

Opportunities for Enhancing Winemaking Processes by Employing High Power Ultrasonics Technology: A Review

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ABSTRACT

High power ultrasonics (HPU) technology has been used in the food industry to develop effective methods for food production, and to reduce operational costs and improve product quality with large-scale commercial applications since the 1990s. While high intensity energy produced by HPU has been successfully applied to many processes in food production, its application to winemaking is still at an experimental stage. However, as a disruptive industry changer, HPU technology offers an enormous range of possibilities to improve the wine industry's competitive position through lower cost, higher quality, and the delivery of cleaner, fresher, and more appealing wines to the customer. It will enable wineries to create more sustainable, transformative and innovative solutions to enhance winemaking processes.

Keywords: *Acoustic cavitation; barrel sanitation; Brettanomyces; high power ultrasonics; winemaking processes.*

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1. INTRODUCTION

The application of high intensity HPU is constantly evolving – most likely due to its powerful functional properties, low impact on the environment and non-invasiveness. HPU has been exploited for several food and beverage processes to reduce or eliminate the need for chemicals and/or heat [1–4]. The use of ultrasonication involves lower running costs, ease of operation, and efficient power output that does not need sophisticated machinery [5,6].

Ultrasound consists of sound waves which are perceived as mechanical vibrations in solid material or fluid are commonly divided into three frequency ranges, namely, low frequency power ultrasound (20-100 kHz), high frequency ultrasound (100 kHz - 1 MHz) and diagnostic ultrasound (1 – 10 MHz) [5]. HPU applications which use frequencies from 20-100 kHz are too high to be detected by the human ear [7]. At this range, the sound waves generate large cavitation bubbles resulting in higher temperatures and pressures in the cavitation zone [5]. HPU uses intensities higher than 1 Watt cm⁻² which are disruptive and have mechanical, chemical and/or biochemical effects [8]. These energies are used to modify the physicochemical properties and enhance the quality of various food systems during processing [8,9].

The effect of ultrasound is attributed to the process known as the acoustic cavitation phenomenon when sound waves are introduced into liquid [9]. The sound waves which act as a source of vibrational energy, alternately compresses, and stretches the liquid's structure. This process of compression and rarefaction of the medium and the subsequent collapse of the bubbles comprises the well-known phenomenon of cavitation, the most important effect in HPU [10]. Within the imploding micron-sized cavitation bubbles temperatures of 5000° C (5273 Kelvin) and pressures of up to 2000 atmospheres (203 MPa) are reached, which in turn produce very high shear energy waves and turbulence in the cavitation zone [11]. Typically, the high-shear energy wave that results in the liquid medium and at the surface of solid boundaries can travel at about 570 km/h. HPU produces nominal power density outputs above 8 W and up to 200 W per liter which are achieved with electrical energy inputs of 1 kW and above [3,5]. The most important parameters affecting the cavitation are ultrasonic frequency and temperature [12].

Research into the effects of ultrasound on grapes, wine, and spoilage microorganisms, notably the work on barrel sanitation by the authors [3,13–16], has been occurring over the past 20 years. The primary focus of this paper is to provide an insight into the rapidly growing field of ultrasonics and the possibility of HPU to influence, change and/or improve processes in wine production.

2. APPLICATION OF HPU TO THE FOOD INDUSTRY

The uses and potential uses of HPU in the food industries have been extensively reviewed [4,5,8,10,17–21]. Applications include extraction of intracellular material, cleaning of fresh produce and inert surfaces, removal of biofilms, emulsification, crystallization, drying and dehydration, texture modification, food cutting, filtration, separation, viscosity reduction or acceleration, inactivation of microorganisms, activation or inactivation of enzymes, disruption of cells, degassing and defoaming, extrusion, acceleration of energy transfer (heating, freezing and thawing), and enhancement of any process dependent upon diffusion and fermentations. The widespread applicability of ultrasound as a non-thermal technology in heat-sensitive foods is because it retains sensory, nutritional, and functional characteristics along with enhanced shelf life and product structure, microbial safety [22], and removal of microbial biofilms [23].

3. OPPORTUNITIES FOR THE APPLICATION OF HPU TO CURRENT WINEMAKING PROCESSES

Numerous HPU applications that have been developed for food processing could potentially replace traditional methods in wine production or enhance conventional winemaking processes. Table 1 summarizes winemaking processes and winery operations that are amenable to HPU treatment.

To date, only its application to barrel sanitation and extraction of compounds from red grape musts has been adopted by the wine industry at a commercial level [13]. Jiranek et al. [17] outlined the opportunities in wine production, particularly in wine microbiology where ultrasound could bring about desirable changes. Clodoveo et al. [24] provided an overview of possible ultrasonic applications for red wine production and suggested that emerging technologies offered better products to

customers and guaranteed higher profit for the industry.

3.1 Extraction

High-intensity ultrasound increases the efficiency and speed of extraction processes for many food components, such as oils, flavorings, pigments, bioactive ingredients, including antioxidants and essential oils [8]. The extraction of colour and flavour from grape skins and musts is an important process which determines the final composition of a red wine. It is generally accepted that an increase in wine color density is not only more desirable in the finished wines but also correlated with an increase in aroma intensity and wine quality. Bates and Patist [3] reported research undertaken by the current authors of an overall gain of 25% anthocyanins and 19% in red colour density over the untreated control. In the trials, red must was treated with HPU (2 kW unit) in a flow cell at 25 L/min and 50% amplitude. The results demonstrated that HPU significantly increased the extraction of anthocyanins in addition to the increase of colour density. This application of HPU to freshly crushed must demonstrates wine quality improvements, and likely elicits advancements in process-cost efficiency and additional wine quality parameters.

Research into the impacts of HPU treatments during grape crushing was also conducted by Bautista-Ortin et al. [25]. They found that grapes treated with HPU during crushing yielded twice the concentration of tannins and similar anthocyanin levels after only three days maceration on skins, when compared to untreated grapes macerated for eight days. They highlighted the benefits of reduced maceration times and possibly of maturation of wines using ultrasound. Their results concur with the efforts of Ferraretto and Celotti [26] who found that HPU changed tannin concentrations but conversely, treatment by HPU in this work did not impact anthocyanin concentrations. Curko et al. [27] discovered that HPU treatment induced changes to phenolic composition, aroma and colour of young wines made from Cabernet Sauvignon, Merlot and Plavac mali, specifically increasing polymerisation of phenolic compounds, typically associated with wine aging. Maza et al. [28] developed their own continuous ultrasound system to improve the extraction of polyphenolic compounds from Syrah grapes. The resulting treatment gave up to 59% recovery of total quantified phenolic compounds using 3000 W/L

in only three minutes of HPU processing. The reduction in time of a wine production phase such as maceration that normally takes up to 10 days, is highly attractive as it reduces pressure on valuable tank space, reduces workload and even significantly reduces refrigeration expense at the busiest and most expensive part of the season. El Darra et al. [29] applied ultrasound to Cabernet Franc grape pomace to compare the extraction of polyphenolic compounds. They too found that HPU increased and accelerated the extraction of anthocyanins and other desirable phenolic compounds from the pomace, and enhanced colour density of the resulting post-fermentation wine. In comparison, research by Barba et al. [30] demonstrated that HPU treatment not only increased extraction yields of anthocyanins and total phenolic compounds, but they found a more equitable ratio of anthocyanin to phenolic compounds, indicating that these treatments were extracting a more comprehensive group of phenolic compounds, not simply releasing the anthocyanins from berry skins, which allows for more substantive wines to be produced with reduced maceration times, better structure and greater aging potential. The findings of Caldas et al. [31] concurred when they demonstrated that using ultrasound to treat red grape pomace, phenolic compounds were extracted in greater amounts in a shorter time as compared to mechanical extractions. Margean et al. [32] discovered that in addition to its microbial load reduction, HPU-treated juices yielded increased L-ascorbic acid and soluble solids, accompanied by a slight reduction in pH. This was considered beneficial to quality as the HPU treatments enhanced extraction of functional compounds from the valuable grape resource. Furthermore, they concluded that HPU was favourable as a replacement to thermal techniques which require juices to be heated above 60°C and thermally shocked by then reducing temperature quickly [32] – both of which are detrimental to juice and resulting wine quality [33].

When considering the extraction and retention of anthocyanins in grape juice, Tiwari et al. [34] compared physical HPU treatments with prediction models. The models were strongly predictive ($p < 0.05$) and had low standard deviation and high coefficients of determination for both anthocyanins and color. They found significant retention of anthocyanins in experimental juices treated with HPU, and these anthocyanins correlated with juice color parameters. These results indicate a strong

benefit of using HPU to enhance juice processing while maintaining anthocyanin and resulting stronger color that consumers prefer. Lieu and Le [35] treated Cardinal grape juice with HPU to compare with enzymatic maceration. Results demonstrated that HPU increased extraction yields and reduced time required to yield the same amounts as enzymatic extractions. Furthermore, they concluded that HPU treatments increased extraction of grape sugars, acids and phenolic compounds, which in the case of anthocyanins increased color density of the treated juices.

Overwhelmingly, literature and reports, lab and field trials all present the strong case in favour of treating grapes, juice, and pomace with HPU to increase extraction of desirable colour, flavour and even aroma compounds or precursors and thus improve quality of resulting products, in addition to reducing residence time in fermentation tanks during critical harvest periods.

3.2 Removal of Surface and Sub-surface Contaminants

Cleaning is among one of the first applications of ultrasound in the food industry, having been first used for cleaning hard materials in the 1950's [36]. Ultrasound achieves accelerated surface cleaning by dissolution and the physical erosion of materials by cavitation, micro-jetting, and shear wave transmission across the surface [5]. For crystalline solids, dissolution by a solvent (water) breaks the crystal structure up into atoms, ions, or molecules. High pressure microjets that impact on the surface favours the dissolution of compounds and the release of particles (including microorganisms) adhered to the solid material [37]. Solid surfaces have irregularities and pores that limit the cleaning efficacies of traditional systems. However, ultrasound can access those areas, enhancing the release of contaminants and biofilms [4]. In liquid systems, acoustic streaming ($\sim 10 \text{ cm s}^{-1}$) has a significant role in the cleaning process by increasing dissolution of soluble material, and the transportation of detached insoluble solids.

When ultrasound is introduced into a volume of water (e.g. in a barrel), thousands of unstable high-pressure micron-sized bubbles that are formed implode, releasing high-intensity energy [5]. This cavitation enables erosion of deposits and biofilms, microbial cell destruction,

extraction, heat and mass transfer, degassing, particle size reduction to occur. The 'brushless scrubbing' in ultrasonic cleaning can reach normally inaccessible places in objects with complex internal cavities that would otherwise be extremely difficult to clean [38]. Increasing the cavitation in cleaning liquid increases the ultrasonic cleaning effect [39].

When an ultrasonic sound wave passes through a solid medium, it produces a series of alternating contractions and expansions, a phenomenon known as the "sponge effect", which facilitates the transfer of matter within the medium surrounding the solid [4]. In porous materials, the sponge effect of ultrasound assists in the exit of air from the pores which are replaced with the surrounding solution [41]. The mechanical stress can cause the formation of microchannels in the interior of the solid, which favors mass transfer process. Studies by Breniaux et al. [42] revealed that HPU enabled tartrates to be effectively removed from the wood structure during HPU treatment. Energy produced by ultrasound pervades every part of the barrel [4] and as far as wine can travel and thus is able to remove particulate matter or biofilms from the pores, crevices, cracks, grooves and spaces between staves, and destroy *Brettanomyces* and other microbes – all of which are unachievable by current physical and chemical applications. Porter et al. [43] demonstrated the ability of HPU to remove 98.0% of tartrate volume from the surface and subsurface of oak staves up to a depth 2 mm at 60° C and > 85.0% of tartrates from stave surfaces. Their use of X-ray tomography confirmed earlier findings by Yap [15] that HPU treatments (4 kW at 20 kHz) were able to significantly reduce tartrate deposits on barrel staves by up to 99.0%.

Yap et al. [44] studied the cleaning action of ultrasound on dirty barrel staves using a 400 W HPU unit. Fig's. 1A, 1B and 1C show the removal of hardened potassium bitartrate deposit encrusted on the stave of a 2-year-old white wine barrel in 6 minutes in 60° C water. Photomicrographs show that ultrasound physically disrupts and removes biofilms adhering to the surfaces of solid materials in a similar manner [45,46]. Further studies by Yap et al. [14] showed that the hardened tartrated deposits on 6-year barrel staves were completely removed by sonicating them in 60°C water with a 1 kW HPU unit for 3-10 minutes.

Table 1. Summary of potential applications of HPU to processes in wine production

	HPU treatment objective / outcome	Winemaking process / production step / winery operation / substrate	Reference
1	Low temperature drying	Harvested grapes	[47–57]
2	Extraction and/or enhancement of bioactive, functional, phenolic compounds, and enhancement in juice production	Seeds, pomace / marc	[58,59,26-29,60-64,32]
		Crushing, must transfer, cold settling, thermovinification, cold maceration, extended post-fermentation maceration, primary fermentation on skins (at various stages of fermentation in tanks or barrels)	[3,58,62,26,28,29,25,65-67]
3	Inactivation of oxidative enzymes (polyphenoloxidase and laccase), activation of glycosidases, pectinases, and immobilized enzymes	Crushing, pressing, must transfer, cold settling, thermovinification, alcoholic fermentation, malolactic fermentation, secondary fermentation in sparkling wine production	[68–77]
4	Destruction of precursor compounds (e.g., hydroxycinnamic acid, p-coumaric acid) required by <i>Brettanomyces</i> to form 4-ethylphenol)	Red must post-crushing, red wine post-fermentation	[78]
5	Inactivation or destruction of <i>Brettanomyces</i> and other spoilage yeasts and bacteria	Crushing, juices, musts and wines; during must and wine transfers, thermal pasteurization, cold maceration, and extended post-fermentation maceration; at the end of primary fermentation; cleaning and sanitizing winery equipment, grape bins, tools, lines, hoses and bottling lines; cleaning oak planks and inserts; barrel sanitation and rejuvenation of <i>Brettanomyces</i> -infected barrels; during maturation, during storage and/or ageing of red wines in barrels or tanks; prior to bottling	[4,13,17,59,79-86,40-42,36,87–88]
6	Enhancement of biocidal action of chemical agents	Cleaning and sanitizing winery equipment, tools, lines, and hoses	[18,86]
7	Removal of solid deposits and biofilms from surfaces and sub-surfaces	Cleaning oak planks and inserts, barrel sanitation, equipment cleaning	[4,8,13,14,23,86,40–42,89,90]

	HPU treatment objective / outcome	Winemaking process / production step / winery operation / substrate	Reference
8	Reduction of foam (de-foaming)	Yeast propagation, primary fermentation (white wine production), barrel fermentation	[3,49,91–94] https://cavitus.com/products/foamcontrol/
9	Cleaning surfaces of membrane and pad filters and, enhancement of filtration rate	Juice and wine clarification, bottling and packaging	[8,55,95–99]
10	Viscosity reduction of high viscosity juices (e.g. juices from botrytised grapes)	Pre-fermentation extraction of high-sugared juices/musts from grapes infected by <i>Botrytis cinerea</i>	[5,100,101]
11	Enhancement /stimulation of yeast growth rate and fermentation rate	Juices, musts, primary fermentation, yeast propagation, slow or stuck fermentations, secondary fermentation in sparkling wine production	[102–107]
12	Enhancement of tartrate crystallization, yeast flocculation and sedimentation	Clarification and stabilization in white wine production	[108,109]
13	Yeast lees stirring (bâtonnage), enhancement of yeast autolysis	Barrel fermentation, maturation of white wine, secondary fermentation in sparkling wine production	[62,110–112]
14	Stimulation, enhancement, or inhibition of malolactic fermentation	Post-primary fermentation, wine maturation in barrels	[28,29,82,103–106,110]
15	Enhancement of ageing process	Barrel or tank maturation of white and red wines, bottle ageing	[110,113–118]
16	Degassing (removal of dissolved carbon dioxide)	Wine in storage in stainless steel tanks, bottling and packaging	[14,94,104]
17	Disinfection of recycled wash-water in packaging operations	Cleaning and sanitizing winery equipment and bottling lines	[119]
18	Enhancement of organic matter decomposition, cell breakdown, biomass destruction, sludge volume reduction, sedimentation/clarification, flocculation, sludge de-waterability; water/effluent disinfection and re-use, reduction in COD and BOD, degassing	Wastewater and effluent treatment and management	[80,119,120]

Biofilms are known to form when a liquid is in contact with an inert surface and any microbial cells within the liquid become attracted to the surface and adhere to it [121]. Biofilms are made up of extracellular polymeric substances (EPS), microbial cells, minerals, biogenic particulate materials, and organic and inorganic pollutants [122]. Biofilms are found on stainless steel surfaces of food processing equipment and wine tanks [123]. Lebleux et al. [124] postulated that the ability of *B. bruxellensis* to form biofilm was a potential resistance strategy to grow under stressful conditions in the winemaking environment.

Fink et al. [90] used ultrasound to remove a *Bacillus cereus* biofilm from polyurethane conveyor belts in bakeries. Mott et al. [125] was able to remove biofilms from the internal surfaces

of 7 cm and 50 cm water-filled glass tubes using ultrasound at 350 kHz, 150 kHz and 20 kHz. Oulahal-Lagsir et al. [126] showed that ultrasound (40 kHz for 10 seconds) could remove biofilms of *Bacillus stearothermophilus*, from stainless equipment in a beef processing plant.

Yap [15] found that HPU (4 kW, 60° C water) was more effective in removing solid deposits from the surfaces of 1- and 3-year barrels compared to high pressure hot water (HPHW) (1000 or 2000 psi with 60° C water for 5 minutes). HPU removed 99.0% of the deposits from the surfaces of 1-year old barrels in 5 minutes, as compared to less than 20% removal in 5 minutes and up to 50-80% after 12 minutes by HPHW. The typical appearance of a barrel before and after cleaning with HPU is shown in Figs. 2A and 2B respectively.

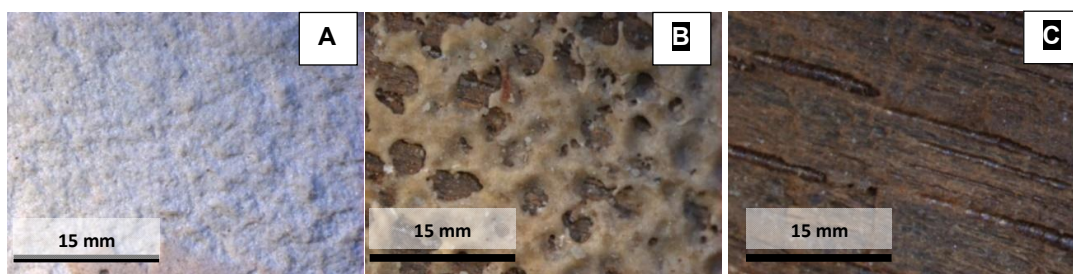


Fig. 1A. Photomicrographic appearance of 2-year-old white wine barrel stave surface before HPU treatment, covered by an impenetrable layer of potassium bitartrate (KHT). Scale bar at 15 mm; **Fig. 1B.** Erosion of KHT layer during sonication. The collapse of cavitation bubbles near solid surfaces which form high-pressure microjets [127] project on to the surface of solid deposits, leading to the dissolution of solid compounds and release of the solid particles (including microbial cells adhered to the solid, and formation of pores in the hard tartrate layer and biofilm. Approximately 50% of the KHT was removed in 3 minutes in 60° C water. Scale bar at 15 mm; **Fig. 1C.** KHT completely removed from the surface of the stave in 6 minutes. Scale bar at 15 mm. Photos by Andrew Yap

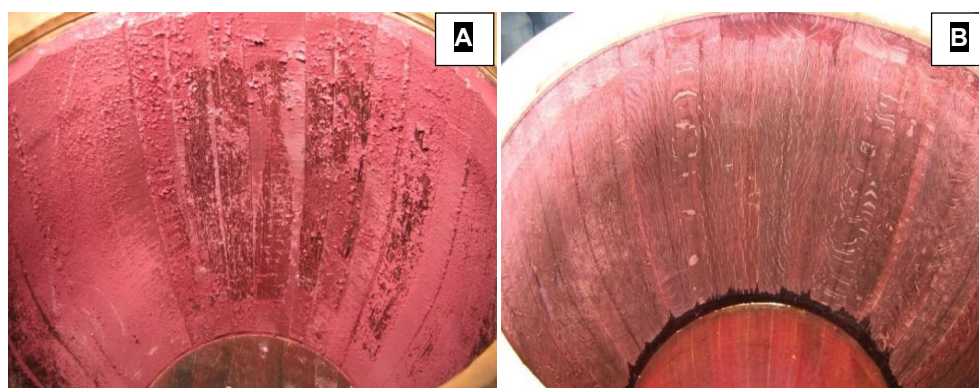


Fig. 2A. A 5-year-old barrel before cleaning by HPU. The surface was covered with a thick, hard, and crusty tartrate deposit; **2B.** Surface of barrel after treatment by HPU (8 mins at 60 °C). The solid deposit on the surface of the staves was uniformly removed. Photos by Andrew Yap

The efficacy of HPU to remove solid deposits from the interior surfaces of 8 1- and 3-year old red wine barrels (225L) were tested at a large Californian winery [13]. Four barrels of each age was cleaned by a 4 kW beta prototype HPU barrel cleaner. 1- and 3-year-old barrels were sonicated at 60° C for 5 and 8 mins, respectively. Data from the trial confirmed unequivocally that HPU was a highly effective and reliable way of removing solid deposits from barrels. Initial surface area covered by the deposits in 1-year old barrels ranged from 10-30%. Following HPU cleaning, all deposits were removed (100% reduction). In the 3-year old barrels, deposits were reduced by 84-94%.

3.3 Inactivation and Enhancement of Microbial Growth

The effectiveness of HPU for inactivating vegetative microbial cells and spores have been reviewed [4,8]. Acoustic cavitation and microstreaming resulted in increased permeability of membranes, selectivity loss, cell membrane thinning [80] and breaks in cell membranes, releasing intracellular content and enzymes [128]. Energy, intensity, pressure, velocity, and temperature were the main parameters affecting HPU. The cell-killing effect of HPU increased with the inclusion of heat, at temperatures somewhat below those generally regarded as necessary for pasteurization [36]. Mawson and Kai [36] believed that the mechanism probably involved softening microbial cell wall polymers and associated cell membranes to facilitate the sonoporation of the cell surface.

Luo et al. [84] exposed six yeast species commonly associated with winemaking to HPU (320-340 W/L power output) at 23-25° C for 20 minutes in separate batches. Decreases in viability following ultrasound treatments occurred for all yeast strains, although some yeasts were more susceptible than others. Both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* viability decreased to 20% in the saline control and 75% in grape juice.

The effect of ultrasound (20 kHz, wave amplitude 71-110 μm) on *Saccharomyces cerevisiae* cells was studied at 35, 45 and 55° C in Sabouraud broth at pH 3.0 and 5.6 [129]. The resistance of the yeast decreased as ultrasonic wave amplitude increased, while the reduction of pH did not affect ultrasound yeast sensitivity.

Structural studies performed in cells sonicated at 45° C and 95.2 μm of wave amplitude indicated the treatment provoked puncturing of cell walls with leakage of content as well as damage at subcellular level.

Yap et al. [130] studied the efficacy of HPU in destroying viable *Brettanomyces bruxellensis* yeast cells present on the surface and in the subsurface of oak staves of 1- and 3-year-old American oak barrels. A 4 kW HPU unit was used to introduce ultrasound in a 225 mL barrel filled with 60° C reverse osmosis water. The number of viable *Brettanomyces bruxellensis* cells present on the surfaces (0-2 mm) and in sub-surfaces (2-4 mm) before and after cleaning was determined by cultural methods. Treatment times were 5, 8 and 12 minutes at 60° C. The results showed that a 5-minute treatment by HPU at 60° C was able to kill all *B. bruxellensis* cells, on the surface and to up 4 mm below the surface of the oak staves. Schmid et al. [16] studied the efficacies of HPU to sanitize oak barrels, at both the surface and subsurface. 1- and 3-year old American oak stave pieces that were infected with *Brettanomyces bruxellensis* in the laboratory were attached to the bilge of the barrel, HPU treatments were carried out in 40° C, 50° C and 60° C water with a 4 kW HPU unit (for 5, 8 and 12 minutes for 1-year old wood; 8, 12 and 15 minutes for 3-year wood) in an open barrel (one head removed). HPU reduced the *Brettanomyces* population on 1-year old staves to about 50-200 cells/mm³ at all temperatures and time points. The control (untreated) stave showed a population of about 6,000 cells/mm³ of wood. No culturable cells were detected after 12 minutes exposure at 50° C, or for any treatment time at 60° C. Significant decreases in culturable cells occurred at 2 mm for all temperatures, with no culturable cells detected after 12 minutes at 50° C, or at any time at 60° C. Like the 1-year-old wood, sonication of the 3-year-old wood reduced the surface culturable cell count by several orders of magnitude after 8 minutes of treatment at 40° C. The culturable cells from the subsurface also decreased significantly with no culturable cells detected after 15 minutes at 50° C or after any treatment duration at 60° C.

HPU was shown to be highly effective in destroying *Brettanomyces* cells in heavily infected wine barrels in a commercial winery in the Napa Valley [13]. A total of 12 infected 2-, 3- and 4-year barrels were subjected to HPU treatment at 60° C for 8, 10 and 12 minutes, respectively. Initial viable cell numbers present in

the barrels ranged from 80,640 to 4.7 million cells per mL. Barrels treated by HPU showed dramatic reductions in cell numbers, viz. 99.96 to 100%, as determined by plating and 94.2 to 99.3% by Scorpion Microbial Assay [13].

In a study by Gracin et al. [87] *Brettanomyces* in a young wine subjected to HPU (400 W, 24 kHz, 100 μ m amplitude) in continuous flow system showed an 89.1-99.7% reduction of cell numbers at 30° C and 40° C, respectively. Van Wyke [88] used HPU to kill *Brettanomyces bruxellensis* (WLP 650 strain) cells by passing a suspension in red wine (12.9% v/v) through a flow vessel at a flow rate of 0.73 mL per second to ensure a residence time of 20.5 seconds. The resultant acoustic power using a 200 W HPU unit was 10.8 W/mL and the temperature of the treatment vessel was 40° C. After a processing time of 30 minutes the cell numbers was reduced by 1.9 log.

The wine barrel has become a unique ecological niche for viable *Brettanomyces* cells and biofilms [131]. Conventional sanitization processes require physical contaminants to be removed from surfaces prior to the application of a disinfecting agent to destroy viable microbial cells from the cleaned surfaces. HPU does not require the use of chemicals and simultaneously removes solid deposits from surfaces and destroys *Brettanomyces* and other microbes, unlike all conventional methods which require two-steps [13].

Low levels of essential nutrients and survival factors lead to slow or stuck fermentations. HPU can stimulate growth of yeast cells by redistributing nutrients in the fermenting juice or must. Ultrasound treatment facilitates the entry of nutrients into the cell by removing toxic substances which clog up the cell walls. Low intensity ultrasound can improve mass transfer of nutrients through the boundary layer or through the wall and membrane [103,105]. Matsuura et al. [104] showed an increase in the fermentation rate of sake, beer, and wine, when a relatively low intensity ultrasound was applied to the fermentation. The fermentation periods were reduced to 50-64% in wine, beer, and sake when ultrasound was applied at 30 mW/cm². *Saccharomyces cerevisiae* yeast growth was stimulated by ultrasound at 30° C in 200 mL starter broth at intensities of 0.2, 0.4 and 0.8 W/cm² and frequency of 20 kHz [132]. In studies by Shokri et al. [133] it was found that low intensity ultrasound increased viable cell count,

specific growth rate and fermentative activities of *Leuconostoc mesenteroides* (PTCC 1663) (20% and 30% amplitudes for 3 min and 5 min). The plate count, cell membrane permeability, and specific growth rate were increased in the ultrasonicated samples by 0.41–0.84 log CFU/mL, 8.83–28.48%, and 12.7–35.5%, respectively, compared to the reference samples.

3.4 Activation and Inactivation of Enzymes and Precursor Compounds

Ultrasound has been known to inhibit the activity of enzymes and precursor compounds [134]. Studies have shown that ultrasound could deactivate enzymes, such as lipases, proteases, peroxidase, polyphenoloxidase (PPO) and pectinesterase [71]; however, there have been few studies on the use of HPU to enhance enzyme activity. The inactivation of enzymes by HPU is mainly the result of protein denaturation, by shear forces resulting from the formation and collapse of cavitating bubbles. Cervantes-Elizarraras et al. [69] found that ultrasound treatment of blackberry juice inactivated all microorganisms being evaluated at 50° C for 17 minutes and increased enzyme inactivation and antioxidant activity in comparison to pasteurized juice. From pre-fermentation, through to fermentation, post-fermentation and aging in wine production, enzymes catalyse many reactions. Originating from the grape, yeasts, bacteria, and fungi the enzymes have a strong influence on the final organoleptic quality of the wine. The application of HPU alone or in combination with various levels of sulphur dioxide and ascorbic acid may provide a better and cheaper solution in controlling oxidation in juices and wine by PPO and laccase. The enzyme laccase which is produced by *Botrytis cinerea* promotes very rapid oxidation and browning of juices and wine. The successful employment of HPU without the use of heat and chemicals could result in the production of higher quality juices and wine and considerable savings.

Grape-derived aroma and flavour compounds are present as free volatiles and, in part, as sugar-bound precursor including glycosides. Glycosides contain aroma and flavor aglycones. The release of aglycones from glycoconjugates, through the application of HPU could accelerate the formation of odor-active volatiles. Hydroxycinnamic acids (HCA) released from grape skins is the precursor required by *Brettanomyces* to produce 4-ethylphenol. It is

possible that by applying ultrasound to the must, the precursor compound could be destroyed, or its concentration reduced drastically without ill-effects to the wine. The destruction of p-coumaric acid in red wines by ultrasound in barrels could also prevent the formation of 4-ethylphenol.

3.5 Filtration, Viscosity Reduction, Drying and Crystallization

In wine-processing there is an absolute requirement to remove suspended solids from white juices and red and white wines. Ultrasound can improve filtration by causing the agglomeration of fine particles and supply sufficient vibrational energy to the system to keep the particles partly suspended and therefore leave more free channels for solvent elution [134]. The use of ultrasound in conventional membrane filtration has been shown to improve process efficiency [135]. Ultrasound can be applied to increase or decrease the viscosity of food and beverage products [3]. A problem associated with *Botrytis*-infected grapes is the high viscosity of the juice which can only be extracted by pressing. With the addition of enzymes such as pectinases, the viscosity of the juice decreases, enabling the pressability of the pulp and obtaining higher juice yields. The employment of HPU could assist in reducing the viscosity of the juice [101]. High-intensity airborne ultrasonic waves have been used to increase the drying rate of materials without affecting the main characteristics and quality of the product. The acoustically assisted hot air-drying process permits the use of lower temperatures for drying heat-sensitive materials [57]. High-intensity airborne ultrasound causes microstreaming at the interfaces that reduce the diffusion boundary layer, increases mass transfer, and accelerate diffusion [50]. The "sponge effect" enhances the diffusion of water from the interior of the product to the surface. Musielak et al. [51] have reviewed the enhancement of food drying by ultrasound. Studies with airborne ultrasound systems have shown reduction times by 20-30% at low temperatures and low air velocities. Ultrasound has been proven to be extremely useful in the crystallization process since it can initiate seeding and control subsequent growth in a saturated or supercooled medium [136]. It has been reported that ultrasound can be used to clarify wine by the precipitation of potassium bitartrate [137]. Ultrasonic treatment reduced precipitation time from 10 days to 1.5 hours.

3.6 Maturation and Ageing of Wine, Yeast Lees Stirring and Autolysis

Lukic et al. [117] applied laboratory HPU treatments with an ultrasonic bath and ultrasonic sonotrode to young red wine styles to determine the impact on aging and aroma. They discovered that after six months, HPU treated wines contained higher levels of antioxidants (sulphur dioxide and glutathione) and had no impact on aroma or colour of the wines. When applied to the wine, ultrasound induced chemical and structural changes in its composition that resemble those that occur after many years of natural ageing [113–115]. Chang [114] found that ultrasound at 20 kHz shortened the process of aging of rice alcoholic beverage from one year (control) to one week. The taste quality of the accelerated-aged beverage was similar to the control (conventional method of production). It has been proven that ultrasound can significantly increase the extraction of polysaccharides from an aqueous solution of fungi without changing their molecular weight profiles [138]. Del Fresno et al. [110] studied the application of HPU to extract polysaccharides in wine production. HPU treatments were imposed for 5 minutes, twice a week for five weeks post-fermentation. Tempranillo grapes were treated in 2 L flasks with headspace and lyophilized lees and oak chips. This aggressive regime resulted in increased levels of dissolved oxygen in what was a very small sample with nearly 4% ullage by volume. They concurred that cellular lysis was the functional result of HPU treatments, and that this treatment advanced the rate and increased the concentration of polysaccharides available in the resulting wines. Furthermore, they identified impacts on anthocyanins and volatile compounds – both critical compounds to manage in wine production [110].

Yeast lees stirring in barrels is one of the processing steps in the production of medium- to full-bodied dry white table wines and some red table wine styles. During the barrel ageing process, interactions between yeasts, wood and wine occurs [139]. Stirring of yeast cells (*bâtonnage*) which promotes release of polysaccharides and mannoproteins is a slow process. Yeast autolysis, an enzymatic process which is influenced by pH, alcoholic content and temperature, may take approximately 12 months to occur naturally [140]. HPU is an efficient method of stirring and re-suspending yeast cells and autolyzing them by breaking down their cell walls. Ferraretto et al. [62]

postulated that ultrasound could reduce the ageing time of wines when they found that the treatment of fermentation lees prompted the lysis of yeast cells and rapid release of colloids, polysaccharides and mannoproteins.

Martín et al. [141] compared a classical yeast autolysis at 25° C with light lees which had spent more than one year in barrels and which was lysed by sonication (22 W/L, 18° C) in a model wine. The ultrasound-assisted yeast lysis increased the concentrations of proteins and polysaccharides in the model wine due to the release of the compounds from the yeasts. Ultrasound also led to a higher cell disruption, such that after 20 hours of ultrasonic treatment, no viable cells were found. The morphological changes in cells were examined by scanning electron microscopy to verify the effect of ultrasound on yeast cells. Compounds released from the autolyzed cells add to the complexity of wine. Ultrasound could be applied to enhance the autolysis of yeast cells in sparkling wine production. The secondary effect of applying HPU in an oak barrel with the wine *situ* is to remove cells and tartrate crystals that have deposited on the walls of the barrel, thus enhancing direct contact between wine and oak surfaces.

3.7 Fresh Water Effluent and Wastewater Treatment

Winery wastewater can have significant environmental impacts when discharged to watercourses [142,143]. Ultrasound have been applied to the treatment of sewage, effluent/waste food, effluent/sludge by breaking down microbiological cells to enhance biodegradation during anaerobic digestion, reduce sludge volume, enhance biogas recovery, and reduce retention time in ponds/digestors [144].

The benefits of ultrasonic pre-treatment with application to contaminant removal has also been considered for other areas, such as distillery wastewater [145], although a conventional ultrasonic bath was used in the experimental analysis. Zhang et al. [146] found that acoustic cavitation improved the microbial activity of activated sludge for wastewater treatment. By applying a frequency of 25 kHz, power density of 0.2 W/mL and duration of 30 seconds, the sludge oxygen utilization rate was increased by 28%, biomass growth rate increased by 12.5% and wastewater COD and

total nitrogen removal efficiency increased by 5-6%.

4. CONCLUDING REMARKS

The volume of research to study the effects of ultrasound on the grape and its products, winery microorganisms and winemaking processes has been increasing steadily over the past two decades; however, more work is necessary to gain a better understanding of the correlation between the effects of ultrasound and winemaking processes to predict changes in quality. Optimisation of the operating parameters will be the major objective for the application of HPU. The application of HPU technology to several operations that occur during vintage, such as the extraction of intracellular compounds from grape skins, during cold settling, cold maceration, fermentation, cap management, extended fermentation, and draining and juice clarification, will limit time on skins, eliminate the use of enzymes, activate or inactivate enzyme activities when required, eliminate processing steps, enable better pressability (therefore fewer pressings), and speed up settling and extraction rates (thus resulting in better utilization of tank space). Overall, the application of HPU to operations that take place during the vintage period would lessen the pressures of time and space constraints. Cleaning and sanitizing tanks, lines and equipment using current technologies cost millions of dollars and take a considerable amount of time. HPU would replace chemicals and shorten cleaning times. Wineries would be incentivised to adopt HPU technology on an industrial scale as there would be product quality improvements.

The employment of HPU to solve the foremost microbiological problem [15] caused by *Brettanomyces* that has been plaguing the global wine industry [147] is the most significant technological development in the history of winemaking. The application of HPU has resulted in the reduction of spoiled red wines following barrel maturation, improvement of wine quality, and extended use of barrels. Many wineries have saved millions of dollars by avoiding the production of spoiled wines and disposal of infected barrels. HPU has enabled *Brettanomyces*-infected barrels to be restored to a healthy state and their lives extended by up to four years (Neil Pike pers. comm. 2017). To date, HPU has remained unchallenged as the most advanced, powerful, and highly effective technology for barrel sanitation.

Ultrasonic processing is establishing itself as a significant food-processing technology with the capability for large commercial scale-up and good payback on capital investment [8,148]. As an environmentally friendly, safe, efficient, and non-thermal technology, it provides, inter alia, large savings in terms of the cost of labour, chemicals, additives, electricity, water, equipment, and barrels. HPU provides cutting-edge, innovative, and valuable technology; however, a lack of awareness of its potential has limited its uptake. Producers of natural, organic, and biodynamic wines should be especially interested in the application of HPU as is a natural way to support barrel and winery sanitation and extraction of desirable compounds among many other functions in the winemaking process. In summary, it is envisaged that HPU would be employed in the wine industry in the foreseeable future to solve and/or manage many production problems, and change and/or improve winemaking processes, wine quality and winery sustainability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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