Identification And Quantification of Odorous Compounds From Adhesives Used In Food Packaging Materials By Headspace Solid Phase Extraction And Headspace Solid Phase Microextraction Coupled To Gas Chromatography Olfactometry Mass Spectrometry.

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Abstract.

Adhesives are often responsible for off-flavors in food in contact with packaging. The aim of this investigation was to identify by GC-O-MS the odorous compounds in five different types of adhesive (hotmelt, vinyl acetate ethylene, starch, polyvinyl acetate and acrylic) used in food packaging. In order to obtain a substantial number of compounds, they were extracted by two complementary extraction methods: HS-SPE and HS-SPME. Fifteen minutes extraction time using PDMS fiber for hotmelt adhesive and DVD/CAR/PDMS fiber for the other adhesives were the best conditions for defining a representative solvent-free adhesive extract using a rapid and simple D-GC-O technique. Thirty three compounds were identified by GC-O-MS. These include butyric acid, acetic acid, methyl butyrate, 1-butanol and nonanal, which were present in most of the adhesives under study producing cheesy, rancid, sour, medicinal and green aromas, respectively. The concentrations were determined, the most abundant compound being acetic acid with concentrations from 22.9 to 8,930 μg/g of adhesive.

1. Introduction

Adhesives are commonly used in multilayer materials for food packaging. In most applications, although they are not in direct contact with the food, their constituents can migrate through the multilayer materials and contaminate it [1,2] as has already been shown in previous studies [3-5].

Packaging materials can also contain a large number of compounds responsible for off-flavors deriving from the degradation of the materials or the manufacturing process, including printing, coating and lamination. Off-flavors are also produced by the interaction between food and packaging. These off-flavors can modify the organoleptic properties of food and produce a negative effect on the quality of the product [6-13]. For food companies, this can lead to an increase in production costs or a possible loss of brand confidence and market share.

Traditionally, the control of odors in the packaging and food industries has been carried out by a trained panel. However, this technique is not valid for identifying the individual compounds responsible for odors and thus for correcting and eliminating the problem [14]. Only an overall perception of the odor and the absence or presence of undesired odors can be detected by a panel. The electronic nose, which is an attractive tool for the quality control of odors, only allows a comparison of the odor response of a sample to that obtained from a reference sample. It can identify neither the odor nor the individual compound responsible for it. Gas chromatography – olfactometry (GC-O) includes a sniffing port in which the human nose acts as an odor detector. When this device is combined with GC-MS, the system as a whole becomes a powerful tool for identifying the individual compound responsible for an odor. This technique was proposed by Fuller et al. [15], where successful detection of active compounds was achieved by sniffing the effluent during gas chromatography. This methodology has proved to be a valuable method for the selection of odor active compounds from a complex mixture, as the human nose is a much more sensitive detector than the conventional “chemical” detectors for such compounds which can be present in very low concentrations [16]. Using this technique, it is common to have very different profiles of odor active compounds than those of the chemical compounds registered in the chromatogram. The odorants frequently provide a higher signal than the non-odorants because of the higher sensitivity of the human nose compared to instrumental detectors.

Although this technique has been used for identifying the aroma-producing compounds in many types of foodstuffs, including wine and spirits, their identification in food packaging materials and specifically the influence of adhesives contained in them has not

yet been tackled. This is surprising given that companies producing packaging materials are obliged to exercise control over these materials which can spoil packaged food and cause serious complaints from customers and consumers. Even though this issue is usually more closely related to food quality than safety, it should be remembered that consumer perception of odors is perhaps one of the main reasons for complaints.

Within the framework of packaging materials, adhesives constitute an important source of chemicals. Formulas specifically developed to glue different materials together in an efficient manner contain many different substances. As adhesives are not usually in direct contact with food, their contribution to the quality and safety packaged food has not been adequately explored. There is no European legislation concerning adhesives in contact with food and thus very few studies of adhesives have appeared in the literature in the last five years. The European Research Project MIGRESIVES provided a considerable amount of information concerning different types of adhesives and their contribution to migration to food, but no mention was made of off-flavors or odorant compounds that can migrate to food. The main objective of this paper, therefore, is to study the contribution of several adhesives to off-flavors present in several packaging materials, and to provide appropriate protocols for the identification by GC-O-MS of the odorous compounds in different types of adhesives used in a series of multilayer packaging materials. For this purpose, several analytical and sensory techniques have been developed, applied and validated using a wide variety of adhesives and samples. The results obtained are shown and discussed.

2. Materials and methods

2.1. Reagents

The standards 1-butanol (71-36-3), p-xylene (106-42-3), p-cymene (99-87-6), nonanal (124-19-6), propanoic acid (79-09-4), naphthalene (91-20-3), benzaldehyde (100-52-7), toluene (108-88-3), hexanal (66-25-1), paraldehyde (123-63-7), butyl acrylate (141-32-2), 1-hexanol (111-27-3), cyclohexanol (108-93-0), methyl benzoate (93-58-3), allyl benzoate (583-04-0), butyric acid (107-92-6), methyl butyrate (623-42-7), acetic acid (64-19-4), ethyl acetate (141-78-6), methyl methacrylate (80-62-6), butyl propanoate (590-01-2), styrene (100-42-5), 2-octanone (111-13-7), 2-ethylhexyl acetate (103-09-3), 2-ethyl-1-hexanol (104-76-7), camphor (76-22-2), 1-octanol (111-87-5) and 4-tert-butylphenol (98-54-4) were purchased from Sigma-Aldrich Química S.A (Madrid, Spain). All were of analytical quality. Dicloromethane and acetic acid, both of HPLC

grade, were supplied by Scharlau Chemie S.A (Sentmenat, Spain). An alkane standard solution C8-C20 at 40 µg/g in hexane was used to calculate retention indexs. A solution of 4-tert-butylphenol at 1000 µg/g in methanol was used as an internal standard solution. The SPME fibers were supplied by Supelco (Bellefonte, PA, USA).

2.2. Adhesive samples and laminates.

Different types of adhesives commonly used in food packaging were obtained: three vinyl acetate ethylene (VAE) adhesives, three hotmelt (HM), one starch, one acrylic (ACR) and one polyvinyl acetate (PVA).

All the adhesives were water based with the exception of the HM. The VAE_01 and PVA adhesives contained triacetin as a plasticizer, while the VAE_02 and VAE_03 adhesives were manufactured with diethylene glycol dibenzoate as a plasticizer. Tackifiers and an antioxidant were present in the starch and acrylic adhesives, but details of their formulas cannot be given for reasons of confidentiality.

Three hotmelt adhesives were supplied. Hotmelt 1 (HM1) was based on ethylene vinyl acetate (EVA) and hotmelt 2 (HM2) on a polyolefin enriched with propene. No precise information was provided for hotmelt 3. These adhesives are solid polymers (films, granules or pellets) at room temperature. To manufacture the laminates, the hotmelt adhesives are first heated at 160-180 ºC. Once melted, they are applied and extended on a substrate forming a uniform layer. Afterwards, a second substrate is placed on this surface and some pressure is applied to form the laminate. For this study, the hotmelt adhesives were heated at 160ºC until they were melted and they were then applied on a flat surface (silicone paper) and cooled to room temperature, simulating the cured process.

For the selection of the most odorant adhesive, the hotmelt and VAE adhesives were also studied as part of multilayer laminates. The laminates were market samples provided by different European companies with the structure [Cardboard (CB)–adhesive–Cardboard (CB)]. Most were not printed and were manufactured with different amounts of adhesive per m^2 of laminate. The substrates used for their manufacture were also separately provided and were of different grammage and thickness. The laminates studied were as follows:

- Lam_01: CB (350 g/m^2 and 502 µm) - VAE_01 (31.8 $g_{adhesive}$/m^2 laminate) - CB
- Lam_02: CB (350 g/m^2 and 479 µm) - VAE_02 (49.1 $g_{adhesive}$/m^2 laminate) - CB
- Lam_03: CB (300 g/m^2 and 485 µm) - VAE_03 (30.7 $g_{adhesive}$/m^2 laminate) - CB

- Lam_04: CB (380 g/m^2 and 380 µm) - HM_01 (31.3 $g_{adhesive}/m^2$ laminate) - CB
- Lam_05: CB (380 g/ m^2 and 380 µm)- HM_02 (31.3 $g_{adhesive}/m^2$ laminate) - CB
- Lam_06: CB (400 g/ m^2 and 570 µm)- HM_03 (68.2 $g_{adhesive}/m^2$ laminate) - CB

CB signifies cardboard, HM hotmelt and VAE vinyl acetate ethylene.

For the rest of the work, the adhesives were individually studied.

2.3. Selection of the most odorant adhesives and their sensory analysis.

The main objective of this work was the identification of the odorous compounds within each type of adhesive. As several hotmelt and VAE adhesives were available, the most odorous adhesive within each group of hotmelt and VAE were initially selected by a triangular test. Three products were shown to the assessors of which two were identical and one was different. The assessors were asked to distinguish which product they believed was the odd one out.

The triangular test was carried out with the laminates described above (Lam_01, Lam_02, Lam_03, Lam_04, Lam_05 and Lam_06) as manufactured (CB-adhesive-CB) and the different substrates without the adhesive used to produce each laminate.

It consisted of finding the different odorous vial among three vials containing 1*5cm^2 of laminate or substrate cut into strips. This difference depends on the odorant capacity of each adhesive.

For this purpose, one panel formed by 20 panelists, previously trained with adhesive odors, was asked to identify the different vial from among three colourless vials (capacity 70 mL) where either one vial or two contained 1*5 cm^2 of laminate cut into strips and two vials or one contained two substrates with the same surface also cut into strips. This test was carried out at two temperature values: room temperature (22 ºC) and at 40 ºC.

The number of successful identifications allowed us to know if there was a significant difference (statistical tables for triangular test [17]) between the laminate and the substrate and, therefore, to choose the most odorant adhesives.

Once the most odorant adhesives for each group were selected, a descriptive analysis was carried out to identify the sensory attributes (1g in colorless vials). This was done by a team of 6 assessors, all of whom had previously carried out the triangular test with a high success rate.

2.4. Direct gas chromatography olfactometry (D-GC-O)

The D-GC-O method was used to perform the representativeness test on the global odor of the HS-SPME extracts in order to select the best extraction conditions. This recent technique consists of connecting a deactivated capillary column between the injector and sniffing port of a GC system in order to avoid chromatographic separation so that the aroma compounds arrive simultaneously at the sniffing port. The equipment used was a CP-3800 Varian equipped with the sniffing port ODO I supplied by SGE (Ringwood, Australia) with a short capillary of untreated silica (20 cm x 0.32 mm i.d) from SGE analytical science (Madrid, Spain).

The parameters of the D-GC-O device were as follows: injection system, splitless mode; injector temperature, 250ºC; oven temperature, 100ºC; carrier gas, helium with a flow rate of 1 mL/min.

For this assay, 5 grams of the adhesives (VAE_02, HM_01, starch, ACR and PVA) were introduced into 20 mL vials. They were extracted by HS-SPME with different fibers and different times. These extracts were introduced in successive sequence into the GC port where the odorous compounds were thermally desorbed with the conditions above described. The compounds arrived simultaneously at the sniffing port where the assessor perceived, evaluated and compared the resulting global odor with the adhesive. This study was repeated six times, each one with a different assessor. The best extraction conditions (fiber and time) were selected.

2.5. GC-O-MS

2.5.1. Identification.

For the identification of the single odor compounds, the adhesives were firstly extracted by two methodologies: HS-SPE and HS-SPME (conditions previously selected by D-GC-O). The two extracts were then analyzed by GC-O-MS where the compounds were separated in the chromatographic column and evaluated (retention time, intensity and odor) at the sniffing port by six panelists. Simultaneous chemical identification was achieved in the MS detector.

The equipment used was a CP-3800 Varian gas chromatograph system (Madrid, Spain) connected to a Saturn 2000 series (Madrid, Spain) with an ion trap mass detector and sniffing port ODO I supplied by SGE (Ringwood, Australia). Chromatographic separations were carried out on a BP-20 (30 m x 0.25 mm x 0.25 μm) from SGE analytical science (Madrid, Spain). The oven temperature program was as follows; initial

temperature 40°C (5 min), heating rate of 10 °C/min to 220 ºC, then held at 220ºC for 10 minutes. Helium was used as carrier gas at 1 mL/min flow. The ionization was performed by electronic impact and the ion trap temperature was 220 C. The electron multiplier voltage was 1600V. Acquisition was carried out in SCAN mode (45-350 m/z).

For the analysis of HS-SPE extracts, 1µL of sample was injected in splitless mode and the following injection conditions were used: initial temperature of 30 ºC for 0.15 min followed by a heating rate of 200 ºC/min to 250 ºC with 25 psi as pulse pressure. The split valve was opened 2.5 min after injection.

For HS-SPME extraction, 5 grams of the adhesive (VAE_02, HM_01, starch, ACR and PVA) were placed in a 20 mL vial each and the following extraction conditions were applied: extraction temperature, 40ºC; extraction time 15 min (previously selected by D-GC-O as described above); DVB/CAR/PDMS fiber was used for the extraction of VAE_02, starch, ACR and PVA adhesives and PDMS fiber was used for the HM_01 adhesive (previously selected by D-GC-O as described above). These extracts were desorbed in the injection port at 250ºC for 2 min with a splitless time of 2.5 min.

2.5.2. Quantification.

For the quantification of the odorous compounds, the adhesives (VAE_02, HM_01, starch, ACR and PVA) were analyzed by HS-SPME coupled to GC-O-MS. A CP-3800 Varian gas chromatograph system (Madrid, Spain) connected to a Saturn 2000 series (Madrid, Spain) with an ion trap mass detector was used under the same conditions as those described above for the identification. The acquisition in this case was carried out in SIM mode and the characteristic ions used for quantification purposes are shown in Table 2.

The same conditions were used for the HS-SPME extraction of adhesives (VAE_02, HM_01, starch, ACR and PVA) as for calibration curves with the standards.

2.6. Extraction of volatile odorous compounds from adhesive

The extraction of odorous compounds from the previously selected adhesives (VAE_02, HM_01, starch, ACR and PVA) was carried out by the two methodologies described below.

2.6.1. Solid phase extraction (SPE) in headspace mode

A standard SPE cartridge (0.8 cm internal diameter and 3 mL internal volume) filled with 400 mg of LiChrolut EN resins was first washed with 20 mL of dichloromethane and dried with desiccant air (negative pressure of 0.6 bar, 10 min). The cartridge was placed on the top of a bubbler flask containing about 50 mL of cured adhesive (54.85 grams of VAE_02, 68.0 grams of HM_01, 55.50 grams of Starch, 60.05 g PVA and 61.60 g ACR) at a constant temperature of 40 ºC. A controlled gentle stream of nitrogen (500 mL/min) was passed through the headspace for 140 min to carry all the volatile compounds out of the flask to the SPE cartridge. The volatile compounds released by the adhesive in each case were trapped in the cartridge containing the sorbent. After 140 min, the cartridge was removed and dried by letting N_2 pass through it. The analytes were eluted with 3.2 mL of dichloromethane with 5% methanol. This process was used in previous works to extract odorous compounds from wine samples [18-20]. The final extract was concentrated under a stream of pure N_2 to a final volume of 500 µL. Five replicates of each adhesive were used for subsequent identification.

2.6.2. Extraction by solid phase microextraction (SPME) in headspace mode

A rapid and simple technique was developed for evaluating the sensory quality of the SPME extracts using the direct gas chromatography-olfactometry (D-GC-O) technique described above. Different types of fibers and extraction times were tested. The assays were as follows:

Selection of appropriate fiber

The first step was the selection of the most appropriate SPME fiber for each adhesive. Four fibers with different polarity and thickness were tested:

- Divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber of 50/30 µm
- Polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber of 65 µm
- Polydimethylsiloxane (PDMS) fiber of 100 µm
- Polyacrylate (PA) fiber of 85 µm

The fiber selection was carried out by direct gas chromatography olfactometry (D-GC-O) where four SPME extracts (four fibers studied for each adhesive at 40 ºC and 15 min of extraction) were introduced in successive sequences into the GC port without a chromatographic column. As in these conditions there was no chromatographic separation, the aroma compounds arrived all together at the sniffing port. Here, for each

SPME extract, a trained panel of six assessors perceived and evaluated the resulting global odor, which was compared to the sample of adhesive. First, the assessors sniffed the reference adhesive (5 grams) contained in a colorless vial. They memorized the odor and compared it with the global aroma obtained at the sniffing port. The different extracts were ranked according to their similarity to the reference using a 10 point scale ranging from 0 (far from the reference) to 10 (close to the reference).

Selection of extraction time

Once the fiber was selected, the extraction time was optimized.

For this purpose, the adhesives were extracted with the fiber at different times (1, 5 and 25 min) and these different extracts were analyzed by D-GC-O applying the same criteria as described above. The values more similar to the adhesive sample were compared to the values previously obtained using the same fiber at 15 minutes of extraction. The extraction time with the highest similarity value was chosen for further study.

Once the extraction conditions (fiber and time) were selected, the adhesives were extracted by HS-SPME with the methodology described above. Five HS-SPME adhesive extracts were used for subsequent identification.

2.7. Identification of odorous compounds from the extract of adhesives

To determine the odorous compounds in each adhesive, one microliter of HS-SPE extract and HS-SPME extract obtained under the extraction conditions selected previously for each adhesive were injected into the previously described GC-O-MS.

Six panelists were able to detect the individual odorous compounds eluted from the chromatographic column and describe their odor notes. Besides, the intensity of these odorous compounds was recorded using a scale from 1 to 3 units, where 1 corresponded to the weakest odor (low intensity), 2 was a clear perception of odor (strong intensity) and 3 corresponded to extremely strong intensity of odor. Fractional values were also allowed. Afterwards, the modified frequency MF (%) was calculated to determine the most important odorous compounds from each extract applying the following equation [21]:

$$MF(\%) = [F(\%)xI(\%)]^{0.5}$$

where F(%) was the percentage of panelists who had detected the odorous compounds and I(%) was the percentage of intensity calculated by the average of the values of intensity given by all the panelists divided by three.

The odorous stimuli detected with a MF(%) higher than 50 were considered as representing the most important compounds present in each adhesive extract. The compounds found in more than one adhesive were also studied even if their MF(%) was lower than 50.

Once the MF(%) was calculated and the most important odorous stimulus for each extraction and each adhesive were selected, the identification was carried out. The retention indexs of these compounds were calculated using a series of n-alkanes prepared in hexane (C10 –C30) injected under the same chromatographic conditions. These retention indexs and their organoleptic characteristics were compared to the compounds with the same values found in the literature [8,22-29]. Additionally, mass spectral matches obtained for unknown peaks from NIST and WILEY mass spectra libraries were also used for identifying the compounds. Thus, a list of likely candidates was obtained for each odorous stimulus found in each extract and in each adhesive.

Finally, the pure candidates prepared in dichloromethane were injected under the same chromatographic conditions (GC-O-MS). To confirm the identification, these candidates had to match the unknown odorous compounds in terms of their retention indexs, odor characteristic and the mass spectrum.

2.8. Determination of the initial concentration profile (CP_0) of odorous compounds from adhesives

Once the odorous compounds extracted for each adhesive were identified, their initial concentrations were calculated. For this purpose, the adhesives (VAE_02, HM_01, starch, ACR and PVA) were analyzed by HS-SPME coupled to GC-O-MS (15 min and PDMS fiber for HM_01 and DVB/CAR/PDMS for the other adhesives). The HS-SPME extraction for the quantification proved to be a fast, very sensitive and free-solvent technique.

Before the initial concentrations were calculated, a study was carried out to avoid the matrix effect for quantification in the water based adhesives (VAE_02, Starch, PVA and ACR). For this purpose, the adhesives were diluted in different proportions (1, 5, 10, 50, 100 and 200) and analyzed with DVB/CAR/PDMS fiber by HS-SPME-GC-O-MS. The

signal obtained was compared with that obtained when the adhesives were diluted in the same proportions and spiked with the previously identified compounds. The increase in the signal was compared with the signal obtained when 100% water samples were spiked at the same concentration level. The dilution factor was selected on the basis of the minimum water dilution obtained with minimum matrix effects and maximum sensitivity of each sample for achieving recoveries over 90% for all odorous compounds[4].

For this assay, aliquots of 5 g of each solution were placed in headspace vials and 10 µL of solution A were added as an internal standard.

After that, the CP_0 was calculated. For building the calibration curves, solutions of different concentrations of the identified compounds were prepared in purified water. Aliquots of 5 mL of each solution were placed in headspace vials and 10 µL of solution A were added as internal standard. Three replicates of each sample were prepared and analyzed with DVB/CAR/PDMS by HS-SPME-GC-O-MS.

The initial concentration of the HM_01 adhesive was determined in another way, because this adhesive was solid at room temperature. In this case, to determine the CP_0 a standard addition procedure was carried out. For this purpose, 5 grams of the pure adhesive were heated at 160 ºC (to be cured) and, once melted, 10 µL of solution containing different concentrations of the compounds identified for this adhesive and also 10 µL of solution A as an internal standard were spiked. Three replicates of each sample were prepared and analyzed with PDMS (previously selected for this adhesive) by HS-SPME-GC-O-MS.

3. Results and discussion

The aim of this work was to identify and quantify the odorous compounds in five different types of adhesives (HM, VAE, starch, PVA and ACR). As several HM and VAE adhesives were available, firstly the most odorant adhesives of each type were selected by a triangular test. Once these adhesives were selected, they (HM_01, VAE_02, starch, PVA and ACR) were extracted by two techniques (HS-SPE and HS-SPME) in order to obtain the highest number of odorous compounds. Previous to the identification, the conditions of HS-SPME extraction (fiber and time) for each adhesive were selected by D-GC-O. After that, they were identified by GC-O-MS where six panelists evaluated their time of retention, odor and intensity, and they were confirmed by the pure standard. Once the identification was carried out, their initial concentration in the adhesive was calculated by HS-SPME-GC-O-MS.

3.1. Selection of the most odorant adhesive for each type of adhesives and their sensory analysis

Firstly, the most odorous adhesive within each group of hotmelt and VAE adhesives was selected through a triangular test.

For the adhesives VAE_01, VAE_02 and VAE_03, there were no significant differences between the laminate (Lam_01, Lam_02 and Lam_03 respectively) and their substrates at room temperature. However, significant differences were found at 40 ºC, for the Lam_01 ($p< 0.4$), Lam_02 ($p<0.01$) and Lam_03 ($p<0.01$), so the adhesives VAE_02 and VAE_03 were the most odorous within the group. However, VAE_02 was selected because its success rate in the triangular test was higher at both temperatures (room and 40 ºC) than for the adhesive VAE_03 (13 successes compared to 12, respectively, at 40ºC).

The same occurred for the HM adhesives where the differences between the substrate and the laminate were not found at room temperature, while at 40 ºC significant differences for the adhesives Lam_04 ($p<0.001$), Lam_05 ($p<0.05$) and Lam_06 ($p<0.05$) were found. Therefore, the adhesive HM_01 was selected for the study.

Once the VAE_02 and HM_01 were selected as the most odorous adhesives within each type, a qualitative descriptive analysis was carried out for all the adhesives. The sensory attributes were assessed. White glue, plastic, pungent and paint odor were found for the adhesive VAE_02. For the adhesive HM_01, rubber tire, woody, depilatory wax, phenolic and leather odors were described. For the starch adhesive, rancid, ferment, white glue and paint odor were assigned. For the PVA adhesive, the odor attributed was a very pungent odor like vinegar and, finally, for the ACR adhesive moss, humidity and camphor were assigned as the main sensory properties.

3.2. Extraction by head space solid phase microextraction

Before carrying out the identification, the conditions for HS-SPME extraction were optimized.

Selection of the appropriate SPME fiber

Figure 1 shows the results of the similarity scale calculated as the average similarity values between the four SPME global odors with respect to the adhesive of reference

given by the different panelists by D-GC-O. The highest similarity values were between 6.3 and 8.4.

For the HM_01 adhesive, the most representative extracts were obtained from the PDMS, PDMS/DVB and DVB/CAR/PDMS fibers (similarity values of 6.3, 5.5 and 4.5, respectively). Thus, PA fiber (polar phase) provided the worst representative extract whose similarity value was significantly lower. The PDMS fiber was selected because it gave the highest similarity value. This is consistent with findings in a previous work to extract the volatile migrant compounds from hotmelt adhesives [3] in which PDMS showed the best performance. This fiber extracts the compounds of low polarity, which could be the unknown odorous compounds.

For the VAE_02, the optimum SPME fiber that provided the highest odor extract was the DVB/CAR/PDMS, whose value was significantly higher. For the rest of the fibers, the similarity values were below the score of 2.3. The same trend was found for starch and PVA adhesives for which clearly the best extract was obtained by the DVB/CAR/PDMS fiber (6.8 and 8.4, respectively). However, for the ACR adhesive the most representative extracts were obtained by the DVB/CAR/PDMS and PDMS fibers. DVB/CAR/PDMS was selected due to its higher similarity value.

The DVB/CAR/PDMS fiber, which has a structure with micropores, mainly extracted the low molecular weight compounds. These could be the odorous compounds and for this reason this proved to be the best fiber in most cases.

Summarizing, the optimum fibers selected were the PDMS fiber for the HM_01 adhesive and the DVB/CAR/PDMS for the rest of the adhesives.

Selection of extraction time

Once the fiber for each adhesive was selected, the extraction time was optimized. Figure 2 shows the average similarity values between the extracts and the reference sample for different extraction times. For all the adhesives, there were significant differences between 1 and 5 minutes versus 15 and 25 min. Longer extraction times achieved higher scores (similarity ranges from 5.7 to 8.5) than short extraction times (rate ranges from 1.2 to 4.6). As significant differences were not obtained between 15 and 25 minutes of extraction, 15 minutes was the selected extraction time.

3.3. Identification of odorous compounds from the adhesives extracted by HS-SPE and HS-SPME coupled to GC-O-MS

Figures 3, 4, 5, 6 and 7 show the chromatograms of HM_01, VAE_02, starch, PVA, and ACR adhesives, respectively, analyzed by GC-O-MS and extracted by HS-SPE or HS-SPME. The compounds with odor characteristics are indicated in the figures with numbers ordered by their retention index (Table 1). Thirty three compounds detected had characteristic odors either with MF(%)>50 or MF(%)<50, but they were all selected because they were found in more than one adhesive using the same extraction technique. Table 1 shows the odor compounds identified for each adhesive (with their retention indexs) and their MF(%) obtained for each extraction technique. Some compounds found in these adhesives showed a higher MF(%) when they were extracted by the HS-SPE technique than by HS-SPME, such as p-xylene detected in the VAE adhesive, with values of MF(%) from 65.3% to 50.3%. By contrast, 1-octanol found in the PVA adhesive showed values of MF(%) higher with the HS-SPME technique than with HS-SPE

Some compounds were detected only by one extraction technique, which emphasizes the importance of using two extraction techniques. These can be seen as complementary techniques for identification in this case. For example, some compounds whose retention indexs were lower than 1092 (where the solvent was detected) were only detected by the HS-SPME extract, because this technique is solventless and thus no solvent delay is required in MS. The solvent prevents the analyst from sniffing the odorous compounds when the SPE extracts are directly injected.

Using two complementary extraction techniques, the number of odorous compounds detected increased and consequently the list of possible migrant compounds to food also increased.

The compounds identified were several acids such as acetic, propanoic and butyric acid, which provided common organoleptic characteristics to the adhesives such as sour, vinegar, rancid and cheese aromas. Ester compounds such as methyl butyrate with cheese aroma and allyl and methyl benzoate with sweet aroma were also identified. Aldehyde compounds such as hexanal and nonanal with grass and green aromas were also found in several adhesives, or alcohol compounds such as cyclohexanol and 1-butanol with camphor and medicine aromas.

In the HM_01 adhesive, odor descriptors such as pine, herb and woody were repeated, which could be produced by calamenene and longifolene compounds. These compounds are present in the essential oils coming from the resin used for the manufacture of this kind of adhesive [3,26-28]. Some of the sensory attributes described above for this adhesive were depilatory wax or woody, which could come from these compounds.

In the VAE_02, three compounds, p-xylene (sweet), benzaldehyde (bitter almond) and one unknown compound with a plastic odor at RI 1797 were found which were not present in the rest of the adhesives. This latter compound could be responsible for the plastic aroma found by the assessors in the descriptive analysis. Two other sensory attributes were white glue and pungent, which could come from acetic acid. Toluene could be responsible for the paint attribute described above.

In the starch adhesive, three compounds (paraldehyde, propanoic acid and allyl benzoate) were found which did not appear in the other adhesives. The propanoic acid compound and the higher MF(%) of butyric acid may be responsible for the rancid aroma found by the descriptive analysis. The aromas of white glue and paint found in this adhesive could also come from acetic acid and toluene, respectively.

In the PVA adhesive, the most important sensory attribute described above was the vinegar odor, which could come from acetic acid, whose MF(%) was the highest of the set of adhesives by both extraction techniques.

In the ACR adhesive, camphor (camphor), 1-octanol (mushroom, moss) and one unknown compound with a mushroom odor at RI 1216 were found which were not present in the rest of the adhesives. These could be responsible for the camphor, moss and humidity aromas found by the assessors in the descriptive analysis.

3.4. The initial concentration profile, CP_0, of the odor compounds in the adhesives

Once the odorous compounds were identified, their initial concentrations were calculated. These assays were carried out by HS-SPME-GC-O-MS.

Previously, the dilution factor was selected in order to minimize the matrix effects and to obtain the maximum sensitivity in each adhesive (VAE_02, starch, ACR and PVA). To achieve recoveries over 90% for all the odor compounds, the adhesives were water diluted 1/100 (w/w).

To build the calibration curve, the compounds found for these adhesives were spiked in pure water at different concentrations. Analytical parameters of the HS-SPME-GC-O-MS (DVB/CAR/PDMS fiber) method and the ions used for their quantification are shown in Table 2. Good results were obtained in terms of linearity, limits of detection (LOD) and reproducibility. LOD values were between 0.03 ng/g (naphthalene) and 5.02 micrograms/g (butyric acid). RSD values were between 2.03 and 15.1%.

To quantify the compounds found in the HM_01 (1-butanol, p-cymene, nonanal, acetic acid and naphthalene), a standard addition procedure was carried out. The analytical

parameters of the HS-SPME-GC-O-MS (PDMS fiber) method and the ions used for their quantification are also shown in Table 2. Again, good results were obtained in terms of linearity. RSD values were between 6.8 and 14.2%.

Due to the difficulty in finding the standards for longifolene, calamenene and methyl butyrate, these were quantified using naphthalene as a standard for longifolene and calamenene, and butyric acid to quantify methyl butyrate.

The concentrations of the identified odor compounds, expressed as ng of compound per g of adhesive, are shown in Table 2. As would be expected, there is a clear relationship between the concentration and the MF(%) for each compound found. For instance, the concentration of toluene, whose MF(%) increased depending on the type of adhesive, from 13.6 in starch adhesive to 68.3 in VAE_02 adhesive, increased from 0.07 to 277 ng/g adhesive, as Table 1 shows. The same tendency was observed for 1-butanol identified in all the adhesives. Its MF(%) increased from 9.2 to 100, depending on the type of adhesive, increasing its concentration from < 8810 to 60.300 ng of compound per g of adhesive.

Comparing different compounds with the same MF(%), the relationship between the MF(%) and the concentration disappeared since compounds with similar MF(%) had different concentrations. For example, acetic and butyric acids had similar MF(%) in the Starch adhesive and VAE_02 for the SPE extract (65.0 and 65.3, respectively, as shown in Table 1) while their concentrations were totally different with values of 52600 and <16700 ng/g, respectively, as shown in Table 2. This fact can be explained by their different odor thresholds. While acetic acid had 0.363 (mg/m^3) according to Devos et al. or 0.145 (mg/m^3) according to SchiMFFan et al [30], the butyric acid threshold is 0.0145 (mg/m^3) according to Devos et al. or 0.00389 (mg/m^3) according to SchiMFFan et al [30,31]. This means that butyric acid will be better perceived by the human nose than acetic acid as its odor threshold is lower, and therefore with a lower concentration its MF(%) will be higher.

The most abundant compound in all the adhesives was acetic acid. Its concentration ranged from 22900 to 8930000 ng/g adhesive depending on the type of adhesive. The PVA adhesive had the highest concentration, this being consistent with the attributes of the assessors who had assigned pungent and vinegar odors in the sensory analysis. The other major odorous compounds were p-cymene (28300 ng/g) in the HM_01 adhesive, ethyl acetate (464000 ng/g) and 1-butanol (60300 ng/g) for the PVA adhesive, and methyl

methacrylate (56200 ng/g), 2-ethylhexyl acetate (68500 ng/g) and 2-ethyl-1-hexanol (514000 ng/g) for the ACR adhesive.

Conclusion

The odorous compounds from adhesives (hotmelt, vinyl acetate ethylene, starch, polyvinyl acetate and acrylic) commonly used in food packaging materials have been identified and quantified. Two extracts of these adhesives obtained by two different methodologies, HS-SPE and HS-SPME (the type of fiber and extraction time being optimized for each adhesive by a simple and rapid D-GC-O method) have been analyzed by the GC-O-MS method. This has proved to be a useful and reliable tool to identify a great number of odorous compounds in these adhesives. Thirty three compounds with characteristic odors were identified; some of them, such as butyric acid, acetic acid, methyl butyrate, 1-butanol and nonanal, were present in most of the adhesives. The most abundant compound was acetic acid with a concentration range between 22900-8930000 ng/g with a sour and vinegar aroma. We can conclude that the two extraction methodologies are complementary, as several compounds were trapped either in the SPE cartridge or in the SPME fiber but not in both. Besides, this study emphasizes the importance of identifying a large number of the chemical compounds responsible for off-flavors coming from adhesives. This leads to a possible way forward for adhesive companies to reformulate and replace these odorous compounds by other odorless compounds or to reduce their concentrations in order to avoid their migration into food and the consequent undesirable organoleptic changes.

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Table 1: Identified odorous compounds with its retention index (RI) and its odor description perceived by the different assessors. Its modified frequency (%MF) by HS-SPE and HS-SPME extraction in five different types of adhesives (HM_01, VAE_02, Starch, PVA and ACR)

				%MF (HM_01)		%MF (VAE_02)		%MF (Starch)		%MF (PVA)		%MF (ACR)	
N	RI	Compound	Odor description	SPE	SPME	SPE	SPME	SPE	SPME	SPE	SPME	SPE	SPME
1	907	Ethyl acetate	Fruity, sweet								54.4		
2	1005	No identified (n.i)	Pungent		50.3								
3	1022	Methyl methacrylate	Sharp fruity										81.6
4	1035	Toluene	Paint				68.3		13.6				
5	1077	Hexanal	Grass, fat						51.8		64.5		
6	1092	Paraldehyde	Pungent, disagreeable						53.6				
7	1098	P-xylene	Sweet			65.3	50.3						
8	1120	Butyl propanoate	Earthy, sweet										64.5
9	1168	1-butanol	Medicine		9.2		16.3		28.9	54.9	100	57.7	
10	1170	Butyl acrylate	Pungent fruit						50.9				100
11	1216	N.i	Mushroom									84.7	
12	1241	Styrene	Gasoline, balsamic									52.7	59.7
13	1280	P-cymene	Gasoline, solvent	52.6									

14	1285	2-octanone	Herb, resin								85.0		
15	1359	1-hexanol	Resin, green				54.0			83.3			
16	1382	2-ethylhexyl acetate	Sharp										76.42
17	1400	Nonanal	Fresh, green	58.3		44.7		33.2					
18	1411	Cyclohexanol	Camphor				51.0			57.7			
19	1427	N.i	Disagreeable, woody	50.9	78.3								
20	1466	Acetic acid	Sour, like vinegar	62.4	68.9	76.1	79.1	65.0	69.3	100	100	79.9	62.3
21	1487	2-ethyl-1-hexanol	Green										100
22	1491	Camphor	camphor									64.5	
23	1536	Propanoic acid	Rancid					50.2					
24	1539	Benzaldehyde	Bitter almond			53.8	51.5						
25	1553	1-octanol	moss, mushroom									72.7	85.0
26	1570	Longifolene	Woody , pine	54.0	50.0								
27	1626	Butyric acid	Rancid, cheese	45.3		65.3		71.4		40.8		60.9	
28	1627	Methyl benzoate	Aromatic, sweet				31.2		43.0				
29	1689	Methyl butyrate	Cheese	37.4		38.2		35.2					
30	1785	Naphthalene	Tar, mothball	47.3				31.5				52.7	
31	1797	N.i	Plastic, glue			50.0							

32	1823	Allyl benzoate	Sweet, floral				50.2	59.1
33	1835	Calamenene	Herb spice	55.3	51.1			

Table 2: Initial concentration profile for the identified odorous compounds, CP_o expressed as ng of compound per g de adhesive for each adhesive, the analytical parameters of the HS-SPME-GC-O-MS method (with DVB/CAR/PDMS fiber and with PDMS fiber annoted by *)

Compounds	**Quant. ion**	**HM_01 ng/gadh**	**VAE_02 ng/gadh**	**Starch ng/gadh**	**PVA ng/gadh**	**ACR ng/gadh**	**Equation**	**R^2**	**Linear range (ng/g)**	**LOD (ng/g)**	**LOQ (ng/g)**	**RSD (%)**
Ethyl acetate	43				464000		y= 0.017x+0.049	0.991	450-49600	135	450	8.35
Methyl methacrylate	69					56200	y=1.928x+0.005	0.999	5.69-1070	1.71	5.69	6.57
Toluene	91		277	0.07			y=70.06x+0.228	0.999	0.06-106	0.05	0.06	5.17
Hexanal	44			277	1200		y=5.566+0.034	0.994	4.87-111	1.46	4.87	11.3
Paraldehyde	45			175			y=0.101x+0.019	0.998	27.8-1920	8.35	27.8	7.38
P-xylene	91		425				y=451.1x+0.124	0.997	0.26-99.3	0.07	0.26	2.03
Butyl propanoate	57					122	y=65.53x+0.224	0.998	2.31-1140	0.71	2.31	13.9
1-butanol	56		<LOQ	<LOQ	60300	8950	y=0.002x+0.015	0.997	8810-95600	2640	8810	9.74
1-butanol*	56	885					y=0.0008x+0.007*	0,998				8.77
Butyl acrylate	55			11.5		8230	y=92.22x+0.037	0.998	0.88-88.1	0.26	0.88	14.6
Styrene	104					445	y=459.2x+0.103	0.999	0.36-86.3	0.11	0.36	7.46
P-cymene	119						y=4.268x-0.305	0.994	0.58-4250	0.17	0.58	5.56
P-cymene*	119	28300					y=0.0053x+0.1315*	0.989				8.77
2-octanone	43				326		y=59.56x-0.031	0.999	0.37-87.2	0.11	0.37	8.89

1-hexanol	56		7480		11500		y=0.643x-0.009	0.991	93.8-24500	28.2	93.8	5.33
2-ethylhexyl acetate	43					68500	y=208.3x-0.029	0.999	0.29-89.7	0.09	0.29	4.33
Nonanal	57		405	158			y=37.02x-0.008	0.994	2.12-193	0.64	2.12	13.6
Nonanal*	57	2630					y=0.0217x+0.2714*	0.985				6.88
Cyclohexanol	57		4420		4390		y=0.087x+0.002	0.997	91.7-1080	27.5	91.7	15.1
Acetic acid	43		429000	52600	8930000	72700	y=0.041x+0.012	0.997	130-9320	38.8	130	14.6
Acetic acid*	43	22900					y=0.033x+0.0612*	0.989				12.3
2-ethyl-1-hexanol	57					514000	y=6.680x+0.259	0.999	9.93-894	2.98	9.93	14.6
Camphor	95					6080	y=0.698x-0.052	0.991	2.98-1030	0.99	2.98	6.99
Propanoic acid	74			<LOQ			y=0.007x+0.014	0.984	2410-95900	724	2410	3.53
Benzaldehyde	106		6390				y=4.927x-0.054	0.999	31.2-1020	9.35	31.2	13.2
1-octanol	56					1350	y=3.141x-0.026	0.999	19.1-954	5.75	19.1	9.06
Longifolene[a]	161	1430										
Butyric acid	60	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	y=0.041-0.147	0.996	16700-99100	5020	16700	12.1
Methyl benzoate	105		1810	6941			y=17.06x-0.119	0.997	9.85-1150	2.96	9.85	2.85
Methyl butyrate[b]	43	<LOQ	<LOQ	<LOQ								
Naphthalene	128	2510		429			y=410.6x+0.046	0.998	0.10-98.5	0.03	0.10	12.2
Naphthalene*	128						y=0.6104x+1.7662*	0.985				4.30

Allyl benzoate	105		4150	y=17.27x-0.396	0.997	2.67-1120	0.80	2.67	3.93
Calamenene[a]	159	1340							

* Standard addition

[a] Quantified with naphthalene* as standard

[b] Quantified with butyric acid as standard

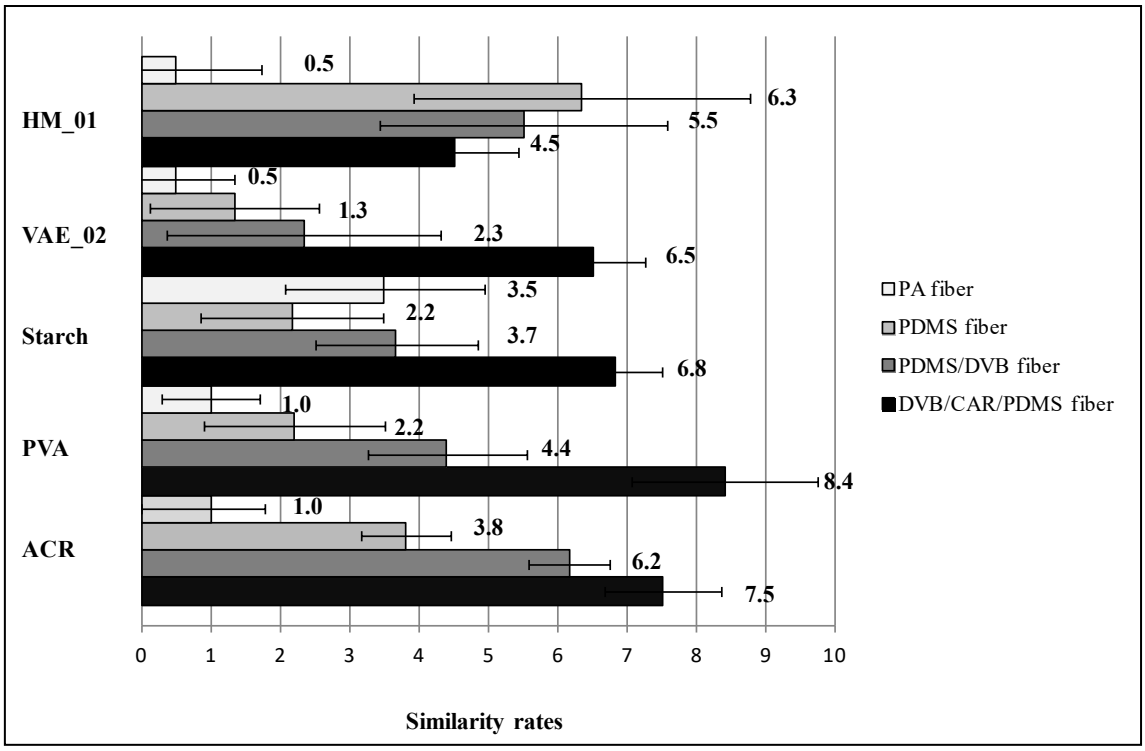

Figure 1: Similarity rates obtained with several SPME fibers by sensory panel of 6 assessors; the scale ranges from 0 (far from the reference) to 10 (close to the reference)

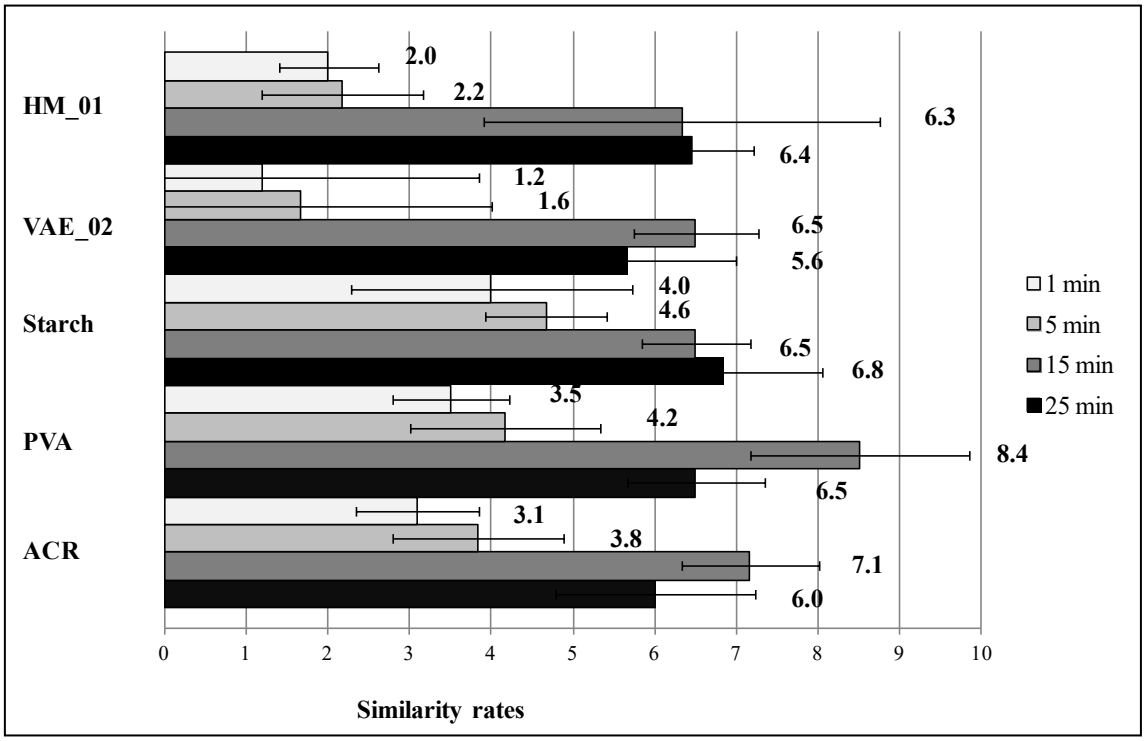

Figure 2: Similarity rates obtained with different extraction times in SPME samples by sensory panel of 6 assessors; the scale ranges from 0 (far from the reference) to 10 (close to the reference)

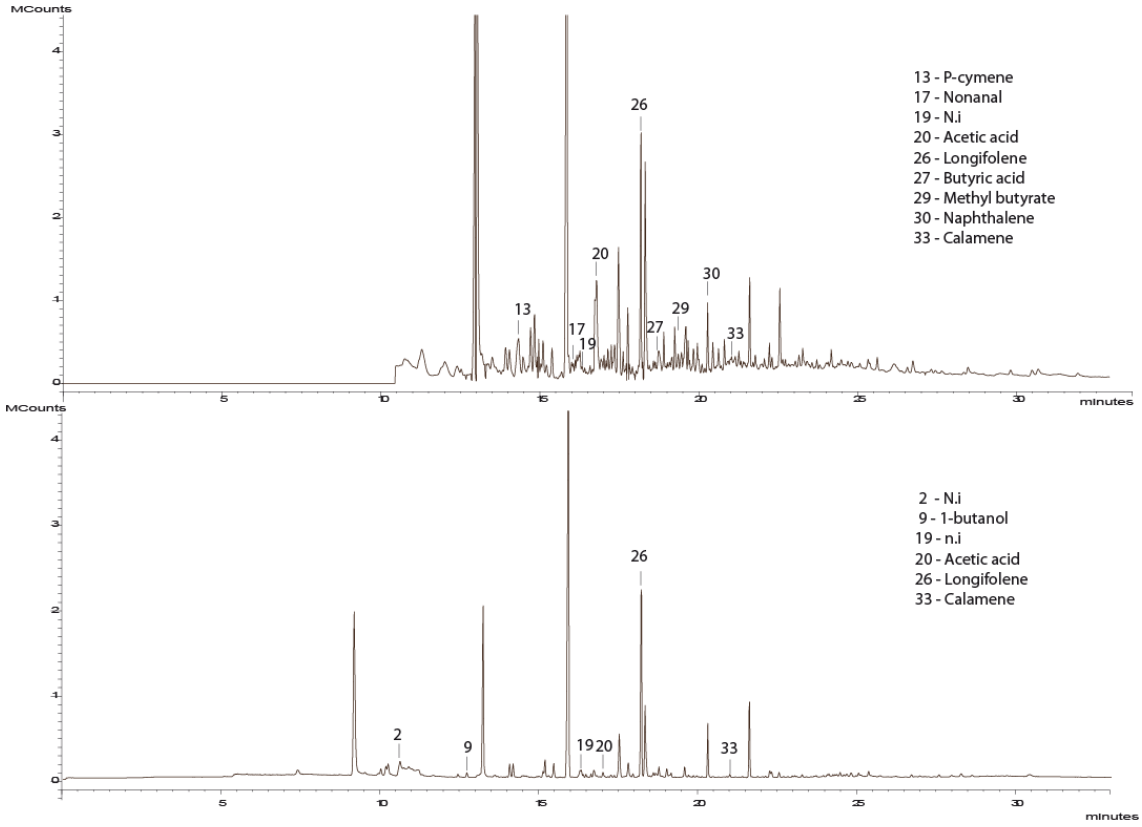

Figure 3: Chromatogram of the HM_01 adhesive analyzed by HS-SPE-GC-MS (on the top) and HS-SPME-GC-MS using a PDMS fiber (on the bottom)

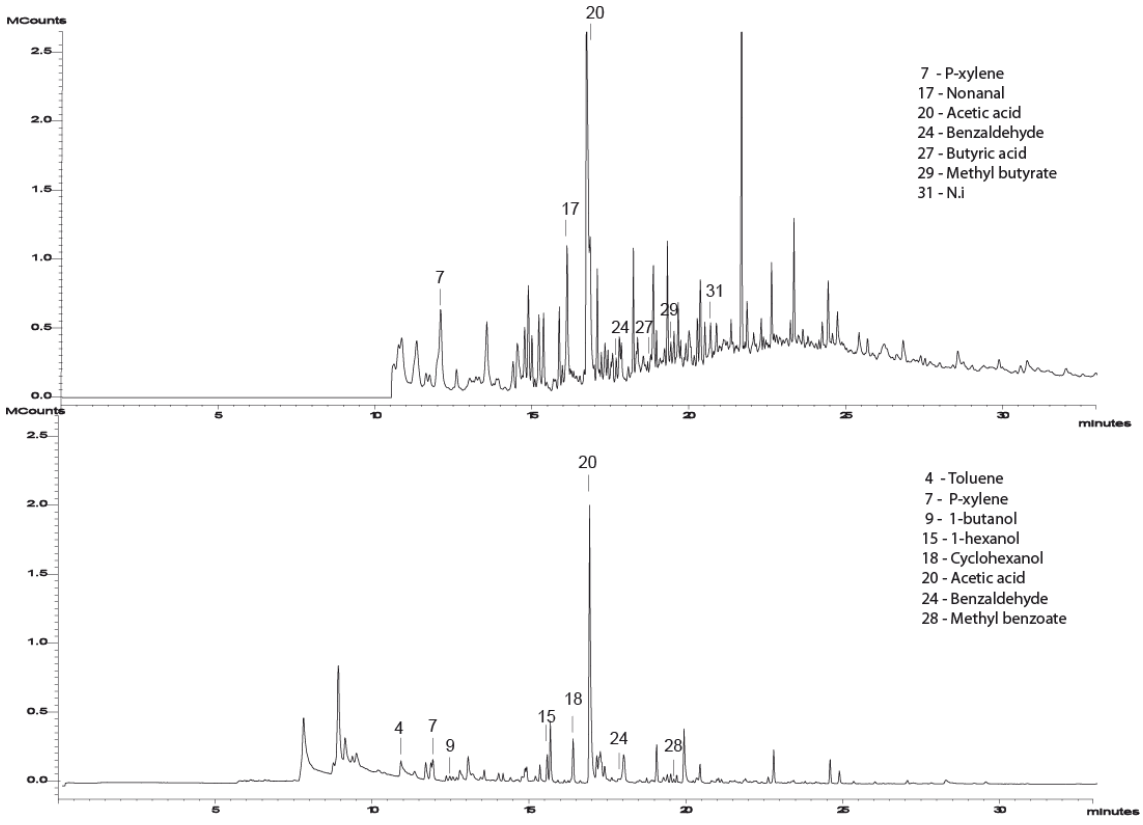

Figure 4: Chromatogram of the VAE_02 adhesive analyzed by HS-SPE-GC-MS (on the top) and HS-SPME-GC-MS using a DVB/CAR/PDMS fiber (on the bottom)

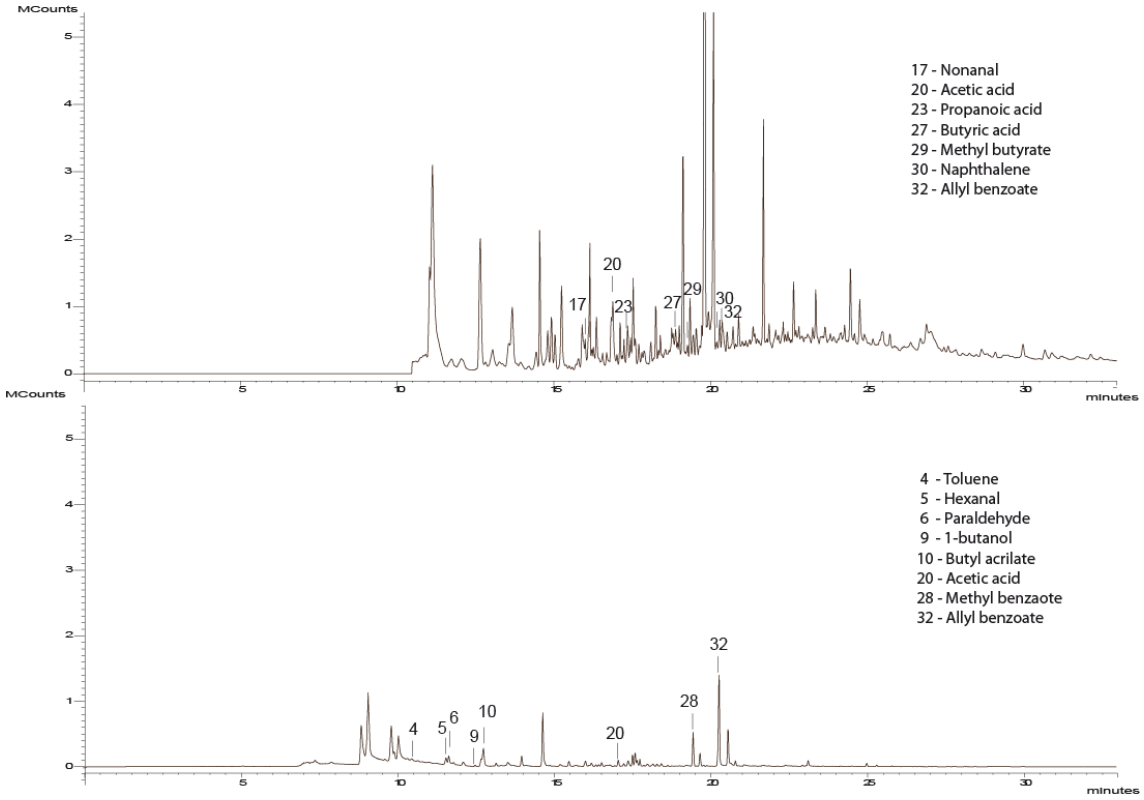

Figure 5: Chromatogram of starch adhesive analyzed by HS-SPE-GC-MS (on the top) and HS-SPME-GC-MS using a DVB/CAR/PDMS fiber (on the bottom)

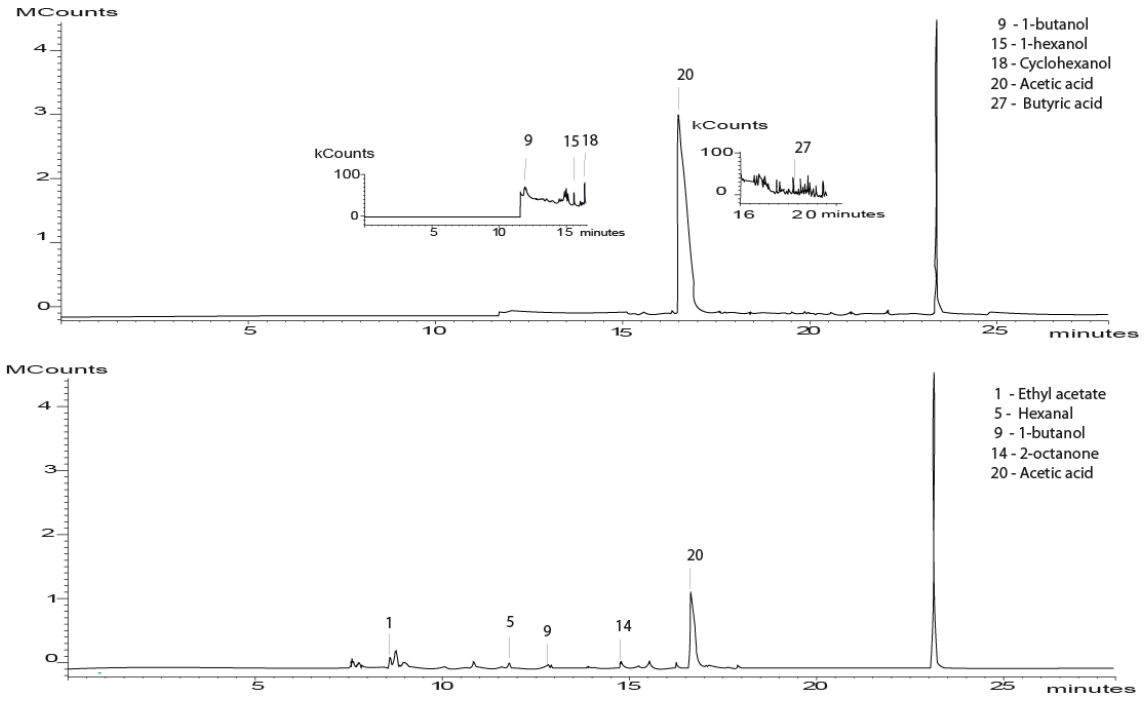

Figure 6: Chromatogram of the PVA adhesive analyzed by HS-SPE-GC-MS (on the top) and HS-SPME-GC-MS using a DVB/CAR/PDMS fiber (on the bottom)

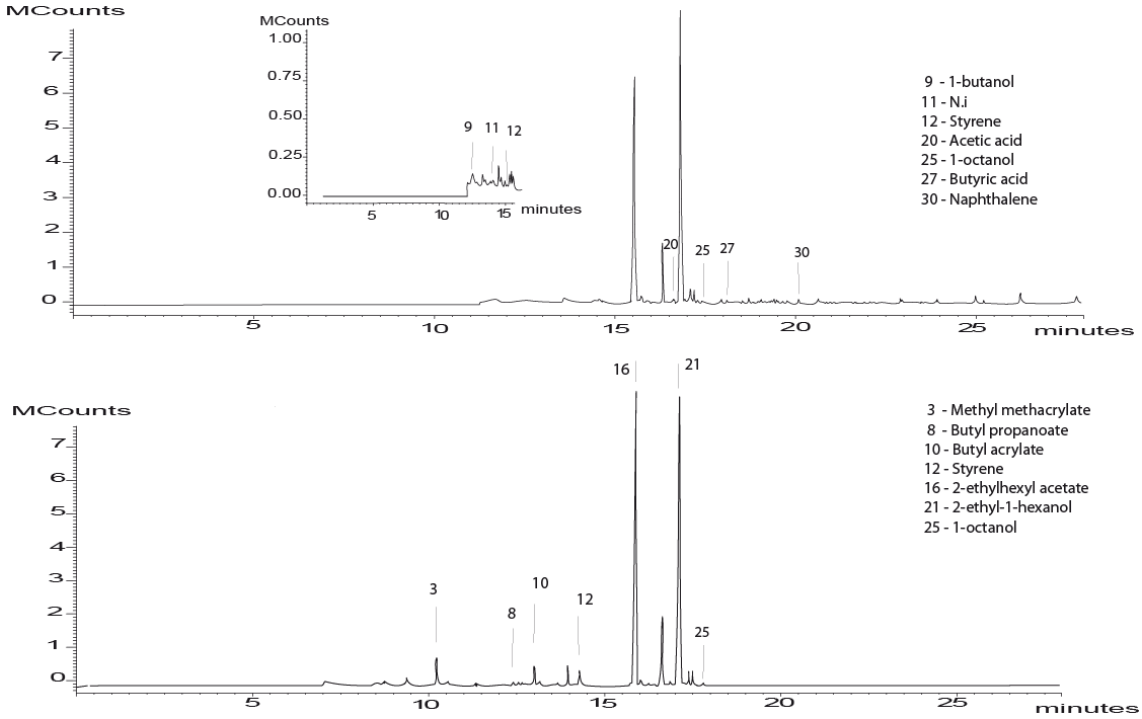

Figure 7: Chromatogram of the ACR adhesive analyzed by HS-SPE-GC-MS (on the top) and HS-SPME-GC-MS using a DVB/CAR/PDMS fiber (on the bottom)