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Original Paper

Solid-phase extraction method for determination of volatile compounds in traditional balsamic vinegar

A solid-phase extraction method for the determination of volatile compounds in traditional balsamic vinegar (TBV) has been developed. The optimisation has been carried out using a two-level factorial design expanded further to a central composite design. LiChrolut-EN SPE cartridges were used and the optimised analytical conditions were: 7 g of TBV diluted 1:4, 5 mL of washing water, and elution with 10 mL of dichloromethane. The linear ranges and detection and quantitation limits were adequate for the analysis of the studied compounds in TBV. The accuracy of the method was also studied: recoveries up to 80% and repeatability values lower than 15% were obtained for the majority of the studied compounds. Two TBVs of different age were analysed by the optimised method.

Keywords: Factorial design / Solid-phase extraction / Traditional balsamic vinegar / Volatile compounds

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1 Introduction

Traditional balsamic vinegar (TBV) of Modena and Reggio-Emilia is a typical product of the Emilia-Romagna region in the north of Italy. It is manufactured from the juice of white grapes (typically, Trebbiano grapes) boiled down to approximately 50% of its original volume to obtain a concentrated must, which is then fermented and subjected to a slow aging process which concentrates the flavours. The flavour intensifies over decades, with the vinegar being kept in fine wooden casks, and the final product is sweet, viscous, and highly concentrated. TBV is very labour-intensive to produce; while it ages and gradually evaporates thanks to wood porosity, the liquid is transferred to successively smaller casks made of different woods, becoming more concentrated with each transfer. Oak, mulberry, chestnut, cherry, juniper, ash, and acacia are the most commonly used woods, each of them with a typical porosity and aromatic impact. At the end of the process, the vinegar is taken from the smallest cask, each cask having been filled with the contents of the preceding (larger) cask and the original cooked must having been added to the largest cask. TBV is appreciated world wide for its extremely high certified quality. Its physicochemical properties have therefore been studied by several authors with regard to carboxylic acids [1], phenolic acids [2], furanic compounds [3], microbiology [4,5], heavy metals [6], as have the relationships between different parameters [7 – 9].

In complex matrices subjected to aging processes, study of the aroma plays a fundamental role in determining the quality of the final product. In the case of TBV, due to its complexity only few publications related to the analysis of volatile compounds exist [10 – 13]. Consequently, even today the extraction and concentration of volatile compounds in TBV constitute a problem that has still not been satisfactorily resolved. In particular, the high percentage of acetic acid present in TBV represents a serious problem, either for the chromatographic response, due to its elevated signal, or for the SPE extraction process, because acetic acid itself acts as a solvent [14].

The present paper describes the development (optimisation and validation) of a solid-phase extraction method with subsequent capillary gas chromatographic – mass spectrometric detection applied to the determination of volatile compounds in TBV. Optimisation of the extraction conditions has been carried out by means of an experimental design. This design allows the simultaneous variation of all experimental factors studied, and the detection of possible interactions among them that could influence the final result but are not detectable by classical experimental methods [15, 16]. Taking into account the complexity (an extraordinary density and the previously mentioned high content of acetic acid) of

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Abbreviations: TBV, traditional balsamic vinegar

our matrix, sample amount, eluent volume, washing water volume, and dilution of the sample were evaluated. The effects of these parameters were investigated using a two-level factorial design expanded further to a central composite design. The juxtaposition of a two-level design with a star design (the centres of the two designs coincide) gives the mentioned composite design. It is one of the most useful designs for estimating a multifactor response surface and has been previously used with excellent results for the study of volatile compounds and pesticides in Sherry wine vinegars [17, 18].

2 Experimental

2.1 Vinegar samples

Due to the high number of experiments needed for the optimisation and the shortage of TBV available, a mix composed of TBV (40%) and industrial balsamic vinegar (60%) was used to optimise the SPE extraction conditions and to determine the performance characteristics of the method. After optimisation and validation, two different samples of TBV with different aging histories were analysed using this methodology.

2.2 Chemicals and reagents

Two different types of SPE packings were studied; LiChrolut-EN cartridges (200 mg) were purchased from Merck (Darmstadt, Germany), C18 Isolute cartridges (1 g and 2 g) were purchased from IST (Hengoed, Mid-Glamorgan, UK).

The aroma standards used in this study were supplied by Aldrich (Milano, Italy) and Sigma Chemicals (St. Louis, MO, USA).

Individual concentrated solutions of each compound were prepared by weight in absolute ethanol. A global solution containing all the compounds was prepared by dilution of each individual solution in a unique volumetric flask. This global solution was used to prepare the working ones for the calibration curves and the performance characteristic. They were prepared in dichloromethane in order to reach a volume of 0.5 mL by evaporation in a stream of nitrogen before the injection in GC–MS. All the working solutions were stored a 4°C.

2-Octanol was employed as internal standard (514 mg/L in absolute ethanol).

2.3 Sample preparation

The extraction cartridges were conditioned with 4 mL of dichloromethane, followed by 4 mL of methanol and 4 mL of water.

After optimisation, extraction was carried out by passage of 7 g of sample, diluted 1:4 in Milli-Q water at around 1.5 mL/min, through the SPE cartridge. After this step, and without taking the cartridge to dryness, a process of washing with 5 mL of water was carried out. After the washing process the cartridge was dried by passage of air. The compounds were recovered by elution with 10 mL of dichloromethane. The eluted sampled were stored at −18°C for at least 24 h in order to freeze out the remaining water. Then the samples were filtered and 100 μL of internal standard (IS) solution were added to the filtered sample. Finally, a volume of 0.5 mL was attained by evaporation in a stream of nitrogen.

2.4 Instrumentation

Two μL of the extract were injected in the splitless mode (splitless time 0.60 min) into a Thermo Finnigan Trace GC ultra gas chromatograph (San Jose, CA), equipped with a Thermo Finnigan Trace DSQ selective mass detector, a merlin microseal injector, and a Stabilwax fused silica capillary column (Restek, Bellefonte, PA; 30 m, 0.25 mm id and 0.25 μm film thickness), under the following working conditions: GC grade helium as carrier gas at a flow rate (constant flow, vacuum compensation) of 1.0 mL/min, 62 kPa; column temperature program, 35°C heated, at 3°C/min, to 100°C, and then heated at 5°C/min to 240°C (held for 10 min). The injection temperature was 250°C. Detection was carried out by electron impact mass (EI) in the full scan mode, using an ionisation energy of 70 eV, transfer line 220°C. The mass acquisition range was 30–400 amu.

Peak identification was carried out using the NIST 2.0 and Wiley 7.0 libraries by comparison of mass spectra and confirmed by retention times of standards. Quantitative data for the identified compounds were obtained by measuring the relative peak area of their quantifying ions in relation to that of the IS (2-octanol).

2.5 Experimental design

The Statgraphics Statistical Computer Package "Statgraphics Plus 5.1" was used for the statistical treatment of the data.

In this study, a sequential exploration of the response was used and carried out in two stages. In the first stage, the relative influence of the factors and their interactions was studied for the total chromatographic area obtained. Four factors were selected as potentially affecting the SPE extraction: sample amount; eluent volume; washing water volume; and dilution of the sample.

Considering this, a 2^{4-1} factorial design was chosen, which involved eight experiments, in duplicate, carried out in random order to avoid the effect of lurking variables. The values corresponding to the high (+), and low (−) points for each factor, chosen on the basis of previous experiments and bibliographic data, are shown in Table 1.

Table 1. Factor levels for the extraction condition optimisation.

Factor	Low (−)	High (+)	Centre	Axial (−α)	Axial (+α)
Sample amount (g)	2	7	4.5	1.28	7.73
Eluent volume (mL)	10	50			
Washing water volume (mL)	5	30	17.5	1.41	33.59
Sample dilution	4	8	6	3.43	8.57

In the second stage, this two-level factorial design was expanded to a star design. A central composite design (CCD, with $\alpha = 1.287$) was obtained, since the centres of the two separate designs were coincidental. Table 1 lists the values assigned to each factor.

3 Results and discussion

3.1 Cartridge selection

Before the factorial design, a preliminary study was carried out to decide which cartridge could be the most suitable.

Our starting point was hence an SPE method for TBV [10], in which silica-based C18 and dichloromethane were used as cartridge packing and eluent, respectively. Even if not validated, that method appeared to be adequate for such a complex matrix.

On the other hand, new SPE packing materials have emerged in recent years, in which silica has been substituted by polymeric resins, offering versatile and promising selectivity.

In particular, LiChroluth-EN resins have been already successfully used in wine analysis for the extraction of a wide range of volatile compounds [19 – 21].

Based on these considerations, three different commercial SPE cartridges (LiChrolut EN; C18-1 g, C18-2 g) were tested. Mean conditions: 5 g of sample (diluted 1:5), 15 mL of water for the washing step, and 20 mL of eluent, were used for the EN and C18-1 g cartridges whereas for the C18-2 g cartridge, in order to preserve the same sample/absorbent proportion, double values of the parameters were used. LiChrolut EN and C18-2 g cartridges showed approximately the same results in terms of total chromatographic areas (data not shown), but taking into account the higher sample amounts required and therefore the longer extraction time needed with the C18-2 g, EN cartridges were finally selected.

3.2 Extraction condition optimisation

Sample amount, eluent volume, washing water volume, and dilution of the sample were evaluated to achieve the best overall analytical conditions. Because the aim of the work was to develop an extraction method which covered the highest possible number of compounds, the total relative chromatographic area was used as experimental response for the optimisation.

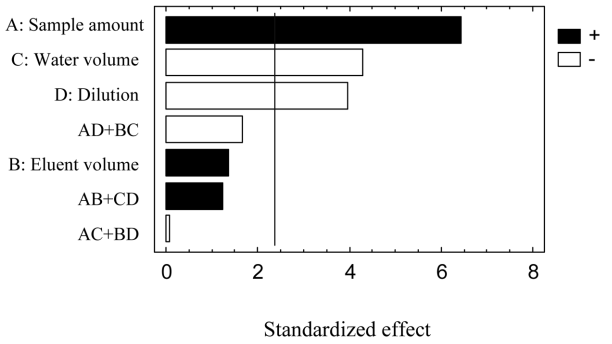

Figure 1. Pareto chart of the main effects in the factorial 2^{4-1} design for the volatile compounds studied. The line represents the significant limit.

3.2.1 Screening by a 2^{4-1} factorial design

The initial screening design detects which variables have the greatest influence on the experimental response.

The data obtained were evaluated by ANOVA at the 5% significance level. These results are shown in bar chart format with the effects sorted in rank order (Fig. 1). Sample amount, washing water volume, and dilution of the sample were significant parameters, whereas the eluent volume did not exert a significant effect (at $p < 0.05$). The weight of sample was the most influential variable and showed a positive sign while washing water volume and sample dilution showed a negative sign. The interactions between the factors did not affect the SPE efficiency statistically, as can be seen in Fig. 1.

3.2.2 Optimisation by a central composite design

Because the eluent volume does not have a statistically significant effect, it was not considered in the study. It was set at 10 mL, the minimum value used in the previous design, in order to minimise the use of environmentally toxic solvent and to assure short extraction times.

The three parameters used in the central composite design (CCD) were: sample amount; washing water volume; and sample volume. The axial values for these parameters are located on a sphere surrounding the two-level factorial design (Table 2).

As expected from the screening experiments, according to the CCD the most influential variable was the sample amount, with a positive sign (Table 2). The washing volume is also significant and with a negative sign, which was a predictable result. Before undertaking the experimental design, the possibility of not using water at

Table 2. Main effects and interactions in the central composite design.

Effect	*F* ratio	*p* value	Sign
A: Sample amount	68.12	0.0000[A]	+
B: Washing water volume	7.88	0.0106[A]	−
C: Sample dilution	3.00	0.0982	−
AA	0.35	0.5604	+
AB	3.96	0.0598	+
AC	0.26	0.6180	+
BB	9.61	0.0054[A]	+
BC	5.17	0.0335[A]	+
CC	0.12	0.7340	−

[a] Values are significant at $p < 0.05$.

all had been checked in several experiments, but some significant problems were encountered. The enormous amount of acetic acid remaining in the extract changed the separation behaviour of the cartridges and hampered the correct integration of the peaks emerging between 19.0 and 21.5 min (including the IS). To overcome these problems, a minimum water volume was found to be mandatory and this parameter was included in the experimental design. Although the dilution of sample was not statistically significant, there existed a two factor interaction between washing volume and dilution taking into account the *p*-values and *F*-ratios of the ANOVA analysis (Table 2). Figure 2 shows the response surface graph obtained by plotting washing volume *vs.* sample dilution. It can be seen that when small amounts of washing water volume are used, lower dilutions provide higher extraction efficiencies.

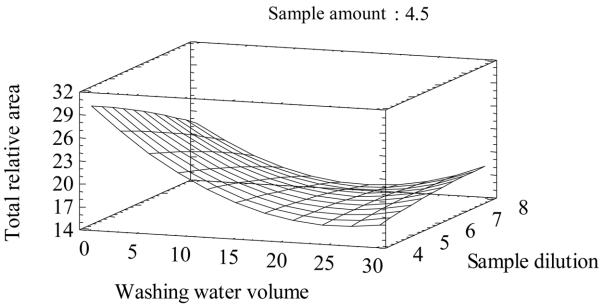

Figure 2. Estimated response surface obtained using the central composite design by plotting washing water volume versus sample dilution.

After considering all the main factors and their interactions, the best conditions for extracting volatile compounds of TBV are: 7 g of sample, 5 mL of washing water, a dilution of 1:4, and 10 mL of eluent. These conditions, therefore, represent an optimal compromise between extraction efficiencies, SPE elution time, reduced volumes of organic solvent, and reasonable cost in terms of sample amount needed for analysis (considering that the commercial price of TBV ranges between 80 and 120 €/100 mL).

3.3 Performance characteristics

A total of 21 compounds, covering different chemical families were studied. To choose representative compounds for each chemical class (Table 3), published data on volatile composition of TBV were taken into consideration [10 – 13].

Table 3. Characteristics of the calibration curves.

Compound	Quantifying ions (*m/z*)	Studied range (μg/L)	r^2	Linearity (LOL,%)	Slope ± S.D.	Intercept ± S.D.
Isoamyl alcohol	55, 70	72.45 – 3622,86	0.9995	98.87	0.0075 ± 0.0001	0.0036 ± 0.0009
3-Hydroxy-2-butanone[a]	43, 45	0.36 – 18.06	0.9989	98.66	0.0137 ± 0.0002	0.0470 ± 0.0118
Ethyl lactate	45	37.02 – 1851.43	0.9988	98.60	0.0162 ± 0.0002	0.0047 ± 0.0015
3-Ethoxy-1-propanol	59, 71	69.48 – 3474.27	0.9991	98.74	0.0052 ± 0.0001	0.0026 ± 0.0008
Furfural[a]	95, 96	3.57 – 357.51	0.9940	96.53	0.0075 ± 0.0003	0.4469 ± 0.2757
Benzaldehyde	105, 106	3.53 – 176.79	0.9996	99.15	0.0578 ± 0.0005	0.0011 ± 0.0003
γ-Butyrolactone[a]	42, 86	0.71 – 35.74	0.9998	99.29	$0.0048 \pm 3 \times 10^{-5}$	0.0134 ± 0.0033
Isovaleric acid[a]	60	1.42 – 71.36	0.9958	97.36	0.0055 ± 0.0001	0.1445 ± 0.0374
Diethyl succinate	101, 129	0.60 – 7438.57	0.9964	97.89	0.0247 ± 0.0005	0.0136 ± 0.0125
Ethyl 2-phenylacetate	164	3.42 – 341.64	0.9976	97.17	0.0033 ± 0.0001	0.0002 ± 0.0001
Guaiacol	109, 124	8.43 – 842.86	0.9999	99.40	0.0334 ± 0.0002	− 0.0002 ± 0.0003
Benzylalcohol	79, 108	44.23 – 4422.86	0.9972	98.01	0.0196 ± 0.0004	0.0082 ± 0.0050
2-Phenylethanol[a]	91, 122	1.44 – 72.15	0.9976	98.01	0.0128 ± 0.0002	0.3661 ± 0.0658
Whiskey lactone	99	12.29 – 1228.57	0.9971	97.79	0.0117 ± 0.0003	0.0011 ± 0.0009
Maltol	126	70.46 – 7045.71	0.9985	98.54	0.0123 ± 0.0002	− 0.0009 ± 0.0036
Eugenol	164	6.6 – 660	0.9999	99.41	0.0163 ± 0.0001	− 0.0001 ± 0.0001
4-Ethylphenol	107, 122	14.63 – 1462.86	0.9994	99.07	0.0437 ± 0.0004	− 0.0002 ± 0.0017
Syringol	139, 154	3.62 – 7257.14	0.9998	99.41	0.0216 ± 0.0001	− 0.0020 ± 0.0024
5-Hydroxymethyl-2-furaldehyde[a]	97, 126	15.30 – 1529.71	0.9956	96.15	$0.0012 \pm 4 \times 10^{-5}$	0.2879 ± 0.2436
Phenylacetic acid[a]	91, 136	0.07 – 7.18	0.9973	97.88	0.0166 ± 0.0003	− 0.0146 ± 0.0070
Hexadecanoic acid	73, 129	69.48 – 3474.29	0.9994	98.62	$0.0013 \pm 2 \times 10^{-5}$	0.0015 ± 0.0002

[a] mg/L.

Table 4. Performance characteristics of the method.

Compound	Detection limit (LOD, μg/L)	Quantitation limit (LOQ, μg/L)	Recovery (%)	Repeatability (RSD,%)	Reproducibility (RSD,%)
Isoamyl alcohol	20.18	67.25	–	8.32	4.19
3-Hydroxy-2-butanone	35.65	118.83	<50	21.38	18.04
Ethyl lactate	12.71	42.36	76.37	6.46	2.27
3-Ethoxy-1-propanol	19.53	65.11	<50	8.48	1.76
Furfural	53.03	176.77	113.73	1.55	2.96
Benzaldehyde	1.05	3.52	104.97	8.65	5.55
γ-Butyrolactone	168.04	560.13	<50	12.41	13.35
Isovaleric acid	496.42	1654.72	114.91	2.67	0.50
Diethyl succinate	4.13	13.77	85.10	6.73	8.57
Ethyl 2-phenylacetate	1.71	5.71	76.50	7.10	7.71
Guaiacol	1.93	6.45	76.36	12.14	7.74
Benzylalcohol	14.87	49.56	107.45	7.17	10.50
2-Phenylethanol	125.93	419.78	118.68	7.97	7.54
Whiskey lactone	3.01	10.03	86.42	7.95	18.76
Maltol	15.56	51.86	89.53	19.71	18.73
Eugenol	1.21	4.02	82.17	11.20	25.63
4-Ethylphenol	1.82	6.08	92.01	7.60	18.92
Syringol	0.35	1.17	61.07	21.75	21.41
5-Hydroxymethyl-2-furaldehyde	791.06	2636.87	–	19.71	24.70
Phenylacetic acid	29.72	99.06	<50	21.80	23.67
Hexadecanoic acid	20.95	69.85	<50	13.49	21.79

–: Not studied.
<50: Value lower than 50% of recovery.

3.3.1 Calibration, linearity

Five levels of concentration were tested in triplicate; these concentrations covered the concentration ranges expected for these compounds in TBV.

One or two majority ions were used to calculate the (volatile compound/internal standard) ion peak area ratio for each studied volatile compound (Table 3). The correlation coefficients were good (r^2 >0.99) for all the studied compounds. To corroborate the excellent linearity values obtained, the "on-line linearity (LOL) = 100 − RDS(b)" was calculated for each compound, with values higher than 96%. RSD(b) is the relative standard deviation of the slope expressed as a percentage. All the characteristics of the calibration curves appear in Table 3.

3.3.2 Detection and quantitation limits

The Alamin Computer Program was used for calculation of the detection and quantitation limits from the calibration curves constructed for each volatile compound [22].

The limits of detection (three times the relative standard deviation of the analytical blank values calculated from the calibration curve) and quantitation (ten times the relative standard deviation of the analytical blank values calculated from the calibration curve) obtained (Table 4) are low enough to determine these compounds in traditional balsamic vinegar samples according to the values found for them in the bibliography [10].

3.3.3 Accuracy: recovery, repeatability and reproducibility

In order to calculate the accuracy of the method, a study of recovery was carried out. Known concentrations of the volatile compounds were spiked to a sample and the concentrations before and after the addition were both determined. All the experiments were performed in triplicate. On the evidence of these concentrations, the percentage of recovery for each studied compound was calculated (Table 4). The majority of the compounds presented reasonably high values of recovery (higher than 75%). Compounds such as hexadecanoic acid, phenylacetic acid, 3-hydroxy-2-butanone, γ-butyrolactone, and 3-ethoxy-1-propanol presented lower values of recovery (<50%). Considering the high solubility of these compounds in water, the washing step could have negatively affected their recovery values; however, it is an essential step because of the necessity of reducing the amount of acetic acid in the samples. The isoamyl alcohol concentration level found in the used matrix was out of range. These high values for isoamyl alcohol came from the wine vinegars employed in the elaboration of industrial balsamic vinegar [13] which constituted part of the matrix, while the calibration focused on the minor levels present in the TBV. On the other hand, 5-hydroxymethyl-2-furaldehyde presented saturated chromatographic peaks that could not be precisely measured. The error arising in the measurement produced unstable recovery values for this compound. For these reasons, the recovery

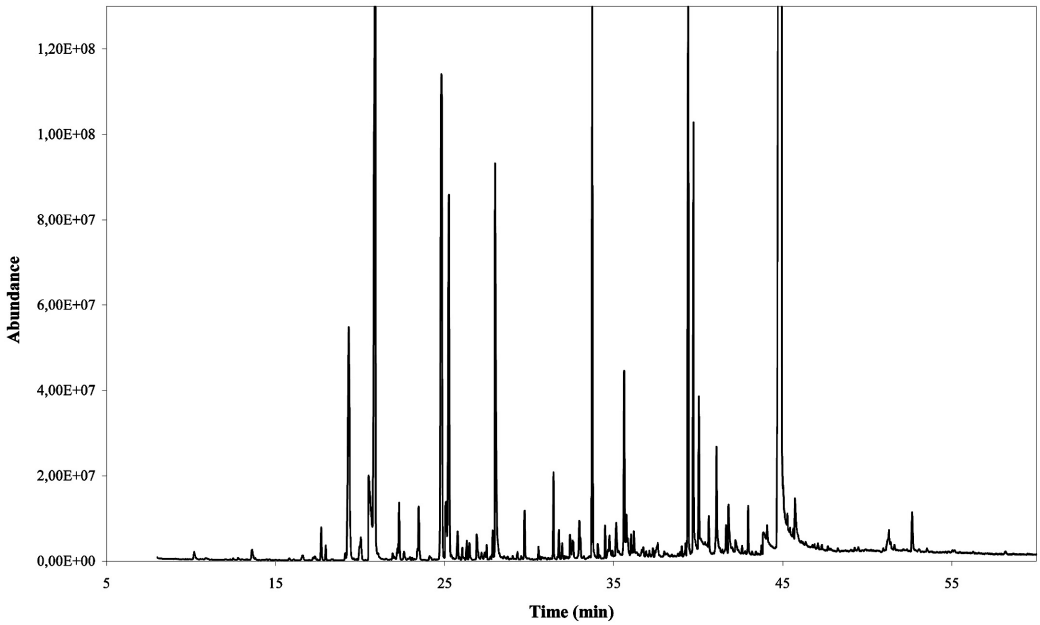

Figure 3. Total ion chromatogram obtained for TBV2 by means of SPE–GC–MS. Retention times (min): Isoamyl alcohol (10.79); 3-hydroxy-2-butanone (13.53); ethyl lactate (16.01); 3-ethoxy-1-propanol (17.26); furfural (20.8); benzaldehyde (22.8); γ-butyrolactone (26.29); isovaleric acid (27.96); diethyl succinate (27.96); ethyl 2-phenylacetate (30.73); guaiacol (32.52); benzylalcohol (32.94); 2-phenylethanol (33.71); whiskey lactone (34.47); maltol (34.76); eugenol (38.77); 4-ethylphenol (39.01); syringol (40.63); 5-hydroxymethyl-2-furaldehyde (44.75); phenylacetic acid (45.74); hexadecanoic acid (50.93).

of isoamyl alcohol and 5-hydroxymethyl-2-furaldehyde was not studied.

The intra-day (repeatability) and inter-day (reproducibility) precision of the method were calculated by means of five extractions of the sample performed at the same time and another five performed on different days. As can be seen in Table 4, the relative standard deviations (RSD) for the intra-day precision ranged between 1.55% and 21.80% (the higher RSD being verified for the compounds with lower recoveries) whereas the inter-day precision showed values of RSD between 0.50% and 25.63%. However, the majority of compounds gave values below 15% while, the high values of RSD, above all in the intra-day study, had also been reported by other authors [19].

In summary, taking into account recovery, repeatability, and reproducibility, the developed method reached an acceptable level of accuracy for the study of volatile compounds in TBV.

3.4 Determination of volatile compounds in TBV

Two different TBVs with different aging periods were analysed in triplicate using the developed method. TBV1 corresponds to a traditional balsamic vinegar with a minimum of 12 years of aging, while TBV2 is an "extravecchio" traditional balsamic vinegar, aged for at least 25 years. The mean results are presented in Table 5. Figure 3 shows the total ion chromatogram of the most aged sample (TBV2) with the retention times of all the studied compounds.

The compounds present in higher concentrations (in the order of mg/L) were 3-hydroxy-2-butanone, furfural, γ-butyrolactone, isovaleric acid, 2-phenylethanol, 5-hydroxymethyl-2-furaldehyde, and phenylacetic acid. The other volatile compounds identified in the samples presented concentrations in the order of μg/L. Although 3-hydroxy-2-butanone and isovaleric acid are majority compounds of TBV, higher concentrations thereof have been found in other vinegars such as Sherry wine vinegars [23]. Furanic compounds such as furfural and 5-hydroxymethyl-2-furaldehyde are mainly formed in the must cooking stage of the elaboration process as a consequence of sugar degradation [7], but their concentrations increase with the aging process, as our experimental results seem to confirm. Other compounds derived from the wood leakage process such as whiskey lactone, eugenol, and syringol, show an increase of concentration from TBV1 to TBV2.

On the other hand, the concentrations of ethyl esters such as diethyl succinate decrease due to the shift in esterification equilibrium caused by the small amount of ethanol and probably also due to volatilisation [10]. The amounts of alcohols such as isoamyl alcohol and 3-ethoxy-1-propanol also decrease in TBV with aging.

The results obtained from this study will lead to further research focused on the study of TBV aroma, increas-

Table 5. Studied volatile compounds mean concentrations (μg/L) found in two real samples of TBV. Mean of three replicate analyses.

Compound	TBV1	TBV2
Isoamyl alcohol	237.63	100.03
3-Hydroxy-2-butanone[a)]	1.70	0.556
Ethyl lactate	126.05	<L.D.
3-Ethoxy-1-propanol	390.69	218.41
Furfural[a)]	75.24	90.22
Benzaldehyde	10.42	12.15
γ-Butyrolactone[a)]	0.86	1.37
Isovaleric acid[a)]	35.50	24.93
Diethyl succinate	21.37	<L.D.
Ethyl 2-phenylacetate	24.02	<L.D.
Guaiacol	<L.D.	12.43
Benzylalcohol	171.38	353.33
2-Phenylethanol[a)]	9.89	14.12
Whiskey lactone	70.78	1025.81
Maltol	446.28	791.35
Eugenol	8.13	23.49
4-Ethylphenol	47.09	94.90
Syringol	19.96	25.71
5-Hydroxymethyl-2-furaldehyde[a)]	728.37	1456.47
Phenylacetic acid[a)]	2.71	2.79
Hexadecanoic acid	160.38	286.13

[a)] mg/L.
TBV1: Aged for at least 12 years.
TBV2: Aged for at least 25 years.
<L.D.: Below detection limit.

ing the number of studied compounds. This could provide the information necessary for a clear understanding of the changes that take place during the elaboration process of this complex product.

4 Concluding remarks

The conditions for the analysis of volatile compounds in TBV by SPE – GC – MS have been optimised by means of a statistical approach. Under the optimised conditions developed in this study, SPE can be considered an appropriate technique for the extraction of this type of compound from matrices with the level of complexity of TBV. The detection and quantitation limits, and the accuracy obtained are adequate for the quantification of the studied compounds.

In view of the obtained results, the developed method can be used for further research related to the volatile composition of TBV, increasing the number of studied compounds.

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The authors declared no conflict of interest.

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