Determination of Safranal from Saffron (*Crocus sativus* L.) by Thermal Desorption−Gas Chromatography

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Thermal desorption−gas chromatography was applied to samples of saffron to quantify the safranal content that is responsible for the distintive aroma. Safranal constituted 72% of the volatile fraction. Using β-cyclocitral as internal standard and submitting the sample to a temperature of 70 °C for 8 min, 83% of the total was determined with the first desorption, 95% with the first and second desorptions, and 100% with three consecutive desorptions.

Keywords: *Saffron; safranal; thermal desorption−gas chromatography*

INTRODUCTION

Picrocrocin ($C_{16}H_{26}O_7$) is responsible for the bitter taste of saffron. By submitting this substance to hydrolysis and dehydratation, it is possible to obtain safranal (Buchecker et al., 1973; Pfander and Schurtenberger, 1982; Curró et al., 1984; Himeno and Sano, 1987; Iborra et al., 1992; Narasimhan et al., 1992; Castellar et al., 1993). Safranal also appears during the dehydration, manipulation, and storage of the fresh product (Himeno and Sano, 1987). Safranal, the principal substance responsible for the aroma of saffron, has the molecular formula $C_{10}H_{14}O$, which corresponds to 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde (Corradi and Micheli, 1979a).

Safranal is determined commercially according to ISO standards (1993) by measuring the absorbance at 330 nm of an aqueous solution of saffron. However, this technique is not necessarily the best one to use because safranal is not very soluble in water and because, at the wavelength mentioned, the cis isomers of the crocetin derivates are also absorbed. Other methods used to determine safranal include high-performance liquid chromatography (HPLC) or gas chromatography (GC). Given the volatile nature of this compound, the best technique is GC following an extraction step. Zarghami and Heinz (1971) use extraction with diethylic ether at a low temperature; Römer and Renner (1974) use distillation−extraction with diethyl ether−pentane followed by concentration of the extract by distillation; Sujata et al. (1992) use thin-layer chromatography (TLC) followed by dissolution in diethylic ether. All the methods mentioned involve extensive manipulation of the sample that can lead to errors, such as the appearance of substances that are not found in the commercial product. To avoid these errors, at least partially, Curró et al. (1986) use GC with headspace. The latter method has the disadvantage that the saffron must be submitted to a thermal treatment 1 h before being injected into the chromatograph. This heat can lead to alteration of aromatic composition as well as unnecessarily prolonging the analysis time.

Recently, we developed a new method for determining wine aromas by thermal desorption (TD) followed by GC (Salinas et al., 1994). This method, which had already been applied to solid drug samples (Glover, 1991), involves very little sample manipulation and is the method that best resembles what is involved when we use our sense of smell. It consists of introducing the sample into a metal tube coupled to the interior of an oven and passing an inert gas through the tube. The gas carries with it the volatile compounds that are retained in an adsorbent trap at low temperature. After rapid heating, the compounds are liberated and automatically injected into the chromatograph column. The purpose of this work, then, is to determine safranal in a commercial sample of saffron by TD−GC.

Table 1. Commercial Characteristics of the Saffron Used

characteristic	value
coloring strength	232.50
bitterness	99.70
safranal	30.02
total ash, % (m/m)	5.76
moisture and volatile matter,[a] % (m/m)	11.45
total length (cm)	2.416
style length (cm)	0.700
stigma length (cm)	1.716
total mean mass (g)	1.8×10^{-3}

[a] At 105 °C.

MATERIALS AND METHODS

Sample. Freshly harvested (1993) stigmas of *Crocus sativus* L. were dried and ground in the traditional way of La Mancha (Spain), which consists of subjecting the sample to a slow dehydration over wood-fire hot ashes until the weight has been reduced to ∼1/5 of initial weight. The commercial characteristics of the saffron are shown in Table 1. According to the ISO standards (1993), the saffron was Quality I, and according to the Spanish MEH standard, (1986) it belonged to "Mancha" quality and was of prime commercial value (Alonso et al., 1987). The samples were kept for 24 h in a drier containing dry silica gel (Merck), with the humidity indicator (completely blue) at 40 °C. During this drying time, the mass was reduced by 10.74%. The saffron was then ground in a rotary grinder and kept in a hermetically sealed container at −20 °C until analysis.

Internal Standard. β-Cyclocitral ($C_{10}H_{16}O$, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde) was used as the internal standard and was supplied by Sigma with a purity of 70%. The internal standard was diluted 1:20 with pure

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Table 2. Conditions of Thermal Desorption

parameters of thermal desorption	A	B
desorption temperature (°C)	150	70
desorption time (min)	4	8
cold trap low (°C)	−20	−30
cold trap high (°C)	200	200
injection time (s)	50	50
transfer line temperature (°C)	200	200
cold trap packing (Tenax-GC 60 mesh) (g)	0.02	0.02

ethanol (Merck), and the solution was added to the saffron in the proportion of 1 μL/0.1 g of sample.

Analytical Conditions of Thermal Desorption. Known amounts of sample of saffron (0.1−0.01 mg) were introduced into a metal tube (88.9 mm in length × 6.35 mm o.d. × 4.50 mm i.d.), the ends of which were blocked with glass wool. The packed tube was introduced into a Spantech TD-4 thermal desorber coupled to a gas chromatograph by a capillary connector. After various preliminary assays, the thermal desorption conditions A and B shown in Table 2 were used.

Gas Chromatographic Analysis. Gas chromatographic analysis was performed with a Perkin-Elmer GC-8310 instrument equipped with a flame ionization detector, a PTV (programmable temperature vaporizer) injector, and SGE column (50 m × 0.22 mm, i.d.) containing fused silica and BP-21 stationary phase (polyethylene glycol acid treated). The conditions were as follows: carrier gas, helium at 30 psig; temperature program, 80 °C, 3 °C/min, 180 °C, 2 min; split ratio, 90:1; detector, 230 °C; program of injector, −0.02 relay 1 ON, −0.01 PTV 200 °C. A Perkin-Elmer Nelson-2600 integrator was used to determine the peak area for quantitative analysis.

Mass Spectrometric Analysis. To confirm peak identification, the TD and GC were coupled to a Perkin-Elmer Q-Mass-910 (EI 70 ev). Column and chromatographic conditions were as just mentioned for GC analysis. Compounds were identified by comparison of their spectra with those of the NIST/EPA/MSDC Mass Spectral database Version 3.02.

RESULTS AND DISCUSSION

Two of the chromatograms performed on saffron according to conditions B of thermal desorption are shown in Figure 1: (a) chromatogram without β-cyclocitral and (b) chromatogram with β-cyclocitral added. Peak 1 corresponds to safranal [mass spectral data *m/z* (%) 91(100), 107(93), 105(53), 79(48), 121(46), 39(43), 77(42), 65(28), 150(26)] and peak 2 corresponds to the β-cyclocitral (IS). To check the response of the method, samples of saffron with a mass of between 0.1 and 0.01 g were used with both conditions A and B. In both cases, the graphical representation of the peak areas of safranal versus the mass of sample used show a linear relationship, with coefficients of variation of >0.99 and significance levels of <0.01. This result means that the greater the quantity of safranal in the tube, the greater the peak area and that the amount of this compound in the sample can be determined by this method. The peak areas corresponding to the β-cyclocitral and safranal contained in 0.1-g samples of saffron submitted to conditions A and B and desorbed eight times are shown in Table 3. The percentage of both compounds determined in each desorption compared with the total amounts determined in all the desorptions is also shown.

The β-cyclocitral is almost totally recovered in the first desorption with both conditions, although slightly more with conditions B than A. Using conditions A, 34.18% of the safranal content was detected in the first desorption and then roughly the same percentage was determined in all successive desorptions. When conditions B were applied, 67.36% of the safranal was detected in the first desorption. The following desorptions led to diminishing quantities of safranal being detected, although the quantity was almost constant from the fourth desorption onward. These data show that with both conditions, the thermal treatment to which the sample was submitted produced additional safranal compared with the amount present at the outset (the increase being greater at higher temperatures) and that from the second desorption onward with conditions A and from the fourth desorption onward with conditions B, any safranal detected is that which has been freshly formed. According to Pfander and Schurtenberger (1982), Curró et al. (1984), and Himeno and Sano (1987), among others, the safranal may be formed from picrocrocin. These authors also cite acid or basic hydrolysis conditions, in addition to the high temperature, as a possible reason for safranal production.

Because safranal formation is greater with conditions A than B, if determinations are carried out in the former, the error will be greater because in the first desorption a substantial quantity of safranal may have been formed. For this reason, conditions B were chosen. If we suppose that in the first desorption with conditions B that the amount of safranal formed is practically negligible and if we subtract the mean value of the peak areas of additional safranal (desorptions 4−8) from the areas obtained in the second and third desorptions, we conclude that 100% of original safranal of the sample can be determined with the first three desorptions, 95% with the first two desorptions, and 83% with the first desorption.

The chromatograms of the saffron samples obtained applying conditions B with no added β-cyclocitral give a mean safranal content of 72% of the total volatile fraction, a value similar to that obtained by Curró et al. (1986) and higher than those values of 47 and 60.3%

Table 3. Peak Areas of Safranal and β-Cyclocitral in Eight Desorptions

desorption no.	conditions A				conditions B			
	a[a]	b[b]	c[c]	d[d]	a[a]	b[b]	c[c]	d[d]
1	283.91	95.58	254.55	34.18	147.14	98.54	170.61	67.36
2	5.65	1.90	70.15	9.42	1.11	0.74	31.73	12.52
3	2.20	0.74	68.02	9.13	0.40	0.27	16.99	6.71
4	1.78	0.60	68.85	9.25	0.29	0.19	8.18	3.22
5	1.13	0.38	69.73	9.36	0.13	0.09	7.87	3.11
6	0.98	0.33	71.69	9.63	0.10	0.07	6.44	2.54
7	0.79	0.27	72.84	9.78	0.09	0.07	7.35	2.90
8	0.57	0.19	68.84	9.24	0.06	0.04	4.12	1.63

[a] The peak area of β-cyclocitral. [b] The percentage of β-cyclocitral compared with the total from eight desorptions. [c] The peak area of safranal. [d] The percentage of safranal compared with the total from eight desorptions

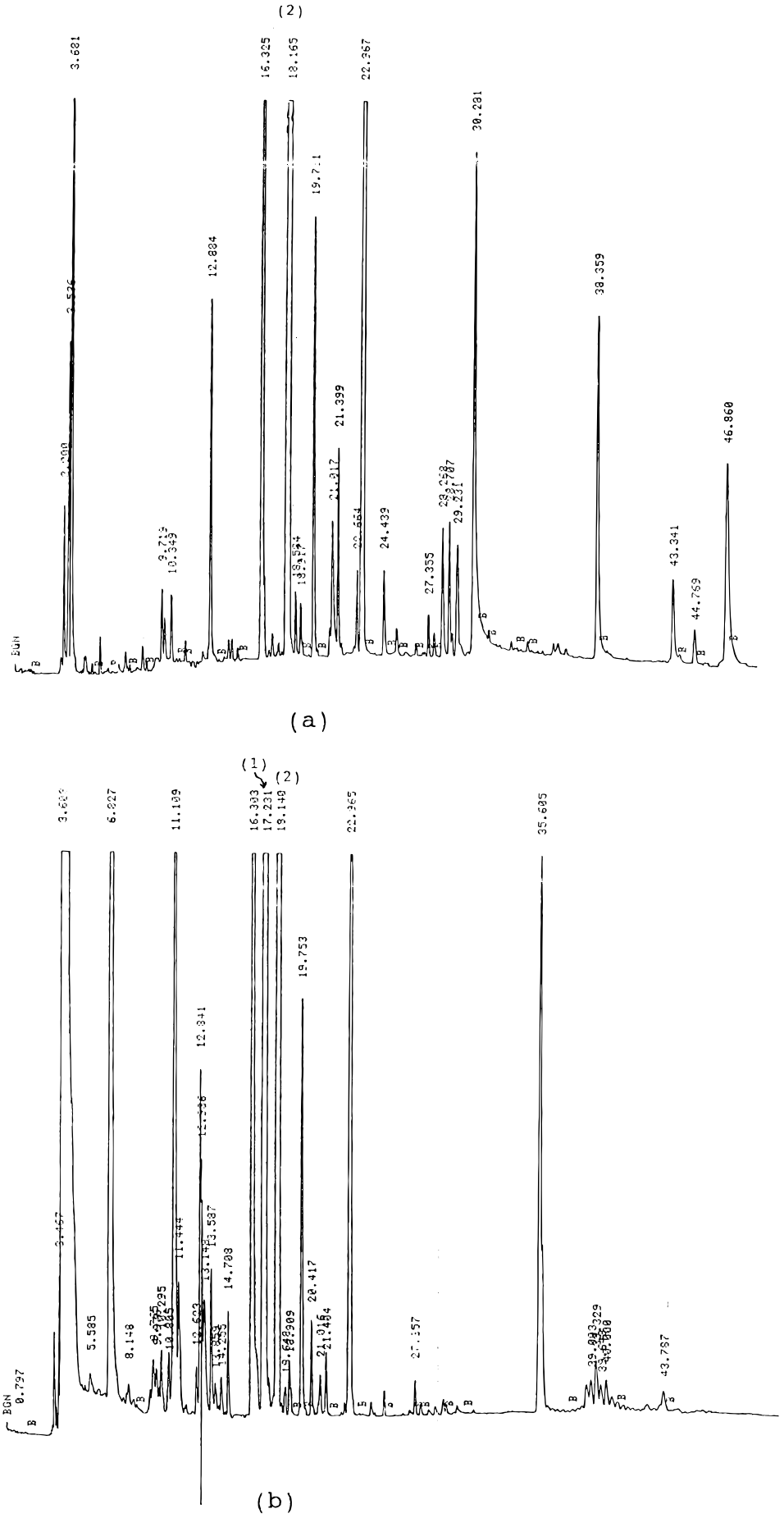

Figure 1. Chromatograms of saffron obtained using conditions B (a) without β-cyclocitral and (b) with β-cyclocitral added: (**1**) β-cyclocitral (IS); (**2**) safranal.

obtained by Zarghami and Heinz (1971) and by Rödel and Petrzika (1991), respectively.

In the quantification of safranal, we used β-cyclocitral, which has a similar chemical structure. For this reason, we consider the same response factor for both compounds. Bearing in mind the considerations just discussed, we use the peak area corresponding to β-cyclocitral to calculate the response factor (9.9945×10^{-5} mg/unity area). With this factor and considering the areas of the peaks of safranal of the first three desorptions (less the safranal formed in the second and third desorption), we obtained a safranal content of 206.47 mg/kg.

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