ESTER PRODUCTION IN ALCOHOLIC FERMENTATION

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ABSTRACT

Esters are volatile organic compounds (VOCs) produced by a wide range of microorganisms, they are responsible for the aromatic profile of beverages (wines, sparkling wines, beers, cachaças, etc.). Understanding these metabolic pathways enables the improvement of the fermentation processes increasing the desired aromas in different beverage. The variation in the aroma of a drink depends on the profile of esters present, which may vary with the strain of yeast used in fermentation. Considering that, amino acids can be biotransformed by yeast into higher alcohols and then into esters, the addition of amino acids to the fermentation wort can affect the production of esters and improves variation in the sensorial profile of the drink. This work aims to produce and extract higher alcohols and esters derived from amino acids during alcoholic fermentation using the strain *Saccharomyces cerevisiae* CA11 from the working collection of the Microbiology laboratory at UNESP in São José do Rio Preto. The methodology used will be *in situ* solid phase micro extraction (HS-SPME). The chromatographic analysis is done using gas chromatography coupled with a flame ionization detector (GC-FID).

Keywords: yeasts. *Saccharomyces cerevisiae*. volatile organic compounds. amino acids, esters

1 INTRODUCTION

Yeasts have been used as precursors to several biotechnological processes for thousands of years, mainly in industries such as the beverage industry and the biofuels industry[1], generating billions in revenue[2,3,4,5]. Currently, there is a great demand for innovation in the diversity of flavors and aromas offered by the beverage industry. This innovation can be achieved through the diversification of organisms used in fermentation processes, as well as the change of the fermentative parameters[6]. The compounds responsible for the variation of the organoleptic profile of beverages are volatile organic compounds, which are highly dependent on the species and strain of the yeast used[6,7]. Among these compounds, we can highlight the higher alcohols and esters as the compounds that contribute the most to the variation of this profile[8]. Esters are synthetized from higher alcohols as their precursor, which in turn have as precursors the amino acids. Some studies indicate that the supplementation of the growth medium with amino acids can results in a positive change in the sensory profile of beverages such as beers, wines, sparkling wines and cachaça[9,10]. That change is desirable from an economic standpoint, seeing how the market is in need for innovation[6] and amino acid supplementation is a cheap and easy way to fulfill that need. The present work aims to obtain a qualitative relationship between the amino acids addition to the must and the ester formed, that data could be applied directly in the beverage industry, which, as discussed previously, is very desirable economically.

2 MATERIAL & METHODS

The yeast *Saccharomyces cerevisiae* CA11 was used. Yeast cultivation takes place in YEPD medium (1%, 2%, 2%), which is autoclaved for 15 min at 120 °C. The fermentation medium to produce volatile compounds was a combination of a nitrogen base by Sigma-Aldrich (Y0626) supplemented with glucose (10%) as a carbohydrate source and with the amino acids (150 ppm). The medium is subsequently filtered with a 0.22 Micron filter.

Initially, the cultivation of the pre-inoculum was made, which is monitored until it reaches 1 x 10^8 cells/ml, with the aid of a Neubauer counting chamber. After the predetermined cell density is reached, there is the centrifugation of this pre-inoculum at 4000 RPM for 15 min at 4 °C. The biomass was suspended in 10% of the total volume, in sterile distilled water. This suspension transferred into the Vials containing the fermentative medium at 10% mass/volume concentration. The vials were closed and incubated without agitation, for 12 hours and placed in a freezer until subsequent analyzes are made.

The methodology for the analyses was the *in-situ* headspace solid-phase microextraction. The vial, containing the fermentative medium, was heated for 20 minutes at 80 °C. Subsequently, there was the insertion of the fiber, which captures and concentrate the volatile organic compounds present in the Vial's headspace. The heating continues for another 10 minutes with the fiber exposed inside the Vial. Subsequently, the fiber was inserted inside a PerkinElmer 600 gas chromatograph coupled to a Clarus 680 Turbo Matrix flame ionization detector (GC-FID). The fiber was exposed in the injector for 1 minute and then removed. The total time of the run is 38 minutes.

3 RESULTS & DISCUSSION

So far, three amino acids have been tested. They are lysine, leucine and valine. In total there are 2 control groups for each amino acid, one biotic control group without the addition of amino acids and one abiotic control group with the addition of amino acids. They allow us to be certain that the biotransformation is not spontaneous and is, in fact, caused by the *S. cerevisiae* CA11 metabolism. The three chromatograms generated allows us to compare the actual fermentation with the two control groups.

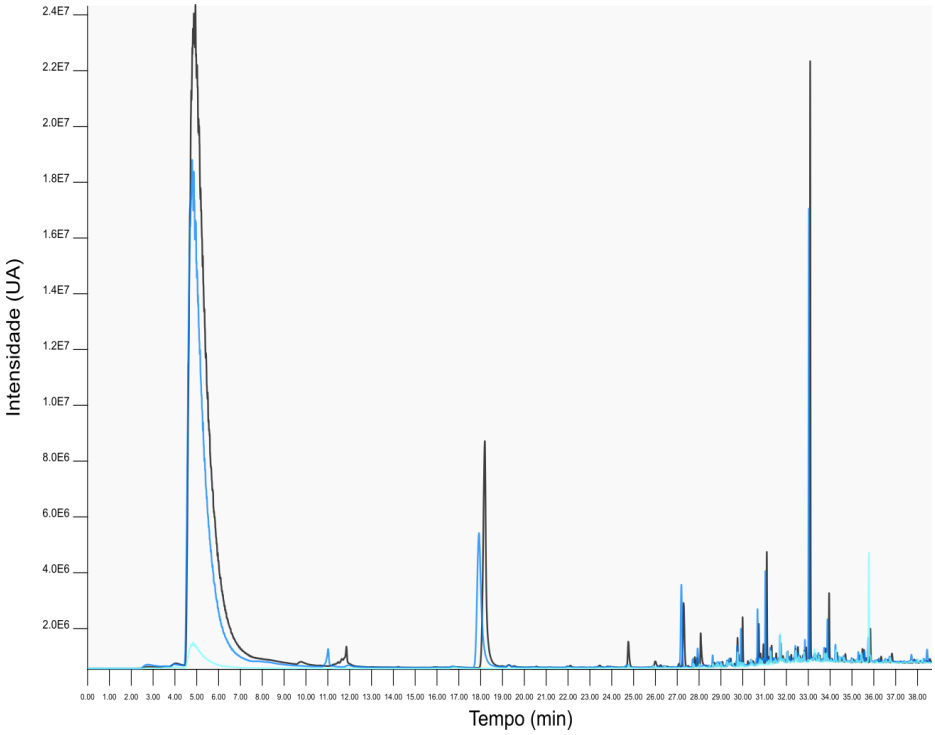

Figure 1 – Chromatogram regarding lysine. In dark blue we have the fermentation with de amino acid; in black we have the biotic control group without the addition of the amino acid; in light blue we have the abiotic control group with the addition of the amino acid.

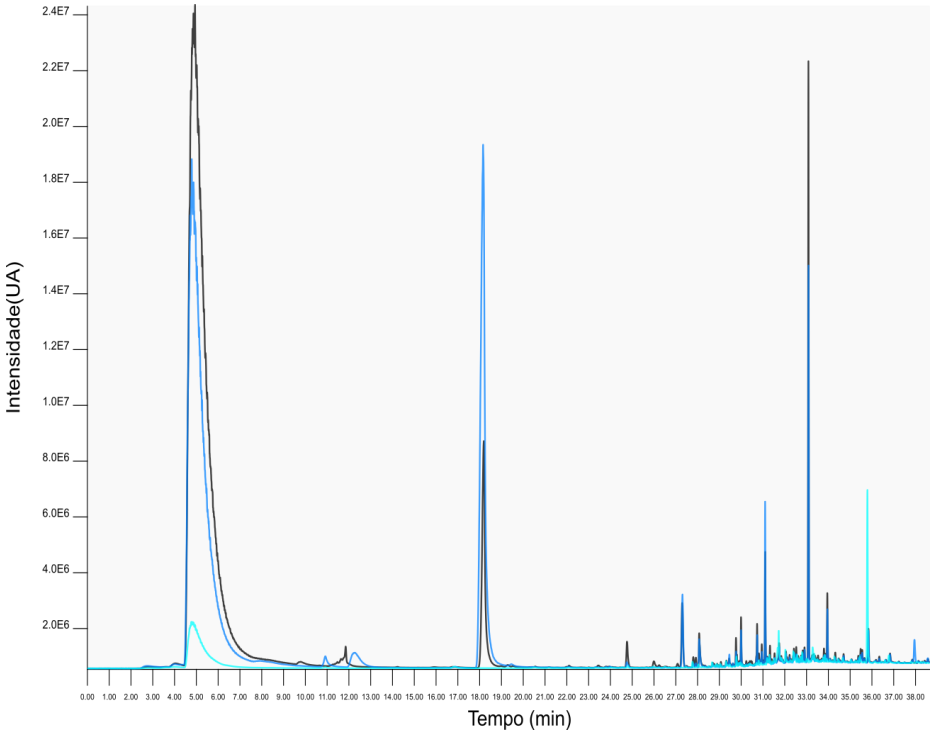

Figure 2 – Chromatogram regarding leucine. In dark blue we have the fermentation with de amino acid; in black we have the biotic control group without the addition of the amino acid; in light blue we have the abiotic control group with the addition of the amino acid.

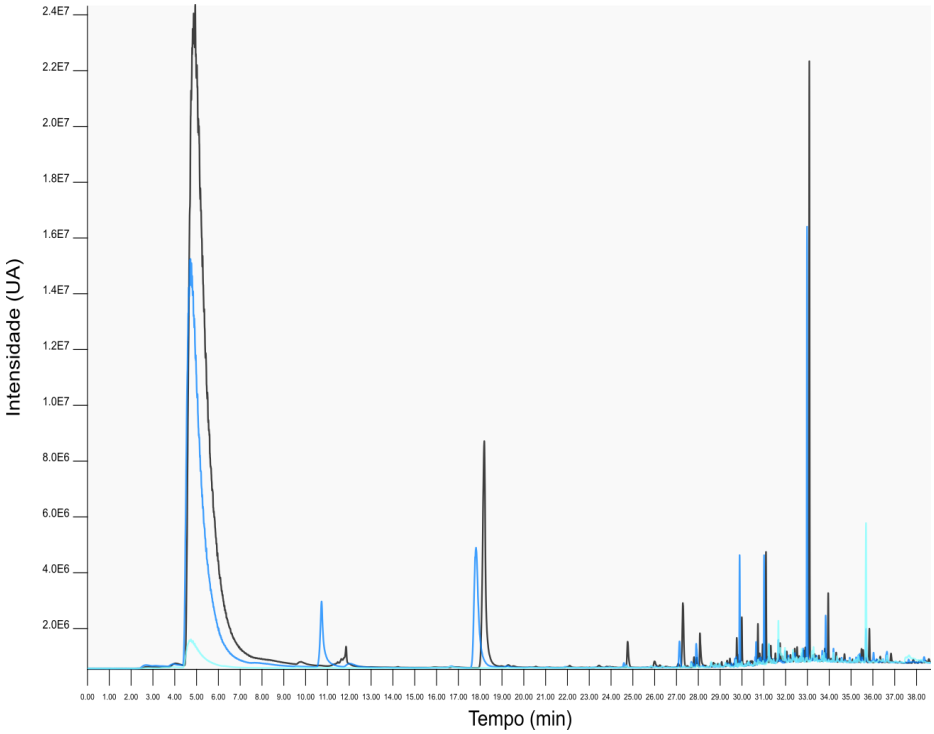

Figure 3 – Chromatogram regarding valine. In dark blue we have the fermentation with de amino acid; in black we have the biotic control group without the addition of the amino acid; in light blue we have the abiotic control group with the addition of the amino acid.

As we can see, with the addition of different amino acids we can observe the synthesis of different compounds. The biosynthetic pathway responsible to produce higher alcohols and esters is well known and is named the Ehrlich Pathway[1,9]. In it, we see amino acids suffering transamination into alpha-ketoacids which undergo decarboxylation into a fusel aldehyde that is reduced into a higher alcohol. That higher alcohol reacts with an acetyl-CoA resulting in an ester[10]. Our results are in conformity with the existing literature in the sense that the supplementation had an effect in the aromas formed. It is visible in the chromatograms that we have a diversity of new compounds that arose from the supplementation. One example is the fermentation supplemented with Leucine. In it we can see an increased peak around the 18-minute mark. We believe that this compound is isoamyl alcohol. This higher alcohol is responsible for a pungent smell, but, more importantly, it can act as the precursor for the synthesis of isoamyl acetate, which is a very desirable aroma in spirits such as cachaça, for example[9]. We also see a trend in the production of ethanol (around the 5-minute mark), that chemical is reduced in the amino acid supplemented fermentations. That is sound when it comes to the biochemistry, seeing as pyruvate is deviated from ethanol production to create higher alcohols and esters in the Ehrlich Pathway, which deeply impacts the final flavor and is very desirable from an organoleptic point of view.

4 CONCLUSION

In conclusion, we can observe that the profile of each fermentation is very different from each other and from each respective control groups, pointing towards the idea that different compounds are being formed in different quantities depending on the amino acid added. We can attest this by looking at the peaks and areas of the chromatograms generated. These partial results are very promising in the sense that we can already see that a minor and cheap change in the fermentation wort can heavily influence the production of volatiles compounds, which is very useful from an industrial standpoint. These results warrant future analyzes, which will be carried out in a later date.

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