Improving the implantation of non-*Saccharomyces* yeasts in winemaking by UHPH processing

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Abstract. The use of unconventional yeasts, also called non-*Saccharomyces* yeasts, is a hot topic in current enology due to the improvement that can be produced in the sensory profile during fermentation. However, the use of these yeasts have problems due to difficulties in the implantation and competition with wild *Saccharomyces* yeasts. Ultra-High Pressure Homogenization (UHPH) has demonstrated to be a powerful tool to eliminate microorganisms in grape must, specially yeast and bacteria even at low in-valve temperatures. UHPH can be considered a non-thermal technology with protective effect on sensitive molecules as terpenes and anthocyanins. The preprocessing of must by UHPH leaves it free of yeast with an optimal sensory quality, being this a perfect situation to inoculate non-*Saccharomyces* yeasts. We have fermented UHPH musts and controls with several non-*Saccharomyces* species (*Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, *Hanseniaspora vineae*), reaching in most of the UHPH treatments better implantations of the inoculated yeasts and a complete elimination of wild yeasts. The impact of the UHPH treatment is a better expression of the metabolic and enzymatic activities of the non-*Saccharomyces* yeasts. That effect enhances the sensory quality and facilitates the use of non-*Saccharomyces* yeasts at industrial scale.

1 Introduction

The use of non-*Saccharomyces* is common in wine industry to improve sensory profile, by specific features of non-conventional yeasts in the formation of fermentative esters, the release of varietal bonded aroma like terpenes or thiols, the formation of derived stable pigments or the release of cell wall polysaccharides and mannoproteins [1,2]. Some specific yeast species are now being produced as dry yeasts and extensively used for acidification (*Lachancea thermotolerans*) [3], aromatic expression (*Metschnikowia pulcherrima*, *Torulaspora delbrueckii*, *Hanseniaspora vineae*) [4-6], and volume and roundness (*Torulaspora delbrueckii, Hanseniaspora vineae*). Most of them are weaker than *Saccharomyces* cerevisiae and frequently more sensitive to SO_2, therefore the competence with wild *Saccharomyces* is unsuccessful and they have a lot of implantation problems. If the implantation fails, what often happens, we are obtaining low benefits and wasting money.

Ultrahigh pressure homogenization (UHPH) is a technique that pumps the grape juice at very high pressure normally 300 MPa (potential range 200-600) until depressurization across an especial valve (Fig. 1) [7]. UHPH technology is at industrial level under international Patent EP2409583 by YPSICON TECHNOLOGIES (https://www.ypsicon.com/). UHPH has been approved by OIV RESOLUTION OIV-OENO 594B-2020 (https://www.oiv.int/public/medias/7587/oiv-oeno-594b-2020-en.pdf).

In valve the fluid together with its colloidal particles, microorganisms and enzymes are affected by intense shear efforts and extreme impact strengths producing nanofragmentation to an average size of 400-500 nm [8]. This means the elimination of microorganisms, the inactivation of enzymes and stabilization of the colloidal structure [9,10].

Moreover, the process is very gentle with grape quality because that even when very high temperatures can be reached at valve (80-150 ºC), the very short time <0.2 seconds for the full process avoid the formation of thermal markers as HMF and the degradation of anthocyanins, terpenes and thiols [8,10]. Additionally, the inactivation of PPOs produces wines protected against oxidation and browning and with better antioxidant capacity [8,9].

Figure 1. UHPH valve and nanofragmentation effect (https://www.ypsicon.com/).

The combined antimicrobial and antioxidative enzymes of UHPH makes it a very powerful alternative to SO_2. What is interesting because of the controversial features of this additive.

2 UHPH and new biotechnologies

The total microbial control in musts processed by UHPH, with the removal of wild microorganisms, let us to better implant non-*Saccharomyces* yeasts with positive impact in sensory profile but weak fermentative performance. Furthermore, the inactivation of PPOs by UHPH helps to work without or with very low levels of SO_2 what normally favors growth and development of non-*Saccharomyces* yeasts because many of them have low resistance to sulphites. Currently, the use of non-*Saccharomyces* is a trend in enology because they help to enhance sensory quality and differentiation [2]. Many selection programs are focused in the obtention of optimal strains of non-*Saccharomyces* to improve color, to enhance the fermentative aroma, to release varietal thiols or terpenes, to improve body or structure and many other possibilities [11].

Additionally to non-*Saccharomyces* yeasts, the use of yeast-bacteria coinoculations to produce simultaneous alcoholic and malolactic fermentations, or the use of non-*oenococcus* LAB as *Lactiplantibacillus plantarum* to degrade malic acid in musts are innovative applications that are growing up in current wine biotechnology.

UHPH helps to produce high efficient coinoculations and fermentations with non-*Saccharomyces* yeasts showing more complex metabolic profiles with impact in sensory perception and also enhance the release of varietal aroma by the expression of enzymatic activities.

3 Material and methods

Must has been sterilized by UHPH at 300 MPa reaching 80 °C during the in-valve step. Processing time is <0.2 seconds. Yeast has been inoculated at 7 log CFU/mL. SO_2 was used in some controls at 40 mg/L.

Lactic acid and other metabolites were analyzed by enzymatic analysis or FTIR. Thiols were analyzed by GC-MS.

4 Effect of UHPH in alcohol reduction and acidification with *Lachancea thermotolerans*

Must sterilization by UHPH facilitates the total implantation of *Lachancea thermotolerans* in grape must and an efficient acidification by the production of lactic acid, and as the lactic acid precursor is glucose, a concomitant reduction of alcoholic degree higher than 1% v/v is produced (Fig. 2). In controls with or without SO_2 the lower implantation avoids the expression of the metabolism of *L. thermotolerans* driving the normal metabolic flow of sugars mainly to ethanol.

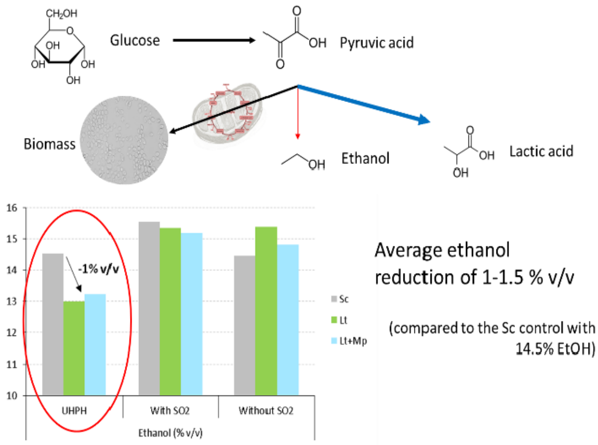

Figure 2. Ethanol reduction in musts processed by UHPH and fermented with high implantation of *L. thermotolerans* by deviation of the glycolytic metabolism towards the production of lactic acid.

Also, it can be observed how the higher alcoholic degree is reached in SO_2 controls because of the removal of the non-*Saccharomyces* and the successful implantation of *Saccharomyces* with high fermentative power. In the controls without SO_2 the effect is less significant for the competition between wild *Saccharomyces* and wild non-*Saccharomyces*.

Concerning lactic acid production in controls with and without SO_2 its formation is marginal because of the low implantation of *L. thermotolerans*, with minor differences with the *Saccharomyces* fermentations and concentrations <0.5 g/L (Fig. 3). However, in UHPH musts the implantation of *L. thermotolerans* is optimal and the production of lactic acid exceeds 5 g/L. And even a synergetic +1.5 g/L can be observed when *Lachancea* and *Metschnikowia pulcherrima* are used in co-fermentation, in agreement with previous research works [12].

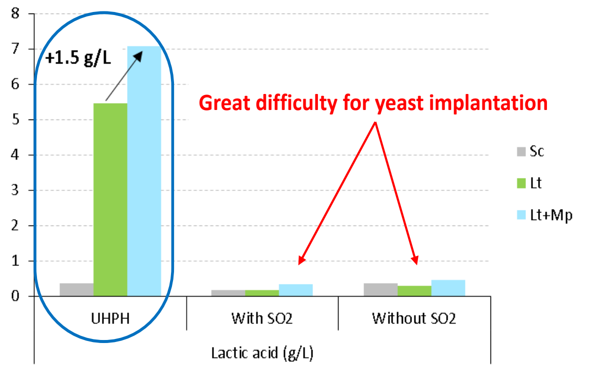

Figure 3. Lactic acid contents in musts processed by UHPH and fermented with high implantation of *L. thermotolerans*.

The intense acidification produced by *L. thermotolerans* in UHPH processed musts have a clear effect in pH reduction and additionally a synergic effect can be observed when it is used in co-fermentation with *M. pulcherrima* (Fig. 4). The pH reduction can be correlated approximately with 0.1 pH units per g/L of lactic acid. Furthermore, can be observed that wines without biological acidification are at pH 4 what means a clear microbiological instability with many possibilities of future microbial spoilage.

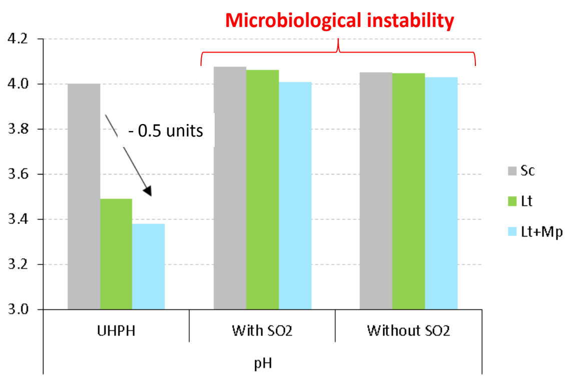

Figure 4. pH effect by the biological acidification with *L. thermotolerans*.

Therefore, the use and high implantation in UHPH processed musts of *Lachancea thermotolerans* is a powerful technology/biotechnology to improve the problems of global warming in wine quality. With the reduction of alcoholic degree, pH control, and the enhancement of fruity aroma increasing wine freshness.

5 Effect of UHPH in the release of thiols using non-*Saccharomyces* yeasts

Musts from Verdejo variety (*Vitis vinifera* L.) that is abundant in thiol precursors sterilized by UHPH were inoculated with several non-*Saccharomyces* (*L. thermotolerans*, *Torulaspora delbrueckii*, *Hanseniaspora vineae* and *M. pulcherrima*) to see the effect of implantation in the aroma profile.

The thiol 3-mercaptohexanol (3MH) is described with smell to rhubarb, passion fruit, gooseberry, guava and citrus and its odor threshold is 0.8 ng/L. The higher levels of 3MH were found in the fermentations by *L. thermotolerans* with concentrations >500 ng/L, what shows the potential of this specie in the revelation of thiol compounds [13]. The other species ranged between 400 and 100 ng/L. The effect of UHPH was quite significant producing for most of the strains except for *H. vineae* increase of the concentration in the range 24-75%.

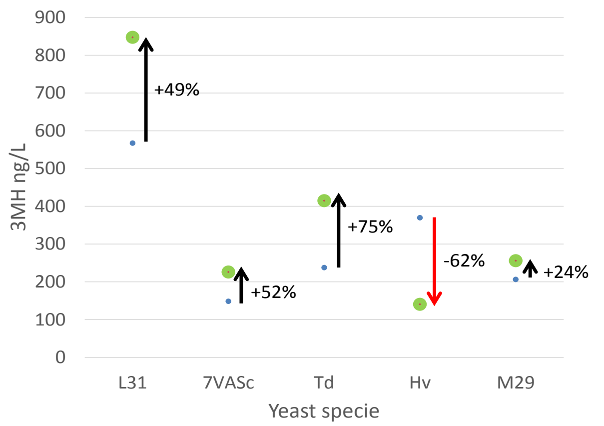

Figure 5. Concentrations of 3MH in wines from musts processed by UHPH (green dots) and controls (blue dots).

Other thiols as 4MMP and 4MMPOH were not detected in most of the samples and concerning the 3MHA the concentrations were quite variable and did not follow a clear pattern.

6 Conclusions

UHPH is a powerful tool to protect wines and to increase the expression of metabolic profiles or enzymatic activities from *non-Saccharomyces* yeasts. Many times, winemakers use expensive cultures of non-*Saccharomyces* yeasts with interesting impact in sensory profile but with low competitiveness with wild yeasts. So, the implantation rate is a very significant indicator of success. It can be clearly observed that must pasteurization by UHPH facilitates the acidification by *L. thermotolerans* and the expression of varietal aromas by several species improving wine quality especially in areas affected by global warming. UHPH opens many possibilities in winemaking.

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