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PHENOLIC COMPOSITION OF BOBAL RED WINES ELABORATED WITH PRE-FERMENTATIVE COLD MACERATION

COMPOSIÇÃO FENÓLICA DOS VINHOS TINTOS DA CASTA BOBAL FEITOS COM MACERAÇÃO PRÉ-FERMENTATIVA EM FRIO

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SUMMARY

The vinification technique called pre-fermentative cold maceration is used to enhance the anthocyanins diffusion from the skins to the must, increasing the pigments extraction. In this study, the influence of the pre-fermentative cold maceration treatments (3 and 5 days) and the time of maceration (7 or 14 days) on color characteristics and phenolic composition of Bobal red wines made from grapes harvested at different phenolic maturity was studied. The results showed the higher the phenolic maturity the higher polymerized tannins, ethanol index and the lower astringency. The pre-fermentative cold maceration enhanced berry compounds extractability, but led to wines with less polymerized anthocyanins and tannins. Moreover wines with higher tannin concentration, total polyphenols and polymerization degrees were produced with longer maceration times. On the contrary a decrease in colour and anthocyanins concentration was observed. Regarding sensory analysis, it has been highlighted the importance of grape maturity and pre-fermentative cold maceration length on the wine quality.

RESUMO

A técnica de vinificação chamada de maceração pré-fermentativa em frio é utilizada para aumentar a difusão de antocianinas das peliculas da uva para o mosto, aumentando por sua vez a extração de pigmentos. Neste trabalho pretendeu-se estudar a influência da maceração pré-fermentativa em frio (3 e 5 dias) e o tempo de maceração (7 ou 14 dias) sobre as características da cor e da composição fenólica dos vinhos tintos de casta Bobal feitos a partir de uvas colhidas em maturação fenólica diferente. Os resultados mostraram que quanto maior a maturação fenólica, maior a quantidade de taninos polimerizados, maior índice de etanol e menor adstringência. Esta técnica permitiu aumentar consideravelmente o extração dos compostos fenólicos da uva; contudo pode levar a que haja menor grau de antocianinas e taninos polimerizados nos vinhos. Além disso, os vinhos com maior concentração de taninos, polifenóis totais e graus de polimerização foram produzidos com maior tempo de maceração. Por outro lado foi observada uma diminuição da cor e da concentração de antocianinas. Em relação à análise sensorial, destaca-se a importância da maturação da uva e da duração da maceração pré-fermentativa em frio na qualidade do vinho.

Key words: grape maturity, cold maceration, phenolic composition, Bobal red wines.
Palavras-chave: maturação da uva, maceração em frio, composição fenólica, vinhos tintos da casta Bobal.

INTRODUCTION

The grape maturation is a complex process which involves biosynthesis, transport, storage and transformations of different components. Compounds such as sugars and organic acids or phenols and flavor precursors are involved in grape quality. Therefore it is important to understand the grape development during ripening in order to define the date of harvest (Diaz-Plaza *et al*., 2000; Rodriguez-Vila, 2000; Souquet *et al*., 2000; Brenon *et al*., 2005; Ribereau-Gayon *et al*., 2006).

The pre-fermentative maceration is defined as the period of time from the filling into tanks with the crushed grapes to the beginning of the alcoholic fermentation. This technique, also known as cold soaking or cryomaceration, is being increasingly used by enologists worldwide in order to improve some important quality characteristics of wines, such us color and aroma (Blouin *et al*., 2000; Gómez-Plaza *et al*., 2001; Parenti *et al*., 2004; Koyama *et al*., 2007; Gómez-Miguez *et al*., 2007; Gil-Muñoz *et al*., 2009). During the pre-fermentative period the extraction of polyphenols from the skins to the must take place in the absence of ethanol (Feuillat, 1996). The cold needed to achieve the low temperature can be obtained in diverse manners, being probably the most widely the addition of dry ice (solid carbon dioxide) (Heredia *et al*., 2010).

Traditionally made in Burgundy due to the low harvest temperatures, this technique has a considerable effect on the wine quality, enhancing the aromatic fraction of Pinot noir wines. Retali (2004) noted that pre-fermetative cold maceration in Nielluccio grapes led to wines with greater aromatic expression and quality, where the anthocyanins are mainly extracted at this step. However when the alcohol content increases a slightly decrease by degradation, absorption or structure modification was observed.

It is well known that this technique was designed to improve the extraction of pigments, tannins and aromas from grape skins to the wine. The underlying idea is that aqueous extraction should improve wine color although previous studies have provided contradictory results. The results obtained in several studies have shown that a cold soak alone has either no effect or a negative effect on the phenolic composition (Watson *et al*., 1995; Feuillat, 1996; Heatherbell *et al*., 1997; Okubo *et al*., 2003; Marais, 2003 a,b). Moreover several authors note the positive influence if this technique on the final composition and sensory quality (Parenti *et al*., 2004; Álvarez *et al*., 2006; Gómez-Miguez *et al*., 2007; Koyama *et al*., 2007; Gil-Miñoz *et al*., 2009; Gordillo *et al*., 2010; Heredia *et al*., 2010).

One of the main features in the red wine making process is the presence of skins and seeds. Red winemaking is thus a very complex process where an overlapping between alcoholic fermentation and maceration take place. This phenomenon, which is responsible for wine color, is not simply an extraction procedure, as numerous reactions in the grape phenolics also occur that deeply influence the color intensity and stability of the wine (Sims and Bates, 1994). The length of skin maceration is the first factor that affects this phenomenon, although maceration affects the extraction not only of phenolic compounds but also of other compounds (proteins, polysaccharides) that may participate in condensation reactions (Glories, 2001; Gómez-Plaza *et al*., 2001; Gónzález Neves *et al*., 2003).

In recent years, some winemaking techniques have also being tested in order to elaborate red wines (Sacchi *et al*., 2005). However the knowledge of the influence of these techniques on the final Bobal red wines composition and sensory quality has not been well studied yet, so only a few data can be found in the literature. For instance Gómez Gallego *et al*. (2012a) stated that wines made with Moravia agria or Bobal, two red single cultivars in danger of extinction in Castilla-La Mancha, are a viable alternative to traditional grape varieties, increasing the offer to the consumer, when the phenolic composition, chromatic characteristics, antioxidant activities and sensory profile were tested. Gómez-García Carpintero *et al*. (2011) also demonstrate that Bobal wines present a complex chemical profile with a wealth of aromas in its aromatic composition. Gómez Gallego *et al*. (2012b) studied the phenolic composition and sensory properties of five minor red grape varieties from Castilla-La Mancha region (Bobal, Moravia agria, Moravia dulce, Rojal y Tortosí). The study suggested that, in order to see how the compounds studied in these grapes are transferred to the elaborated wines and affects their sensory properties; future work verifying the effect of the wine making process will be needed.

The Bobal grape variety was selected, on the one hand, because it is the most widely used in the Designation of Origin Utiel-Requena (Valencia, Spain) with more than 70% of the total grapevine area of this region and, on the other hand, because despite being used traditionally in the production of rosé and red "doble pasta" wines and being sold mostly in bulk during the last few years Bobal grapes have been used to produce high quality aged wines shown the high potential of the grapes (Méndez, 2005; Sánchez, 2008; Gómez-García Carpintero *et al*., 2011). The main objective of this study was to assess the pre-fermentative cold maceration influence on the color and phenolic composition of Bobal red wines. In this sense the definition of chemical and phenolic characteristics of the wines produced by two maceration times and at different grape maturity levels has been studied. The knowledge of the most important phenolic parameters may profit from the selection of the best winemaking procedure and the optimum date of harvest in order to increase wine quality.

MATERIAL AND METHODS

Grapes of *Vitis vinifera*, cv. Bobal were harvested in 2008 from 40 years old vines at the Coloraos experimental vineyard located in Requena (Valencia, Spain) at two different maturity levels, spaced one week. Grapes were carefully harvested into 15 kg boxes and transported to an experimental wine production center.

The grapes were destemmed and crushed, and the must was homogenized and distributed to 50 L stainless steel tanks. Sulfur dioxide was added (5 g/hL) prior to carry out the different vinification methods. Alcoholic fermentation was induced by inoculation with *Saccharomyces cerevisiae* (2056 Uvaferm Rhone) at the rate of 20 g/hL. To ensure the development of malolactic fermentation selected *Oenococcus oeni* lactic acid bacteria (Starin 31 ITV France Lallemand) were inoculated at the end of alcoholic fermentation.

Cold pre-fermentative maceration process consisted of two stages: a first stage where the refrigeration of the grapes takes place in a cold storage-room at 10 ºC for 3 or 5 days, followed by 7 or 14 days of traditional maceration-fermentation (between 20-25 ºC) - Figure 1. After the cold maceration period was completed the temperature of the tanks was rapidly brought to 20 ºC to allow the starting of alcoholic fermentation. Punching was carried out once a day during the fermentation process. For the wine conservation 30 mg/L sulfur dioxide was added. At the end of fermentation the wines were raked and stored in a cold room at 5 ºC for two months. All wines were elaborated in triplicate.

Analytical essays

Enological parameters such as degree Beaumé (ºBé), pH or total acidity (mg/L tartaric acid) or alcohol (% v/v) were determined according to official EU methods (Reglamento 440/2003).

Color intensity (CI) and total polyphenol index

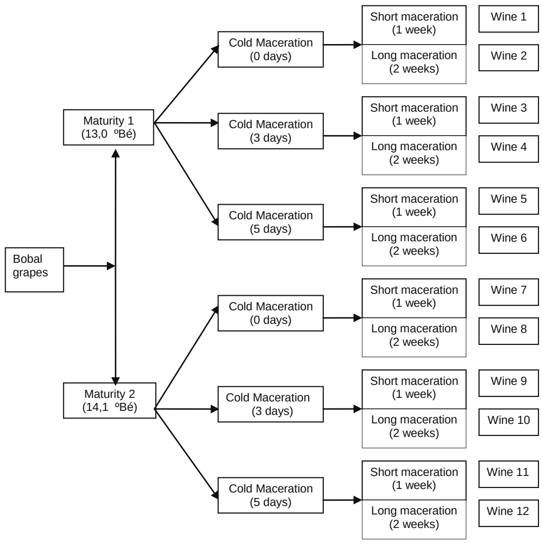

Figure 1 - Experimental design
Desenho experimental

(TPI) was determined by spectophotometric methods (Blouin 1992). Quantification of antocyanins was done by high performance liquid chromatography (HPLC) using the modified method as described by Boido *et al*. (2006). Concentrations were determined using the external standard method and their factor responses were deduced from their respective calibration curves. Quantification of condensed tannins were done by precipitation with methyl cellulose (Sarneckis *et al*., 2006). Polyvinylpolipyrrolidone (PVPP) and astringency was estimated by the method referenced by Llaudy *et al*. (2004). Hydrochloridric (HCl), ethanol (EtOH), polymerization, dyalisis, and ionization (Glories, 1984), and DMACH (Vivas *et al*., 1994) indexes were determined. Each analysis was performed in triplicate.

Sensory analysis

The sensory panel consisted in 9 judges with considerable experience in sensory analysis. Sensory profile was determined using six descriptors (color, aroma intensity and aroma quality, taste intensity and taste quality and final assessment). The panelists used a 10-point scale to rate the intensity of each attribute. Assessment took place in a standard sensory analysis chamber, equipped with separate booths and wine tasting glasses.

Statistical analysis

To study the influence of the several factors analysis of variance (ANOVA) was performed. Principal Component Analysis (PCA) was carried out and was applied to discriminate among the means of phenolic and traditional parameters in the wines according to the different winemaking techniques.

Analysis of variance (ANOVA) and Principal Component Analysis (PCA) were applied, in order to evaluate whether significant differences among the samples exist as well as to select the variable that most influence the differences between them. LSD test was used to separate the means (α-value < 0.01) when the ANOVA test was significant. For the statistical treatment of the data the Statgraphics Plus 5.1 software was used.

RESULTS AND DISCUSSION

General composition of must

Means values of must standard parameters for maturity 1 (October 15th) and maturity 2 (22th October) respectively are: degree Beaumé (13.0±0.05 and 14.1±0.07), pH (3.40 ± 0.02 and 3.48 ± 0.03), total acidity (6.18 ± 0.20 and 6.10 ± 0.15 g/L tartaric acid). The data obtained was concordant with previous studies carried out in Bobal grapes (Gómez-García Carpintero *et al*., 2011). These analyses revealed that the grapes were in good conditions to be harvested and elaborated. As was to be expected total acidity and pH of musts was higher and lower, respectively, in the grapes harvested first (Blouin and Gimberteau, 2004). With regards to the results it should be pointed out that, despite the high degree Beaumé observed in both dates of harvest, the cultivar is able to keep higher values of acidity displaying the great results of this variety in the Utile-Requena region edapho-climatic conditions.

Composition of the wines

Table I shown the results obtained for the enological parameters of the different wines elaborated. Three conventional parameters and twelve spectral phenolic parameters were quantified due to their significance in red wines (Spranger *et al*., 2004).

Generally speaking the higher TPI, CI, anthocyanins and tannin concentration values were observed for the wines elaborated with grapes from maturity 1 (Wines 1-6). These results are not in agreement with other studies (Bautista *et al*., 2003; Llaudy *et al*., 2005) where better results were observed at higher maturity grapes level. An overall view of these data indicates probably overripened grapes where the harvest was carried out in the latest date (maturity 2).

In addition wines where pre-fermentative cold maceration (PCM) was applied for 3 days shown the higher phenolic concentration (TPI), however wines that reach the highest colour intensity and anthocyanin concentration were those cold soaked for 5 days. In previous studies this technique (PCM) has been successfully tested and has resulted in an improved CI (Gómez-Plaza *et al*., 2001; Parenti *et al*., 2004; Koyama *et al*., 2007; Gil-Muñoz *et al*., 2009) and anthocyanin concentration (Gómez-Míguez *et al*., 2007; Álvarez *et al*., 2009; Gordillo *et al*., 2010).

The pre-fermentative cold maceration for 3 or 5 days increase the anthocyanin concentration between 13%

TABLE I

Mean values and standard deviation of phenolic parameters in the wines

Valores médios e desvio padrão dos parâmetros fenólicos em vinhos

	Wine 1	Wine 2	Wine 3	Wine 4	Wine 5	Wine 6	Wine 7	Wine 8	Wine 9	Wine 10	Wine 11	Wine 12
Grape maturity	October 15th						October 22th					
Cold soak	0 days		3 days		5 days		0 days		3 days		5 days	
Maceration time	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks	1 week	2 weeks
Alcohol (% v/v)	14.30±0.15	14.35±0.20	14.25±0.25	14.32±0.24	14.30±0.15	14.30±0.20	15.30±0.30	15.15±0.20	14.98±0.22	15.17±0.41	15.37±0.23	15.37±0.37
TA (g/L)	6.23±0.25	6.15±0.30	6.27±0.45	6.23±0.27	6.08±0.18	6.15±0.52	6.30±0.12	6.15±0.41	6.47±0.16	5.59±0.19	6.10±0.05	6.04±0.23
PH	3.41±0.04	3.41±0.01	3.41±0.02	3.39±0.02	3.40±0.01	3.38±0.03	3.41±0.02	3.41±0.01	3.53±0.01	3.52±0.02	3.50±0.04	3.50±0.02
TPI	44.7±3.2	44.7±3.1	52.8±2.4	50.7±1.6	48.1±1.1	49.2±0.5	33.1±0.2	38.0±4.3	34.8±2.4	40.0±1.3	44.6±1.2	46.3±0.5
CI	13.5±0.9	11.6±0.3	12.6±0.2	10.7±0.3	13.3±0.2	13.4±0.2	7.2±0.7	7.9±0.5	8.8±0.4	6.8±0.2	10.7±0.4	11.1±0.1
Anthocyan.(mg/L)	431.3±3.5	234.0±1.7	395.7±3.0	321.7±2.1	405.5±3.3	413.8±2.0	172.3±1.8	196.8±1.2	282.6±1.9	174.3±1.6	254.1±2.6	308.4±2.5
Tannins (g/L)	2.17±0.02	2.27±0.01	2.16±0.13	2.35±0.17	1.72±0.02	2.57±0.06	1.92±0.07	1.78±0.08	1.23±0.13	1.57±0.07	1.11±0.10	1.48±0.11
HCL index	33.0±0.9	36.7±0.2	35.0±1.0	19.8±2.3	20.3±1.8	24.5±3.0	39.4±0.2	44.5±0.7	29.3±0.6	23.5±1.2	20.1±2.2	18.0±2.7
Ethanol index	39.3±0.2	35.9±1.0	22.0±3.1	22.7±1.4	12.7±2.2	22.8±2.2	41.1±0.7	32.4±0.1	37.1±1.3	42.6±3.6	31.9±0.8	31.1±3.3
Astringency index	55.4±1.6	58.0±1.7	57.6±3.4	54.5±3.3	62.6±3.8	39.1±3.1	22.4±0.6	48.5±1.4	23.5±2.9	32.7±3.4	27.8±3.0	20.8±0.6
PVPP index	45.2±3.5	38.6±3.2	25.6±1.6	22.5±2.6	28.2±2.2	25.9±3.7	45.8±0.4	46.4±0.5	37.3±2.1	41.2±2.8	44.3±3.4	33.5±3.7
Polymer. index	33.3±0.9	33.0±0.3	33.3±1.1	38.1±1.5	31.7±1.5	43.9±3.2	31.7±3.2	35.4±2.4	34.0±0.4	42.1±3.2	34.7±2.5	40.7±1.2
Ionizati. index	21.2±0.2	22.7±0.8	23.0±1.3	19.9±1.0	21.1±0.6	21.9±0.7	20.8±1.1	19.8±0.7	29.6±1.3	28.2±2.0	29.4±2.4	28.7±2.2
DMACH index	42.5±3.2	38.3±2.3	34.4±1.1	39.2±1.8	37.9±3.2	36.9±1.5	40.1±3.6	39.9±3.2	31.7±3.1	33.8±1.3	40.9±3.7	29.8±1.6
Dialysis index	31.7±1.5	33.8±1.7	34.8±1.8	39.0±1.7	32.4±2.1	19.3±1.7	40.4±2.9	49.4±1.6	37.3±1.5	36.2±3.1	36.4±3.0	34.0±3.1

and 33% respectively, in concordance with other studies (Cuasnon, 1999a; Zamora, 2003) where an increase between 52% and 17%, respectively was observed, although dry ice was used in the pre-fermentative step.

On the other hand the tannin concentration remains unaffected in the cold soaked wines, being the not cold soaked, the wines that show the highest tannin content. In red wines the target of cold pre-fermentative maceration is to enhance the water-soluble compounds in the absence of ethanol, since as the cap has not been formed the contact between the solid parts and the must is better (Gómez-Miguez *et al*., 2007), therefore a tannin, alcohol soluble compounds, extraction should not be expected (Sacchi *et al*., 2005).

Proanthocyanidins in grape berry are located in skin and seeds. The skin proanthocyanidins are located in the vacuoles of skin cells, then the extraction during the pre-fermentative maceration process, requires the cells walls to be broken, to allow their vacuole content to be extracted or to diffuse into the wine. Moreover the extraction of the proanthocyanidins of the seed needs longer maceration time, because the lipids present in the seed must be eliminated first, which is normally done by the increasing content of ethanol (Glories and Saucier, 2000). In conclusion skin tannins are early extracted and give volume and smoothness, but in the presence of alcohol, seed tannins are also extracted which provide rougness and astringency (Ribereau-Gayon *et al*., 2006).

The contradictory results obtained shown the extraction of the phenolic compounds in the pre-fermentative step was highly dependent on several factors such as grape variety and maturity, soil, climate and vinification techniques (Auw *et al*., 1996), as well as by the proanthocyanidin concentration, the composition of the berry cell walls, and the processing method (Busse-Valverde *et al*., 2010).

The wines elaborated with higher grape maturity and cold soaked shown the higher proportion of anthocyanins in colored forms (flavilium cation), measured by the ionisation index. In addition the higher grape maturity wines have higher tannin-polysaccharide molecules (ethanol index), decreasing considerably their concentration in pre-fermentative macerated wines.

In terms of stability, the higher grape maturity wines shown a higher percentage of anthocyanins linked with tannins (A-T), determined by the PVPP index, being found the highest values in the not cold soaked wines. The higher anthocyanin extraction in the cold soaked wines led to less anthocyanin-tannin molecules. The ratio between these compounds did not allow achieving good anthocyanin stabilization. Otherwise the higher maceration time the higher anthocyanins mean degree of polymerisation, measured by the polymerization index, showing the cold macerated wines the highest values, as has been reported by several authors (Gordillo *et al*., 2010; Heredia *et al*., 2010).

The wines made with more mature grapes shown higher dialysis index, stating higher proportion of, anthocyanin or polysaccharide, linked tannins molecules, suggesting polymerization reaction during ripening. These results were particularly relevant in the non pre-fermentative macerated wines. On the other hand these wines led to a higher tannin mean degree of polymerisation, measured by the HCL index. Finally the most astringent wines are those made with the less mature grapes.

Effect of grape maturation

Mean and standard deviation values of the phenolic parameters according to the different date of harvest appear in the Table II. The statistical analysis was realized considering all the wines made from grapes harvested in both dates tested. Independent of the vinification technology used the effect of the grape maturity in wine composition was assessed. In these wines, the effect of the grape ripeness produces a significant increase in TPI, CI, anthocyanin and tannin concentration and gelatin index in wines harvested first. The results show that the maturation level has a great impact on the phenolic composition of Bobal wines. Saint Cricq de Gaulejac *et al*. (1998) mentioned that the optimal grape maturity level occurs when the anthocyanins decrease start, i.e., at the beginning of the overripening period when the degradation of the berry cells starts.

TABLE II
Effect of grape phenolic maturity on phenolic parameters
Efeito da maturação fenólica da uva nos parâmetros fenólicos

	Maturity 1 October 15th	**Maturity 2 October 22th**
TPI	48.36±2.0 b	39.47±1.7 a
CI	12.52±0.4 b	8.75±0.4 a
Anthocyan.(mg/L)	367.00±2.6 b	231.42±1.9 a
Tannins (g/L)	2.21±0.07 b	1.52±0.09 a
HCL index	28.28±1.5	29.13±1.3
Ethanol index	25.90±1.7 a	36.08±1.6 b
Astringency index	54.53±2.8 b	29.28±2.0 a
PVPP index	32.67±2.8 a	41.42±2.2 b
Polymer. Index	35.56±1.4	36.43±2.2
Ionizati. Index	21.63±0.8 a	26.08±1.6 b
DMACH index	38.20±2.2	36.03±2.8
Dialysis index	31.83±1.7 a	38.95±2.5 b

Different letters within the same row indicates the existence of statistical significant differences.

Anthocyanins synthesis starts during veraison and remains active throughout grape ripening. For this reason anthocyanins accumulate in the skins during ripening (Fernández-López *et al*., 1992; Lanaridis and Bena-Tzourou, 1997). However anthocyanin concentration may decrease slightly during overripening (González-San José *et al*., 1990; Cacho *et al*., 1992). The concentration of some anthocyanin monoglucosides such as malvidin-3-glucoside and peonidin-3-glucoside usually increase during maturation, while the others anthocyanin monoglucosides tend to decrease during ripening. Simultaneously acylated anthocyanins tend to increase throughout ripening, although in some cases they decrease at the end of the process (Canals *et al*., 2005). Theoretically wines obtained from well-ripened grapes have more color and are richer in anthocyanins than those obtained from unripened grapes.

Generally speaking the proanthocyanidin concentration is highest at veraison. Later, proanthocyanidin concentration decrease after some time before complete ripeness, when it remains relatively constant. At the same time the main degree of polymerization increases throughout ripening (Canals *et al*., 2005). The significant differences obtained in the wines made from second harvested grapes could be due by a varietal factor where overripened grapes were used, although the evolution of the phenolic composition of Bobal grapes has not been researched yet. The decrease in the anthocyanin and tannin concentration led to a decrease in the TPI and therefore to the CI, parameter highly related to the phenolic composition of the elaborated wines.

No significant difference in the HCL, polymerization and DMACH indexes was found between both dates of harvest. Otherwise higher anthocyanins that contribute to the wine color (Ionisation index), and linked anthocyanin-tannin compounds (PVPP index) were found in the wines from more mature grapes. It has been well reported that the higher A-T molecules the higher color stability, keeping the anthocyanins in solution, avoiding precipitation phenomenon (Boulton, 2001), but several studies have recently questioned the stability of this reactions (Cheynier *et al*., 2006). Ethyl linked anthocyanins are less stable than anthocyanins in aqueous solution at wine pH because of acid-catalyzed cleavage of their ethyl bounds. Reactions of the cleavage products led to flavanyl-pyranoanthocyanins, which change color from red to tawny and increase resistant to sulfite bleaching.

The more mature grapes have shown the higher dialysis and ethanol indexes values. As was to be expected greater amounts of high polymerized tannins were observed in the wines made from grapes harvested last. Polysaccharides are present in concentrations ranging from 0.5 to 1.5 g/L. Wine polysaccharides play an important role in mouthfeel properties of wine even by providing increased viscosity or by modulating astringency. More specifically there is evidence that polysaccharide fractions are likely to contribute a fullness sensation to wine inducing a greater decrease on the attributes associated with astringency (Vidal *et al*., 2004).

Effect of pre-fermentative cold maceration

The use of pre-fermentative cold maceration allows better structured wines, richer in phenolic and aromatic compounds, thus preventing a strong connection with the area of production (Álvarez *et al*., 2006). Moreover this technique can potentially promote a selective and significant extraction of phenols, these results in a less aggressive taste and reduces de use of sulphur dioxide as anti-microbic and anti-oxidant (Amati *et al*., 1992).

Mean and standard deviation values of the phenolic parameters according to the pre-fermentative cold maceration treatment appear in the Table III. Independent of the vinification technology used the effect of pre-fermentative cold maceration technique in wine composition was assessed. Two different maceration periods were assayed: 3 and 5 days. A wine elaborated following the traditional vinification method, as a control, was also carried out. The influence of this technique on the final phenolic composition led to significant changes in most of the parameters studied.

According to these variables, wines were affected in general by the pre-fermentative treatment used. These results are similar to those found by several authors (Cuasnon, 1999b; Zamora, 2004; Casassa *et al*., 2005).

condensation or copigmentation reactions between anthocyanins and other polyphenols are also responsible for the color of young wines (Francia-Aricha *et al*., 1997; Darias-Martín *et al*., 2001). Only in the 5 days cold macerated wines, where the anthocyanin concentration is higher, the CI has significant differences, stating that a long pre-fermentative period is required in order to decisively influence the Bobal wines chromatic characteristics. As it was reported above several authors found better color where pre-fermentative maceration was applied, being probably one of the most important features of this vinification technique.

TABLE III

Effect of pre-fermentative cold maceration on phenolic parameters

Efeito da maceração pré-fermentativa em frio sobre os parâmetros fenólicos

	Prefermentative cold maceration		
	0 days	**3 days**	**5 days**
TPI	40.13±2.7 a	44.58±1.9 b	47.05±0.8 b
CI	10.05±0.6 a	9.73±0.3 a	12.13±0.2 b
Anthocyan.(mg/L)	258.60±2.1 a	293.58±2.1 b	345.45±2.6 c
Tannins (g/L)	2.04±0.05 b	1.83±0.13 a	1.72±0.07 a
HCL index	38.40±0.5 c	26.90±1.3 b	20.73±2.4 a
Ethanol index	37.18±0.5 c	31.10±1.5 b	24.63±2.4 a
Astringency index	46.08±1.3 c	42.08±3.2 b	37.58±2.6 a
PVPP index	44.00±1.9 b	31.65±2.3 a	32.96±3.3 a
Polymer. Index	33.35±1.7	36.88±1.6	37.75±2.0
Ionizati. Index	21.13±0.7 a	25.18±1.4 b	25.27±1.5 b
DMACH index	40.20±3.1 b	34.78±1.8 a	36.38±2.5 a
Dialysis index	38.75±1.9 b	36.83±2.0 b	30.53±2.3 a

Different letters within the same row indicates the existence of statistical significant differences

The TPI and the anthocyanin concentration shown the higher content in wines after 5 days of skin maceration, where significant differences between both cold maceration treatments appeared for the anhtocyanin content. Moreover the CI only shows significant differences where the pre-fermentative treatment is extended to 5 days. Otherwise the cold soaked wines shown lower values for the tannin concentration where the not macerated wines have significant differences displaying the higher amounts of these compounds. These results are in concordance with Retali (2004), who found no differences between the tannin concentration in cold soaked Niellucio wines, while an increase in CI, anthocyanin concentration and TPI was detected.

During the vinification of red grapes, the length of the maceration step, together with the effect of other parameters, such as temperature, stirring of the fermentative medium and wine making process have a considerable influence of the final phenolic content of the wine. Moreover it is known that the maceration temperature greatly affects the transfer of polyphenols from the skins to must (Gil-Muñoz *et al*., 1999). The results suggest higher maceration temperatures are needed in Bobal wines on order to obtain higher tannins concentration.

It is well known that wine color is not only due to free monomeric anthocyanins. New pigments formed after

In the pre-fermentative step the extraction of the phenolic compounds takes place in the absence of ethanol because this low maceration temperature prevent yeast from starting the fermentation process. Anthocyanins and tannins are the compound most directly responsible for the color in red wine. Anthocyanins are easily soluble in water and are dissolved from the moment maceration begins, whereas tannins are more soluble in alcohol so, their extraction depends on fermentation. Therefore, the longer cold pre-fermentative maceration treatment the higher anthocyanin concentration. Five days cold maceration led to wines with better chromatic characteristics.

A significant negative effect was found in the pre-fermentative macerated wines. The control wines have shown higher ethanol, gelatin, PVPP and dialysis indexes, where an increase in the cold maceration length led to wines with significant lower values. By contrast the cold soak gives wines where highly colored anthocyanins concentration was present. A higher mean degree of tannin polymerisation, determined by the DMACH index, was also detected.

Unlike it was observed by several authors, who found a better phenolic interaction when pre-fermentative techniques were assayed (Álvarez *et al*., 2009, Gordillo *et al*., 2010; Heredia *et al*., 2010), in the Bobal wines elaborated this technique did not provide an increase in the interactions between the compounds that decisively influence the chromatic and sensory wine properties. This behavior could be done by the low tannin concentration observed in the pre-fermentative cold macerated wines. An optimum ratio between anthocyanins and tannins is needed in order to achieve a better color stability (Zamora, 2003).

During storage and ageing, wine color changes from bright red to a reddish brown hue. This is attributed to the formation of new, more stable, polymeric pigments proceeding from reaction between anthocyanins and other phenolic compounds, including, flavan-3-ol monomers and polymers (Castillo-Sánchez *et al*., 2008). It is well known that the formation of polymeric pigments between anthocyanins and flavanols allow the preservation of wine color (Thorngate and Singleton, 1994; Rivas Gonzalo *et al*., 1995; Bakker and Timberlake, 1997). Accordingly the low tannin concentration observed in the wines do not led to high anthocyanin stabilization. The longer the pre-fermentative cold maceration period the lower anthocyanin-tannin interaction.

Effect of the maceration time

The phenolic compounds present in the grape berry are transferred to the must during alcoholic fermentation process. Not only an extraction procedure takes place, as a numerous reactions in the grape phenolics also occur that deeply influence the color intensity and stability of the wine. The length of skin maceration is the first factor that affects this phenomenon, although maceration affects the extraction not only of phenolic compounds but also of other compounds (proteins and polysaccharides) that may participate in condensation reactions.

The concentration of phenolic compounds and spectral data of the wines where different maceration times were tested are shown in Table IV. The increase of the maceration time from 1 week to 2 weeks allowed to wines with significant higher TPI and tannin concentration, but otherwise a significant decrease in the color and anthocyanin concentration was observed in the longer macerated wines. Moreover no significant differences were found in the parameters which determine the reactions between the main wine phenolic compounds. As it was expected only the 2 weeks macerated wines shown a higher polymerisation index, stating a polymeric pigments increase due to the anthocyanin reactions, with other structures since anthocyanins are implicated in copigmentation complexes, chemical reactions (oxidation, covalent linking) and polymerization reactions of that transform the monomeric anthocyanins into polymeric pigments (Álvarez *et al*., 2009).

TABLE IV
Effect of maceration time on phenolic parameters
Efeito do tempo de maceração nos parâmetros fenólicos

	Maceration time	
	1 week	**2 weeks**
TPI	43.01±1.8 a	44.82±1.9 b
CI	11.02±0.5 b	10.25±0.3 a
Anthocyan.(mg/L)	323.58±2.7 b	274.83±1.9 a
Tannins (g/L)	1.72±0.08 a	2.00±0.13 b
HCL index	29.52±1.1	27.83±1.7
Ethanol index	30.68±1.4	29.58±1.9
Astringency index	41.55±2.6	42.27±2.5
PVPP index	37.73±2.4	34.68±2.8
Polymer. Index	33.12±1.6 a	38.87±2.0 b
Ionizati. Index	24.18±1.2	23.53±1.2
DMACH index	37.92±3.0	36.32±2.0
Dialysis index	35.50±2.1	35.28±2.1

Different letters within the same row indicates the existence of statistical significant differences.

Longer contact time is related to a greater concentration of anthocyanins in wines, although some studies have found no direct relationship between the length of maceration and the final anthocyanin content, as a part of the anthocyanins may be fixed in the solids or maybe reduced to a colorless forms. Flavan-3-ol and tannin concentration also increase during skin fermentation, and it has been found that tannins continue to be extracted after anthocyanin extraction has reached a maximum (Gómez-Plaza *et al*., 2001).

Principal Component Analysis

In order to verify the results obtained a Principal Component Analysis (PCA) was tested. This procedure extracts the dominant patterns in the data matrix in terms of a complementary set of scores and loading plots. This treatment permits us a reduction of dimensionality, a data exploration finding relationships between objects, estimating the correlation structure of the variables and investigating how many components are necessary to explain the greater part of variance with a minimum loss of information. When PCA is performed on auto-scaled matrix data the principal component loading are eigenvectors of the correlation matrix.

PCA was performed on the autoscaled data (8 samples and 15 variables) using the Statgraphics software package in order to provide partial visualization of the data set in a reduced dimension, and four principal components with eigenvalues higher than one accounting for 84.24% of total variance were obtained. From the loadings of the variables (Table V) mainly alcohol (% v/v), pH, ethanol and PVPP index are the positive dominant features in the PC1, by contrast TPI, CI and anthocyanin and tannin concentration are the main negative ones, accounting for 43.89%

TABLE V

Loadings in the features on the first four principal components

Carga nos recursos dos primeiros quatro componentes principais

	PC1	PC2	PC3	PC4
Alcohol (% v/v)	0,3589	-0,0500	-0,0094	-0,0828
TA (g/L)	-0,0890	0,2073	0,5811	0,2753
PH	0,3051	-0,2730	0,1823	-0,0071
TPI	-0,3175	-0,2101	-0,0409	-0,2033
CI	-0,3311	-0,1176	0,2186	0,1925
Anthocyanins (mg/L)	-0,3196	-0,1472	0,2560	0,2020
Tannins (g/L)	-0,2961	0,1504	-0,3657	0,2622
HCL index	0,0475	0,4490	-0,0219	0,1800
Ethanol index	0,2925	0,1370	-0,1610	0,4472
Astringency index	-0,3029	0,1814	-0,0543	-0,3124
PVPP index	0,2784	0,2556	-0,0252	0,2200
Polymer. Index	0,0120	-0,3458	-0,5348	0,1544
Ionizati. Index	0,2431	-0,3531	0,2236	0,0904
DMACH index	-0,0974	0,3663	-0,1015	0,0300
Dialysis index	0,2099	0,2841	0,0634	-0,5640
% of variance	43,89	24,05	9,60	6,71

of the total variability. HCL, PVPP, DMACH and Dialysis indexes dominate in the PC2, being pH, polymerization and ionization indexes the dominant in the negative side, accounting the second component for 24.05%.

In Figure 2, where the scores of each wine samples were examined in a two dimensional plot of the two first principal components (67.93% of the total variability) a complete separation of samples into two groups was found according to different date of harvest. The first group, in the positive part of the PC1 is mainly composed by samples from the earliest date of harvest. The second group in the negative part of the graph is made up of samples where grapes were harvested last.

The PCA shown a better phenolic concentration in the wines harvested first, where the presence of the main phenolic compounds were significantly higher and also a deeply increase in the wine color was found. Moreover higher astringency was detected in these wines. Is needed to be pointed out the importance of the date of harvest in the phenolic composition and chromatic characteristics of Bobal red wines, being the overripening a potential wine quality key factor. On the other hand due to the varietal factor complementary enological techniques might be needed in other to reduce the wine astringency and make wines well accepted by the consumers. It is well known by the Utiel-Requena enologists the unripe, aggressive, harsh tannins that Bobal grapes usually shown even in a perfect phenolic maturity conditions.

In addition a complete separation of samples into two groups was found according to pre-fermentative maceration technique. The first group, in the positive part of the PC2 is composed by the not cold macerated control wines. The second group in the negative part of the PC2 is made up of samples where the pre-fermentative cold maceration was applied for 3 or 5 days. The PCA do not let to a separation between the length of the pre-fermentative step.

From the loading of the variables (Table V) the PCA shown a better phenolic interaction in the not cold soaked wines, where the HCL, PVPP, DMACH and Dialysis indexes are the main dominant features. Otherwise pH and ionization and polymerization indexes are the main dominant features in the cold soaked wines. These results indicate the higher anthocyanin extraction in the cold soaked wines which led to an increase in the anthocyanins, which contributes to the wine color, with an increase in the reactions involving these compounds.

Better tannin extraction is required in cold soaked Bobal wines in order to increase the optimum ratio tannin-anhtocyanin. The higher anthocyanin concentration reached needs a higher tannin presence when better color stabilization was desirable. High tannin levels may stabilize the anthocyanins and wine color but on the other hand may contribute to excessive astringency (Sims and Bates, 1994; Gómez-Plaza *et al*., 2001).

Finally the PCA do not allow separate the wines according to the length of the skin maceration. No separation of the samples was found; therefore it has no sense to extend the maceration step, avoiding future

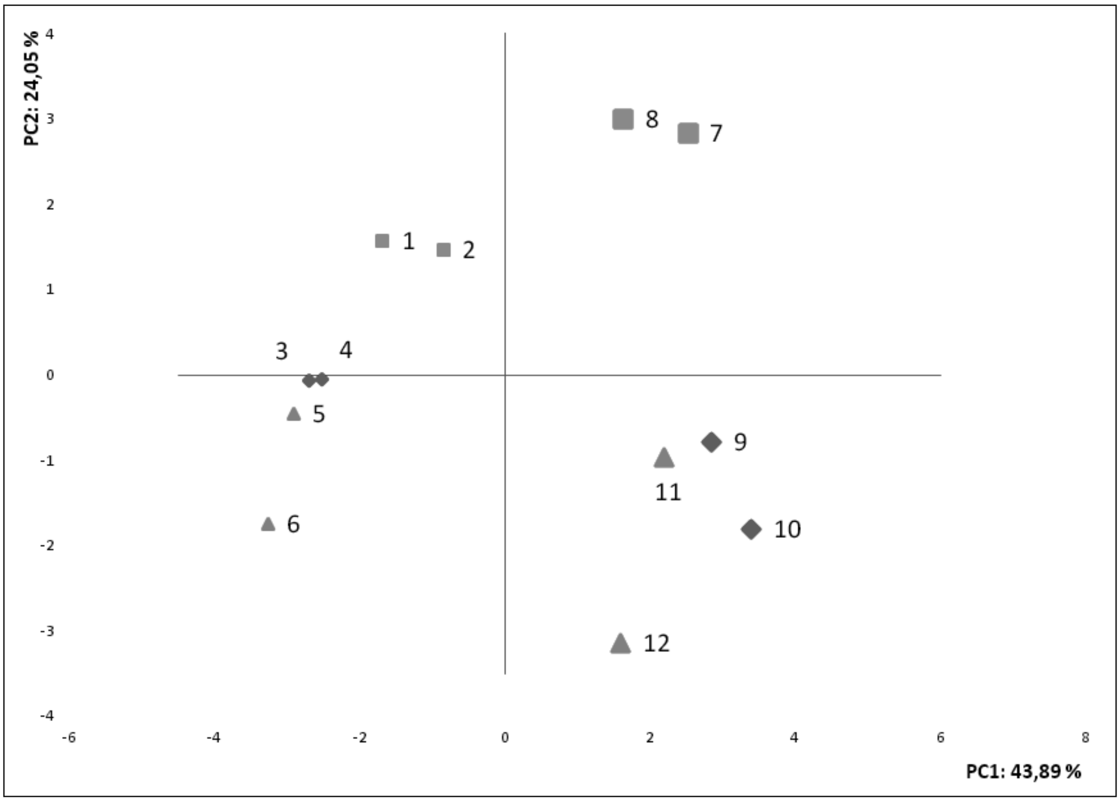

Figure 2 - Scores of the samples in the plane defined in the first two principal components. PC1 and PC2 (■ control wines ♦ 3 days cold soaked wines ▲ 5 days cold soaked wines; small symbols: 15th October harvest; big symbols: 22th October harvest).

Pontuação das amostras no plano definido nas primeiras duas componentes principais PC1 e PC2 (■ Vinhos controlo ♦ Vinhos com 3 dias de maceração pré-fermentativa em frio ▲ Vinhos com 5 dias de maceração pré-fermentativa em frio: símbolos pequenos: Colheita de15 Outubro; símbolos grandes: Colheita de 22 de Outubro).

problems in wine quality. On the other hand higher tannin concentration was observed when samples were subjected to analysis of variance (ANOVA). Suggesting better tannin concentration obtained by extending the maceration length with probably future better anthocyanin stabilization. Bobal variety is characterized in the Utiel-Requena wine region by their high amount of anthocyanins that led to good chromatic characteristics, and then even losing some anchocyanins content, greater color stability could be accomplished by extending the maceration period.

Sensory evaluation of wines

The sensory panel did not observe significant differences in the wines after malolactic fermentation for sensorial aroma intensity and quality and taste intensity, where wines from two different dates of harvest were assessed (Table VI). Wines from grapes harvested first achieved a significant greater score for the sensory color as was observed above. Otherwise wines from grapes harvested last obtained better scores for the attributes taste quality and final assessment where better phenolic interactions took place. Finally, we suggest that a slightly phenolic concentration loose could be assumed in order to obtain wines better rated by the consumers.

Not significant differences in wines macerated for 1 or 2 weeks were observed by the sensory panel. As we reported above better tannin concentration can be achieved in order to increase phenolic stability, although it has no sense increase the maceration time in wines that are not going to be aged, because wines with shorter maceration time led to better chromatic characteristics.

Only the attribute taste quality shown significant higher values when the pre-fermentative cold maceration was applied for 5 days. Moreover the cold macerated wines shown the highest values for the final assessment, where the longer the pre-fermentative step the higher the scores achieved. No differences in the sensory wine color were observed, despite the higher antchocyanin concentration in the cold soaked wines. Blouin *et al.* (2000) reported not differences between the pre-fermentative macerated and the control in a sensory evaluation of Merlot and Cabernet Sauvignon wines, stating that this technique does not always achieve good results in the sensory evaluations.

CONCLUSIONS

The maturation level has a significant impact on the phenolic composition of Bobal wines. Higher phenolic concentration and wine colour was observed in wines harvested earlier, moreover these wines shown higher astringency. Otherwise better interactions between polyphenols were also found in the wines

TABLE IV

Influence of grapes maturity, maceration time and pre-fermentative cold maceration on the mean values of wines sensory analysis.

Influência da maturação das uvas, do tempo de maceração e da maceração pré fermentativa em frio nos valores médios dos atributos sensoriais de vinhos.

Factor	Time	Colour	Aroma Intensity	Aroma Quality	Taste Intensity	Taste Quality	Final assessm.
Grape matutity	Maturity 1	7.68±0.11b	6.50±0.21	6.07±0.33	6.76±0.17	6.24±0.14a	6.37±0.24a
	Maturity 2	7.03±0.43a	6.63±0.42	6.43±0.38	6.76±0.26	6.71±0.23b	6.95±0.24b
Maceration time	1 week	7.38±0.49	6.71±0.30	6.40±0.40	6.79±0.23	6.42±0.30	6.68±0.48
	2 weeks	7.33 ±0.45	6.42±0.31	6.10±0.34	6.73±0.21	6.53±0.33	6.65±0.29
Cold maceration	0 day	7.32±0.52	6.40±0.41	6.20±0.15	6.82±0.28	6.27±0.22a	6.50±0.24
	3 days	7.41±0.52	6.63±0.19	6.40±0.35	6.86±0.17	6.59±0.24b	6.65±0.23
	5 days	7.34±0.45	6.40±0.36	6.15±0.60	6.59±0.05	6.56±0.39b	6.84±0.58

Different letters within the same column indicates the existence of statistical significant differences.

from more mature grapes. An overripenes should be taken into account in order to avoid phenolic material decrease, and an additional enological treatment may be necessary with the objective to reduce wine astringency. Pre-fermentative maceration technique led to wines with lower tannin concentration although an increase in total polyphenols and wine colour was also observed. A significant negative effect was found in the parameters that state the phenolic reactions in the cold soaked wines. The results suggest higher maceration temperatures are needed in Bobal wines on order to obtain higher tannins concentration which probably increase the reaction between polyphenols. The maceration length did not highly affect the polyphenol interaction, moreover an increase on the tannin concentration was also observed in the 2 weeks macerated wines. If a slight decrease in the anthocyanin concentration can be assumed an extended maceration could be done in order to improve tannin concentration and future possible phenolic stability. The wines better valued by the sensory panelist were those from grapes harvested last and pre-fermentative cold macerated.

REFERENCES

Álvarez I., Aleixandre J., García M.J., Lizama V., 2006. Impact of prefermentative maceration on the phenolic and volatile compounds in the Monastrell red wines. *Anal. Chim Acta*, **563**, 109-115.

Álvarez I., Aleixandre J.L., García M.J., Lizama V., Aleixandre-Tudó J.L., 2009. Effect of the prefermentative addition of copigments on the phenolic composition of Tempranillo wines after malolactic fermentation. *Eur. Food Res. Technol.*, **228**, 501-510.

Amati A., Carnicini A., Monti R., Zironi R., 1992. Vinificazione per macerazione delle vinacce e temperatura controllata influenza del tempo e della temperatura in impianto pilota. *Vignevini*, **9**, 29.

Auw J.M., Blanco V., O'Keefe S.F., Sims, C.A., 1996. Effect of processing on the phenolics and color of Cabernet Sauvignon: Chambourcin and Noble wines and juices. *Am. J. Enol. Vitic.*, **47**, 279-286.

Bakker J., Timberlake C.F., 1997. Isolation, identification, and characterization of new color-stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.*, **45**, 35.

Bautista A.B., Perez L.J., Romero I., Fernández J.I., López J.M., Gómez, E., 2003. Influencia del estado de maduración de la uva de variedad Monastrell en el color de los vinos. In: *II Congreso Nacional de Ciencia y Tecnología de Alimentos*.

Blouin J., 1992. Manuel pratique d´analyse des mouts et des vins. Chambre d´Agriculture de la Girone, France.

Blouin J., Gimberteau G., 2004. *Maduración y madurez de la uva*. Ed. Mundi Prensa, Madrid.

Blouin J., Papet N., Stonestreet E., 2000. Étude de la structure poli phénolique des vins rouges par analyses physico-chimiques et sensorielles. *J. Int. Sci. Vigne Vin*, **34**, 33-40.

Boido E., Alcalde-Eon C., Carrau F., Dellacassa E., Rivas Gonzalo J.C., 2006. Aging effect on the pigment composition and color of *Vitis vinifera* L. cv. Tannat wines. Contribution of the main pigment families to wine color. *J. Agric. Food Chem.*, **54**, 6692-6704.

Boulton R., 2001. The copigmentation of anhocyanins and its role in the color of red wine. A Critical Review. *Am. J. Enol. Vitic.*, **52**, 67-87.

Brenon E., Bernard N., Zebic, O., Deloire, A., 2005. Maturité du raisin: Propoposítión d´une méthode utilisant le volume des baies comme indicateur. *Rev. Œnolog. Techniq. Vitivinic.*, **117**, 52-54.

Busse-Valverde N., Gómez-Plaza E., López-Roca J.M., Gil-Muñoz R., Fernández-Fernández J.I., Bautista-Ortín A., 2010. Effect of different enological practices on skin and sees proanthicyanidins in three varietal wines. *J. Agric. Food Chem.*, **58**, 11333-11339.

Cacho J., Fernandez P., Ferreira V., Castells J.E., 1992. Evolution of five anthocyanidin-3-glucosides in the skin of the Tempranillo, Moristel, and Garnacha grape varieties and influence of climatological variables. *Am. J. Enol. Vitic.*, **43**, 244-248.

Canals R., Llaudy M.C., Valls J., Canals J.M., Zamora F., 2005. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.*, **53**, 4019-4025.

Casassa F., Sari S., Avagnina S., Diaz M., Jofré V., Fanzone M., Catania C., 2005. Influencia de técnicas de maceración sobre la composición polifenólica, aromática y las características organolépticas de vinos cv. Merlot. In: *X Congreso Latinoamericano de Viticultura y Enología* (Actas p. 3089). Bento Gonçalves. Brasil.

Castillo-Sánchez J.X., García-Falcón M.S., Garrido J., Martínez-Carballo E., Martins-Dias L.R., Mejuto X.C., 2008. Phenolic compounds and color stability of Vinhão wines: Influence of wine making protocol and fining agents. *Food Chem.*, **106**, 18-26.

Cheynier V., Dueñas-Paton M., Salas E,, Maury C., Souquet J., Sarni Machado P., Fulcrand H., 2006. Structure and properties of wine pigments and tannins. *Am. J. Enol. Vitic.*, **57**, 298-305.

Cuasnon M., 1999a. Une nouvelle technique: La macération prefermentaire à froid- Extraction à la niège carbonique. 1ª partie. *Rev. Œnolog. Techniq. Vitivinic.*, **92**, 26-30.

Cuasnon M., 1999b. Une nouvelle technique: La macération prefermentaire à froid- Extraction à la niège carbonique. 2ª partie. *Rev. Œnolog. Techniq. Vitivinic.*, **93**, 28-30.

Darias-Martín J., Carrillo M., Díaz E., Boulton R.B., 2001. Enhancement of wine colour by prefermentation addition of copigments. *Food Chem.*, **73**, 217–220.

Díaz-Plaza M., Lorente E.M., Reyero J.M., Pardo F., Salinas M.R., 2000. Aportación al estudio de la maduración de varias viníferas tintas cultivadas en la D.O. Jumilla. *Viticultura-Enología Profesional*, **68**, 37-46

Fernández-López J. A., Hidalgo V., Almela L., López Roca J.M., 1992. Quantitative changes in anthocyanin pigments of Vitis Vinifera cv Monastrell during maturation. *J. Sci. Food Agric.*, **58**, 153-155.

Feuillat M., 1996. Vinification du Pinot noir en Bourgogne par macération préfermentaire à froid. *Rev. Fr. Oenol.*, **82**, 29-31.

Francia-Aricha E.M., Guerra M., Rivas-Gonzalo J.C., Santos-Buelga C., 1997. New anthocyanin pigment formed after condensation with flavanols. *J. Agric. Food. Chem.*, **45**, 2262-2266.

Gil-Muñoz R., Gómez-Plaza E., Martínez A., López-Roca J.M., 1999. Evolution of phenolic compounds during wine fermentation and post-fermentation: Influence of grape temperature. *J. Food Compos. Anal.*, **12**, 259-272.

Gil-Muñoz R., Moreno-Pérez A., Fernández-Fernández J.I., Martínez-Cutillas A., Gómez-Plaza E., 2009. Influence of low temperature prefermentative techniques on chromatic and phenolic characteristics of Syrah and Cabernet Sauvignon wines. *Eur. Food Res. Technol.*, **228**, 777–788.

Glories Y., 1984. La couleur des vins rouges. Mesure, origine et interpretation. *Connais. Vigne Vin*, **18**, 253-271.

Glories Y., 2001. Caracterization du potenciel phenolique: adaptation a la vinification. *Prog. Agric. Viticol.*, **118**, 347-350

Glories Y., Saucier C., 2000. Tannin evolution from grape to wine. Effects on wine taste. In *The ASEV 50th Anniversary Annual Meeting*; Ratz, J., Ed., ASEV, Davis, CA.

Gómez Gallego M.A., Gómez García-Carpintero E., Sánchez-Palomo E., Hermosin Gutierrez I., González Viñas M.A., 2012 a. Study of phenolic composition and sensory properties of red grape varieties in danger of extinction from the Spanish region of Castilla-La Mancha. *Eur. Food Res. Technol.*, **234**, 295-303.

Gómez Gallego M.A., Gómez García-Carpintero E., Sánchez-Palomo E., González Viñas, M.A., Hermosin Gutierrez I. 2012 b. Oenological potential, phenolic composition, chromatic characteristics and antoixidant activity of red-single cultivar wines from Castilla-La Mancha. *Food Res. Int.*, **48**, 7-15.

Gómez Míguez M., González-Miret M.L., Heredia F.J., 2007. Evolution of colour and anthocyanin composition of Syrah wines elaborated with pre-fermentative cold maceration. *J. Food Eng.*, **79**, 271-278.

Gómez-García Carpintero E., Sánchez-Palomo E., González-Viñas M.A., 2011. Aroma characterization of red wines from cv. Bobal grape variety grown in la Mancha region. *Food Res. Int.*, **44**, 61-70.

Gómez-Plaza E., Gil-Muñoz R., López-Roca J.M., Martínez-Cutillas A., Fernández-Fernández J.I., 2001. Phenolic compounds and colour stability of red wines : Effect of skin maceration time. *Am. J. Enol. Vitic.*, **52**, 266-270.

González Neves G., Ferrer M., Carbonneau A., Moutounet M., 2003. Adaptación de la vinificación en tinto en función del potencial polifenólico de las uvas. *Agrociencia*, **1**, 59-67.

González-San José M.L.; Barron L.J.R., Diez C., 1990. Evolution of anthocyanins during maturation of Tempranillo grape variety, Vitis Vinifera, using polynomial regression models. *J. Sci. Food. Agric.*, **51**, 337-343.

Gordillo B., Lopez-Infante M.I., Ramirez-Pérez P., Gonzalez-Miret M., Heredia F., 2010. Influence of prefermentative cold maceration on the color and anthocyanin copigmentation of organic tempranillo wines elaborated in warm climate. *J. Agric. Food Chem.*, **58**, 6797-6803.

Heatherbell D., Dicey M., Goldsworthy S., Vanhanen L., 1997. Effect of prefermentation cold maceration on the composition, colour and flavor of Pinot noir wine. *Proceedings of the 4th International Symposium on Cool Climate Viticulture and Enology.* VI: 10-17. New York State Agricultural Experimental Station, Geneva, New York.

Heredia F, J., Escudero-Gilete M.L., Hernanz D., Gordillo B., Melendez-Martinez A.J., Vicario, I.M., Gonzalez-Miret M.L., 2010. Influence of the refrigeration technique on the color and phenolic composition of Syrah wines obtained with pre-fermentative cold maceration. *Food Chem.*, **118**, 377-383.

Koyama K., Goto-Yamamoto N., Hashizume K., 2007. Influence of maceration temperature in red wine vinification on extraction of phenolics from berry skins and seeds of grape (*Vitis vinifera*). *Biosci. Biotechnol. Biochem.*, **71**, 958–965.

Lanaridis P., Bena-Tzourou I., 1997. Etude des variations des anthocyanes pendant la maturation des raisins de cinq cépages rouges, cultivés en Grèce. *J. Int. Sci. Vigne Vin*, **31**, 205-212.

Llaudy M.C., Canals R., Canals J., Rozés N, Zamora F, 2004. New method for evaluating astringency in red wines. *J. Agric. Food Chem.*, **52**, 742-746.

LLaudy M.C., Canals R., Cabanillas P., Canals J.M., Zamora F., 2005. La maceración prefermentativa en frío. Efectos en la extracción de color y los compuestos fenólicos, e influencia del nivel de maduración de la uva. ACE. *Revista de Enología Artículos Científicos*, 60.

Marais J., 2003 a. Effect of different wine-making techniques on the composition and quality of Pinotage wine. I. Low-temperature skin contact prior to fermentation. *S. Afr. J. Enol. Vitic.*, **24**, 70-75.

Marais J., 2003 b. Effect of different wine-making techniques on the composition and quality of Pinotage wine. II. Juice/skin mixing practices. *S. Afr. J. Enol. Vitic.*, **24**, 76-79.

Méndez J.V., 2005. *Estudio de la maduración fenólica y antocianica en uvas tintas de Bobal para diferentes condiciones agrológicas*. Tesis Doctoral. Universidad Politécnica de Valencia, Spain.

Okubo K., Goto-Yamamoto N., Okazaki N., 2003. Effect of prefermentation cold soak on extraction of anthocyanin during red wine making. *J. Brew. Soc. Japan.*, **98**, 193-200.

Parenti A., Spugnoli P., Calamai L., Ferrari S., Gori C., 2004. Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *Eur. Food Res. Technol.*, **218**, 360–366.

Retali E., 2004. Macération préfermentaire à froid: application à une vendange de Nielluccio. *Rev Fr. Œnol.*, **209**, 16-18.

Ribéreau-Gayon P., Glories Y., Maujean A., Dubourdieu D., 2006. *Phenolic compounds. Handbook of Enology : Vol. 2. The chemistry of wine, stabilization and treatments.* Chichester : John Wiley and Sons, Ltd., p. 141-204.

Rivas Gonzalo J.C., Bravo-Haro S., Santos-Buelga C., 1995. Detection of compounds formed through the reaction of malvidin-3-monoglucoside. *J Agric Food Chem.*, **43**, 1444–1449.

Rodríguez Villa J.C., 2000. Buenos vinos: comencemos por la uva madura. *Viticultura-Enología Profesional*, **66**, 6-11.

Sacchi K., Bisson L., Adams D., 2005. A review of the effect of winemaking techniques on phenolic interaction in red wines. *Am. J. Enol. Vitic.*, **53**, 197-206.

Saint Cricq de Gaulejac N., Vivas N., Glories Y., 1998. Maturité phénolique: définition et contrôle. *Rev. Fr. Œnol.*, **173**, 22-25.

Sánchez N., 2008. *Influencia de diferentes factores agrológicos y tecnológicos sobre la mejora de la calidad de los vinos tintos de Bobal.* Tesis Doctoral, Universidad Politécnica de Valencia, Spain.

Sarneckis C.J., Dambergs R.G., Jones P., Mercurio M., Herderich M.J., Smith P.A., 2006. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimized tool for grape and wine analysis. *Aust. J. Grape Wine Res.*, **12**, 39-49.

Sims C.A., Bates R.P., 1994. Effects of skin fermentation time on the phenols, ellagic acid sediment and sensory characteristics of a red Vitis rotundifolia wine. *Am. J. Enol. Vitic.*, **45**, 56-62.

Souquet J.M., Cheynier V., Moutounet M., 2000. Les proanthocyanidines du raisin. *Bull. OIV*, **73**, 835-836, 601-609.

Spranger M.I., Clímaco M.C., Sun B., Eiriz N., Fortunato C., Nunes A., Leandro C., Avelar M.L., Belchior A.P., 2004. Differentiation of red winemaking technologies by phenolic and volatile composition. *Anal. Chim. Acta*, **513**, 151.

Thorngate J.H., Singleton V.L., 1994. Localization of procyanidins in grape seeds. *Am. J. Enol. Vitic.*, **45**, 259-262.

UE, *Official Methods to Wine Analyses*, Reglamento 440/2003

Vidal S., Francis L., Williams P., Kwiatkowski M., Gawel R., Cheynier V., 2004. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chem.*, **85**, 519–525.

Vivas N., Glories Y., Lagune L., Saucier C., Agustin M., 1994. Estimation of the polymerisation level of procyanidins from grapes and wines by use of p-dimethylaminocinnalamdehyde. *J. Int. Sci. Vigne Vin*, **28**, 319-336.

Watson B.T., Price S.F., Valladao M., 1995. Effect of fermentation practices on anthocyanin and phenolic composition of Pinot noir wines. Abstr. *Am. J. Enol. Vitic.*, **46**, 404.

Zamora F., 2003. *Elaboración y crianza del vino tinto: Aspectos científicos y prácticos*. Mundi Prensa AMV, Madrid.

Zamora F., 2004. La maceración prefermentativa en frío de la uva tinta. *Enólogos*, **32**, 36-39