TARGETED AND UNTARGETED MASS SPECTROMETRY-BASED METABOLOMICS APPROACH FOR THE CHARACTERIZATION OF GREEK ORGANIC WINES

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INTRODUCTION

The tight relationship between nutrition and health has emerged an intense research concerning bioactive compounds in foods. Wine contains high levels of antioxidants, compounds that provide beneficial health effects, and has been recognized to be responsible for health-promoting properties when consumed in moderation. Bioactive constituents are directly related to the quality of wines and their characteristic profile can be influenced by many factors including grape variety, geographical origin and type of farming[1]. Although organic viticulture is a wide-spread form of organic agriculture, there is a very limited number of studies correlating the wine bioactive constituents' profile to different agricultural and winemaking practices[2]. In this work, white organic wines from Malagouzia, Savatiano and Assyrtiko varieties, were analyzed using UPLC-QTOF/MS after a rapid sample preparation. Targeted and untargeted screening methods were used to identify and quantify several wine bioactive constituents, mainly phenolic compounds. These results were compared with the ones obtained from the analysis of conventional white wines and the effect of the farming type in the metabolic profile was evaluated.

EXPERIMENTAL

Centrifugation

Filtration RC 0.22 μm

Injection **LC-QToF/MS**

Wine samples

✓ All wine samples were sourced from wineries in the region of **Attica**, Greece.

✓ All **organic** samples have a **certificate** of organic production

✓ **Pool samples** from the varieties Assyrtiko, Malagouzia and Savatiano were analyzed, deriving from vinification of the grapes in **industrial scale** (batch quantity **greater than 4000L**)

Instrumentation: UHPLC-QToF/MS
Column: Acclaim C18, 2.1x100 mm, 2.2 μm, (Dionex-Thermo Scientific), **ESI:** Negative (-)
Gradient elution program: (A) 5 mM ammonium acetate in H_2O/MeOH 90/10, (B) 5 mM ammonium acetate in MeOH
Acquisition mode: broad-band Collision Induced Dissociation bbCID, (MS & MS/MS) (Col. Energy MS: 4eV, MS/MS: 25eV)

Bruker Maxis Impact™

RESULTS & DISCUSSION

TARGET SCREENING

Target list included **25** phenolic compounds

Identification Criteria
deltaRT ≤ 0.1 min
Accuracy: Error ≤ 5 mDa
Isotopic fit: ≤ 100 mSigma
MS/MS fragments
Ion Intensity > 200
Area > 800

Bruker TASQ Client

	Organic Assyrtiko 1 pool sample	Conventional Assyrtiko 1 pool sample	Organic Malagouzia 5 pool samples (mean value)	Conventional Malagouzia 1 pool sample	Organic Savatiano 1 pool sample	Conventional Savatiano 3 pool samples (mean value)
	Concentration (mg/L)					
Caffeic acid	0.35	0.42	**0.47**	**0.11**	1.2	0.84
Catechin	**19**	**5.5**	**4.1**	**1.7**	5.3	5.3
Epicatechin	**8.2**	**1.4**	1.3	1.0	1.4	1.3
Ferulic acid	0.38	0.86	0.43	0.30	0.53	0.52
Hydroxytyrosol	1.7	1.5	1.9	0.80	1.9	2.9
Quercetin	**5.4**	**0.61**	0.28	-	-	0.40
Resveratrol	**0.83**	**0.10**	0.064	0.057	0.12	0.12
Taxifolin	0.32	0.11	0.12	0.091	0.16	0.14
Tyrosol	**29**	**7.9**	9.2	7.8	**17**	**9.3**
p-coumaric acid	1.0	0.68	1.9	0.78	0.84	1.1
salicylic acid	0.35	0.08	0.26	0.14	0.57	0.52

NON-TARGET SCREENING

✓ **Suspect list** included **86** natural compounds existing in wine (**FooDB** database)

✓ **Predicted Retention Times** were obtained using an in-house **QSRR** prediction model[3]

✓ *Identification Criteria*
deltaRT ≤ 2 min, *Accuracy*: Error ≤ 5 mDa, **Isotopic fit**: ≤ 100 mSigma, **MS/MS fragments**

Isoquercetin

■ Assyrtiko conventional wine
■ Assyrtiko organic wine

MS Spectra

MS/MS Spectra

$C_{21}H_{20}O_{12}$
Mass accuracy: **0.08 mDa**
Isotopic fit: **5.4 mSigma**

Compound	Isoquercetin
Predicted t_R	5.1 min
Experimental t_R	5.6 min
Δt_R	-0.5 min

$[C_{15}H_9O_7]^-$
Mass accuracy: **0.3 mDa**
Isotopic fit: **50.9 mSigma**

Fig. 1: XIC, MS and MS/MS spectra of Isoquercetin in organic Assyrtiko wine from Attica.

CONCLUSIONS

✓ **Target** and **non-target** screening were used to characterize Greek organic wines and to identify markers indicating the type of farming

✓ Overall **35** compounds were detected and identified with target and suspect screening

✓ The concentrations of **catehin**, **epicatehin, tyrosol, quercetin** and **resveratrol** proved to be substantially higher in organic wines of Assyrtiko, Malagouzia and Savatiano varieties.

✓ **Isoquercetin** was detected only in organic wines & can be used as a potential biomarker for Assyrtiko variety.
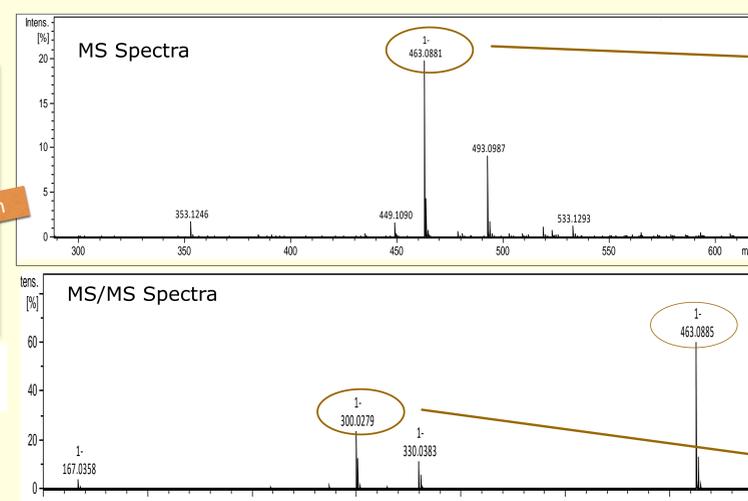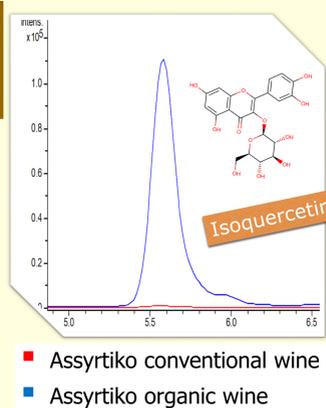
AKNOWLEDGMENTS

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