

Extraction, Separation and Identification of Volatile Organic Compounds from Paprika Oleoresin (Spanish Type)

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In the present work the extraction, separation, identification, and quantification of VOCs in paprika oleoresin were performed. Five different methods were used for extraction: simultaneous distillation–extraction (SDE) at atmospheric and reduced pressures, static and dynamic headspace, and purge and trap. The results obtained were compared and assessed. Separation, identification, and quantification were carried out by HRGC-FID and HRGC-MS. Samples of red pepper from Murcia (Spain) were obtained by industrial extraction. All treated samples are manufactured as paprika oleoresin to be used as a colorant. The VOCs identified in paprika oleoresin, which have also been found in other foods, are reported.

Keywords: *Paprika oleoresin; simultaneous distillation–extraction (SDE); dynamic headspace; purge and trap; static headspace; HRGC-MS; VOCs; Capsicum annum*

INTRODUCTION

Red pepper belongs to the Tubiflora order of the Solanaceae family. The species *Capsicum annum* L. is of a great agricultural interest. Paprika oleoresin is its lipidic extract, obtained as a red sticky oil, with a characteristic red pepper flavor (Cabeza et al., 1992).

The interest in paprika oleoresin is mainly due to its colorant power. This property has a number of applications in food, cosmetic, pharmaceutical industries, etc., and is used to replace other synthetic colorants, given the nontoxic properties of paprika oleoresin. Carotenoids and xanthophylls (ketonic and hydroxylic derivatives of carotenes) confer a red color to the oil and are found in plant chromophores as colloids.

A number of studies have been carried out on paprika oleoresin, most of them focusing on its application as a colorant. It should be pointed out that changes in the flavor of some foods may occur on the addition of oleoresin. In this work we analyzed its flavor content to the exclusion of its colorant properties. The volatiles from the oleoresin of the African type of *Capsicum frutescens* were isolated by SDE both at atmospheric pressure and under vacuum (Keller et al., 1981). Several studies on the volatile compounds from *Capsicum* have been also carried out (Chitwood et al., 1983; Govindajaran, 1986; Wu and Liou, 1986).

Paprika oleoresin samples were obtained by routine industrial-scale extraction of red pepper from Murcia, Spain. We applied five extraction methods and listed the identified VOCs. Industrial production of paprika oleoresin shows strong variations in VOC content due to solvent elimination below lawful levels, by means of distillation and steam distillation with high vacuum. Analyses were performed on the same oleoresin sample in order to compare the extraction methods.

EXPERIMENTAL PROCEDURES

Extraction of VOCs. Five extraction procedures were carried out in order to obtain a clean extract prior to gas chromatographic analyses, thereby obviating the need for

further clean-up. (Parliment, 1986; Paul Thomas, 1993). The extraction methods tested were simultaneous distillation–extraction (SDE, Likens–Nickerson method) at atmospheric and low (160 mmHg) pressures, dynamic and static headspace, and purge and trap.

The SDE method was performed in the low-density solvent configuration, (Likens and Nickerson, 1964; Nickerson and Likens, 1966). The SDE at low pressure was carried out to avoid possible thermal degradation (Hiatt et al., 1994). Hexane was used as an extraction solvent (6 mL, without subsequent concentration) for both working procedures. The extraction times were 6 h in both cases, and the paprika oleoresin/water ratio was 1:2. Each extraction was carried out with 150 g of paprika oleoresin. For the extractions at low pressure, a CO₂–acetone trap between the extractor and the vacuum pump maintained the temperature from –80 °C to –20 °C, thereby avoiding leaks of solvent and volatile compounds. The SDE method at low pressure reduced the boiling point of water by 40 °C.

Dynamic headspace and purge and trap were carried out by using nitrogen as the stripping gas and carbon disulfide as the desorption solvent, without subsequent concentration (Núñez et al., 1984; Abeel et al., 1994), at 30, 50, and 80 °C. The best results were achieved at around 80 °C for both extraction methods. Nitrogen was introduced across the surface of the sample in the dynamic headspace and through the sample in the purge and trap, in both cases at 60–90 mL/min. Activated coconut carbon was used as retention material in cartridges (ORBO-32) with two adsorbent beds (of 400 and 200 mg). A backup bed determined if VOC breakthrough occurred on the front adsorbent and ensured the reliability of the method. To carry out the extraction, 30 g of paprika oleoresin was placed in a flask with an entrance for the nitrogen and an exit for the activated carbon cartridge. The extraction took 12 h in all cases.

Static headspace extraction was carried out by placing 2 g of sample in a vial of 43 mL capped with a septum and heating it at different temperatures for 1 h (Cole and Woolfenden, 1992). The sample was injected with a syringe of 1 mL in the gas chromatograph with the injector in the split mode. The best results were obtained between 80 and 100 °C, but no further significant compounds were detected, despite the rapid and simple application of this method.

All the analyses were performed on a CE 8000 Series gas chromatograph equipped with a split-splitless injector and a flame ionization detector (FID). A DB-1 column, 30 m in length, 0.251 mm i.d., with a 0.25 μm film, and helium as the

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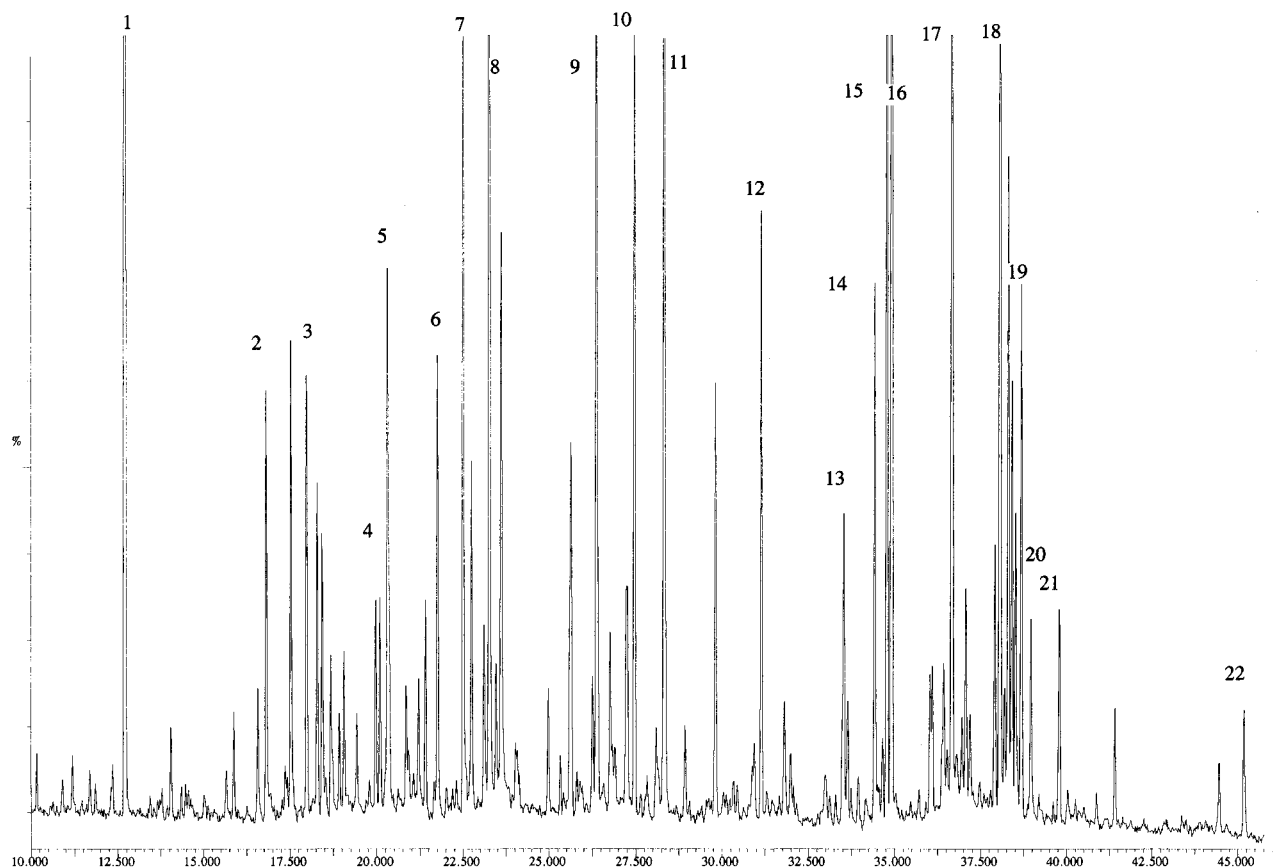


Figure 1. Chromatogram of the SDE extraction at reduced pressure including the main identified VOCs. Peak identification: 1, 1,3-dimethylbenzene; 2, benzaldehyde; 3, 6-methyl-5-hepten-2-one; 4, limonene; 5, 2,2,6-trimethylcyclohexanone; 6, 4-methylbenzaldehyde; 7, tetramethylpyrazine; 8, (*E*)-6-methyl-3,5-heptadien-2-one; 9, 2,4-dimethylbenzaldehyde; 10, safranal; 11, β -cyclocitral; 12, tridecane; 13, 1,2-dihydro-1,1,6-trimethylnaphthalene; 14, α -cubebene; 15, tetradecane; 16, β -elemene; 17, geranylacetone; 18, β -ionone; 19, α -selinene; 20, methyl 10-methylundecanoate; 21, dihydroactinidiolide; 22, methyl tetradecanoate.

carrier gas at 1 mL/min were used. Injection was split (1:16) at 250 °C. The injection volume was 1–3 μ L in the dynamic headspace, the purge and trap, and the SDE methods and 1 mL in the static headspace. The temperature program ranged from 35 °C (6 min) to 250 °C (2 min) at 3 °C/min. The detector temperature was set at 275 °C.

Identification of VOCs. Identification was carried out by HRGC-MS on a VG TS-250 double focusing sector mass spectrometer coupled to a KONIK Series 3000 gas chromatograph and a Fisons MD800 quadrupole mass spectrometer coupled to a CE 8000 Series gas chromatograph. A DB-5 column, 30 m in length, 0.25 mm in i.d., with a 0.25 μ m film was used. Injection was split (1–3 μ L, 1 mL in static headspace injection) and on-column (1 μ L). The carrier gas was helium, and the temperature program ranged from 60 °C to 275 °C at 5 °C/min. Spectra were obtained on positive-ion electron ionization mode at 70 eV, scanning from m/z 45 to 450 at 1 s/scan, and compared with those there in the NIST/NBS library (62235 spectra). Some components were identified by matching their spectral data with those of the reference compounds. Figure 1 shows a chromatogram with the peaks related to the main identified VOCs. HRGC-FID analyses were performed on a CE 8000 Series gas chromatograph in order to assess the efficiency of the extractions.

Quantification of VOCs. Quantification was performed on extracts obtained with the atmospheric-pressure Likens–Nickerson extraction method. Nerol ((*Z*)-3,7-dimethyl-2,6-octadien-1-ol) was used as an internal standard. The analytical instrumentation used and conditions set were the same as those cited in Identification of VOCs. The total VOC content was calculated by atmospheric pressure SDE extraction of 486.8 g of paprika oleoresin using pentane as the solvent in order to allow fast and easy vaporisation.

RESULTS AND DISCUSSION

The five extraction methods were applied to samples from the same industrial extraction, and consequently the results were comparable. Some VOCs which can be observed at the end of the HRGC-FID chromatograms using the SDE extraction at atmospheric pressure did not appear when the process was carried out at reduced pressure. Despite the application of higher temperatures at atmospheric pressure, the former method resulted in the extraction of more VOCs.

However, the SDE at reduced pressure must not be discarded for the extraction of VOCs of paprika oleoresin, as this method enables us to obtain the most volatile of the VOCs, which give the most aromatic characteristics to the oleoresin. No thermal degradation of VOCs was detected in the results obtained with the two SDE methods.

As expected, better results in terms of the amount and number of VOCs obtained were achieved in the HRGC-FID chromatograms from purge and trap than in the those using the dynamic headspace method. The greatest yield from the extractions at the highest temperatures can be attributed to the increase in pressure of each volatile compound, and the best mobility inside the paprika oleoresin is due to the diminution of viscosity.

Static headspace is a very rapid method to extract a few characteristic VOCs from paprika oleoresin and to obtain a pattern which allows us to assess the product by the presence of specific VOCs. The biggest drawback is the small number of VOCs that can be isolated. This

Table 1. Identified Compounds in the Samples of Paprika Oleoresin

compound	best extraction method ^a	concentration (mg/kg) ^b	identification ^c
Hydrocarbons			
<i>m</i> -xylene [1,3-dimethylbenzene]	ABCDE	11.5	MS/RC
limonene [1-methyl-4-(1-methylethenyl)cyclohexene]	BD	0.6	MS/RC
α -ocimene [3,7-dimethyl-1,3,7-octatriene]	B	<0.1	MS
terpinolene [1-methyl-4-(1-methylethylidene)cyclohexene]	B	<0.1	MS/RC
tridecane	AB	1.2	MS/RC
1,2-dihydro-1,1,6-trimethylnaphthalene	ABCD	0.8	MS
1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene	B	<0.1	MS
α -cubebene [1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)naphthalene]	ABCD	1.8	MS
β -elemene [1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-cyclohexane]	ABCD	11	MS/RC
Tetradecane	AB	5.3	MS/RC
caryophyllene [4,11,11-trimethyl-8-methylenebicyclo-[7.2.0]undec-4-ene]	B	<0.1	MS
eremophyllene [1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)naphthalene]	B	<0.1	MS
β -guaiene [1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)azulene]	A	<0.1	MS
α -selinene [1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)naphthalene]	AB	4	MS
pentadecane	AB	<0.1	MS/RC
Alcohols			
1,3-butanediol	D	<0.1	MS
7-octen-4-ol	BD	<0.1	MS
2-phenylethanol	CD	<0.1	MS/RC
Aldehydes			
hexanal	AB	<0.1	MS/RC
(<i>E,E</i>)-2,4-heptadienal	AB	<0.1	MS
2,4-decadienal	B	<0.1	MS
benzaldehyde	B	1.3	MS/RC
4-methylbenzaldehyde	AB	1.3	MS
2,4-dimethylbenzaldehyde	ABCD	1.8	MS
safranal [2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde]	ABCD	1.6	MS
β -cyclocitral [2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde]	ABCD	2.2	MS/RC
Ketones			
6-methyl-5-hepten-2-one	ABD	0.9	MS
(<i>E</i>)-3,7-octadien-2-one	B	<0.1	MS
2,2,6-trimethylcyclohexanone	BCD	1.2	MS
(<i>E</i>)-6-methyl-3,5-heptadien-2-one	ABC	5.2	MS
1-(methylphenyl)ethanone	ABCD	<0.1	MS
α -ionone [(<i>E</i>)-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one]	ABC	0.4	MS/RC
dihydro- β -ionone [4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone]	ABCD	0.6	MS
geranylacetone [(<i>E</i>)-6,10-dimethyl-5,9-undecadien-2-one]	ABCD	7.9	MS/RC
β -ionone [(<i>E</i>)-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one]	BD	7.8	MS/RC
(<i>E,E</i>)-6,10,14-trimethyl-5,9,13-pentadecatrien-2-one	ABC	0.4	MS
Acids			
dodecanoic acid	A	<0.1	MS/RC
tetradecanoic acid	ABC	<0.1	MS/RC
hexadecanoic acid	ABC	<0.1	MS/RC
Esters			
methyl salicylate	B	<0.1	MS/RC
methyl 10-methylundecanoate	ABC	0.9	MS
methyl tetradecanoate	ABC	0.8	MS
Lactones			
dihydroactinidiolide [(<i>R</i>)-5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4 <i>H</i>)-benzofuranone]	ABCD	0.9	MS
Pyrazines			
tetramethylpyrazine	ABCD	2.9	MS

^a Extraction methods: A: SDE at atmospheric pressure. B: SDE at low pressure (160 mmHg). C: purge and trap at 80 °C. D: dynamic headspace at 80 °C. E: static headspace. ^b Quantification obtained using SDE method at atmospheric pressure. (This method provides the highest yield of VOCs). ^c Identification methods: MS: mass spectrometry, compared with NBS and Wiley mass spectral data bases. RC: comparison with authentic reference compound.

method is not very suitable for paprika oleoresin, but it could be considered for the analysis of the VOCs with a low boiling temperature. Its main advantage is the absence of solvent which interferes with the first peaks of the chromatograms.

Table 1 shows all the VOCs identified in paprika oleoresin grouped according to the chemical substitu-

ents, maintaining the elution order in the chromatogram. The best extraction methods are indicated for each compound. The quantification results are also shown.

The treatments to eliminate the solvent (hexane, dichloromethane) in the industrial process of obtaining paprika oleoresin lead to a considerable diminution of

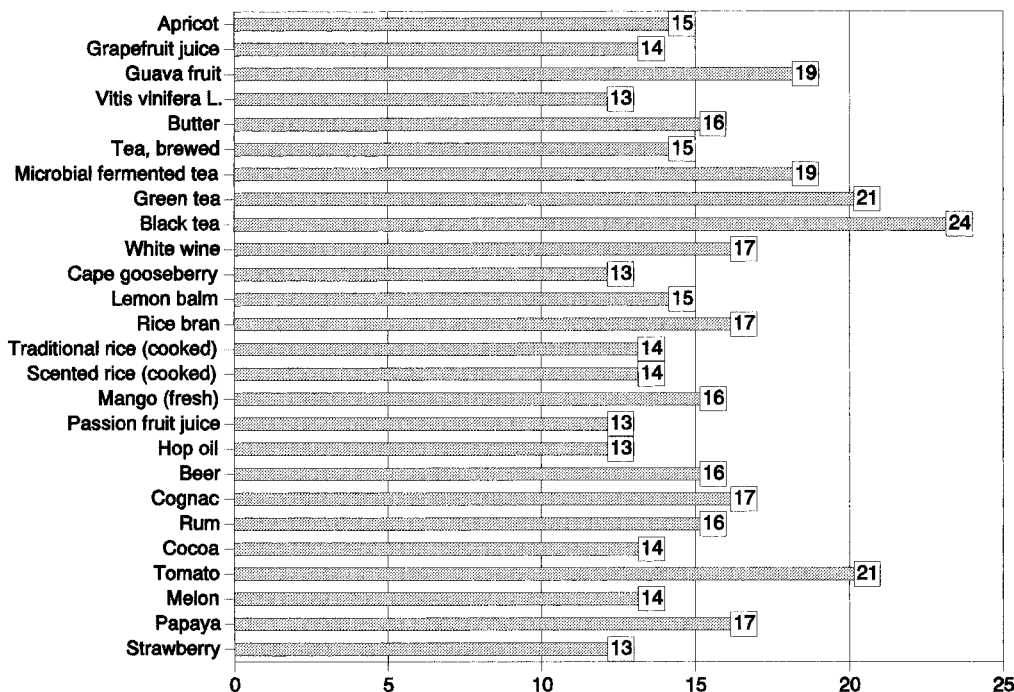


Figure 2. Number of volatile organic compounds identified in paprika oleoresin and previously identified in another foods.

VOCs and to a large discrepancy in the total amount of these compounds. Bearing this in mind, a content of 0.0545 g of VOCs (0.0112% w/w) was estimated in the paprika oleoresin used.

The presence of carotenes in red pepper is of importance in the identification of VOCs cited in Table 1, β -carotene being the most characteristic. Its polyenic chain undergoes oxidation easily, yielding cyclic and noncyclic products containing often an oxygenated functional group in a trimethylcyclohexane ring or an oxygenated functional group in an allylic chain. So, the presence of 1,2-dihydro-1,1,6-trimethylnaphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene, α -ionone, β -ionone, dihydro- β -ionone, dihydroactinidiolide, 2,6,6-trimethylcyclohexanone, and β -cyclocitral suggests that β -carotene may be considered to be its precursor. Safranal may also be considered to be a carotene derivative (Weeks, 1986). The presence of 6-methyl-5-hepten-2-one, (*E*)-3,7-octadien-2-one, (*E*)-6-methyl-3,5-heptadien-2-one, geranylacetone and (*E,E*)-6,10,14-trimethyl-5,9,13-pentadecatrien-2-one is due to the fact that they are carotenoid derivatives (Buttery and Ling, 1992). Identification of 2-phenylethanol could be explained via transformation of the amino acid phenylalanine (Buttery and Ling, 1992). Benzaldehyde and related volatile compounds can be explained as products of hydrolysis of glucosides (Buttery and Ling, 1992).

In general, although pyrazines are present in small quantities in natural samples, their contribution to the flavor of these samples is very considerable. Their source is a Maillard reaction, e.g., tetramethylpyrazine (Parliment, 1989).

The following VOCs are derivatives from lipids: hexanal, (*E,E*)-2,4-heptadienal, 2,4-decadienal, and 7-octen-4-ol, and dodecanoic, tetradecanoic, and hexadecanoic acids (Tressl et al., 1981).

Figure 2 shows the foods in which the VOCs present in paprika oleoresin have been previously identified (Maarse and Visschser, 1992). Thus, the number of the VOCs identified in a given food and in paprika oleoresin is correlated.

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Received for review April 18, 1996. Revised manuscript received February 10, 1997. Accepted February 10, 1997.[®]

JF960266I

[®] Abstract published in *Advance ACS Abstracts*, April 1, 1997.