Enzymatic protein stabilisation

By Michael Sobe and Sebastian Bausinger

Protein precipitation is a major problem, mainly in white and rosé wines. This is caused by the aggregation of proteins. In the process the proteins dissolved in the wine group together to form precipitates or floccules. Protein aggregation is affected by changing conditions, such as the pH value, storage temperature and phenolic compounds (Claus et al. 2014).

Since the International Organisation of Wine and Wine (OIV) approved aspergillopepsin I for winemaking, for the first time the user has a reliable and reasonably priced enzymatic method of protein stabilisation at their disposal. As a result, use of aspergillopepsin I in combination with heat treatment and subsequent filtration to remove turbidity-forming proteins from white, rosé and sparkling wines has been included in the OIV Codex. Adoption into EU law took place as a result of amendment of Regulation EU 2019/934 on 27 October 2021.

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Enzymatic protein stabilisation

The OIV Resolution describes a process developed at an Australian Wine Research Institute (AWRI) in which an acid protease obtained from Aspergillus niger, aspergillopepsin I, reliably degrades grape proteins during a heating stage (Marangon et al. 2012). In the process aspergillopepsin I acts on thermolabile chitinases and thaumatin-like proteins, which play a decisive role in turbidity formation. The grapes form these proteins as a defence against fungi (Tattersall et al. 2001). The concentration depends on the grape variety, the climatic conditions and the vineyard's pathogen content. Climatic conditions are particularly influential as drought stress and rot cause protein contents to rise (Meier et al. 2016).

Mode of action

As shown in Figure 1, the heating stage at 65-70 °C is required for both optimum enzyme action and for the thermolabile proteins which, in their native state are globular and are therefore resistant to proteolytical degradation, to unfold (Water et al. 1992). In the globular state it is difficult for aspergillopepsin I to reach the interfaces, resulting in only minor proteolytic cleavage. After the proteins develop, the interfaces are exposed and intense proteolytic cleavage takes place. In addition, the high temperature causes the necessary deactivation of the enzyme.

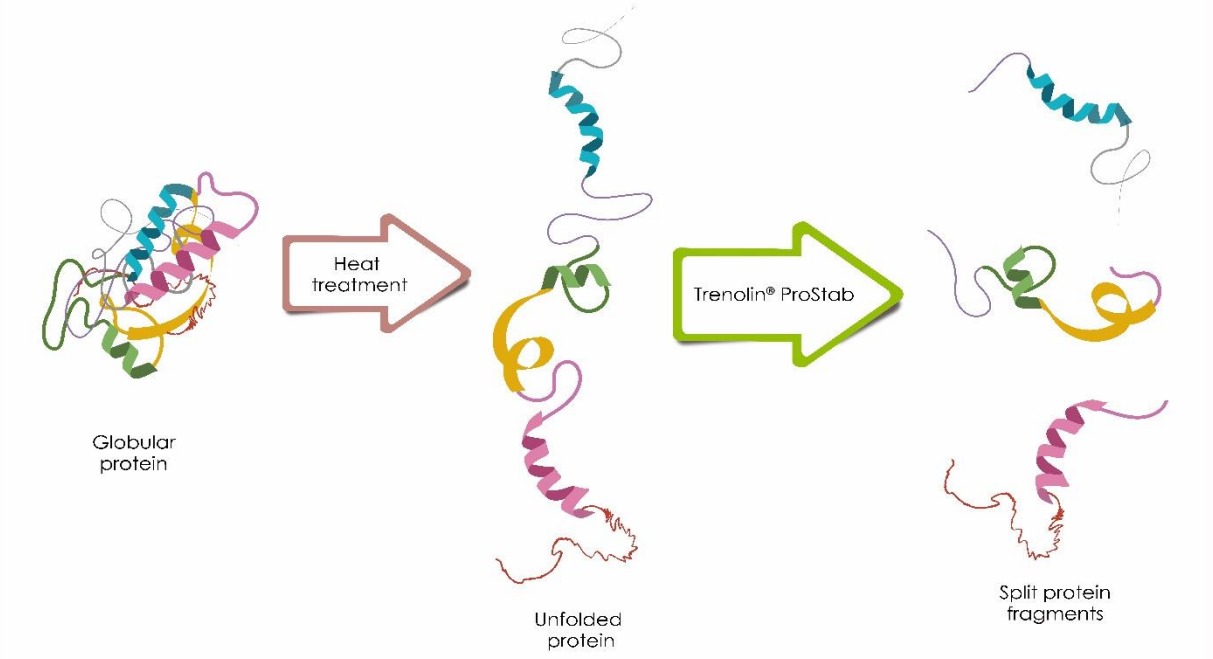

Figure 1. Proteolytic cleavage

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Sensory factors

It has heretofore been assumed that heat treatment of white musts may have a detrimental effect on wine quality. Marangon et al did not, however, find any negative sensory effects upon appropriate energy input by brief heating. Immediate cooling down of the must has a protective effect (Jaeckels et al. 2016). A sensory assessment of the resulting wines revealed no negative effects at all.

Trials

Between 2017 and 2020, Erbslöh Geisenheim GmbH conducted numerous trials using aspergillopepsin I. Figure 2 shows trials from Germany and France conducted using Trenolin® ProStab. The trials compared the protein stability of wines without heat treatment, with heat treatment and heat treatment combined with the addition of enzymes. Temperature and length of heating were adjusted to the winemaking scenario in question. Protein stability was determined by heat test (4h, 85 °C). Wines that exhibit a turbidity of < 2 NTU after the heat test are deemed to be protein stable.

In the case of heat treatment without the addition of enzymes, it was possible to significantly reduce the turbidity-causing proteins in five out of six wines, compared to the control version without heat treatment. It was not possible, however, to achieve total protein stabilisation in any of the wines.

In the case of heat treatment with the addition of enzymes, it was possible to significantly reduce the turbidity-causing proteins compared to the versions without heat treatment and with heat treatment. In four out of six wines it was possible to achieve complete protein stabilisation.

Enzymatic protein stabilisation

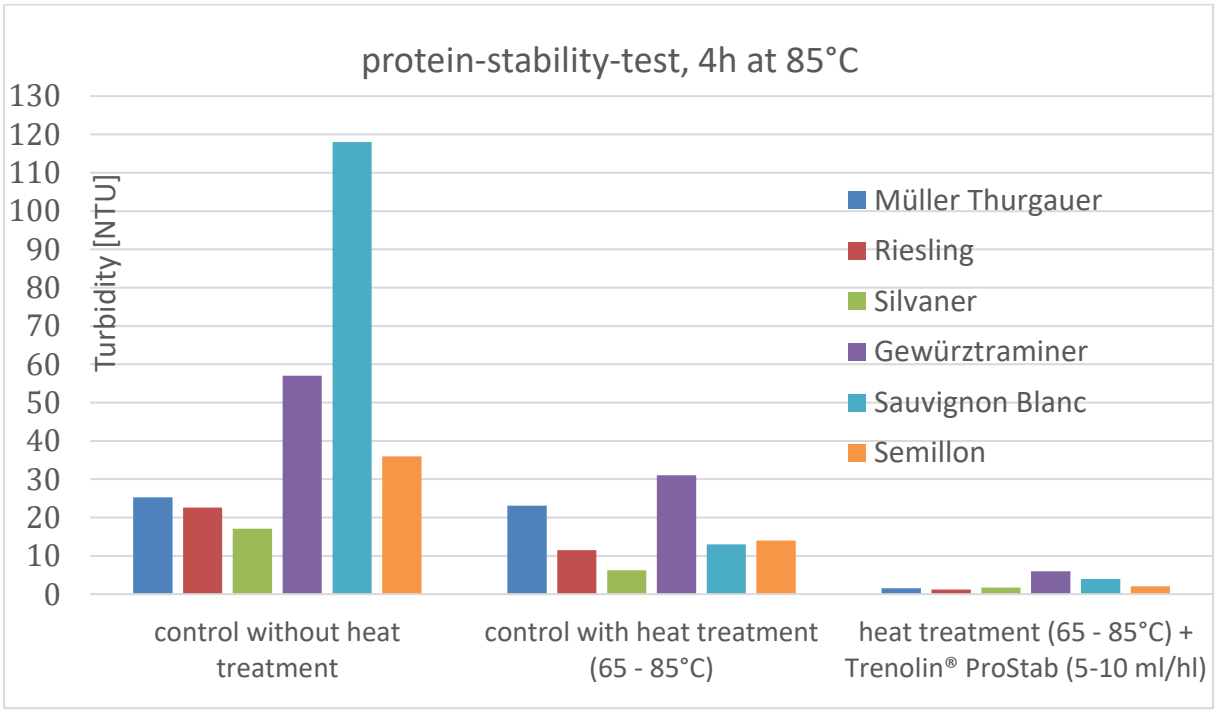

Figure 2. Trenolin® ProStab Trials 2017-2020, Germany and France

Use

Unfolding of thermolabile proteins is reversable, so for this reason aspergillopepsin should be added before heating the musts. The must is heated over a period of 1-2 minutes at a temperature of 65-70 °C. The musts' protein contents are occasionally subject to great fluctuations, so the following parameters must be considered to obtain the correct dosage of aspergillopepsin I: vintage, grape variety protein content and vineyard pathogen loading. The must should be cooled again as quickly as possible after heating.

After use a laboratory heat test to confirm the protein stability achieved in the treated wine is obligatory. If necessary, bentonite can be used as a treatment to achieve complete stability.

Protective colloids, such as CMC or metatartaric acid, not only react with thermolabile, but also other proteins. If these products are used for wine stabilisation, a BentoTEST which also covers all the proteins should also be conducted.

Enzymatic protein stabilisation

Advantages of enzymatic protein stabilisation

- Prevents protein turbidity from forming in wine
- Labour time and cost savings
- No wine losses
- No change in the wine's aroma and colour
- Improved filtration properties
- Reduced foaming
- Liquid formulation is easy to use

Summary:

Aspergillopepsin I offers users a reliable and reasonably priced enzymatic method of protein stabilisation. The energy costs incurred differ from case to case, but can be partly offset by omission of bentonite treatment, savings in work time and greater flexibility in winemaking. For establishments that have the option of heating musts, this produces interesting possibilities with regard to early bottlings.

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