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ENVIRONMENT CONTROLLED FORMATION KINETICS OF COMPLEXES OF MALVIDIN-3-*O*-GLUCOSIDE WITH POLYPHENOLS

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ABSTRACT. The kinetics of formation of malvidin-caffeic acid and malvidin-catechin complexes was studied by time-dependent recording of the photo-luminescence (PL) signal of malvidin. The molecular environment was changed according to the fermentation process in red wines: water – ethanol mixtures with up to 14 %vol of ethanol was applied as binary solvent. Two reaction channels were examined according to the order of complex formation. In the first case, the aqueous solutions of malvidin and polyphenols were mixed, then the ethanol content was elevated according to the actual grade of fermentation. In the second case, the stock solutions are prepared as binary solvent mixtures. Our results show a faster formation of the complexes in the former case. Kinetic parameters show that the activation energy of the first reaction channel is lower and the frequency factor is higher, supporting a higher reaction rate. These observations are applicable to a wide range of chemistry where the molecular environment is composed of binary solutions. In particular, it has significant consequences for winemaking procedures, where one method of color improvement is based on the association of the species examined in this work.

Keywords: malvidin; polyphenol; copigmentation; photoluminescence

INTRODUCTION

Our recent work showed that below and above 8%vol. ethanol content of wines the π-π stacks of malvidin-polyphenol complexes are quite different [1]. A monomolecular solvation shell exists below 8%vol. When the ethanol content jumps above this margin, the solvation shell changes into a bimolecular environment (water-ethanol), which results in much more stable complexes. Using these results we were able to suggest a modified winemaking technology: whole grape bunches are pressed and the obtained white juice fermented.

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The skins are stored under reductive conditions in the freezer until the alcohol content exceeds the above mentioned critical concentration of 8%vol. Then the skins are thawed and put back into the juice, afterwards the wine is fermented to dryness. However, our analyses show that, although the stability of the color is much higher compared to the control samples, the evolution of the wine's color is much slower by applying the new winemaking procedure.

To understand the possible background of this phenomenon we examine the kinetics of the formation of malvidin-polyphenol complexes as a function of the alcoholic content of the samples. Malvidin-3-glucoside as anthocyanin and caffeic acid and catechin, respectively, as polyphenols were chosen for these studies. According to our previous results [1-5] different reaction pathways were followed by appropriate preparation of the samples as described below.

RESULTS AND DISCUSSION

The formation kinetics of the complexes of malvidin and polyphenols were examined by measuring the photoluminescence (PL) intensity of malvidin at 395 nm. Sample preparation was performed under the protocol described in the b) and c) subsections listed in the experimental part. The main difference between the b) and c) series is as follows: in the b) series of the samples one part of the complexes is formed in pure aqueous environment and then this solvation shell is changed to water-ethanol clusters by adding ethanol to the solutions. In the series c) the complex formation already occurs in the solutions, which contain 12%vol. ethanol right from the beginning. Therefore, complexes can only be formed via association of malvidin and the corresponding polyphenol, whose binary solvent shell has already been built-up.

Figures 1 shows the change of the PL signal plotted against time during formation of malvidin - caffeic acid complexes. Similar time-dependence of PL change was observed in the case of formation of malvidin-catechin complexes. It can be clearly seen that in both cases of the copigmentation process, the complex formation in water was found to be faster by an order of magnitude compared to that in water-ethanol mixtures. This result is surprising at first sight, since water-ethanol mixtures are known to have a lower permittivity (~69.8 (12%vol. ethanol)) than the pure aqueous solutions (~78.5). Since the π-π interactions, which stabilize the malvidin-polyphenol complexes, were found to be stronger in a low permittivity solvent, the forces inducing the formation of such complexes have to be also increased resulting in a faster rate of complex formation. The dielectric properties of the bulk solutions are the same in these two cases and so, the stability of the complexes is the same after the thermodynamic equilibrium is reached.

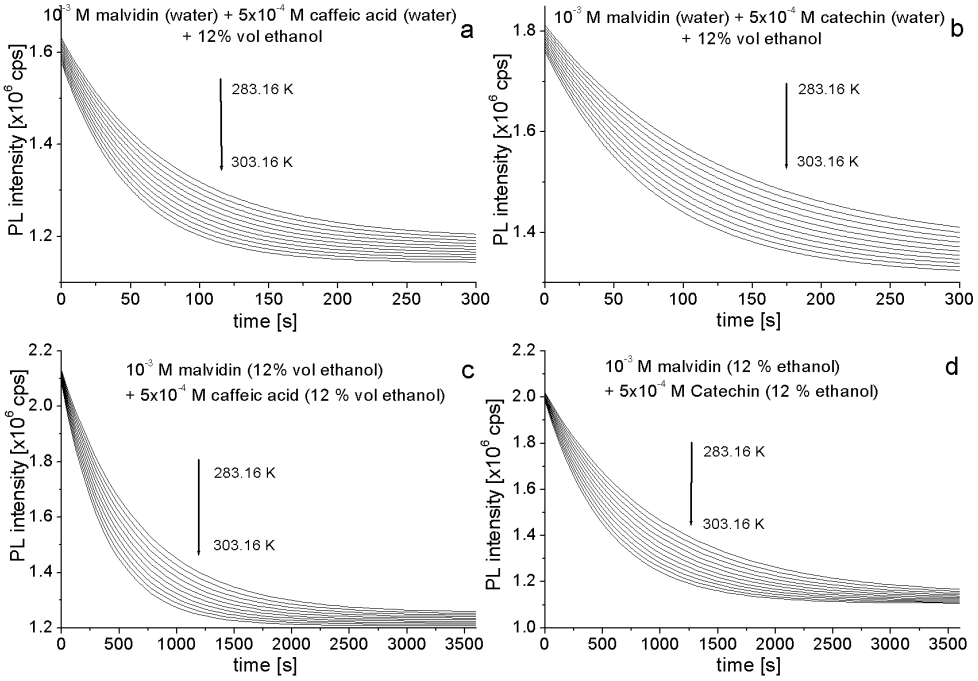


Figure 1. Change of the PL signal plotted against time during formation of malvidin - caffeic acid (left) or malvidin – catechin (right) complexes

Table 1. Arrhenius parameters of the complex formation reactions of compound **1** with compound **2a** or **2b**

Reaction	ΔE (kJ/mol)	A (s^{-1})
1 (water) + **2a** (water) + 12%vol. ethanol	-16.46	13.69
1 (water) + **2b** (water) + 12%vol. ethanol	-17.46	12.88
1 (12%vol. ethanol) + **2a** (12%vol. ethanol)	-21.43	13.46
1 (12%vol. ethanol) + **2b** (12%vol. ethanol)	-22.17	12.25

However, to give a proper description of this unexpected property we have to consider that the solvation shell of the interacting species has to be broken (or has to be removed, at least in part) prior to complex formation. Therefore, the difference observed in the formation kinetics is mainly due to the energy barrier of the complex formation (refer table 1).

Note that the reactants are the same in both cases and the shape of the PL spectra is always identical. This property supports the idea that no significant conformational changes of the interacting molecules occurs under the effect of the change in the molecular environment. The decreased formation rate of the complexes in the water-ethanol mixtures highlights that the desolvation in alcoholic solutions needs more activation energy, i.e. the solvation shell in the water-alcohol mixture is more stable compared to the solvation shell in the pure aqueous solutions.

CONCLUSIONS

Formation kinetics of malvidin polyphenol copigmentation complexes through two reaction channels were studied in water ethanol mixtures by time-dependent recording of photoluminescence (PL) signal of malvidin. In the case of the first channel, the malvidin polyphenol complexes are formed in water first and then their solvation shells are altered according to the changed composition of the bulk solutions after diluting the aqueous solution with ethanol. Through the second channel, the stock solutions are prepared as binary solvent mixture, therefore, the bicomponent solvation shells of the interacted species are formed prior molecular association. Our results show a faster formation of the complexes in the former case. Evaluation of the Arrhenius parameters of the reaction shows that the activation energy of the first reaction channel is lower and the frequency factor is higher, supporting faster reaction rate. These observations are applicable in wide scale of chemistry where the molecular environment is composed by binary solutions. The weak interactions between the aromatic molecules can be highly affected by the molecular environment, especially in condensed phase and in multicomponent solutions [6,7]. Therefore big effort has been taken to understand the properties of complex solutions using the particular physico-chemical properties of the pure components [8,9]. These works focus on two characteristics of the solvent molecules: either by the associative [10] or non-associative [11] behavior or by their similar [12] or quite different [13] permittivity characters. The results presented here highlight the importance the structure of the solvation shell and the necessity of its description at molecular level.

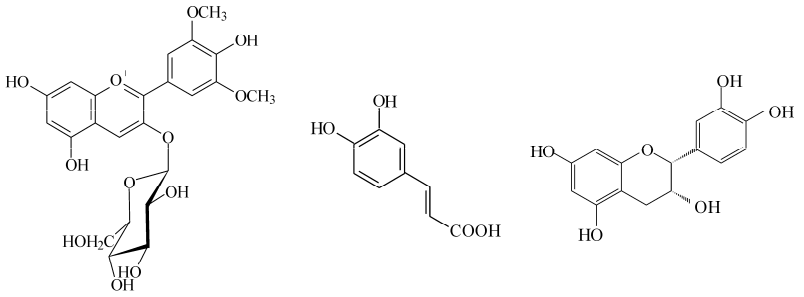
EXPERIMENTAL SECTION

Material. Anthocyanin: **1** malvidin-3-*O*-glucoside: MW=494.87 g/mol. Colorless polyphenol: **2a**: caffeic acid, MW=180.16 g/mol and **2b**: catechin, MW=290.28 g/mol (ref. figure 2).

Polyphenol standards were purchased from Extrasynthese (Genay, France) and used as received. Ethanol (spectroscopic grade, anhydrous, Panreac, Spain) was used without further purification.

Sample preparation. Three series of samples were prepared as follows:

a) The interaction between malvidin and the copigment was investigated by means of the Job's method. Detailed information about this method is described elsewhere. Briefly, stock solutions of **1**, **2a** and **2b** of 0.002 M were prepared in buffer (sodium acetate [0.06 M] and phosphoric acid [0.02M]). The pH was adjusted to 3.2 by addition of 0.1M HCl. To determine the stoichiometry of the complexes and the thermodynamic parameters of the complex formation, stock solutions of malvidin-3-glucoside (1) and stock solutions of the polyphenols (**2a** or **2b**) were mixed at eleven different molar ratios. Concentration range is chosen according to the typical concentration of malvidin in grapes, which is around 10^{-3} M … 10^{-4} M. The concentration of the colorless copigments (**2a**, **2b**) is varied in the same concentration. Note that these copigment concentrations are higher by an order of magnitude than that of the natural polyphenol content of grapes and wines (which falls in the interval of 10^{-4} M…10^{-5} M). However, the Job's method requires relatively high concentrations of both substances. The ethanol content of the samples was varied in the range of 0…20%vol. in steps of 1%vol. Although wine alcohol content normally ranges between 10 and 14%vol., we chose this large range for two reasons: i) to investigate the reactions during fermentation (i.e., increasing alcohol content from 0 to 14%vol.) and ii) to include the situation of products with higher alcohol content than wine, such as fruit liquors, in which color stabilization might be of similar interest.



1 **2a** **2b**

Figure 2. Chemical structures of malvidin-3-O-glucoside and the colorless polyphenols caffeic acid and catechin

b) $4x10^{-3}$ M malvidin and $2x10^{-4}$ M polyphenol stock solutions were prepared in aqueous buffer as described above. Then these solutions were mixed in 1:1 ratio in stirred cuvette located in the sample holder of the fluorometer. Using this procedure, the concentrations were $2x10^{-3}$ M malvidin and 10^{-3} M polyphenol. After one minute stirring, the PL signal did not change indicating that the chemical equilibrium was formed. Hereupon the samples were diluted with 24%vol. ethanol reaching final concentrations of 10^{-3} M malvidin 1, $5x10^{-4}$ M polyphenol **2a** or **2b** and 12%vol. ethanol. The time dependence of the PL signal at 395nm was then recorded.

c) $2x10^{-3}$ M malvidin and 10^{-3} M polyphenol stock solutions were prepared in alcoholic aqueous phosphate buffer as described above, so each solution contained 12%vol. ethanol. These solutions were then mixed in a stirred cuvette located in the fluorometer's sample holder and the PL signal was recorded.

Apparatus. The steady-state PL spectra were recorded on a Fluorolog t3 spectrofluorometer (Jobin-Yvon/SPEX, Longjumeau, France). For data collection a photon counting method with 0.2 s integration time was used. Excitation and emission bandwidths were set to 1 nm. Front face detection was used to eliminate the inner filter effect. DataMax 2.20 software was applied for the data evaluation.

Excitation wavelength was 350 nm and the emission spectra recorded within the 380 nm … 600 nm range. The measurements were carried out at five different temperatures (15, 20, 25, 30 and 35°C), each in five replicates. Following the Job's procedure, the stability constants and also the thermodynamic parameters were determined.

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