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Use of New Methods for Hygienization and Sanitation of Wooden Barrels in the Alcoholic Beverages Industry

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Abstract

The study presents a review of the importance and implementation of some basic and new techniques to minimize pollution during the aging of wine and alcoholic beverages. Microbial contamination in the alcoholic beverage industry, mainly in aging environments, is often underestimated, more than 1 million unwanted microorganisms have been detected mainly in wooden barrels grouped into: yeasts, lactic acid bacteria, acetic acid bacteria and gram-negative anaerobic and anaerobic tolerant bacteria . The most dominant are: Brettanomyces_bruxellensis, Pediococcus_damnosus, Acetobacter.sp, Paenibacillus.sp. etc. that penetrate very easily into the porous structure of wood. Standard cleaning and hygienization schemes require the use of chemical preparations such as: Na_2CO_3 1% by spraying the walls at 40-50 °C, rinsing with water, sanitizing with chlorine-active substances, 700 mg/hl SO_2 or steam against mold, washing with alkaline materials 40-50ºC. New hygienization and sanitation techniques, such as the use of cold plasma, enzymes or ozone, aim to replace chemical preparations that damage wooden containers and affect the organosensory indicators of drinks during aging in them. These innovative treatments affect the deep cleaning of the barrels without any direct contact with the product. Cold plasma treatment is fast, facilitates the removal of stains from the surface of the barrel, accelerates the oxidation process, facilitates the decomposition of microorganisms such as bacteria and fungi, has no residual effects on wine and spirits. Minimizing product exposure reduces the risk of microbial contamination during the processing and aging of alcoholic beverages. Furthermore, cold plasma does not require chemical additives or adsorbents to be effective in killing microorganisms because it uses atmospheric oxygen as an oxidizing agent.

Keywords: hygienization, cold plasma, ozone, sanitation, barrels

1.Introduction

Cleaning is the process of removal (chemical or mechanical process) of mineral and organic waste that may contain microbial load or that promote the development of microorganisms degrading the alcoholic product, from the surfaces of containers and equipment. While sanitization is considered the reduction of the viable population of microbial cells. Cleaning removes at least 90% of microorganisms, sanitation is to destroy them and minimize their deposition in other places [1]. These complex biofilm deposits form quickly and are more tightly bound than biofilm alone [2]. Chemical cleaners are more valuable for eliminating bacteria attached to the surfaces of equipment than disinfectants, EDTA (ethylene-diamine-sodium-tetraacetate) component of the detergent improves the ability to break biofilms [3]. Depending on the type of microorganisms and the process in which they are involved, membrane disruption, macromolecule dysfunction and metabolic inhibition occur [4]. The use of cleaning products can lead to the production of unpleasant tastes, corrosion of the material, low rinsing and ecological risk [5]. Chlorine-based disinfectants have been shown to be very effective against yeasts, but since they are corrosive and may produce unpleasant odors, their use

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is controversial [6]. The effectiveness of sanitation procedures should be evaluated, and it should be ensured that if a surface is not cleaned satisfactorily, the problem can be identified and corrective action can be taken. [7] The use of wooden barrels is inevitable in the oenological industry and that of alcoholic beverages, Oak is usually chosen by the barrel-maker as the preferable woodspecies due to its mechanical properties, permeability, contributionto characteristic aromas, and usage tradition [8]. Aging of alcoholic beverages in wooden barrels is expected to bring important organoleptic changes. [9]. The impact on organosensory characteristics as a result of aging in wooden containers depends on many factors including: type of wood, contact time, pre-treatment of wood and barrels during production [10]. Since the wood is porous, it forms a semipermeable barrier between the alcoholic beverages and the outside environment, enabling respiration [11]. The wooden barrels are reusable until the new aromatic profile that we want to give to the alcoholic drink that ages in [12]. Reusing barrels can introduce a potential microbial contamination that can affect the quality of the final product, since wooden barrels represent a suitable habitat for the development of microorganisms, mainly fungi and bacteria that develope and remain in the barrel after use. [13]. These microorganisms, are grouped into lactic acid bacteria such as Lactobacillus and Pediococcus [14] or in acetic acid bacteria such as Acetobacter and Gluconobacter [15], and are mainly located on the inner surfaces of the used barrels, deeper in the wood, mainly in the joints, cracks and in the joints of the staffs [16]. During maturation some microorganisms can penetrate the wood (even up to 1,2 cm deepth) during the aging process it can contaminate the next beverage [17]. Brettanomyces spp. is one of the most important undesirable microorganisms that can survive and thrive inside wooden barrels it acts in aerobic conditions and produces acetic acid, isovaleric acid, acetyl-tetrahydro-pyridine, 2-ethyl-tetrahydro-pyridine, etc. which are related to mold in wine and undesirable odor and taste [18]. Hygiene is particularly important in the maturation of drinks with a low alcohol content of approximately 8% vol.a.a. Polluting microorganisms tolerate up to 10% vol a.a - 16% vol. a.a. [19]. The purpose of the study is to present a summary that highlights the importance of implementing some basic and new techniques to minimize pollution during the aging of wine and alcoholic beverages in wooden barrels.

2. Material and Methods

This paper refers to a literature review which highlights the need to use new eco-friendly methods to clean and sanitize the environments, equipment and containers used in the alcoholic beverage production industry. Also to group all the main techniques that are applied at different levels in the units of production and storage of alcoholic beverages. This is to come to the aid of Albanian producers in such a way that they have a choice to apply with the aim of cleaning and sanitizing wooden dishes, at a low cost.
Alcoholic beverages are easily affected, microbial touch will mainly bring high amounts of volatile acidity, deterioration of taste, this is seen by the presence of the compound tetrahydropyridine (derived from lysine and ethanol) produced by Brettanomyces spp, and Pichia strains in the maturation phase. S. cerevisiae, S. Pombe, D. bruxellensis, Z. bailii may produce phenols from phenolic acids, these volatile phenols are very dangerous at higher concentrations [20]. There are various sources of barrel contamination, such as from 2,3,4,6-tetrachloroanisole TCA and pentachloroanisole PCA, chlorophenols are biomethylated to the corresponding chloroanisoles by O-phenylmethylase. Wooden barrels can be remotely contaminated through the atmosphere if they are stored in environments where these contaminants are concentrated. [21]. Several types of treatments are used in wineries to disinfect barrels, such as chemical agents or physical agents, UV radiation, hot water, microwaves, ultrasound etc showing different levels of efficiency [22]. Microwave treatment enables only a 35% reduction of Brettanomyces spp. [23]. The objective of this review is to provide an overview of the sanitation methods used in alcoholic beverage production system from conventional techniques such as the use of sulfur dioxide and steam to alternative and new approaches using Cold Plasma, ozone and high power ultrasound.

2.1. An overview of the cleaning system in the beverage industry.

Cleaning chemicals are available in acidic and alkaline formulations. Alkaline cleaners are much more widely used, basic chemicals are more effective than acids in removing soils from the effect of alkaline hydrolysis with fatty acid compounds [24].

Table 1. Cleaning and sanitizing agents used. Ingredients in proprietary formulations are included when disclosed by producer.

Cleaning Agents	Dose	Sanitizing Agents	Dose
- Potassium hydroxide [KOH]	10 g/L	- Peracetic acid [C2H4O3] + hydrogen peroxide [H2O2]	0.1 g/L+10 g/L
- Potassium hydroxide [KOH] - Sodium hydroxide [NaOH] - Proprietary potassium caustic blend (85-90% potassium hydroxide [KOH]) - Proprietary sodium caustic blend (40-45% sodium hydroxide [NaOH])	20 g/L	- Peracetic acid [C2H4O3] -Potassium bisulfate	0.2 g/L~0.1 g/L
		[KHSO4] + Hydrogen peroxide [H2O2]	2.7 g/L+10 g/L
		- Potassium bisulfate [KHSO4] + hydrogen peroxide [H2O2]	5.4 g/L+10 g/L
		- Hydrogen peroxide [H2O2]	10 g/L
- Proprietary cleaner blend (6% sodium carbonate [Na2CO3],	170 g/L	- Proprietary quaternary ammonium blend (10% alkyl dimethyl alkyl benzyl ammonium chlorides)	2.0 g/L
- potassium carbonate [K2CO3], - alkyl dimethyl alkyl benzyl ammonium chlorides,	6%	- Chlorine dioxide [ClO2]	0.1 g/L
- ethylenediaminetetraacetic acid [C10H16N2O8],	4.9%	- Chlorine Dioxide [ClO2]	0.01 g/L
		- Chlorine Dioxide [ClO2]	0.005 g/L
		-Potassium bisulfate [KHSO4]	1.4 g/L~2.7 g/L
- 6.3% hydrogen peroxide [H2O2])	6.3%		
- Proprietary sodium alkaline detergent (sodium percarbonate [$C_2H_6Na_4O_{12}$]-based blend)	10 g/L	- Potassium bisulfate [KHSO4] + citric acid [C6H8O7]	2.7 g/L+ 20 g/L
		- Potassium bisulfate [KHSO4]	5.4 g/L
		- Citric acid [C6H8O7]	20 g/L
- Proprietary potassium alkaline detergent (potassium carbonate [K_2CO_3]-based blend)	20 g/L	- Proprietary iodine-based sanitizer (≤ 5% iodine [I2], ≤ 5% hydroiodic acid [HI])	1.5 g/L
- Proprietary alkaline bio-cleaner (blend of coconut fatty acid, coconut oil, palm oil, sodium benzoate [$C_7H_5NaO_2$], and organic silicone)	17.5 g/L		

Wood has a porous nature that allows liquids with all their components to penetrate, leaving behind deposits of organic and inorganic components, rinsing with water under pressure is insufficient to remove them. The cleaning cycle must be thorough or any subsequent attempts at sanitization will be rendered ineffective in an unclean barrel [25]. Barrel sanitization cycles aim to manage populations of yeast and bacteria inhabiting the barrel wood. B. bruxellensis is by far the most commonly studied and is variably quoted as capable of penetrating barrel wood at depths up to 6-8 mm, 4-8 mm, 8 mm, 9 mm [26]. Yeasts and bacteria are found in 12 mm depth in barrel wood [27]. A wide range of barrel sanitization methods have been studied, such as *Hot water, Steam, Ozone, High-pressure ultrasound, Microwave technology, and Peracetic acid* [28]. High temperatures ≤50 °C and treatments to reduce ph are applied to good sanitation, this have a profound effect on the physiological and structural properties of microorganisms, such as the impact on their membranes, DNA, ribosomes, proteins and enzymes. [29].

2.2. Specific treatments.

- ***Sulfur dioxide***: It chemically reacts with nucleic and fatty acids in the cell, causing cell lysis. Affects Brettanomyces spp., AAB, LAB; and acts on the inner surface (0-4 mm), underground (4-8 mm). Prevents oxidation (gaseous SO_2, can reduce emission of volatile substances (aqueous SO_2) [30]. The use of sulfur dioxide (SO_2) is the most used in the alcoholic beverage industry to protect and preserve barrels from contamination by microorganisms. SO_2 is used in two ways: combustion of sulfur and gas directly from SO_2 under pressure [31]. For dry storage of barrels, this hygienic method is usually applied every 3 or 4 weeks and the amount of sulfur burned varies between 3-9 g/100 L barrel [32]. SO_2 can be applied in solution during wet storage of the barrels, for this the barrels are filled with a water solution of 200 mg/L potassium metabisulfite ($K_2S_2O_5$) acidified with 3 g/L citric acid [33]. During wet barrel storage, desirable wood aroma compounds are depleted, this does not occur when SO_2 is used in the form gases [34]. SO_2 can react with oak wood components to form lignosulfuric acid, which can release hydrogen sulfide, giving off strong rotten egg odors, or it can form musty-smelling thiopyrazines [35]. In addition to its antimicrobial action, SO_2 has an antioxidant effect, binds to dissolved oxygen and inhibits the action of oxido-reductases present in wood, such as tyrosinase and laccase, resulting in the reduction of the chemical oxidation of phenolic and certain aromatic compounds. [32].

- ***The high power ultrasound (HPU)*** treatment process consists of filling the barrel with water (heated to 40, 60 or 80 °C by an autonomous system) and then inserting the sonotrode, to emit ultrasound waves, in the hole of the bump, thus allowing the water pressure inside the barrel (0.3 bar).This is a technique based on the conversion of electrical energy into ultrasonic sound waves (20 kHz-10 MHz), outside the range of human hearing (16-20 kHz) [36]. It is effective in killing yeasts, gram-negative bacteria and to a lesser extent gram-positive bacteria, since Gram-positive bacteria have a thick peptidoglycan layer, which makes them more resistant to HPU [37] HPU affects B. bruxellensis even to a depth of 9 mm in contaminated oak wood with processing parameters set at 60 °C / 6 minutes at 3.8 kW [36]. Regarding wood properties, it was found that HPU does not have any negative effect on the extracted wood components [38].

- *HPHW high pressure hot water treatment*: it is the most common cleaning technique, barrels are filled with hot water under variable temperature 60°C-90 °C) and pressure 0.1-70 bar, the hygiene of the barrels depends on the temperature and pressure applied, the treatment time depends on the degree of contamination [39]. It was observed that Brettanomyces bruxellensis could not be recovered if a temperature of 70 °C for 20 minutes or 80 °C for 15 minutes was applied even when the yeast was placed in the oak wood container at a depth of 4-9 mm. [40].

- *Steam treatment*: used to disinfect all surfaces in the processing unit. The sanitization of oak barrels with this technique is done by passing steam through the hole of the barrel for at least 10-30 minutes, as the treatment time increases, the best sanitization effect is achieved [41]. Steam treatment for 5 and 10 minutes at 47.4 °C and 57.5 °C, respectively, at a depth of 8 mm serves to disinfect the wood of the barrel, while the application of a temperature of 75 °C for 9 minutes reaches a depth of 9 mm, eliminating Brettanomyces spp. [42].

- *Ultraviolet radiation*: Ultraviolet (UV) radiation with a wavelength of 200-280 nm is widely used as a disinfection technique with a wide range of applications, such as air disinfection, surface disinfection and liquid sterilization. This technique acts on microorganisms by inactivating them as a result of nucleic acid damage by dimerization of neighboring thymine molecules, mainly affecting reproduction [43]. This technique is used in the cleaning of surfaces other than wood, because the porosity of wood protects microorganisms from direct radiation, UV radiation is only useful for killing microbial populations fixed on the surface of wooden containers [44].

- *Oxidizing agents*: Oxidizing agents: sodium percarbonate and peracetic acid: are agents used to disinfect wooden barrels, peracetic acid, PAA has a broad antimicrobial spectrum, ease of application and completely biodegrades into harmless products [45]. Depending on the concentration, temperature, pH and amount of organic material, the efficiency of PAA treatment is also evaluated, pH lower than 8.2 increases the disinfection efficiency, this technique for disinfection of wooden barrels is limited [27], treatment with 200 mg. /L PAA /7 days is found to damage Brettanomyces cells, the soaking treatment with 200 mg/L PAA reduces the concentrations of the main odor components in the wooden barrels, this may be due to the decrease in pH. Applied concentrations of sodium percarbonate perhydrate ($2Na_2CO_3 \cdot 3H_2O_2$) range from 1-1.5 gr/l for normal or undamaged barrels to 3 gr/l for problem barrels[46]

Figure 1. Cleaning, Detartrating and Disinfection of Barrels: After decanting, proceed and rinse using a high pressure machine and cold water, duration of steam cycle depending on the age and status of the barrel, proceed again and rinse with cold water.

2.3. New efficient alternative techniques for hygiene.

- *Ozone:* The use of Ozone (O_3) forms an attractive alternative to more traditional techniques for the microbial control of wooden barrels. O_3 is a broad-spectrum antimicrobial agent that is effective against a wide range of microorganisms such as bacteria, fungi, yeasts, viruses, bacterial and fungal spores. It inactivates microorganisms by progressive oxidation of vital cellular components, causing irreparable damage to cell membrane fatty acids, proteins, and DNA [47]. O_3 can be applied as a gas or dissolved in water, it has a very short storage time of no more than 20-30 minutes, its degradation rate depends on: temperature, pH and organic matter in the solution, negative ions are produced during treatment with a germicidal effect that protects the wooden container, the gas enters the pores and spaces of the network formed by the pores, the aromatic compounds are oxidized. O_3 is part of production environment disinfection protocols in cleaning soil and tartrates and disinfecting the interior surface of wood, but is insufficient to inactivate yeast and bacteria deep in the wood. If B. bruxellensis-derived phenolic character is detected in a barrel, winemakers will discard the affected barrel to limit the risk of spreading the infection [48].

- *Dry ice blasting*: This is a sanitizing technique being used in the food industry. Here, CO_2 dry ice at -78.5°C is used to remove solid waste from barrels and is based on three effects: mechanical, thermal and diffusion. The thermal effect occurs when the CO_2 grains cool down immediately and make the wood surface brittle, the mechanical effect is attributed to the kinetic energy of the CO_2 grains, and the diffusion effect is related to the sublimation of CO_2 [49]. During impact, the dry ice grains sublimate, detaching the material from the barrel surfaces for cleaning, this is an environmentally friendly technique as it creates chemical-free waste. It has been shown that dry ice blasting of contaminated oak wood surfaces resulted in a 97.8–100% reduction of B. bruxellensis and Lactobacillus brevis [50]. It has also been observed that blasting in dry ice has a positive impact on the organoleptic properties of barrel-aged wine, after treatment the aromatic tones of wood and vanilla increase, which is attributed to higher concentrations of eugenol and cis- and trans-lactones [51]. This is because blasting removes thin layers of wood (0.5 – 0.8 mm) [37].

- *Cold plasma:* Atmospheric pressure cold plasma (APCP) technology is considered as a valuable new alternative to solve the difficulties associated with the disinfection of wooden barrels. APCP is a non-equilibrium, ionized gas formed at ambient temperature and atmospheric pressure [51]. So far, reactive oxygen species "ROS" and reactive nitrogen species "RNS" are thought to be the main molecules responsible for the biocidal effect [52]. This technology consumes only compressed air and electricity to generate plasma that occurs at atmospheric pressure and room temperature, APCP does not produce toxic chemicals or waste, this technique is valuable to improve the microbiological quality of a wide variety of foods without changing their physico-chemistry. Its biocidal effect properties on food-borne bacteria, molds and yeasts have been seen [53]. APCP treatment on the surface of oak wood contaminated with Brettanomyces showed that it can be considered as an effective and sustainable alternative to burning sulfur for sanitizing the wood barrel [54]. The types and amount of ROS, RNS, UV

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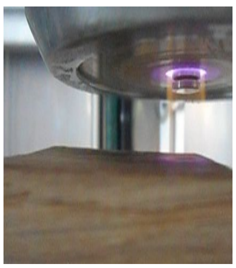
radiation, charged particles, electric field and heat and their synergistic effect have been suggested as the most likely cause for APCP-induced microbial inactivation [55]. In fact, it is known that the characteristics and concentrations of ROS and RNS generated during APCP treatments depend on the type of plasma gas used [52]. Several studies showed that –OH generated with APCP caused significant damage to the cell membrane of B. bruxellensis yeasts that directly affects their permeability and their osmotic balance. [55]. These alternative technologies have been studied to replace sulfur combustion, their effect is similar, but they have followed different paths [56]. Application of APCP in a 12-pass treatment with different plasma power (90 W and 500 W) and plasma gases (air, nitrogen and argon) resulted in total inactivation of yeasts after air and nitrogen plasma treatments, but have lower effect on bacteria. Reactive oxygen and nitrogen species generated during the plasma generation process appear to play a major role in microbial inactivation [57] With this treatment, morphological modifications on the surface of the wood have not been evidenced [55], which happens with the usual treatments with steam, SO_2 or with the treatment of the environment with solutions containing chlorine, which would lead to the damage of the wood layer and the formation of TCA, which leads to the deterioration of the quality and aromas of alcoholic beverages.



Plasma Gas	Gas flow	Plasma Power	Temperature Average
Argon	40 slm	90 W	33.7 ^{0}C
Air	60 slm	500 W	54.3 ^{0}C
Nitrogen	60 slm	500 W	53.0 ^{0}C

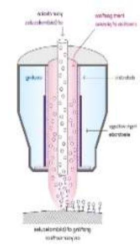


Figure 2. Plasma treatment process of one oak wood fragment and the condition of Plasma treatment [56]]

3. Results and Discussion

Sanitizers play an essential role in protection from bacterial contact or wild yeasts in alcoholic beverages. All cleaning and disinfection preparations are composed of active chemicals. All containers for storing and aging alcoholic beverages must be sanitized before use, among them wooden barrels, due to the porous nature of the building material, must go through special sanitization cycles. The hygienization cycles of wooden barrels aim to manage populations of microorganisms such as yeasts, bacteria and indigenous microflora that are fixed in the wood of the barrel.

The most used method is the use of SO_2; although Regulation 98/8/CE2 does not allow the use of this method. The amount of Brettanomyces in wooden barrels is also significantly reduced by high-pressure ultrasound treatment of barrels filled with hot water, but requires access to specialized equipment. High power ultrasound combined with heat at 60ºC/6 minutes has been shown to eliminate Brettanomyces yeast up to 9 mm deep in wood. The HPU was emitted inside the barrel at a frequency of 20 kHz, 3.8 kW. During HPU treatment the surface area of the wood and the kinetics of oxygen desorption can be affected, indicating that the tartrate has been removed, the wettability of the wood can also be affected, depending on the temperature and duration of exposure. The use of steam ensures a full degree of elimination of microorganisms on different surfaces. Dry steam is an effective means of killing Brettanomyces b., without the need to undergo chemical treatment. The process is carried out for a short time (15 minutes at a minimum temperature above 100ºC).

The new methods that are used for hygiene and that have a direct effect on the reduction or disappearance of the populations of microorganisms spoiling alcoholic beverages are treatment with ozone, cold plasma under atmospheric pressure (APCP) that have a direct effect on the disinfection of oak wood. The studies carried out have shown that these treatments affect the elimination of bacteria such as: Pediococcus pentosaceus and Acetobacter pasteurianus or Brettanomyces bruxellensis yeasts from wooden barrels (this is also related to the degree of their pollution). It has been concluded that the APCP treatment is a (low-cost) technology used for food disinfection without loss of organoleptic or physico-chemical properties, but improves the microbiological quality of a wide range of foods, of plant and animal origin.

All new treatments are shown not to have an impact on the degradation of the wood of the barrels, they have an impact on the reduction and disappearance of microflora, protecting not only the physico-chemical indicators of alcoholic beverages but also maintaining or improving their organoleptic aspect.

4. Conclusions

New hygienization and sanitation techniques, such as the use of cold plasma, enzymes or ozone, aim to replace chemical preparations that damage wooden containers and affect the organosensory indicators of drinks during aging in them. The use of steam ensures a full degree of death throughout the wine storage and filling system.

Special importance is given to the use of these methods to minimize the use of chemical preparations which, even if used in the environment, have an impact on wooden containers, creating TCA contamination. These innovative treatments affect the deep cleaning of the barrels without any direct contact with the product. Cold plasma treatment is fast, facilitates the removal of stains from the surface of the barrel, accelerates the oxidation process, facilitates the decomposition of microorganisms such as bacteria and fungi, has no residual effects on wine and spirits. Minimizing product exposure reduces the risk of microbial contamination during the processing and aging of alcoholic beverages. Furthermore, cold plasma does not require chemical additives or adsorbents to be effective in killing microorganisms because it uses atmospheric oxygen as an oxidizing agent.

The advantages of using a clean and eco-friendly technology for barrel sanitation include consistent and repeatable results with no chemical residue that reduces sulfur dioxide and taint-causing or hazardous chemicals while lowering labor, energy and barrel costs.

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