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THE INFLUENCE OF pH AND LATE MICROOXYGENATION ON SOURNESS, BITTERNESS AND ASTRINGENCY OF RED WINE

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Introduction

In Mediterranean regions, pH values are often considered too high and acidification by addition of tartaric acid is often used. A decrease in pH allows better control of wine oxidation, physico–chemical stability, microbiological development, SO2 additions (1), and affects its sensory characteristics. Correction of pH by acidification is difficult to obtain with random results up to date, principally because high levels of pH are normally related to high levels of potassium ions (K+) and not only to organic acid contents. To decrease K+ contents and consequently pH, an electromembrane process with bipolar membranes has been recently tested in wine (2). This separative technique, under the effect of a continuous current and selective membranes (cationic and bipolar), permits the extraction of K+ ions and the free acid/salified acid equilibrium is displaced towards the acid form, thus lowering pH.

Sourness, astringency and bitterness are three of the main sensory attributes of red wine. Different parameters such as alcohol (3-6), sweetness, viscosity (7), tannin and proanthocyanidin composition and concentration (4,8), type and concentration of organic acids (9-11) and pH (5,6,10) have been shown to affect these sensory attributes in model solutions and wine. Sourness of wines (4,12) and acid aqueous solutions (10,13) is dependent of pH, and is also elicited by the concentration and anion species of the acid (10).

Lowering the pH of wines (3,6,12) or model solutions (12) has no effect on bitterness. Adding acid has also been reported to increase the astringency of wine (5,12) and model solutions (10,12). Astringency elicited by acids is related to pH in aqueous solutions (10,13,14), and in white wine, but not in red wine (14).

Wine micro-oxygenation may increase coarseness and colour intensity in a first phase, to later soften tannins (15). After a 6 month treatment of 0.75 to 3 ml/l/month micro-oxygenation, moutounet et al. (16) observed a positive action over the hard and drying expression of red wines, together with a gain of structure.

The evolution of native anthocyanins and their conversion to ethyl-bridged anthocyanin–flavanol adducts following wine microoxygenation have been reported (17). Involvement of tannins in these reactions may explain in part the decrease of astringency in wines.

This research was conducted primarily to investigate the effects of a pH decrease obtained by an electromembrane process on sourness, bitterness and astringency perception in red wine after a 9 months storage. This was achieved by descriptive sensory analysis where all three sensory attributes are evaluated at the same time to simulate a real wine tasting.

Materials and Methods

Wine preparation. Seven months after wine-making (Pech Rouge-INRA, Gruissan), a Petit verdot wine (anthocyanins: 276.68 mg/L; proanthocyanidins: 1079 mg/L) was selected for its high pH value (3.9). Fifteen hL were treated with an electromembrane technique to obtain a pH 3.5 wine that was used to fill three 1 hL tanks. The pH 3.9 wine was distributed in 6 tanks (1 hL).
All wines were maintained at 15°C during 9 months in a controlled temperature room. Three of the pH 3.9 tanks were treated with 2 mL/L/month of oxygen during the last 3 months of storage. **Physico-chemical analysis.** HPLC anthocyanin analyses and spectrometry measurements (absorbance at 280, 420, 520 and 620 nm, Colour Intensity (CI) and hue) were performed as described previously (17). Proanthocyanidin analysis was performed by acid-catalysed cleavage in the presence of phloroglucinol followed by HPLC analysis (18). This analysis gives access to total proanthocyanidin content, their mean degree of polymerisation (mDP), and percentage of constitutive units (i.e. catechin, epicatechin, epicatechin gallate, epigallocatechin).

**Sensory evaluation.** 19 paid judges, 13 female and 6 male, between 19 and 57 years of age, were selected on the basis of interest, availability and good sensory capacities after a preliminary selection test which included rating, discrimination and difference tests. Ten of the nineteen judges had previous experience in wine sensory evaluation.

Two initial sessions of 9 triangular tests each were performed to see if there were differences between experimental repetitions and treatments and, prior to formal testing, judges were trained during 5 sessions of 2 hours each for the attributes of sourness, astringency and bitterness. At each training session, in which the three attributes were rated in aqueous solutions (deionised and microfiltered MilliQ water) and red wine, low and high reference standards for sourness (2.5 g/L of citric acid in a 1% ETOH solution), astringency (1 g/L of Alum in a 1% ETOH solution) or bitterness (5-10 mg/L of quinine in a 1% ETOH solution) were presented. To give the judges further orientation in bitterness, four other standards were added to the training: 1 g/L of phenylalanine, 0.4 g/L of caffeine, 5 mg of quinine sulphate and 5 mg/L of quinine HCL. Judges were given feedback on their performance at the end of each session. A total of 16 ranking tests (tests were repeated according to results), 3 recognition tests, and 5 discrimination tests were conducted, and 13 different standards were used.

The formal sensory evaluation was conducted in the Sensory Evaluation Laboratory of the INRA at Montpellier (France) in individual computerized booths. A complete factorial design of 2 pH levels (3.5 and 3.9) and two micro-oxygen treatments (0 and 2 mL/L/month) gave 9 samples per session which were presented to each judge in a monadic service after being balanced by a Latin Square design to avoid the effects of presentation order and first order carry over effects (19). The session was repeated three times, yielding a total of 27 samples. At each session, a reference wine was presented before the formal evaluation.

The 10 mL wine samples were presented in black OIV standardised wine glasses to avoid colour interaction in the sensory response. Wines were evaluated under standardised daylight (Norme V09-105AFNOR, 1995) For better discrimination and for avoiding carry over effects, judges used a sip and spit protocol with a pectin rinse. The Judges then rated maximum astringency, bitterness and acidity on an unstructured 10-cm line scale, anchored by the terms low and high. **Data analysis.** Intensity ratings were subjected to mixed model Analysis of Variance (ANOVA) were judges were treated as a random effect. Principal Components Analysis (PCA) was carried out on the correlation matrix of the mean ratings of sensory attributes with no rotation. The preparation of sessions, sensory descriptive ratings and statistical analysis was performed by FIZZ V.2.1 software (Biosystemes, Couternon, France).

**Results**

**Sensory evaluation.** As shown in Figures 1 and 2, the diminution of 0.4 points of pH significantly enhanced the perception of sourness (p <0.001***), effect that has been extensively described in literature (4,10,12,13), and significantly reduced bitterness (p <0.001***), contrary to Kallithraka et al (12) who observed that bitterness of wines was not affected by pH (12). Other authors have
reported that an increase from pH 3.2 to pH 3.8 of a de-alcoholised white wine concentrate had no significant impact in bitterness; in contrast, as pH was raised from 2.9 to 3.2, bitterness was significantly enhanced (4).

In this study, no significant impact of a 0.4 point difference in pH was observed for astringency. A decrease of pH has been reported to enhance astringency in model solutions (10,12,13), and in a de-alcoholised red wine blend, a difference of 0.6 points of pH (3.2 vs 3.8) overall astringency was enhanced (p<0.05*) (5), but this effect has not always observed in red wine (14). Late micro-oxygenation had no effect over acidity, bitterness or astringency. The increase in wine coarseness (15) was not confirmed under these experimental conditions.

**Physico-chemical analyses.**

Wines at pH 3.5 presented significantly lower levels of anthocyanins (p <0.01**) and dry extract (p <0.05*), possibly due to the removal of cations by the electrodialysis treatment. They also showed lower values of absorbance at 620 nm (p <0.01**) and hue (p <0.01**) than pH 3.9 wines, and significantly higher levels of absorbance at 420 nm (p <0.01**) and 520 nm (p <0.01**) and of colour intensity (p <0.01**). This along with the significantly larger area of the hump measured in the HPLC profile at 520 nm in the pH 3.5 wine (p <0.05*), suggests that...
Anthocyanins underwent different reactions at pH 3.5 and 3.9, as observed earlier in model solutions (20). These different reactions may also partly explain the lower anthocyanin content in the more acidic wine.

No difference was observed between the two pH levels for 280 nm absorbance, total polyphenol index (TPI), proanthocyanidin content and qualitative composition (mDP, % epicatechin gallate (%gall) and % epigallocatechin (%egc) units), meaning that the treatment did not modify tannin composition.

Micro oxygenated wines presented significantly lower proanthocyanidin concentration (p < 0.01**), and significantly higher levels of absorbance at 420 and 620 nm, colour intensity (p < 0.05*). These differences did not have any incidence on sensory properties.

**Conclusions**

Decreasing wine pH values by 0.4 points using an electromembrane technique reduced bitterness and enhanced acidity of a Petit verdot red wine, while no effect on astringency was observed, which are important results in terms of practical winemaking.

Late micro-oxygenation did not affect astringency, acidity or bitterness in this experiment. Differences in the pigment composition were induced by the electromembrane treatment, either directly by removal of anthocyanin flavylum ions or indirectly, different anthocyanin reactions being favoured at each pH value. Whether these changes are related to taste properties (especially enhanced bitterness of the higher pH wine) remains to be investigated. Tannin composition was not modified by the electromembrane treatment. Slightly lower tannin levels were found in micro-oxygenated wines but this was not detected by sensory analysis.

**Literature Cited**