EXTRACTION TIME COURSE OF SELECTED RED COLOR COMPONENTS FROM GRAPE POMACE

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Abstract

The color components of red wine affect to large extent the sensory characteristic (e.g. color and astringency) and the antioxidant property of wine. Besides, these polyphenolic compounds extracted from pomace represent an important source as food colorants and nutraceuticals.

In this preliminary study the time course of extraction of selected color components of red grape pomace, including free anthocyanins, polymeric pigments and copigmented anthocyanins was investigated. The ratio liquid/solid was set between 0.5/1 to 5/1 (w/w) and the extraction occurred in buffer with 12% alcohol to simulate the winemaking process.

The extraction of free anthocyanins showed a two-stage curve, whereas polymeric color increased through the same period of time, the final content being approximately stable. Free anthocyanins and polymeric pigments both increased within 24 hours.

This finding suggested that both classes of compounds were already present in the pomace at beginning of the extraction process. High color extraction was achieved by decreasing the ratio liquid/pomace from 5/1 to 0.5/1. Using the ratio 5/1of white wine/pomace, i.e. simulating the actual vinification condition, after seven days of extraction the white wine turned its color into rosé with the following values: total color 0.880 AU, free anthocyanins 0.594 AU, polymeric color 0.286 AU, and almost none copigmentation.

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J. COMMODITY SCI. TECHNOL. QUALITY *2010, 49* (II) 121 - 131

The main effect of the solvent-to-solid ratio was to increase the solubility and equilibrium constants and thus improve anthocyanin and polymeric pigment content to a maximum at the highest solvent-to-solid ratio.

Riassunto

Il colore dei vini rossi è riconducibile alla presenza di sostanze coloranti di natura polifenolica, in particolare gli antociani ed i loro derivati polimerici. Tali composti nel vino contribuiscono alle proprietà sensoriali (colore, astringenza), commerciali e nutrizionali dei vini.

In questo studio preliminare è stato osservato l'andamento dell'estrazione dalle vinacce di antociani liberi, pigmenti polimerici ed antociani copigmentati. A tal fine è stato variato il rapporto liquido/solido in un interballo tra 0,5/1 e 5/1 (p/p). L'andamento delle curve di estrazione varia in funzione del tipo di composto (i.e. antociani e pigmenti polimerici), fase liquida e rapporto solido/liquido. La maggiore estrazione di antociani è stata ottenuta con un rapporto liquido/solido pari a 0,5/1.

Interessante la presenza nelle vinacce di pigmenti polimerici in notevole quantità a dimostrazione del fatto che tali composti sono in parte già presenti nei vini già in fase di fermentazione. Utilizzando un rapporto liquido/solido di 5/1, ovvero simulando le reali condizioni di vinificazione, dopo sette giorni di estrazione è stato possibile ottenere un vino rosato con i seguenti parametri: colore totale 0,880 UA, antociani liberi 0,594 UA, colore polimerico 0,286 UA, e quasi assenza di copigmentazione.

L'effetto principale del rapporto liquido/solido è stato quello di aumentare la solubilità e le costanti di equilibrio e quindi migliorare l'estrazione di antociani e pigmenti polimerici.

Keywords: anthocyanins, copigmentation, polymeric pigments, wine.

Introduction

The color components of red wine give an important contribution to the sensory characteristic (e.g. color and astringency) and the antioxidant property of wine (1). Besides, a variety of health promoting products obtained from by-products of the grape and wine industry has recently been introduced to the market (2).

The color of red wine is due principally to anthocyanins extracted from the skin of grape berry (3). As the fermentation progresses, after

reaching the maximum there immediately begins a visual color decrease (4), with no such magnitude of reduction in individual anthocyanins (5).

The extraction phenomena is complex and to explain the drop of red color in wine several hypothesis have been formulated, including (i) reduction of free anthocyanins to colorless flavenes (6); (ii) adsorption of anthocyanins by yeasts and stems; (iii) coupling of anthocyanins with tannins (7); (iv) change in equilibria between colored and non-colored anthocyanin forms (8), and (v) loss in copigmentation due to the increase in ethanol content (9).

In particular, copigmentation, a phenomenon that produces a color enhancement resulting from association of anthocyanins with a non-colored copigments, has recently been reviewed in wine (10). Copigmentation is of critical importance in understanding the relationship between grape composition and wine color, and the variation in color during winemaking. Despite the presence of alcohol, copigmentation can still occur, although to a reduced extent (11), and it is therefore expected that copigmentation generally occurs in young red wines, for as long as monomeric anthocyanins remain (12).

In this study the time course of extraction of selected color components of red grape, including free anthocyanins, polymeric pigments and copigmented anthocyanins, was investigated.

Materials and Methods

About 8 tons of red grape cv. Sangiovese in good sanitary conditions was fermented and at the end of the maceration process the pomace, i.e. skins and seeds, was collected to extract the residual pigments in laboratory scale according to literature (13). Fixed aliquots of pomace were mixed with Sangiovese red wine, Trebbiano white wine and buffer solution (12% ethanol, pH 3.60 potassium bitartrate buffer, 60 mg/L of total SO_2) resulting in four comparative trials as follows:

A) ratio liquid/pomace = 0.5/1 (w/w);
B) ratio liquid/pomace = 1/1 (w/w);
C) ratio liquid/pomace = 2/1 (w/w);
D) ratio liquid/pomace = 5/1 (w/w). This ratio almost reproduced the usual winemaking condition.

The term *liquid* phase includes (i) white wine, (ii) red wine, and (iii) buffer solution. Thus for each trial (A, B, C, and D) three liquid

phases were compared. The wines contained 60 mg/L of total SO_2, and were adjusted to pH 3.60 and 12% ethanol before the experiment.

Extraction trials were run in laboratory using a glass bottle of 1 liter volume (8.5 cm diameter; 16 cm height, with screw closure) placed onto a horizontal shaker (mod. 709, ASAL, Milano, Italy) operating at 75 r.p.m., and set at 30 °C. Aliquot of liquid (10 ml) were sampled with time, centrifuged at 4500 g × 15 min at 5 °C (Allegra R21, Beckman, Milano, Italy), then the absorbance was measured at 520 nm by a spectrophotometer (UV 1240, Shimadzu, Milano, Italy) using the Boultons' assay (14) as previously described in details (15).

Briefly, the color measures at 520 nm were made after the addition of acetaldehyde (total color), of sulfur dioxide (polymeric color), and a dilution of 1:19 in a 12% ethanol, pH 3.6 potassium bitartrate buffer, corrected for the dilution (copigmentation).

The free anthocyanins were calculated by the difference between total and polymeric color. The present method does not use of a low pH reading and thereby eliminates the effect of pH on polymeric color from the calculation of total anthocyanins.

The trial A (ratio liquid/marc = 0.5/1, w/w) was sampled only once at the end of the extraction process.

Results and Discussion

Among the three solutions used in the extraction test only Sangiovese red wine already contained red pigments as follows: total color 1.014 AU, free anthocyanins 0.792 AU, polymeric color 0.222 AU. None copigmentation was present in the 1 year old red wine.

Figures 1 and 2 show the changes in the absorbance values at 520 nm during the time course of extraction with red and white wine, respectively.

Extraction with buffer solution matched the results obtained with white wine (data not shown). Moreover, taking into account the initial level of red color in Sangiovese wine, the result obtained with red and white wine were almost similar.

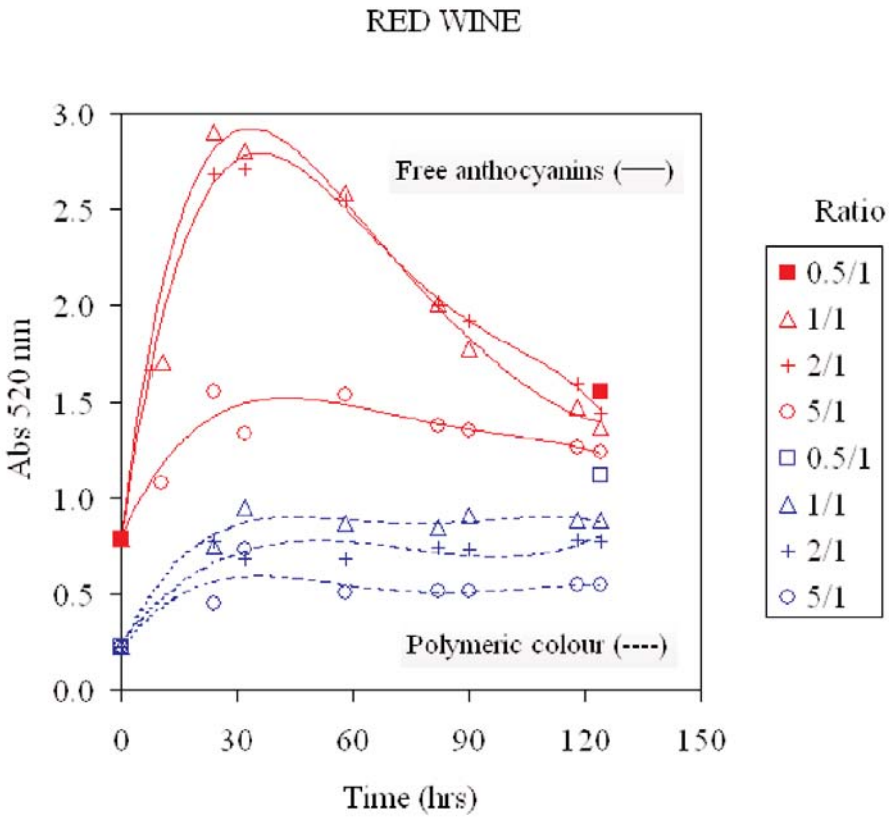

Fig. 1 - Change in absorbance at 520 nm during the extraction of free anthocyanins (), and polymeric color (---) from pomace by red wine. Ratio red wine/pomace 0.5/1 (); 1/1 (); 2/1 (+); 5/1 (O).

In both cases, the absorbance due to free anthocyanins increased during the initial period of extraction reaching the maximum within 24 hours. After that time the color decreased of approximately 53% and 47% for red and white wine, respectively. The general trend observed in the extraction experiment resembled the classic red wine fermentation (5-6, 9, 16).

It is important to remind that in our experimental conditions (i) no fermentation occurred, (ii) only pomace was present, (iii) the extraction occurred at pH 3.6 and 12% ethanol.

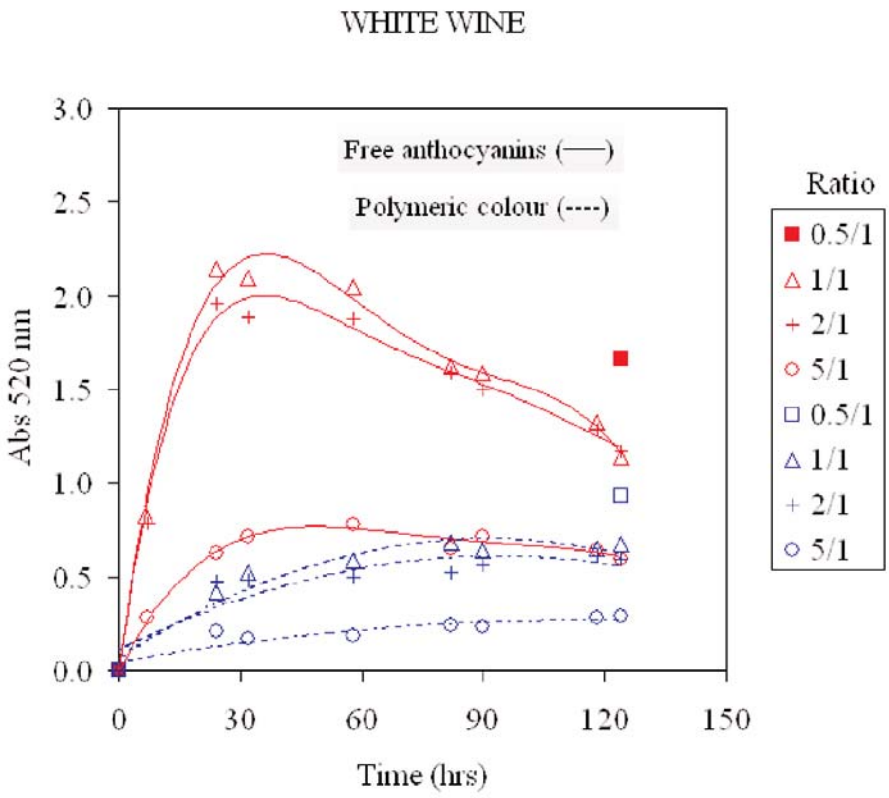

Fig. 2 - Change in absorbance at 520 nm during the extraction of free anthocyanins (), and polymeric color (---) from pomace by white wine. Ratio white wine/pomace 0.5/1 (đ); 1/1 (Đ); 2/1 (+); 5/1 (O).

High color extraction was achieved by increasing the ratio liquid/pomace from 5/1 to 2/1. Using the ratio 5/1of white wine/pomace, i.e. simulating the actual vinification condition, after seven days of extraction the white wine turned its color into red with the following values: total color 0.880 AU, free anthocyanins 0.594 AU, polymeric color 0.286 AU, and almost none copigmentation.

The main effect of the solvent-to-solid ratio was to increase the solubility and equilibrium constants and thus improve anthocyanin and polymeric pigment content to a maximum at the highest solvent-to-solid ratio.

According to Cacace and Mazza (17) the increase of total phenolic and anthocyanin yields with the increase of the solvent-to-solid ratio is

consistent with mass transfer principles. The driving force during mass transfer within the solid is considered to be the concentration gradient, which is greater when a higher solvent-to-solid ratio is used, resulting in an increase of the diffusion rate.

The extraction of free anthocyanins showed a two-stage curve, whereas polymeric color increased through the same period of time, the final content being approximately stable.

Free anthocyanins and polymeric pigments both increased within 24 hours. This finding suggested that both classes of compounds were already present in the pomace at beginning of the extraction process.

Two mechanisms probably regulate the color extraction, (i) the partitioning of free anthocyanins between liquid and solid phase, and (ii) the quickly coupling of anthocyanins with tannins (18).

Despite the importance of polymeric pigments, the mechanisms, the products and the kinetics of polymer formation in wine are only partially known (19-20).

Taking into account the reduction of pH values (0.1→0.05 unit) observed at the end of the extraction, and considering that anthocyanins increase their absorbance at low pH, the data herein presented might be underestimated. However, the wines contained 60 mg total SO_2/l that counterbalanced the pH effect, depending on the acetaldehyde content.

As expected copigmentation was high in red wine, followed by white wine and buffer solution. A linear contribution of copigmentation to the total color of wine was found at distinct level of liquid/pomace ratio (Figure 3).

White wine and buffer solution required an high content of pomace (ratio liquid/solid = 1/1), whereas 50% of pomace was needed for red wine (ratio liquid/solid = 2/1).

The extent of copigmentation can be increased by increasing either the concentration of total anthocyanin or of cofactor or both.

In particular, Sangiovese is low in copigmentation probably due to the lack of acylated forms of the non-malvidin pigments.

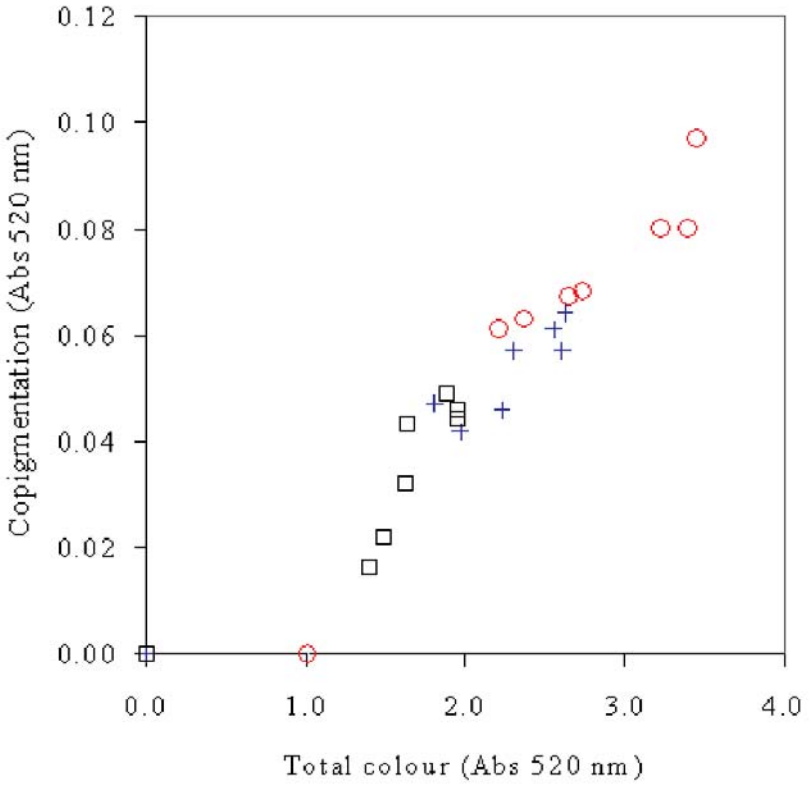

Fig. 3 - Relationship between copigmentation and total color. Legend: red wine (O) ratio wine/pomace: 2/1; white wine (+) ratio wine/pomace: 1/1; and buffer solution (□) ratio buffer/pomace: 1/1.

The result of this study can be useful on industrial scale to improve the winemaking process and the recovery of phenolics from pomace as well. In particular, commercial red winemaking procedure can benefit by measuring the classic optical density at 520 nm together with additional selected parameters including copigmentation, polymeric pigments and tannins. In fact, in grapes and wines, anthocyanins can be either as free

monomers or combined with other phenolics to form small and large polymeric pigments (21). It is well known that polymerization can affect the properties of the pigments (e.g. increasing color stability, and reducing astringency). Polymerization begins in the berry during maturation, then tannin polymerization continues in wine until an anthocyanin molecule binds the terminal unit. Therefore, the ratio of anthocyanins-to-tannins seems among the driving parameters for pigments polymerization.

In conclusion, this preliminary study highlight the need of specific extraction models, methods of analysis and parameters to match the consumers' preference through the optimization of the winemaking process and to fit the high quality standard of natural colorants as food additives and functional food ingredients.

Received 20 May, 2010
Accepted 15 July, 2010

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