USE OF ULTRA-HIGH PRESSURE HOMOGENIZATION IN WINEMAKING TO CONTROL MICROBIAL POPULATIONS IN GRAPE MUSTS. EFFECTS IN ANALYTICAL AND SENSORY QUALITY

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Introduction

Ultra-High Pressure Homogenization (UHPH) is a high throughput and reliable technique able to sterilize liquid foods (Loira et al, 2018). UHPH can be considered a cold sterilization technique and it is able to work with refrigerated fluids. Currently there are available UHPH devices able to process fluids in continuous mode at a working flow of 10,000l/h with one single HP-Pump, but associating several pumps the processing capacity can be increased (http://www.ypsicon.com, Fig 1A, B, Fig 2). Particulate and sparkling fluids can also be processed by UHPH sterilization, so musts with some turbidity or small colloidal particles can be treated. Particle size must be lower than 0.5 mm and after the UHPH treatment they have an average size of 100-300 nm. As final size is higher than 100 nm, it is unnecessary to consider special nanomaterial regulations. Concerning sparkling products, the possibility to process carbonated juices or fluids open an interesting window in the sterilization of sparkling wines, beers and ciders, but also facilitating the stopping of fermentation in musts during fermentation to keep residual sugars in partially fermented beverages.

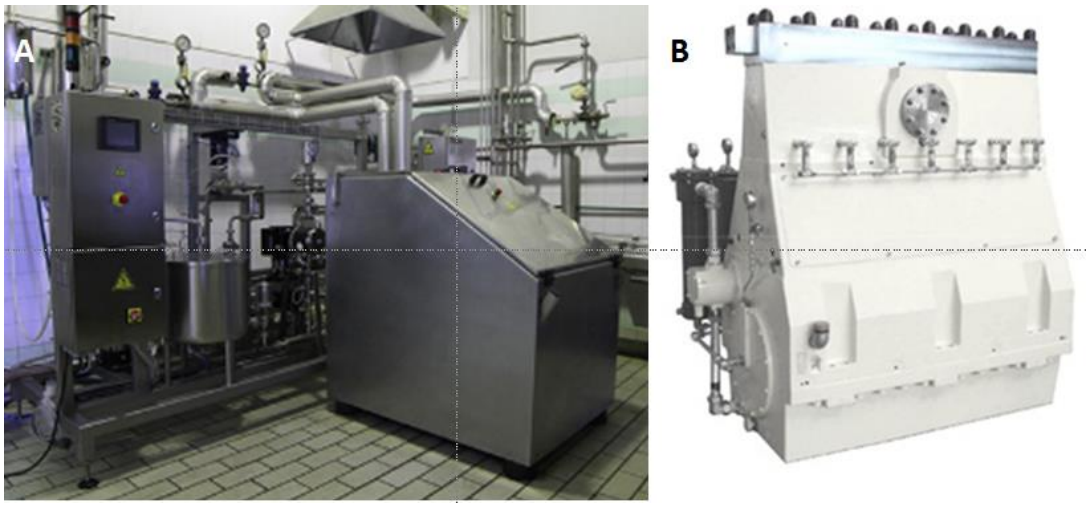

Fig 1. 200-400 MPa continuous technology Ypsicon(A). UHPH pump 10,000 l/h (B).

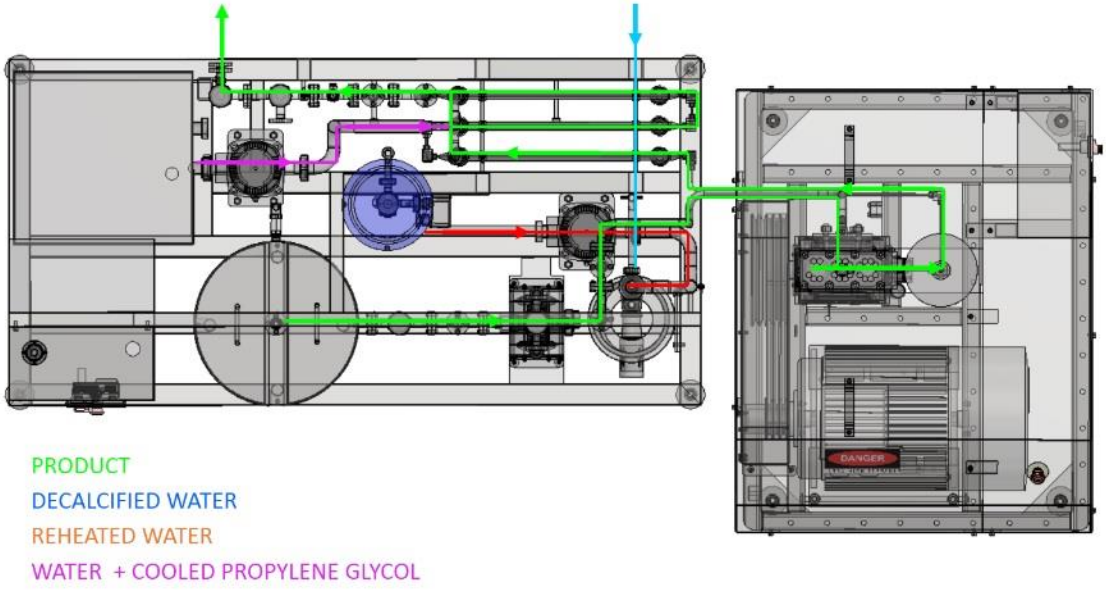

Fig 2. UHPH system section showing the product flow.

The modern UHPH pumps are able to process fluids at 300 MPa with an oscillation of just 1MPa (http://www.ypsicon.com). Even when fluid increases the temperature reaching 98 ºC when cross the valve because of the strong impact and shear forces, the adiabatic expansion post valve reduces the temperature producing a global temperature increment lower than 5ºC (Fig 3). The 98 ºC is produced during less than 0.2 s not affecting the sensory quality of product without covalent bonds breakage, and therefore, without impact in pigments and aromatic molecules. So, UHPH can be considered a gentle technique for the management of delicate varietal musts.

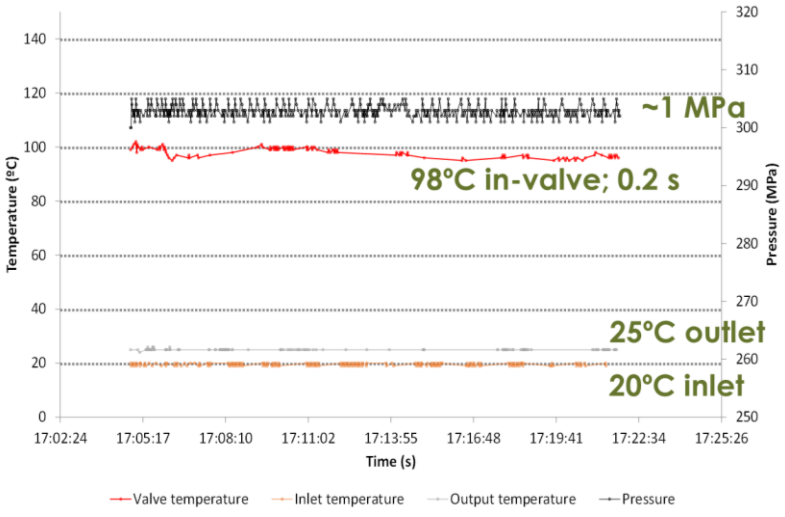

Fig 3. Thermal and pressure conditions during the in-valve flow (adapted from complementary material published in Loira et al, 2018*).*

The effect of extreme pressure (300 MPa) before the valve decreasing until in-pipe flow pressure after the valve (slightly higher to atmospheric pressure 0.1MPa) produces intensive depolymerisation of fluid components including background microorganisms. Size reduction to 100-300 nm also is produced in living cells which cells structures are strongly fragmented. The sterilization affects to yeast and moulds, bacteria and spores. The intense disintegration of particles also reduces the size of colloidal or suspended particles in turbid fluids producing an effective homogenization of the fluid structure. In turbid fluids the colloidal structure remains stable and without colloidal precipitations even after the fermentation (Fig 4) because of the low and homogenous size particle obtained.

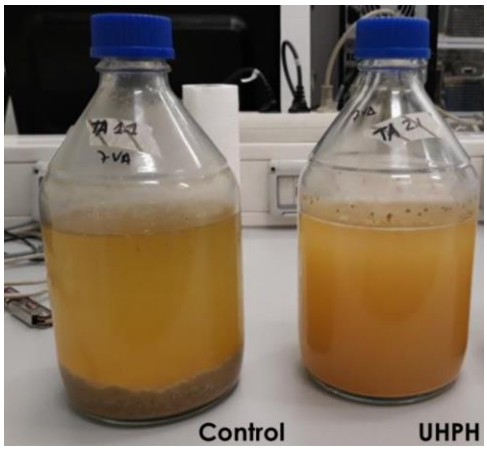

Fig 4. Control and UHPH treated musts after fermentation.

Material and Methods

White musts from *Vitis vinifera* L. were settled at 4 °C. Clean must was separated in three batches: i) sulfited (35 mg/L of total SO_2), ii) UHPH processed and iii) control must. UHPH sterilization was performed at 300MPa-150 L/h in a machine patented by UAB (EP2409583, Universidad Autónoma de Barcelona) and manufactured by Ypsicon Advance Technologies (Barcelona, Spain). Musts were fermented at lab scale in 2 L flasks inoculated at 5-logCFU/mL with *S. cerevisiae*. Microbial counts were done by plating before and after the fermentation in YEPD (yeasts and moulds), Agar-Lysine (non-*Saccharomyces* yeasts), MRS (bacteria)

Oenological parameters were measured by infrared spectroscopy OenoFoss™ (FOSS Iberia, Barcelona, Spain), organic acids and residual sugars were measured enzymatically using an Y15 enzymatic autoanalyzer (Biosystems, Barcelona, Spain) and ethanol quantification was done by liquid chromatography with refractive index detection (LC-RI).

Volatile compounds were analysed by gas chromatography with flame ionization detection (GC-FID), using an Agilent Technologies 6850 GC equipped with an integrated flame ionization detector. Separation was performed in a DB-624 column (60m×250 µm×1.40 µm) column.

Colour and phenols were determined by the use of a UV–visible (UV–Vis) spectrophotometer 8453 from Agilent Technologies™ (Palo Alto, CA, USA) with a photodiode array detector.

A preference sensory evaluation test was developed to assess the quality of the wines. A panel of nine experienced tasters evaluated the wines. The blind tasting took place in the tasting room, provided with fluorescent lighting and presenting samples in random order.

Results and discussion

Microbial counts were similar in sulfited and untreated musts. *Saccharomyces* yeasts were in average 1×10^6 CFU/mL, similar values were obtained for non-*Saccharomyces*. The bacterial counts were in average 7×10^3 CFU/mL. No yeasts neither bacteria were detected after the UHPH processing. Hence, at the end of fermentation, only the inoculated yeast was found in UHPH-processed must, with a total absence of non-*Saccharomyces* highlighting the effectiveness of the UHPH.

After fermentation, wines reached an alcoholic strength of 10% v/v ethanol, as expected, according to the initial amount of sugars. The residual sugars were below 0.2 g/L. Malic acid remained above 2 g/L with absence of lactic acid indicating the absence of malolactic fermentation. Levels of acetic acid were very low (0.2 g/L in UHPH and sulfited treatments) indicating the purity of the fermentation.

Sulfited wines showed the highest levels of volatile compounds, but mainly due to the concentration of higher alcohols, producing a winey aroma typical of low quality wines. UHPH-processed wines had lower concentrations of these compounds, showing fruity or varietal smells. Higher concentrations of esters were measured in wines from UHPH-processed must producing fruity smells and increasing the aromatic complexity.

A high inactivation for polyphenol oxidase (PPO) activity was obtained in the UHPH treatment compared to the control. Considering 100% of PPO activity in the control, UHPH sample decreased up to 90% their PPO activity.

The UHPH wine was better evaluated in global quality, but especially in aromatic profile, and described as fruitier by the tasters than either untreated or sulfite added controls what also is in accordance with the higher values of esters measured by GC-FID.

Conclusions

UHPH is a fast and highly effective technique to eliminate wild microorganisms, in a cold and gentle way, producing sterile musts and facilitating the implantation of yeast starters. This is especially interesting in the new fermentation biotechnologies using non-*Saccharomyces* yeasts or co-inoculation with lactic acid bacteria. Also helps to reduce SO_2 levels.

References

Loira, I., Morata, A., Bañuelos, M. A., Puig-Pujol, A., Guamis, B., González, C., Suárez-Lepe, J. A. (2018). Use of Ultra-High Pressure Homogenization processing in winemaking: Control of microbial populations in grape musts and effects in sensory quality. Innovative Food Science and Emerging Technologies 50, 50-56.

http://www.ypsicon.com/main.asp?Familia=173&Subfamilia=270&cerca=familia&pag=1

Abstract

Ultra-High Pressure Homogenization (UHPH) is a high throughput and effective technique useful to sterilize fluid foods at low temperatures or even under refrigeration temperatures. The shear forces and impact across the valve are able to eliminate whatever kind of microorganisms without affecting sensory quality. A white must (Vitis vinifera L.) was processed at 300 MPa (inlet temperature 20 °C, in-valve 98 °C, outlet 25 °C, time in valve 0.02 s) and compared with two untreated controls, a must that underwent a spontaneous fermentation (without sulfites) and another must added with 35 mg/L of total SO_2. All were inoculated with the same Saccharomyces cerevisiae strain. UHPH treatment led to the total elimination of grape microorganisms considering an initial population of 6-log CFU/mL of wild yeasts and fungi, and approximately 4-log CFU/mL of background bacteria. In a parallel assay, UHPH-processed must without inoculation showed absence of fermentation for several months at 18 °C. The musts UHPH processed showed a lighter appearance (10%) before fermentation compared to the control. Triangular test showed the existence of sensory differences between the wines obtained and the preference tests showed that the judges found the UHPH-wine more fruity (3.5 out of 5 compared with 2 in controls). Industrial relevance of UHPH is that allow must processing reducing sulfite addition and controlling wild and spoilage microorganisms. This opens new opportunities in the use of emerging fermentation biotechnologies such as the use of non-Saccharomyces yeasts and the yeast-bacteria co-inoculations.