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Optimization of Thyme Volatiles Retention by Refined Corn Oil Using Response Surface Methodology

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Abstract: ***Response surface methodology was used to optimize thyme volatiles retention by refined corn oil. The effects of two parameters namely mixing time and thyme flowers quantity, on volatiles retention in corn oil, were studied. Essential oil (EO) composition of thyme flowers and flavoured oil volatile compounds were analyzed by GC-MS. The fitted mathematical model allowed us to plot response surfaces as well as isoresponse curves and to determine optimal retention conditions. Thyme flowers EO was dominated by phenol fraction (67.30%). Monoterpene hydrocarbons attained 28.23% and carvacrol was the main component constituting 67% of thyme EO followed by γ-terpinene (7.83 %) and p-cymene (5. 88 %). Thus, three responses were studied: γ-terpinene, p-cymene and carvacrol retention by refined corn oil. Flavoured oil major volatiles were p-cymene (0.060 – 0.341 mg/ml), α-thujene (0.042 – 0.306 mg/ml) and γ-terpinene (0.011 – 0.202 mg/ml). The carvacrol was one of the minor volatile compounds of the flavoured oil ranging from 0.001 mg/ml to 0,020 mg/ml. Results clearly indicated that flowers quantity was the main factor influencing the γ-terpinene, p-cymene and carvacrol retention by refined corn oil. The selected optimal conditions were: Flowers quantity 5 g and mixing time 25 min. In these optimal conditions γ-terpinene, p-cymene and carvacrol yields can reach about 0.202 mg/ml, 0.341 mg/ml and 0.020 mg/ml, respectively. Oil enriched with thyme volatiles may have more biological activities and increased oxidative stability then refined one.***

Keywords: Response surface methodology, thyme flowers, volatiles, central composite design, corn oil.

1. Introduction

Seed oil composition has been studied extensively [1]. Recently, more interest is given to its minor compounds (volatiles, chlorophylls, phenolics…) [2]. In order to improve quality of crude oils, refining process is necessary to remove undesirable compounds (phospholipids, free fatty acids, pigments and volatile compounds) responsible for off-flavours; although some unwanted non-volatile compounds might remain at the end of the process [3]. To improve taste oil and to satisfy consumer preferences, one of the key discoveries was the incorporation of aromatic plants essential oils such as lavender, thyme and menthe [4],[5]. These essential oils (EOs) or some of their constituents were characterized by several biological activities, including antimicrobial and antioxidant ones [6]. Among plant extracts, *Thymus* species are widely used in the food industry as herbal teas, flavouring agents (condiment and spice), aromatic, and medicinal plants [7], [8]. They have also been used as carminative, diuretic, urinary disinfectant and vermifuge [9]. The antioxidant activity of genus *Thymus* members, including the well known specie, *Thymus capitatus*, has been widely reported [10], [11].The main objective of this study was to optimize refined corn oil aromatization by *Thymus capitatus* flowers EO. To the best of our knowledge, there are no published data dealing with corn oil supplementation with thyme EO. An efficient way of optimization might be to systematically create prototypes around the key ingredient levels of the product via some type of response surface experimental design [4]. Response surface methodology (RSM) is a collection of mathematical and statistical techniques that make a full description of independent variables effect in the vicinity of the optimum conditions [12], [13]. Several classes of treatment structures can be used as RSM experiments [14]. The most widely used class is very similar to a factorial experiment.

The aim of this study was to look for the experimental conditions leading to the maximum thyme volatiles retention by refined corn oil. As many factors can influence volatiles retention yield, central composite design (CCD) was applied to fit and exploit a mathematical model representing the relationship between the responses (γ-terpinene, *p*-cymene and carvacrol yields) and variables (mixing time and flowers quantity).

2. Materials and Methods

2.1. Plant material

Refined corn (*Zea mays* L.) oils were purchased from a local refinery located at Oued Ellil (North West of Tunisia) and stored at cold (4°C) in the dark. Fresh thyme flowers were collected in June 2009 at full flowering stage from the Mornag Mont (North of Tunisia). They were air-dried and stored at room temperature away from humidity.

2.2. Oil treatment with Natural Herbs

Thyme dried flowers were incorporated into refined corn oil in a mixing agitator at a rate of 1, 3 and 5g in 40 ml of oil, for 5, 15 and 25 min, after optimized conditions. The mixture was centrifuged in order to remove solid residue. After, flavoured oils were filtered and an internal standard (6-methyl-5-hepten-2-one) was added. Volatile compounds were extracted by dynamic headspace method with splashed gas N_2 (Strip-trap). Nitrogen gas was bubbled through 40 ml of oil at a flow rate corresponding to a constant pressure of 0.4 bar.

2.3. Thyme Essential Oil Extraction.

Three lots of 100 g of air-dried flowers were separately hydrodistilled for 90 min (time fixed after a kinetic survey during 30, 60, 90, 120 and 150 min). The volatile compounds of the oil were collected in diethyl ether using liquid–liquid isolation. All experiments were done in triplicates and results were expressed in percentages.

2.4. GC-FID analysis

Flavoured corn oil volatiles, were analyzed by GC using a Hewlett- Packard 6890 apparatus (Agilent technologies, Palo Alto, California, USA), equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polyethylene glycol capillary column (HP Innowax, 30m x 0.25 mm i.d, 0.25 µm film thickness) and an apolar HP-5 column (30 m x 0.25 mm, 0.25 µm film thickness) were used. The flow of the carrier gas (N2) was 1.6 ml/min and the split ratio in the injector was 60:1. The analysis was performed using the following temperature program: oven temperature isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C min-1152 , and isotherm at 205 °C during 10 min. Injector and detector temperatures were held, respectively, at 250 and 300 °C. Surfaces of peaks and percentages of the different compounds were determined using the same HP chemstation cited above.

2.5. GC-MS analysis

A HP 5890 series II coupled to a HP 5972 mass spectrometer with electron impact ionization (70eV) and a HP-5MS capillary column (30 m x 0.25 mm, 0.25 µm film thickness) were used. Column temperature was programmed to rise from 50°C to 240 °C at a rate of 5 °C/min; transfer line temperature was 250 °C. The carrier gas was helium with a flow rate of 1.2 ml/min and a split ratio of 60:1. Scan time and mass range were 1s and 40-300 m/z respectively.

2.6. Compounds identification

The identification of volatile components was assigned by comparison of their retention indices (RI) relative to (C_8–C_{22}) n-alkanes with those of literature or with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library 167 of the GC–MS data system and other published mass spectra [15].

2.7. Experimental design.

When many factors affect a desired response, it can be an exhausting task to optimize a process. Therefore, response surface methodology (RSM) can be an effective tool for optimizing the response [16]-[19]. Response surface methodology is defined as statistical method that uses quantitative data from appropriate experimental design to determine optimal conditions [20].
RSM was selected for the present study to optimize thyme volatiles retention in refined corn oil. The individual and interactive effects of the mixing time (X_1: 5, 15 and 25 min) and flowers quantity (X_2: 1, 3 and 5 g), on γ-terpinene, *p*-cymene and carvacrol retention (Y_1, Y_2 and Y_3, respectively) as responses (Dependent variables), were studied. In order to determine the significant experimental variables and develop a response surface for the optimization of corn oil aromatization, the major factors mentioned above were further investigated by CCD. The experimental range for each factor was selected on the basis of results obtained from preliminary experiments carried out by CCD [14]. The levels of the independent variables, mixing time (X_1) and flowers quantity (X_2), were coded as: +1 and -1, representing the maxima and minima for each parameter in the CCD.

A second order polynomial model was fitted for γ-terpinene, *p*-cymene and carvacrol retention, giving an equation of the following form:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 + e \qquad (1)$$

Where (Y) is the calculated response function, X_1 and X_2 are the levels of the independent variables, β_0 is the intercept term, β_1 and β_2 are the linear coefficients, β_{11} and β_{22} are the quadratic coefficient, β_{12} is the interaction coefficient and e is the global error . Nemrod-w software package was used for the regression analysis of the experimental data obtained [21]. Fit quality of the polynomial model equation was expressed by the determination coefficient R^2, and its statistical significance was checked by an F-test. The significance of the regression coefficient was tested by a t-test. Significance level was given as *** $P< 0.001$, ** $P< 0.01$, * $P< 0.05$. Differences with p-value superior to 0.05 were not considered significant. For CCD validation, optimum conditions were fixed on the basis of the data obtained from experimental design.

Table 1 presents independent variables levels in coded and encoded form according to the experimental design, and the responses for all experiments.

Table1. Experimental matrix for the central composite design

Run	X_1	X_2
1	-1.0000	-1.0000
2	1.0000	-1.0000
3	-1.0000	1.0000
4	1.0000	1.0000
5	-1.0000	0.0000
6	1.0000	0.0000
7	0.0000	-1.0000
8	0.0000	1.0000
9	0.0000	0.0000
10	0.0000	0.0000
11	0.0000	0.0000
12	0.0000	0.0000
13	0.0000	0.0000

3. Results and Discussion

3.1. Refined Corn Oil Aromatization

Thirty-one components were identified in the *Thymus capitatus* EO, amounting to 99.02% of the total oil, which are listed in Table 2 in order to their elution.

EO was characterized by its richness in phenols (67.30%) with carvacrol the major compound (67%), followed by monoterpene hydrocarbons which attained 28.23% mainly represented by γ-terpinene (7.83%) and *p*-cymene (5.88%). The monoterpene alcohol, terpinene-4-ol, was also found at high proportion of 4.46%. Other compounds are present with relatively low levels of between 1% and 2%: α-thujène (1.23%), myrcene (1.68%), α-terpinene (1.55%), 1,8 cineole (1.95%), linalool (1.12%) and β-caryophyllene (1.46%). Our results are in agreement with the chemotype carvacrol which characterizes the Tunisian *Thymus capitatus* already studied by Hedhili et al. [23] and Bounatirou et al. [8]. Thus, by studying the chemical composition of *Thymus capitatus* EO, these authors have mentioned that it was composed mostly by carvacrol (54-84, 6%), p-cymene (1, 9-17%), γ - terpinene (2-14%) and β-caryophyllene (1-9%). Moreover, Hazzit et al. [23], reported that *Thymus pallescens* EO was characterized by high levels of carvacrol (44.4-57.7%), p-cymene (10.3 - 17.3%) and γ-terpinene (10.8-14.2%) which represent the major compounds of the EO.

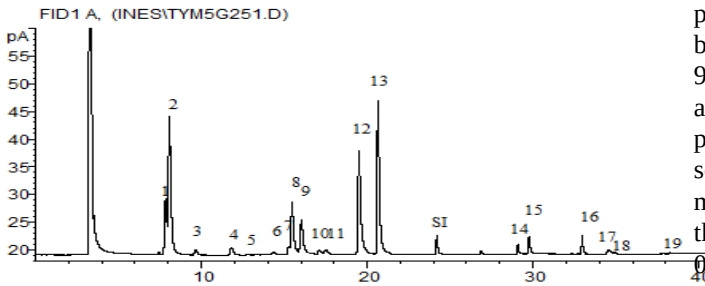

Figure 1: GC profile of flavoured corn oil volatile compounds (Mixing Time: 25 min; Flowers Quantity: 5 g).

The peak numbers correspond to the following: 1. α-pinene, 2. α-thujene, 3. Camphene, 4.β-pinene, 5. 1 octen-3ol, 6. δ-3-Carene, 7. α –phellandrene, 8. Myrcene, 9. α-terpinene, 10. Limonene, 11. 1.8-cineole, 12. γ-terpinene, 13. *p*-cymene, 14. Trans-sabinene hydrate, 15. Camphor, 16. Linalol, 17. Terpinene-4-ol, 18. β-caryophyllene, 19. Borneol, 20. Carvacrol, SI. Internal Standard

Fig 1 showed that twenty components were identified in flavoured oil representing 99.4 % to 99.77% of the total aroma.

Table 2: Essential oil composition (%) of *Thymus capitatus* dried flower and flavoured oil (optimal conditions: 5 g, 25 min)

Compounds [A]	Thyme	Thyme
α-thujene	1.23 ± 0.11[b]	**24.06 ± 0.03[a]**
α-pinene	0.50 ± 0.01[b]	8.16 ±0. 01[a]
Camphene	0.12 ± 0.02[b]	1.10 ±0.00[a]
1 octen-3ol	0.07 ± 0.00[b]	0.27 ±0.00[a]
Sabinene	0.57 ± 0.07[b]	-
β-pinene	0.18 ± 0.00[b]	1.65 ±0.00[a]
Myrcene	1.68 ± 0.03[b]	8.68 ±0.01[a]
α –phellandrene	0.14 ± 0.01[b]	0.75 ±0.01[a]
δ-3-Carene	0.49 ± 0.01[a]	0.5 ±0.01[a]
α-terpinene	1.55 ± 0.03[b]	6.28 ±0.02[a]
p-cymene	**5.88 ± 0.02[b]**	**26.47 ±0.03[a]**
Limonene	0.18 ± 0.01	0.63 ±0.00[a]
1-8 cineole	1.95 ± 0.17[a]	0.78 ±0.01[b]
E- β –Ocimene	0.14 ± 0.04[b]	-
Trans-sabinene hydrate	0.24 ± 0.01[b]	0.74 ±0.00[a]
γ-terpinene	**7.83 ± 0.06[b]**	**14.30 ±0.02[a]**
Linalol	1.12 ± 0.03[b]	1.38 ±0.02[a]
Camphor	0.05 ± 0.00[b]	1.58 ±0.01[a]
Borneol	0.37 ± 0.02[a]	0.09 ±0.00[b]
Terpinen-4-ol	**4.46 ± 0.02[a]**	0.59 ±0.01[b]
Teroinolene	0.18 ± 0.01[a]	-
Geraniol	0.25 ± 0.02[a]	-
acetate de linalyle	0.04 ± 0.00[a]	-
Thymol	0.31 ± 0.03[a]	-
Carvacrol	**66.99 ± 0.01[a]**	**1.56 ±0.01[b]**
Eugenol	0.09 ± 0.00 [a]	-
acetate de geranyle	0.26 ± 0.01[a]	-
acetate de neryle	0.15 ± 0.01[a]	-
β-caryophyllene	1.46 ± 0.04[a]	0.16 ±0.01[b]
Germacrene D	0.22 ± 0.01[a]	-
Caryophyllene oxide	0.32 ± 0.02[a]	-
Total	**99.02 ± 0.01[b]**	

From thyme flowers EO already studied, only 64.5% of the identified compounds have migrated in the flavoured oil. In addition, although thyme EO was characterized by high percentage of phenols (67.30%), flavoured oil was dominated by monoterpene hydrocarbons which represented 89.88 % to 92.59 % of the total aroma, followed by monoterpene alcohols (4.84 % - 3.07 %), ketones (1.53 % - 2.02 %), phenols (0.84 % - 2.84 %) and esters (0.78 % - 1.49 %) and sesquiterpene hydrocarbons (0 % - 0.44%). Flavoured oil major volatiles were *p*-cymene (0.060 – 0.341 mg/ml), α-thujene (0.042 – 0.306 mg/ml) and γ-terpinene (0.011 – 0.202 mg/ml). The carvacrol which was the major volatile compound of thyme flowers EO, become one of the minor volatile compounds of the flavoured oil ranging from 0.001 mg/ml to 0,020 mg/ml.

Concerning oil aromatized by thyme no data was available for its volatiles composition. However, the high affinity of monoterpene hydrocarbons to oil was mentioned by others. In fact, Cabrera [24] indicated that monoterpene hydrocarbons are apolar compounds followed by ethers, esters, ketones, alcohols and aldehydes, and confirmed that phenols were the most polar ones. Thus, due to the high hydrophobic matrix used in this study and lipophilic character of the monoterpene hydrocarbons this class had a higher affinity to corn oil than the others [25]. This could explain the high proportion of monoterpene hydrocarbons in oil and the low retention of phenols such as carvacrol despite the fact that it is the major compound of thyme EO.

Based on earlier studies, some constituents of plants, particularly terpenoids, have been reported to be useful in the management of inflammatory diseases [26]. In fact, some monoterpene hydrocarbons (β-pinene and γ-terpinene), have been found active as radical scavengers and antioxidant compounds [7]. Moreover, the presence of phenolic compounds in the flavoured oil could be of great interest because of their physiological function, including antioxidant, antimutagenic and antitumour activities [27] and which potentially have beneficial implications for human health [28]. Indeed, antioxidant activity of the phenolic compounds is characterized by having redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers and also have metal chelation properties [29], [30]. Although, phenolic volatiles, mainly carvacrol was weakly represented in our experiment, it might contribute to antioxidant property of the flavoured oil [31].
Thus, due to carvacrol, γ-terpinene, and *p*-cymene importance in thyme flowers EO and because of their several benefits, this study was focused on the optimization of the retention of these three compounds by refined corn oil using response surface methodology to determine optimal conditions of oil aromatization.

3.2. Response measurements

A total of 13 experiments with different combinations of the two variables were carried out according to the conditions indicated in Table 3. Response values (γ-terpinene, *p*-cymene and carvacrol yields) are reported in the last column of this table. The highest carvacrol content in the flavoured oil (0.020 mg/ml) was observed at runs number 4 and 8, where the factors mixing time and flowers quantity were used at their levels X_1 (+1, 0) and X_2 (+1) respectively. This value was about 20 fold higher than that observed at run number 1, where the related factors were used at their lower levels for X_1 (-1) and X_2 (-1). Also, the highest γ-terpinene and *p*-cymene contents that characterized the flavoured oil (0.202 mg/ml and 0.341 mg/ml, respectively) were observed at run number 4, where the independent variables mixing time and flowers quantity, were used at their higher levels X_1 (+1) and X_2 (+1), respectively. These values were about 18.3 (for the γ-terpinene yield) and 5.6 (for *p*-cymene yield) fold higher than those observed at run number 1, where the related factors were used at high levels for X_1 (-1) and X_2 (-1).

Table 3: Experimental conditions of the central composite design and the corresponding experimental responses

N° Exp	X_1	X_2	Y_1	Y_2	Y_3
1	-1	-1	0.011	0.062	0.001
2	1	-1	0.05	0.06	0.005
3	-1	1	0.146	0.211	0.015
4	1	1	0.202	0.341	0.02
5	-1	0	0.119	0.166	0.012
6	1	0	0.175	0.231	0.018
7	0	-1	0.043	0.101	0.004
8	0	1	0.196	0.316	0.02
9	0	0	0.17	0.238	0.017
10	0	0	0.169	0.24	0.017
11	0	0	0.171	0.241	0.017
12	0	0	0.174	0.24	0.017
13	0	0	0.1655	0.2377	0.0165

3.3. Validation of the Model

The analysis of variance for the fitted model showed that the regression sum of squares was statistically significant at the level 99.9% and the lack of fit is not significant. The regression coefficients and the analysis of the variance (ANOVA) indicate the high significance of the model (Table 4).

Table 4: Experimental conditions of the central composite design and the corresponding experimental responses

	Y_1		Y_2		Y_3	
Term	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
β_0	0.16912	***	0.23922	***	0.01689	***
β_1	0.02517	***	0.03217	***	0.00250	***
β_2	0.07333	***	0.10750	***	0.00750	***
β_{11}	-0.02017	***	-0.04043	***	-0.00183	***
β_{22}	-0.04767	***	-0.03043	***	-0.00483	***
β_{12}	0.00425	7.2%	0.03300	***	0.00025	6.8%

The coefficient of determination R^2 is used in the context of statistical models whose main purpose is the prediction of future outcomes on the basis of other related information. It is the proportion of variability in a data set that is accounted for by the statistical model and provides a measure of how well future outcomes are likely to be predicted by the model [32]. The R^2 value is always between 0 and 1. The closer the R^2 is to 1.0, the stronger the model and the better it predicts the response [33]. Variables Y_1, Y_2 and Y_3, yielded R^2 values of 0.998, 0.999 and 0.997, respectively. These high R^2 values showed the good agreement between the experimental results and the theoretical values predicted by the model [34] ($R^2_{Pred\ Y1}$ = 0.992; $R^2_{Pred\ Y2}$ = 0.999; $R^2_{Pred\ Y3}$ = 0.987 for Y_1, Y_2 and Y_3, respectively). Values of the adjusted determination coefficient were also very high to advocate for a high significance of the model [33] ($R^2_{Adj\ Y1}$ = 0.996; $R^2_{Adj\ Y2}$ =

0.999; $R^2_{Adj\ Y3}$ = 0.987). The statistical results obtained for the dependent variables (Y_1–Y_3) suggest that the model is appropriate for the data, as most of the factors and the interactions considered in the experimental design were significant at $p < 0.05$.

3.4. Statistical Analysis of Coefficients

The significance of each coefficient was determined by P-values which were listed in Table 4. The ANOVA analysis of the optimization study indicated that X_1, X_2, X_1^2 and X_2^2 were more significant ($P < 0.001$) than the effect of the interaction between the two independent variables (X_{12}). Mixing time and flowers quantity had a positive effect on γ-terpinene, *p*-cymene and carvacrol retention yields. However, the coefficient β_1 (0.0252, 0.0322 and 0.0025, for Y_1, Y_2 and Y_3, respectively) was lower than β_2 (0.0733, 0.1075, 0.00750 for Y_1, Y_2 and Y_3, respectively) at the $P < 0.001$ probability level, therefore the factor X_2 (Flowers quantity) was the most important factor affecting γ-terpinene, *p*-cymene and carvacrol retention by corn oil while the overall effect of X_1 was negligible because of the negative quadratic effect obtained ($\beta_1>0$; $\beta_{11}<0$). Hypothesis of no global significance factor X_1, is verified by examining response, that are well spread along the horizontal axis representing X_1. Negative quadratic effect was also obtained for flowers quantity at the $P < 0.001$ probability level ($\beta_{22} < 0$). This effect gave the appearance of curved curvilinear response surface to have optimal conditions for each response. The coefficient β_{12} of X_1X_2 interaction was found to be not significant ($P > 0.05$) for Y_1 and Y_3. Conversely, positive quadratic effects were obtained for mixing time and flowers quantity at the $P < 0.001$ probability level for Y_3 ($\beta_{12} = 0.033$, at $P < 0.001$)

The final equations for optimization of corn oil aromatization parameters derived from the application of the method (after eliminating non-significant terms) are given below:

$$Y_1 = 0.1691 + 0.0252\ X_1 + 0.0733\ X_2 - 0.0202\ X_1^2 - 0.0477\ X_2^2 + 0.0042\ X_1X_2 \quad (2)$$

$$Y_2 = 0.2392 + 0.0322\ X_1 + 0.1075\ X_2 - 0.0404\ X_1^2 - 0.0304\ X_2^2 + 0.0330\ X_1X_2 \quad (3)$$

$$Y_3 = 0.0169 + 0.0025\ X_1 + 0.0075\ X_2 - 0.0018\ X_1^2 - 0.00487\ X_2^2 + 0.0002\ X_1X_2 \quad (4)$$

3.5. Interpretation of the Response Surface Model

The relationship between the responses and the experimental variables can be illustrated graphically by plotting three-dimensional response surface plots (Fig. 2). The vertical axes show γ-terpinene, *p*-cymene and carvacrol retention yield (Y_1, Y_2 and Y_3, respectively), and each of the two horizontal axes represents the two independent variables (mixing time (X_1) and flowers quantity (X_2)). The topography of these response surfaces are also illustrated by isoresponse contours representing lines of constant response in a two variable plane. Such plots are helpful in studying the effects of the variation of the factors in the domain studied and consequently, in determining the optimal experimental conditions [35]. In Fig. 2, the examination of the isoresponse contours and three-dimensional plots showed that γ-terpinene, *p*-cymene and carvacrol retention by corn oil increased when increasing flowers quantity and mixing time. Sample presenting the most significant γ-terpinene, *p*-cymene and carvacrol contents (0.202 mg/ml, 0.341 mg/ml and 0.020 mg/ml, respectively) is that containing the highest flowers amount (5 g) and flavouring for the longer period of mixing time (25 min).

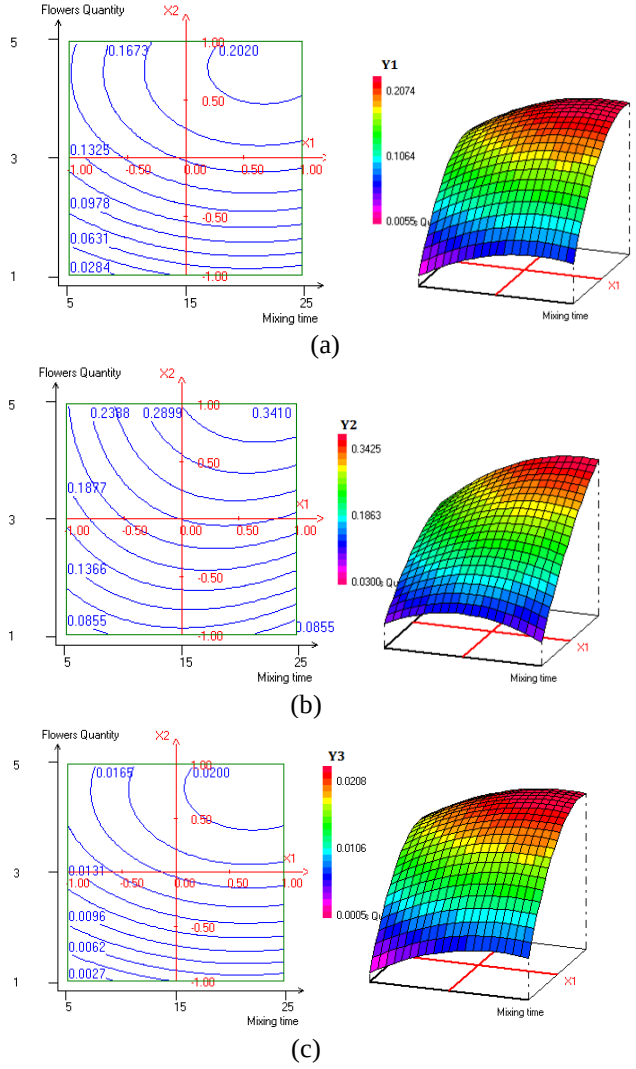

Figure 2: Three-dimensional response surface and contour plots for the effect of incubation time and peel quantity on γ-terpinene (a), *p*-cymene (b) and carvacrol (c) retention in refined corn oil.

Optimal conditions selected by the software NEMRODW were: Mixing time: 25 min and flowers quantity: 5 g. Under these conditions, the expected values of the γ-terpinene, *p*-cymene and carvacrol yields were Y_{1op} = 0.20403 mg/ml, Y_{2op} = 0.34102 mg/ml. and Y_{3op}= 0.02057 mg/ml, respectively.

4. Conclusion

This work has revealed that monoterpene hydrocarbons were abundant in the flavoured oil forming 92.59 % of the total aroma mainly represented by *p*-cymene, α-thujene and γ-terpinene. Thyme flowers major volatiles were carvacrol, γ-

terpinene and *p*-cymene. Due to the several biological activities of these volatile compounds, the response surface methodology was a useful tool to determine the optimal experimental conditions of their retention by refined corn oil. Aroma retention by refined corn oil increased significantly with increasing peel quantity and mixing time. The selected optimal conditions (Mixing time: 25 min and flowers quantity: 5 g) have been checked and confirmed. This enriched oil may have important biological activity. In fact, thyme EO and its volatile compounds exhibit a range of biological activities. A future study will focus on the influence of the γ-terpinene, *p*-cymene and carvacrol retention on phenolic composition of the flavoured corn oil and its antioxidant activities.

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