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Modeling of enzymatic production applied to the extraction of essential oils

Eloutassi Noureddine*[1], Nechad Imane[1], Louaste Bouchra[2], Yemni Majda[1], Amal Lahkimi[1], Chaouch Mahdi[1]

[1]*Laboratory of Materials Engineering and Environment, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco*

[2]*Biotechnology Laboratory, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco*

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Abstract

The catalytic power of enzymes is no longer a secret for agro-food, cosmetic, pharmaceutical and other industries, they use enzymatic catalysts in the chain of processing of raw material. In this work, we tested the possibility of improving the yield of essential oil of aromatic and medicinal plants by the use of an enzymatic process coupled with extraction. The plant tested is *Rosmarinus officinalis*, while the extraction method is the hydro distillation coupled to the pectinases. The production of the pectinase enzyme was obtained from the supernatant of strain *Aspergillus Niger* culture medium , who grew up in the wastes of the hydro distilled plant as the only source of carbon. In order to better optimize the production of the enzyme, the investigation of the effects of the different factors on the performance is proving a crucial step. Given the narrow number of factors in production, screening was paramount for the collection of influence factors. Then, modelling by experiment plans allowed us to establish a practical mathematical model that would increase the yield positively. The use of the enzymatic catalyst proved to be of great practicality, it induced improvements in the performance of the essential oil with an increase of 25% and the reduction of the processing time by 50%. Therefore, the rate of hydrodistillation of *Rosmarinus officinalis* biomass treated by enzymatic preparation has improved.

***Corresponding Author:** Eloutassi Noureddine ✉ imanenechad5@hotmail.com

Introduction

Rosemary (*Rosmarinus officinalis)* covers extensive areas in Morocco (USAID 2006, MAPM 2013, Irzanawie. 2013, CAT 2015, HCEFLD 2015, Bachiri and *al.* 2016), it is one of the most exploited plants as an aromatic, medicinal and culinary plant. This plant has been widely used in traditional medicine and cosmetics, it is also used as a natural food preservative and flavoring agent. Its essential oils have been the subject of several studies that have shown that they have antimicrobial, antioxidant and anti-inflammatory properties that makes *R. officinalis* a plant of great interest in the food and medical industries (Hussain 2010, Rafie 2017,)

Moreover, because of their enormous catalytic potential, enzymes are the main economic actors in agri-food development (Perry 2008, Plouffe, 2010, Linden 2012, Gouzi 2014). Pectinases constitute the group of enzymes able of degrading pectic polymers present in plant cell walls (Fogarty & Kelly 1982). They are used in the treatment of juice (extraction and clarification), the treatment of alcoholic beverages, the extraction of vegetable oil and other food industries (Hoa 2013, Pinheiro 2017).

In the context of green chemistry, the objective of this research work has been towards the optimization of extraction of essential oils from *Rosmarinus officinalis* using the pectinase enzyme extracted from the wild strain of *Aspergillus Niger* as an enzymatic catalyst to Improve performance and quality, while reducing the cost and time of biotreatment., the effectiveness of a new ecological approach for the extraction of essential oils intended for the pharmaceutical industry and which may also interest other industrial fields such as perfumery and agro-food.

Materials and methods

Essential Oil Extraction by hydrodistillation

The extraction of essential oils was carried out by Hydrodistillation type Clevenger. Essential oils were separated by settling. Then they were kept in small opaque vials and stored at 4 °c before use.

Extraction of essential oil by distillation after enzymatic treatment

The effect of pectinolytic enzymes on the hydrodistillation yield of rosemary leaves essential oils, was evaluated by the addition of concentrate from ultrafiltration (cell Amicon 52, membrane Diaflo PM 10) of the supernatant of the *Aspergillus Niger EF97* culture medium, containing pectin as inducing enzymes pectinolytic (Jayani *et al.*, 2005). 20ml of the concentrate, was added to 100g of *Rosmarinus officinalis*at the time of the grinding of the plant biomass (10 min), and the yield of the essential oil was measured after the hydrodistillation

Conditions of the enzyme production

Strain Aspergillus Niger was isolated via preliminary microbiological assays; its optimum growth is achieved on a food-based waste medium. Mold is sporulated on Potato dextrose agar medium (PDA) incubated at 30°C for 7 days. The PDA medium contains 1 g/l of L-phenylalanine, 3 g/l yeast extract, 5g/l sodium chloride, 1g/l bipotassium phosphate, 12g/l Agar, pH is adjusted to 7 by NaOH (0.5 M). The spores were peeled by the addition of 50ml of sterile distilled water with vigorous agitation using a magnetic bar. Spore suspension was subsequently inoculated into culture and production medium of *Aspergillus Niger*.

The enumeration of the spores is carried out by the Thomas cell (0.0025 mm2/0.1 mm) and by measuring the absorbance at 650 nm, referring to a calibration curve based on a series of dilutions of a mother solution of spores.

The cultures were produced in a volume of 100ml of medium contained in 250ml Erlenmeyer containing the treated cellulose enriched by the yeast extract. A spore suspension of 109 spores/ml was used as an inoculum subjected to an incubation temperature of 30°C, coupled with agitation for 72h in a Marie Bath. Thus, samples of the culture medium were collected to evaluate the specific activities of the pectinolytic enzyme.

Enzymatic activity of pectinolytic enzymes

The Polygalacturonase activity (PG) was evaluated by the rate of reducing sugars released using the Miller Method (Miller 1959). In a buffer solution containing pectin (50mm citric acid). An enzymatic unit (PG) is defined by the amount of enzymes that releases 1 µmol of Galactironique acid per hour per ml at PH = 5.

The Pectinesterase activity (PE) was evaluated by the method of Kertesz (Kertesz, Z. 1955). An enzymatic unit (PE) is defined by the amount of enzymes that releases 1µmol of carboxyl Group per ml per min.

The Pectin lyase Activity (PL) was evaluated by the method of Albersheim (Albersheim and *et al.,* 1960). An enzymatic unit (PL) is defined by the amount of enzymes that increases the absorbance from 0.55 to 235nm for one minute at 25°C and PH 6.5.

Characterization of substrate after enzymatic production

At the end of the fermentation, the biomass is separated by filtration on smooth paper of Whatman (N° 2, high resolution and diam. 150mm). Its dry weight is determined after rinsing with distilled water and drying in an oven set at 105°C.On the filtrate, the enzymatic activity is determined by the reducing power of D-glucose released during enzymatic hydrolysis of waste from lignocellulosic plants. The dosage of reducing sugars is determined by a colorimetric reaction due to the presence of 3, 5 dinitrosallycilic acid.

The enzymatic activity is expressed by the international unit corresponding to 1 µmol of D-glucose released per minute at 40°C and at pH= 5. Then, 0.5ml of the enzyme extracts are added 0.5ml of substrate (cellulose solution).

The mixture is incubated at 40°C for 30min. The reaction is stopped by adding 1ml of dinitrosallycilic acid, followed by heating at 100°C for 5 min. After cooling in an ice bath, 10ml of distilled water is added. The absorbance is read at 540 nm against white (prepared from the denatured enzymatic extract at 100°C).

The concentration of the corresponding reducing sugars is determined from a calibration curve established with concentrations of D-glucose varying from 0 to 2mg/ml.

Results and discussion

Pectinolytic activity

The method of extraction of essential oils is an elemental link in the optimization chain, it plays a key role in the quantification of the yield and the chemical characterization of the extracts obtained. The hydrodistillation is one of the oldest methods of extraction of essential oils, it gives volatile products easily analysable by the techniques of separation, it is a relatively simple and less costly technique. This extraction method resulted in yields that evolve in conjunction with distiller dimensions to reach a threshold of 1.2g of essential oils per 100g of the plant.

This allowed us to optimize the geometric form and thermodynamic conditions, in the ultimatum of the development of an automated distillation system, which will be the key tool in the saving of heat energy dissipated in the heating, the Control of the amount of water used in the condensation and subsequently, limiting the loss of essential oils by working in a closed system. This leads us to say that, by proving to be more efficient than the separate generator distillation, the hydrodistillation would be the appropriate alternative that could overcome the techno-economic constraints associated with the extraction of essential oils in Morocco. In order to control and improve local production of essential oils, we were required to perform batch kinetics to determine maximum yield and total yield (Fig. 1) in the presence and absence of pectinases enzymes. The enzymatic units used are summarized in table 1.

The concentrate contains three types of pectinolytic activity, respectively: Polygalacturonase (PG), Pectinesterase (PE), and Pectinlyase (PL). The quantities of pectinolytic enzymes expressed in enzymatic units and obtained after concentration of the supernatant of the culture medium by ultrafiltration are similar to those used in agro-processing industries.

These enzymes, which act in synergy, degrade the pecto-cellulosic wallof plant cells and thus facilitate the extraction of essential oils by hydrodistillation.

Table 1. Pectinolytic enzymes produced by the strain Aspergillus Niger EF97 used in the extraction of essential oils from R. officinalis.

Pectinolytic activity	Supernatant	Concentrated
Polygalacturonase PG (U/ml)	9.8	92
Pectinesterase PE (U/ml)	2.5	21
Pectinlyase PL (U/ml)	1.9	17

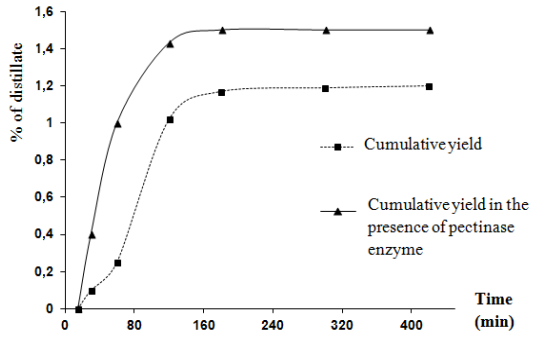

Fig. 1. Kinetics of production of essential oils from *Rosmarinus officinalis* leaves.

Fig. 1 illustrates the kinetics of distillation and the cumulative production of essential oils in the presence and absence of pectinases enzymes. Considerable variation was obtained based on the length of the distillation. The pectinolytic enzymes reduced the distillation time and increased the weight yield of essential oils relative to *R. officinalis* biomass.

Optimization of the conditions of production of the enzyme by plan of experiments

Hadamard Matrix

The optimization was established in the first place by the screening plan. This allows the selection of factors having a significant effect on the growth of *Aspergillus Niger* EF97 and the production of pectinolytic enzymes. Then to evaluate the influence of k factors at two-level on the response studied with a number of experiments n such that N = k-1 N is a multiple of 4 and can be between 4 and 100).

The Hadamard matrices were used. The matrixs of experiments of Hadamard (Hadamard 1893), Allow to estimate the "weight" (aj) of k factors in N experiments, with a variance: var (a_j) = σ 2 /N. The Hadamard matrix are orthogonal and have an element of 1 or-1, established by circular permutation from a base generator (table 2).

Table 2. The Hadamard matrix for the study of 11 factors with 12 experiments. X_1 = pH, X_2 = Agitation, X_3 = cellulose, X_4 = Yeast Extract, X_5 = Cellulose Solution, X_6 = $NaNO_3$, X_7 = CaCl2, X_8 = $ZnCl_2$, X_9 = (NH_4) 2 SO_4, X_{10} = KH_2PO_4, X_{11} = Tween 80.

Test number	Factors										
	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11
1	+	+	-	+	+	+	-	-	-	+	-
2	-	+	+	-	+	+	+	-	-	-	+
3	+	-	+	+	-	+	+	+	-	-	-
4	-	+	-	+	+	-	+	+	+	-	-
5	-	-	+	-	+	+	-	+	+	+	-
6	-	-	-	+	-	+	+	-	+	+	+
7	+	-	-	-	+	-	+	+	-	+	+
8	+	+	-	-	-	+	-	+	+	-	+
9	+	+	+	-	-	-	+	-	+	+	-
10	-	+	+	+	-	-	-	+	-	+	+
11	+	-	+	+	+	-	-	-	+	-	+
12	-	-	-	-	-	-	-	-	-	-	-

The matrix used is represented in table 2 with 12 experiments and 11 factors each line represents the different experiments and each column the factors tested. The factors of the last experiment being always taken at level-1. The upper (+) and lower (-) levels of all the factors tested are reported in table 3 while the tests are carried over into the test matrix as table 2.

Table 3. The factors tested and their corresponding concentrations.

Factors	Level (+)	Level (-)
X1 : pH	6	5
X2 : Agitation	200 rpm	100 rpm
X3 : cellulose	24 g/l	0 g/l
X4 : Yeast Extract	5 g/l	0 g/l
X5 : Cellulose Solution	36 g/l	0 g/l
X6 : $NaNO_3$	36 g/l	0g /l
X7 : $CaCl_2$	1 g/l	0 g/l
X8: $ZnCl_2$	0,02 g/l	0 g/l
X9: $(NH_4)_2$ SO_4	0,5 g/l	0 g/l
X10: KH_2PO_4	0,1 g/l	0 g/l
X11 : Tween 80	0.3 %	0 %

Statistical analysis of Hadamard matrix

After completion of the tests constructed from the matrix of HADAMARD, the results of the enzymatic activity were obtained, which allowed us to treat them

statistically in order to be able to know the preponderant factors, and propose thereafter, a mathematical model that will help us to estimate the weights of the different factors.

- $Y=\beta 0+ \beta 1X1 + \beta 2X2 + \beta 3X3 + \beta 4X4 + \beta 5X5 + \beta 6X6 + \beta 7X7 + \beta 8X8 + \beta 9X9 + \beta 10X10 + \beta 11X11 + \varepsilon$
- Statistical analysis of the plan of experiments

Table 4. Quantity of ß-Galactosidase and biomass produced according to the Hadamard plan.

Test number	Factors											Enzyme activity (µmole/min)	Biomass (g/l)
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}		
1	+	+	-	+	+	+	-	-	-	+	-	8691	20,42
2	-	+	+	-	+	+	+	-	-	-	+	8922	43,35
3	+	-	+	+	-	+	+	+	-	-	-	8753	22,00
4	-	+	-	+	+	-	+	+	+	-	-	8323	24,02
5	-	-	+	-	+	+	-	+	+	+	-	8662	32,48
6	-	-	-	+	-	+	+	-	+	+	+	8331	25,25
7	+	-	-	-	+	-	+	+	-	+	+	8252	5,55
8	+	+	-	-	-	+	-	+	+	-	+	8654	19,92
9	+	+	+	-	-	-	+	-	+	+	-	8693	06,65
10	-	+	+	+	-	-	-	+	-	+	+	8013	19,25
11	+	-	+	+	+	-	-	-	+	-	+	9635	31,22
12	-	-	-	-	-	-	-	-	-	-	-	8001	09,24

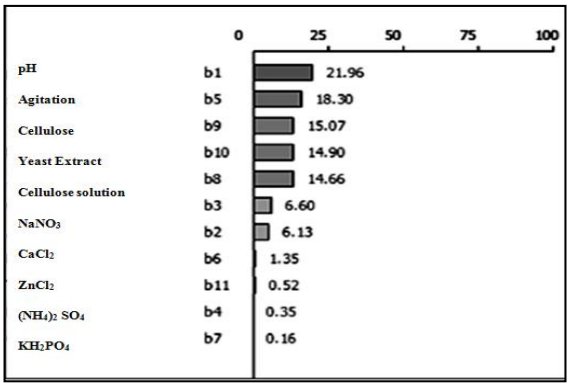

Fig. 2. The Weight frequency chart.

Pareto chart

The Pareto chart, commonly known as the Law of 80-20, is a tool for classifying the causes of a problem in descending order to highlight the main causes of the problem. So it's a visual decision-making medium. The Pareto chart has allowed us to know the factors that have an influence of more than 80% on our response which is other than enzymatic activity in order to be able to do a screening is to retain only these influencing factors. The cumulative Pareto chart supports the results given by the weight frequency chart, by classifying the factors according to their contribution in the variation of the response and to identify the most influential factors by accumulating the weights of the latter. It is widely accepted that 20% of the effort brings about 80% of the results. Thus, the pH, cellulose Solution, $ZnCl_2$, $(NH_4)_2SO_4$et KH_2PO_4, prove to be the most influencing factors in viscosity.

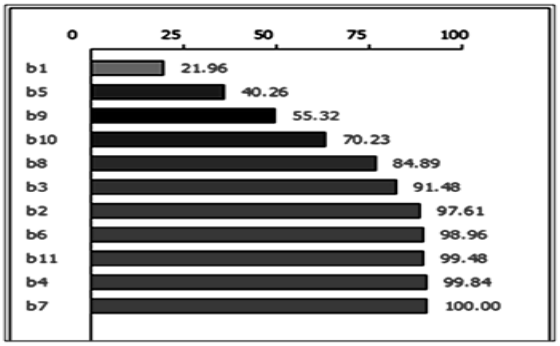

Fig. 3. The accumulated Pareto chart.

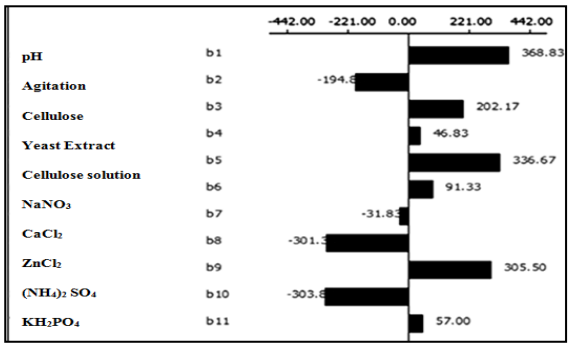

Fig. 4. Factorial weight of each factor on our surveyed response.

Analysis by the NEMRODW software

After conducting the experiments and carefully measuring their responses, they were involved in the experimentation plan or in the matrix of experiments. The results obtained are processed by the NEMRODW software.

Nemrodw (Nemrodw, 2000) is a software that is exclusively dedicated to the construction and analysis of experience plans. Like any highly specialized software, it has the advantage of being quickly usable to process a study carried out using an experience plan. The graph of the factorial weights obtained by the NEMRODW software allows us to decrease the weight of the input factors which are also the factors

studied such as pH, cellulose solution, $ZnCl_2$, $(NH_4)_2$, SO_4 and KH_2PO_4which one seeks to analyze an influence in a plan of experiments.

The response is the magnitude measured at each trial, the plan is to determine what factors influence it or how it evolves. It is the stage of screening of factors that consists in quickly searching, among a set of potentially influential factors, those that are actually in an experimental field set. After treatment of the various factors by the diagrams (Fig. 1 and 2) and the graph (Fig. 3), only those influencing the enzymatic activity of 84.89% were selected and represented in table 5. Then, simple linear regression was passed to evaluate the quality of our model by the coefficient of determination R2 is the correlation coefficient shown in the following table.

Table 5. Experiment matrix for 5 factors influencing enzymatic activity.

Factors	level (+)	level (-)
pH	6	5
Cellulose Solution	36 g/l	0 g/l
$ZnCl_2$	0,02 g/l	0 g/l
$(NH_4)_2 SO_4$	0,5 g/l	0 g/l
KH_2PO_4	0,1 g/l	0 g/l

Table 6. Simple linear regression coefficients.

Statistics regression	Coefficient
Correlation	0,92
R^2 determination	0,85
Observations	12

The counting of the results indicates a correlation coefficient of the order of 0.92 which means that we have an alignment between our response and the other factors. In addition, a correlation coefficient of 0.85 was obtained, which shows that our model is able of predicting 85% of the observations studied.

Analysis by ANOVA

ANOVA is a very useful method for analyzing the results of controlled experiments carried out in the laboratory or in the field. Generally speaking, the objective of an analysis of variance (ANOVA) is to test the significant differences between the mean of several independent groups of observations. The ANOVA table breaks down the variability in the number of contributions due to the various factors. The contribution of each factor is measured after elimination of the effects of the other factors and the values of the probabilities test the statistical significance of each of them.

If one of the probabilities is less than 0.05, this factor has a statistically significant effect on number at the confidence level of 95.0%. Conversely, if the probability value is greater than 0.05, this factor does not have a statistically significant effect. We used ANOVA as a modeling tool to be able to verify the assumptions of the existence of a significant slope or not and thus to know the significance of each factor studied separately.

Table 7. Modeling of enzyme production by (ANOVA).

Analysis of variance	Degree of liberty	Sum of squares	Average Squares	F_{cal}	P value
Regression	5	6309950,33	1261990,067	6,73	0,0189
Residues	6	1123546,66	187257,77		
Total	11	7433497			

The variable regression at a probability less than 0.05 which leads us to say that there is a significant slope then the interpolation of the factors on the right allows us to estimate the response. Following this deduction, we spent analysis of the variance of factors such as pH, Cellulose solution, $(NH_4)_2$, SO_4, KH_2PO_4, and $ZnCl_2$ (Table 8).

Table 8. Significance of factors.

	Coefficients	Statistics t	P value
Constant	4483,33	3,197	0,018
pH	737,66	2,952	0,025
Cellulose Solution	18,70	2,695	0,035
$(NH_4)_2 SO_4$	1222	2,445	0,046
KH_2PO_4	-6076,66	-2,433	0.042
$ZnCl_2$	-30133,33	-2,412	0.049

The results obtained clearly show that there is a significant weight on the response studied, since the probability is less than 0.05. So according to the statistical tests already mentioned, we can then postulate our model by replacing the coefficients by their calculated values is the factors by their levels.

Y = 4483,33 + 737,66 X1 + 18,70 X5 + 1222 X8 - 6076.66 X9 - 30133,33 X10.

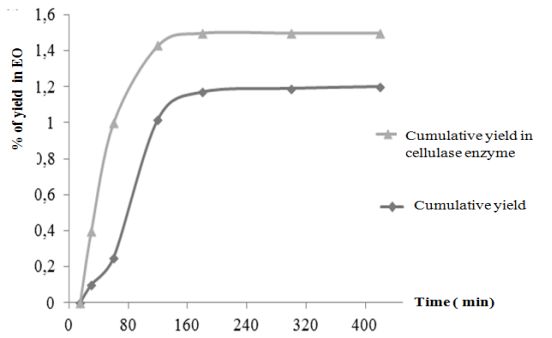

Fig. 5. Kinetics of production of essential oils from *Rosmarinus officinalis* leaves in the presence of cellulase enzymes.

Table 9. Essential oil distillation with and without use of cellulase.

Test	Yield EO	Cellulase (U/ml)	Yield EO
1	1,19	0	1,20
2	1,20	1,4	1,25
3	1,18	2,8	1,29
4	1,22	4,2	1,32
5	1,21	5,6	1,40
6	1,20	7,02	1,42
7	1,22	9.8	1,45

The hydrodistillation of *Rosmarinus officinalis* results in a cumulative yield of 1.2 g/100g, whereas the scenario is no longer the same with the use of Cellulases. These enzymes that have an effect on plant material degradation, are proving substantial in the process of optimizing the extraction of essential oils by increasing yield to 1.45 g/100g. Results confirmed by statistical analysis and predictive model.

Conclusion

Better optimization of the extraction of essential oils is always useful and stimulating, especially by acting in the best measures of respect for the environment. This is the case with this pilot study, extraction of essential oils by the use of pectinolytic enzymes. The hydrodistilation technique coupled with the use of pectinases as enzymatic catalysts offers significant advantages over other conventional methods, it has increased yield to 1.45 g/100g of dry matter, also , we were able to model the process of hydrodistillation of the species *Rosmarinus officinalis*. These improved results, are of great relevance for the extraction of essential oils by thus bringing, by their usefulness and their usability, a new element of knowledge in the technique of hydro distillation will make the exploitation. As simple as it is profitable. The use of pectolytic enzymes would also be a comparative advantage in the enhancement of the plant biomass of rosemary.

References

Albersheim P, Neukom H, Deuel H. 1960. Uber die Bildung von ungesattigten Abbauprodukten durch ein pekinabbauendes Enzym. Helv. Chim. Acta **43(5),** 1422-1426.
DOI:10.1002/hlca.19600430525

Bachiri G. Echchegadda J. Ibijbijen L. Nassiri. 2016. Etude Phytochimique Et Activité Antibactérienne De Deux Espèces De *Lavande* Autochtones Au *Maroc*: «Lavandula stoechas L. et Lavandula dentata L. European Journal of Scientific Research **12(30),** October 2016.

Benabdelkader. 2012. Bioddiversité,, Bioactivité et Biosynthèse des Composes Terpéniques Volatils des Lavandes Ailées,, *Lavandula stoechas* Sensu Lato, un Complexe d'Espèces Méditerranéennes d'Intérêt Pharmacologique. Doctoral thesis 2012 Université Jean Monnet-Saint Etienne Faculté Des Sciences Et Techniques Ecole Doctorale: Sciences, Ingénierie et Santé.

Benayad N. 2013. Évaluation de l'activité insecticide et antibactérienne des plantes aromatiques et médicinales Marocaines. Extraction de métabolites secondaires des champignons endophytiques isolés de plantes Marocaines et activité anticancéreuse. Doctoral thesis. Université Mohammed V – Agdal Faculté Des Sciences Raba 2013, 1.

Chahboun N, Esmail A, Abed H, Barrahi M, Amiyare R, Berrabeh M, Oudda H, Ouhssine M. 2015. Evaluation de l'activité bactériostatique d'huile essentielle de la *Lavandula officinalis* vis-à-vis des souches d'origine clinique résistantes aux antibiotiques . J. Mater. Environ. Sci **6(4),** 1186-1191.

Fechtal M, Tahiri T, Benabid A, Benrahmoune Z. 1996. Estimation de la biomasse du Romarin (*Rosmarinus officinalis* L.) dans le centre occidental du Rif. Ann. Rech. For. Maroc. T **(29),** 50-56.

Fleurentin J, Hoefler C, Mortier F, Pelet JM. 1986. Pharmacologie des extraits de jeunes pousses et de plante entière de *Rosmarinus officinalis*. Journel of Phytotherapy **20,** pp 19- 29.

Fogarty MV, Kelly CT. 1983. Pectic enzymes. In: Fogarty, M.W. (Ed.), Microbial Enzymes and Biotechnology. Applied Science Publishers, London p. 131-182.

Gouzi. 2014. Extraction et caractérisation biochimique des polyphénol oxydases de champignons et leur application en biocatalyse supportée. Doctoral thesis, L'université Pierre Et Marie Curie www.tel.archives-ouvertes.fr/tel-01158909/document

Hadamard J. 1893. Résolution d'une question relative aux déterminants», Bulletin des sciences mathématiques, vol. **17,** p. 240-246.

HCEFLD haut commissariat aux eaux et forets et la lutte contre la désertification. 2015. Stratégie nationale de développement des plantes aromatiques et médicinalesspontanées. http://unctad.org/meetings/ en/Presentation/ditc-ted-20102015-NGER-Morocco-BABA-42.pdf

Hoa BT, Hung PV. 2013. Optimization of nutritional composition and fermentation conditions for cellulase and pectinase production by *Aspergillus oryzae* using response surface methodology. International Food Research Journal **20(6),** pp. 3269-3274.

Hussain AI, Anwar F, Chatha SAS, Jabbar A, Mahboob S. 2010. *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian Journal of Microbiology **41,** 1070-1078.

Jayani RS, Saxena S, Gupta R. 2005. Microbial pectinolytic enzymes: a review". Process Biochem **40,** 2931-2944.

Kertesz Z. 1955. Pectic enzymes. In:Methods inenzymology (Vol. 1), Ed.S.P. Colowik & N.O.Kaplan. Academic press, New York, USA. 158.

Linden G. 2012. Transformation des produits alimentaires par les enzymes. Univ abderahmane mira de bejaia/bibliob1.

Mario DL, Sanna-Passino G, Demontis S. 2002. "Essential Oil Formulations Useful as a New Tool for Insect Pest Control. "Pharm. Sc.iTech **3, 2,** 1-11.

Miller GL. 1959. Use of dinitrosalicylic acid reagentfor determination of reducing sugar›› Analytical Chemistry, vol. **31,** p. 426-428.

Penchev. 2010. Étude des procédés d'extraction et de purification de produits bioactifs à partir de plantes par couplage de techniques séparatives à basses et hautes pressions. Thèse de doctorat" Institut National Polytechnique de Toulouse 2010.

Perry MC. 2008. Evaluation de la curcumine comme agent anti-cancéreux dans le traitement des tumeurs cérébrales. Mémoire de maîtrise, université du Québec à Montréal https://archipel.uqam. ca/874/1/m10213.pdf

Peyron L. 1984. Production marocaine des plantes aromatiques". J Parf. Cosm **55**, 35-39.

Pinheiro VE, Desagiacomo CCV, Michelin M, Maller A, Monteiro LMO, Jorge JA, Polizeli MDLTD. 2017. Neosartorya glabra polygalacturonase produced from fruit peels as inducers has the potential for application in passion fruit and apple juices. Brazilian Journal of Food Technology **20,** pp.1-11.

Plouffe, 2010. Étude Du Potentiel Anti-Inflammatoire De La Quercétine Et De La Sésamine Sur Les Cellules Microgliales N9 Activées Par Trois Neurotoxines: Le Lipopol Ysaccharide, Le Méthyl-1-Phényl-4-Pyridinium Et Le Paraquat" L'université Du Québec À Trois-Rivières 2010. http://depot-e.uqtr.ca/ 1408/1/030135151.pdf

Rafie H, Soheila H, Grant E. 2017. A Novel Therapeutic Agent for Antioxidant, Antimicrobial, Anticancer, Antidiabetic, Antidepressant, Neuroprotective, Anti-Inflammatory, and Anti-Obesity Treatment". Biomed J Sci & Tech Res **1(4).** BJSTR.MS.ID.000371.

Riyaha H. 2013. Valorisation des plantes aromatiques et médicinales: Etude du potentiel chimique et antibactérien des huiles essentielles de Rosmarinus officinalis (sauvage et domestiqué). Mémoire de Master, Faculté des sciences et techniques Fès Maroc 2013.

Selmaoui KA, uazzani Touhami A. Mouria R, Benkirane and Douira A. 2017. Détection de l'activité enzymatique pectinolytique et cellulolytique des champignons responsables de la pourriture des pommes en conservation. International Journal of Innovation and Scientific Research Vol. 30 No. **2,** pp. 242-250.

Shimi H, Cillard J. 1994. Autoxydation de l'huile d'argan. *Arganiaspinosa* L. du Maroc."Sciences des Aliments **14,** 117-124.

USAID. 2016 Agence américaine pour le développement international:" Projet filiere des plantes Aromatiques et medicinales " JUIN 2006, www.agrimaroc.net/PAM-AAI.pdf