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# Extraction of Essential Oil from Laurel Leaves by Using Microwaves

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**Abstract:** The effects of microwave power and time in solvent-free microwave extraction (SFME) on the yield and composition of the essential oil obtained from laurel (*Laurus nobilis* L.) leaves were studied. The extraction was also performed by hydrodistillation as a control. Specific gravities and refractive indices of the essential oils obtained by different methods and at various conditions were also examined. The main constituent of laurel essential oil was 1,8-cineole (630–730 mg/mL). Essential oils obtained by SFME and hydrodistillation were comparable with respect to both yield and composition while the process time was reduced by 55–60% when SFME was used.

**Keywords:** Aroma compounds, essential oil, *Laurus nobilis* L., solvent-free microwave extraction (SFME)

## INTRODUCTION

*Laurus nobilis* L. (*Lauraceae*), is an evergreen tree or shrub which is native to the Mediterranean region and Turkey (1). Its leaves, which have been used as a spice since antiquity primarily because of its essential oil content, are harvested principally in Turkey from wild growing plants (2). Dried laurel leaves and their essential oil are widely used as flavor enhancers for foods such as meats, soups, sauces, confectionery (3),

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and fish (4). Additionally, it is known that the essential oil of laurel is widely used in the perfume and soap industries (1), as well as in drugs (5).

In general, essential oils have antibacterial and antioxidant activities and may also exhibit antifungal, antiviral, antiparasitic, and insecticidal properties depending on their components. Several studies have evaluated the antimicrobial activity of laurel essential oil (6,7), and also the antioxidant properties of some of the leaf extracts (8,9). Therefore, laurel is used in the food industry as a food preservative (5). Improvement in the production technology of essential oils is quite important to improve the overall yield and product quality. Recently, application of microwave heating for the isolation and analysis of the essential oils has been subject of interest. Dry plant material and substantial amount of water placed in a Clevenger apparatus was heated inside a microwave oven in microwave-assisted hydrodistillation (1). A new technique called ''solvent-free microwave extraction'' (SFME) which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the essential oils in fresh plant materials has been developed (10). This method has been used to obtain essential oils from different spices, herbs, and cardamom seed (10–12). Essential oil extraction was performed by introducing an insulated microwave coaxial antenna inside the extraction flask containing dry laurel leaves and water (13). However, there are no studies in literature about the solvent-free microwave extraction of essential oils from laurel.

The aim of the study is to determine the possibility of using SFME in the extraction of essential oil from laurel. The effects of microwave power and extraction time on the yield and composition of the final product are investigated. Specific gravity and refractive index of the essential oils were also examined. The results are compared with those obtained with the hydrodistillation method.



## MATERIALS AND METHODS

### Materials

The dried laurel (*Laurus nobilis* L.) leaves were obtained from Kütaş (Kütaş Tarim Ürünleri Diş Tic. San. A.Ş., İzmir, Turkey). The leaves were cut into small pieces having sauter mean diameter of 2103.4 μm.

The standard materials used for the qualitative and quantitative analysis of the essential oil constituents were α-pinene, γ-terpinene, methyl eugenol, p-cymene, cuminaldehyde (Fluka, Ronkonkoma, NY, US), β-eudesmol (Fluka, Tokyo, Japan), 4-terpineol, bornyl acetate, terpinolene, eugenol (Fluka, Buchs, Switzerland), limonene, myrtenal

(Fluka, Neu-Ulm, Germany), β-caryophyllene (Fluka, Madrid, Spain), borneol, α-terpineol, camphor, β-pinene, 1,8-cineole, linalool (Sigma-Aldrich, Steinheim, Germany), and camphene (Supelco, Bellefonte, PA, USA). Nonane, which was used as an internal standard, was purchased from Fluka (Buchs, Switzerland). Sodium sulfate anhydrous was purchased from Riedel-de Haën (Seelze, Germany).

## Hydrodistillation

In conventional hydrodistillation, Clevenger apparatus was used. A hemispherical heater (Termal Laboratory Equipments, Istanbul, Turkey) with a maximum power of 200 W was used in the experiments. Laurel leaves and water were placed in the apparatus at a dry plant material: water ratio of 1:10. Distillation was performed for different times and for each distillation time, experiments were conducted twice.



## Solvent-Free Microwave Extraction (SFME)

A domestic microwave oven with an interior cavity size of $29 \times 37 \times 40$ cm was used in the experiments (White-Westinghouse, Pittsburg, USA). The maximum power of the oven was 622 W which was measured using IMPI-2L test (14). The microwave oven was modified by drilling a hole at the top. A flat bottom flask having a capacity of 1000 mL was placed in the oven and connected to the Clevenger apparatus through the hole.

Before SFME, 150 g of laurel leaves were soaked in 700 mL distilled water at room temperature for 1 h in order to hydrate the external layers of the plant material since dried leaves were used. Then, the excess water was drained off. The moistened plant material was placed in a flat-bottom flask connected to a Clevenger apparatus and the process was started. The SFME process was performed at different power levels (622 W and 249 W) and for different extraction times. For each condition, experiments were replicated twice.

## Analysis of Essential Oil

### Yield

Essential oil yield was expressed in terms of the volume of the oil collected in mL per gram of dry plant material.

## Composition

The essential oils obtained at different conditions were collected in amber colored vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen, and kept at 4°C until being analyzed. Identification and quantitative analysis of essential oil components were performed using gas chromatography (Agilent Technologies 6890 N Network GC System, Palo Alto, CA, US) and gas chromatography coupled to mass spectrometry (Agilent Technologies 6890 N Network GC System coupled to Agilent Technologies 5973 Network Mass Selective Detector, Palo Alto, CA, US). In order to perform quantitative analysis with Flame Ionization Detector (FID) at the same time with the component characterization of Mass Selective Detector (MSD), a two hole ferrule was used in which two columns were placed. By this way, injection of the sample from one injection block was distributed equally into two columns. The capillary columns used for both of the analysis were HP-5MS (30 m × 0.25 mm × 0.25 μm) with a 5% phenyl methyl siloxane stationary phase. GC-MS conditions were as follows: carrier gas, He; flow rate, 1.2 mL/min; splitless; injection volume 1 μL; injection temperature 250°C; oven temperature program, holding at 60°C for 5 min, and rising to 210°C with 2°C/min; MSD transfer line temperature, 230°C; MSD quadrupole temperature, 150°C; ionization temperature, 230°C; ionization mode, electronic impact at 70 eV. Solvent delay was for 4.5 min. The GC analysis was performed with the following conditions: flow rate, 0.8 mL/min; FID temperature, 260°C; make-up gas type, He with a make-up flow rate of 45 mL/min.

For the quantitative analysis of laurel essential oil, calibration solutions composed of eugenol, γ-terpinene, cuminal, 1,8-cineole, β-pinene, p-cymene, β-caryophyllene, bornyl acetate, methyl eugenol, α-pinene, linalool, α-terpineol, myrtenal, 4-terpineol, camphor, β-eudesmol were prepared in seven different concentrations and injected into the columns with the GC method given above. Nonane was used as an internal standard. A calibration curve ($r^2 \geq 0.998$) was obtained for each component in the solution, which was used for the quantitation of the corresponding components in the laurel essential oil. For the quantitation of the other constituents of laurel essential oil, the approach of Schoenmakers, Oomen, Blomberg, Genuit, and Van Velzen (15) was used. Following this approach, monoterpene hydrocarbons, phenols, alcohols, ketones, aldehydes, esters, ethers and sesquiterpenes in the essential oil of laurel were quantified using the relative response factors of β-pinene (∼1.23), eugenol (∼1.48), 4-terpineol (∼1.43), camphor (∼1.34), myrtenal (∼1,18), bornyl acetate (∼1.6), 1,8-cineole (∼1.46), and β-caryophyllene (∼1.26), respectively. For the quantitation of lactones the relative response factor of β-caryophyllene (sesquiterpene) was used.

The components of the essential oils were identified by comparison of their retention times with those of available authentic standards and with library matching of their mass spectra (NIST98, Wiley7n, Flavor2). The data were analyzed by a software program, MSD ChemStation (G1701 DA D.02.00.275).

### Specific Gravity and Refractive Index of Essential Oil

Specific gravity of the essential oils obtained at different conditions were calculated by dividing the weight of 10 μL essential oil to that of 10 μL distilled water. Weight measurements were made in triplicate using a highly sensitive balance with an accuracy of ±0.00001 g (Denver Instrument, Gottingen, Germany) at 22 ± 2°C.

Refractive index measurements were made in triplicate using the Bellingham Stanley Ltd. RFM 330 refractometer (Kent, England). Measurement temperatures were 25 ± 2°C.



### Statistical Analysis

The results (essential oil yields, concentrations of the essential oil compounds at different chemical classes, and specific gravity and refractive index of the essential oil) were statistically evaluated by one way analysis of variance (ANOVA). The differences between different extraction conditions with respect to yield, concentrations of the essential oil compounds at different chemical classes, and specific gravity and refractive index of the essential oil obtained at final extraction time were determined. Whenever a significant difference was obtained, a Tukey pairwise comparison test ($p \leq 0.05$) was performed.

## RESULTS AND DISCUSSION

### Essential Oil Yield

Effects of microwave power level and duration of the SFME process on the essential oil yield of laurel were investigated. Laurel leaves were soaked in water before SFME since dried leaves were used in the experiments as mentioned in the materials and methods section. The amount of water absorbed by dry laurel leaves was about 135% of its initial weight when soaked in water for 1 h. The variation of the essential oil yield (mL oil/g laurel) with time during hydrodistillation and SFME processes can

be seen in Fig. 1. Maximum yields obtained using SFME at 622 W and 249 W power levels were 0.0235 and 0.022 mL oil/g laurel, respectively. In hydrodistillation method, the maximum yield was found to be 0.022 mL oil/g laurel. According to one-way ANOVA, no significant difference was obtained between these values ($p \leq 0.05$).

It was found that the time needed for the complete extraction of essential oil of laurel at 622 W power was 85 min, while it was 130 min in 249 W power. An increase in microwave power increased the pressure gradient which enhanced the extraction and reduced the process time. In the case of hydrodistillation, the process time was 195 min. Therefore, the extraction time seems to be reduced by 55–60% in the case of SFME. The reason for the reduction in the time of the extraction process in SFME method than in hydrodistillation is the higher pressure gradient formed inside the plant material during microwave heating. In microwave heating, large amounts of interior heating result in increased moisture vapor generation inside the food which creates significant interior pressure and concentration gradient (16).



## Composition

Figure 2 shows the total ion chromatogram of the laurel essential oil. The composition of the essential oil of laurel obtained by SFME and conventional hydrodistillation methods are given in Table 1. Due to

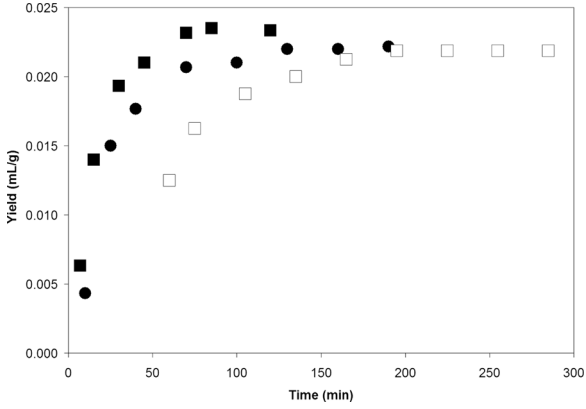


***Figure 1.*** Variation of essential oil yield of laurel during hydrodistillation and solvent-free microwave extraction (SFME) at different power levels (■, SFME-622 W power[a]; ●, SFME-249 W power[a]; □, hydrodistillation[a]) (*means extraction conditions with different letters are significantly different when maximum yield values were considered, $p \leq 0.05$).

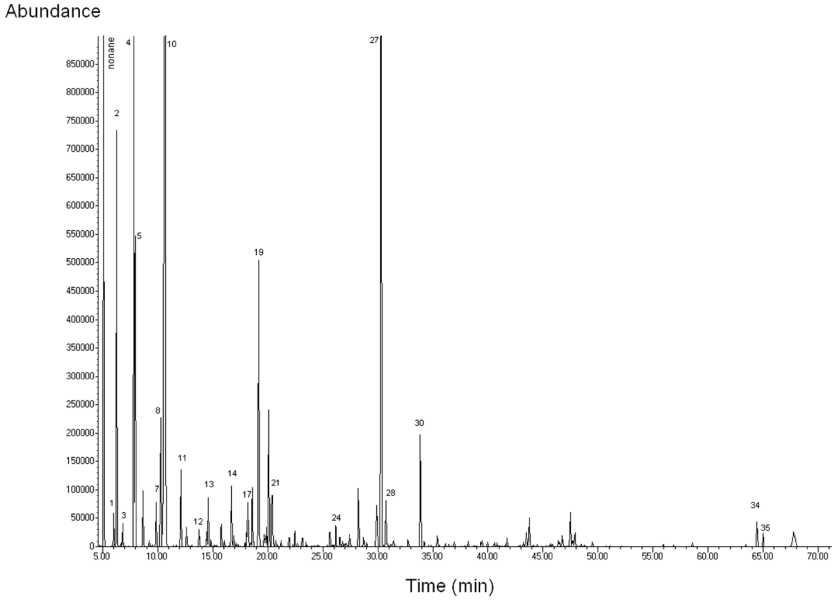


***Figure 2.*** Total ion chromatogram (obtained by GC-MS analysis) of the laurel essential oil extracted by SFME at 622 W power level (1: α-thujene, 2: α-pinene, 3: camphene, 4: sabinene, 5: β-pinene, 7: α-terpinene, 8: p-cymene, 10: 1,8-cineole, 11: γ-terpinene, 12: terpinolene, 13: linalool, 14: pinocarveol/trans-pinocarveol, 17: pinocarvone, 19: 4-terpineol, 21: myrtenal, 24: bornyl acetate, 27: α-terpinyl acetate, 28: eugenol, 30: methyl eugenol, 34: dehydrocostuslactone (?), 35: erementhin (?).

the lack of some of the authentic compound standards, only about 90% of the essential oil could be characterized. The composition of the essential oils obtained by both of the methods were found to be almost the same. The main components of the essential oil of laurel were determined as 1,8-cineole (630–730 mg/mL oil) followed by α-terpinyl acetate (90–115 mg/mL oil), sabinene (45–55 mg/mL oil), α-pinene (30–40 mg/mL oil), 4-terpineol (35–40 mg/mL oil) and β-pinene (~30 mg/mL oil). It was also found that the essential oil was composed mainly of oxygenated compounds (≥75%) while monoterpene hydrocarbons constituted about ≥15% of the essential oil, respectively.

In the case of both hydrodistillation and SFME, concentrations of the monoterpene hydrocarbons increased, while that of oxygenated compounds decreased with time slightly (Table 1).

The oxygenated compounds detected in laurel essential oil were found to be mainly composed of ethers (70–75%), especially of 1,8-cineole (~70%). The rest of the oxygenated constituents of the oil were

**Table 1.** Concentrations of the compounds present in the essential oil of laurel obtained by different methods

| | | | | Concentration (mg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hydrodistillation | | | SFME-622 W | | | SFME-249 W | | |
| No | Compounds | RT 1 (min)[a] | RT 2 (min)[b] | 60 min | 75 min | 195 min | 7 min | 15 min | 85 min | 10 min | 25 min | 130 min |
| 1 | α-thujene | 6.043 | 9.852 | 2.45 | 2.19 | 3.45 | 1.70 | 1.84 | 3.29 | 2.00 | 2.56 | 2.67 |
| 2 | α-pinene | 6.287 | 10.179 | 19.52 | 24.24 | 38.98 | 9.73 | 19.98 | 38.89 | 9.72 | 20.04 | 32.62 |
| 3 | camphene | 6.837 | 10.909 | 1.94 | 2.55 | 2.87 | 1.29 | 1.92 | 2.92 | 1.57 | 1.92 | 2.43 |
| 4 | sabinene | 7.875 | 12.186 | 39.50 | 43.16 | 47.03 | 25.59 | 41.58 | 53.45 | 26.36 | 41.02 | 46.69 |
| 5 | β-pinene | 7.986 | 12.348 | 20.40 | 24.26 | 32.35 | 11.11 | 20.34 | 33.28 | 12.17 | 20.88 | 30.21 |
| 6 | α-phellandrene | 9.273 | 13.868 | — | — | 1.58 | — | — | — | — | 1.31 | 0.90 |
| 7 | α-terpinene | 9.882 | 14.580 | 3.05 | 3.26 | 4.92 | 1.60 | 2.18 | 4.46 | 2.08 | 2.15 | 4.25 |
| 8 | p-cymene | 10.300 | 15.051 | 10.43 | 11.30 | 12.79 | 6.73 | 9.48 | 13.41 | 7.54 | 10.33 | 12.70 |
| 9 | limonene | | 15.325 | — | — | — | — | — | — | — | — | 8.17 |
| 10 | 1,8-cineole | 10.708 | 15.507 | 933.17 | 843.32 | 630.24 | 853.90 | 878.79 | 731.75 | 1011.91 | 874.09 | 653.05 |
| 11 | γ-terpinene | 12.133 | 17.120 | 5.77 | 6.50 | 8.89 | 3.21 | 3.98 | 8.18 | 2.68 | 4.46 | 8.74 |
| 12 | terpinolene | 13.795 | 18.953 | 1.57 | 1.63 | 2.92 | — | 1.08 | 2.30 | — | 1.19 | 2.60 |
| 13 | linalool | 14.627 | 19.878 | 2.22 | 2.69 | 2.40 | 2.47 | 2.99 | 2.17 | 2.27 | 2.97 | 2.48 |
| 14 | pinocarveol/ trans-pinocarveol | 16.729 | 22.116 | 10.26 | 11.44 | 9.83 | 11.12 | 13.46 | 11.30 | 9.09 | 13.04 | 12.07 |
| 15 | camphor | 16.946 | 22.355 | 1.18 | 1.18 | 0.89 | 1.36 | 1.29 | — | — | 1.17 | 1.03 |
| 16 | sabina ketone | 18.090 | 23.405 | 3.44 | 4.23 | 3.67 | 5.77 | 6.02 | 4.40 | 4.47 | 5.46 | 5.72 |
| 17 | pinocarvone | 18.233 | 23.680 | 8.54 | 8.49 | 6.30 | 9.00 | 9.52 | 8.03 | 7.93 | 9.53 | 8.03 |
| 18 | borneol | 18.476 | 23.984 | 7.26 | 9.16 | 8.81 | 9.93 | 11.58 | 11.46 | 6.80 | 7.96 | 12.25 |

(*Continued*)

**Table 1.** Continued

| No | Compounds | RT 1 (min)[a] | RT 2 (min)[b] | Concentration (mg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hydrodistillation | | | SFME-622 W | | | SFME-249 W | | |
| | | | | 60 min | 75 min | 195 min | 7 min | 15 min | 85 min | 10 min | 25 min | 130 min |
| 19 | 4-terpineol | 19.207 | 24.649 | 33.33 | 38.23 | 36.78 | 28.46 | 36.81 | 40.47 | 24.93 | 36.40 | 42.56 |
| 20 | α-terpineol | | 25.756 | 1.85 | 2.15 | 2.22 | 2.27 | 2.83 | 2.90 | 1.35 | 2.49 | 3.06 |
| 21 | myrtenal | 20.430 | 25.877 | 10.10 | 10.92 | 10.09 | 9.63 | 12.34 | 12.50 | 8.35 | 11.55 | 12.49 |
| 22 | cuminal | 23.168 | 28.966 | — | 3.27 | 3.06 | — | 3.79 | 3.43 | — | 3.50 | 3.50 |
| 23 | carvone | 23.517 | 31.030 | 2.49 | 2.23 | 2.75 | 1.40 | 1.95 | 3.01 | 1.95 | 2.62 | 3.33 |
| 24 | bornyl acetate | 26.197 | 31.656 | 3.56 | 4.24 | 4.54 | 3.00 | 4.31 | 4.83 | 3.20 | 4.05 | 4.37 |
| 25 | cumic alcohol | 26.557 | 31.935 | — | — | 1.62 | — | 1.48 | 2.64 | — | 1.18 | 2.47 |
| 26 | pseudolimonene | 28.225 | 33.636 | 4.89 | 5.37 | 7.14 | 3.87 | 5.73 | 8.02 | 3.16 | 5.75 | 8.64 |
| 27 | α-terpinyl acetate | 30.301 | 35.676 | 45.69 | 64.40 | 90.28 | 40.76 | 68.17 | 107.30 | 35.08 | 66.68 | 115.32 |
| 28 | eugenol | 30.719 | 36.107 | 5.84 | 8.01 | 12.15 | 7.49 | 11.08 | 16.36 | 5.41 | 8.52 | 17.32 |
| 29 | elemene (?) | 32.752 | 38.223 | — | — | — | — | — | 1.31 | — | — | 1.93 |
| 30 | methyl eugenol | 33.864 | 39.078 | 5.32 | 9.23 | 12.08 | 6.80 | 10.08 | 14.76 | 5.05 | 8.09 | 14.93 |
| 31 | β-caryophyllene trans/cis-methyl | 34.214 | 39.793 | — | — | — | — | — | 2.28 | — | — | 3.10 |
| 32 | isoeugenol | 39.382 | 44.690 | — | — | — | — | — | 1.63 | — | — | 1.00 |
| 33 | β-eudesmol dehydrocostuslacto | 47.696 | 53.238 | — | 3.26 | 4.04 | — | — | 4.84 | — | — | 3.89 |
| 34 | ne (?) | 64.429 | 69.523 | — | — | 0.97 | — | — | 5.15 | — | — | 1.83 |
| 35 | eremanthin (?) | 64.990 | 70.082 | — | — | — | — | — | 3.05 | — | — | — |
| | % of total | | | 95.03 | 93.55 | 91.26 | 92.91 | 92.21 | 88.69 | 93.35 | 91.86 | 88.75 |

[a]Retention time in min on HP-5MS column obtained by MSD; [b]Retention time in min on HP-5MS column obtained by FID.

determined as alcohols, aldehydes, ketones, esters, lactones, and phenols (Table 1). Among these, although it was very small, only ethers and ketones showed a reduction in their amounts as the process continued. Since ethers constituted the main components of the oxygenated compounds present in the laurel essential oil, the decrease with time in the amount of oxygenated components must be primarily due to the decrease in the amount of ethers, especially 1,8-cineole. They might have been lost due to some degradation reactions caused by high temperature and/or hydrolytic effects.

Figure 3 shows the variation of monoterpene hydrocarbons and oxygenated compounds detected in the laurel essential oil with respect to different methods at the end of the extraction process. No significant difference in the amount of monoterpene hydrocarbons and oxygenated compounds was observed between the methods and power levels studied ($p \leq 0.05$).



## Specific Gravity and Refractive Index of Laurel Essential Oil

The mean values for the specific gravities and refractive indices of the laurel essential oil extracted by hydrodistillation, SFME at 622 W and at 249 W power levels were given in Table 2. Specific gravity and refractive index values of the laurel essential oil were similar to the values

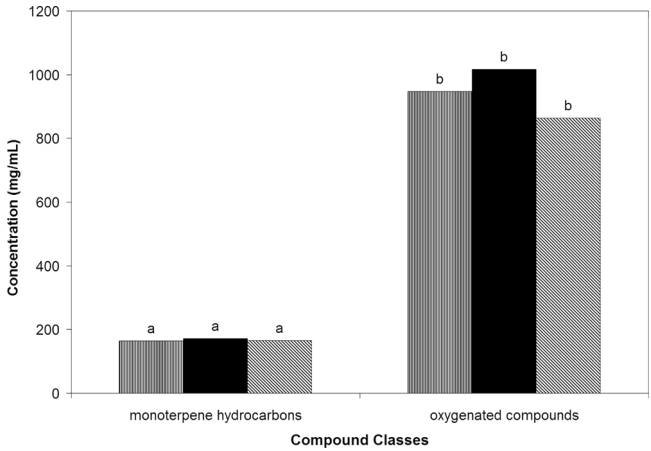


***Figure 3.*** Variation of concentrations of monoterpene hydrocarbons and oxygenated compounds in the essential oil of laurel with respect to different extraction methods (||||, SFME-249 W power; ■, SFME-622 W power; ⩙, hydrodistillation) (*means bars with different letters within each compound class are significantly different, $p \leq 0.05$).

**Table 2.** Specific gravity and refractive index of the essential oil of laurel obtained at different conditions

| | Hydrodistillation | SFME (249 W) | SFME (622 W) |
|---|---|---|---|
| Specific gravity (at $22 \pm 2°C$) | $0.86^{a*}$ | $0.86^{a}$ | $0.87^{a}$ |
| Refractive index ($25 \pm 2°C$) | $1.46^{b}$ | $1.47^{b}$ | $1.47^{b}$ |

*Means physical properties with different letters within each row are significantly different, $p \leq 0.05$.

given in literature (17). According to one-way ANOVA, no significant difference within each physical properties were found between the methods of extraction and power levels ($p \leq 0.05$).

## CONCLUSION

No significant differences were obtained in the maximum essential oil yields obtained by SFME and hydrodistillation. Compositions of the laurel essential oils obtained by both of the methods were found to be similar. It can be concluded that SFME is a good alternative for the extraction of essential oils from laurel since it provided essential oils of almost the same quality with conventional hydrodistillation while reducing the time of the process drastically.



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