

Identification by GC-O and GC-MS of New Odorous Compounds in Natural Rubber

Niramol Juntarachat,¹ Nicolas Bouvier,² Jean-Paul Lepoutre,² Aurélie Roland,²
Jérôme Sainte-Beuve,³ Françoise Granet,⁴ Jean-Michel Salmon,² Peggy Rigou,² Pascale Chalier¹

¹Université de Montpellier 2, UMR-IATE, Ingénierie des Agropolymères et Technologies Emergentes, cc023, pl E. Bataillon, 34095, Montpellier Cedex 05, France

²INRA, UMR Sciences Pour l'œnologie, Plateau Technique d'analyse des Composés Volatils, Bâtiment 28, 2, place Pierre Viala, 34060 Montpellier Cedex 01, France

³CIRAD, UMR IATE, Bâtiment 33 Campus INRA - Supagro, 2 Place Viala, 34060 Montpellier Cedex 01, France

⁴Manufacture Française des Pneumatiques Michelin, 23 Place des Carmes, 63040, Clermont-Ferrand, France

Correspondence to: P. Chalier (E-mail: chalier@univ-montp2.fr).

ABSTRACT: Natural rubber products emit malodorous compounds, which can contaminate the air and water of the inhabited surroundings of the production factories. Analytical methods such as SPME coupled with GC-MS and GC-O-FID were used to elucidate the composition and olfactory impact of volatile compounds released from two various grades of natural rubber submitted to different coagulation processes. Forty-three volatile compounds were identified and amongst 16 of them that had never been reported. The volatile compounds belonged to different chemical classes and were the result of enzymatic and microbial action as well as thermal degradation. Among the new compounds identified, a terpene (limonene), benzyl derivatives, or trimethylamine were found in both grades of natural rubber. In contrary phenyl acids, fatty acid esters, or 2,6 dimethoxyphenol were specific to one grade of natural rubber and may result from the coagulation process. The use of GC-O analysis emphasized the contribution of short-chain fatty acids to the malodorous odour generated but also evidenced the participation of trimethylamine and 2,6 dimethoxyphenol. At last, it was demonstrated that the presence of phenyl acids in one natural rubber could partially mask the obnoxious odour. © 2013 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* 130: 1863–1872, 2013

KEYWORDS: rubber; properties and characterization; applications

Received 17 October 2012; accepted 3 April 2013; Published online 7 May 2013

DOI: 10.1002/app.39356

INTRODUCTION

Natural rubber, arguably the most important polymer produced by plants, is used in many thousands of products and is an essential raw material in the tyre industry because of its specific properties. The leading role of natural rubber in our modern civilization has direct repercussions on the development of the natural rubber-producing sector in countries such as Thailand, Indonesia, or Malaysia, but also for the downstream sector of the supply chain in China, Europe, and the United States.

Raw natural rubber is a solid product obtained from coagulation, crumbling, and drying of latex, a milky colloid produced by certain plants, among which *Hevea brasiliensis* is the most productive tree. Coagulation of latex can be either natural and occur directly in the cup used to recover the latex from the tree or controlled by the addition of acetic or formic acid. In natural coagulation, micro-organisms naturally present in latex play a major

role, including during the maturation phase that can last about a month. Natural coagula are then washed, blended, made into crumbs, and dried in order to obtain a product containing less than 0.5% water and therefore suitable for manufacturing.¹ The initial latex contains rubber particles made of high molecular weight poly (*cis*-1, 4-isoprene), which amounts to 20–45% of the total mass of the latex, along with nonisoprene compounds and serum. The nonisoprene elements, which amount to around 5% in weight compared to dry rubber, mostly consist of proteins, lipids,² carbohydrates, and inorganic constituents.³

Throughout the processes of drying, storage, mastication, curing, mixing, and vulcanization, offensive odours are emitted from the raw material and natural rubber product that cause environmental and social concerns because of water and air contamination in the inhabited surroundings.⁴ Low molecular weight fatty acids such as acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids were identified as the major odorous and offensive

compounds emitted by gazes from natural rubber factories or by natural rubber samples.^{5–9} The strength of the odour depends on the rubber quality and drying process and the lower the rubber quality the stronger the malodour is. Other compounds responsible for the odours were found such as aliphatic and aromatic hydrocarbons, aldehydes, and ketones probably derived from lipid oxidation and derivatives containing nitrogen and sulphur that may result to protein oxidations.^{8,9}

Whilst liquid effluents are successfully treated in almost all production factories, few odour treatment systems are efficient at the moment. In order to find adapted solutions that cope with the generation of malodour compounds in the atmosphere, it is important to get a precise identification of those compounds and to determine their olfactory impact.

Few works have focused on the identification of volatile compounds emitted from rubber manufacturing. The identification of compounds released in a rubber factory atmosphere was performed using a trapping system on charcoal followed by solvent extraction of the compounds adsorbed and GC/MS analysis.⁵ Other works report the direct extraction of volatile compounds from raw rubber sample using solvent extraction⁹ or direct static headspace sampling.^{6–8} This latter extraction method consists in heating the rubber sample in a vial until the volatile compounds vaporize into the gas phase and reach equilibrium. An aliquot of the gas phase is then sampled with a syringe and injected into a GC-MS instrument.¹⁰ The main advantages of this method are the simplicity, rapidity, and suitability for routine analyses. However, this method lacks sensitivity, as it cannot concentrate the molecules in the gas aliquot injected in the GC/MS and therefore compounds present at low concentrations cannot be identified.

Solid phase micro-extraction (SPME) combined with static headspace overcomes this disadvantage by concentrating the volatile and semivolatile compounds vaporized in the headspace on an appropriate fiber coated with an extracting phase. After adsorption and absorption of the volatiles onto the SPME fiber, the fiber is introduced in the injection port and the volatiles directly desorbed in the injector. SPME technique was successfully applied to the analysis of additives or degradation products of polymer and for the identification and quantification of compounds present at very low concentration.¹⁰

The aims of this article are therefore: (i) to use SPME in a static headspace mode to trap and concentrate volatile compounds present at high and low concentrations in two grades of natural rubber and that are released in the atmosphere under certain conditions, (ii) to identify these compounds by gas chromatography coupled with mass spectrometry, (iii) to determine their sensory impact and level using Gas Chromatography coupled with Olfactometry (GC-O), and (iv) to establish a correlation between volatile compounds emitted and curing process.

EXPERIMENTAL

Natural Rubber Samples

Two grades of natural rubber were studied each one being differently processed, thus allowing to study the impact of the process on the volatile compound composition. The Technically

Specified Rubber 10 (TSR10, 10 indicating the impurity rate, i.e., 0.1%) was obtained from latex naturally coagulated. The coagulate was subjected to crumb processing, continuous washing by water, and drying at 115°C for 4 h. The Technically Specified Rubber 5 Constant Viscosity (TSR5CV, 5 indicating 0.05% of impurities) was obtained by controlled coagulation of the latex using acetic acid with a solution of neutral hydroxylamine sulphate. The coagulate was subjected to crumb processing, continuous washing by water, and drying at 120°C for 3 h. All dried rubber crumbs were then pressed into rubber bales.

Sample Preparation

Three grams of natural rubber was cut into small pieces and introduced into sealed glass vials (20 mL) just before analysis. All solid-phase micro-extractions were performed using a 50/30 μm , 2 mm divinylbenzene (DVB) /carboxen (CAR)/ polydimethylsiloxane (PDMS) fiber (Supelco, Bellefonte, USA). This bipolar fiber allows the extraction of compounds with different polarities. In addition, the layered coating of the fiber with both DVB and CAR allows larger analytes to be trapped in DVB layers and smaller analytes in CAR, thus expanding the molecular weight of analytes that can be extracted and enabling extraction of analytes at trace levels. Therefore use of this fiber allows extraction of a wide range of volatile and semi volatile compounds with different polarities and molecular weight. This fiber appears as the best choice for screening different volatile compounds in rubber.

For the extraction of volatile compounds by SPME, three different temperatures of volatilization were tested. The samples were equilibrated at 30°C, 120°C, and 160°C during 10 min and the fiber was then exposed to the headspace during 30 min in order to trap the volatile compounds. For GC-O analysis, the samples were equilibrated at 120°C for 10 min and the fiber exposed to the headspace during 60 min. The fiber was then introduced in the GC injector and compounds directly desorbed at 250°C for 5 min.

GC/MS Analysis

An Agilent gas chromatograph 5973 series equipped with a DB-WAX analytical fused-silica column (30 m \times 0.25 mm \times 0.25 μm film thickness from J&W Scientific, Folsom, CA) coupled with 5989A mass selective detector, was used. Compounds were injected by thermal desorption at 250°C during 5 min in the injector in a splitless mode. The oven temperature was held at 40°C for 5 min, ramped to 250°C at a rate of 4°C/min and maintained at 250°C for 10 min. The carrier gas was Helium 6.0 (Air Product), with a flow-rate of 1.5 mL/min. The electron impact (EI) energy was 70 eV and the quadrupole temperature was set at 250°C. Detection was carried out in full scan mode covering a mass range (m/z) of 50–450 amu.

Description of the Panel and Execution of the GC-O/FID Analysis

The panel was composed of seven panellists (five women and two men aged 23–55) who were recruited among the staff and students of our laboratory. The GC-O experiments were performed as detailed in the Table I. Sniffing the chromatographed effluent was performed in one session of 1 h after which no odour was detected at the olfactory detector outlet (ODO). The

Table I. GC-O/FID Plan of Experiments and Panellist

Day of analysis	GC-O panellist	Rubber type
1	A+B	TSR5CV
3	C+D	TSR5CV
5	E+B	TSR10
10	C+F	TSR10
10	D+G	TSR10
11	C+F	TSR5CV

session was divided in two and panellists changed after 30 min in order to avoid tiredness of the participants. The panellists sat comfortably with their noses placed in the nosepiece of the ODO flushed with humidified air in order to prevent dryness of the mucous membrane. The panellists were asked to give a qualitative description of the odour in their own words at the moment they smelt it and the intensity of the odour from 1 (low) to 5 (high). At the same time, an assistant wrote down the retention time. Three replicates were performed on each rubber type sample. A Hewlett Packard HP gas chromatograph 5890 series II, with a double system of detection olfactometry port and flame ionization detector (FID), equipped with a DB-WAX analytical fused-silica column (30 m × 0.25 mm × 0.25 μm film thickness from J&W Scientific, Folsom, CA) was used. The injection of compounds was conducted by thermal desorption at 250°C during 5 min in the injector in a splitless mode. The oven temperature was held at 60°C for 5 min, ramped to 250°C at the rate of 4°C/min and maintained at 250°C for 10 min. Hydrogen 6.0 (Air Product), was used as a carrier gas at 1.5 mL/min. The temperature of the FID was held at 300°C and it was supplied with 30 mL/min of Hydrogen 6.0 (air product) 300 mL/min of air, and 30 mL/min of nitrogen as a make-up gas. At the end of the column, a flow division system conducted the eluted compounds in the FID detector and in the olfactory detector outlet.

Identification of Volatile Organic Compounds and Their Related Sensory Activity

The identification of volatile organic compounds (VOCs) present in the TSRs was performed by comparing the mass spectra of the unknown compounds with those in the Wiley library 275.L (Agilent Technologies). Attribution was performed on peaks showing a signal to noise S/N > 3 and identification of the compound was valid when the confidence rating of mass spectra comparison was superior or equal to 95. This attribution was further confirmed using relative retention indices or by injection of reference compounds. The linear retention indices (I_s^T) of compounds identified by GC/MS and compounds found to have an olfactive impact by GC-O/FID were calculated and compared using the following Van den Dool & Kratz equation¹¹ and after a standard solution of alkanes of C₆ to C₂₄ was analyzed by GC/MS and GC-O/FID using the same GC conditions:

$$I_s^T = 100 \times \left[n + (N - n) \frac{t_{r(\text{unknown})} - t_{r(n)}}{t_{r(N)} - t_{r(n)}} \right]$$

This study focused on the odours that were repetitive and well described.

RESULTS AND DISCUSSION

Identification of Volatile Compounds in Natural Rubber by GC-MS

The aims of this part were to elucidate the volatile compounds released from two grades of natural rubber (TSR10, TSR5CV) using SPME as the extraction and concentration method and to evaluate the impact of the temperature on compounds volatilization in the headspace. The analytical method used and the temperature tested were chosen in order to mimic the real conditions of rubber processing in order to give a good representation of the volatile compounds released in the atmosphere.

The identified compounds released at 30°C, 120°C, and 160°C are summarized in Table II. These three temperatures correspond to the different natural drying and storage temperatures, the drying temperature occurring in manufactures and the vulcanization temperature. A total of 43 compounds were clearly identified and amongst them 16 had never been reported in literature. Other compounds were also present but only in trace making their identification difficult. The qualitative comparison of data obtained from the two grades of natural rubber showed little variability and 31 compounds were found common to both samples. Identified volatile compounds belonged to different chemical classes including groups previously described⁵: volatile carboxylic acids, alcohols, aldehydes, and aliphatic ketones or aromatic hydrocarbons, phenols, derivatives containing nitrogen or sulphur, but also phenolic acids and fatty acid esters. Their occurrence could be explained by microbial or enzymatic degradation, thermic degradation and by redox reactions. Indeed, rubber is prone to the development of microorganisms as it contains high quantities of lipids, proteins, and carbohydrates. Numerous species were identified in latex and its derivative products and it was demonstrated that the quantity of micro-organisms affected the physical properties and structure of processed natural rubber.¹² Furthermore, it was not surprising to find volatile compounds arising from fermentation of amino-acids, lipids, or sugars. However, some components are clearly produced during the drying process by thermal degradation but both microbial and thermal pathways are responsible of the release of other.

Carboxylic acids (C2 to C18) were the major compounds found in both samples and fatty acids of low molecular weights were identified in other studies as responsible for mal-odours in natural rubber.^{6,8,9} The presence of acetic acid could be because of the residual amount after its addition during coagulation treatment but may also arise from carbohydrate fermentation, such as propionic acid. These two acids give an indication of the extent of micro-organism activity in the latex.¹³ The fatty acids result from the lipid hydrolysis during coagulation and drying, free fatty acids account for more than 20% of lipids extracted from dry rubber.³ As previously described,³ linoleic acid is the most important fatty acid followed by oleic and stearic acids. Isobutanoic and isovaleric acids may occur from amino-acid metabolism (valine and leucine, respectively) by micro-organisms such as *Streptomyces*, positive gram bacteria known to degrade natural rubber.¹⁴ Oxidation products of fatty acids such as low chain aldehydes and 2-heptanone were also identified in both grades of natural rubber. Hexanal and pentanal are

Table II. Compounds Identified in Natural Rubbers TSR10 and TSR5CV at Three Different Temperatures (30°C, 120°C, and 160°C)

N°	Aroma compound	I _T ^S Literature	I _T ^S GC-MS	Sample					
				TSR10			TSR5CV		
				Temperature °C					
30	120	160	30	120	160				
1	Trimethylamine	677 ^a	684	-	-	+	-	-	+
2	Butanal	832 ^a	846	+	-	-	+	-	-
3	Pentanal	935 ^a	955	+	+	+	+	+	+
4	Toluene	1042 ^a	1020	+	+	+	+	+	+
5	Hexanal	1067-1093 ^a	1062	+	-	-	-	-	-
6	Methyl valerate	1087 ^a	1068	+	+	+	+	+	+
7	Ethyl benzene	1124 ^a	1105	+	-	-	+	-	-
8	<i>p</i> -Xylene	1149	1113	-	-	-	+	-	-
9	Ethyl valerate	1147-1170 ^a	1149	+	-	-	-	-	-
10	2-Heptanone	1160 ^a	1159	+	+	+	+	+	+
11	Limonene	1188 ^c	1172	+	-	-	+	-	-
12	Isoamyl alcohol	1210 ^b	1195	+	-	-	-	-	-
13	1-Pentanol	1244 ^a	1218	+	-	-	-	-	-
14	Ethyl hexanoate	1230 ^b -1244-1270 ^a	1239	+	-	-	+	-	-
15	1,2,4 Trimethylbenzene	1293 ^a	1257	+	-	-	+	-	-
16	Tridecane	1300 ^a	1272	-	+	+	-	-	-
17	Acetic acid	1434-1449-1452 ^a	1421	+	+	+	+	+	+
18	Benzaldehyde	1525 ^a	1502	+	+	+	-	+	+
19	Propanoic acid	1523 ^a	1540	+	+	+	+	+	+
20	Isobutanoic acid	1584 ^b	1569	+	+	+	+	+	+
21	Butanoic acid	1622 ^b 1628-1650 ^a	1631	+	+	+	+	+	+
22	Isovaleric acid	1686-1691 ^a	1669	+	+	+	+	+	+
23	Sesquiterpene	-	1727	+	+	+	+	+	+
24	Valeric acid	1721 ^a (FFAP)	1747	+	+	+	+	+	+
25	Hexanoic acid	1858 ^b 1863-1872	1847	+	+	+	+	+	+
26	Benzyl alcohol	1869 ^b	1863	-	+	+	-	+	+
27	Benzothiazole	1951 ^a	1929	-	+	+	-	-	-
28	Phenol	2008 ^a	2001	+	+	+	+	+	+
29	2-pyrrolidone	2020 ^a	2014	+	+	+	+	+	+
30	Methyl myristate	2034 ^a	2026	+	+	+	-	-	-
31	Octanoic acid	2060 ^b 2083-2098 ^a	2050	+	+	+	+	+	+
32	<i>p</i> -Cresol	2077/2100 ^a FFAP	2107	+	+	+	-	-	-
33	Nonanoic acid	2158 ^b 2202 ^a	2160	-	+	+	-	-	-
34	Methyl palmitate	2182 ^d	2195	-	+	+	-	-	-
35	2,6-Dimethoxyphenol	2296-2307 ^a	2250	-	-	-	-	+	+
36	Benzoic acid	2420 ^b	2435	+	+	+	+	+	+
37	Dodecanoic acid	2449 ^b 2517 ^a	2477	-	+	+	-	+	+
38	Phenyl acetic acid	2543 ^a	2552	-	+	+	-	-	-
39	3-Phenylpropanoic acid	2650 ^a	2624	-	+	+	-	-	-
40	Palmitic acid	2886 ^b	2906	-	+	+	-	+	+
41	Stearic acid	3140 ^a	3109	-	+	+	-	+	+
42	Oleic acid	3184 ^a	3141	-	+	+	-	+	+
43	Linoleic acid	3181 ^a	3200	-	+	+	-	+	+

New identified compounds are in bold. I_T^S are extracted from ^apherobase data base <http://www.pherobase.com> and when column was not DBwax is was specified, ^bSelli et al Food Chemistry, 2004,85,207-213, ^cBerlinet et al J. Science food and Agric., 2006,86, 2206-2212, ^dBlazevic & Mastelic, Flavour and Fragr. J. 2008,23,278-285.

generally described in food products containing lipids as the result of auto-oxidation and photo-oxidation of linoleic acid present in both samples.¹⁵ 2-heptanone can be formed from octanoic acid by a derivative pathway of β -oxidation involving micro-organisms such as fungi.¹⁶ Pentanol could come from the reduction of pentanal or from the isomerization of isoamyl alcohol (3-methyl-1-butanol).¹⁷ It was the first time that isoamyl alcohol was identified in rubber samples, but it was found only in the TSR10. As reported in Table III, its formation involved micro-organism or enzyme action by direct degradation of leucine through the Ehrlich pathway.¹⁷

Several esters that have never been reported in rubber were identified in both samples. Methyl or ethyl esters of short or long fatty acids such as methyl valerate, ethyl valerate, ethyl hexanoate, methyl myristate, and methyl palmitate were found in TSR10; whereas only ethyl hexanoate was present in TSR5CV. However, methyl ester of hexanoic acid and methyl esters of long-chain fatty acids such as palmitic or oleic acids have been previously identified in cup lump rubber.^{8,9} These esters could result from the microbial or enzymatic action of an esterase on the corresponding acids during the coagulation.

Using the highest temperature, i.e. 160°C, trimethylamine (TMA), a volatile basic nitrogen compound was identified in both samples. A similar compound, ethylamine was previously identified in TSR10.⁶ TMA which is responsible of characteristic fishy off-odour is found in fish or seafood products spoiled by *Shewanella putrefaciens* or *Photobacterium phosphoreum*.¹⁸ Its formation is explained by the reduction of TMA-N-oxide in anaerobic conditions (Table III).

Among compounds recurrently present in both samples, aromatic compounds were well represented with the presence of phenol, *p*-cresol, ethyl benzene, trimethyl benzene, toluene, benzoic acid, benzyl alcohol, and benzaldehyde. These compounds might result from thermal degradation of lignin and of fatty acid esters¹⁹ or from microbiological degradation of aromatic amino-acid such as phenylalanine.

If phenol and toluene were previously identified in different grades of natural rubber such as TSR5 and TSR10 or in ribbed smoked sheet (RSS), ethyl benzene, and trimethyl benzene were only described in RSS sample. They result from a smoking operation specific to the manufacture of RSS natural rubber and contribute to its smoky odour.⁶ Their presence in both samples suggested that the drying process was responsible for their formation.

Benzaldehyde, benzoic acid, and benzyl alcohol were identified for the first time in rubber. The production of benzaldehyde could be explained by the oxidation of the phenyl acetaldehyde formed (i) from phenyl pyruvate after phenylalanine transamination (microorganism pathway), (ii) from phenylalanine Strecker degradation in the Maillard reactions (thermal degradation).^{19–21} Benzoic acid and benzyl alcohol might be the result of oxidation and reduction of the aldehyde, respectively (Table III).

Some aromatic compounds appeared as specific to one particular rubber. High amount of phenyl acids were found in TSR10.

Phenyl acetic acid the corresponding Strecker acid of phenyl acetaldehyde²² had already identified in cup lump extract,⁹ but 3-phenyl propionic acid (hydrocinnamic acid) had never reported in natural rubber. Surprisingly, phenyl acetaldehyde, a potential precursor of many compounds, was not identified in samples from this article. The absence of aldehyde in TSR10 and TSR5CV and the absence of acid in samples could be explained by the respective ratio between aldehyde and acid that depends on reaction parameters such as pH, oxygen, and also other reactions.²¹ The 3-phenyl propionic acid could be released by thermal degradation of lignin.

The *p*-xylene and 2,6-dimethoxyphenol were only identified in TSR5CV; whereas the *p*-cresol occurred only in TSR10. However, this last compound had previously been observed in other grades of natural rubber such as TSR5 and TSR20 but never in TSR10.⁸ This phenol may result to the thermal or microbial degradation of phenolic acids and lignin. The 2,6-dimethoxyphenol had never been reported in natural rubber. It had been described as a potential pyrolysis product of a phenolic acid, sinapic acid.¹⁵ *o*-Xylene was described in RSS samples and 2-methoxyphenol was found in a deproteinized natural rubber sample.⁶ The occurrence of volatile compounds into natural rubber depends on numerous parameters such as the raw material origin, the applied process to obtain rubber from latex and their chemical identification depends on their concentration, the limits of detection, and the accuracy of analytical methods, thus explaining the differences found from one study to another studies.

Benzothiazole, a heterocyclic compound containing both sulphur and nitrogen and characterized by a rubber odour, was only detected in TSR10. However, a thiazole has been previously reported in RSS samples.⁶ Thiazoles formation involves the combination of hydrogen sulphide and ammonia released from the thermal degradation of amino-acids with aldehydes and dicarbonyl compounds originated from lipid degradation²³ (Table III). They normally appear in cooked fish such as sardines and mackerel rich in polyunsaturated fatty acids.

Two terpenic compounds were found in both grades of natural rubber; limonene that had never been reported in natural rubber and one sesquiterpene that could not be precisely identified. However, the presence of a monocyclic terpene, camphene had already been observed in the components from cup lumps.⁸ They may occur from thermal degradation of *cis* 1,4 polyisoprene, the raw material of natural rubber²⁴ or thermal oxidation of lipids²³ (Table III). 2-pyrrolidone had never been reported in literature as a volatile compound released from natural rubber. This molecule found in certain cooked foods could result from the Maillard reaction between sugar and asparagine or glutamine.²⁵

It should be noted that some compounds previously identified in natural rubber were not found in the present study. The origin of latex (Thailand) but also the coagulation process, the nature and rate of contamination by micro-organisms, the drying process and the time and condition of storage may explain the absence of some compounds. Samples of TSR10 and TSR5CV

Table III. Hypothetical Formation Pathway of the New Volatile Compounds Found in Rubber Samples

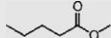

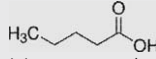

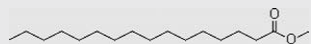
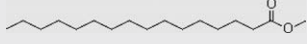

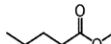
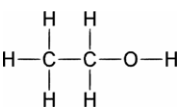
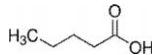
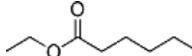
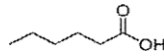
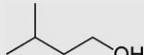
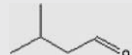
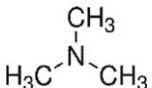
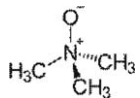
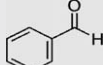
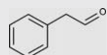




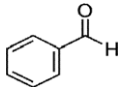
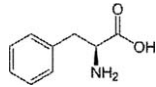
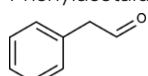
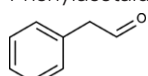
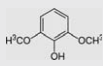
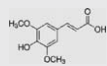
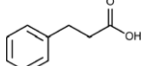
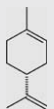
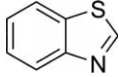

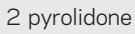
Pathway	Volatile compound	Specific pathway	Reactants
Microbial and enzymatic degradation	Methyl valerate 	Esterification of a fatty acid with methanol 	Valeric acid 
	Methyl myristate 		Myristic acid 
	Methyl palmitate 		Palmitic acid 
	Ethyl valerate 	Esterification of a fatty acid with ethanol 	Valeric acid 
	Ethyl hexanoate 		Hexanoic acid 
	Isoamyl alcohol 	Ehrlich pathway from leucine	Isovaleraldehyde 
	Trimethylamine 	Reduction by anaerobic bacteria	Trimethylamine-N-oxide 
	Benzaldehyde 	Oxidation (amino-oxidase)	Phenylalanine via Phenylacetaldehyde 
Oxidation Reduction	Benzoic acid 	Oxydation	Benzaldehyde 
	Benzyl alcohol 	Reduction	Benzaldehyde 
Thermal Degradation	Benzaldehyde 	pyrolysis	Phenylalanine 
			Phenylacetaldehyde 
			
	2,6 dimethoxyphenol 	pyrolysis	Sinapic acid 
	3 phenyl propionic acid 	Thermal oxidation	lignin
	Limonene and sesquiterpene 	Thermal oxidation β scission	Lipids cis 1,4 polyisoprene
	Benzothiazole 	Thermal degradation of amino-acid and lipids	Hydogen sulfide (H ₂ S) + Amonia (NH ₃) + Aldehydes or dicarbonyl compounds 
	2 pyrrolidone 	Maillard	Sugar + Asparagine or glutamine

Table IV. Optimal SPME Conditions Obtained by Factorial Analysis for the Selected Compounds Extracted from the Rubber TSR5CV

Compounds	MW (g/mol)	BP (°C)	Optimal temperature (°C)	Optimal equilibration time (min)	Optimal extraction time (min)
Pentanal	86	103	30	21	30
Acetic acid	60	118-119	110.3	120	60
Hexanal	100	119-124	30	120	60
Propionic acid	74	141	120	120	60
2-Heptanone	114	149-150	102.4	10	60
Isobutanoic acid	88	155	120	99.7	60
Butanoic acid	88	163.5	100	120	30
Isovaleric acid	102	176-177	65.6	120	60
Valeric acid	102	186-187	103	10	30
Hexanoic acid	116	205	96.3	10	30
Palmitic acid	256	351-352	120	85.7	60

MW, molecular weight; BP, boiling point.

had undergone a different coagulation process, natural against controlled, which may explain the poorer aromatic profile of TSR5CV compared to TSR10. One major difference between both samples is the presence of isoamylic alcohol, and fatty acid esters in TSR10 only. These compounds are of microbiological origin and have a positive impact on the odour of rubber. However, both grades of natural rubber released compounds from microbial and thermal degradation. Concerning products of thermal degradation *p*-xylene and 2,6 dimethoxyphenol were specifically found in TSR5CV, whereas TSR10 contained phenyl acids, *p*-cresol, and benzothiazole. It was shown that during the initial stage of maturation, micro-organisms enhanced the sensitivity of thermal oxidation explaining the presence of compounds derived of thermal treatment in TSR10.¹¹ To control the coagulation process, hydroxylamine sulphate is added in TSR5CV. This compound is known to react with aldehydes and acts as radical scavenger. It could explain the formation of 2,6 dimethoxyphenol or the absence of some compounds such as benzothiazole.

Study of HS-SPME Parameters That Affect Extraction of Compounds

Identification of volatile compounds in rubber were previously carried out using direct headspace gas.¹ However, it was demonstrated that this method might lack sensitivity towards compounds present at very low concentrations. In addition, those compounds, although in low concentration may have an important odour impact that would be omitted when the analytical methods lack sensitivity. Therefore, we considered the efficiency of SPME to overcome such a problem and SPME parameters such as temperature of extraction, time of extraction, and equilibration time were tested in order to evaluate their impact on all compounds of interest.

Effect of Temperature. It clearly appeared that temperature greatly influenced the extractability of the volatile compounds present in rubber. For all samples, 30–40 compounds were identified at the three temperatures tested (see Table II). Lower

temperature (30°C) greatly favored the extraction of low molecular weight aldehydes, esters, and alcohols, short-chain fatty acids and their corresponding esters and the very volatile benzene, ethyl benzene, toluene, and *p*-xylene (BTEX). However, higher temperature of 120°C favored the extraction of higher molecular weight such as the medium and long-chain fatty acids and their corresponding esters, as well as benzyl compounds. Lastly, in the same conditions of equilibrium and extraction time (i.e. 10 and 30 min, respectively), the TMA could only be detected at the very high extraction temperature of 160°C. This compound may have a strong affinity to the rubber matrix and requires a high temperature to be volatilized in the headspace or a longer time of extraction at a lower temperature. This result is interesting as the TMA has a very strong odour impact.

Study of the Combined Effect of SPME Parameters Using Experimental Design. The combined effect of equilibration time (10, 30, 60 min) and extraction time (30, 60 min) was investigated at low and high temperatures of extraction (30°C, 60°C, 120°C) on a limited number of specific volatile compounds using the experimental design statistical method. The study was performed on both the TSR5CV and TSR10 natural rubber in order to evaluate possible matrix effect. Tables IV and V report the optimal extraction conditions obtained by multivariate analysis that was performed with the Statgraphics Plus 5.0 software.

The results obtained showed that SPME efficiency for the extraction of a large panel of compounds from a specific matrix is variable and it is difficult to obtain just one optimal SPME condition for the extraction. Efficiency of the SPME depends not only on the volatility of the compounds, but also on the specific interactions that the compound undergoes with the matrix and its affinity to the fiber. The carboxylic acids are highly polar compounds that can easily create hydrogen bonding with other compounds from the matrix, explaining why they are more difficult to extract than aldehydes. In addition, short aldehydes are generally more volatile than fatty acids. Although

Table V. Optimal SPME Conditions Obtained by Factorial Analysis for the Selected Compounds Extracted from the Rubber TSR10

Compounds	MW (g/mol)	BP (°C)	Optimal temperature (°C)	Optimal equilibration time (min)	Optimal extraction time (min)
Acetic acid	60	118–119	83	78.1	60
Propionic acid	74	141	73.5	67.6	60
Pentanal	86	103	30	61.3	60
Butanoic acid	88	163.5	71	67.4	60
Isobutanoic acid	88	155	75.7	10	60
Hexanal	100	119–124	70.9	10	60
Isovaleric acid	102	176–177	69	68.5	60
Valeric acid	102	186–187	74.2	66.6	60
Hexanoic acid	116	205	79	67	30
Phenylacetic acid	132	265.5	120	62.3	30
Phenylpropanoic acid	144	280	120	27.2	30
Palmitic acid	256	351–352	120	10.1	60

MW, molecular weight; BP, boiling point.

both matrixes are natural rubber, each grade is characterized by different physical–chemical properties and shows specific interactions with the different compounds, explaining why different optimal conditions are obtained for a same compound on a different rubber. For example, higher temperatures are required to extract most part of fatty acids from TSR5CV. This result suggests that interactions of fatty acids with the polyisoprene chain are stronger in natural rubber obtained from controlled coagulation than in that obtained by natural coagulation.

Because it was difficult to obtain similar optimal conditions for all compounds and for both grades of natural rubber, the surface response methodology was used to look at the experimental conditions that suit most selected compounds. The conditions obtained were the following: equilibrium time 10 min, temperature of extraction 120°C, and extraction time 60 min. It was checked that under these conditions TMA could be extracted and olfactory study was also performed under the same optimal conditions of extraction.

Identification of Malodorous Compounds in Natural Rubber by GC-O-FID. To study the sensory impact of volatile compounds released from TSR10 and TSR5CV, samples were heated at 120°C. This temperature permitted relatively good extraction of most compounds, and corresponds to the drying temperature used in the industrial rubber process that seems to be the critical step where obnoxious odours are released.

All analyses were performed in triplicate as described in experimental section. Among all the odours identified by the panel, we mainly focused on those perceived with the highest intensity by all panellists. The volatile compounds responsible for the odours described by the panellists were identified following the IOFI statement on the identification of flavouring compounds. First, compounds identification was made by comparison of the retention indices obtained by GC-O and GC/MS and the reference indices already published. This identification was further confirmed with spectra obtained by mass spectrometry analysis of the rubber.

Table VI. Olfactory Impact Obtained by GC-Olfactometry Analysis of TSR5CV Sample

Compound	Odour description	Literature odour	Intensity	$I^T_{S \text{ literature}}$	$I^T_{S \text{ GC-MS}}$	$I^T_{S \text{ GC-O}}$	% of OAV
Trimethylamine	Rotted fish	Fishy, ammonia	4	609	614	625	1.38
2-Heptanone	Cream, butter, vanilla	Cheesy, fruity, creamy	4	1178	1159	1161	3.46
Acetic acid	Vinegar, acid	Vinegar	4	1415	1421	1422	30.75
Propionic acid	Feet, vinegar	Pungent, vinegar	3	1528	1540	1530	4.73
Butanoic acid	Vomit	Unpleasant butter, cheese, dairy, rancid	4	1602	1631	1638	18.1
Valeric acid	Feet, acid, chemical	Unpleasant, penetrating, rancid	4	1750	1736	1794	28.6
Octanoic acid	Citrus fruits, fruity	Fatty, fruity	3	2050	2065	2065	8.08
2,6 Dimethoxyphenol	Tar, marker, leather	Sweet, phenol smoky, medicinal, balsamic	4	2269	2250	2235	4.94

OAV, odorant active compound.

Table VII. Olfactory Impact Obtained by GC-Olfactometry Analysis of TSR10 Sample

Compound	Odour description	Literature odour	Intensity	I _S ^T _{CG-MS}	I _S ^T _{CG-O}	% of OAV
Trimethylamine	Rotted fish	Fishy, ammonia	3	677	677	0.075
Hexanal	Fruity	Fruity, apple	4	1062	1065	0.50
Acetic acid	Vinegar	Vinegar	5	1421	1417	22.2
Benzaldehyde	Floral, fruity and spicy	Bitter, spicy almond, sweet, burning	3	1502	1576	1.85
Isobutanoic acid	Vomit, feet	Unpleasant odour, butter, cheese	4	1568	1598	2.65
Butanoic acid	Vomit, feet, acid, unpleasant	Unpleasant butter, cheese, dairy, rancid, sharp	5	1635	1622	12.05
Isovaleric acid	Disagreeable floral, rotted fruity	Disagreeable, sour, rancid, cheesy	4	1669	1646	1.53
Sesquiterpene (not identified)	Green, crushed mint	-	3	1727	1730	0.265
Valeric acid	Vomit, feet, sour, acid, malodours	Unpleasant, penetrating, rancid	4	1747	1736	9.7
Octanoic acid	Disagreeable fruity	Fatty, fruity	4	2056	2094	14.80
p-Cresol	Chemical product, detergent	Medicinal	4	2107	2076	0.27
Phenylacetic acid	Honey, floral	Sweet, honey, floral	5	2552	2557	30.75
3-Phenylpropanoic acid	Honey, floral	Floral, sweet fatty, rose musk, cinnamon	5	2624	2647	3.36

OAV, odorant active volatile.

The characteristic odours identified in the TSR5CV and TSR10 are reported in Tables VI and VII. It appears that fatty acids with low molecular weights such as acetic acid, propionic acid, butanoic acid, and valeric acid are the main compounds responsible for the unpleasant smell of rubber. These acids accounted for over 50% of the odorant active compounds and they were already reported as being responsible for bad smells during the rubber process or for odorous emission from agricultural wastes such as swine facilities.^{5-9,26} However, it is the first time that the presence of TMA and 2,6-dimethoxyphenol is reported as contributing to the negative odours generated in rubber process.

Regarding the TSR10 rubber, we noticed that this sample had a stronger odour than the TSR5CV at room temperature. Consequently, more odorous compounds were identified by the panel (15 against eight) in TSR10 and with higher odour intensity. Fatty acids with low molecular weights and TMA were also identified for their strong obnoxious odour but, propionic acid, which significantly contributes to the smell of the TSR5CV, was not identified in the TSR10. However, other compounds such as hexanal, phenylacetic acid, and 3-phenylpropionic acid were identified as compounds contributing positively to the TSR10 rubber odour only. The specific odours of both phenyl acids are well representative of the overall odour of this rubber at room temperature. Moreover, both compounds accounted for 34% of odorant active components against 12% for malodorous butanoic acid.

Table VIII reports, in an ascending order, the odour thresholds, expressed in gm^{-3} , of few compounds identified in natural rubber. As expected, the malodorous compounds such as TMA and

Table VIII. Odour Threshold of Some Volatile Compounds Identified in Rubber Samples

Volatile compounds	Odour threshold (mg m^{-3}) ^a
Butanoic acid	0.00389
Trimethylamine	0.00589
Phenylacetic acid	0.00724
p-Cresol	0.00832
Isovaleric acid	0.0105
Ethylbenzene	0.0129
Valeric acid	0.0204
Lauric acid	0.0204
Octanoic acid	0.0240
Hexanal	0.0575
Hexanoic acid	0.0603
Isoamyl alcohol	0.162
Benzaldehyde	0.186
Acetic acid	0.363
Phenol	0.427
Trimethylbenzene	0.776
Pentanol	1.70
p-Xylene	2.14
Toluene	5.89
Benzene	12

^a Refs. [26, 27].

fatty acids have much lower values than other compounds, thus explaining their strong impact in odorous emissions of natural rubber. However, phenyl acetic acid exhibits a particularly low odour threshold and despite its low volatility, it contributes positively to the odorous emissions of rubber and may hide the obnoxious odour emitted. In addition, its low odour threshold explained its odourant impact perceived at ambient temperature, whereas it was not detected by SPME-GC-MS analyses at 30°C.

CONCLUSION

The use of HS-SPME method coupled with GC-MS analysis has brought improvements in the knowledge of aromatic volatile compounds present in natural rubber and to predict the volatile compounds that can be released in the atmosphere during rubber process. The HS-SPME carried out at different extraction temperatures allowed to identify 16 new compounds such as long-chain fatty acids or fatty acid esters, but also compounds unexpected in rubber such as isoamyl alcohol, benzyl alcohol, benzaldehyde, benzoic acids, and phenyl acids, that could not be identified with simple HS extraction.

Enzymatic and microbial degradation as well as thermal degradation and oxidation of the raw material during rubber process could explain the diversity of the volatile compounds identified in both grades of natural rubber. The chemical classes of the volatile compounds found in each rubber varied with the coagulation process used. Consequently, the aromatic profile of TSR10 (natural coagulation) was richer than the one of TSR5CV (controlled coagulation). A rigorous comparison between compound identification by GC-MS and odour identification by GC-O-FID highlighted the compounds responsible for the different pleasant and disturbing odours perceived from rubber. The phenyl acids with a pleasant honey odour were found only in TSR10. These compounds had a strong impact on odorous emissions of rubber and partially masked the obnoxious odour because of short fatty acids. Besides these acids, TMA and 2,6 dimethoxyphenol also contribute strongly to the malodorous odour.

This article based on an optimized sample preparation by HS-SPME for the extraction and concentration of volatile compounds and the identification of their structure and odour impact by chromatography coupled with spectrometric and olfactometry methods has contributed to the knowledge of odorous components emitted by natural rubber. This method can be adapted to the analyses of malodorous emissions at different stages of rubber processing, targeting the most odorant active compounds. This would help in finding the adapted solutions to minimize their impact on farmers in plantations, workers in factories, and citizens. This method can also be used to analyze the odorant volatiles emitted by any polymers.

REFERENCES

1. Companon, P., Ed. *Techniques Agricoles et Production Tropicales*; Maisonneuve, Larose: **1986**; p 543.

2. Liengprayoon, S. PhD thesis, (Montpellier), **2008**.
3. Vaysse, L.; Bonfils, F.; Thaler, P.; Beuve, J. S. in *Sustainable Solutions for Modern Economies*, Hofer, R., Ed.; BB Hardback: Germany, **2008**.
4. Tekasakul, P.; Tekasakul, S. *J. Aerosol Res.* **2006**, *21*, 122.
5. Isa, Z. *Plant. Bull.* **1993**, *215*,56.
6. Hoven, V. P.; Rattanakaran, K.; Tanaka, Y. *Rubber Chem. Technol.* **2003**, *76*, 1128.
7. Hoven, V. P.; Rattanakaran, K.; Tanaka, Y. *J. Appl. Polym. Sci.* **2004**, *92*, 2253.
8. Sakdapipanich, J.; Insom, K. *Kgk-kautschuk gummi kunststoffe*, **2006**, *56*, 382 .
9. Danteravanich, S.; Yonglaoyoong, S.; Sridang, P.; Wisunthorn, S.; Penjamras, P. I. *Proceedings of International Natural Rubber Conference*, Cambodia, **2007** 13–17 November.
10. Bart, J. C. J. *Polym. Test.*, **2001**, *20*,729.
11. Intapun, J.; Sainte Beuve, J.; Bonfils, F.; Tanrattanakul, V.; Dubreucq, E.; Vaysse, L. *J. Appl. Polym. Sci.* **2010**, *118*, 1341.
12. Van Den Dool, H.; Kratz, D. J. A. *J. Chromatogr.*, **1963**, *11*, 463.
13. Galli V.; Olmo N.; Barbas C. *J. Chromatogr. A*, **2002**, *942*, 367.
14. Heisey, R. M.; Papadatos, S. *Appl. Environ. Microbiol.*, **1995**, *61*,3092.
15. Belitz, H. -D.; Grosch, W. *Food Chemistry*; Springer Verlag: Berlin, Heidelberg, New York, **1999**.
16. Chalier, P.; Crouzet, J. *Food Chem.*, **1998**, *63*, 447.
17. Cann, A. F.; Liao, J. C. *Appl. Microbiol. Biotechnol.*, **2010**, *85*, 893.
18. Gram, L.; Ravn, L.; Rasch, M.; Bartholin Bruhn, J.; Christensen, A. B.; Givskov, M. *Int. J. Food Microbiol.*, **2002**, *78*, 79.
19. Herbinet, O.; Glaude, P. -A.; Warth, V.; Battin-Leclerc, F. *Combust. Flame*, **2011**, *158*, 1288.
20. Okrasa, K.; Guibe-Jampel, E.; Plenkiewicz, J.; Therisod, M. *J. Mol. Catal. B Enzym.*, **2004**, *31*, 97.
21. Chu, L. F.; Yaylayan, V. A. *J. Agric. Food Chem.*, **2008**, *56*, 10697.
22. Hofmann, T.; Munch, P.; Schieberle, P. *J. Agric. Food Chem.*, **2000**, *48*, 434.
23. Chung, H.; Choi, A.; In Hee, C.; Young-Suk, K. *Eur. J. Lipid Sci. Technol.*, **2011**, *113*, 1481.
24. Chen, F.; Quian, J. L. *Fuel*, **2002**, *81*, 2071.
25. Stadler, R. H.; Verzegnassi, L.; Varga, N.; Grigorov, M.; Studer, A.; Riediker, S.; Schilter, B. *Chem. Res. Toxicol.*, **2003**, *16*, 1242.
26. Schiffman, S. S.; Bennett, J. L.; Raymer, J. H. *Agric. Forest Meteorol.*, **2001**, *108*, 213.
27. Devos, M.; Patte, F.; Rouault, J.; Laffort, P.; Van Gemert, L. *J. Standardized Human Olfactory Threshold*; Oxford University Press: New York, **1990**.