



# Pasteurization of Beer by Non-Thermal Technologies

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The pasteurization of beer occurs at the end of the industrial production, after fermentation. Generally, a mild thermal process (60°C) is employed aiming to inactivate the fermenting yeast and potential spoilage microorganisms, thus extending the beer shelf-life at room temperature. The heat treatment negatively affects the original beer freshness and flavor. In this study, beer pasteurization using emerging non-thermal technologies, namely, high-pressure processing (HPP), pulsed electric fields (PEF), and ultrasound (US), was reviewed, including the effect on microbial inactivation and beer quality. The combination of non-thermal methods with mild heat for more efficient pasteurization of beer was also reviewed. All technologies caused microbial inactivation in beer. However, room temperature HPP treatment was the most efficient method, delivering the minimum 15 PU (pasteurization units) to beer after seconds (e.g., 300 MPa for 27 s), as opposed to thermal and TS treatments which required several minutes, while causing a negative impact on beer sensory. As expected, PEF + heat caused a higher microbial inactivation than PEF alone, and yeast ascospores were more resistant than vegetative yeast cells. Non-thermal PEF (35–45 kV/cm) caused 3–5.8 log reductions in vegetative bacteria. Studies on thermal assisted PEF and ultrasound combined with low heat (50–55°C) showed processing times in the magnitude of microseconds for PEF pasteurization and 0.5–2 min (depending on the temperature) for TS pasteurization. With respect to impact of these technologies on beer quality, HPP, thermosonication (TS), high pressure homogenization (HPH), and dense phase CO<sub>2</sub> (DPCD) treatments revealed less effect on beer sensory properties, better retaining the freshness of original beer, compared to thermally processed beers.

**Keywords:** HPP, high pressure processing, PEF, pulsed electrical field, ultrasound, quality, microbial inactivation, yeast

## INTRODUCTION TO BEER PASTEURIZATION

Beer is an alcoholic beverage, which is produced by yeast fermentation of the sugar obtained from malted cereal grains (e.g., barley and wheat). A Sumerian tablet found in Mesopotamia dated 6,000 years ago is the oldest evidence of beer production (Mirsky, 2007; Nelson, 2014). Global beer production amounted to about 1.91 billion of hectolitres in 2018, from 1.30 billion hectolitres in 1998, which demonstrates beer as a high demand beverage in the world (Statista, 2020). The production of beer consists of four stages: malting in which barley (or other cereal) is converted to malt, mashing where hops are added and malt is converted to wort by boiling, fermentation of sugars

to ethanol (yeast pitching), and post-fermentation operations. The hops added during production are responsible for the bitter flavor and contribute to its natural preservation. The beer ingredients (water, cereal, hops, and yeast) can be combined in different ways to create different styles of beers such as ale, lager, stout, pilsner, etc. Ale and lager are the two major classes of beers: an ale beer ferments with top-cropping *Saccharomyces cerevisiae* at temperatures around 15–20°C, and a lager beer is fermented by bottom-cropping yeasts such as *Saccharomyces carlsbergensis* (*pastorianus*) or *Saccharomyces uvarum* at temperatures ranging between 8 and 13°C (Hardwick et al., 1995; Hornsey, 2003). Regions such as the Senne Valley in Belgium still use wild yeasts for spontaneous fermentation.

The fermenting yeast is the dominant microorganism present in beer. Regardless of the pasteurization method, existent microorganisms which survived the process can occasionally grow during storage and negatively affect the quality of beer. As opposed to pitching yeasts, wild yeasts are recognized as redundant yeasts throughout fermentation in beer production. The major groups of wild yeasts that can spoil beer belong to the genus *Zygosaccharomyces*, *Saccharomyces*, and *Brettanomyces*. According to Suzuki (2011), the microbial instability in beers is often caused by lactic acid bacteria of genera *Lactobacillus* and *Pediococcus*, and strict anaerobic bacteria belonging to genera *Pectinatus* and *Megasphaera*. Other spoilage organisms, particularly found in unpasteurized draft beers, are *Micrococcus* and *Zymophilus* bacteria, and moulds (Lawrence, 1988; Esmaeili et al., 2015).

The main post-fermentation operations of bottled or canned beer are clarification/filtration, filling, and pasteurization, which are followed by labeling, box packing, and palletizing. Keg beers might be pasteurized before the keg filling operation. Filtration is one of the oldest non-thermal methods employed by breweries before filling (bottling/canning). The shelf-life of filtered beer is much shorter in comparison to thermal pasteurized beers since some spoilage organisms still remain in the beer after filtration (Curtis, 1968). Then, a process of thermal pasteurization occurs in the industrial production of bottled beer, aiming to extend beer shelf life through the inactivation of undesirable spoilage microorganisms that will otherwise promote unwanted chemical reactions (Priest, 2003; Priest, 2006; Priest and Stewart, 2006; Wray, 2015). As beer also contains carbon dioxide and alcohol, and is made bitter with hops, all of which are natural antimicrobials, a mild thermal pasteurization is effective for its stabilization at room temperature (Silva and Gibbs, 2009) and to ensure instead of ensuring there are no issues with pathogenic microorganisms.

The term pasteurization comes from the scientist Louis Pasteur, who first used thermal pasteurization method, coincidentally to preserve beer. Since then, a variety of beverages and foods (e.g., fruit juices, and milk) have been preserved/stabilized this way to extend shelf-life. The intensity of a thermal pasteurization process is measured in pasteurization units or PU, where 1 PU is defined as 1 min treatment at 60°C (Baselt, 1958; Portno 1968; Tsang and Ingledew, 1982; Reveron et al., 2003; Reveron et al., 2005; Milani et al., 2015b). A 15 PU treatment is the minimum recommended for beer pasteurization,

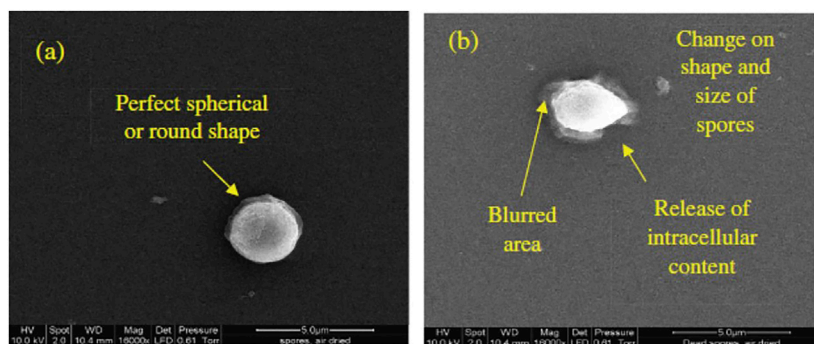
resulting in desired inactivation of spoilage yeasts and bacteria in beer (Del Vecchio et al., 1951; Portno, 1968; European Brewery Convention, 1995). However, the beer industry applies a more severe pasteurization process (e.g., 120–300 PU) to avoid spoilage due to on-going modifications in traditional beer composition such as alcohol-free and less bitter beers (Silva et al., 2014).

Since the conventional thermal process can negatively affect the beer flavor (Milani and Silva, 2016), emerging non-thermal pasteurization technologies like high pressure processing (HPP), pulsed electric fields (PEF), and power ultrasound (US) alone or combined with mild heating have been researched (Walkling-Ribeiro et al., 2011; Evelyn and Silva, 2015, 2016a, 2016b; Milani et al., 2015a; Sulaiman et al., 2015; Milani et al., 2016; Milani and Silva, 2016, 2017). The main objective of this work was to review the application of emerging non-thermal technologies to pasteurize beer, and the effect on beer quality parameters and key beer microorganisms.

## BACKGROUND ON CONVENTIONAL THERMAL PASTEURIZATION EFFECTS ON BEER QUALITY

Appearance (e.g., turbidity and haze), aroma and flavor, mouth feel, strength, specific gravity, and alcohol concentration are some of the beer quality parameters controlled in breweries (Eckhardt, 1989; Varnam and Sutherland, 1994; Ogle, 2007). The lightstruck character is a beer off-flavor defect caused by light. This phenomenon can be minimized using amber glass, bottles, or cans (Milani et al., 2015b). Changes in beer chemical composition during beer processing, ageing, and storage alter its sensory attributes (Vanderhaegen et al., 2006).

Craft beers produced in microbreweries are usually not pasteurized so that original properties of the beer produced are retained until consumption. However, this type of artisanal beer needs to be cold stored and distributed. Thermal treatment is the most common pasteurization method used in industrial large-scale breweries. As heat can have a negative impact on beer quality, some studies have reported the effect of thermal process on different quality characteristics of beer (Bernstein and Laufer, 1977; Vanderhaegen et al., 2003; Vanderhaegen et al., 2006; El Gharras, 2009; Lund and Andersen, 2011). Bernstein and Laufer (1977) concluded the formation of 2-furfural during beer thermal pasteurization is one of the most significant parameters causing beer staling and the loss of freshness. The presence of polyphenolic compounds is related to beer sensory characteristics, as these types of compounds are mainly responsible for the degree of bitterness or sweetness and mouthfeel in beer (El Gharras, 2009). Lima et al. (2009) revealed that thermal treatment dramatically affects the phenolic content of different foods which results to significant difference in sensory properties of the beer. Lund and Andersen (2011) concluded that the overall volatile profile of unpasteurized beers contained more fruity character and less staling volatile esters, suggesting that pasteurized beer has lower sensory acceptance than the fresh, non-thermally treated beer. Härnultv and Larsson (1992) studied the influence of thermal



**FIGURE 1** | Environmental scanning electron microscopy (eSEM) images of *Saccharomyces cerevisiae* free spores (a) untreated live (b) HPP treated dead (16,000 × magnification) (Reprinted from *International Journal of Food Microbiology*, Vol. 263, pg. 23, Siti N.M. Rozali Elham A. Milani, Rebecca C. Deed, Filipa V.M Silva, *Bacteria, mould and yeast spore inactivation studies by scanning electron microscope observation*, with permission from Elsevier).

pasteurization and membrane filtration on the quality parameters of Swedish lager beer and recorded a staling effect on the thermally pasteurized beers at high PU levels compared to filtered non-heated beer. The thermal pasteurization process can generate off-flavors, as beer is a delicate beverage. With respect to flavor, the possibility of using a method of pasteurization with no heat or less heat would be welcomed by the brewing industry (Folkes, 2004; Milani, 2016). The following sections explore alternative non-thermal methods for beer pasteurization.

## HIGH PRESSURE PROCESSING OF BEER

### High Pressure Processing Overview

High pressure processing (HPP) is one of the most significant innovations in food processing in recent decades. Pressures in the range of 200–600 MPa can inactivate microorganisms in foods and beverages (Figure 1). This technique retains the food's natural freshness and quality parameters such as aroma, color, and important food components while destroying the spoilage bacteria, yeasts, and moulds in beverages (e.g., beer, wine, juices) or solid foods. Consequently, HPP technology is a breakthrough in processing solid or liquid foods. Nowadays, HPP food processing is being applied on ever-increasing commercial basis (Balda, 2018). In 2019, a semicontinuous unit appropriate for beverages was designed by Hiperbaric, which was named HPP “in bulk” process, to differentiate from the original HPP “in pack” process (Hiperbaric, 2021). The HPP “in bulk” allows the use of any type of packaging material, as aseptic filling is carried out after the process. Additionally, the combination of HPP with heat allows inactivation of microbial spores which are resistant to HPP at room temperature (Evelyn and Silva, 2015; Evelyn and Silva, 2016a, 2016b, 2017, 2019; Uchida and Silva, 2017; Silva and Evelyn, 2018). The high pressure thermal assisted processing (HPTP) is not applied industrially, although there are several research studies using lab scale units (e.g., 2 L HPP vessel). Although non-linear inactivation of microorganisms by HPP or HPTP has been reported, generally microbial inactivation increases when one

instead of any of the three main processing parameters increases: pressure, temperature, or holding time (Farkas and Hoover, 2000; Silva et al., 2012; Milani et al., 2016; Milani and Silva, 2016; Silva and Evelyn, 2018).

Commercial HPP batch vessel volumes range in size from 30 to 600 L. A common process cycle first loads the vessel with pre-packaged product, then fills the rest of the vessel void space with water, which acts as the pressure-transmitting fluid. Then, the vessel closes, and the expected process pressure is obtained through adding water that is delivered *via* an intensifier. After keeping the product at the target pressure for the pre-determined time, the vessel is decompressed by releasing the pressure (Balasubramaniam et al., 2008).

There are several studies on the mechanism of microbial inactivation *via* HPP, which is the result of a combination of factors. The cell membrane is the primary site for pressure-induced microbial inactivation (Figure 1). Microorganisms are known as resistant to selective chemical inhibitors because of their capability to eliminate such agents from the cell, through the cell membrane. However, once the membrane is injured, this capability is lost. Furthermore, HPP leads to some alterations in cell morphology (Rozali et al., 2017) (Figure 1), protein denaturation, and biochemical reactions inside the cell. HPP denature major microbial enzymes and disrupt ribosomes (Linton and Patterson, 2000). Larger injury of the microorganisms results from quick alterations in intracellular-extracellular differences at the membrane interface, leading to higher inactivation rate (Palou et al., 1998).

Clearly, there are opportunities for innovative use and new food products (Ferrentino et al., 2015). According to Balda (2018), more than 300 HPP units were installed and operating in food industries by 2016, with an annual production ratio of ≥250 thousand tons. HPP processed products available in the food market are mainly fruit juices, pre-cooked meals like meat and fish, and other beverages and smoothies (Mújica-Paz et al., 2011; Buzrul, 2012; Balda, 2018; Houška and Pravda). HPP food and beverages have longer shelf life than untreated products, and can meet customer's quality expectations providing better taste and preservative-free healthier products (Nunes et al., 2017). At present time, one of the main disadvantages of this technology is

**TABLE 1** | Effect of HPP on beer quality.

Beer type	HPP pressure (MPa)	HPP time (min)	Beer quality	Reference
Pale lager beer	300	5	No changes in colour, foam stability haziness and aromatic compounds. Increase in haziness	Fischer et al. (1999)
	700	5		
Pale ale beer Mild ale beer	600	5	Color and permanent haze of pale ale beer were not affected by HPP as opposed to thermal pasteurized beer (60°C, 10 min). No effect on main chemical constituents of the beer	Castellari et al. (2000)
Lager beer Pilsner beer	500	5	No change in the turbidity	Fischer et al. (2002)
Lager beer	300	5	The bitterness of HPP beer is the same as untreated beer while thermal pasteurization increased the bitterness	Buzrul et al. (2005)
Ale beer Lager beer	600	0.5	No significant difference in the overall flavour of HPP and untreated beers	Milani and Silva (2016)
Cloudy wheat beer	600	5	As opposed to thermal treatment HPP retained the original colloidal haze of the untreated beer	Yin et al. (2016)
Lager beer	250	5	No change in the original beer extract, alcohol content, apparent extract, pH and color Higher foam stability during 2 months storage of the HPP beer compared to the untreated beer	Štulíková et al. (2020)
	550	5		

the high capital investment, limiting HPP application to high value products. Additionally, the fact of being initially designed in batch mode is not advantageous for beverages.

## High Pressure Processing Impact on Beer Quality

**Table 1** presents a chronological review of past studies of the effect of HPP on beer properties/quality. Although in a few reports information is missing, all treatments are expected to be non-thermal ( $T < 45^{\circ}\text{C}$ ). For example, Milani and Silva (2016) obtained temperatures  $\leq 36^{\circ}\text{C}$  for HPP compression times of 15, 26, 45, and 60 s for 200, 300, 400, and 600 MPa, and noted a higher increase of temperature for higher pressures (longer compressions). Fischer et al. (2006) applied HPP at 300 and 700 MPa for 5 min on mash, wort, and pale lager beer. Compared to an untreated mash sample, the content of dissolved protein in HPP treated mash increased as the pressure increased. Moreover, the fermentation degree dropped with the increasing pressure and no changes in pH value were registered. For the HPP treatment of wort the results revealed HPP treatment could increase the bitterness more than thermal treatment. The results on pale lager beer samples showed that 300 MPa HPP treatment for 5 min did not significantly change the color, aromatic compounds, and foam stability, while a 700 MPa treatment for 5 min increased the beer haziness. Pale ale and mild ale beers were submitted to 600 MPa treatment for 5 min by Castellari et al. (2000) and compared to thermally treated beer. Color and permanent haze of pale ale beer were not affected by HPP as opposed to thermally pasteurized beer. Also, there was no effect of HPP on the main constituents of the beer. Permanent haze

was more influenced by the stabilizing process in pale ale than in mild ale. Moreover, the results of HPP treatment (500 MPa and 5 min) on lager and pilsner beers that Fischer et al. (2002) carried out revealed no changes on HPP beer turbidity. Buzrul et al. (2005) concluded that HPP treatment of lager beer (300 MPa for 5 min) did not alter the bitterness of the original untreated beer, while thermal pasteurization increased the bitterness. This outcome emphasises the advantage of HPP compared to thermal processing with respect to a pasteurized beer flavor attribute. Recent studies of Milani and Silva (2016) also demonstrated no significant differences in the overall flavor of beers (ale and lager) submitted to 600 MPa for 30 s and untreated beers. In addition, preference testing confirmed no preference between the untreated and HPP treated beers. HPP processing at 600 MPa for 30 s (holding pressure phase) was performed to ensure a minimum treatment of 15 PU (equivalent to 15 min at  $60^{\circ}\text{C}$ ) recommended for beer pasteurization (Milani et al., 2015a). Likewise, preference tests confirmed no preference between the untreated and HPP-treated beers.

Yin et al. (2016) sensorial studies on cloudy wheat beer revealed that original beer colloidal haze was retained with HPP treatment (600 MPa, 5 min). Moreover, analysis by dynamic light scattering showed that HPP treatments at 500 MPa/10 min demonstrated more uniform and smaller particle sizes than untreated beer, that leads to a better haze stability during beer storage. Štulíková et al. (2020) analyzed the quality of lager beer after HPP treatment at 250 and 550 MPa for 5 min. Both beers presented higher foam stability for 2 months storage compared to untreated beer. The original beer extract, alcohol content, apparent extract, pH, and colour did not change



**TABLE 2** | HPP inactivation of yeasts and bacteria in beer.<sup>a</sup>

Beer type	Microorganism	Beer alcohol content (% alc/vol)	Processing pressure (MPa)	Processing time (min)	Log reduction	References
Lager beer	<i>Saccharomyces cerevisiae</i> ascospores	4.8	400	5	4.5	Milani and Silva (2016)
			300	5	3.2	
		7	400	5	5.2	
			300	5	3.0	
Model beer	<i>Lactobacillus plantarum</i>	5	300	5	2.0	Gaunzle et al. (2001)
		10	300	5	4.0	
Wheat beer	Total yeasts	4.9	600	5	5.6	Yin et al. (2016)

<sup>a</sup>The HPP processing temperature was  $\leq 25^{\circ}\text{C}$ ; thus, a non-thermal HPP treatment was applied in all studies.

with the HPP process at 250 MPa. The foaming characteristics and colloidal stability of beer can be enhanced with HPP (Pérez-Lamela et al., 2002).

Overall HPP of beer can better retain the original flavor of the beer than thermal pasteurization, although for certain beers and at very higher pressures ( $\geq 700$  MPa), the HPP can increase the beer haziness.

## High Pressure Processing Microbial Inactivation in Beer

As mentioned previously, different types of microorganisms could contaminate beer during the brewing process. HPP pasteurization treatment often uses pressures up to 600 MPa (87,000 psi) for a particular holding time (Farkas and Hoover, 2000; Anon 2006; van Wyk and Silva, 2017a; van Wyk and Silva, 2017b; van Wyk et al., 2018). A review of the studies of HPP inactivation of yeasts and bacteria in beer is summarized in **Table 2**. Yin et al. (2016) studied the total yeast count of wheat beer (4.9% alc/vol) after 5 min processing of beer at 600 MPa and recorded 5.6 log reduction. Milani and Silva (2016) studied the non-thermal HPP *S. cerevisiae* ascospores as the most heat resistant form of spoilage yeasts for inactivation of beer. After 5 min HPP processing at 300 and 400 MPa of 4.8% alc/vol beer, 3.2 and 4.5 log reductions of *S. cerevisiae* ascospores were recorded, respectively. The minimum pasteurization is achieved in both HPP processes, as 15 PU is equivalent to 1.34 log reductions in the strain used by Milani and Silva (2016). For 7.0% alc/vol beer, 3.0 and 5.2 log reductions were achieved, respectively. Gaunzle et al. (2001) also studied on the effect of HPP on the inactivation of *Lactobacillus plantarum* in different alcohol level beers. They used a model beer using 5 and 10% alcohol and concluded that ethanol enhanced the effect of pressure on the inactivation rate of *L. plantarum* to maximum 5 log reduction of *L. plantarum* at 300 MPa and 5 min of treatment time at room temperature.

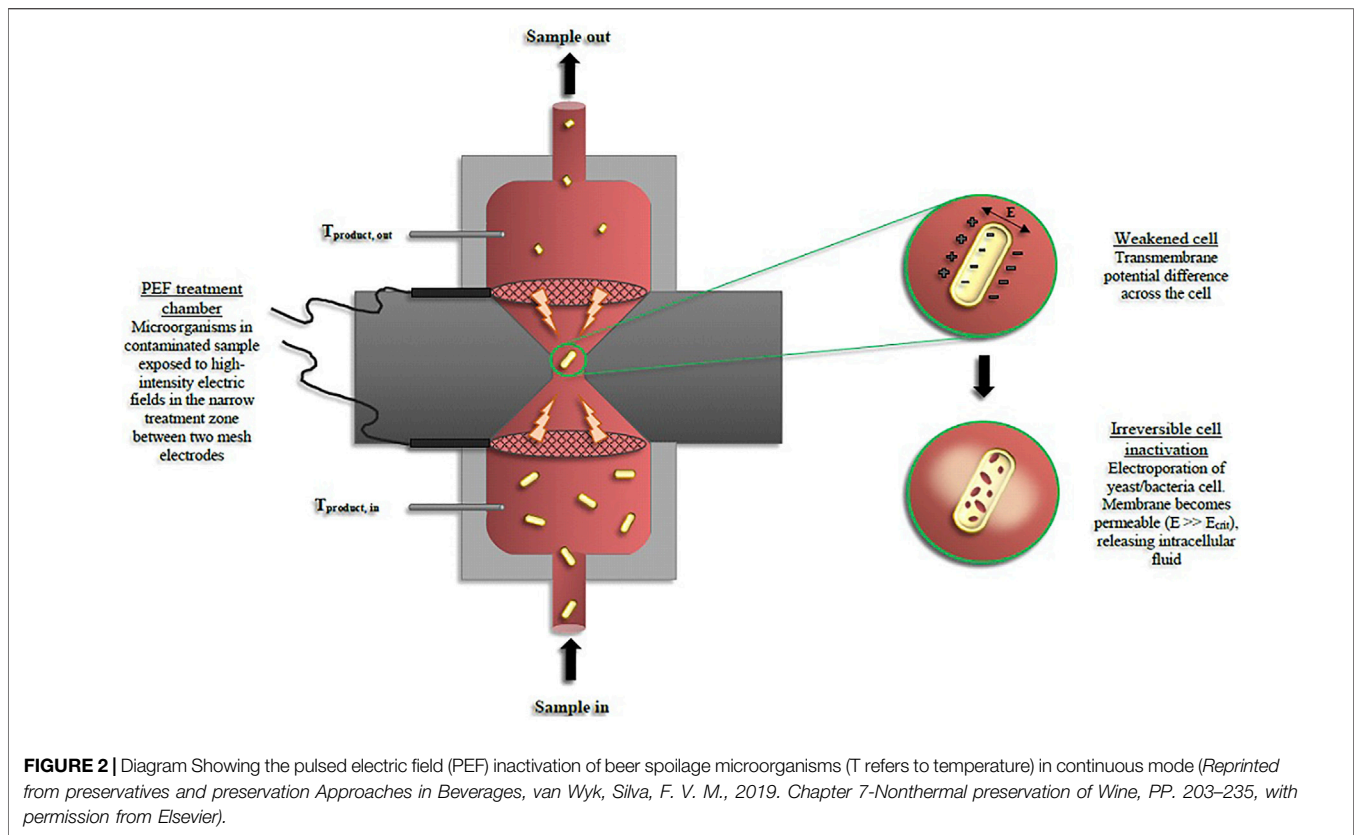
Referring to these results, HPP technology demonstrated an efficient method for beer pasteurization. The environmental scanning electron microscope (eSEM) images of *S. cerevisiae* free spores before and after being submitted to 600 MPa HPP showed the loss of the spore round shape (integrity) with

intracellular contents release, with viability loss confirmed by plating (Rozali et al., 2017).

## PULSED ELECTRIC FIELDS PASTEURIZATION OF BEER

### Pulsed Electric Fields Overview

Sale and Hamilton (1967) initiated research on pulsed electric fields (PEF) in the sixties. From 1990 to date, PEF was investigated broadly in studies that mainly focused on the PEF disinfection of water and pasteurization of liquid food (Toepfl et al., 2006). Having low processing temperature and extremely short residence time, in the magnitude of microseconds, PEF leads to effective microbial inactivation and retention of product quality (Sulaiman et al., 2017). The permeabilization of the cellular tissue by PEF in microseconds could be developed to replace or complement conventional thermal pasteurization techniques. There is a great potential for PEF application to beverages, namely, beer and wine, as PEF can be set in continuous mode (Milani et al., 2015a; van Wyk et al., 2019a) (**Figure 2**). A typical continuous PEF system is based on a high voltage pulse generator with a treatment chamber and a suitable beverage handling system (pump, tube), as well as controlling devices to monitor inlet and outlet temperatures, electric field intensity, pulse frequency, and duration. Liquid food product is pumped through the treatment chamber, mainly in continuous mode, where two electrodes can be placed with different configurations, according to the chamber design (e.g., parallel, coaxial, and collinear). The high voltage electrical pulses are conveyed to the electrodes, and an electric field is generated between the two electrodes. While flowing within this gap between the 2 electrodes (the treatment chamber), the liquid food is submitted to the pulsed electric fields generated. The electric field intensity is the ratio between peak voltage and the gap between electrodes. Note that the  $\text{CO}_2$  present in the beer does not conduct the electric energy, possibly causing sparks. This is a process and risk issue to control during treatment. The main process parameters for PEF treatments are the electric field intensity, shape and width of the pulse, frequency of pulses, residence time (calculated from the flow rate and the volume of the treatment chamber), beverage electrical conductivity, and treatment temperature (which is the outlet temperature,  $T_{\text{out}}$ ). A PEF treatment increases the



temperature of the beverage due to Joule effect. A non-thermal PEF process (maximum  $T_{out}$  40–45°C) can be assured by strict control PEF processing conditions and by lowering the initial temperature of the beverage (van Wyk et al., 2019b). The real treatment time can be calculated from the frequency and width of the PEF pulses, the flow rate of the liquid food product that is pumped into the treatment chamber, and its volume. The selection of conditions for these parameters determines the final lethal effect on the microbial population (Abram et al., 2003; Altunakar and Barbosa-Canovas, 2011; Milani et al., 2015a).

## Pulsed Electric Fields Impact on Beer Quality

The main quality concern in the breweries is an off-flavor developed in beer exposed to light, that generates skunky off-notes in the beer, referred as the “lightstruck character” (Marsili et al., 2007). The 3-methyl-2-butene-1-thiol and organic sulphur compounds are responsible for this skunky smell, which limits the beer’s shelf life. The main reason for bottling beers in dark glasses or aluminium cans is to avoid or reduce the development of lightstruck character in beer during storage. Milani et al. (2015a) assessed the lightstruck character in nine different PEF beers (45 kV/cm, 70  $\mu$ s), by comparing them with a control (untreated beer sample) and a lightstruck beer sample (exposed to sunlight for 8 h). The sensory panel could not detect differences between aroma and flavour of control, PEF, and

lightstruck for Pilsner and dark ale beers indicating that these do not have a tendency to develop the lightstruck character with PEF treatment, probably due to the beers dark color. However, the panel could detect the lightstruck flavor/aroma in the rest of light lager beers treated by PEF. Hence, it is important to select the beer and optimize the PEF processing conditions to avoid the development of this and other undesirable flavors. A trained panel of 25 judges detected differences in the beer flavor and mouth feeling, with a significantly lower rating for PEF beer exposed to a treatment of 41 kV/cm for 175  $\mu$ s treatment time (Evrendilek et al., 2004) compared with control samples (untreated beers). Some panellists detected a metallic mouth feeling, which was probably associated with the migration of compounds from the PEF electrodes to the beverage during the treatment. Oziemblowski et al. (2017) observed lower bitterness and turbidity of PEF processed beer submitted to 200, 300, and 400 pulses in comparison to unpasteurized beer, which could be considered as a beneficial effect of PEF pasteurization of beer.

Overall pulsed electric fields can develop the lightstruck character in certain beers, depending on the processing conditions.

## Pulsed Electric Fields Microbial Inactivation in Beer

During the PEF treatment, the electric field inactivates the microorganisms in foods by increasing the transmembrane potential in the cell membrane until electroporation takes place (the permeabilization of the cell and organelles membranes), and

**TABLE 3** | PEF inactivation of yeasts in beer.<sup>a</sup>

Microorganism	Beer type	Alcohol content (% alc/vol)	Electrical field intensity (kV/cm)	Treatment time (μs)	T (°C)	Log reduction	References
<i>Saccharomyces cerevisiae</i> (ascospore)	Lager	0.0	45	70	Non-thermal	0.2	Milani et al. (2015a)
	Lager	4.0			53	0.9	
	Ale	7.0			Non-thermal	1.1	
				52	2.9		
				Non-thermal	2.2		
				53	4.0		
		Lager	3.5	35	574	Non-thermal	
			35	1145	55	≥6.8	
			45	402	Non-thermal	≥6.8	
<i>Saccharomyces uvarum</i> (vegetative)	Keg	nr	22	216	Non-thermal	4.1	Evrendilek et al. (2004)
<i>Rhodotorula rubra</i> (vegetative)	Keg	nr	22	216	Non-thermal	4.3	Evrendilek et al. (2004)

<sup>a</sup>T is the processing temperature, measured at the outlet of the PEF treatment chamber; Non-thermal means only the effect of PEF in the microorganism, as there is no heating of the beer, T below 44°C; nr—not reported.

**TABLE 4** | PEF inactivation of bacteria in beer.

Microorganism	Beer type	Alcohol content (% alc/vol)	Electrical field intensity (kV/cm)	Treatment time (μs)	T (°C)	Log reduction	References
<i>Lactobacillus plantarum</i> (vegetative)	Model beer	nr	35	nr	Non-thermal	>3.0	Ulmer et al. (2002)
	Lager	3.5	35	574	Non-thermal	1.8	Walkling- Ribeiro et al. (2011)
			45	402	Non-thermal	3.2	
	45		804	52	6.5		
<i>Pediococcus damnosus</i> (vegetative)	Keg	nr	41	175	Non-thermal	4.7	Evrendilek et al. (2004)
	Keg	nr	41	175	Non-thermal	5.8	Evrendilek et al. (2004)
<i>Salmonella enterica</i> (vegetative)	Lager	3.5	35	574	Non-thermal	2.0	Walkling- Ribeiro et al. (2011)
			45	536	Non-thermal	4.0	
			45	804	49	5.7	
<i>Bacillus subtilis</i> (vegetative cells)	Keg	nr	41	175	Non-thermal	4.8	Evrendilek et al. (2004)
	Lager	3.5	35	574	Non-thermal	1.8	Walkling- Ribeiro et al. (2011)
			45	536	Non-thermal	3.0	
			45	402	49	3.4	

<sup>a</sup>T is the processing temperature, measured at the outlet of the PEF treatment chamber; Non-thermal means only the effect of PEF in the microorganism, as there is no heating of the beer, T below 43°C; nr—not reported.

subsequent cell death (Heinz et al., 2001). Since PEF pasteurization can be operated in a continuous mode, it has the potential to be implemented in the beverage industry at a commercial scale to preserve drinks. Some studies have investigated the effect of PEF or temperature-assisted PEF on the inactivation of microbial populations in beer. **Table 3** shows results of PEF studies carried out with yeasts in beers, while **Table 4** shows studies conducted with bacteria in keg and lager beers. Milani et al. (2015a) studied the non-thermal PEF and thermal assisted PEF pasteurization of beer with different alcohol contents to inactivate the heat resistant *S. cerevisiae* ascospores. PEF treatment of 45 kV/cm electric field for 70 μs (real treatment time) at room temperature for 0.0, 4.0, and 7.0% alc/vol beers and recorded 0.2, 1.1, and 2.2 log reductions of *S. cerevisiae* ascospores, respectively. Thermally assisted PEF at 52–53°C (45 kV/cm, 70 μs) presented a higher spore inactivation: 0.9, 2.9, and 4.0 log reductions in 0.0, 4.8,

and 7.0% alc/vol beers, respectively. The other studies presented in **Table 3** were carried out with vegetative forms of yeasts. Walkling-Ribeiro et al. (2011) studied non-thermal and temperature assisted PEF treatment of 3.5% alc/vol beer to inactivate the *S. cerevisiae* vegetative cells. Non-thermal PEF processes of 35 kV/cm for 574 μs and 45 kV/cm for 402 μs caused 3.8 and ≥6.8 log reductions of *S. cerevisiae* vegetative cells, respectively. Thermal assisted PEF treatment of beer at 55°C, 35 kV/cm for 1145 μs resulted in ≥6.8 log reduction of *S. cerevisiae* vegetative cells. Evrendilek et al. (2004) studied the effect of PEF on vegetative yeasts and obtained 4.1 log reductions of *Saccharomyces uvarum* and 4.3 log reductions of *Rhodotorula rubra* in a keg beer using a 10°C cooling water bath to ensure a non-thermal process (22 kV/cm, 216 μs).

PEF inactivation of *Lactobacillus plantarum* vegetative bacteria was studied by Ulmer et al. (2002) in model beer (**Table 4**). The

results showed >3.0 log reduction of bacteria during the non-thermal PEF at 35 kV/cm electric field intensity. Evrendilek et al. (2004) studied the effect of non-thermal PEF treatment (41 kV/cm and 175  $\mu$ s) on three different bacteria *L. plantarum*, *Pediococcus damnosus*, and *Bacillus subtilis* vegetative cells in keg beer and recorded 4.7, 5.8, and 4.8 log reductions, respectively. Walking-Ribeiro et al. (2011) carried out non-thermal and thermally assisted PEF treatment of *L. plantarum*, *Salmonella enterica*, and *B. subtilis* vegetative bacteria in 3.5% alc/vol lager beer. A non-thermal PEF (35 kV/cm and 574  $\mu$ s) caused similar inactivation in the three bacteria: 1.8, 2.0, and 1.8 log reductions for *L. plantarum*, *S. enterica*, and *B. subtilis*, respectively. A non-thermal PEF treatment of higher electric field (45 kV/cm, 402  $\mu$ s) led to a higher inactivation of *L. plantarum* (3.2 log reductions). Thermally assisted PEF at 52°C, 45 kV/cm, 804  $\mu$ s caused a higher log reduction of 6.5. Regarding *S. enterica*, the same authors recorded 4.0 log reduction after a non-thermal PEF process of 45 kV/cm and 536  $\mu$ s and 5.7 log reduction at 49°C, 45 kV/cm, and 804  $\mu$ s. With respect to *B. subtilis*, 3.0 log reductions were achieved at room temperature (45 kV/cm and 536  $\mu$ s) and 3.4 log reductions at 49°C (45 kV/cm and 402  $\mu$ s) were registered, respectively. In resume the higher the PEF temperature, the higher the microbial inactivation. Same conclusions were taken by a few PEF-thermal studies carried out with other beverages and foods (Jayaram et al., 1992; Zhang et al., 1995; Vega-Mercado et al., 1997).

The effect of microorganism size on PEF inactivation could be demonstrated by comparing bacteria and yeast inactivation submitted to the same PEF conditions (Walking-Ribeiro et al., 2011). While the three different bacteria were reduced by a magnitude of approximately 2 logs after a PEF treatment of 35 kV/cm for 574  $\mu$ s (Table 4), 3.8 decimal reductions were obtained with the yeast *S. cerevisiae*. Vegetative yeasts are larger (4–6  $\mu$ m) than bacteria, and thus more susceptible to PEF than bacteria. The review also showed that it is more difficult to inactivate yeast spores by PEF than vegetative yeast cells (Table 3).

## POWER ULTRASOUND TREATMENT OF BEER

### Ultrasound Overview

Ultrasound are sonic waves of the same physical nature as sound, but with frequencies above the range of human hearing (20–100 kilo Hertz, kHz). Based on frequency difference and sound intensity, ultrasound waves are classified in two categories. High frequency ultrasound (diagnostic ultrasound) operates at frequencies of 2–20 MHz with sound intensities in the range of 0.1–1 W/cm<sup>2</sup>. Food quality assessment, medical imaging, and non-devastating inspection are examples in which high frequency ultrasound is used. Lower frequencies (20–100 kHz) with a sound intensity of 10–1000 W/cm<sup>2</sup> are employed in power ultrasound (also called high intensity ultrasound). Because of high energy level, power ultrasound is considered appropriate to be used in the food industry for microbial destruction and equipment disinfection (Baumann et al., 2005, 2009; Ugarte et al., 2006). Power ultrasound processing, often called sonication, operates

through a liquid medium which can be the food or water containing a solid food (Figure 3). Nowadays, ultrasound technology can be used more widely in food pasteurization applications. The main US applications for food processing at a commercial scale are emulsification, size reduction, crystallization, and extraction (Feng and Yang, 2011).

### Ultrasound Impact on Beer Quality

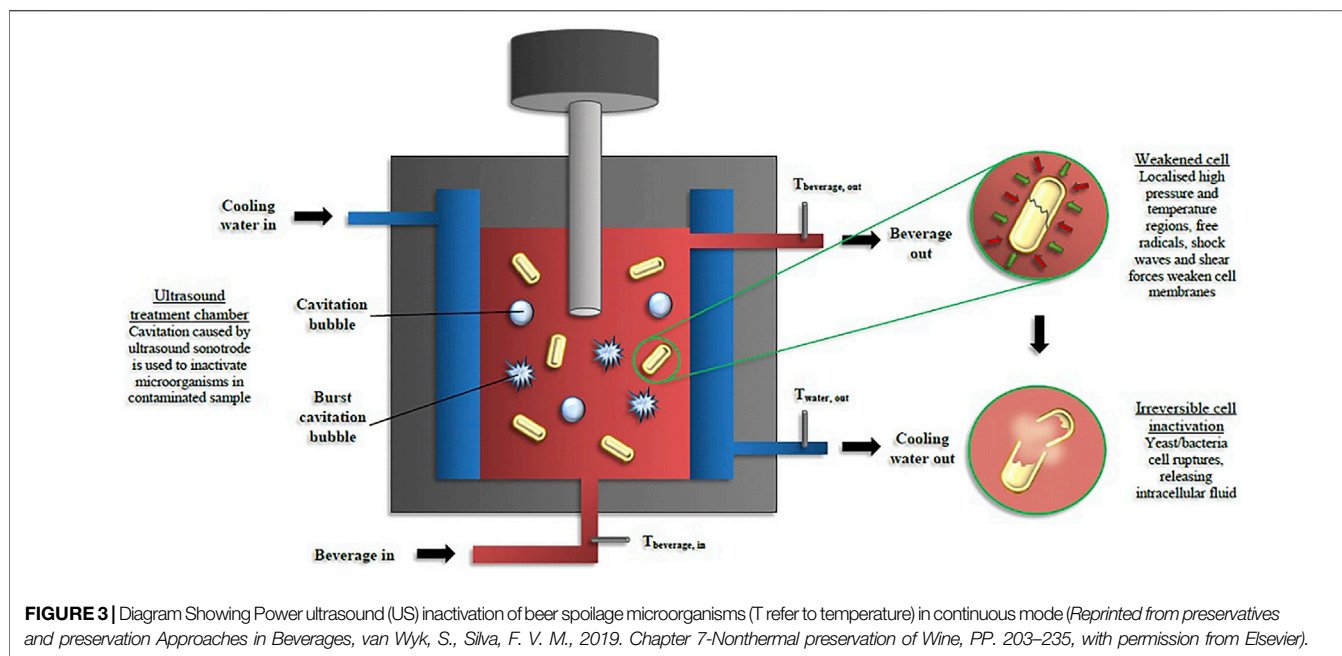
Ultrasound of 1 MHz has been applied for wines, whiskey, and spirits, and results showed that it has changed the alcohol/ester balance creating an effect similar to ageing (Mason et al., 1996). With respect to applications to beer industry, at the beginning of the mashing process, US increases the beer yield and can reduce the fermentation time. This process increases the oxidation in fermented products, which leads to a better flavor and maturation. It also improves the hygiene of beer defoaming and defoaming operations before bottling (Chemat and Khan, 2011). Deng et al. (2018) did not record significant changes on physicochemical and sensorial properties of Chinese lager beer submitted to the following US and thermosonication (TS, simultaneous exposure to US and heat) conditions: 2.7 W/ml acoustic power density, temperatures of 40, 50, and 60°C, for 2 min treatment time. However, a higher acoustic power density (10.8 W/ml) and temperature (75°C) TS process for 20.5 s, equivalent to a 15 PU process, developed an unacceptable haze in ale and lager beers (Silva et al., 2015). Borsato et al. (2019) concluded that sonication of malted barley increased the size of the starch granules, and a better quality was obtained when malted barley was exposed to TS compared with thermally treated or untreated malted barley. Xiao et al. (2020) showed that the main parameters (ethanol content, original gravity, pH, bitterness, and viscosity) of wheat beer were scarcely affected by TS (at 50°C and 2.7 W/ml for 3 min). Meanwhile, TS-treated beer had higher colloidal haze and foam stability than thermally (60°C-15 min) pasteurized beer. Dynamic light scattering analysis showed that TS treatment resulted in a smaller and more uniform particle size, thus having a positive effect on the desired beer haze stability.

Overall, ultrasound technology seems to have potential for beer pasteurization. An increase in the stability of beer turbidity and haze was observed and the overall beer quality was not much affected by the US process. In addition, this technology can also be applied to improve the yield of other operations (e.g., mashing) and speed up the beer production.

### Ultrasound Microbial Inactivation in Beer

Microbial inactivation mechanism of ultrasound can be explained through the effect of cavitation on microbial cell walls (Figure 3). Water jets of liquid, generated by the asymmetric implosion of transient cavitating bubbles, may cause severe cell envelope damage and cleavage of the texture of the polymeric materials of the cell walls (Evelyn and Silva, 2020). In terms of the chemical effects, transient cavitation can create OH- and H radicals and hydrogen peroxide. In addition, stable cavitating bubbles can generate microstreaming alongside the bubble and create high hydrodynamic shear stresses, which cause cell membrane damages. Many studies have been carried out to investigate the application of power ultrasound on the inactivation of





different yeasts and bacteria in foods and beverages (Adekunte et al., 2010; Bauman et al., 2005; Char et al., 2010; and; Suslick and Nyborg, 1990), including wine (Luo et al., 2012; Gracin et al., 2016).

Milani and Silva (2017) studied the effect of power ultrasound treatment with and without heat on lager beers pasteurization. A pasteurization of 15 pasteurization units, equivalent to 1.34 log reductions in *S. cerevisiae* ascospores, was achieved in 4.8% alc/vol beer when batch mode 16.2 W/ml thermosonication at 50°C for 1.9 min or TS at 55°C for 26 s was applied. On the other hand, 16.2 W/ml US without heat at room temperature (23°C) required 20 min for the same level of pasteurization. Deng et al. (2018) demonstrated the inactivation of yeast and spoilage bacteria (including lactic acid bacteria and aerobic bacteria) in lager beer by 2.7 W/ml TS at 50°C and 60°C for 2 min. Xiao et al. (2020) showed that the microbiological stability of TS-treated wheat beer (at 50°C and 2.7 W/ml for 3 min) was comparable with that of the heat-treated sample (at 60°C for 15 min).

The studies demonstrated US and ultimately TS at 50–55°C are efficient methods to pasteurize beer.

## OTHER NON-THERMAL TECHNOLOGIES

### Dense-Phase CO<sub>2</sub>

DPCD is known as a non-thermal technique that inactivates spoilage microorganisms and enzymes through carbon dioxide molecular effect at a pressure of below 50 MPa while maintaining the freshness, sensory attributes, and nutritional qualities of food (Damar and Balaban, 2006). Fraser (1951) was the first scientist demonstrating the microbial inactivation by gas pressure of various types of bacteria. The mechanism of microbial inactivation by DPCD is not well known. However, researchers have demonstrated that various processes can be involved in the microbial inactivation (Fraser

1951; Daniels et al., 1985; Lin et al., 1991; Nakamura et al., 1994; Ishikawa et al., 1995): intra- and extracellular pH decrease due to the acidic effect of CO<sub>2</sub>; proteins and ions precipitation; inhibition of biological activities; and modifications of cell walls that cause cell rupture and extraction of cellular substances, leading to intracellular electrolyte balance disorder (Erkmen and Karaman, 2001).

Some studies with different foods DPCD pasteurization have been carried out (Haas et al., 1989; Arreola et al., 1991; Ishikawa et al., 1997; Shimoda et al., 1998; Erkmen and Karaman, 2001; Folkes, 2004; Kincal et al., 2005; Damar and Balaban, 2006). Apart from fruit juices, DPCD was applied to alcoholic beverages like beer. Folkes (2004) studied the effect of DPCD on microbial inactivation and sensorial parameters of beer and found no significant difference between the untreated beer and DPCD treated beers. Dagan and Balaban (2006) studied the pasteurization of beer by DPCD to inactivate vegetative yeasts and got a maximum 7.38 log reduction of yeasts at 26.5 MPa, 21°C, 9.6% CO<sub>2</sub>, and 4.77 min residence time. With respect to beer quality, haze was reduced by DPCD pasteurization from 146 nephelometric turbidity units (NTU) to 95. The aroma and flavor of treated beer were not significantly different from fresh beer. Foam capacity and stability were minimally affected by CO<sub>2</sub> processing, although changes would most likely be unnoticed by consumers. These results revealed that DPCD technology is an effective treatment for beer pasteurization while retaining the aroma and freshness of beer. This cold pasteurization technique can be used as an alternative method by the industry as it could preserve the nutritional and haze characteristics of beer during storage.

### High Pressure Homogenization

High pressure homogenization (HPH) is another non-thermal technology which is widely investigated for food pasteurization (Puig et al., 2008; Franchi et al., 2011; Franchi et al., 2013; Comuzzo et al., 2015). In HPH, the fluid food is exposed to a

forced pressure and rapid acceleration of media in a thin gap during a continuous process e.g., 200 m/s at 340 MPa. Then, a sudden drop of pressure causes cavities, shear, and friction of microbial cells that leads to microbiological inactivation (Franchi et al., 2011; Franchi et al., 2013). Franchi et al. (2013) studied the effect of HPH treatment on microbial inactivation and quality parameters of beer. He ran HPH treatment on Pilsen beer inoculated by lactic acid bacteria, acetic bacteria, and yeasts. *Lactobacillus delbrueckii* was the most resistant microorganism, requiring 250 MPa for a 6 log reduction. Furthermore, Franchi et al. (2011) claims that according to the molecular conformational changes in polysaccharides and proteins of beer, this process promotes a positive effect on beer quality and improves beer color. Also, an addition of a small amount of lysozyme (50 mg/L) would enable a reduction in the level of pressure to 150 MPa, with energy and costs savings. Overall, HPH processing has the potential of cost-effective commercial application in the beer industry.

## Ultraviolet Irradiation

Ultraviolet treatment is another promising technology that is widely used for food pasteurization as an alternative for heat treatment (Bintsis et al., 2000). In the food industry, UV irradiation is mainly used for milk, juices, and cider preservation (Basaran et al., 2004; Quintero-Ramos et al., 2004; Matak et al., 2005; Koutchma, 2019). Main parameters in UV-C processing are the time and doses of applied. According to Bintsis et al. (2000), the most efficient wavelengths for pasteurization are between 200 and 280 nm, so called as UV-C. The DNA of virus, bacteria, fungi, yeasts, and molds is destroyed through the exposure to irradiation and hence the reproduction process of microbial cells is stopped (Koutchma, 2019).

Some researchers carried out studies to investigate the effect of UV irradiation on beer pasteurization and its sensory properties. Lu et al. (2010) optimized the optical-fiber distribution density and thin-film thickness for treating beer by UV. The apparatus could reduce more than 4 log of inoculated *Lactobacillus brevis* in draft beer at UV doses of 9.7 mJ/cm<sup>2</sup>, while the reduction of *S. cerevisiae* was not as efficient. They concluded that naturally contaminating lactic acid bacteria and Enterobacteriaceae could be effectively inactivated in draft beer. Moreover, the results of Hosseini et al. (2011) on UV processing of non-alcoholic beer demonstrated that beer spoilage microorganisms were totally inactivated at pulse UV wavelength of 266 nm. Mezui and Swart (2010) studied the sensory parameters of UV treated beer, and reported the formation of lightstruck flavor in beer processed by low ultraviolet light irradiation (UV-C).

While successful microbial inactivation in beer by UV has been reported, more sensory studies are needed to assess the impact of this process on the formation of the lightstruck character in different beers.

## COMPARISON OF TECHNOLOGIES AND FINAL REMARKS

This review demonstrated the advantages and limitations of novel non-thermal technologies in terms of beer quality and microbial inactivation compared to conventional thermal processing. The CO<sub>2</sub> present in beer can cause technological problems, and this issue needs to be addressed (e.g., sparks in PEF treatment). In general PEF, thermosonication, HPP, HPH, and DPCD treatments revealed less effect on beer sensory properties, retaining more of the nutritional and freshness of original beer, compared to thermally processed beers.

Non-thermal HPP inactivation of *S. cerevisiae* ascospores in beer is much faster (few seconds for 15 PU) than thermosonication (few minutes) and thermal pasteurization (15 min) (Milani et al., 2016; Evelyn et al., 2017). The high pressure (HPP) process is more efficient than thermal treatment for equivalent yeast inactivation, retaining the original beer flavour and requiring less energy (HPP—77 kJ/L vs 60°C thermal—189 kJ/L) (ICHEM, 2016; Milani et al., 2016). The most recently developed commercial HPP process “in bulk” followed by aseptic filling allows the use of glass bottles or cans for beer packaging while keeping the freshness of the original non processed beer. Power ultrasound at room temperature presented insufficient microbial inactivation, while thermal assisted sonication (thermosonication) was successful for beer pasteurization (15 PU) and also increased the stability of beer turbidity and haze. For both direct contact or indirect US systems, as the distance from the wave source increases, the efficiency of microbial inactivation decreases. US treatment PEF and UV-C developed the undesirable lightstruck character in beer, with a negative impact in beer sensory quality.

Some of the alternative methods can achieve higher microbial log reductions, with shorter processing times, and less energy requirements compared with the conventional thermal processing, while keeping the freshness of the raw beer.

The research on non-thermal pasteurization of other alcoholic beverages is expanding (van Wyk et al., 2019a). More research is needed to compare capital investment, costs of operation, energy consumption, and sustainability of different technologies. Some of these technologies are not commercially available yet at a larger scale. Therefore, more studies at pilot and industrial scales are needed to elucidate the industrial feasibility of the non-thermal technologies reviewed.

## AUTHOR CONTRIBUTIONS

Both authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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