sulfite modification induces a significant unfolding of the native structure that altered 2-nonanone binding.

These data demonstrate that the binding of flavor compounds to β-Lg is affected by the conformational states of proteins as reported earlier for bovine serum albumin (Kinsella, 1980) and soy proteins (Damodaran and Kinsella, 1981a,b). Chemical or physical changes that alter the conformational states produce marked changes in the flavor-binding characteristics of proteins. Future research should be directed toward obtaining a better understanding of the relationship between protein structure and the binding of flavor molecules. In this regard β-Lg is a good model for food protein because its structure and conformation are very well described (Sawyer et al., 1985; Creamer, 1983; McKenzie, 1971). In addition β-Lg has good binding properties as summarized in Table II. Noteworthy from the data (Table II) is its extremely high affinity for retinol, which reflects a considerable homology between the structures of β-Lg and retinol binding protein (Perviaz and Brew, 1985) and may reflect a physiological function of β-Lg as a carrier or retinol (Perviaz and Brew, 1985).

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Registry No. 2-Heptanone, 110-43-0; 2-octanone, 111-13-7; 2-nonanone, 821-55-6.

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Principal Constituents of Black Truffle (*Tuber melanosporum*) Aroma

Thierry Talou,* Michel Delmas, and Antoine Gaset

A dynamic headspace technique was employed for isolating black perigord truffle (*Tuber melanosporum*) aroma volatiles using a Tenax trap. The volatiles eluted from the trap by heat desorption were analyzed by capillary gas chromatography and coupled capillary gas chromatography–mass spectrometry. A total of 14 volatiles was identified, from which 9 compounds could be described for the first time as black perigord truffle aroma constituents. Quantitatively, alcohols were predominant.

Truffles are subterranean edible fungi (order Tuberales) that grow in various parts of Europe, particularly in France. The black perigord truffle (*Tuber melanosporum*) is a blackish fruit with finely veined flesh.

Its flavor is particularly praised by mushroom fanciers. The aroma impression has been described as slightly sulfurous (Andreotti and Casoli, 1968), but little has been published about black perigord truffle volatiles.

Recent studies were carried out by Ney and Freitag

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(1980), who identified several alcohols, an amine, and a sulfur compound after steam distillation, and by Claus et al. (1981), who showed the presence of a steroidal pheromone explaining, according to the authors, the ability for pigs to detect truffles underground. But no aromagram of black perigord truffle has been published.

Additional work (Fiecchi et al., 1967) has also been done exclusively on one volatile constituent of white truffle (*Tuber magnatum*).

We report here the identification of the principal volatile constituents of black perigord truffle aroma using a Tenax-trapping technique.

EXPERIMENTAL SECTION

Materials. Fresh black perigord truffles (*Tuber melanosporum*) were collected directly from the field prior to having been exposed to freezing temperatures and were analyzed the day after gathering. Some samples were obtained from commercial sources available in France (Pebeyre Ltd., Cahors, France), but in this case they were kept a few days in cold storage.

Sample Preparation. Weights of brushed truffles analyzed ranged from 15 to 20 g. Truffles were fully ripe (Montant et al., 1983; Kulifaj, 1984), and therefore they had their characteristic aroma. Samplings were carried out in truffle flesh and intact analyzed.

Headspace Sampling Using the DCI System. The method used the new gas chromatographic device DCI (desorption–concentration–introduction) based on dynamic headspace analysis, available from Delsi Instruments (Gregoire and Samoun, 1982).

A 200-mg sample of truffle was put into the crucible of the desorption oven, located upstream of a Tenax trap (200 mg of Tenax GC, 60–80 mesh, packed into 7-cm-length by 2-mm-i.d. steel tube). At ambient temperature (25 °C), at low pressure (1.5 psi), and for 5 min, a 20 mL/min flow of He moved the equilibria to desorption of aroma volatiles to be analyzed. Desorbed and diluted in scavenging gas, they were then concentrated and trapped in the Tenax trap, cooled at −30 °C by circulation of liquid nitrogen. By switching a rotative valve, carrier gas flowed through the trap toward the GC column. By rapid thermal desorption at 240 °C, aroma volatiles were then directly transferred from the trap into the GC column, and the GC program was simultaneously started.

In Figure 1, the schematic diagram of the DCI system is represented.

Capillary Gas Chromatography (HRGC). A DCI system coupled with a GIRDEL 30 gas chromatograph, fid equipped, with a coated fused silica UCON 75H90000 capillary column (50 m, 0.32 mm (i.d.), film thickness 0.2 μm) was used. The temperature program was isothermal at 30 °C for 5 min, 30–50 °C at 2°/min, and 50–160 °C at 5°/min. Column inlet pressure was 11 psi of He and the split 50 mL/min. Flow rates for detector gases were 25 mL/min H_2 and 500 mL/min air, respectively. Detector temperature was kept at 200 °C, and attenuation varied from 8 to 128 × 10^{-11} aps.

Quantitative HRGC determination was carried out on an ENICA 10 integrator.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A GIRDEL 31 gas chromatograph equipped with a DCI system was coupled by a glass line tubing interface to a NERMAG R10-10B mass spectrometer (quadrupole mass filter instrument). The system was connected on-line to a data processing system provided by a Digital Equipment Corp. PDP8 computer, using SIDAR software, including the library of mass spectral data NIH/EPA.

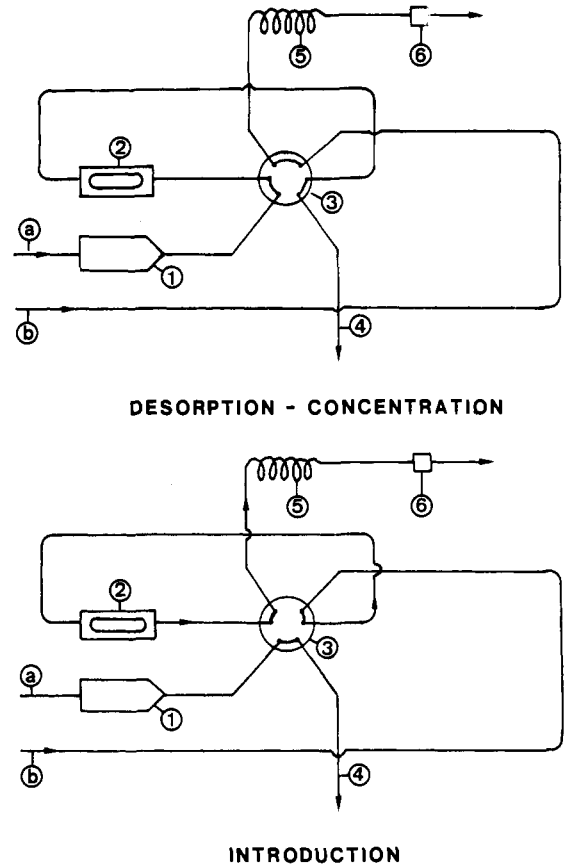

Figure 1. Schematic diagram of the apparatus: (a) scavenger gas; (b) carrier gas; (1) desorption oven; (2) Tenax trap; (3) switching valve; (4) gas vent; (5) GC column; (6) fid detector.

GC conditions were as follows: temperature, isothermal for 10 min at 30 °C and then from 30 to 160 at 3°/min; column inlet pressure, 7 psi; split, 30 mL/min.

Significiant MS operational parameters: electron ionization voltage, 70 eV; source temperature, 200 °C; electron multiplicator tension, 1.9 kV; emission current, 200 μA; integration time, 1 ms/uma. For optimum sample transfert, an interface temperature of 200 °C was adopted.

Data system facilities such as background subtraction, various scale expansions, and manual deconvolution of unresolved GC peaks were extensively employed in evaluating the mass spectral data.

Authentic Compounds. Authentic chemical samples for comparison were obtained from a commercial source (Aldrich). For all of them, identities were verified by spectral (MS) means and comparison with published data (Stenhagen et al., 1974).

RESULTS AND DISCUSSION

Separate HRGC–MS analysis were carried out on 10 different fresh black perigord truffles (five of which were provided by Pebeyre Ltd.) harvested during the period March 1985–March 1986. This was to ensure that the qualitative analysis was consistent and that some idea could be obtained concerning the quantitative variation.

The results of HRGC separation of black perigord truffle volatiles are outlined in Figure 2. GC peaks gave mass spectral data that, on inspection, indicated the presence of more than one component. Further manipulations of the data, using in particular the background subtraction and the manual deconvolution of these peaks, enabled generation of pure spectra of the components, substantially free of contamination, hence facilitating interpretation.

The compounds identified by HRGC and HRGC–MS

Table I. Volatiles Identified in Black Perigord Truffles by HRGC and HRGC–MS

peak no.[a]	compound	mass spectral data[b]	rel %
	Alcohols		
8	ethanol	31, 45, 46, 29	30–40
9	2-butanol[c]	45, 31, 59, 29	0.2–0.4
10	1-propanol	31, 29, 42, 27	1–2
11	2-methyl-1-propanol	43, 33, 42, 41	17–24
12	2-methyl-1-butanol[c]	41, 29, 57, 56	12–20
13	3-methyl-1-butanol[d]	41, 29, 42, 55	tr
	Aldehydes		
1	acetaldehyde[c]	29, 44, 43, 42	3–5
4	2-methylpropanal[c]	43, 41, 72, 27	5–7
6	2-methylbutanal[c]	41, 57, 29, 58	3–4
7	3-methylbutanal[c,d]	44, 41, 43, 29	tr
	Ether		
14	anisole[c]	108, 78, 65, 39	0.05–0.1
	Ketones		
3	acetone[c]	43, 58, 42, 39	5–10
5	2-butanone[c]	43, 72, 29, 27	2–2.5
	Sulfur Compound		
2	dimethyl sulfide	42, 62, 45, 46	2–6

[a] The peak numbers correspond to numbers in Figure 1. [b] The four most intense peaks are represented. [c] Reported for the first time as a black perigord truffle aroma constituent. [d] Tentatively identified by mass spectral data alone.

are listed in Table I, together with the range of relative percent of components found (based on HRGC peaks area). First, the mass spectra obtained were compared with those stocked in the NIH/EPA library. But compounds were considered as being identified only if their mass spectral data and HRGC retention data were consistent with that of authentic samples run on the same instruments.

In total, six alcohols, four aldehydes, one ether, two ketones, and one sulfur compound could be identified, and nine compounds were described for the first time as black perigord truffle aroma constituents.

But none of them was characteristic of black perigord truffle (*T. melanosporum*) aroma in the same way bis(methylthio)methane was for the white truffle (*T. magnatum*) (Fiecchi et al., 1967).

Unlike the synthetic aromatization of white truffle flavored edible oil that only involved this compound (Rigoli, 1982), the use of all the identified constituents made it necessary to actually obtain nature-identical Black Truffle flavoring products (Talou et al., 1986).

Qualitatively, the majority of the compounds listed in Table I have been identified previously in edible mushrooms aroma (Pyysalo, 1976) and, in general, in raw vegetables (Teranishi et al., 1981).

Quantitatively, the alcohols occupied a special place among the black truffles volatiles (60–85% of the total amount). We noticed that the relative concentration of alcohols increased with the period of cold storage although the aroma itself was not really altered.

Some volatiles compounds identified after steam distillation of truffles by Ney and Freytag (1980) were different from those found in the present work of headspace analysis, in particular 3-methylbutylamine. But damage to the natural material (steam distillation would cause extreme damage) gave rise to considerable structure degradation, i.e. protein degradation, and 3-methylbutylamine was known as one of its byproducts (Fenaroli, 1975).

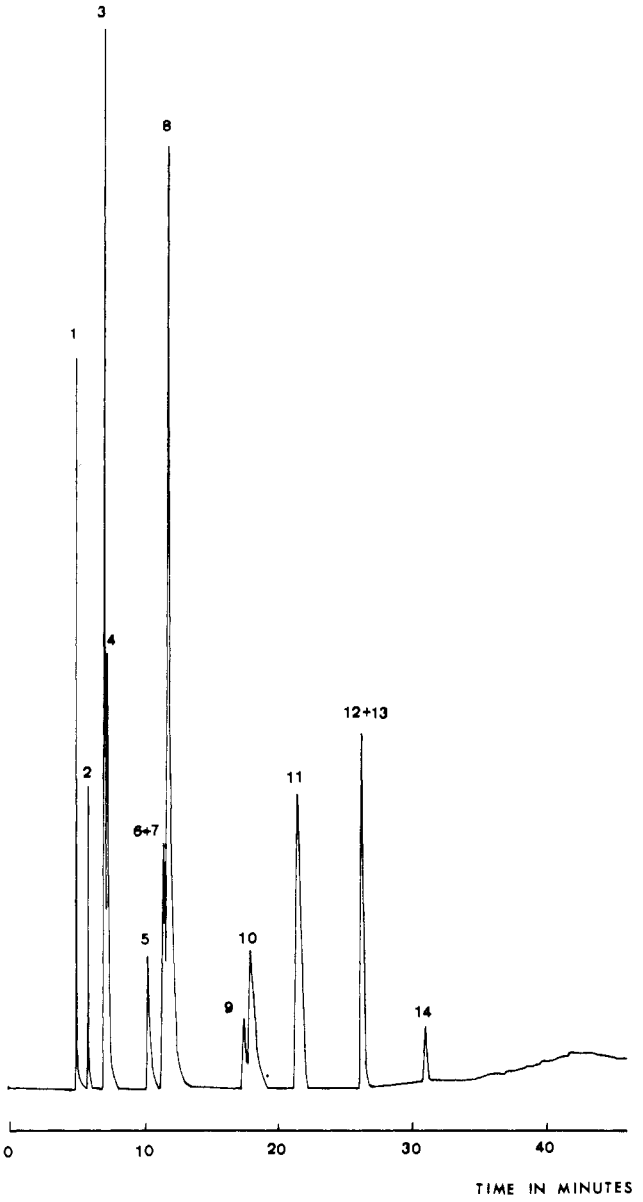

Figure 2. HRGC separation of black perigord truffle volatiles (after Tenax trapping) on a 50 m × 0.32 mm coated fused silica capillary column UCON 75H90000, df 0.2 μm. The peak numbers correspond to the numbers in Table I.

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Registry No. Ethanol, 64-17-5; 2-butanol, 78-92-2; 1-propanol, 71-23-8; 2-methyl-1-propanol, 78-83-1; 2-methyl-1-butanol, 137-32-6; 3-methyl-1-butanol, 123-51-3; acetaldehyde, 75-07-0; 2-methylpropanal, 78-84-2; 2-methylbutanal, 96-17-3; 3-methylbutanal, 590-86-3; anisole, 100-66-3; acetone, 67-64-1; 2-butanone, 78-93-3; dimethyl sulfide, 75-18-3.

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Rapid Sample Preparation Method for HPLC Analysis of Capsaicinoids in Capsicum Fruits and Oleoresins

Vincent K. Attuquayefio and Ken A. Buckle*

A fast miniblender and commercially available Sep-pak filter cartridges have enabled sample preparation time of capsicum fruit for capsaicinoid analysis to be reduced from several hours to about 5 min. Sample purity is also greatly improved, with almost all the coloring matter and other lipid contaminants removed. The procedure gives a recovery of about 98%, and the results for capsaicinoids content are in agreement with more elaborate, time-consuming techniques. A typical extraction and HPLC analysis is about 25 min/sample.

For the objective analysis of the pungent principles (capsaicinoids) of capsicum fruits, sample preparation should provide complete extraction of the principles without interfering coextractives. In most cases this requires extensive and costly sample preparation and thus enhances the popularity of the less objective organoleptic methods for pungency assessment (Todd, 1958; Hartman, 1970; Todd et al., 1977).

As part of an extensive examination of the capsaicinoids in fresh and processed capsicums, a rapid method for capsaicinoid extraction, cleanup, and separation by high-performance liquid chromatography (HPLC) was sought. Capsicum samples extracted in chloroform or other solvents produce extracts containing a variety of interfering materials that affect resolution and sensitivity, necessitate column flushing after every run, and tend to reduce column life (Chiang, 1986). To assure faster analysis, longer column life, and more uniform resolution over several runs, a sample preparation technique was developed that reduced the level of interfering contaminants yet still enabled rapid and accurate analysis.

MATERIALS AND METHODS

Preliminary Experiments. Fresh samples of capsicum fruits (*Capsicum annuum* var. Yatsubusa) were obtained from capsicum plants grown in a glass house at The University of New South Wales, Kensington, Australia (UNSW). Samples were sun-dried and freeze-dried at the UNSW, and oleoresin capsicum was donated by Mauri Foods Australia Pty Ltd. Preliminary experiments indicated that direct high-speed blending of capsicum fruit tissue was effective in extracting the capsaicinoids. Subsequently, acetone, chloroform, methanol, methanol/0.1 N HCl (80:20, v/v), methanol/1% acetic acid (90:10, v/v), and acetonitrile were examined from the point of view of yield, ease, and effectiveness of extract cleanup. Parameters studied included capsaicinoid content and color of extract, the nature of the extracts with respect to the position of the coarse particulates, and the degree of chromatographic interference produced by the extract.

The HPLC procedure for capsaicinoids used a Waters Associates liquid chromatograph equipped with a 6000A pump, a U6K injector, an RCM module fitted with C_{18} Rad-Pak column (8-mm i.d., 5-μm pore size), Lambda-Max 480 detector set at 280 nm and 0.01 aufs, and mobile phase of methanol/water (63:37, v/v) at a flow rate of 3.5 mL/min.

Standard solutions of capsaicin (10 mg %, Merck) were prepared in the extracting solvent diluted with different volumes of water to give serial polarity gradings. The standards were introduced in 10-mL portions onto the Waters Associates Sep-pak filters previously conditioned in a solvent and the effluents assayed for the presence of capsaicinoids. The solvent containing no trace of capsaicinoids was of the desired polarity. A lower polarity solvent was chosen to remove selectively the capsaicinoids from the Sep-pak while avoiding the elution of relatively nonpolar constituents such as pigments and lipids. The Sep-pak containing capsaicinoids was washed with acetonitrile/water mixtures of lower polarity than the established wash solvent. The effectiveness of these solvents in eluting the capsaicinoids was determined by assaying a further 1.0 mL of acetonitrile wash from the Sep-pak for residual capsaicinoid.

Final Sample Preparation Method. *Extraction.* Dehydrated ground capsicum (1.0 g) or 10 g of fresh capsicum is blended with 10 mL of acetonitrile for 2 min. For oleoresins, 0.1 g of sample is dissolved in 2.0 mL of hexane and the capsaicinoids are transferred into 10 mL of acetonitrile by solvent partitioning. A 1.0-mL aliquot of the acetonitrile extract is taken for cleanup as with ground and fresh samples.

Cleanup. A C_{18} Sep-pak is conditioned with about 5 mL of acetonitrile followed by 5 mL of double-distilled water.

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