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Effect of the addition of ammonium and amino acids to musts of Airen variety on aromatic composition and sensory properties of the obtained wine

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Abstract

Musts from Airen grapes to which ammonium (100, 300 mg/l) and amino acids had been added (doubling the level of amino acids in that must) were fermented with three different yeast strains. Statistical treatments showed that the strain of yeast is the major factor affecting wine volatile composition, but must nitrogen supplementation also has an influence. The addition of any source of nitrogen to the must reduces the contents in the wine of β-phenylethanol (ca. 65% reduction), methionol (ca. 70%) and isoamyl alcohol (40–65%) and increases wine content of propanoic acid by 30–130%. Wines from musts supplemented with ammonium are richer in ethyl lactate and c-3-hexenol and wines supplemented with amino acids are richer in γ-butyrolactone and isobutanol. From the sensory point of view, must supplementation with ammonium brings about a decrease in sulphury notes and an increase in citric flavour. The effect of amino acid supplementation depends on the yeast strain. In one case, the effect is similar to that of ammonium supplementation; in the others an increase in fruity and fusel notes was obtained.

Keywords: Must; Wine; Ammonium; Amino acids; Aroma; Yeast

1. Introduction

The aromatic complexity of wines varies, depending on the variety of grape used, the aromas produced during the fermentation and those developed during the aging process. The production of both, aromas and spoilage compounds, by the yeasts can be significantly affected by winery practices such as clarification, airation, yeast strains, nutrient addition and temperature of fermentation.

It is a common practice in vineyards to supplement the must with diammonium phosphate (DAP), urea or yeast nutrients to prevent problems related to nitrogen deficiency: slow and stuck fermentations and SH_2 production. This addition must follow some criterion, since the addition of large amounts of ammonium in the must can result in later problems. Wines with higher amounts of residual nitrogen run greater risks of microbiological instability and production of ethylcarbamate.

The nitrogen composition of the must affects fermentation kinetics, the production of aromatic and spoilage compounds, of ethanol and glycerol (Albers, Larsson, Liden, Niklasson, & Gustafsson, 1996) and also urea, the main precursor of ethylcarbamate, a carcinogen present in wine. The two main sources of yeast-assimilable nitrogen are primary amino acids and ammonium (Butzke, 1998). Several studies on the effect of ammonium addition on most of these parameters have been carried out, but little is known about its influence on the formation of most aromatic compounds.

In particular, SH_2 production is usually controlled in vineyards by the addition of DAP to must. Jiranek, Langridge, and Henschke (1995) have demonstrated that, when there is little ammonium in the exponential phase, more SH_2 is produced than when there is ammonium present. Recently, Marks, van der Merwe, and van Vuuren Hennie (2003), using genetic studies,

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demonstrated that assimilable nitrogen is a key factor in the regulation of SH_2 during alcoholic fermentation.

On the other hand, the addition of ammonium to musts with a low nitrogen level produces an increase in the fermentation speed during the exponential phase, with a significant increase of biomass, but it does not prolong the time during which the cells can multiply (Monteiro & Bisson, 1992; Ough, 1964). If there were high nitrogen concentrations in the must, Jiranek, Langridge, and Henschke (1991) observed that the differences in the fermentation speed of different yeasts were smaller than when the musts were poor in nitrogen.

Increase of assimilable nitrogen in must grape, by means of vinegrape fertilization, or by addition of ammonium, produces a diminution in the formation of fusel alcohols (Vos & Gray, 1979; Rapp & Versini, 1991; Ough & Bell, 1980; Castor, 1954).

Webster, Edwards, Spayd, Peterson, and Seymur (1993) have demonstrated that the addition of a good source of nitrogen to a must affects yeast metabolism and the fermentation bouquet, yielding a "cleaner" but less complex wine. They also found that wines from fertilized vines contain more esters and were different by sensory analysis.

The yeast strain is another factor to consider in the formation of the fermentation aroma. Some authors find that, the yeast used affects aromatic compounds, esters, and alcohols, which modify the fruity notes of the wines obtained (Perez-Coello, Briones Perez, Ubeda Iranzo, & Martin Alvarez, 1999). They mainly find differences related to the distinctive capacity of yeasts to assimilate nitrogen. The yeasts with greater demand for nitrogen produce higher concentrations of esters during the fermentation, and those with lesser demands produce greater concentrations of higher alcohols (Perez-Coello et al., 1999; Torrea, Fraile, Garde, & Ancin, 2003). Nevertheless, Ubeda and Briones (2000) find that, although the amounts of volatiles formed are different, there are no clear sensory differences between the wines obtained.

On the other hand, the studies conducted by Marchetti and Guerzoni (1987), with 16 different yeast strains, clearly indicate that the composition of the initial must influences the aroma of the wine obtained to a greater extent that the yeast strain used.

Considering the widespread use of ammonium addition and active dry yeasts in vineyards, it is necessary to know the effect that such an addition may have on the aroma of the wine and the influence of different yeasts commonly used in wine production. This article describes the study of 15 wines made with Airen, the largest *vitis vinifera* cultivar in Spain. The must was supplemented with different amounts of ammonium and/or amino acids, and three different yeast strains were used in the study.

2. Materials and methods

2.1. Reagents and standards

The pure reference compounds used in the quantitative analysis of volatile compounds were purchased from Aldrich (Gillingham, UK), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), Poly Sciences (Niles, IL), Lancaster (Strasbourg, France), and Chemservice (West Chester, PA). Individual amino acids were from Sigma. Diammonium phosphate and formaldehyde were obtained from Panreac S.A. (Barcelona, Spain). Water was obtained from a Milli-Q purification system (Millipore, Beldford, MA).

2.2. Samples and vinification

2.2.1. Grapes

Must from *vitis vinifera* grapes of Airen variety was used. Two types of experiments were performed:

2.2.2. Experiment 1

Must Airen (Airen 1) was divided into three batches of 600 ml. Each batch consisted of three samples, each inoculated with active dry yeasts *Saccharomyces cerevisiae*: Fermicru AR2 (L1), Stellevin NT116 (L2) and LW LVCB CT1+ (L3) obtained from DSM Food Specialties Oenology S.A.S. (France). The first batch was the control (no ammonium added), samples from the second batch were supplemented with 100 ppm of ammonium, and samples from the third with 300 ppm. The experiment was replicated.

2.2.3. Experiment 2

The concentrations of individual amino acids in the Airen 1 must were measured; then, solutions of each one of the amino acids were prepared and added to the must in order to double the original concentration of each amino acid. This batch was identified as Airen 2. A second batch was prepared by further addition of 100 ppm of ammonium to this amino-acid enriched must. Both batches were divided into three samples and each sample was inoculated with one of the three aforementioned yeasts. The experiment was replicated. All the samples used in the study are presented in Table 1.

Table 1
FAN in all the musts used in the present study

Musts	FAN (mg N/l)
Airen 1 (A1)	175
Airen 1 + 100 ppm ammonium	240
Airen 1 + 300 ppm ammonium	375
Airen 2 (A2 = A1 + amino acids)	274
Airen 2 + 100 ppm ammonium	342