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Characterisation of Italian vinegar by pyrolysis–mass spectrometry and a sensor device ('electronic nose')

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Industrially made vinegar 'Aceto Balsamico di Modena' and traditionally produced vinegar 'Aceto Balsamico Tradizionale di Modena e di Reggio Emilia' were analysed by means of pyrolysis–mass spectrometry (Py–MS) and a sensor technique ('electronic nose'). Both methods allow a fast classification (typically about 5 min). While the 'electronic nose' is analysing the volatile compounds of the samples simultaneously with 32 sensors, Py–MS applies thermal decomposition of the samples, subsequently analysing the pyrolysate with a mass spectrometer. Both techniques were demonstrated to be capable of discriminating between the two groups of vinegar. Although the number of samples available for this study did not seem to be sufficient for detailed analysis, both methods indicated possible discrimination of the samples within the group of 'Aceto Balsamico Tradizionale di Modena' regarding the age of the sample.

INTRODUCTION

Besides vinegar from red and white wine, there is the traditional balsamic vinegar from Modena and Reggio Emilia that has been recognised as the product of a specific region in Italy (Law No. 93, 1986). The matrix for fermentation is must of local grapes, such as Trebbiano, being concentrated to at least one-third of its initial volume. The fermentation is carried out by a slow ageing process by changing barrels of various wood types and sizes (Vitali, 1995; Seidemann, 1996). There are two commercially available balsamic vinegar types on the market: 'Aceto Balsamico Tradizionale di Modena' and 'Aceto Balsamico di Modena' (industrially made). The traditional type is made from cooked grape must matured by progressive concentration. Ageing is performed in a series of casks of different wood for at least 12 years (Turtura *et al.*, 1991; Giudici *et al.*, 1992; Stacchini *et al.*, 1990; Giudici, 1990). Balsamic vinegar is comparable with vinegar made by the traditional 'solera' system used for 'Jerez' wines in Spain (Garcia-Parrilla *et al.*, 1994). Such vinegars have in common the requirement to be aged for variable periods of at least 12 years. Some methods concerning the characterisation of wine vinegar are described in the literature. They are based on analysis of phenolic acids and volatile compounds in Spanish vinegar (Garcia-Parilla *et al.*, 1994; Troncoso *et al.*, 1987; Blanch *et al.*, 1992), and on cluster analysis using important parameters such as acidity, ash content, alcohol, glycerol etc. for differentiation of vinegar from wine (Guerrero *et al.*, 1994).

Characterisation of balsamic vinegar was done by analysis of organic acids (Giudici *et al.*, 1994), glycerol (Plessi *et al.*, 1988), gluconic acid (Giudici, 1993) and other chemical parameters (Antonelli *et al.*, 1990). Another useful tool for the evaluation of age and quality of balsamic vinegar is the ratio of D/L-amino acids such as alanine, aspartic acid and phenyl alanine in which racemisation occurs after the lysis process of the micro-organisms responsible of the fermentation process (Chiavero *et al.*, 1995).

Pyrolysis–mass spectrometry (Py–MS) and sensor technique instruments, such as 'electronic noses', are analytical fingerprinting techniques. Py–MS has been shown to be a versatile and rapid method of ascertaining the quality and composition of food such as orange juice (Aries *et al.*, 1986), Scotch whisky quality (Reid *et al.*, 1993), virgin olive oils (Goodacre *et al.*, 1993) and recently for the characterisation of wines (Montanarella *et al.*, 1996) and vegetable fats used for chocolate production (Anklam *et al.*, 1996*a*). The mass spectra hereby obtained show regions of more or less unique patterns

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that can be correlated to the samples analysed. The 'electronic nose' used for this investigation has 32 analytical sensors which are used to investigate the vapour phase of samples. It has been shown to be a suitable method for characterisation of various foods such as wines from different origins (Aromascan, 1996) or cheeses (Aromascan, 1996; Anklam *et al.*, 1996*b*). Each of the sensors differs somewhat regarding its response towards molecular properties such as size or polarity.

In this paper we present an approach for the application of Py–MS and an 'electronic nose' used for the rapid identification of the two types of balsamic vinegar. In Table 1 some of the important features of both methods are summarised.

Both methods were chosen because they provide the advantage of a fast classification of samples. However, none of the methods allow an identification of the species (fragments or molecules) contributing to the signal obtained. The identification would require other, more time-consuming, techniques such as headspace gas chromatography or pyrolysis–gas chromatography–MS, where the species are separated prior to their detection. For this investigation the focus was placed on short analysis time and reduced time for sample preparation. The aim here is to focus on the use of the two methods for a rapid identification of vinegar.

MATERIALS AND METHODS

Samples

Thirteen vinegars labelled 'Aceto Balsamico di Modena', (obtained from local supermarkets) and nine 'Aceto Balsamico Tradizionale di Modena' (furnished by producers of the traditional balsamic vinegar of Modena) were analysed. To investigate the influence of different producers of the 'Aceto Balsamico Tradizionale di Modena', a second set of samples was analysed with Py–MS comprising all the samples above and an additional 18 samples from another two producers.

Pyrolysis–mass spectrometry (Py–MS)

Pyrolysis–mass spectrometry was performed on a Rapyd-400 (Horizon Instruments Ltd, Sussex, UK) based on a quadrupole mass analyser and employs Curie-point pyrolysis. Vinegar samples were pipetted onto metal foils (Curie point 530°C) which were subsequently inserted into small glass tubes and dried in an oven at 70°C for 10 min. The foils were pushed into the tube using a stainless-steel depth gauge so as to lie 10 mm from the mouth of the tube. Finally, Viton O-rings were placed on the tubes. The pyrolysis temperature (530°C) was held for 3 s and the resulting pyrolysate was ionised at 25 eV. The mass range was scanned between m/z 40 and 350. The expansion chamber temperature was 160°C, while the temperature of the ion source was maintained at 200°C. Five replicates of each sample were analysed.

Data handling

The normalised data were processed using the GENSTAT package supplied by the manufacturer. Using canonical variance analysis (CVA), (MacFie *et al.*, 1978) a set of masses was determined showing the significant correlation regarding the grouping of samples. Using the signals of these masses, principal component analysis (PCA) (Marriott, 1974) was applied for a dimension reduction. Data were reduced by keeping only those principal components whose eigenvalues accounted for more than 0.1% of the total variance.

'Electronic nose'

An 'electronic nose' system (A8S with MKII sample station, AromaScan, UK) based on 32 sensors was applied. Each of the sensors differs in its response with respect to hydrophoby, size or polarity of the molecules in the gas phase. One hundred microlitres of vinegar was put on a support of filter paper in a plastic bag. It was then filled with air with a controlled level of humidity and after equilibration analysed by the instrument. The sampling port was cleaned between each analysis with an aqueous 2% butanol solution in order to eliminate any error due to cross-contamination. Forty percent of reference humidity and 50% of bag humidity were applied during the analysis. Reference air was used to virtually set the detector signal to zero. In Table 2 the sampling procedure details are listed.

Data handling

For data analysis the software supplied by the manufacturer was used. Database files were constructed from averaged data collected between 220 and 240 s. Data are presented as compressed data, each point representing a single sample run. The graphs were generated by an algorithm known as Sammon mapping (Sammon,

Table 1. Characteristics of the pyrolysis–mass spectrometry and the 'electronic nose'

	Pyrolysis–MS	'Electronic nose'
Analyte	Pyrolysate of the whole sample	Volatile compounds
Detector	Mass spectrometer	Sensors (made of polymers)
Analysis time	< 2 min	5 min
Number of replication	Typically 3	Typically 1–2
Data evaluation	Multivariate data evaluation	
Compound identification	No identification of contributing species possible	

Table 2. Sampling procedure, the first four parameters are describing the valve sequence applied during analysis

Parameter	
Reference	10 s
Sample	240 s
Wash	1 s
Reference	10 s
Reference air humidity (rel.)	40%
Bag air humidity (rel.)	50%
wash solution	2% butanol in water

1970), where the signals of 32 sensors, or 'fingerprints' are compressed to two or three dimensions to visualise the underlying differences between the samples.

RESULTS AND DISCUSSION

Two kinds of vinegar, 'Aceto Balsamico di Modena' (A) and 'Aceto Balsamico Tradizionale di Modena' (B), were analysed by means of Py–MS applying the combined canonical variances (CV) and principal component (PC) analysis on the spectra. Figure 1 represents the loadings of the first three PCs calculated from the signals of 22 significant masses as determined by CV. The two groups of vinegar were well separated as shown in Fig. 1.

The samples labelled 'A' consist of 13 industrially manufactured vinegar samples of 'Aceto Balsamico di Modena', produced by nine different manufacturers. The production process involved includes ripening of up to 6 years. The samples labelled 'B' consist of nine samples of 'Aceto Balsamico Tradizionale di Modena', ripened for 5, 7, 9, 11, 13 (two samples), 15, 18, and 25 years, respectively. These samples were obtained from the same farm and were produced according to the law protocol for the traditional vinegar (Law No. 93, 1986).

Five replicates of each sample were analysed. The diversity of the industrially made samples (labelled 'A') causes a rather large dispersion of these samples in Fig. 1. Furthermore, the difficulties controlling the pyrolysis process itself contribute to this dispersion, which is, however, an intrinsic property of the method mainly caused by a non-uniform spread of the liquid on the metal foils used.

The vinegar 'Aceto Balsamico Tradizionale di Modena' (labelled 'B' in Fig. 1) shows a rather compact cloud, despite the variations in the ripening process involved. However, all these samples were obtained by only one manufacturer. Knowing that the group of 'Aceto Balsamico Tradizionale' (labelled 'B' in Fig. 1) contained vinegar samples between 5 and 25 years old, the possibility of a further differentiation within this group was investigated. Figure 2 represents again the loadings of the first three PCs calculated from the signals of 17 significant masses as determined by CV. 'Aceto Balsamico di Modena' (industrially manufactured) is again labelled 'A', while the vinegar group called 'Aceto Balsamico Tradizionale di Modena' is further divided in two groups: samples up to the age of 11 years are labelled 'B', and the samples ranging from 13 to 25 years are labelled 'C'.

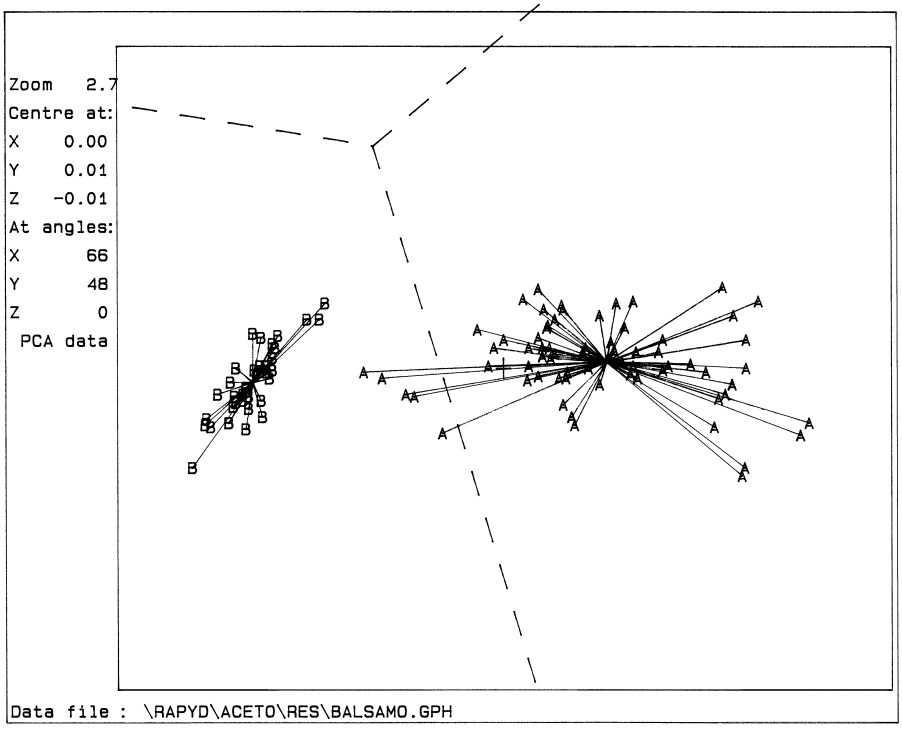

Fig. 1. Py–MS graph showing the two groups of vinegar samples analysed: (A) the industrially made 'Aceto Balsamico di Modena'; and (B) 'Aceto Balsamico Tradizionale di Modena'. Five replicates of each sample were analysed. 3D graph of the loading vectors was obtained by the PCA based on the signals of 22 masses.

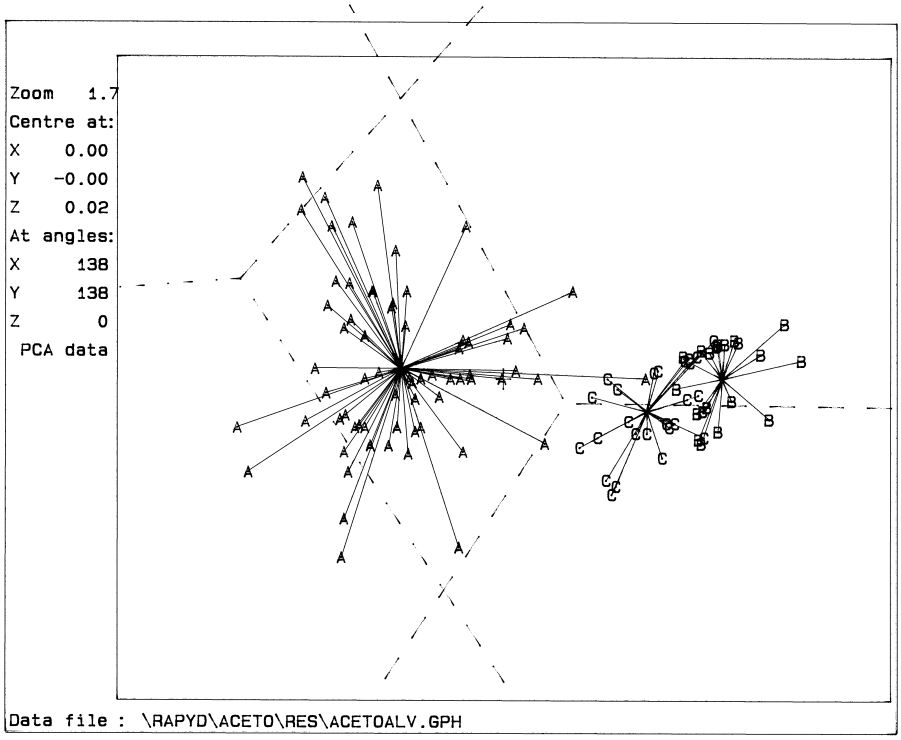

Fig. 2. Py–MS graph showing the industrially made 'Aceto Balsamico di Modena' (A), 'Aceto Balsamico Tradizionale di Modena' from 5 to 11 years old (B), and 'Aceto Balsamico Tradizionale di Modena' from 13 to 25 years old (C). Five replicates of each sample were analysed. 3D graph of the loading vectors was obtained by the PCA based on the signals of 17 masses.

Figure 2 indicates a possible discrimination of the samples within the group 'Aceto Balsamico Tradizionale di Modena' with regard to the ripening process. The results given in Fig. 2 indicate that a further differentiation of the samples with regard to the ripening process is possible. However, the limited number of samples available for this study is not sufficient for more detailed analysis. Considering the results given in these figures, Py–MS appears to be a fast and versatile method for discriminating between the traditionally made 'Aceto Balsamico Tradizionale di Modena' and the industrially made 'Aceto Balsamico di Modena'. From the results it can be deduced that three replications are sufficient. This has also been documented in the literature, e.g. for other matrices.

To investigate the influence of different producers of the 'Aceto Balsamico Tradizionale di Modena' on the differentiation of the two kinds of balsamic vinegar, 18 samples from a further two producers were analysed. Figure 3 shows the result of the combined canonical variance/principal component analysis. The data are represented by cluster analysis, that is according to the similarity of their mass spectra. The similarity is 100% at the left side of the graph and decreases vs the right-hand side. Groups of equal similarity are joined vertically by parentheses. The horizontal dots are introduced to guide the eyes. Three groups are clearly distinguishable. The industrially manufactured balsamic vinegars build one group (the top one in Fig. 3) and the other two groups consist of traditionally manufactured balsamic vinegar only. Five of the younger samples (< 10 years) of the traditional made balsamic vinegar are grouped together at the bottom of the graph, all of them were stored in barrique barrels (small wood barrels, usually for 100 litres or less, made of oak), while all other traditionally made vinegars are stored in 'normal' wood barrels. Except for this phenomenon, no significant differences within the group of traditionally made balsamic vinegars of three different producers could be found.

Analysis by the 'electronic nose' was also performed with the same two sets of balsamic vinegar samples as in Fig. 2. Figure 4 shows the 2D graphs of 'Aceto Balsamico di Modena' (●) and 'Aceto Balsamico Tradizionale di Modena' (■).

Preliminary tests revealed a rather good reproducibility of the 'electronic nose'. Thus, only one measurement for each sample is shown in Fig. 3. Detailed data analysis revealed that the 5 year old sample of 'Aceto Balsamico Tradizionale di Modena' could not be discriminated from the industrially made samples, using this technique. To enhance the discrimination of the older samples from the industrially made ones this sample was excluded from further data evaluation. The group of traditional samples (■) seems to be sub-divided into two groups: samples from 7–13 years old are in the left corner of the 2D graph, and the samples from 13 to 25 years old are on the bottom right side of the 2D graph. Figure 3 shows that the two 13 year old 'Aceto Balsamico Tradizionale di Modena' samples are placed in two different groups. This particular phenomenon

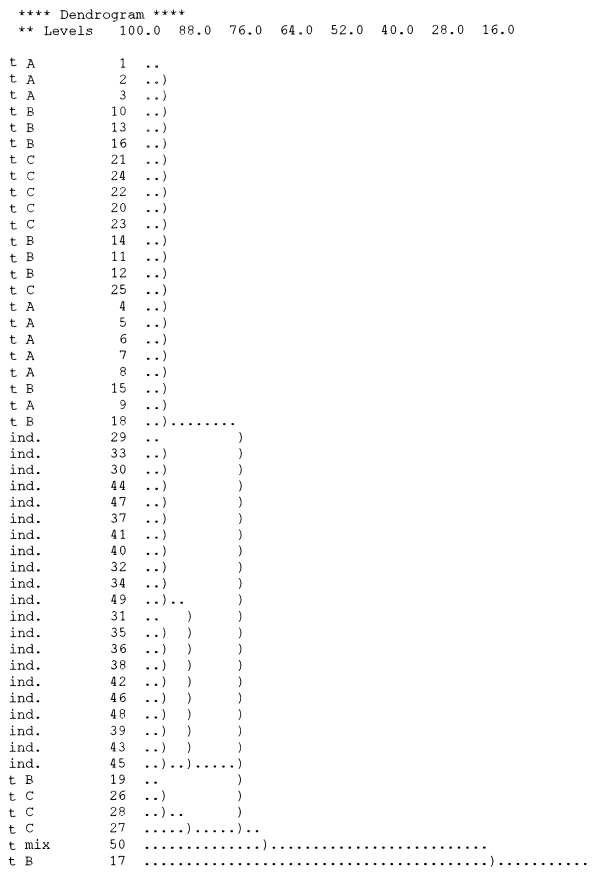

Fig. 3. Result of the CVA/Factor analysis of industrially and traditionally made balsamic vinegars, visualised as a dendrogram obtained by cluster analysis. Traditionally made vinegars are labelled with a '*t*' followed by the code of the producers and the age of the samples. Industrially made samples are labelled as 'ind.'. Samples are grouped by parentheses according to their similarity. The more similar the samples the closer are the parentheses on the left side (left edge 100% similarity). Dots are introduced only to guide the eye.

could not be fully explained, but differences in the production process are presumed to play a major role. A parameter to be considered is the nature of the wood used for the vinegar ripening, as it could influence the aroma. Young samples were preferentially stored in oak barrels, while ageing continued in barrels of chestnut, cherry, ash and mulberry wood. All the industrially made 'Aceto Balsamico di Modena' are placed at the top right of the 2D graph.

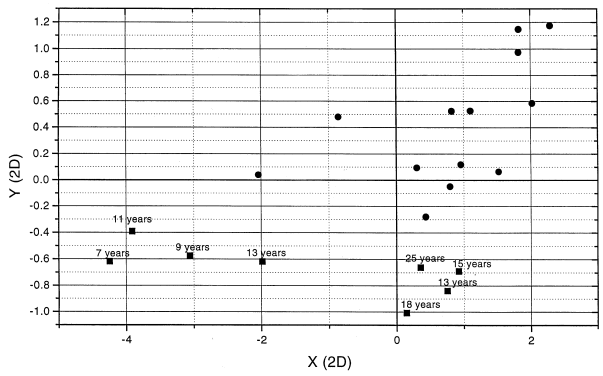

Fig. 4. 2D graph of the industrially made 'Aceto Balsamico di Modena'(●) and 'Aceto Balsamico Tradizionale di Modena' (■).

Although the number of samples available for this study appears to be insufficient for detailed analysis, it is obvious that the method could be used for a distinct discrimination of the traditionally made 'Aceto Balsamico di Modena' from the industrially made ones. In addition, it would appear possible (according to Fig. 3) to discriminate the samples within the group 'Aceto Balsamico Tradizionale di Modena' on the basis of the sample.

CONCLUSIONS

The scope of this investigation has been, to show the suitability of Py–MS and an 'electronic nose' for a fast and reliable classification of vinegars. Our results suggest that both methods are able to provide a fast (analysis time about 5 min) and versatile method for discriminating industrially manufactured 'Aceto Balsamico di Modena' from 'Aceto Balsamico Tradizionale di Modena'. Using either Py–MS or the 'electronic nose', it seems to be possible to discriminate the samples within the group 'Aceto Balsamico Tradizionale di Modena' on the basis of the age of the sample. Despite the rather low number of traditionally made vinegars, the results seem to indicate that Py–MS and the 'electronic nose' can be rapid analytical tools for the discrimination of industrially made 'Aceto Balsamico di Modena' from traditionally made 'Aceto Balsamico Tradizionale di Modena'. The extension of this approach to a larger number of vinegars and, especially, the creation of a data base of adequate size for Py–MS results on traditional balsamic vinegar is foreseen.

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