Use of SPME–GC–MS in the Study of Time Evolution of the Constituents of Saffron Aroma: Modifications of the Composition During Storage

Maurizio D'Auria[1,*], Giacomo Mauriello[1], Rocco Racioppi[1], and **Gian Luigi Rana[2]**

[1]Dipartimento di Chimica, Università della Basilicata, Via N. Sauro 85, and [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Viale dell'Ateneo Lucano, 85100 Potenza, Italy

Abstract

The effect of storage on the composition of saffron aroma is studied. Six samples of saffron from different areas of Italy are analyzed by solid-phase microextraction–gas chromatography–mass spectrometry. Samples 1, 2, and 3 are derived from cultivations of *Crocus sativus* in the zone of Salerno (Southern Italy) from 2000 to 2002. Samples 4, 5, and 6 are derived from cultivations in Sardinia, Italy (from 1998, 2000, and 2001, respectively). In all samples, 3,5,5-trimethyl-2-cyclohexen-1-one; 3,5,5-trimethyl-2-cyclohexen-1,4-dione; safranal; and 2,4,4-trimethyl-6-hydroxy-3-carboxaldehyde-2,5-cyclohexadien-1-one are found. 5,5-Dimethyl-2-methylene-1-carboxaldehyde-3-cyclohexene; 3,5,5-trimethyl-1,4-cyclohexandione; and β-ionone are found with nonanal, dihydro-β-ionone, and 2,6-di-*t*-butylphenol. Safranal is the main component in all of the samples. The most important changes are in the presence of alcohols and aldehydes and oxidation products of the major terpenoids components. Furthermore, the presence of safranal—the most important constituent of the flavor—changes during the time, increasing during 3 years, then decreasing after 5 years.

Introduction

The solid-phase microextraction (SPME)–gas chromatographic (GC)–mass spectrometric (MS) determination of volatile organic compounds in saffron was recently reported on (1,2). Saffron comes from the dried red stigmata of *Crocus sativus* L. flowers and was formerly used as a dyestuff and in medicine. The value of Saffron is determined by three main secondary metabolites: crocin and its derivatives, picrocrocin, and safranal, which are responsible for color, taste, and odor (3–5). Traditional medicine used saffron in the treatment of numerous illness, including tumors. In the last few years, antitumoural properties of both crude saffron extracts and its main components, both in vitro and in vivo, were reported (6–10).

Safranal is one of the main components of saffron essential oil, determining its aroma. It is a monoterpene aldehyde, formed in saffron by hydrolysis from picrocrocin during drying and storage (11,12). Difficulties encountered in the extraction and biochemical analysis of saffron secondary metabolites may be primarily attributable to their degradation. Crocin and picrocrocin, which are biosynthesized in plant cells, tend to naturally degrade in the cells of stigmas during flowering, drying, storage, and extraction.

In this study, the seasonal variation in saffron deriving from both Salerno (Southern Italy) and Sardinia has been examined. In the first case samples collected in the years 2000, 2001, and 2002 were examined. In the second case, samples collected in 1998, 2000, and 2001 were examined. The object of this work is to study the flavor variability during the year. The possible effects caused by storage of the spice were also studied.

In order to carry out this type of research, SPME was used, as in previous work in this field (1,2). This method, after little more than 10 years from the introduction, has been demonstrated to be a powerful technique for qualitative and quantitative determination of volatile constituents in a natural matrix (13).

Experimental

Dried samples of saffron derived from cultivation of *Crocus sativus* in the zone of Salerno (Southern Italy) were used. A sample was obtained from cultivations of 2000, one from cultivations of 2001, and the latter from cultivations of 2002. Dried samples of saffron derived from cultivations in Sardinia, Italy (San Gavino, Cagliari) in the years 1998, 2000, and 2001, respectively, were also used.

A 100-µm polydimethylsiloxane–SPME module (57300-U, Supelco, Bellefonte, PA) was used. The fiber was maintained over the sample (0.1 g) in a 20-mL vial at 36°C for 20 min. The analyses were carried out with an HP 6890 plus GC equipped with a Phenomenex Zebron ZB-5 MS capillary column (30-m × 0.25-mm i.d., 0.25-µm film thickness) and HP 5973 mass selective detector (mass range, 15–800 amu; scan rate, 1.9 scans/s; and electron microscopy voltage, 1435). Helium at 0.8 mL/min was

* Author to whom correspondence should be addressed: email dauria@unibas.it.

used as the carrier gas. The injector was splitless at 250°C. A desorption time of 0.4 min was used. The detector was maintained at 230°C. The oven was maintained at 40°C for 2 min, and the temperature was then increased to 250°C (8°C/min). Finally, this temperature was maintained for 10 min. The mass spectra were identified by comparison with spectra in Wiley 6N and NIST98 libraries. All analyses were carried out in triplicates. The observed standard deviation was ± 0.02 of the percent area.

Results and Discussion

Three samples were derived from cultivations of *Crocus sativus* in the zone of Salerno (Southern Italy), from cultivation of 2000, 2001, and 2002. The results of the analyses are reported in Table I (Figure 1 collects the structures of all the identified compounds). The aim of this work was to study the evolution during storage of the components of aroma. SPME does not allow for a quantitative

Table I. Volatile Components of Saffron

	Retention time (min)	Compound	Sample (area %)					
			Salerno			Sardinia		
			2000	2001	2002	1998	2000	2001
1	2.24	2-Methylpropanal	0.02	0.16	0.03			
2	2.35	Acetic acid	0.05	0.09		0.17		
3	2.39	3-Methylbutanal			0.03			
4	2.98	2-Methylbutanal	0.04		0.04			
5	3.74	1-Pentanol			0.02			
6	4.32	Hexanal	0.03	0.19	0.24	0.02	0.11	0.14
7	6.05	1-*t*-Butylcyclopentadiene	0.08				0.12	
8	6.47	Heptanal		0.11	0.21			
9	6.73	2(5H)-Furanone	0.16	0.40	0.89			
38	6.74	4,4-Dimethylcyclopentenone					0.10	
10	8.73	Octanal			0.08			
11	9.15	β-Phellandrene			0.02			
12	9.23	6-Methyl-5-hepten-2-one		0.02	0.02			
13	9.56	3,5,5-Trimethylcyclohex-3-en-1-one		6.61	8.45			1.64
14	9.77	2,5-Dimethyl-2,4-hexadiene	0.06					
15	10.02	1-(1-Methylethyl)-4-methyl-2,4-cyclohexadiene						
16	10.07	1,3,5-Trimethylbenzene	0.02				0.03	
17	10.62	Benzaldehyde			0.01			
39	10.78	1,1,3-Trimethylcyclopentadiene				0.21		
18	10.83	Nonanal		0.38	0.50		0.22	1.48
40	10.87	6-Methyl-3,5-heptadien-2-one				0.14		
19	10.92	5,5-Dimethyl-2-methylene-1-cyclohexylcarbaldehyde	3.50	3.06		0.75	1.06	
20	11.11	2-Phenylethanol			0.06			
21	11.20	3,5,5-Trimethylcyclohexenone	1.77	6.37	4.18	10.26	9.36	5.79
22	11.52	4-(1-Methylethyl)-benzaldehyde	0.06					
23	11.65	3,5,5-Trimethylcyclohex-2-en-1,4-dione	0.63	3.78	1.09	6.16	6.80	4.57
24	12.11	3,5,5-Trimethylcyclohexan-1,4-dione	0.28	3.10	3.54	3.14	1.84	2.23
25	12.67	2-Hydroxy-3,5,5-trimethylcyclohexenone	0.10	0.10			0.34	
26	12.83	Safranal	83.97	59.13	49.63	46.74	60.42	41.13
41	13.12	2-Isopropylidene-3-methylhexa-3,5-dien-1-al				0.67	0.13	7.07
27	13.15	2,7,7-Trimethyl-2,4-cycloheptadien-1-one	0.10			2.58	0.90	
28	14.29	2-Hydroxy-3,5,5-trimethylcyclohex-2-en-1,4-dione	0.14				0.61	
29	14.64	4-Hydroxy-3,5,5-trimethylcyclohex-2-enone		0.63	0.48			5.75
42	14.87	2,4,5-Trimethylbenzaldehyde				0.18		
30	16.11	2,6,6-Trimethyl-4-hydroxycyclohexa-1,4-dien-3-on-1-carbaldehyde	0.85	6.71	5.62	5.77	2.89	7.56
31	16.88	Dihydro-β-ionone	0.09	0.56	0.26	0.10	0.25	
32	16.98	Dihydro-β-ionol			0.18			
43	17.02	(E)-6,10-Dimethyl-5,9-undecadien-2-one				0.04		
33	17.34	2,6-Di-(1,1-dimethylethyl)-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one			0.06	0.06		
34	17.53	2,6,6-Trimethyl-4-hydroxycyclohexen-1-carbaldehyde	0.73					
35	17.63	β-Ionone	0.09	0.58	0.22	0.11	0.12	
36	17.72	Pentadecane	0.03		0.02	0.10		
37	18.03	2,6-Di-(1,1-dimethylethyl)-phenol	0.03	0.35			2.31	

estimation of the compounds adsorbed. These difficulties arose from a differential absorption of the different compounds on the fiber (2). However, considering that the absorption of a single component on the fiber was the same in all the samples, this method allows us to obtain a description of the concentration modifications of single components.

All of the samples showed the presence of 2-methylpropanal (**1**). The presence of hexanal (**6**) and 2(5H)-furanone (**9**) was observed. Some minor components were found only in the sample of 2000, such as: 2,5-dimethyl-2,4-hexadiene (**14**); 1-(methylethyl)-4-methyl-2,4-cyclohexadiene (**15**); 4-(methyl ethyl)-benzaldehyde (**22**); and 1,3,5-trimethylbenzene (**16**). 6-Methyl-5-hepten-2-one (**12**) was found in both the samples of 2001 and 2002. 3-Methylbutanal (3), pentanol (**5**), β-phellandrene (**11**), benzaldehyde (**17**), phenylethanol (**20**), dihydro-β-ionol (**32**), and 2,6-di-*t*-butyl-4-methyl-4-hydroxy-2,5-cyclo hexadienone (**33**) were found only in the sample of 2002.

Besides these minor components, the same approach can be used for the main components. Thus, in the samples of 2001 and 2002, a relevant amount of 3,5,5-trimethyl-3-cyclohexen-1-one (**13**) was found, and samples of 2000 and 2001 contained 5,5-dimethyl-2-methylene-1-carboxaldehyde-3-cyclohexene (**19**). All of the samples showed the presence of 3,5,5-trimethyl-2-cyclohexen-1-one (**21**) (Figure 2) (the highest quantity was found in the sample of 2001) and 3,5,5-trimethyl-2-cyclohexen-1,4-dione (**23**) (in this case the highest quantity was found in the sample of 2002). 3,5,5-Trimethyl-2-hydroxy-2-cyclohexen-1-one (**25**) was found in the samples of 2000 and 2001, and 2,6,6-trimethyl-1,4-cyclohexandione (**24**) (Figure 3) was found in all of the samples (the highest amount was found in the sample of 2002). The main component of the flavor, as expected, was safranal (**26**) (it is the main component in all the samples, but in sample 1 it represents approximately 84% of the chromatographic area).

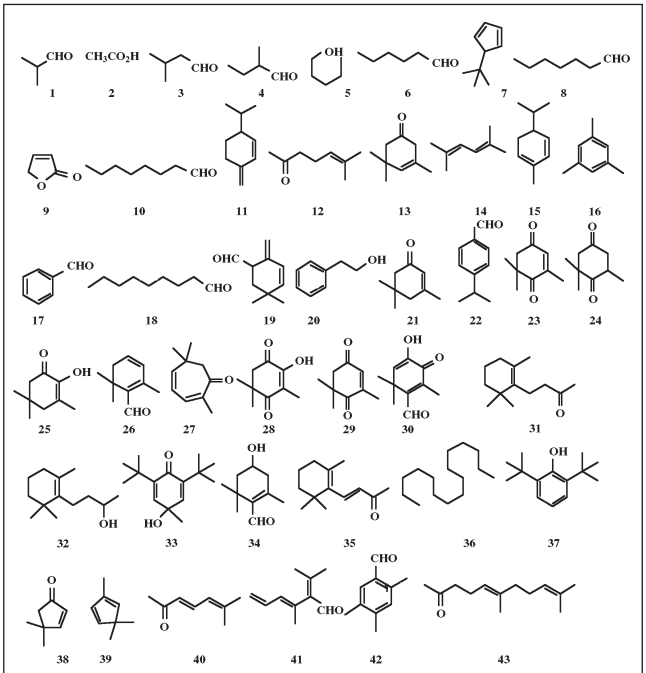

Figure 1. Chemical structure of the compounds identified by the SPME analysis of saffron from different sites in Southern Italy.

In the sample of 2000, small amounts of 2,7,7-trimethyl-2,4-cycloheptadien-1-one (**27**), 3,5,5-trimethyl-2-hydroxy-2-cyclohexen-1,4-dione (**28**) and 2,6,6-trimethyl-4-hydroxy-1-carboxaldehyde-cyclohexene (**34**) were detected. Furthermore, the samples of 2001 and 2002 contained 3,5,5-trimethyl-4-hydroxy-2-cyclohexen-1-one (**29**). Finally, all of the samples contained 2,6,6-trimethyl-4-hydroxy-1,4-cyclohexadien-3-one-1-carboxaldehyde (**30**), dihydro-β-ionone (**31**), and β-ionone (**35**).

The secondary components of the aroma are primary alcohols and aldehydes. Primary alcohols were found only in the sample of 2002, though they were absent in the samples of 2000 and 2001. The amount of nonterpenoid aldehydes shows a similar trend: in the sample of the year 2000, only three compounds were found; four were found in the sample of 2001; and eight compounds were found in the sample of 2002. These compounds can easily be oxidized, and these oxidation processes can reduce the presence of aldehydes in the flavor.

2(5H)-Furanone (**9**) diminishes during storage. In this case, hydrolytic processes can be responsible for the disappearance of this compound. A similar trend was found when examining the presence of 3,5,5-trimethyl-3-cyclohexen-1-one (**13**). Also, in this case, the highest amount of the compound can be found in the youngest sample. This compound can isomerize into the more stable 3,5,5-trimethyl-2-cyclohexenone (**21**). In this case, a maximum cannot be observed in the 2002 sample, and the highest amount was found in 2001. Degradative processes are probably responsible for the amount in the 2001 sample. Diminishing

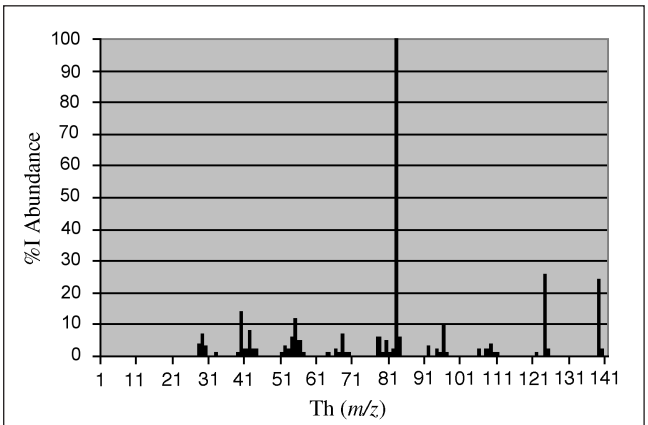

Figure 2. Mass spectrum of 3,5,5-trimethylcyclohexenone found in a saffron sample from Salerno (Italy).

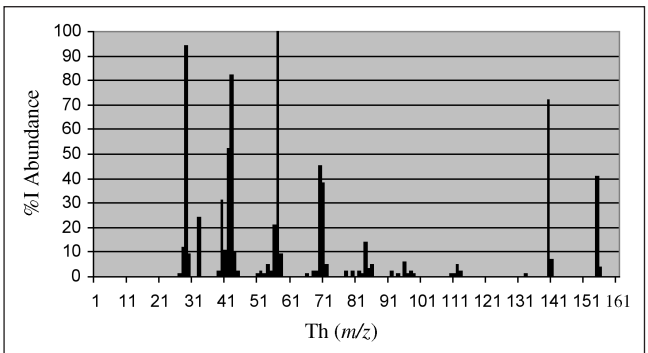

Figure 3. Mass spectrum of 2,6,6-trimethyl-1,4-cyclohexandione found in a saffron sample from Salerno (Italy).

amounts as a function of the age of the sample were also observed for 2,2,6-trimethyl-1,4-cyclohexandione (**24**).

A completely different behavior can be observed in safranal (**26**). The amount of this compound increased during the time. Probably, this behavior is connected to the progressive decomposition of picrocrocin.

The other samples (Table I) were derived from cultivations in Sardinia, Italy (San Gavino, Cagliari) in the years of 1998, 2000, and 2001. The sample of 2000 showed the presence of little amounts of hexanal (**6**) (present in trace also in the sample of 1998); 1-*t*-butyl-cyclopentadiene (**7**); 4,4-dimethyl-cyclopentenone (**38**); and 1,3,5-trimethylbenzene (**16**). In the sample of 2001, 3,5,5-trimethyl-3-cyclohexen-1-one (**13**) was found, though in the samples of both 1998 and 2000, 5,5-dimethyl-2-methylene-1-carboxaldehyde-3-cyclohexene (**19**) was found. All of the samples contained **26**, **21**, **23**, **24**, and a new compound, 2-isopropyliden-3-methyl-3,5-hexadienal, which was never found in saffron (**41**). The sample of 2000 showed the presence of 3,5,5-trimethyl-2-hydroxy-cyclohexen-1-one (**25**). Obviously, the main component of these samples was safranal (**26**). Also, **30** was present in large amount in all samples. 2,7,7-Trimethyl-2,4-cycloheptadien-1-one (**27**), dihydro-β-ionone (**31**), and **35** were detected in the samples of 1998 and 2001. Compound **25** and 2,6-di-*t*-butylphenol (**37**) were found only in the sample of 2000, and **29** was found only in sample of 2001.

In the samples from Sardinia, primary alcohols were not found. Also, the number of aldehydes was lower than in the other samples. The total amount decreased during the time, in agreement with the previously reported data. Only the sample of 2001 contained **13**, a compound that can easily isomerize to **21**.

An interesting consideration can be made on the amounts of compounds **23**, **24**, and **29**. Compound **29** is a precursor of **23** and was present only in the sample of 2001. In the same sample, the amount of **23** was relatively low in comparison with the amounts in the other samples. The maximum was observed in the sample of 2000, though an increase of the reduction product **24** was observed in the sample of 1998.

Safranal was also the most abundant component in the samples from Sardinia. Also in this case, its amount was higher in the sample of 2000 than in that of 2001; however, in the sample of 1998, the presence of safranal decreases. The age of the sample influences the presence of this important component; although during the initial years safranal increased, degradation processes reduced the amount of it over an extended time period. It is noteworthy to mention the high quantity of the aldehyde **41** in the sample from cultivations of 2002. Finally, cycloheptadienone **27** was not present in the youngest sample while its amount increased over time.

Conclusion

This work clearly shows that the composition of the flavor components in saffron changes with the time. The most important modifications are the presence of alcohols and aldehydes and the presence of oxidation products of the major terpenoid components. Furthermore, the presence of safranal, the most important constituent of the flavor, changes over time, showing that it increases during 3 years. However, after this period, after 5 years, the amount of safranal decreases. The trend observed in this study could depend on the seasonal variation of volatile components in saffron. However, the results reported here show that the same trend can be observed in samples derived from different sites and years. Therefore, natural changes in the concentration of the aroma components can be ruled out, and storage can be considered as responsible for the observed behavior.

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