

Available online at www.sciencedirect.com

ScienceDirect

Resource-Efficient Technologies 3 (2017) 12–21

www.elsevier.com/locate/reffit

Research paper

Extraction of green absolute from thyme using ultrasound and sunflower oil

Dima Mnayer^{a,b,*}, Anne-Sylvie Fabiano-Tixier^a, Emmanuel Petitcolas^a, Karine Ruiz^a,
Tayssir Hamieh^{b,c}, Farid Chemat^a

^a Avignon University, INRA, UMR408, GREEN Extraction Team, 84000 Avignon, France

^b Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, 6573 Dekwaneh, Lebanon

^c Laboratory of Materials, Catalysis, Environment and Analytical Methods (MCEMA), Faculty of Sciences, Doctoral School of Sciences and Technology (EDST), Lebanese University, 6573-14 Beirut, Lebanon

Received 16 November 2016; received in revised form 7 December 2016; accepted 26 January 2017

Available online 15 February 2017

Abstract

Absolute is the plant aroma isolate mostly used in the food and fragrance sectors. The use of organic solvents constitutes the most commonly used method for obtaining this aroma. However, this technique may leave trace amounts of solvents which are considered undesirable for these industries. In this work, a new green extraction approach was implemented using ultrasound (US) with sunflower oil (SO) as a natural solvent to produce green absolute from thyme (*Thymus vulgaris*). US optimal conditions for absolute yield were investigated using response surface methodology (RSM) and compared to conventional SO (SO-CV) and hexane (Hex-CV) extractions. The absolutes were analyzed by GC–MS for their chemical composition and tested for their antioxidant activities (total phenols, DPPH and frying test).

Optimized conditions obtained by RSM for absolute yield were $T = 50\text{ }^{\circ}\text{C}$, $t = 22\text{ min}$, $P = 98\text{ W}$. The US using SO as solvent offers important advantages: shorter extraction time, increase of 47% in absolute yield compared to SO-CV extraction. Although the absolute obtained by hexane extraction provided improved yield (8.64 g/100 g DW), it contained around 75% of waxy materials. GC–MS analysis showed no remarkable variation of the chemical composition of the absolutes compared to those obtained by hexane extraction. Moreover, the US extraction allowed the highest recovery of monoterpene phenols thymol and carvacrol (86.2%). The absolute obtained by SO-US was free from waxes and organic solvent residues and exerted the highest antioxidant activity. Results show that ultrasound extraction using SO is a good alternative. It suggests the possibility of the production of green absolutes on pilot and industrial scale.

© 2017 Tomsk Polytechnic University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Green extraction; Ultrasound; Natural solvent

1. Introduction

The food and fragrance industries are interested in the plant aroma compounds such as concrete and absolute. The concrete is the plant extract with characteristic odor and flavor; it consists mainly of volatile compounds and non-volatile waxy substances obtained by petroleum solvents such as hexane or pentane. Then, the concrete is dissolved in ethanol solution to reduce the waxy components which disturb the formulation,

allowing the formation of the absolute, the most faithful aroma to the plant scent [1]. However, this extraction method may leave trace amounts of organic solvents, which are considered undesirable for these industries, because they are harmful to human health and the environment [2].

At the same time, there is a big interest in green chemistry to seek out for green extraction techniques that may reconcile the challenges set by the economics, the society and the environment. The green chemistry is intended to develop extraction processes, which reduce the energy consumption, allow the use of natural solvents and ensure a safe and high quality product [3,4].

Different absolute extractions using petrochemical solvents have been reported for flowers such as *Narcissus pseudonarcissus*

* Corresponding author. Avignon University, INRA, UMR408, GREEN Extraction Team, 84000 Avignon, France. Fax: 00961 1 510870.

E-mail address: d_mnayer@yahoo.fr (D. Mnayer).

Nomenclature

Abbreviations

| | |
|-----|------------------------------|
| Abs | Absolute |
| CV | Conventional |
| EO | Essential oil |
| Hex | Hexane |
| OC | Oleo-concrete |
| RSM | Response surface methodology |
| SE | Soxhlet extracts |
| SO | Sunflower oil |
| US | Ultrasound |

[5], *Michelia Champaca* Linn [1], *Tagetes patula* L. [6], and *Mimusops elengi* L. [7].

Thyme (*Thymus vulgaris*) is a perennial herbaceous shrub belonging to the Lamiaceae family. It is widespread throughout the Mediterranean region [8]. This aromatic plant is extensively used for food flavoring and is well known for its various beneficial effects, e.g., antiseptic, carminative, antimicrobial, and antioxidative properties [9]. Most of these biological properties are mediated by thymol and carvacrol, the main monoterpene phenol components of the thyme.

Different extraction techniques were conducted on thyme. The essential oil was obtained by steam distillation [10,11] and hydrodistillation [12,13]. The aroma active compounds of thyme were obtained by other extraction methods such as microwave assisted hydrodistillation [14], supercritical fluid extraction [15], solid phase microextraction, pressurized liquid extraction, Soxhlet extraction [16] and by the use of different solvents [17].

Although the different characteristics of thyme extracts were extensively studied, so far no reported works on absolute of thyme have been conducted yet for food application.

Ultrasound (US) has been used for various processes in the food industry. The technique is fast and allows the reduction of solvents, thus resulting in a more pure product and higher yields. This method has been applied to extract food components such as antioxidants [18], aromas [19] and other organic and mineral components from a variety of matrices [20–23]. The extra vibration of US improves the cavitation process and the contact surface that occurs between sample matrix and liquid solvent phase causing the rupture of cell walls, consequently enhancing the recovery yield of available extractable cell material [24,25]. The US was used for the recovery of aromatic compounds from thyme by ultrasound-assisted dynamic extraction [26]. It was also used in the thyme drying process [27] and in the steam distillation of thyme EO [28].

In this work, a new green procedure using ultrasound, an inexpensive and easy-to-use method for green absolute extraction from thyme (*Thymus vulgaris*), was conducted. Sunflower oil (SO) was used as a substitute to organic solvents: a process which is in line with green extraction concepts. The US extraction method was optimized using response surface methodol-

ogy (RSM) and compared to conventional SO and hexane extractions. The chemical compositions of the absolutes were analyzed by gas chromatography–mass spectrometry (GC-MS). The total phenolic compounds Folin-Ciocalteu, the antioxidant test 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total polar material tests (TPM) were also conducted on absolutes obtained by different extraction methods. The objective of this study was the implementation for the first time of a new green approach to produce green absolute from thyme for food industry using ultrasound apparatus and sunflower oil as an environmentally friendly solvent.

2. Materials and methods

2.1. Plant material and chemicals

Sunflower oil was obtained from a local supermarket in Avignon province (France), *Thymus vulgaris* (chemotype thymol) was purchased from Flore en thym, SAS (south of France). Ethanol (high purity 99.8%) and hexane were obtained from VWR, (EC), Folin Ciocalteu phenol reagent was purchased from chem-lab (Belgium), DPPH reagent and thymol were supplied by Sigma Aldrich (Germany), methanol was bought from Merck (Germany), and ethanol (96%) was supplied by Brenntag (Belgium).

2.2. Extraction procedures

Solid–liquid ratio: The different ratios (plant material/solvent) vary with the type of solvent and plant material used. In this work, a ratio of 1/10 (w/w) was selected for the different absolute extraction techniques. For a ratio above 1/10, the dry matter absorbed all of the available solvent and increased in volume resulting consequently in a lower yield. The combination of high absolute yields and higher amount of available solvent was chosen since the ultrasound apparatus requires a minimum amount of free solvent for extraction procedures.

Conventional extraction of absolute with hexane and ethanol (Fig. 1a): Ten grams of thyme dry matter were soaked in hexane (100 ml) and submitted to heating reflux for 2 hours. The solution was filtered. The hexane was evaporated on a rotary evaporator at 40 °C giving the waxy residue called concrete. Ethanol (high purity 99.8%) (20 ml) was added to the concrete, warmed at 50 °C for 5 min to get a homogeneous mixture. Filtration of the precipitate followed by evaporation of ethanol at 40 °C gave a green absolute. Extractions were performed in triplicates and mean yield values, expressed in g/100 g of thyme dry weight (DW), were reported.

Extraction of absolute with sunflower oil and ethanol (Fig. 1b): Ten grams of thyme dry matter were soaked in 100 ml sunflower oil, so-called “oleo concrete (OC)”. The OC was then extracted by US and Conventional methods (CV). Then, the oleo concrete was filtered; ethanol (high purity 99.8%) (250 ml) was added to the solution, agitated vigorously and allowed to stand for 1 hour. The ethanolic solution was collected, partly evaporated and refrigerated at –14 °C for 12 hours to eliminate SO residue. The supernatant was collected and evaporated at 40 °C giving a yellow extract, the absolute. Extractions were

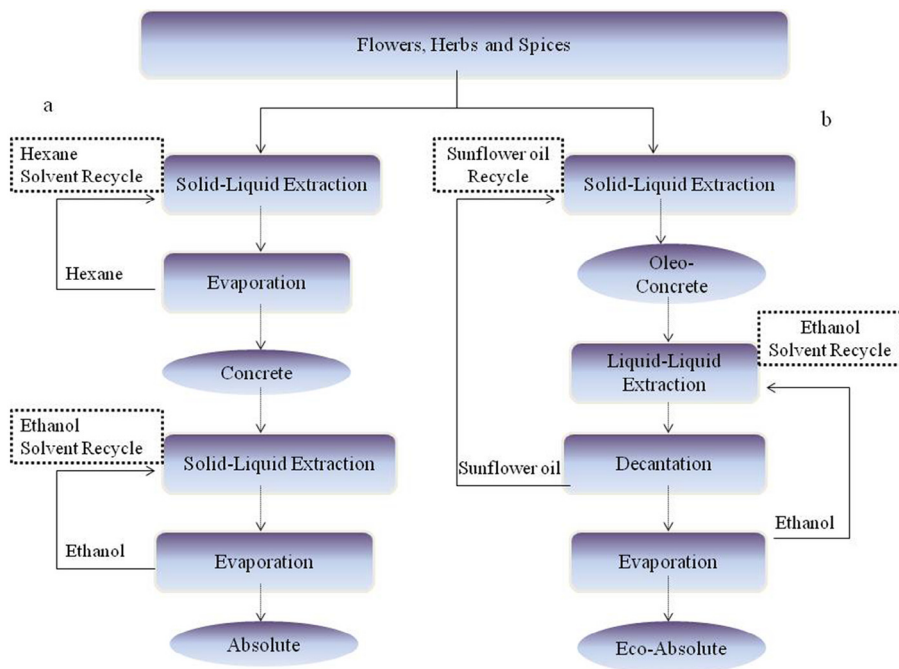


Fig. 1. (a) Conventional extraction of absolutes using hexane and ethanol; (b) green extraction of eco-absolute using sunflower oil and ethanol.

performed in triplicates and the absolute yields (g/100 g DW) were reported.

SO ultrasound (SO-US) and conventional (SO-CV) extractions: US extractions were performed on OC solutions in a Pyrex reactor with 4×12.5 cm internal dimension and maximal capacity of 250 ml. The ultrasonic device used was a titanium alloy microprobe of 6 mm tip (Ultrasonic Processor, Fisher Bioblock Scientific, France) operating at a maximum frequency and power of 20 kHz and 130 W respectively. The double-layered mantle (with water circulation) allowed the control of extraction temperature by cooling/heating systems (Ministat 125, Germany). US extraction was compared to conventional extraction by replacing the microprobe with magnetic agitation.

2.3. Experimental design

In order to investigate the influence and relevance of the operating parameters required during US extractions, a Central Composite Design (CCD) was used to analyze absolute yields from thyme leaves [29]. Three independent factors (namely temperature (T), sonication time (t) and ultrasonic power (P)) were evaluated, as well as eventual interaction between these variables.

The limit values of each variable range were chosen as function of limitations of ultrasonic apparatus (minimum and maximum power available in the device from 32 to 98 W, temperature of extraction for absolutes 10–50 °C (the aromatic compounds might degrade above 50 °C) and time of sonication (from 7 to 23 min). Results involved a total of 20 experiments; including six replications at the center point to evaluate experimental error measurement, and randomized to avoid effects of extraneous variables.

Variables were coded according to Eq. (1), where X_i is the coded value, x_i the real value of a variable, \bar{X}_i the real value of a variable at the center point, and Δx_i the step change:

$$X_i = \frac{x_i - \bar{x}_i}{\Delta x_i} \quad (1)$$

Experimental data for predicting absolute yields have then been represented using a second order polynomial Eq. (2) as follows:

$$Y = \beta_{o+} + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j>i}^{n-1} \beta_{ij} X_i X_j \quad (2)$$

where Y is the response variable absolute yield (g of absolute per 100 g of thyme DW). The results were analyzed using the Statgraphics XV[®] software.

2.4. Kinetic study

Kinetic studies were conducted to compare the absolute yields obtained by SO-US and SO-CV extractions according to the optimum values of temperature, sonication time and ultrasonic power, obtained by the experimental design. Kinetics were performed at different extraction times (5, 15, 20 and 30 min). The results were plotted as function of time. The experiments were carried out three times and the absolute yields obtained by both SO extractions were compared to those obtained by Hex-CV method.

2.5. GC analysis

The absolutes obtained by conventional hexane (Abs-Hex-CV), by conventional SO (Abs-SO-CV), and by ultrasound extractions (Abs-SO-US) at optimal conditions were analyzed by GC to identify their chemical constituents.

Samples were subjected to a standard dilution 10% (w/w) in ethanol 96%. A filtration in Nylon Syringe filters (0.45 μm) was realized for the entire collection of samples before GC injection. GC analysis was carried out on an Agilent 7890 gas chromatograph coupled with a flame ionization detector (FID) equipped with an automatic liquid sampler. Two types of capillary column were used: VF-1 and VF-WAX (20 m \times 0.15 mm \times 0.15 μm) from Agilent. Hydrogen was used as carrier gas at a constant flow rate of 1 ml/min; the oven temperature was programmed as follows: it started at 60 $^{\circ}\text{C}$ then increased by 15 $^{\circ}\text{C}/\text{min}$ until 250 $^{\circ}\text{C}$ for 7.5 min. Injector and detector temperatures were set at 250 $^{\circ}\text{C}$. The internal normalization method was used for quantification of the components of the extracts.

GC–MS analysis was carried out on an Agilent 6890 gas chromatograph coupled with a 5973N mass selective detector equipped with an automatic liquid sampler. The tentative identification was realized using the combination of a polar (VF-WAXms) and an apolar (VF-1 ms) capillary column of 20 m \times 0.15 mm \times 0.15 μm from Agilent. The carrier gas was helium at a constant flow rate of 0.8 ml/min and the oven temperature was programmed as follows: it started at 60 $^{\circ}\text{C}$ then increased by 10 $^{\circ}\text{C}/\text{min}$ until 250 $^{\circ}\text{C}$ for 11 min. A 1 μl injection was made in split mode with a ratio of 1:200 with an injector temperature of 250 $^{\circ}\text{C}$. The temperature of MS source and quadrupole were 230 $^{\circ}\text{C}$ and 150 $^{\circ}\text{C}$ respectively and the mass scan is 40–400 amu. Compounds were tentatively identified using NIST 2005 library and Payan Bertrand internal library of mass spectra.

2.6. Total phenol content

The samples obtained at optimal conditions were studied for their total phenol content (TPC) using Folin Ciocalteu reagent [30].

A volume of 100 μl of the filtered ethanolic solution of absolutes (5 mg/ml) was introduced into test tubes followed by 1250 μl Folin-Ciocalteu reagent and 1 ml of a 10% Na_2CO_3 solution. The tubes were allowed to stand for 1 hour in the dark. The absorbance was determined using a spectrophotometer (Jenway 6300, UK) at 760 nm against a calibration curve. The results were expressed as mg thymol equivalent per g of absolute. Each assay was carried out in triplicates.

2.7. DPPH free-radical-scavenging assay

The antioxidant activity of the absolutes was measured by bleaching the purple colored methanolic solution of the stable radical DPPH [31].

The absolutes were prepared in ethanol (high purity 99.8%) from a stock solution of 5 mg/ml).

Two milliliters of fresh methanolic solution of DPPH (6×10^{-5} M) were added. The samples were shaken in the dark for four hours.

The decrease in absorbance at 517 nm was determined using a spectrophotometer (Jenway 6300, UK) for all samples. All the samples were tested in triplicate.

The inhibition percentage of the DPPH radical was calculated according to the formula of Yen and Duh [32]:

$$I = (A_0 - A_i) / A_0 \times 100$$

where I = DPPH inhibition (%), A_0 = absorbance of control sample ($t = 0$) and A_i = absorbance of the tested sample ($t = 4$ h).

2.8. Frying oil test

The antioxidant effect of the absolutes under heating conditions was studied by the determination of Total Polar Materials (TPM). The objective of this test consisted of studying whether the addition of any of these extracts would increase the shelf life of fried sunflower oil.

Each of the absolutes (0.80 g) was added separately to sunflower oils (100 ml) and the samples were heated under domestic frying conditions, i.e. 180 ± 5 $^{\circ}\text{C}$ during several hours [33]. The temperature was monitored by a thermocouple (ATC-300) inserted directly into the domestic deep-fat electric fryers. All samples were evaluated before the first heating sessions and every 1 h of heating until oil discard using a cooking oil tester (Testo 270, Testo Sàrl, Forbach, France) which measures the TPM value. A maximum value of 25% is tolerated in accordance with the French law (Article 3-3 of decree No 86–857 of 18/07/86). This maximal legal content of TPM in frying oils, including hydrolysis products (diglycerides, monoglycerides and free fatty acids) and a complex distribution of oxidation products encompassing polymers, is formed at temperatures below 180 $^{\circ}\text{C}$ (French law No 86–857). The TPM value, usually assessed in restaurants and the agrofood industry by fast commercial tests (mostly based on colorimetric readings), has proven to correlate well with values obtained by official standards [33].

3. Results and discussion

3.1. Experimental design studies

Three key variables that affect extraction of absolute yield were studied in a central composite design, namely: ultrasonic power, temperature and sonication time. Ultrasonic power ranged from 32 to 98 W. The chosen ultrasonic power limits were in function of regulation limitations in the ultrasonic apparatus. Moderate temperatures were chosen with a range of 10–50 $^{\circ}\text{C}$ since appropriate temperature setting is necessary to avoid destruction of aromatic compounds as well as provide an efficient application of US (US effects are known to decrease with temperatures higher than 50 $^{\circ}\text{C}$). Moreover, the increase in cavitation phenomena is directly proportional to the increase in the system temperature. However, at very high temperatures, a decrease in shock waves is observed, diminishing the effect of US [34], therefore, a maximum temperature of 50 $^{\circ}\text{C}$ was chosen. Finally, the sonication time range chosen (from 7 to 23 min) was short yet competitive with conventional extraction, showing a potential future industrial application.

Table 1
ANOVA for absolute yield in the CCD.

| Source | Sum of squares | Df | Mean square | F-ratio | P-value |
|---------------------|----------------|----|-------------|---------|---------------|
| A: temperature | 0.0789702 | 1 | 0.0789702 | 26.99 | 0.0004 |
| B: ultrasonic power | 0.0539701 | 1 | 0.0539701 | 18.45 | 0.0016 |
| C: sonication time | 0.0633429 | 1 | 0.0633429 | 21.65 | 0.0009 |
| AA | 0.0188115 | 1 | 0.0188115 | 6.43 | 0.0296 |
| AB | 0.00056448 | 1 | 0.00056448 | 0.19 | 0.6698 |
| AC | 0.00149605 | 1 | 0.00149605 | 0.51 | 0.4909 |
| BB | 0.0195737 | 1 | 0.0195737 | 6.69 | 0.0271 |
| BC | 0.00220448 | 1 | 0.00220448 | 0.75 | 0.4057 |
| CC | 0.00645025 | 1 | 0.00645025 | 2.20 | 0.1684 |
| Total error | 0.0292591 | 10 | 0.00292591 | | |
| Total (corr.) | 0.267839 | 19 | | | |

$R^2 = 0.890$; R^2_{adj} (adjusted for Df) = 0.792.

Since after a certain time cavitation bubbles do not continue to absorb energy to grow and collapse [35] and the usual time used for US extraction in the industry are usually not longer than 60 min [36], 23 min was chosen as maximum limit. These three controlled variables were studied in a multivariate study with 20 experiments.

3.1.1. Results for absolute yield

Coded experiments and responses obtained for each run of the central composite design were studied. The responses varied widely as a function of parameter settings of the experiments (from 1.975 to 5.428 g of absolute per 100 g of thyme dry matter). Significance and suitability of the design were then studied using a variance analysis (ANOVA) in Table 1. Statistical significance of each effect (including interaction terms, linear and quadratic effects) was tested by comparing the mean square against an estimate of the experimental error. Depending on the degree of freedom (Df) involved, F-ratio can be calculated (ratio of the mean squared error to the pure error). With a confidence level of 95%, F-ratio significance can be evaluated using the *p*-value column (significant effects have been typed in bold).

Five effects were found significant at a 95% confidence level in the experimental domain studied. Linear effects of the three key variables (T, P, t) appear to be highly significant. The quadratic effects of the temperature (T^2) and the ultrasonic power (P^2) were also significant but less important than the linear ones.

The lack of significance of the cross-product terms (P.T, T.t, P.t) suggests the absence of interactions between variables. The experimental data obtained from the CCD allowed us to determine an empirical relationship linking response studied (absolute yield) and key variables involved in the model (in coded units). Thus, a second order polynomial equation was obtained:

$$Y = 4.81 - 0.030T - 0.102P - 0.157t + 0.002 T^2 + 0.92 \times 10^{-3} P^2$$

where Y represents absolute yield (expressed in g per 100 g of dry weight), P represents the applied ultrasonic power, t is the sonication time and T is the temperature in coded units. Only significant variables were shown ($p < 0.05$). The applied model

appears to be adequate for our experimental results at the 95% confidence level. More than 89% of the variability of responses was explained (R^2 statistics > 0.890), asserting a good accuracy and ability of the established model within the limits of the range used [37]. R^2_{adj} is a regression coefficient adjusted for the number of coefficient involved in the model; it allows comparison between models with different numbers of independent variables and allows testing the level of suitability to the regression coefficient. Its value (> 0.79) indicates a high degree of correlation between observed and predicted data.

3.1.2. Optimization of ultrasound extraction

A graphical representation can be introduced in order to visualize the significant relationship linking levels of variables and response studied (absolute yield). Fig. 2 depicts three-dimensional plots, each plot highlighting the response behavior function of two variables with the third variable fixed to its central point. The most influential variables are the linear terms of temperature (T), sonication time (t) and ultrasonic power (P): absolute yield increases as temperature, sonication time and ultrasonic power increase. The quadratic effect of ultrasonic power (P^2) and temperature (T^2) has been illustrated on these surfaces but with a less predominant influence as observed in the Pareto chart (presence of weak surface curvature when temperature and ultrasonic power increase).

Fig. 2 optimal settings for absolute yield maximization were 98.63 W for ultrasonic power, 50.18 °C for temperature and 22 min for sonication time. The absolute yield (8.31 g of absolute per 100 g of thyme dry weight) predicted by the model was verified experimentally since the highest yield was obtained using the optimized settings with a value of 5.92 g/100 g DW.

This shows the importance of optimizing parameters when a modification is done on one or various parameters such as solid/liquid ratio, temperature or solvent. The absolutes were obtained and optimized by US extraction, showing the viability of this procedure with great yields using sunflower oil as solvent.

3.2. Kinetic study

To evaluate the impact of ultrasound extraction in optimized conditions obtained from the response surface method, a comparison study was carried out between SO-US and SO-CV

Table 2

Absolute yields obtained by different extraction methods.

| | Yield \pm SD (g/100 g DW) |
|------------|-----------------------------|
| Abs-Hex-CV | 8.64 \pm 0.23 |
| Abs-SO-US | 5.93 \pm 0.35 |
| Abs-SO-CV | 4.03 \pm 0.25 |

SD, standard deviation.

methods (Fig. 3). At 20 min, the extraction phase started to reach a plateau, which marked the end of both extraction processes. From Fig. 3, it is possible to observe that ultrasound extraction increased in absolute yield by more than 47% compared to the conventional one (4.03 and 5.93 g of absolute per 100 g DW for SO-CV and SO-US respectively). The comparison shows a clear improvement of the extraction, which is attributed to ultrasonic cavitation, since it is the only variable of treatment that differs in both experiments. This result shows that US extraction allowed a better yield recovery which is in accordance with different studies [25,38]. Indeed, the collapse of the cavitation bubbles of the ultrasonic probe destroys the glands of the aromatic plant, releases their content into the medium, and increases consequently their extraction yield [24,39].

3.3. Absolute yield and quality

Absolute yields obtained by both SO-US and SO-CV extractions at 30 min were compared to conventional hexane extraction (Hex-CV) (Table 2). The highest absolute yield was obtained by Abs-Hex-CV with a value of 8.64 g/100 g DW, followed by Abs-SO-US (5.93 g/100 g DW) whereas Abs-SO-CV had the lowest yield (4.03 g/100g DW).

Although the conventional hexane extraction gave improved yield, the absolute obtained by this method contained about 74.27% of waxy components.

The waxes were detected by precipitation in ethanolic solution below $-1\text{ }^{\circ}\text{C}$ overnight. In fact, their limited solubility causes clouding and renders the absolute inappropriate for some formulations [1]. Although filtration was conducted to remove the majority of waxes, a high percentage was observed. Indeed, different studies reported that absolute was not totally free from waxes when extracted by conventional extraction solvent such as pentane or hexane [1,6,7,40]. However, in the absolutes obtained by both SO extractions, no precipitated waxes were found after refrigerating the ethanolic absolute solutions.

As a result, the absolute obtained by hexane was higher in yield but was lower in quality than the ones obtained by both SO extractions. The sunflower oil used as natural solvent gave a totally dewaxed absolute which is more suitable for food industry.

3.4. GC-FID and GC-MS identification

GC-MS analysis was conducted to identify thyme absolutes and compare the performance of the different extraction methods (Fig. 4).

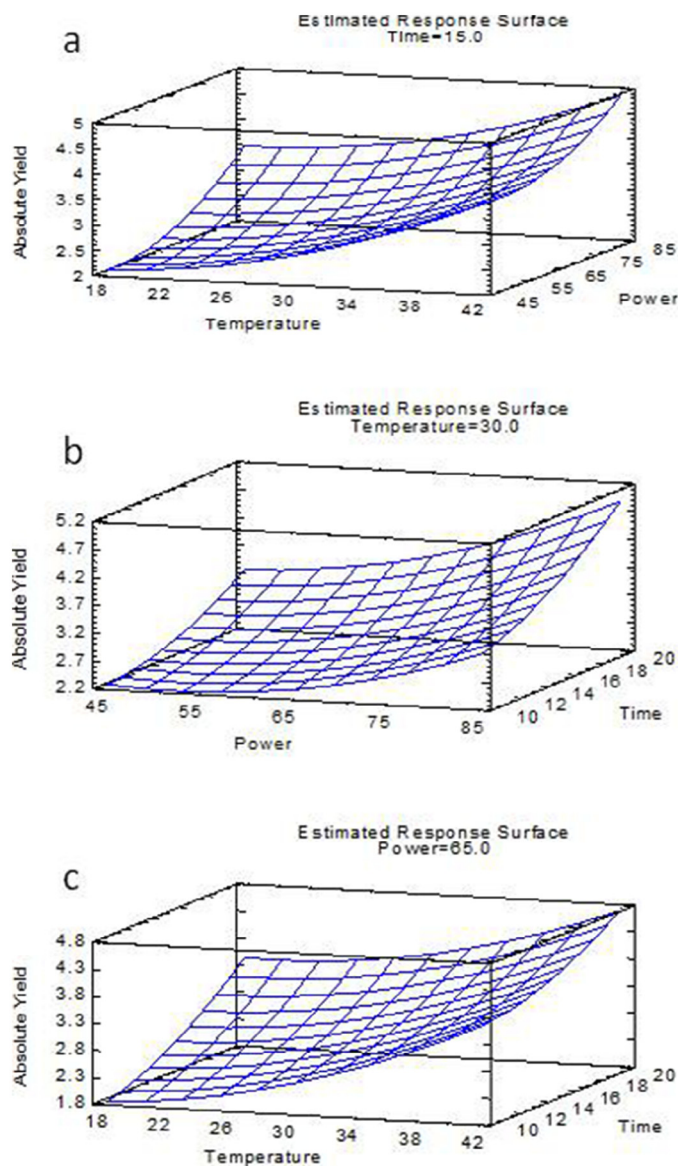


Fig. 2. Optimization of ultrasound absolute extraction by sunflower oil: absolute yield investigation in the multivariate study: (a) absolute yield as a function of temperature and power, (b) absolute yield as a function of time and power and (c) absolute yield as a function of temperature and time.

In the absolute extracted with Hex-CV, two monoterpene phenols were identified representing 85.6% of the volatile part of the extract having thymol as the major one ($79.6\% \pm 1.1$) followed by carvacrol ($6.0\% \pm 0.4$).

The GC-MS analysis of the absolutes obtained by SO-US identified two compounds which constitute 86.2% of the volatile part of the extract. Thymol was the main component representing $80.5\% \pm 1.3$, followed by carvacrol ($5.7\% \pm 0.2$).

GC-MS of the absolute obtained by SO-CV method showed also the presence of these two monoterpene phenols representing 63.4% of the volatile part of the extract but with a lower amount for thymol ($59.7\% \pm 0.7$) and carvacrol ($3.7\% \pm 0.1$) compared to other absolutes.

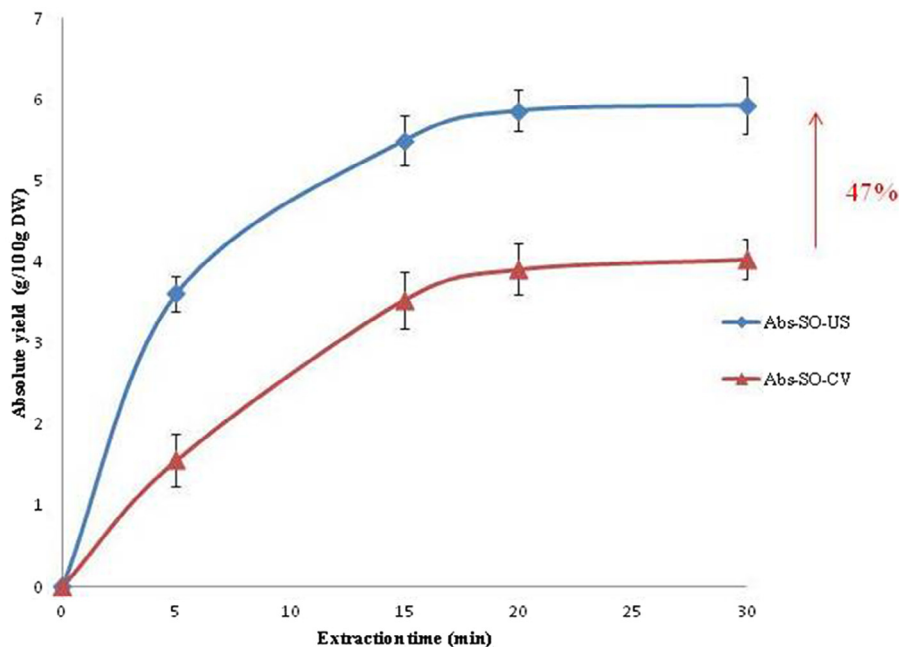


Fig. 3. Comparison between conventional (CV-▲) and ultrasound extractions (US-◆).

These results show that the absolute extraction methods were selective for the recovery of monoterpene phenols unlike other components such as terpenes; this might be due to their low solubility in the different solvents used or to their degradation during the extraction processes.

Moreover, the highest concentration of thymol, a marker monoterpene phenol in thyme that plays an important role in the overall biological activity of the plant or its extracts [41],

was observed in absolute obtained by US (80.5%) followed by Abs-Hex-CV (79.6%) whereas the lowest amount was found in the Abs-SO-CV (59.7%).

In addition to that, a moderate variation in the chemical composition of the Abs-SO-US and Abs-SO-CV compared to the Abs-Hex-CV was observed. Specifically, the profile obtained in the Abs-SO-US was relatively similar to that presented by Abs-Hex-CV since they recovered approximately the

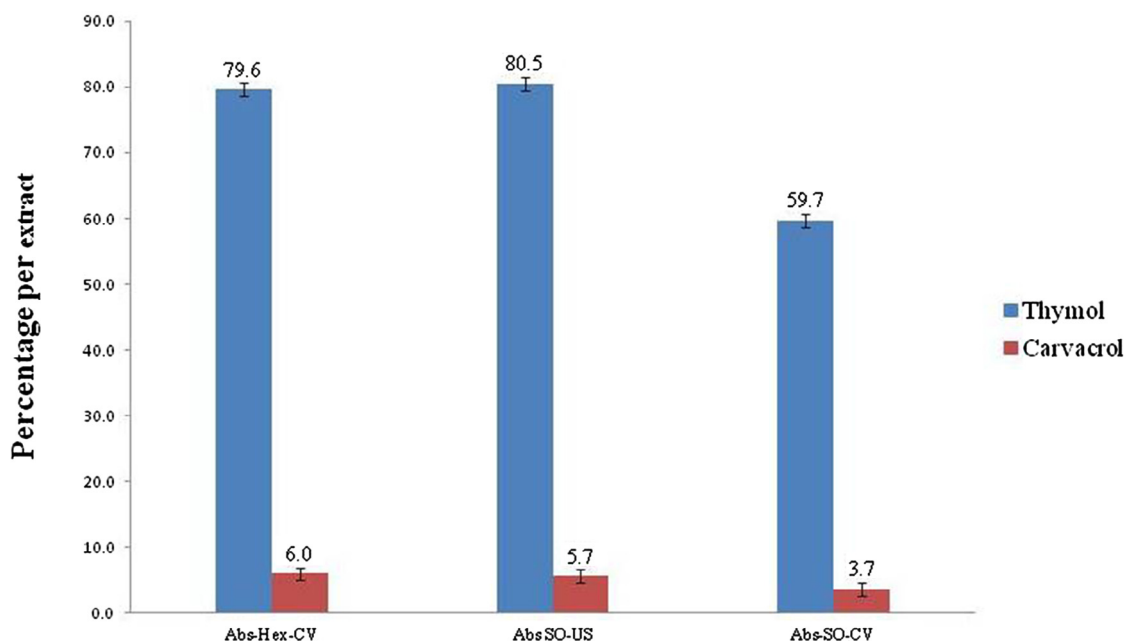


Fig. 4. Chemical composition of thyme absolutes by GC-MS.

Table 3
Total phenol contents of Abs-Hex-CV, Abs-US-SO and Abs-CV-SO.

| | TPC thymol equivalent (mg/g)* |
|------------|-------------------------------|
| Abs-Hex-CV | 123.53 ± 1.11 |
| Abs-SO-US | 171.22 ± 0.71 |
| Abs-SO-CV | 73.64 ± 0.95 |

* Values are mean ± standard deviation (n=3).

same thymol and carvacrol amounts (80.5% and 79.6% for thymol and 5.7% and 6.0% for carvacrol in Abs-SO-US and Abs-Hex-CV respectively).

Results show the effectiveness and the selectivity of SO-US in extracting the monoterpene phenols (thymol and carvacrol) resulting in a more pure extract. The controlled temperature used in US extraction facilitated the recovery of these thermo-sensitive compounds. Thus, the US using SO as solvent is the best extraction method since no remarkable variation in the chemical composition of the absolute was observed compared to Hex-CV.

3.5. Total phenolic content

The Total Phenolic Contents (TPC) of the absolutes obtained by different extraction methods are presented in Table 3. The highest phenol content was observed in Abs-SO-US with a value of 171.22 mg thymol equivalent/g. The Abs-Hex-CV had a lower TPC (123.53 mg thymol equivalent/g) whereas the Abs-SO-CV showed the lowest amount of TPC (73.64 mg thymol equivalent/g).

Data on the total phenol content of thyme absolute are not available. However, different studies on thyme extracts have showed their rich content in phenols and flavonoids [17,42,43].

The highest phenol content of Abs-SO-US might be attributed to thymol and carvacrol contents in its composition (86.2%), followed by Abs-Hex-CV (85.6%) whereas the Abs-SO-CV had the lowest content (63.4%). It might be also related to other phenolic compounds present in Abs-SO-US which have increased its antioxidant activity.

This result allowed us to conclude that ultrasound extraction did not degrade phenols; on the contrary, their concentrations were increased compared to the non-sonicated samples. In fact, ultrasonic degradation of phenols is normally slow in comparison with other volatile aromatic compounds that diffuse more readily into the cavitation bubble for decomposition [44].

3.6. DPPH radical scavenging activity

The hydrogen atom donating ability of the absolutes was tested using the stable free radical DPPH. Table 4 shows the concentrations of each absolute required to scavenge DPPH represented as inhibition percentage. A concentration-dependent scavenging activity was found for the studied absolutes. All absolutes were able to reduce the stable free radical 2,20-diphenyl-1-picrylhydrazyl (DPPH) to the yellow diphenylpicrylhydrazine with varying degrees of scavenging capacities. Great bleaching action (from purple to yellow) reflected a higher antioxidant activity and thus a lower IC₅₀ (Table 4). Sonicated absolutes presented a lower IC₅₀ (0.95 mg/ml), representing a better antioxidant activity for those samples when compared to the activity of extracts obtained by Hex-CV (1.80 mg/ml) and SO-CV extractions (2.03 mg/ml). This is in agreement with its higher concentration of phenolic contents, which confirmed the usual correlation between antioxidant activity and TPC [45].

The antioxidant activity of these extracts might be mainly attributed to their monoterpene phenol compounds, which represent the main constituents of the absolutes. It is possible to observe that the antioxidant activity of the absolutes is mainly dependent on thymol concentration in their composition. In fact, different studies have showed the high antioxidant activity of this component [46,47] which is in accordance with our results. Therefore, SO-US is an efficient method for absolute extraction with high antioxidant capacity.

3.7. Frying oil test

The possible use of thyme absolutes in the frying process for extending the shelf life of sunflower oil was conducted by monitoring the TPM values during heating sessions at 180 °C.

Table 5 shows the heating hours required to reach a maximum tolerance TPM of 25% in the sunflower oil samples.

All samples (sunflower oil used as control and its mixture with each absolute) display similar TPM results since the total heating times required to reach the maximal TPM value of 25% were quite close (ranging from 10 to 12 h).

The addition of any of the thyme absolutes did not increase the shelf life of the SO. This might be explained by the prolonged heating process which caused the degradation of the natural antioxidants [26,48].

Table 4
Antioxidant activity of absolutes at different concentrations measured by DPPH method.

| | Inhibition DPPH (% ± SD) | | | | | | IC ₅₀ (mg/ml) |
|------------|--------------------------|--------------|--------------|--------------|--------------|--------------|--------------------------|
| Abs-SO-US | 0.25 mg/ml | 0.5 mg/ml | 0.75 mg/ml | 1 mg/ml | 1.25 mg/ml | 1.5 mg/ml | 0.95 |
| | 14.61 ± 0.86 | 31.28 ± 0.33 | 41.34 ± 1.62 | 50.70 ± 0.95 | 61.83 ± 0.55 | 77.59 ± 1.48 | |
| Abs-Hex-CV | 0.5 mg/ml | 1 mg/ml | 1.5 mg/ml | 2 mg/ml | 2.5 mg/ml | 3 mg/ml | 1.80 |
| | 19.40 ± 2.54 | 35.31 ± 0.70 | 44.05 ± 0.66 | 53.72 ± 0.50 | 65.00 ± 1.40 | 75.14 ± 1.86 | |
| Abs-SO-CV | 15.24 ± 0.99 | 25.01 ± 1.32 | 40.09 ± 0.11 | 49.19 ± 1.32 | 60.79 ± 0.09 | 70.72 ± 0.59 | 2.03 |

SD, standard deviation.

Table 5
Frying time for 25% TPM for sunflower samples.

| | SO | SO+Abs-Hex-CV | SO+Abs-SO-US | SO+Abs-SO-CV |
|-----------------------------|----|---------------|--------------|--------------|
| Frying time for 25% TPM (h) | 12 | 12 | 10 | 12 |

SO, sunflower oil used as control; SO+Abs-hex-CV, sunflower oil added with absolute using hexane extraction, SO+Abs-SO-US, sunflower oil added with absolute using ultrasound extraction with sunflower oi; SO+Abs-SO-CV, sunflower oil added with absolute using conventional extraction with sunflower oil.

4. Conclusion

A green extraction method is always useful and challenging with only few modifications in the chemical composition and characteristic of the molecules. The ultrasound technique using sunflower oil as solvent offers important advantages over SO-CV and Hex-CV methods: shorter extraction time, good selectivity and higher recovery of monoterpene phenols, pure absolute free from waxy components and organic solvent residues representing the strongest antioxidant activity and giving rise to a purer aroma, more similar and faithful to the natural scent of the target plant. The use of sunflower oil as natural solvent facilitates the industrial application for absolute as a natural ingredient in food formulations. Thus, this new green approach is of great interest for green chemistry. It provides a good alternative to conventional extraction; it can be used for the extraction of valuable compounds from a large number of aromatic plants which are widely used not only in food, but also in fragrance and aromatherapy sectors. This suggests the possibility of the production of green absolutes using ultrasound technology on pilot and industrial scales.

References

[1] P.K. Rout, S. Naik, Y.R. Rao, Liquid CO₂ extraction of flowers and fractionation of floral concrete of *Michelia champaca* Linn, *J. Supercrit. Fluids* 56 (2011) 249–252.
 [2] Y.-C. Yang, M.-C. Wei, S.-J. Hong, Ultrasound-assisted extraction and quantitation of oils from *Syzygium aromaticum* flower bud (clove) with supercritical carbon dioxide, *J. Chromatogr. A* 1323 (2014) 18–27.
 [3] S. Armenta, S. Garrigues, M. de la Guardia, Green analytical chemistry, *TrAC Trends in Analytical Chemistry*. 27 (2008) 497–511.
 [4] Y. Li, A.S. Fabiano-Tixier, M.A. Vian, F. Chemat, Solvent-free microwave extraction of bioactive compounds provides a tool for green analytical chemistry, *TrAC Trends in Analytical Chemistry*. 47 (2013) 1–11.
 [5] C. Remy, *Narcissus in perfumery*, in: G.H. Hanks (Ed.), *Narcissus and Daffodil: The Genus Narcissus*, 2004, Taylor and Francis e-library, London, 2004, p. 452.
 [6] O. Prakash, P.K. Rout, C.S. Chanotiya, L.N. Misra, Composition of essential oil, concrete, absolute and SPME analysis of *Tagetes patula* capitula, *Ind Crops Prod.* 37 (2012) 195–199.
 [7] P.K. Rout, D. Sahoo, L.N. Misra, Comparison of extraction methods of *Mimusops elengi* L. flowers, *Ind Crops Prod.* 32 (2010) 678–680.
 [8] K.L. Goodner, K. Mahattanatawee, A. Plotto, J.A. Sotomayor, M.J. Jordán, Aromatic profiles of *Thymus hyemalis* and Spanish *T. vulgaris* essential oils by GC–MS/GC–O, *Ind Crops Prod.* 24 (2006) 264–268.
 [9] S.-J. Lee, K. Umamo, T. Shibamoto, K.-G. Lee, Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties, *Food Chem.* 91 (2005) 131–137.

[10] A. Grigore, I. Paraschiv, S. Colceru-Mihul, C. Bubueanu, E. Draghici, M. Ichim, Chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods, *Romanian Biotechnol Lett* 15 (2010) 5436–5443.
 [11] M.C. Rota, A. Herrera, R.M. Martínez, J.A. Sotomayor, M.J. Jordán, Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils, *Food Cont* 19 (2008) 681–687.
 [12] A. Ghasemi Pirbalouti, M. Hashemi, F.T. Ghahfarokhi, Essential oil and chemical compositions of wild and cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L, *Ind Crops Prod.* 48 (2013) 43–48, doi:10.1016/j.indcrop.2013.04.004.
 [13] S. Shabnum, M.G. Wagay, Essential oil composition of *Thymus Vulgaris* L. and their uses, *J Res Develop.* 11 (2011) 83–90.
 [14] M.-T. Golmakani, K. Rezaei, Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from *Thymus vulgaris* L, *Food Chem.* 109 (2008) 925–930.
 [15] M.R. García-Risco, G. Vicente, G. Reglero, T. Fornari, Fractionation of thyme (*Thymus vulgaris* L.) by supercritical fluid extraction and chromatography, *J. Supercrit. Fluids* 55 (2011) 949–954.
 [16] A. Dawidowicz, E. Rado, D. Wianowska, M. Mardarowicz, J. Gawdzik, Application of PLE for the determination of essential oil components from *Thymus vulgaris* L, *Talanta* 76 (2008) 878–884.
 [17] M.H.H. Roby, M.A. Sarhan, K.A.-H. Selim, K.I. Khalel, Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts, *Ind Crops Prod.* 43 (2013) 827–831.
 [18] M. Virot, V. Tomao, C. Le Bourvellec, C.M.C.G. Renard, F. Chemat, Towards the industrial production of antioxidants from food processing by-products with ultrasound-assisted extraction, *Ultrason. Sonochem.* 17 (2010) 1066–1074.
 [19] T. Xia, S. Shi, X. Wan, Impact of ultrasonic-assisted extraction on the chemical and sensory quality of tea infusion, *J. Food Eng.* 74 (2006) 557–560.
 [20] E. Roselló-Soto, C.M. Galanakis, M. Brnčić, V. Orlien, F.J. Trujillo, R. Mawson, et al., Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions, *Trends Food Sci. Technol.* 42 (2015) 134–149.
 [21] K.G. Zinoviadou, C.M. Galanakis, M. Brnčić, N. Grimi, N. Boussetta, M.J. Mota, et al., Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties, *Food Res. Int.* 77 (2015) 743–752.
 [22] O. Parniakov, E. Apicella, M. Koubaa, F.J. Barba, N. Grimi, N. Lebovka, et al., Ultrasound-assisted green solvent extraction of high-added value compounds from microalgae *Nannochloropsis* spp, *Bioresour. Technol.* 198 (2015) 262–267.
 [23] M. Koubaa, H. Mhemdi, F.J. Barba, S. Roohinejad, R. Greiner, E. Vorobiev, Oilseed treatment by ultrasounds and microwaves to improve oil yield and quality: an overview, *Food Res. Int.* 85 (2016) 59–66.
 [24] Z. Pan, W. Qu, H. Ma, G.G. Atungulu, T.H. McHugh, Continuous and pulsed ultrasound-assisted extractions of antioxidants from pomegranate peel, *Ultrason. Sonochem.* 18 (2011) 1249–1257.
 [25] M. Vinatoru, An overview of the ultrasonically assisted extraction of bioactive principles from herbs, *Ultrason. Sonochem.* 8 (2001) 303–313.
 [26] J. Roldangutierrez, J. Ruizjimenez, M. Luquedecastro, Ultrasound-assisted dynamic extraction of valuable compounds from aromatic plants and flowers as compared with steam distillation and superheated liquid extraction, *Talanta* 75 (2008) 1369–1375.
 [27] J. Rodriguez, E.C. Melo, A. Mulet, J. Bon, Optimization of the antioxidant capacity of thyme (*Thymus vulgaris* L.) extracts: management of the convective drying process assisted by power ultrasound, *J. Food Eng.* 119 (2013) 793–799.
 [28] R. Kowalski, J. Wawrzykowski, Effect of ultrasound-assisted maceration on the quality of oil from the leaves of thyme *Thymus vulgaris* L, *Flavour Fragr. J.* 24 (2009) 69–74.

- [29] M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar, L.A. Escaleira, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, *Talanta* 76 (2008) 965–977.
- [30] V. Singleton, J. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic.* 16 (3) (1965) 144–158.
- [31] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of free radical method to evaluate antioxidant activity, *Lebensmittel Wissenschaft Und Technologie*. 28 (1995) 25–30.
- [32] G.C. Yen, P.D. Duh, Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species, *J. Agric. Food Chem.* 42 (1994) 629–632.
- [33] S. Casal, R. Malheiro, A. Sendas, B.P.P. Oliveira, J.A. Pereira, Olive oil stability under deep-frying conditions, *Food Chem. Toxicol.* 48 (2010) 2972–2979.
- [34] J.P. Lorimer, T.J. Mason, Sonochemistry. Part 1-The physical aspects, *Chem. Soc. Rev.* 16 (1987) 239.
- [35] E. Ozcan, Ultrasound assisted extraction of phenolics from grape pomace. Middle East Technical University, The graduate School of Natural and Applied Sciences, Ph.D., Chemical Engineering, Ankara. 2006.
- [36] F. Chemat, X. Zill-e-Huma, M.K. Khan, Applications of ultrasound in food technology: Processing, preservation and extraction, *Ultrason. Sonochem.* 18 (2011) 813–835.
- [37] H. Mirhosseini, C. Tan, N. Hamid, S. Yusof, Effect of Arabic gum, xanthan gum and orange oil on flavor release from diluted orange beverage emulsion, *Food Chem.* 107 (2007) 1161–1172.
- [38] S. Samaram, H. Mirhosseini, C.P. Tan, H.M. Ghazali, Ultrasound-assisted extraction and solvent extraction of papaya seed oil: crystallization and thermal behavior, saturation degree, color and oxidative stability, *Ind Crops Prod.* 52 (2014) 702–708.
- [39] S. Veillet, V. Tomao, F. Chemat, Ultrasound assisted maceration: an original procedure for direct aromatisation of olive oil with basil, *Food Chem.* 123 (2010) 905–911.
- [40] P.K. Rout, S.N. Naik, Y.R. Rao, G. Jadeja, R.C. Maheshwari, Extraction and composition of volatiles from *Zanthoxylum rhesta*: comparison of subcritical CO₂ and traditional processes, *J. Supercrit. Fluids* 42 (2007) 334–341.
- [41] M. Hudaib, E. Speroni, A.M. Di Pietra, V. Cavrini, GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle, *J. Pharm. Biomed. Anal.* 29 (2002) 691–700.
- [42] R. Chizzola, H. Michitsch, C. Franz, Antioxidative properties of *Thymus vulgaris* leaves: comparison of different extracts and essential oil chemotypes, *J. Agric. Food Chem.* 56 (2008) 6897–6904.
- [43] N. Zeghad, R. Merghem, Antioxidant and antibacterial activities of *Thymus vulgaris* L, *Med Arom Plant Res JI* 1 (2013) 5–11.
- [44] P. Chowdhury, T. Viraraghavan, Sonochemical degradation of chlorinated organic compounds, phenolic compounds and organic dyes – a review, *Sci. Total Environ.* 407 (2009) 2474–2492.
- [45] L.S. Artajo, M.P. Romero, J.R. Morelló, M.J. Motilva, Enrichment of refined olive oil with phenolic compounds: evaluation of their antioxidant activity and their effect on the bitter index, *J. Agric. Food Chem.* 54 (2006) 6079–6088.
- [46] S. Aman, S. Moin, M. Owais, M.U. Siddiqui, Antioxidant activity of thymol: protective role in AAPH-induced hemolysis in diabetic erythrocytes, *Int J Pharm Sci Invention* 2 (2013) 55–60.
- [47] A. Esmaeili, A. Khodadadi, Antioxidant activity of a solution of thymol in ethanol, *Zahedan J Res in Med Sci* 14 (2012) 14–18.
- [48] A. Tomaino, F. Cimino, V. Zimbalatti, V. Venuti, V. Sulfaro, A. De Pasquale, et al., Influence of heating on antioxidant activity and the chemical composition of some spice essential oils, *Food Chem.* 89 (2005) 549–554.