Aroma transition from rosemary leaves during aromatization of olive oil

Mustafa Yılmazer a,*, Sermin Gökşu Karagöz b, Gulcan Ozkan c, Erkan Karacabey c

a Experimental and Observational Student Research and Practice Center, Suleyman Demirel University, Isparta, Turkey
b Faculty of Science and Art, Department of Chemistry, Suleyman Demirel University, Isparta, Turkey
c Faculty of Engineering, Department of Food Engineering, Suleyman Demirel University, Isparta, Turkey

1. Introduction

Olive fruit and its related products, especially extra-virgin olive oil, are popular products in the Mediterranean countries because of their delicious taste, pleasant aroma, and nutritional benefits [1–3]. These products have their own characteristic aroma and taste, which differentiate them from other similar products. Thus, the aroma profile of any olive product plays a significant role in its quality evaluation and product characterization. The main aroma compounds that migrate from olive fruits to oil are trans-2-hexenal, hexanal, and cis-3-hexenal [4,5]. In recent times, aromatized olive oil has been gaining increasing attention in the olive oil industry, because the main objective of aromatization is to produce alternative tastes for consumers. Aromatized olive oil is generally produced by small scale producers (boutique manufacturers). Herbs and aromatic plants are extensively used in aromatization due to their strong aromas. Rosemary (Rosmarinus officinalis L.; Family: Lamiaceae) is one of the popular plants used in the aromatization of olive oil due to its beneficial effect on health and significant nutritional potential with...
high antimicrobial, anti-diabetic, and antioxidant effects [6–10].

α-Pinen, 1,8-cineole, camphene, camphor, p-cymene, myrcene, limonene, and β-caryophyllene have been reported as the main volatile compounds responsible for the strong aroma of rosemary [7,11]. Different techniques are used to induce transition of compounds of interest from aromatic plants and herbs into olive oil. These, in most cases, involve mixing their extracts with oil or adding these herbs/plants to the oil. However, these methods are reported to have some disadvantages such as turbidity, overdosage [12], and extraction of undesirable constituents (waxes and bitters) [1]. By contrast, some aromatization techniques involve direct addition of ground and/or whole-plant materials into olive or olive paste during the crushing and malaxation steps, respectively. However, these methods also cause some problems, which should be resolved prior to obtaining standard aromatized olive oil. For example, in the crushing step, it is not easy to adjust the concentration of aromatic plant added due to the nonhomogenous distribution of leaves, woody parts, and limited time available for transition. In the malaxation step, kneading parameters have a significant effect on transition of target compounds from natural source to olive oil [4,13,14]. Previous studies have indicated that temperature and time are important variables affecting the malaxation step, and thus both should be considered and well adjusted [4,14,15]. Although there are studies on aromatized olive oils, to the best of our knowledge none of these studies has examined the influence of malaxation parameters and herb amount on the aroma profile of aromatized olive oil.

The main objective of this study was to evaluate the transition of aroma compounds from rosemary and olive fruit to the final oil under the influence of malaxation parameters and amount of herb.

2. Material and methods

2.1. Study material

Gemlik olive, a commercial cultivar, was used as the raw material in this study. The aromatic plant rosemary (R. officinalis) was cultivated in the research and application fields of Agricultural Faculty of Süleyman Demirel University, Isparta, Turkey. Rosemary was ground and sieved using a 1-mm sieve. Samples were stored in a sealed plastic bag at 4°C until further use. Analytic standards (α-pinen, myrcene, p-cymene, camphor, 1,8-cineole, and camphene) were purchased from Sigma-Aldrich Co. Ltd. (St Louis, MO, USA), limonene was purchased from Fluka (Steinheim, Germany), and hexanal and trans-2-hexenal were purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Experimental design

A central composite design was chosen to model the variation in compounds of interest in the aroma profile as a function of malaxation conditions for each of the following: temperature, time, and rosemary amount at five levels with 18 runs including four central points. Independent variables were temperature (X1), time (X2), and rosemary amount (X3). The area of each major aroma compound (α-pinen, 1,8-cineole, camphene, camphor, p-cymene, myrcene, and limonene) was the dependent variable in this study. The range and levels of independent process variables with coded values and corresponding responses, which are experimentally obtained, are presented in Tables 1 and 2. Response surface methodology was used to evaluate the effects of process parameters and to produce the corresponding models. Experimental data were analyzed using Minitab Software (Minitab version 16.1.1; Minitab, Inc., State College PA, USA). Full quadratic second-order regression model including the linear, quadratic, and two-factor interaction effects was used for the prediction of process conditions towards targets (Equation 1).

\[ Z = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j \]  

(1)

where:

- \( Z \) is the dependent variable;
- \( X \) is independent variables;
- \( \beta_0 \) is the constant coefficient;
- \( \beta_i \) is the linear coefficient (main effect);
- \( \beta_{ii} \) is the quadratic coefficient; and
- \( \beta_{ij} \) is the two-factor interaction coefficient.

Response surfaces of predicted values obtained using proposed models were plotted in the studied variable ranges using the Sigma Plot Software (SPSS Inc., Chicago, IL, USA). Model adequacy was evaluated by considering parameters of \( R^2 \) value and lack-of-fit test.

2.3. Extraction of olive oil

Olive oil was extracted according to the experimental design of the malaxation process (Table 1) using the Abencor method [16]. The aromatized oil obtained was filtered using cotton and anhydrous sodium sulfate. The filtered oils were stored in amber glass bottles at 4°C without headspace until further analysis.

2.4. Determination of aroma profile of aromatized olive oil by solid-phase microextraction–gas chromatography/mass spectrometry

A 2-g sample was weighed in a 15-mL vial closed by a silicone septum. The sample was placed on a heating block at 45°C and held for 15 minutes to achieve temperature equilibrium. A Carboxen/polydimethylsiloxane manual solid-phase microextraction (SPME) fiber (75-μm Fused Silica, Supelco Ltd., Bellefonte, PA, USA) was inserted into the vial and kept for 30 minutes at 45°C to absorb volatile compounds from olive oil. The fiber was then inserted into the injection port of a gas chromatograph for 5 minutes at 250°C for the desorption of aroma compounds. Gas extraction/mass spectrometry (GC/MS) analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with an MS-QP2010 plus a mass spectrometer.
Shimadzu Corporation, Kyoto, Japan). The analyses conditions are as follows: column, Rxi-5Sil MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Restek, Bellefonte, PA, USA); temperature program, from 40°C (2 minutes) to 250°C (5 minutes) at 4°C/min; injection temperature, 250°C; inlet pressure, 83.5 kPa; carrier gas, He ([linear velocity (u]): 44.2 cm/s); injection mode, split (10:1); MS interface temperature, 250°C; MS mode, electron ionization; detector voltage, 1.5 kV; mass range, 35–450 m/z; scan speed, 1428 u/s; interval, 0.30 seconds (2 Hz). Data handling was made through GCMSsolution 2.5 (Shimadzu).

GC/MS analysis was accomplished in the scan mode in the 40–300 amu mass range. Volatile compounds were identified by comparison of their retention indices (RIs) and mass spectra with analytic standards (hexanal, (E)-2-hexenal, α-pinene, myrcene, p-cymene, limonene, camphor, 1,8-cineole, and camphene), and in some cases matched with Wiley-NIST, Flavour and Fragrance Natural and Synthetic Compounds mass spectra library search and Kovats RIs. RI was calculated for each compound using a homologous series of C7–C30 n-alkanes.

### Table 1 – Three-factor, five-level central composite design used for response surface methodology and corresponding percent areas of characteristic aroma compounds in olive oil.

| Run order | Factor 1, X1 | Factor 2, X2 | Factor 3, X3 | Characteristic aroma compounds in olive oil (%) |
|-----------|-------------|-------------|-------------|-----------------------------------------------|
|           | Temperature (°C) | Time (minutes) | Rosemary concentration (%) | Hexanal     | (E)-2-Hexenal |
| 1         | 45 (1.68)     | 50 (0)      | 1.000 (0)   | 4.65        | 9.06         |
| 2         | 29 (–1)       | 30 (–1)     | 0.405 (–1)  | 7.32        | 16.82        |
| 3         | 29 (–1)       | 30 (–1)     | 1.595 (1)   | 3.96        | 6.63         |
| 4         | 35 (0)        | 83.6 (1.68) | 1.000 (0)   | 4.40        | 9.64         |
| 5         | 41 (1)        | 70 (1)      | 1.595 (1)   | 4.87        | 6.96         |
| 6         | 35 (0)        | 50 (0)      | 0.000 (–1.68) | 27.85   | 45.35        |
| 7         | 29 (–1)       | 70 (1)      | 0.405 (–1)  | 9.02        | 20.28        |
| 8         | 35 (0)        | 50 (0)      | 1.000 (0)   | 6.01        | 6.46         |
| 9         | 35 (0)        | 50 (0)      | 1.000 (0)   | 4.85        | 8.26         |
| 10        | 29 (–1)       | 70 (1)      | 1.595 (1)   | 4.25        | 7.42         |
| 11        | 41 (1)        | 30 (–1)     | 1.595 (1)   | 5.18        | 7.83         |
| 12        | 25 (–1.68)    | 50 (0)      | 1.000 (0)   | 7.08        | 10.71        |
| 13        | 35 (0)        | 50 (0)      | 1.000 (0)   | 5.16        | 8.73         |
| 14        | 41 (1)        | 70 (1)      | 0.405 (–1)  | 8.88        | 18.96        |
| 15        | 35 (0)        | 50 (0)      | 2 (1.68)    | 3.85        | 5.74         |
| 16        | 35 (0)        | 16.4 (–1.68) | 1.000 (0)  | 5.67        | 11.28        |
| 17        | 35 (0)        | 50 (0)      | 1.000 (0)   | 3.40        | 9.88         |
| 18        | 41 (1)        | 30 (–1)     | 0.405 (–1)  | 10.52       | 17.05        |

*Randomized.

### Table 2 – Area (real value × 10⁻⁶) of major aroma compounds of rosemary detected in aromatized olive oil.

| Run order | α-Pinene | Myrcene | p-Cymene | Limonene | Camphor | 1,8-Cineole | Camphene |
|-----------|----------|---------|----------|----------|---------|-------------|----------|
| 1         | 3.434    | 17.083  | 1.56     | 4.826    | 5.540   | 5.485       | 1.916    |
| 2         | 1.415    | 6.341   | 50.00    | 2.150    | 3.902   | 2.763       | 0.750    |
| 3         | 3.498    | 31.743  | 3.529    | 9.996    | 6.750   | 8.461       | 1.908    |
| 4         | 2.485    | 22.250  | 2.410    | 6.642    | 5.332   | 6.303       | 1.318    |
| 5         | 2.812    | 34.740  | 3.512    | 11.181   | 7.136   | 9.127       | 1.537    |
| 6         | 0.082    | 0.131   | 0.101    | 0.091    | 0.132   | 0.090       | 0.000    |
| 7         | 1.522    | 8.390   | 0.601    | 2.156    | 3.065   | 2.352       | 0.793    |
| 8         | 1.744    | 18.830  | 1.524    | 5.751    | 4.783   | 4.217       | 0.933    |
| 9         | 2.126    | 25.417  | 2.055    | 7.982    | 5.136   | 4.790       | 1.133    |
| 10        | 2.430    | 42.624  | 3.687    | 13.240   | 6.410   | 5.937       | 1.317    |
| 11        | 2.322    | 41.354  | 3.088    | 13.533   | 6.667   | 5.920       | 1.286    |
| 12        | 1.906    | 23.971  | 1.879    | 7.657    | 4.825   | 4.487       | 1.025    |
| 13        | 1.924    | 26.478  | 2.010    | 8.319    | 4.943   | 4.745       | 1.001    |
| 14        | 1.371    | 12.479  | 0.952    | 3.922    | 3.272   | 2.871       | 0.690    |
| 15        | 2.460    | 51.703  | 3.879    | 17.380   | 7.593   | 5.744       | 1.343    |
| 16        | 1.825    | 23.657  | 1.817    | 8.004    | 4.942   | 4.488       | 0.944    |
| 17        | 1.502    | 23.666  | 1.815    | 8.145    | 4.745   | 4.153       | 0.804    |
| 18        | 1.271    | 10.136  | 0.778    | 3.233    | 3.057   | 2.591       | 0.638    |

*Randomized.
3. Results and discussion

Different techniques are available for the aromatization process. In this study, aromatized olive oil was produced by mixing ground rosemary leaves and crushed olive paste during the malaxation stage. Olive oil was aromatized using rosemary to produce an oil product having a different aroma profile compared with raw olive oil. Results of SPME–GC/MS analysis revealed the presence of more than 45 volatile compounds from olive fruit and/or rosemary. The major aroma compounds of olive oil are hexanal (27% of the total area of the aroma profile for olive oil) and (E)-2-hexanal (45% of the total area of the aroma profile for olive oil; Table 1). However, these aroma compounds were not included in the interested aroma profile of aromatized olive oils (Table 2), because they are not characteristic compounds in the transition of aromas from rosemary and their amounts in total drastically decreased with the addition of rosemary (Table 1 and Fig. 1). Instead, seven other aroma compounds, whose percentage in the total area was higher than 1%, were selected as major compounds according to peak area comparison and analyzed in the remaining part of this study. These seven aroma compounds (α-pinene, 1,8-cineole, camphene, camphor, p-cymene, myrcene, and limonene) are also the major compounds reported for rosemary essential oil profile [7,8,11].

The calculated areas of seven aroma compounds are presented in Table 2. During the evaluation of trial number 6, which did not include rosemary addition during the malaxation step, only camphene was not detected in olive oil; in other words, the other aroma compounds simultaneously come from both olive fruits and rosemary. The transition of each aroma compound into the aromatized olive oil was investigated in terms of malaxation conditions and rosemary addition. Regression analysis conducted for all responses of interest was significant (p < 0.05; Table 3). Analysis of variance of regression revealed that a full quadratic second-order regression model is able to predict the area of each aroma compound with high success (Table 3). Determination of coefficient value is higher than 0.8, and lack-of-fit test was found to be insignificant for all models (p > 0.05; Table 3). Thus, it can be concluded that the response surface analysis would be a suitable tool to explain the transition of each essential oil of interest from olive fruit and rosemary into olive oil under the influence of process conditions and rosemary addition.

Statistical analysis indicates the significance of each model parameters. Results point to the strong effect of rosemary addition, which has an influence on the transition of its aroma compounds to olive oil. Kneading conditions (temperature and time) did not induce any significant variation in transition (Table 3) in contrast to the common expectation that malaxation temperature and time change the transition of aroma compounds of interest from rosemary. The studied range of temperature and time of kneading was determined according to the corresponding suggested levels in the olive oil industry (<45°C and <90 minutes). Thus, these insignificant influences of temperature and time on the studied ranges could be explained by their insufficient levels to affect the transition of aroma compounds from rosemary to olive oil. Response surface for myrcene was drawn as a function of temperature and rosemary concentration, where malaxation time was kept constant (50 minutes; Fig. 2). Variations in the remaining aroma compounds of interest as a function of malaxation conditions and rosemary addition (not shown) were found to follow the similar trend, as can be seen in Fig. 2. The strong effect of rosemary concentration on aroma compounds of interest is clear; however, temperature does not cause any change in the transition of target compounds or only limited variations are seen.

![Graph](image-url)  
**Fig. 1** – Change in percent area of major characteristic aroma compounds in olive oil for each trial.
Regression coefficients of predicted models for the investigated responses of virgin olive oil aromatized by rosemary.

| Variable | Coefficient | X-Piene | Myrcene | p-Cymene | Limonene | Camphor | 1,8-Cineole | Camphene |
|----------|-------------|---------|---------|----------|----------|---------|-------------|----------|
| $\beta_0$ | 1,822,151* | 23,578,502* | 1,839,406* | 7,600,796* | 4,888,310* | 4,445,382* | 967,013* |
| $\beta_1$ | 179,308** | -263,267** | -138,068** | -48,019** | 209,631** | 326,935** | 106,625** |
| $\beta_2$ | 90,812** | 742,167** | 133,859** | -86,470** | 82,915** | 443,654** | 47,131** |
| $\beta_3$ | 1,166,997* | 24,567,441* | 2,040,131* | 8,070,846* | 3,289,907* | 3,493,213* | 669,096* |
| $\beta_11$ | 865,161** | -2,848,475** | 49,517** | -1,352,828** | 436,852** | 864,207** | 511,149** |
| $\beta_{12}$ | 349,711** | -425,029** | 394,039** | -271,429** | 388,776** | 1,269,094** | 171,391** |
| $\beta_{13}$ | -534,355** | 2,538,187** | 269,714** | -1,141,401** | -885,807** | -1,210,053** | -288,304** |
| $\beta_{12}$ | 543,049** | -6,114,938** | 386,261** | -1,719,495** | 472,831** | 2,247,731** | 297,578** |
| $\beta_{13}$ | -174,471** | -2,250,365** | -168,207** | -480,169** | 269,814** | 105,536** | -65,483** |
| $\beta_{23}$ | -277,105** | -139,412** | 378,142** | 69,227** | 84,970** | 286,984** | -153,586** |
| Model | *** | * | *** | ** | **** | *** | *** | *** |
| Quadratic | *** | * | * | * | * | * | *** | *** |
| Cross product | * | * | ** | * | * | * | ** | ** |

Lack-of-fit

| $R^2$ | 0.83 | 0.96 | 0.98 | 0.95 | 0.98 | 0.91 | 0.84 |

** Significant at $p < 0.001$. 
** Not significant ($p > 0.05$). 
*** Significant at $p < 0.05$. 
**** Significant at $p < 0.01$. 

* Polynomial model $Z = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \sum_{j=1}^{4} \beta_{ij} X_i X_j$, where $\beta_0$ is the constant coefficient, $\beta_i$ is the linear coefficient (main effect), $\beta_{ij}$ is the quadratic coefficient, and $\beta_{ij}$ is the two factors interaction coefficient.

Conflicts of interest

All contributing authors declare no conflicts of interest.

References

[1] Moldão-Martins M, Beirao-da-Costa S, Neves C, Cavaleiro C, Salgueiro L, Beirao-da-Costa ML. Olive oil flavoured by the essential oils of Mentha × piperita and Thymus mastichina L. Food Qual Prefer 2004;15:447–52.

[2] Morales MT, Luna G, Aparicio R. Sensory and chemical evaluation of winey-vinegary defect in virgin olive oils. Eur Food Res Technol 2000;211:222–8.

[3] Stark AH, Madar Z. Olive oil as a functional food: epidemiology and nutritional approaches. Nutr Rev 2002;60:170–6.

[4] Kalua CM, Bedgood Jr DR, Bishop AG, Prenzler PD. Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. J Agric Food Chem 2006;54:7641–51.

[5] Kalua C, Allen M, Bedgood D, Bishop A, Prenzler P, Robards K. Olive oil volatile compounds, flavour development and quality: a critical review. Food Chem 2007;100:273–86.

[6] Chipault J, Mizuno G, Hawkins J, Lundberg W. The antioxidant properties of natural spices. J Food Sci 1952;17:46–55.

[7] Zaouali Y, Bouzaine T, Boussaid M. Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem Toxicol 2010;48:3144–52.

[8] Ojeda-Sana AM, van Baren CM, Elechosa MA, Juárez MA, Moreno S. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. Food Control 2013;31:189–95.
[9] Ding Y, Wang S-Y, Yang D-J, Chang M-H, Chen Y-C. Alleviative effects of litchi (Litchi chinensis Sonn.) flower on lipid peroxidation and protein degradation in emulsified pork meatballs. J Food Drug Anal 2015;23:501–8.

[10] Yen H-F, Hsieh C-T, Hsieh T-J, Chang F-R, Wang C-K. In vitro anti-diabetic effect and chemical component analysis of 29 essential oils products. J Food Drug Anal 2015;23:124–9.

[11] Jamshidi R, Afzali Z, Afzali D. Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. Am Eurasian J Agric Environ Sci 2009;5:78–81.

[12] Gambacorta G, Faccia M, Pati S, Lamacchia C, Baiano A, La Notte E. Changes in the chemical and sensorial profile of extra virgin olive oils flavored with herbs and spices during storage. J Food Lipids 2007;14:202–15.

[13] Aguilera MP, Beltran G, Sanchez-Villasclaras S, Uceda M, Jimenez A. Kneading olive paste from unripe ‘Picual’ fruits: I. Effect on oil process yield. J Food Eng 2010;97:533–8.

[14] Inarejos-García AM, Gómez-Rico A, Salvador MD, Fregapane G. Influence of malaxation conditions on virgin olive oil yield, overall quality and composition. Eur Food Res Technol 2009;228:671–7.

[15] Ranalla A, Pollastri L, Contento S, Lucera L, Del Re P. Enhancing the quality of virgin olive oil by use of a new vegetable enzyme extract during processing. Eur Food Res Technol 2003;216:109–15.

[16] Martinez J, Munoz E, Alba J, Lanzón A. Informe sobre utilización del analizador de muestras Abencor. Grasas Aceites 1975;26:379–85.