Determination of Volatile Components in Fresh, Frozen, and Freeze-Dried Padrón-Type Peppers by Gas Chromatography–Mass Spectrometry Using Dynamic Headspace Sampling and Microwave Desorption

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Abstract

"Padrón-type" peppers are a small variety of *Capsicum annuum* cultivated mainly in Galicia, Spain. To compare the effects of freezing and freeze-drying on the volatile components of Padrón-type peppers, preserved samples are analyzed by means of dynamic headspace sampling on an adsorbent followed by microwave desorption into a gas chromatograph equipped with a mass spectrometric detector. Sixty-five compounds are identified, including hydrocarbons, terpenes, alcohols, phenols, ethers, aldehydes, ketones, esters, pyrroles, pyrazines, and sulfurous compounds. Fresh whole, homogenized, and freeze-dried peppers have characteristic volatile-component profiles, whereas frozen peppers have a highly variable volatile-component profile.

Introduction

The climate and isolated nature of the Atlantic coastal region around Padrón in Galicia (northwest Spain) have proven ideal for the cultivation of several small varieties of green peppers (*Capsicum annuum* L.) belonging to the Solanaceae family (1). These peppers, cultivated in the Padrón area (and elsewhere in Spain), are designated "Padrón-type" peppers. They are generally shaped like a cone or truncated cone and have a slightly rugose, shiny, green skin marked with three or four ridges that converge towards the apex. Typically, they are commercialized and eaten when immature, 3–5 cm in length, and 1–2 cm in breadth at the base (2). Their flavor is generally not "hot", although some varieties and cultivars contain higher levels of capsaicin, the principal hot component of peppers formed during fruit maturation.

The traditional quality parameters for capsicum fruits are color and pungency. Currently, however, flavor is gaining importance as a quality parameter for fresh fruit and vegetables (3). In view of this, volatile-component profiles of Padrón-type peppers cultivated in Galicia are determined in this work, com- paring the results of peppers preserved by hand- or vacuum-packing and freezing at –22°C with those preserved by freeze-drying and storing at room temperature. In each case, volatile components were determined one month after preservation.

In previous studies of related fresh and processed capsicum fruits (4–7), extraction procedures such as steam or vacuum distillation, solvent extraction, and static headspace sampling have allowed identification of more than 125 different volatile components. Some of the limitations inherent to these extraction procedures (the formation of artifacts, the need to pre-concentrate samples prior to analysis, and the possibility of sample contamination by solvents) can be overcome using dynamic headspace sampling methods (purge and trap). In this work, the volatile components of Padrón-type peppers were extracted using a purge-and-trap method, adsorbed onto graphitized carbon black, and rapidly desorbed by means of microwave heating. The principles and characteristics of this technique have been described (8), and its application to the analysis of various volatile compounds has been reported (9–13). The use of microwave energy for this purpose is especially attractive, because it allows more uniform heating of the sample than traditional thermal methods and can selectively desorb components according to their individual microwave absorption capacity (8). The desorbed volatile components were identified by gas chromatography–mass spectrometry (GC–MS).

Experimental

Apparatus and operating conditions

Whole peppers were sealed in polyethylene bags under a vacuum by means of a Henkovac 1900 (Howden Propak, Melbourne, Australia) apparatus operating at 5.33 kPa (40 mm Hg) for 16 s and subsequently frozen at –22°C for 1 month. Fresh peppers were homogenized in a Moulinex Minipro (Moulinex, Guipúzcoa, Spain) at low speed for 5 min. Homogenized

peppers were freeze-dried in a Liolabore 3 Telstar (Teistard, Tarrasa, Spain) apparatus operating at 0.1 mm Hg between –40 and 30°C, then stored in airtight flasks for one month.

The volatile components of the peppers were determined using a Rektorik (Geneva, Switzerland) MW-1A microwave sampler coupled via a heated interface to a GC–MS system (Fisons model 8000 with MSD 800, Manchester, UK). The output from the detector was fed into a personal computer running Masslab software (version 1.1). The operating conditions were as follows: column, J&W Scientific (Folsom, CA) DB-5 (30 m × 0.25-mm i.d. fused-silica capillary column with a 1-µm film of 5% diphenyl-polysiloxane–95% dimethybpolysiloxane as stationary phase); carrier gas, helium; flow rate, 2.45 mL/min; average linear velocity, 35 cm/s; head pressure, 0.8 bar adjusted at the MW-1A; split, 30-mL/min; injection mode, microwave desorption; interface temperature, 220°C; backflush delay, 20 s; desorption power, 7 (arbitrary units); bypass delay, 6 s; desorption time, 0.8 s; MSD interface temperature, 250°C; source temperature, 200°C; mode, electronic impact at 70 eV; mass range, m/z 35–200; scan delay time, 0.45 s; interscan delay time, 0.05 s; GC oven temperature, 60°C for 1 min to 220°C at 4°C/min, held for 3 min (45 min total).

Trap material

The traps used for the sampling were ceramic Rektorik 1-010B and 1-010R trapping tubes (10 cm × 3.5-mm i.d.) containing approximately 60 mg graphitized carbon black and activated charcoal, respectively. Prior to sampling, the traps were conditioned by thermally desorbing any adsorbed trace contaminants three times with microwave energy (purge mode). The released contaminants were flushed via the split vent. A desorption GC–MS test run was performed in order to monitor the efficiency of the cleanup process.

Samples

Padrón-type peppers (*Capsicum annuum* L.) were obtained from a commercial plantation in northwest Spain (Herbón, Corufia). They were planted in open fields in March and harvested in July. A total of six 1-kg lots of peppers were sampled in order to evaluate interlot variability. The volatile compounds of fresh peppers (whole and homogenized) were determined on the day of collection.

Preservation procedures

Subsamples (200 g) of the lots of harvested peppers were placed in polyethylene bags. Six such lots were sealed manually, expelling as much of the air as possible, and another six lots were mechanically sealed under vacuum. These lots were deep-frozen at –22°C. Another six lots were freeze-dried.

Dynamic headspace sampling of volatile components

Padrón-type peppers (15 g

whole fresh pepper, 15 g frozen whole pepper, 2 g homogenized fresh pepper, or 0.2 g freeze-dried pepper) were transferred to an impinger flask for dynamic headspace sampling of volatile compounds (Figure 1). The flask was heated in a water bath at 40°C, and nitrogen was passed through the sample for 1 h at 60 mL/min onto a trap containing graphitized carbon black, where any volatile components were adsorbed. An activated charcoal trap was placed before the sample to remove artifacts derived from the nitrogen line.

Analysis of volatile components

The trapped components were thermally desorbed using microwave energy (350 MHz for 0.8 s) and directed into an in-line GC capillary column without the need for an additional retrapping unit. The microwave generator supplied energy to a rectangular cavity. After the introduction of the trap into the cavity, the air remaining in the trap was purged with carrier gas. Upon microwave heating, the substances were desorbed and swept across a heated interface into the capillary column within a few seconds. Because the adsorbent was fixed in the ceramic trapping tubes by quartz wool, microwave energy heated the sorbent material only.

After each run, the traps were desorbed once more to ensure that all trapped material was injected into the column. Just before another sample collection, the trap was baked again using microwave energy to reduce interferences. Some typical total ion chromatograms of volatile components are shown in Figure 2.

Identification of volatile components

Components were identified by comparing their mass spectra with the Wiley (New York, NY) mass spectra library (version 1.4). In all, 65 compounds were identified with a resemblance percentage of 85% (Table I).

Comparison of volatile-component profiles

The chromatographic profiles of the volatile components identified for various samples were compared using analysis of variance (ANOVA) implemented by the computer program SPSS for Windows (SPSS, Chicago, IL). Specifically, peak areas per gram of sample were compared in order to assess the effects of various factors and their two-way interactions on
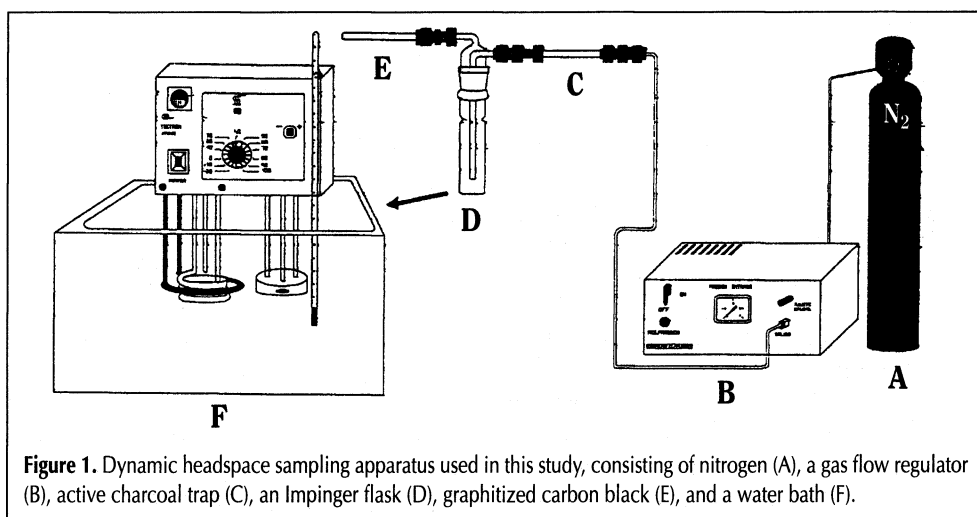
Figure 1. Dynamic headspace sampling apparatus used in this study, consisting of nitrogen (A), a gas flow regulator (B), active charcoal trap (C), an Impinger flask (D), graphitized carbon black (E), and a water bath (F).

Figure 2. Total ion chromatograms of volatile components in whole (A), homogenized (B), vacuum-packed and frozen (C), hand-packed and frozen (D), and freeze-dried (E) Padrón-type peppers. Compounds are identified by the peak numbers shown in Table I.

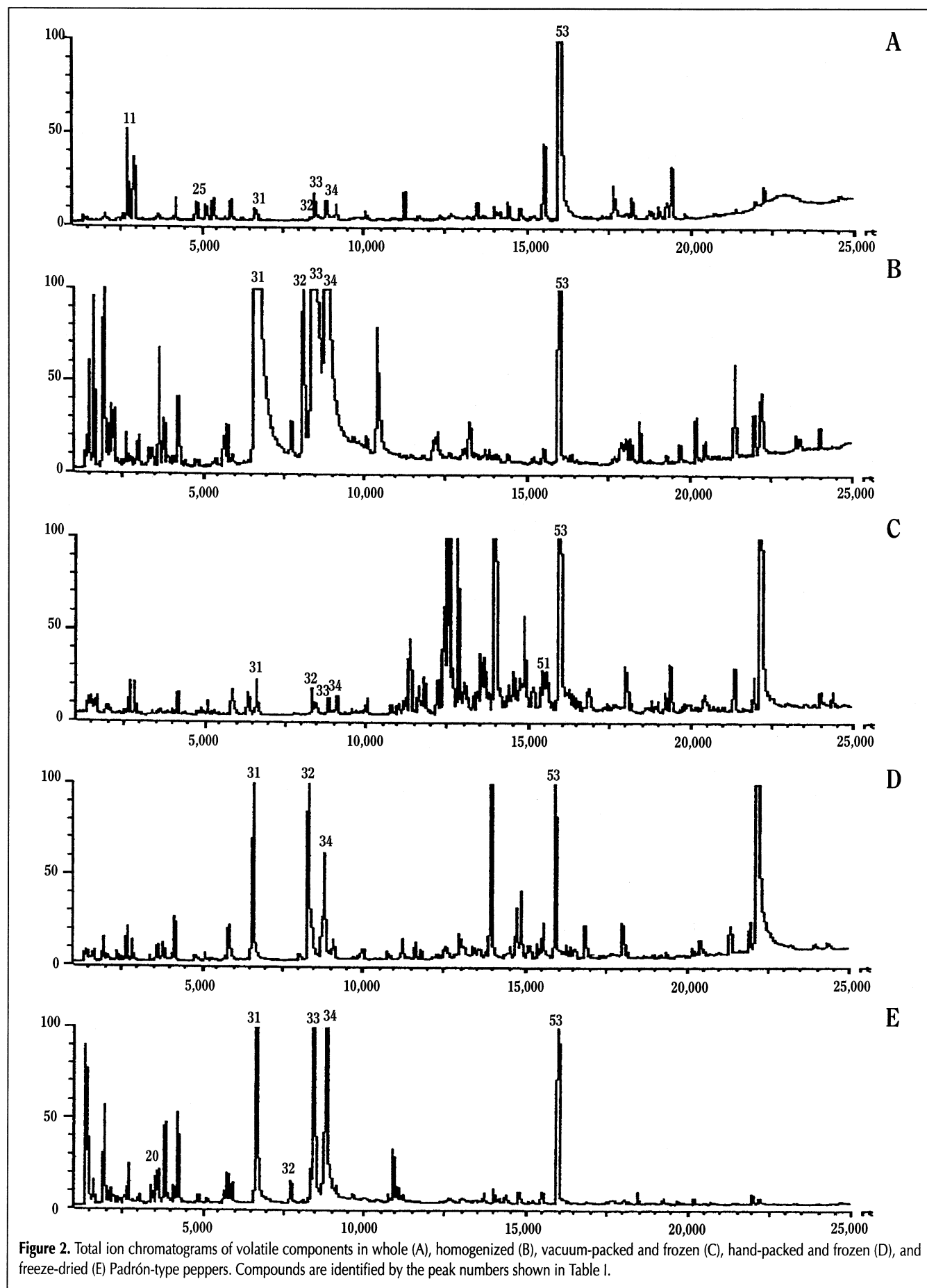**Table I. Volatile Components Identified in Whole (W), Homogenized (H), Vacuum-Packed and Frozen (V), Hand-Packed and Frozen (HP), and Freeze-Dried (F) Padrón-Type Peppers by Dynamic Headspace Sampling–GC–MS***

Number	Retention time	Compound name	W	HP	V	H	F	Type†	Wiley Library number
1	1.459	1-Propene		+				10	59
2	1.609	1,3-Butadiene		+				10	118317
3	1.717	Ethanol	+					1	94
4	1.901	1-Pentene		+	+	+	+	10	350
5	1.926	2-Pentene		+				10	351
6	1.934	Pentane	+		+	+	+	10	118774
7	2.009	2-Methyl-1,3-butadiene=isoprene	+	+	+	+	+	10	118589
8	2.126	1,3-Pentadiene		+		+		10	118584
9	2.243	1,3-Cyclopentadiene		+				10	259
10	2.626	1-Hexene		+				10	119201
11	2.712	Hexane	+	+	+	+	+	10	1018
12	2.912	2-Methyl-furan	+		+	+		8	663
13	2.968	1-Hexyne		+			+	10	119077
14	3.043	1,3-Pemadiene 3-methyl		+			+	10	683
15	3.043	2,4-Hexadiene					+	10	678
16	3.360	2-Hexen-4-yne		+				10	600
17	3.418	3-Methyl-butanal=isovaleraldehyde					+	2	119356
18	3.643	Benzene	+	+	+	+	+	10	119006
19	4.094	3-Pentanone		+				3	119370
20	4.196	Heptane	+		+	+		10	120425
21	4.253	2-Ethyl-furan		+			+	8	1503
22	4.378	1,4-Dioxane	+	+	+	+		8	119546
23	4.477	Cyclohexanone					+	3	120065
24	4.854	Methyl cyclohexane	+					10	120140
25	5.146	1-Methyl-1H-pyrrole	+	+	+	+	+	9	119054
26	5.346	Dimethyl disulfide	+		+	+		11	119805
27	5.653	1-Pentanol		+			+	1	1169
28	5.753	2-Penten-1-ol		+			+	1	975
29	5.897	Methyl-benzene	+		+	+	+	10	1321
30	6.346	Ethyl-2-hexene-1			+			10	3570
31	6.639	Hexanal	+	+	+	+	+	2	120294
32	8.396	2-Hexenal	+	+	+	+	+	2	1687
33	8.483	3-Hexen-1-ol	+	+	+	+	+	1	120364
34	8.866	1-Hexanol	+	+	+	+	+	1	2364
35	9.150	1,2-Dimethyl-bencene	+		+	+		10	2699
36	10.415	2,4-Hexadienal		+				2	1487
37	10.790	Isocitronellen			+	+		5	9653
38	11.242	2,6-Dimethyl-1,7-octadien				+		10	9645
39	11.268	Tricyclene	+		+	+		5	124417
40	11.640	α-Pinene	+		+	+	+	5	124388
41	11.807	3,7-Dimethyl-1,6-octadiene				+		10	9644
42	12.266	2,3,6-Trimethy-1,4,6-heptatriene	+					10	8984
43	12.599	1-Ethyl-4-methyl-benzene			+	+		10	5057
44	12.957	Phenol		+		+		7	1389
45	13.119	p-Menth-2-ene				+		5	9707
46	13.569	Pinane			+	+		5	9740
47	13.699	2-Pentyl-furan					+	8	9517
48	13.966	Decane			+	+		10	125045
49	14.458	Carane			+	+		5	124650
50	15.162	1,3,5-Trimethyl-benzene				+		10	5060
51	15.476	Limonene	+	+	+	+	+	5	124342
52	15.550	1,8-Cineole			+	+		5	126660
53	15.987	δ-3-Carene	+	+	+	+	+	5	124411
54	16.850	Dihydro-myrcenol	+		+	+		5	16451
55	17.631	α,α-Dimethyl-benzenemethanol	+					1	124244
56	17.897	Linalool	+	+	+			5	126535

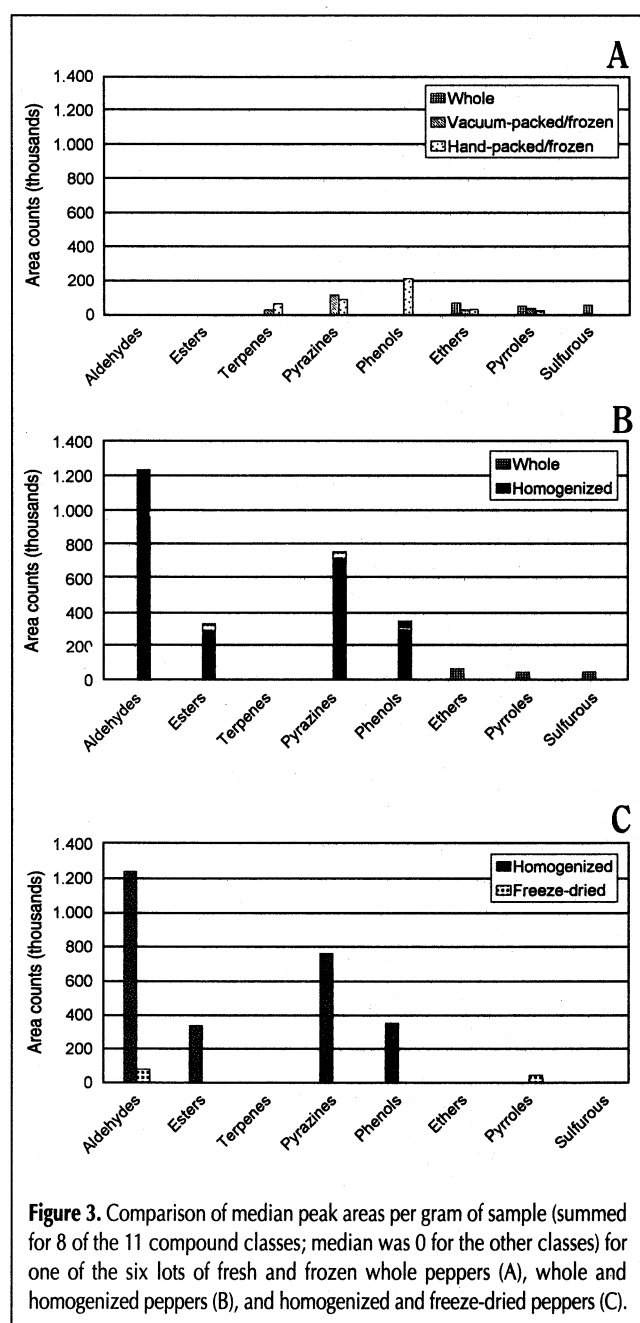* A plus symbol (+) denotes that the compound was detected.
† Component classes: 1, alcohol; 2, aldehyde; 3, ketone; 4, ester; 5, terpene; 6, pyrazine; 7, phenol; 8, ether; 9, pyrrol; 10, hydrocarbon; and 11, sulfur.

Table I (continued). Volatile Components Identified in Whole (W), Homogenized (H), Vacuum-Packed and Frozen (V), Hand-Packed and Frozen (HP), and Freeze-Dried (F) Padrón-Type Peppers by Dynamic Headspace Sampling–GC–MS*

Number	Retention time	Compound name	W	HP	V	H	F	Type†	Wiley Library number
57	18.002	Undecane			+			10	126867
58	18.206	Nonanal	+		+			2	125003
59	18.461	Isobutyric acid hexyl ester		+			+	4	23227
60	19.282	2,6-Dimethyl-2,4,6-octatriene	+		+		+	10	9113
61	19.729	Butanoic acid 3-hexenylester		+			+	4	22325
62	20.373	2-Tridecenal			+			2	34000
63	21.347	2-Isobutyl-3-methoxypyrazine	+		+	+		6	20259
64	22.002	Dodecane					+	10	123225
65	22.196	Methyl-salicylate		+	+	+	+	4	14253

* A plus symbol (+) denotes that the compound was detected.
† Component classes: 1, alcohol; 2, aldehyde; 3, ketone; 4, ester; 5, terpene; 6, pyrazine; 7, phenol; 8, ether; 9, pyrrol; 10, hydrocarbon; and 11, sulfur.

Figure 3. Comparison of median peak areas per gram of sample (summed for 8 of the 11 compound classes; median was 0 for the other classes) for one of the six lots of fresh and frozen whole peppers (A), whole and homogenized peppers (B), and homogenized and freeze-dried peppers (C).

the volatile-component profile. Three different analyses were performed, and the controlled factors were as follows: volatile component (65 levels), type of preservation treatment (3 levels: fresh, frozen in vacuum-sealed bags, frozen in hand-sealed bags), and sample lot (6 levels); volatile component (65 levels), state of fruit (2 levels: whole fresh fruits, homogenized fresh fruits), and sample lot (6 levels); volatile component (65 levels), preservation treatment applied to homogenized fruit (2 levels: homogenized fresh fruits [i.e., no preservation] and freeze-dried fruits), and sample lot (6 levels).

Later, to reduce the number of data to be compared, the lot with the median peak area per gram of sample was chosen from among the 6 lots subjected to each preservation treatment; for these samples, peak areas per gram for 11 classes of components were summed. These data are displayed in Figure 3.

Results and Discussion

One source of the volatile compounds responsible for the flavor of capsicum fruits is the rapid hydrolytic and oxidative degradation of endogenous unsaturated lipids (mainly those containing linoleic or linolenic acid) that accompanies tissue disruption (7). Indeed, hexanal, 2-hexenal, hexanol, and 3-hexenol (compounds identified in Padrón-type peppers) are known to be the principal volatile products of disruption of the tissues of bell peppers (6). These six-carbon alcohols and unsaturated aldehydes, which have been described as "grassy" and reminiscent of "crushed leaves" (3), are thought to form by enzyme-catalyzed oxidative cleavage because the content in them is considerably decreased by the addition of hydroperoxide quenchers, such as stannous chloride (6). Other compounds whose formation is favored by tissue disruption are 2-methyl- and 2-ethylfuran, both of which are derived from linolenic acid (6).

The only pyrazine identified in Padrón-type peppers was 2-isobutyl-3-methoxypyrazine, which has as distinct "pepper" aroma (14,15). This pyrazine has also been found in other varieties of capsicum (3–5,7).

Another important group of detected aromas was the terpenes, which are often used as food flavorings because of their diverse and usually pleasant characteristics (15). All of the

samples contained δ-3-carene, a common component of many spices (3,16) and the major monoterpene detected in other capsicum varieties (16).

Among the ester components, the most abundant was methyl salicytate, which was detected in all but the whole peppers. This compound is thought to derive from the breakdown of amino acids (9).

All three ANOVA analyses indicated that the volatile-component profile of the samples was significantly affected by the preservation treatment and, as would be expected, the nature of the volatile components. The interaction between these two independent variables was also significant ($p \leq 0.05$). Thus, each preservation treatment affects the volatile-component profile differently and could feasibly give rise to a characteristic volatile-component profile.

Variation among lots was only significant ($p \leq 0.05$) in the first ANOVA analysis (i.e., whole fresh peppers and hand- or vacuum-packed frozen peppers). The two-way interactions involving the factor "lot" (lot by volatile component and lot by preservation treatment) were also significant. This variation among lots would make it difficult to observe a characteristic volatile-component profile for frozen peppers.

In the characteristic volatile-component profiles for fresh whole or homogenized peppers and for freeze-dried peppers, certain compound types dominated. The profiles for the typical samples in Figure 3 clearly show which compounds these were and how their pepper content varied. There were three important changes. First, freezing whole fresh peppers led to increases in their terpene and pyrazine content and the appearance of phenols in hand-packed frozen peppers. Second, the homogenization of peppers led a to complete loss of volatiles, such as ethers, pyrroles, and sulfurous compounds, but not pyrazines. Concurrently, there were marked increases in pepper contents in aldehydes, esters, pyrazines, and phenols. Third, the compound types whose contents increased upon homogenization (i.e., aldehydes, esters, pyrazines, and phenols) virtually disappeared after freeze-drying, whereas pyrrole content increased.

Conclusion

The technique of GC–MS with dynamic headspace sampling and microwave-induced thermal desorption proved highly suitable for the identification of volatile components of Padrón-type peppers, allowing the identification of characteristic volatile-component profiles for fresh whole, homogenized, and freeze-dried peppers and a highly variable volatile-component profile for frozen peppers. There are no data for the volatile components of Padrón-type peppers available for comparison. Nonetheless, the profiles obtained here showed little evidence of artifacts that are often detected when foodstuffs are analyzed following extraction with solvents or methods such as steam distillation. This technique should be suitable for the identification of volatile components in other fruits, the monitoring of flavor quality, and the development of new cultivars with specific flavor characteristics.

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