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Original Article

Aroma transition from rosemary leaves during aromatization of olive oil

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ABSTRACT

The aroma profile of aromatized olive oil was determined in this study. The primary objective was to investigate the transition of major aroma compounds from rosemary and olive fruit during the kneading step of olive oil production by response surface methodology. For this purpose, temperature, time, and amount of rosemary leaves were determined as independent variables. The results indicated that temperature and time did not affect the transition of target compounds, but rosemary leaves addition had a strong influence on transition, especially for characteristic aroma compounds of this herb. Adequacies of developed models were found to be high enough to predict each aromatic component of interest.

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

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high antimicrobial, antidiabetic, and antioxidant effects [6–10].

α-Pinene, 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 coextraction 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-pinene, 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 (X_1), time (X_2), and rosemary amount (X_3). The area of each major aroma compound (α-pinene, 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).

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

where:

Z is the dependent variable;
X is independent variables;
β_0 is the constant coefficient;
β_i is the linear coefficient (main effect);
β_{ii} is the quadratic coefficient; and
β_{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 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

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[a]	Factor 1, X_1	Factor 2, X_2	Factor 3, X_3	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

[a] Randomized.

(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 ($\bar{u}$): 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, *trans*-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 Kováts RIs. RI was calculated for each compound using a homologous series of C7–C30 *n*-alkanes.

Table 2 – Area (real value × 10^{-6}) of major aroma compounds of rosemary detected in aromatized olive oil.

Run order[a]	α-Pinene	Myrcene	*p*-Cymene	Limonene	Camphor	1,8-Cineole	Camphene
1	3.434	17.083	1.656	4.826	5.540	5.485	1.916
2	1.415	6.341	1.265	2.150	3.392	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	34.470	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	8.519	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	8.154	4.745	4.153	0.804
18	1.271	10.136	0.778	3.233	3.057	2.591	0.638

[a] 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.

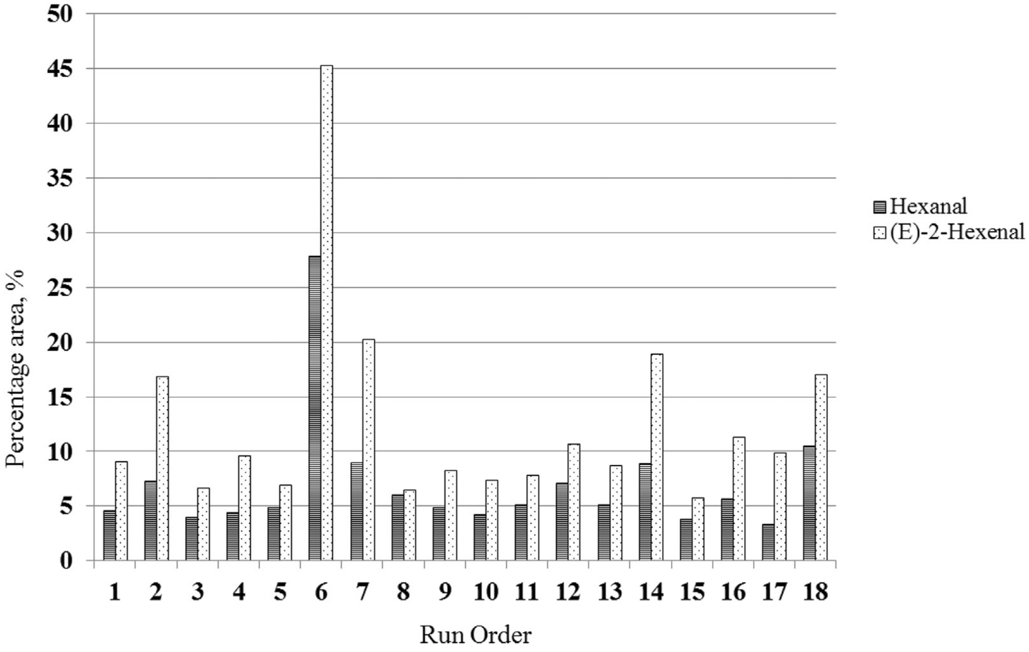

Fig. 1 – **Change in percent area of major characteristic aroma compounds in olive oil for each trial.**

Table 3 – Regression coefficients of predicted models for the investigated responses of virgin olive oil aromatized by rosemary.

Variable[a]	Coefficient						
	α-Pinene	Myrcene	*p*-Cymene	Limonene	Camphor	1,8-Cineole	Camphene
β_0	1,822,151*	23,578,502*	1,839,406*	7,600,796*	4,888,310*	4,445,382*	967,013*
β_1	179,308**	−263,267**	−138,068**	−48,019**	209,631**	326,953**	106,625**
β_2	90,812**	742,167**	133,859**	−86,470**	82,915**	443,654**	47,131**
β_3	1,166,997*	24,567,441*	2,040,131*	8,070,846*	3,289,907*	3,493,213*	669,096*
β_{11}	865,161**	−2,848,475**	49,517**	−1,352,828**	436,852**	864,207**	511,149***
β_{22}	349,711**	−425,029**	394,039**	−271,429**	388,776**	1,269,094**	171,391**
β_{33}	−534,355**	2,538,187**	269,714**	1,141,401**	−885,807***	−1,210,053**	−288,304**
β_{12}	543,049**	−6,114,938**	386,261**	−1,719,495**	472,831**	2,247,731***	297,578**
β_{13}	−174,471**	−2,250,365**	−168,207**	−480,169**	269,814**	105,536**	−65,483**
β_{23}	−277,105**	−139,412**	378,142**	69,227**	84,970**	286,984**	−153,586**
Model	***	*	*	*	*	****	***
Linear	****	*	*	*	*	*	****
Quadratic	**	*	**	**	***	**	**
Cross product	**	**	**	**	**	**	**
R^2	0.83	0.96	0.98	0.95	0.98	0.91	0.84
Lack-of-fit	0.116	0.385	0.464	0.346	0.082	0.036	0.090

* Significant at $p \leq 0.001$.

** Not significant ($p > 0.05$).

*** Significant at $p \leq 0.05$.

**** Significant at $p \leq 0.01$.

[a] Polynomial model $Z = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j$, where β_0 is the constant coefficient, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient.

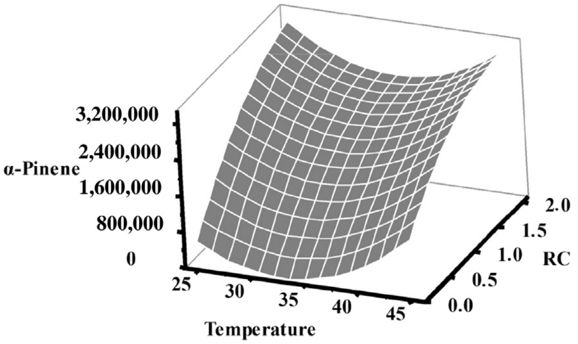

Fig. 2 – Change in area of α-pinene in the aroma profile of aromatized olive oil under effects of rosemary concentration (RC, %) and temperature (°C). Time was kept constant (50 minutes).

Under the light of these findings, it could be concluded that the transition of aroma compounds from rosemary to olive oil is irrespective of the studied malaxation conditions when aromatic material is mixed with olive paste during the malaxation step instead of the crushing step or during infusion in oil. This provides an opportunity for manufacturers, especially when they produce aromatized olive oil at the condition similar to the ones used in this study. Absence of effect or limited effects of malaxation conditions on aroma compound transition from rosemary to olive oil indicate a more controllable process in terms of aromatization. In other words, malaxation conditions could be out of consideration for this purpose, and therefore, rosemary concentration is the only parameter that can achieve desired aromatic characteristics irrespective of whether the cold or hot-pressed olive oil method is chosen.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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