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Characterization of the components of a salty smoke flavouring preparation

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A solid smoke flavouring preparation had a heterogeneous appearance and microscopy revealed transparent crystalline particles, vegetable matter, irregular brown particles and dark brown globular particles. A quantity of globular particles was separated from a fraction of the total sample. Fourier Transform infrared spectra of the total sample and of the globular particles, as well as of their residues and extracts in dichloromethane were studied. The insoluble fraction of the flavouring preparation in dichloromethane was again extracted with distilled water and infra-red spectra of its residue and extract were also studied. It was inferred that, in addition to compounds responsible for the flavour, vegetable matter, NaCl, fatty acids and fatty esters and a carrier (presumably made of saccharides) were used in the manufacture of this flavouring preparation. Globular particles of the flavouring preparation were composed of fatty acids and fatty esters, carrier and flavour compounds. Gas chromatography/mass spectrometry and gas chromatography with flame ionization detection techniques were used to study the soluble fraction (in dichloromethane) of the flavouring preparation. Phenol derivatives were the main components responsible for the smoke flavour. In addition to the flavouring compounds arising from wood pyrolysis, flavouring compounds derived from plants were also found. Fatty acids and fatty esters, detected by infra-red spectroscopy were shown to be the main components of the soluble fraction. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The use of smoke condensates instead of the traditional smoke procedure, is increasing in the food industry for economic and health reasons, because polycyclic aromatic hydrocarbons, some of which are carcinogens, can be eliminated before their use (Simko & Brunckova, 1993; Simko *et al.*, 1994). In fact the smoke flavour is used, in many cases, as a novel flavour in several foods, not previously smoked.

To the best of our knowledge, studies of the composition of smoke condensates presently used in the food industry are few (Fiddler *et al.*, 1970*a,b*; Fujimaki *et al.*, 1974; Radecki *et al.*, 1976, 1977; Baltes & Söchtig, 1979; Radecki & Grzybowski, 1981; Potthast & Eigner, 1988; Wittkowski *et al.*, 1990).

In addition, most of the studies of smoke condensates are only related to the identification of the compounds responsible for flavour whereas usually the other components of the flavouring preparation are not considered, with the exception of polycyclic aromatic hydrocarbons, which may be potential contaminants (Gomaa *et al.*, 1993; Yabiku *et al.*, 1993).

However, information about all the components that constitute smoke flavouring preparations is important, in order to establish relations with their organoleptic properties, with their storage stability and with the organoleptic properties of the smoked food. Bearing this in mind, we previously studied an aqueous liquid smoke condensate and a solid smoke flavouring preparation (Guillén *et al.*, 1995; Guillén & Manzanos, 1996).

We study here a salty smoke flavouring preparation of a different appearance and with different organoleptic properties from those previously studied. Fourier Transform infra-red spectroscopy (FTIR) was used to study the total sample and one part of the sample, as well as the residues and the extracts obtained using, successively, dichloromethane and distilled water as solvents. Observation by microscope of the flavouring preparation and of some of its solid fractions was also carried out. The volatile fraction of the dichloromethane extract was studied by gas chromatography/mass spectrometry (GC/MS) and gas chromatography (GC) with flame ionization detector (FID).

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MATERIALS AND METHODS

Sample, solvents and standard compounds

The subject of study is a commercial solid smoke flavouring preparation, used in the Spanish food industry. Dichloromethane and distilled water were used as solvents. The organic solvent was selected for its high effectiveness in extracting polycyclic aromatic compounds (Guillén *et al.*, 1991; Guillén, 1994) and aromatic compounds in general and for its high volatility. Standard compounds used for the identification and quantification in the gas chromatographic study are asterisked in Table 2 (see below) and were available from Aldrich, Fluka and Sigma.

Extraction of the flavouring preparation

The extractions were carried out by means of an ultrasonic bath. First, dichloromethane was used as a solvent and the residue of this extraction was then again extracted using distilled water as a solvent. Using the same organic solvent and methodology, a selected part (dark brown globular particles) of the flavouring preparation was extracted. The flavouring preparation and the solid fractions of different extractions were examined using a Nikon microscope.

Infra-red spectra

Fourier Transform infra-red spectra were obtained of the solid smoke flavouring preparation, of the dark brown globular particles and of their residues and extracts using, successively, dichloromethane and water as solvents. 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 (Guillén *et al.*, 1992). FTIR spectra were recorded on a Nicolet Magna 550 spectrometer with DTGS KBr detector, adding 32 interferograms obtained at a resolution of 4 cm^{-1}. The spectrometer was operated with Nicolet OMNIC software and the spectra were corrected for scattering. Quantitative data were obtained from the corrected area of certain bands by using OMNIC software.

Gas chromatography–mass spectrometry and gas chromatography studies

The gas chromatographic-mass spectrometric study was performed using a Hewlett–Packard chromatograph, model 5890 series II, equipped with a selective detector 5971(MS) and a Hewlett–Packard Vectra 486/66U computer. A fused-silica capillary column (30 m long, 0.25 mm diameter), coated with a non-polar stationary phase (Hewlett–Packard-5, cross-linked 5% phenyl methyl silicone) was used. The temperature program began at 50°C (0.5 min) and increased 2°C/min until 280°C was reached, Helium was used as gas carrier. Injector and detector temperatures were 250 and 280°C, respectively. The injection technique used was splitless. The volume of sample injected was close to 1 μl. Mass spectra were recorded at an ionization energy of 70 eV. Components were identified by their retention times, by comparing their mass spectra with those in a commercial library (Wiley 138K, Mass Spectral Database, 1990) and in many cases by using standards, as previously (Guillén *et al.*, 1995; Guillén & Manzanos, 1994, 1996).

A Hewlett–Packard gas chromatograph Model 5890 series II, equipped with a flame ionization detector and a Hewlett–Packard 3395 integrator, was used for the quantitative study. A fused-silica capillary column (30 m long, 0.32 mm i.d.), coated with a non-polar stationary phase (Hewlett–Packard-5) was used. The temperature of the detector was 300°C. The other conditions of the chromatographic run were the same as those used for GC/MS. The quantification was carried out using external standards. The response factors of all compounds, asterisked in Table 2, were determined as before (Blanco *et al.*, 1992). For the quantification of compounds not available commercially, response factors of compounds of similar nature were used. Each stage of this experimental section was replicated several times in order to increase the accuracy of the results.

RESULTS AND DISCUSSION

The flavouring preparation (S) appeared heterogeneous. Observation by microscope showed several components, irregular brown particles, globular dark brown particles of different sizes (GP), transparent crystalline particles and several kinds of particles of vegetable matter.

Fourier transform infra-red spectroscopy study

The infra-red (IR) spectrum of S (Fig. 1a) had bands corresponding to OH groups (3429 cm^{-1}), olefinic and/or aromatic CH bonds (3009 cm^{-1}), very intense bands of CH_3 and CH_2 groups (2928–2854 cm^{-1}), and carbonyl groups of esters (1748 cm^{-1}), acids, aldehydes, or ketones (1710 cm^{-1}), as well as C–O–C groups (1160 cm^{-1}) (Günzler & Böck, 1975; George & McIntyre, 1987).

GP components were selected and studied by IRTF. The spectrum (Fig. 1b) has basically the same bands as observed in Fig. 1a, showing that the globular particles have the same functional groups as the total sample. Intensity of the bands of the spectra 1a and 1b in Table 1, however shows some differences between the spectra. The proportion of compounds having OH groups was smaller in GP than in S and GP was richer than S in compounds having olefinic and/or aromatic CH bonds, aliphatic CH bonds and carbonyl groups.

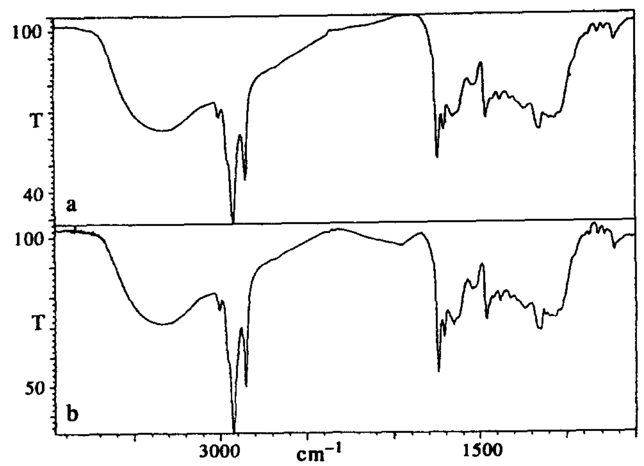

Fig. 1. Infra-red spectra of: (a) the flavouring preparation S; and (b) the globular particles of the flavouring preparation GP.

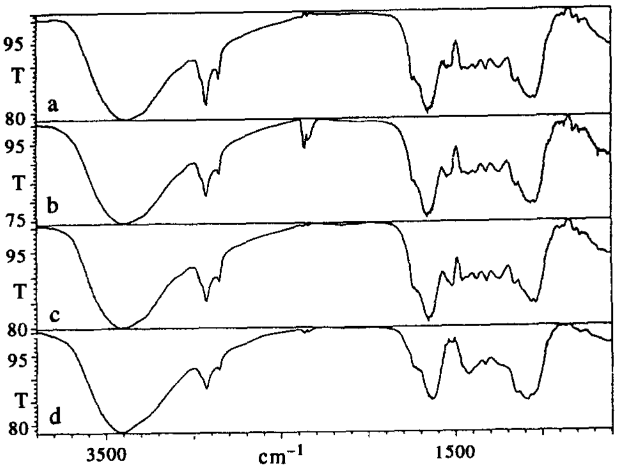

Fig. 2. Infra-red spectra of: (a) SDI; (b) GPDI; (c) SDIWI; and (d) SDIWS.

Table 1. Ratio between FTIR absorbances of some functional groups of S and GP

Ratio	S	GP
Abs. 3009 cm^{-1}	113.7	101.8
Abs. 2928–2854 cm^{-1}	2.1	1.8
Abs. 1748 cm^{-1}	14.4	12.8
Abs. 1710 cm^{-1}	78.1	66.1

The flavouring preparation S was extracted with dichloromethane. Microscopic observation of the insoluble fraction (in dichloromethane) of the flavouring preparation (SDI) revealed transparent crystalline particles, brown particles of different sizes and vegetable matter. GP components selected from the total sample, were also extracted with dichloromethane. The insoluble fraction (in dichloromethane) of the GP components (GPDI) was formed of brown particles. Infra-red spectra of SDI and GPDI (Fig. 2(a,b)) were similar, showing that the main components, in both samples, observable by infra-red spectroscopy, are the same. These spectra show, compared to those of S and GP (Fig. 1(a,b)), a decrease in the intensity of some bands and almost a disappearance of others. The bands at 3417 cm^{-1}, 1650 cm^{-1} and 1060 cm^{-1} are intense, but the bands of olefinic and/or aromatic CH bonds and the bands at 1748 and 1712 cm^{-1} almost disappeared in spectra 2a and 2b. The bands for aliphatic CH_2 and CH_3 are smaller than in spectra 1a, and 1b. The maximum of the C–O–C group band was shifted from 1160 cm^{-1} in spectra 1a and 1b to 1060 cm^{-1} in spectra 2a and 2b. From these results it can be inferred that the main components of the globular particles and of the total sample, insoluble in dichloromethane and observable by IR spectroscopy, are the same in SDI and GPDI and their nature is close to that of the saccharides.

The residue of the extraction of S in dichloromethane (SDI) was extracted with distilled water. The spectrum of the insoluble fraction in water (SDIWI), which was formed of brown particles in addition to vegetable matter, is given in Fig. 2c. Spectra 2a, 2b and 2c were similar, showing that the main component of SDIWI is the same as that in SDI and GPDI. The IR spectrum of the soluble fraction in water (SDIWS), which is formed of crystalline transparent particles of NaCl and light brown particles, is given in Fig. 2d. The CH_3 and CH_2 bands were smaller than those of the spectra 2a, 2b and 2c, and this spectrum shows a different fingerprint region between 1550 and 1000 cm^{-1}. The organic component of this sample could also be a water-soluble saccharide.

The IR spectrum (Fig. 3a) of the soluble fraction in dichloromethane of S, designated as SDS, that presumably contains the compounds responsible for the flavour in sample S, has pronounced bands of olefinic and/or of aromatic CH bonds, aliphatic CH_3 and CH_2 bonds, and carbonyl C=O bonds. Also the rocking

Table 2. Compounds detected in the extract of the flavouring smoke preparation in dichloromethane, together with their concentrations in the solid smoke flavouring in mg/kg. Asterisked compounds were used as standards for identification and quantification

Compound	(mg/Kg)
Phenol derivatives	
phenol*	4.7
2-methylphenol*	12.3
3-methylphenol* and 4-methylphenol*	11.5
2,6-dimethylphenol*	13.5
2-ethylphenol*	8.8
2,4-dimethylphenol* and 2,5-dimethylphenol*	14.4
3-ethylphenol*	10.4
2,3-dimethylphenol*	13.1
Guaiacol derivatives	
2-methoxyphenol(guaiacol)*	29.2
2-methoxy-4-methylphenol*	29.0
2-methoxy-4-ethylphenol*	12.4
2-methoxy-4-vinylphenol*	0.8

Table 2—*contd.*

Compound	(mg/Kg)
2-methoxy-4(2-propenyl)phenol(eugenol)*	13.7
2-methoxy-4-propylphenol*	3.1
2-methoxy-4(1-propenyl)phenol(isoeugenol)*	6.8
2-methoxy-4(1-propenyl)phenol(isoeugenol)*	22.4
4-hydroxy-3-methoxybenzaldehyde(vanillin)*	26.3
1-(4-hydroxy-3-methoxyphenyl)ethanone*	10.8
1-(4-hydroxy-3-methoxyphenyl)-1-propanone	9.0
Syringol derivatives	
2,6-dimethoxyphenol(syringol)*	97.7
2,6-dimethoxy-4-methylphenol*	44.6
2,6-dimethoxy-4-ethylphenol	22.3
2,6-dimethoxy-4-(2-propenyl)phenol*	18.6
2,6-dimethoxy-4-propylphenol	9.1
2,6-dimethoxy-4-(1-propenyl)phenol	3.9
4-hydroxy-3,5-dimethoxybenzaldehyde*	41.7
2,6-dimethoxy-4-(1-propenyl)phenol(isomer)	2.2
1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone*	34.2
1-(4-hydroxy-3,5-dimethoxyphenyl)-1-propanone	24.4
1-(4-hydroxy-3,5-dimethoxyphenyl)-2-propanone	12.4
Pyrocatechol derivatives	
1,2-dihydroxybenzene*	14.7
3-methoxy-1,2-dihydroxybenzene*	38.0
Carbonyl derivatives	
2-furancarboxaldehyde*	20.3
4-hydroxy-4-methyl-2-pentanone*	2.7
1-acetoxy-2-propanone	6.0
5-methyl-2(3H)furanone*	1.6
2-methyl-2-cyclopenten-1-one*	1.6
1-(2-furanyl)-ethanone*	1.9
2(5H)furanone*	79.8
2(3H)dihydrofuranone	4.4
5-methyl-2-furancarboxaldehyde*	4.9
3-methyl-2-cyclopenten-1-one*	3.5
3-methyl-1,2-cyclopentanedione(cyclotene)*	33.4
3-hydroxy-2-methyl-4H-pyran-4-one(maltol)*	45.5
3-ethyl-1,2-cyclopentanedione	16.2
Alkyl-aryl ethers	
1,4-dimethoxybenzene*	6.8
Terpene and sesquiterpene derivatives	
γ-terpinene*	1.2
linalool*	6.0
camphor*	1.8
exo-borneol*	3.8
endo-borneol*	4.7
α-terpineol*	30.4
anethole*	1.2
neryl acetate*	2.1
geranyl acetate*	4.6
trans-caryophyllene*	2.6
ar-curcumene	0.6
α-zingiberene	1.8
β-bisabolene	1.6
β-sesquiphellandrene	0.9
Acids and esters	
methyl hexadecanoate	103.6
hexadecanoic acid(palmitic acid)*	676.4
methyl 9,12-octadecadienoate	38.1
methyl 9-octadecenoate	79.5
9,12-octadecadienoic acid (linoleic acid) and 9-octadecenoic acid*	8896.0
Others	
squalene*	143.1

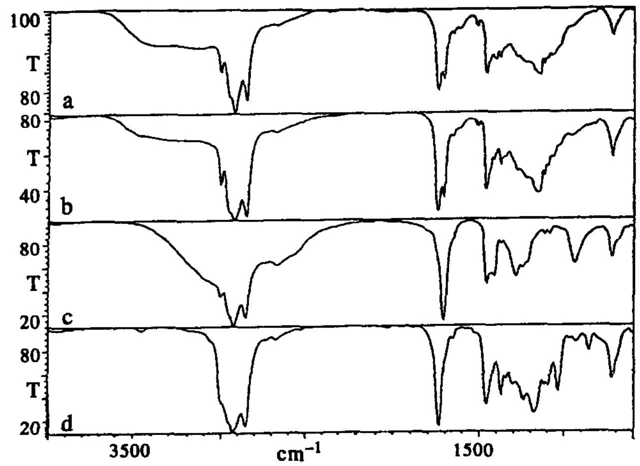

Fig. 3. Infra-red spectra of: (a) SDS; (b) GPDS; (c) oleic acid; and (d) ethyl oleate.

band of CH_2 bonds at 724 cm^{-1}, characteristic of $(CH_2)_n$ group with $n > 7$, is intense. From spectrum 3a it can be inferred that the main components of the extract are substances which have a large aliphatic chain with some unsaturation and which may contain carbonyl (esters, acids, aldehydes or ketones) groups. In addition a weak but wide band at 3474 cm^{-1}, characteristic of OH bonds, and a fairly intense band due to C–O–C groups at 1160 cm^{-1} are observed.

The IR spectra of mixtures of compounds responsible for the flavour in smoke flavourings previously studied (Guillén *et al.*, 1995; Guillén & Manzanos, 1996), has bands of OH, CH aromatic, CH_3 and CH_2 aliphatic, carbonyl and C–O–C groups. All these groups are present in spectrum 3a. However, neither the CH_2 rocking band, nor the aliphatic and carbonyl bands were found in the spectra of the previously studied preparations, at such intensity as found in spectrum 3a. A shift in the maximum of the C–O–C bands is also observed in spectrum 3a, in relation to those of the previously studied smoke flavourings. The IR spectrum of the flavouring components of the previously studied smoke flavouring has the characteristic pattern of the bands of the C–O–C group characteristic of syringol and syringol derivatives with maxima at 1218 and 1108 cm^{-1}, showing that these substituted phenols are the main components which contain C–O–C bonds in the mixture. However, the C–O–C band of SDS in spectrum 3a shows a maximum close to 1160 cm^{-1}, that can be attributed to the C–O–C group from ester molecules.

For these reasons it was inferred that SDS is formed, apart from by the characteristic compounds responsible for the smoke flavour of the preparation, by compounds related to fatty acids and esters. Spectra 3c and 3d, corresponding to oleic acid and ethyl oleate respectively, support this assumption, and we consider that these compounds could be the main components in SDS. For this reason the IR spectrum is not useful for obtaining quantitative data for the proportion of typical functional groups of components responsible for the smoke organoleptic properties of this flavouring. However, the IR spectrum shows the presence of other substances, soluble in dichloromethane, used in the manufacture of this flavouring preparation.

The IR spectrum of the dichloromethane extract of the selected globular particles (GPDS) (Spectrum 3b) is also similar to that of 3a. This fact suggests that the globular particles are made of fatty esters and acids together with brown particles carrying some flavour compounds.

From these results it could be concluded that, in addition to compounds responsible for the smoke flavour, vegetable matter, NaCl, fatty acids and fatty esters, and a carrier (this latter presumably made of saccharides) were used in the manufacture of this preparation.

Gas chromatography/mass spectrometry and gas chromatography studies

With the aim of knowing the components responsible for the organoleptic properties of this preparation, the composition of SDS was studied by GC/MS and by GC with FID detection. In Table 2 the compounds identified are given, together with their concentrations in the solid preparation in mg/kg obtained using GC with FID detector.

Aldehydes, lactones, ketones, diketones and furan and pyran derivatives constitute the carbonyl derivatives. This group represents approximately 2% of all compounds determined by gas chromatography. Among these compounds, 2-furancarboxaldehyde, 2(5H)-furanone, cyclotene and maltol are the main components.

Substituted phenols are the major components responsible for the flavour of this condensate. They represent approximately 6% of the compounds determined by gas chromatography. All the important substituted phenols, from the flavour point of view, are present. Syringol and syringol derivatives are the main components, followed by guaiacol and guaiacol derivatives. Phenol and alkyl phenol derivatives are in low concentration. Also, a methyl benzyl ether, 1,4-dimethoxybenzene, in very small concentration, has been detected.

All the compounds mentioned above have been considered responsible for smoke flavour (Fujimaki *et al.*, 1974; Maga, 1988; Kim *et al.*, 1974; Baltes *et al.*, 1981; Toth & Potthast, 1984; Potthast, 1988).

In addition to the cited compounds which can be generated in the wood pyrolysis process, other compounds were found. The first group is composed of terpene and sesquiterpene derivatives. These compounds, which constitute approximately 0.6% of the compounds quantified by gas chromatography, are probably contained in the observed vegetable matter and are extracted with dichloromethane. The presence of zingiberene (warm, woody), *ar*-curcumene (woody), β-sesquiphellandrene (woody), and bisabolene (woody, balsamic flavour) could be associated with comminuted roots of ginger (Ashurst, 1991; Menut *et al.*, 1994). The other components contribute to enrich the flavour of the preparation with several notes (γ-terpinene: light, citrus, herbaceous; linalool: light, lavender; camphor: fresh, camphoraceous; *endo*-borneol: camphoraceous, earthy, pine; α-terpineol: sweet, floral, lilac; anethole: strong, sweet, anise; neryl acetate: fruity, floral, rose; geranyl acetate: fruity, floral, rose; *trans*-cariophyllene: spicy, woody flavour).

The second group of compounds foreign to pyrolytic processes are the fatty acids and fatty esters and squalene, compounds also detected by infra-red spectroscopy as forming part of the globular particles of the flavouring preparation. Squalene fatty acids and esters constitute 91.6% of the compounds determined by gas chromatography in SDS.

It is evident that the flavouring components of this salty smoke flavouring preparation are compounds contributing smoke flavour, and others, arising from vegetable matter, help to produce a specific global flavour. Among the smoke components only substituted phenols are in noteworthy concentrations, but smaller than those found in other smoke condensates. On the other hand, the presence of fatty acids and esters in this flavouring preparation greatly influences its organoleptic properties.

These results indicate that the manufacture of this preparation is much more complex than those previously studied. Flavouring compounds coming from plants and fatty acids and esters are only present in the preparation here studied. In addition, the difference observed in the organoleptic properties between this preparation and those previously studied is justified by the difference in composition.

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