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DETECTION OF ADDITIONAL COMPOUNDS WITH FLAVOURING POTENTIAL OF WINES

STURZA R., NEJINSKII A., LOZACOVICI D., MARCOCI L.

Moldavian National Center of Alcoholic Beverages Testing,

Summary: The study has been focused on the comparative analysis of various analytical techniques for the injection of samples applied to detect the additives with flavouring potential that are used to obtain illicitly the "Muscat" and "Isabela" wines, by implementing the GC/MS method with injection of liquid samples directly into the capillary column, using the "Head-space" method and the solid phase microextraction (SPME).

Key Words: GC/MS, Head-space, SPME, Wine and Flavours

INTRODUCTION

The wine aroma is a basic element of the quality of wine obtained from grapes. Aroma compounds are most closely associated with the volatile fraction of food [1]. The study of the volatile fraction in enological products has become necessary and is more than sufficiently justified, considering these compounds make a major contribution to the consumer's overall perception of the quality of particular food and drink products. In fact, these complex volatile compounds largely determine the acceptance or rejection of many products by the consumer. In addition, product characteristics known as "off-flavours", caused by the presence of volatiles that give rise to disagreeable odour and flavours, often imply microbial contamination; therefore the study of volatiles becomes part of the larger subject of food safety [2].

In other words, the wine flavour results from a harmonious compound of several chemicals having different origin and structure. The main focus is on the terpenoids substances that are capable to release smelling volatile substances (terpenes) through hydrolysis. The methoxypyrazines, some compounds with thiol functions, phenolic acids also belong to this category. Through decarboxylation they form the aldehydes, alcohols, and later esters, which can have the flavour of flowers or fruits [3].

The determination of the volatile fraction is normally performed by gas chromatography (GC), a technique which in recent years has made great advances. Given that SPME is very appropriate for application in the field of volatile compounds, this technique is being widely used for the characterization of wines [4]. Bonino and others [5] utilized HS-SPME for the extraction of aroma compounds characterizing a Piedmont wine (Ruché) derived from a non-aromatic wine. The most aromatic monoterpenes, are in the form of monoterpenic alcohols, namely linalool, α-terpineol, nerol, geraniol, citronellol, o-trienol, which express the rose flavour [6], [7]. The citronellol and linalool are the most aromatic, playing an important role in the formation of Muscat varieties flavour, where their concentration is higher the perception threshold [8].

In order to improve the gustatory and olfactory properties of grape wines or in order to give them certain characteristics, the wine aromatisation often is carried out illegally by adding different flavoured substances both of plant and synthetic origin. The usage of any type of flavoured elements, of synthetic origin included, for the natural wine production is prohibited by the EU and RM legislation [9].

The objective of this research was to compare the efficiency of various analytical techniques for the injection of samples used to detect the additives with flavouring potential that are utilised for the illicit obtaining of "Muscat" and "Isabel" wines on the basis of raw material wine with neutral aromatic potential.

MATERIALS AND METHODS

For the analysis, the raw material wine of „Mixture of European white varieties" and „Mixture of European red varieties" type were used, to which have been added naturally identical aromatizers of "Muscat" (white wine) and "Isabel" (red wine) type from commerce, in the recommended amount (1:10000). Subsequently, the comparative analysis of the composition of raw material wine and of the "variety" wine has been carried out using the GC/MS method by injecting liquid samples directly into the capillary column, the „*head-space*" (HS) method and the solid phase microextraction (SPME). All tests have been carried out using the Shimadzu GC system coupled with a single quadrupole mass-

spectrometer GCMS-QP2010 Plus equipped with the three-dimensional automated system for the injection of samples AOC-5000 (GCMS-QP2010 Plus xAOC-5000).

In the case of injection of samples by the HS and SPME methods, the 20 ml vials have been used, in which 10 ml of sample and 4 g of NaCl were administered. For the solid phase microextraction (SPME), the 100μm Carboxen-PDMS fibre was used, which provides the extraction of volatile and semi-volatile compounds on a concentration range from tenths of ppb up to tens of ppm [10].

For the identification, the general library of NIST-5 mass spectra and the FFNSC 1.3, a library which was specially developed for flavours and fragrances (available from Shimadzu Europa GmbH) were used. The accuracy of displacement has been verified according to the library of Covatz retention indices (MLRI). The analysis of the experimental data was carried out with the GC/MS Solution software (Shimadzu), which contains the SCAN/SIM options (Fast Automated Scan/SIM Type (FASST); creation of automatic SIM (Scan/SIM) table (COAST).

RESULTS AND DISCUSSIONS

The comparative analysis of the composition of raw material wine of "Mixture of white European varieties" and "variety" types, by adding naturally identical aromatizers of "Muscat" type, carried out using the GC/MS method with the injection of liquid samples directly into the capillary column, indicates the presence of unessential differences. The reason of this phenomenon consists in the matrix effect of the majority components of wine - ethanol, organic acids, esters, and, in particular, glycerine, which has an extremely large trace. This fact demonstrates the minor sensibility of the GC/MS method with the injection of liquid samples directly into the capillary column for the analysis of wine components with flavouring potential [11].

The "*headspace*" method refers to the determination of volatile organic substances in the gaseous phase, which are in equilibrium with those from liquid or solid phase [12]. This technique of introducing the sample is used when the interest components are situated in non-volatile and dirty matrices or that contain large amounts of water.

The differential analysis of the composition of raw material wine and of "variety" wine, made by the administration of "Muscat" naturally identical aromatizer using the GC/MS method with the injection of samples by HS method, demonstrates the presence of some significant differences (*figure 1*).

In the "Muscat" wine, the presence of a range of monoterpenes ($C_{10}H_{16}$) is identified: *beta*-myrcen (2,6,7-octatriene), *p*-cymen, D-limonen, *beta* (Z, E)-ocimene and terpineol. All these monoterpenes were identified in grapes of flavoured varieties [13], although these are formed in insignificant amounts and are not of interest in the olfactory field. At the same time, they are part of the composition of essential oils extracted from exotic and flavoured plants: limonen - from lemons and oranges, terpinol - from coriander, myrcenelum - from Myrcia acris plant, ocimenum – from basil leaves, p-cymen - from camphor tree wood. The presence of an insignificant trace, from the point of view of intensity, but identified with certainty (camphor) demonstrates once again that the concerned monoterpenes were not formed in grapes, but come from essential oils extracted from plants (but in any case are not of synthetic origin).

The terpene monohidroxilic alcohols (terpenols) are the most important compounds with flavouring potential, because these represent volatile free flavours from aromatic grapes. From the quantitative point of view, they represent about 40-50% of dosed volatile aromatic substances, having a very low threshold of volatile perception, of 0,1-0,5 mg/L of wine [14]. The main terpenols are linalool, geraniol, nerol, citronelol, ho-trienol and α -terpineol.

In the case of "Muscat" wine, obtained by using the naturally identical aromatizer, the presence of S-linalool and of ethyl ester of linalool was identified. The S-linalool trace abundance is maximum compared to other compounds with flavouring potential. It is known that namely this tertiary acyclic terpenic alcohol possesses muscatel flavour.

The linalool presence has been identified in the essential oils of many plants (linaloil - a tree in Central America, orange, bergamot, rose, etc.). In the grapes of "Muscat" varieties, the linalool represents 53.4% of the total of terpenic alcohols, nerol and geraniol, that always accompany the linalool, represent only 17,6-31,8% [14]. In the examined case, the presence of nerol and geraniol acetate were detected, the abundance of traces being insignificant. This acyclic terpenic alcohol is present in essential oils of rose, lavender, citronel. By oxidation this is converted into aldehyde

(geranial), with an odorous impact much weaker than geraniol.

As the purpose of this research was to compare the sensibility of the analytical methods for samples injection in detecting the compounds with wine flavouring potential, the analysis of raw material wine and of "variety" wine with samples injection by the solid phase microextraction (SPME) was carried out. The differential scanning of chromatographic traces for the basic wine and for the "variety" flavoured wine demonstrated the presence of the following compounds (figure 2).

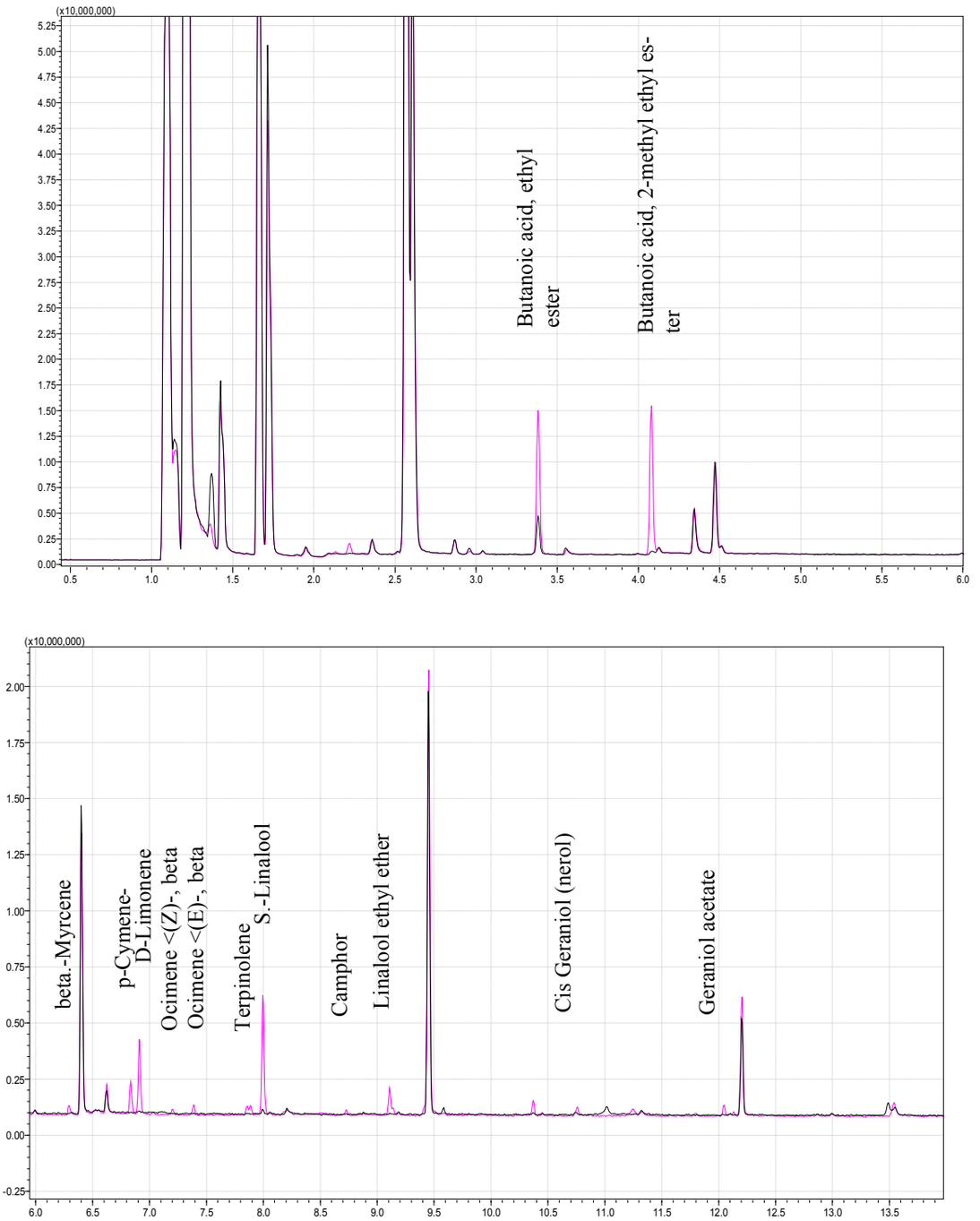

Figure 1. The identification of differences between the composition of raw material wine (white) and "Muscat" wine using the GC/MS method with sample injection by HS method

SPME is a solvent less extraction technique that can be used to extract analytes from both liquid and solid matrices. The quantitative use of this technique has been proven with the automatic robot. The

use of SPME for the analysis of flavours and off-flavours in food and beverages is an important preparation tool.

In addition to the compounds detected by the "Headspace" sample injection method, the presence of following monohidroxylic terpenic alcohols was reported: terpineol, alpha-terpineol and 1-terpinen-4-ol. The presence of nerol acetate was also additionally detected. The presence of these compounds with flavouring potential, although in insignificant quantities, demonstrates the natural origin of the used aromatizer (essential oils).

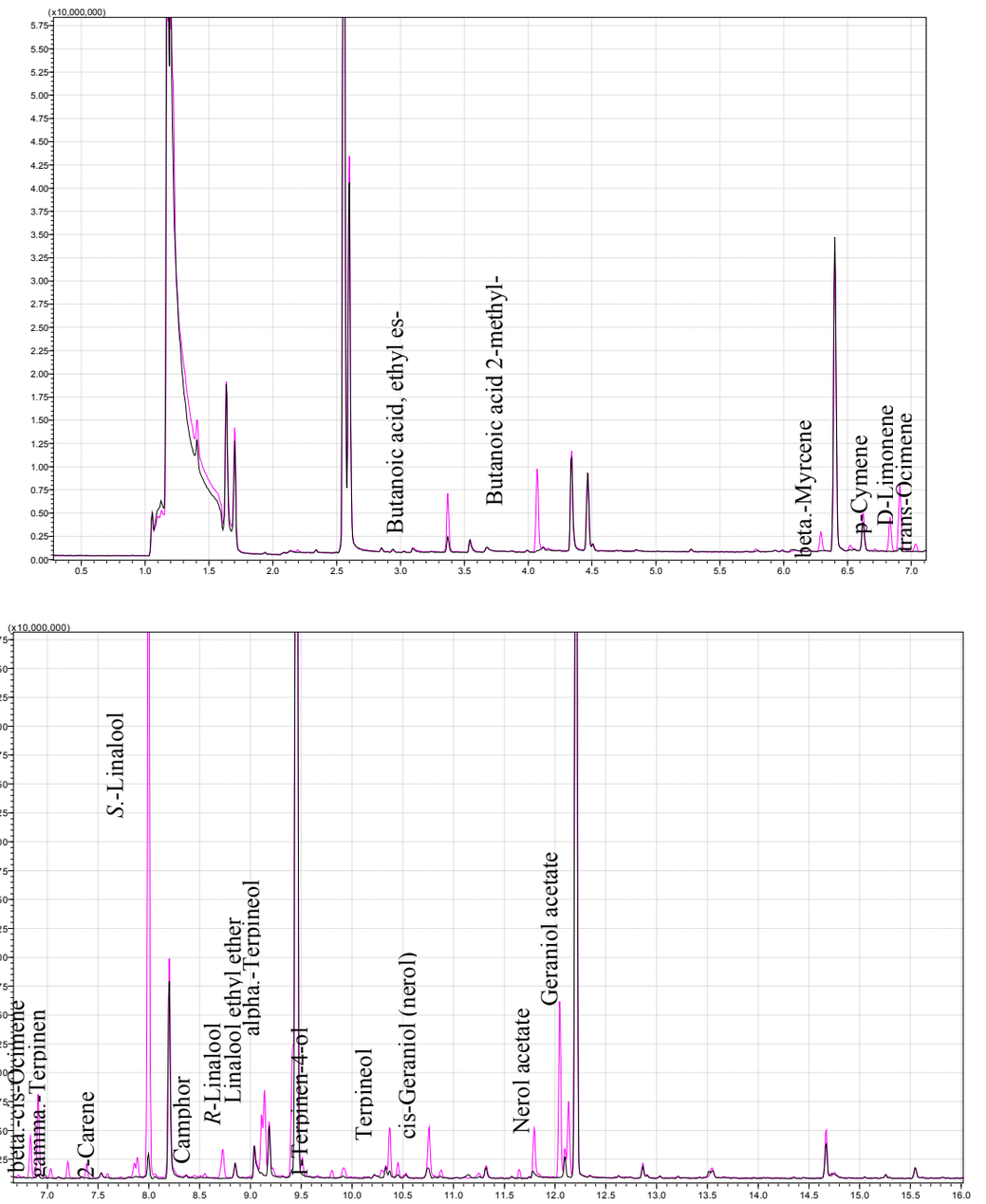

Figure 2. The identification of differences between the composition of raw material wine (white) and “Muscat” wine using the GC/MS method with samples injection by the solid phase microextraction (SPME)

The intensity of chromatographic traces in the case of SPME method is considerably higher. Thus, the beta-linalool trace in the case of SPME is saturated. The alpha-terpineol trace, masked in the case of "Headspace" method by the ethyl decanoat trace, a less volatile ester, but present in significant

quantities in the raw material wine, is detected in the case of samples injection using the SPME method. The chromatograms comparison for the "variety" wine obtained by these two methods of samples injection indicates that the "Headspace" method, although it can be applied successfully to the analysis of compounds with wine flavouring potential, has certain drawbacks – the vapour phase composition varies essentially from the liquid phase composition. The volatility of components has a predominant role in this case.

The differential scanning of chromatograms of the raw material wine and of the Izabela "variety" wine, obtained by the administration of "naturally identical" aromatizer (according to commercial features) demonstrated the presence of the following traces. The presence of methylanthranilat is attested - a component of the primary flavours of American hybrid grapes. The abundance of this trace is prevalent among the registered flavouring components.

The ethyl izobutirat was found, an ester with a nuance of fruit and fusel flavour, which is characteristic for the wines of Muscatel type, with a very low threshold of olfactory perception - 0.02 mg/L. Its presence in the composition of flavouring substances is not characteristic for the wines of Vitis Labrusca varieties. As well, two significant traces of enantilic and butyrate ether <3-methylbutil, 2-methyl-> were recorded - two chemicals of synthetic origin with pineapple flavour (enantilic ether) and fruit caramel (butyrate < 3-metilbutil-, 2-methyl->). The hexilic ester of the butanoic acid, present in extremely low quantity (pineapple flavour) can be of synthetic origin, but is also detected in the composition of natural wines [15].

The presence of the p-Cresol, 2,6-di-tert-butyl- compound, an antioxidant agent of synthetic origin, restricted to food products, demonstrates eloquently the synthetic origin of the concerned aromatizer. This compound, which manifests allergic effect and is suspected to be carcinogenic, is not part of the flavouring compounds, but is included in the composition of the synthetic aromatizer for its antioxidant effect. 3 - phenyl-2-cyclohexene-1-ona also represents a synthetic compound that could be used as a solvent for p-Cresol, 2,6-di-tert-butyl.

CONCLUSIONS

The carried out research demonstrated that the usage of the GC/MS method with sample injection by "Headspace" method and with the solid phase microextraction (SPME) can be successfully applied to identify the aromatic profile of wines, to detect the wine counterfeiting (by using naturally identical and synthetic aromatizers). It is also relatively easy to detect the origin of the aromatizer. The sensitivity of the method decreases in the order to use the following analytical techniques for samples injection:

SPME> „Headspace" >> injection of liquid samples directly into the capillary column

The GC/MS method with the injection of liquid samples directly into the capillary column can not be applied to identify the aromatic profile of wines, as in this case the effect of the matrix leads to the decrease of sensibility in the detection of substances that are present in very small quantities. The GC/MS method with the samples injection by the solid phase microextraction provides a higher sensibility than the method of samples injection by application of the "*Headspace*" technique.

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