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Analytical Methods

Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity

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Abstract

Grape seed oil (*Oleum vitis viniferae*) representing a promising plant fat, mainly used for culinary and pharmaceutical purposes as well as for various technical applications, was subject of the present investigation. HS-SPME-GC–MS was applied to study volatile compounds in several seed oil samples from different grape oils. The triacylglycerol (TAG) composition of these oils was analyzed by MALDI-TOF-MS/MS. In addition the total phenol content and the antioxidant capacity (using TEAC) of these oils were determined. The headspace of virgin grape oils from white and red grapes was dominated by ethyl octanoate (up to 27.5% related to the total level of volatiles), ethylacetate (up to 25.0%), ethanol (up to 22.7%), acetic acid (up to 17.2%), ethyl hexanoate (up to 17.4%) and 3-methylbutanol (up to 11.0%). Triacylglycerol composition was found to be dominated by LLL (up to 41.8%), LLP (up to 24.3%), LLO (up to 16.3%) and LOO (up to 11.7%), followed by LOP (up to 9.3%) and LOS/OOO (up to 4.3%). Total phenol content ranged between 59 μg/g and 115.5 μg/g GAE. Antioxidant capacity (TEAC) was analyzed to range between 0.09 μg/g and 1.16 μg/g.

Keywords: Grape seed oil (*Oleum vitis viniferae*); Volatiles; Triacylglycerols; Total phenols; Antioxidant capacity

1. Introduction

Grapes, the berries of *Vitis vinifera* L. ssp *sativa*, are used for various utilizations since ancient times. Also today, they are of worldwide interest for nutritional purposes including raw and dried consummation, wine production, but also extracts of their peels and seeds are used in pharmaceutical applications with advertised health beneficial properties due to polyphenolic and especially interesting resveratrol content. Production of grapes generally is situated in moderate-warm climate zones, e.g. Italy (9,256,814 mt/year), France (6,787,000 mt/year), USA (6,414,610 mt/year), Spain (5,880,800 mt/year) but also China (5,698,000 mt/year) in 2006 (FAO, 2006).

From an ecological point of view, the complete utilization of grapes including the grape pomace as byproduct from producing wine is an important aspect in waste reduction. Furthermore obtaining valuable products from grape skin and seeds – known for providing beneficial substances for lowering incidence of atherosclerosis and coronary heart diseases based on their typical fatty acid composition and their content of high valuable polyphenols – is a welcome side-effect (Peschel et al., 2006). Pomace consists of 20–26% grape seeds, 7.8–11% protein and 10–20% fatty oil depending on pressing conditions (Bockisch, 1993; Schieber, Muller, Rohrig, & Carle, 2002). A careful but fast drying of the pomace

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after production of wine is needed to achieve high valuable grape seed oils with characteristic smell and taste as well as poly phenolic compounds. Otherwise the quality of the resulting oil may be affected by various microorganisms disturbing balanced smell and taste (Matthaeus, 2006).

Grape seed oil is composed of average 90% poly- and monounsaturated fatty acids, which are responsible for its value as nutritive edible oil, particularly of linoleic acid (58–78%, 18:2n-6) followed by oleic acid (3–15%, 18:1n-9) and minor amounts of saturated fatty acids (10%). Unrefined oils contain bioactive compounds including tocopherols (5–52 mg/100 g) and numerous phenolic components, consisting of low and high molecular plant phenolics that may contribute to beneficial effects of vegetable oils (Bockisch, 1993; Firestone, 1999; Morin, 1996). Furthermore an unusual high smoke point (about 190–230 °C) has been reported on grape seed oil, making it suitable for cooking at high temperature (Morin, 1996).

Investigating the volatile profile and the TAG composition of the oils with methods, which have already been successfully applied on the characterization analysis of various poppy oils, linseed and camelina oils (Krist, Stuebiger, Bail, & Unterweger, 2006a; Krist, Stuebiger, Unterweger, Bandion, & Buchbauer, 2005), may provide information about processing conditions and raw material (Buchbauer, Boucek, & Nikiforov, 1998) and could be applied in terms of quality control in order to identify purity or blending of these oils, as was already shown for the detection of added sunflower oil to virgin poppy seed oils (Krist, Stuebiger, Bail, & Unterweger, 2006b). TAG profiling allows the rapid characterization of oils based on the simultaneous detection of all TAG species present within a certain oil sample (Jakab, Nagy, Heberger, Vekey, & Forgacs, 2002). MALDI-MS provides a very useful technique for the semiquantitative analysis of minute amounts of oils within very short time (Ayorinde, 2000; Belgacem, Bowdler, Brookhouse, Brancia, & Raptakis, 2006; Lay, Liyanage, Durham, & Brooks, 2006). Novel types of MALDI tandem time-of-flight (TOF/TOF) mass spectrometers facilitate the direct structural analysis of lipid molecules (e.g. their fatty acid composition) by MS/MS experiments using collision-induced dissociation (CID) (Belgacem et al., 2006; Jackson, Wang, & Woods, 2005). Furthermore according to various publications dealing with the content of phenolic compounds, especially investigated in olive oils as well as in grapes (Hrncirik & Fritsche, 2004; Yilmaz & Toledo, 2004), our grape seed oil samples were characterized by analyzing the total phenol content by using Folin–Ciocalteau colorimetric method (Pastrana-Bonilla, Akoh, Sellappan, & Krewer, 2003). The trolox equivalent antioxidant capacity (TEAC) of the grape seed oils was analyzed in order to evaluate their potential use as nutraceutical (functional) foods. As cold pressing process does not involve chemicals or heat prior to or during oil production procedure, it is therefore known that cold pressed seed oils may contain phytochemicals including natural antioxidants (Kornsteiner, Wagner, & Elmadfa, 2006).

Grape seed oil represents a promising plant fat. Astonishingly nothing is yet known about the volatile composition, the TAGs and the antioxidant capacity of this oil type. As it seems to be worthwhile to investigate these parameters within the scope of fundamental research and with regard to quality assurance, the aim of the presented study was to characterize different grape oil types (refined and unrefined) in terms of volatile compounds (by using HS-SPME-GC–MS), triacylglycerols (using TAG profiling) and their total phenol content and antioxidant capacity.

2. Materials and methods

2.1. Chemicals

Reference compounds for SPME-Analysis were obtained from Aldrich (Milwaukee, USA) and Fluka (Darmstadt, Germany). Folin–Ciocalteau reagent (2 N) and Gallic acid (90% purity) were purchased from Sigma (St. Louis, MO). Methanol grade Chromasolv was purchased from Fluka. Double deionized water (DDH_2O) was used for reaction, Sodium Carbonate (min. 99%) was purchased from Loba Feinchemie (Fischamend Austria), TAS – total antioxidant status – kit and control serum was provided by Randox (Ardmore, UK).

2.2. Samples

Investigated oils were obtained from local producers, providing virgin cold pressed unfiltrated grape seed oils, produced in 2006 and stored at 4 °C prior to analysis to avoid oxidation processes (Table 1).

2.3. SPME-GC–MS Analysis

Headspace volatiles from various grape seed oils, which have not been investigated prior to this study, were analyzed by headspace solid phase micro extraction (HS–SPME). SPME compatible vials containing 10.0 g oil each were tightly sealed with aluminium foil and an aluminium cap and extracted isothermally for 10 h to produce sufficient amounts of analytes at room temperature (22 °C) by using a pre-conditioned Supelco 57348 2 cm, 50/30 μm DVB/Carboxen/PDMS Stable-Flex fiber. Before and after each oil sample blank values were analyzed. After sampling had been carried out, the SPME device was placed immediately into the GC–MS instrument. For separation of volatile compounds a 60 m × 0.25 mm (inside diameter) RTx-5 (Restec) non-polar column, with a film thickness of 0.25 μm was attached to a Hewlett-Packard HP-6890 model gas chromatograph equipped with an HP-5972 mass selective detector. The following column temperature program sequence was used. The initial temperature of 38 °C was held for 1 min and then increased at a rate of 2.5 °C min^{-1} to 175 °C. From this point the temperature was increased at a rate of 50 °C min^{-1} to 220 °C, which

Table 1
Samples

Samples	Origin	Grape variety	Conditions of seed treatment and oil production
A	Bavaria, Germany	Unknown	Cold pressed oil, refined
B	Styria, Austria	Various	Sun-dried (10 h) grape-seeds, cold pressed from seeds that were repeatedly pressed before
C	Burgenland, Austria	"Welschriesling" (white wine pomace)	Grape seeds were heated to 60 °C for 30 min before cold pressing process
D	Burgenland, Austria	"Chardonnay" (white wine pomace)	Cold pressing process
E	Styria, Austria	"Schilcher" (white wine pomace)	Grape seeds were heated to 60 °C for 30 min before cold pressing process
F	Burgenland, Austria	"Merlot" (red wine pomace)	Cold pressing process
G	Burgenland, Austria	"Cabernet-Sauvignon" (red wine pomace)	Cold pressing process
H	Burgenland, Austria	"Zweigelt" (red wine pomace)	Cold pressing process
I	Styria, Austria	"Zweigelt" (red wine pomace)	Grape seeds were heated to 60 °C for 30 min before cold pressing process

was held for 2 min. The injector port temperature was 250 °C. After 4 min of using splitless mode, the split ratio was set to 1:40 to expurgate the GC–MS-system. A constant flow of 1 ml/min was applied (carrier helium 5.0). The transfer line temperature was 250 °C, resulting in an ion source temperature of approximately 160 °C. An ionization voltage of 70 eV was used for electron impact ionization. Mass spectra were recorded with a scan range of 10–300 amu. Volatile compound identification was carried out by using Wiley 275, NBS 75 K and in-house mass spectra libraries and partly by co-injection of reference compounds. In addition retention indices of the sample compounds were determined on the basis of homologue *n*-alkane hydrocarbons analyzed under identical GC–MS conditions.

2.4. Triacylglycerol analysis

2.4.1. Sample preparation

Grape seed oils were dissolved in pure chloroform at a concentration of 1 mg/mL. A freshly prepared matrix solution consisting of $Na_4[Fe(CN)_6]$ suspended in methanol containing 5 vol.% glycerol was used (Zollner, Stubiger, Schmid, Pittenauer, & Allmaier, 1997). After spotting 0.5 μL of this suspension on the sample plate an equal volume of the oil sample was spotted above, which immediately forms an opaque layer by evaporation of the solvents covering the target spot. This preparation is ready for direct mass spectrometric analysis. For each oil four samples were taken. MALDI-analysis were done in triplicate.

2.4.2. Mass spectrometry

MALDI mass spectra were measured using a novel type AXIMA-TOF2 (Kratos-Shimadzu, Manchester, UK) reflectron time-of-flight mass spectrometer equipped with a nitrogen laser (337 nm, 3 ns pulse width) (Belgacem et al., 2006). All measurements were performed in the positive ionization reflectron mode using delayed ion extraction. The delay time was adjusted to *m*/*z* 1000. The ion acceleration voltage was set to 20 kV and the reflectron detector was operated at 24 kV. For MS/MS experiments helium was used as collision gas by floating the CID cell to $\sim 5 \times 10^{-6}$ mbar in contrast to the normal vacuum conditions within the analyzer part of the instrument ($\sim 1 \times 10^{-7}$ mbar). Each MS and MS/MS spectrum represents the accumulation of 500–1000 single-laser-shots.

2.5. Total phenols (TP)

The quantitative determination of total phenols was analyzed by using the Folin–Ciocalteau colorimetric method, based on the reaction of the reagent with the functional hydroxyl groups of phenols. This method requires extraction of the phenols from the sample, a calibration curve using a pure phenolic compound (e.g. gallic acid) and the measurement of absorbance after colour reaction. Extraction of phenols was carried out according to a method presented by Parry et al. (2005) using 1.0 g of each oil. For extraction of oils 90:10 methanol:water (3 ml) was added, followed by vortex for 4 min and centrifugation for 5 min at 3000 u min^{-1}. The extraction procedure was carried out three times for each oil. All methanolic extracts were combined and concentrated until dryness. Before measurement of total phenols, the dry matter was dissolved in 10:90 methanol:water (1 ml). The resulting antioxidant solution was stored in the dark at −20 °C until analyzing. The TP content was analyzed according to the Folin–Ciocalteau reagent method (Singleton, Orthofer, & Lamuela-Raventos, 1999; Singleton & Rossi, 1965). Two hundred microliters of sample extract were filled into a test tube along with 8.2 mL of water and 0.5 mL of Folin–Ciocalteau reagent. After 5 min 1.0 ml of sodium carbonate (10%) were added, mixed and allowed to stand for 60 min. Absorption at 765 nm was measured in a Shimadzu UV–Vis Spectophotometer (Shimadzu UV – 1201 V, Korneuburg, Austria) against a blank sample.

The TP content was expressed as gallic acid equivalents (GAE) in milligrams per gram of oil, using a standard

curve generated with 50 μg, 100 μg, 200 μg, 300 μg and 500 μg gallic acid per 100 ml.

2.6. Antioxidant capacity

The antioxidant capacity (AC) of grape seed oil extracts was measured using the trolox equivalent antioxidant capacity (TEAC) assay, introduced by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993) and Rice-Evans and Miller (1994) and modified to a ready-to-use kit (TAS-Kit) provided by Randox (Ardmore, UK). This assay is based on the samples' antioxidants ability to react with a specie of free radicals especially generated.

Trolox, a vitamin E analogue, was used as standard. $ABTS^{\cdot+}$ radical cation was generated by the interaction of ABTS (2,2′-Azino-di-[3-ethylbenzthiaoline sulphonate]) with a peroxidase (metmyoglobin) and H_2O_2.

Measurement of the TEAC was achieved by comparing decreased absorption after using 20 μl of grape seed oil extract, reagent blank or trolox standard, respectively with the reagent composition as follows: ABTS® 610 μmol/l and peroxidase (metmyoglobin) 6.1 μmol/l as "chromogen solution", hydrogen peroxide (in stabilized form) in a "substrate solution" at 250 μmol/l and "standard" Trolox solution contained 1.82 mmol/l. Reagent blank was measured by using DD_2O, chromogen solution and substrate; standard trolox was constituted from standard, chromogen solution and substrate furthermore samples were composed of the oil extract, chromogen solution and substrate.

Absorbance was monitored at 600 nm 3 min after the addition of reactant at a temperature of 37 °C (incubation of the sample). The decrease in absorption after addition of reactant substrate containing hydrogen peroxide in phosphate buffer and subsequent incubation at 37 °C was used to calculate the TEAC value. TEAC value is expressed as mmol/l and converted to μg/g. A higher TEAC value of a sample is related to a stronger antioxidant capacity (Lee & Yen, 2006).

Data on total phenol content (TPC) and antioxidant capacity (AC) were reported as mean ± standard deviation (SD) from triplicate determinations for each grape seed oil samples. For each grape seed oil three samples were taken.

Analyses of significant group differences in TPC and AC were conducted (SPSS for Windows, Version Rel. 10.0.7., 2000, SPSS Inc., Chicago, IL) by using the non-parametric Mann–Whitney-U test to identify differences among means. Statistical significance was declared at $p \leqslant 0.05$.

3. Results and discussion

3.1. Volatile compounds

The volatile fraction of nine different grape seed oils were trapped by SPME and subsequently analyzed by GC–MS in order to identify volatile compounds. A large number of substances consisting mainly of short chain acids, alcohols, esters, as well as flavour active aldehydes and ketones were detected.

Grape seed oil (A) passed through a refining process after pressing to enhance storage time. This process obviously led to a lower number of identifiable substances (eight compounds) in these oil samples. In the headspace of the samples taken from grape seed oil (B), which was gained from seeds pressed several times before, only nine substances could be investigated (results are shown in Table 2). In contrast to the lower number of volatiles in samples (A) and (B), in further analyzed virgin grape oils (C–I) 27–33 volatiles were detected in each oil sample. Cold pressing conditions are known for resulting in lower yields of oil in comparison to solvent-extraction methods, but in contrast to high yield solvent-extracted or refined oils, virgin oils contain more diverse alcoholic compounds, acids and subsequently esters which are known to be aroma active.

The headspaces of oil samples (A) and (B) contained almost identical compounds. Here acids e.g. acetic acid (A: 3.0%, B: 10.2% of total peak area) and hexanoic acid (A: 2.1%, B: 1.9%) were identified, which are well known for giving a sour, pungent fatty and sweaty aroma. These compounds, also present in all other investigated grape oil samples, can be considered as being typical constituents of fatty products (Jelen, Obuchowska, Zawirska-Wojtasiak, & Wasowicz, 2000).

Furthermore degradation due to oxidation processes, activity of enzymes and microbes, processing conditions as well as storage results in flavour active carbonyls and alcohols like pentanal, hexanal, heptanal and 2-heptanone, also identified in oil samples (A) and (B). The outstanding high content of hexanal, representing the main component in both refined grape seed oils ((A): 25%, (B) 30%) results presumably from degradation of linoleic acid, grape oils' main fatty acid. The very high content of this substance can probably be concluded from the processing conditions including refining, as lower contents of hexanal were identified in the group of virgin grape oils (C)–(I).

Most components identified in samples (A) and (B) were also investigated in the analyzed grape oils (C)–(I), respectively. *Trans*-2-Hexenal and *trans*-2-heptenal representing flavour active compounds formed by enzymatic actions are known to provide fruity, green, leafy notes and were detected in the majority of grape oils, as well as components like hexanoic acid, *trans*-2-hexenal, heptanal and 2-heptanone with comparable amounts in both groups of oils (refined and unrefined).

Terpenes detected in all groups of analyzed grape oils were α-pinene and limonene, which are known to be naturally occurring smell intense compounds in fatty products and may be used to differentiate between types of oils as was already shown for the detection of adulteration of poppy seed oils with sunflower oils (Krist et al., 2006b).

In the group of virgin grape seed oils produced from pomace of white wine production, seed oil (C) from "Welschriesling" high contents of acetic acid, ethylacetate,

Table 2
Volatile compounds of analyzed grape seed oil varieties based on HS-SPME-GC–MS measurements

RI[a]	Component listed in order of increasing RI	CAS – NO	Percentage of peak area related to the total level of volatile compounds[d]								
			Grape seed oil A	Grape seed oil B	Grape seed oil C	Grape seed oil D	Grape seed oil E	Grape seed oil F	Grape seed oil G	Grape seed oil H	Grape seed oil I
503	Ethanol[b]	[64–17–5]			6.6	2.4	13.1	Traces	6.4	9.4	22.
600	Acetic acid[b]	[64–19–7]	3.0	10.2	17.2	6.6	10.9	0.8	7.4	1.1	7.6
613	Ethyl acetate[b]	[141–78–6]			25.0	11.4	9.1	8.5	0.4	16.2	20.0
699	Pentanal[b]	[4630–07–3]	2.8	4.8	0.5			1.1			
711	3-Hydroxy-2-butanone[c]	[513–86–0]			4.0	0.7	0.8	0.2	0.7		0.9
717	Diethylacetal[c]	[105–57–7]				1.1	0.8	0.1	0.9	0.7	0.7
737	3-Methylbutanol[b]	[123–51–3]			3.4	11.0	9.0	5.4	10.5	6.8	6.7
744	2-Methylbutanol[c]	[137–32–6]			1.7	4.3	3.8	2.8	4.2	3.1	2.5
756	Pentanol[c]	[71–41–0]	0.8			0.4	0.1	0.8	0.3		0.1
758	Isobutyl acetate[c]	[110–19–0]			0.6	0.2	0.4	0.2	0.3	0.2	0.4
778	2,3-Butandiol[c]	[513–85–9]			1.1	0.9	0.8	0.4	1.1	0.6	0.4
782	1,3-Butandiole[c]	[107–88–0]			1.2	0.3	0.2	0.2	0.4		0.2
800	Hexanal[b]	[66–25–1]	25.0	30.7	1.4		1.8	7.5	3.0		
857	*Trans*-2-hexenal[c]	[6728–26–3]	0.1	0.6	0.5		tr	0.7			tr
872	Hexanol[b]	[111–27–3]			11.0	0.9	2.8	2.5	0.5	1.1	2.0
876	Isoamyl acetate[b]	[13–92–2]			2.6	5.2	4.9	2.7	5.2	2.9	3.3
883	2-Methyl butyl acetate[c]	[624–41–9]			0.7	1.9	1.4	0.8	1.6	0.9	1.0
888	2-Heptanone[b]	[110–43–0]	0.6	1.8	0.1	0.7	tr	1.3	0.2	0.5	tr
893	Styrene[b]	[100–42–5]			0.2	0.6	0.8	0.6	0.5	0.7	0.7
905	Hheptanal[b]	[111–71–7]	0.4	0.9	0.1			0.3	0.1	0.3	
918	*y*-Butyrolactone[b]	[96–48–2]			0.6	0.2	0.2		0.3	0.2	0.1
934	α-Pinene[b]	[80–56–8]	tr	tr	0.2	tr	0.5	0.8	tr	tr	tr
936	Benzaldehyde[b]	[100–52–7]			0.3	0.9	0.2	0.4	0.5	0.3	0.3
956	*Trans*-2-heptenal[b]	[18829–55–5]	1.3	7.2	1.4	0.7	0.4	9.2	0.8	0.3	
980	1-Octen-3-ol[b]	[339–86–4]			0.8		0.3	2.8	0.3	0.1	0.2
986	Hexanoic acid[b]	[142–62–1]	2.1	1.9	0.4	2.5	0.9	2.6	1.9	0.6	0.7
993	2-Pentylfuran[b]	[3777–69–3]		1.9			0.1	2.1	0.4		
997	Ethyl hexanoate[b]	[123–66–0]			1.9	13.6	17.4	12.7	12.7	11.9	10.7
1008	Hexyl acetate[b]	[142–92–7]			2.3	0.4	0.3	1.3	0.4	tr	0.8
1031	Limonene[b]	[5989–27–5]	0.3	0.6	0.2	tr	0.2	0.5	0.1	tr	tr
1037	3-Octen-2-one[b]	[18402–82–9]			0.1	0.2	0.1	0.9	0.2	0.1	
1060	*Trans*-2-octenal[b]	[2548–87–0]			0.2	0.8	0.2	2.8	0.8	0.3	0.1
1097	Ethyl heptanoate[c]	[106–30–9]				0.2	0.1		0.2	0.2	0.1
1118	Phenethylalkohol[c]				2.2	6.8	2.4	2.8	6.5	4.3	3.5

1179	Octanoic acid[b]	[127-04-2]			0.2	0.3	0.3	0.2	
1195	Ethyl octanoate[b]	[106-23-1]	14.4	8.9	4.9	11.8	27.5	12.1	

[a] Retention indices calculated using a non-polar RTx-5 column.
[b] Compound identified by retention index and mass spectra correlation with reference volatiles (authentic samples).
[c] Compound identified by comparing mass spectra libraries (commercially available: Wiley 275, NIST and custom made in-house libraries).
[d] Percentage of peak areas calculated from GC–MS analysis using a non-polar RTx-5 column. The average quantitative composition was determined by internal normalization. For each grape seed oil variety three samples and three replications for each sample were used.

hexanol, hexylacetate and 2.3-butandiole were identified, whereas in grape seed oil (D) from "Chardonnay" higher contents of 3-methylbutanol, isoamyl acetate, 2-heptanone, benzaldehyde, ethyl hexanoate, and ethyl octanoate as well as lower contents of 3-hydroxybutanone compared to grape oil (C) were identified. Among the volatile compounds deriving from the group of virgin grape oils numerous alcoholic and esterified compounds were detected, e.g. 2.3-butandiole (0.4–1.1%) could be observed within all virgin grape oils originally deriving from fermentation of grapes. This substance is used as a quality marker compound in wines (Hagenauer-Hener, Henn, Dettmar, Mosandl, & Schmitt, 1990). Furthermore alcoholic compounds 3-methylbutanol, 2-methylbutanol as well as hexanol, caused by fermentation and degradation processes, were identified in the virgin grape oils with high amounts. Also flavour active esters like ethylhexanoate, well known for giving a fruity powerful wine like aroma, as described in Gurbuz, Rouseff June, and Rouseff Russell (2006) and ethyloctanoate, were only identified in virgin grape oils (except C), too, giving a fruity, floral and brandy scent. 2-Methylbutanol (2.5–4.2%) and 3-methylbutanol (5.4–10.5%) were especially presented in grape oils from red wine production. *Y*-butyrolactone associated with roasted notes, was detectable with highest amounts in oil samples (C) gained from previously heated seeds.

3.2. Triacylglycerols

MALDI-MS analysis revealed that grape seed oils consist of seven abundant TAG species containing mainly linoleic (18:2), oleic (18:1), palmitic (16:0) and stearic (18:0) acid to a lesser extent. Altogether these components comprise more than 90% of the oils (see Table 3). The most prominent components were detected at *m*/*z* 877 and 901, which were identified by MS/MS analysis to consist of dilinoleoyl–palmitoylglycerol (C52:4, LLP) and trilinoleoylglycerol (C54:6, LLL), respectively. Peaks exhibiting the same carbon number (CN) but differing only in double bonds (ND) increments (2 Da) were found to be composed of linoleoyl–oleoyl–palmitoylglycerol (*m*/*z* 879, LOP), dioleoyl–palmitoylglycerol (*m*/*z* 881, OOP), dilinoleoyl–oleoylglycerol (*m*/*z* 903) and dioleoyl–linoleoylglycerol (*m*/*z* 905, OOL) as major TAG components of all grape seed oil varieties (Table 3). Beside these directly identified TAG, the possible composition of the acyl groups of minor components within the TAG profile could be calculated based on the known fatty acid composition of grape seed oils (Firestone, 1999) (Table 3). As is obvious from these calculations, many of these components may be composed of several TAG molecules with different fatty acid residues but exhibiting same *m*/*z* values (isobaric components). Due to the prevalence of linoleic and oleic acid within grape seed oil these TAG most likely contain at least one of these unsaturated fatty acids. Relative quantification of such components could be done only together and would need further chromatographic separation. Neverthe-

Table 3
Triacylglycerol composition of grape oil varieties based on detection by MALDI-MS

TAG *m/z* $[M+Na]^+$	Fatty acid composition[a]	Components known from literature[b]	CN:ND[c]	TAG composition calculated from peak intensity (% ± standard deviation)[d]								
				Grape seed oil A	Grape seed oil B	Grape seed oil C	Grape seed oil D	Grape seed oil E	Grape seed oil F	Grape seed oil G	Grape seed oil H	Grape seed oil I
847.6	16:1/16:1/18:3		50:5		0.3 ± 0.0	0.3 ± 0.4	0.1 ± 0.1	0.3 ± 0.3	0.9 ± 0.9	0.2 ± 0.3	0.4 ± 0.2	0.2 ± 0.1
849.6	16:1/16:1/18:2 16:1/16:0/18:3		50:4		0.3 ± 0.1	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.0		0.4 ± 0.1	0.3 ± 0.1
851.6	16:0/16:1/18:2 16:0/16:0/18:3		50:3		0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.2	0.1 ± 0.2		0.1 ± 0.1	0.1 ± 0.1
853.6	16:1/16:1/18:0 16:0/16:0/18:2		50:2	0.3 ± 0.5	0.9 ± 0.2	0.6 ± 0.1	0.9 ± 0.0	0.5 ± 0.1	0.3 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
855.6	16:0/16:1/18:0 16:0/16:0/18:1		50:1	0.2 ± 0.4	0.2 ± 0.1	0.1 ± 0.1	1.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.1
861.6	17:1/16:1/18:3		51:5		0.2 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.8 ± 0.4	0.7 ± 0.4	0.8 ± 0.4	0.6 ± 0.2
863.6	17:1/16:1/18:2 17:0/16:1/18:3 17:1/16:0/18:3		51:4		0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.3	0.5 ± 0.2	0.3 ± 0.1
875.6	16:0/18:2/18:3 16:1/18:1/18:3 16:1/18:2/18:2		52:5		0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0		0.5 ± 0.1	0.4 ± 0.0
877.6	16:0/18:1/18:3 16:0/18:2/18:2[f] 16:1/18:0/18:3 16:1/18:1/18:2	LPL (17.0%)	52:4	20.3 ± 1.9	19.8 ± 0.6	23.6 ± 1.6	21.4 ± 1.2	22.4 ± 1.0	20.7 ± 1.4	20.2 ± 0.5	19.7 ± 1.8	24.3 ± 1.1
879.6	16:0/18:0/18:3 16:0/18:1/18:2[f] 16:1/18:0/18:2 16:1/18:1/18:1	LOP (+ LSL) (15.9%)	52:3	9.3 ± 7.0	4.8 ± 0.6	3.3 ± 0.2	5.3 ± 0.3	4.8 ± 0.1	3.7 ± 0.4	4.6 ± 0.2	5.2 ± 1.1	3.6 ± 0.2
881.6	16:0/18:0/18:2 16:0/18:1/18:1 16:1/16:1/20:0 16:1/18:0/18:1	LSP OPO (<0.1%)	52:2	1.5 ± 1.3	1.8 ± 0.2	0.7 ± 0.2	4.0 ± 0.3	0.5 ± 0.1	0.7 ± 0.3	3.1 ± 0.1	2.3 ± 0.4	1.0 ± 0.1
883.7	16:0/16:1/20:0 16:0/18:0/18:1 16:0/16:0/20:1 16:1/18:0/18:0		52:1			0.1 ± 0.1		0.2 ± 0.2	0.1 ± 0.1			
885.7	16:0/16:0/20:0 16:0/18:0/18:0		52:0	0.5 ± 0.6	0.4 ± 0.3	0.5 ± 0.9	0.3 ± 0.1	0.1 ± 0.2	1.1 ± 1.3	0.8 ± 1.3	1.0 ± 1.3	0.2 ± 0.2
899.6	18:1/18:3/18:3 18:2/18:2/18:3		54:7		0.6 ± 0.1	0.8 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.4	0.6 ± 0.1	0.7 ± 0.1
901.6	18:0/18:3/18:3 18:1/18:2/18:3 18:2/18:2/18:2[f]	LLL (35.8%)	54:6	34.6 ± 2.4	34.9 ± 0.3	41.8 ± 0.8	35.3 ± 0.5	39.2 ± 1.2	37.3 ± 2.1	37.0 ± 2.7	36.1 ± 2.2	41.4 ± 1.3
903.6	18:0/18:2/18:3 18:1/18:1/18:3 18:1/18:2/18:2[f]	LOL (21.0%)	54:5	16.3 ± 0.7	17.6 ± 1.9	13.8 ± 1.2	14.0 ± 0.2	14.4 ± 0.5	15.1 ± 0.8	14.8 ± 0.8	14.5 ± 1.0	13.8 ± 0.7
905.6	18:0/18:1/18:3 18:0/18:2/18:2 18:1/18:1/18:2[f]	LSL OLO (10.3%)	54:4	11.7 ± 0.7	11.3 ± 0.4	9.1 ± 0.6	10.1 ± 0.6	10.6 ± 0.5	11.0 ± 0.6	10.1 ± 0.7	10.4 ± 0.2	9.5 ± 0.6

907.6	18:0/18:0/18:3 18:0/18:1/18:2 18:1/18:1/18:1	LOS OOO (<0.1%)	54:3	4.3 ± 0.8	2.9 ± 0.4	1.4 ± 0.3	2.8 ± 0.5	2.1 ± 0.2	1.8 ± 0.2	3.1 ± 0.4	2.2 ± 0.5	1.3 ± 0.0
909.6	18:0/18:0/18:2 18:0/18:1/18:1		54:2	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	0.6 ± 0.4	0.5 ± 0.5	0.6 ± 0.3	0.2 ± 0.2
931.6	18:2/18:3/20:0 18:2/18:2/20:1		56:5	0.2 ± 0.4	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.2	0.4 ± 0.4	0.5 ± 0.5	0.5 ± 0.1
933.6	18:1/18:2/20:1 18:1/18:3/20:0 18:2/18:2/20:0		56:4	0.3 ± 0.5	0.7 ± 0.2	0.6 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	1.7 ± 0.4	1.1 ± 0.4	1.2 ± 0.4	0.4 ± 0.4
935.7	18:0/18:2/20:1 18:0/18:3/20:0 18:1/18:1/20:1 18:1/18:2/20:0		56:3	0.1 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.1
937.6	18:0/18:1/20:1 18:0/18:2/20:0 18:1/18:1/20:0		56:2	0.1 ± 0.2	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.2	0.3 ± 0.1	0.1 ± 0.1
939.6	18:0/18:0/20:1 18:0/18:1/20:0		56:1		0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1		0.1 ± 0.1		0.1 ± 0.1	0.1 ± 0.1
961.6	18:2/18:2/22:0 18:2/20:1/20:1 18:3/20:0/20:1		58:4		0.1 ± 0.1							
963.6	18:1/20:1/20:1 18:2/20:0/20:1 18:3/20:0/20:0		58:3			0.1 ± 0.1	0.1 ± 0.1				0.1 ± 0.1	
965.6	18:0/18:2/22:0 18:0/20:1/20:1 18:1/18:1/22:0 18:2/20:0/20:0		58:2			0.1 ± 0.1						
			LLL/OOO[e] quotient	8.1	11.8	30.1	12.4	18.4	20.8	11.8	16.2	32.0

[a] Composition calculated based on known fatty acid composition of grape seed oil (Firestone, 1999) and confirmed by MS/MS analysis.
[b] Confirmed in literature based on results from liquid chromatography (Barron et al., 1988).
[c] Total number of carbon atoms (CN) and double bonds (ND) of the acyl residues. TAG with odd carbon number (*m/z* 861, 863) contain margaric (17:0) and margaroleic acid (17:1), respectively.
[d] Values represent the mean ± SD of triplicate measurements. The individual peak intensity was normalized to total ion signals in the mass range between *m/z* 840–980; Peak intensities were corrected for isotopic contribution according to the 20% rule (Lay et al., 2006). For isobaric TAG the numbers correspond to the sum response of all components with same m/z value.
[e] The quotient is a direct measure for the linoleic vs. oleic acid ratio of the oils.
[f] If possible, the position of acyl groups is only indicative.

less, most of these TAG comprise only trace amounts of the oils (<0.5%).

Comparison between the grape seed varieties showed only marginal differences of the relative abundances of the homologues TAG components (equal *m/z* values) from the mass spectra. Interestingly, despite this fact, the content of linoleic acid versus oleic acid containing TAG of the oils appeared to be quite different as can be illustrated by the LLL/OOO quotient (see Table 3). Highest ratios were found from oil samples (C) and (I) related to the variety "Welschriesling" from Burgenland (Austria) and "Zweigelt" grown in Styria (Austria), respectively. The observed differences may be most likely the result of different vine varieties and vegetation conditions, as it is well known that climatic conditions have a considerable influence on the oleic–linoleic acid balance of plant oils (Lajara, Diaz, & Diaz Quidiello, 1990). Considering such regional variations our results from MALDI-MS analysis were in very good agreement with literature data obtained by different analytical methodology (Barron, Celaa, Santa-Maria, & Corzo, 1988).

Our research group recently analyzed a variety of seed oils exhibiting high contents of linoleic acid containing TAG (Krist et al., 2006b). Since the grape seed oils also contain high levels of trilinoleoylglycerol (34–41%), we compared the TAG profiles with those of poppy seed oils analyzed during one of our previous investigations. As can be seen in Fig. 1 the mass profiles of grape seed and poppy seed oils were nearly indistinguishable from each another within the range of uncertainty.

3.3. Total phenols and antioxidant capacity

Published data of different single grape parts show the highest amounts on phenols in skin and pulp (Pastrana-Bonilla et al., 2003). Phenol contents of our analyzed grape seed oils range within literature data of olive oils presented by Hrncirik and Fritsche (2004) and Montedoro et al., 1992. In our investigation the extraction of the oil samples based on the Parry method was applied in order to analyse the total phenol content and the antioxidant capacity in a subsequent following step. The amount of phenolic components originally occurring in the oily matter was analyzed 59.0–115.5 μg/g GAE in extracted grape seed oils (see Table 4). Lower contents of total phenols were investigated in grape oils (A)–(D) representing the group of refined and treated grape oils as well as virgin grape oils from white wine production, respectively compared to oils (E)–(I) from red wine production.

The antioxidant capacity of the oil extracts was analyzed with amounts ranging from 0.09 to 1.16 μg/g of oil (see Table 4). Refined and treated as well as grape oils produced

Table 4
Total phenol content and antioxidant capacity of grape oil varieties

	TPC[a] (μg/g) ± SD[b]	TEAC[c] (μg/g) ± SD[b]
Grape seed oil A	70 ± 0.01	0.14 ± 0.0
Grape seed oil B	68 ± 0.03	0.52 ± 0.01
Grape seed oil C	63.5 ± 0.005	0.09 ± 0.01
Grape seed oil D	59 ± 0.02	0.90 ± 0.07
Grape seed oil E	105.5 ± 0.06	0.43 ± 0.03
Grape seed oil F	108 ± 0.002	0.87 ± 0.00
Grape seed oil G	115.5 ± 0.005	1.16 ± 0.02
Grape seed oil H	103.5 ± 0.0 6	0.72 ± 0.21
Grape seed oil I	69.5 ± 0.06	0.73 ± 0.32

[a] Total phenol content analyzed as gallic acid equivalent (GAE) μg/g of oil, values are the average of triplicates.
[b] SD Standard deviation.
[c] Antioxidant capacity analyzed as TEAC (Trolox Equivalent Antioxidant Capacity) [μg/g] of oil, values are the average of triplicates.

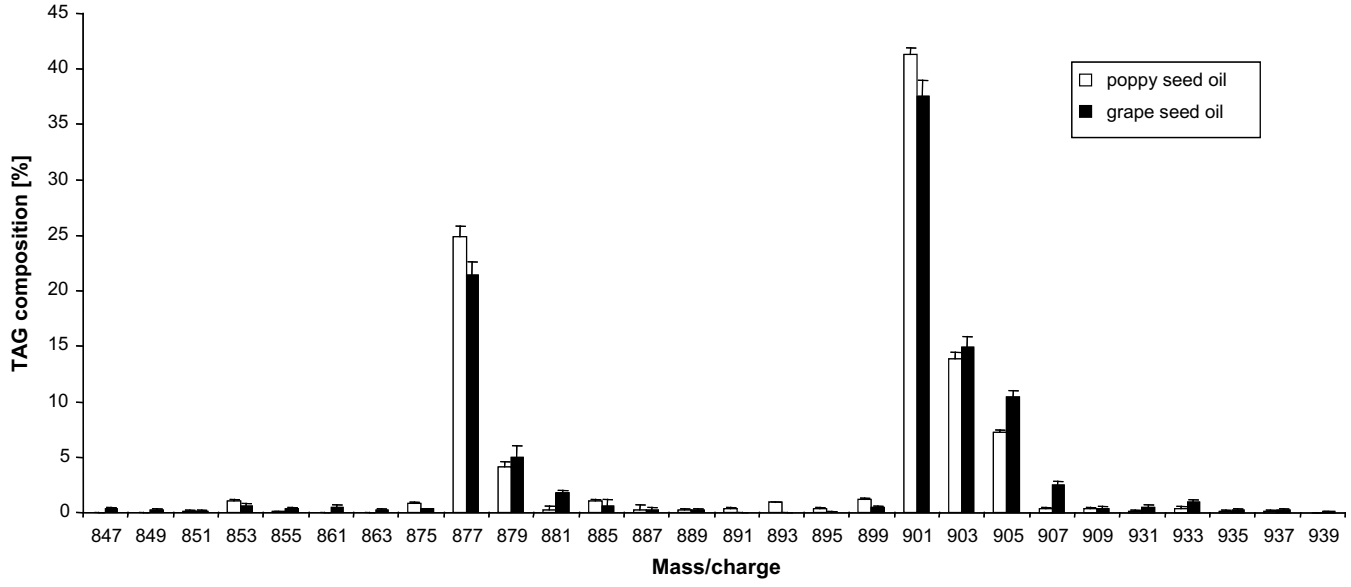

Fig. 1. Comparison of MALDI mass profiles of grape seed and poppy seed oils. Very similar TAG profiles were observed between both types of oils. TAG at *m/z* 877, 901, 903 and 905 were identified by MS/MS analysis to comprise homologous fatty acid compositions. Values displayed represent the mean ± SD of several grape seed ($n = 9$) and poppy seed ($n = 3$) oil varieties grown in Austria.

from seeds of white grapes were analyzed with lower TC in comparison to virgin grape oils from seeds of red gapes. Identification of the present phenols will be part of further investigations in order to show which phenols are reacting with Trolox.

4. Conclusion

According to our analysis of volatile compounds, total phenols and antioxidant capacity based on TEAC of various grape seed oils, we can conclude that virgin grape seed oils produced from grapes of red wine production (''Merlot'', ''Cabernet-Sauvignon'' and ''Zweigelt'' of different denominations) contain a higher number of volatiles. Also the total phenol content was higher. Furthermore these oils were analyzed with a higher TEAC value compared to grape oils from grapes of white wine production (''Welschriesling'' and ''Chardonnay''). From the nutritional aspect based on measurements of total antioxidant capacity and total phenols, virgin grape oils produced from pomace of grapes from red wines are to be preferred. In contrast to volatiles and phenol content of grape seed oils, TAG profiles of these oils appeared to be very consistent between the different seed varieties, also independent of the producing conditions.

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