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ARTICLE

Phenolic profile and anti-inflammatory activity of sixteen Table Grape (*Vitis vinifera* L.) varieties

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Several studies have shown that fresh grape and its derivatives contains phenolic compounds having antioxidant and health promoting effects, particularly in relation to cardiovascular system. In this study, two methods were developed to characterize sixteen varieties of table and wine grapes: 1) a LC-MS method to identify major and minor phenolic compounds; and 2) a HPLC-DAD method to quantify the most representative compounds. Sixty-seven molecules belonging to different classes of phenolic compounds were identified: anthocyanins, flavan-3-ols, flavonols, stilbenes and organic acids. In parallel, the free radical scevnging activity and the anti-inflammatory activities of the 16 grape varieties were evaluated. The results showed a good correlation between total phenolic content and the biological activity. Extracts from Exalta and Albarossa grape varieties were the most active in reducing IL-8 release by gastric epithelial cells (IC_{50}=8.48 µg/mL and 6.68 µg/mL, respectively), a biomarker of inflammatory processes. The observed biological activities where mainly associated with skin and seed extracts/portions. The interest in studying table grapes and their non-fermented derivatives as source of healthy compounds has increased in the last years and our findings suggest that table grapes and their fresh derivatives, in addition to wine, could be involved in the health promoting effects of the Mediterranean diet.

Introduction

Grape (*Vitis vinifera* L.) is one of the most widely produced crops in the world, with approximately 75 million tons produced every year; about 41% are produced in Europe, 29% in Asia and 21% in the Americas. About 45% of grape production is used as such or fresh derivatives, while the remaining 55% is fermented for wine production.[1]

Since the first observations of the "French paradox",[2] wine has been extensively studied for its health promoting effects, particularly in relation to cardiovascular system. A crucial role of phenolic compounds in these positive properties has been also hypothesized. Nevertheless, the wine market has recently shown a decreased trend due to the frequent misuse/abuse of alcoholic beverages also in young people; this social problem was faced in December 2009 by WHO with the publication: "Strategies to reduce the harmful use of alcohol: draft global strategy".[3] This "new" market situation has stimulated a significant increase of interest in non-fermented products (in particular, table grapes), as a potential alternative source of phenolic compounds. It is notable that among vine products, up to 81% of not-fermented grapes are consumed as fresh grapes. Studies performed among consumers have shown an increased preference for seedless varieties, which are also more suitable for industrial processing. From a nutritional point of view, despite the relative high content of sugars (mean 15-18 g/100 g fresh weight) and calories (65 kcal/100 g fresh weight), grapes are good sources of manganese (3% RDA), potassium (11% RDA), vitamins B6 (3% RDA), B1 (6% RDA), and C (3% RDA).[4]

In addition to nutritional aspects, grapes are among the richest sources of polyphenols; among them, flavonoids are the most abundant with higher contribution by flavanols, flavonols and anthocyanins (red varieties). Grape skins and leaves contain anthocyanins and flavonol, whereas pulp and seeds contain mainly proanthocyanidins and organic acids. Flavonoids and non-flavonoid compounds are widely present in fruits and vegetables and it has been shown they can express a broad spectrum of beneficial properties for human health, including antioxidant and anti-inflammatory properties.[5]

Oxidative stress and inflammation are closely related factors for the induction of pathophysiological processes, one of which can be modulated by the other. As a consequence, these two conditions can be found at the same time in many pathological conditions, including metabolic syndrome and neurodegenerative disorders.

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Several studies have been performed to evaluate the modulatory properties of flavonoids on those inflammatory factors, which are responsible for different chronic diseases; however, only few studies have been focused on the effects of grapes and their derivatives on gastric inflammation.[6,7] Gastritis is one of the most common gastrointestinal inflammatory disorders, frequently associated with the bacterium *Helicobacter pylori*. During *H. pylori* infection, gastric epithelial cells show higher levels of cytokines including IL-1β, TNF-α, and IL-8, a potent chemokine playing a key role in gastric diseases. This response is highly dependent on the NF-κB activation, leading to the transcription of several pro-inflammatory mediators and to the worsening of the inflammatory conditions. The search for new compounds capable to modulate positively these mechanisms could prevent a prolonged inflammation state with positive effects on human health.

Polyphenols could represent good candidates for this purpose, but the effect on gastric inflammation of polyphenols from fresh grapes has not been investigated so far, even though data on grape seeds or skin extracts have showed promising applications.

On these bases, the objective of the present study was the chemical characterization by HPLC-DAD-ESI-MS^n of sixteen different grape varieties with the evaluation of their antioxidant potential and gastric anti-inflammatory activity. The results obtained with the whole fruit will be compared to those deriving from isolated skin and pulp to define the relative contribution to the biological properties and to identify the most promising varieties.

Materials and Methods

Materials

Acetonitrile, methanol, formic acid and water (LC-MS grade) were purchased from VWR International (Fontenay-sous-Bois, France). Folin-Ciocalteu's reagent, sodium carbonate and reagents used for biological assays were from Sigma Aldrich (Germany). The commercial standards at disposal were: flavonols (quercetin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide, kaempferol and kaempferol-3-*O*-glucoside), flavan-3-ols (epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, procyanidin B2), the stilbene resveratrol and trans-piceid; these standard as well as the reactive 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) were from Sigma-Aldrich (Steinem, Germany). Organic acids (caftaric acid, ferulic acid, chlorogenic acid and caffeic acid) and anthocyanins (delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and malvidin-3-*O*-glucoside) were from Extrasynthèse (Lyon, France). All standards were stored according to the supplier's instructions and then solubilized at concentration of 1 mg/mL in a water:methanol mixture (1:1, v/v), and diluted to a final concentration of 10 μg/mL and injected in the chromatographic system.

Samples

Thirteen table grapes (two red and four white seed-containing varieties, three red and three white seedless varieties), harvested in 2015, were included in this study. Three wine varieties were studied in parallel for comparison. Grape varieties and their origin are reported in Table 1.

Table 1. Grape varieties included in this study and their origin*

Typology	Cultivar	Origin
Red table grapes	"Pasiga" 157/16	CRA Conegliano Veneto, Italy
	"Red Flame" 155/19	CRA Conegliano Veneto, Italy
	"Red Globe"	Beja, Portugal
	"Beauty seedless" 155/7	CRA Conegliano Veneto, Italy
	"King's Ruby seedless" 157/20	CRA Conegliano Veneto, Italy
	"Nerona" 157/17	CRA Conegliano Veneto, Italy
Red wine grapes	"Nebbiolo"	CRA Asti, Italy
	"Barbera"	CRA Asti, Italy
	"Albarossa"	CRA Asti, Italy
White table grapes	"King's Husainy" B. 158/2	CRA Conegliano Veneto, Italy
	"Sugarone" B. 155/5	CRA Conegliano Veneto, Italy
	"Exalta" B. 155/3	CRA Conegliano Veneto, Italy
	"Sultanina" 156/5	CRA Conegliano Veneto, Italy
	"Canner Seedless" B. 155/10	CRA Conegliano Veneto, Italy
	"Centennial seedless" 155/13	CRA Conegliano Veneto, Italy
	"Sugarone"	Beja, Portugal

*A specimen voucher of each grape variety was labeled and stored at Department of Pharmacological and Biomolecular Sciences (University of Milan), Lab. Food Chemistry and Toxicology.

Samples preparation

Aliquots of 50 g of berries from each type of grape were weighed, homogenized and freeze-dried. All samples were maintained at -20°C till the analysis.

For spectrophotometric and chromatographic analysis, 0.4 g of each homogenate grape sample were mixed with 3 mL of a methanol:water (1:1, v/v) mixture, and sonicated for 15 minutes by using a ultrasonic bath; red and white grape samples were centrifuged for 15 minutes at 3000 and 8000 r.c.f. (relative centrifugal force), respectively. The supernatant was collected and filtered on 0.45 μm filters (Millipore, Billerica, MA, USA). Grape precipitates were extracted again with 2 mL of methanol:water mixture, the supernatants were combined and brought to volume (5 mL) with the same extraction mixture.

Total polyphenol content assay

Total polyphenol content was determined according to the Folin–Ciocalteu's method, as reported by Singleton and Rossi.[8] Aliquots of 300 μL prepared as described above were solubilized in 1 mL of a 50:50 (v/v) water:methanol solution, mixed in test tubes with 1.5 mL of Folin–Ciocalteu's reagent diluted 10 times, and 1.2 mL of 7.5% (w/v) sodium carbonate. After 30 min, the absorbances were measured at 765 nm in a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA).

The polyphenol content was calculated using a standard curve of gallic acid, and results were expressed as equivalents of gallic acid in mg/g.

HPLC-DAD-ESI-MS^n analysis

A HPLC method combined with electrospray ionization mass spectrometric (ESI-MS) and with Diode Array Detector (DAD) has been set up. The analytic platform was composed by a Surveyor LC system, which was connected to an LCQ Advantage mass spectrometer through a Finnigan IonMax electrospray ionization (ESI) source assembled with a high flow stainless steel emitter (Thermo Fisher Scientific, Rodano, MI, Italy). Full instrument control and data analysis were provided by Xcalibur software (version 2.0.7, Thermo Fisher Scientific, Rodano, MI, Italy). The chromatographic column was a Synergi 4u MAX-RP 80A (250x2.0 mm 4 μm) (Phenomenex, Torrance, CA, USA).

The analysis was performed using a linear gradient elution at a flow rate of 0.3 mL/min, where the elution phases had the following composition: A) water:acetonitrile:formic acid 96.99:3:0.01 (v/v/v); and B) acetonitrile:water:formic acid 50:49.99:0.01 (v/v/v). The gradient was programmed as follows: 0-15 min: 94-70 % A, 15-30 min: 70-50 % A, 30-35 min: 50-10 % A, 35-38 min: 10% A isocratic, 38-48 min: 10-94 % A.

The mass spectrometer operated in electrospray for negative ions (ESI^-) using nitrogen as a sheath gas. The capillary temperature was 275 °C, source voltage was 3.50 kV, sheath gas flow was 35 arb and sweep gas flow was 10 arbs. The collision energy, for MS^2 scans was 60 %.

Chromatograms were recorded at 200-800 nm. Different classes of phenolic compounds (anthocyanins, flavan-3-ols, proanthocyanidins, flavonols, stilbenes and phenolic acids) were identified by their UV spectra recorded with a DAD detector, by their MS spectra and their corresponding MS^n fragments. The class of phenolic acids and flavonoids identified were detected at 280-520 nm. More details are reported in the Electronic Supplementary Information.

Database matching. The following three different on-line databases were used: in order to identify the phenolic compounds under study: 1) "Phenol-Explorer", which contains information about approximately 500 polyphenols, available at http://phenol-explorer.eu; 2) "Metabolome", built from 6850 molecules, available at http://www.hmdb.ca and 3) "Massbank", which was helpful for comparing the fragmentation pattern of some phenol compounds. It was available at http://www.massbank.jp.[9–11]

Quantitative HPLC-DAD analysis. A HPLC method combined with a Diode Array Detector (DAD) has been set up and validated;[6] the method was applied to grape samples to quantify anthocyanins (delphinidin-3-*O*-glucoside, cyanindin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside) and other flavonoids (caftaric acid, rutin, hyperoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide, kaemferol-3-*O*-glucoside). In addition, recovery and stability of phenolic compounds were evaluated. The recovery of phenolic compounds was evaluated adding the matrix with the different class of phenolic compounds at three different concentrations (low, medium, high).[12] Short and long term stability of polyphenols was assessed at room temperature and at -20 ° C for 30 days.

Stock solutions of anthocyanins were diluted in methanol:water (50:50, v/v) at the final concentration of 100 μg/mL, and then diluted with 0.1 N HCl to obtain the working solutions at concentrations ranging from 0.1 to 12.5 (μg/mL).

The anthocyanin separation was performed using a HPLC equipment Thermo (San Josè, CA, USA). The instrument consisted of a pump (P2000, Thermo Separation products, Sam Josè, CA, USA), an interface (SN4000, Thermo Separation products, Sam Josè, CA, USA) UV detector (975-UV), a Diode Array Detector (6000 LP, Thermo Separation products, Sam Josè, CA, USA, an injection valve (Rheodyne, Cotati, CA, USA) with a 20 μL loop. The chromatographic column was a Synergi 4u MAX-RP 80A (250x4.60 mm 4 μm) (Phenomenex, Torrance, CA, USA) Iso-Disc Filters PTFE 0.45 μm were from Supelco Analytical (Bellefonte, PA, USA).

The analysis was performed using a gradient elution at a flow rate of 0.8 mL/min, where: A) water:acetonitrile:formic acid 87:3:10 (v/v/v); and B) acetonitrile:water:formic acid 50:40:10 (v/v/v). The gradient was set as follows: 0-15 min: 94-70%, A, 15-30 min: 70-50% A, 30-35 min: 50-10% A, 35-38 min: 10% A isocratic, 38-48 min: 10-94% A. The detection was at 520 nm.

Stock solutions of caftaric acid, rutin monohydrate, hyperoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide, kaemferol-3-*O*-glucoside, epicatechin (EC) and epicatechin-gallate (ECG) were prepared in methanol:water (50:50, v/v) at the final concentration of 200 μg/mL. Solutions were diluted with 0.1 N HCl in order to obtain working solutions with concentrations ranging from 0.1 to 50 (μg/mL). Finally, they were stored at -20 °C, till the analysis.

The analysis of this group of flavonoids was performed by using the same instrumentation described above but with a different chromatographic column (Synergi 4u MAX-RP 80A - 250x2.0 mm 4 μm- Phenomenex, Torrance, CA, USA).

The analysis was performed using the gradient elution described above, but detection was at 360 nm, apart from EC and ECG, detected at 280 nm.

Measurement of free radical scavenging capacity

DPPH assay The radical scavenging activity, in the paper indicated also with the term antioxidant acitivity (AOA), was assessed using 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH).[13,14] Aliquots of 1 mL of DPPH (Sigma Aldrich, Germany) solubilized in methanol (5 mg/100 mL) were mixed with 0.5 mL of each sample prepared as described above. The absorbance was measured after 30 minutes at 517 nm. The results were expressed as mg/g Trolox equivalents (TE).

Oxygen Radical Absorbance Capacity (ORAC) Assay. The assay was applied according to the method proposed by Ou et al. and modified by Davalos et al..[15,16] The assay was carried out using a Victor X3 plate reader (Perkin Elmer, USA) equipped with a fluorescence detector set at excitation and emission wavelengths of 484 and 528, respectively. Analyses were performed in phosphate buffer (pH 7.4, 75 mM). Peroxyl radicals were generated using AAPH (12 mM) and fluorescein (70 nM) was used as substrate. Trolox (6.25 – 50 μM) or samples

(400 μg/mL) were added and, after shaking, values were registered every minute for 120 min at 37°C. The area under the curve (AUC) was calculated by integrating the relative fluorescence curve. The net AUC was calculated by subtracting the AUC of the blank. The final ORAC values were determined from the linear regression equation of Trolox concentrations and expressed as μM Trolox/mL.

***In vitro* anti-inflammatory activity**

Preparation of the water extracts from grapes. Aliquots of 2 g of freeze-dried fruits (see methods) were extracted for four 4 hours with 20 mL of deionized water at room temperature, under stirring and dark conditions. Then, the mixture was filtered, and the recovered material was subjected to a further extraction for 16 hours with the same volume of deionized water under the same conditions. Then, the aqueous extracts were combined, frozen and immediately freeze-dried. The extracts were stored in aliquots at -20°C until the biological assays.

Skin, pulp and seeds from Exalta and Albarossa varieties were manually separated and extracted following the method previously described.

Cell culture. Human epithelial gastric cells (AGS) were grown in 75 cm^2 flask (Euroclone S.p.A, Pero, Italy) using DMEM F12 medium supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, 2 mM L-glutamine, and 10% heat-inactivated FBS (Euroclone S.p.A, Pero, Italy). AGS cells were incubated under a humidified atmosphere containing 5% CO_2.

Cytotoxicity assay. AGS cells were seeded in 24-well plates (30000 cells/well), after 72 hours they were co-treated with increasing concentrations of extract and the stimulus TNFα (10 ng/mL) for 6 hours. The integrity of cell morphology was analyzed before and after the treatment through microscopic inspection. Cell viability was measured at the end of the treatment by MTT method. Culture medium was removed from the plate and 200 μL of MTT solution was added in each well for 45-60 minutes. The absorbance was read at 550 nm using a microplate reader (iMarkTM Microplate Absorbance Reader, Bio-Rad). No evidences of cytotoxicity were observed in AGS cells treated for 6 hours with the extracts at the concentrations used for testing biological activity.

IL-8 release measurement. AGS cells were seeded in 24-well plates (30000 cells/well), after 72 hours they were co-treated with the extract (5-100 μg/mL) and the stimulus TNFα (10 ng/mL) for 6 hours. IL-8 released in the culture medium was measured through the ELISA Development Kit (Peprotech Inc., London, UK). Corning 96 well EIA/RIA plates from Sigma-Aldrich (Milan, Italy) were coated with the antibody provided in the ELISA Kit and incubated overnight at room temperature. After blocking the reaction with 1% albumin solution, each sample (200 μL) was added into wells for 2 hours at room temperature. The amount of IL-8 was detected by spectrophotometry (λ: 450 nm, 0.1 s) using biotinylated and streptavidin–HRP conjugate antibodies, and evaluating the 3,3',5,5'-tetramethylbenzidine (TMB) substrate reaction. Quantification of IL-8 contained in each sample was performed using an optimized standard curve supplied in the ELISA Kit (8.0 – 1000.0 pg/mL). EGCG 20 μM was used as reference inhibitor of IL-8 release.

Biological assays were performed in triplicate.

Statistical analysis. All data are expressed as mean ± SD of at least three independent assays performed in duplicate; data were analyzed by unpaired one-way analysis of variance (ANOVA) followed by Bonferroni as post-hoc test. Statistical analysis was done using GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). *$p < 0.05$ was considered statistically significant. IC50 was calculated using GraphPad Prism 6.00 software.

RESULTS AND DISCUSSION

Total polyphenol content

Total phenolic content (TPC) in red and white grape samples ranged, respectively, between 0.44 ±0.02 and 7.94 ±0.19 mg gallic acid equivalents/g of fresh weight (Figure 1). These values are in agreement with data from scientific literature, where a TPC content between 0.70 and 5.7 mg gallic acid equivalents/g is reported.[17–19] As expected, TPC of red wine varieties was higher than that from red table grapes: Albarossa showed the highest polyphenol content (7.94 ± 0.19 mg/g) followed by Barbera and Nebbiolo (4.08 ± 0.25 and 3.85 ± 0.07, respectively).

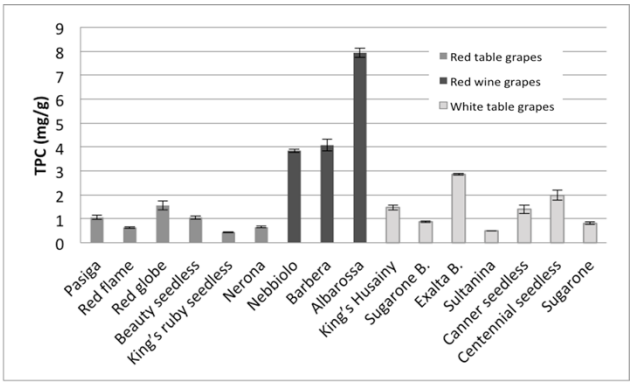

Figure 1. TPC (mg/g of gallic acid equivalent) in grape samples.

Phytochemical characterization of grapes by HPLC-DAD-ESI-MS^n and HPLC-DAD

Grapes are characterized by a complex polyphenol profile. Due to the similar spectral characteristic of individual compounds belonging to the same subclasses and the limited commercial availability of reference compounds, HPLC coupled to mass spectrometry was the strategic tool for the complete characterization of samples.

The phenolic profile of sixteen varieties of grapes (including red and white varieties) has been investigated by HPLC-DAD-ESI-MS^n. This method allowed the identification of 25 anthocyanins, 18 flavan-3-ols, 18 flavonols, 3 stilbenes and 3 organic acids. Table 2 shows the distribution of polyphenol classes and their relative abundance in the individual varieties included in the study.

The specific LC-MS data of the identified compounds are reported in the Electronic Supplementary Information (Tables

1S, 2S, 3S, 4S). In addition, HPLC-DAD and MS/MS spectra of one red (Albarossa) and one white (Exalta) grapes are reported in Figures 1S and 2S, respectively.

Considering anthocyanins, 25 compounds were separated by LC-MS: five of them were identified as the 3-*O*-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin. Additionally, 20 further compounds were detected and identified as acylated (including acetyl, coumaroyl and caffeoyl esters) or di-glucoside anthocyanins, in agreement with previous studies.[20] Generally, wine varieties showed the highest

Table 2. Classes of polyphenols and their relative abundance in grapes samples

Anthocyanins																	
Typology		**Red table grapes**						**Red wine grapes**			**White table grapes**						
Nr.	**/ Samples**	**PS**	**RF**	**RG**	**BEs**	**KRs**	**NR**	**NB**	**BR**	**AL**	**KH**	**SC**	**EX**	**SL**	**CAs**	**CEs**	**SG**
1	*Malvidin-3-O-glucoside*	++	++		+++	++	++	+++	++	+++							
2	*Malvidin-caffeoyl-hexoside*	+++			+++	+	+++			+++							
3	*Malvidin-caffeoyl-hexoside*	++			++		++	++		+++							
4	*Malvidin-cumaroyl-hexoside*	+++			+++	+	+++	+++	+++	+++							
5	*Malvidin-cumaroyl-hexoside*	++			++		++	++	++	++							
6	*Malvidin-acetyl-hexoside*	+++			+++		+++	++	+++	+++							
7	*Malvidin-acetyl-hexoside*	++			++				++	++							
8	*Delphinidin-3-O-glucoside*							+	+++	+++							
9	*Delphinidin-coumaroyl-hexoside*	+							+++	+++							
10	*Delphinidin-caffeoyl-hexoside*		+++					+									
11	*Delphinidin-caffeoyl-hexoside*		+++					+									
12	*Delphinidin-acetyl-hexoside*								+++								
13	*Cyanidin-3-O-glucoside*			+		++		+++	+++	+++							
14	*Cyanidin-coumaroyl-hexoside*								+++	+++							
15	*Cyanidin-coumaroyl-hexoside*		+						++								
16	*Petunidin-3-O-glucoside*	+	+			++	+	++	+++	+++							
17	*Petunidin-coumaroyl-hexoside*	+++					+++		+++	+++							
18	*Petunidin-caffeoyl-hexoside*	+++								+++							
19	*Petunidin-caffeoyl-hexoside*									+							
20	*Petunidin-acetyl-hexoside*								+++								
21	*Peonidin-3-O-glucoside*	+++	+++	++	+++	+++	+++	+++	+++	++							
22	*Peonidin-coumaroyl-hexoside*	+++			+++	+++	+++	+++	+++	+++							
23	*Peonidin-coumaroyl-hexoside*	++					++	++	++	++							
24	*Peonidin-caffeoyl-hexoside*						+++										
25	*Peonidin-acetyl-hexoside*							+++	+++								
Flavan-3-ols																	
26	*C (catechin)*	+		+++				+++	+++	+++	+++	++	+++		++		++
27	*EC (epicatechin)*			+++				+++	+++	+++	++	++	++				+
29	*ECG (epicatechin gallate)*			++						+++							
31	*Proanthocyanidin dimer*	+++	+++	+++	++	++	++	++	+++	++	+++	+++	+++	++	+++	+++	+++
32	*Proanthocyanidin dimer*			++	++	+		++	++	+	+		+		+		+
33	*Proanthocyanidin dimer*			++				+++			+++	++					+++
34	*Procyanidin B2 dimer*		++	++				+++	+++	+++	++	++	++				+++
35	*Proanthocyanidin dimer (-gallate)*	++		+				+	++	+++			+++		++	++	
36	*Proanthocyanidin dimer (-gallate)*	+++	+++	+++				+++	++	+++			+++		+++	++	
37	*Proanthocyanidin dimer (-gallate)*									+						+	
38	*Proanthocyanidin dimer (-gallate)*			+				+	+	++							
39	*Proanthocyanidin trimer*	++	+	+++	++	++	+	+		++	++	+	++		+	++	+
40	*Proanthocyanidin trimer*					+	++								+	+	
41	*Proanthocyanidin trimer*	+++	+++	+++	+++	+++	+++	++	+++	++	+++	+++	+++	+++	+++	+++	
42	*Proanthocyanidin trimer*	++	+	++			+	+		+	++	+	+		++		
43	*Proanthocyanidin trimer*	+++	++	+++				+++	+++	+++	+++	+++	+++	+	++	++	
Flavonols																	
44	*Quercetin*							+							++	+	
45	*Quercetin-3-O-galactoside*	+	+	+	+	+	+	+	+	+		+		+	+	+	+
46	*Quercetin-3-O-glucoside*	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+++	+++	+++
47	*Quercetin-3-O-glucuronide*	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	++
48	*Quercetin-3-O-rutinoside*	++	+++	+			+	+++	++	+		+			+++	+	
49	*Kaempferol*							++							+++	+	
50	*Kaempferol-galactoside*		++					++	+	+					++	+	+
51	*Kaempferol-3-O-glucoside*		+++	+	++			+++	++	++					+++	++	++
52	*Kaempferol-glucuronide*		++					+							+++	++	
53	*Kaempferol-rutinoside*		+++												+++		
54	*Isorhamnetin-hexoside*	++	++		++	++	++	+++	+	+	++	+++	+		+	+	+++
55	*Trihydroxyflavone-riboside*	+	+		++		+				+	+		+		+++	+++
56	*Trihydroxyflavone-riboside*	+++	+		+++	+++	+++	+	++	+++	+++	+++	++	+++	+	+++	+++
57	*Trihydroxyflavone-hexoside*				++							++	+			+	++
58	*Syringetin-hexoside*	+++			+++	++	+++	++	+++	+++							
59	*Myricetin-hexoside*	++	++		++	++	++	+++	++	++							
60	*Myricetin-hexoside*	+++							+++	+++							

61	*Dimethylquercetin-hexoside*	+++			+++	+++	+++	+++	+++	+++							

Table 2. Classes of polyphenols and their relative abundance in grapes samples (*continue*)

Stilbenes and organic acids																	
Typology		**Red table grapes**						**Red wine grapes**			**White table grapes**						
Nr. / Samples		**PS**	**RF**	**RG**	**BEs**	**KRs**	**NR**	**NB**	**BR**	**AL**	**KH**	**SC**	**EX**	**SL**	**CAs**	**CEs**	**SG**
62	*trans*-Resveratrol																
63	*cis*-Resveratrol			+	++			+									
64	*trans*-Piceid								+++	+++					+	+	
65	Caftaric acid	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
66	*trans*-coutaric acid								+	++							
67	*cis*-coutaric acid								++	+					++		+

PA: Pasiga; **RF**: Red Flame; **RG**: Red Globe; **Bes**: Beauty seedless; **KRs**: King's Ruby seedless; **NE**: Nerona; **NB**: Nebbiolo; **BR**: Barbera; **AL**: Albarossa; **KH**: King's Husainy; **SC**: Sugarone (from Conegliano); **EX**: Exalta; **SL**: Sultanina; **CAs**: Canner Seedless; **CEs**: Centennial seedless; **SG**: Sugarone (from Beja)

+++ = high relative abundance; **++** = medium relative abundance; **+** = low relative abundance

relative abundance of anthocyanin compounds. In all grapes analyzed (table and wine varieties), the most representative anthocyanins were peonidin-glucoside and malvidin-3-*O*-glucoside; the former was detected in all red varieties, whereas malvidin-3-*O*-glucoside was present in all samples except for Red Globe. Flavan-3-ols dimers and trimers were quite abundant and equally distributed in all grape samples: flavan-3-ols monomers were less present; only catechin, epicatechin and epigallocatechin monomers were identified. Proanthocyanidin dimers showed a relative high abundance in all grape samples, while proanthocyanidin trimers were significant in all grape varieties except for Sugarone (from Portugal). Apart from proanthocyanidin trimers, a similar flavan-3-ols pattern was observed in Sugarone grapes coming from two different countries (Italy and Portugal).

In red table grapes the most common flavonols were quercetin galactoside, quercetin glucoside, quercetin glucuronide and quercetin rutinoside as reported previously.[17,21] A similar pattern was observed in the red wine grapes, which were rich in flavonol compounds. Generally speaking, the white varieties showed the lowest relative abundance in flavonols and the distribution of these phenolics was quite homogeneous, being quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide and trihydroxyflavone-riboside the most abundant.

As for stilbenes, resveratrol was found only in its cis-isomer form and only in a small number of red varieties: in red grapes, it was identified only in Red Globe, Beauty Seedless and Nebbiolo varieties. Apart from Nebbiolo, all wine varieties showed a high abundance of trans-piceid, which was found also in white table grapes Canner Seedless and Centennial Seedless, but not in Sugarone (from Portugal). This variability is in agreement with the scientific literature, which reports controversial data regarding the content of resveratrol, piceid and their isomers.[21] Only few studies investigated the resveratrol and piceid content in non-fermented grape beverages. One of them reported that the content of resveratrol glycosides (piceid) in berry skins was from two to four times higher than that of the aglycone forms, and that it was more soluble during juice extraction due to their sugar moiety.[22,23]

Finally, caftaric acid (an organic acid) was present in all samples, as reported previously.[17] Trans- and cis-coutaric were detected only in wine grapes, apart from Nebbiolo.

After the first screening, the most abundant compounds were quantified by HPLC-DAD (Tables 3-4). The method was previously validated according to Di Lorenzo et al.[5,12]

In addition, recovery and stability of the phenolic compounds were assessed according to FDA recommendations.[12] Recovery was always >90%, and the percentage of variation after short and long-term stability evaluation was always within ±15%. A significant difference between wine and table grapes varieties was found in terms of delphinidin-3-*O*-glucoside content, which was particularly abundant only in wine grapes. Malvidin-3-*O*-glucoside was the most abundant in the table grape varieties Pasiga (41.87±1.25 μg/g), King's Ruby (24.00±0.49 μg/g), Nerona (71.70±2.71 μg/g), Beauty Seedless (141.68±5.63 μg/g) and in wine cultivar Albarossa (1067.40±34.66 μg/g) and Barbera (653.78±12.55 μg/g).

Table 3. Anthocyanin content (μg/g) of red grape samples (mean±SD)

Samples	**DP (μg/g) Mean±SD**	**CY (μg/g) Mean±SD**	**PT (μg/g) Mean±SD**	**PO (μg/g) Mean±SD**	**MV (μg/g) Mean±SD**
Pasiga	1.27 ±0.06	1.80 ±0.01	3.69 ±0.10	5.65 ±0.28	41.87 ±1.25
Red Flame	2.76 ±0.31	14.05 ±1.11	2.51 ±0.17	9.17 ±0.43	6.83 ±0.33
Red Globe	8.94 ±0.06	30.61 ±0.32	7.49 ±0.04	39.34 ±1.80	16.83 ±0.50
Beauty seedless	6.90 ±0.13	3.10 ±0.08	20.92 ±0.81	19.06 ±1.73	141.68 ±5.63
King's Ruby	4.20 ±0.34	5.77 ±0.09	6.16 ±0.12	13.18 ±0.15	24.00 ±0.49
Nerona	1.48 ±0.05	2.26 ±0.05	4.07 ±0.22	21.08 ±1.00	71.70 ±2.71
*Nebbiolo**	26.35 ±1.17	65.92 ±1.48	31.44 ±1.58	202.00 ±12.38	142.46 ±5.75
*Barbera**	325.54 ±9.15	135.19 ±2.64	322.50 ±3.00	104.51 ±2.82	653.78 ±12.55
*Albarossa**	433.69 ±6.96	161.03 ±2.33	472.84 ± 8.37	123.80 ±0.80	1067.40 ±34.66

DP: delphindin-3-*O*-glucoside; **CY:** cyanidin-3-*O*-glucoside; **PT:** petunidin-3-*O*-glucoside; **PO:** peonidin-3-*O*-glucoside; **MV:** malvidin-3-*O*-glucoside

* red wine grapes

Table 4. Flavonoid content (μg/g) of grape samples (μg/g fresh weight) (mean±SD)

	Samples	Caftaric acid (μg/g) Mean ±SD	Rutin (μg/g) Mean ±SD	Hyperoside (μg/g) Mean ±SD	Quercetin-3-*O*-Glucoside (μg/g) Mean ±SD	Quercetin-3-*O*-Glucuronide (μg/g) Mean ±SD	Kaempferol-3-*O*-glucoside (μg/g) Mean ±SD	EC (μg/g) Mean ±SD	ECG (μg/g) Mean ±SD	Total[a] (μg/g) Mean ± SD
Red table grapes	*Pasiga*	14.89 ±0.36	N.D.§	N.D.°	8.35 ±0.28	N.D.#	0.28 ±0.06	-	-	23.52±0.46
	Red flame	5.91 ±0.12	1.71 ±0.04	1.54 ±0.01	16.44 ±0.20	4.45 ±0.12	2.77 ±0.09	-	-	32.82 ±0.28
	Red globe	10.54 ±1.06	1.49 ±0.09	1.60 ±0.10	14.87 ±0.76	10.01 ±0.44	1.58 ±0.09	-	-	40.09 ±1.39
	Beauty seedless	10.93 ±0.46	N.D.§	0.06 ±0.02	5.82 ±0.05	6.56 ±0.24	4.21 ±0.06	-	-	27.58 ±0.53
	King's ruby seedless	4.59 ±0.16	N.D.§	N.Q.°	0.79 ±0.03	0.417 ±0.003	0.14 ±0.002	-	-	5.94 ±0.16
	Nerona	7.63 ±0.19	N.D.§	N.D.°	1.33 ±0.01	2.48 ±0.05	0.49 ±0.01	-	-	11.93 ±0.20
Red wine grapes	*Nebbiolo*	17.90 ±0.55	1.60 ±0.18	6.02 ±0.39	48.08 ±2.80	8.39 ±0.91	8.02 ±0.42	-	-	90.01 ±3.05
	Barbera	N.D.^	1.14 ±0.09	2.42 ±0.35	23.94 ±1.85	21.05 ±1.27	1.35 ±0.09	-	-	49.90 ±2.27
	Albarossa	N.D.^	N.D.§	N.D.°	12.88 ±1.39	6.69 ±0.19	0.83 ±0.08	-	-	20.4 ±1.41
White table grapes	*King's Husainy*	7.22 ±0.05	N.D.§	N.Q.°	4.00 ±0.03	4.23 ±0.06	0.17 ±0.02	23.99 ±0.36	N.D.***	39.61 ±0.37
	Sugarone B.	4.05 ±0.18	N.D.§	N.D.°	2.25 ±0.02	0.75 ±0.03	0.53 ±0.01	65.58 ±0.24	N.D.***	73.16 ±0.30
	Exalta B.	7.69 ±0.08	N.D.§	N.Q.°	1.70 ±0.04	5.93 ±0.01	N.D.*	N.D.**	34.50 ±6.10	49.82 ±6.10
	Sultanina	9.47 ±0.78	N.D.§	N.Q.°	0.12 ±0.003	1.34 ±0.002	N.D.*	N.D.**	53.87 ±1.06	64.80 ±1.32
	Canner seedless	14.21 ±0.73	2.29 ±0.07	8.12 ±0.27	63.80 ±1.46	21.61 ±0.06	21.94 ±0.30	19.41 ±2.19	N.D.***	151.38 ±2.76
	Centennial seedless	15.36 ±0.07	0.54 ±0.01	0.92 ±0.03	9.65 ±0.48	20.67 ±0.98	1.07 ±0.06	50.75 ±4.40	N.D.	98.96 ±4.53
	Sugarone	9.03 ±0.49	N.D.§	1.03 ±0.05	9.87 ±0.34	N.D.#	1.22 ±0.09	72.31 ±3.15	N.D.***	93.46±3.21

[a] Total content of phenol compounds measured by HPLC; ^ < LOD (43.5 ng/mL); § < LOD (4.3 ng/mL); °< LOD (3.3 ng/mL); # < LOD (4.9 ng/mL); * < LOD (4.0 ng/mL); **< LOD 0.012 ng/mL; *** <LOD 0.009 ng/mL

Cyanidin-3-*O*-glucoside and petunidin-3-*O*-glucoside were the less abundant anthocyanins both in table and wine grape varieties. Compared to other table grapes, Red Flame was the variety showing a lower anthocyanin content.

Excluding anthocyanins, phenolic compound content ranged between 5.94 (King's Ruby seedless) and 151.38 μg/g (Canner seedless) (Table 4). Total phenol content measured by HPLC was lower than TPC measured by Folin-Ciocalteu's assay. This difference can be due to: 1) the aspecific nature of Folin-Ciocalteu's assay, affected by some interfering molecules such as sugars, ascorbic acid, aromatic amines, leading to a possible overestimation of the total phenol content; 2) other compounds could contribute to the total phenol content, as in the case of procyanidins dimers and trimers detected by LC-MS analysis, which were relatively abundant in some white varieties (e.g. Exalta and Canner seedless, see Table 2). Unfortunately, pure compounds to quantify these molecules are not commercially available.

Figure 2 represents HPLC-DAD and MS/MS spectra of the four most representative phenolic compounds: A) Epicatechin B) Procyanidin B_2; C) Malvidin-3-*O*-glucoside; D) Quercetin. Generally speaking, flavonols and flavan-3-ols were the most abundant compounds. Quercetin-3-glucuronide and epicatechin were the most frequently detected molecules, which were present mainly in white table grapes and, in particular, in the seedless varieties Canner and Centennial.

Kaempferol-3-*O*-glucoside was equally distributed in red and white table grapes (0.14-4.21 μg/g and 0.17-1.22 μg/g, respectively). Only exception was the seedless variety Canner, where the concentration was significantly higher (21.94 ± 0.30 μg/g).

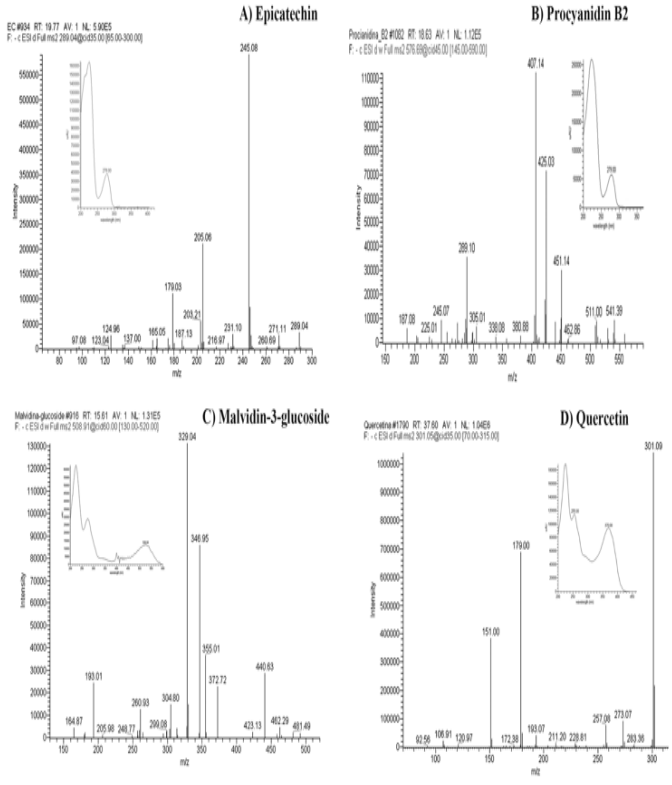

Figure 2. HPLC-DAD and MS/MS spectra of the four most representative phenolic compounds: A) Epicatechin B) Procyanidin B2; C) Malvidin-3-glucoside; D) Quercetin More details are reported in the Electronic Supplementary Information, Tables 2S.

Significant amount of ECG was present only in two white table grape samples, Sultanina and Exalta (53.87 µg/g and 34.50 µg/g, respectively), while in the other varieties this compound was not detectable (< LOD 0.012 and 0.009 µg/mL for EC and ECG, respectively). In red varieties flavan-3-ols monomers were not quantified due to interference with anthocyanins.

Free radical scavenging capacity

DPPH assay (Trolox equivalents). Figure 3 shows the free radical scavenging capacity measured in red and white grape samples (fresh fruit). The red wine varieties showed the highest free radical scavenging activity (10.92 ± 0.08; 7.45 ± 0.08; 4.88 ± 0.18 mg/g
for Albarossa, Barbera and Nerona, respectively), followed by the white grape Exalta (3.41 ± 0.04 mg/g). Among white varieties, even Centennial seedless and King's Husainy showed a significant antioxidant activity (1.51 ± 0.01 mg/g and 1.24 ± 0.01 mg/g, respectively), higher than some red varieties. The lowest content was found in the white table grape Sultanina (0.31 ± 0.002 mg/g) and in the red table grape King's Rubyseedless (0.31 ± 0.001 mg/g). Samples with the highest content of phenolic compounds showed the highest antioxidant activity; these parameters were highly correlated (R^2>0.95) (not shown).

ORAC assay. Results obtained by ORAC assay are reported in Figure 3, where AUC obtained analyzing grape samples (400 µg/mL) are compared with the positive control (Trolox 24 µM). The red wine varieties Albarossa and Barbera showed the highest free radical scavenging activity, followed by the red grape Beauty Seedless and the white grape Canner Seedless. In addition, Centennial, Sultanina and Exalta showed a significant antioxidant potential. The results do not totally match with data from DPPH assay; in fact, among white varieties Exalta and King's Husainy were the most active for their antioxidant capacity. These differences could be due to the different chemical reactions involved in the two tests. Moreover, many antioxidants that react quickly with peroxyl radicals (ORAC) can react slowly or even be inert to DPPH due to steric inaccessibility.[12]

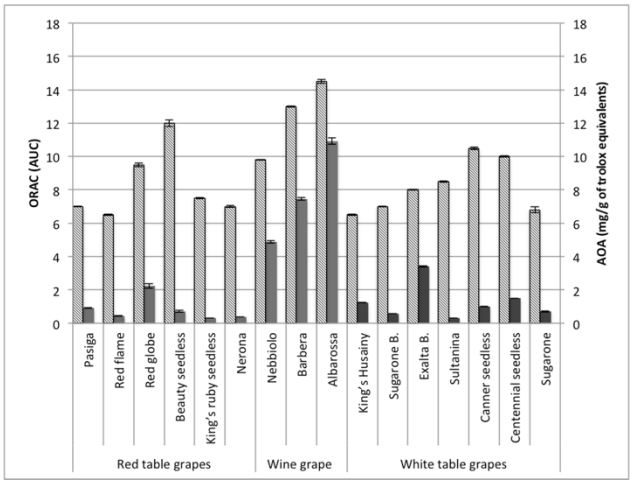

Figure 3. DPPH radical scavenging activity (AOA, mg/g Trolox equivalents) and ORAC values (AUC) of grape samples obtained analysing grapes at 400 µg/mL

***In vitro* anti-inflammatory activity**

Effect on the TNFα-induced IL-8 release in human epithelial gastric cells. The sixteen grape water extracts were tested for their ability to inhibit TNFα-induced IL-8 release, a chemokine widely involved in the gastric inflammation. Screening was performed in AGS cells at the concentration of 50 µg/mL. Among white grapes included in the study, Exalta showed the highest inhibitory activity whereas Albarossa was the most active among red grapes. The extracts (50 µg/mL) were able to decrease IL-8 release of about 85% and 75%, respectively (Figure 4).

On the basis of these findings, AL and EX water extracts were

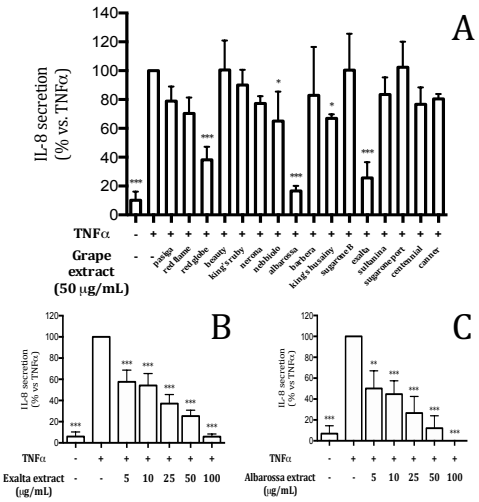

Figure 4. A: Effect of sixteen grape water extracts on the TNFα-induced IL-8 release. AGS cells were treated for 6 h with the stimulus TNFα (10 ng/mL) and the extract at the concentration of 50 µg/mL. IL-8 released in the culture medium was measured with an ELISA assay. The graphs show the means ± s.d. of at least 3 experiments performed in duplicate. *p < 0.05, ***p < 0.001 vs. TNFα alone. According to the literature, EGCG 20 µM was used as the reference inhibitor of IL-8 release. Effect of Exalta (B) and Albarossa (C) grape extracts (whole fruit) on the TNFα-induced IL-8 release. AGS cells were treated for 6 h with the stimulus TNFα (10 ng/mL) and the extract at different concentrations (5-100 µg/mL). IL-8 released in the culture medium was evaluated with an ELISA assay. The graphs show the means ± s.d. of at least 3 experiments performed in duplicate. **p < 0.01, ***p < 0.001 vs. TNFα alone. According to the literature, EGCG 20 µM was used as the reference inhibitor of IL-8 release.

selected for further studies. In order to study more deeply the inhibitory activity on IL-8 release, assays were performed using AGS cells with increasing concentrations of the extracts (5 - 100 µg/mL) in the presence of TNFα for 6 hours. Biological assays did not show significant variability between experiments. As shown in Figure 4, both Exalta and Albarossa showed great inhibitory effect on the TNFα-induced IL-8 release, with low IC50s (8.48 µg/mL and 6.68 µg/mL for Exalta and Albarossa, respectively).

Contribution of each part of the fruit to the IL-8 inhibitory effect in AGS cells. The next step of the study was to investigate the contribution of the different parts of Exalta and Albarossa grapes (skin, pulp and seeds) to the inhibitory activity observed with the whole fruit. Exalta and Albarossa from skin, pulp and seeds were prepared as reported in Materials and Methods section.

As shown in Figure 5, extract from skin and seeds of Exalta and Albarossa determined a dose dependent inhibition of TNFα-induced IL-8 secretion. Extract from Exalta skin inhibited the IL-8 release with an IC_{50} of about 5-fold lower than that from Albarossa skin (IC_{50}: 9.77 μg/mL and 51.47 μg/mL respectively), while the IC_{50} of extract from Exalta seeds was about 2-fold higher than that from Albarossa seeds (IC_{50}: 8.31 μg/mL and 4.58 μg/mL respectively). These results could be partially explained by the high amounts of anthocyanins in Albarossa skin (Table 3) and flavonols in Exalta, seeds (Table 2 and Table 4). Both anthocyanins and flavonols (mainly procyanidins) showed anti-inflammatory activity in previous studies, with the inhibition of NF-kB pathway in a concentration-dependent pattern.[24,25] Relative abundance of procyanidins and flavan-3-ols dimer and trimers showed by LC-MS analysis supports the hypothesis of a role of these compounds in the observed biological activity. Even though Exalta is a seedless variety, it contained little undeveloped seeds, probably deriving from the not complete partenocarpic process. This characteristic could contribute to the particular abundance of flavonols in Exalta grape.

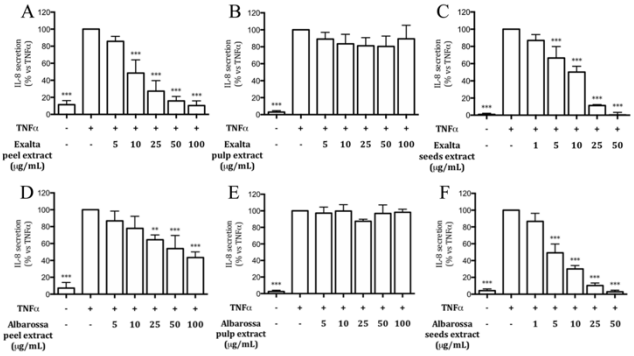

Figure 5 Effect of skin, pulp and seeds extract from Exalta (A-B-C) and Albarossa (D-E-F) cultivar on the TNFα-induced IL-8 release. AGS cells were treated for 6 h with the stimulus TNFα (10 ng/mL) and increasing concentrations of each extract (5-100 μg/mL). IL-8 released in the culture medium was evaluated with an ELISA assay. The graphs show the means ± s.d. of at least 3 experiments performed in duplicate. **p < 0.01, ***p < 0.001 vs. TNFα alone. According to the literature, EGCG 20 μM was used as the reference inhibitor of IL-8 release.

No effect on TNFα-induced IL-8 release was observed with extracts from Exalta and Albarossa pulp, even at the highest concentration tested (100 μg/mL).

Conclusions

The aim of the study was the chemical characterization of different *Vitis vinifera* L. (grape) varieties and the measure of the relative free radical scavenging activity and anti-inflammatory activities.

The LC-MS analysis allowed a preliminary characterization of samples included in the study, with the identification of 67 phenolic compounds including anthocyanins, flavan-3-ols, flavonols, stilbenes and organic acids. After the screening analysis, the most abundant compounds were quantified by HPLC-DAD method.

Among anthocyanins, malvidin-3-*O*-glucoside and peonidin-3-*O*-glucoside were the most abundant compounds in red grape samples. Proanthocyanidins were identified by MS^n fragmentation; among them, dimers and trimers of flavan-3-ols were the most abundant in table grape varieties containing seeds. With regards to flavonols, quercetin and kaempferol derivatives were widely distributed in all varieties analysed. Among the water extracts tested, those from the wine variety Albarossa and the table grape Exalta inhibited significantly TNFα-induced IL-8 secretion and expression in human gastric epithelial cells. In agreement to these data, Albarossa and Exalta were particularly rich in polyphenols (7.94±0.19 and 2.86±0.045 mg/g, respectively). Taking into consideration the results obtained from the chromatographic analysis, a significant contribution to the high polyphenol content of Exalta could be due to the high presence of procyanidin dimers and trimers (as shown in Table 2). The most active grapes were also among the varieties with the highest antioxidant potential (measured by DPPH assay). As for the anti-inflammatory activity, grape varieties can be classified from the most active to the least active in the following way: Albarossa >Exalta>Red Globe>Nebbiolo>King's Husainy. For the free radical scavenging activity the rank was: Albarossa>Barbera>Nebbiolo>Exalta>Red Globe. This correlation between the two biological assays suggests that active components in grapes could counteract the oxidative stress occurring during inflammatory process *in vivo.* Furthermore, the free radical scavenging capacity could be part of the mode of action of the observed anti-inflammatory activity. However, further studies are needed to investigate this aspect in more detail.

Considering the mean serving of grape in Mediterranean countries corresponding to 200 g, it is possible to calculate that the active dose observed in *in vitro* test can be easily reached at gastric level, also taking into consideration the dilution operated by gastric fluids (about 20 mL). The effect at gastric level observed in *in vitro* test can be reasonably extrapolated at the *in vivo* situation, since no metabolic changes of active molecules is done at this level.

The results described in this paper show that, when consumed regularly, some varieties of table grapes could contribute significantly to the intake of active molecules and in particular of phenolic derivatives, which could counteract oxidative stress and modulate inflammatory processes in the stomach, a district so frequently involved in inflammation.

Conflicts of interest

There are no conflicts to declare

Acknowledgements

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Notes and references

1 FAO-OIV, *FAO-OIV FOCUS 2016 Table and dried grapes*, *FAO-OIV FOCUS 2016 Table and dried grapes*, 2016.

2 S. Renaud and M. de Lorgeril, Wine, alcohol, platelets, and the French paradox for coronary heart disease, *Lancet*, 1992, **339**, 1523–1526.

3 World Health Organization, World Health Statistics 2 0 0 9, *World Heal. Stat.*, 2009, **1**, 29.

4 The Commission of European Communities, COMMISSION DIRECTIVE 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions, *Off. J. Eur. Union*, 2008, **L 285/9**, 9–12.

5 C. Di Lorenzo, M. Dell'agli, E. Colombo, E. Sangiovanni and P. Restani, Metabolic syndrome and inflammation: a critical review of in vitro and clinical approaches for benefit assessment of plant food supplements., *Evid. Based. Complement. Altern. Med.*, 2013, **2013**, 782461.

6 E. Sangiovanni, C. Di Lorenzo, E. Colombo, F. Colombo, M. Fumagalli, G. Frigerio, P. Restani and M. Dell'Agli, The effect of in vitro gastrointestinal digestion on the anti-inflammatory activity of Vitis vinifera L. leaves, *Food Funct.*, 2015, **6**, 2453–2463.

7 V. Georgiev, A. Ananga and V. Tsolova, Recent advances and uses of grape flavonoids as nutraceuticals, *Nutrients*, 2014, **6**, 391–415.

8 V. L. Singleton, J. A. Rossi Jr. and Rossi J A Jr., Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic.*, 1965, **16**, 144–158.

9 V. Neveu, J. Perez-Jiménez, F. Vos, V. Crespy, L. du Chaffaut, L. Mennen, C. Knox, R. Eisner, J. Cruz, D. Wishart and A. Scalbert, Phenol-Explorer: an online comprehensive database on polyphenol contents in foods., *Database (Oxford).*, 2010, **2010**, 1–9.

10 M. Arita and K. Suwa, Search extension transforms Wiki into a relational system: A case for flavonoid metabolite database., *BioData Min.*, , DOI:10.1186/1756-0381-1-7.

11 H. Horai, M. Arita, S. Kanaya, Y. Nihei, T. Ikeda, K. Suwa, Y. Ojima, K. Tanaka, S. Tanaka, K. Aoshima, Y. Oda, Y. Kakazu, M. Kusano, T. Tohge, F. Matsuda, Y. Sawada, M. Y. Hirai, H. Nakanishi, K. Ikeda, N. Akimoto, T. Maoka, H. Takahashi, T. Ara, N. Sakurai, H. Suzuki, D. Shibata, S. Neumann, T. Iida, K. Tanaka, K. Funatsu, F. Matsuura, T. Soga, R. Taguchi, K. Saito and T. Nishioka, MassBank: A public repository for sharing mass spectral data for life sciences, *J. Mass Spectrom.*, 2010, **45**, 703–714.

12 U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, Bioanalytical Method Validation Guidance, *Biopharmaceutics*, 2018, 25.

13 Brand-Williams, M. E. Cuvelier and C. Berset, Use of a Free Radical Method to Evaluate Antioxidant Activity, *Food Sci. Technol.*, 1995, **28**, 25–30.

14 L. P. Leong and G. Shui, An investigation of antioxidant capacity of fruits in Singapore markets, *Food Chem.*, 2002, **76**, 69–75.

15 B. Ou, M. Hampsch-Woodill and R. L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe, *J. Agric. Food Chem.*, 2001, **49**, 4619–4626.

16 A. Dávalos, C. Gómez-Cordovés and B. Bartolomé, Extending Applicability of the Oxygen Radical Absorbance Capacity (ORAC-Fluorescein) Assay, *J. Agric. Food Chem.*, 2004, **52**, 48–54.

17 E. Cantos, J. C. Espín and F. A. Tomás-Barberán, Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS, *J. Agric. Food Chem.*, 2002, **50**, 5691–5696.

18 C. Aubert and G. Chalot, Chemical composition, bioactive compounds, and volatiles of six table grape varieties (Vitis vinifera L.), *Food Chem.*, 2018, **240**, 524–533.

19 C. Cassino, V. Gianotti, F. Bonello, C. Tsolakis, M. C. Cravero and D. Osella, Antioxidant Composition of a Selection of Italian Red Wines and Their Corresponding Free-Radical Scavenging Ability, *J. Chem.*, , DOI:10.1155/2016/4565391.

20 L. Narduzzi, J. Stanstrup and F. Mattivi, Comparing Wild American Grapes with Vitis vinifera: A Metabolomics Study of Grape Composition, *J. Agric. Food Chem.*, 2015, **63**, 6823–6834.

21 E. S. Lago-Vanzela, R. Da-Silva, E. Gomes, E. García-Romero and I. Hermosín-Gutiérrez, Phenolic composition of the Brazilian seedless table grape varieties BRS clara and BRS morena, *J. Agric. Food Chem.*, 2011, **59**, 8314–8323.

22 S. Vincenzi, D. Tomasi, F. Gaiotti, L. Lovat, S. Giacosa, F. Torchio, S. Río Segade and L. Rolle, Comparative study of the resveratrol content of twenty-one italian red grape varieties, *South African J. Enol. Vitic.*, 2013, **34**, 30–35.

23 A. I. Romero-Pérez, R. M. Lamuela-Raventós, C. Andrés-Lacueva and M. De La Carmen Torre-Boronat, Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content, *J. Agric. Food Chem.*, 2001, **49**, 210–215.

24 X. Terra, V. Pallarés, A. Ardèvol, C. Bladé, J. Fernández-Larrea, G. Pujadas, J. Salvadó, L. Arola and M. Blay, Modulatory effect of grape-seed procyanidins on local and systemic inflammation in diet-induced obesity rats, *J. Nutr. Biochem.*, 2011, **22**, 380–387.

25 F. Blando, N. Calabriso, H. Berland, G. Maiorano, C. Gerardi, M. A. Carluccio and Ø. M. Andersen, Radical scavenging and anti-inflammatory activities of representative anthocyanin groupings from pigment-rich fruits and vegetables, *Int. J. Mol. Sci.*, 2018, **19**, 1–15.

