Fatty Acid Profile of New Varieties of Grape Seed Oils Based on NMR Data and Their Authentication

MIHAELA TOCIU[1], MARIA CRISTINA TODASCA[1]*, MIHAELA MIHALACHE[1], VICTORIA ARTEM[2], ANAMARIA HANGANU[3]

[1] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, 1-7 Polizu Str., 011061, Bucharest, Romania

[2] Research Centre for Viticulture and Enology Murfatlar (RCVE), 2 Bucuresti Str., Murfatlar, Constanta

[3] Romanian Academy, Centre of Organic Chemistry Costin D. Nenitescu, 202B Splaiul Independentei, 060023, Bucharest, Romania

Grape seeds from five known red grape sorts and two new local sorts, obtained by hybridization of red and white varieties, have been valorized as vegetal oils, through good yield extraction. The fatty acid content the of obtained oils was determined by GC-FID, as methyl esters, and directly by ^{1}H-NMR spectroscopy. The obtained grape seed oils have a high content in linoleic acid (over 70%) and a relatively low quantity of saturated fatty acid (stearic and palmitic). Both measurement methods gave similar results confirming the accuracy of the spectroscopic method, which is less time and reagent consuming. Based on the fatty acid profile (FAP) of the oils, determined by either FT-IR or ^{1}H-NMR and using the Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) statistical methods, the discrimination between the classical known grape sorts and the new local varieties could be achieved. The results show that such method is appropriate for discovering possible adulteration of the grape oils, valuable products due to the essential unsaturated acid content.

Keywords: grape seed oil, fatty acid profile, PCA, HCA, NMR spectroscopy

Management and disposal of large amounts of wastes produced by the food-processing industry generate serious environmental problems [1]. The wastes resulting from the wine industry are a source of raw materials, which may be used to manufacture new valuable products. The wine industry wastes are of interest for us, as Romania is a known wine producer, being ranked in 2015 as twelfth at global and sixth at European level [2].

The grape seeds constitute a great part of the residue (~15%) resulted from wine production [3]. Converting such remaining materials into added-value bio-products represents a good a method to control wastes from wine industry. Such valorization is compulsory for a cost effective policy, as well as for environmental reasons [4]. A proper way of valorization seems to be the production of grape seed oils [5, 6]. The interest for these oils is due to their numerous applications, in various fields: pharmaceutical industry, cosmetics, cooking, etc. The oil extracted from grape seeds has exceptional therapeutic properties [7, 8] due to the high content of unsaturated fatty acids [9] together with significant quantities of polyunsaturated fatty acids.

Also, the grape seed oil contains vitamin E with a known antioxidant activity [8], having neuroprotective and antitumor effects and being able to lower the cholesterol levels. This oil is rich in phytosterols that may exert anti-arteriosclerotic activity [10]. The aqueous extracts prepared from grape seeds may have antibacterial and antioxidant activities [11].

In this context, it is worthwhile mentioning that the determination of the authenticity of these oils is very important due to fraud affecting the well-being of consumers [12]. Adulteration of food products involves the replacement of high cost ingredients with lower grade and cheaper substitutes [13].

This paper aims to characterize a number of grape seed oils, according to the fatty acid profile (FAP), by chromatographic and spectroscopic methods. These oils have been obtained by extraction from different traditional red grape varieties: Burgund (**BU**), Cabernet Sauvignon (**CS**), Merlot (**ME**), Pinot Noir (**PN**) and Feteasca Neagra (**FN**) as well as, new local varieties: Cristina (**CR**) and Mamaia (**MA**), created at the Research Centre for Viticulture and Enology Murfatlar (RCVEM). The study was conducted in order to prove the efficiency of the extraction method and to compare the accuracy of the oil FAP resulted by different analytic methods. The FAP determination can also help to establish the grape variety, based on the PCA method [14] confirming the oil authenticity.

Experimental part

Material and methods

The *samples* are represented by the grape seeds from 14 different samples of red authentic varieties - both traditional (international varieties: Burgund (**BU**), Cabernet Sauvignon (**CS**), Merlot (**ME**), Pinot Noir (**PN**) and national Romanian variety: Feteascã Neagrã (**FN**)) as well as new local varieties: Cristina (**CR**), Mamaia (**MA**), created at RCVE Murfatlar, were collected from the crops of years 2010 (samples marked with 1) and 2011 (samples marked with 2) of Murfatlar vineyard Dobrogea (a southeastern region of Romania). The seed samples were provided by Murfatlar Viticulture Research Station of Constanta, the RCVE Murfatlar. Before being processed seeds were cleaned of plant residues.

Deuterated chloroform ($CDCl_3$) (min. 99.8%) and petroleum ether 40-60 °C fraction (p.a.) were purchased from Merck (Darmstadt, Germany). The solvents and BF_3-MeOH 10-14% complex have been purchased from Sigma - Aldrich (Steinheim, Germany).

The oils from all the grape seeds have been extracted with light petroleum ether in a Soxhlet device, according to the known standard procedure (ISO 659:2009). The oils were transformed in methyl esters by treatment with methanol and BF_3 as catalyst, according to the standard method (ISO 12966-4:2015). The mixture of fatty acid

* *email: cristina.todasca@chimie.upb.ro; Phone: +40214023887*

methyl esters (FAMEs) was analyzed by GC the components being identified with the help of the Supelco certified reference material, containing 37 FAME Mix species. The analyses were performed with an Agilent Technologies 7890 A instrument, with FID detector, on a poly(biscyanopropyl)siloxane capillary column, having the following characteristics: 100 m length, 0.25 mm inner diameter, 0.2 μm film thickness (Supelco SP™ 2560). The working temperature program has been oven initial temperature 140 °C to final temperature 240°C. The analyses were performed in triplicate. The average values are presented in Table 1.

FT-IR spectra were recorded on a Bruker Equinox 55 Spectrometer, with a ZnSe [ATR (Attenuated Total Reflectance) crystal] spectral window 600 to 4000 cm^{-1}, and resolution of 2 cm^{-1}, at room temperature (~25°C).

The ^{1}H-NMR spectra of the extracted oils were recorded on a Bruker Avance III 400 spectrometer, operating at 9.4 Tesla, corresponding to the resonance frequency of 400.13 MHz for the ^{1}H nucleus, equipped with a direct detection four nuclei probe head and field gradients on z axis. Samples were analyzed in 5 mm NMR tubes (Wilmad 507), prepared by dissolving the grape seed oil in $CDCl_3$, in a ratio of: 2/8 (V/V) and has been used the TMS as internal standard. Typical parameters for ^{1}H-NMR spectra were: 45° pulse, 2.05 s acquisition time, 6.4 KHz spectral window, 16 scans, 26 K data points.

The experimental data have been used in statistical analyses, such as: PCA (Principal Component Analysis) and HCA (Hierarchical Clustering Analysis). This has been performed using the XLStat 2015 software (Addinsoft).

Results and discussions

The oil was obtained by a continuous extraction of the grape seeds, using a Soxhlet standard method (Material and methods). The amount of oil extracted from 100 g of grape seeds ranges from 7.96 ± 0.09 g for the **MA** variety 2010 to 13.95 ± 0.05 g for the **CR** variety 2010. There are variations of the resulted oil quantity depending on grape variety and crop year (fig. 1).

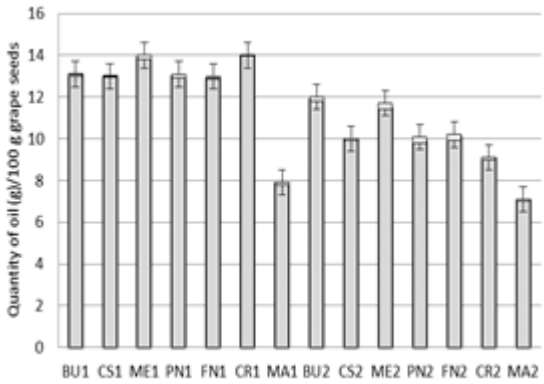

Fig. 1 Amount of oil extracted for each grape seed variety: **BU, CS, ME, PN, FN, CR, MA**

The FAP was established for each oil sample by GC-FID and NMR analyses

In the GC-FID procedure, the components identification was performed by comparing the retention time for each peak with those of a well-known standard mixture of 37 fatty acid methyl esters (Supelco 37 FAME Mix). In the standard mixture the exact concentration of each component is known. Signals were calibrated taking into account the concentration of each component of the standard mixture, correlated with the detector's response.

The grape seed oils were also analyzed by the NMR method described in a previous paper [15]. ^{1}H-NMR spectra were of similar shape, but differ in signal intensities and integral's values. The NMR analysis of the vegetable oils consists in a system of chemometric equations [16] allowing the determination of the FAP in terms of classes by tri-unsaturated fatty acids (linolenic acid), di-unsaturated fatty acids (linoleic acid), mono-unsaturated fatty acids (mainly oleic acid) and saturated fatty acids (palmitic and stearic acids) for each analyzed sample.

The results obtained by the NMR method have been confirmed by the GC-FID standard method. The FAP of the oils, obtained by the NMR and the standard GC-FID method are presented in table 1.

According to the experimental data (table 1), all the seven types of the red grape seed oils (**BU**, **CS**, **FN**, **ME**,

Table 1
FATTY ACID COMPOSITIONS OF THE GRAPE SEED OILS ESTABLISHED BY NMR AND GC-FID METHODS

Sample*	GC-FID	NMR	GC-FID	NMR	GC-FID	NMR	GC-FID	NMR
	saturated (%)		mono-unsaturated (%)		di-unsaturated (%)		tri-unsaturated (%)	
BU1	12.52	12.54	20.35	20.35	66,73	66.72	0.40	0.40
BU2	12.59	12.49	17.85	17.85	69.32	69.34	0.30	0.33
CS1	12.97	12.94	16.83	16.83	69.81	69.83	0.39	0.40
CS2	13.00	13.03	12.87	12.86	73.77	73.75	0.36	0.37
FN1	12.95	12.89	19.76	19.77	66.94	66.94	0.40	0.40
FN2	12.29	12.36	16.53	16.54	70.79	70.76	0.35	0.33
ME1	11.58	11.59	15.52	15.51	72.56	72.57	0.33	0.33
ME2	11.33	11.35	14.02	13.99	74.30	74.30	0.35	0.37
PN1	12.25	12.20	16.93	16.93	70.51	70.54	0.31	0.33
PN2	11.62	11.70	15.81	15.79	72.29	72.24	0.29	0.27
CR1	11.77	11.74	16.76	16.74	71.13	71.19	0.34	0.33
CR2	11.96	11.97	15.34	15.32	72.35	72.35	0.35	0.37
MA1	10.23	10.22	19.50	19.51	69.97	69.97	0.31	0.30
MA2	10.06	10.07	16.53	16.53	73.07	73.07	0.35	0.33

**1 - for 2010 crop and 2 - for 2011 crop*

Sample	Saturated* (% molare)	Mono-unsaturated* (% molare)	Di-unsaturated* (% molare)	Tri-unsaturated* (% molare)
CR	11.9±0.16	16.0±1.00	71.8±0.82	0.35±0.03
CH	10.6±0.11	18.1±1.48	71.1±1.62	0.27±0.05
MA	10.1±0.11	18.0±2.11	71.5±2.19	0.32±0.02
ME	11.5±0.17	14.7±1.07	73.4±1.22	0.35±0.03
MO	12.5±0.33	16.2±1.51	71.0±1.82	0.35±0.03

** Average values on 2010 and 2011 crops ± standard deviation*

Table 2
FATTY ACID COMPOSITION (^{1}H-NMR) OF **CR** AND **MA** SORTS AND SOME OF THEIR ASCENDANTS

PN, **CR**, **MA**) subjected to this study contain about the same amount of tri-unsaturated fatty acid (linolenic acid), around 0.35%. A slightly larger amount was obtained for **BU**, **CS** and **FN** varieties. The average amount of di-unsaturated fatty acid (linoleic acid) in the grape seed oil samples is 74.30%. A larger amount was observed in case of **ME** variety. In the case of local varieties (**CR** and **MA**) the amount of di-unsaturated fatty acid is equal or larger than the average value of the traditional varieties samples. The oils contain around 17% mono-unsaturated fatty acids; a larger amount was detected for the **BU** variety. The average amount of saturated acids is 11%; the smallest amount being in the **MA** variety. These results are in agreement with literature data concerning FAP of grape seed oils. According to literature, the grape seed oils are composed of 90% unsaturated fatty acids, mainly linoleic acid (58-78%, 18:2 ω-6) followed by oleic acid (3-15%, 18:1 ω-9), plus linolenic acid (0.3-0.6%, 18:3 ω-3), and minor amounts of saturated fatty acids (10%), such as: palmitic acid 7.2-8.5% and stearic acid 3.8-3.9% [17]. Similar results are obtained even by a supercritical fluid extraction of the oil [12, 18].

The new **CR** grape sort resulted by the hybridization of **BN** (Babeasca Neagra) and **CH** (Chardonnay) varieties. The **MA** variety was created by hybridization of varieties **ME** x **BN** x **MO** (Muscat Ottonel). The **BN** plant constitutes the support for the new varieties, being a climate adapted specie, not used for wine production. There is no information on the seed oil produced by this species. The new grapes are intended to produce superior quality red wines preserving some characteristics of parents. The FAP of these new varieties are different from those of their parent species (table 2).

A slight enhancement of unsaturated fatty acid content may be noticed for the **MA** variety. This makes the corresponding grape seed oils more suitable for the human consumers, as an unsaturated fatty acid source.

Principal Component Analysis

Determination of the authenticity of oils and fats by manufacturers is very important as fraud affects the well-being of the consumers. Adulteration of food products involves the replacement of high cost ingredients with lower grade and cheaper substitutes [19]. The grape seed oil is a healthy food due to the high content in unsaturated vegetable fatty acids [20] and can be a possible subject for adulteration.

The discrimination between different types of edible oils and fats was achieved by a number of spectral methods also the GC-FID data [21] was used to distinguish the type of vegetable oils.

The differences between grape seed oils from local varieties and traditional oils were established first by applying the PCA method to the FT-IR spectral data. The FT-IR region relevant for this study is the spectral section set 1800-900 cm^{-1}, were are situated the absorption bands for fatty acids fingerprint. The overlapped FT-IR spectra, for the 7 varieties of grape seed oils subjected to this study, shows small differences (fig. 2). For accurate analysis the FT-IR data, turned into a vector with 19 variables, were subjected to statistical analyses (PCA and HCA).

A good separation of the grape seed oils created at RCVE Murfatlar from the other genuine traditional oils is obtained (fig. 3a). The new local varieties cluster against the traditional red varieties; this could be also noticed from the HCA. A well-defined group was formed, separated from the specific areas of the red traditional varieties (fig. 3b).

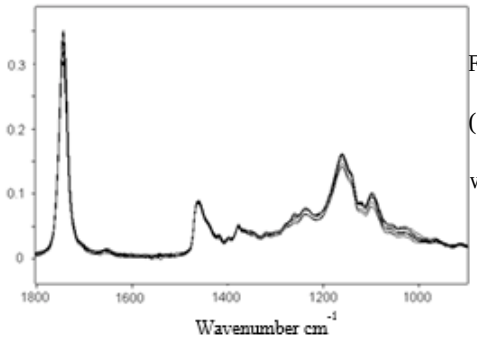

Fig. 2 Overlapped FT-IR spectra (1800-900 cm^{-1}) for the different varieties of grape seed oils

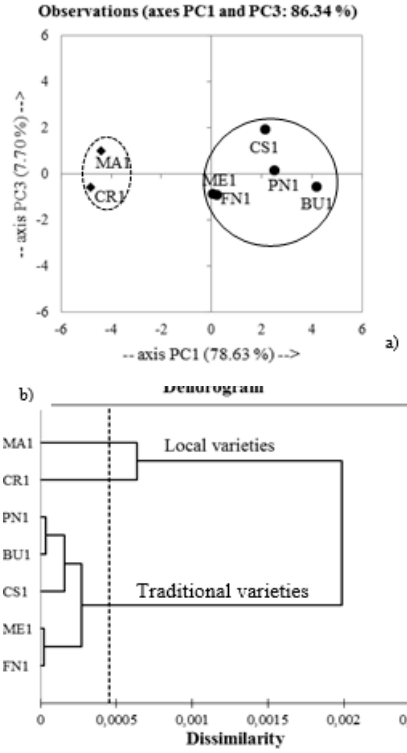

Fig. 3 Statistical analysis (PCA (a) and HCA (b)) of IR data for the grape-seed oils (obtained from 2010 crop)

The NMR spectroscopy analysis may be also applied for compositional analysis and structural identification of functional components in foods [22]. As shown before, and confirmed by the experimental results presented in this paper, the data obtained based on the ^{1}H-NMR analysis are comparable to the ones determined chromatographically. This method of analysis, correlated with PCA [23], was used to evaluate the oil composition [24].

The ^{1}H-NMR spectra of 7 varieties of grape seed oils is presented in figure 4. The integral values of signal A-J are used in the chemometrical computations (fig. 4).

The NMR spectral data were also subjected to a PCA analysis and the results are presented in figure 5. A very

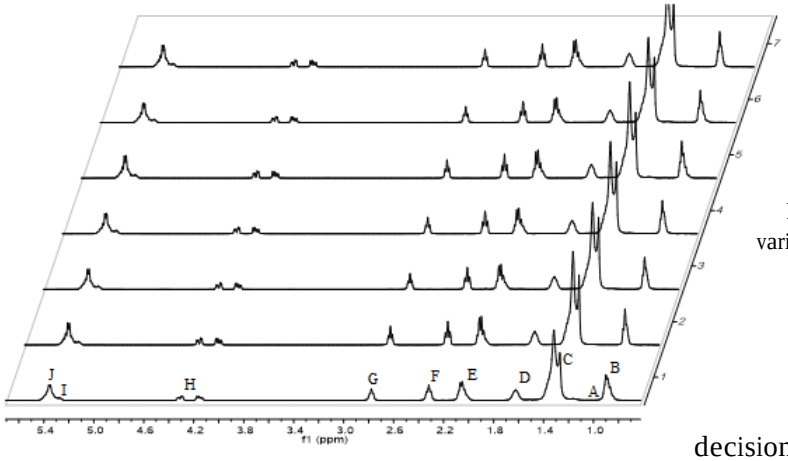

Fig. 4 The ^{1}H-NMR spectra for the red grape seed oils varieties (**CR** - 1, **ME** - 2, **FN** - 3, **BU** - 4, **MA** - 5, **CS** - 6, **PN** - 7)

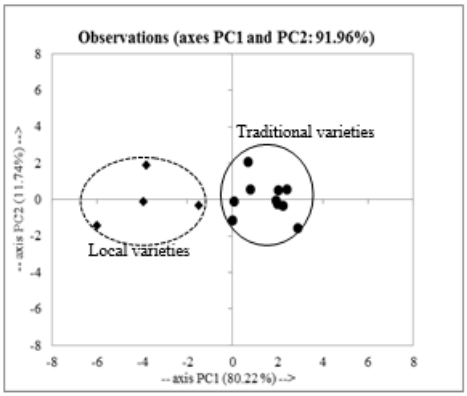

Fig. 5 The graphical representation of the principal component scores PC1/PC2 for the red grape seed oils (**BU, CS, ME, PN, FN, CR, MA**) - crops 2010 and 2011

good discrimination between oils extracted from grape seeds from the varieties created at RCVEM and the traditional varieties is obtained. Thus, grape seed oil varieties **CR** and **MA** is found in the quadrant I and IV, while oils extracted from red traditional grape seeds are placed in quadrant II and III. Sharing a commune ancestor (**BN** grape variety) makes the two newly created varieties (**MA** and **CR**) to group together, differently from the classical red varieties cultivated in Murfatlar vineyard.

Conclusions

A number of red grape seeds varieties produced by Murfatlar, a vineyard situated in the south-east of Romania, have been valorized by oil extraction. The extraction method of the oil seeds gave better or similar yields than the other methods specified in literature.

The fatty acid composition of the oils has been determined using statistical computations and the ^{1}H-NMR data, the results being similar with the standard GC-FID. Thus, the NMR method, which is less time consuming and demands fewer chemicals, seems appropriate for the FAP determination with proper results for grape seed oils.

Based on statistical analysis (PCA and HCA) of their experimentally determined FAP, the oils produced from the two new varieties obtained by hybridization were characterized in comparison with those obtained from the already known (traditional) species and the results offer a good discrimination of the two newly created varieties.

The newly created **MA** variety contains the smallest amount of saturated fatty acids, holding a good prospective for food applications.

The oils extracted from all grape seeds samples could be differentiated according to their variety on the basis of their FAP and specific spectral fingerprint. Accordingly, decisions of the optimal use (as food or as cosmetic ingredient) for the obtained oils may be completed.

Reference

1.FAVA, F., TOTARO, G., DIELS, L., REIS, M., DUARTE, J., BESERRA CARIOCA, O., POGGI-VARALDO, H. M., SOMMER FERREIRA, B., N. Biotechnol., **32**, 2015, p. 100.
2.*** OIV (International Organization of Vine and Wine), 2015. World viticulture situation, http://www.oiv.int.
3. MIRONEASA, S., LEAHU, A., CODINA, G.G., STROE, S.G., MIRONEASA, C., Journal of Agroalimentary Processes and Technologies, **16**, nr.1, 2010, p.1.
4. BARBIERI, L., ANDREOLA, F., LANCELLOTTI, I., TAURINO, R., Waste Manag., **33**, 2013, p. 2307.
5. FERNANDES, L., CASAL, S., CRUZ. R., PEREIRA, J.A., RAMALHOSA, E., Food Res. Int., **50**, 2013, p. 161.
6. ANTOCE, A.O., COJOCARU, G.A., Rev. Chim.(Bucharest), **66**, no. 10, 2015, p. 1567.
7. SABIR, A., UNVER, A., KARA, Z.J., Sci. Food Agr., **92**, 2012, p. 1982.
8. DAVIDOV-PARDO, G., MCCLEMENTS, D.J., Food Chem., **167**, 2015, p. 205.
9. SANTOS, L.P., MORAIS, D.R., SOUZA, N.E., COTTICA, S.M., BOROSKI, M., VISENTAINER, J.V., Food Res. Int., **44**, 2011, p. 1414.
10. YU, J., AHMEDNA, M., Int. J. Food Sci. Technol., **48**, nr. 2, 2013, p. 221.
11. ADAMEZ, J.D., SAMINO, E.G., VALDES SANCHEZ, E., GONZALEZ-GÓMEZ D., Food Control, **24**, 2012, p. 136.
12. AI, F.F., BIN, J., ZHANG, Z.M., HUANG, J.H., WANG, J.B., LIANG, Y.Z., YU, L., YANG, Z.Y., Food Chem., **143**, 2014, p. 472.
13. ŠMEJKALOVÁ, D., PICCOLO, A., Food Chem., **118**, 2010, p. 153.
14. BARBIERI, S., BENDINI, A., VALLI, E., TOSCHI, T.G., J. Food Comp. Anal., **44**, 2015, p. 186.
15. HANGANU, A., TODASCA, M.C., CHIRA, N.A., MAGANU, M., ROSCA, S., Food Chem., **134**, 2012, p. 2453.
16. CHIRA, N.A., TODASCA, M.C., NICOLESCU, A., ROSCA, A., NICOLAE, M., ROSCA, S.I., Rev. Chim.(Bucharest), **62**, no. 1, 2011, p. 42.
17. DELLA CORTE, A., CHITARRINI, G., DI GANGI, I.M., MASUERO, D., SOINI, E., MATTIVI, F., VRHOVSEK, U., Talanta, **140**, 2015, p. 52.
18. LACHMAN, J., HEJTMANKOVA, A., TABORSKY, J., KOTIKOVA, Z., PIVEC, V., STRALKOVÁ, R., VOLLMANNOVÁ, A., BOJÒANSKÁ, T., DIDIN, M., LWT - Food Sci. Technol., **63**, nr. 10, 2015, p. 620.
19. PASSOS, C.P., SILVA, R.M., DA SILVA, F.A., COIMBRAB, M.A., SILVA, C.M., Chem. Eng. J., **160**, 2010, p. 634.
20. DE LORGERIL, M., SALEN, P., BMC Medicine, 10:50, 2012.
21. POPESCU, R., COSTINEL, D., DINCA, O.R., MARINESCU, A., STEFANESCU, I., IONETE, R.E., Food Control, **48**, 2015, p. 84.
22. MARCONE, M.F., WANG, S., ALBABISH, W., NIE, S., SOMNARAIN, DI., HILL, A. Food Res. Int., **51**, 2013, p. 729.
23. DIRAMAN, H., SAYGI, H., HI^{a}IL, Y., Eur. Food Res. Technol., **233**, 2011, p. 403.
24. ALONSO-SALCES, R.M., HOLLAND, M.V., GUILLOU, C., Food Control, **22**, 2011, p. 2041

Manuscript received: 9.03.2017