Nadiya Rashid Malik et al. 2016, Volume 4 Issue 1
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Optimization of Process Parameters in Extraction of Thyme Oil Using Response Surface Methodology (RSM)

[1]Nadiya Rashid Malik, [2]K. C. Yadav, [3]Anurag Verma

Abstract

Thyme is a labiate plant whose essential oil has demonstrated medicinal properties. The aim of this study was to evaluate the feasibility of replacing n-hexane with methanol for the extraction of oil from Thymus vulgaris and to optimize extraction time and temperature using Response Surface Methodology(RSM) by implementing central composite design-face-centered to obtain optimal yield of thyme oil using modified methanol extraction technique. For methanol solvent, the effect of the maceration process on extraction yield under reduced pressure was also evaluated. Higher oil yields were obtained when methanol soaked thyme leaves were macerated causing burst of cells and thus increasing the area for material exchanges. The optimal values of variables with methanol extraction were determined to be 45^{0}C for 3 h with a response yield of 20 ml. 1 HNMR results showed that Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol ; isomeric with carvacol ,found in thyme essential oil .These compounds have shown anti-inflammatory, antioxidant ,antibacterial ,antifungal and immunomodulatory properties.

Keywords: Maceration ,Methanol, Nuclear Magnetic Resonances (NMR) spectroscopy , Response Surface Methodology (RSM), Rotary Vacuum Evaporator, Thyme , Thymol.

Introduction

Nowadays consumer awareness regarding the use of synthetic chemical additives , foods preserved with natural additives have become boon to food technologists and in turn to consumers .Many natural compounds (thyme, basil ,clove and oregano) are gaining interest and are generally recognized as safe(GRAS).Use of Thyme dates back to 3500 BC by Sumerians and Egyptians .Its spread to Europe was due to Romans , as they used it to give aromatic flavors to liqueurs & cheese. Thyme is the general name for the many herb varieties of the Thymus species, all of which are native to Europe and Asia. Common or garden thyme is considered the principal type, and is utilized commercially for flowering and ornamental purposes. Thyme is native to the Western Mediterranean region, extending to south-eastern Italy. Thyme is a culinary Labiatae herb characterized by its volatile oil. Health promoting properties of these species have been attributed to their inherent secondary metabolites namely Volatile and phenolic compounds (Jordan et al., 2013). Extract of thyme rich in phenolic acids and flavonoids such as cafeic acid, syringic acid, genistic acid and luteolin with strong antioxidant activities have been proposed to be used as preservation for certain foods and pharmaceutical products (Chizzola et al.,2008). The antioxidant and antibacterial activities of the basic components of the essential oils of thyme (carvacrol and thymol) have been demonstrated. Due to their biological activities, essential oils of thyme are widely used in various cosmetics industries and found as a component of disinfectant and insecticides (Bousbia et al., 2009). Hexane extraction, the most common type of solvent extraction, requires expensive equipment to handle the solvent and to ensure worker safety measure because hexane is a highly volatile solvent. Hexane is classified as a hazardous air pollutant by the US Environmental Protection Agency and they thus consider vegetable oil extraction plants to represent a potential major source. It is estimated that 0.7 kg of hexane per ton of seed is released into the environment (United State Environment Protection

Agency, 2005). Exposure to hexane at 125ppm for 3 months causes peripheral nerve damage, muscle wasting, and atrophy (Agilent Technology, MSDS, Agilent Technology, 2008). For this reason, alternative solvent extraction methods are needed that eliminate the use of toxic compounds such as hexane (Tyson et al., 2004) Thus, market demand for the Green solvents is getting high. Extraction is the important step to obtain the valuable volatile oil. As per changing technologies the extraction techniques were also changed and modified to extract essential oils. These changes were led by the advancements in the scientific era. However, various problems are encountered during extraction of thyme essential oil which have direct impact on the quality and yield. Economic & environmental aspects are also affected. These problems seem to overshadow the genuine health benefits. Potential use of different green solvents , namely methanol, can be made for extraction of essential oils for environment related issues .These solvents are generally recognized as safe (GRAS) and approved by U.S. Food and Drug Administration as pharmaceutical and food additive.

High pressures increase rate of extraction and yield but significant amount of waxes are co-extracted and essential oil content is reduced .On the other hand, low pressures reduce boiling points of solvents facilitating faster extraction rates at lower temperatures. Rotary Vacuum evaporator has been used to extract the thyme oil as it yields superior quality essential oil and higher yields due to zero loss to surroundings or Vacuum.

The Response Surface Methodology (RSM) is important in designing, formulating, developing, and analyzing new scientific studying and products. It is also efficient in the improvement of existing studies and products. The most common applications of *RSM* are in Industrial, Biological and Clinical Science, Social Science, Food Science, and Physical and Engineering Sciences. It is a collection of mathematical and statistical techniques useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery 2005). For example, the content of essential oil of a herb is affected by a certain amount of temperature x1 and time x2. The essential oil can be extracted under any combination of treatment x1 and x2. Therefore, temperature and time can vary continuously. When treatments are from a continuous range of values, then a Response Surface Methodology is useful for developing, improving, and optimizing the response variable. In this case, the content of essential oil *y* is the response variable, and it is a function of temperature and time. It can be expressed as

$$y = f(x1, x2) + e$$

The variables x1 and x2 are independent variables where the response *y* depends on them. The dependent variable *y* is a function of x1, x2, and the experimental error term, denoted as *e*. The error term e represents any measurement error on the response, as well as other type of variations not counted in *f*. It is a statistical error that is assumed to distribute normally with zero mean and variance s. (Nuran Bradley., 2007). NMR is the most powerful analytical tool available currently. NMR allows characterization of a very small amount of sample (10mg), and does not destroy the sample (non-destructive technique). NMR spectra can provide vast information about a molecule's structure and is the only way to prove which compound is being analysed. NMR is used in conjunction with other types of spectroscopy and chemical analysis to fully confirm a complicated molecule's structure. A simple NMR spectrum plots absorption on the y axis, and magnetic field strength on the x axis (Scale is in parts per million, and usually goes from 0- 10 ppm). The different positions of NMR absorptions are described as chemical shifts (δ).A chemical shift is defined as the difference in parts per million (ppm) between the resonance frequency of the observed proton and that of the tetramethylsilane (TMS) hydrogens. TMS is the most common reference compound in NMR, it is set at δ=0ppm.

Recently, the World Health Organization has urged a reduction on salt consumption in order to reduce the incidence of cardiovascular diseases. By reducing the salt levels on processed foods, it is necessary to use other additives that ensure the preservation of food, hence, is generated a research field in safe foods development that contains natural ingredients displaying no detrimental effect on health. The use of essential oils as antibacterial additives ,antioxidant, antifungal which are aromatic compounds obtained from vegetal material (flowers, shoots, seeds, leaves, twigs, bark, grasses, wood, fruits or roots) makes it possible. Thyme oil shows almost all these effects in food, thus making it possible to keep food "Safe, Natural & Healthy". (Deans Sg Et Al., 1993).

Hence considering all the potential benefits, following objectives were taken up for this study

1). To extract thyme oil from *Thymus vulgaris*.

2) To optimize time and temperature by using response surface methodology (RSM).

3) To evaluate the quality of extracted thyme oil.

Materials and Methods

Rotary Vacuum Evaporator (RVE)

A laboratory Rotary Vacuum Evaporator (BUCHI TYPE) in the department of Food Process Engineering Lab, Vaugh School of Agricultural Engineering and Technology, SHIATS, Allahabad, was used for extraction of thyme oil. A picture of the Rotary Vacuum Evaporator is shown in Fig 2.1 It is used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. Rotary Vacuum evaporators function because lowering the pressure above a bulk liquid lowers the boiling points of the component liquids in it. It consists of two round bottomed flasks, condenser, heating water bath, bump traps and metal clips. The solvent collection flask is emptied prior to use to prevent mixing of chemicals. The flask with the solution is placed on rotary evaporator and bump traps are used to prevent splashing of solution into condenser. Metal clips are used to secure the flask and the bump trap. The dial on the motor is used to control speed of flask rotation (0-220 rpm). The Vacuum ON/OFF control is managed by turning a stop cock at top of condenser.The flask is lowered into the water bath by using a handle . The solvent starts collecting on the condenser and drip into the receiving flask. The temperature of heating bath is controlled by an adjustable dial indicating temperatures. Liquid solvents can be removed without excessive heating of sensitive solvent solute combinations. Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents such a n –hexane, methanol or ethyl acetate from compounds which are solid at room temperature and pressure. It also allows removal of a solvent from a sample containing a liquid compound if there is minimal co evaporation (azeotropic behavior), and a sufficient difference in boiling points at the chosen temperature and reduced pressure. A oil sealed rotary vane type high Vacuum pump(Discharge : 50 l/min) was was used to create the Vacuum in the system The pump consists of rotor, with two spring loaded vanes mounted eccentrically in the stator body, which rotates inside a statinary casing. The level of Vacuum created is indicated by a Vacuum gauge. A control panel is provided with ON/OFF switch mains.

Figure 2.1: Rotary Vacuum Evaporator

Experiments

Sample collection

The dried leaves of *Thymus vulgaris* were purchased from ˋˋCortus India Private limited", New Delhi. The dried thyme leaves were in hygienic condition and were not affected by any damage and spoilage.

Experimental design

Response Surface Methodology (RSM) was used for designing the experiments (Khuri and Cornell, 1987). RSM used in this study was a central composite face-centered design involving two different factors : Temperature and Time . The extraction of essential oil from *Thymus vulgaris* was accessed based on the face-centered experimental plan as shown in table 2.2.2The results were analyzed using Analysis Of Variance (ANOVA) given in results table 3.2(b)by design expert 8 software. Three-Dimensional plots & their respective contour plots were obtained based on the effect of the levels of two factors on the Response 1(Yield) were studied. The optimum region was also identified based on the main parameters in overlay plot. The experiment was repeated randomly and each result was compared with predicted values to determine the model adequacy.

Table 2.2.2. Effect of Temperature and Time on oil Yield

Standard	Run	**Factor 1** Temperature (˚C)	**Factor 2** Time (h)	**Response 1** Yield R1(ml)
7	1	37.5	2	10
4	2	45	3	20
13	3	37.5	2.5	13.5
10	4	37.5	2.5	13.5
5	5	30	2.5	8.5
12	6	37.5	2.5	13.5
11	7	37.5	2.5	13.5
1	8	30	2	7
3	9	30	3	9
8	10	37.5	3	14
6	11	45	2.5	18
2	12	45	2	16
9	13	37.5	2.5	13.5

Preparation of thyme extract

Thyme plant extracts were obtained by Rotary Vacuum Evaporator (RVE) The dried thyme leaves (40g) were extracted with methanol solvent (400 ml) at 29˚C by dipping the leaves in methanol for two days. Dipping was followed by maceration in mortar and pestle for about an hour. The whole mixture was then transferred to rotary Vacuum evaporator flask at different time temperatures using RSM software generated runs. Vacuum was created in Rotary evaporator by running Vacuum pump after certain time intervals for efficient oil extraction at lower temperatures. Oil containing traces of solvent were collected in the separate round bottom flask attached to rotary Vacuum evaporator. Methanol was evaporated by subjecting the oil-solvent mix to heat in water bath at 70˚C for about an hour . thyme oil was thus extracted on the principle of difference in boiling points of solvent and oil. The obtained extracts were kept in the dark at 4˚C until they were used.

(a)

b)

Figure 2.2: (a) Macerated methanol Thyme extracts (b) Thyme oil

Analysis of moisture content

Initial moisture contents of the thyme leaves were determined by the hot air oven method. It was worked out by weighing 5g sample accurately and subjected to oven drying at 110°C for 4 -6h. Oven dried samples were cooled in desiccators and weighed. The drying was repeated until the constant weights were obtained .The resultant loss in weight was calculated as % moisture content (A.O.A.C 1990).

$$\frac{\text{Difference in dry weight}}{\text{Weight of sample}} \times 100 \qquad [1]$$

Analysis of Crude fat

Sample (5g) was weighed accurately in thimble and defatted with n-hexane in soxhlet apparatus for apparatus for 2 h. The resultant ether extract was evaporated and crude fat content was calculated as per A.OA.C, 1990 method.

Crude fat%=

$$\frac{(\text{Weight of flask+oil}) - \text{Weight of Flask}}{\text{Weight of sample}} \times 100 \text{ .... [2]}$$

Total Ash

Total ash was determined according to A.O.A.C (1990). Sample (5g) was weighed into a crucible and ignited at low flame till all the material was completely become smokeless. Then it was kept in muffle furnace for 6h at 600°C and further cooled in desiccators and weighed. This was repeated till 2 consecutive weights were constant and percentage ash was calculated.

$$\text{Ash\%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \text{ .... [3]}$$

Statistical Analysis

ANOVA

The analysis of variance of the data obtained was done completely Randomized Design (CRD) for different treatments as per the methods given by (Panse and Sukhatme., 1967) for values of Ash Moisture and Crude fat content. The experiment was conducted by adopting completely randomized Fisher in 1923 gives an appropriate method capable of analyzing the variation of population variance. The significant affect of treatment was judged with the help of 'F' (variance ratio). Calculated F value was compared with the table value of F at 5% level of significance. If calculated value exceeded the table value the effect was considered to the significant. The significance of the study was tested 5% level.

t = $\sqrt{(n-2)} / \sqrt{(1-1/2^2)}$

S.Ed. = MESS / r × t ×s

C. D = S.Ed × t 5 % at e. d. f.

Where,

t = distribution of observation

r = co-efficient of correlation

n = no. of observation

S.Ed. = standard error of difference

e.d.f = error of degree of freedom

C.D = critical difference

MESS = error mean sum of square

Response Surface Methodology (RSM)

RSM was used for designing the experiments (Khuri and Cornell, 1987) .RSM used in this study was a central composite face-centered design involving two different factors: Temperature and Time. The extraction of essential oil from *Thymus vulgaris* was accessed based on the face-centered experimental plan as shown in table -----.The results were analyzed using Analysis Of Variance (ANOVA) given in table ---- by design expert 8 software. Three-Dimensional plots & their respective contour plots were obtained based on the effect of the levels of two factors on the Response 1 (Yield) were studied. The optimum region was also identified based on the main parameters in overlay plot. The experiment was repeated randomly and each result was compared with predicted values to determine the model adequacy.

Nuclear Magnetic Resonance (NMR) Spectroscopy

H NMR spectroscopy for structural analysis of modified methanol extracted thyme oil was carried out on a Bruker 500 MHz spectrophotometer using $CDCl_3$ as solvent. ^{1}HNMR spectra of solution CDCl3 was calibrated to tetramethylsilane (TMS) as international standard.

Results and Discussions

Proximate composition of Thyme leaves

Proximate composition generally represents the nutritional quality of product. It is necessary to observe the proximate composition of dried leaves so as to judge the effect on final product after utilization as an ingredient. The proximate composition of Thyme leaves was determined using ANOVA (analysis of variance) and presented in the following table 3.1.

Table 3.1 Proximate composition of thyme leaves

Sample	**Ash**	**Fat**	**Moisture**
T1	2.40	15.33	9.07
T2	2.32	15.30	9.45
T3	2.30	15.35	9.48
T4	2.30	15.30	9.49
Mean	**2.33**	**15.32**	**9.3725**
F- test	S	S	S
S. Ed. (±)	0.006	0.008	0.010
C. D. (P = 0.05)	0.013	0.017	0.022

The data represented in the above table describes composition of major constituents of Thyme. The Average moisture content of thyme leaves was found to be 9.37 ± 0.010.. The mean value of crude fat and ash of dried Thyme leaves was observed to be 15.32 ± 0.006 and 2.33±0.006 respectively. Fig 3.1.1 shows the differences in proximate composition of four sample (T1, T2, T3 and T4) determinations undertaken for analysis.

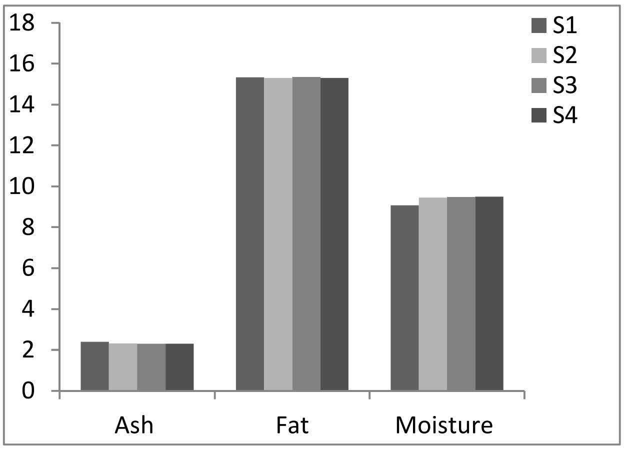

Figure 3.1.1: Differences in proximate compositions of four samples

Analysis of Thyme oil extracted at different intervals of time and temperature using Response Surface Methodology (RSM)

Response surface Model was developed to represent the data and to find the optimal conditions of parameter values in extracted oil. The solvent extracted oil at different experimental conditions given in table 2.2.2 above. The extracted oil ranged from 7 ml to 20 ml. The maximum value was at experimental conditions of 45^{0}C for 3 h. whereas the minimum value was at 30^{0}C for 2h. The extraction Process was based on the difference in boiling points of solvent and oil. The data on extracted oil under different experimental were analyzed using ANOVA. The model was tested for their adequacies to describe response surface with following results in table 3.2(a) and 3.2(b) with the final equations in terms of coded and actual factors.

Table 3.2(a) ANOVA Results of Response Function

Std. Dev.	**0.46**	**R-Squared**	**0.9910**
Mean	13.08	Adj R-Squared	0.9846
C.V. %	3.53	Pred R-Squared	0.9304
PRESS	11.59	Adeq Precision	41.948

Table 3.2(b) ANOVA Results of Response Function

SOURCE	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob >F**
Model	164.93	5	32.99	154.53	<0.0001
A-Temp	145.04	1	145.04	679.46	<0.0001
B-Time	16.67	1	16.67	78.08	<0.0001
AB	1	1	1.00	4.68	<0.0001
A2	0.35	1	0.35	1.62	0.0672
B2	2.22	1	2.22	10.40	0.2442
Residual	1.49	7	0.21		0.0146
Lack of Fit	1.49	3	0.5		
Pure Error	0	4	0.00		
Cor Total	166.42	12			

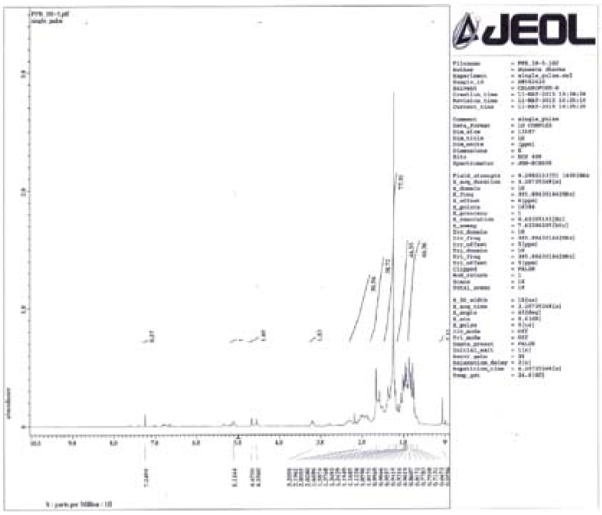

Figure 3.2.1(a) Response Surfaces Plot showing the effect of temperature and time on the extraction of thyme oil

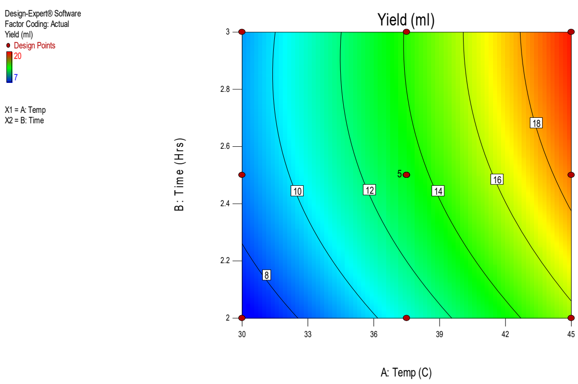

Figure 3.2.1(b) Contour Plot showing the effect of temperature and time on the extraction of thyme oil

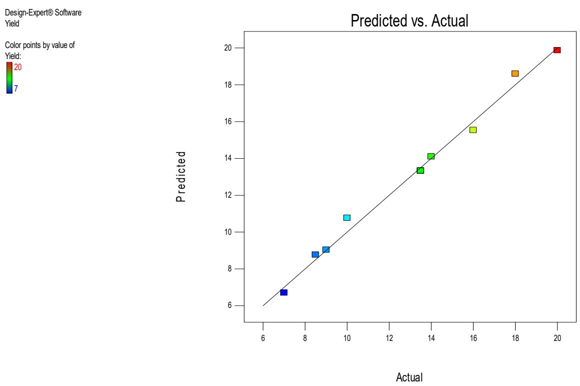

Figure 3.2.1 (C) Graph showing prediction and actual values

Nuclear Magnetic Resonance (NMR) spectroscopy

HNMR of modified methanol extracted thyme oil showed good agreement with reported results of thyme oil as shown in Fig 3.3. The spectrum were recorded (CDC13, 500MHz) had δ (ppm) 1.50 (d, 6H, 2×CH_3), 2.19(s, 3H, CH_3) 3.20 [sept, 1H, CH $(CH_3)_2$], 4.5 (br, 1H, OH), 6.63 (s, 1H, aromatic, H-6), 6.75 (d, 1H, aromatic). Based upon results and comparison of the spectra with the corresponding standard spectra of thymol (reported) the hexane extracted compound was identified as thymol, 2-isopropyl-5-methylphenol.

Conclusions

Based on the experimental results reported herein, the followings conclusions were made:

1. Sincere efforts were made to explore the potential of thyme oil in food products The Mean nutritional composition of dried thyme leaves showed that dried thyme leaves contain Moisture 9.37%, Ash 2.33% and Crude Fat 15.32%.
2. Experiments were conducted to optimize the process parameter for extraction of thyme oil by modified rotary Vacuum evaporator technique using methanol as solvent optimized by Response surface methodology (RSM).
3. The extracted oil content values ranged from 7ml to 20 ml. The maximum value was at experimental conditions of 3h at 45^0C temperature. The minimum value was found at 2h at 30^0C. The removal of oil in case of solvent extraction process was based on principle difference in boiling points of solvent and oil. It was observed that the yield of essential oil incremented with increase in temperature and yield may decrease with increase in pressure.
4. In the present investigation attempts were made to study the effect of grinding of methanol soaked dried leaves in mortar and pestle. Grinding helped to reduce the size of leaves and increase the surface area that contributes to oil diffusion during extraction .Grinding once was sufficient to obtain a suitable particle size and hence maximum oil yield in thyme oil extraction.
5. It was concluded that Thyme oil contains Thymol (2-isopropyl-5-methyl phenol) as the main monoterpene phenol with antifungal, antibacterial and antioxidant properties as reported in ^{1}H NMR spectroscopy .^{1}HNMR of methanol extracted thyme oil showed good agreement with reported results of hexane extracted thyme oil. The spectrum were recorded(CDC13,500MHz)hadδ(ppm)1.50(d,6H,2×CH

$_3$),2.19(s,3H,CH_3)3.20[sept,1H,$CH(CH_3)_2$],4.5(br,1H,OH), 6.63(s,1H,aromatic,H-6),6.75 (d,1H,aromatic).

Acknowledgements

The authors would like to acknowledge the Department of Food Process Engineering, Vaugh School of Agricultural Engineering and Technology, SHIATS, for supporting this Project.

References

[1] Jordan, M.J., Maria, V.L., Rota, C., Loran, S., Sotomayor, J.A., 2013. Effect of bioclimatic area on the essential oil composition and antibacterial activity of Rosmarinus officinalis L. Food Control 30, 463–468.

[2] Chizzola, R., Michitsch, H., Franz, C., 2008. Antioxidative properties of Thymus vulgaris leaves: comparison of different extracts and essential oil chemotypes. J.Agric. Food Chem. 56, 6897–6904.

[3] Bousbia, N., Vian, M.A., Ferhat, M.A., Petitcolas, E., Meklati, B.Y., Chemat, F., 2009. Comparison of two isolation methods for essential oil from rosemary leaves: hydrodistillation and microwave hydrodiffusion and gravity. Food Chem. 114, 355–362.

[4] Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – a review. Food Chem. Toxicol. 46, 446–475.

[5] Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., 2007. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link. Food Chem. 105, 146–155.

[6] Kisko, G., Roller, S., 2005. Carvacrol and p-cymene inactivate Escherichia coli O157:H7 in apple juice. BMC Microbiol. 5, 36, http://dx.doi.org/10.1186/1471-2180-5-36.

[7] Fadli, M., Saad, A., Sayadi, S., Chevalier, J., Mezrioui, N.-E., Pages, J.-M., Hassani, L., 2012. Antibacterial activity of Thymus maroccanus and Thymus broussonetii essential oils against nosocomial infection – bacteria and their synergistic potential with antibiotics. Phytomedicine 19, 464–471.

[8] Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. 94, 223–253.

[9] Deans, S.G. and G. Ritchie, 1987. Antibacterial properties of plant essential oils. Int. J. Food Microbiol., 5: 165–180

[10] Douglas, M. H., & McGimpsey, J. A. (1994). Seasonal variation in essential oil yield and composition from naturalized Thymus vulgaris L.

[11]Dorman H. J. D., Deans S. G. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308–316 10.1046/j.1365-2672.2000.00969.

[12] FDA, Department of health and human services, title 26,vol 6,parts 500-599.

[13] Govaris A., Botsoglou E., Sergelidis D., Chatzopoulou P. S. (2011) Antibacterial activity of oregano and thyme essential oils against Listeria monocytogenes and Escherichia coli O157, H7 in feta cheese packaged under modified atmosphere. Food Sci. Technol. 44, 1240–1244.

[14] Grieve and Mrs. Maud. (2008) Thyme .A Modern Herbal © copyright protected 1995-2014 Botanical.com.

[15] Ivan Angelov, David Villanueva Bermejo, Roumiana P. Stateva, Guillermo Reglero, Elena Ibanez and Tiziana Fornari (2013) Extraction of thymol from different varieties of thyme plants using green solvents. Journal of Engineering Science and Technology EURECA 2013 Special Issue August (2014) 79 – 88 © School of Engineering, Taylor's University 79

[16] Kuok Loong NG, Puteri Farah Wahida, Chien Hwa Chong(2014) Optimisation of extraction of thymol from plectranthus amboinicus leaves using response surface methodology .Journal of Engineering Science and Technology EURECA 2013 Special Issue August (2014) 79 – 88 © School of Engineering, Taylor's University.

[17] Nuran Bradley (2007) The response surface methodology . Indiana university south bend.

[18] Ashnagar, A, Gharib Naseri, N and Ramazani, Characterization of the major chemical compounds found in Thymus vulgaris plant grown wildly in Chahar Mahal and Bakhtiari province of Iran .International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN : 0974-4304Vol. 3, No.1, pp 01-04, Jan-Mar 2011.

[19] Khuri A.I., Cornell J.A. (1987), Response Surfaces. Marcel Dekker, New York.

[20] PANSE, V.G. AND SUKHATME, P.V (1967),.Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research, New Delhi.

[21] Montgomery Douglas C (2005). Design and Analysis of Experiments Response Surface Method and Designs (New Jersey, John Wiley and Sons, Inc).

[22] Hagenmaier, R.D (1974). Aqueous processing of full-fat sunflower seeds: yields of oil and protein. J. Am. Oil Chem. Soc. 51, 470–471.

[23] Kim, S.H. (1989). Aqueous extraction of oil from palm kernel. J. Food Sci, 54, 491-492.

Author's details

[1]Student, Department of Food Process Engineering, Sam Higginbottom Institute of Agricultural Sciences and Technology, UP, India, nadiyamalik21@gmail.com

[2]Assistant Professor, Department of Food Process Engineering, Sam Higginbottom Institute of Agricultural Sciences and Technology, UP, India, kailashnature@gmail.com

[3]Student, Department of Food Process Engineering, Sam Higginbottom Institute of Agricultural Sciences and Technology, UP, India, anuragver123@gmail.com.

Copy for Cite this Article- Nadiya Rashid Malik, K. C. Yadav, Anurag Verma, "Optimization Of Process Parameters In Extraction Of Thyme Oil Using Response Surface Methodology (RSM)', *International Journal of Science, Engineering and Technology,* Volume 4 Issue 1: 2016, pp. 248- 256.