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Towards the use of grapevine by-products for reducing the alcohol content of wines

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ABSTRACT

Increasing alcohol content in wines and growing public concern over alcohol-induced health problems have made alcohol management* a concept of growing importance in the wine industry. In the current study, the possible use of grape rachis to reduce alcohol content in wine was studied by introducing different concentrations of sterilized rachis to vials containing commercial wines of different types. Ethanol content determined by HPLC showed a decrease of up to 8.25% which correlated with rachis concentration. Quality assessment done by HPLC, GC, Spectrophotometry and a sensory analysis panel revealed some negative effects on wine quality. To better understand the factors causing the rachis-induced ethanol reduction, its main constituents were macerated separately with a commercial wine. Ethanol content, determined by HPLC, showed a significant decrease caused by maceration with lignin. These results suggest grape rachis and lignin as potential tools for a sustainable approach to the alcohol management of wines.

* Alcohol management – A global term for the set of techniques aimed for achieving a balanced wine by maintaining a moderate alcohol content.

Keywords: *Alcohol reduction; Alcohol management; Dealcoholization; Lignin; Grapevine by-products; Whole cluster.*

1. INTRODUCTION

Wine is a complex matrix composed of a large number of components, with its major two components being water and ethanol. In recent years, excessive ethanol levels in wines have become a growing issue. Ethanol levels have been continually rising [14], hand in hand with the growing awareness of its harmful effects [30, 28].

Among the main hypotheses for the cause of documented increase in ethanol content, global warming, which have been shown to advance harvest dates and increase berry sugar content at harvest [4, 7, 8], is repeatedly mentioned.

In the lack of an existing alcohol management strategy, an increase in must total soluble solids content (TSS) could directly translate to an increase in the ethanol content of the wine in the same magnitude.

Ethanol has a major effect on the sensorial characteristics of a wine. Increasing ethanol concentrations in wine have been shown to induce mouth sensations described as velvet, viscose, heat and bitterness [25] and hotness, drying and roughing in a white wine model [27]. Increasing ethanol concentrations in the wine causes an increase in the solubility of aroma compounds in the liquid phase which decreases their concentration and alters the relative composition in the headspace over the wine, chemically modifying wine's aroma [13, 20].

Viticultural strategies for alcohol management are focused on obtaining mature berries with lower sugar content. The displacement of vineyards to colder areas as in higher latitudes or altitudes was offered by Webb [16] as a suitable adaptation strategy to global warming and increasing berry TSS, while the selection of new varieties which will obtain lower sugar content at maturity of all other parameters, is in the focus of long term projects as the VDQA [1]. Other vineyard strategies focus on management techniques as modifying leaf area to crop ratio using trimming and defoliation [11, 19].

Enological strategies for alcohol management include decreasing TSS levels in the must at the pre-fermentative stage, decreasing the ethanol yield resulting from the fermentation process and removing ethanol from the wine at the post-fermentative stage. Pre-fermentative approaches include the use of enzymes as glucose oxidase that can convert glucose to non-fermentable molecules [5], nanofiltration membranes to exclude sugars, as examined by Garcia-Martin [24] and, where legal, the addition of water. Some methods for decreasing the ethanol yield of a fermentation process include the use of specially selected yeasts with increased sugar-to-ethanol conversion rate [9] and metabolic rerouting to redirect yeast carbon flux into end points other than ethanol. The later can be achieved by genetic engineering [2] or the addition of metabolic inhibitors such as furfural [3]. Methods for the removal of ethanol from the wine include advanced distillation techniques such as the spinning cone column and membrane osmotic distillation, described by Wright and Pyle [12] and Diban [22], respectively.

Alcohol management at the post-fermentative stage offers the possibility to carefully adjust the ethanol content to achieve equilibrium with all other parameters of the finished wine, informally known in the industry as achieving a “sweet spot”.

Whole cluster fermentation, traditionally practiced in several wine regions in the world for the enhancement of wine quality, was found to significantly decrease final ethanol content in wine [10]. The mechanism involved in this partial dealcoholization was not extensively investigated and was estimated to be a result of dilution from water defusing out of the rachis during the process [10]. Whether the incorporation of grape

rachis in the fermentation process affects wine quality negatively or positively is, in part, subjective and highly dependent on wine characteristics. Major influences of this technique on final wine quality, as found in the literature, are listed in table1. Some findings may offer a great challenge for the possible use of rachis in the partial dealcoholization of wine.

Table1. Measured effects of fermentation with rachis on wine quality.

Effect on wine quality	Reference
Decrease in ethanol content	Ribereau-Gayon *et al.,* 1976
Decrease in total acidity	
Increase in tannin concentration	
Decrease in wine color intensity	
Increased astringency	M. Isabel Spranger *et al.,* 2004
Increased methoxypyrazines concentration	Hashizume and Samuta., 1997

Lignin is the most abundant natural polymer next to cellulose and exists in plant cell walls as one of the major constituents [29]. It is naturaly abundant in the grapevine, making up to 340mg g-1 of grape rachis dry weight [17]. The adsorption of different alcohols on softwood lignin was investigated by Yang, Ladisch and Ladisch [31]. Alcohols were found to bind through combined hydrophobic and hydrophilic interactions with the complex structure of lignin, containing high number of alkyl and hydroxyl groups. For being a by-product of the paper and bio-diesel industry, lignin is a relatively inexpensive material, thus its possible use for partial wine dealcoholization offers potential advantages, as in the case of the grape rachis. The possible use of natural constituents of the grapevine as tools to cope with increasing ethanol content in wine is an opportunity from an environmental, economic and communicational point of view. As known by the author, the use of lignin for the partial dealcoholization of wine was never previously investigated.

This study focuses on the partial dealcoholization effect of grape rachis, aiming to better understand its effect on wine quality, the mechanism involved and optimizing its potential use in the wine industry. For this goal, grape rachis and its major constituents were applied by post-fermentative maceration with commercial wines. The effect of this process on final ethanol content was measured and major quality parameters were analyzed and compared.

2. EXPERIMENTAL SECTION

2.1. Maceration of wine with grape rachis.

Grape rachis material was obtained from imported Thompson seedless table grapes (South Africa), purchased at "Mercamadrid" wholesale market, Spain. All grape rachis were manually separated from the grapes, cut in 1-3 cm pieces, weighed and added to the trial flasks to start the trial on the same day. Samples were dried (70°C, 10 days) and measured for dry weight in order to calculate water content of the grape rachis. All trials were done by triplicates. Four different wines (Table2) were tested for the influence of maceration with two different rachis concentration established as 5% and 10% by weight, and a control treatment not including rachis. The rachis used was calculated to have a water content of 59.67% by weight. Prepared grape rachis were distributed into 100ml flasks and sterilized (121°C, 20 min), 80ml of wine was poured in each flask in a laminar flow cabinet, the flasks were sealed using air-tight rubber caps and stored at 17ºC for 30 days in darkness until analysis.

Table2. Wines macerated with grape rachis.

Wine type	Denomination of Origin	Alcohol% by vol.*
Verdejo (white, dry)	Rueda, Spain	13.0
Fino (sherry)	Montilla-Moriles, Spain	15.0
Jumilla (red, dry)	Jumilla, Spain	12.5
Toro (red, dry)	Toro, Spain	14.5

Table3. Compounds used in the maceration treatments.

Compound	Origin	Concentrations (g L^{-1})
Lignin (Alkalined)	CYMIT Quimica, Spain	10, 25, 50
Lignin (Dealkalined)	CYMIT Quimica, Spain	10, 25, 50
Apple Pectin	Fluka, Sigma-Aldrich, Switzerland	10, 25, 50
Cellulose	Chromatography paper 1 (Whatman, England)	10, 25, 50
Dry rachis	Thompson seedless, South Africa	25, 62.5, 125

2.2. Adsorption by stem constitutes.

Five different compounds were tested for their possible effect on the ethanol content of wine (Table 3). For this, dry red wine (D.O Ribera del Duero, Spain) was macerated with three different concentrations of the tested compounds. Concentrations used were defined relatively to an average rachis concentration in a *Vitis Vinifera L.* cluster estimated as 5% of total cluster weight, rachis water content of 55% by weight and biomass composition of the rachis, as shown by Ping [17], determined for lignin and Cellulose as 34% and 36% by weight, respectively. Lignin and

pectin products used were in the form of dry powder. Treatments were established at 100%, 250% and 500% of this value with the control treatment containing only wine. Dry rachis was obtained from the same batch as described, following a drying process (70ºC, 10 days), and was crushed to a powder. 60ml vials containing 50ml of wine and tested compounds were well stirred and stored at ambient temperature for 8 days before analysis.

2.3. Ethanol quantification.

Ethanol was analyzed by LC-RI (Liquid chromatography-Refractive index detection) using a Waters 600E (Maryland, USA) equipped with a 717p auto sampler device and a 2414 Refractive Index Detector. The separation was performed using a reverse phase C18 column Eclipse XDB 4.6 x 150 mm, 5 µm size particle (Agilent, PA, CA). The solvent was MiliQ water in isocratic mode at a flow of 0.4 ml/min. The temperature was set at 30ºC in column and detector. The calibration was done with an external standard of ethanol and glucose (Panreac, Barcelona, Spain). Samples were injected after filtration at 0.45 µm membrane filters of cellulose methyl ester (Tecknokroma, Barcelona, Spain). The volume of injection was 2 µl.

2.4. Anthocyanin determination.

The concentration of anthocyanins in the different samples was analyzed using a Waters (Milford, MA) HPLC chromatograph equipped with a 600-MS controller, a 717 plus auto-sampler, and a 996 photodiode-array detector. Gradients of solvent A (water/formic acid, 90:10, by vol.) and solvent B (water/methanol/formic acid, 45:45:10, by vol.) were used in a reverse-phase Kinetex C18 column (Phenomenex, Torrance, CA, USA) (100 x 4.6 mm; particle size 2.6 µm) as follows: 10% B (0.8 ml min-1) from 0 to 3 min, 10–50% B linear (0.8 ml min-1) from 3 to 18 min, 50% B from 18 to 20 min, 50–10% B linear (0.8 ml min-1) from 20 to 21 min, and re-equilibration of the column from 21 to 23 min. Detection was performed by scanning in the 500–600 nm range. Quantification was performed by comparison against an external standard at 525 nm and expressed as a function of the concentration of malvidin-3-O-glucoside (Extrasynthèse, Genay, France). 100µL samples, previously filtered (0.45 µm membrane filters made of cellulose methyl ester) (Tecknokroma, Barcelona, Spain), were injected into the HPLC apparatus. Determinations were made in triplicates. The following anthocyanins were identified: delphinidin, cyanidin, petunidin, peonidin, and malvidin, for their 3-glucoside (glycosylated), pyruvic (vitisins), acetyl glycoside (acetylated), cinnamoyl (p-coumaryl) (coumarylated) and vinylphenolic pyroanthocyanins (VPAs) (solely for malvidin) derivates. The different anthocyanins were identified by their retention time relative to that of the major anthocyanin in *Vitis Vinifera L.*, malvidin-3-O-glucoside, and by comparing their UV-visible absorption spectra with the literature [21]. Correlation analysis was performed to establish relationships between variables. All calculations were made using the PC Statgraphics 5.0 software package (Graphics Software Systems, Rockville, MD).

2.5. Volatile compounds

Volatile compounds were determined using an Agilent Technologies 6850 gas chromatograph (Network GC System) equipped with an integrated flame ionisation detector (GC-FID), as described by Morata [3]. A DB-624 column (60 m x 250 µm x 1.40 µm) was employed, calibrated with the following compounds as external standards: acetaldehyde, methanol, 1-propanol, 1-butanol, 2-butanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethyl acetate, 2-phenylethyl alcohol, diacetyl, ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl butyrate, ethyl lactate and hexanol. 4-methyl-2-pentanol was used as an internal standard (all compounds from Fluka, Sigma–Aldrich Corp. Buchs SG, Switzerland). Injector temperature was 250ºC and detector temperature was 300ºC. The column temperature was 40ºC for the first 5 min, rising linearly by 10ºC min-1 until reaching 250ºC; this temperature was then maintained for 5 min. Hydrogen was used as the carrier gas; this was provided by a hydrogen generator (LNI Schmidlin SA, Geneva, Switzerland). The flow rate was 22.1 L min-1, injection split ratio was 1:10 and detection limit 0.1 mg L-1. One hundred µL of internal standard (concentration of 500 mg L-1) were added to 1 mL test samples and filtered using a syringe membrane filters (0.45 µm pore size) (Teknokroma, Barcelona, Spain). They were then placed in 2 mL glass vials sealed using a PTFE/silicon septum. 1µL of the filtrate was injected into the GC apparatus.

2.6. Spectrophotometry.

An Agilent 8453 UV-Visible Chem Station diode array spectrophotometer (Santa Clara, USA) was used for the color analysis. Samples were analyzed in a 1mm path length quartz cuvette and a range of 200-1100nm. Absorbance in 420, 520 and 620 nm was measured. Color intensity was calculated as the sum of absorbance at the three wavelengths, while tonality was calculated as the ratio between absorbance at 420nm and 520nm.

2.7. Sensory analysis.

Sensory evaluation was carried out using a tasting panel which consisted of seven participants and included PhD and MSc students of the Viticulture and Enology departement as well as the lab staff. Tasting samples were centrifuged at 5,000 rpm for 5 min at 10ºC in 50ml falcon tubes. Tastings took place in the department's assigned tasting room, using white light, with tasters located in separate tables. All wines were served at a temperature of 16ºC using 25ml samples in clear, tulip-shaped tasting glasses. Wines were presented in random order.

Statistical analysis was done using PC Statgraphics plus v.5 (statistical graphic corporation, Rockville, MD, USA). ANOVA tests were performed using a level of significance of $p \leq 0.05$.

3. RESULTS SECTION

3.1. Maceration of wine with grape rachis.

Final ethanol content given in Table4 shows a linear effect of increasing grape rachis concentrations in all four wines tested. A decrease in ethanol content of about 1% by vol. was measured in all wines when 10% (w/w) of grape rachis was macerated with the wines. An assessment of the effect of this treatment on wine quality is given below.

Table4. Final ethanol content in the different treatments.

Wine	Treatment			Regression line formula	R^2
	SR	5%	10%		
Verdejo	12.94 ± 0.17 [a]	12.55 ± 0.1 [b]	11.98 ± 0.08 [c]	y = -0.478x + 13.445	0.9882
Fino	14.66 ± 0.08 [a]	14.13 ± 0.13 [b]	13.51 ± 0.1 [c]	y = -0.5748x + 15.251	0.9987
Jumilla	12.73 ± 0.04 [a]	12.25 ± 0.06 [b]	11.68 ± 0.09 [c]	y = -0.5257x + 13.271	0.9975
Toro	13.98 ± 0.07 [a]	13.52 ± 0.05 [b]	12.98 ± 0.02 [c]	y = -0.5012x + 14.497	0.9984

Ethanol content as measured in four different wines (Verdejo, Fino, Jumilla and Toro) where three treatments with different grape rachis concentrations were applied: No rachis ('SR'), 5% by weight ('5%') and 10% by weight ('10%'). Values are average ± S.D. Values in the same row marked by different letters are significantly different ($p<0.05$, n=3). Regression line formulas and R^2 values were calculated using Microsoft Excel 2010, for each wine separately.

3.2. Wine color. The analyses of the absorbance measured in 420, 520 and 620nm, for all four wines, are given in Figure 1.

The effect of rachis maceration on wine color clearly differed between white and red wines. In the white wines examined, the absorbance pattern of the samples implied a dramatic increase in the yellowish (420 nm) and reddish (520 nm) color of the samples, with absorbance values increasing up to 5 and 3 folds respectively (Verdejo wine).

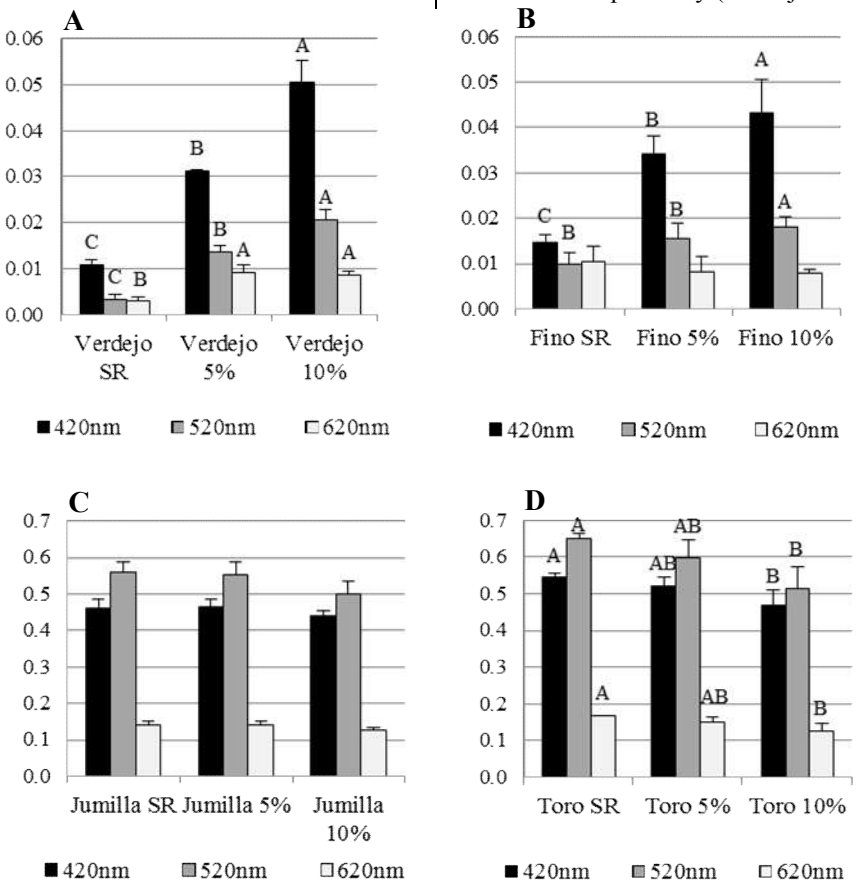

Figure1. Absorbance values as measured in four different wines: Verdejo, Fino, Jumilla and Toro (marked as A, B, C and D respectively) where three treatments with different grape rachis concentrations were applied: No rachis ('SR'), 5% by weight ('5%') and 10% by weight ('10%'). Wavelengths measured were: 420nm, 520nm and 620nm. Columns with the same color, marked by different letters represent significantly different values. ($p<0.05$, n=3, apart from Toro SR and Verdejo SR where n=2).

This might be caused by deepening yellow color deriving from rachis-extracted phenolic compounds, as well as possible browning due to the chemical oxidation of extracted tannins and their modification in the wine [6, 23, 26]. In the red wines examined, the absorbance pattern of the samples implied a slight decrease in all measured spectra, found significant only in the "Toro" wine samples. Color intensity was found to significantly decrease in the "Toro" wine implying a hypochromic effect on wine color. Tonality is normally used to indicate the development of color towards orange tones during the process of wine aging. This value was found to significantly increase in both red wines in response to maceration with grape rachis, implying a bathochromic shift in wine color towards orange tones. Figure 2 compares the major anthocyanin groups as measured in the different treatments. Figure 3 displays a principle component analysis (PCA) of the measured values. A clear separation between the control and the 10% (w/w) treatment was achieved for both wines and showed a general trend of decrease in anthocyanins. A significant decrease was measured in the quantity of coumarylated anthocyanins and VPA's in both wines, while in the "Toro" wine a significant decrease was additionally found in glycosidic anthocyanins and in the sum of anthocyanins in the wine as a result of rachis maceration. These results support the assumption that the hypochromic and bathochromic shifts in the

color of the red wines, are partly attributed to anthocyanin adsorption by the rachis, as previously suggested by Ribereau-Gayon [26].

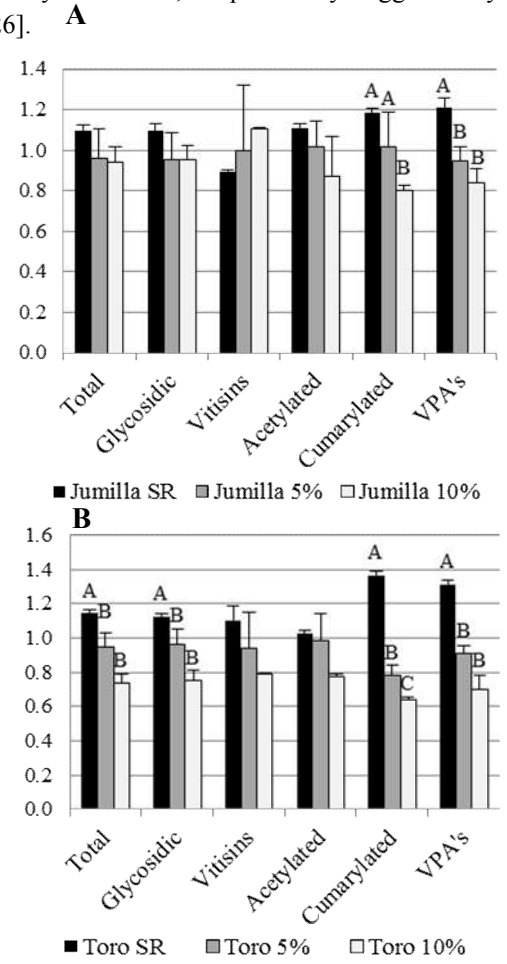

Figure 2. Comparison of major anthocyanin groups as measured in two red wines: Jumilla and Toro (marked as A and B respectively), where three treatments with different grape rachis concentrations were applied: No rachis ('SR'), 5% by weight ('5%') and 10% by weight ('10%'). Normalization was done with the respective average value per anthocyanin group. Values presented are average values ± S.D. Columns marked by different letters within the same anthocyanin group, represent significantly different values ($p<0.05$, $n=3$ apart from Toro 10%, where $n=2$).

3.3. Fermentative aroma. Measured concentrations of fermentation-derived volatile compounds in all four wines are given in figure4. Significant differences were found uniquely in the "Toro" wine, and include a decrease in higher alcohols such as methyl-2-butanol-1 and in esters of ethyl acetate and ethyl lactate. In an overall view the lack of a significant and persistent trend, caused by grape rachis maceration on fermentative aromas of the commercial wines, could serve to negate the hypothesis of dilution as the main cause for the rachis-mediated ethanol reduction as presented in this work.

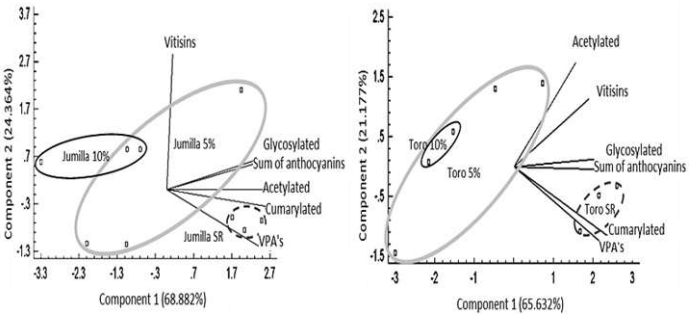

Figure 3. Principle component analysis of major anthocyanin groups as measured in two red wines: Jumilla and Toro (marked as A and B respectively), where three treatments with different grape rachis concentrations were applied: No rachis ('SR' (dotted line)), 5% by weight ('5%' (thick gray line)) and 10% by weight ('10%' (black line)).

3.4. Sensory analysis. The results of sensory evaluation, given in figure 5, display a clear and persistent image of the negative effect of maceration with grape rachis on wine quality, as perceived by the tasting panel. The effect on the "Verdejo" wine seems more pronounced, maybe owing to its lighter style. In all wines a similar trend was recorded, as attributes associated with quality, declined and astringency increased. The negative effect on the quality of aroma can likely be attributed to the addition of rachis-derived aromas as methoxypyrazines [15] which are perceived as negative, rather than the adsorption or dilution of positive ones. The increase in astringency is the probable consequence of tannin extraction from the rachis and is in accordance with Ribereau-Gayon [10] and Spranger [18].

3.5. Maceration of wine with major stem constitutes. Results given in table5 show a significant effect of dry rachis and both types of lignin in decreasing the ethanol content of the wine. Alkalined lignin (Lignin A) was found to be the most efficient, achieving the strongest effect (-6%) using the medium concentration (25g/L). These results are in accordance with Yang, Ladisch and Ladisch [31] as previously cited and should be included in the hypothesis of the mechanism of alcohol reduction by the grape rachis, as was measured in the trials presented in this work. Studies of the effect of lignin on wine sensorial quality were not conducted due to the lack of a food grade lignin product and should be included in further studies.

Table 5. Final ethanol content in wine macerated with stem constituents

Treatment	Compound content			
	Non	Low	Medium	High
Cellulose	13.88 ± 0.06	13.79 ± 0.02	13.72 ± 0.15	13.68 ± 0.11
Dry rachis	13.88 ± 0.06 ^{a}	13.66 ± 0.1 ^{bc}	13.73 ± 0.1 ^{ab}	13.51 ± 0.15 ^{c}
Lignin A	13.88 ± 0.06 ^{a}	13.66 ± 0.05 ^{b}	**13.06** ± 0.15 ^{c}	13.09 ± 0.1 ^{c}
Lignin D	13.88 ± 0.06 ^{a}	13.57 ± 0.07 ^{b}	13.64 ± 0.05 ^{b}	13.43 ± 0.06 ^{c}
Pectin	13.88 ± 0.06	13.78 ± 0.11	13.75 ± 0.15	-

Final ethanol concentrations as measured in a red wine (D.O Ribera del Duero, Spain), after maceration with increasing concentrations (low, medium and high) of five compounds. Ethanol content of the control wine is given in 'Non' column. Values are average ± S.D. Values in the same row, marked by different letters are significantly different ($p<0.05$, $n=3$). Pectin in high concentration was not measured due to high viscosity.

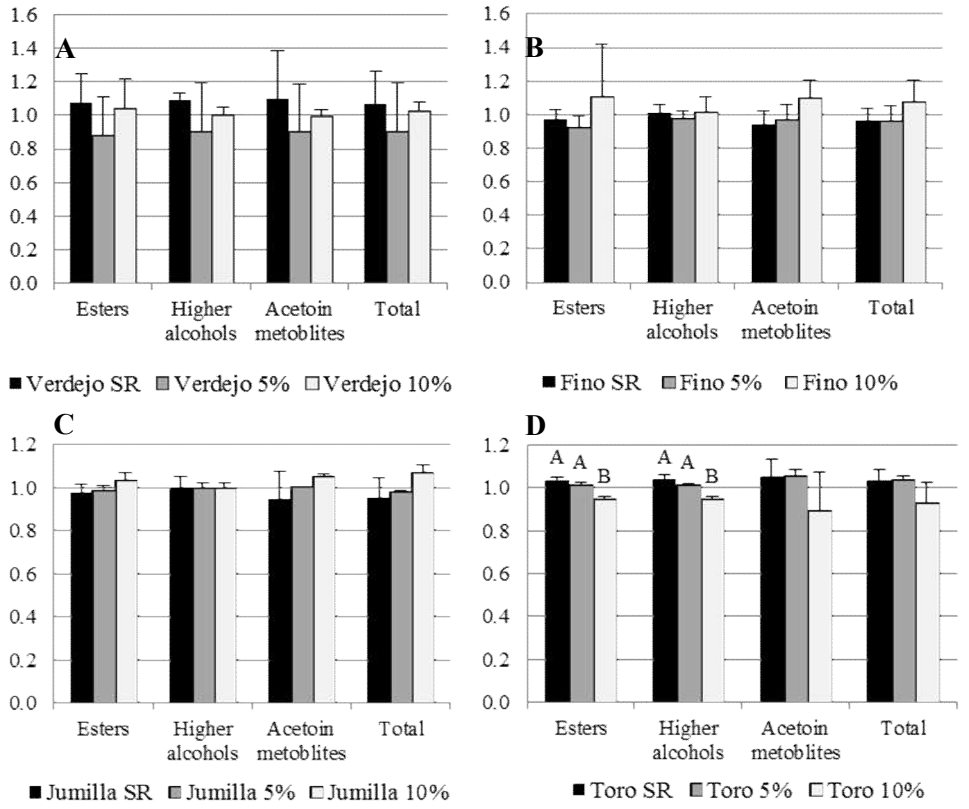

Figure 4. Comparison of major fermentative aroma groups as measured in four wines: Verdejo, Fino, Jumilla and Toro (marked as A, B, C and D respectively) where three treatments with different grape rachis concentrations were applied: No rachis ('SR'), 5% by weight ('5%') and 10% by weight ('10%'). Normalization was done with the respective average value per compound group. Values presented are average values ± S.D. Columns marked by different letters within the same wine and fermentative aroma group, represent significantly different values ($p<0.05$, n=3 apart from Toro 10%, where n=2).

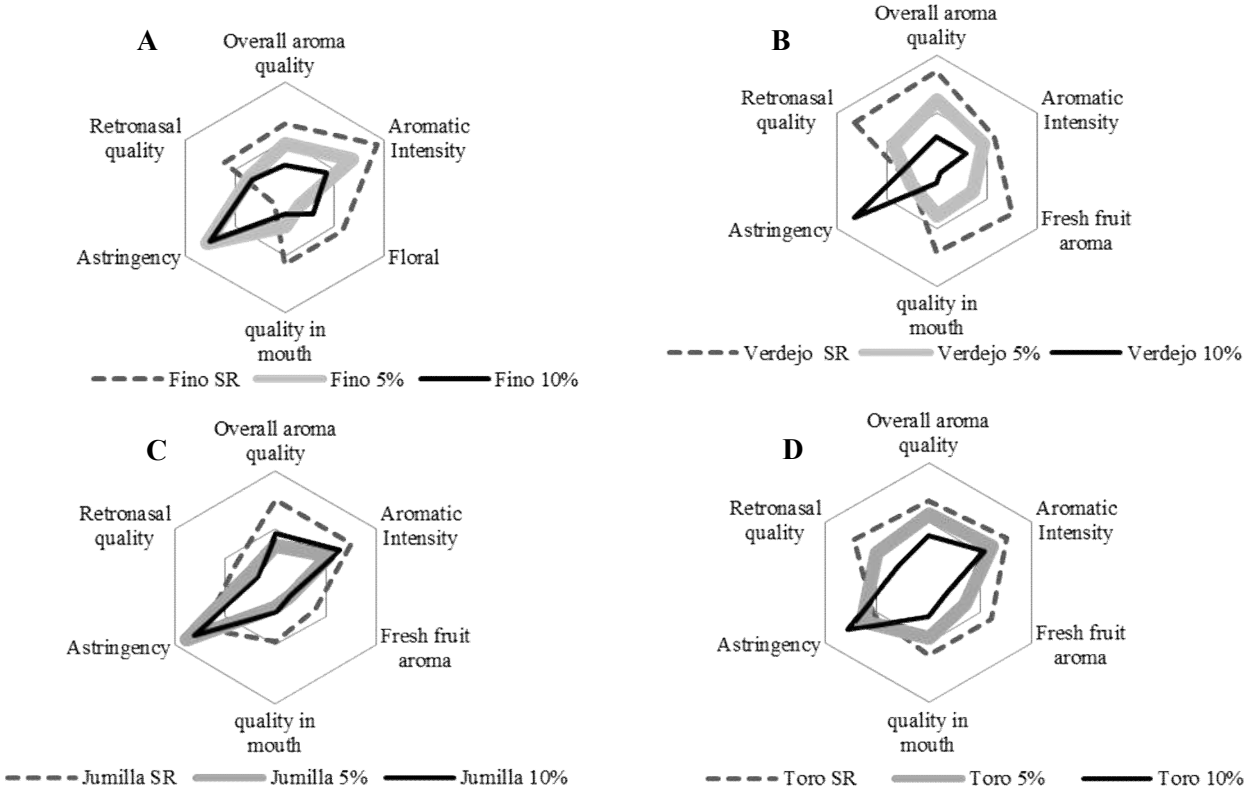

Figure 5. Main sensory attributes as estimated by a tasting panel (n=7) for 4 commercial wines: Verdejo, Fino, Jumilla and Toro (marked as A, B, C and D respectively), where three treatments with different grape rachis concentrations were applied: No rachis (SR), 5% by weight (5%) and 10% by weight (10%). Each attribute was rated using a scale of 1 (low) to 6 (high). Values are increasing from the center of the plot (1) to its perimeter (6).

4. CONCLUSIONS

The introduction of grape rachis at the post-fermentative stage has shown a strong correlation between rachis concentration and reduction in ethanol content. Statistically significant reduction in ethanol levels was visible in all tested concentrations and equal for all tested wines, reaching up to 1% ethanol by vol in the wine. However, negative effects on wine quality included changes in color intensity and tonality as well as reduction in anthocyanins content. In a sensory analysis test, all treated wines were perceived as inferior when compared to control samples, rated as less fruity and more bitter and astringent. These findings offer a great challenge for the possible use of grape rachis in alcohol management. Further understanding of the mechanism by which the rachis contributes to the measured decrease in ethanol content could serve to increase the efficiency of its use and minimize negative effects on wine quality. Contrary to previous works, this study could not attribute the reduction in ethanol content solely to a dilution phenomenon. The lack of clear evidence for dilution, combined with the signifcant decrease in ethanol content achieved by the use of lignin may suggest that the adsorption of ethanol by the ligneous constituent of the rachis should be considered as a major contributor to the phenomenon. Further research is needed in order to study the impact of lignin incorporation on wine quality and maximize its effectiveness in the adsorption of ethanol. This may offer a promising tool for the alcohol management of wines.

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7. CONFLICT OF INTEREST

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