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Study of the components of a solid smoke flavouring preparation

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The study of the components of a solid smoke flavouring preparation was carried out using several techniques. The sample was extracted by means of an ultrasonic bath and by successively using dichloromethane and water as solvents. Observation by microscope, of the initial sample and the residues of extractions, indicate that the carrier of this preparation consists of two components insoluble in dichloromethane. One of these consist of small transparent globular particles, and the other of irregular particles of a gold colour, the latter is soluble in water. Fourier Transform infra-red spectra of the solid smoke flavouring preparation, its extracts and residues, were obtained and studied. From these it can be concluded that the two components of the carrier are saccharides, one of them similar to starch and the other with reducing ability owing to the presence of carbonyl groups. Also, from the infra-red spectrum of the flavouring mixture extracted with dichloromethane, ratios between the absorbances of carbonyl and phenol functional groups are obtained, and compared with those of an aqueous smoke flavouring. Finally, the composition of the volatile fraction of the extract in dichloromethane was studied by gas chromatography–mass spectrometry and gas chromatography techniques. Carbonyl compounds, namely ketones, aldehydes, and furan and pyran derivatives, are the major components and constitute approximately 60% of the volatile fraction of the extract. Also, phenol derivatives are present in the sample. Guaiacol and syringol derivatives constitute approximately 25% of the volatile fraction. Some compounds, not previously detected in wood smoke, were identified in this flavouring. However, polycyclic aromatic hydrocarbons were not detected in the sample. Quantitative data from gas chromatography and infrared spectroscopy agree, and show the close relationship between the composition of the soluble fraction in dichloromethane and the odour of the sample.

INTRODUCTION

Smoke flavouring preparations used in the food industry can appear to be different and to have different organoleptic properties. Their physical states can be liquid or solid, and their appearance can also be very different owing to the carrier and components used in their manufacture. Their organoleptic properties are determined by their flavour components and by the concentration of each in the mixture. In addition, interactions between the carrier and the flavour compounds are responsible for the aromas of the various preparations. Finally, interactions between food components and components of the flavouring preparations determine the flavour and the final aroma of the smoked food.

The use of smoke condensates to give smoke flavour to foods has been suggested as a means of avoiding the contamination of smoked foods by polycyclic aromatic hydrocarbons (PAHs) from the traditional means of smoking. However, recent studies have shown the presence of PAHs in some commercial smoke flavourings (Gomaa *et al.*, 1993; Yabiku *et al.*, 1993).

For these reasons, the study of the components of smoke and smoke condensates has been the subject of interest in the past and continues to be so (Fiddler *et al.*, 1970*a*; Fujimaki *et al.*, 1974; Radecki & Grzybowski, 1981; Wittkowski *et al.*, 1981, 1990; Maga, 1988; Potthast, 1988; Potthast & Eigner, 1988).

Recently we have characterized the volatile components of the soluble fraction, in dichloromethane, of an aqueous smoke flavouring preparation (ASFP) used in the Spanish food industry (Guillén *et al.*, 1995). In this paper we study a solid smoke flavouring

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preparation (SSFP) by means of Fourier Transform infra-red spectroscopy (FTIR) and gas chromatography–mass spectrometry (GC–MS) and gas chromatography (GC). The aim of this study is to obtain information about the volatile components responsible for flavour and the components which constitute the carrier of this preparation. This information could be useful, not only from a methodological point of view but also to establish relationships between sensory effects changes during storage, and the organoleptic properties of smoked foods manufactured using this preparation. At the same time, this report examines the testing of the limitations and the usefulness of the several techniques used in the study of mixtures of a similar nature.

MATERIALS AND METHODS

Sample, solvents and standard compounds

The subject of study is a solid smoke flavouring preparation, used in the Spanish food industry. Dichloromethane and distilled water were used as solvents for the extraction of this solid smoke flavouring. The organic solvent was selected, not only for its high effectiveness in extracting polycyclic aromatic compounds (Guillén *et al.*, 1991; Guillén, 1994) and aromatic compounds in general, but also for its high volatility, and for its absorption in infra-red spectroscopy at 954 and 714–680 cm^{-1}. The flavouring preparation and the residues of the extractions were examined by a Nikon microscope. All compounds indicated with (s) in Table 2 (see below), available from Aldrich, Fluka or Sigma, were used as standard compounds in the chromatographic study.

Extraction of the smoke flavouring preparation

The extractions were carried out by means of an ultrasonic bath. First, dichloromethane was used as solvent, and the residue of this extraction was then again extracted using distilled water as solvent.

Infra-red spectroscopy

Fourier Transform infra-red spectra were obtained of the solid smoke flavouring preparation, and of its residues and extracts. Films deposited on KBr pellets were used to obtain the spectra of extracts in dichloromethane, and pellets of mixtures of the solid samples and KBr were used to obtain the spectra of the solid samples, as in previous studies (Guillén *et al.*, 1992*a*). FTIR spectra were recorded on a Nicolet Magna 550 spectrometer with DTGS KBr detector, co-adding 32 interferograms obtained at a resolution of 4 cm^{-1}. The spectrometer was operated under Nicolet OMNIC software and the spectra were corrected for scattering. Semi-quantitative data were obtained from the area of several bands.

Gas chromatography–mass spectrometry and gas chromatography

The gas chromatographic–mass spectrometric study was performed using a Hewlett-Packard chromatograph, model 5890 Series II, equipped with a Mass Spectrometer Selective Detector 5971, and a Hewlett-Packard Vectra 486/66U computer. A fused-silica capillary column (30 m long and 0.25 mm diameter), coated with a non-polar stationary phase (Hewlett-Packard-5 cross-linked 5% phenyl methyl silicone) was used. The temperature programme began at 50°C (0.5 min) with an increase of 2°C min^{-1} up to 280°C (15 min), and He was used as the gas carrier. Injector and detector temperatures were 250 and 280°C, respectively. The injection technique used was splitless. The injection volume was 1 μl. Mass spectra were recorded at an ionization energy of 70 eV. Components were identified by their retention times, by their mass spectra, by comparing their mass spectra with those in a commercial library (Wiley138k, Mass Spectral Database, 1990) and, in some cases, using standards, as in previous studies (Guillén *et al.*, 1992*b*, 1995). Guillén & Manzanos, 1994).

A Hewlett-Packard gas chromatograph model 5890 series II, equipped with a Flame Ionization Detector (FID) and a Hewlett-Packard 3395 integrator, was used for the quantitative study. A fused-silica capillary column (30 m long, 0.32 mm internal diameter), coated with a non-polar stationary phase (Hewlett-Packard-5, cross-linked 5% phenyl methyl silicone), was used. The gas chromatographic conditions were the same than in the GC–MS study.

RESULTS AND DISCUSSION

Observation by microscope

Original sample, SSFP, and residues of successive extractions in dichloromethane, DI, and in water, WI, were examined by microscope. The SSFP sample consists of two components observable by microscope. One is formed of irregular particles of several sizes and golden colour, GP, and the other is formed by small transparent globular particles, TP, that surround the former. The residue of the extraction, using dichloromethane as solvent, has the same components. However, the residue of the subsequent extraction, using water as solvent, is formed only by the small transparent globular particles. The conclusion of these observations is that the carrier of this preparation is formed by two components both insoluble in dichloromethane, of which only one is insoluble in water.

Fourier Transform infra-red spectroscopy study

Figure 1(a)–(c) shows the FTIR spectrum of the SSFP sample and the spectra of its residue DI and extract DS,

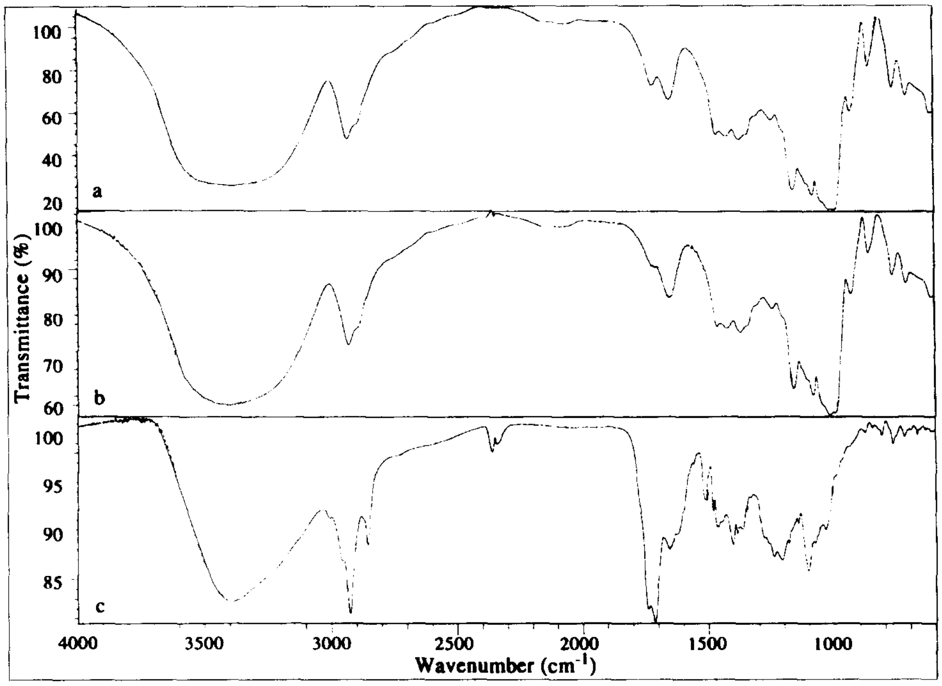

Fig. 1. Infra-red spectra from 4000 to 600 cm^{-1} of (a) the solid smoke flavouring preparation SSFP, (b) its residue DI and (c) extract DS using dichloromethane as solvent.

respectively, obtained using dichloromethane as solvent. Assuming that the dichloromethane is able to dissolve all the compounds responsible for flavour, the spectrum shown in Fig. 1(b) will correspond to the carrier and the spectrum shown in Fig. 1(c) to the mixture of the flavouring compounds.

Spectra in Fig. 1(a) and (b) are very close. Both show a broad OH stretching vibration band at 3430 cm^{-1} approximately. In addition, bands of symmetrical and asymmetrical vibrations of methyl and methylene groups, and a band of the carbonyl group are also present. In addition, asymmetrical and symmetrical C–O–C stretching vibrations are observed at 1160, 1081 and near 1000 cm^{-1}. The C–O stretching vibration of C–O–H groups could also contribute to this last band. The only detectable difference between both spectra is the different intensity of the carbonyl group band at 1715 cm^{-1}. This band has a higher intensity in the SSFP sample than in the DI sample. The great similarity between these spectra indicates that the flavouring compounds extracted with dichloromethane are present in the flavouring preparation at very low concentration, as can be expected, and also that some of the flavouring components have absorptions at the same wavelengths as the carrier.

Figure 2(a) and (b) shows the spectra of the extract WS, and the residue WI, obtained from the sample DI, using water as solvent. The spectra are very similar, and the only appreciable difference is the band of the carbonyl group present in the WS sample and absent in the WI sample. The spectrum shown in Fig. 2(b) is very similar to the spectrum shown in Fig. 2(c) (wheat starch). Dextrin also has a very similar spectrum to those shown in Fig. 2(b) and (c). From these results it can be inferred that the WI sample, which corresponds to TP component of the carrier, is a saccharide very close to starch because in addition, it acquires a blue colour in the presence of an I_2/I^- solution. However, the GP component of the carrier (the spectrum shown in Fig. 2a of the WS sample) is presumably a reducing saccharide because of the carbonyl group present in its molecule.

Figure 1(c) shows the FTIR of the extracted fraction of the flavouring preparation DS, using dichloromethane as solvent. From this spectrum, information about the flavouring mixture can be obtained. In Fig. 1(c) one can observe the presence of the OH functional group, of phenol derivatives and of alcohols, that produces the stretching vibration band at 3400 cm^{-1}. This band can also include absorptions of the OH bonds of carbonyl compounds, which can undergo keto–enol tautomerism, for example cyclotene. Likewise, a shoulder at 3009 cm^{-1} and bands at 2926 and 2853 cm^{-1} corresponding to C–H aromatic and aliphatic bonds, respectively, are also observed. The C–H olefinic stretching vibration can also contribute to the band at 3009 cm^{-1}. Carbonyl functional groups are also present in the sample, which give a band at 1714, and shoulders at 1741 and 1774 cm^{-1}, owing to ketones and aldehydes, esters and lactones, respectively.

The possibility of obtaining useful semi-quantitative data for comparative studies from FTIR spectra (George & McIntyre, 1987; Guillén *et al.*, 1992*a*) is known. The FTIR spectrum of the soluble fraction in dichloromethane of the previously studied ASFP sample (Guillén *et al.*, 1995) has almost the same main functional groups as the flavouring fraction of the SSFP sample. However, the spectra of both flavouring

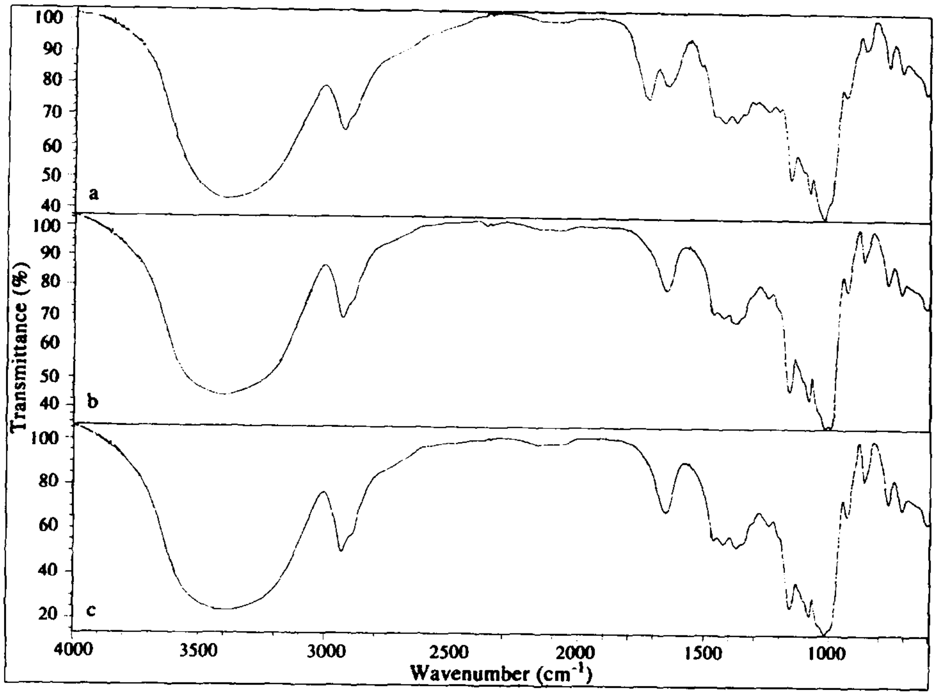

Fig. 2. Infra-red spectra from 4000 to 600 cm^{-1} of (a) the extract WS, (b) the residue WI of the DI sample using water as solvent, together with (c) the spectrum of wheat starch.

mixtures show differences in the intensities of the bands, indicating the different concentration of several functional groups.

Table 1 shows ratios between absorbances of some functional groups in both samples. It can be observed that the ratio between the absorbances of OH and of aliphatic C–H (or carbonyl) functional groups is smaller in the SSFP sample than in the ASFP sample. This fact shows that the concentration of the OH functional group is smaller in the SSFP sample than in the ASFP sample, and that the concentration of the C–H aliphatic (or carbonyl) functional group is higher in the SSFP sample than in the ASFP sample. These results indicate a higher concentration of phenol derivatives and a smaller concentration of carbonyl derivatives in the aqueous liquid smoke than in the solid smoke flavouring preparation. This fact is associated with the great difference between the odours of both samples.

Gas chromatographic–mass spectrometric and gas chromatographic studies

The soluble fraction in dichloromethane of the SSFP sample, designated as DS, which is considered to contain the mixture of flavouring components, was also studied by the GC–MS technique. Compounds of similar volatility to dichloromethane were not considered in the study. However, it is well known that this technique provides for the identification of compounds even when the chromatographic separation is not sufficient to afford an accurate quantification (Wittkowski *et al.*, 1981). Table 2 lists the identified components grouped by families of compounds, together with the proportion of the major components in the sample obtained by gas chromatography with FID detector.

Table 2 shows the presence of carbonyl derivatives which, in general, contribute a caramel or burnt sugar aroma to the overall flavour (Fiddler *et al.*, 1970*b*) [3-methyl-2-cyclopenten-1-one: somewhat sweet, grassy; 2-ethyl-2-cyclopenten-1-one: grassy (Kim *et al.*, 1974); 3-methyl-1,2-cyclopentanedione: like either brandy or caramel, an odour and taste such as hydrolyzed vegetable protein, smoked ham, cloves (Tóth & Potthast, 1984; Potthast *et al.*, 1988)].

Also some furan and pyran derivatives have been detected. These compounds have in general been considered responsible for the softening of the heavy aromas usually associated with phenolic compounds (Kim *et al.*, 1974). Radecki *et al.* (1977) have found a sweet, fragrant and floral aroma in some furan derivatives. Some compounds, such as 2-furancarboxaldehyde and 5-methyl-2-furancarboxaldehyde, are known to contribute to the overall flavour with sweet, bread-like, caramel-like, and sweet-spicy, warm and slightly caramel-like notes, respectively (Kim *et al.*, 1974). Also lactones

Table 1. Ratio between FTIR absorbances of some functional groups of the soluble fraction in dichloromethane of a solid smoke flavouring preparation (SSFP) and an aqueous smoke flavouring preparation (ASFP) previously studied

Ratio	SSFP	ASFP
O–H absorbance/ aliphatic C–H absorbance	6	11
O–H absorbance/ carbonyl C = O absorbance	6	8

and pyranones are components which make an important contribution to the smoke flavour [3-methyl-2(5H)-furanone: sweet, like burnt caramel (Kim *et al.*, 1974); 3-hydroxy-2-methyl-4H-pyran-4-one: caramel, butterscotch (Buckingham, 1994)].

The main compounds of these groups in the sample are: 4-hydroxy-4-methyl-2-pentanone (4%), 1-(acetoxy)-2-propanone (3%), 2-hydroxy-3-methyl-2-cyclopenten-1-one (11%), 3,5-dimethyl-1,2-cyclopentanedione (3%), 2-furancarboxaldehyde (5%), 5-(hydroxymethyl)-2-

Table 2. Compounds identified in the volatile fraction of the extract in dichloromethane of the solid smoke flavouring. Compounds identified using standards are indicated by (s). Proportion of main components in brackets

Family		Compound(%)
Aldehydes and ketones		
	(s)	4-hydroxy-4-methyl-2-pentanone (4%)
		1-(acetoxy)-2-propanone (3%)
	(s)	2-methyl-2-cyclopenten-1-one
	(s)	3-methyl-2-cyclopenten-1-one
		1-(acetoxy)-2-butanone*
		methylcyclohexanone*
		2-methyl-1-penten-3-one*
		2-ethyl-2-cyclopenten-1-one
Diketones		
	(s)	3-methyl-1,2-cyclopentanedione(cyclotene) (11%)
		3,5-dimethyl-1,2-cyclopentanedione* (3%)
		5,5-dimethyl-1,2-cyclopentanedione*
		3-ethyl-1,2-cyclopentanedione
Furan and pyran derivatives		
	(s)	2-furancarboxaldehyde (5%)
	(s)	1-(2-furanyl)ethanone
	(s)	5-methyl-2-furancarboxaldehyde
	(s)	5-(hydroxymethyl)-2-furancarboxaldehyde (3%)
	(s)	2(5H)-furanone (16%)
	(s)	5-methyl-2(3H)-furanone (3%)
		5-methyl-2(5H)-furanone
		3-methyl-2(5H)-furanone
	(s)	3-hydroxy-2-methyl-4H-pyran-4-one
Phenol derivatives		
	(s)	phenol (4%)
	(s)	2-methylphenol
	(s)	3-methylphenol
	(s)	4-methylphenol
		dimethylphenol
	(s)	3-ethylphenol
	(s)	2-methoxyphenol (guaiacol) (3%)
	(s)	4-methyl-2-methoxyphenol (4%)
		4-ethyl-2-methoxyphenol (2%)
	(s)	4-hydroxy-3-methoxybenzaldehyde (vanilline)
	(s)	1-(4-hydroxy-3-methoxyphenyl)ethanone
		1-(4-hydroxy-3-methoxyphenyl)-2-propanone
	(s)	2,6-dimethoxyphenol (syringol) (6%)
		4-methyl-2,6-dimethoxyphenol (1%)
		4-ethyl-2,6-dimethoxyphenol
		4-(1-propenyl)-2,6-dimethoxyphenol
		4-(2-propyl)-2,6-dimethoxyphenol
	(s)	4-hydroxy-3,5-dimethoxybenzaldehyde
	(s)	1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone
		1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone
		3-methoxypyrocatechol
Miscellaneous		
	(s)	4-methyl-1-(1-methylethyl)-3-ciclohexen-1-ol* (2%)
	(s)	1,4-dimethoxybenzene
		4-methoxybenzenemethanol*
	(s)	2-methyl-2-butenoic acid
		1,2-ethyl diacetate*
		methyl-4-oxopentanoate*

furancarboxaldehyde (3%), 2(5H)furanone (16%), and 5-methyl-2(3H)-furanone (3%). Aldehydes, ketones, diketones, furan and pyran alkylderivatives, constitute approximately 60% of the sample.

In addition to the above mentioned compounds, phenol derivatives are also present. A pungent odour is attributed to phenol, methyl- and some dimethyl-phenol derivatives, and a cresolic odour is associated with some dimethyl and other alkylphenol derivatives. The odour of guaiacol is described as sweet and smoky and somewhat pungent, and that of 4-methyl and 4-ethyl guaiacol as sweet and smoky. A smoky odour is also attributed to syringol, and mild, heavy and burnt notes are attributed to the odour of its 4-alkyl derivatives (Kim *et al.*, 1974). The odour and the taste of some phenol derivatives in Table 2 have been described with considerable accuracy by Baltes *et al.* (1981), and by Tóth and Potthast (1984).

The main components of these group of compounds are: 2-methoxyphenol (3%), 4-methyl-2-methoxyphenol (4%), 4-ethyl-2-methoxyphenol (2%), 2,6-dimethoxyphenol (5%), 4-methyl-2,6-dimethoxyphenol (1%), 4-ethyl-2,6-dimethoxyphenol (0.5%). Phenol derivatives in general constitutes approximately 25% of the total sample.

Finally, an ether, an acid, and some alcohols and esters have been detected in the volatile fraction of this solid smoke flavouring preparation. However, no hydrocarbons have been found in this sample. Compounds marked with an asterisk in Table 2 have not been previously detected in wood smoke (Maga, 1988).

Obviously all the compounds in Table 2 contribute to the overall flavour of this smoke flavouring preparation, and many of them were also present in the previously studied aqueous liquid smoke flavouring. However, the major difference between the SSFP and the ASFP volatile fractions appears to be in the different concentrations of the common components.

This difference is shown by data for the proportions of several groups of compounds in both samples. In the SSFP sample, the proportion of compounds including carbonyl functional groups is approximately 60% and the proportion of phenol derivatives is approximately 25%. Some authors have stated that carbonyl derivatives are present in smoke in a small concentration and they have been considered as minor compounds (Girard, 1991). However, in the ASFP sample, phenol derivatives constitute approximately 65% of its volatile fraction. This fact, also shown by the FTIR technique, explains the difference in odour between the two smoke condensates.

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