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Yeast strain and nitrogen supplementation: Dynamics of volatile ester production in Chardonnay juice fermentations

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• This article covers two experiments. In the first experiment, the researchers studied how ester production varied across a number of strains of *Saccharomyces cerevisiae*. In the second experiment, they studied how supplementing the juice with nitrogen affected ester production.

• **Esters** –formed by the action of yeast during alcoholic fermentation- are the product of the condensation of an *acid* and an *alcohol*. They derive their names from these two components, mentioning the alcohol first, then the acid (Ex. ethyl acetate is the condensation of *ethanol* and *acetic acid*). When the acid involved is a fatty acid, the resulting esters are called *fatty acid esters*.

• The authors tracked 7 **aroma compounds** –all esters- belonging to two categories: 1) *acetate esters* (ethyl acetate, isoamyl acetate, hexyl acetate), and 2) fatty acid ethyl esters (ethyl butyrate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate). The **strains** tested were six commercial *S. cerevisiae* strains and one *S. bayanus* strain (more correctly identified as *S. cerevisiae* var. *bayanus*). Their actual names are M1, Simi White, T 306, CY 3079, DV-10, ICV 254 D, and Uvaferm CEG. The **sources of nitrogen** tested were diammonium phosphate, or DAP, (added at 170 mg nitrogen/L) and a mix of amino acids added at 170 mg N/L, in the relative proportions naturally appearing in juice.

Acetate esters	Fatty acid ethyl esters
(C2) Ethyl acetate	Ethyl butyrate (C4)
(C3) Isoamyl acetate	Ethyl hexanoate (C6)
(C6) Hexyl acetate	Ethyl octanoate (C8)
	Ethyl decanoate (C10)

Number of carbons in either alcohol or acid indicated in parenthesis

• The authors measured the production of esters at different times during the fermentations with the help of two analytical techniques: *gas chromatography with flame ionization detection*, and *solid-phase microextraction* (or SPME). These techniques allowed the authors to identify: 1) which strains tended to accumulate esters most quickly (*accumulation rate*), 2) the concentration of esters at which the rate of accumulation dramatically slowed or stopped (*maximum concentration*), 3) the time during fermentation when most of the production took place (early or late in the fermentation), and 4) how much of each ester existed in the finished wine (*final concentration*).

• Please note that, as the authors emphasize, accumulation rate, maximum concentration, and final concentration, are not simply a function of ester formation, but also reflect degradation (hydrosysis + volatilization). In addition, a yeast may produce enormous amounts of an ester at the beginning, but completely stop its production towards the end. For this reason, we will sometimes have to refer to these parameters separately, even though "**final concentration**" is obviously especially important as it will determine the contribution of that ester to the aroma of the wine at the end of fermentation.

• **Results Experiment 1: Effect of different strains on ester formation.**
i- ***Acetate esters*****.** 1) The rates of formation and maximum concentrations of acetate esters varied 2- to 3-fold across strains. M1 had some of the highest values while Simi White, CY 3079 and T 306 had the lowest. 2) The maximum acetate ester concentrations were reached at the midpoint of fermentation, when CO_2 production (weight loss) was highest. 3) Of the three acetate esters studied, only isoamyl acetate was above its aroma-perception threshold at the end of the fermentations.

• **ii-** ***Fatty acid ethyl esters***. 4) In general, M1 and 254 D tended to produce some of the highest final concentrations of fatty acid ethyl esters, and Simi White produced some of the lowest. (One exception to the above was the higher molecular weight ester, ethyl decanoate, which was very low in M1 fermentations.)

• **Results Experiment 2: Effect of different nitrogen supplementations on ester formation.**
The authors selected 3 of the strains above to study the effect of nitrogen supplementation: a high-, a moderate-, and a low-ester producer (M1, 254 D, and Simi White, respectively). The Chardonnay juice used in the study had total assimilable nitrogen levels within those recommended for optimal fermentation (200 to 500 mg/L). Now, let's see the results of Experiment 2. 1) Of the 3 strains tested, only 254 D showed an effect of nitrogen addition on ester formation, therefore, the following results focus on this strain. 2) The fermentation with added DAP ("+DAP") had a shorter initial lag phase and finished fermentation approximately 2 days earlier than the fermentation with added amino acids ("+AA) or the fermentation with no additions (control).

• **i-** ***Acetate esters.*** 3) In general, the +DAP treatment showed the highest final concentrations of acetate esters, and the +AA treatment the lowest. (Hexyl acetate was the exception this time, which showed the same final levels in all treatments - +DAP, +AA, and control.)

• **ii-** ***Fatty acid ethyl esters***. 4) Once again, and in general, the +DAP showed the highest final concentrations of fatty acid ethyl esters, and the +AA treatment the lowest. (The two highest molecular weight esters –ethyl octanoate and ethyl decanoate- were an exception. Ethyl octanoate showed no significant differences across treatments. And ethyl decanoate was highest for the control.) 7) At the end of the fermentation, all of the esters studied –both acetate esters and fatty acid ethyl esters- were above the aroma threshold with the exception of ethyl acetate, hexyl acetate and ethyl decanoate.

• In summary, the authors found significant differences in ester formation depending on nitrogen source and yeast strain. Strains M1 and 254 D tended to form higher ester concentrations, and strain Simi White tended to form lower ester concentrations. Addition of nitrogen as DAP tended to produce higher ester concentrations than addition as an amino-acid mix – but only for fermentations conducted with 254D and using a juice that was not nitrogen deficient. The authors were unable to find a correlation between the rate of ester accumulation and final ester concentrations; or between maximum ester concentration and final ester concentrations. Even though more work is needed, winemakers may be able to optimize ester formation by varying yeast strain and nitrogen source.

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