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# Chemometric tools to highlight non-intentionally added substances (NIAS) in polyethylene terephthalate (PET)

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ABSTRACT

In an effort to identify non-intentionally added substances (NIAS), which is still a challenging task for analytical chemists, PET pellets, preforms and bottles were analyzed by an optimized headspace solid phase microextraction coupled to gas chromatography–mass spectrometry (HS–SPME/GC–MS). Fingerprints obtained by the proposed method were analyzed by three chemometric tools: Principal Components Analysis (PCA), Independent Components Analysis (ICA) and a multi-block method (Common Components and Specific Weights Analysis CCSWA) in order to extract pertinent variations in NIAS concentrations. Total ion current (TIC) chromatograms were used for PCA and ICA while extracted ion chromatograms (EIC) were used for CCSWA, each ion corresponding to a block. PCA managed to discriminate pellets and preforms from bottles due to several NIAS. Volatiles like 2-methyl-1,3-dioxolane, ethylene glycol, ethylbenzene and xylene were responsible for the discrimination of pellets and preforms. Less volatile compounds like linear aldehydes and phthalates were responsible for the discrimination of bottles. ICA showed more specific discriminations especially for bottles and pellets while CCSWA managed to discriminate preforms. The proposed methodology, combining HS–SPME/GC–MS with chemometric tools proved its efficiency in highlighting NIAS in PET samples in a relatively simple and fast approach compared to classical techniques.



## 1. Introduction

Polyethylene terephthalate (PET) has become the most widely used packaging material world-wide for beverages, replacing other packaging materials such as PVC, glass and metal. The reason for this development is the excellent properties of PET which is lightweight, shatter resistant, transparent, and recyclable [1]. Nowadays, PET bottles are used for soft drinks, mineral waters, energy drinks, vegetable oils and ice teas as well as for more sensitive beverages like beer, wine and fruit juices [2].

In recent years, concerns about the safety of packaged foods have increased noticeably. Those worries are related to the leaching of chemicals from packaging material into the packaged food. In fact, plastic packaging contains many additives such as antioxidants, stabilizers, lubricants, colorants, anti-statics and many other processing aids which may migrate into the packaged food. Yet, all these substances, intentionally added to the polymer, are well known and documented in the European commission regulation EU 10/2011 [3] on plastic materials intended to come into contact with food. This regulation establishes a positive list of those compounds authorized for use in plastic formulations and manufacturing and provides migration limits for quite a number of molecules.

However, concerns are rising nowadays about unknown substances called "non-intentionally added substances" or NIAS [4]. This category of molecules is defined by the same regulation as "impurities in the substance used or reaction intermediates formed during the production process or decomposition or reaction products". This new EU regulation [3] gives much importance to NIAS and specifies that "the notion of risk due to the substance, concerns not only the substance itself but also the impurities in this substance and any reaction or degradation product", putting emphasis on the gravity of the problem caused by the appearance of NIAS in food packaging materials.

NIAS in PET have many origins. The first is impurities in the substances used in the manufacture of PET. In this case, the investigation may be difficult due to the potentially hazardous character of the contamination. These impurities may be introduced along with additives and colorants master-batches, with solvents, catalysts, polymerization and production aids, etc.

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External contamination from the surrounding environment and the storage conditions of all the substances used may also be a source of impurities. Acrylic adhesives used in food packaging to form the geometric shape of the package as well as to stick labels on the packages are also a possible source of NIAS which can migrate and reach the packaged food [5]. Jickells et al. [6] mentioned that benzene in PET can come from the use of t-butyl perbenzoate as an initiator in the manufacture of the polymer. Hexanedioic acid polymer with 1,3-benzenedimethanamine (called MXD6) used as barrier material in PET bottles [7] can also generate NIAS [8]. Phthalates (DEHP, DBP, BBP and DOP) have been identified in PET packaging materials marketed in Australia [9].

The second origin of NIAS, according to the definition provided by the EU 10/2011 [3], is products resulting from the degradation of the polymer as well as its additives during processing. In fact, during PET manufacturing, several degradation and decomposition reactions can occur. High temperatures and the presence of oxygen in the PET can promote thermo-mechanical and thermo-oxidative reactions generating numerous NIAS in the polymer and contributing to changes in its chemical structure. Romaõ et al. [10] showed that the main degradation products of bottle-grade PET are oligomers, cyclic and linear diacidic end groups, along with diethylene glycol (DEG). Volatile organic compounds such as aldehydes (acetaldehyde, formaldehyde, and benzaldehyde), aliphatic hydrocarbons ($C_1$–$C_4$), aromatic hydrocarbons (benzene, toluene, ethylbenzene, xylene, and styrene), esters (vinyl benzoate, methyl acetate) were identified in PET samples subjected to temperatures between 200 and 300 °C, applied generally in the production and processing [11].

Along with those that have been done on the packaging material itself, many studies have investigated the interaction of PET packaging with foodstuffs, especially bottles with mineral water [12–14] and several substances have been identified, mainly aldehydes (acetaldehyde and formaldehyde), phthalates (DMP, DEP, DBP, DIBP, BBP, DEHP, DNOP, etc.) and volatile organic compounds (benzene, toluene, xylene, ethylbenzene, etc.). In some cases, the origin of the substances found in bottled drinking water has not been clearly established but what is sure is that NIAS in PET can migrate and contribute to the presence of these molecules in the water [15]. Some of these molecules such as 2-ethylhexanol, benzaldehyde and acetophenone have been also identified by Ducruet et al. [16] in strawberry syrup after 250 days of storage in PET packaging.

For the manufacture of PET bottles, injection blow-molding is the preferred process. Amorphous preforms are obtained by processing PET granules (pellets) then preforms are stretched by a blow-molding process to achieve bi-axially oriented bottles [15]. Each step of this process can introduce and generate NIAS in the polymer posing a risk of unacceptable migration of chemical substances from PET bottles into foodstuffs in contact. Thus, a compliance work must investigate NIAS at each step of the manufacturing chain and each product must be seen as a component in the final food contact material [17].

Certificates must assure the safety of the starting substances as well as the product during the process. Therefore screening tests should be applied for the determination of any unwanted substances in food packaging materials. Conventionally, gas chromatography coupled to mass spectrometry has been one of the most powerful tools to identify volatile and semi-volatile NIAS in polymers [5,8]. But, achieving a follow up of these unknown substances at each step of the production process of a bottle is not very simple to accomplish and is still a challenge.

Chemometric methods have great potential for the treatment of chemical data such as those acquired from chromatography coupled to mass spectrometry, yet much attention must be paid to the preparation of the data set. In fact, the analytical data matrices used in chemometrics can come from two sources: (1) signals coming directly from the chromatographic instrument (fingerprinting) or (2) data from derived information such as measured signal intensity or composition/concentration results (profiling). The transformation of the chromatographic signal into compositional data (profiling) may pose some problems as valuable information may be lost. On the other hand, although rarely done, by using the entire chromatogram (fingerprinting), it is possible to retain all the information [18].

In this context, the aim of our study is to develop a simple and fast methodology, combining fingerprints obtained by an optimized head space/solid phase microextraction (HS–SPME) coupled to gas chromatography/mass spectrometry (GC–MS) along with chemometrics in order to highlight volatile and semi-volatile NIAS in PET at each step of the production process of a bottle.

## 2. Materials and methods

### 2.1. Samples

Samples of virgin PET pellets, preforms and bottles were supplied by a company specialized in the manufacturing of PET preforms and bottles, mainly for beverages, mineral water and edible oil. Two batches of samples were collected, each consisting of virgin pellets, preforms and bottles allowing us to obtain a set of $n=6$ samples. Batches will be labeled R and J in reference to the pellet's origin.

The PET samples were ground in an IKA Labortechnik A10 S2 universal laboratory mill (Germany) in presence of liquid nitrogen, in order to increase the surface area and thus to improve the extraction efficiency. Samples were cooled using liquid nitrogen to prevent any degradation during grinding.

### 2.2. HS–SPME optimization

#### 2.2.1. Experimental design

A face centered cubic central composite design (2nd degree with cubic domain) was carried out to optimize the parameters affecting the HS–SPME procedure [19–21]. The experimental design consisted of a model with 50 experiments including six measures in the central point of the experimental domain. The optimization criterion was the maximization of the number of compounds extracted by the fiber (number of chromatographic peaks detected). This criterion was chosen with respect to our main goal which is to highlight as many NIAS as possible in our PET samples. Variables optimized were the nature of the fiber coating, the sample amount, the incubation time, the extraction time, the extraction temperature and the desorption time. Table 1 shows the experimental levels employed for the central composite design and Table 2 shows the experimental matrix with detailed conditions for all the experiments which were done randomly. All statistical calculations were carried out with the software NEMRODW (version 2007-03).

**Table 1**
Experimental domains in the design for HS–SPME optimization.

| Extraction parameters | Experimental domain |
|---|---|
| Fiber coating | PDMS (100 μm); CAR/PDMS (75 μm); DVB/CAR/PDMS (50/30 μm) |
| Sample amount | 1–3 g |
| Incubation time | 2–5 min |
| Extraction time | 10–30 min |
| Temperature | 70–80° C |
| Desorption time | 2–5 min |

**Table 2**
Matrix of the experimental design.

| Experiment | Sample amount (g) | Incubation time (min) | Temperature (°C) | Extraction time (min) | Desorption time (min) | Fiber coating |
|---|---|---|---|---|---|---|
| 1 | 1.0 | 2.0 | 70 | 10 | 2.0 | 100 μm PDMS |
| 2 | 3.0 | 2.0 | 70 | 10 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 3 | 1.0 | 5.0 | 70 | 10 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 4 | 3.0 | 5.0 | 70 | 10 | 2.0 | 100 μm PDMS |
| 5 | 1.0 | 2.0 | 80 | 10 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 6 | 3.0 | 2.0 | 80 | 10 | 2.0 | 100 μm PDMS |
| 7 | 1.0 | 5.0 | 80 | 10 | 2.0 | 100 μm PDMS |
| 8 | 3.0 | 5.0 | 80 | 10 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 9 | 1.0 | 2.0 | 70 | 30 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 10 | 3.0 | 2.0 | 70 | 30 | 2.0 | 100 μm PDMS |
| 11 | 1.0 | 5.0 | 70 | 30 | 2.0 | 100 μm PDMS |
| 12 | 3.0 | 5.0 | 70 | 30 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 13 | 1.0 | 2.0 | 80 | 30 | 2.0 | 100 μm PDMS |
| 14 | 3.0 | 2.0 | 80 | 30 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 15 | 1.0 | 5.0 | 80 | 30 | 2.0 | 50/30 μm DVB/CAR/PDMS |
| 16 | 3.0 | 5.0 | 80 | 30 | 2.0 | 100 μm PDMS |
| 17 | 1.0 | 2.0 | 70 | 10 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 18 | 3.0 | 2.0 | 70 | 10 | 5.0 | 100 μm PDMS |
| 19 | 1.0 | 5.0 | 70 | 10 | 5.0 | 100 μm PDMS |
| 20 | 3.0 | 5.0 | 70 | 10 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 21 | 1.0 | 2.0 | 80 | 10 | 5.0 | 100 μm PDMS |
| 22 | 3.0 | 2.0 | 80 | 10 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 23 | 1.0 | 5.0 | 80 | 10 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 24 | 3.0 | 5.0 | 80 | 10 | 5.0 | 100 μm PDMS |
| 25 | 1.0 | 2.0 | 70 | 30 | 5.0 | 100 μm PDMS |
| 26 | 3.0 | 2.0 | 70 | 30 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 27 | 1.0 | 5.0 | 70 | 30 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 28 | 3.0 | 5.0 | 70 | 30 | 5.0 | 100 μm PDMS |
| 29 | 1.0 | 2.0 | 80 | 30 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 30 | 3.0 | 2.0 | 80 | 30 | 5.0 | 100 μm PDMS |
| 31 | 1.0 | 5.0 | 80 | 30 | 5.0 | 100 μm PDMS |
| 32 | 3.0 | 5.0 | 80 | 30 | 5.0 | 50/30 μm DVB/CAR/PDMS |
| 33 | 1.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 34 | 3.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 35 | 2.0 | 2.0 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 36 | 2.0 | 5.0 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 37 | 2.0 | 3.5 | 70 | 20 | 3.5 | 75 μm CAR/PDMS |
| 38 | 2.0 | 3.5 | 80 | 20 | 3.5 | 75 μm CAR/PDMS |
| 39 | 2.0 | 3.5 | 75 | 10 | 3.5 | 75 μm CAR/PDMS |
| 40 | 2.0 | 3.5 | 75 | 30 | 3.5 | 75 μm CAR/PDMS |
| 41 | 2.0 | 3.5 | 75 | 20 | 2.0 | 75 μm CAR/PDMS |
| 42 | 2.0 | 3.5 | 75 | 20 | 5.0 | 75 μm CAR/PDMS |
| 43 | 2.0 | 3.5 | 75 | 20 | 3.5 | 100 μm PDMS |
| 44 | 2.0 | 3.5 | 75 | 20 | 3.5 | 50/30 μm DVB/CAR/PDMS |
| 45 | 2.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 46 | 2.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 47 | 2.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 48 | 2.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 49 | 2.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |
| 50 | 2.0 | 3.5 | 75 | 20 | 3.5 | 75 μm CAR/PDMS |

In order to estimate the optimal HS–SPME conditions, a desirability function is used. In fact, when multiple responses are evaluated by an experimental design, it is unlikely that optima obtained for the different responses are identical. In this situation, the desirability concept is generally used, finding an acceptable compromise so that all responses validate the experimental specifications or restrictions imposed by the user [22].

### 2.2.2. HS–SPME manual extraction

The optimization step was done on ground PET pellets. The SPME was performed manually with a SPME holder (Supelco). The SPME fibers used in this optimization (Supelco, USA) were conditioned in accordance with the manufacturer's instructions. Samples were placed in 20 mL glass vials which were transferred to a hot plate. Then the fiber was exposed to the vapor phase for the chosen extraction time (according to the experimental design). At the end of this time, the fiber was inserted into the needle and subsequently introduced into the injection port of the gas chromatograph. The desorption of the analytes from the fiber coating was done at 250 °C.

### 2.2.3. Apparatus

A CE GC 8000 TOP Gas Chromatograph with flame ionization detector was used with a 30 m × 0.32 mm i.d. × 1 μm DB5 column (J & W Scientific, USA). The GC operating conditions were as follows: injection temperature 250 °C (splitless mode), oven temperature was held at 40 °C for 5 min then increased to 130 °C at 3 °C $min^{-1}$, then to 250 °C at 6 °C $min^{-1}$ and kept at this temperature for 25 min. The carrier gas was hydrogen at a constant pressure of 50 KPa and a flow rate of 1 mL $min^{-1}$.

## 2.3. HS–SPME/GC–MS conditions

Using optimal conditions, ground PET pellets, preforms and bottles were analyzed using the CombiPal multipurpose sampler (CTC analytics, USA) with the SPME configuration. For each

sample, triplicates were carried out. Therefore 18 samples were analyzed in total (9 for each batch: R and J). An Agilent 6890 gas chromatograph interfaced to a 5975 mass spectrometer was used. Chromatographic separations were carried out with a 30 m × 0.32 mm × 1 μm DB5MS capillary column. The GC operating conditions were as follows: injector temperature 250 °C (splitless mode), oven temperature was held at 35 °C for 5 min then increased to 130 °C at 3 °C $min^{-1}$, then to 250 °C at 6 °C $min^{-1}$, then to 320 °C at 7 °C $min^{-1}$ and kept on this temperature for 1 min. The carrier gas was helium at a constant pressure of 64 KPa and a constant flow rate of 1 mL $min^{-1}$. The transfer line temperature was set at 320 °C. The detector was a quadrupole mass spectrometer. For ionization, an Electron Impact (EI) ion source at 70 eV was used. In order to maximize the detectable compounds, Total Ion Current (TIC) mode was operated in the mass range of 29–600 *m*/*z*. All identifications were done based on MS-library Wiley 7, NIST05s. Compounds were identified by matching their mass spectra to the library with a matching criterion over 90%. Retention times of pure standards were obtained for confirmation.

### 2.4. Chemometric methods

Three chemometric tools were applied in this study: Principal Components Analysis (PCA), Independent Components Analysis (ICA) and a multi-block analysis method: Common Components and Specific Weights Analysis (CCSWA). PCA and ICA were applied on the Total ion current (TIC) chromatograms while CCSWA was used with blocks, each corresponding to a different extracted ion chromatogram (EIC) or *m*/*z*.

#### 2.4.1. Principal Components Analysis (PCA)

The first chemometric tool applied on the data set was Principal Component Analysis (PCA). PCA is a multivariate statistical method that calculates a set of new orthogonal axes or variables known as principal components (PCs) and which are linear combinations of the original variables. The PCs are uncorrelated with one another and each successive PC is calculated so as to be orthogonal to all the others and to contain as much of the remaining variability in the data set as possible [23]. Briefly, PCA produces orthogonal components by decomposing the initial data matrix **X** into a matrix product $\mathbf{T}.\mathbf{P}^{\mathbf{T}}$ (the "$^{\mathbf{T}}$" in $\mathbf{P}^{\mathbf{T}}$ means "transposed matrix"). The **T** matrix is commonly called the scores matrix. The matrix **P**, called the loadings matrix, shows which variables are responsible for patterns found in scores **T** [24]. In our case, the data consisting of 18 TIC chromatograms was exported in ASCII format to build the data matrix (18 chromatograms × 10,277 variables). Data treatment was done using MATLAB version R2007b (The MathWorks, Natick, USA). Before any manipulation of the data, the TICs matrix was normalized by rows and centered by columns, in order to eliminate any uncontrolled intensity variation in the chromatograms and to highlight the differences between the samples.

#### 2.4.2. Independent Components Analysis (ICA)

ICA is based on the construction of latent variables, called Independent Components (ICs), which are linear combinations of the original variables. The ICs are assumed to correspond to the signals of the pure sources present in the analyzed mixtures. The hypothesis used to enable the extraction of the "pure source signals" is that these vectors are statistically independent, whereas Principal Components Analysis (PCA) is based on calculating orthogonal vectors maximizing the variance extracted from the data.

ICA aims to extract pure signals from mixtures which means that the latent variables obtained are easier to interpret and useful for qualitative and quantitative analysis of mixtures [25].

The general model of ICA can be described as

$$\mathbf{X} = \mathbf{A}.\mathbf{S}$$

Where **X** is an $n \times m$ matrix that denotes $n$ measured signals and comprising $m$ variables (X: matrix of observed signals). **A** is an $n \times d$ mixing matrix of unknown coefficients, related to the corresponding proportions and **S** is a $d \times m$ matrix denoting "pure" source signals.

The goal of ICA is to estimate a demixing matrix $\mathbf{W}=\mathbf{A}^{-1}$ so that the "pure" component signals can be recovered from the measured mixed signals by $\mathbf{S}=\mathbf{W}\mathbf{X}$.

In this study, the Joint Approximate diagonalization of Eigenmatrices (JADE) algorithm was used to calculate **W** [26]. JADE uses joint diagonalization of matrices calculated from fourth-order cumulants of the data and does not require any gradient searches, thus avoiding the convergence problems encountered with other procedures [27]. The optimal number of ICs was calculated by the mean of ICA-by-blocks procedure [28].

The same data set used in PCA was treated by ICA, using MATLAB version R2007b (The MathWorks, Natick, USA).

#### 2.4.3. Multi-block data analysis

Gas chromatography coupled to mass spectrometry generates a large amount of data for each analyzed sample. Different data tables can be obtained corresponding to ions with different *m*/*z* ratios which can be extracted from GC–MS measurements carried out on each sample. In this situation, Common Components and Specific Weights Analysis (CCSWA) [29] provides a powerful tool to take into account the information contained in matrices corresponding to different *m*/*z* values.

This method determines a common space describing the dispersion of all the data sets; each table having a specific weight (or 'salience') associated with each dimension in this common space. Significant differences in the values of saliences for a given dimension reflect the fact that the dimension contains different amounts of information from each block [28].

In our case, the data consisted of 255 tables corresponding to 255 extracted ion chromatograms (*m*/*z*) from the initial GC–MS measurements carried out on each of the 18 samples. Extracted *m*/*z* ranged between $m/z=40$ and $m/z=294$. The choice of this range is explained by the fact that Electron Impact ionization (EI) generates low mass ranges and molecular ions are generally weak or absent. In addition, chemical compounds studied in our case are all volatiles and semi-volatiles with a relatively low molecular mass. All the data was stored in cubic array (18 samples × 10,277 variables × 255 *m*/*z*) which was treated as 255 blocks of size 18 × 10,277.

## 3. Results and discussions

### 3.1. Optimization of the HS–SPME method

The most important parameters affecting the HS–SPME procedure were considered in our optimization in order to improve the extraction of NIAS from PET samples (pellets, preforms and bottles). A Central composite design was used for the optimization. It is one of the most useful designs for estimating a multifactor response surface while keeping to a minimum the number of experiments. The optimization criterion was the maximization of the number of extracted compounds (number of chromatographic peaks). The six replicates at the central point were performed to estimate the experimental error and to detect the lack of fit [22].

Response surfaces obtained by the central composite design are illustrated in Fig. 1. The response surface in Fig. 1A shows a maximum response for an extraction time approaching its maximum value and an extraction temperature near the middle of its domain of variation. Fig. 1B shows that the response is maximal for a maximum incubation time. The desorption time and the sample amount showed no significant effect on the studied response (Fig. 1C).

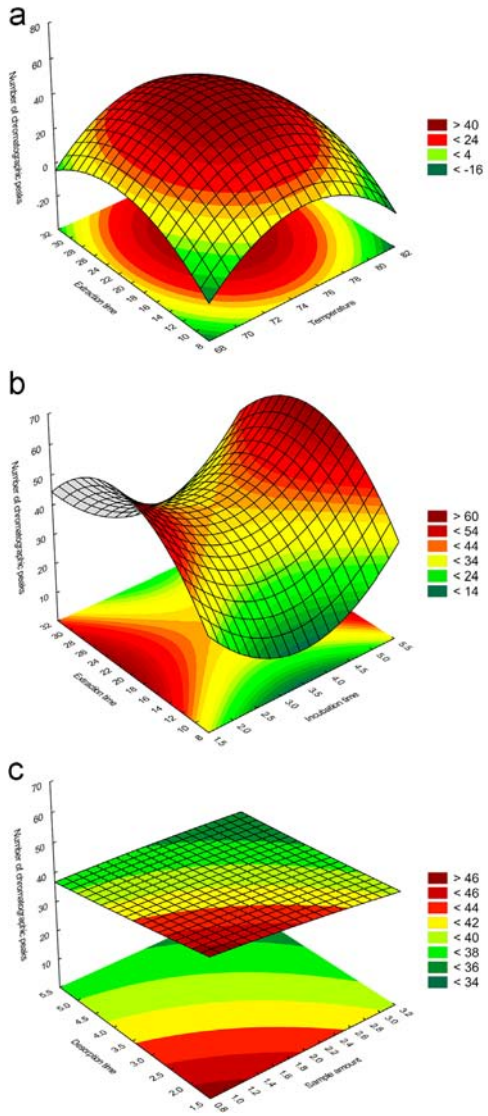


**Fig. 1.** Response surfaces obtained using the central composite design by plotting extraction time versus temperature (A), versus incubation time (B) and desorption time versus sample amount (C).

When implementing the desirability function, which aimed at maximizing the number of extracted compounds from the PET while respecting a good compromise between the optimized parameters, the selected optimal conditions were: fiber coating: DVB/CAR/PDMS (50/30 μm), sample amount: 2.9 g, incubation time: 5 min, extraction time: 29 min, extraction temperature: 77 °C and desorption time: 4.9 min. These optimal conditions were used for the screening analysis of all PET samples.

The repeatability of the optimal extraction conditions was also tested on ground PET pellets for $n=6$ repetitions. Chromatographic peak areas were considered as the repeatability criterion, using the major peaks of the TIC chromatograms. The same major peaks were considered each time and the repeatability was calculated for each of the major peaks chosen. The repeatability values ranged between 7.82 and 14.81 with an average value of $10.64 \pm 2.8\%$.

Although the optimization of the HS–SPME procedure was carried out with ground PET pellets, it can be assumed that similar conditions could be applied to ground PET preforms and bottles.

### 3.2. Chemometric methods applied on GC–MS fingerprints of PET samples

Fig. 2 shows an example of the TIC chromatograms obtained for PET pellets, preforms and bottles. The averaged information in the 18 TIC chromatograms (retention time range 1–66.67 min, 10,277 variables) was analyzed by both Principal Components Analysis (PCA) and Independent Components Analysis (ICA). For the Common Components and Specific Weights Analysis (CCSWA), the data set consisted of 255 blocks of extracted ion chromatograms (10,277 variables) for the 18 samples.

#### 3.2.1. Principal Components Analysis (PCA)

PCA (with 6 principal components) was performed on the analytical data set of the 18 TIC chromatograms. $PC_1$ and $PC_2$ (first and second principal components) were chosen to represent the information because the maximal amount of variance in the data set and its direction are often explained by the first PCs [23]. Fig. 3A shows the principal component projection plot of $PC_1$ and $PC_2$ scores of 18 chromatograms (pellets, preforms and bottles from two batches R and J). It was found that $PC_1$ and $PC_2$ extracted 72% of the total variance in these samples. From the scatter points, the samples could be classified into four groups indicating a clear

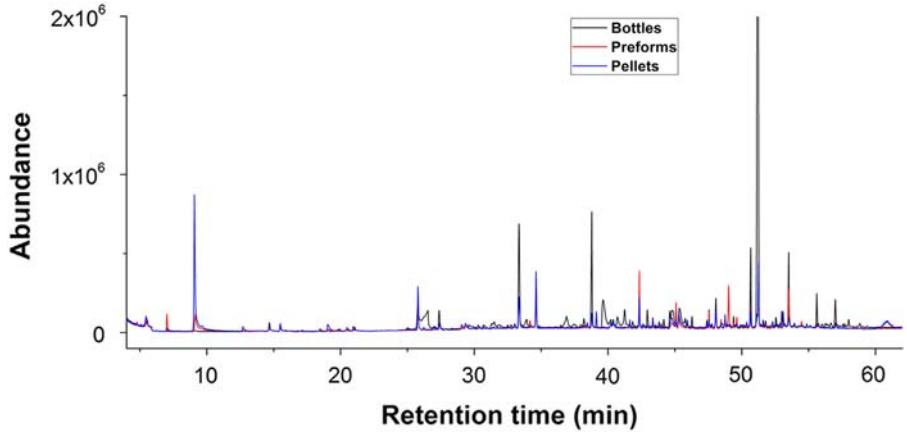


**Fig. 2.** Example of TIC chromatograms obtained by HS–SPME/GC–MS of ground PET pellets, preforms and bottles.

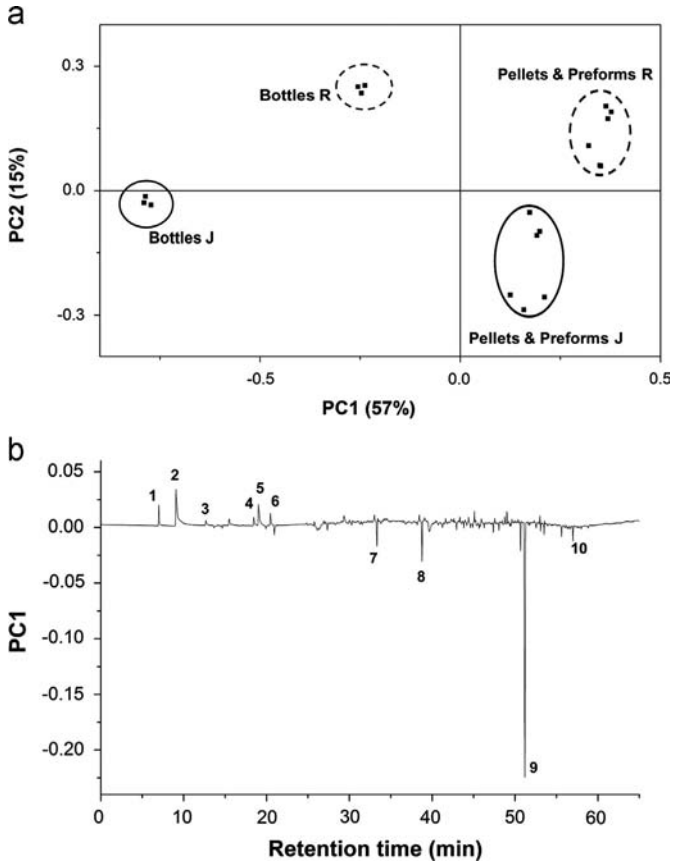


**Fig. 3.** Principal component projection plot of $PC_1$ and $PC_2$ scores (A) and loadings of $PC_1$ (B). (1): 2-methyl-1,3-dioxolane; (2): ethylene glycol; (3): toluene; (4): ethylbenzene; (5 and 6): xylene isomers; (7): nonanal; (8): decanal; (9): diethyl phthalate; and (10): diisobutyl phthalate.

separation of pellets and preforms from bottles along $PC_1$ and a differentiation between the two batches R and J along $PC_2$. In order to highlight the volatile compounds responsible for this discrimination, $PC_1$ loadings are represented in Fig. 3B.

This clear discrimination of pellets and preforms from bottles could be interpreted as a consequence of the loss (positive peaks in $PC_1$ loadings) of the more volatile compounds (2-methyl-1,3-dioxolane, ethylene glycol, toluene, ethylbenzene and xylene isomers) during the blowing step (preform for bottle transition). These NIAS, generated in PET pellets and preforms, as a result of degradations during processing, were lost due to their high volatility, through blowing, which takes place at high temperatures. In addition, the apparition (negative peaks in $PC_1$ loadings) of linear aldehydes (nonanal, decanal) and phthalates (diethyl phthalate, diisobutyl phthalate) in bottles also contributed, in a marked way, to the discrimination.

In fact, PCA gave valuable information about the discrimination between different samples. However, no specific information can be extracted since this technique is based on calculating orthogonal vectors that correspond to the maximum dispersion of the samples in the multidimensional space of the original variables. For this reason, the loadings vectors tend to be mixtures of the signals of all the NIAS that had an impact on this dispersion. ICA, which extracts statistically independent vectors, related to the pure 'source' signals, was therefore applied to the data set in order to obtain signals that are chemically easier to interpret.

### 3.2.2. Independent Components Analysis (ICA)

Independent Components Analysis with 5 Independent Components (ICs), as determined by ICA-by-Blocks, was applied to the TIC chromatogram matrix. We present only the figures of the ICs corresponding to significant signals (Fig. 4). Each IC plot shows the scores values according to the corresponding IC. The same scores are represented in the three plots: the first one is labeled with the three repetitions done on each sample, the second, with the two batches (R and J) and the third, with the nature of the sample (pellets: G, preforms: P and bottles: B). The ICs signals are represented in the lower part of the same figure. These signals are directly related to signals of chemical compounds extracted from the mixture in the initial GC–MS fingerprints.

In the case of ICA, of the five independent components, three contained reliable information and thus the data set could be decomposed into three signals related to chemical compounds present in the initial mixture of signals. This fact appears clearly in $IC_1$ (Fig. 4A) which shows that bottles J are discriminated from the rest of the samples due to diethyl phthalate (DEP), which exists in large amounts in these bottles, while the discrimination of bottles R is slightly influenced by this phthalate. On the other hand, $IC_2$ (Fig. 4B) showed that the discrimination of bottles R along with bottles J is due to linear aldehydes (heptanal, nonanal and decanal). $IC_5$ (Fig. 4C) showed that ethylene glycol (EG) is responsible for the discrimination of pellets J. These interpretations were not clear in PCA and this shows the capacity of ICA to extract pure signals from mixed GC–MS signals and the specificity in the identification of NIAS responsible for the discrimination of each group of samples.

### 3.2.3. Multi-block data analysis

In order to extract additional information from the GC–MS data, CCSWA was applied using the ComDim implementation in the *SAISIR* toolbox [30]. This analysis was performed using 6 Common Components (CCs). Four of the 6 CCs contained relevant information. The scores of $CC_1$, $CC_2$, $CC_4$ and $CC_6$ are represented in Fig. 5. The same scores are represented in three plots. The first one is labeled with the batch types (R and J), the second with the nature of the sample (G: pellets, P: preforms and B: bottles) and the third with the three repetitions.

Looking at the scores of the first and the second CCs we can clearly see the discrimination of bottles of the two batches R and J from the rest of the samples (Fig. 5). In addition, $CC_2$ shows discrimination between the two batches of bottles (R and J). These interpretations were also clear in ICA and even in PCA. However, $CC_4$ and $CC_6$ demonstrate new discriminations which were not clear in either PCA or ICA.

In $CC_4$ (Fig. 5) we can notice a discrimination of preforms J from the rest of the samples. The values of the saliences corresponding to $CC_4$ (Fig. 6) show that the major ions influencing this discrimination are $m/z$=191, 192, 206, 221, 277 and 292 with saliences of more than 0.6. When we investigate the extracted ion chromatograms corresponding to the $m/z$ 191, 192 and 206 for the J pellets we can clearly identify the NIAS responsible for this discrimination as 2,4-bis(1,1-dimethylethyl) phenol. In fact, the mass spectrum of this compound, under Electron Impact ionization (EI), presents the following $m/z$ as its major fragmentations: 41, 57, 191, 192 and 206. This compound is a degradation product of the antioxidants Irgafos 168 and Irganox 1010 [31]. Ions corresponding to $m/z$=221, 277 and 292 were related to compounds which were difficult to identify, having low matching percentages.

$CC_6$ (Fig. 5) shows a discrimination of pellets R (positive scores) and preforms R (negative scores) from the rest of the samples. According to the saliences of $CC_6$ (Fig. 6), the data tables contributing to this discrimination correspond to $m/z$=48, 91, 92, 106, 117, 118, 119, 120, 134 and 272 (scores above 0.1). After the investigation of the extracted ion chromatograms corresponding

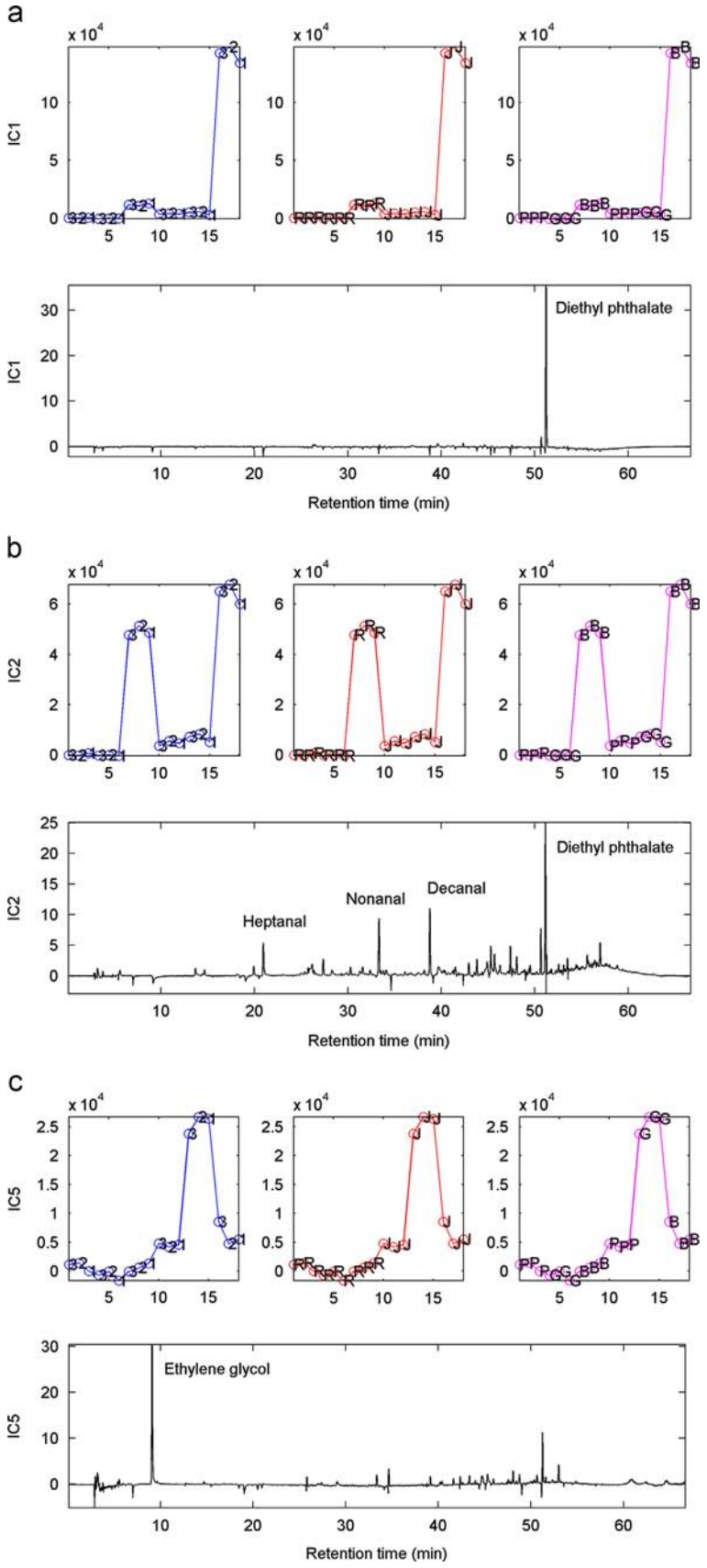


**Fig. 4.** Independent component projection plots of $IC_1$ (A), $IC_2$ (B) and $IC_5$ (C). Loadings are represented in the same figures.

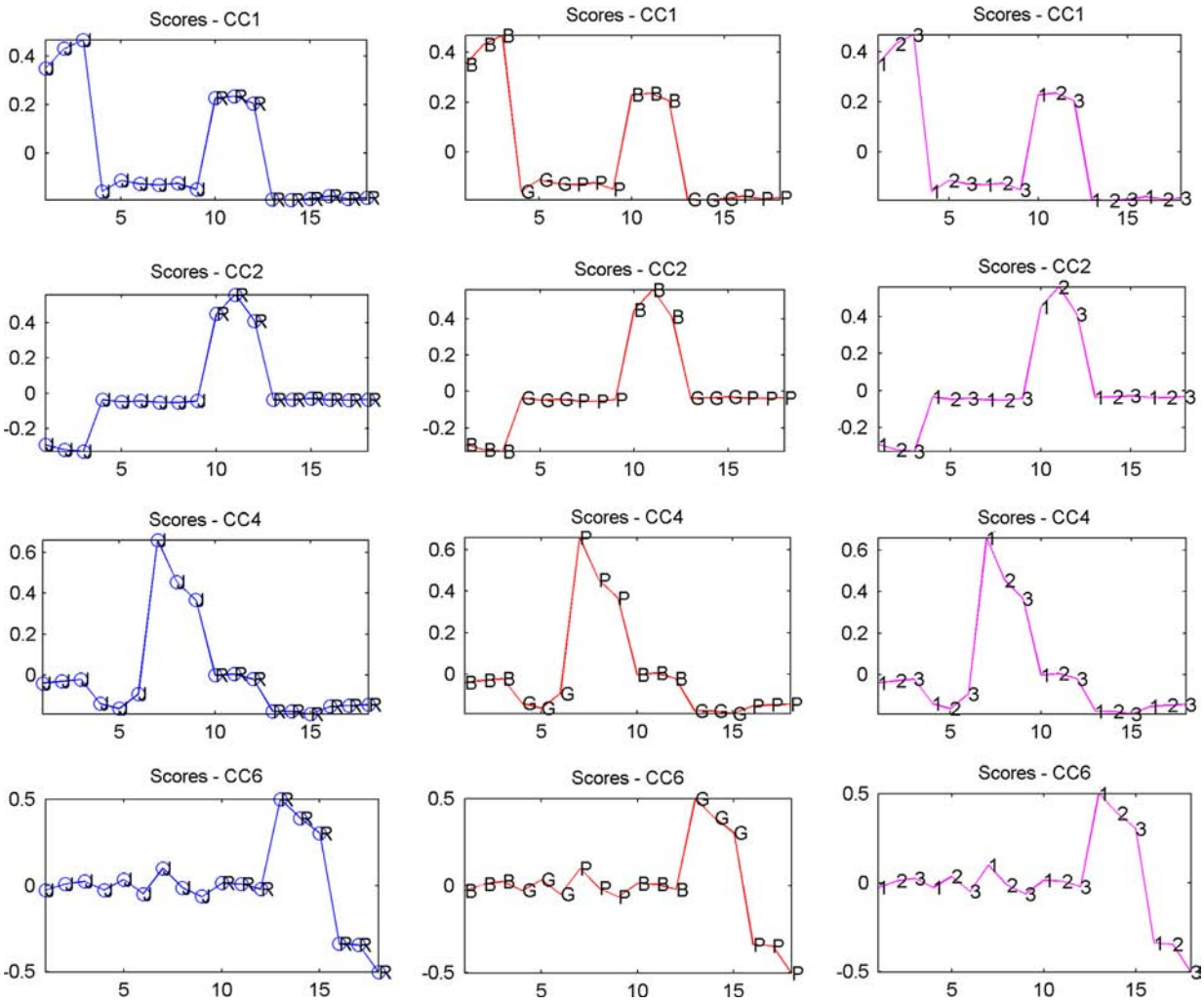


**Fig. 5.** CCSWA scores on $CC_1$, $CC_2$, $CC_4$ and $CC_6$ related to the batches types (R and J), the nature of the samples (G: pellets; P: preforms and B: bottles) and the repetitions ($n=3$).

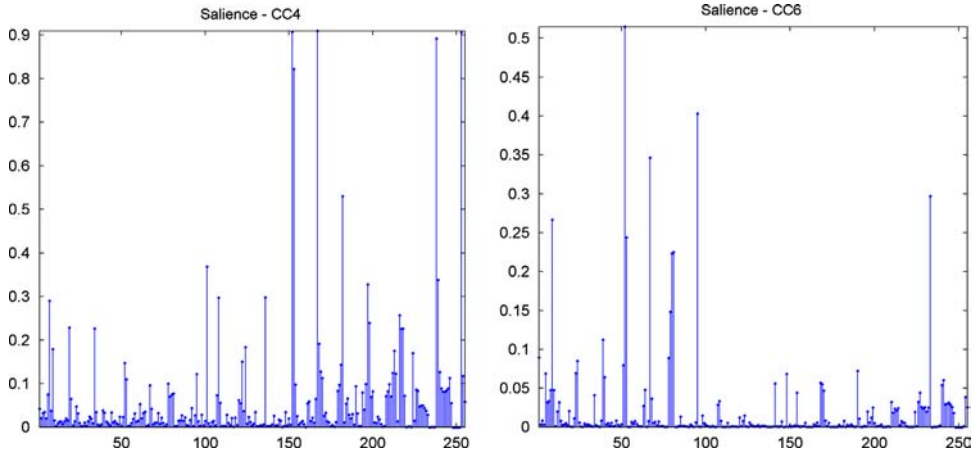


**Fig. 6.** Saliences corresponding to the 255 $m/z$ according to $CC_4$ and $CC_6$.

to the selected $m/z$ for pellets and preforms R, the following compounds were identified as responsible for the discriminations: 2-methyl-1,3-dioxolane ($m/z=48$); toluene, ethylbenzene and xylene isomers ($m/z=91$, 92, 106) and dichlorobenzene ($m/z=117$, 118) with a maximal contribution for compounds with an ion $m/z=91$ which could be toluene, ethylbenzene or xylene isomers.

### 3.3. Discussion about the origin of the discriminating compounds

The origin of the discriminating molecules in PET pellets, preforms and bottles is probably NIAS generated or introduced during the process. In our study, acetaldehyde, which is one of the main thermal degradation products of PET, was not identified but 2-methyl-1,3-dioxolane, which is the acetal formed by the reaction

of acetaldehyde and free ethylene glycol, is present, being an indicator of the initial amounts of acetaldehyde in PET [32]. Residual acetaldehyde is an important marker for the industry in the selection of PET grades. Removing acetaldehyde from PET is important as this aroma compound can migrate from the PET leading to sensorial deterioration of foods or beverages in contact. Acetaldehyde has a distinctive odor and taste and has a low sensory threshold level especially in mineral water where its migration is detectable at low concentrations of 10–20 ppb [33]. Acetaldehyde is generated during process and to reduce its concentration into the final resin, stripping process could be used after melt-phase polymerization [32]. However, our results showed that the blowing step could also contribute to lowering the level of acetaldehyde and its products in bottles.

Linear aldehydes may be thermo-oxidation products of polyethylene waxes used as lubricants in PET [16]. These molecules can easily migrate into food in contact with PET bottles and, like acetaldehyde, have a low sensory threshold level.

Ethylene glycol (EG) is a residual monomer as well as a degradation product of PET. Its presence in PET pellets depends on the polymerization conditions [10]. EG is generated by vinyl ester end group poly-condensation reactions; also by intermolecular and intra-molecular trans-esterification reactions between PET oligomers [34]. Toluene, ethylbenzene and xylene are thermal degradation products of PET samples subjected to high temperatures [11] similar to those used in the manufacturing process of the PET bottle.

The presence of phthalates (DEP, DIBP) in tested PET bottles is very controversial and many theories could be proposed to explain their presence. As seen above, the manufacture of PET bottles involves several steps, each with the potential for introducing contaminants. However polyethylene terephthalate (PET) is a semi-crystalline polymer belonging to the family of polyesters which has a glass transition around 75 °C. So during steps at ambient temperature, contamination by volatile compounds such as phthalates are not promoted as PET is vitreous where diffusion of organic compounds is very low [35]. In contrast, critical steps during process are steps which take place at temperatures higher than the glass transition, where diffusion of organic compounds is exacerbated. As a consequence, hazardous contamination may occur in PET processing during the blowing step which takes place at temperatures higher than the glass transition. Thus, the blowing step is the most critical one during the production process of a PET bottle leading to the loss of the more volatile compounds which may be beneficial by reducing the level of the more volatile NIAS such as 2-methyl-1,3-dioxolane (indicator of acetaldehyde) but in opposite could cause contamination by phthalates from external devices (pumping, tubing, purity of compressed air, etc.).

## 4. Conclusion

Three chemometric tools (PCA, ICA and CCSWA) were used in this study in order to identify NIAS in PET during the production process of a bottle. Samples consisted of PET pellets, preforms and bottles from two independent batches. A central composite design was carried out to optimize the HS–SPME of ground PET samples taking into account the major influential factors. Combining chemometrics with the data set obtained by the HS–SPME/GC–MS of PET samples proved to be very useful and fulfilled the aim of our study. The investigation of the scores and the loadings obtained in each chemometric tool used, enabled us to identify several NIAS responsible for the discriminations between the different samples and thus allowed us to understand some of the phenomena occurring during the production process of the PET bottle. Blowing appeared to be the most critical step during processing leading to the loss of the more volatile compounds and introducing several contaminations, especially phthalates. This fact was highlighted in PCA, ICA and CCSWA through the discrimination of bottles from pellets and preforms. ICA showed more specific discriminations as the discrimination of bottles J due to DEP and pellets J due to EG. CCSWA applied on 3D data set, separated into blocks, highlighted discriminations of pellets and preforms R as well as preforms J, which were not clear in either PCA or ICA. All compounds responsible for the highlighted discriminations were NIAS generated during the process and introduced along with it. The results obtained by this combination of chemometric tools such as PCA, ICA and CCSWA along with HS–SPME/GC–MS data of polymer samples generated relevant information for the identification of NIAS during processing.

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## References

[1] S.K. Pandey, K.H. Kim, Ecotoxicol. Environ. Saf. 74 (2011) 527–532.
[2] F. Welle, Resour., Conserv. Recycl. 55 (2011) 865–875.
[3] Commission regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come in contact with food. Official Journal of the European Commission.
[4] J. Muncke, J. Steroid Biochem. Mol. Biol. 127 (2011) 118–127.
[5] E. Canellas, P. Vera, C. Domeno, P. Alfaro, C. Nerin, J. Chromatogr. A 1235 (2012) 141–148.
[6] S.M. Jickells, C. Crews, L. Castle, J. Gilbert, Food Addit. Contam. 7 (1990) 197–205.
[7] F. Welle, F. Bayer, R. Franz, Packag. Technol. Sci. 25 (2012) 341–349.
[8] R. Franz, F. Welle, Investigation of non-intentionally added substances (NIAS) in PET bottles and closures (Poster Presentation), in: Proceedings of the 4th International Symposium on Food Packaging, 19–21 November, 2008, Prague, Czech Republic.
[9] D. Balafas, K.J. Shaw, F.B. Whitfield, Food Chem. 65 (1999) 279–287.
[10] W. Romao, M.F. Franco, Y.E. Corilo, M.N. Eberlin, M.A.S. Spinacé, M.A.D. Paoli, Polym. Degrad. Stab. 94 (2009) 1849–1859.
[11] M. Dzięcioł, J. Trzeszczynski, J. Appl. Polym. Sci. 81 (2001) 3064–3068.
[12] S.V. Leivadara, A.D. Nikolaou, T.D. Lekkas, Food Chem. 108 (2008) 277–286.
[13] D. Amiridou, D. Voutsa, J. Hazard. Mater. 185 (2011) 281–286.
[14] X.L. Cao, J. Chromatogr. A 1178 (2008) 231–238.
[15] C. Bach, X. Dauchy, M.C. Chagnon, S. Etienne, Water Res. 46 (2012) 571–583.
[16] V. Ducruet, O. Vitrac, P. Saillard, E. Guichard, A. Feigenbaum, N. Fournier, Food Addit. Contam. 24 (2007) 1306–1317.
[17] K. Grob, J. Stocker, R. Colwell, Food Control 20 (2009) 476–482.
[18] J.M. Bosque-Sendraa, L. Cuadros-Rodrigueza, C. Ruiz-Samblasa, A. Paulina de la Matab, Anal. Chim. Acta 724 (2012) 1–11.
[19] Y. Vander Heyden, C. Perrin, D.L. Massart, Optimization strategies for HPLC and CZE, Handbook of Analytical Separations, Volume 1, Separation Methods in Drug Synthesis and Purification, Elsevier, Amsterdam163.
[20] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997.
[21] D.C. Montgomery, Design and Analysis of Experiments, 4th ed., John Wiley, New York, 1997.
[22] M.A. Bezerra, R.E. Santelli, E.P. Oliveira, L.S. Villar, L.A. Escaleira, Talanta 76 (2008) 965–977.
[23] E.C. Shin, B.D. Craft, R.B. Pegg, R.D. Phillips, R.R. Eitenmiller, Food Chem. 119 (2010) 1262–1270.
[24] C.B.Y. Cordella, T. Tekye, D.N. Rutledge, R. Leardi, Talanta 88 (2012) 358–368.
[25] G. Wang, Q. Ding, Z. Hou, Trends Anal. Chem. 27 (2008) 368–376.
[26] J.F. Cardoso, A. Souloumiac, IEE Proc.-F 140 (6) (1993) 362–370.
[27] F. Ammari, C.B.Y. Cordella, N. Boughanmi, D.N. Rutledge, Chemom. Intell. Lab. Syst. 113 (2012) 32–42.
[28] D. Jouan-Rimbaud Bouveresse, A. Moya-González, F. Ammari, D.N. Rutledge, Chemom. Intell. Lab. Syst. 112 (2012) 24–32.
[29] G. Mazerolles, M. Hanafi, E. Dufour, D. Bertrand, E.M. Qannari, Chemom. Intell. Lab. Syst. 81 (2006) 41–49.
[30] Dominique Bertrand, Christophe Cordella, SAISIR package. Free toolbox for chemometrics in the Matlab, Octave or Scilab environments, 2011. Available from: ⟨http://www.chimiometrie.fr/saisir_webpage.html⟩.

[31] J. Alin, M. Hakkarainem, Polym. Degrad. Stab. 97 (2012) 1387–1395.
[32] S.R. Kesaboina, A.L. Elizabeth, S.A. Jabarin, Polym. Eng. Sci. 52 (2012) 1271–1283.
[33] K.E. Özlem, Int. J. Food Sci. Technol. 43 (2008) 333–338.
[34] J.D. Badía, E. Strömberg, A. Ribes-Greus, S. Karlsson, Anal. Chim. Acta 692 (2011) 85–95.
[35] P.Y. Pennarun, P Dole, A. Feigenbaum, J. Appl. Polym. Sci. 92 (2004) 2845–2858.