Asian Journal of Chemistry; Vol. 23, No. 12 (2011), 5197-5201

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Phenolic Contents of Romanian Wines with Different Geographical Origins

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(*Received*: 14 August 2010; *Accepted*: 4 August 2011) AJC-10252

Phenolic compounds in wine are important because they contribute to the colour, taste and body of the wine. The skin and seeds of the grape berry are rich in phenolic compounds. Some have strong antioxidant and anticancer activities and they are routinely consumed in the human diet in significant quantities. Wines from four different Romanian grape cultivars were analyzed in order to determine their phenolic contents. For the analysis, reversed phase-high performance liquid chromatography (RP-HPLC) coupled with diode array detection was used. The most abundant phenolic substance detected was (+)-catechin and (-)-epicatechin as flavonoid and gallic acid as a phenolic acid. As a result, it was determined that types and concentrations of phenolics changed according to the wines from different cultivars.

Key Words: Phenolic compounds, Wines, RP-HPLC, Direct injection.

INTRODUCTION

The phenolic compounds of red wines are substances which play an important role in several sensory properties such as colour, flavour, astringency and hardness[1]. They manifest a wide range of beneficial health effects including antiinflammatory, antiviral, anti-carcinogenic and antiatherogenic activities[2]. These protective health effects derived from the consumption of wines have been attributed to the antioxidant character of phenolics[3].

The type and concentration of the phenolic compounds in wine depends on grape variety, ripening, atmospheric conditions, viticulture and vinification techniques[4,5]. Generally, the major determinant factor for the variation in the polyphenolic content of different red wines throughout the world is probably the amount of sunlight to which the grapes are exposed during cultivation[6]. Phenolic compounds are also significant in white wines, where they occur at much lower concentrations.

Currently, there are only few research works on individual polyphenols content in Romanian wines[7], even if there are many studies on phenols *i.e.*, the total phenolic contents and antioxidant activities of several wine samples[8,9]. In order to identify the phenolic compounds in wine samples[10,11], the high performance liquid chromatography (HPLC) technique has been generally used. To determine the wine phenolics, different extraction methods including solid-phase extraction with C_{18} or strong anion exchange anionic cartridges, liquid-liquid extraction with different organic solvents have been applied. For this study, wine samples were directly injected to HPLC. Thus, this method allows the determination of phenolic compounds in wines without any prior purification. Despite the wealth of information on wine in general, it appears to be poor information on phenolic compounds of Romanian wines obtained from different grape cultivars.

The aim of this work is to characterize the content of poliphenolic compounds in wine that included phenolic acids (*e.g.*, gallic acid, caffeic acid, syringic acid, vannilic acid, *p*-coumaric acid, *trans*-cinnamic acid, ferrulic acid), flavan-3-ols [*e.g.*, (+)-catechin, (-)-epicatechin], resveratrol, rutin by reversed phase high-performance liquid chromatography (RP-HPLC) with PDA detector using the direct quantitative determination. The second goal of this work is to obtain a comparison of polyphenolic content amongst white and red wines and amongst wines from different geographical origins of Romania.

EXPERIMENTAL

A total of 16 wine samples (12 red and 4 white) from the four Vine and Wine Research-Development Station (SCDVV): Valea Calugareasca, Murfatlar, Bujoru, Iasi from three main wine regions of Romania *i.e.*, Muntenia, Dobrogea, Moldova were analyzed. A list of all wines analyzed in this study is

TABLE-1
LIST OF THE ANALYZED WINE SAMPLES

No.	Variety	Type	Wine making cultivars	Region
1	Feteasca Neagra	Red	SCDVV Valea Calugareasca	Muntenia
2	Feteasca Neagra	Red	SCDVV Murfatlar	Dobrogea
3	Feteasca Neagra	Red	SCDVV Bujoru	Moldova
4	Cabernet Sauvigon	Red	SCDVV Valea Calugareasca	Muntenia
5	Cabernet Sauvignon	Red	SCDVV Murfatlar	Dobrogea
6	Cabernet Sauvignon	Red	SCDVV Bujoru	Moldova
7	Cabernet Sauvignon	Red	SCDVV Iasi	Moldova
8	Pinot Noire	Red	SCDVV Valea Calugareasca	Muntenia
9	Pinot Noire	Red	SCDVV Murfatlar	Dobrogea
10	Merlot	Red	SCDVV Valea Calugareasca	Muntenia
11	Merlot	Red	SCDVV Bujoru	Moldova
12	Merlot	Red	SCDVV Murfatlar	Dobrogea
13	Feteasca Alba	White	SCDVV Bujoru	Moldova
14	Feteasca Alba	White	SCDVV Iasi	Moldova
15	Muscat Ottonel	White	SCDVV Valea Calugareasca	Muntenia
16	Muscat Ottonel	White	SCDVV Murfatlar	Dobrogea

presented in Table-1. The HPLC analysis was performed without any particular treatment except filtration through membrane filters 0.45 µm.

All standards (gallic acid, (+)-catechin, caffeic acid, chlorogenic acid, (-)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, resveratrol, rutin and *trans*-cinnamic acid) were purchased from Sigma-Aldrich (Steinheim, Germany). Stock solutions of all the standards were prepared in methanol. Working standards were made by diluting the stock solutions in the same solvent. Both stock and working standards were stored at 4 ºC until further use. Glacial acetic acid and methanol were both LC grade and were obtained from Merck. Double distilled water (Smart 2 Pure system, TKA) was used throughout. Retention time at 278 nm wavelength for each standard are shown in Table-2.

TABLE-2
RETENTION TIME (t_R) AT 278 NM FOR STANDARD COMPOUNDS

No.	Standards	t_R (min)
1	Gallic acid	9.002
2	(+)-Catechina	20.987
3	Caffeic and Chlorogenic acid	27.928
4	(-)-Epicatechina	29.873
5	Syringic acid and vanillin	31.233
6	*p*-Coumaric acid	37.613
7	Ferrulic acid	42.238
8	Resveratrol	52.302
9	Rutin	53.028
10	*trans*-Cinnamic acid	54.995

Detection method: Phenolic compounds were evaluated by reversed phase-high performance liquid chromatography (RP-HPLC) with direct injection. Chromatographic analysis was carried out with a Thermo Finnigan Surveyor Plus equipped with a Surveyor photodiode array detector (PDA), Surveyor autosampler, Surveyor LC Pump (quaternary gradient) and Chrome Quest Chromatography Workstation.

Separation were performed at 30 °C with Aquasil C_{18} (5 µm, 250 mm × 4.6 mm) column. The flow rate was 1 mL min^{-1} and injection volume 10 µL. Gradient elution of two solvents was used: Solvent A consisted of: acetic-water (2:98 v/v), solvent B: methanol and the gradient programme used is given Table-3.

TABLE-3
SOLVENT GRADIENT CONDITIONS WITH LINEAR GRADIENT

Time (min)	A %	B %
Initial	100	0
5	90	10
20	80	20
35	70	30
50	60	40
55	80	20
60	100	0
65	100	0

The wine samples, standard solutions and mobile phases were filtered by a 0.45 µm pour size membrane filter. The amount of phenolic compounds in the extracts was calculated as mg/L wine using external calibration curves, which were obtained for each phenolic standard. Each determination was carried out in triplicate and the mean and standard deviations were reported.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of the standard mixture, red wine "Cabernet Sauvignon" and white wine "Riesling Italian". Content of the individual polyphenols in wines from three wine-producing regions of Romania are reported in Tables 4-7. Due to incomplete separation between caffeic acid and chlorogenic acid, syringic acid and vanillin, we made the quantification in wine sample, only for other phenolics.

The results obtained confirm a variation in the phenolic content amongst wines tested, due to their different geographical origin, grape variety and type of wine, as expected. The major differences were observed in wine samples obtained from different cultivars (Fig. 2). The obtained ranges are in agreement with the values reported in available literature[12-14].

The most abundant phenolic substances detected were (-)-epicatechin and (+)-catechin as a flavonoid and gallic acid as a phenolic acid. The values ranged from 8.51 to 83.22 mg/L for (-)-epicatechin, from 6.95 to 61.10 mg/L for (+)-catechin

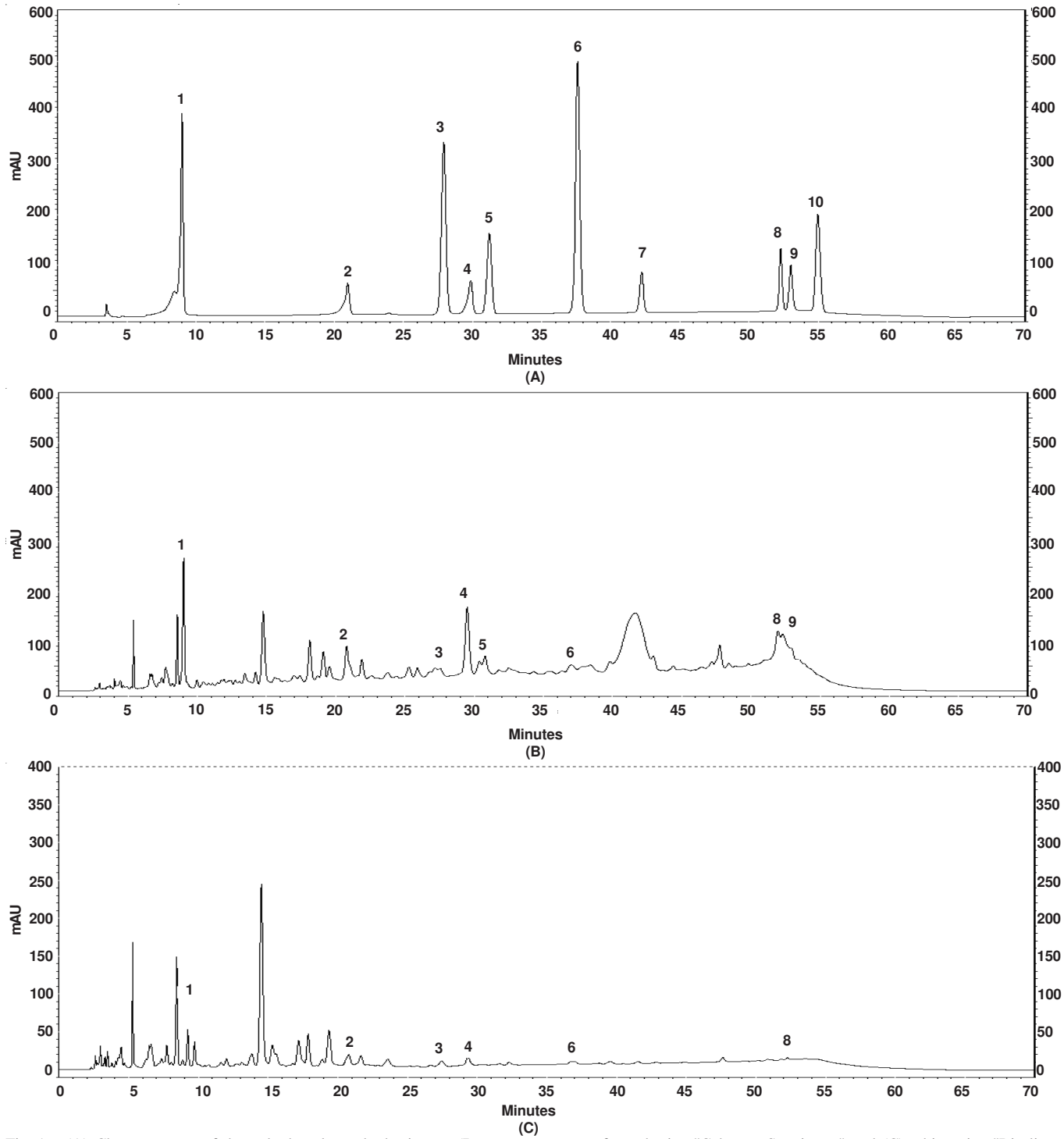

Fig. 1. (A) Chromatogram of the polyphenol standard mixture, (B) Chromatogram of a red wine "Cabernet Sauvignon" and (C) white wine "Riesling Italian" at 278 nm

TABLE-4
PHENOLIC COMPOUNDS IN WINES FROM SCDVV VALEA CALUGAREASCA (MUNTENIA REGION) (mg/L)

No.	Compound	Red wine				White wine
		Feteasca Neagra	Cabernet Sauvignon	Pinot Noire	Merlot	Muscat Ottonel
1	Gallic acid	22.16 ± 0.3	25.05 ± 0.2	21.85 ± 0.4	22.72 ± 0.3	5.90 ± 0.1
2	(+)-Catechina	23.18 ± 0.2	26.00 ± 0.3	61.10 ± 0.5	43.49 ± 0.4	4.90 ± 0.3
3	(-)-Epicatechina	25.60 ± 0.4	83.22 ± 0.4	42.60 ± 0.3	68.40 ± 0.3	10.54 ± 0.4
4	*p*-Coumaric acid	2.47 ± 0.1	2.20 ± 0.1	1.75 ± 0.1	2.21 ± 0.2	1.39 ± 0.1
5	Resveratrol	2.10 ± 0.1	2.03 ± 0.1	2.60 ± 0.2	2.65 ± 0.1	0.44 ± 0.1
6	Rutin	2.27 ± 0.2	1.24 ± 0.2	1.50 ± 0.1	4.66 ± 0.2	2.03 ± 0.1

TABLE-5
PHENOLIC COMPOUNDS IN WINES FROM SCDVV MURFATLAR (DOBROGEA REGION) (mg/L)

No.	Compound	Red wine				White wine
		Feteasca Neagra	Cabernet Sauvignon	Pinot Noire	Merlot	Muscat Ottonel
1	Gallic acid	36.65 ± 0.3	22.60 ± 0.2	34.35 ± 0.4	14.86 ± 0.1	17.54 ± 0.2
2	(+)-Catechina	7.11 ± 0.2	8.86	6.95 ± 0.1	15.69 ± 0.3	21.22 ± 0.3
3	(-)-Epicatechina	8.51 ± 0.2	47.61 ± 0.5	12.38 ± 0.1	42.17 ± 0.3	7.91 ± 0.2
4	*p*-Coumaric acid	12.11 ± 0.3	5.69 ± 0.1	7.62 ± 0.2	3.57 ± 0.2	4.22 ± 0.1
5	Resveratrol	1.50 ± 0.2	3.27 ± 0.1	1.03 ± 0.1	5.26 ± 0.2	0.95 ± 0.1
6	Rutin	2.34 ± 0.3	2.61 ± 0.2	1.13 ± 0.3	1.01 ± 0.1	1.27 ± 0.1

TABLE-6
PHENOLIC COMPOUNDS IN WINES FROM SCDVV BUJORU (MOLDOVA REGION) (mg/L)

No.	Compound	Red wine			White wine
		Feteasca Neagra	Cabernet Sauvignon	Merlot	Feteasca Alba
1	Gallic acid	76.69 ± 0.5	16.25 ± 0.3	34.79 ± 0.4	0.69 ± 0.1
2	(+)-Catechina	13.21 ± 0.2	11.66 ± 0.3	23.81 ± 0.1	4.52 ± 0.3
3	(-)-Epicatechina	15.55 ± 0.3	71.68 ± 0.6	80.95 ± 0.5	5.60 ± 0.2
4	*p*-Coumaric acid	4.06 ± 0.2	1.16 ± 0.2	2.93 ± 0.1	0.17 ± 0.1
5	Resveratrol	2.02 ± 0.1	1.64 ± 0.3	5.81 ± 0.2	0.37 ± 0.1
6	Rutin	2.34 ± 0.2	4.42 ± 0.1	6.68 ± 0.3	Not detected

TABLE-7
PHENOLIC COMPOUNDS IN WINES FROM
SCDVV IASI (MOLDOVA REGION) (mg/L)

No.	Compound	Red wine	White wine
		Cabernet Sauvignon	Feteasca Alba
1	Gallic acid	11.49 ± 0.2	2.96 ± 0.1
2	(+)-Catechina	26.00 ± 0.3	14.31 ± 0.2
3	(-)-Epicatechina	44.03 ± 0.2	9.52 ± 0.3
4	*p*-Coumaric acid	1.83 ± 0.1	Not detected
5	Resveratrol	1.51 ± 0.1	Not detected
6	Rutin	1.35 ± 0.1	Not detected

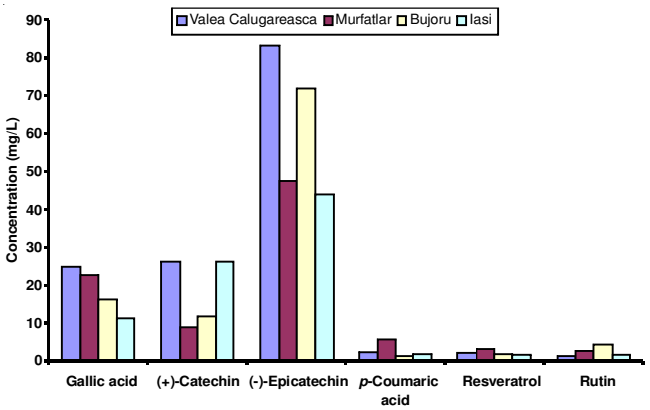

Fig. 2. Phenolic content of Cabernet Sauvignon wine from different region

and from 11.49 to 76.69 mg/L for gallic acid in the all red wine samples. For white wine the values ranged from 5.6 to 10.54 mg/L for (-)-epicatechin, from 4.52 to 21.22 mg/L for (+)-catechin and from 0.69 to 17.54 for gallic acid. (+)-Catechin was also found to be the most abundant phenolic compounds in Greek wines (11.80-40.00 mg/L)[15].

Variations in flavonol content of individual wines may be explained by several factors. It has long been known that the increased biosynthesis of polyphenols, especially flavonols, is greatly influenced by sunlight exposure and temperature, so it would be normally expected that the wines made from grapes, which are grown in warmer, sunnier areas, have a higher level of flavonols. The "Cabernet Sauvignon" wine from SCDVV Valea Calugareasca shows the highest (+)-catechin and (-)-epicatechin concentration and this probably because the area receive strong exposure to the sun and fresh winds during the day.

Low concentration of flavonols in the white wines was expected since these compounds are present mainly in grape skin, while in the production of white wines, skin-contact maceration is either avoided or allowed for a very short period[16]. Concentration of catechin in white wine "Muscat Ottonel" from SCDVV Murfatlar fell within the range of concentration in red wines.

The stilbene *trans*-resveratrol, a compound with multiple health benefits, was found in all wines samples, except in Feteasca Alba, Moldova region and amounts were comparable with the concentration range found in the literature[17].

Red wines were found to have higher concentrations of *trans*-resveratrol (1.03-5.81 mg/L) compared with those produced from white varieties (0.37-0.95 mg/L). It was also interesting that red wines "Merlot" produced from different cultivars (SCDVV Valea Calugareasca, SCDVV Murfatlar and SCDVV Bujoru), appeared to contain important amounts of *trans*-resveratrol.

Rutin was found in all red wine ranged from 1.01 to 6.68 mg/L, in white wine was not determinate, except "Muscat Ottonel" from SCDVV Murfatlar and SCDVV Valea Calugareasca. *p*-Coumaric acid was grater in red wine sample (1.16-12.11 mg/L) in comparison with white wine (0.17-4.22 mg/L). On the other hand, in this study, ferrulic acid and *trans*-cinnamic acid were not detected in any wine samples.

Conclusion

The results of the study showed that in the analyzed wines the most abundant polyphenols were gallic acid, catechin and epicatechin. The results obtained have confirmed a variation in the phenolic content amongst white and red wines and also amongst wines made from grapes of different geographical origin.

According to our best of knowledge, there are no detailed data regarding the composition of phenolic compounds in Romanian wine obtained several grape cultivars, so this preliminary study contributes new knowledge of the composition of the wines of different grapes. Further studies regarding to identification of other interest polyphenolic compounds in wine, are necessary to confirm the differences observed.

REFERENCES

1. J.L. Rubichaud and A.C. Noble, *J. Sci. Food Agric.*, **53**, 343 (1990).
2. L.H. Yao, Y.M. Jiang, J. Shi, F.A. Tomás-Barberán, N. Datta and R. Singanusong, *Plant Foods for Human Nutrition*, **59**, 113 (2004).
3. C. Rice-Evans and L. Packer, Flavonoids in Health And Disease, Marcel Dekker, New York, USA (1998).
4. D.M. Goldberg, A. Karumanchiri, E. Tsang and G.J. Soleas, *Am. J. Enol. Viticult.*, **49**, 23 (1998).
5. M.A. Rodríguez-Delgado, G. González-Hernández, J.E. Conde-González and J.P. Pérez-Trujillo, *Food Chem.*, **78**, 523 (2002).
6. V. Rastija, G. Srecnik and M. Medic-Šaric, *Food Chem.*, **115**, 54 (2009).
7. D. Preda, L. Leopold, F. Ranga, F. Fetea, N. Pop and C. Socaciu, *Buletin USAMV-CN*, **62**, 338 (2006).
8. I.D. Nistor, N.D. Miron, A. Gradinaru and A.M. Dospinescu, *Roman. Techn. Sci. Acad.*, **1**, 220 (2007).
9. F. Busuricu, T. Negranu-Parjol, D.P. Balaban, A. Popescu and A. Anghel, *Innov. Roman. Food Biotechnol.*, **2**, 10 (2008).
10. E. Revilla and J.M. Ryan, *J. Chromatogr. A*, **88**, 461 (2000).
11. M. Lopez, F. Martinez, C. Del Valle and M. Miro, *J. Chromatogr. A*, **922**, 359 (2001).
12. P.B. Andrade, G. Mendes, V. Falco, P. Valentão and R.M. Seabra, *Food Chem.*, **73**, 397 (2001).
13. E. Kilinç and H. Kalkan, *J. Wine Res.*, **14**, 17 (2003).
14. S. Kallithraka, E. Tsoutsouras, E. Tzourou and P. Lanaridis, *Food Chem.*, **99**, 784 (2006).
15. C. Proestos, A. Bakogiannis, C. Psarianos, A.A. Koutinas, M. Kanellaki and M. Komaitis, *Food Control*, **16**, 319 (2005).
16. D.P. Makris, S. Kallithraka and P. Kefalas, *J. Food Composit. Anal.*, **19**, 396 (2006).
17. M. Gerogiannaki-Christopoulou, P. Athanasopoulos, N. Kyriakidis, I.A. Gerogiannaki and M. Spanos, *Food Control*, **17**, 700 (2006).