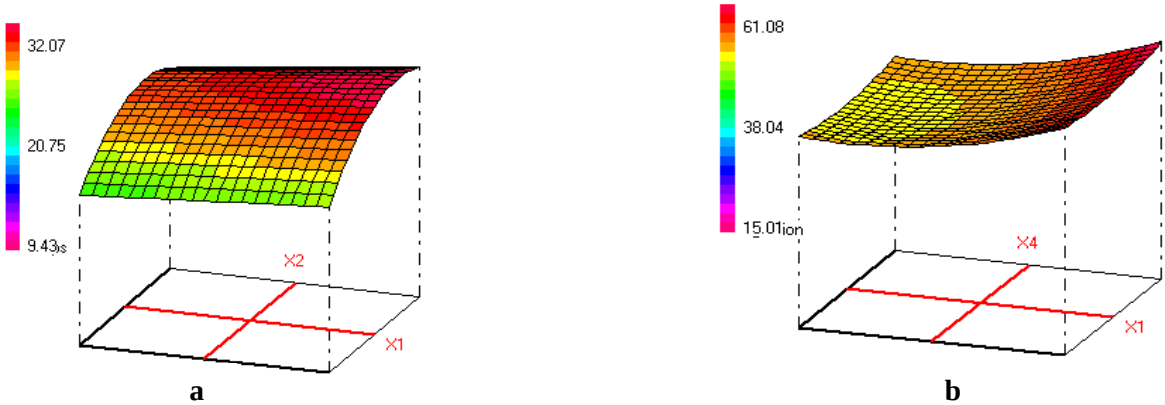
c- Effects of extraction variables on phenol and flavonoids content

Three-dimensional (3-D) plots for extraction yield as function of extraction temperature and time at pH = 7, agitation = 280 rpm and ratio = 50 are given in Fig. 5. It can be seen that the extraction time demonstrated an exponential increase on the TP and TF content as shown in Figures 2 and 3. The effect of temperature also displayed an exponential increase within the range of 30–60 °C.



a **b** **c**

Figure 2: Response surface for total phenolics in function of temperature and time (a), temperature and pH (b) and temperature and agitation (c)



a **b**

Figure 3: Response surface for total flavonoids as function of temperature and time (a), temperature and ratio (b)

ANOVA showed that the effects of all variables: incubating temperature, extraction time, pH, agitation, and liquid-to-solid ratio were significant and quadratic models were obtained for predicting the responses. The optimal conditions were obtained at a temperature of 58 °C, extraction duration of 54 min, a pH of 8, an agitation speed of 246 rpm, and a liquid-to-solid ratio of 77:1.

Heat has been found to enhance the recovery of phenolic compounds as described by Durling *et* al. and Da Silva *et* al. (19,20). Increased temperature might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions in the plant material, and for this reason the extraction temperature had a positive linear effect. Although, it should be noted that high temperatures could also decompose phenolic compounds already extracted or even involve the breakdown of these molecules still remaining in the plant matrix (21, 22).

Extraction time is a major parameter affecting the extraction efficiency of bioactive compounds from plants. Indeed, it can be observed that TP and TF yields were improved by increasing the extraction duration from 1 min to 50 min, but tend to decrease beyond 50 min. Several studies have confirmed that prolonging the time of extraction could denature the phenolic compounds already extracted (23). As for the effect of pH, and according to table 4, the quadratic effect of the pH on the TP and TF extracted revealed that the bioactive molecule extracted remained constant at acidic to near neutral pH (6.5) but decreased gradually as the alkalinity increased to pH 10. Similar results were observed by Chethan & Malleshi, (24). In fact, they reported that acidic to neutral extract from finger mill were rich in benzoic acid derivatives (gallic acid, proto-catechuic acid, and p-hydroxy benzoic acid) and cinnamic acid (p-coumaric acid, syringic acid, ferulic acid, and trans-cinnamic acid). However, only gallic and proto-catechuic acids were detected on the alkaline extract. As a result of the fact that more phenolic compounds can permeate into the solvent by diffusion, increasing the stirring speed could linearly increase the extraction rate of those molecules (25).

Table 4: Regression coefficients of the predicted second-order model for the response variables, total phenolics (Y1) and total flavonoid (Y2)

Model parameters	Y1 (phenol level)	Y2 (flavonoid level)
b0	52.427[a]	28.204[a]
Linear effects		
b1	2.978[a]	1.093[a]
b2	7.998[a]	2.701[a]
b3	0.402[NS]	0.852[a]
b4	1.428[a]	0.462[a]
b5	-0.762[b]	1.386[a]
Quadratic effects		
b11	2.934[a]	-0.059[NS]
b22	-6.986[a]	-3.048[a]
b33	-5.446[a]	-0.729[b]
b44	1.479[c]	-0.613[c]
b55	-2.061[b]	0.438[NS]
Interaction effects		
b12	0.238[NS]	0.324[b]
b13	-0.576[c]	-0.250[c]
b23	1.205[a]	0.338[b]
b14	0.320[NS]	0.161[NS]
b24	0.596[c]	0.251[c]
b34	0.220[NS]	0.047[NS]
b15	0.915[b]	-0.074[NS]
b25	-0.286[NS]	0.104[NS]
b35	0.630[c]	0.302[b]
b45	-1.144[a]	0.086[NS]

Statistically significant at [a]$p < 0.001$, [b]$p < 0.01$, [c]$p < 0.05$, and [NS]: non-significant.

By increasing the liquid to solvent ratio, the TP tend to decrease. These results are conflicting with mass transfer principle, by reason of concentration gradient which is supposed to be higher when a high liquid to solid ratio is used, leading to enhance the diffusion. Similar results were found by Şahin & Şaml (26). Indeed, the authors reported a decrease of phenols yields from OLE with the increase of liquid to solid ratio. On the other hand, the amount of TF compounds tends to increase with the liquid to solid ratio. In fact, more flavonoid compounds can permeate into the solvent under higher solvent-to-material ratio conditions (27). A non significant effect was observed in TF extraction yield with further increase in liquid to solid ratio, probably because of the high solubility of flavonoid compounds in water and their easy extraction with a week liquid to solid ratio.

Conclusion

The experimental designs were found to be adequate to describe and predict the extraction process of phenolic compounds from olive leaves. Optimal extraction conditions were found to occur when the following set of parameters were used: temperature = 58 °C, extraction duration = 54 min, pH=8, agitation speed = 246 rpm and the liquid-to-solid ratio = 77.

References

1. Perez-Martinez P., Lopez-Miranda J., Blanco-Colio L., Jimenez C.B.Y., Moreno J.A., Delgado-Lista J., Egido J., Perez-Jimenez F., The chronic intake of a Mediterranean diet enriched in virgin olive oil, decreases nuclear transcription factor _B activation in peripheral blood mononuclear cells from healthy men. *Atherosclerosis*. 194 (2007) e141.
2. Meot-Duros L., Magne C., Antioxidant activity and phenol content of Crithmum maritimum L. leaves. *Plant Physiol.* Biochem. 47 (2009) 37-41.
3. Ben Sghaier M., Skandrani I., Nasr N., Franca M.G. D., Chekir-Ghedira L., Ghedira K., Flavonoids and sesquiterpenes from Tecurium ramosissimum promote antiproliferation of human cancer cells and enhance antioxidant activity: A structure–activity relationship study. *Environ. Toxicol. Pharmacol*. 32 (2011) 336-348.
4. Japón-Luján R., Luque de Castro M.D., Superheated liquid extraction of oleuropein and related biophenols from olive leaves. *J. Chromatogr. A.* , 1136 (2011) 185-191
5. Gucci R., Lombardini L., Tattini M., Analysis of leaf water relations in leaves of two olive (Olea europaea) cultivars differing in tolerance to salinity. *Tree Physiol.* 17 (1997) 13-21.
6. Fernández-Escobar R., Moreno R., García-Creus M., Seasonal changes of mineral nutrients in olive leaves during the alternate-bearing cycle. *Sci. Hortic.* 82 (1999) 25-45.
7. Ciafardini G., Zullo B.A., Microbiological activity in stored olive oil. *Int. J. Food Microbiol*. 75 (2002) 111-118.
8. Sifaoui I., López-Arencibia A., Martín-Navarro C. M., Chammem N., Reyes-Batlle M., Mejri M., Abderabba M., Piñero J. E., Activity of olive leaf extracts against the promastigote stage of *Leishmania* species and their correlation with the antioxidant activity. *Exp. Parasitol.*, 141 (2014) 106-111.
9. Bouknana D., Hammouti B., Salghi R., Aouniti A., Sbaa M., Physicochemical Characterization of Olive Oil Mill Wastewaters in the eastern region of Morocco, *J. Mater. Environ. Sci.* 5 (2014) 1039-1058.
10. Meftah H, Latrache H, Hamadi F, Hanine H, Zahir H, El louali M., Comparaison des caractéristiques physicochimiques des huiles d'olives issus de différentes zones de la région Tadla Azilal (Maroc) [Comparison of the physico-chemical characteristics of the olive oil coming from different zones in Tadla Azilal area (Morocco)]. *J. Mater. Environ. Sci.* 5 (2014) 641-646
11. Zahari A, Tazi A, Azzi M., Optimisation des conditions de traitement des margines par un superoxydant K3FexMnyO8 [Optimization of treatment conditions of Olive Oil Mill Wastewater by superoxidant K3FexMnyO8], *J. Mater. Environ. Sci.,* 5 (2014) 2484-489
12. Lamzira Z, Ghabbour N, Rokni Y, Thonart P, Chihib N.E, Berrabah M, Asehraou A., Characterization of Phenolic Profile of Moroccan Picholine Olive Variety, *J. Mater. Environ. Sci.* 5 (2014)490-497.
13. Taamalli A., Arráez-Román D., Barrajón-Catalán E., Ruiz-Torres V., Pérez-Sánchez A., Herrero M., Ibañez E., Micol V., Zarrouk M., Segura-Carretero A., Fernández-Gutiérrez A., Use of advanced techniques for the extraction of phenolic compounds from Tunisian olive leaves: Phenolic composition and cytotoxicity against human breast cancer cells, *Food Chem. Toxicol*. 50 (2012) 1817-1825.
14. Luque de Castro M. D., Garcıa-Ayuso L. E., Soxhlet extraction of solid materials: an outdated technique with a promising innovative future, *Analytica chimica acta*, 369 (1998) 1-10.
15. Škerget M., Kotnik P., Hadolin M., Hraš A.R., Simonič M., Knez Ž., Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities, *Food Chem*. 89 (2005) 191-198.
16. Jia Z.S., Tang M.C., Wu J.M., The determination of flavonoid contents in mulverry and their scavenging effects on superoxide radicals, *Food Chem*. 64 (1999) 555-559.

17. Dewanto V., Wu X., Adom K., Liu R.H., Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity, *J. Agric. Food Chem*. 50 (2002) 3010–3014.
18. Al-Farsi M.A., Lee C.Y., Optimization of phenolics and dietary fibre extraction from date seeds, *Food Chem.*, 108 (2008) 977–985.
19. Durling N.E., Catchpole O.J., Grey J.B., Webby R.F., Mitchell K.A., Foo L.Y., Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures, *Food Chem*. 101 (2007) 1417-1424.
20. Da Silva J.F.M, De Souza M.C., Matta S.R., De Andrade M.R.M., Vidal F.V.N., Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities, *Food Chem*. 99 (2006) 431.
21. Chan S.W., Lee C.Y., Yap C.F., Wan Aida W.M., Ho C.W., Optimisation of extraction conditions for phenolic compounds from limau purut (Citrus hystrix) peels, *Int. Food Res. J.* 16 (2009) 203- 213.
22. Saha J., Biswas A., Chhetri A., Sarkar P.K., Response surface optimisation of antioxidant extraction from kinema, a Bacillus-fermented soybean food, *Food Chem*. 129 (2011), 507–513.
23. Juntachote T., Berghofer E., Bauer F., Siebenhandl S., The application of response surface methodology to the production of phenolic extracts of lemon grass, galangal, holy basil and rosemary, *Int. J. Food Sci. Tech*. 41 (2006) 121-133.
24. Chethan S., Malleshi N.G., Finger millet polyphenols: Optimization of extraction and the effect of pH on their stability, *Food Chem*. 105 (2007) 862-870.
25. Zhou Y., Gao F., Zhao Y., Lu J., Study on the extraction kinetics of phenolic compounds from petroleum refinery waste lye, *J. Saudi Chem. Soc*. 18 (2014) 589-592.
26. Şahin S., Şaml R., Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology, *Ultrason Sonochem*. 20 (2013) 595-602.
27. Prasad N.K., Chun Y., En Y., Zhao M., Jiang Y., Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp, *Innov. Food Sci. & Emerg. Technol.* 10 (2009) 155-159.