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# Three-way principal component analysis of the volatile fraction by HS-SPME/GC of aceto balsamico tradizionale of modena

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**Abstract**

The present research is aimed at monitoring the evolution of the volatile organic compounds of different samples of aceto balsamico tradizionale of modena (ABTM) during ageing. The flavouring compounds, headspace fraction, of the vinegars of four *batterie* were sampled by solid phase microextraction technique (SPME), and successively analysed by gas chromatography. Obtaining a data set characterized by different sources of variability such as, different producers, samples of different age and chromatographic profile. The gas chromatographic signals were processed by a three-way data analysis method (Tucker3), which allows an easy visualisation of the data by furnishing a distinct set of graphs for each source of variability.

The obtained results indicate that the samples can be separated according to their age highlighting the chemical constituents, which play a major role for their differentiation.

The present study represents an example of how the application of Tucker3 models, on gas chromatographic signals may help to follow the transformation processes of food products.


*Keywords:* ABTM; Vinegar; HS-SPME; Tucker; Multi-way data analysis; VOC

## 1. Introduction

Aceto balsamico tradizionale of modena (ABTM), is one of the most representative Italian artisan gastronomic food and, despite its limited production, it is known and commercialized all around the world. In 2000, this product obtained the protected denomination of origin (PDO) certification from the European Commission, because of its typical and unique production procedures and the well-defined geographical area of production [1]. ABTM is traditional balsamic vinegar, which is obtained by alcoholic fermentation and acetic bio-oxidation of condensed cooked grape musts. The fermented cooked must is aged for a long time (at least 12 years), in a set of barrels (batteria) composed by a variable number (from 5 to 10) of wooden casks of different volumes. During the ageing process the liquid in each barrel is kept constant by transferring a certain amount of vinegar from one barrel to another in a decreasing progression with a procedure known as "topping up" [2]. In the first year the vinegar is mainly transformed by a microbial activity and successively by enzymatic and chemical reactions.

Due to this production method, which implies a wide transformation of the raw materials, the characterization of ABTM of different ages, coming from the several barrels of a batteria, could be extremely interesting and useful for the authentication of the product and for the comprehension of the phenomena that take place during the ageing period. Investigations about samples coming from different batterie may be helpful to discriminate products of various ages and to differentiate the marketable one, which is at least 12 years old, from the younger ones. In fact, studies about ABTM, usually take into account only the old product and only few works [2–5] have dealt with different aged samples.

The volatile organic compounds, which concur to the olfactory characteristics, have a fundamental importance for the final

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evaluation of the product and, probably, they may play a considerable role in differentiating vinegars on a time base scale, i.e. vinegars of different age. Therefore, one of the aims of the present research is to monitor the evolution of the volatile organic compounds of ABTM samples during ageing. Besides all the analytical techniques used for the flavour composition characterization, the gas chromatography (GC) equipped with head space solid phase microextraction technique [6,7] (HS-SPME) may represent an innovative breakthrough and an a hyphenated approach when linked with chemometric signals processing tools [8].

In the present work, a three-way data set has been analysed, as the ABTM ageing process is affected by different sources of variability, such as the different ageing of vinegar samples, the different batterie and the chromatographic profile. Traditional bilinear chemometrics methods, such as principal component analysis (PCA), may allow extracting useful information from higher order data sets, i.e. data characterized by more than two sources of variability, by using unfolding procedures but, on the other hand, unfolding may be often unfavorable and. it has been recently demonstrated that the use of an *N*-way approach can significantly improve the visualization and interpretation of multi-way data [9,10]. In this particular case, the advantage offered by three-way analysis is due to the possibility of not overwhelming the intra-batteria variability with the inter-batterie variability, as a results it will be possible to depict both the similar evolution of vinegar samples with ageing inside each series of barrels and the distinctive features among batterie due to the traditional conduction procedure.

Tucker3 method is one of the most popular *N*-way methods [9,11], and it can be fairly considered as a natural extension of bilinear PCA. Multi-way methods have been successfully applied to the analysis of different kinds of instrumental data, mainly spectroscopic ones [12–15]. On the other hand, very few applications on gas chromatograms have been reported [13,15] and therefore it is also of interest to test the performance of these methods on this experimental data set.

The data have been organized as a three-way array: samples × HS-SPME/GC-FID signal points × batterie. Owing to this set-up it should be possible: (i) to stress out how the changes of the volatile organic fraction (different composition and/or concentration) is reflected in the ageing process; (ii) to obtain an overview of the relationships between the volatile fraction and the vinegars age and finally and (iii) to assess similarities and dissimilarities among the investigated batterie.

Furthermore, a comparative analysis of the results obtained by Tucker3 analysis with PCA unfolding has been accomplished.

## 2. Materials and methods

### 2.1. Sampling

Four different batterie were analyzed, namely batterie "O", "R", "Sa" and "Sd". Each batteria is composed by six barrels. The first barrel (1) contains the oldest vinegar product (the marketable ABTM) and the last barrel (6) the youngest one.

Among the investigated cask series, batteria "O" is the oldest one, being started 50 years ago, while batteria "Sd", which was started about 7 years ago, is the youngest one. Finally, batterie "R" and "Sa" were started 10 and 18 years ago, respectively.

From each barrel, of different size, different wood and different product ageing, a test sample of 100 mL was obtained following the described procedure; five aliquots of 100 mL of product were taken at different depth and homogenized to obtain a barrel representative sample (500 mL). Successively, an aliquot of 100 mL (test sample) was taken while the rest of the vinegar (400 mL) was re-introduced in the barrel.

All the samples were stored in polystyrene boxes at 4 °C in order to prevent any matrix modification such as fermentation or other chemical modifications. Before analysis, samples were kept at room temperature with continuous mixing to return the product to its initial condition.

### 2.2. Head space solid phase microextraction (HS-SPME) analysis

The volatile organic composition was monitored by sampling the headspace of the vinegar by using the solid phase microextraction technique (HS-SPME). The experimental set up and analytical procedure has already discussed in a previous work [8]. An aliquot of 1.00 mL of each sample was transferred by a calibrated syringe in a 10 mL flask equipped with a screw-top quick fit adaptor and a Teflon-coated silicone septum. The flask was held in a warm water bath at 40.0 °C for 1 h to allow equilibrium between the sample and headspace, prior to SPME sampling. The fibre was then inserted into the sample container through the septum and exposed to the headspace for 15 min. Finally, it was desorbed in the GC injector for 4 min by splitless injection mode.

The analysis of all the samples was twice replicated, with the exception of the samples of batteria "O", because of their extremely low amount.

### 2.3. Gas chromatography (GC)

Samples were analyzed with a Varian 3400 Gas Chromatograph provided with flame ionization detector (FID) and a non-polar fused silica capillary column (CPSil 5MS; length 60 m; internal diameter 0.25 mm; film thickness 1 μm) was used. Helium was used as carrier gas (column head pressure was 28 PSI; linear velocity was 25 cm $s^{-1}$ at 200 °C).

The injector temperature was 240 °C and the split valve was closed for 4 min during sample injection and then opened for the rest of the chromatographic run. The injector was equipped with a special deactivated SPME glass insert (0.75 mm internal diameter). The GC oven starting temperature was 55 °C (5 min); then it was increased at 4 °C $min^{-1}$ to 150 °C, and at 8 °C $min^{-1}$ to 250 °C and finally at 10 °C $min^{-1}$ to 270 °C (5 min). The FID temperature was set at 280 °C.

The chromatograms were acquired at constant sampling rate, $\nu = 20$ Hz, for a total time of 59 min and 15 s, by using the software Chromeleon© Version 4.12 (distributed by SOFTRON GmbH).

In order to rationalize the chemical transformation occurring during the ageing process of ABTM, gas chromatography–mass spectrometry (HS-SPME/GC–MS) analysis was performed on some representative ABTM samples, using the same experimental condition optimized in a previous work [8], allowing the identification of some of the main chromatographic peaks. The GC–MS system used was formed by a 3400 Varian GC coupled to a Finnigan SSQ710AMS capable of electron impact ionization. The identification of unknown compounds was based on matches to the NIST and GP libraries, supplied by Finnigan.

### 2.4. Data analysis

Because of high dimensionality of collected HS-SPME/GC-FID signals, data analysis was performed on the reduced chromatograms, obtained after a sub-sampling of 1/8 of the collected points, since the chromatographic profile was unaltered, i.e. after sub-sampling peak width at half height, typically measuring 0.06 min, was not altered.

Furthermore, the first 3 min and the last 10 min were cut because there were no peaks at all, resulting in an HS-SPME/GC-FID signal vector of 7018 points.

The main issue with this data set is to capture the interrelationships among objects (samples), variables (HS-SPME/GC-FID signal points) and the variation occurring along the series. Thus, taking into account the different samples age together with the different features and age of the ABTM batterie, the structure of the data set is characterized by three different sources of variability. Multi-way models lead to an easier identification of the effects present in each of the three modes and allow a simultaneous analysis of the different sources of variation.

Therefore, the data set was organized as a three-way array, in which the three modes are constituted by the samples, the HS-SPME/GC-FID signal points and the batterie, with dimensionality 6 samples × 7018 HS-SPME/GC-FID signal points × 7 batterie (4 batterie plus 3 replicate batterie).

Centering across the first mode (samples) was applied as data pretreatment to remove the constant difference among the samples.

As far as scaling is concerned, working with unscaled data seems preferable since in this case there is the risk of dramatically up weighting components present in very small amount and showing little variation (such as spectral background or baseline). However, given the presence of major and minor constituents, it is desirable to give to the different volatile compounds a comparable influence in the data analysis. In order to do this, the data set was scaled within the second mode (HS-SPME/GC-FID signal points) by using a block scaling procedure called "block-adjusted non-scaled data" [4,16]. In practice, this pretreatment requires to subdivide the HS-SPME/GC-FID signals in different regions (blocks) whose values will be scaled in order to attain the same block-variance after pretreatment. The subdivision in blocks is shown in Fig. 1a, where a representative HS-SPME/GC-FID signal is reported. The vertical line represents the subdivision in two blocks/regions. The variables

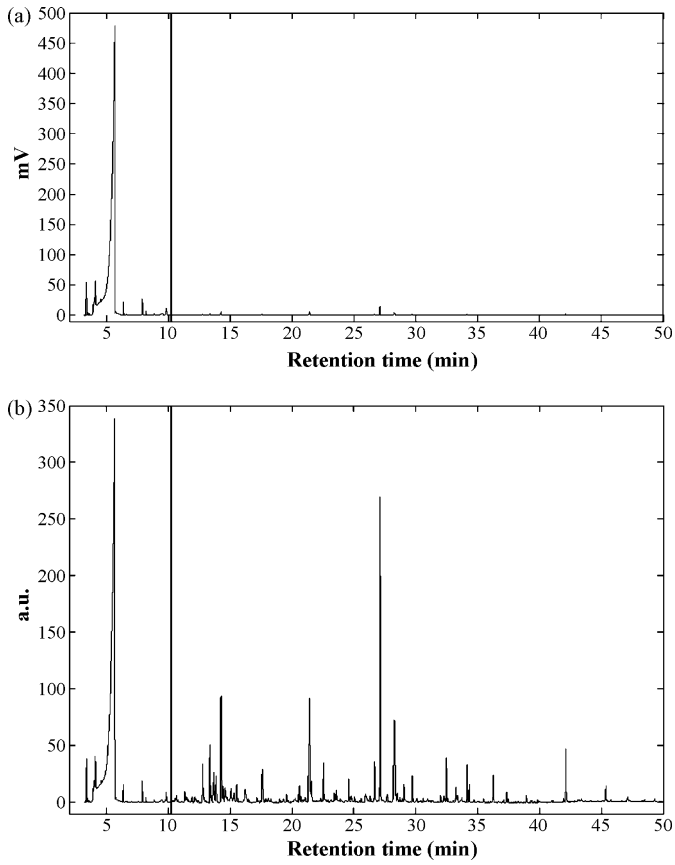


Fig. 1. (a) Representative chromatogram of an ABTM sample. The vertical line divides the signal in the two regions selected for performing the block-scaling pretreatment; (b) the corresponding 'blockscaled' signal.

belonging to the same block are equally weighted and the corresponding pretreated signal is reported in Fig. 1b. Thus, this pretreatment allows minor constituents that could play a fundamental role in the ageing process, to contribute to the model without altering the relative scale of variables belonging to the same block.

Finally, explained variation and 'core array analysis' were used to estimate the number of significant factors (F) and the Tucker3 models were calculated under no constrains.

### 2.5. Tucker3 model

Tucker3 is a decomposition method and it can be fairly considered as a generalization of singular value decomposition (SVD) to higher order array.

In this section a short introduction is given, referring the reader to the literature [9,17,18] for a detailed description of the properties and characteristics of this multi-way technique.

Briefly a three-way array $X$, of dimension $I \times J \times K$, is decomposed into triplets of loadings vectors. Each triplet is called component or factor (F) or latent variable (LV).

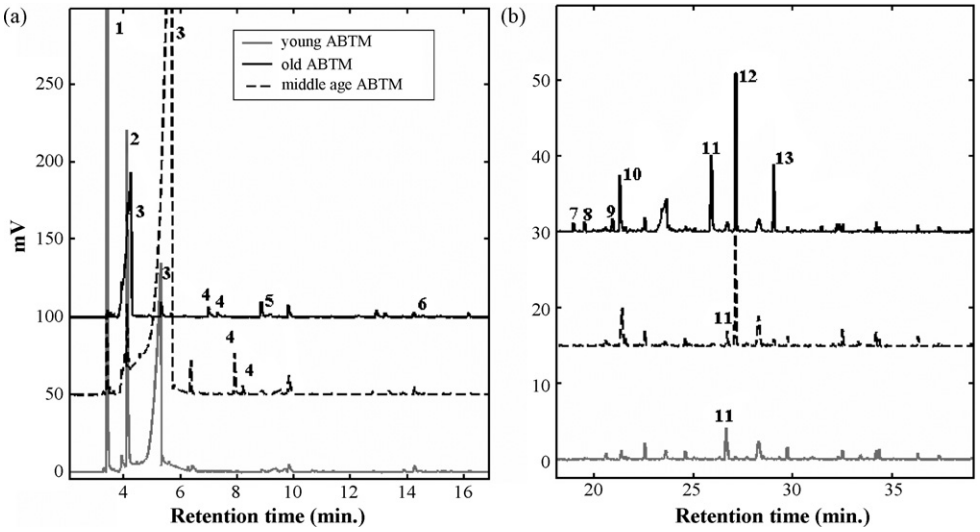


Fig. 2. Different zoom of representative chromatographic regions, the identified chemical species follow: (a) (1) ethanol; (2) ethyl acetate; (3) acetic acid; (4) 2,3-butanediol; (5) furfural; (6) 5-methyl furfural; (b) (7 and 8) isomers of 2,3-butanedioldiacetate; (9) 1,3-propanedioldiacetate; (10) 2-phenyl-ethanol; (11) 5-hydroxymethyl furfural; (12) 2-phenyl-acetate; (13) 5-acetoxymethyl furfural.

The decomposition can be mathematically expressed by the following equation:

$$x_{ijk} = \sum_{p=1}^{P}\sum_{q=1}^{Q}\sum_{r=1}^{R} t_{ip} w^{j}_{jq} w^{k}_{kr} g_{pqr} + e_{ijk}$$

where $t_{ip}$, $w^{j}_{jq}$ $w^{k}_{kr}$ are the elements of the first mode score matrix $T$ ($I \times P$), and of the second, $W^J$ ($J \times Q$), and the third modes, $W^K$ ($K \times R$), weights, respectively. $e_{ijk}$ is a residual term containing all the unexplained variation.

$P$, $Q$ and $R$ are the number of factors extracted for each mode; $g_{pqr}$ is the element of the core array $G$ of order $P \times Q \times R$.

The core array $G$ is composed of singular values; it represents the value by which the single component product is weighted. Therefore, the value and the sign of each core element, give information about the entity of the interaction among the components of the different modes. The squared elements of the core array are proportional to the variation explained by the combination of the factors corresponding to their indices, i.e. if $g_{112}$ is the largest core element, special attention in interpreting the model has to be given to the interaction between Factor 1 of mode 1, Factor 1 of mode 2 and Factor 2 of mode 3.

### 2.6. Unfolding PCA

In the case of unfolding PCA the data array has been unfolded to a bi-dimensional data matrix: $IK$ (barrels and batterie, on rows) $\times J$ (GC signal points, on columns) of dimension $42 \times 7018$. Prior to PCA the same variables block scaling procedure, as for the Tucker3 model, was applied as data pretreatment.

### 2.7. Software

Tucker3 results have been obtained by using the $N$-way Toolbox [19] for MATLAB©. PCA unfolding has been carried out by using the PLS-toolbox 3.5 (distributed by Eigenvector Research Inc., Wenatchee, WA).

## 3. Results and discussion

In this work, the main organic compounds, which concur to the volatile fraction, were identified through the reference mass spectra libraries; furthermore, the identification was supported by comparing the retention times with those reported in a previous work [8].

The behavior of the volatile fraction in the several vinegar samples is extremely interesting as it reflects the three main phases that can be identified during the ageing process, corresponding to fermentation, maturation and ageing.

Fig. 2 reports overlaid representative chromatograms of a young, a middle age and an old ABTM sample coming from the same batteria. For a better graphical visualization of the chromatograms, two distinct plots a and b, covering the 2–17 and 18–40 min regions, respectively, have been done. A different shift degree on the $Y$-axis among the three chromatograms (young, middle age and old) has been used in Fig. 2a and b, moreover a zoom of the $Y$-axis is applied in Fig. 2b. The main chromatographic peaks visible in Fig. 2a and b, have been identified as ethanol (no. 1, Rt $\approx$ 3.4 min), ethyl acetate (no. 2, Rt $\approx$ 4.17 min) and acetic acid (no. 3, Rt $\approx$ 5 min), which is the major product of acetic bio-oxidation. Although these organic compounds are the principal species detected in traditional balsamic vinegars, many other molecules concur to the organoleptic properties and to the characterization of this product, such as the two isomers of 2,3-butanediol (no. 4, Rt $\approx$ 5–8 min), furfural (no. 5, Rt $\approx$ 8.8–9.2 min) and 5-methylfurfural (no. 6, Rt $\approx$ 14–14.5 min). In the retention times interval from 18 to 20.5 min are present low intensity peaks, corresponding to the two isomers of 2,3-butanediol diacetate (nos. 7 and 8). Furthermore, the compounds **1**–**3** propanediol diacetate (no. 9, Rt $\approx$ 20.5 min), 2-phenyl-ethanol (no. 10, Rt $\approx$ 21.3 min),

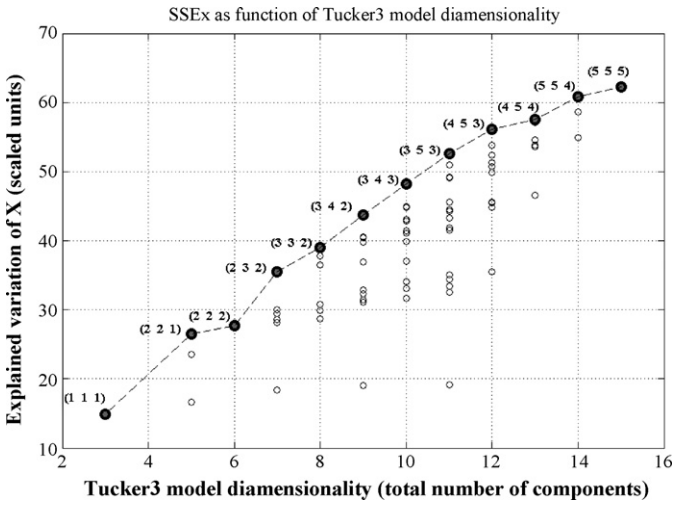


Fig. 3. Variance explained (%) by the different Tucker3 models expressed with respect to scaled data, the labels report the dimensionality of the Tucker3 model with respect to each mode, while on the *x*-axis the global model dimensionality is reported. For the sake of clarity, labels are reported only for the models on the edge.

5-hydroxymethyl-furfural (no. 11, Rt ≈ 25.9 min), 2 phenylacetate (no. 12, Rt ≈ 27.1 min) and 5-acetoxymethyl-furfural (no. 13, Rt ≈ 29 min) were also detected in the vinegars.

In order to assess the degree of variability of the different organic compounds during vinegar ageing and the similarity/dissimilarity among batterie a Tucker3 analysis was employed on data arranged as a three-way array of 6 samples × 7018 HS-SPME/GC signal points × 7 batterie dimensions.

In order to choose the best complexity of the Tucker3 model, i.e. the number of factors for each mode, the tuck test routine [9,19] was applied. The results are shown in Fig. 3, and according to the best compromise between good fit and a low number of factors, a [2 3 2] Tucker3-model, explaining 54% of total variance with respect to raw data, have been chosen. Although the [3 3 2] Tucker3-model explains more variance than the [2 3 2] model, the analysis of the core array elements of both the models pointed out that the third factor (F3) in mode 1 is not necessary (results not shown). Table 1 reports the main core array elements of the chosen model. For the sake of clarity, the squared elements of the core array are proportional to the variation explained by the combination of the factors corresponding to their indices. For example the (1 1 1) is the largest core element, which alone represents the 25% of the explained variance of the core array. This means that the interaction between Factor 1 (F1) of the first, second and third modes, is the most important one and it has to be mainly taken into account in the interpretation of the model.

Table 1
Largest core entries for the [2 3 2] Tucker model

| Index of the core elements | Explained core variance (%) | Value of the core elements |
|---|---|---|
| [1 1 1] | 25 | 2111 |
| [2 2 1] | 19 | 1825 |
| [1 3 2] | 18 | 1779 |

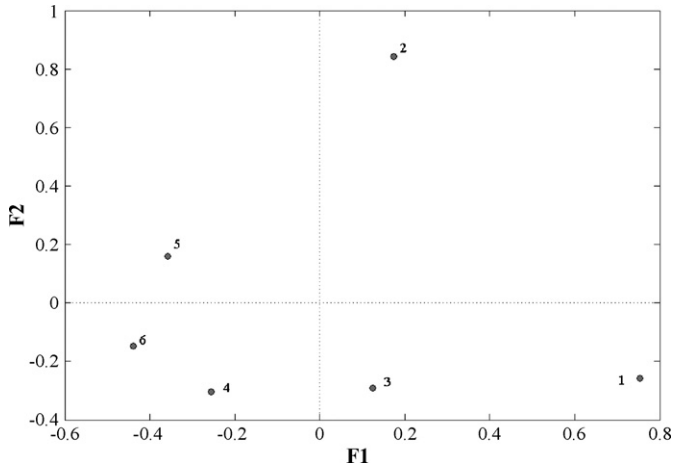


Fig. 4. Scores plot of mode 1 (samples) for the first two factors (results of Tucker3 applied on block scaled HS-SPME/GC-FID signals).

A careful analysis of the first two factors (F1 versus F2) scores plot of mode 1 (Fig. 4) shows that the first factor (F1) describes quite well the evolution of the ageing process of the several batterie, differentiating the degree of ageing of the vinegar samples. In fact, vinegar samples are well ordered on the basis of their age on the first factor, decreasing their scores values from the oldest sample to the youngest one.

Fig. 5 reports the second mode (HS-SPME/GC-FID signals) loadings plot of the first three factors.

Taking into account the highest absolute loadings values, the differentiation of the vinegar samples is strongly influenced by four main chromatographic regions corresponding to the following retention times—(i) Rt: 3–6 min; (ii) Rt: 12–15 min; (iii) Rt: 20–23 min; and (iv) Rt: 25–30 min.

The interpretation is based on loadings plots combined with the information in the core array elements. The first important core element (1 1 1) indicates interaction among Factor 1 (F1) of all the three modes. Considering the positive value of this interaction term, it could be said that the volatile compounds belonging to the chromatographic regions with highest loading

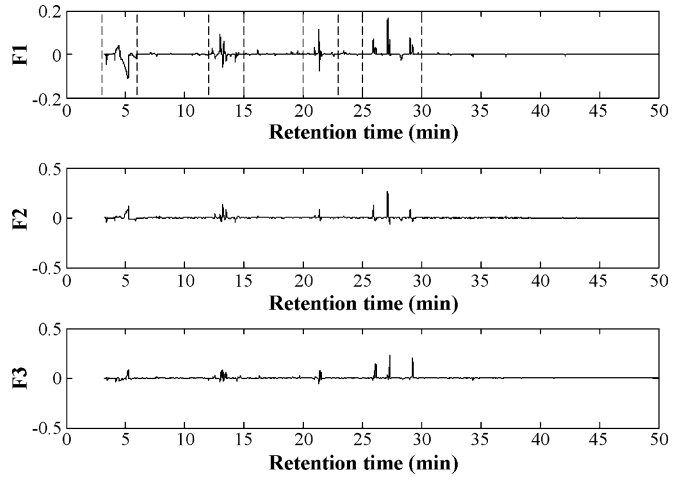


Fig. 5. Loadings plot of mode 3 (batterie) for the first two factors (results of Tucker3 applied on blockscaled HS-SPME/GC-FID signals).

values (referred to F1) are generally present in lower concentration in the younger samples (negative F1 scores values) and increase from middle to oldest samples, respectively.

In general, the compounds which concur to the volatile fraction of the product go through a variation when passing from the young to the middle age samples, while they have a tendency to concentrate during ageing, because of the loss of water.

Now, owing to the constancy of the overall instrumental conditions, i.e. chromatographic elution and detection, together with the experimental set up, namely the sample and flask volumes, temperature, conditioning, sampling time and SPME fibre, some further considerations might be carried out from a deeper evaluation of the chromatographic profiles reported in Fig. 1 and the Tucker loadings plots (Figs. 4 and 5).

In particular, the young samples are characterized by remarkable amount of ethanol and acetic acid (negative F1 loading value), which decreases with age and is no more present in old vinegars (positive F1 value). This behavior probably depends on the fermentation and bio-oxidation processes, which take place only in the young barrels. Initially, yeasts ferment sugars to alcohols, especially ethanol, which is successively oxidized to acetic acids by acetic acid bacteria. During ageing, the amount of acetic acid diminishes, probably because of a slow evaporation of this species.

The behavior of ethyl acetate, that is one of the species detected in young samples is similar to ethanol (they have both a negative loadings value in the respective modes). The ethyl acetate may be obtained from condensation reaction of ethanol and acetic acid. It is rather abundant during the first phase of maturation and is extremely reduced with time.

In the second chromatographic zone, Rt: 12–15 min, it has not been possible to assert the identity of the peaks on the basis of the libraries matches.

As far as 5-methyl-furfural is concerned (Rt: 14–14.5 min), its amount is relatively consistent (modest contribution to loadings of mode 2) in all samples in spite of the occurrence of the evaporation process, confirming also for this species the presence of a transformation/conversion process [20–22].

From a comparison between the F1 Tucker scores mode (samples mode) and the third zone of Fig. 4a, 2-phenyl-ethanol undergoes a concentration process during ageing.

Finally, phenyl-acetate, 5-hydroxymethyl-furfural (5HMF), and 5-acetoxymethyl-furfural is other significant molecule. The two compounds belonging to furfural family, 5HMF and 5-acetoxymethyl-furfural are produced during the cooking of the grape must to obtain the starting raw materials for ABTM, namely condensed cooked must [20]. 5HMF is present in all the samples (Fig. 2), but its concentration is definitely higher in old samples, mainly because of water evaporation, as well as 5-acetoxymethyl-furfural, that is mainly produced during ageing (both contribute with a positive loadings in F1 of mode 2). Finally, phenyl-acetate is present in low concentration in young samples and its amount significantly increases in middle age and old samples.

In addition, it is interesting to observe the third mode (batterie) loadings plot (Fig. 6): the batterie "Sa" and "O" lie on the extreme positions along the first and second factors, respectively. The second (2 2 1) and third (1 3 2) important core array elements seem to particularly highlight the peculiarity of the two oldest batterie.

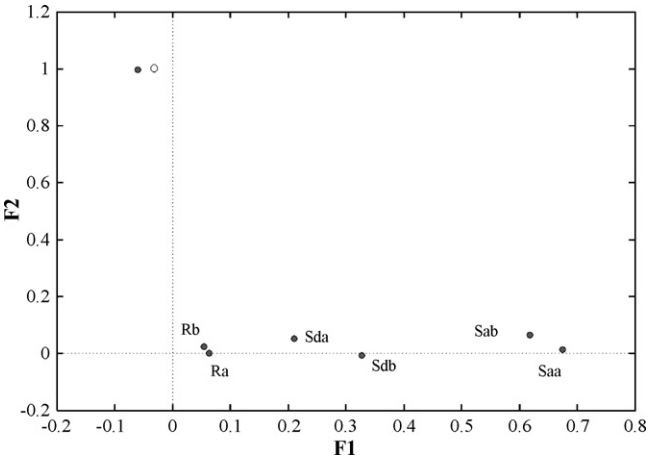


Fig. 6. Loadings plot of mode 2 (GC-signals) for the first two factors (results of Tucker3 applied on blockscaled HS-SPME/GC-FID signals).

As regards the interaction described by the (2 2 1) core array element, batteria Sa is quite peculiar, mainly because of sample 2, which has the highest amount of 2-phenyl-acetate and acetic acid and this determines its extreme position on high F2 values in mode 2 loadings plot.

The (1 3 2) core array element highlights the peculiar features of "O" batteria (the oldest one). The fact that Factor 2 of "batteria mode" interacts with Factor 1 of "barrel mode" can be interpreted as the ageing inside the barrel depends on whether the product starts to age in the oldest batteria "O" or in the younger ones. Considering Factor 3 of "retention time mode" are mainly influential the higher contents of phenyl-acetate, 5-hydroxymethyl-furfural (5HMF), and 5-acetoxymethyl-furfural species in oldest "O" samples with respect to the other batterie.

Although batteria "O" is the oldest ones, its position does not follow the ageing order along F1 in Fig. 6. This behavior could be due to a shift on retention times values for its samples with respect to the corresponding samples of the other batterie, as shown in Fig. 7, where a zoom of the chromatographic regions

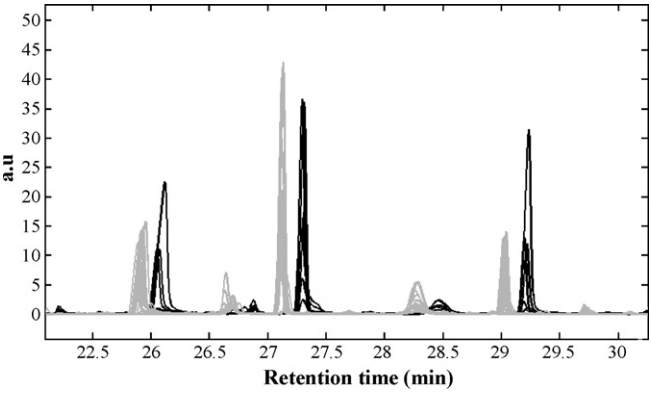


Fig. 7. The chromatograms of the oldest samples (1) of each batteria are reported overlaid. The black solid line refers to the chromatogram of batteria "O" oldest sample.

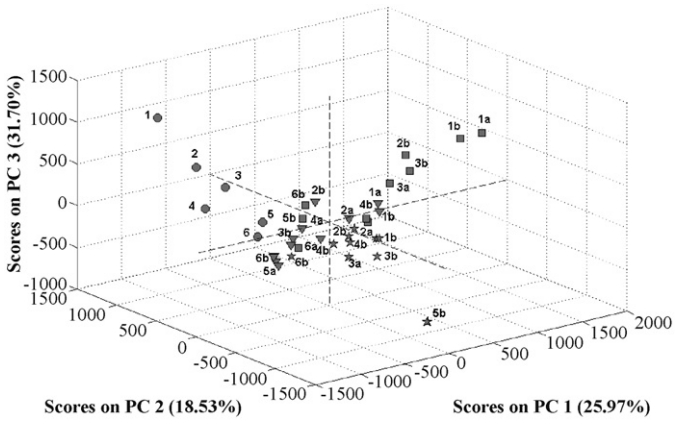


Fig. 8. Scores plot for the first three principal components (results of PCA-unfolding analysis applied on blockscaled data). The % of explained variance by each component is reported on parenthesis and it is expressed with respect to raw data. Labels from 1 to 6 refer to casks, replicates are labelled with 'a' and 'b' letters. Symbols: circles, batteria "O"; stars, batteria "R"; squares, batteria "Sa" and triangles, batteria "Sb".

between 25 and 31 min of the raw HS-SPME/GC-FID signals (grey lines) of all the batterie, together with the batteria "O" (black lines) is reported. "O" batteria's samples are actually shifted with respect to the other ones and the shift problem is more evident among the Factor 3 loadings value.

In order to deal with the shift issue an attempt to use PARAFAC2 method has been done, but neither using the "retention times × samples × batterie" nor the "retention times × batterie × samples" arrangement we did get any better result. To consider the eventual performance of different alignment algorithms as preprocessing step will be dealt in a future work.

However, it has to be noticed how Tucker3 model has been able to hold the major information on the first two factor and living out the shifting problem mainly in Factor 3.

In order to highlight the advantages offered by a three-way approach, the Tucker3 results have been compared with standard two-dimensional PCA analysis. PCA has been performed by unfolding the 6 vinegar samples × 7018 HS-SPME/GC-FID signal points × 7 batterie data array to a bi-dimensional matrix with all the vinegar samples on the rows (42 samples resulting from 6 samples × 7 batterie) and the HS-SPME/GC-FID signal points on the columns.

The same pretreatment of the three-way case has been applied in order to obtain a more fair comparison among bi- and three-dimensional tools. A three principal components model, explaining 68% of the total data variance, seemed appropriate taking into account the screen plot (not shown) and the information that can be gathered by the corresponding scores plots.

Fig. 8 reports component 1 (PC1) versus component 2 (PC2) versus component 3 (PC3) PCA score plot. The samples have been labelled in the same way as for the three-dimensional case, i.e. the label 1 refers to the oldest samples and the label 6 to the youngest ones. The replicates are labelled with a and b letters. The several batterie are represented with different symbols, as reported in the legend of Fig. 8. Even if a good discrimination is observed for batteria "O", with all of its vinegar samples totally located on the right side of Fig. 8, an higher degree of overlap among the others batteries is evident. Furthermore, it is rather difficult to extrapolate any kind of information as far as the ageing process of ABTM samples is concerned due to overlapping among the different barrels. In fact, due to the batterie peculiarities, it is reasonable that 'young' samples coming from old batterie are somehow similar to 'old' samples that belong to young batterie. Concluding, PCA analysis mainly highlights the inter-batterie differences and not the intra-batteria ones, giving limited information about the ABTM ageing process.

As far as PCA loadings plot is concerned, the most influential chromatographic peaks are substantially the same with respect to Tucker3 mode 2 loading plot and thus PCA loadings plots have not been reported.

Summarizing, adopting unfolding strategy does not give any information as far as ABTM ageing process is concerned since the patterns in the scores plot are less recognizable, thus there is an obvious advantage by using methods, which takes into account the true tri-dimensional structure of the data set.

## 4. Conclusions

This work is focused on the analyses of the volatile compounds of ABTM samples of different age, coming from different batterie. Tucker3 model was applied for the analyses of the whole signal data, performing a block scaling pretreatment to emphasise the importance of minor constituents. The batterie showed an analogous trend in the evolution of the volatile organic compounds observable in the first mode scores plot, where samples are grouped according to vinegar age. It was possible to observe that the compounds which concur to the volatile fraction of the product are extremely transformed from the young to the middle age samples, besides they have a tendency to concentrate during ageing, because of the natural loss of water.

Generally, the separation of vinegar samples of different age is due both to the different amount of acetic acid, ethyl acetate and ethanol (that are the principal products of the alcoholic fermentation and bio-oxidation processes) and of furfurals and other minor compounds.

Moreover, it was observed that the three-way method is more efficient for the study of these types of data than unfolding PCA, as it offers an easier visualisation of the data structure, in particular capturing the trends which are common to the different batterie in the samples mode plot and the variability due to the different batterie in the third mode loadings plot.

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