

## Effects of pre-fermentative maceration conditions on Zalema wine colour and composition

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### ABSTRACT

Nine different white wines were elaborated applying different skin contact times (2, 4, 6, 8, 12, 18 and 24 hours) and temperatures (5, 10 and 20 °C). The colour and phenolic composition of the wines were determined at the different winemaking stages. Although extended skin contact (maceration time  $\geq 12$  hours) increased significantly the levels of phenolics, some of them were important compounds as antioxidant agents, such as caffeic acid. Related to wine colour, when the skin contact process was longer (12, 18 and 24 hours) at low temperatures (5 and 10 °C) the wines visually had an acceptable colour ( $L^*$  values higher than 90 CIELAB units;  $a^*$  values ranging from -0.86 to 1.32 units;  $b^*$  values ranging from 5.40 to 21.30 units). So an exhaustive control of the skin contact conditions (time and temperature) is vital to reduce browning in white wines due to this vinification technique.

### 1. INTRODUCTION

In winemaking industry, different oenological practices are applied (pressing, racking, clarification, filtration...) in order to obtain quality wines. These practices are able to modify the wine composition and its sensory properties, such as flavour and colour. In this sense, skin contact (maceration) process, has been widely researched for over thirty years in order to improve the quality of white wines. Skin contact can be defined as a pre-fermentation process applied to wine elaboration: the skins of crushed and destemmed grapes are macerated in their own juice at controlled conditions (time and temperature) prior to pressing. Controlling skin contact conditions is vital to obtain high quality white wines<sup>1</sup>. Previous results have shown a greater extraction of phenolic compounds due to this oenological practice<sup>2</sup>, which are responsible for some of the major organoleptic properties of wines, in particular colour and astringency. In this sense, these compounds contribute to colour stability because they can act as oxidation substrates in white wines. So colour changes in white wines due to browning, such as the loss of bright pale-yellow colour, might occur when the total phenolic content is too high. However, a wide range of studies has shown that there is no direct cause-effect relationship between the polyphenol content and the colour of the wine, because other factors such as redox processes, variations in pH, etc. intervene. On the other hand, many polyphenol-rich white wines have antioxidant characteristics similar to those of red wines, so they can also help to prevent arteriosclerosis and coronary heart diseases<sup>3</sup>.

The objective of this study was to evaluate the influence of different skin contact conditions (time and temperature) on the colour properties and the phenolic content of Zalema white wines.

### 2. METHODS

*Vitis vinifera* cv. Zalema grapes were processed in the same way in each skin contact assay carried out, which were classified in two groups (SH and LL) according to the skin contact conditions: SH=Short times (2, 4, 6, 8 hours), High temperatures (20 °C); LL=Long times (12, 18, 24 hours), Low temperatures (5, 10 °C). In order to achieve the lowest skin contact temperatures (5 and 10 °C), an additional experimental refrigerator equipment was used. This equipment consisted of a refrigeration unit (HITSA-TOPAIR, mod RAE-101, Madrid, Spain) and a recirculation of must and skins system (V. Navas, mod VNIP206/2, Ciudad Real, Spain). Wine samples were periodically taken immediately after fermentation until the moment of bottling.

Individual phenolic compounds were analyzed by HPLC with direct injection of the sample, except filtration (0.45 µm pore size) before injection (100 µL). An Agilent 1100 series (Palo Alto, CA, USA) chromatograph equipped with a reversed phase Nova-pack C18 column (30 cm x 3.9 mm ID) and a diode array detector was used. Identification of individual compounds was carried out by comparing their retention times and spectra with those of original standards. Quantitative determinations were carried out with a standard external calibration method. Wavelengths used for quantification were 280 nm for benzoic acids and tyrosol, 320 nm for cinnamic acids and their tartaric esters, and 360 nm for flavonols.

Colour measurements were made with a Hewlett Packard UV-Vis 8452 (Palo Alto, CA, USA) Spectrophotometer, using 10 mm path length glass cells. The samples were filtered prior to the spectrophotometric analysis. The whole visible spectrum (380-770 nm) was recorded ( $\Delta\lambda=2$  nm) and Illuminant D<sub>65</sub> and 10° Observer were used in the calculations as standard conditions. The CIELAB parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*_{ab}$ ,  $h_{ab}$ ), and the CIELUV saturation,  $s^*_{uv}$ , were determined by using the software CromaLab®<sup>4</sup>.

A Linear Discriminant Analysis (LDA) was used to classify wine samples according to colour parameters. On the other hand, one-way analysis of variance (ANOVA) was carried out to establish which phenolic compounds differed significantly between the different skin contact assays.

### 3. RESULTS

**Composition.** Table 1 shows the phenolic content of the obtained wines grouped by the fermentative maceration conditions used in this work. It can be observed that the quantified phenolic compounds, in general, were extracted in greater quantity when long skin contact times were applied. The predominant phenolic compound was caftaric acid, followed by tyrosol, a noncarboxylic phenol. Previous studies have shown that caftaric acid seems not to have a pronounced effect as agent responsible for chemical browning in white wines<sup>6</sup>.

The differences found between the two groups of skin contact conditions assayed (SH and LL) were statistically significant in the case of caffeic acid, p-hydroxybenzoic acid, m-coutaric acid, ethyl galate, catechin, tyrosol, caftaric acid and fertaric acid.

**Table 1:** Concentration (mg/L) of the phenolic compounds detected in the wine samples. Mean values and standard deviation.

Phenolic compound	Skin contact assay <sup>a</sup>		Significance level <sup>b</sup>
	SH	LL	
Protocatechuic acid	0.69 ± 0.46	1.27 ± 0.68	ns
Caffeic acid	0.78 ± 0.07	4.11 ± 1.07	***
Ferulic acid	0.58 ± 0.13	1.44 ± 1.15	ns
Catechin	3.88 ± 0.69	7.69 ± 2.74	*
p-hydroxybenzoic acid	1.08 ± 0.12	4.27 ± 1.27	***
Tyrosol	10.15 ± 5.09	24.77 ± 7.38	*
Quercetin-3-rutinoside	2.59 ± 0.23	4.08 ± 1.64	ns
Quercetin-3-L-rhamnoside	0.67 ± 0.24	0.97 ± 0.28	ns
Quercetin-3-D-galactoside	1.74 ± 0.45	1.40 ± 0.86	ns
Caftaric acid	27.13 ± 0.94	57.14 ± 10.91	*
p-coutaric acid	3.08 ± 0.96	4.94 ± 2.11	ns
m-coutaric acid	1.03 ± 0.15	0.48 ± 0.26	**
Fertaric acid	1.34 ± 0.19	4.20 ± 2.00	*
Ethyl galate	0.50 ± 0.31	1.56 ± 0.37	**

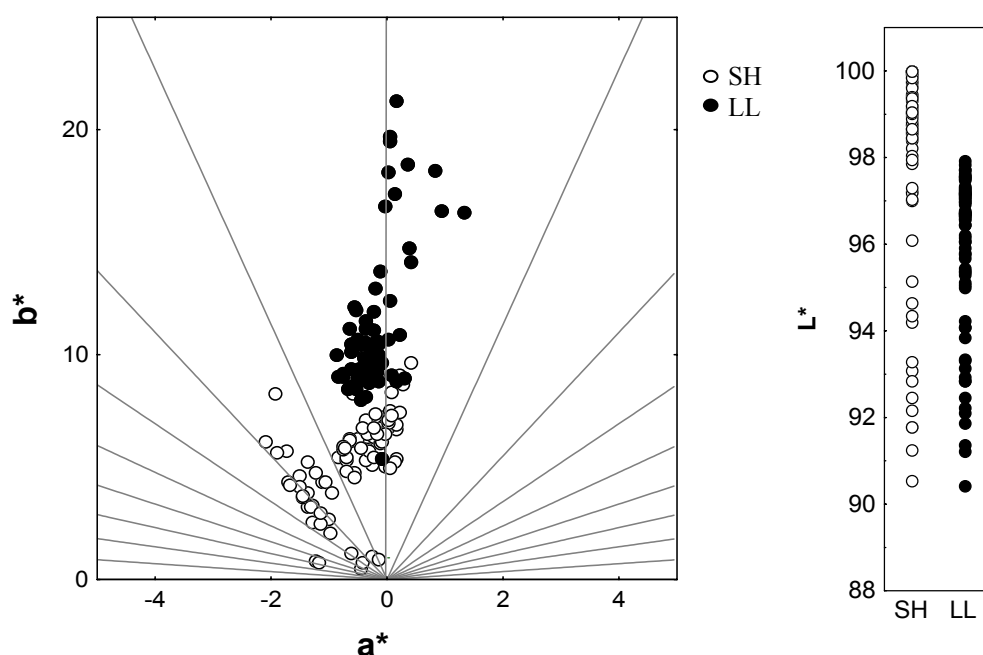
<sup>a</sup> SH: short skin contact times, high temperatures; LL: long skin contact times, low temperatures.

<sup>b</sup> \*, \*\*, \*\*\* denote significances at  $P<0.05$ ,  $P<0.01$  and  $P<0.001$ ; ns: not significant.

As regards the three groups of phenols (hydroxycinnamic acids, flavonoids and benzoic acids), hydroxycinnamates were found as the predominant phenolic group in both types of wine (75 %

of the total phenols quantified in the SH wines and 77 % in the LL wines), in accordance with results reported by other authors<sup>7</sup>. On the other hand, it was found some decreases in phenolic content of the final wines in relation to the previous musts. The major decrease was observed in flavonoids, specially in wines which had long maceration times (mean 23.00 mg/L in musts and 14.14 mg/L in wines). This fact indicates that, approximately, 26 % of total analyzed flavonoids were lost in these wines (LL) along the vinification process.

**Colour.** Wine samples were represented in the ( $a^*b^*$ ) plane (by CIELAB space) (Figure 1). In this Figure, the  $L^*$  values (lightness) are separately given. The analyzed samples were found to have  $a^*$  values ranging from -2.10 to 1.30 units,  $b^*$  values from 0.47 to 21.30 units and hue values near  $90^\circ$ . These values are related to the pale-yellow area of the ( $a^*b^*$ ) plane, almost colourless. It can also be observed a high lightness in all samples, with  $L^*$  values higher than 90 CIELAB units. However, in the ( $a^*b^*$ ) plane, it can be observed clearly separated the two wine groups (SH and LL): SH wine samples (short times, high temperatures) are situated closer to the origin of coordinates, with a more pale-yellow colour and a lesser intense colour.



**Figure 1:** Representation in the ( $a^*b^*$ ) plane of Zalema wine samples obtained from the skin contact assays carried out in this work, and their  $L^*$  (lightness) values.

In order to confirm the colour difference between both sample groups, the total colour difference was calculated using the following expression:  $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$  (as the Euclidean distance between two points in the three-dimensional space defined by  $L^*$ ,  $a^*$  and  $b^*$ ). It was obtained that the mean colour difference between both sample groups was higher than 3 units ( $\Delta E^*_{ab} = 7.19 \pm 1.73$ ), which indicates that the colour differences between wine samples of the different skin contact groups assayed (SH and LL) were easily detected by the human eye<sup>5</sup>.

A linear discriminant analysis was carried out in order to explore which of the variables were better for discriminating among the different skin contact sample groups. This statistical technique was also able to differentiate the two sample groups (SH and LL) as above, according to their colour characteristics. The angular coordinates of the CIELAB space ( $L^*$ ,  $C^*_{ab}$  and  $h_{ab}$ ) and the saturation index of the CIELUV space ( $s^*_{uv}$ ) were used as discriminant variables (independent variables). The rest of the colour variables were not included in the discriminant analysis because they would cause redundant results. It was found that  $C^*_{ab}$ ,  $h_{ab}$  and  $s^*_{uv}$  were able to discriminate between both groups of samples with high levels of significance ( $p < 0.001$ ). When applying the classification functions to

sample wines, 93% of the samples were correctly assigned: the percentages of prediction were about 89% for SH wine samples (short times, high temperatures) and about 99% for LL wine samples (long times, low temperatures).

#### **4. CONCLUSIONS**

The final wines obtained after applying different pre-fermentative maceration conditions were significantly different according to their colour and their phenolic composition. With long skin contact times (12, 18 and 24 hours) and low temperatures (5 and 10 °C), the browning and the intense colour of the wines were higher, although it was considered an acceptable colour of a white wine ( $L^*$  values higher than 90 CIELAB units;  $a^*$  values ranging from -0.86 to 1.32 units;  $b^*$  values ranging from 5.40 to 21.30 units). As expected, the total phenolic content in these wines was also higher, showing caffeic acid and p-hydroxybenzoic acid the major significant differences. It has been previously reported that caffeic acid is considered a strong antioxidant against LDL oxidation, so it contributes to the beneficial health effects of these wines<sup>8</sup>.

#### **References**

1. J. J. Darias-Martín, O. Rodríguez, E. Díaz and R. M. Lamuela-Raventós, "Effect of skin contact on the antioxidant phenolics in white wine", *Food Chemistry* 71, 483-487 (2000).
2. P. Ho, F. S. S. Rogerson, S. J. Watkins, M. D. Silva, T. A. Hogg and I. Vasconcelos, "Effect of skin contact and oxygenation of musts on the composition of white port wines", *Sciences des Aliments* 19(6), 687-699 (1999).
3. V. Katalini, M. Milos, D. Modun, I. Musi and M. Boban, "Antioxidant effectiveness of selected wines in comparison with (+)-catechin", *Food Chemistry* 86, 593-600 (2004).
4. F. J. Heredia, C. Álvarez, M. L. González-Miret and A. Ramírez, "CromaLab<sup>®</sup>, análisis de color", Registro General de la Propiedad Intelectual. SE-1052-04. Sevilla. Spain (2004).
5. M. Melgosa, M. M. Pérez, A. Yebra, R. Huertas and E. Hita, "Algunas reflexiones y recientes recomendaciones internacionales sobre evaluación de diferencias de color", *Óptica Pura y Aplicada* 34, 1-10 (2001).
6. M. Mayén, R. Barón, J. Mérida and M. Medina, "Changes in phenolic compounds during accelerated browning in white wines from cv. Pedro Ximénez and cv. Baladi grapes", *Food Chemistry* 58, 88-95 (1997).
7. C. Betés-Saura, C. Andrés-Lacueva and R. M. Lamuela-Raventós, "Phenolics in white free run juices and wines from Penedes by high-performance liquid chromatography: changes during vinification", *Journal of Agricultural and Food Chemistry* 44, 3040-3046 (1996).
8. D. P. Makris, E. Psarra, S. Kallithraka and P. Kefalas, "The effect of polyphenolic composition as related to antioxidant capacity in white wines", *Food Research International* 36, 805-814 (2003).