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Analytical Methods

Highlight on the problems generated by *p*-coumaric acid analysis in wine fermentations

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Abstract

p-Coumaric acid is a natural hydroxycinnamic acid existing in grapes and wine. It is the precursor of the 4-ethylphenol molecule through the bioconversion reaction by *Brettanomyces* yeast. Chromatographic methods are the most common techniques to detect *p*-coumaric acid. It is known that this acid is highly unstable in analysis and fermentation experiments. This paper highlights the problems occurring in *p*-coumaric acid analysis in wine fermentation conditions when studying its bioconversion. First, it was shown that *p*-coumaric acid was unstable at elevated temperature. On the other hand, it was found that in our experimental conditions *p*-coumaric acid reacted with ethanol. This work revealed also that the *p*-coumaric acid is partially adsorbed on *Brettanomyces* yeast, certainly on cell walls. Because of these phenomena the quantity of *p*-coumaric acid which can participate to the bioconversion into ethylphenol decreases.

Keywords: *p*-Coumaric acid; Chromatographic analysis; Temperature; Adsorption; Ethanol; *Brettanomyces*; Fermentation

1. Introduction

p-Coumaric acid is a natural hydroxycinnamic phenolic acid that exists in wine in an esterified form with tartaric acid, the tartaric *p*-coumaroyl ester. It can also exist as glucose heteroside (Ribereau-Gayon, Glories, Maujean, & Dubourdieu, 1998). In grapes, *p*-coumaric acid is a polyphenol precursor, especially for flavonoids, flavones and flavonols (Hrazdina, Parsons, & Mattick, 1984). This acid is equally a crucial substrate for enzymes to create resveratrol (Goldberg, Tsang, Karumanchiri, & Soleas, 1998). In grape juice, the *p*-coumaric acid can reach a concentration of 60 mg/l (Chatonnet, Viala, & Dubourdieu, 1997), but it does not reach more than 8 mg/l in wine (Goldberg et al., 1998).

During the fermentation, *p*-coumaric acid is liberated due to the action of esterase activity in yeast and it seems nowadays that the sequential action of two enzymes on the free *p*-coumaric acid could be the principal cause of ethylphenol production in wine (Baumes & Cordonnier, 1986; Chatonnet & Boidron, 1988; Dubois, Brule, & Illic, 1971; Etievant, 1981; Schimidzu & Watanabe, 1982). First hydroxycinnamate decarboxylase changes the hydroxycinnamic acid into a hydroxystyrene (vinylphenol) (Edlin, Narbad, Gasson, Dickinson, & Lloyd, 1998), which is then reduced to ethyl derivatives by vinylphenol reductase (Dias, Pereira-da-Silva, Tavares, Malfeito-Ferreira, & Loureiro, 2003). Moreover, many authors proved that ethylphenol occurrence is due to the presence of *Brettanomyces* sp. yeast and its spore *Dekkera* (Chatonnet, Dubourdieu, Boidron, & Pons, 1992; Dias et al., 2003; Edlin, Narbad, & Lloyd,

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1995). In fact, the first enzyme, hydroxycinnamate decarboxylase, exists within a large number of bacteria and yeast, but the second, vinylphenol reductase, is only performed by a few yeast species like *Brettanomyces* sp.

Yeast of the genus *Dekkera*/*Brettanomyces*, especially *D. bruxellensis* species, is able to produce important amount of ethylphenols in wine, mainly 4-ethylguaiacol and 4-ethylphenol. The problem is that 4-ethylphenol molecule is associated in sensorial analysis to horse sweat smell or mousy taint flavours. Because of its bad organoleptic impact on wine, *Brettanomyces* sp. is considered as contaminating yeast.

In the literature, the problem is mentioned over and over, certainly because the quality of wine and productivity in winemaking are nowadays main factors than they were with the increasing competitive world wine market. Ethylphenol presence in wine is responsible of great economical loss and its production is much more detected (Chatonnet et al., 1997).

This is a main reason to stop this phenomenon in winemaking industries. It is very difficult to avoid *Brettanomyces* sp. growth in wine first because of the low hygiene level which can be reached in winemaking, second because of the resistance and growth of this yeast in strict conditions (low levels of sugar, high levels of ethanol) (Suárez, Suárez-Lepe, Morata, & Calderón, 2007). A way to struggle against ethylphenol production would be to avoid the bioconversion of *p*-coumaric acid into ethylphenols rather than avoiding *Brettanomyces* presence.

Thus, we must have a full understanding of the bioconversion mechanism. Authors usually show out the ethylphenol occurrence in wine versus *Brettanomyces* presence, without mentioning *p*-coumaric acid when studying its bioconversion. Yet, it would be very useful to have kinetics of *p*-coumaric acid bioconversion into vinylphenol, then into ethylphenol. Equally, the following up of the *p*-coumaric acid would be interesting in order to provide valuable data in yield calculation. So, according to us, it appears absolutely necessary to follow up products, substrates and have good methods to measure them.

The most common techniques that detect *p*-coumaric acid in its media are chromatographic methods. Medawar (2003) showed a difference between the initial concentration of the *p*-coumaric acid added to fermentation and the one detected by HPLC just after adding the acid. We noticed the same problem while we were studying the bioconversion of *p*-coumaric acid into ethylphenols in our experiments. These observations made us to search for the origin of this *p*-coumaric acid loss. What does coumaric acid turn to in a wine (synthetic) medium?

In this field of art, authors pointed out several mechanisms of physical, chemical or biochemical reactivity of hydroxycinnamic acids in wine mediums. First, Medawar (2003) suspected that high sterilisation temperature is responsible of the *p*-coumaric acid disappearance. In addition, it could be a solubility problem since *p*-coumaric acid is not easily soluble in water, neither in synthetic wine medium. That is why, before being added, it should always be dissolved into pure ethanol which is the most common solvent (Tuzen & Ozdemir, 2003).

Moreover, the *p*-coumaric acid, as all the phenolic compounds do, can undergo a polymerization reaction or an electrophilic addition with other wine components, especially an electrophilic addition with ethanol that gives ethoxyphenols in wine ageing (Dugelay, Beaumes, Guntata, Razungles, & Bayonove, 1995; Seyhane E., 1994). Several authors showed that *p*-coumaric acid can undergo an oxidation (Bagchi, Grag, Krohn, Bagchi, & Tran, 1997; Herrera, Pulgarin, Nadtochenko, & Kiwi, 1998), an esterification (Dugelay, Guntata, Sapis, Beaumes, & Bayonove, 1993), or an amination (Clark, 2000) under different conditions. These bibliographical observations lead to ask questions about the reactivity of *p*-coumaric acid in a fermentation medium. On the other hand, when O'Neill, Christov, Botes, & Prior (1996) exposed an HPLC method for cinnamic acid detection, they claimed that the correction should be done in function of each medium analysis, because of the adsorption of the hydroxycinnamic acids on different medium components, as wheat bran in their case of study. We know as well that phenolic compounds can adsorb on yeast cell walls (Medina, Boido, Dellacassa, & Carrau, 2005; Morata, Gomez-Cordoves, Colomo, & Suárez, 2005; Morata et al., 2003; Rizzo, Ventrice, Varone, Sidari, & Caridi, 2006).

All these phenomena can generate loss of *p*-coumaric acid in a wine synthetic medium. The goal of this paper is not to discuss the bioconversion of *p*-coumaric acid into ethylphenol, but to evaluate the chemical instability and the physical availability of *p*-coumaric acid under our oenological conditions, to determine the exact quantity which can really participate in the bioconversion.

2. Materials and methods

2.1. p-Coumaric acid detection

Standard of *p*-coumaric acid used for all experiments was acquired from Aldrich–Sigma society. As presented in the market, *p*-coumaric acid has a white crystalline powder aspect. All other chemicals were reagent grade or better. Two ways have been employed to detect *p*-coumaric acid:

- *UV detection*: the *p*-coumaric acid absorption was carried out on a UV spectrophotometer at 305 nm in quartz cell. The *p*-coumaric acid belongs to the hydroxycinnamic acid family well known for its absorbance in the UV region of the spectra because of the mesomeric effect of the double bonds, and their phenolic cycle (Seyhane, 1994). The polyphenols are detectable then at low concentration (1–10 μM) (Barthelmebs, Divies, & Cavin, 2000).
- *HPLC detection*: the *p*-coumaric acid analysis was carried out by HPLC. The ODS-2 5u™ (Waters®) column followed a Spherimarge ODS-2™ pre-column at 30 °C.

The automatic injector was used in a full loop of 20 μl, with a mobile phase composed of: water 77%, acetonitrile 23%. Formic acid was added in the concentration of 0.12 g/l to regulate the pH at 3.5. The flow was fixed at 0.7 ml/min. The detector used was a UV spectrophotometer. The detection was done at 305 nm. *p*-Coumaric acid retention time was 12.5 min.

2.2. Stability tests of the p-coumaric acid with experimental conditions

In order to study the day light and oxygen effect on the *p*-coumaric acid in our experimental conditions, two 40 mg/l solutions of *p*-coumaric acid were prepared in Erlenmeyer flasks of 500 ml containing 300 ml of liquid. The first solution was a synthetic wine medium with 10% v/v ethanol; the second was a hydroalcoholic solution containing distilled water with 10% v/v ethanol. Both of the solutions were stirred for 10 days at ambient temperature, closed inside the Erlenmeyer flasks. Each solution was monitored by UV spectrophotometry at 305 nm.

On the other hand, to study the *p*-coumaric acid thermodegradation at high temperature, two 40 mg/l solutions of *p*-coumaric acid dissolved into a synthetic wine medium were prepared into 50 ml volume Pyrex Shott containers. They were kept in darkness without any contact with air. Two temperatures were tested: ambient temperature (approximately 20 °C) and 75 °C obtained using a heating plate. The changes in absorbance at 305 nm were detected in time. Absorbance changes of the *p*-coumaric acid solution were equally tested before and after autoclaving for 20 min at 120 °C.

2.3. Reactivity tests of the p-coumaric acid with media components

To study the *p*-coumaric acid dissolution in ethanol, 10 mg/l of the acid were added either after being dissolved or not into ethanol to synthetic wine media containing 10% v/v ethanol. The absorbance of the acid was followed up in time by UV spectrophotometer in quartz cells at 305 nm for 120 h.

On the other hand, *p*-coumaric acid was either being added or not to different media to study its reactivity with ethanol as a solvent or with tartaric acid. These different media had the following composition: Medium A (Hydroalcoholic: water/ethanol 10% v/v), Medium B (water–20 mg/l of *p*-coumaric acid), Medium C (pure ethanol–20 mg/l of *p*-coumaric acid), Medium D (Hydroalcoholic: water/ethanol 10% v/v–20 mg/l of *p*-coumaric acid), Medium E (synthetic wine medium 10% v/v ethanol), Medium F (synthetic wine medium 10% v/v ethanol–20 mg/l of *p*-coumaric acid), Medium G (tartaric acid solution–20 mg/l of *p*-coumaric acid). The solutions were followed up by UV analysis with a sweeping wavelength between 250 and 350 nm.

2.4. Nuclear magnetic resonance (NMR) and infra red (IR) analysis

Two solutions of *p*-coumaric acid were prepared and tested by NMR to know whether a reaction occurs or not between *p*-coumaric acid and tartaric acid or between *p*-coumaric acid and ethanol. The first contained 10 mg/l of *p*-coumaric acid dissolved in ethanol; the second contained 10 mg/l of *p*-coumaric acid dissolved in tartaric acid solution. In both solutions, pH was adjusted to 3.5 as in our oenological conditions. The NMR spectrum of the crystalline *p*-coumaric powder was done after dissolution in DMSO at 10 mg/l concentration in order to be compared to other spectra. The NMR tests were recorded at 300 MHz on a Brucker spectrometer. Chemical shifts are expressed in part per million relative to tetramethylsilane. NMR solvent was pure DMSO.

On the other hand, infra red analysis of the pure *p*-coumaric acid and the one dissolved into ethanol, where the pH was regulated at 3.5, were acquired on a Shimadzu-8300 Fourier Transform infra red spectrophotometer.

2.5. Adsorption test of p-coumaric acid on Brettanomyces yeasts

One strain of yeast was isolated from Buzet winery, situated in the south west of France. It was identified genetically as *Brettanomyces bruxellensis*. The *Brettanomyces* yeast was scraped from a Petri dish where it was stored at 4 °C.

The yeast culture medium was a synthetic wine medium that had the following composition: glucose 20 g/l, yeast extract 0.5 g/l, $(NH_4)_2SO_4$ 0.5 g/l, citric acid 0.3 g/l, malic acid 3 g/l, tartaric acid 2 g/l, $MgSO_4$ 0.4 g/l, KH_2PO_4 5 g/l, glycerol 6 g/l. Ethanol was added up to 10% v/v after autoclaving. Culture of *Brettanomyces* was inoculated at 3.10^6 cell/ml yeast concentration. Culture was carried out in 500 ml Erlenmeyer flask with 300 ml of liquid medium stirred at 250 rpm. After inoculation, culture flasks were incubated at 30 °C.

Fermentation was carried out in order to have different population concentration of *Brettanomyces* yeast at different stages of yeast growth.

p-Coumaric acid was added in the fermentation described above after dissolution in 1 ml of pure ethanol, to have respectively the concentration of 5, 10 or 20 mg/l. Two minutes after adding *p*-coumaric acid in each solution, a sample was taken, centrifuged, and then the liquid part was tested on HPLC to detect the *p*-coumaric acid concentration, and on GC/MS to detect the vinylphenol and ethylphenols content. Three synthetic wine mediums (T1, T2 and T3) free of *Brettanomyces* yeast were prepared containing, respectively, 5, 10 and 20 mg/l of *p*-coumaric acid and

served as a witness test. All these experiments were done at a temperature of 30 °C.

2.6. Yeast counting

Biomass concentration was followed up by two analytical techniques. A correlation between the optical density of yeast suspension at 620 nm and cell dry weight gives weight concentration, and the use of a Thoma hemacytometer gives a concentration in number of cells. The percentage of viable cells is obtained using the ethylene blue staining method, with an error of 8%.

3. Results and discussion

3.1. p-Coumaric acid stability in the air oxygen under day light

The results showed that there was no significant change in absorbance after 10 days. It seems that the *p*-coumaric acid is stable in daylight, at ambient temperature and in the presence of oxygen under experimental conditions.

3.2. Thermodegradation test of p-coumaric acid

The purpose of this test was to study the stability of the *p*-coumaric acid with temperature. The results showed that there was no change in UV absorbance during 30 h under ambient temperature, while the absorbance decreased when the temperature was 75 °C (Fig. 1). The absorbance decreased by 26% (from 0.65 down to 0.481) when the *p*-coumaric acid solution was autoclaved. As a control, the absorbance of a wine synthetic medium, free from the acid, was followed up before and after autoclaving. The absorbance remained constant: 0.3 abs value.

These tests showed that *p*-coumaric acid in solutions, particularly in natural wine under oenological conditions, is quite stable at ambient temperature. On the other hand, it is unstable when temperature increases. We confirm the results of Bagchi et al., 1997 and Herrera et al., 1998. Practically, it would be important to add the *p*-coumaric acid in synthetic wine media after autoclaving in order to be sure that all the *p*-coumaric acid will be available in its natural form for enzymatic reactions.

3.3. Reactivity tests of the p-coumaric acid in the media

It is difficult to dissolve *p*-coumaric crystalline powder in pure distilled water. When it is dissolved, the *p*-coumaric acid takes filamentous form within 40 h. Authors proposed in their work the dissolution of *p*-coumaric acid in ethanol which is a potent solvent. On the other hand, Tuzen and Ozdemir (2003) proposed the dissolution of *p*-coumaric acid into hydroalcoholic solutions.

The observations showed that *p*-coumaric acid dissolves slower in hydroalcoholic solutions than in pure ethanol solution (Fig. 2). But they showed also that there was a loss of *p*-coumaric acid quantity relative to 0.2 absorbance unit, when dissolving into pure ethanol, which leads to think that a probable ethanol/*p*-coumaric acid reaction could take place. The study of *p*-coumaric acid UV absorbance in the media detailed in the material and methods part (Media A–F), confirmed this hypothesis. It gave the following results.

In the range studied, Medium A showed no absorption. Medium B showed two absorption bands at 285 nm and 305 nm. On the other hand, media C and D showed remarkable hypsochrome and hypochrome effect. The maximum wavelength absorption decreased from 284 nm (Medium B) to 248 nm (Medium C), and the band became larger.

The hypochrome effect could result from a destabilization of the mesomeric system which minimizes the absorption probability of the carbonyl group. The hypsochrome effect could result even from the polarity change of the solvent or from any change in the *p*-coumaric acid structure. Moreover, this peak shift occurred more slowly (24 h) in Medium D, where the medium had a little ethanol concentration (10% v/v ethanol). These observations lead to think that some reactions between the *p*-coumaric acid and the ethanol might take place and that the concentration of ethanol participates in its kinetics.

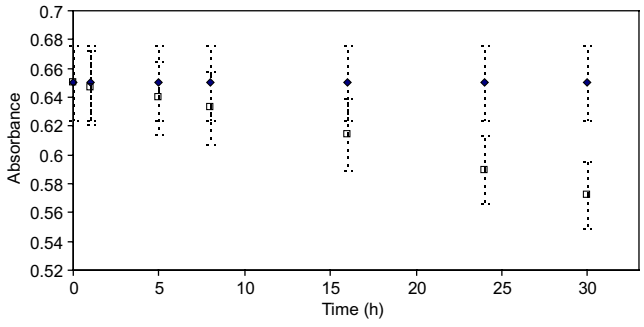

Fig. 1. Absorbance as a function of time at ambient temperature 20 °C (♦) and 75 °C (□) in synthetic wine medium.

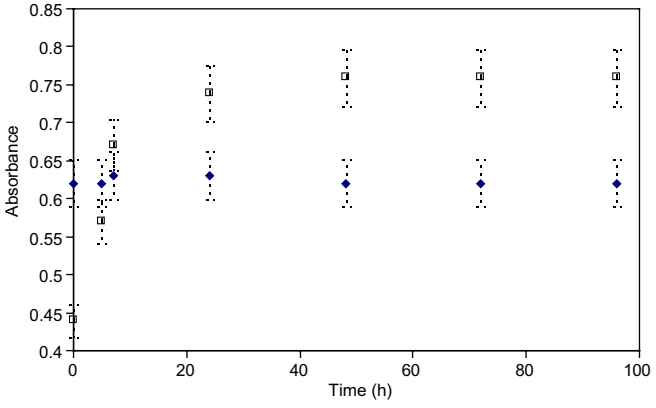

Fig. 2. Kinetic profile of the dissolution of *p*-coumaric acid in ethanol (♦) and synthetic wine medium (□).

Comparing Mediums E and F (synthetic wine medium without and with *p*-coumaric acid), *p*-coumaric acid absorption bands were found at 284 and 305 nm. A 248 nm band was found after 24 h in the media F. As in the Medium D, a reaction between *p*-coumaric acid and ethanol was suspected.

To study the eventual esterification in the Medium G between *p*-coumaric acid and tartaric acid, wavelength was fixed at 305 nm, and the absorbance variation was followed up in time. The absorbance remained constant during a whole week, when the solution was kept in darkness without any contact with air oxygen, at ambient temperature.

These observations showed that this acid did not present any reactivity with tartaric acid neither with any of the synthetic wine medium in our conditions.

To understand the reality of the hypsochrome and hypochrome effect observed with ethanol, a NMR and IR analysis were carried out.

3.4. NMR and IR reactivity tests of the p-coumaric acid

Many shifts occurring in spectra of Fig. 3a (pure *p*-coumaric acid) and spectra of Fig. 3b (*p*-coumaric acid in ethanol – pH 3.5) are discussable basing on organic chemistry references (Mc murry, 2000). The chemical shifts of *p*-coumaric acid phenolic cycle in addition to the two hydrogens of the vinylic bond are found at 7.5 ppm (multiplet), at 6.7 ppm (doublet) and at 6.3 ppm (doublet). The big difference is presented in spectrum 3b where we can find the characteristic signals of ethanol at 3.4 ppm (quartet) and at 1 ppm (triplet). Another quartet found at 3.7 ppm integrating 2 protons, as well as another 3 protons at 1 ppm led to conclude that an ethylic ester was formed in the medium.

The results of the Infrared spectroscopy confirmed as well the presence of an ester in the medium when the *p*-coumaric was dissolved in pure ethanol where the pH was 3.5. In fact, two characteristics IR absorptions made the carboxylic group easily identifiable when the *p*-coumaric acid was not dissolved in ethanol. The O–H bond of the carboxyl group gave rise to a very broad absorption over the range 2500–3500 cm^{-1}, and the carbonyl bond showed absorption at 1680 cm^{-1}. On the other hand, two specific absorptions revealed an ester function when the *p*-coumaric was dissolved in ethanol. The first was situated in the same range of the carbonyl of the carboxylic acid, and the other was an intense absorption around 1240 cm^{-1}, showing a C–O bond.

The difference between spectra A (pure *p*-coumaric acid – Fig. 3a) and B (*p*-coumaric acid in ethanol – Fig. 3b) as well as the absorbance in the infrared spectroscopy discussed above, elucidated a probable esterification between ethanol and *p*-coumaric acid occurring in the medium.

The identification of the probable product of the reaction is outside the aim of this paper. It could be revealed

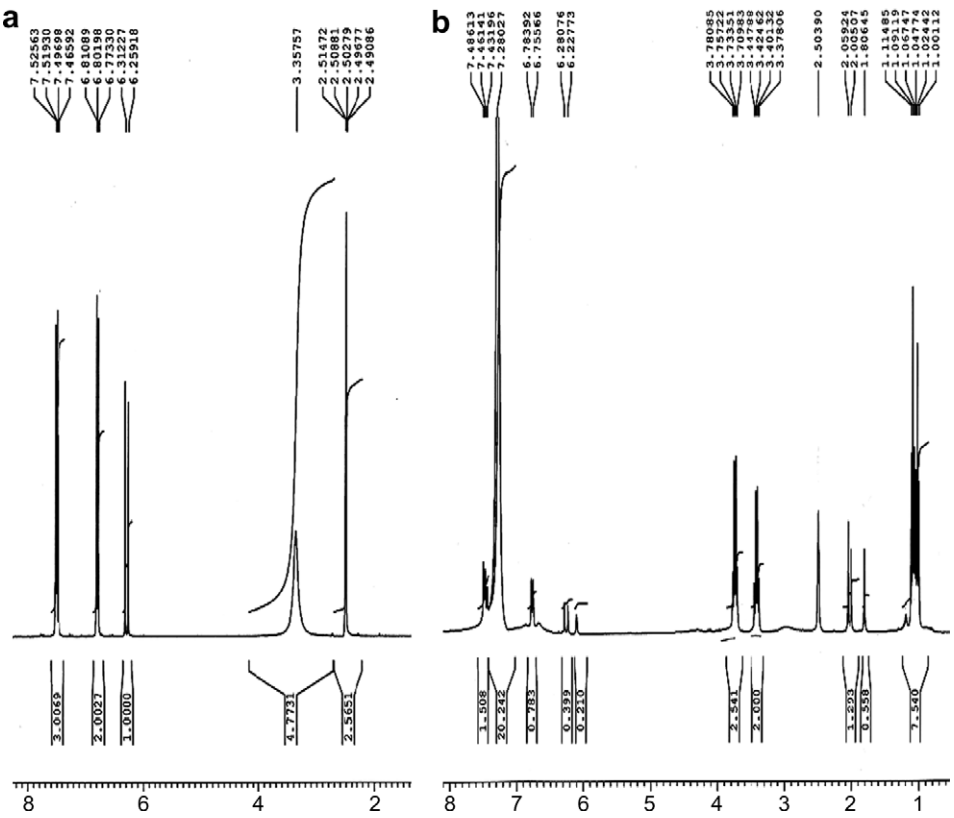

Fig. 3. NMR spectra of *p*-coumaric acid without (a) and with ethanol (b).

with more accurate analysis, and will be discussed in future work. In this case of study, the important matter is to be conscious of the presence of a reaction between ethanol and *p*-coumaric acid in the experimental conditions even if this reaction extent is limited.

On the other hand, the following up of the same *p*-coumaric/tartaric acid solution, by NMR after one month, showed no changes in the spectra which reveals that the *p*-coumaric acid did not react with the tartaric acid in our conditions. This result confirmed the UV analysis of media G.

3.5. Adsorption tests of p-coumaric acid

The yeast concentration in the sample and the *p*-coumaric acid concentration, added and found just after adsorption on yeast, are resumed in Table 1. Results were compared for the solutions A, C and E, where the *Brettanomyces* sp. population was different but the concentration of the *p*-coumaric acid added was the same. All the samples tested were free of vinylphenol and ethylphenols.

The acid loss was found to be higher when population concentration was higher: 48% of loss for E and 9% for A.

On the other hand, using the results found for the solution B, C and D, where the adsorbent mass of the population concentration was the same, but the *p*-coumaric acid concentration added different, an isotherm was established, based on the Langmuir equation of adsorption in solution. Because the samples were taken only after 2 min of adding *p*-coumaric acid in the medium, yeasts could be considered as a constant solid adsorbent.

The Langmuir equation for solutions has the followed expression (Avom, Ketcha Mbadcam, Matip, & Germain, 2001):

$$Q_e = (Q_o K_L C_e)/(1 + K_L C_e)$$

where Q_e is the adsorbed quantity (mol/g) when theoretical equilibrium is established, C_e the concentration when equilibrium is established (mol/l), Q_o the maximum capacity of adsorption (mol/g) and K_L the Langmuir constant (empirical constant in function of temperature and the studied system). Knowing that the substance quantity adsorbed by the system is $X = C_i$ (initial concentration) $- C_e$ (concentration at equilibrium), and that $Q_e = X/m$, where m is the adsorbant mass.

The $Q_e = f(C_e)$ curve is presented in Fig. 4.

Table 1
p-Coumaric acid loss in adsorption tests

Solution	Yeasts concentration (cell/ml)	*p*-Coumaric acid added (mg/l)	*p*-Coumaric acid found after adsorption (mg/l)	*p*-Coumaric acid loss after adsorption (%)
A	3×10^6	10	9.1	9
B	360×10^6	5	3.4	32
C	360×10^6	10	8.4	16
D	360×10^6	20	16.8	16
E	450×10^6	10	5.2	48

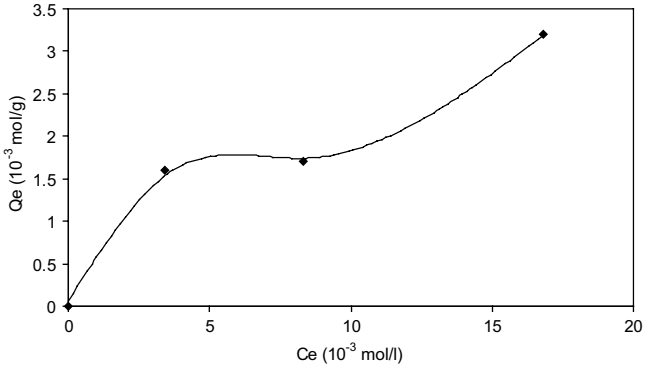

Fig. 4. Adsorption isotherm of *p*-coumaric acid $Q_e = f(C_e)$ at 30 °C.

This curve is assimilated to a Langmuir type II isotherm. The adsorption of *p*-coumaric acid on *Brettanomyces bruxellensis* yeast is assimilated to a liquid/solid adsorption in our conditions. These results showed that *p*-coumaric acid adsorbed on *Brettanomyces* sp., does not give neither vinylphenol nor ethylphenol occurrence in the medium, which induced a non absorption of coumaric acid by this yeast when the samples were taken (after 2 min). These results joined the bibliographical analysis concerning the polyphenolic adsorption on yeasts cell wall (Medina et al., 2005; Morata et al., 2003; Morata et al., 2005; O'Neill et al., 1996; Suárez et al., 2007).

In our case of study, the adsorption increased when the *Brettanomyces* sp. population was higher. This phenomenon explains the difference found between the initial concentration of *p*-coumaric acid detected and the one added.

4. Conclusion

p-coumaric acid is an important component to study in wine because its bioconversion by *Brettanomyces* sp. into ethylphenols damages the quality product. Nevertheless, in oenological conditions *p*-coumaric acid can disappear in different ways (Fig. 5). It is very important then to know the exact quantity of coumaric acid that can participate in this bioreaction in the purpose of calculating its real yield.

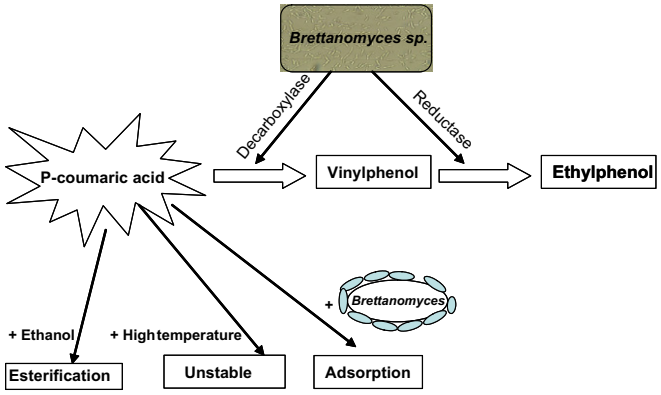

Fig. 5. *p*-Coumaric acid disappearance in experimental wine conditions.

First, it was shown in this paper that *p*-coumaric acid was stable at oenological temperature (20–30 °C) but unstable at high temperature. Therefore, it is not recommended to autoclave it in solution.

Our RMN and IR spectra showed that the *p*-coumaric acid could react with ethanol, as all polyphenols of wine do. This reaction seems to be an esterification and its rate becomes higher when ethanol concentration increases in the medium. More studies should take place in order to determine the exact form of the reaction product.

Although the *p*-coumaric acid is naturally esterified with tartaric acid in natural wine, it was not subjected to an esterification with tartaric acid in our synthetic wine medium.

On the other hand, this work showed that *p*-coumaric acid solutions can adsorb on *Brettanomyces* yeast. In our conditions, this adsorbance corresponds to the type II Langmuir isotherm. The adsorption of phenolic compounds is a classical problem observed with *Saccharomyces* yeast as mentioned in our introduction. In our work, we showed for the first time as we know a true adsorption phenomenon of the *p*-coumaric acid on the *Brettanomyces* yeast.

Finally, in our experimental conditions, only 50–90% of *p*-coumaric added in the beginning of the fermentation is available for the bioconversion into ethylphenol. Adsorption on *Brettanomyces* walls seems to be responsible of the major loss. More studies should lead us to a better understanding of this adsorption phenomenon.

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