CHR HANSEN

Improving food & health

Frequently Asked Questions and Answers

1. **What are hydroxycinnamic acids?**
   - Hydroxycinnamic acids are organic acids naturally present in grapes and wine.
   - They are predominantly found in the tartaric acid bound form in grapes, must and young wines.

2. **Is the hydroxycinnamic acid concentration dependent on the grape variety?**
   - Yes it is. There are three different hydroxycinnamic acids namely ρ-coumaric acid, ferulic acid and caffeic acid. The ratio and concentration of these depend on the grape variety, growth conditions, acidity of the wine, fermentation profile, maturation time and temperature. Therefore, it is hard to predict the concentration of hydroxycinnamic acids that will end up in the final wine. However, in general ferulic acid tends to be the least abundant.

3. **How do the hydroxycinnamic acids liberate from the tartaric acid bound form?**
   - Hydroxycinnamic acids can be liberated from tartaric acid by chemical hydrolysis of the ester bonds, due to the acid conditions in the wine or they can be liberated by enzymatic hydrolysis *via* cinnamoyl esterase enzyme activity.

4. **Which microorganisms can have active cinnamoyl esterase enzymes?**
   - The research until now has found that some *Oenococcus oeni* and Lactobacillus spp. strains can have cinnamoyl esterase activity during the malolactic fermentation (MLF) in wine. However, no research has yet looked into the cinnamoyl esterase activity of other wine related bacteria such as Pediococcus spp. or Acetobacter spp.
   - No yeast specie has been found to be cinnamoyl esterase positive. Whereas, some fungi have been found to have cinnamoyl esterase activity e.g. Aspergillus spp.

5. **Do hydroxycinnamic acids have a taste, flavour or other direct impact on the palate of the wine?**
   - No, hydroxycinnamic acids do not have and taste, flavour or other direct impact on the palate of the wine.

6. **Do all wines contain hydroxycinnamic acids?**
   - Yes, all wines contain hydroxycinnamic acids to some extent.

7. **Can hydroxycinnamic acids bind/react with other compounds in the wine?**
    - Yes, they can bind to various compounds (anthocyanins, oxygen, Fe(III), ethoxy radicals, ethanol, and glutathione).

8. **Why are hydroxycinnamic acids of interest in relation to winemaking?**
    - Hydroxycinnamic acids can react with certain compounds in the wine, which may have a positive effect on colour stabilisation, when co-pigmenting to the colour compounds (anthocyanins).
    - Hydroxycinnamic acids can have an antioxidant effect (scavenging oxygen).
    - Hydroxycinnamic acids can also be metabolised into volatile phenols by certain Brettanomyces spp. However, this requires a high Brettanomyces ssp. infection of a Brettanomyces ssp. strain that has the specific hydroxycinnamic acid to volatile phenol metabolism. Not all Brettanomyces spp. are able to metabolise hydroxycinnamic acids into volatile phenols.

9. **Can all strains of Brettanomyces spp. metabolise hydroxycinnamic acids into volatile phenols?**
    - No, not all strains of Brettanomyces spp. can metabolise hydroxycinnamic acids into volatile phenols. It is only certain strains that can. Also, it requires 10^5 cfu/ml of *Brettanomyces* before they start this metabolism.
    - The conditions of the wine will affect the metabolism of hydroxycinnamic acids into volatile phenols e.g. the higher the pH the higher potential efficiency of the metabolism.

10. **Can other yeast metabolise hydroxycinnamic acids into volatile phenols?**
    - Yes, other yeast species have the same ability. However, only Brettanomyces* spp. appear to be able to produce high concentrations of volatile phenols under oenological conditions.
    - All *Saccharomyces cerevisiae* wine yeasts have the gene, *PAD1*, which transcribes a phenolic acid decarboxylase that -when expressed in its active form- can convert hydroxycinnamic acids into volatile phenols. However, the enzyme in *Saccharomyces cerevisiae* yeast is not active enough to create a problem in wine.

11. **Which volatile phenols can the hydroxycinnamic acids be metabolised into?**
    - ρ-Coumaric can be de-carboxylated into 4-ethylphenol (4-EP).
    - Ferulic acid can be de-carboxylated into 4-ethylguaiacol (4-EG).
    - Caffeic acid can be de-carboxylated into 4-ethylcatechol (4-EC).

12. **Are there other precursor compounds in wine, which can be metabolised by Brettanomyces ssp. into volatile phenols?**
    - Yes, ethyl coumarate can also be metabolised into 4-ethylphenol (4-EP) by some Brettanomyces spp.

13. **How fast is the chemical hydrolysis of hydroxycinnamic acid from the bound form, during winemaking?**
    - This is hard to say, the chemical hydrolysis process will depend on the pH, temperature etc. of the wine. However, the hydrolysis mainly happens during the maturation and storage of wine.

14. **When is the hydroxycinnamic acids liberated during malolactic fermentation (MLF) when a cinnamoyl esterase positive strain is present?**
    - The latest research indicates that the enzymatic hydrolysis happens at the end of the MLF.

15. **Is this cinnamoyl esterase activity common during spontaneous malolactic fermentation (MLF)?**
    - Yes, according to published literature cinnamoyl esterase activity is quite common during spontaneous MLF. A spontaneous MLF is normally conducted by a large variety of different lactic acid bacteria (Lactobacillus, Pediococcus and Oenococcus ssp.) this diversity largely increases the risk of some of the strains being cinnamoyl esterase positive. Some strains of both Lactobacillus ssp. and Oenococcus ssp. have been identified to be cinnamoyl esterase positive, whereas no research has yet been published on any Pediococcus ssp. strains.

16. **Which malolactic cultures from Chr. Hansen have cinnamoyl esterase activity?**
    - We now classify the strains which have cinnamoyl esterase activity as cinnamoyl esterase positive. In the Viniflora® range, Oenos and CiNe™ are cinnamoyl esterase positive.

17. **Have all the malolactic cultures from Chr. Hansen been tested for cinnamoyl esterase activity?**
    - Yes, the whole Viniflora range has been tested. Therefore, we know that Oenos 2.0, CH11, CH16, CH35 and NoVA™ are cinnamoyl esterase negative, meaning they do not have the cinnamoyl esterase activity.

18. **Are there other commercial cultures for malolactic fermentation which are cinnamoyl esterase positive?**
    - Yes. We have at Chr. Hansen conducted a survey of several commercial malolactic cultures and we have identified other commercial malolactic cultures, not coming from Chr. Hansen, which are cinnamoyl esterase positive.

19. **Is cinnamoyl esterase activity an issue in wines which are not contaminated with Brettanomyces ssp?**
    - No, hydroxycinnamic acids only have a potential negative impact on the sensory profile of a wine if a Brettanomyces ssp.** infection occurs.

20. **Is cinnamoyl esterase a defect in malolactic bacteria?**
    - No, it is not a defect but rather a feature which can potentially be used as a positive in winemaking. Both the anti-oxidant capacity of hydroxycinnamic acids in white wines as well as the potential positive effect on red wine colour needs further investigations.

21. **What is Chr. Hansen's recommendation on using cinnamoyl esterase positive strains (Oenos and CiNe™) and cinnamoyl esterase negative strains (NoVA™, CH11, CH16, CH35, Oenos 2.0)?**
    - With our current knowledge we recommend that Oenos and CiNe™ are used for white, rosé and red wines with no barrel contact – to stabilise colour and protect the wines from oxidation.
    - For wines where there is a risk of Brettanomyces ssp.* infection**, particularly wines which have barrel contact, we recommend using Oenos 2.0, CH16, CH11, CH35, or NoVA™, depending on the other grape / wine parameters, in order to minimise the potential impact when a *Brettanomyces* infection occurs.

*When writing *Brettanomyces* it refers to both Brettanomyces spp. and Dekkera spp. as they are the same genus. *Brettanomyces* and *Dekkera* differ in their reproduction method, *Brettanomyces* is asexual (anamorph) and *Dekkera* is sexual (teleomorph), but this has no practical impact when considering a wine spoilage.

**A *Brettanomyces* infection can be defined as when you start identifying viable cells of *Brettanomyces*. However, the infection will only start having an impact on the sensory profile of the wine when there is approx. 10^5 – 10^6 cfu/mL of Brettanomyces spp. with the metabolism to produce volatile phenols, as the metabolism of volatile phenols from hydroxycinnamic acids is only initiated in the specific *Brettanomyces* yeast, when the cell counts reach 10^5 – 10^6 cfu/mL of *Brettanomyces*.