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Reduction of acetic acid in grape must and wine by using microbiological-technical processes

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Initial situation

Acetic acid is perceived as an off-flavour already from 0.5 g/L or 0.6 g/L in white and red wines. Below the sensory threshold, acetic acid is perceived as disturbing and can also mask the varietal aroma of a wine. The formation of ethyl acetate, which is particularly noticeable from a sensory point of view and is often described as a solvent note, is to be regarded as a direct consequential damage due to its formation in the wine from acetic acid. The acetic acid problem for the wine producer therefore already plays out well below the legal limits for acetic acid of 1.08 g/L and 1.2 g/L in white and red wines respectively.
In the winemaking process, acetic acid is already formed in the grapes, primarily by acetic acid bacteria of the Acetobacter and Gluconobacter genera. These are often found as secondary infections in damaged grapes. In recent years, the strong occurrence of the cherry

vinegar fly Drosophila suzukii in particular has led to severe phytosanitary problems and predicts a tense situation for German winegrowers in the coming years.
Membrane-supported methods of reducing acetic acid in affected wines have been tested for several years, but with only moderate success. However, an approval within the EU is not in sight and, moreover, would not be legal for the treatment of wines above the limit values. Moreover, this method is technically unsuitable for treating must. There are approaches to break down the acetic acid present in the must by yeasts already during fermentation. Individual experiments have shown that suitable wine yeasts can in principle break down acetic acid under certain fermentation conditions. However, there was still no practical suitability because the complex factors leading to stable acetic acid degradation were largely unknown. The decisive factor seems to be above all the sugar content during fermentation, which within a certain framework stimulates the wine yeasts to use acetic acid as an alternative energy source. The possibilities of this stimulation are also strongly dependent on the genetic make-up of the respective wine yeast strain and have only been partially researched.
With the so-called fed-batch process, which has been used successfully for decades in biotechnology, for example, a process-technological approach exists for the targeted fermentation of wine must under strict control of desired target parameters.
This technology was to be used in the research project in order to create stable framework conditions for active acetic acid reduction by yeasts, and to offer practical wineries a reliable way of avoiding problematic acetic acid levels in the end product already during fermentation and before the wine stage.

The aim of the research project was to ensure an analytically and sensory relevant reduction of the acetic acid content in must and wine by developing a modified fermentation management. Innovative microbiological-technical strategies such as fed-batch fermentation were to be used for this purpose. The properties of certain wine yeasts to break down acetic acid should be researched and made usable for winemaking. The elucidation of molecular biological principles of the acetic acid metabolism of yeasts should also create a rapid screening option for yeast strains with regard to their acetic acid degradation capacity. In a complementary technological strategy, these molecular biological findings should be implemented in the modification of classical fermentations and the fed-batch fermentation process.

Findings

The threshold determination showed that the threshold values for ethyl acetate were variety-independent, while significant variety-dependent differences were determined for acetic acid. The screening of 31 *S.cerevisiae* strains revealed a broad spectrum ranging from strains with tendencies to form to degradation of acetic acid. However, the results that stood out positively in this context when the yeast strain DV10 was used in 2017 proved not to be reproducible in later years, as did other strains that had previously stood out positively. The potential candidates were tested in the meantime in the further series of trials. Experiments on the refermentation of wines with elevated acetic acid levels showed that this did not lead to the desired degradation without pre-treatment of the yeast fermentation starter, so possible manipulations of acetic acid metabolism were explored in further experiments. In the meantime, molecular biological characterisation had shown on the one hand that there is a direct correlation between acetic acid degradation and the expression of the *ACS1*, *ACS2* and *FPS* genes involved in acetic acid metabolism. On the other hand, it could be shown that in the absence of sugars, the *ACS1*, *ACS2* and *FPS* genes are only overexpressed 10-14 days after yeast addition and that the overexpression of *ACS1* is many times higher than *ACS2*. Therefore, degradation experiments were carried out with sugar-free pre-cultured *S. cerevisiae*. However, this procedure alone did not stimulate acetic acid degradation as desired. Other parameters such as pH value, pantothenic acid supply and inoculation dosage did not lead to activation of the acetic acid metabolism of the yeast cells either. High inoculation doses of 250 g/hl even led to an additional formation of acetic acid.

In a non-saccharomyces screening, *Metschnikowia pulcherrima* and to a lesser extent *Candida guillermondii* and *Candida zeytanoides* were identified as potential strains for acetic acid degradation. These can serve as a starting point for future research on acetic acid management and can already be incorporated into cellar management advice. In the context of molecular biological characterisation, a method for stabilising biological samples was also developed, which enables the exchange of fermentation samples in future research projects between research centres or cooperation partners.

On the cellar technological side, the fed-batch process also allowed traditional *Saccharomyces cerevisiae* yeasts to degrade large amounts of acetic acid, regardless of whether acetic acid was already present in the must or added during fermentation. Under favorable conditions, degradation reached over 80% at acetic acid concentrations of 1-1.2 g/l. Nevertheless, the results also demonstrate that the potential of the fed-batch process can only be effectively realized with an adequate supply of nutrients. The addition of thiamine was essential to avoid significant pyruvic acid (pyruvate) formation in the presence of high acetic acid concentrations. The relevance of other vitamins and cations requires further

study. Fed-batch fermentations were also more effective in reducing acetic acid when applied on a larger scale (20 l containers) compared to batch fermentations. It was shown that due to the greater system complexity of fed-batch processes, higher demands must be placed on system robustness. This further development can form the basis for appropriate plant engineering in the context of integrated acetic acid management, from which numerous wineries will benefit in the long term.

Economic importance

In Germany, a total of 7.7 million hl of wine were produced in 2019, 63 % of which was white wine. The German wine industry, which includes 18,700 wineries, consists exclusively of small and medium-sized enterprises that do not have their own research resources. Therefore, the acetic acid problem could not be tackled strategically so far, and reductions in quality due to acetic and ethyl acetate sting up to the loss of marketability had to be accepted. High acetic acid contents (~0.6-0.7 g/L) even below the legal thresholds quickly lead to delisting or rejection by buying commission agents in view of the fierce competition in the No. 1 wine importing country. The losses can be estimated at 2-5 million € p.a. The results of the research have created a basis for recommending practices to wineries in the context of cellar management advice that contribute to avoiding and reducing high acetic acid levels. In addition to the wine industry itself, plant manufacturers in beverage technology as well as manufacturers of process analytical technologies can profit directly from the research results by opening up or expanding new fields of activity. Manufacturers of pure yeasts can also react to the requirements of wine producers and develop or market corresponding products, especially due to the rapid selection possibilities using molecular biological markers.

Publications (Selection)

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2020 Salvadé, Jaccard, Pernet and Mira de Orduña (2020) Real-time quantification of sugars and ethanol during wine fermentation using an innovative miniature spectral analyser Journal of Wine Research

2021 Scharfenberger-Schmeer and Mira de Orduña (2021) Acetic acid metabolism of oenological Saccharomyces and non-Saccharomyces yeast in model grape must at various initial sugar and acetic acid concentrations OENO One (in progress)

Further Information

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Förderhinweis

…ein Projekt der Industriellen Gemeinschaftsforschung (IGF)

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