Effect of Drying Method on the Volatiles in Bay Leaf (*Laurus nobilis* L.)

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The effect of different drying treatments on the volatiles in bay leaf (*Laurus nobilis* L.) was studied. Simultaneous distillation extraction (SDE) and solid-phase microextraction (SPME) were compared by gas chromatography–mass spectrometry (GC–MS) of the volatile components in bay leaves. SDE yielded better quantitative analysis results. Four drying treatments were employed: air-drying at ambient temperature, oven-drying at 45 °C, freezing, and freeze-drying. Oven drying at 45 °C and air-drying at ambient temperature produced quite similar results and caused hardly any loss in volatiles as compared to the fresh herb, whereas freezing and freeze-drying brought about substantial losses in bay leaf aroma and led to increases in the concentration levels of certain components, e.g., eugenol, elemicin, spathulenol, and β-eudesmol.

INTRODUCTION

The bay leaf (*Laurus nobilis* L.) is a perennial, nondeciduous tree grown in temperate climates, particularly in Mediterranean countries. Dried bay leaves and the essential oil they contain have a strong, spicy aroma, and they are therefore widely used as flavor enhancers for foods such as meats, soups, sauces, and confectionery (*1*).

The chemical composition of the essential oil in bay leaves of different origins has been studied by different researchers. 1,8-Cineole was the major component in all cases, with percentages ranging between 30% and 56%, followed by linalool, α-terpinyl acetate, and several monoterpene hydrocarbons such as β-pinene and sabinene. Benzene compounds (eugenol, methyl eugenol, and elemicin), present in percentages ranging between 1% and 12%, are responsible for the spicy aroma of bay leaves and are extremely important factors determining the sensory quality of bay leaves (*2–4*).

Drying is commonly employed in preparing spices for market, as some spices can contain up to 75–80% water, and water levels need to be lowered to less than 15%. In the countryside, the household method of drying in the shade, or, better still, in well-ventilated rooms is still in use today, but industrial-scale drying is carried out in convection ovens. Drying of spices inhibits microorganism growth and forestalls certain biochemical changes; but at the same time it can give rise to other alterations that affect spice quality, such as changes in appearance and alterations in aroma caused by losses in volatiles or the formation of new volatiles as a result of oxidation reactions, esterification reactions, etc.

Changes taking place in the volatile compounds present in spices and other plants have been studied by different workers who have shown that the changes depend on several factors: primarily the drying method and the biological characteristics of the plant concerned.

Reductions in the total quantities of essential oils have been reported, amounting to 36–45% in sweet basil, 23–33% in marjoram, and 6–17% in oregano during drying at ambient temperature (*5*).

Drying in a convection oven also produces losses in volatiles, with the losses varying according to the drying temperature and drying time employed (*6, 7*). Increases in the quantities of certain compounds normally present in the spice (*8–10*) or the formation of new compounds have in some cases been observed after drying, probably as a consequence of oxidation reactions, hydrolysis of glycosylated forms, or the release of substances following the rupture of cell walls (*11*).

Accordingly, processing (grinding, drying, etc.) not only brings about a reduction in overall spice aroma but may also result in qualitative changes by giving rise to a secondary aroma in addition to the original aroma of the fresh plant.

Using freeze-drying as the drying treatment has been reported to result in changes that are less pronounced, and the spice has been observed to retain features that are closer to the characteristic appearance and aroma of the fresh plant (*6, 12, 13*).

The method used to extract and analyze the volatiles can also influence the results. The traditional method of extracting essential oils from plants, steam distillation, primarily collects the most volatile components, whereas solvent-based extraction methods are capable of extracting substances spanning a broader range of volatilities, depending on the solvent employed.

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Table 1. Volatile Compounds Identified in the Fresh Bay Leaf Extracts and Percentages Extracted Using SDE and SPME

compound	retention time	SDE mean ($n = 4$)	SDE RSD (%)	SPME mean ($n = 4$)	SPME RSD (%)
(1) α-thujene	9.03	0.44	10.5	traces	
(2) α-pinene	9.28	4.33	9.5	0.85	27.9
(3) camphene	9.68	1.06	11.6	traces	
(4) sabinene	10.37	5.80	4.9	2.48	33.0
(5) β-pinene	10.54	3.47	5.4	traces	
(6) 1,8-cineole	12.29	33.28	1.0	43.56	10.4
(7) γ-terpinene	13.14	0.46	8.8	0.27	11.4
(8) *trans*-thujan-4-ol	14.19	0.43	8.2	0.29	30.1
(9) α-terpinolene	14.54	0.16	2.8	traces	
(10) linalool	17.06	24.53	2.7	26.70	4.2
(11) terpinen-4-ol	17.41	2.28	8.9	0.86	9.7
(12) α-terpineol	18.44	4.09	6.7	4.95	12.7
(13) nerol	19.09	0.29	7.8	0.09	18.1
(14) *trans*-geraniol	19.25	0.11	1.4	traces	
(15) linalool acetate	20.04	0.46	8.0	0.80	47.7
(16) borneol acetate	21.48	1.67	3.8	2.27	28.9
(17) eugenol	21.56	3.00	12.8	1.23	32.9
(18) terpinyl acetate	22.31	8.16	3.1	11.75	40.7
(19) methyl eugenol	22.44	4.64	4.7	3.12	3.4
(20) elemicin	25.01	0.43	5.1	traces	
(21) spathulenol	25.91	0.55	8.3	0.41	49.0
(22) β-eudesmol	27.05	0.37	7.9	0.29	12.3

Simultaneous distillation extraction (SDE) has been widely used in analyzing the volatiles of herbs and plants (*14*). Supercritical fluid extraction (SFE) offers an advantage over SDE, in that the substances extracted can be altered by making minor variations in the pressure and temperature conditions of the extraction fluid (*15*).

Headspace analysis methods, such as purge and trap, direct thermal desorption and solid-phase microextraction (SPME), are fast and simple, they detect the most volatile substances, and hence are not always comparable to the methods mentioned above (*16, 17*).

The present study compared two different methods of extracting the volatiles from fresh bay leaves, SDE and SPME, with a view to determining which was more suitable for examining the effect of different drying methods on the volatiles in this spice.

MATERIALS AND METHODS

Samples. Fresh bay leaves were collected in the province of Toledo (Spain) in the month of September. The initial sample was divided into five batches. One was stored chilled at 5 °C for analysis fresh. The remaining batches were immediately dried using one of the following different drying methods tested: (a) drying at ambient temperature in a dark, well-ventilated room for three weeks (mean temperature 25 °C; mean relative humidity 39%); (b) drying in a convection oven at 45 °C for 14 h; (c) freezing at −18 °C for 24 h; or (d) freeze-drying in a Telstar model Cryodos-50 freeze-drier at a temperature of −53.2 °C and a pressure of 1.1×10^{-2} mB for 24 h. For freezing and freeze-drying, the bay leaves were first chopped into small pieces of approximately 0.5 cm^2.

The drying conditions employed in each of these methods were selected after conducting trials to achieve the percentage moisture content (9%) satisfying ASTA (American Spice Trade Association) requirements using the lowest possible temperature and shortest possible time.

Extraction and Concentration of Volatiles. *Solid-Phase Micro-extraction (SPME).* The SPME method was previously optimized (*18*). An amount of 200 mg of chopped fresh bay leaves with 1 μL of ethyl caprate (1.130 g/L) added as internal standard was used. Adsorption in the headspace was performed using a 100-μm poly(dimethylsiloxane) fiber at 60 °C for 30 min. Desorption then took place in the gas chromatograph injector at 250 °C for 5 min with the sample in splitless

mode for 0.5 min. Four replications of each extraction were performed for each sample.

Simultaneous Distillation-Extraction (SDE). A microscale simultaneous distillation extraction apparatus (Chrompack, Middelburg, The Netherlands) was used as previously described (*19*). An amount of 2 g of chopped bay leaves in 60 mL of water with 100 μL of ethyl caprate added as internal standard was extracted under atmospheric conditions for 2 h using dichloromethane as the extraction solvent. The extracts obtained were concentrated to approximately 0.5 mL using nitrogen and were stored frozen at −18 °C for gas chromatographic analysis.

Analysis of Volatiles. An amount of 1 μL of extract was analyzed using a Hewlett-Packard G 1800 B GCD System with a mass detector (Hewlett-Packard, Palo Alto, CA). The column was an SPB-1 (Supelco) methyl silicone column (50 m × 0.25 mm, 0.25 μm film thickness). Column temperature was 70 °C (3 min), increased at 4 °C/min to 120 °C, and increased at 8 °C/min to 250 °C. Injector temperature was 250 °C. Transfer line temperature was 280 °C. Mass detector conditions were as follows: electronic impact (EI) mode at 70 eV; source temperature 178 °C; scanning rate 1 scan/s; mass acquisition range 35−350.

Identification of the components was performed by comparing the mass spectra with those on record in the Wiley G 1035 A library, and using authentic standards when it was possible. Quantitative determinations were carried out under the assumption that component response factors were the same as the response factor for the internal standard.

Statistical Analysis. Principal component analysis (PCA) and the Student−Newman−Keuls test (SPSS Program, 2000) were used to evaluate the significance of the difference between the various drying treatments.

RESULTS AND DISCUSSION

Table 1 presents the results of the analysis of fresh bay leaves using SDE and SPME. In all, 22 substances were quantified. The results have been expressed in percentage, and the coefficient of variation values are also given. **Figure 1** depicts the chromatograms for each of the two methods.

Qualitatively the composition of the extracts obtained by each of these methods was similar, with the major component being 1,8-cineole, followed by linalool, terpinyl acetate, and the monoterpenes sabinene and α-pinene. This composition was similar to the composition of bay leaves grown in Spain described in the literature (*4*). Two substances belonging to the
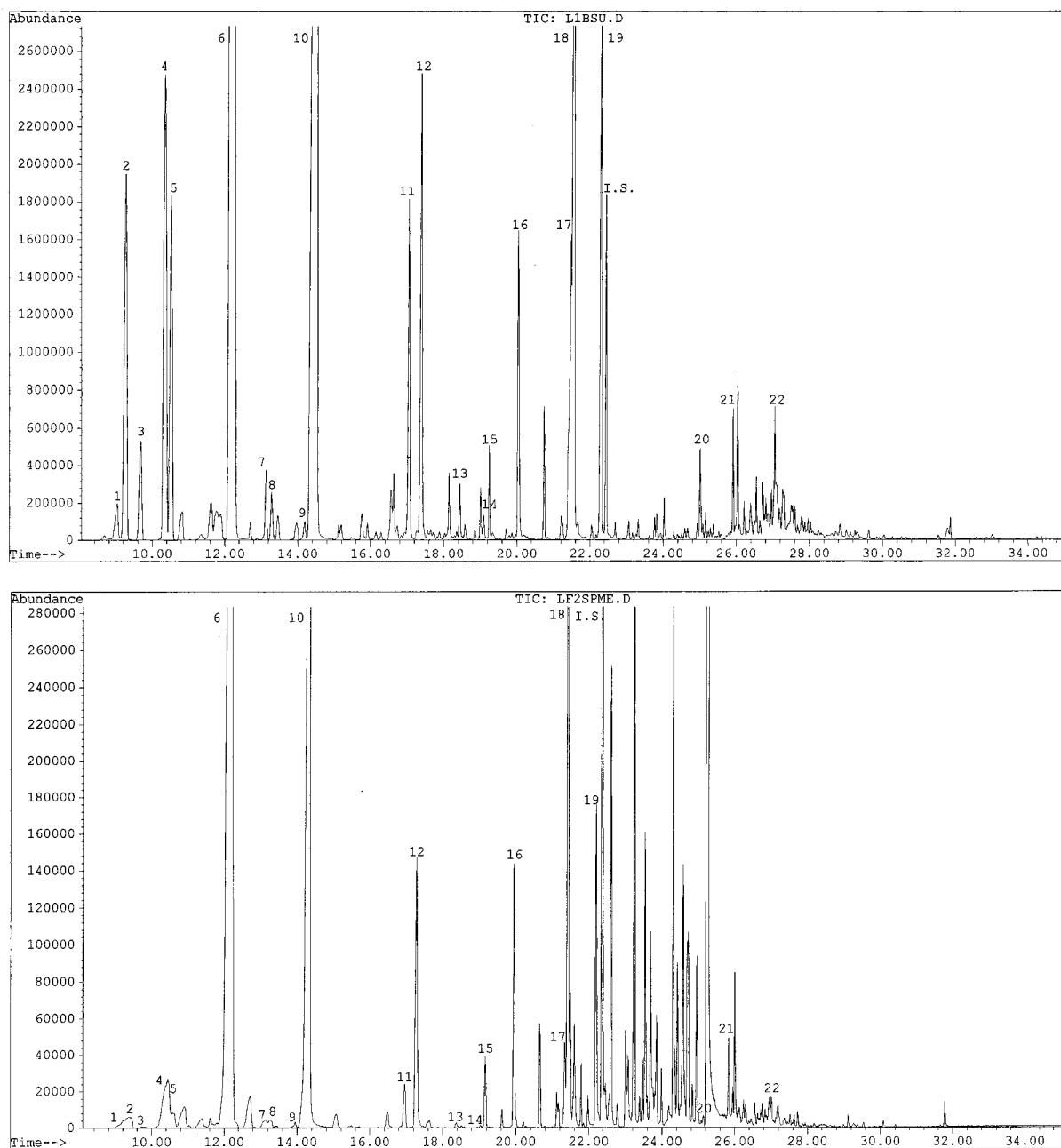
Figure 1. Chromatograms of volatile compounds extracted from fresh bay leaves (*Laurus nobilis* L.) using (a) Simultaneous Distillation Extraction (SDE) and (b) solid-phase microextraction (SPME). For compound listing, see **Table 1**.

group of oxygenated sesquiterpene derivatives, spathulenol and β-eudesmol, were also tentatively identified. Neither of these compounds has been previously described as bay leaf components in the literature consulted.

The extracts obtained by SDE were rich in hydrocarbon monoterpenes (15.7%) in comparison with those from SPME (3.6%), whereas the oxygen terpene percentage was higher in SPME (95.7%) compared with that in SDE (83.4%). However, in peppermint, SPME tends to extract a higher amount of the more volatile monoterpenes than the extraction techniques with solvents (*17*). These differences are due to the matrix effect in releasing volatile compounds as each spice has a characteristic plant tissue structure. In the case of bay leaf, extraction yields were higher for SDE, and the coefficient of variation values were also better, making SDE a suitable method for quantitative analysis (*14, 16*), especially in the case of fresh samples in which the volatile concentration levels are lower.

Table 2 shows the concentrations (in μg/g dry weight) for the fresh bay leaves and the sample batches dried by the methods tested in this study extracted using SDE, again with the resulting coefficient of variation values. The table also gives the results of the Student−Newman−Keuls test for comparison of the means. On the whole, the results indicate that there was little alteration in the concentrations of volatiles between the fresh spice and the bay leaves that were air-dried at ambient temperature or oven-dried at 45 °C. The concentrations of certain oxygenated terpenes, such as 1,8 cineole, linalool, and geraniol, decreased slightly after air or oven drying, but the only decrease that was more pronounced was for terpinyl acetate (45%). Eugenol underwent an increase on the order of 60% for all of these drying methods, and because of the spiced aroma of this compound, this increase could have repercussions on the sensory perception of dried samples.

The decline in the concentration of the volatiles after air-

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Table 2. Concentrations (SDE Analysis) of Volatile Components (μg/g Dry Weight) Extracted from Fresh Bay Leaves and from Batches Dried Using the Different Methods Tested

compound	fresh mean (*n* = 4)	fresh RSD (%)	air-dried at ambient temp. mean (*n* = 4)	air-dried at ambient temp. RSD (%)	oven-dried at 45 °C mean (*n* = 4)	oven-dried at 45 °C RSD (%)	frozen mean (*n* = 4)	frozen RSD (%)	freeze-dried mean (*n* = 4)	freeze-dried RSD (%)
α-thujene	34.2a	13.3	37.2a	6.5	36.0a	16.3	25.6b	4.5	22.5b	9.5
α-pinene	338.2a	12.0	355.3a	3.8	350.0a	14.5	193.4b	5.2	165.7b	10.0
camphene	83.4a	12.6	87.0a	8.3	78.7a	14.0	16.5b	5.6	14.9b	8.8
sabinene	448.4a	9.0	478.5a,b	4.8	534.2b	13.9	333.7c	5.4	295.3c	6.8
β-pinene	269.2a	8.7	270.8a	2.7	272.8a	10.8	159.6b	4.2	137.9b	8.0
1,8-cineole	2515.8a	3.9	2172.2b	1.7	2349.4c	4.2	1796.8d	3.6	1621.2e	4.8
γ-terpinene	35.7a	8.1	35.1a	4.5	32.7a	7.1	33.0a	1.7	27.8b	7.6
trans-thujan-4-ol	32.4a	8.2	35.0a,b	4.3	38.2b	9.0	28.2b	3.1	26.0c	4.1
α-terpinolene	11.7a	5.5	10.8a	4.8	11.2a	7.1	10.9a	2.8	9.4b	7.8
linalool	1822.6a	6.8	1708.3a	10.8	1522.3b	7.6	403.7c	4.6	339.1c	2.0
terpinen-4-ol	173.2a	5.6	146.9b	7.6	140.9b	3.0	146.8b	3.6	132.1b	2.7
α-terpineol	308.7a	5.6	278.6b	3.3	324.3a	4.2	96.1c	4.2	86.3c	2.4
nerol	21.9a	6.7	23.3a	3.7	22.8a	10.6	15.4b	6.1	13.0c	1.4
trans-geraniol	8.2a	5.4	7.8a	13.9	6.6b	1.4	5.4c	5.5	4.6d	4.5
linalool acetate	33.2a	13.4	39.3b	11.6	30.9a	10.1	6.2c	10.4	5.7c	4.8
borneol acetate	124.6a	1.6	99.6b	7.5	102.9b	9.5	13.1c	2.6	12.1c	4.4
eugenol	222.5a	15.6	451.0b	11.7	445.0b	8.6	431.7b	4.9	445.5b	6.3
terpinyl acetate	602.5a	5.7	318.6b	11.2	353.8b	13.7	489.7c	6.0	343.5b	10.2
methyl eugenol	341.2a	8.7	322.5a	3.5	318.7a	10.4	133.8b	4.9	125.5b	2.5
elemicin	32.0a	7.2	30.6a	9.9	30.8a	6.5	75.3b	2.8	64.2c	2.8
spathulenol	41.8a	6.2	35.3a	11.0	37.7a	4.4	117.9b	5.4	105.5c	3.3
β-eudesmol	27.3a	4.3	23.2b	6.7	23.6b	5.9	70.2c	3.1	54.5d	3.6

a Different letters (a, b, c, d, e) in the same row indicate statistical differences at the 0.05 level according to the Student–Newman–Keuls test.

drying depends on the type of compound and spice concerned, as well as on drying time and drying temperature (*7*). Certain researchers have observed only slight alterations in the volatile composition of rosemary and wormwood after drying at ambient as well as in temperature and at 30 °C (*20–22*) parsley (*23*) and bay leaves (*24*). However, other workers have reported lower levels of monoterpenes and higher levels of certain sesquiterpenes in dill and in ginger (*6, 10*).

In contrast, there were substantial losses in volatiles in the frozen and freeze-dried bay leaves, exceptions being eugenol, elemicin, and the two sesquiterpenes spathulenol and β-eudesmol, whose concentration levels increased. The increases recorded may be attributable to rupture of the plant cells in which the volatiles are stored. Increases in certain compounds have been recorded in different spices by some other researchers after freeze-drying (*6, 7, 13*), whereas losses in other volatile components have been reported to be lower than those indicated by our findings. The significance of freeze-drying conditions on losses of volatiles in dill has been pointed out (*11*).

When principal component analysis (PCA) was applied to the data presented in **Table 2**, the first two principal components explained nearly 78% of the variance between the samples. Principal component 1 (PC1) alone explained 60% of the variance among the sample batches. **Figure 2** plots the samples on the coordinate grid defined by the first two principal components and shows that PC1 separated the fresh samples and the air-dried and oven-dried batches from the frozen and freeze-dried batches. The substances that were positively correlated with PC1 (**Table 3**) experienced decreases in the frozen and freeze-dried samples and included α-terpineol, linalool, linalool acetate, methyl eugenol, and others. On the other hand, the substances that were negatively correlated with PC1 underwent increases in those same batches, these substances being β-eudesmol, elemicin, and spathulenol.

Principal component 2 (PC2) was of lesser importance, explaining only 17% of the variance among the sample batches. It separated the fresh bay leaves from all the other batches that
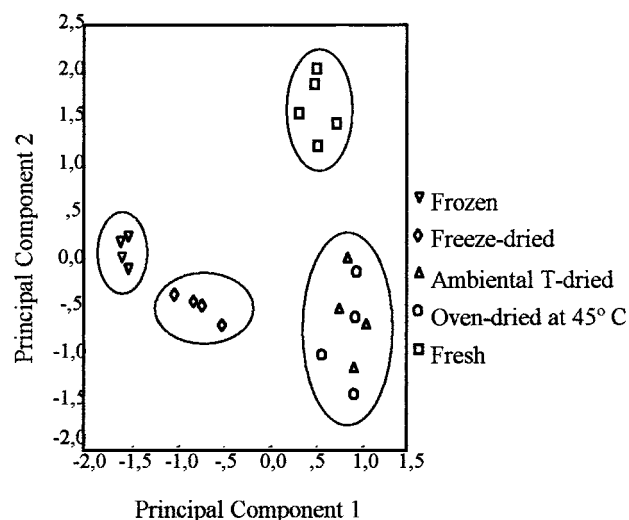

Figure 2. Plot of samples of fresh and dried bay leaf on the coordinate grid defined by principal components 1 and 2.

had undergone any of the drying treatments. The substances most closely correlated with this principal component were eugenol, which increased in all the dried batches, and terpinyl acetate, which decreased after drying.

In other spices such as dill, thyme, and sage, freeze-drying yielded batches that were more similar to the fresh samples than were the air-dried and oven dried batches (*7, 11, 22*), though other researchers found no sensory differences between different drying treatments (*12*).

In summary, air-drying of bay leaves at ambient temperature and oven-drying at 45 °C, brought about small losses in the volatile components. Consequently, oven-drying may be a good method for preserving the sensory characteristics of this spice, in that it can be completed in a shorter time and under more closely monitored conditions than other drying methods. Both

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Table 3. Volatiles Most Closely Correlated with Principal Components 1 and 2

PC	volatile compound	loading[a]	% explained variance	% cumulative variance
1	β-eudesmol	−0.987	60.905	60.905
	elemicin	−0.972		
	spathulenol	−0.971		
	linalool acetate	0.921		
	α-terpineol	0.919		
	methyl eugenol	0.918		
	camphene	0.913		
	linalool	0.902		
	borneol acetate	0.900		
2	eugenol	−0.945	17.348	78.253
	terpinyl acetate	0.924		

[a] Only those volatiles with absolute correlation coefficient values greater than 0.90 have been included.

freezing and freeze-drying produced greater losses in the volatiles contributing to bay leaf aroma, though they also brought about increases in certain other, less volatile substances that may also be involved in the overall aroma of this spice.

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