Targeted analysis of intra and extracellular media of three winemaking strain of yeast

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1- Introduction

For the yeasts, tryptophan is a nitrogen source that together with other compounds constitutes the yeast assimilable nitrogen. In fact, the nitrogen content exerts a regulation effect both in rate and in its end. Lack of nitrogen has been pointed as one of the main reasons of sluggish and sluggish fermentations [1].

Pathways related to aromatic amino acids (tryptophan, tyrosine and phenylalanine) are of particular interest, some compounds implicated in these pathways are known to contribute to quality, flavor and aroma of wine and other fermented foods. For this reason the amounts of metabolites in a cells or produced by them represents integrative information about cellular function and therefore, link phenotype of a cell in response of environmental changes. Moreover, sample preparation is a crucial step to guarantee the integrity of the metabolites present in the samples. Due to, the metabolite concentrations reflect the last reaction of yeast system to a genetic or environmental change. For this reason, in the present work it has decided to improve an intracellular extraction method at low temperature to preserve the metabolites levels and avoid changes.

The goal of this work was to explore the yeast pathways related to tryptophan and the production of compounds that could affect wine quality. In the context to elucidate deeper the evolution of the metabolites involved in these pathways, an UPLC-ESI-MS/MS method was developed for the analysis of 38 compounds related to tryptophan. The method was used to analyse the extra and intra cellular extracts produced by the fermentation of three strain of yeast (RED FRUIT, QA03 and Torilpura dellaTri). Each fermentation was repeated six times, and sampling was performed after 2, 5, and 15 days. The kinetics of 23 compounds for the extracellular and 18 for the intracellular samples provided known and novel information, helpful to understand better the metabolism of the three studied yeasts.

2-Materials and Methods

The samples obtained as shows the Figure 1, were subject to cold glycerol quenching [2], and then to a low temperature extraction with cold methanol [2], replacing a freezing/thaw cycle by 1 min of sonication in a bath of ice. Before analysis with Xevo TQMS System (Waters, UK) mass spectrometer, the samples were clean up with SPE as shows Figure 3.

3- Results

The Figure 4 shows the PCA of extracellular and intracellular samples separated by sampling days. Phenylpyruvic (Ph) acid and tryptophol (TOL) are two compounds whose, exert the higher influence over the separation of intracellular from extracellular samples, due to their contribution in the loading between factors and variables (Figure 5). TOL is the compound which exerts the major influence over the difference of the intracellular samples. Ph-Py has the highest weight over the extracellular samples.

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