Journal homepage: http://fens.usv.ro/index.php/FENS
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XXI, Issue 3 - 2022, pag. 198 - 208

EXTRACTION OF NATURAL ANTIOXYDANTS FROM ALGERIAN *ROSMARINUS OFFICINALIS* L. WITH GRAS-SOLVENT AND KINETIC STUDY

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Received 29th July 2022, accepted 29th September 2022

Abstract: *The objectif of this study was to improve extraction of natural antioxidants (NAs) found in Algeria Rosmarinus officinalis* L. *using cleaner extraction processes. To verify the single factors effects, a Factorial design (FD) was used to study the impact of the main process variables (time, solid-to-liquid ratio, and temperature) on extraction efficiency. Total Polyphenol Content (TPC), Total Flavonoids Content (TFC) and Antioxidant Activity (AA) were measured to control the extraction process efficiency on different experimental conditions. The best experimental result was obtained at 30°C for 30 min and 0.05 g/mL. The value of TPC, TFC and AA was respectively, 121.80 mg GAE/g DW, 44.01 mg QE/g DW and 96.36 %. The correlation between TPC, TFC and AA remains unclear. The extraction of polyphenols was shown to obey first order kinetic with a 0.045 min^{-1}coefficient for the fast period and 0.05 min^{-1} for the slow period. Finally, the plant morphology investigated by SEM revealed physical changes on plant material.*

Keywords*:* *Rosmarinus officinalis L.*, NAs, modeling, kinetic.

1. Introduction

In food industry, synthetic antioxidants (SAs) [butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ)...] have been used for a long time as food's preserved [1]. However, these products cause many problems to the food quality (degradation) and the human health when consumed. Many recent studies have focused on their high toxicity [2]. Moreover, research was conducted to replace these products with substances derived from plants. For these reasons, scientists aimed to substitute SAs by natural antioxidants (NAs). They are extracted from plants by cleaner extraction processes. The major classes of compounds with antioxidant activity are: vitamins (C and E), carotenoids, flavonoids, phenolic acids, tannins, lignans and stilbenes [3]. NAs can be extracted from plants and substitute the synthetic antioxidants, source of toxicity [4]. Among these plants, rosemary, which is very widespread plant in the Mediterranean region was used as food preservative [5]. For these reasons, some studies on this spice and its antioxidant properties were conducted and reported.

Several techniques were used to extract NAs from plants. These include conventional and non-conventional extractions: solid-liquid extraction, heated reflux extraction, ultrasound- assisted extraction, microwave-assisted extraction, supercritical fluid extraction, accelerated, and batch extraction technique. The latter is preferred because it always gives good outcome.

DOI: https://doi.org/10.4316/fens.2022.019

The aim of this work was the valorization of Algerian plant *Rosmarinus officinalis* L. and improvement of the extraction process using hydroalcoholic mixture. Only a few papers about the plant used in this work are available, which motivated the actual study. Furthermore, another motivation for the study was the scarce information on the application of design of experiment (DOE) in the polyphenols extraction from *Rosmarinus officinalis* L., and also the impact of extraction on plant microstructure.

In this study, factorial design (FD) was used to verify the single factors effect's (time, solid-to-liquid ratio and temperature) on extraction efficiency. TPC, TFC and AA were measured to control the efficiency of extraction process on different experimental conditions. The interactions between factors were determined using RSM. The extraction kinetics was also determined. Finally, the morphology of plant was analyzed using SEM before and after extraction. The effect of plant microstructure on the extraction mechanisms was discussed in detail in this paper.

2. Matherials and methods

2.1. Characterization of plant matrix

Rosmarinus officinalis L. was collected in Algiers (Algeria) during the month of March. To preserve the molecules' greatest integrity, the leaves were cut off the stems and let to dry at room temperature for three weeks. The dried leaves were finely powdered in a mortar with a sieve of 1.12 mm after being crushed into little pieces. The powder was stored protected from light and moisture for subsequent use.

2.2. Reagents and chemicals

Folin-Ciocalteu reagent, Gallic acid Quercetine and (DPPH) (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) were purchased from Sigma–Aldrich. Aluminum chloride was obtained from Merck. Additionally, EtOH of 96% purity (Rieldel-de Haen), MeOH (sigma-Aldrich), Xylen (Cheminova PRS) and sodium carbonate (BHD chemicals Ltd) were used.

2.3. Analysis

2.3.1. Total Polyphenols Content

According to Singleton et *al*. [6], TPC was determined by Folin-Ciocalteu and expressed as Gallic acid equivalents (mg GAE/g DW). Using a spectrophotometer (UV-VIS SECOMAM S250), absorbance was measured at 760 nm. The results were calculated using a standard Gallic acid curve of (1–30 µg/mL).

2.3.2. Total Flavonoids Content

The Miliauskas technique [7] was used to measure TFC, which was expressed as quercetin equivalents (mg QE/g DW). A spectrophotometer (UV-VIS SECOMAM S250) was used to detect absorbance at 430 nm. The results were calculated using a standard curve of quercetin (1-40 µg/mL).

2.3.3. DPPH scavenging assay

The ability of the extracts to scavenge DPPH radical was assessed spectrophotometrically as described by Que et *al*. [8].

2.3.4. Morphology analysis

The samples were observed using a scanning electron microscope (Jeol JSM 6360LV) with a maximum resolution and voltage (50 nm, 30kV). These observations serve to locate precisely the sites producing active molecules and to visualize any modification after extraction.

2.4. Experimental design and statistical analysis

FD was used to study the effects of three variables: extraction time (X_1), solid-to-liquid ratio (X_2) and temperature (X_3). The low and high levels each factor in the experimental design are showed in Table 1. The variables responses (TPC, TFC and AA) were expressed individually as a function of the independent variables. The experimental design presented eight (8) combinations (Table 2), including three replicates of the central point in order to estimate pure error and to assess the fit lack of the proposed models. All the experiments were performed randomly. A first-order polynomial equation (1) was used to express TPC (Y_1), TFC (Y_2) and AA (Y_3) of rosemary as a function of independent variables as-follow:

$$Yi = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 12X1X2 + \beta 13X1X3 + \beta 23X1X2 \quad (1)$$

Where Y_i represents the response variables, β_0 is the model constant, β_i and β_{ij} are the linear and interaction coefficients respectively. Xi and Xj are the levels of the independent variables.
The model appropriateness was also evaluated by the coefficient model (R^2) and adjusted coefficient model (R^2_{Adj}) and also by the statically significance model (P-value model) properly tested by ANOVA.

3. Results and discussion

3.1 Choice of solvent

NAs are usually extracted by conventional solvent extraction methods. Ethanol (EtOH) was used instead of methanol due to its high toxicity. Moreover, EtOH has been classified since 2012 as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration, USA). However, it is not recommended to use it alone when extracting NAs from plants because it causes the deshydratation of vegetable cells. However, NAs are usually linked to sugar. EtOH can be blended with water in any proportion. Studies showed that TPC yield increased by increasing water content [9]. EtOH (80 %) was selected as solvent in the actual study (batch extraction).

3.2. Fitting model

Three factors that may affect the experimental responses were selected as independent variables at three levels. The minimum, central, and maximum levels for each factor and the different independent variables of the extracts are listed in Table 1. The experiments were performed according to the design of experiments shown in Tables 2. The same table displays observed and predicted responses on two levels. Statistical analysis of experimental data was performed by using the Statgraphics plus software version 5.1.

Table 1.

Coded/real levels used in Factorial Design of Algerian *Rosmarinus officinalis* L. batch extraction.

Coded levels	-1	0	+1
Time (min) (X_1)	30	60	90
Solid-to-liquid ratio (g/mL) (X_2)	0.05	0.1	0.15
Temperature (°C) (X_3)	30	45	60

Table 2.

Factorial design of three variables with their experimental and predicted responses of Algerian *Rosmarinus officinalis* L. batch extraction.

Run	Coded variable			TPC (mg GAE/gDW)		TFC (mg QE/gDW)		AA (%)	
	X1	X2	X3	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	+1	+1	+1	80.65	88.53	19.13	18.86	92.68	95.92
2	-1	+1	+1	34.40	31.33	12.43	12.46	95.08	96.30
3	+1	-1	+1	110.06	106.99	40.20	40.23	53.01	54.23
4	-1	-1	+1	95.45	103.33	42.10	41.83	48.83	52.07
5	+1	+1	-1	20.91	17.84	13.98	14	47.27	48.49
6	-1	+1	-1	12.15	20.03	11.73	11.46	91.14	94.38
7	+1	-1	-1	55.12	62.99	38.85	38.58	51.00	54.24
8	-1	-1	-1	121.80	118.73	44.01	44.03	96.36	97.58
9	0	0	0	76.51	68.72	25.71	27.68	79.01	74.15
10	0	0	0	70.21	68.72	29.01	27.68	81.06	74.15
11	0	0	0	78.64	68.72	27.31	27.68	80.20	74.15

The results obtained indicate that the levels of TPC ranged from 12.15 mg GAE/g DW to 121.80 mg GAE/g DW. The best results related to the lower levels were observed in run 8 at 121.80 mg GAE/g DW followed by 110.06 mg GAE/g DW on run 3. The levels of TF ranged from 12.43 mg QE/g DW to 44.01 mg QE/g DW. The best results were observed in run 8 at 44.01 mg QE/g DW followed by 42.10 mg QE/g DW in run 4. For AA the level ranged from 47.27 % to 96.36 %, the best results were observed also in run 8 at 96.36 % followed by 95.08 % in run 2.

Overall, run 8 gave the best values for the three responses. It is about 121.80 mg GAE/g DW for polyphenols, 96.36% for AA and for flavonoids a value of 44.01 mg QE/g DW. These results indicate the presence of high content of polyphenols. Similar results were obtained using the same reagent on Turkish rosemary [10], and it varied from 147.3 to 34.1 mg GAE/g DW. The authors pointed out that the extract composition changes according to many factors (e.g., the type of sample, location, time, etc). The physicochemical nature of the individual polyphenols in the extract has probably more contribution to the AA against the TPC.

We noted that some researchers have shown lower phenolic contents of *Rosemary* extract [11]. The results for TPC, TFC and AAO tudied by analysis of variance (ANOVA) were summarized in Table 3.

For TPC, four effects have *P*-values less than 0.05, indicating that they are significantly different from zero at the 95% confidence level. For TFC and AA, two effects and four effects have *P*-values less than 0.05. In the present study, the values of R^2 were 96.4%, 99.7% and 96.1% for TPC, TFC and AA respectively. The values of R^2_{Adj} were 90.9 %, 98.9% and 90.3% for TPC, TFC and AA respectively. The Durbin-Watson (DW) values for TPC, TFC and AA were 1.9, 1.96 and 1.56 respectively. Since the DW value is greater than 1.4, there is no serious auto correction in the residuals.

Table 3.
Analysis of variance (ANOVA) for TPC, TFC and AA of Algerian *Rosmarinus officinalis* L.batch extraction.

Variable response	Source of variation	Sum of square	Df	Mean square	F-Ration	p-Value
TPC	X_1	1.08045	1	1.08045	0.01	0.9264
	X_2	6863.23	1	6863.23	61.38	0.0014*
	X_3	1528.49	1	1528.49	13.67	0.0209*
	X_1 X_2	1433.27	1	1433.27	12.82	0.0232*
	X_1 X_3	1763.59	1	1763.59	15.77	0.0165*
	X_2 X_3	356.445	1	356.445	3.19	0.1487
TFC	X_1	0.446512	1	0.446512	0.29	0.6168
	X_2	1455.03	1	1455.03	956.28	0.0000*
	X_3	3.49801	1	3.49801	2.30	0.2040
	X_1 X_2	32.04	1	32.04	21.06	0.0101*
	X_1 X_3	7.43051	1	7.43051	4.88	0.0916
	X_2 X_3	5.13601	1	5.13601	3.38	0.1400
AAO	X_1	955.938	1	955.938	24.53	0.0077*
	X_2	740.548	1	740.548	19.01	0.0121*
	X_3	1.83361	1	1.83361	0.05	0.8389
	X_1 X_2	3.23851	1	3.23851	0.08	0.7874
	X_1 X_3	1035.35	1	1035.35	26.57	0.0067*
	X_2 X_3	1125.04	1	1125.04	28.88	0.0058*

The effect of extraction factors is presented in Figure 1. Pareto chart traduces ANOVA for operating parameters and their combinations. It shows their own effects from the most to the least significant level at 5%. The vertical line indicates the statistical level.

Figure 1a shows that for TPC the interaction of X_2 has a higher effect, followed by the interaction of (X_1/X_3), the effect of X_3 and of (X_1/X_2) respectively. The effect of the extraction factors on TFC is presented in Figure 1b. This figure shows that one parameter (X_2) has a significant effect. Figure1c shows that for AA, the interaction of (X_2/X_3) has a higher effect. It is followed by the interaction of (X_1/X_3), the effect of X_1 and of X_2 respectively.

Regarding the TPC and TFC, the solid-to-liquid ratio has a higher effect. For AA, the solid-to-liquid ratio has also an effect but it is not the higher effect. The solid-to-liquid ratio has an effect because increasing the solvent proportion leads to increase of the concentration gradient, which increases the diffusion of solid compounds in the solvent. In the actual condition, temperature had an effect for TPC extraction. This result was confirmed by many authors [12]. Increasing temperature accelerates mass transfer, which increases cellulose activity and polyphenols solubility [12]. Some previous work has

shown that extraction temperature was the main parameter. The increase of temperature implies directly an increase of diffusion coefficient and solubility of the compounds. Up the temperature of 50 °C phenolic compounds can be degraded completely]. The time had no significant effect on TFC and AA but not on TPC.

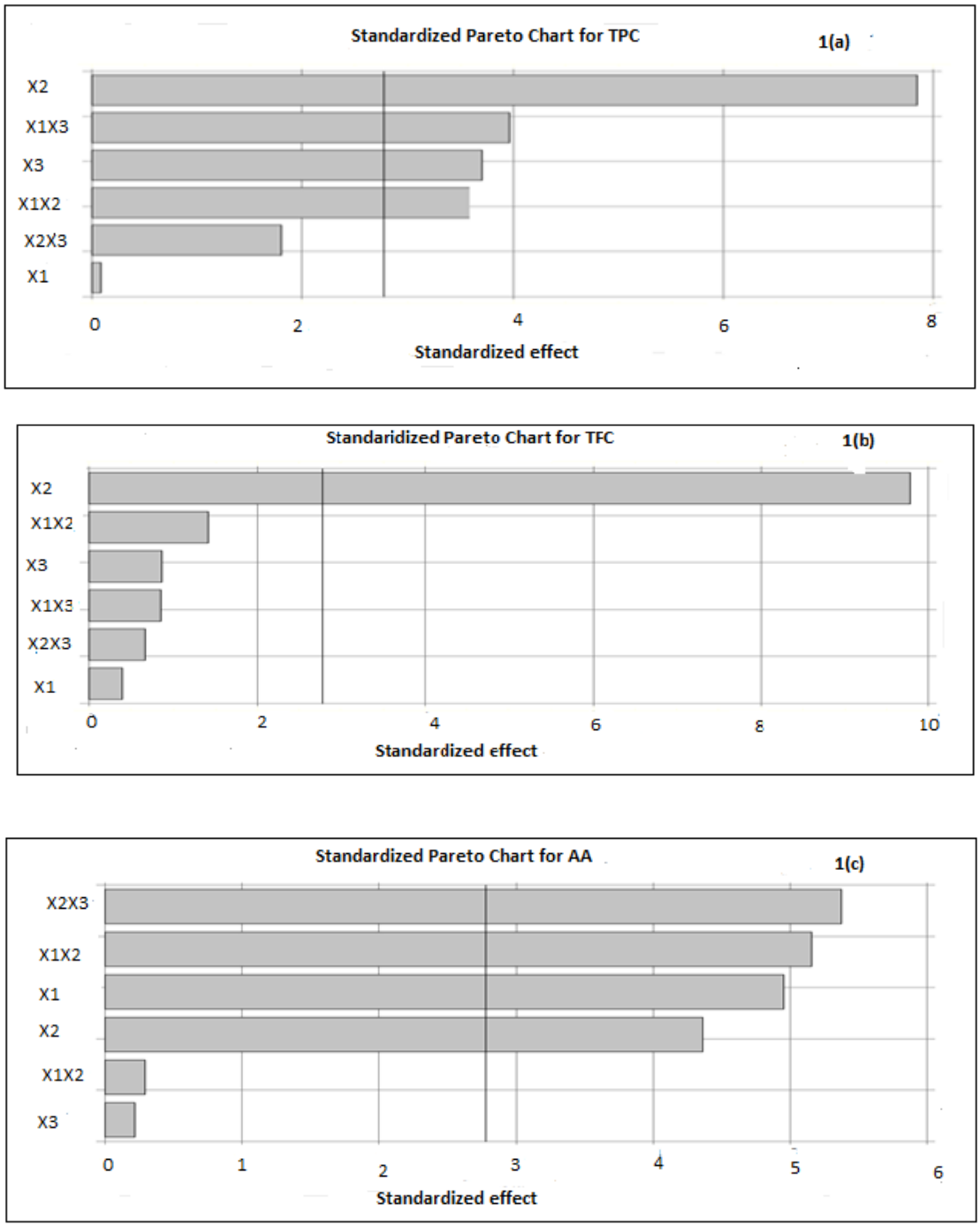

Fig. 1. Pareto Chart for TPC, TFC and AA effects from *Algerian Rosmarinus officinalis* L. batch extraction (a): Pareto Chart for TPC, (b): Pareto Chart for TFC and (c): Pareto Chart for AA.

The fitted mathematical models (Y_1, Y_2, and Y_3) for TPC, TFC and AA with 95% for coefficient level are given in Eq 2, 3 and 4 respectively:

$$Y1 = 68.72 + 0.37X1 - 29.29X2 + 13.82X3 + 13.38X1X2 + 14.85X1X3 + 6.68X2X3 \quad (2)$$

$$Y2 = 27.68 + 0.24X1 - 13.43X2 + 0.66X3 + 2.00X1X2 + 0.96X1X3 + 0.80X2X3 \quad (3)$$

$$Y3 = 74.15 - 10.93X1 + 9.62X2 + 0.48X3 - 0.64X1X2 + 11.37X1X3 + 11.86X2X3 \quad (4)$$

The values of R^2 for TPC, TFC and AA were 96.4%, 99.7% and 96.1% respectively. It indicates that there is a good correlation between the experimental and predicted data.

3.3. Correlation between the responses of TPC and TFC

Figure 2 does not show a relationship between AA, TPC and TFC. This result is in agreement with investigations on AA of plant extracts by other authors [13]. It is due probably to synergetic effects between the extracted products. Figure 3 does not show a relationship between TPC and TFC. However, the correlation between TPC, TFC and AA remains unclear. The recovery of AA may indicate that this is only partially related to the compounds observed here. It may also indicate the presence of other chemicals involved in its activity. Antioxidants present in rosemary extracts are not limited only to polyphenols [14]. Moreover, it is important to consider the occurrence of synergism between the chemical compounds in the extract. AA dependes on chemical structure and the interaction between antioxidant substances [15]. Previous studies indicated that the correlation degree of AA depends not only on the TPC, but also on the extracts composition.

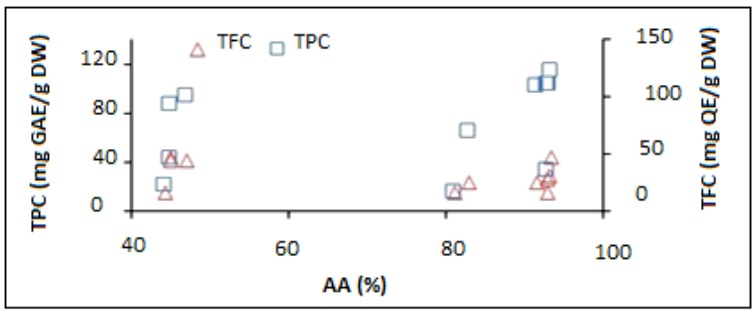

Fig. 2. AA plotted according to TPC and TFC of extracts from *Algerian Rosmarinus officinalis* L. batch extraction.

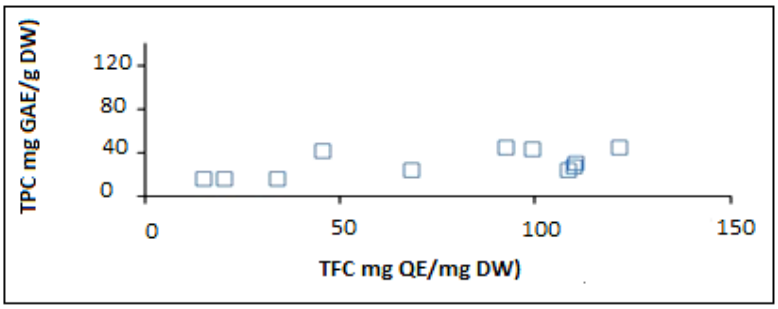

Fig. 3. Relationship between TPC and TFC of extracts from *Algerian Rosmarinus officinalis* L. batch extraction.

Thus, kinetic examination is required to generate detailed data, which could be used for comparison of the efficiency of various extraction methodologies and for the engineering of improved extraction processes. We will examine the kinetics of polyphenols in next section.

3.4. Kinetics studies

To identify the model that better described polyphenols extraction. Concentration values were plotted as a function of *t*. The best model fitted to the extraction kinetics using non-linear regression was described by the following equation:

$$[Y = a(1 - e^{-bx})] \quad (5)$$

This suggested that the extraction of polyphenols as a function of *t* can be adequately predicted by Equation (6). It represents first-order kinetics [16, 17].

$$[C_t = C_\infty(1\text{-}e^{-kt})] \quad (6)$$

Where C_t is the concentration of total polyphenols at time *t*, C_∞ the final concentrarion of total polyphenols, and *k* is the apparent first-order rate constant of extraction.

The extraction process follows first-order kinetic, with a fast period from 0 to 20 min and a slow period from 20 to 100 min of extraction.

When $\ln[C_\infty/(C_\infty - C)]$ is plotted against time (Figure 4), the points fall on two interesting straight lines, the first with a relatively steep slope and the second with a relatively shallow one. The points of intersection of $\ln[C_\infty/(C_\infty - C)]$ vs *t* plot the fast and the slow stages are termed transition points.

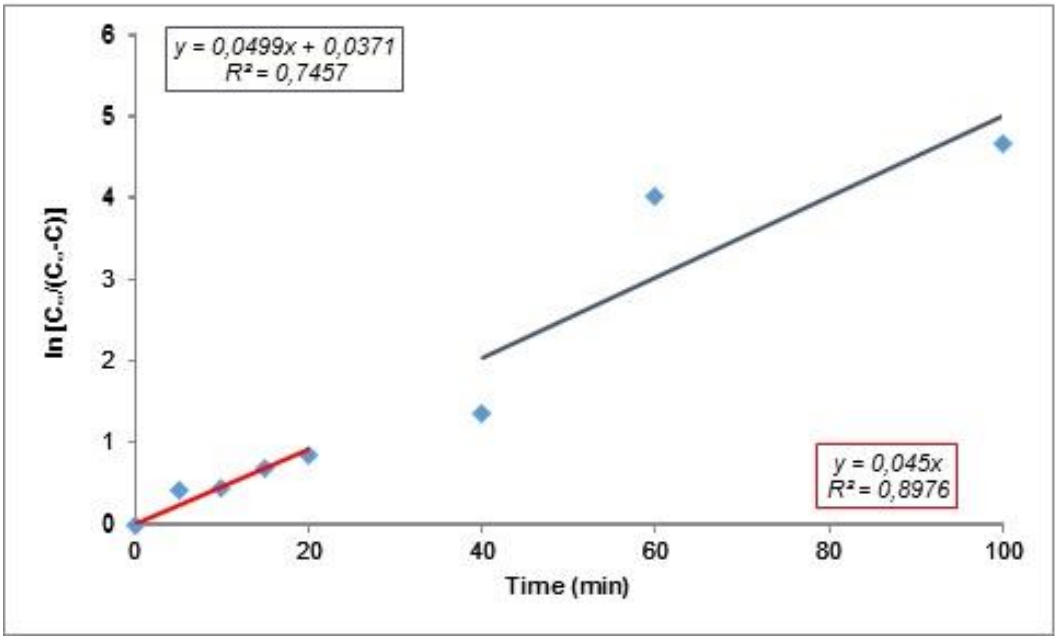

Fig. 4. Kinetics of the fast and the slow stage of TPC extraction and respective constants of Algerian *Rosmarinus officinalis* L. batch extraction.

Indeed, the coefficient at the fast period was 0.045 min^{-1} and of 0.050 min^{-1} for the slow period.

3.5. Morphology

Algerian *Rosmarinus officinalis* L. leaves morphologies were analyzed by SEM

(Quanta 200, FEI Company; France) before and after extraction processes, as illustrated in Figure 5 and Figure 6 respectively. It can be clearly observed a change in the morphological structure of the plant.

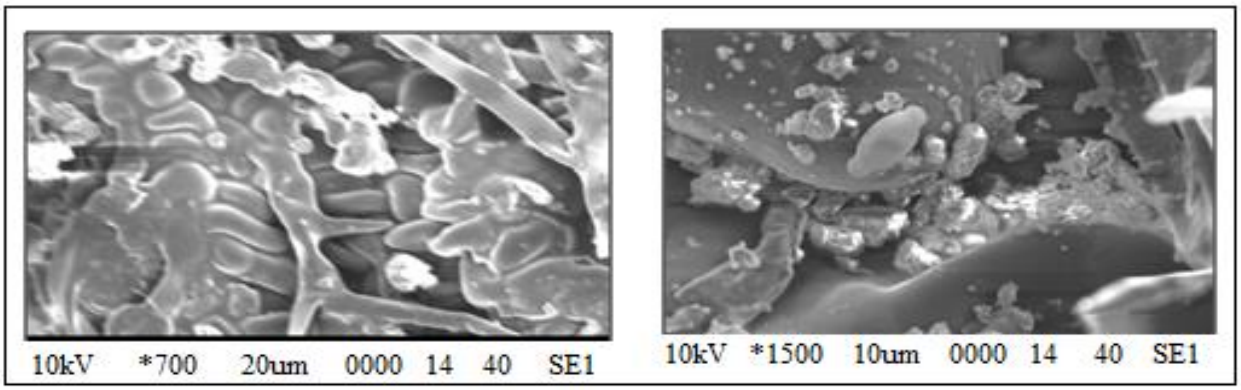

Fig. 5. SEM images of fresh Algerian *Rosmarinus officinalis* L. leaf before batch extraction process

These observations show the presence of many non-glandular trichomes commonly called hair-bearing. Some of them are unicellular, simple and conical in shape. Others are multicellular and branched [18, 19]. However, only glandular trichomes can synthesize and contain the essential oil which can also have antioxidant activities. After extraction, physical changes are observed on the plant material as seen on Figure 6. Secretarial channels micrograph shows that extraction process was enough to cause damage on cells and their walls. We observe a bursting of the walls of the cuticle of glandular hair and a dehydration of the leaves. This can be explained by a rise in temperature in the in-situ cellular structures. It induced an increase of internal pressure of these cells and caused their auto destruction by emptying them of their contents. The changes observed for extraction process are clearly showing that cells were damaged during extraction process. Similar effects were reported in the literature [20].

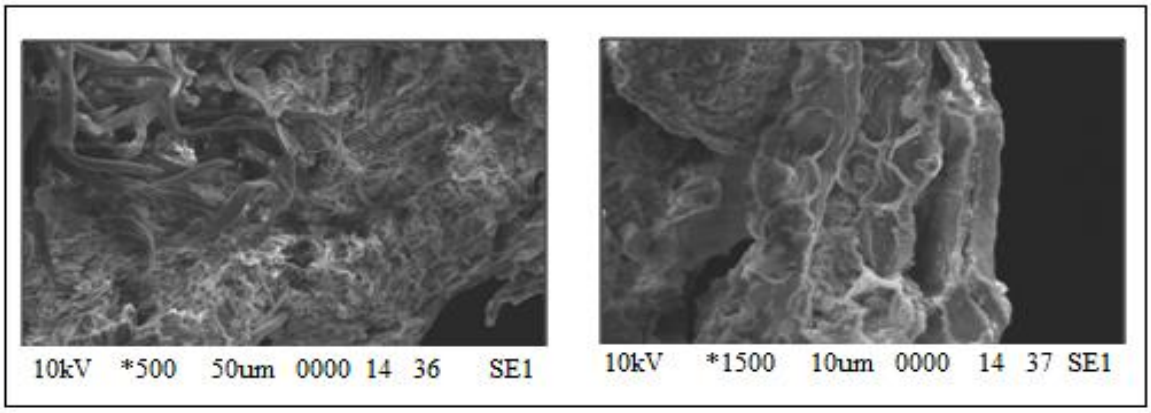

Fig. 6. SEM images of fresh Algerian *Rosmarinus officinalis* L. leaf after batch extraction process

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Volume XXI, Issue 3 - 2022, pag. 198 - 208

4. Conclusion

Algerian *Rosmarinus officinalis* L. leaves was extracted in batch mode with Gras-solvent (ethanol 80%). The selection of solvent is mainly related with the future use of the extract. The effect of the main process variables (time, solid-to-liquid ratio, temperature) on the yield was studied. The characteristic models released from each response are first-order linear models with interactions. The investigation of the effects of each factor on the three selected responses shows that solid-to-liquid ratio presents the overriding factor for two responses (TPC, TFC). Indeed, the best experimental result was obtained at 30°C for 30 min and 0.05 g/mL. The value of TPC, TFC and AA was respectively 121.80 mg GAE/g DW, 44.01 mg QE/g DW and 96.36%. The correlation between TPC, TFC and AA remains unclear. The polyphenols extraction follows first order kinetic with coefficient of 0.045 min^{-1} for fast period and 0.05 min^{-1} for the slow period. The morphology investigations by SEM revealed physical changes on plant material. Antibacterial activity of extracts will be tested in prospect.

5. Acknowledgements

The authors thank the colleagues from laboratory for their continuous interest, support and discussion. Their insight and expertise have been highly appreciated. Many thanks also to A. Naitbouda for his SEMsupport.

6. References

[1]. FRANCO, D., RODRÍGUEZ-AMADO, I., AGREGÁN, R., MUNEKATA, P. E. S., VÁZQUEZ, J. A., BARBA, F. J., & LORENZO, J. M. Optimization of antioxidants extraction from peanut skin to prevent oxidative processes during soybean oil storage. *LWT*, 88: 1-8, (2018)

[2]. MOGHADDAM, M., MIRAN, S.N.K., PIRBALOUTI, A. G., MEHDIZADEH, L., & GHADERI, Y. Variation in essential oil composition and antioxidant activity of cumin (Cuminum cyminum L.) fruits during stages of maturity. *Industrial Crops and Products*, 70: 163-169, (2015)

[3]. ACOSTA-ESTRADA, B. A., GUTIÉRREZ-URIBE, J. A., & SERNA-SALDÍVAR, S. O. Bound phenolics in foods, a review. *Food Chemistry*, 152: 46-55, (2014)

[4]. GRANATO, D., NUNES, D. S., & BARBA, F. J. An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal. *Trends in Food Science & Technology*, 62: 13-22, (2017).

[5]. SEBRANEK, J.G., SEWALT, V.J.H., ROBBINS, K., & HOUSER, T.A. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat science*, 69: 289-296, (2005)

[6]. SINGLETON., V. L. & ROSSI., J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16: 144-158, (1965)

[7]. MILIAUSKAS, G., VENSKUTONIS, P. R., & VAN BEEK, T. A. Screening of radical scavenging activity of some medicinal and aromatic plants. *Food Chemistry*, 85: 231-237, (2004)

[8]. QUE, F., MAO, L., & PAN, X. Antioxidant activities of five Chinese rice wines and involvement of phenolic compounds. *Food Research International*, 39: 581-587, (2006)

[9]. ZHANG, B., YANG, R., & LIU, C. Z., Microwave-assisted extraction of chlorogenic acid from flower buds of Lonicera japonica Thunb. *Separation and Purification Technology*, 62: 480-483, (2008)

[10]. YESIL-CELIKTAS, O., GIRGIN, G. O. Z. D. E., ORHAN, H., WICHER, H.J., BEDIR, E., & VERDAR SUKAN, F. Screening of free radical scavenging capacity and antioxidant activities of Rosmarinus officinalis extract with focus on

location and harvesting times. *European Food Research and Technolo9gy,* 224: 443-451, (2007)
[11]. WOJDYLO, A., OSZMIANSKII, J., & CZELERYS, R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105: 940-949, (2007)
[12]. D'ALESSANDRO, L. G., KRIAA, K., NIKOV, I., & DIMITROV, K. Ultrasound assisted extraction of polyphenols from black chokeberry. *Separation and purification technology*, 93: 42-47, (2012)
[13]. HERRERO, M., PLAZA, M., CIFLUENTES, A., & IBÁÑEZ, E. Green process for the extraction of bioactives from rosemary: chemical and functional characterization via ultraperformance liquid chromatography-tandem mass spectrometry and in-vitro assays. *Journal of chromatography A*, 1217: 2512-2520, (2010)
[14]. IBARRA, A., CASES, J., BILY, A., HE, K.., BAI, N., ROLLER, M., COUSSAERT, A., & RIPOLL, C. Importance of extract standardization and invitro/ex vivo assay selection for the evaluation of antioxidant activity of botanicals: A case study on three Rosmarinus officinalis L. extract. *Journal of Medicinal Foods*, 13: 1167-1175, (2010)
[15]. GEORGETTI, S. R., CASAGRANDE, R., SOUZA, C.R.F., OLIVIERA, W. P., & FONSECA, M. J. V. Spray drying of the soybean extract: Effects on chemical properties and antioxidant activity. *LWT- Food Science and Technology*, 41: 1521-1527, (2008)
[16]. HERODEZ, S.S., HADOLIN, M., ŠKERGET, M., & KNEZ, Z. Solvent extraction study of antioxidants from Balm (Melissa officinalis L.) leaves. *Food Chemistry* 80: 275-282, (2003)
[17]. SPIRO, M., & JAGO, D.S. Kinetics and equilibria of tea infusion. Part 3. Rotating disc experiments interpreted by steady–state model. *Journal of the Chemical Society, Farady Transactions*, 78: 295-305, (1982)
[18]. MARIN, M., KOKO, V., DULETIC-LAUSEVIC, S., MARIN, P. D., RANCIC, D., & DAJIC-STEVANOVIC, Z. Glandular trichoms on the leaves of Rosmarinus officinalis: Morphology, Stereology and histochemistry. *South African Journal of Botany*, 72: 378-382, (2006)
[19]. BOTTEGA, S., & CORSI, G. Structure, secretion and possible functions of calyx glandular hairs of Rosmarinus officinalis L. (Labiatae), *Botanical Journal. Of the Linnean Society*, 132: 325-335, (2000).
[20]. PARE, J.R.J. & BELANGER, J.M.R. Instrumental method in Foods analysis; Elseiver Science, Eds, Amsterdam, ISBN: 9780080534770, (1997).