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Sanitation of wooden barrels for ageing beer – a review

The use of wooden barrels is a traditional technique for fermentation and maturation of beer, most prominently present in the production of traditional sour beers like Belgian lambic beer and red-brown acidic ales. In recent years, barrel ageing of beer is also gaining an increased interest to add notes of wood or aromas from the previously matured beverage to the beer. However, insufficient cleaning and sanitation of barrels can result in microbial spoilage which may have a detrimental impact on beer quality, e.g. caused by wild yeasts like *Brettanomyces*, acetic acid bacteria and lactic acid bacteria. Therefore, in order to control the microbial load of the barrel, it is important to properly clean and sanitise the barrels. To date, to our knowledge no systematic review has been published on the available sanitation techniques for wooden beer barrels. Here, we provide a comprehensive overview of various chemical and physical cleaning and sanitation methods that are commonly applied in breweries like sulphur dioxide, steam and hot water. In addition, we discuss a number of alternative methods that are gaining interest and popularity such as ozone and high-power ultrasound. We address their advantages and drawbacks, emphasising their ability to eradicate spoilage microorganisms and influences on the extraction of typical wood associated flavours from the barrels. Finally, limitations in existing knowledge are discussed and areas that merit further study are identified, including combinations of different treatments.

Descriptors: barrel; beer; *Brettanomyces*; sanitation; sulphur dioxide; wood

1 Introduction

Wooden barrels hold an important place in the history of human civilisation. As a predominant form of shipping container they greatly facilitated the trade and logistical activities of all kinds of bulk goods, including petroleum oils, nails, soap and glue [1]. Besides being both durable and easy to handle, wooden barrels also appeared to provide positive effects on the foods and beverages that were transported, such as meat and fish, vegetables, olives, syrups, beer and wine. In particular, the Greeks and Romans already bought wine in wooden barrels from the Gauls around 300 BC, as they preferred the wood-aged wine to their own wine [2]. Although oak (*Quercus*) has become the preferable wood for barrel manufacturing, the first barrels were made of readily available and relatively bendable wood species such as pine (*Pinus*), poplar (*Populus*) and palm (Arecaceae). To make the wood species used more workable and less susceptible to fractures, heat was applied over time, either by steam or through direct contact with fire [3]. Today, 2.5 million new barrels are annually produced worldwide with wine and whisky industry as main purchasers [3]. However, whereas the traditional wooden barrel has been drummed out of service in the beer industry for practical reasons, in recent years there is a revived interest in wood maturation of beer [4]. Ageing of beer in wooden barrels may yield important organoleptic changes, leading to complex, tasteful beers with notes of wood or aromas from the previous beverage that has been matured in the barrel [3, 5, 6]. The precise organoleptic effects of wood ageing depend on many factors, including the type of wood, contact time, region of origin, and method of wood treatment, i.e. drying process and especially the degree of toasting applied during manufacture [7–9]. Furthermore, due to its porosity, the wood forms a semi-permeable barrier between the beer and the environment, allowing a tailored exchange of gases [10].

Oak is usually chosen by the barrel-maker as the preferable wood species due to its mechanical properties, permeability, contribution to characteristic aromas, and usage tradition [11]. To a lesser extent other wood types like chestnut (*Castanea*), cherry (*Prunus*), acacia (*Acacia*) and walnut (*Juglans*) are used. Today, the beer industry most of the time reuses barrels from wine and whisky industry, where barrels may be used only once [3]. Furthermore, barrels used for beer ageing are often reused for maturing the next batch of beer [3]. Most of the time, barrels are reused until the barrel no longer significantly contributes to the aroma and flavour profile of successive batches [11, 12]. The practice of reusing barrels has both advantages and disadvantages. Major advantages include

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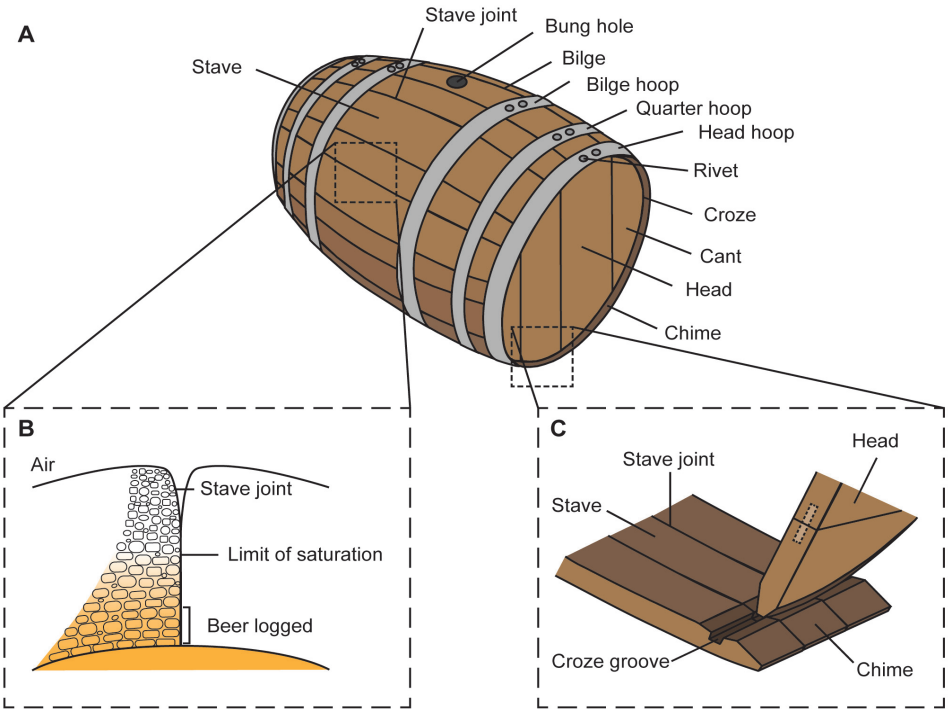

Fig. 1 **Wooden barrel anatomy (A) with close captions onto the positions that are the most challenging to clean and disinfect. These positions are joints between the staves in the barrel's body (B) and the joint between the croze groove and the barrel's head (C). As beer penetrates the wood, it transfers along microbes which afterwards become inaccessible for many physical or chemical agents while cleaning and sanitising the barrel**

lower purchase costs of used barrels, as well as the fact that flavours from the beverage previously matured in the barrel can be imparted into the beer [3]. One of the most important disadvantages of reusing barrels is potential microbial contamination which can affect the quality of the end product through undesirable souring and/or development of off-flavours, resulting in significant economic losses. Wooden barrels represent a suitable habitat for diverse microorganisms, including fungi and bacteria that have developed and remained in the barrels after previous usage [13]. Indeed, while new barrels generally do not harbour many fungi and bacteria [14], the interior surfaces of used barrels are often loaded with several microbes, both at the surface and deeper in the wood, especially in the joints, cracks and crevices of staves [15] (Fig. 1). It is known that throughout maturation certain microorganisms may have penetrated the wood, sometimes even up to 1.2 cm depth [16], which may contaminate the next maturing beverage. These microbes are of great concern for the brewing industry, particularly if they are associated with spoilage and contaminate new batches of beer after refilling.

The yeast *Brettanomyces* is one of the most important undesirable microorganisms that can survive and thrive inside wooden barrels. *Brettanomyces* yeasts are generally associated with spoilage in winemaking [17], although they also occur during barrel ageing of beer [18]. *Brettanomyces* spp. produce acetic acid under aerobic conditions, as well as the rancid-smelling isovaleric acid, and acetyltetrahydropyridine (ATHP) and 2-ethyltetrahydropyridine (ETHP), which are associated with mousiness in wine [19]. Furthermore, the yeast produces volatile phenols (4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG)) that can negatively impact wine and beer quality. Depending on their concentration the resulting flavours are described as "phenolic", "leather", "horse sweat", "stable", "smoked", and "bacon" [20–24]. Nevertheless, the same compounds are considered essential contributors to the flavours of lambic [25], American coolship ale [26] and various Belgian red-brown acidic ale beers [27]. Other microorganisms that often inhabit wooden barrels include acetic acid bacteria (AAB) [28, 29] and lactic acid bacteria (LAB) [30]. Acetic acid bacteria of the genera *Acetobacter* and *Gluconobacter* are the most frequent contaminants of barrel-aged beer. They can spoil beer through the oxidation of ethanol to acetic acid, providing harsh sourness, effectively transforming beer into vinegar [31]. Lactic acid bacteria, with *Lactobacillus* and *Pediococcus* as the most commonly reported genera in finished beer, produce lactic acid, and show high degrees of ethanol and hop tolerance, by which they can thrive well during barrel ageing and contribute to beer spoilage [32]. *Pediococcus* spp. are also known to cause beer "ropiness", a temporary flaw due to the production of exopolysaccharides [33] leading to more viscous beer, characterised by the formation of strands in extreme circumstances. Nevertheless, despite their spoilage-causing capabilities, just like *Brettanomyces* spp., AAB and LAB are generally desired in traditional sour beers [30, 34]. In other beer production processes, they can lead to major economic losses and inconsistent quality of the final product [35].

Therefore, effective cleaning and sanitation of barrels are essential when wooden barrels are reused to limit microbial contamination [15] and avoid undesirable flavours in the final beer. Cleaning involves the removal of organic and inorganic deposits from the barrels, which may contain microbes or promote microbial proliferation, and is usually performed with mechanical actions or by chemical means [36]. Sanitation refers to the reduction of the microbial load to acceptably low numbers [37], and efficacy can depend on numerous parameters such as the sanitation method, contact time, and the presence of remaining materials on the wood surface to be treated. Additionally, sanitation efficacy is affected by the age of the barrels, the contamination degree and wood surface defects such as blisters or cracks [15]. Generally, proper cleaning enhances the efficacy of subsequent sanitation. Sanitation is especially important in the maturation of beverages with a low to intermediate alcohol content (< 8 % ABV), as several barrel-associated microorganisms are able to grow under these conditions. Moreover, both LAB and AAB have been shown to be tolerant against higher ethanol percentages (> 10 % ABV) [32, 38–40], while tolerance up to 15–16 % ABV has been reported for some *Brettanomyces* strains [41].

Table 1 Summary of physical and chemical cleaning and sanitation methods for wooden beer barrels

Method		Mode of antimicrobial action	Affected microbes[a]	Penetration depth[b]	Impact on wood volatiles concentration	Cost and ease of use	References
Physical method							
	High pressure and hot water	Thermal inactivation, mechanical removal	*Brettanomyces* spp., AAB[c] (presumably LAB[d])	Interior surface and subsurface (upon longer treatment times)	Decreases key volatiles	Expenses for energy production, water readily available, safety measurements needed	[15], [52-56]
	Steaming	Thermal inactivation	*Brettanomyces* spp.	Interior surface and subsurface	Decreases key volatiles	High investment costs (steam generator) and associated production costs, safety measurements needed	[55], [57-62]
	Ultraviolet radiation	Irreversible DNA damage (dimerisation of adjacent thymines)	*Brettanomyces* spp., AAB[c]	Interior surface	-[e]	High initial investment costs	[64-67]
	Dry ice	Thermal inactivation, mechanical and expansive impact	*Brettanomyces* spp., LAB[d]	Surface and progressively deep into the wood	Increases in eugenol and cis- and trans-oak lactonesf	Expensive instruments, safety measures for ears needed	[69-71]
	High power ultrasound	Inactivation by high localised temperatures and pressure	*Brettanomyces* spp.	Interior surface and subsurface	-[e]	Expensive equipment, high investment costs	[60], [73], [74-80]
	Shaving and re-toasting	Thermal inactivation, mechanical removal	*Brettanomyces* spp., AAB[c], LAB[d]	Interior surface and subsurface	Decreases in 4-EP and 4-EGg	Inexpensive, ease of use	[3], [82-85]
Chemical method							
	Sulphur dioxide	Chemical reaction with nucleic and fatty acids in the cell, causing cell lysis	*Brettanomyces* spp., AAB[c], LAB[d]	Interior surface and subsurface	Unchanged, prevents oxidation (gaseous SO_2, can diminish extraction of volatiles (aqueous SO_2)	Inexpensive, ease of use, safety measurements needed	[59], [86-92], [94], [96-99]
	Sodium percarbonate and peracetic acid	ROS[h] formation inactivates enzymes and lipoproteins in cell membrane, causing cell lysis	*Brettanomyces* spp., AAB[c], LAB[d]	Interior surface and subsurface	Decreases in key volatiles concentration	Inexpensive, ease of use	[15], [62], [100-105], [107]
	Ozone	ROS[h] formation inactivates enzymes and lipoproteins in cell membrane, causing cell lysis	*Brettanomyces* spp., AAB[c], LAB[d]	Interior surface	Decreases gentisic acid	Expensive, specialised equipment needed, safety measures needed	[50], [108-115]

[a] Affected microbes for which studies are available
[b] Interior surface (0-4 mm) and subsurface (4-8 mm)
[c] Acetic acid bacteria
[d] Lactic acid bacteria
[e] Not addressed
[f] In wine
[g] 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG)
[h] Reactive oxygen species

The desired outcome of sanitation is cell death, an event whereby normal cellular functions, including respiration, metabolism, cell growth and proliferation are terminated [42]. To this end, high temperatures and pH-lowering treatments are often applied. In general, temperatures of 50 °C and above have profound effects on the structural and physiological properties of microorganisms, with membranes, RNA, DNA, ribosomes, proteins and enzymes all being affected [43, 44]. Likewise, changes in pH can lead to cell death through the disruption of pH homeostasis, cell membrane integrity and fluidity, metabolic regulation, and macromolecule repair [45–47]. Many methods applied, including several that are discussed in this review, rely on lowering of the pH, which may be particularly effective against yeasts and fungi [48, 49]. In barrels, the joints between the staves in the barrel's body and the head are the most challenging positions to clean and disinfect [50] (Fig. 1). Furthermore, the presence of precipitated deposits on the inner surfaces of the barrels complicates cleaning and sanitation. Numerous methods for barrel cleaning and sanitation for wine production are available and have been recently reviewed [15]. Thus far, and to the best of our knowledge, no systematic overview exists that provides a comprehensive summary of barrel sanitation methods for maturation of beer. In this review article, we present an integrated list of physical and chemical cleaning and sanitation methods and discuss their efficacies, advantages and disadvantages (summarised in Table 1). Furthermore, we indicate a number of areas that merit future research.

2 Physical cleaning and sanitation methods

2.1 Hot water and high-pressure hot water treatment

The most common cleaning technique is rinsing with hot water and high-pressure hot water (HPHW) treatment, since water is readily available in the breweries. Furthermore, it dissolves the beer stone (a precipitate of calcium oxalate and organic material that deposits on the barrel surface) [51], while leaving behind no chemical residues [52]. Nevertheless, from a safety perspective, it has to be noted that its use necessitates great caution due to the high temperatures involved. Furthermore, production of energy to heat the water is relatively expensive, and often lengthy treatments have to be employed. Barrels are filled with hot water either by static or rotating spray heads under variable temperature and pressure conditions, ranging from 60 to 90 °C and from 0.1 to 70 bars, respectively. Sanitation efficacy is dependent on the applied temperature and pressure, in combination with the treatment time and degree of contamination [15]. To prevent excessive growth of spoilage organisms as well as drying out of the barrels which would compromise their integrity [3], it is recommended to apply hot water and HPHW treatment as soon as possible after the vessel has been emptied. Both hot water treatment and HPHW treatment have been shown to be effective for decreasing the number of bacteria that occur on the wood surface, like AAB [53], when hot water of 85 to 88 °C is applied for 20 minutes. In the case of wood-penetrating microorganisms, like *Brettanomyces* yeasts, however, its effectiveness seems to be dependent on the depth at which the microorganisms occur and the sampling protocol used to evaluate the treatment's efficacy. Sampling can be performed at the surface of the barrel only (non-destructive sampling) or deeper in the wood (destructive sampling) [15]. A treatment with hot water at 60 °C for 19 minutes was found to reduce presumptive *Brettanomyces* populations in previously used barrels with more than 8 log units as determined after rinsing the barrel with sterile peptone water and plating on Dekkera/*Brettanomyces* Differential Medium (DBDM) [54]. A more recent study using a destructive sampling method (sawing staves into cubes) in combination with plating on DBDM [55] revealed that *Brettanomyces bruxellensis* could not be recovered from cubes heated in water at 70 °C for 20 minutes, or 80 °C for 15 minutes when the yeast was present in oak at depths of ≤ 4 mm. Longer heating times (70 °C for 30 minutes or 80 °C for 20 minutes) were required if *B. bruxellensis* was present at depths of 5 to 9 mm within cubes made from staves [55]. A drawback of these hot water based methods, however, is that they may exert negative effects on the amount of volatile compounds released from the wood after refilling the barrels. It has been found that a hot water treatment at 82 °C as short as 5 minutes resulted in a significant decrease of key oak volatile concentrations, mainly furfural and 5-methylfurfural [56].

2.2 Steam treatment

Steam treatment is another frequently employed technique within the beer industry to sanitise wooden barrels, as well as filters, hoses and tanks. Steam is generated by a pressurised electric generator, which significantly decreases water usage compared to HPHW, although it is accompanied with higher costs, i.e. for the hot water generator and associated electrical power. As with hot water treatment, its handling needs to be taken with care. For barrel sanitation, steam is generally blown through the bunghole into the barrel for 10 to 30 minutes. Nevertheless, longer steaming periods have been shown to provide improved sanitation [57]. In order to have sufficient sanitation efficiency during steaming of the barrels, it is important that the treatment time is long enough so that the heat can penetrate deep enough into the wood to kill the wood-inhabiting microbes. Wood has a low thermal conductivity, which may protect the microorganisms from the heat [58], generating so-called "cold spots" where microbial cells are not completely inactivated [55]. Steaming for 5 and 10 minutes resulted in a temperature of 47.4 °C and 57.5 °C, respectively, at a depth of 8 mm, which was found to be enough to kill *B. bruxellensis*, suggesting that both protocols may be sufficient to sanitise wooden barrels [59]. However, it has to be noted that barrel steaming generally proves to be inadequate to eradicate cells from the sides of stave surfaces, and the joints between staves [60]. This problem can be avoided by dismantling the barrel into staves and steam them as separate units. Nevertheless, this is a time-consuming and labour-intensive procedure. Furthermore, it may compromise the integrity of the barrel upon reassembly. Previous research has shown that when staves are steamed, a temperature as high as 75 °C can be achieved after 8 to 9 minutes in the staves at a depth of 9 mm, whereby *Brettanomyces* was successfully eradicated [61]. Similarly to HPHW treatment, steaming generally lowers the concentration of furfural, 5-methyfurfural and phenolic oak volatiles [62]. Despite the availability of numerous studies, more research is still needed to elucidate the efficacy of steaming, not only for yeasts like *Brettanomyces*, but also for AAB and LAB.

2.3 Ultraviolet irradiation

Irradiation with ultraviolet (UV) is a disinfection technique with a variety of applications, such as air disinfection, surface disinfection and liquid sterilisation [63]. The most effective wavelength range to kill off microorganisms is located between 200 and 280 nm, so-called "short wavelength UV light" (UV-C), with a maximum effect at 254 nm [63]. Microorganisms are inactivated by UV light as a result of damage to nucleic acids by dimerisation of adjacent thymine molecules [64]. As a result, the microorganisms are unable to perform vital cellular functions, including reproduction [65]. However, microorganisms differ in sensitivity to UV light, with yeasts generally being more resistant to UV irradiation than bacteria [66]. Although this technique is already commonly used in diverse applications such as the food industry and medical facilities, creating sterile workplaces or for sterilising waste and drinking water, so far, only little is known about its efficacy for barrel sanitation. A study by Guzzon and colleagues (2017) showed that UV irradiation was the least effective sanitation treatment tested in barrels, including treatments like steam and ozone. On average, only 35 % of the plate-culturable *Brettanomyces* spp. and AAB present in the barrels were eliminated after a 30 minute treatment with a 36 W UV lamp. This is probably due to the porous nature of wood, which shields cells from direct radiation. Therefore, UV radiation is most probably only useful to kill surface-located microbes [67].

2.4 Dry ice blasting

Dry ice blasting is an innovative barrel sanitation technique that

is already used in the food industry for the removal of residues in food containers and metal ovens [68]. With this technique, dry ice granules (CO_2) at – 78.5 °C are used as a blasting medium to remove solid residues from the barrels from which the lid has to be disassembled. The process is based on a combination of three effects, including thermal, mechanical and expansive effects. Upon impact, the thermal effect shows up when the CO_2 pellets immediately cool and embrittle the wood surface. Next, the mechanical effect comes into play and is attributed to the kinetic energy of the CO_2 pellets, and finally the expansive effect is based on the sublimation of CO_2 [69]. The grains of dry ice sublimate during impact, leaving only detached material behind, which has to be removed from the barrel surface. This makes dry ice blasting an environmentally friendly technique that creates very little waste and no chemical residue. A study by Costantini and colleagues (2016) showed that dry ice blasting of contaminated oak wood surfaces resulted in a 97.8 to 100 % reduction in *B. bruxellensis* and *Lactobacillus brevis* as determined by the contact plate method [70]. However, contact plates are known to result in poor recovery rates for porous materials like wood, and mainly sample surface microbes. This study also showed that dry ice blasting has a positive influence on the organoleptic properties of wine matured in the barrels after treatment. Specifically, dry ice blasting enhanced the aromatic tones of wood and vanilla, attributed to higher eugenol and *cis*- and *trans*-oak lactones concentrations [70]. This is most probably due to the fact that the blasting also removes thin wood layers (0.5 – 0.8 mm) which exposes the underlying toasted wood to the barrel content [70]. As a downside, dry ice blasting requires special emphasis on labour safety. For example, dry blasting generates sound pressure levels of 70 to 110 dB, thus ear protection while handling the dry ice is needed [71]. Further, the generation of the granules requires special equipment which represents high initial investment.

2.5 High power ultrasonics

High power ultrasonics (HPU) is a technique in which electrical energy is converted into ultrasonic sound waves (20 kHz to 10 MHz), which fall outside the range of human hearing (16 to 20 kHz) [72]. When formed in liquid, HPU forms cavitation bubbles which generate high localised temperatures (> 5000 °C) and pressure (order of 50 MPa) upon collapse [73, 74]. The exact physicochemical mechanism by which microorganisms are inactivated by HPU is not yet fully understood. However, it is generally believed that its activity results from the cavitation that causes mechanical effects leading to cell wall and membrane damage. Furthermore, cavitation can lead to sonochemical reactions, which may give rise to the production of antimicrobial hydrogen peroxide [75]. Several studies have already demonstrated that HPU is effective for the killing of different microorganisms, especially yeasts [76, 77]. However, there is a clear difference in effectiveness for different microorganisms. In bacteria, the technique is especially effective against gram-negative bacteria and to a lesser extent against gram-positive bacteria. A possible explanation is that gram-positive bacteria have a thicker peptidoglycan layer, which makes them more resistant to HPU [78, 79]. The same could be said for yeasts, where the composition of the cell wall may differ significantly between different species [75]. In the study by Schmid and colleagues, complete removal of culturable *B. bruxellensis* cells was shown in surface (0 – 2 mm) and subsurface samples (2 – 4 mm), obtained after 12 minutes of HPU exposure at 50 °C and 60 °C, respectively [80]. Additionally, it was demonstrated that HPU impacts *B. bruxellensis* even up to a depth of 9 mm in contaminated oak wood with processing parameters set at 60 °C for 6 minutes at 3.8 kW [60]. With regard to wood properties, it was found that HPU does not have any adverse effects on the extractable components of the wood [80]. However, despite these promising results, additional research is required regarding its efficacy as a sanitation method for the purpose of beer ageing.

2.6 Barrel shaving and/or re-toasting

Barrel shaving is a technique that entails the removal of approximately 5 to 6 mm of the barrel interior surface by shaving, planning or routering disassembled barrels [81]. It is a centuries-old technique, which is currently receiving renewed attention in the brewing industry and beyond it. Barrel shaving is mainly applied when most of the aromatic components have already been extracted from the wood and one wants to expose the next batch of beer to a new wood surface [3]. However, as the toasting effect of toasted barrels reduces with wood depth, shaving often necessitates re-toasting to re-establish the desired toasting degree [3]. Nevertheless, although this method may prolong the lifespan of a barrel, the extractive characteristics are often not identical to new barrels. Advantageously, the removal of affected wood also removes microorganisms that occur on and in the scraped wood, thereby reducing the risk of contamination [50]. The re-toasting also contributes to the microbiological control of the vessel. Depending on the cooperage, during the process of toasting the vessel reaches a temperature from 150 to 250 °C that will kill most of the remaining microbes [82, 83]. This was also indirectly concluded in the study of Pollnitz and colleagues (2000) where decreased levels of 4-EP and 4-EG were observed after wood shaving and re-toasting, which are the main volatiles produced by *B. bruxellensis* [84]. However, there are also obstacles when applying this technique. Wine, beer or other beverage residues that remain in the barrel after the surface has been shaved, are toasted along with it and can cause undesirable aromas and off-flavours afterwards [85]. In addition, there is a danger that the integrity of the wood structure may be destroyed or damaged which can cause leakage.

3 Chemical sanitation methods

3.1 Reducing agents – sulphur dioxide

Usage of sulphur dioxide (SO_2) is probably the most widely applied method in the brewing industry to protect and preserve wooden barrels against microbial growth since it is inexpensive and easy to use. There are two ways SO_2 is used, including burning of elemental sulphur and direct gassing by pressurised SO_2. Burning sulphur reacts with oxygen to form SO_2 until all oxygen in the vessel has been consumed [86]. For dry storage of the barrels, this sanitation method is commonly applied every 3 to 4 weeks and the amount of sulphur burned ranges between 5 and 20 g per 225 L barrel [59, 87]. Sulphur dioxide is an antimicrobial agent and has an inhibitory effect on diverse microorganisms, among which are the most common beer spoilage organisms (*Brettanomyces* spp., AAB and LAB) [88]. It exerts its microbiocidal activity by

crossing the microbial membrane and disrupting the activity of enzymes and other proteins in the cell by changing the pH of the cytoplasm [89, 90]. Furthermore, it chemically reacts with nucleic acids [91] as well as fatty acids [92] in the cell, thereby killing the cell. Additionally, SO_2 initiates cell death processes by depleting the intracellular pool of ATP, decreasing the cytoplasmic pH and interacting with NAD^+/$NADP^+$ [93]. As an alternative, SO_2 can be applied in solution during wet preservation of the barrels. In this case, the barrels are filled with a (cold) water solution of 200 mg/L of potassium metabisulphite ($K_2S_2O_5$) acidulated with 3 g/L of citric acid [17]. A major disadvantage of wet preservation of barrels is that desirable wood aroma compounds are generally depleted [3, 94], which is not the case when SO_2 is used in the gaseous form [62]. Sulphur dioxide can also react with oak wood constituents and form lignosulphurous acid, from which hydrogen sulphide may be released, reminiscent of rotten egg. The hydrogen sulphide in turn can react with pyrazines in toasted wood to form musty smelling thiopyrazines [95]. In addition to its antimicrobial action, SO_2 has an antioxidant effect. In the presence of catalysts it binds with dissolved oxygen and inhibits the action of oxidoreductases that occur naturally in wood, such as tyrosinase and laccase, resulting in decreased chemical oxidation of phenolic and certain aromatic compounds [87, 96, 97]. On the other hand, in most beers the antimicrobial capacity of SO_2 is weak. For a typical beer, having a pH around 4 to 5 [98], most of the SO_2 occurs in a bound state causing loss of antimicrobial effectiveness [99]. Finally, caution should also be exercised when applying SO_2 since the gas is irritant to the eyes, nose and throat. Its usage hence requires adequate ventilation.

3.2 Oxidising agents-sodium percarbonate and peracetic acid

In some cases, wooden barrels are sanitised using oxidising chemicals such as sodium percarbonate and peracetic acid (PAA), both belonging to the group of peroxide-based compounds. Properties which have made PAA a preferable chemical for sanitation are its broad antimicrobial spectrum, ease of implementation, and its complete biodegradability into harmless products [100]. The efficacy of PAA treatment depends on factors like concentration, temperature, pH, and the amount of organic material [101]. A pH below 8.2 (which corresponds to its isoelectric point (pK_a)) will result in increased disinfection efficacy [102, 103]. Disinfection in aqueous solutions is based on the formation of reactive oxygen species (ROS), which cause a range of effects, such as the oxidation of sulfhydryl (–SH), disulphide (S–S), and double bonds in proteins, enzymes and other biomolecules. It also causes the disruption of the chemiosmotic function of the cytoplasmic membrane and transport through ruptured cell walls [103]. However, a major disadvantage associated with the use of PAA is an increased effluent organic content with high chemical oxygen demand (COD) [100]. So far only few studies have investigated the applicability of PAA to sanitise wooden barrels [104]. It was found that a 200 mg/L PAA treatment for seven days resulted in no detectable *Brettanomyces* cells by using plate cultivation. Although not investigated for wooden barrels, in stainless steel tanks PAA was shown to also have excellent sanitation efficacy against AAB and LAB, achieving complete sanitation [105]. However, examining the effect on the volatile composition of oak wood revealed that a 24-hour soak with 200 mg/L PAA significantly reduced the concentrations of key aroma compounds such as 5-hydroxymethylfurfural, 4-EG, eugenol, *cis*- and *trans*-isoeugenol, and 4-methylsyringol. The exact mechanism as to how PAA results in diminished volatile concentrations remains unclear so far [62], but may be due to enhanced volatile extraction due to lowered pH [6].

Perhydrate sodium percarbonate ($2Na_2CO_3 \cdot 3H_2O_2$) is another oxidising agent used for barrel treatment in both beer and wine industry. Applied concentrations of commercial products range from 1 to 1.5 g/L for undamaged normal barrels to a maximum dosage of 3 g/L for problematic barrels, i.e. barrels described as “gone-off” due to extensive use [106]. When dissolved in water, it causes similar cellular damage as PAA [107]. Manufacturers recommend a 24-hour soak with sodium percarbonate in cold or hot water to treat contaminated barrels. Hot water, however, causes a faster dissipation of oxygen. The antimicrobial activity of sodium percarbonate is dependent on the applied concentration, level of contamination, pH, temperature, and exposure time [15]. Despite its frequent application in wineries, surprisingly only very little is known about the efficacy of sodium percarbonate as a barrel biocide or a disinfectant in general. More research is needed in order to elucidate its antimicrobial effectiveness and to determine its influence on the wood structure and on the volatile compounds released from the wood.

3.3 Ozone

Because of recent technological progress through the development of ozone generators (based on dielectric barrier discharge [108]), the use of ozone (O_3) forms an attractive alternative to more traditional techniques for the microbial control of wooden barrels. Ozone is a triatomic oxygen molecule which is formed by the addition of free radical oxygen to molecular oxygen (O_2). Ozone has to be prepared on site and ozone generators that produce the free radical oxygen necessitate a lot of energy, which can be achieved using the corona discharge method which is rather expensive for small scale brewery operations [109]. It is a broad spectrum antimicrobial agent which is effective against a wide range of microorganisms, including bacteria, fungi, yeasts and viruses, as well as bacterial and fungal spores. Ozone inactivates microorganisms by the progressive oxidation of vital cellular components, causing irreparable damage to the fatty acids in the cell membrane, proteins and DNA [109–111]. Ozone oxidises sulfhydryl groups of amino acids of enzymes and polyunsaturated fatty acids to hydroxyperoxides [110], which ultimately leads to cell inactivation by cell lysis [109]. Ozone can be applied both as gas or in liquid form (dissolved in water), and the treatment is relatively simple and fast. Most importantly, as cell lysis is its main mechanism of action, microorganisms cannot develop resistance towards it [112]. However, due to its short half-life, ozone cannot be stored and must be produced on site. The half-life of ozone in distilled water at 20 °C is 20 to 30 minutes [113], and its degradation rate is dependent on environmental factors such as temperature, pH and organic matter in solution or COD [50, 111]. Gaseous ozone is more stable and has a half-life of approximately 12 hours in atmospheric air [114]. The half-life of ozone decreases with increasing temperature and pH. Nevertheless, previous research showed that gaseous ozone at 17 °C still has relevant biological

Box 1: Casestudy on synergestic effects of sanitation technqiues

We investigated the sanitation efficacy of the two sanitation techniques that are most commonly used in the Belgian brewing industry (unpublished survey), including pressurised hot water and the combination of pressurised hot water and sulphur dioxide (Fig. 2A). Experiments were performed using contaminated oak disks which had been in contact with a light blond beer (5.4 % ABV and 19.85 ppm iso-α-acids) for 40 weeks, and which had been stored at 4 °C for 50 days prior to sanitation. Besides re-used oak disks, new oak disks were included in the experiment, which were artificially inoculated with a number of wood-associated bacteria (*Acetobacter* sp., *Bacillus* sp., *Brevibacillus* sp., *Gluconobacter* sp.) and yeasts (*Brettanomyces bruxellensis, Debaryomyces hansenii, Saccharomyces cerevisiae, Saccharomyces pastorianus, Wickerhamomyces anomalus*) (Fig. 2A). To this end, the oak disks were soaked in a cell suspension of the test microorganisms at a concentration of 10^6 CFU/ml for each individual microbial species for one week before treatment. Four out of five re-used disks and newly inoculated disks were treated with pressurised hot water (80 °C, 3 bar, 30 s) (Fig. 2A). After drying the treated disks overnight, two out of four re-used and newly inoculated disks were submitted to an additional sulphur treatment by burning sulphur wicks in a closed container and leaving the wooden disks exposed to the sulphur gasses for one night (Fig. 2A). As a control, one re-used and one newly inoculated disk were included without prior sanitation. Next, all ten disks were mounted onto standard 60 L stainless steel vessels which were filled with light blond beer (5.4 % ABV and 19.85 ppm iso-α-acids, the same beer as was previously used for the re-used disks), mimicking real maturation conditions (Fig. 2A). The efficacy of each sanitation technique was evaluated by taking swab samples of the disk surface immediately after treatment. Each swab sample was then vortexed in 5 mL physiological water (0.85 % NaCl) for 30 seconds, followed by the plating of a 10-fold dilution series of each cell suspension on two media, including (i) plate count agar supplemented with 200 ppm cycloheximide, which was incubated both aerobically and anaerobically at 25 °C, used to evaluate the total number of aerobic and anaerobic bacteria, respectively, and (ii) yeast extract peptone glucose agar supplemented with 100 ppm of chloramphenicol, incubated aerobically at 25 °C, used to evaluate the total number of yeasts. Further, after two weeks of maturation of the beer, microbial growth was monitored by plating beer samples on the same media. Following centrifugation (3500 × g for 15 minutes at 4 °C), the cell pellet was dissolved in 5 mL physiological water and a 10-fold dilution series was plated. A pairwise Tukey t-test was performed to detect significant differences between treatments.

Results revealed that the microbial load after sanitation of the new oak disks has significantly decreased in terms of culturable aerobic bacteria, anaerobic bacteria and fungi for both sanitation methods (Fig. 2B). In contrast to the inoculated new disks, the overall microbial load on the surface of the re-used disks was quite low at the start of the experiment and remained low or became undetectable after sanitation. The effect of both sanitation techniques on re-used disks was only significant for culturable fungi. In this respect, it should be noted that the re-used disks were stored at 4 °C for 50 days between the two runs of beer maturation which may have had a substantial impact on the initial microbial load. Whereas swab samples provide a good indication of the microbial load on the surface of the wood, it is known that throughout maturation certain microorganisms may have penetrated the wood, sometimes even up to 1.2 cm depth [16], which may contaminate the next maturing beer. For this reason, beer samples were also analysed after two weeks of wood maturation, since the microbial count of those samples is influenced by both the microorganisms residing on the wood surface and the microorganisms that inhabit the wood at a larger depth. As shown in figure 2C, no significant differences were detected in the aerobic bacterial, anaerobic bacterial and fungal cell counts of the inoculated new disks. In contrast, the aerobic and anaerobic bacterial cell counts of the re-used wooden disks were significantly lower for the disks that were sanitised by pressurised hot water and the disks that were treated with pressurised hot water and sulphur dioxide. Additionally, these results indicate that even though no significant differences in bacterial cell counts were found at the surface of the treated and untreated re-used disks, the sanitation methods seem to have influenced the bacteria that resided inside the pores of the wood. Likewise, these results reinforce that swab samples of wood do not necessarily provide an accurate measure of the microbial load residing in the wood and caution should be taken as to not disregard the microorganisms that have penetrated the pores of the wood. Finally, these results indicate that there was no significant difference between the applied sanitation methods. No supplementary effect of sulphur dioxide was found on top of the effect of the pressurised hot water.

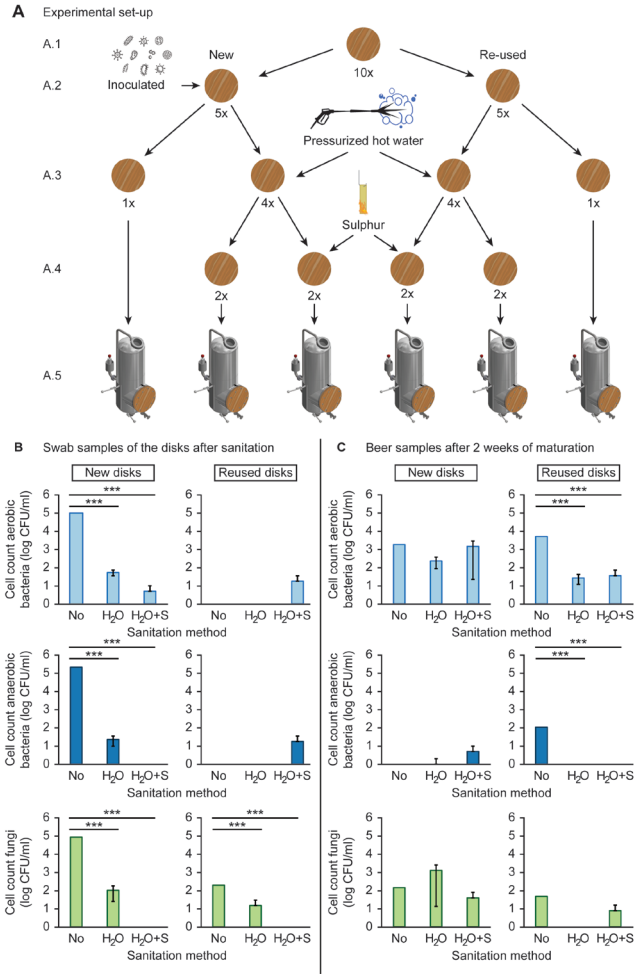

Fig. 2A **Overview of the experimental set-up**

Fig. 2B **Aerobic bacterial cell count, anaerobic bacterial cell count and fungal cell count from swab samples of the wooden disks, which were taken for each treatment, including (i) no sanitation, (ii) sanitation with pressurized hot water, and (iii) sanitation with pressurized hot water and sulphur dioxide**

Fig. 2C **Aerobic bacterial cell count, anaerobic bacterial cell count and fungal cell count from beer samples after two weeks of wood maturation using disks that were sanitized using different methods, including (i) no sanitation, (ii) sanitation with pressurized hot water, and (iii) sanitation with pressurized hot water and sulphur dioxide**

activity [67]. A study in which the effect of COD on the antimicrobial activity of ozone was investigated revealed that a COD equal to or more than 9 mg/L led to the immediate degradation of ozone after only 5 minutes, where it no longer had biological significance. In contrast, at a COD value between 0.01 and 1 mg/L, the aqueous ozone concentration remained around 2 mg/L after 20 minutes, assuring antimicrobial activity [115]. Therefore, to guarantee antimicrobial activity of ozone, it must be ensured that the water in which the ozone is dissolved has been stripped of organic components as much as possible. Additionally, in the same study the effect of ozone on extracted wood components was investigated by comparing wines aged with the addition of untreated wooden chips and gaseous ozone-treated wooden chips [115]. Out of 29 tested phenols, a statistically significant decrease was only observed for gentisic acid upon exposure to the ozone-treated wood. Gentisic acid is an aromatic carboxylic acid that is known for its anti-oxidising effect and is generally present in trace amounts [87]. Ozone could therefore be an effective alternative for the disinfection of wooden barrels if the environmental factors are taken into account. However, when handling ozone, it is important to bear in mind that it is a toxic gas which primarily affects the respiratory tract. The symptoms of ozone toxicity include headache, dizziness, burning sensation in the eyes and throat, and coughing [109].

4 Conclusions and future outlook

To conclude, in this review we presented an overview of diverse methods employed for the cleaning and sanitation of wooden barrels. Nevertheless, it remains difficult to answer the question which method is the best to use, as there is still a lack of data regarding the efficacy and economical aspects of several of the techniques discussed. Moreover, sometimes even contradictory results regarding efficacy are presented. A potential explanation of this stems from different sampling methods and different analysis methods applied. More general, there are numerous factors and variables that can influence the outcome of efficacy studies, including test organisms, natural or artificial wood contamination, destructive or non-destructive sampling methods, and culture-dependent or culture-independent analysis methods. The latter is particularly important as many microorganisms, including *Brettanomyces* spp., are known to enter a viable but non-culturable (VBNC) state [116], especially when subjected to physical and chemical stressors [117, 118]. The majority of the studies so far use culture-dependent techniques to evaluate sanitation efficacy, often using contact plates. However, contact plates are known to result in very poor recovery rates for porous materials like wood [119]. Thus it is predicted that they are not able to truly assess effects on microbes that occur in the deeper wood layers. This can be circumvented by using techniques that sample deeper in the wood, including wood shaving. As several microbes are not amenable to cultivation in laboratory conditions [120], culture-independent techniques may be used to investigate microbial presence before and after sanitation. However, it should be noted that DNA-based detection techniques cannot differentiate between living and dead cells [121]. Instead, methods targeting mRNA are better suited to measure living or active cells [123]. Furthermore, instead of focusing on particular microbial populations, deep sequencing methods enabling in-depth characterisation of microbial communities [122] can be used to evaluate effects on entire communities [14, 25, 124, 125].

Methods like ozone treatment are gaining increasing interest as sanitation method, and seem to exhibit great potential. However, high investment costs and the fact that there is still a lot of research needed to gain more insight into their sanitation efficacy might make brewers hesitant when it comes to implement ozone for barrel sanitation. In the meantime, it is likely that HPHW and SO_2 will remain the preferred option for brewers since they are not as expensive and have been shown to be quite effective to clean and sanitise barrels against main spoilage microbes like *Brettanomyces* yeasts, LAB and AAB. Although several techniques are available and most of them have been tested separately, it is reasonable to assume that they may exert synergistic effects when applied in combinations. This approach has already been shown to be useful in the food industry. For example, synergistic effects in killing the food-borne pathogen *Staphylococcus aureus* in oyster mushrooms were reported when pairing UV radiation with sanitisers, including ethanol, hydrogen peroxide and sodium hypochlorite [126]. Synergism was also observed between hydrogen peroxide and seventeen mineral and organic acids against several food-borne bacterial strains [127]. Finally, similar effects were seen for inactivation of free-living bacteria and treatments of biofilms in municipal water, where UV radiation yielded synergistic effects when paired with either hypochlorite, hydrogen peroxide, or peracetic acid [128]. We recently evaluated the sanitation efficacy of a combination of HWHP and SO_2 (Box 1), a combination which is commonly applied by brewers to clean and sanitise wooden barrels. Our results suggest that this approach is more effective than each of the methods on its own, which reinforce the potential of combining different methods to get synergistic effects.

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