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HPLC-MS, GC-MS and GCxGC-MS Characterization of a Georgian Saperavi Wine Obtained by Qvevri Winemaking Method

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Summary: *Saperavi, a Georgian wine obtained in Qvevri, was characterized by HPLC-DAD-MS, GC-MS and GCxGC-MS/TOF and compared with Sangiovese. Saperavi showed a high content of polyphenols (512 mg/L), of which 33% of anthocyanins, and a wide range of volatile compounds, also terpenes associated with aroma of flowers, citronella and wood.*

Keywords: *Polyphenols, volatile compounds, circular viticulture, 2D fingerprint*

Introduction

The quality of a wine and consequently the valorisation of the production area depends on numerous components, whose presence/absence and quantity plays a considerable role. The perception of the aroma of a wine depends on the simultaneous perception of a large number of volatile compounds and the coupling of sensory analysis to GC-MS (Gas Chromatography-Mass Spectrometry) analysis can provide useful indications for an adequate evaluation of the aroma [1]. Phenolic compounds are always present and they contribute to the sensory and chemical quality of the final product [2] in addition to their potential beneficial effects on human health [3]. The HPLC-DAD-MS (High-performance liquid chromatography with diode-array detection and mass spectrometry) was the elective technique for the identification and quantification of phenolic compounds in food samples. In this work the characterization of phenolic and aromatic compounds in a Georgian wine, cultivar Saperavi, vinified and refined in Qvevri, is reported. The vinification technique in Qvevri, amphorae constantly buried in the ground where the temperature remains constant throughout the year, has been recognized UNESCO world heritage. Saperavi wine was compared with Sangiovese wines stored in amphorae. HS-SPME-GC×GC-MS fingerprint analysis was also applied and provided a sensitive method for the direct comparison and chemical visualization of wine volatile components. In addition, antioxidant and antiradical activity of Saperavi wine was evaluated using Folin-Ciocalteau and DPPH·.

Experimental

The VOCs (volatile Organic compounds) profile was determined by SPME (Solid-Phase MicroExtraction)-GC-MS. 1 ml of wine was placed in a 25 ml vial and then 4 ml of water and 2 g of NaCl were added. An Internal Standard (IS) in suitable amount was added to each sample. SPME Conditions: absorption of VOCs at 80°C (for 30-min) on a trivalent fiber Carboxen PDMS DVB 1 cm,

followed by desorption at 280°C and, then, analysis by GC/MS. An Agilent 7890 a GC equipped with a 5975C MSD was used. The analyte separation was achieved with a column Agilent DB InnoWAX 50m, 0.20 µm id, 0.40 µm df. Chromatographic condition: initial temperature 40°C for 0,5 min, then 6°C min-1 up to 260°C. This temperature was maintained for1 min.
A tentative compounds identification was performed by comparing Mass spectra of each peak with those reported in mass spectral databases. The peak areas were normalized over the area of the opportune IS.
For GCxGC-MS analysis VOCs were absorbed exposing a 2-cm trivalent SPME fiber as described in GC-MS analysis. An Agilent 7890a GC equipped with a 5975C MSD was used and comprehensive GC×GC analyses were carried out on an Agilent GC 7890B, with an Agilent flow modulator system, coupled to an TOF-DS Markes detector. The analytes separation was achieved with a InnoWAX (20mx0.18mmx0.2) coupled with a HP-5MS (5mx0.35mm).
The wine samples for HPLC-DAD-ESI/MS analyses were prepared as follows: 50.0 mL of wine were concentrated under vacuum and rinsed with 30.0 mL of water at pH 1.7 by HCOOH. Each solution was defatted with n-hexane and extracted by liquid-liquid extraction three times, each with 20.0 mL of ethyl acetate (AcOEt). The obtained fractions were vacuum concentrated and rinsed in 2.0 mL (AcOEt fraction) or 5.0 mL (aqueous fraction) of H_2O/HCOOH pH 1.7. The analyses were carried out using an HP-1100 liquid chromatograph equipped with a DAD detector and interfaced with an Agilent TOF MS equipped with an ESI source (Agilent Corp, Santa Clara, CA, USA) like previously described [4]. Quantification of the single compounds was performed by HPLC-DAD using five-point regression curves built with the available standards. Curves with an r^2>0.9998 were considered. Calibration was performed at the wavelength of the maximum UV-Vis absorbance, by applying the correction of molecular weights. The determinations of the polyphenol contents were carried out in triplicate; the results are given as means and the standard error was <5%.

Results

Wine aroma perception deals with a large number of Volatile Compounds and the use of GC-MS can be useful for a correct evaluation of the aroma. The GC-MS chromatogram of Saperavi wine showed a wide number of VOCs. Alcohols, Esters, Acids, Aldehydes and Terpenes were determined. Terpenes are present in very small concentrations, yet they have a considerable impact on the organoleptic properties of grapes and wines. In this wine linalool, α-terpineol, β-Citronellol, α-Terpinene, γ-Terpinene, p-Cymene and Terpinolene were found. Only traces of a minor number of terpenes were found in a Sangiovese wine stored in amphorae, compared to Saperavi. Saperavi did not show phenols associated with unpleasant aroma descriptor, on the contrary of Sangiovese.
The use of HS-SPME-GC×GC-MS analysis permitted the creation of a comprehensive template matching fingerprinting. This method considers, as comparative feature, each individual 2D peak together with its time coordinates, detector response and MS fragmentation pattern, and includes them in a sample template that is created by the analyst and can be used to compare plots from different samples directly and comprehensively.
The HPLC-DAD-MS/TOF analysis led to the identification and quantification of anthocyanins, flavonols, hydroxycinnamic acids, stilbenes, procyanidins and

other phenolic acids. The total of secondary metabolites was [illegible] where the anthocyanins were 167.2 mg/L. Sangiovese wines aged in amphorae showed an average total content of 200 mg/L.

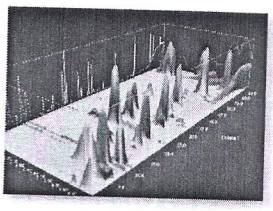

Fig. 10. 3D view of GCxGC/TOF of Saperavi wine

Conclusions

The characterization of this wine is part of a project about circular [illegible] Georgian territory. The primary aim of circular viticulture is to [illegible] network of companies and research institutes to create a closed and [illegible] chain in the wine sector in order to evaluate the quality of the vineyard, [illegible] environmental and management parameters aimed at producing [illegible] wines and also use grapes to produce functional foods such as juices, [illegible] grape seed oil and other nutraceuticals. It is also planned the [illegible] secondary raw materials and waste products for the production of [illegible] fractions with high biological activity, which can be used in the food, [illegible] phytotherapeutic and agronomic sectors as well as innovative materials. The sustainable exploitation allows the final use of exhausted materials to produce sustainable energy.

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References

1. A. Romani et al. *1st MS-WINE DAY*. Bagno a Ripoli (FI), 16-17 Aprile [illegible]
2. A. Baiano et al. *Food Chemistry*, 146 (2014), pp. 226–233
3. T.R. Watkins editors, *Wine nutritional and therapeutic benefits*, American Chemical Society 1997.
4. A Romani et al. *Journal of Pharmaceutical & Biomedical Analysis*, [illegible] pp. 415-20