

Use of Response Surface Methodology (RSM) for the Identification of the Best Extraction Conditions for Headspace Solid-Phase Micro Extraction (HS-SPME) of the Volatile Profile of cv. Arbequina Extra-Virgin Olive Oil

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The effect of the experimental conditions on assessing the global volatile profile of extra virgin olive oils (EVOO) by headspace solid-phase micro-extraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS) is studied to obtain maximization of the total peak areas of the compounds. Response Surface Methodology (RSM) is applied to Arbequina EVOO, and the influence of oil quantity, extraction time, and extraction temperature on the total area and extraction of the major desirable compounds is analyzed. The experimental data are adequately fitted into second-order polynomial models with non-significant lack of fit ($p > 0.05$) and coefficients of determination (R^2 and R^2 -adjusted) higher than 0.88 and 0.78, respectively. A strong similarity is found between the predicted and experimental values. Furthermore, the surface plots show that the extraction of the volatile compounds is favored with increasing extraction temperature, time, and oil quantity. The extraction conditions to obtain the maximum response of “green” volatile compounds from Arbequina olive oil by HS-SPME placed in 50 mL vials are 4.6 g of oil, 43 min, and 59 °C.

Practical Applications: The findings of the present work show that specific conditions of HS-SPME/GC-MS affect the extraction of volatile compounds from cv. Arbequina olive oil and establish the optimum extraction parameters to more efficiently determine the global profile of this fraction, taking into account the organoleptic characteristics of the cultivar. The results also contribute to the knowledge of the volatile profile of the monovarietal olive oil cv. Arbequina, which is one of most cultivated and consumed types of olive oil worldwide.

1. Introduction

The aroma of olive oils is attributed to a complex mixture of volatile compounds, mainly aldehydes, alcohols, ketones, hydrocarbons, and esters. Compounds with five or six carbon atoms represent the most important fraction and are generated from polyunsaturated fatty acids by the lipoxygenase pathway (LOX).^[1] The production of volatile compounds occurs in low amounts during the climacteric period of olives and at a high level through the oil extraction process, particularly during the crushing and malaxation steps.^[1,2]

Sensory properties play an important role in discriminating different types of edible oils, oil categories, and monovarietal olive oils. In this sense, volatile compounds are a decisive characteristic used to check olive oil quality and to assess the degree of oxidation.^[2] Consequently, these compounds affect the sensory perception of the oil and consumer acceptability.^[2–4]

The headspace solid phase microextraction (HS-SPME) technique consists of extracting volatile substances by exposing a fiber coated with a stationary phase (adsorbent) to the vapor phase (headspace)

in equilibrium with the olive oil contained in a thermostatted vial and sealed with a perforable septum. The analysis is performed by thermal desorption by inserting the fiber directly into the GC injector at a suitable temperature.^[1]

Nevertheless, only an adequate SPME sampling condition enables high efficiency and sensitivity for this method of analysis.^[5] In this way, optimal microextraction conditions are linked to sample quantity, temperature, time, and the type of fiber.^[5]

Several studies have been published on the use of HS-SPME to characterize the profiles of volatile compounds of olive oils.^[6–13] However, a wide range of conditions have been used concerning quantity (1–20 g),^[14–15] time (10 min–6 h),^[8–14] and temperature of extraction (25–100 °C).^[5–14] For this reason, comparisons among published data and the definition of a sensorial profile of olive oils. Currently, a complete validation of the current methodologies is

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still lacking, and none of the methods based on SPME–GC can be considered fully established; therefore, quantification of volatiles may be subjected to significant errors that hinder sensory interpretation from the chemical data.^[13] Thus, standardizing the extraction conditions, taking into account the specificities of each monovarietal olive oil, is an interesting challenge that has not been undertaken before.

Response Surface Methodology (RSM) is a mathematical and statistical tool that has been applied to study the effects of multiple factors and their interactions on one or more response variables and is very effective in reducing the required number of experiments with high reproducibility.^[16] Even though this methodology has been successfully applied to different types of foods such as lettuce, olive paste and beef,^[17–19] few studies exist on olive oil or mixtures of olive oil with other food ingredients.^[16,20–21] To our knowledge, until now, RSM has not been applied to the extraction conditions of olive oil volatiles.

Among the available monovarietal olive oils, cv. Arbequina is cultivated in different regions and is popular worldwide.^[15,22] It is appreciated due to its organoleptic characteristics such as fresh, herbal and green; tomato flavor, with notes of apple and banana; and sweet almond and artichoke undertones, in general with a low-medium intensity of pungency and bitterness.^[3,9,12,15,23]

Thus, the present study aimed to model and examine the effect of HS-SPME extraction conditions, i.e., the sample quantity, time and temperature, on obtaining the maximization of total peak areas from the major volatile compounds of monovarietal Arbequina olive oil. For this purpose, RSM was used.

2. Experimental Section

2.1. Sample

Monovarietal Arbequina extra virgin olive oil (EVOO) was used in the present study. The oil was directly obtained from producers from the 2014/2015 harvest and was stored protected from light at 4 °C until analysis. The Arbequina orchard was located in the Valladolid province (Spain), and harvesting of the fruits occurred at 2.5 on the ripening index. The olive oil was classified as extra virgin olive oil according to legal requirements (European Regulation 2568/91). Analyses were previously performed in our laboratory, showing free acidity of 0.2%, expressed in oleic acid, a peroxide value of 3.7 mEq O₂/kg, $K_{232} = 1.50$, $K_{270} = 0.16$, $\Delta K = -0.01$, a median intensity of sensory defects = 0 and a median intensity of fruity positive attributes >0 in sensory analysis.

2.2. Volatile Headspace Solid-Phase Microextraction (HS-SPME)

For the headspace solid-phase microextraction (HS-SPME), a 2 cm fiber coated with divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 μm) from Supelco (Bellefonte, USA) was used. This type of fiber has been widely used for determination of volatiles in olive oils, showing good

effectiveness and reproducibility and a high affinity for compounds of low and medium molecular weights.^[5,24,28] The olive oil sample was placed in 50 mL vials to avoid any contact with the fiber and to provide efficient extraction, according to the method used in previous studies.^[29] Then, 4-methyl-2-pentanol was added as an internal standard (100 ppm in methanol; 10 μL), and the vials were sealed with a polypropylene cap with a silicon septum. The volatiles were released at the selected extraction temperature in a water bath and vigorously stirred with a stir bar (350 rpm) for the time required to allow equilibration in the headspace. After the equilibrium time (5 min), the DVB/CAR/PDMS fiber was exposed over a precise extraction time for volatiles adsorption and immediately inserted into the injection port of the GC system for thermal desorption and reconditioning (10 min at 280 °C). Experiments were performed at different conditions of sample quantity, extraction temperature, and extraction time (see below).

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Conditions

Chromatographic analysis was performed on a Shimadzu GC-2010 Plus equipped with a mass spectrometer Shimadzu GC/MS-QP2010 SE detector. A TRB-5MS (30 m × 0.25 mm × 0.25 μm) column (Teknokroma, Spain) was used. The injector was set at 220 °C, and the manual injections were made in splitless mode. The mobile phase consisted of helium (Praxair, Portugal) at a linear velocity of 30 cm s⁻¹ and a total flow of 24.4 mL min⁻¹. The oven temperatures were the following: 40 °C during the first min, followed by an increase to 220 °C at a rate of 2 °C min⁻¹, and then maintained at 220 °C for 30 min. The ionization source was maintained at 250 °C with an ionization energy of 70 eV and an ionization current of 0.1 kV. All mass spectra were acquired by electron ionization. The ionization was left off during the first 2 min. The MS spectra fragments were compared with those obtained from a database (NIST 11) and with the GC retention index. Furthermore, retention indices were obtained using commercial n-alkanes C₇–C₃₀ (Sigma–Aldrich, St. Louis, USA) by direct splitless liquid injection (1 μL), with all further conditions of GC and MS set as those used for the volatile analysis. The identification was also performed considering a 90–95% spectrum match for tentative identification and a match of at least 98% for identification. For semi-quantification purposes, the areas of the chromatographic peaks were determined by integrating the reconstructed chromatogram from the full scan chromatogram using the ion base for each compound (m/z intensity 100%). For optimization purposes, each individual ion peak area and the total area were calculated.

2.4. Experimental Design and Data Analysis

The Response Surface Methodology (RSM) of Minitab[®] software (version 16, Coventry, England) was used to determine the best extraction conditions for the major volatiles of the cv. Arbequina extra virgin olive oil. A Central Composite Design (CCD), full-factorial ($\alpha = 1.682$) with three independent factors, namely, the quantity of oil (X_1), time (X_2), and temperature (X_3), was applied.

The response variables were the total areas of the peaks and the areas of the main compound peaks, namely those of Z-3-hexenal, E-2-hexenal, Z-3-hexen-1-ol, 1-hexanol, Z-3-hexen-1-ol acetate, hexyl acetate, and E-2-hexen-1-ol acetate. **Table 1** shows the range and center point values of the three independent variables that have been coded to 5 levels: -1.682 , -1 , 0 , $+1$, and $+1.682$. The experimental design consisted of six axial points at a distance of ± 1.682 from the center, with six replicates of the central point used to determine the experimental error for data reproducibility and eight cube points (**Table 2**). The experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. Furthermore, each point of the CCD was carried out in duplicate, as described previously, to optimize the extraction antioxidant properties.^[18]

The experimental data from the CCD was analyzed using response surface regression and fitted to a second-order polynomial model, as indicated in Equation (1):

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (1)$$

where Y is the predicted dependent variable, β_0 is the model constant that fixes the response at the central point of the experiment (intercept), β_i is the regression coefficient for the linear effect terms, β_{ii} is the quadratic effect term, β_{ij} is the interaction effect term of variables i and j , and X_i and X_j are the independent variables. The adequacy of the model was predicted through analysis of variance (ANOVA) including the F -ratio, which established the lack-of-fit of the model and the determination coefficient (R^2) and adjusted R^2 . Additionally, the experimental and predicted values for each dependent variable were compared.

2.5. Application of the Optimized Conditions to cv. Arbequina Olive Oil Samples

The selected conditions were applied to five different mono-varietal Arbequina olive oil samples. The samples were directly obtained from the producers and were stored protected from light at 4°C until analysis. The samples were from different geographical origins. Four samples were from Spain, namely samples 1, 2, and 3 from Seville and sample 4 from Granada. Sample 5 was from Rio Grande do Sul (Brazil). All samples were

classified as extra virgin olive oil according to legal requirements (European Regulation 2568/91 and further amendments).

3. Results and Discussion

3.1. Effects of SPME Conditions on the Volatile Composition of cv. Arbequina Olive Oil

In olive oil, six-carbon straight-chain compounds (C6) are among the most important compounds for aroma, as aldehydes and alcohols are mainly linked to green-fruity notes and esters are linked to fruity and aromatic flavors.^[24–26]

In the present work, the main volatiles found were C6 compounds, including aldehydes (Z-3-hexenal, E-2-hexenal), alcohols (1-hexanol, Z-3-hexen-1-ol), and the corresponding esters (Z-3-hexen-1-ol acetate, hexyl acetate, and E-2-hexen-1-ol acetate). Our results are in concordance with those of previous studies showing a high content of C6 compounds in cv. Arbequina olive oils from the Mediterranean basin.^[9–10,12,15,22] According to Pérez et al.,^[25] the content of C6 is on average 2–160 times higher than that of the rest of the groups of volatile compounds in the oils. All of the volatile compounds identified in the present work were related with positive sensorial characteristics, such as almond, apple, astringent, banana, bitter, fresh, green-fruity, green leaves, pungent, sweet, and tomato.^[1,3,27] Additionally, E-2-hexenal is also considered as an index of freshness for olive oils.^[4,28]

In the analyzed cv. Arbequina olive oil, the seven most identified representative volatile compounds were Z-3-hexenal, E-2-hexenal, Z-3-hexen-1-ol, 1-hexanol, Z-3-hexen-1-ol acetate, hexyl acetate, and E-2-hexen-1-ol acetate (**Table 2**).

Regarding the experimental values of total area, it was observed that the highest peak area occurred for condition 9 (1.3 g/30 min/50 °C), and slightly lower values were found for the conditions 7 (4 g/40 min/60 °C) and 18 (2 g/40 min/60 °C). In all cases, high temperatures (50–60 °C) and long extraction times (30–40 min) were effective in providing high total peak areas.

Moreover, condition 7 (4 g/40 min/60 °C) showed the highest peak values for E-2-hexenal, Z-3-hexen-1-ol acetate, hexyl acetate, and E-2-hexen-1-ol acetate. On the other hand, the best condition for extracting Z-3-hexenal, Z-3-hexen-1-ol, and 1-hexanol compounds was 17 (4.7 g/30 min/50 °C). In this sense, the results obtained indicated that the peak area increased with increasing temperature and time.

Furthermore, nonanal was detected in all conditions tested (data not shown) with between 0.09 – 2.6 ng g^{-1} of olive oil. This compound, when detected in higher quantities than in the present assay, has been associated with signs of rancidity.^[1,2] Other studies also reported low values of nonanal in Arbequina olive oils.^[10,15] At the same time, the temperature conditions applied in the present work seem to not be responsible for the low content of nonanal. Previously, Vichi^[30] reported that an extraction temperature of 60°C did not alter volatile phenols but improved their extraction efficiency.

In addition, hexanal was not detected in our analytical conditions, similar to the results of a recent study performed on commercial olive oils in Spain, Italy, and France.^[31]

Table 1. Coded and uncoded (true) values of variables of CCD model.

	Coded units				
	-1.682	-1	0	1	$+1.682$
	True values				
Oil, X_1 (g)	1.3	2.0	3.0	4.0	4.7
Time, X_2 (min)	13.18	20.00	30.00	40.00	46.82
Temperature, X_3 (°C)	33.18	40.00	50.00	60.00	66.82

Table 2. Observed responses and predicted values for total area and major volatile peaks compounds in cv. Arbequina olive oil.

Condition	Oil (g)	Time (min)	T (°C)	Total area (x10 ⁸)		Z-3-hexenal (x10 ⁷)		E-2-hexenal (x10 ⁷)		Z-3-hexen-1-ol (x10 ⁷)		1-hexanol (x10 ⁶)		Z-3-hexen-1-ol acetate (x10 ⁷)		Hexyl acetate (x10 ⁶)		E-2-hexen-1-ol acetate (x10 ⁶)	
				E*	P	E*	P	E*	P	E*	P	E*	P	E*	P	E*	P	E*	P
1	3.0	30.00	50.00	6.48	6.28	1.10	1.19	3.32	3.27	1.13	1.06	2.55	2.39	3.05	2.92	1.63	1.58	0.99	0.95
2	2.0	40.00	40.00	6.38	5.70	1.03	1.03	2.92	3.27	0.91	0.90	2.01	2.00	2.25	2.59	1.19	1.44	0.71	0.61
3	4.0	20.00	40.00	4.93	5.51	1.16	1.19	2.35	2.64	0.84	0.78	1.82	1.71	1.33	1.68	0.61	0.65	0.45	0.52
4	2.0	20.00	40.00	5.09	5.70	0.92	1.03	2.02	2.37	0.68	0.67	1.51	1.45	1.18	1.68	0.55	0.56	0.40	0.49
5	3.0	30.00	50.00	6.31	6.28	1.27	1.19	3.32	3.27	1.06	1.06	2.45	2.39	2.84	2.92	1.50	1.58	0.93	0.95
6	3.0	30.00	33.18	5.11	5.04	1.22	1.26	2.36	3.22	0.78	0.77	1.65	1.66	1.28	1.60	0.62	0.76	0.38	0.44
7	4.0	40.00	60.00	6.81	6.07	1.17	1.19	3.59	3.49	1.09	1.08	2.42	2.39	4.62	4.69	2.69	2.59	1.56	1.69
8	4.0	20.00	60.00	5.76	6.07	1.17	1.19	3.06	3.09	1.03	1.04	2.29	2.33	2.97	2.71	1.65	1.72	0.96	0.83
9	1.3	30.00	50.00	6.92	6.60	0.87	0.88	2.70	2.73	0.82	0.85	1.83	1.89	2.58	2.92	1.47	1.42	0.81	0.72
10	2.0	20.00	60.00	6.02	6.45	0.94	0.95	2.76	2.69	0.90	0.87	2.01	1.93	1.51	2.71	0.84	1.53	0.52	0.86
11	3.0	13.18	50.00	4.83	5.77	1.01	1.07	2.28	2.31	0.82	0.83	1.81	1.85	1.46	1.70	0.69	0.84	0.52	0.54
12	3.0	30.00	66.82	6.24	6.31	0.96	1.12	3.08	3.32	0.93	0.94	2.04	2.05	4.04	4.24	2.20	0.24	1.52	1.46
13	3.0	46.82	50.00	6.71	5.77	1.15	1.07	3.44	3.40	1.06	1.05	2.39	2.36	3.74	4.14	2.08	0.23	1.28	1.36
14	3.0	30.00	50.00	6.02	6.28	1.14	1.19	3.13	3.27	1.01	1.06	2.28	2.39	2.69	2.92	1.40	1.58	0.92	0.95
15	3.0	30.00	50.00	6.09	6.28	1.18	1.19	3.18	3.27	1.03	1.06	2.25	2.39	2.77	2.92	1.49	1.58	0.87	0.95
16	4.0	40.00	40.00	5.76	5.32	1.23	1.27	3.18	3.67	1.05	1.07	2.36	2.40	2.22	2.59	1.15	1.62	0.69	0.86
17	4.7	30.00	50.00	5.96	5.96	1.30	1.28	3.44	3.41	1.14	1.13	2.60	2.56	2.77	2.92	1.48	1.73	0.89	0.91
18	2.0	40.00	60.00	6.78	6.45	0.95	0.95	2.97	3.08	0.90	0.96	1.93	1.99	3.95	4.69	2.29	2.41	1.41	1.44
19	3.0	30.00	50.00	6.29	6.28	1.18	1.19	3.29	3.27	1.04	1.06	2.36	2.39	3.08	2.92	1.75	1.58	0.97	0.95
20	3.0	30.00	50.00	6.47	6.28	1.26	1.19	3.37	3.27	1.08	1.06	2.49	2.39	3.07	2.92	1.68	1.58	1.03	0.95

* Average of duplicate extractions.
E, experimental values; P, predicted values.

3.2. Model Fitting Using Response Surface Methodology

The experimental and predictive values for total area and major volatile compound peak areas for each extraction condition are shown in Table 2. The predicted values were determined according to the quadratic models obtained using the quantity of oil (X_1), time (X_2), and temperature (X_3) as independent variables. Thus, the predicted values (Y) (Z-3-hexenal, E-2-hexenal, Z-3-hexen-1-ol, 1-hexanol, Z-3-hexen-1-ol acetate, hexyl acetate, and E-2-hexen-1-ol acetate) were assessed as a function of the significant linear, quadratic, and interaction effects ($p < 0.05$), as shown in Table 3. The response surface models obtained with a 0.05 level of significance were the following Equations (2–9):

$$Y_{\text{Total area}} = 62.80 - 1.90X_1 + 5.18X_2 + 3.75X_3 - 1.80X_2^2 - 2.13X_3^2 \quad (2)$$

$$Y_{\text{Z-3-Hexenal}} = 119 + 11.8X_1 - 4.01X_3 - 3.81X_1^2 - 4.00X_2^2 \quad (3)$$

$$Y_{\text{E-2-Hexenal}} = 32.7 + 2.02X_1 + 3.23X_2 + 2.27X_3 - 0.705X_1^2 - 1.46X_2^2 - 1.95X_3^2 - 1.25X_2X_3 \quad (4)$$

$$Y_{\text{Z-3-Hexen-1-ol}} = 106 + 8.52X_1 + 6.60X_2 + 5.04X_3 - 2.46X_1^2 - 4.13X_2^2 - 7.10X_3^2 - 4.75X_2X_3 \quad (5)$$

$$Y_{\text{1-Hexanol}} = 23.9 + 1.99X_1 + 1.52X_2 + 1.17X_3 - 0.601X_1^2 - 1.02X_2^2 - 1.91X_3^2 - 1.23X_2X_3 \quad (6)$$

$$Y_{\text{Z-3-hexen-1-ol Acetate}} = 29.2 + 7.24X_2 + 7.83X_3 + 2.67X_2X_3 \quad (7)$$

$$Y_{\text{Hexyl Acetate}} = 158 + 9.21X_1 + 43.9X_2 + 48.6X_3 + 14.9X_1X_3 + 16.4X_2X_3 \quad (8)$$

$$Y_{\text{E-2-Hexen-1-ol Acetate}} = 95.2 + 5.50X_1 + 24.4X_2 + 30.1X_3 - 4.79X_1^2 + 7.00X_1X_3 + 11.6X_2X_3 \quad (9)$$

Table 3. ANOVA for the quadratic models developed to describe the influence of oil quantity (X_1), time (X_2), and temperature (X_3) on total area and major volatile compounds peaks from cv. Arbequina olive oil.

Term constant	Total area ($\times 10^7$)		Z-3-hexenal ($\times 10^5$)		E-2-hexenal ($\times 10^6$)		Z-3-hexen-1-ol ($\times 10^5$)		1-hexanol ($\times 10^5$)		Z-3-hexen-1-ol acetate ($\times 10^6$)		Hexyl acetate ($\times 10^4$)		E-2-hexen-1-ol acetate ($\times 10^4$)	
	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p	Coefficient	p
Constant	62.80	0.000	119	0.000	32.7	0.000	106	0.000	23.9	0.000	29.2	0.000	158	0.000	95.2	0.000
X_1	-1.90	0.004	11.8	0.000	2.02	0.000	8.52	0.000	1.99	0.000	1.87	0.148	9.21	0.035	5.50	0.025
X_2	5.18	0.000	3.28	0.076	3.23	0.000	6.60	0.000	1.52	0.000	7.24	0.000	43.9	0.000	24.4	0.000
X_3	3.75	0.000	-4.01	0.036	2.27	0.000	5.04	0.000	1.17	0.001	7.83	0.000	48.6	0.000	30.1	0.000
X_1^2	0.51	0.286	-3.81	0.040	-0.705	0.010	-2.46	0.019	-0.601	0.038	-1.11	0.651	-4.65	0.236	-4.79	0.040
X_2^2	-1.80	0.005	-4.00	0.033	-1.46	0.000	-4.13	0.001	-1.02	0.002	-1.38	0.095	-7.60	0.066	-3.11	0.158
X_3^2	-2.13	0.002	-3.56	0.052	-1.95	0.000	-7.10	0.000	-1.91	0.000	-1.17	0.284	-6.78	0.095	-1.22	0.561
$X_1 \cdot X_2$	-0.21	0.767	-0.738	0.740	0.324	0.300	0.558	0.647	0.317	0.369	-1.21	0.692	-6.43	0.223	-4.80	0.109
$X_1 \cdot X_3$	0.70	0.330	0.078	0.972	0.416	0.191	0.106	0.930	0.127	0.714	2.51	0.592	14.9	0.013	7.00	0.028
$X_2 \cdot X_3$	-0.39	0.580	-2.00	0.378	-1.25	0.020	-4.75	0.002	-1.23	0.004	2.67	0.009	16.4	0.008	11.6	0.002
Lack-of-fit		0.504	0.637		0.687		0.965	0.919	0.952		0.976	0.229	0.970	0.428	0.975	
R^2	0.952		0.882		0.981		0.964		0.933		0.954		0.940		0.952	
R^2 adjusted	0.908		0.777		0.964		0.933		0.910		0.954		0.940		0.952	

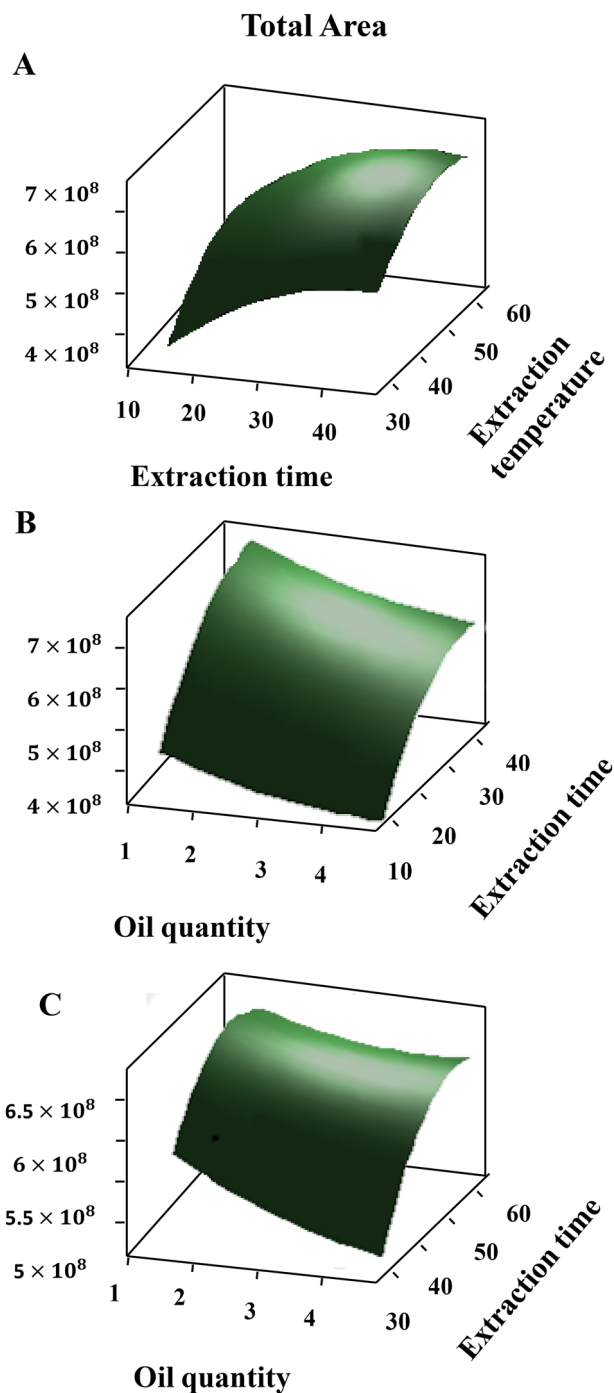


Figure 1. Response surface plots of total area considering middle values for oil quantity (3 g) A), temperature (50 °C) B), and time (30 min) C).

The values obtained experimentally for all response variables were similar to the predicted values (Table 2), indicating that the models fitted the values in a satisfactory way.

The quality of the models for predicting total area and major volatile compounds was evaluated by the lack-of-fit, R^2 and adjusted R^2 values (Table 3). A lack-of-fit indicated by p -values higher than 0.05 indicate that the variation between samples was mainly due to the factors selected for the model and the

pure error.^[18] Furthermore, R^2 and adjusted R^2 values closer to unity indicate that the empirical models satisfactorily fit the real data. All models showed non-significant lack-of-fit values ($p > 0.05$). Additionally, high values for R^2 and adjusted R^2 were observed, ranging from 0.882 to 0.981 and 0.777 to 0.964, respectively.

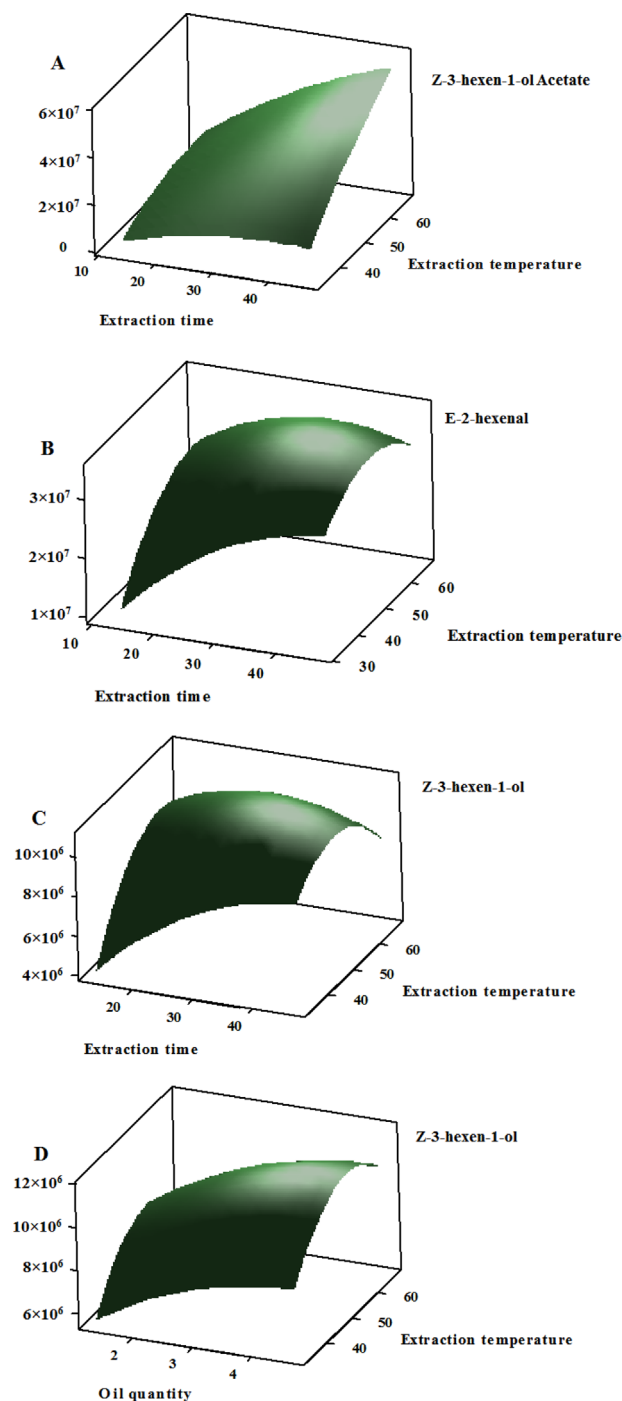


Figure 2. Response surface plots of Z-3-hex-1-ol acetate A), E-2-hexenal B), and Z-3-hex-1-ol C) fixing the middle value for oil quantity (3 g). Response surface plot of Z-3-hex-1-ol at fixing temperature (50 °C) D).

In general, the three factors studied had a significant role in the response variables studied, with the exceptions of oil quantity (X_1) on Z-3-hexen-1-ol acetate and of extraction time (X_2) on Z-3-hexenal. Furthermore, in almost all situations, oil quantity, extraction time, and temperature exerted a positive linear effect on the responses analyzed. Individually, the extraction temperature (X_3) had the most significant impact on Z-3-hexen-1-ol acetate, hexyl acetate, and E-2-hexen-1-ol-acetate, as confirmed by the higher coefficient values observed in Table 3, while the oil quantity showed the highest effect on Z-3-hexen-1-ol and 1-hexanol. On the other hand, the extraction time had the highest effect on the total area and E-2-hexenal.

Some response surface plots of the total area and major volatiles present in cv. Arbequina olive oil are shown in **Figure 1** and **2**. The application of high times and temperatures increased the total area, while the effect of oil quantity was less evident.

The major volatile compounds detected were Z-3-hexen-1-ol acetate and E-2-hexenal. These compounds have been reported previously in some studies of cv. Arbequina olive oils.^[9–10,12,15,22,27] In earlier studies, oils from this cultivar were associated with ripe fruit aroma, slight oily odor, and sweet taste,^[32] but according to García-González et al.,^[22] the market tendency for a high demand of greener and bitterer oils has favored the production of monocultivar Arbequina oils from low-ripened olives. As a result, the sensorial descriptors such as cut-green, tomato and medium bitter taste have increased, which are sensory traits associated with the predominance of Z-3-hexen-1-ol acetate and E-2-hexenal. For these reasons, we selected the surface plots of Z-3-hexen-1-ol acetate and E-2-hexenal to illustrate the behavior of the major compounds (Figure, B). For the former compound, it was stated by the quadratic model (Equation 7) that only time and temperature influenced the extraction of this compound. On the contrary, all factors influenced E-2-hexenal extraction, with the highest linear coefficients being obtained for time and temperature. Thus, in general, the best responses were obtained when longer extraction times and higher temperatures were applied. Nevertheless, in the case of E-2-hexenal, a slight decrease of the total area was observed at 60 °C and at 40 min of extraction time onwards.

Concerning Z-3-hexen-1-ol (**Figure 2C**), upon fixing the quantity of olive oil to 3 g, a similar behavior to that of E-2-hexenal was

Table 4. Validation of the optimal point of extraction.

	D^a	Y Predicted	Y Experimental	Error (%) ^b
Total area ($\times 10^8$)	0.72	6.63	6.73	1.51
Z-3-hexenal ($\times 10^7$)	0.65	1.16	1.21	4.31
E-2-hexenal ($\times 10^7$)	1.00	3.62	3.54	2.21
Z-3-hexen-1-ol ($\times 10^7$)	0.88	1.10	1.06	3.63
1-hexanol ($\times 10^6$)	0.86	2.49	2.42	2.81
Z-3-hexen-1-ol acetate ($\times 10^6$)	1.00	4.62	4.82	4.33
Hexyl acetate ($\times 10^6$)	0.97	2.67	2.80	4.87
E-2 hexen-1-ol acetate ($\times 10^6$)	1.00	1.56	1.77	13.46

^a) D refers to the individual desirability.

^b) Error (%) was calculated by the equation: $\frac{Y_{\text{experimental}} - Y_{\text{predicted}}}{Y_{\text{predicted}}} \times 100$

Table 5. Application of the optimized conditions to different cv Arbequina olive oils.

Samples	Total area ($\times 10^8$)	E-2-hexenal ($\mu\text{g kg}^{-1}$)	Z-3-hexen-1-ol ($\mu\text{g kg}^{-1}$)	1-hexanol ($\mu\text{g kg}^{-1}$)	Z-3-hexen-1-ol acetate ($\mu\text{g kg}^{-1}$)	Hexyl acetate ($\mu\text{g kg}^{-1}$)
Sample 1	4.53	96.4 ± 3.75	–	30.3 ± 0.42	34.6 ± 2.86	14.2 ± 1.32
Sample 2	5.30	132 ± 1.91	–	69.3 ± 0.48	35.8 ± 1.33	30.8 ± 1.35
Sample 3	4.73	144 ± 38.7	–	28.9 ± 9.52	45.7 ± 8.87	14.1 ± 2.50
Sample 4	8.39	29.6 ± 0.97	133 ± 5.4	–	115 ± 12.3	23.3 ± 2.69
Sample 5	5.19	290 ± 8.78	–	68.04 ± 0.65	34.4 ± 1.16	12.36 ± 0.35

observed. When the oil quantity increased (Figure 2D), the area of the peak of this compound also increased.

Thereby, our results suggest that high values of each one of the three analyzed variables are individually favorable for better extraction; however, for some compounds, the simultaneous application of a higher temperature and longer time of extraction might favor degradation, decreasing the peak area. These results are in concordance with those of Beltran et al.,^[6] who showed a significant increase of volatile compounds in the headspace of olive oil emulsions with increasing temperature. The higher concentration was mainly attributed to the rise of vapor pressure due to temperature increase, which enhances the mass transference of analytes, thereby increasing their concentration in the gas phase.^[5–6]

3.3. Optimization of Volatiles Extraction and Verification of the Models

The optimal extraction point was 4.6 g of oil, 43 min and 59 °C, with a 0.88 combined desirability. The predicted and experimental values of each compound peak, the calculated individual desirabilities (D) and the percentages of error are depicted in Table 4. The scale of the individual desirability function ranges between 0 for a completely undesirable response, and 1 for a fully desired response. Hence, a global desirability value of 0.88 predicted for the present work is quite acceptable to target the ideal extraction conditions to define the volatile profile of cv. Arbequina olive oils. The experimental values were obtained by conducting assays in triplicate under the recommended optimum conditions. Table 4 shows close similarity between the predicted values of the responses and the experimental results with low errors (<15%) and good desirability (0.65–1.00). The high values of desirability showed that our goal to maximize extraction of the volatile compounds studied in the present work was successfully achieved.

3.4. Application of the Optimized Extraction Conditions

The obtained extraction conditions were applied to five Arbequina olive oil samples (Table 5) from different geographical origins. In general, the major observed compound was E-2-hexenal, followed by Z-3-hexen-1-ol acetate and 1-hexanol. However, some particularities were detected, for example, sample 4 exhibited Z-3-hexen-1-ol as the major compound. Thus, the samples showed different volatile profiles, allowing differentiation.

4. Conclusions

Temperature was the extraction condition of HS-SPME/GC–MS that most affected the volatile profile of cv. Arbequina olive oils; however, the time also exerted a strong influence on the extraction of aroma compounds. Furthermore, control of the proposed parameters allows optimal extraction of desirable volatile compounds, improving the total areas of the peaks. The maximum response for all volatile compounds analyzed was obtained using 4.6 g of oil, 43 min, and 59 °C. The results obtained through the developed quadratic models show that RSM was a good and adequate tool to study the behavior of volatiles as they depend on the considered factors to maximize the aroma profile analysis in monovarietal olive oils. However, the extraction conditions must be optimized for each type of olive oil, as different varieties present different volatile compound profiles.

Abbreviations

CCD, central composite design; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane; EVOO, extra virgin olive oil; HS-SPME, headspace solid-phase microextraction; RSM, response surface methodology.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

aroma, extraction conditions, gas chromatography-mass spectrometry, headspace solid-phase microextraction, monovarietal olive oil

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