OPTIMIZATION OF HYDRODISTILLATION OF ESSENTIAL OIL FROM *MENTHA SPICATA* L. BY USING RESPONSE SURFACE METHODOLOGY

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***Abstract*— This study was conducted to optimize the hydrodistillation process of *Mentha spicata* L. essential oil using the response surface methodology. The optimal values of operating parameters (independent variables) such as extraction time (100–240 min) and water volume to plant mass ratio (0.055–0.120) were investigated using a central composite design, which led to only 13 experiments. The response variables were selected based on the highest essential oil yield as well as the carvone ratio, which is the main component of the essential oil. The experimental data were fitted to a linear model for essential oil yield and a modified quadratic model for the carvone ratio. The hydrodistillation time and water volume to plant mass ratio have significant effects ($p<0.05$) on both the essential oil yield and the carvone ratio. The optimal conditions were identified as 145.7 min of extraction time and a 0.105 ml/g water volume to plant mass ratio by the 3D response surface and the contour plots derived from the models. At these predicted conditions, the essential oil yield and carvone ratio were calculated to be 1.383% and 28.541%, respectively. The findings indicate that the response surface approach can be used successfully in the hydrodistillation of *Mentha spicata* L.**

***Keywords*— *Mentha spicata* L., essential oil, carvone, hydrodistillation, response surface methodology, central composite design**

I. INTRODUCTION

Essential oils are a mixture of volatile compounds extracted from several plant organs. Plants have been used as both basic nutrients and medicines since the existence of humanity. It has been seen that the use of plants as medicines has decreased while examining medical and aromatic compounds in the 20th century. This situation may be explained by technological developments and social and political changes. Due to the synthesis of organic chemicals since the middle of the 19th century, usage of plants as medicine decreased until the 1970s. However, aromatic plants have been re-used as medicines in the 1980s and 1990s because of the awareness of consumers. According to the World Health Organization (WHO) statistics, the number of medicinal and aromatic plants used in the world today is around 20000. Moreover, the amount of essential oil production is 45000 tons per year. In Turkey, 500 different kinds of aromatic plants have been used as medicine and raw materials for scents. Essential oils obtained from these plants have a large market area in the world. Essential oils are some important materials for the perfumery and cosmetics industry and are also used in soap, detergent, and toothpaste production (Sarkic and Stappen, 2018).

Since essential oils have a high economic value and wide usage area, investigation of their chemical structures and biological activation has been inevitable. Moreover, essential oils have various therapeutic benefits because of their antioxidant, anti-inflammatory, antimicrobial and antifungal properties (Giacometti *et al.,* 2018). Besides, essential oils are used to extend the shelf–life of food products due to the risks of using synthetic preservatives (Tongnuanchan and Benjakul, 2014). Pure essential oils contain more than 200 organic volatile compounds (monoterpenes, sesquiterpenes, hydrocarbons, and their oxygenated derivatives, and terpenoids (isoprenoids)) which are called secondary metabolites. Essential oils also include non–volatile residues and flavonoids. Furthermore, the total amount of essential oil may be 0.01–10% of the plant mass. The essential oil of *Mentha spicata* (spearmint) has an important value in world trade and also possesses increasing importance in the pharmaceutical industry.

As essential oils are present in low amounts in plants, the extraction process plays a vital role in both the yield and quality of essential oils. Production of essential oil and using a method for extraction are dependent on the botanical material that is used. On the other hand, the state and form of the material are other factors influential on extraction. The extraction procedure is the most important state in essential oil production. For example, an inappropriate extraction process can cause a loss or alter the activity of chemical signature, and finally, it may result in a loss of bioactivity (Tongnuanchan and Benjakul, 2014). For this reason, various extraction methods have been proposed in the literature. Steam distillation, water distillation, a combination of these, cohabitation, maceration, and effleurage are known as conventional extraction methods. Among these methods, the steam and hydrodistillation methods have been widely used. Water distillation, or in other words, hydrodistillation with a Clevenger type apparatus is a technique included in European Pharmacopoeia (Giacometti *et al.,* 2018). Besides these, innovative approaches for obtaining essential oils have been proposed such as ultrasound, high pressure,

and pulsed electric field–assisted extraction, and these constitute green technologies. Since extraction yields are highly related to the characteristic properties of target molecules such as a hydrophobic/lyophilic structure, using organic solvents is common. Moreover, conventional methods are low–cost methods in comparison to innovative extraction methods. However, conventional extraction methods sometimes require complex, time–consuming, multi-step processes.

Among essential oil extraction studies, Tongnuanchan and Benjakul (2014) proposed different extraction methods. Regarding distillation, the steam distillation, hydrodistillation, and hydro diffusion methods were presented. In the same manner, the solvent, supercritical CO_2, and subcritical water techniques were given among solvent extraction methods. Moreover, solvent–free microwave techniques were also mentioned in their paper. In the study by Almeida *et al.* (2012), different extraction techniques such as sub/–supercritical fluid extraction, hydrodistillation, and the Soxhlet method were used to evaluate the extraction yield, chemical profiles, and antioxidant activity of the extracts. In a study with lavender (*Lavandula angustifolia* L.), three different extraction methods–SC–CO_2, hexane, and hydrodistillation–were compared to evaluate chemical composition, antioxidant and antimicrobial activity (Danh *et al.,* 2013). Essential oils were extracted from *Rosemary, Myrtle,* and *Sour Orange* via hydrodistillation methods by Ammar *et al.* (2014). In their study, modelling of the distillation process was carried out. As microwave radiation has greener features, Desai and Parikh (2015) used this method to extract essential oils from the leaves of lemongrass. Microwave radiation was also compared to hydrodistillation. The Taguchi method was used in the optimization part of their study. In the kinetic part, the desorption model was fitted to experimental data, and the biological activities of lemongrass were also assessed. Another study investigated the effects of different parameters on extraction yield related to the essential oil hydrodistillation extraction process from *Marrubium Vulgare* L. These parameters were heating, drying, grinding, plant and solvent to solid ratio. The second stage was modelling the study with three models from the literature. Besides, the kinetic parameters of these models were estimated with a genetic algorithm. The study conducted by Sodeifian *et al.*, (2017) presented experimental results and mathematical modelling predictions of essential oil from *Eryngium billardieri* with the supercritical CO_2 method (SC–CO_2). In the modelling part of the study, the design of experiments and experimental optimization methods were performed with response surface methodology (RSM). In addition to this, to define the kinetic behavior of the extraction process, a Brunauer–Emmett–Teller (BET) theory with three adjustable parameters correlated the experimental data and model parameters optimized by the simulated annealing algorithm. In another application of the supercritical CO_2 essential oil extraction method, extraction was carried out by Shahsavarpour *et al.* (2017).

The biological activity of essential oils was investigated in the presence of *Candida* species. In the study conducted by Belhachat *et al.* (2018), essential oil extraction from ripe berries of *Pistacia Lentiscus* was carried out. In their study, the ultrasonic pre–treated hydrodistillation method was used. RSM was used to optimize the extraction parameters. For this purpose, the design of experiment method was used based on the Box Behnken Design. Within this concept, extraction time, ultrasonic power, and plant material to water ratio were selected. In the study by Bahmani *et al.* (2018), hydrodistillation with ultrasound was used for the extraction of essential oil from tarragon (*Artemisia dracunculus L.*). Moreover, this method was compared to standard hydrodistillation methods. Gas chromatography analysis was used for determining the essential oil contents. To reduce antioxidant properties, the DPPH radical method was selected. According to the statistical results, the effect of ultrasound power on the extract was not significant. Modi *et al.* (2019) proposed an innovative approach as sono hydrodistillation for the extraction of essential oil from the bark of cinnamon. In the sono hydrodistillation method, ultrasound is combined with hydrodistillation. Energy consumption and CO_2 emission decreased with the sono hydrodistillation method in comparison to the hydrodistillation method.

Response Surface Methodology (RSM) is defined as a powerful optimization method for determining various process parameters and evaluating the interaction effects on response variables (Yolmeh and Jafari, 2017). Moreover, RSM is known as a multivariate statistical technique, and it has been used in the optimization of food processes. When publications in the literature are examined, optimization studies have been found with RSM (Modi *et al.,* 2019). In the study by Kusuma and Mahfud (2016), the response surface methodology was used to optimize the extraction of sandalwood oil with the microwave extraction method. In another study, response surface methodology was used to optimize supercritical fluid extraction of essential oil from pomegranate (*Punica granatum* L.) peel (Ara and Raofie, 2016). In their study, the hydrodistillation method was also used to compare the obtained essential oil content and volatile compounds. In a study conducted by Hosseini *et al.* (2018), response surface methodology was used to optimize heat and ultrasound-assisted extraction of polyphenols from dried rosemary leaves. Sagdic *et al.* (2021), response surface methodology was used to determine the optimum extraction conditions for phenolic compounds from purple basil leaf by conventional solvent extraction and microwave-assisted extraction techniques. Guntero *et al.* (2021) studied the modeling and optimization of the extraction of essential oil of cloves. They were applied to the Taguchi method and factorial design for the experimental design and optimization. In a recent study, process optimization was carried out using the response surface methodology (RSM) to achieve minimum specific energy consumption and maximum moisture diffusion during the drying of melon slices (Tunckal *et al.*, 2022).

In a microwave-assisted hydrodistillation study, RSM was used for the optimization of extraction of essential oils from *Rosmarinus officinalis* L. (Akhbari *et al.*, 2018). Besides the RSM method, other methods such as the Taguchi method were used to optimize parameters for the extraction of essential oils from spearmint (*Mentha spicata* L.) (Ansari and Goodarznia, 2012). Specifically, optimization of essential oil extraction from spearmint with RSM is very limited in the literature. In a study carried out by Bimakr *et al.* (2011), flavonoid compounds were extracted from *Mentha spicat*a L. with supercritical carbon dioxide extraction, and an optimization study was carried out with RSM. In the study by Shrigod *et al.* (2017) the supercritical fluid extraction (SFE) and hydrodistillation methods were compared to determine the quality of the essential oil of *Mentha spicata*. An optimization study for essential oil extraction from oven-dried samples with SFE was conducted with RSM.

To the best of our knowledge, no studies on the application of RSM to the extraction yield of essential oil from *Mentha spicata* L., as well as the carvone ratio, which is the major component of the essential oil, have been published to date, and this is the first report announcing it. The objectives of this study were (i) to investigate the effect of the drying treatment on the essential oil yield and composition of *Mentha spicata* L. (at different drying conditions including shade dying and oven drying at 35ºC and 50ºC), (ii) to model the effect the extraction time and water volume to the mass of plant ratio as independent variables on the changes in yield and main component (carvone) of essential oil as dependent variables of a hydrodistillation process for spearmint leaves by RSM.

II. MATERIAL AND METHODS

A. Plant Material

Mentha spicata L. (spearmint) samples used in these experiments were grown in Battalgazi County, Malatya province, Turkey (altitude 785 m, longitude 38°21'56" E, latitude 38°25'24"N). Fresh aerial parts of spearmint (Fig. 1) were hand–harvested in September 2016. Immediately after being harvested; all specimens were brought to the laboratory. The leaves of plants were separated from stems, then, the samples were stored at 4ºC in a refrigerator until use.

Figure 1: *Mentha spicata* L. samples

B. Sample preparation and drying treatments

The high moisture content of medicinal and aromatic plants makes them unsuitable for long–term storage. Drying aromatic herbs is also important because drying methods have a significant impact on the yield, composition, and content of essential oils. In this sense, since it is necessary to dry the samples for long–term studies, higher essential oil yield, and main component ratio were taken as criteria to decide at which temperature to perform. So, the leaves were randomly divided into three batches for different drying conditions. The first group was dried in shade at about 28ºC, and the other groups were dried in an oven (Nüve FN 500, Turkey) at two different temperatures of 35°C and 50°C, up to the initial moisture content decreased to 10%. After that, they were ready to extract essential oils.

C. Extraction of essential oils

The hydrodistillation method was used to extract the essential oil in a Clevenger–type apparatus and was carried out in two different ways. First, 500 ml of distilled water was poured into 50 g of samples dried in the shade and the oven (at 35°C and 50°C) to decide which drying method to apply, depending on the amount of essential oil and the ratio of the main component of essential oils. Second, it was performed with distilled water containing various volumes for varying exposure times with a constant mass loading of 50 g of the dried sample which both the amount of essential oil and the main component ratio are high for optimization studies. At all of the extraction experiments, dragged oil with boiling water condensed through the cooler and was collected in the capillary tube.

The moisture in the EOs was eliminated by adding about 0.5 g sodium sulfate (Na_2SO_4, ≥99.0%, VWR Chemicals) after the extraction process was done, and the sealed white vials were maintained in the dark at 4ºC until the GC–MS analysis. In each experimental run, the extraction yield of essential oil has been calculated using Eq. 1.

$$\text{Yield}(\%, \text{v/w}) = \frac{\text{Volume of essential oil (ml)}}{\text{Weight of dried sample (g)}} \text{x}100) \quad (1)$$

D. GC–MS Analysis

GC–MS analysis was performed using a Shimadzu 2010 (Shimadzu, Kyoto, Japan) GC equipped with an MS–QP 2010 (Shimadzu, Kyoto, Japan) series mass selective detector. The column that was used was a TRB–Wax (Teknokroma, Barcelona, Spain) fused silica capillary column (30m× 0.25mm i.d. and 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The column was kept at 40°C for 5 min after injection and then stored at 240°C with an increasing rate of 3°C/min for 15 min. The total operating time of the furnace including cooling was 86 min. The injector, transfer line temperature, and ion source temperature were taken as 250°C. The applied ionization voltage was 70 eV, and the mass range was 35–450 m /z. A mixture of n–alkanes (C_8–C_{20} + C_{20}–C_{40}) was used as a reference for the calculation of the retention index (RI) values of each compound (Yilmaztekin, 2014). The separated components were determined by comparing the mass spectra

Table 1. Independent variables and their levels used in the model design

#	Name	Code	Levels				
			-1.414	-1	0	+1	+1.414
1	Extraction time (min)	A	71	100	170	240	269
2	Water volume to the mass of plant ratio (ml/g)	B	0.042	0.055	0.088	0.120	0.133

of the chromatographic peaks to the spectra of NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) and Wiley libraries. Besides, the experimental RI values of the compounds were verified by comparison to the theoretical RI values obtained from the NIST Standard Reference Database (Stein, 2011). Based on the peak area of the GC, the percentage composition of EO was calculated in each case.

E. Optimization of the hydrodistillation process using RSM

Response surface methodology (RSM) was used to establish and optimize hydrodistillation process parameters to the best crude extraction yield and main component ratio. Runs of the experiment were randomly generated by utilizing the statistical software package Design–Expert version 10 (trial). In this study, the independent variables were exposure time (100–240 min., A) and water volume to the mass of plant ratio (0.055–0.120, B), and these parameters were tested at five levels: for codes of –1.414, –1, 0, +1, +1.414 as it is depicted in Table 1.

The experimental data was designed using a two–factor, three-level central composite design (CCD). In this design, 13 treatments were generated with five replicates at the center (0,0) to estimate the pure error. The essential oil volume and amount of carvone were the response variables.

F. Statistical analysis

Analysis of variance (ANOVA) was used to identify the optimum conditions for hydrodistillation of essential oils from leaves of *Mentha spicata* L. ANOVA was carried out to determine how each parameter affects responses and to fit mathematical models to experimental data and to test also the accuracy of the models. Based on the results of the experiments provided by the software, several models were developed. Linear (Eqs. 2a-2b) and quadratic models were proposed for the essential oil volume and carvone ratio, respectively. Moreover, to obtain more accurate results, the quadratic model was modified, and the third–order model (Eqs. 3a-3b) was assessed.

$$Y = b_0 + b_1 \cdot A + b_2 \cdot B \quad (2a)$$

$$Y = b_0 + b_1 \cdot t + b_2 \cdot \left(\frac{\text{water volume}}{\text{mass plant}}\right) \quad (2b)$$

$$Z = b_0 + b_1 \cdot A + b_2 \cdot B + b_{12} \cdot A \cdot B + b_{11} \cdot A^2 + b_{22} \cdot B^2 + b_{112} \cdot A^2 \cdot B \quad (3a)$$

$$Z = b_0 + b_1 \cdot t + b_2 \cdot \left(\frac{\text{water volume}}{\text{mass plant}}\right) + b_{12} \cdot t \cdot \left(\frac{\text{water volume}}{\text{mass plant}}\right) + b_{11} \cdot t^2 + b_{22} \cdot \left(\frac{\text{water volume}}{\text{mass plant}}\right)^2 + b_{112} \cdot t^2 \cdot \left(\frac{\text{water volume}}{\text{mass plant}}\right) \quad (3b)$$

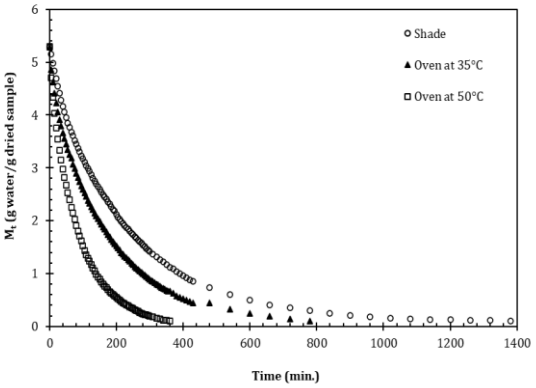

Figure 2: Changing moisture content of samples dried in shade and an oven (35°C and 50°C)

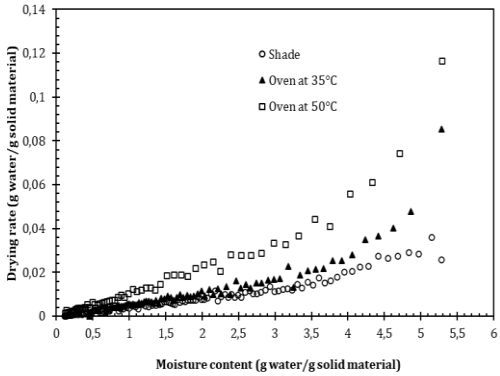

Figure 3: Variation in drying rates in shade and the oven (30°C and 35°C) with change in moisture content

where Y and Z are the responses of the extraction oil yield and the carvone ratio, respectively. B_0, b_1, b_2, b_{12}, b_{11}, b_{22} and b_{112} are constant coefficients of intercept, linear, quadratic, and interaction terms. A and B are coded independent variables.

III. RESULTS AND DISCUSSIONS

A. Drying Treatments: Alteration in Moisture Content and Drying Rate

The change in the moisture content of the samples dried in shade and oven is illustrated in Fig. 2. According to the experimental results, the drying times of the samples were determined as 1380 min in shade, 780 min in the oven at 35°C, and 360 min. in the oven at 50°C. While comparing the drying times of the samples, the minimum drying time was observed at 50°C with the oven as expected. This state may be explained by the fact that the hot air–drying method is very effective in terms of time-saving. As seen in Fig. 2, the drying time was highly influenced by the drying temperature. When the drying temperature increases, the heat transfer rate to the inner parts of food increases, and so, the surface moisture gets evacuated faster.

To determine the effect of the increasing temperature of the samples on the drying rate, the samples dried in shade and an oven with two different temperatures as 35°C and 50°C were investigated (Fig. 3).

While comparing the drying methods, the highest drying rate was observed in the oven drying at 50°C as

Table 2. Essential oil yield (%) obtained from drying treated spearmint samples

Drying condition	Essential oil yield (%, v/w)
Shade	1.7
Oven (35°C)	1.3
Oven (50°C)	0.6

expected. According to the drying experiments, it was determined that the moisture loss rate decreased with time. This situation may be clarified as the drying process takes place only at a decreasing rate. Therefore, it was concluded that mass transfer was the effect of diffusion during the drying of the spearmint samples. When a previous study (Kadam *et al.,* 2011a) was examined, a decreasing speed period was observed for spearmint leaves in hot air drying between 45 and 65°C, and it was also indicated that there was no constant speed period at all drying temperatures. In a study conducted by Doymaz (2006), the author observed a diffusion–controlled drying process between 35 and 60°C for *Mentha spicata* L. In another study by Gunhan *et al.* (2005), it was shown that all drying processes of *Laurusnobilis* leaves were carried out at a decreasing rate. In a study carried out by Kadam *et al.* (2011b), *Ocimum Sanctum* leaves were dried at 55–65°C, and it was stated that there were a decreased rate and a diffusion control drying behavior under all operating conditions. These results obtained from this study were compatible with the literature.

B. Effect of Drying Treatment on The Essential Oil Yield

Hydrodistillation of the spearmint samples dried under different drying conditions resulted in clear light–yellow volatile oils. The ratio of the collected essential oils was calculated on a dry weight basis (%, v/w) and is given in Table 2.

As seen in Table 2, there were considerable differences among the samples taken under different drying conditions. The highest essential oil ratio was obtained from the samples dried in shade. Moreover, as the temperature increased, the ratio of essential oil decreased, so it can be said that loss of essential oil correlates positively with drying temperature. These results were compatible with the literature. For example, Wang *et al.* (2013) dried *Mentha rotundifolia "Variegata"* in shade (at 22°C) and at 60°C, and essential oils from the samples dried at the two temperatures were obtained via hydrodistillation. The essential oil ratios were determined as 0.63% in shade and 0.31% in the oven. In the same manner, Khorshidi *et al.* (2009) dried *Rosmarinus officinalis* L. with three different drying techniques in an oven (45°C), in shade, and the sun and essential oils were extracted via hydrodistillation. It was concluded that the maximum amount of essential oil was obtained by drying in shade (1.8%). In the study conducted by Khalid *et al.* (2008), *Melissa officinalis* samples were dried in the shade, the sun, and at 45°C. The authors used the essential oil ratios of fresh samples to compare the amounts of essential oils. According to their study, the highest essential oil ratio was determined in the fresh samples (0.33%), and secondly, it was detected in the shade–dried samples (0.29%). In the study carried out by Hassanpouraghdam *et al.* (2010), *Ocimum basilicum* samples were dried in the shade, sun, and at 40°C and 60°C. The essential oil ratios were measured as 0.9%, 0.5%, 0.8% and 0.4%, respectively. As a result of the study, drying at high temperatures at 60°C caused volatile oil loss. When an overall assessment is made, an increase in temperature reduces the rate of essential oil, and the result of this paper was compatible with the literature.

C. Effect of Drying Treatment on The Essential Oil Composition

The essential oils extracted using hydrodistillation (using Clevenger) were quantified with GC–MS. The calculated retention indices (RI) and percentages of the components of the spearmint samples dried in different conditions are presented in Table 3.

Based on Table 3, there was no significant effect of different drying methods on essential oil's component type, but the drying method was very effective on the composition in terms of percentage amount. The quantities of two major spearmint components were considered as indicators of a quality essential oil to ease data analysis. The main volatile compounds detected in the spearmint essential oils were D–limonene and carvone.

When the essential oil compositions of the samples dried in shade and in the oven was compared, it was seen that there were significant losses in the amount of carvone and D–limonene in the samples dried in the oven. This situation could depend on chemical transformations during the drying process. Moreover, the change in the concentration was related to the plant family and drying methods. Since our spearmints belonged to the *Lamiaceae* family, the essential oils were stored on the surfaces of the leaves, and so, a loss of volatile compounds is easy.

The essential oils extracted from *Mentha spicata* L. contained significant amounts of carvone and D–limonene. Examining the literature, drying in shade was the most appropriate method for extraction of carvone and D–limonene in high yields (Antal *et al.,*2011; Zheljazkov *et al.,* 2014; Kripanand *et al.,*2015). In parallel to this situation, microwave or hot air–drying causes a significant loss of essential oils. Additionally, hot air–drying damages the chemical composition of essential oils. The results of this paper were compatible with this information; however, the amount of essential oil was different from that reported in the other study. This situation might be explained by climatic conditions, harvest time, or combinations of all these effects.

D. Optimization of the Hydrodistillation Process and Model Development

The highest essential oil yield and main component content are expected from any essential oil extraction method. In this context, when the results obtained in the sections so far are evaluated together, it is found that both were obtained by shade drying in the present study. Hence, the hydrodistillation process was optimized by using the RSM approach with shade–dried samples.

Table 3. The essential oil composition of drying treated of *Mentha spicata* L.

#	Component	RI	Composition of essential oils (%)		
			Dried in shade	Dried in the oven (35°C)	Dried in the oven (50°C)
1	α-pinene	1011	1.40	1.29	1.00
2	Camphene	1054	0.12	0.06	ND
3	β-pinene	1098	1.91	1.67	1.35
4	β-myrcene	1155	1.18	0.80	0.52
5	α-terpinene	1171	0.16	0.11	ND
6	D-limonene	1191	14.55	9.19	6.84
7	1,8-cineole	1203	7.96	8.49	8.12
8	*trans*-2-hexenal	1210	0.22	ND	ND
9	*cis*-β-ocimene	1226	0.97	0.46	0.20
10	γ-terpinene	1238	0.41	0.29	0.15
11	*trans*-β-ocimene	1243	0.50	0.36	0.14
12	β-cymene	1262	0.21	0.19	0.18
13	α-terpinolene	1275	ND	0.06	ND
14	3-octyl acetate	1328	0.25	0.36	0.30
15	3-octanol	1387	1.72	0.97	0.68
16	Hexyl valerate	1437	0.26	0.05	ND
17	1 Octen 3 ol	1442	ND	0.07	0.11
18	*trans*-sabinenehydrate	1460	2.75	2.61	2.40
19	*cis*-3-Hexenyl valerate	1480	0.28	0.07	ND
20	α-Bourbonen	1508	ND	ND	0.14
21	β-Bourbonen	1514	1.40	2.37	2.29
22	Linalool	1538	0.37	0.11	0.14
23	β-Yılangen	1570	ND	0.28	0.34
24	Bornylacetate	1577	ND	0.08	ND
25	β-Elemen	1583	0.56	0.38	0.29
26	β-caryophyllene	1594	3.14	1.48	1.41
27	Terpinene-4-ol	1598	1.54	1.03	1.08
28	*trans*-dihydrocarvone	1605	2.10	3.25	3.09
29	*cis*-dihydrocarvone	1625	ND	0.25	0.34
30	pulegone	1645	ND	ND	0.20
31	Dihydrocarvylacetate	1665	3.12	7.68	7.56
32	α-Terpineol	1692	0.37	0.49	0.44
33	Germacrene D	1706	0.52	0.63	0.63
34	Neohydrocarveol	1720	0.44	1.08	2.37
35	*cis*-carvylacetate	1728	ND	0.26	ND
36	Carvone	1736	35.01	26.42	26.62
37	Dihydrocarveol	1746	6.57	9.55	10.63
38	*trans*-carvylacetate	1764	1.84	5.99	6.17
39	*cis*-carveol	1828	1.39	3.08	6.69
40	Carveol	1859	4.26	4.45	4.65
41	Caryophyllene oxide	1985	0.53	ND	ND
42	*cis*-Jasmone	1941	ND	0.49	0.40
43	Viridiflorol	2083	ND	0.54	0.59
	Total		**97.90**	**97.01**	**98.06**

A central composite design (CCD) was used to determine the effects of time and water volume on the mass of plant ratios on both essential oil yield and main component content. Different extraction time (A: 71, 100, 170, 240, and 269 min) and water volume to mass of plant ratio (B: 0.042, 0.055, 0.088, 0.120, and 0.133 mL/g) were selected for code of -1.414, -1, 0, 1, +1.414, respectively. The results of the various runs are presented in Table 4. The second run had the highest essential oil yield, while the twelfth run had the lowest. The mathematical models describing the essential oil yield of spearmint and carvone ratio in terms of independent variables within the region as the target functions were represented in coded and actual factors by Eqs. 4–7, respectively.

$$EOY = 1.580 + 0.247 \cdot A - 0.196 \cdot B \quad (4)$$

$$EOY = 1.502 + 3.530 \cdot 10^{-3} \cdot t - 6.018 \cdot WPO \quad (5)$$

Table 4. The central composite design with two independent variables and outcomes for both responses.

Run	Independent variables		Responses	
	A: Time (min)	B: water volume to the mass of plant ratio (ml/g)	EO Yield (%)	Carvone ratio (%)
1	170	0.088	1.51	27.42
2	240	0.055	2.1	20.38
3	170	0.088	1.54	26.89
4	170	0.088	1.31	26.95
5	170	0.133	1.45	28.47
6	269	0.088	1.74	23.46
7	240	0.120	2.02	27.01
8	170	0.088	1.22	27.59
9	100	0.055	1.92	24.24
10	170	0.042	1.69	23.63
11	71	0.088	1.12	27.02
12	100	0.120	1.1	29.76
13	170	0.088	1.76	28.67

Table 5. Results of ANOVA from the linear RSM for essential oil yield response

Source	Sum of squares	df*	Mean square	F-values	p-values	
EO Yield:						
Model	0.7945	2	0.3973	7.60	0.0098	Significant
A	0.4885	1	0.4885	9.34	0.0121	
B	0.3060	1	0.3060	5.85	0.0361	
Residual	0.5228	10	0.0523			
Lack of fit	0.4159	6	0.0693	2.59	0.1878	Not significant
Pure Error	0.1069	4	0.0267			
Cor Total	1.32	12				

Table 6. Results of ANOVA from the modified quadratic RSM for carvone ratio response

Source	Sum of squares	df*	Mean square	F-values	p-values	
Carvone:						
Model	78.33	6	13.05	24.86	0.0005	Significant
A	16.95	1	16.95	32.27	0.0013	
B	7.84	1	7.84	14.93	0.0083	
AB	0.31	1	0.308	0.59	0.4728	
A^2	10.69	1	10.69	20.35	0.0041	
B^2	7.73	1	7.73	14.73	0.0086	
A^2B	5.36	1	5.36	10.21	0.0187	
Residual	3.15	6	0.5252			
Lack of fit	0.32	2	0.1611	0.2278	0.8059	Not significant
Pure Error	2.83	4	0.7072			
Cor Total	81.48	12				

***** *Degree of freedom*

$$CR = 27.68 - 1.46 \cdot A + 1.4 \cdot B + 0.278 \cdot A \cdot B - 1.24 \cdot A^2 - 1.05 \cdot B^2 + 1.64 \cdot A^2 \cdot B \quad (6)$$

$$CR = -11.693 + 0.36 \cdot t + 494.186 \cdot WPO - 3.374 \cdot t \cdot WPO - 0.001 \cdot t^2 - 998.225 \cdot WPO^2 + 0.01 \cdot t^2 \cdot WPO \quad (7)$$

where EOY is the essential oil yield, CR is the Carvone ratio, A and t are the time, B and WPO are the Water/Plant ratio.

Interpreting the coefficients' sign and magnitude permits understanding the effects of the variable. Positive coefficients indicate an increase in response as the variable level increases, whereas negative coefficients indicate a decrease when the variable level increases. In this manner, among the linear variables (A and B), A showed a positive effect, while B showed a negative effect on the essential oil yield. But, in the carvone ratio, the situation is the opposite. The quadratic term of A^2B showed the largest effect on the carvone ratio.

The analysis of variance (ANOVA) results for essential oil yield and carvone ratio are given in Tables 5 and 6, respectively. Both models had p values less than 0.05 (p–values<0.05), indicating that model fitness was significant and adequate; also, the proposed models fit the experimental data well, with a non–significant "lack of fit" (p–values>0.05). As seen in Table 5, linear coefficients (A and B) are significant model terms, and the most effective parameter was the time (A) for the yield of essential oil; as seen in Table 6, all parameters (linear coefficients (A and B), interaction coefficients (AB), and quadratic coefficients (A^2, B^2 and A^2B)) except AB (it is acceptable to have only one parameter) in Eq. 6 are significant model terms for carvone ratio, with the most effective being the time (A). Additionally, the F–values of 7.60 for the linear model and 24.86 for the modified quadratic model indicate that they are significant. Generally, a response variable with a high F–value and a low P value has a greater impact on the process (Akhbari *et al.*, 2018).

The coefficient of determinations (R^2), adjusted R^2, and predicted R^2 values show the correctness of the response model, with higher values implying a greater correlation between the experimental data and the model's expected values (Akhbari *et al.*, 2018). But, the essential point to indicate the model's correctness is that R^2 is not considered the major point because adding a variable to the model will enhance R^2's value (Belhachat *et al.*, 2018). The statistical parameters for both models used in the current study are given in Table 7.

The coefficients of determination (R^2) of the models were 0.6031 and 0.9613 for the essential oil yield and for the carvone ratio, respectively. The values of adjusted–R^2 and predicted–R^2 were 0.5237 and 0.2226 for Equation 4 and 0.9227 and 0.8980 for Equation 6, respectively. The predicted R^2 of 0.2226 is not as close to the adjusted R^2 of 0.5237 as one might normally expect.

The software computed these regression coefficients, like the others, for various combinations of the independent variables, and the linear model was determined as the most suitable for the essential oil yield because the predicted R^2 value was negative when other models were applied, including the modified ones. The same parameters for the carvone ratio were in agreement with each other. In the case where there were many non–significant terms in the model, the adjusted R^2 would be much smaller than the R^2 (Belhachat *et al.*, 2018). Since these values are close to each other in both the linear model for the essential oil yield and the modified quadratic model for the carvone ratio, all parameters have a significant effect. Also, these values indicate that observed data and predicted data are highly correlated. The coefficient of variation (C.V.%) defines the level of distribution of the data; the smaller the C.V.%, the better the reproducibility (Bel-

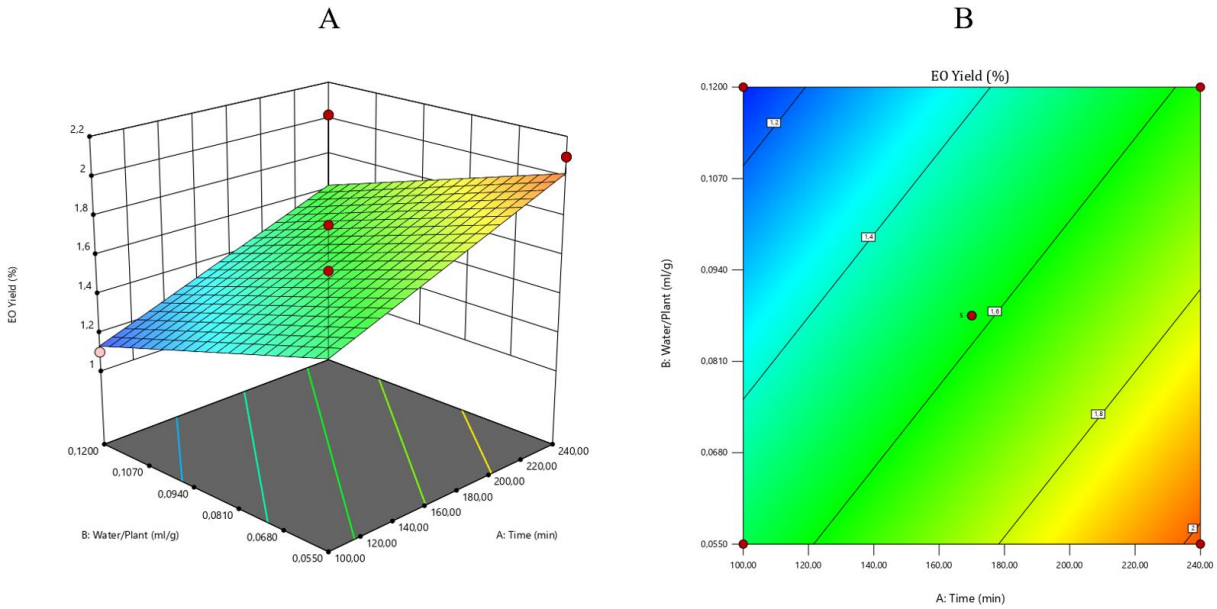

Figure 4: Response surface and contour plots of independent variables on essential oil yield

Table 7. Statistical results of fitting models

Parameter	EO Yield	Carvone Ratio
	Model	
	Linear	Modified quadratic
Std. Dev.	0.2287	0.7247
Mean	1.58	26.27
C.V.%	14.51	2.76
PRESS	1.02	8.31
R^2	0.6031	0.9613
Adjusted R^2	0.5237	0.9227
Predicted R^2	0.2226	0.8980
Adeq Precision	8.0605	16.8984

hachat *et al.*, 2018). According to the small C.V.% in Table 7, the experimental results were accurate and reliable.

A smaller value is predicted for the Predicted Residual Sum of Squares (PRESS), which is a measure of how well a model fits each point in the design. The small values reported in our investigation for both models are acceptable.

Adeq Precision measures the signal–to–noise ratio used for model measurement for accuracy. A ratio greater than 4 is desirable. The ratios of 8.0605 for the linear model and 16.8984 for the modified quadratic model indicate an adequate signal. These models can be used to navigate the design space. All of these statistical parameters indicate that the models are reliable.

A response surface plot of the equation was the best way to demonstrate the effect of any parameter on the essential oil yield and the carvone ratio within the experimental space under consideration. Figures 4 and 5 show 3D and 2D contour plots that illustrate the relationship between independent and dependent variables generated by the linear and modified quadratic models for the essential oil yield and the carvone ratio, respectively.

As illustrated in Fig. 4 (A) and (B), increasing the hydrodistillation time had a greater effect on essential oil yield than increasing the water volume to the mass of plant ratio, which is consistent with the result of Equation 4. Increasing the time from 71 min to 269 min and the water volume to mass the of plant ratio from 0.042 to 0.133 led to yield enhancement of the essential oils (Fig. 4(A)). After 240 minutes, the essential oil yield increased to 2.1 ml with a water volume to mass the of plant ratio of 0.055, according to the 2D contour plot (Fig. 4(B)). This can be interpreted as a longer hydrodistillation time giving heavier and higher boiling point constituents more time to diffuse out of the oil pockets in the leaves, thereby increasing the yield.

The effects of the hydrodistillation time and the water volume to the mass of plant ratio are depicted in Fig. 5 (A) and 5 (B) in 3D and 2D, respectively. Both parameters have significant effects, both linear and quadratic, on the carvone ratio. The highest carvone ratio, with a value of 29.76%, was obtained at the time of 100 min and the water volume to mass the of plant ratio of 0.12 (Fig. 5(A)). According to the 2D contour plot (Fig. 5(B)), the carvone ratio increased to 29.76% after 100 minutes with a water volume to mass the of plant ratio of 0.120. As the water ratio increases in the fixed plant ratio, there is a decrease in the carvone ratio. This was most likely because some volatile components are resistant to hydrolysis when there is a lot of water present. The carvone ratio was affected by increasing time until 153 min and then decreased. Thus, the hydrodistillation time showed both positive and negative effects on the carvone ratio, while the water volume to the mass of plant ratio showed only a positive effect with no linear increase.

All of the aforementioned results demonstrated that a nominal change in extraction time could have a greater impact on the response.

E. Verification of the Optimized Conditions

Optimization of the essential oil yield and the carvone ratio from *Mentha spicata* L. leaves was performed using the Design Expert as a numerical optimizer with the criteria of minimizing time, maximizing water volume to the mass of plant ratio, maximizing essential oil yield,

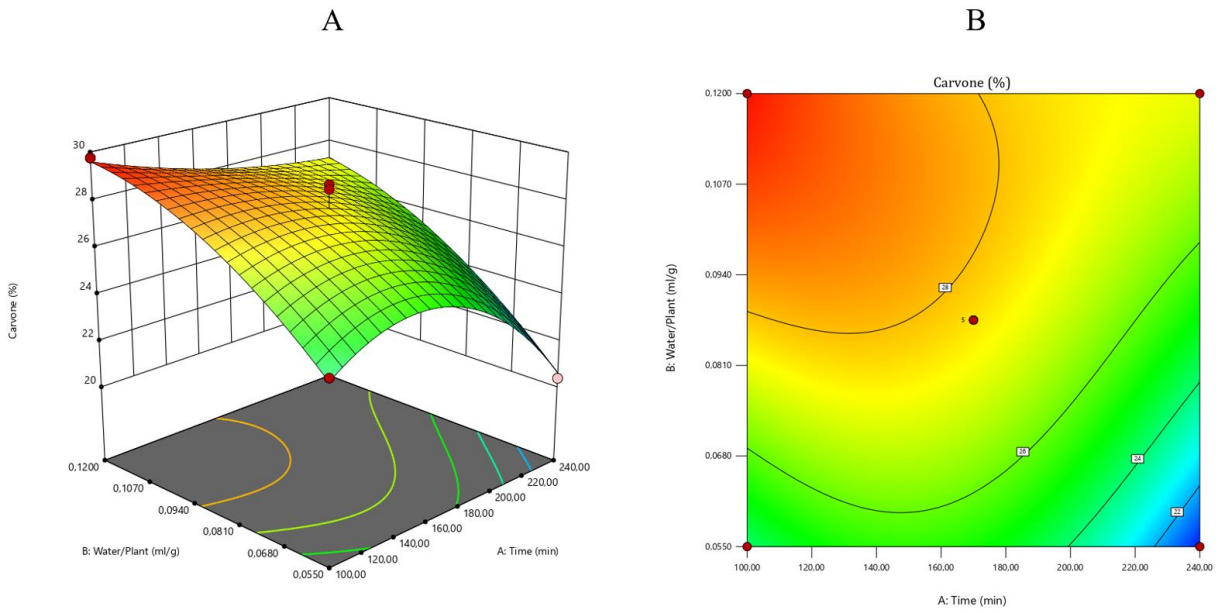

Figure 5: Response surface and contour plots of independent variables on carvone ratio

and maximizing carvone ratio. The optimal conditions were found to have a hydrodistillation time of 145.7 min and a water volume to the mass of plant ratio of 0.105 ml/g. Under these conditions, the essential oil yield and the carvone ratio were determined as 1.383 ml and 28.541%, respectively. To verify the model conditions, three replicate experiments were set up under optimal conditions. Accordingly, mean values of the essential oil yield and the carvone ratio were found to be 1.4±0.13 ml and 28.496±0.56%, respectively, consistent with the prediction of the correlation.

IV. CONCLUSIONS

In the present study, the response surface methodology was used to investigate the optimum hydrodistillation process parameters that could obtain a maximum yield of essential oil and carvone ratio from *Mentha spicata* L. Before optimization, the effects of different drying methods and temperatures on the amount and composition of essential oil extracted from *Mentha spicata* L were investigated. The dried samples' essential oil ratios were detected as 1.7% in shade, 1.3% in the oven (35°C), and 0.6% in the oven (50°C). The chemical composition analysis of the essential oil was performed with GC–MS. The highest value of carvone, which was the main constituent, was obtained in the shade drying conditions, as was the essential oil yield. So, the optimization was applied to the hydrodistillation of shade–dried samples. The essential oil yield was predicted using a linear model. The carvone ratio was predicted using a modified quadratic model, which showed good agreement between predicted and actual values (R^2=0.9613). According to the analysis of variance, the effects of hydrodistillation time and water volume to a mass of plant ratio, and their interactions were found to significantly influence the essential oil production and carvone ratio, and they were statistically significant. The optimal conditions were the hydrodistillation time of 145.7 min and the water volume to the mass of plant ratio of 0.105 ml/g. Under these conditions, the mean value of essential oil yield is 1.4±0.13 ml and the carvone ratio is 28.496±0.56%, which agrees well with the expected results from the CCD approach. This study clearly shows that the RSM successfully reduced the operation time and the water volume to the mass of plant ratio and, hereby, both essential oil yield and carvone ratio can be improved by optimizing. The proposed optimization of the chosen extraction method is also applicable to any other essential oil.

REFERENCES

Akhbari, M., Masoum, S., Aghababaei, F. and Hamedi, S. (2018) Optimization of microwave-assisted extraction of essential oils from Iranian *Rosmarinus officinalis* L. using RSM. *J. Food Sci. Technol.* **55**, 2197–2207.

Almeida, P.P., Mezzomo, N. and Ferreira, S.R.S. (2012) Extraction of *Mentha spicata* L. Volatile Compounds: Evaluation of Process Parameters and Extract Composition. *Food Bioproc. Technol.* **5**, 548–559.

Ammar, A. H. Meniai, A.H. and Zagrouba, F. (2014) Experimental study and modeling of essential oil extraction from plants by hydrodistillation. *Chem. Eng. Technol.* **37**, 1235–1242.

Ansari, K. and Goodarznia, I. (2012) Optimization of supercritical carbon dioxide extraction of essential oil from spearmint (*Mentha spicata* L.) leaves by using Taguchi methodology. *J. Supercrit. Fluids* **67**, 123–130.

Antal, T., Figiel, A., Kerekes, B. and Sikolya, L. (2011) Effect of Drying Methods on the Quality of the Essential Oil of Spearmint Leaves (*Mentha spicata* L.). *Dry. Technol.* **29(15)**, 1836–1844.

Ara, K.M. and Raofie, F. (2016) Application of response surface methodology for the optimization of supercritical fluid extraction of essential oil from

pomegranate (*Punica granatum* L.) peel. *J. Food Sci. Technol.* **53**, 3113–3121.

Bahmani, L., Aboonajmi, M., Arabhosseini, A. and Mirsaeedghazi, H. (2018) Effects of ultrasound pre–treatment on quantity and quality of essential oil of tarragon (*Artemisia dracunculus* L.) leaves. *J. Appl. Res. Med. Aromat. Plants.* **8**, 47–52.

Belhachat, D., Mekimene, L., Belhachat, M., Ferradji, A. and Aid, F. (2018) Application of response surface methodology to optimize the extraction of essential oil from ripe berries of Pistacia lentiscus using ultrasonic pretreatment. *J. Appl. Res. Med. Aromat. Plants.* **9**, 132–140.

Bimakr, M., Rahman, R.A., Ganjloo, A., Taip, F.S., Salleh, L. and Sarker, Z.I. (2011) Optimization of Supercritical Carbon Dioxide Extraction of Bioactive Flavonoid Compounds from Spearmint (*Mentha spicata* L.) Leaves by Using Response Surface Methodology. *Food Bioproc. Tech.* **5**, 912–920.

Danh, L.T., Han, L.N., Triet, N.D.A., Zhao, J., Mammucari, R. and Foster N. (2013) Comparison of Chemical Composition, Antioxidant and Antimicrobial Activity of Lavender (*Lavandula angustifolia* L.) Essential Oils Extracted by Supercritical CO_2, Hexane and Hydrodistillation. *Food Bioproc. Technol.* **6**, 3481–3489.

Desai, M.A. and Parikh, J. (2015) Extraction of Essential Oil from Leaves of Lemongrass Using Microwave Radiation: Optimization, Comparative, Kinetic, and Biological Studies. *ACS Sustain. Chem. Eng.* **3**, 421–431**.**

Doymaz, İ. (2006) Thin–layer drying behaviour of mint leaves. *J. Food Eng.* **74(3)**, 370–375.

Giacometti, J., Bursać Kovačević, D., Putnik, P., Gabrić, D., Bilušić, T., Krešić, G., Stulić, V., Barba, F.J., Chemat, F., Barbosa–Cánovas, G. and Režek Jambrak, A. (2018) Extraction of bioactive compounds and essential oils from Mediterranean herbs by conventional and green innovative techniques: A review. *Food Res. Int.* **113**, 245–262.

Gunhan, T., Demir, V., Hancioglu, E. and Hepbasli, A. (2005) Mathematical modelling of drying of bay leaves. *Energy Convers. Manag.* **46**, 1667–1679.

Guntero, V., Ferretti, C.A., Mancini, P.M. and Kneeteman, M.N. (2021) Optimization of Eugenol Production from Cloves Using Taguchi Design of Experiments. *Lat. Am. Appl. Res.* **51,** 93-99.

Hassanpouraghdam, M.B., Hassani, A., Vojodi, L. and Farsad–Akhtar, N. (2010) Drying Method Affects Essential Oil Content and Composition of Basil (*Ocimum basilicum* L.). *J. Essent. Oil-Bear. Plants.* **13**, 759–766.

Hosseini, H., Bolourian, S., Yaghoubi Hamgini, E. and Ghanuni Mahababadi, E. (2018) Optimization of heat–and ultrasound–assisted extraction of polyphenols from dried rosemary leaves using response surface methodology. *J. Food Process. Preserv.* **42**, e13778.

Kadam, D.M., Goyal, R.K., Singh, K.K. and Gupta, M.K. (2011a) Mathematical modeling of convective thin layer drying of basil leaves. *J. Med. Plants Res.* **5,** 4721–4730.

Kadam, D.M., Goyal, R.K., Singh, K.K. and Gupta, M.K. (2011b) Thin layer convective drying of mint leaves. *J. Med. Plants Res.* **5**, 164–170.

Khalid, K.A., Hu, W. and Cai, W. (2008) The Effects of Harvesting and Different Drying Methods on the Essential Oil Composition of Lemon Balm (*Melissa officinalis* L.). *J. Essent. Oil-Bear. Plants.* **11**, 342–349.

Khorshidi, J., Mohammadi, R., Tabatabaei, M.F. and Nourbakhsh, H. (2009) Influence of drying methods, extraction time, and organ type on essential oil content of rosemary (*Rosmarinus officinalis* L.). *Nature Sci.* **7**, 42–44.

Kripanand, S.M., Guruguntla, S. and Korra, S. (2015) Effect of various drying methods on quality and flavor characteristics of mint leaves (*Mentha spicata* L.). *J. Food Pharm. Sci.* **3**, 38–45.

Kusuma, H.S. and Mahfud, M. (2016) Response Surface Methodology for Optimization Studies of Microwave–assisted Extraction of Sandalwood Oil. *J. Mater. Environ. Sci.* **7**, 2028–2508.

Modi, P.I., Parikh, J.K. and Desai, M.A. (2019) Sonohydrodistillation: Innovative approach for isolation of essential oil from the bark of cinnamon. *Ind. Crops Prod.* **142**, 111838.

Sagdic, O., Karasu, S., Ozkan, K. and Akcicek, A. (2021) Optimization of Phenolic Compounds Extraction from Purple Basil Leaf by Conventional and Microwave Assisted Extraction Method. *Lat. Am. Appl. Res.* **51**, 285-292.

Sarkic, A. and Stappen, I. (2018) Essential Oils and Their Single Compounds in Cosmetics—A Critical Review. *Cosmetics.* **42,** 1–21.

Shahsavarpour, M., Lashkarbolooki, M., Eftekhari, M.J. and Esmaeilzadeh, F. (2017) Extraction of essential oils from *Mentha spicata* L. (*Labiatae*) via optimized supercritical carbon dioxide process. *J. Supercrit. Fluids.* **130**, 253–260.

Shrigod, N.M., Swami Hulle, N.R. and Prasad, R.V. (2017) Supercritical fluid extraction of essential oil from mint leaves (*Mentha spicata*): Process optimization and its quality evaluation. *J. Food Proc. Eng.* **40**, e12488.

Sodeifian, G., Sajadian, S.A. and Ardestani, N.S. (2017) Experimental optimization and mathematical modeling of the supercritical fluid extraction of essential oil from Eryngium billardieri: Application of simulated annealing (SA) algorithm. *J. Supercrit. Fluids.* **127**, 146–157.

Stein, E. (2011) Retention indices, *NIST Chemistry WebBook*, NIST Standard Reference Database Number 69, National Institute of Standards and Technology, Gaithersburg, Md, USA. 2011. http://webbook.nist.gov/.

Tongnuanchan, P. and Benjakul, S. (2014) Essential Oils: Extraction, Bioactivities, and Their Uses for Food Preservation. *J. Food Sci.* **79**, R1231–R1249.

Tunckal, C., Ozkan Karabacak, A., Tamer, C.E., Yolci Omeroglu, P. and Goksel, Z. (2022) Mathematical Modelling and Optimization of Melon Slice Drying with Response Surface Methodology in A Heat Pump Drying System. *Lat. Am. Appl. Res.* **52**, 101-110.

Wang, J., Li, R., Tan, J. and Jiang, Z.T. (2013) Effect of Drying on Essential Oil Yields and Chemical Composition of Pineapple Mint (Mentha rotundifolia 'variegata') from China. *J. Essent. Oil-Bear. Plants*. **13**, 630–635.

Yilmaztekin, M. (2014) Analysis of volatile components of cape gooseberry (*Physalis peruviana* L.) grown in Turkey by HS–SPME and GC–MS, Sci. *World J.* eCollection. **2014**, 796097.

Yolmeh, M. and Jafari, S.M. (2017) Applications of Response Surface Methodology in the Food Industry Processes. *Food Bioproc. Tech.* **10**, 413–433.

Zheljazkov, V.D., Astatkie, T. and Jeliazkova, E. (2014) Drying and Shade Effects on Spearmint Oil Yields and Composition. *HortScience*. **49**, 306–310.

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