ADDITION OF AMINO ACIDS TO GRAPE JUICE OF THE MAUZAC VARIETY: EFFECT ON AROMA PRODUCTION DURING ALCOHOLIC FERMENTATION

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Abstract. The wine's flavor like that of the other fermented drinks is extremely complex. The analyses of the volatile components carried out up to these days reveal the presence of several hundred of substances. In Fact, in the literature, more than 800 flavour compounds have been reported in wines, including higher alcohols, aldehydes, ketones, esters, acids and monoterpenes. The nitrogen composition is claimed by some to be the most important factor influencing the production of aromatic and spoilage compounds. The aim of this work was to describe the effect that the addition of different concentrations of leucine, isoleucine and valine have on the synthesis of aromatic components. The study was conducted on juice of the Mauzac variety. Different amounts of the three amino acids cited were added and their evolution was followed by head space analysis.

Keywords: Alcoholic fermentation, *Saccharomyces Cerevisiae*, amino acids, aroma

INTRODUCTION

Flavour is the most important factor that contributes to the enjoyment of wine. It is composed in part of volatile compound produced during alcoholic fermentation.

Among the volatile carbonyl compounds of wine, the aldehydes are the most important as aroma compounds because of their low sensory threshold values (Bayonove &al., 1998; Rapp, 1998; Schereir, 1979); acetaldehyde is the major component and constitutes more than 90% of total aldehydes content (Rapp & Versini, 1991).

Significant components in young wines are the esters, ethyl acetate being the most important (Bertrand, 1978).

Higher alcohols are quantitatively the largest group of the flavour components in alcoholic beverages; isoamyl alcohols, isobutanol and propanol have been considered to be the most significant (Perez&al., 1999; Torrea & al., 2003; Hernandez Orte & al., 2003)

Many factors have been shown to influence the amounts of the volatile components produced during fermentation, including yeast strain, temperature, pH and nitrogen composition of the must.

The nitrogen composition of the must is of a great importance, it affects fermentation kinetics, the production of aromatic and spoilage compounds, of ethanol and glycerol (Albers, 1996).

The two main sources of yeast assimilable nitrogen are amino acids and ammonium (Butzke, 1998). Several studies on the effect of ammonium or amino acids addition on most of these parameters have been carried out, but little is known about its influence on the formation of the most aromatic compounds.

Webb and Ingraham, (1963) by using a mutant strain of Saccharomyces cerevisiae unable to synthetize the leucine, the valine and the isoleucine, have established that this strain was unable to produce the isobutylic and isoamylic alcohols. This experience

showed that the synthesis of the superior alcohols from carbonhydrates follows the same metabolic pathway as that of the amino acids.

Ferreira, Lopez and Cacho (2000) in their study of young spanish red wines, found that some of the most important differences between young wines of different varieties were due to the presence of isoamyl acetate, isobutyric acid and their ethyl esters, as well as the presence of fusel alcohols, all of which are by products of yeast amino acid metabolism.

Hernendez-Orte, Ibarz and Ferreira (2005), by supplementing must from Airen grapes with ammonium and amino acids, concluded that the content of the wine of isoamyl alcohol reduces (40-65%) and of propanoic acid increases by 30-130%.

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

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

Considering the widespread use of assimilable nitrogen addition, it is necessary to know the effect that such an addition may have on the aroma of the wine.

So, the aim of this article was to describe the effect that the addition of different concentrations of leucine, isoleucine and valine have on the synthesis of aromatic components.

The study was conducted on juice of the Mauzac variety, to which different amounts of the 3 amino acids were added.

The choice of the three amino acids selected for the study and the amounts added were based on previous works found in the literature (Etievant, 1988; Huang, 1991).

The evolution of the above mentioned six aromatic compounds was followed by head space analysis.

MATERIAL AND METHOD

Strain:

The yeast used for this study is Saccharomyces cervisiae strain VL1 commercialized by “Laffort Oenologie” as Dry Active Yeast. The innoculum was prepared from this yeast preserved on agar slants at 4°C. The medium of fermentation was inoculated by a preculture of 12 hours old incubated on 100ml of grape must.

Medium:

Conservation Medium

The yeast strain was preserved on agar slants. The composition of the solid medium was as following:

Agar-agar:	20g
Glucose:	20g
Yeast extract:	10g
Chloramphenicol:	0.1g
H2O q.s.p:	1000ml

Grape must

The grape must used was from "Mauzac" variety, supplied by the ITV-France, Midi-Pyrenees regional station, and has the following characteristics:

Initial concentration of sugar: 195g/l

Concentration in assimilable nitrogen: 89mg N/l (measured with formol titration method)

Turbidity after clearing: 300NTU

Initial pH: 3.2

Fermentation conditions:

Batch fermentations were carried out in 2.O l fermentor. The volume of the medium in the fermentor was 1.6 l. The temperature was regulated at 30°C and the agitation rate was 200 rpm.

The fermentors were inoculated with 3.10^6 cells of viable yeasts per ml taken from a prior culture prepared 15 hours before.

Samples (10 ml) of the fermenting medium were withdrawn regularly in order to determine the fermentation kinetics.

Several fermentations were conducted under the conditions described above. The medium was fortified with reagent grade amino acids prior to inoculation. Three amino acids were used: Leucine, Isoleucine and Valine in

the following quantities: 0, 25, 50, 200 and 500 mg/l.

Analytic methods:

The biomass quantity was determined by the measurement of the optical density at 620nm, in 2mm wide cuvette, using a spectrophotometer U.V- visible double beam Hitachi U-2000. This value was correlated with the one of "dry weight" (dry cell mass kept on a membrane of 0.45 µm after filtering a known volume of the growth medium). The viability was evaluated by counting on Thomas cell through the microscope, after staining by methylen blue (Bonora and Mares, 1982).

The glucose was measured by an enzymatic method using an automatic device (YELLOW SPRING INSTRUMENTS, model 2700 select) containing a glucose – oxydase.

The ethanol was measured by chromatography in gaseous phase with a chromatograph chrompack 437 A (column chrompack poraplot Q wide bore (0.35m x 25m)) provided with a detector with flame ionization. The temperatures of the detector and the injector were 200°C, the vector gas used was the nitrogen with an out put of 8ml/mn. The inside standard was the isopropanol 1% added on the proportion of 2/1 to the sample.

The volatile compounds have been analysed by chromatography in gaseous phase-Head space with a chromatograph 8500 Perkin Elmer (colum BP 20 polar (25m x 0.32mm x 0.5 µm)). The temperature of the cathetometer was 280°C and that of the injector was 260°C. The vector gas used was the nitrogen at a pressure of 25 PSIG.

The analysis of the volatile components was done through a standard solution containing the seven compounds we focused on at the concentration of 100 mg/l.

Each sample was preheated at 60°C during 1 hour before injecting in the head space column (time of pressurization: 0.51mn; time of injection: 0.40mn).

The amino acids, have been analysed by chromatography in liquid phase (Hewlett packard (HP)) according to aminoquant 1090 SYSTEM

RESULTS AND DISCUSSION

Our study deals with the addition of leucine, isoleucine and valine to the gape juice of Mauzac variety during an alcoholic fermentation.

The kinetics established for each one of the fermentations carried out, showed that the differences observed in terms of biomass and ethanol production, are not significant.

Nevertheless, the duration of fermentations of amino acid-supplemented juices was shorter. This observation seems to be in agreement with reports from other authors (Jiranek Langridge, and henschke, 1991).

The effect of amino acids supplementations on the production of the volatile compounds selected can be seen in fig.1, 2 and 3and can allow us to reach these conclusions:

As first, it can be said that each amino acid influences the synthesis of few volatile compounds.

Figure 1 revealed a significant effect of leucine supplementation on the esters and alcohols generation. With more Leucine present in the medium, more ethyl acetate, isoamyl acetate, isobutanol, propanol and isoamyl alcohols were formed.

This would indicate that the addition of leucine enables the yeast to work more efficiently resulting in a better performance with regard to the production of aroma compounds.

These results were confirmed by other experiments done under the same experimental conditions by using a synthetic medium (data not given).

The production of ethyl acetate and isoamyl acetate are not very sensitive to isoleucine additions in the medium. On the other hand, the variations of propanol formations are very small and those of isobutanol and isoamyl alcohols are all the more high as the content of the addition increases (fig.2).

Figure3 lead us to observe a similar trend for the additions of valine as for isoleucine.

The addition of valine at 200mg/l increases in an optimal way the isobutanol production and the formation of isoamyl alcohols was higher in all cases of supplementation.

For acetaldehyde, no great differences were found in the course of its formation in all of the fermentations and supplementations studied. It was therefore deduced that the nitrogen concentration did not have a great influence on its synthesis.

On the other hand, acetaldehyde disappeared during the fermentation, due fundamentally to its combination with anthocyanins and sulphurous compounds.

The increase of the isoamyl alcohols generation observed from the additions of Leucine and isoleucine does correspond well to the metabolism and this would seem to indicate that the yeast used is with lesser demand for nitrogen (Perez-Coello &al., 1999;Torrea&al.,2003)

Also, the effect of both these amino acids addition can be explained by the increased capacity of the yeast to transforme the synthetized α-ketoacids, avoiding their accumulation and latter expulsion to the medium after their reduction to higher alcohols.

For the valine additions, the increase we noticed in the isobutanol formation is optimal with the addition of 200mg/l. It seems that the isobutanol production, besides being linked to the quantity of the valine in the medium, is also linked to the nitrogen concentration that is easily absorbed in the medium.

It may be, either used directly in the protein synthesis, or transformed into leucine, as its metabolism permits.

However, the isoamyl alcohols concentration results from the carbonaceous catabolism in this case, since it is not affected by the addition of valine. The Leucine resulting would be used in the proteins synthesis.

CONCLUSIONS

As a first conclusion, the role played by amino acids supplementations on fermentation kinetics was not evident.

However, the higher alcohols production was influenced in a very specific way. The Leucine and the isoleucine influenced the isoamyl alcohols production (respectively the 3-methyl-butanol and the 2-methyl -butanol).

The isoamyl acetate synthesis followed the production of the 3-methyl butanol-1. As to the isobutanol, even if the leucine and the isoleucine didn't take any part, the valine influenced its production, but for a concentration in supplementation that is around 200mg/l.

For the acetaldehyde and the propanol-1, it had been hard to determine what the influence of the additions of amino acids is.

It clearly appears that yeast uses the amino acids as a nitrogen source when they are present in large quantities.

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