Climate change and its impact on yeast nutrition

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Introduction
Climate change-related weather extremes lead to nitrogen deficiency during grape ripening and thus exacerbate an unfavourable nutrient situation during fermentation. This is best countered by using nutrients to increase yeast fitness. A trend towards direct inoculation without rehydration has, on the other hand, become established.

The decision to avoid additional yeast activation or nutrition does not depend solely on the yeast's characteristics, such as fermentation strength, fructophilia and alcohol tolerance; it also depends on a variety of accompanying circumstances. Variable weather conditions and fungicides place additional pressure on wine yeasts. A natural active ingredient in the yeasts, glutathione, reduces the contaminated must's damaging influence.

This article addresses difficult fermentation conditions, fining agents for clarification, the use of rehydration nutrients containing glutathione as a helpful component in the detoxification of wine yeasts, as well as various approaches to remedy stuck fermentation. The focus here is on activation of yeasts through the simultaneous use of rehydration nutrients, especially glutathione, as a component in the VitaDrive® ProArom activator, as well as fining agent CarboTec, which can help to stabilise the wine's fermentation and aroma despite unfavourable conditions.

Nutrient situation of musts
Amino acids form increasingly as the grapes ripen. However, there is also an increase in proline, which the yeast cannot metabolize under fermentation conditions. In addition to the grape variety, the must's content also depends on the climatic conditions, the type of rootstock used, the soil and how it is worked. The water balance and

fertilization, as well as climate-related infection pressure, also play an important role. The yeast absorbs and metabolises amino acids differently during fermentation. Yeasts are quite selective and prefer certain amino acids (Crépin et al., 2012). During development of Vita*Drive* ProArom, care was taken to increase the availability of these amino acids (see Fig. 1, Group A).
Excessive amounts of ammonium should not be present, however, during rehydration. Since nitrogen compounds (ammonium and amino acids) are necessary for yeast growth, the must can be supplied with DAHP and thiamine, in addition to the rehydration nutrient, until the end of the 2nd fermentation period. In the case of difficult vintages, it is advisable to provide support with complex nutrients, such as Vitaferm® Ultra F3, in order to ensure successful fermentation.

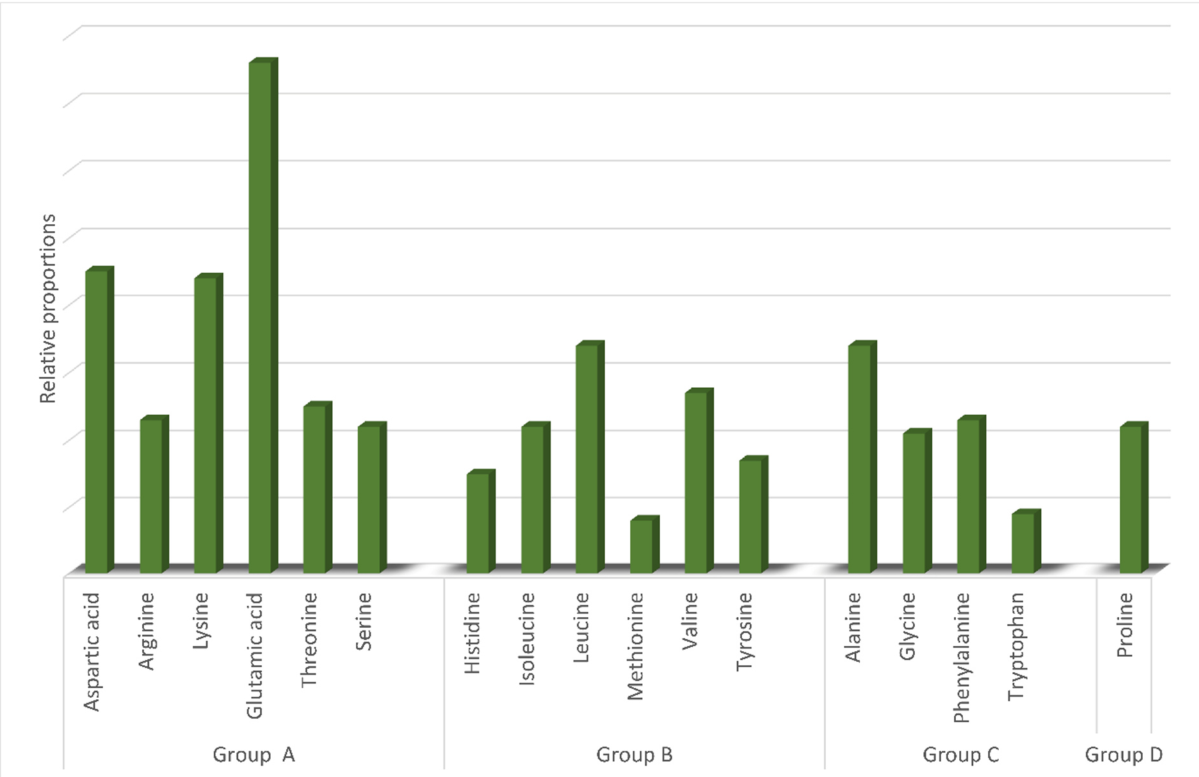

Figure 1: Relative amino acid proportions in Vita*Drive*® ProArom. Yeasts generally prefer nitrogen sources in the following order: Group A > ammonium salts > Group B > Group C > Group D.

Fungal infections and spray residues damage yeast activity and change the aroma of wine
Fungal infections in grapes alter the must's amino acid spectrum. The ratio of glucose to fructose also shifts unfavourably, reducing vitamins and increasing toxin content. The more the harvested crop is burdened, the more difficult it is for the yeast to ferment. The pesticides used to treat the fungus also affect fermentation if residues remain in the must. With a correspondingly long delay, this is of no concern for subsequent fermentation, despite the compounds' chemical diversity. In the case of high fungal pressure, as well as climatically induced different ripening stages on a vine, spray residues can still get into the must, making life difficult for the yeast. Not only the fermentation delay, or stucking alone, is problematic, but also the increased formation of isoamyl and ethyl acetate, which affect the wine's sensory quality (García et al., 2004).

Yeasts react very sensitively to heavy metal ions, especially copper, after consumption of the ammonium supply during fermentation. They can ferment well with copper poisoning, as long as sufficient glutathione, magnesium and zinc are present. Problems occur more frequently with the final fermentation, however, during the last third of the fermentation period. Yeasts with a high ammonium requirement are therefore particularly sensitive (Fröhlich, 2012).

Direct inoculation versus rehydration

Various series of experiments on direct inoculation have shown that it is possible to dispense with rehydration without fermentation delays (Schmidt, 2013). One reason for this is the use of strongly fermenting active dry yeasts. The yeasts are supported by nutrition concepts such as F3, which are applied during production of the yeasts.

But even powerful yeasts can suffer when it comes to nutrient, vitamin or mineral deficiencies. These can best be eliminated during rehydration. This is all the more important if yeasts have been damaged by spray residues.

The standard recommendation is to rehydrate with lukewarm water or must/water mixtures (Mahmud et al., 2010; Singer and Lindquist, 1998). Yeasts become metabolically active faster through rehydration at higher temperatures (< 42°C). The trehalose required for drying, which was produced to protect important enzymes, can now be broken down by the trehalase enzyme. The entire enzyme apparatus is then stabilised with the help of so-called heat shock proteins, and the yeast can start fermenting faster.

The positive effect of glutathione on yeast

An influence on the yeasts cannot be excluded, even if the majority of spray residues from the must can be removed with fining agents (CarboTec). The tripeptide glutathione is used worldwide in the wine industry to protect wine yeasts and aromas. However, the natural molecule has many more functions within the cell than in the must. In addition to its reductive character and its function as a reserve, the yeast uses glutathione primarily to bind toxins such as heavy metals or pesticide residues. The pollutants are enzymatically bound to it and first transported to the central vacuole of the yeast for further excretion. Yeasts with a good glutathione supply show a high vitality (Sharma et al., 2003). Nevertheless, dosages of glutathione-containing products applied during fermentation and not for rehydration, often lead to sulphur off-flavors.

First steps when the yeast becomes weak

If the yeasts are stressed, fermentation does not progress optimally and there are often fermentation delays or fermentation even stops. As a general rule, the glucose/fructose ratio shifts in favour of fructose. Under these conditions, any remaining fructose is metabolized very slowly by yeasts and, depending on the sugar and alcohol content, the wine can be spoiled by the accompanying microbiota. If simple techniques, such as stirring the yeast and adding nutrients no longer help, the old yeast must often be racked off at this stage of fermentation and the still sweet young wine should be prepared for final fermentation. If the medium-chain fatty acids formed under stress are not removed, they effectively prevent the start of fermentation by fresh yeast. Yeast cell wall preparations, such as PuroCell® O, help to bind these fatty acids and supply the yeasts with essential nutrients (see restart protocol at www.erbsloeh.com).

Choosing the right yeast for the start of fermentation

The yeasts described under the oenological term "*Bayanus*" are particularly characterised by low nutrient requirements, strong fermentation activity and high alcohol tolerance. They are the means of choice when fermentation is to be completely finished after restart. Some yeasts, known as fructophilic, have a special transporter equipment, compared to other yeasts, for absorption of the various sugars. Right from the start, yeasts prefer to absorb glucose. The excess fructose is used again in the course of further fermentation. This is achieved by activating various transporters during fermentation. These vary in the different affinities and repressions for the both sugar types. *Bayanus* yeasts have a special ace up their sleeve compared to normal wine yeasts because they have an additional transport system for fructose. The symporter known as FSY1 becomes active at low fructose contents and in the presence of alcohol. i.e. under conditions immediately before the end of fermentation.

In Fig. 2, different fermentation yeasts were examined for the presence of the gene for FSY1. The osmophilic, alcohol-tolerant and fructose-tolerant Oenoferm® X-treme F3 in particular is equipped with this transporter.

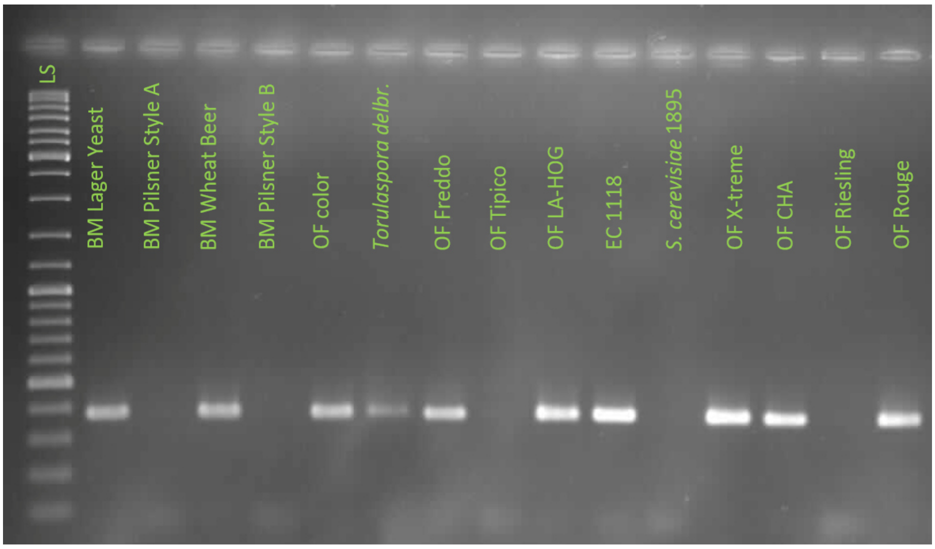

Figure 2: Electrophoretic analysis (agarose gel after ethidium bromide staining under UV light with 256 nm): analysis of a gene fragment of FSY1 in different wine yeasts. Fermenting yeasts, such as Oenoferm® X-treme F3 or Oenoferm® CHA F3 in particular, show the presence of the gene.

Adaptation to fructose

Knowledge of adapted use of yeast's different transporters can be used to remedy stuck fermentation. If yeasts are rehydrated under aerobic conditions and propagated for four hours under stirring, it is possible to demonstrate the effect of sugars fructose (variant A) and sucrose (variant B) on the use of transporters. After four hours of exposure to oxygen, the yeasts have completely metabolized the sugars (see Tab. 1). The yeasts increased in this way were now added to a wine that had stopped fermenting (cf. Fig.3; total sugar: 9.1 g/L, fructose: 6.7 g/L, glucose: 2.5 g/L). A comparison of the fermentation processes shows that the yeast adapted to fructose (variant A) fermented the wine to 3 g/L residual sugar within 18 days, despite the low sugar content. The control (variant B) with sucrose stopped at 6.1 g/L.

Table 1: Treatment variations for stuck fermentation. Adaptation of wine yeast Oenoferm® X-treme F3 to different sugars.

Variants	Treatment
Variant A	Rehydration of 35 g/hL OF X-treme F3 + Vita*Drive* F3 in 100 g/L **fructose** solution (25 min, 37 °C). Then propagate aerobically for 4 h while stirring.
Variant B	Rehydration of 35 g/hL OF X-treme F3 + Vita*Drive* F3 in 100 g/L **sucrose** solution (25 min, 37 °C). Then propagate aerobically for 4 h while stirring.

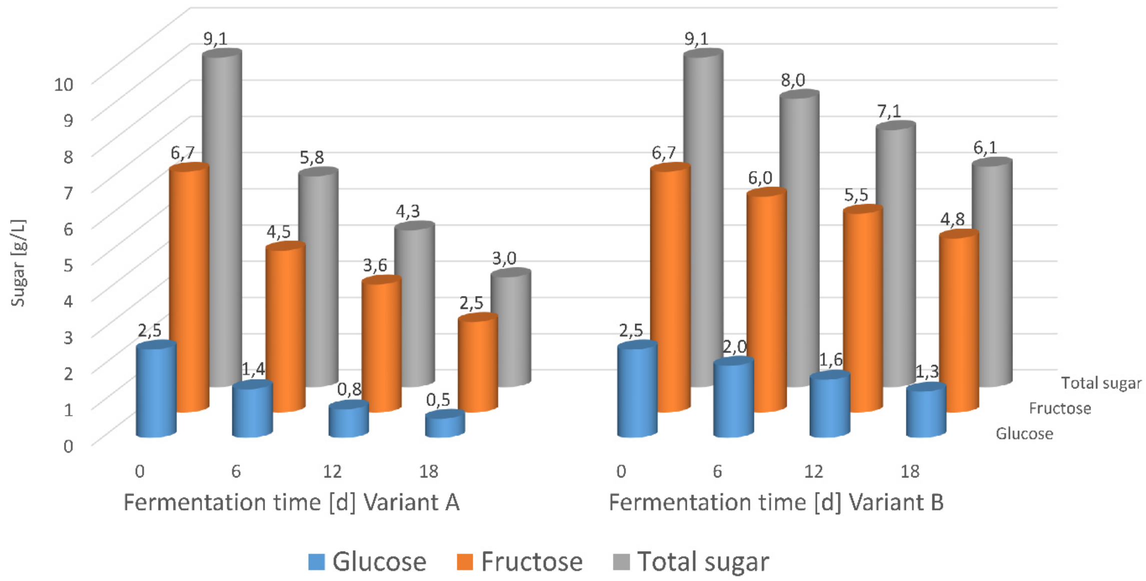

Figure 3: A stuck fermentation (Grenache Noir 2018; 15.3% vol.) was treated using two methods (see Tab. 1). The comparison shows the positive effect of adaptation of yeast to fructose.

Conclusion

Conventional agents such as DAHP and thiamine are often no longer sufficient in order to help the yeasts through climate-induced fermentation problems. With activators such as VitaDrive® ProArom, fermentation stagnation can be avoided by significantly reducing the harmful effects of toxins, pesticides and heavy metals during activation of the yeast. This is best achieved in combination with thermal rehydration. Currently, for the coming harvest, increased spray residues must be expected, which can best be reduced by prophylactic fining with CarboTec. If, however, stuck fermentation also has to be eliminated, a Bayanus yeast with a high fermentation capacity should be chosen. Adaptation to fructose is also feasible, in order to keep the fermentation time as short as possible.

Acknowledgement

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