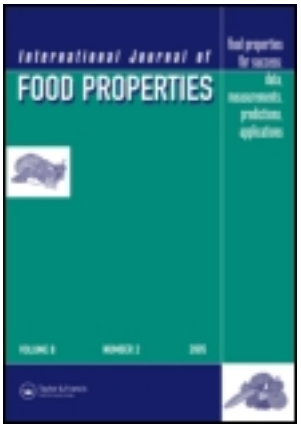

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CHARACTERIZATION OF UVA LONGANESI RED WINE BY SELECTED PARAMETERS RELATED TO ASTRINGENCY

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This research represents a first attempt to chemically characterize wines produced from the autochthonous grape variety, Uva Longanesi, based upon the phenolic compounds responsible for its high astringency as confirmed by preliminary sensory analysis. In addition, a wine produced from Sangiovese, the most popular grape variety in the Emilia-Romagna region, was analyzed for comparative purposes. Results showed that Uva Longanesi wine had a higher pH, alcohol concentration, and total dry extract than the Sangiovese wine. With regards to phenolic constituents, the Uva Longanesi wine had higher color parameters and greater concentrations of total phenolics, including monomeric anthocyanins, small polymeric pigments, and tannins. The phenolic composition of Uva Longanesi wine was found to be responsible for the high reactivity of the wine during fining trials.

Keywords: ***Grape, Wine, Anthocyanin, Flocculation, Sensory properties.***

INTRODUCTION

Consumers are demonstrating an ever increasing interest in foods that reflect their place of origin, particularly with regards to how the local territory is expressed through distinct sensory characters. With regards to the wine industry, this has led to an increasing interest in autochthonous grape varieties (grapes that only exist within a certain region and are native to that area). Uva Longanesi (also known as '*Bursôn*') is an autochthonous grape variety cultivated in the Ravenna province (Emilia-Romagna region, northern Italy), registered under the name of the Longanesi family that discovered the mother vine in the year 1913. Uva Longanesi wine was granted Ravenna Geographic Type Indication appellation status in 2007. The wine is characterized by a robust astringency and high levels of total phenols, including anthocyanins, that make Longanesi wine suitable for aging in wooden barrels.[1,2] To our knowledge, no scientific study has been conducted to characterize this local grape variety, which could be used to benefit winemaking in Emilia Romagna region.

Astringency is one of the most important sensory attributes of red wine; it is elicited by the binding of phenolic compounds, tannins in particular, to salivary proteins, thus producing drying and puckering sensations in the mouth.[3] Given the recent

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evidence regarding the health benefits of some astringent compounds, the underlying mechanisms and the factors influencing astringency perception in wine have been regularly reviewed.[4–10]

The fining of wine with proteins is a standard practice to reduce perceptual astringency through the removal of high molecular weight galloylated procyanidins. However, fining red wine with gelatin reduces, to some extent, the concentration of anthocyanins and the color density.[11–13] Gelatin has been often used as a model protein to measure the reactivity of tannins,[14–16] and the 'gelatin index' is the classic analytical method for estimating astringency in red wine.[17] The binding of tannins to proline-rich proteins has been proposed as an initial step in the development of astringent sensations;[18] a haze monitoring approach has been recently used by sensory scientists to generate predictive models of astringency perception based upon the turbidity generated by polyphenol binding to mucin.[19]

The aim of this preliminary work was to chemically characterize for the first time an Uva Longanesi wine, focusing on the phenolic compounds responsible for its high astringency. A Sangiovese red wine, the most popular grape variety in Emilia-Romagna region, was also analyzed for comparative purposes.

MATERIALS AND METHODS

Wine Samples

Uva Longanesi and Sangiovese di Romagna red grapes (*Vitis vinifera*, L.) were harvested at the technological stage of ripening then immediately destemmed, crushed, dosed with potassium metabisulfite (80 mg/L total SO_2 equivalent), and fermented using a starter (2.5×10^6 cells/mL) of dry yeasts (*Saccharomyces cerevisiae* 404 IMIA strain). Fermentation with maceration was carried out for seven days at a controlled temperature (28°C) in jacketed stainless steel tanks. Additions of diammonium phosphate up to a yeast assimilable nitrogenous compound value of 200 mg/L were carried out at the beginning and after three days of fermentation. Fermentations curves exhibited a normal trend and were followed by malo-lactic fermentation after which the wines were devatted, adjusted to 30 mg/L of free SO_2, and kept at −4°C for 4 weeks to improve tartaric stabilization. At the end of tartaric stabilization, wine was added with sulfur dioxide (free 30 mg/L), filtered using a cartridge line with a decreasing porosity (from 10 to 0.65 μm), then bottled in 0.75-litre glass bottles and the samples stored at room temperature for three months before analysis.

Wine Fining Trial

The phenolic affinity to bind gelatin was assessed according to the wine's ability to generate haze in solution as measured by nephelometry. Food grade enological gelatin (Oliver Ogar, Verona, Italy) was added as a fining agent at different levels (range 1–5 g/L) to 100 mL of wine and the time course of haze development was monitored up to 48 h. Turbidity measurements were performed in 24-mm diameter cuvettes using a model 2100N turbidimeter (Hach, Loveland, CO, USA) in the ratio mode using a tungsten light source and 10 sec signal averaging. Measurements were conducted at room temperature and results were expressed in nephelometric turbidity units (NTU). The turbidimeter was

calibrated according to the supplier's instructions with Formazin Primary Standards (20, 200, 1000, 4000, and 7500 NTU).

Chemical Analysis

The two wines were analyzed for selected parameters according to the literature:

- *Basic analysis*: ethanol, titratable and volatile acidity, pH, sulfur dioxide, and total dry extract were analyzed according to the European Official Methods;[20]
- *Spectrophotometric analysis:* optical densities at 420 and 520 nm,[21] total phenols,[22] small and large polymeric pigments,[23] tannin,[24] gelatin, ethanol, and polymer indexes[25] were measured using a UV mini 1240 spectrophotometer (Shimadzu, Milano, Italy);
- *HPLC analysis:* monomeric phenolic compounds[26] and anthocyanins.[27] Briefly, monomeric phenolics compounds were analyzed with a Jasco high performance liquid chromatography (HPLC) system (Jasco, Tokyo, Japan) equipped with two pumps (PU980) and connected to a diode array (MD1510, Jasco, Tokyo, Japan) detector. Samples were injected with a 10 μL loop using a 7125 valve (Rheodyne, Cotati, CA, USA) onto a Chromolith Performance RP-C18e column (100 mm × 4.6 mm; Merck, Milano, Italy) kept at 30 ± 1°C. Elution conditions consisted of a 2.1 ml/min flow-rate and a multistage binary gradient (A: Methanol–double-distilled water [2.5:97.5, v/v] at pH 3 with H_3PO_4; B: Methanol–double-distilled water [50:50, v/v] at pH 3 with H_3PO_4) as follows (time—A%): 0′—100%; 10′—100%; 15′—82%; 20′—75%; 22′—65%; 34′—0%; 35′—100%. Anthocyanin HPLC separation was carried out with a RP Hypersil C18 column (150 mm × 4.6 mm; Phenomenex, Torrance, CA, USA) using a mixture of 6% aqueous perchloric acid and methanol at a flow rate of 1 ml/min. Samples were filtered through a 0.45 μm PTFE membrane filter before injection (20 μl loop).

Sensory Analysis

Twelve highly experienced judges (age ranging from 23 to 55, 7 males, 5 females) were involved in this study. They were recruited among employers and students at the Department of Food Science (Cesena, Italy) and were trained over the years on recognition of basic stimuli as well as mouth–feel sensation, aroma, and qualitative and quantitative sensory test. In this study, in order to confirm their ability in recognition and scoring of astringency, judges were requested to rank standard solutions at different levels of astringency (alum) and/or bitterness (caffeine) and sourness (citric acid) as well as several wines. Considering the high experience of the judges and the results obtained, training was carried out in two weeks. During the actual testing session, the difference between Longanesi and Sangiovese wines were determined by means of a paired comparison test.[28] Wine samples, labeled with a three-digit code, were presented in standardized tasting glasses containing 30 ml of wine. Each judge was presented with two samples (according to a randomized order) and requested to taste wines from left to right and identify the sample that was higher in astringency. Data were interpreted by means of binomial-based tables.

RESULTS AND DISCUSSION

Wine Composition

The composition of the Uva Longanesi wine was characterized by a higher alcohol concentration, total dry extract, and color parameters (Table 1), thus confirming that the Uva Longanesi grape variety has good potential to produce wines with desired body and structure. HPLC analysis of anthocyanins revealed a distinctive pattern for each wine with a lack of acetylglucoside and coumaroylglucoside anthocyanins in Sangiovese (Fig. 1). Besides Malvidin-3-glucoside, which was the most important anthocyanin in both wines, Uva Longanesi contained Malvidin-3-acetylglucoside, whereas Sangiovese showed Delphinidin-3-glucoside and Petunidin-3-glucoside as major compounds. Assuming the verity of the hypothesis that there is an optimal anthocyanin/tannin ratio to produce the polymeric pigments, the high anthocyanin content of Uva Longanesi wine (Table 2) would require a proportional tannin content to expedite the formation of polymers between tannins and anthocyanins.

As expected, Uva Longanesi wine was also rich in total phenolics, including small polymeric pigments (SPP) and tannins (Table 3) that are most probably responsible for the high astringency of this wine found by the preliminary sensory test. HPLC analysis of simple phenolics in Uva Longanesi confirmed the high content of monomeric compounds, e.g., gallic acid and (−)-epicatechin (Table 2), that contributed to the astringency and bitterness of the wine to a lesser extent. Isomeric configuration can produce significant differences in sensory properties: (–)-epicatechin is considered to be more astringent and bitter than (+)-catechin.[29,30] Hydrocinnamates compounds determined in Uva Longanesi and Sangiovese wines were found at levels too low to make a contribution to astringency or bitterness.[31]

Besides astringent tannins, Uva Longanesi wine was also characterized by a high ethanol index that estimates the tannins linked to proteins and polysaccharides. These are considered as 'good tannins' involved in polymerization reactions that are not expected to increase wine astringency.[32] Both wines showed similar concentrations of large polymeric pigments (LPP) and similar dialysis indexes, which indicate the polymeric compounds with molecular weights >1500 and 3500 Da, respectively. The gelatin index, a measure of the ability of tannins to react with proteins, was higher for Uva Longanesi wine, but the difference with Sangiovese was not as great as expected. More insight into the tannin-protein interactions was obtained from the fining trial.

Table 1 Composition of Uva Longanesi and Sangiovese di Romagna wines for basic parameters.

		Wine	
Parameter	Unit	Longanesi	Sangiovese
pH	—	3.86	3.49
Titratable acidity	g/L	5.07	5.44
Alcohol	vol%	13.6	13.2
Total dry extract	g/L	30.7	25.3
Optical density 420 nm	AU	3.80	2.07
Optical density 520 nm	AU	7.05	3.33

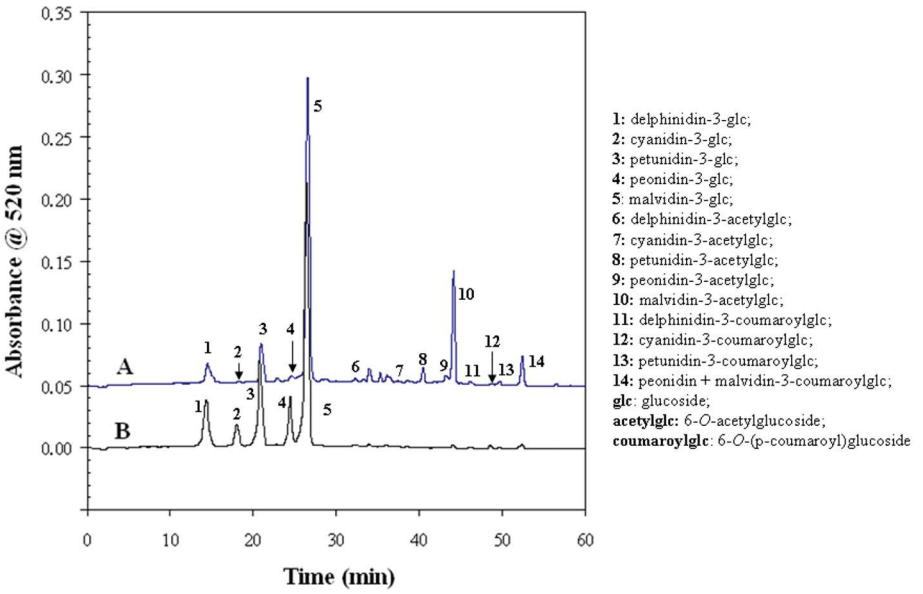

Figure 1 HPLC chromatogram of anthocyanins in Uva Longanesi (top: A) and Sangiovese wines (bottom: B). See Table 2 for peak quantitation (color figure available online).

Wine Fining Trial

Uva Longanesi and Sangiovese wines were treated with enological gelatin (fining agent) at dosages commonly used in winemaking and the time course of haze development up to 48 h was measured. As expected, gelatin concentration affected the haze formation to a large extent due to interaction with polyphenolic compounds of wine. During the fining test, after a rapid initial increase of the haze value, the turbidity curve reached a plateau at 24 h (*data not shown*); thus, the haze value at equilibrium (24 h) was taken for further consideration. Figure 2 shows the turbidity at 24 h with increasing gelatin concentration that appears to follow a positive exponential function versus turbidity. The data for Sangiovese (■) and Longanesi (●) wines overlapped at low gelatin dosage, whereas results follow two distinct patterns above 3 g/L; thus, the optimum ratio of protein to tannin was different between the two wines. According to the model described in the literature,[33,34] the hypothesis that changing the stoichiometry of the aggregates with increasing tannin/protein ratio was formulated.

Several factors affect protein–polyphenol interaction in model systems, including protein/polyphenol ratio, pH, alcohol concentration, and carbohydrates content.[35–38] In particular, a model has been proposed to explain the different NTU values observed with different tannin/protein ratios.[33] The model assumes that there is a fixed number of polyphenol-binding sites in toto, and that a polyphenol has two (or more) 'ends' which can specifically interact with the binding site on the (proline-containing) proteins, thereby allowing a single polyphenol molecule to bridge between protein molecules.[34] If the amount of polyphenol is held constant as the protein increases, haze increases up to a point and then declines. This behavior is peculiar to the interaction between tannins and protein and can be explained by the reversibility of the tannin–protein aggregate formation

Table 2 Composition of Uva Longanesi and Sangiovese di Romagna wines for anthocyanins and simple phenolic compounds.

	Wine	
Parameter (mg/L)	Longanesi	Sangiovese
Phenolic acids		
Protocatechic acid	3.2	2.0
Gallic acid	19.4	6.0
Syringic acid	5.7	3.6
Cinnamic acids		
Caffeic acid	2.4	2.4
Coutaric acid	4.4	3.8
Caftaric acid	17.0	13.9
Flavanols		
(+)-Catechin	5.8	7.7
(−)-Epicatechin	11.1	8.1
Flavonols		
Rutin	2.9	5.1
Myricetin	0.7	0.5
Quercetin	0.3	0.9
Anthocyanins*		
Df-3-glc	12.2	25.5
Cn-3-glc	2.0	9.7
Pt-3-glc	14.8	36.8
Pn-3-glc	4.0	16.1
Mv-3-glc	105	99.4
Df-3-acetylglc	3.0	nd
Cn-3-acetylglc	2.4	nd
Pt-3-acetylglc	5.6	nd
Pn-3-acetylglc	3.8	nd
Mv-3-acetylglc	30.6	nd
Df-3-coumaroylglc	2.6	nd
Cn-3-coumaroylglc	1.9	nd
Pt-3-coumaroylglc	2.7	nd
Pt + Mv coumaroylglc	9.4	nd
Total anthocyanins	200	187

*Df: delphinidin; Cn: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glc: glucoside; acetylglc: 6-O-acetylglucoside; coumaroylglc: 6-O-(p-coumaroyl)-glucoside.

nd: not determined.

Table 3 Composition of Uva Longanesi and Sangiovese di Romagna wines for polymeric pigments and tannins.

		Wine	
Parameter	Unit	Longanesi	Sangiovese
Total phenols	mg/L	2319	1431
SPP	AU	1.15	0.52
LPP	AU	0.65	0.59
Tannins	g/L	1.25	0.49
Gelatin index	%	43	41
Ethanol index	%	16	7
Dialysis index	%	35	35

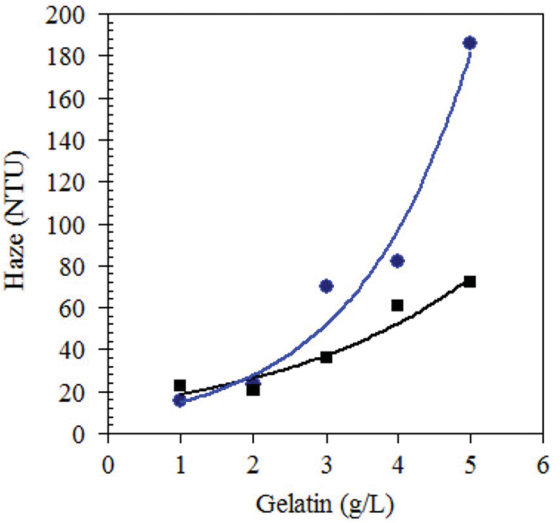

Figure 2 Change in turbidity (NTU) at 24 h as a function of fining treatment of Sangiovese (■) and Longanesi (●) wines with increasing concentrations of gelatin (color figure available online).

and by the changing stoichiometry of the complex formed, resulting from changes in the polyphenol/protein ratio.[6,39] Such a model implies that 'single ended' polyphenols would be expected to block haze formation by competing for proteins and prevent cross-linking.

It is well known that the more reactive species that trigger haze formation are polyphenolic compounds with different degrees of oxidation and polymerization. Simple flavanoids present in wine produce little, if any, haze. However, as the wine ages, some flavanoids oxidize and begin to polymerize increasing the oligomeric and polymeric phenolics (i.e., tannin) able to crosslink protein molecules[13,40–42] and, hence, give rise to haze. Obviously, the final level of polymeric phenolics depends on the rate of their accumulation and degradation in wine. In this view, the high turbidity found with Uva Longanesi wine can be explained due to the different phenolic compounds in that wine.

CONCLUSIONS

Uva Longanesi wine has a high level of phenolic compounds that confer significant astringency. Therefore, winemakers require a specific strategy to avoid the excessive extraction of these astringent compounds. For example, (1) selection of grapes with the desired level of phenolics maturity, (2) avoiding seed breakage during the crushing of the grapes, or (3) short macerations without seeds all could help to limit the extraction of the most astringent phenolics compounds. Additionally, the use of selected oenological gelatins during fining could further decrease wine astringency. The use of appropriate enological procedures could lead to the production of high quality wines amenable to wood aging and be suitable as blend wines as well. Further analyses on a large number of Uva Longanesi grape and wine samples are needed to estimate the compositional variability of this product and its evolution during aging.

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