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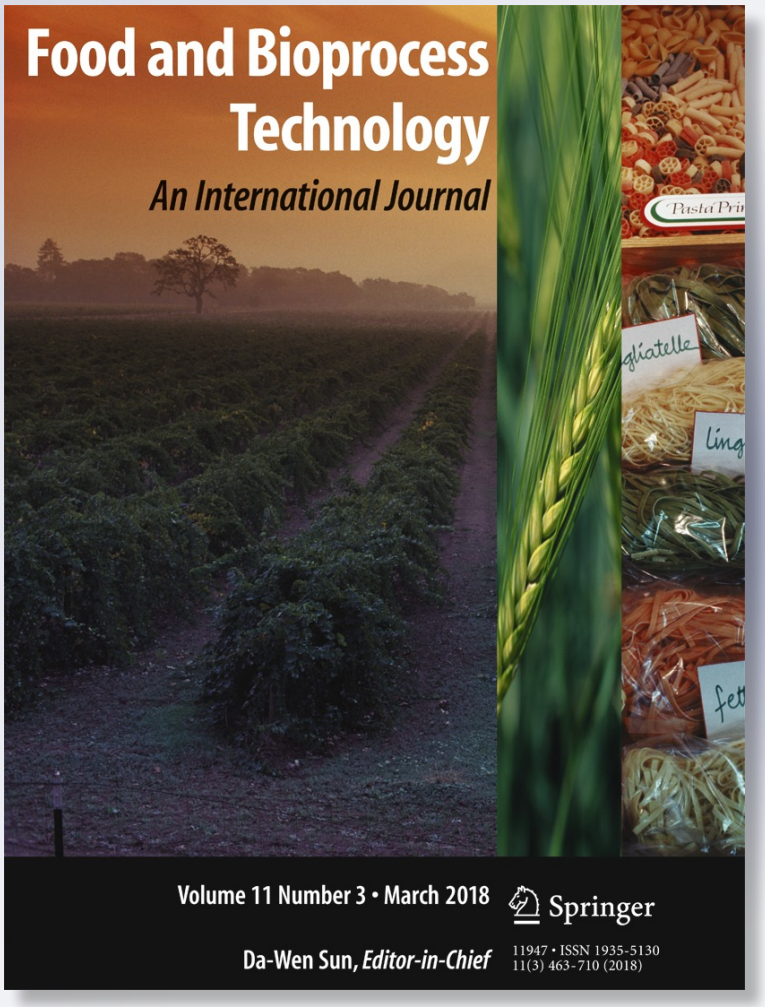

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REVIEW

Non-thermal Technologies as Alternative Methods for *Saccharomyces cerevisiae* Inactivation in Liquid Media: a Review

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Abstract

The ongoing demand for high-quality processed foods which would preserve their natural and fresh-like characteristics has awakened a growing interest in non-thermal technologies. Thanks to their ability to inactivate microorganisms under mild conditions, these technologies avoid drawbacks usually attributed to the use of thermal treatments, such as nutrient loss, off-flavors, and changes in the food's physical and chemical properties. *Saccharomyces cerevisiae* (*S. cerevisiae*) mainly causes spoilage in liquid foods with high sugar content and low pH values. Thus, it is one of the most undesirable microorganisms in the food industry since its presence may lead to important economic losses. This review offers an exhaustive compilation and critical revision of research conducted in the field of *S. cerevisiae* inactivation in liquid media, emphasizing the use of non-thermal technologies, such as high pressure processing, high-power ultrasound, supercritical carbon dioxide, pulsed electric fields, and others. Likewise, using these technologies in combination (the hurdle approach) may enhance their individual effect and significantly reduce the treatment time needed to obtain a given level of *S. cerevisiae* inactivation. In general, non-thermal technologies are proving to be able to successfully inactivate *S. cerevisiae* in liquid media. However, the need for further investigation and complete industrial implementation is made evident throughout this review.

Keywords Emerging technologies · Microorganism inactivation · High pressure processing · High-power ultrasound · Pulsed electric fields · Supercritical carbon dioxide

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Introduction

Saccharomyces cerevisiae is a single-cell budding yeast. It is the most widely studied eukaryotic organism, and it has a characteristic ellipsoidal shape with a 5- to 10-μm large diameter and 1- to 7-μm small diameter (Darby et al. 2012; Walker 1998). *S. cerevisiae* can grow quickly in both defined and complex media such as food systems. It reproduces by means of simple mitosis of both haploid and diploid form of the cells. Nevertheless, under stress conditions, the diploid form can present sporulation through meiosis, thus resulting in four haploid spores which may reproduce as well (Herskowitz 1988).

The cell wall of yeasts is a rigid barrier that mainly controls the transport of material into and out of the cell but also protects the plasma membrane and allows adhesion between cells (Brul et al. 2000; Caballero-Córdoba and Sgarbieri 2000; Osumi 1998; Walker 1998). Cell wall represents 26–32% of the dry weight of the cell, and it consists of approximately 85–90% of polysaccharides and 10–15% of proteins (Nguyen

et al. 1998; Ortuño et al. 2014a). Since cell wall rupture is a fundamental part of the microorganism inactivation process, the characteristics of the cell wall are directly related to the resistance of microorganisms against the inactivation treatments (Liu et al. 2013; Ortuño et al. 2014a; Wu et al. 2015).

The US Food and Drug Administration (FDA) considers *S. cerevisiae* to be GRAS (Generally Recognized As Safe) (FDA 2015). However, this microorganism can represent important economic losses for the food industry due to spoilage, especially in foods with low pH and high sugar content, such as fruit products and alcoholic beverages (Patrignani et al. 2009; Stratford et al. 2000). Food spoilage caused by *S. cerevisiae* is related to its fermentation capacity, resulting in gassiness, turbidity, cloudiness, and off-flavors associated with acetic acid and hydrogen sulfide (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Fitzgerald et al. 2004; Marx et al. 2011).

Thermal processing is an effective inactivation method for microorganisms such as *S. cerevisiae* and certain spores. Nevertheless, elevated temperatures and long treatment times may have detrimental effects on quality of the treated product, including nutrient loss, off-flavors, and deterioration of the bioactive compounds present in the product (Piyasena et al. 2003). On the other hand, chemical preservatives (i.e., salts and organic acids) may lead to low consumer acceptance since food consumers prefer products with natural, safe, and environment-friendly ingredients. Also, chemical preservatives can change the organoleptic properties of the foods and their ingestion has been related to long-term adverse effects on health (Rupasinghe and Yu 2012; Aneja et al. 2014). Consequently, alternative food preservation methods have emerged in order to maintain the nutritional and organoleptic properties of processed products, thus rendering them more attractive to the consumers.

Among these new preservation techniques, non-thermal technologies have been developed to design and manufacture fresh-like products (Nafar et al. 2013). In comparison to thermal processing, the main advantages of these technologies are the reduction of energy consumption, improvement of process safety, preservation of nutritional properties, and increasing global quality of food products (Ross et al. 2003). In the last decade, a large number of investigations have focused on implementing non-thermal technologies in food processes as a way of contributing to a reduction in the $\log_{10}$ of colony forming units (CFU) of microorganisms in liquid food products. These range from widely commercialized and industrialized technologies, such as high pressure processing (Campos and Cristianini 2007; Huang et al. 2017) and high pressure homogenization (Martínez-Monteagudo et al. 2017; Patrignani and Lanciotti 2016), to technologies that are still being tested at laboratory scale, such as high-power ultrasound (Pala et al. 2015; Wu et al. 2015), pulsed electric fields (Donsì et al. 2007a; Milani et al. 2015), supercritical carbon dioxide (Ortuño et al. 2013; Paniagua-Martínez et al. 2016), ultraviolet light (Guerrero-Beltrán et al. 2009; Kaya and Unluturk 2016), and pulsed light (Ferrario et al. 2013, 2015), among others.

In addition, the hurdle approach aims to develop mild processes based on synergistic interactions between different preservation treatments (Leistner 1992; Ross et al. 2003). Combining non-thermal technologies with traditional physical and chemical techniques can produce synergistic antimicrobial effects in food systems (Gómez et al. 2011). According to Ferrario et al. (2015), the selection of non-thermal hurdles is determined by the characteristics of microbial cells and the media in which the microorganisms are embedded (water activity, temperature, pH, and chemical composition).

This paper provides an extensive literature review on the application of non-thermal technologies as alternative methods for *S. cerevisiae* inactivation in liquid media, as well as its critical revision. This review is not only aimed at the scientific community, but it also aspires to provide a comprehensible summary of the available information for technologists and engineers working in the liquid-food industry, for them to get to know the main mechanisms of non-thermal technologies involved in *S. cerevisiae* inactivation and the synergistic effects that can be achieved by combining these technologies. By means of this, the authors of this review hope to help in reducing the information gap that exists between research and industry.

Non-thermal Technologies as Alternative Methods for *Saccharomyces cerevisiae* Inactivation

Chemical Preservatives

Since the Middle Ages, chemical preservatives have been widely used in food applications for their bactericidal, virucidal, fungicidal, antiparasitical, and insecticidal properties. Nowadays, however, synthetic chemical preservatives do not enjoy wide consumer acceptance since their consumption has been linked to serious medical problems, such as cancer (Aneja et al. 2014). Therefore, natural chemical preservatives have been on the rise recently. Table 1 displays the main chemical preservatives studied in the last decade for the *S. cerevisiae* inactivation in liquid media, including essential oils and other chemicals.

Weak acids (sorbic acid, benzoic acid, propionic acid, and sulfites) are among the most common chemical preservatives used in the prevention and control of *S. cerevisiae* in liquid foods. Stratford et al. (2013) investigated the effect of acetic and sorbic acid at different proportions against 25 different *Saccharomyces* strains in a YEPD growth medium. They observed considerable variation in the resistance of the strains (expressed as minimum inhibitory concentration, MIC) to

Table 1 Summary of chemical preservatives used for the inactivation of *Saccharomyces cerevisiae* in liquid media

Media	Chemical preservative	Minimum inhibitory concentration	Reference
Saboraud dextrose 25 °C	Essential oil from lemon	0.027 mg/mL	(Conte et al. 2007)
Wine	Short synthetic peptides	25–100 μM	(Enrique et al. 2007)
Water and malt extract broth	Chlorogenic acid	Values not reported	(Muthuswamy and Rupasinghe 2007)
Dimethyl sulfoxide (DMSO) at 4%	Catechin and phloridzin	25 and 5 mM	
Yeast malt broth	Extract of ethylacetate and Essential oil from citrus peel	0.28 mg/mL 0.56 mg/mL	(Chanthaphon et al. 2008)
Wine	Dimethyl dicarbonate	0.1 mg/mL	(Costa et al. 2008)
Pear juice Orange Juice	D-limonene and a mixture of terpenes from *Maleuca alternifolia*	1, 10, and 25 mg/mL	(Donsì et al. 2011)
Apple juice Milk	Essential oil from lemon	0.377 ± 0.13 μL/mL	(Tserennadmid et al. 2011)
Mixed fruit juices	Essential oil from *Eucalyptus globulus*	1.13 mg/mL	(Tyagi and Malik 2011)
Wine	Pterostilbene Resveratrol Luteolin Ferulic acids Potassium Metabisulphite	0.064 mg/mL 0.25 mg/mL 0.512 mg/mL 0.512 mg/mL 0.512 mg/mL	(Pastorkova et al. 2013)
Apple juice Orange juice	Essential oil from S*capania nemurea*	0.2–0.4 mg/mL	(Bukvicki et al. 2014)
Wine	Carvacrol and thymol	0.128 mg/mL	(Chavan and Tupe 2014)
Mixed fruit juices	Essential oil from lemon	1.13 mg/mL	(Tyagi et al. 2014)
Wine	Chitosan	2 mg/mL	(Elmacı et al. 2015)

both acids. In general, sorbic acid inhibited growth at much lower concentrations (2.3–4.3 mM) than acetic acid (110–180 mM). Interestingly, strains from the same origin showed different MICs, a fact that may have industrial implications. As for another commonly used chemical preservative in the food industry, Costa et al. (2008) tested the potential of the dimethyl dicarbonate (DMDC) to inactivate *S. cerevisiae* in wine. The inactivation effect was dependent on the inoculum size, observing that *S. cerevisiae* was resistant at low inoculation concentration (MIC = 0.1 mg/mL). DMDC was also tested in combination with metabisulphite at different wine volumes. The combination of DMDC and metabisulphite lowered the cell count (no viable cells detected) when 2 mL were essayed, but it was less efficient in tests with larger volumes (50 mL and 1 L). As for other synthetic agents, Enrique et al. (2007) observed the action of 13 short synthetic peptides against the proliferation of *S. cerevisiae* as well as other yeasts in wine. Five of these peptides showed moderate but considerably better antimicrobial activity against *S. cerevisiae* than the rest, with MIC values within a range of 16 to 32 μM. Another set of experiments were performed in a model medium (GPY, yeast extract + peptone + glucose) and wine. The researchers reported that *S. cerevisiae* was completely inactivated when cells were exposed to 25–100 μM peptides in GPY. However, the opposite was observed when peptides were tested in wine. Accordingly, Elmacı et al. (2015) noted that *S. cerevisiae* exhibited excellent resistance to the antimicrobial action of chitosan in laboratory media (MIC>2 mg/mL) even though it extended its lag phase during growth.

On the other hand, essential oils made up of natural compounds, such as eugenol, citral, pinene, thymol, cinnamic acid, carvacrol, and others, are among the most investigated chemical preservatives due to their ability to control the growth of *S. cerevisiae* in liquid media. These properties are influenced by many factors, including botanical source, time of harvesting, stage of development, and method of extraction (Tiwari et al. 2009; Tserennadmid et al. 2011). Depending on type and concentration, essential oils exhibit cytotoxic effects on living cells but are usually nongenotoxic. In some cases, however, changes in intracellular redox potential and mitochondrial dysfunction induced by essential oils can be associated with their capacity to exert antigenotoxic effects (Bakkali et al. 2008). In fact, researchers suggest that *S. cerevisiae* is among the most sensitive yeasts to these chemical preservatives, even though the reduction of count cells is moderate. In this sense, Chanthaphon et al. (2008) found that ethylacetate extract and essential oil from fresh kaffir lime peels (*Citrus* spp.) exhibited MIC values of 0.28 and 0.56 mg/mL, respectively, against *S. cerevisiae* var. sake. The researchers found that the composition of the obtained extract depended on the solvent used. Similarly, lemon extracts (5 to 1000 ppm) exhibited a MIC value of 0.027 mg/mL for *S. cerevisiae* (Conte et al. 2007). Tserennadmid et al. (2011) explored anti-yeast

activities of four essential oils, namely clary sage, juniper, marjoram, and lemon, against *S. cerevisiae* and other yeasts in malt extract medium and apple juice. In this study, lemon essential oil showed to be the most effective against *S. cerevisiae* (MIC = 0.375 ± 0.13 μL/mL) compared to the other studied products. Interestingly, the studied combinations of essential oils (juniper extract with each of the other three) did not reveal differences from pure extracts in *S. cerevisiae* inactivation. Moreover, lemon essential oil significantly ($p < 0.05$) lowered the growth rate of *S. cerevisiae* in clear apple juice while no effect was observed for cloudy apple juice. Even though organoleptic analysis revealed that the taste of clear apple juice was pleasant, the odor was not acceptable for the panelists. Similarly, Tyagi et al. (2014) focused on anti-yeast activity of lemon grass oil, which reduced the cell count of *S. cerevisiae* by 1 $\log_{10}$ in mixed fruit juice at MIC level (1.13 mg/mL). Tyagi and Malik (2011) studied the antimicrobial activity of *Eucalyptus globulus* against 14 food spoilage microorganisms, including *S. cerevisiae*. The MIC of essential oils was determined by agar dilution assay and for *S. cerevisiae*; it was 1.13 mg/mL. The tests revealed that the Gram-negative bacteria were more resistant than the Gram-positive bacteria, possibly due to the more restricted access to the membrane in Gram-negative bacteria, which have lipopolysaccharide cell envelope. On the other hand, Bukvicki et al. (2014) explored the antimicrobial activity of three *Scapania nemorea* liverwort extracts against bacteria and seven different species of yeasts, including *S. cerevisiae*. Extracts were obtained using three solvents: methanol, ethanol, and ethyl acetate. *S. cerevisiae* was found to be the most sensitive yeast to the liverwort extracts (MIC values of 0.2–0.4 mg/mL). The methanol extract showed the best antimicrobial activity. Moreover, methanol liverwort extract was succesfully applied in combination with mild heat to inactivate *S. cerevisiae* in orange and apple juices. Therefore, the good results shown by the methanol liverwort extracts suggest that they can be used in other liquid foods alone or combined with other non-thermal technologies. Chavan and Tupe (2014) tested the antimicrobial activity of carvacrol and thymol against wine inoculated with *S. cerevisiae* found in the grapes commonly used for wine fermentation and other species. Carvacrol and thymol showed a potent inhibitory effect on *S. cerevisiae* from natural microbiota, with MIC values of 0.128 mg/mL for both carvacrol and thymol. The pH used in the tests did not affect the antimicrobial activity of carvacrol and thymol for *S. cerevisiae* even though a value of pH 3.5 seemed to enhance the antifungal activity of the extracts for other yeasts. Donsì et al. (2011) worked in the nanoencapsulation of essential oils for incorporation into fruit juices. They tested D-limonene and a mixture of terpenes extracted from *Maleuca alternifolia*. Emulsions were prepared with sunflower oil and palm oil as organic phases and lecithin, Tween 20, glycerol monooleate, and CLEARGUM® CO 01 as emulsifying agents. Nanoencapsulated terpene mixtures showed a higher *S. cerevisiae* inactivation effect compared to pure terpenes and pure D-limonene (1, 10, and 25 mg/mL, respectively). These findings highlight the importance of the diameter of nanoemulsion droplets as well as the emulsifying agent used.

As for natural chemical preservatives other than essential oils, Pastorkova et al. (2013) investigated the antimicrobial capacity of 15 different phenolic compounds commonly found in grapes. *S. cerevisiae* seem to be most susceptible to pterostilbene (MIC = 0.064 mg/mL), but was also inhibited by resveratrol, luteolin, and ferulic acids (MIC = 0.256, 0.512, and 0.512 mg/mL, respectively). Thus, only pterostilbene and resveratrol achieved better results in *S. cerevisiae* inhibition than potassium metabisulphite (MIC = 0.512 mg/mL). Muthuswamy and Rupasinghe (2007) analyzed the antimicrobial activity of catechin, chlorogenic acid, and phloridzin against *S. cerevisiae* using four different concentrations, 1, 5, 10, and 25 mM. A study was conducted using four solvents for each compound. Chlorogenic acid was dissolved in water and then, sterile warm growth medium was added (malt extract broth) and catechin and phloridzin were dissolved in dimethyl sulfoxide at 4%. The results showed that chlorogenic acid and phloridzin suppressed *S. cerevisiae* completely at 25 and 5 mM, respectively, but catechin did not inhibit its growth at any of the tested concentrations.

As it can be seen in this section, the effectiveness of chemical preservatives in the inactivation of *S. cerevisiae* is generally moderate to high. Moreover, natural chemical preservatives, such as essential oils, are more attractive to consumers yet not as effective in inactivating *S. cerevisiae* in comparison to synthetic chemical preservatives. Additionally, both synthetic and natural chemical preservatives can alter some the organoleptic properties of the final products. In this sense, other non-thermal technologies are being applied to liquid foods in order to inactivate *S. cerevisiae* effectively while maintaining the quality of the product. Nevertheless, some researchers have explored the possibility of combining the effects of chemical preservatives with other non-thermal technologies, aiming to getting the best of both. These technologies, namely high pressure processing, high-power ultrasound, supercritical carbon dioxide, pulsed electric fields, and others, as well as their combination with chemical preservatives, are revised in the following sections of this review.

High Pressure Processing

High pressure processing (HPP) is the most successful commercialized technology among the non-thermal technologies reviewed in this work (Huang et al. 2017). Consumer acceptance of HPP has continuously grown since the quality of pressurized foods may be superior in terms of nutrient retention and sensorial properties when compared to conventional

food preservation technologies like thermal processing (Mújica-Paz et al. 2011). The HPP food market is expected to continue expanding over the next 5–10 years (Jermann et al. 2015).

S. cerevisiae cells have a moderate resistance to lethal pressure effects. HPP inactivates vegetative cells of pathogens and spoilage microorganisms by holding foods at 100–600 MPa up to 10 min (Serment-Moreno et al. 2014). In fruit juice and other liquid media, treatments around 300–400 MPa with pressure holding times under 10 min consistently achieve more than 5 $\log_{10}$ reductions or non-detectable counts (Table 2). In some cases, *S. cerevisiae* may be readily inactivated during come-up time (CUT), which corresponds to the time required to reach the target pressure level (Donsì et al. 2007b; Guerrero-Beltran et al. 2011; Palou et al. 1998). Sub-zero (− 20 to 0 °C) temperatures or temperature levels slightly over room temperature (40–50 °C) have shown that *S. cerevisiae* cells are inactivated in shorter processing times when compared to pressure treatments performed at room temperature (Chen and Tseng 1997; Hashizume et al. 1995; Perrier-Cornet et al. 2005). Studies on the effectiveness of HPP towards *S. cerevisiae* helped clarify the underlying mechanisms of microbial inactivation at high pressure, indicating that several intrinsic (growth stage, microbial strain) and external (solutes, acidity) factors may affect the pressure resistance of microorganisms (Fernandes 2005; Moussa et al. 2013; Patterson 2005).

Intrinsic Factors in HPP Inactivation of *S. cerevisiae*

Cellular membrane disruption is the main inactivation mechanism of microorganisms by HPP since pressure may induce lipid phase transition from gels to hydrated crystal structures that reduce membrane fluidity and may increase the melting temperature of lipids by 10–20 °C for every 100 MPa (Abe 2015; Matsuki et al. 2013; Zulkurnain et al. 2016). Consequently, the cellular membrane structure becomes rigid, resulting in bud formation, scarring, or even the removal of the cellular membrane (Marx et al. 2011). In situ studies have indicated that the cellular membrane may be altered during CUT where a pressure build-up rate of 30 MPa/min to reach 250 MPa resulted in a 10% cellular volume decrease (Perrier-Cornet et al. 1995). Cell volume continued to gradually decrease during pressure holding time up to 15 min. As pressure remained constant, the volume decrease was attributed to the diffusion of water and solutes such as Na^+ and Ca^{2+} ions to the outside of the cell, thus having important implications on physiological processes (Perrier-Cornet et al. 1999). After 15 min, the leakage of intracellular material seemed to be irreversible, as the final cellular volume of *S. cerevisiae* cells was 90% of the volume measured before the pressure treatment (Perrier-Cornet et al. 1995).

In response to pressure, *S. cerevisiae* accelerates the synthesis rate of lipids conferring higher membrane fluidity, as in the case of gene *ERG25* encoding ergosterol biosynthesis, or

Table 2 Inactivation of *Saccharomyces cerevisiae* by high pressure processing (HPP) in liquid media at ambient temperature

Media	*P* (MPa)	*t* (min)	SV_{HPP}	Reference
Mandarin juice	400	10	3.0*	(Ogawa et al. 1990)
Mandarin juice concentrate (40°Brix)	400	10	< 1.0	(Ogawa et al. 1990)
Yeast and mold broth	270	40	7.0*	(Hashizume et al. 1995)
Sabouraud broth	300	CUT	5.0*	(Palou et al. 1998)
Orange juice[a]	400	2–10	4.0–5.0*	(Parish 1998; Zook et al. 1999)
Apple juice[a]	400	10	4.0	(Zook et al. 1999)
Orange juice concentrate (42°Brix)	400	60	3.0	(Basak et al. 2002)
Malt Wickerman media[b]	200	10	4.0–5.0	(Perrier-Cornet et al. 2005)
Applesauce	375	2.5	6.0*	(Chauvin et al. 2006)
Mango puree	345	1	5.6*	(Guerrero-Beltrán et al. 2006)
Pineapple juice	250	CUT	~ 1.0	(Donsì et al. 2007b)
Orange juice	276	CUT	5.7*	(Guerrero-Beltran et al. 2011; Parish 1998; Zook et al. 1999)
	400	0.8–5.0	4.0–5.0*	
Pear nectar	241	1.2	0.5	(Guerrero-Beltran et al. 2011)
Beetroot juice	300	10	3.5	(Sokołowska et al. 2013)
Phosphate buffer	300	10	5.4*	(Sokołowska et al. 2013)
Beer[a]	300	5	~ 5.5	(Milani et al. 2015)

P: pressure, *t* time, SV_{HPP} $\log_{10}$ reductions, * non-detectable counts after HPP treatment, *CUT* come-up time

[a] Inactivation of *S. cerevisiae* ascospores

[b] HPP treatments performed at 0 °C

gene *OLE1* regulating enzyme Δ9-desaturase to yield a higher proportion of unsaturated fatty acids (Fernandes et al. 2004). De Freitas et al. (2012) observed that *S. cerevisiae* mutant with a deleted *OLE1* gene adapted better to pressures 150–200 MPa by providing 18 carbon chain fatty acid with three unsaturations (linoleic acid; C18:3) as compared to solutions containing fatty acids with one (oleic acid; C18:1) or two (linoleic acid; C18:2) unsaturations. In addition to the unsaturation degree, longer fatty acid chains enhance membrane integrity under pressure. Approximately 4.8 $\log_{10}$ CFU/mL of *S. cerevisiae* survived after 200 MPa/30 min if the yeast was grown with 1 mM of oleic acid (C18:1), whereas 2.7 $\log_{10}$ counts were observed when the yeast uptake was based on palmitoleic acid (C16:1) to stabilize the cellular membrane (De Freitas et al. 2012).

The growth stage affects the pressure resistance of *S. cerevisiae*, where spores and vegetative cells in the stationary phase display a higher pressure resistance than microorganisms in the growth phase (Fernandes 2005; Patterson 2005). *S. cerevisiae* ascospores are capable of surviving in orange juice after 10 min at 400 MPa, while 5 min treatments resulted in complete inactivation of vegetative cells for the same pressure level in apple and orange juices (Table 2).

The hindrance of tryptophan intake, a vital amino acid, has been identified as a key step to the inhibition of *S. cerevisiae* growth at 15–25 MPa. The transportation of tryptophan to the inner cellular compartments does not occur spontaneously and pressure denatures proteins related to tryptophan transportation inside the cell, such as permeases Tat1 and Tat2 (Abe and Horikoshi 2000; Suzuki et al. 2013). As a result, exponentially growing *S. cerevisiae* cells become arrested in the first phase of eukaryotic cell division (G_1 phase), as observed during 16 h at 10 MPa (Perrier-Cornet et al. 1995). Another growth inhibition mechanism is related to the removal of the cell cytoskeleton. *S. cerevisiae* cells in the exponential growth phase showed cell division retarded by 3.5–24 h following 100–200 MPa pressure treatments, until yeast cells were capable of restoring the actin filaments that make up the cytoskeleton (Kawarai et al. 2006; Kobori et al. 1995).

External Factors in HPP Inactivation of *S. cerevisiae*

Water activity (a_w) may be among the most influencing factors conferring *S. cerevisiae* pressure resistance. Table 2 shows *S. cerevisiae* survived (1–3 $\log_{10}$ reductions) in mandarin and orange juice concentrates with 40°Brix ($a_w \sim 0.95$), while less than 1 $\log_{10}$ reductions were observed at 300–600 MPa in sucrose solutions with similar soluble solid content (40°Brix; (Chauvin et al. 2006; Goh et al. 2007). Scanning electron microscopy images of *S. cerevisiae* cells 60°Brix ($a_w < 0.9$) sucrose solution closely resembled non-treated cells after processing at 600 MPa/1 min (Goh et al. 2007). The enhanced yeast baroresistance is mainly attributed to the interactions of sucrose with biological macromolecules located on the cellular membrane, which may decrease the melting temperature of lipids and stabilize proteins (Goh et al. 2007). Additionally, Moussa et al. (2013) suggested that cellular membranes of partially dehydrated cells provided additional flexibility to withstand pressure changes. Conversely, membranes of hydrated cells were hypothesized to have a higher mechanical tension as they are fully extended, making them more susceptible to rupture by compression. Salts such as sodium chloride may also provide a protective effect but to a lesser extent, since the protective effects are hypothesized to be related to slower solute diffusion and shrinkage of pores induced by HPP due to differences in osmotic pressure between the cytoplasm and the exterior of the cell, not by interactions with biomolecules as in the case of sucrose (Goh et al. 2007). Studies have suggested that a 10% decrease in *S. cerevisiae* cellular volume may trigger irreversible yeast inactivation, regardless of the applied pressure-time combination or a_w of the surrounding media (Moussa et al. 2013).

Another important factor influencing HPP microbial inactivation is the acidity of the system, since high pressure may decrease pH of foods by inducing the dissociation of weak acids (Samaranayake and Sastry 2010, 2013). The inactivation rate of *S. cerevisiae* remained unaffected by 100–500 MPa in acidified fruit juices and buffers with pH 2.5–5.0, suggesting that the yeast adapts well under high pressure/high acid conditions (Ogawa et al. 1990; Zook et al. 1999). In situ measurements for apple, grapefruit, orange, and tomato juices showed only a slight pH decline (< 0.3 pH units) at 100–800 MPa (Samaranayake and Sastry 2013). At first glance, such a small pH change may seem insignificant to alter the pressure stability of *S. cerevisiae*, but 0.3 pH units at 40–60 MPa were sufficient to diminish the activity of key enzyme vacuolar phosphofructokinase and increase the activity of esterases in the yeast (Abe 2015). Carbon dioxide has been reported to promote the acidification of intracellular compartments where the dissociation of carbonic acid is heavily favored under pressurized systems (Abe 2015; Fernandes 2005). The dissociation of dissolved CO_2 under pressure may explain the higher inactivation levels of *S. cerevisiae* ascospores in beer at 300 MPa (5 $\log_{10}$ reductions) when compared to apple and orange juices at a higher pressure level (4–5 $\log_{10}$ reductions, 400 MPa; Table 2).

High Pressure Processing in Combination with Other Technologies

HPP has been used in combination with natural compounds or other non-thermal food processing technologies (the hurdle approach). Natural antimicrobial agents obtained from essential oils enhance microbial inactivation under high pressure (Gayán et al. 2012). At 176 MPa, the addition of limonene (2200 μg/mL) or α-terpenine (150 μg/mL) lowered the initial

S. cerevisiae load in yeast broth by 3.1 and 4.5 $\log_{10}$, respectively, whereas only 1 $\log_{10}$ reduction was found in the absence of either antimicrobial. According to the authors, both monoterpenes could have acted as an additional stress factor that slowed down the recovery of injured cells (Adegoke et al. 1997).

Simultaneous HPP and pulsed electric fields treatments also significantly enhanced *S. cerevisiae* inactivation at 200 MPa and 10 kV/cm, yielding < 100 CFU/mL in a water-agar suspension (Rzoska et al. 2015). In the "High-Power Ultrasound" section, the effects of high power ultrasound on *S. cerevisiae* are discussed. In the following section (Pulsed Electric Fields), the principles of pulsed electric fields will be explained in more detail.

An interesting combination of HPP with mechanical homogenization, called high pressure homogenization (HPH), is commonly used to inactivate a variety of spoilage microorganisms in liquid foods on an industrial level. However, the research on HPH application for a specific inactivation of *S. cerevisiae* is scarce (Donsì et al. 2009; Maresca et al. 2011; Patrignani et al. 2009; Spiden et al. 2013). In comparison to HPP, which employs pressures up to 600 MPa, HPH operates in the range of 100–350 MPa and sometimes makes use of elevated temperatures (up to 50 °C for vegetative yeast cells and up to 100 °C for spores, at 200 MPa) for very short residence times (< 0.5 s), in order to achieve effective inactivation of microorganisms (Martínez-Monteagudo et al. 2017; Patrignani and Lanciotti 2016). As for the effectiveness of HPH treatment on *S. cerevisiae*-inoculated fruit juices, applying 150 MPa in three consecutive passes and not exceeding 25 °C is sufficient for *S. cerevisiae* inactivation and extension of the product shelf life to 28 days in refrigerated conditions (Maresca et al. 2011).

Among the *S. cerevisiae* inactivation techniques revised in this paper, HPP is evidently the most commercialized and widely studied technology. HPP has been used on a variety of fruit juices and cultivation media. Importantly, a great deal is already known about the underlying mechanisms in *S. cerevisiae* inactivation and growth inhibition by means of HPP, both on cellular and molecular level. However, there are several areas of research that should be addressed in the near future, including the development of suitable HPP kinetic models and the improvement of HPP industrial implementation in terms of higher temperature ranges and continuity of the HPP systems.

Most current HPP designs assume first-order kinetic models, whereas numerous researchers prefer the log-logistic model for HPP microbial inactivation kinetics. However, recent studies have shown their corresponding limitations to describe HPP microbial inactivation kinetics where mathematical expressions like the Weibull, Gompertz, or quasi-chemical models could become better alternatives. The development of in situ sensors will allow scientists to make measurements of foods and biological systems under pressure will also help to validate kinetic models and extend our understanding of HPP.

Furthermore, several technical issues need to be resolved in order to improve the industrial implementation of HPP. The application of sterilizing HPP units in which high pressure is combined with temperature levels from 90 to 130 °C remains a challenge at an industrial level. Moreover, HPP use in liquid media has been limited to batch systems whereas continuous systems are desirable in the food industry to make production more efficient. Therefore, continuous or semi-continuous HPP systems should be developed in order to meet this requirement. Additionally, the use of HPP in liquid media at an industrial scale is still relatively expensive, and therefore, reducing its cost would be desirable.

High-Power Ultrasound

In the last decade, high-power ultrasound technology (HPU) has been widely studied in order to intensify and ensure the quality of various food processes (Anaya-Esparza et al. 2017; Cárcel et al. 2012; Ozuna et al. 2015). Acoustic energy induces a series of synergic effects which can produce microbial inactivation in liquid foods. The cavitation phenomenon is mainly associated with the formation, growth, and implosion of tiny gas bubbles in a liquid when ultrasound propagates through it. Cavitation in liquids might lead to destruction of *S. cerevisiae* cells due to the extreme temperatures (5000 K) and pressures (1000 atm) generated in the cavitation zone (Ozuna et al. 2015; Soria and Villamiel 2010). Moreover, cavitation effects provoke large shearing forces and turbulence in the medium which can affect the internal structure of this yeast. Guerrero et al. (2005) used transmission electron microscopy and reported that HPU treatment (600 W, 20 kHz, 10–25 min, 45 °C; Table 3) induced physical damage in the cell microstructure of *S. cerevisiae* (puncturing and rupturing the cell wall, disruption of organelles, and discontinuity of plasmalemma) which was attributed to intracellular cavitation induced by the acoustic energy. Similar results were observed by Cameron et al. (2008), Liu et al. (2013), and Marx et al. (2011) using frequencies higher than 20 kHz and acoustic powers between 120 and 750 W (Table 3). However, it is important to consider the importance of the acoustic field characterization in HPU application systems with the aim to measure the acoustic energy available in liquid media during the *S. cerevisiae* inactivation process. This would allow to contrast the results obtained with different HPU devices.

In addition to these effects, acoustic energy is associated with the rupture of water molecules generating highly reactive free radicals that may modify biomolecules present in the cell membrane of *S. cerevisiae*. Wu et al. (2015) studied the damage induced by HPU to cell walls and membranes of *S. cerevisiae* by measuring the release of cell wall polysaccharides and intracellular proteins, respectively. Quantitative protein and

Table 3 Inactivation of *Saccharomyces cerevisiae* by high power ultrasound (HPU) in liquid media

Media	Ultrasonic device	Treatment parameters				Maximal inactivation	Reference
		F (kHz)	Ultrasonic energy	*t* (min)	*T* (°C)		
Sabouraud broth (pH 3 and 5.6)	Vibracell VCX600, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 600 W (wave amplitude 71 to 110 μm)	2–30	35–55	> 5 $\log_{10}$	(Guerrero et al. 2001)
Sterile water	Horn-type sonicator	27.5	UED 42 W/mL	3	< 34	~ 2.4 $\log_{10}$	(Tsukamoto et al. 2004a)
	Squeeze film-type sonicator	26.6	UED 0.158 W/mL	60	Values not reported	~ 1.8 $\log_{10}$	
Sterile water	Horn-type sonicator	27.5	OP 176 W	9	< 38	~ 3 $\log_{10}$	(Tsukamoto et al. 2004b)
Sabouraud broth (pH 5.6) + 1000 ppm low weight chitosan	Vibracell VCX600, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 600 W (wave amplitude 95.2 μm)	10–25	45	3 $\log_{10}$	(Guerrero et al. 2005)
Sterile saline solution and UHT milk	Vibracell VCX750, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 750 W (wave amplitude 124 μm)	2.5–10	24–26	2.10–3.62 $\log_{10}$	(Cameron et al. 2008)
Sterile water	Ultrasonic generator (Kaijo TA-4021type; Lot. No. 92D8)	200	OP 200 W	0–90	20	~ 3.9 $\log_{10}$	(Oyane et al. 2009)
Peptone water, squeezed orange, and clarified apple juice	Vibracell VCX600, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 600 W (wave amplitude 95.2 μm, 80%)	5–20	40	~ 1 $\log_{10}$	(Char et al. 2010)
Apple juice	UP400S, Hielscher, USA (Ø = 22 mm)	24	OP 400 W (wave amplitude 120 μm)	30 (pulsed application 0.5 s on/off pulse)	60	7 $\log_{10}$	(Marx et al. 2011)
Pineapple, grape, and cranberry juices	UP400S, Hielscher, USA (Ø = 22 mm)	24	OP 400 W (wave amplitude 120 μm)	0 to 10. For pulsing treatments 5 s on/off pulse	40–60	6.4 $\log_{10}$ (pineapple juice)	(Bermúdez-Aguirre and Barbosa-Cánovas 2012)
Physiological saline	Vibracell VCX750, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 750 W (wave amplitude 124 μm)	1–5	< 35	~ 92%	(Wordon et al. 2012)
Red grape juice	UP200S, Hielscher, Germany	0 to 135	OP 200 W	20–40	25–50	Values not reported	(Nafar et al. 2013)
Pomegranate juice	Ultrasonic liquid processor, Misonix, Inc., USA (Ø = 19 mm)	20	Wave amplitude 24.4 to 61 μm	3–15	25	1.86 $\log_{10}$	(Alighourchi et al. 2014)
Apple, orange, pineapple, strawberry, and red fruit juices	Vibracell VC130, Sonic & Materials Inc., USA (Ø = 5 mm)	20	Power level 20–60% (18.2–54.6 W)	2–6 (pulse 2–6 s)	Values not reported	2.16 ± 0.59 $\log_{10}$	(Bevilacqua et al. 2013)
Pomegranate juice	Vibracell VC505, Sonic & Materials Inc., USA (Ø = 19 mm)	20	OP 500 W (wave amplitude 50, 75, and 100%)	6–30 (pulsed application 5 s on/off pulse)	< 35	1.36 $\log_{10}$	(Pala et al. 2015)
Orange juice	Ultrasonic processor, MPI, Switzerland	20 ± 1	UEI 0.11 to 0.30 W/mm^2	3–15	20–60	~ 4.4 $\log_{10}$	(Samani et al. 2015)
Commercial and natural squeezed apple juices		20		10–30	20–44	2.8 $\log_{10}$ (natural apple juice)	(Ferrario et al. 2015)

Table 3 (continued)

Media	Ultrasonic device	Treatment parameters				Maximal inactivation	Reference
		F (kHz)	Ultrasonic energy	*t* (min)	*T* (°C)		
Sodium chloride (0.25 and 0.5 M)	Vibracell VCX600, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 600 W (wave amplitude 95.2 μm, 80%)	5–30	5–85	Values not reported	(Wu et al. 2015)
	Vibracell VCX750, Sonic & Materials Inc., USA (Ø = 13 mm)		UEI 10, 24, and 39 W/cm^2				
Commercial and natural squeezed apple juices	Vibracell VCX600, Sonic & Materials Inc., USA (Ø = 13 mm)	20	OP 600 W (wave amplitude 95.2 μm, 80%)	30	25	3.7 $\log_{10}$ (commercial juice)	(Ferrario and Guerrero 2016)
Natural orange and pomegranate juices	UP200H, Hielscher, Germany (Ø = 3 mm)	24	Wave amplitude 105 μm UED 33.31 W/mL	30	50	2.81 $\log_{10}$ (pomegranate juice)	(Sánchez-Rubio et al. 2016)
Chinese rice wine	SCIENTZ-950E, Scientz, Ningbo, China (Ø = 12 mm)	20	OP 0–750 W	0–120	30–40	0.76 $\log_{10}$	(Lyu et al. 2016)

F frequency, *t* time, *T* temperature, Ø diameter probe, *OP* output power, *UED* ultrasonic energy density, *UEI* ultrasonic energy intensities

polysaccharide assays showed that polysaccharides were released faster than proteins at low acoustic intensity (10 W/m^2), whereas higher acoustic intensities (24 and 39 W/m^2) had an inverse effect. With increasing temperature, the liberation of cell wall polysaccharides to the medium was favored due to the weakness of the cell wall induced by heat. Likewise, Wordon et al. (2012) found by means of real-time fluorescent flow-cytometry analysis that injuries associated with HPU application were more evident with increasing temperature.

In order to design an optimal microbial inactivation process assisted by HPU, it is necessary to take into account various factors that include process parameters (ultrasonic power, frequency, temperature, volume, among others), physicochemical properties of the food, and the characteristics of other technologies used in combination (the hurdle approach). Guerrero et al. (2001) studied the influence of process parameters (temperature, pH, and amplitude) on HPU-assisted *S. cerevisiae* inactivation. Temperature and wave amplitude were the most significant variables for the ultrasonic inactivation of *S. cerevisiae*. At 35 and 45 °C, the microbial inactivation significantly decreased when the wave amplitude increased from 71.4 to 107.1 μm. However, titanium particles from the probe appeared in the medium when the highest amplitude was assayed. At the highest temperature tested (55 °C), HPU did not contribute to the inactivation of *S. cerevisiae*. Finally, a non-significant interaction between temperature, pH, and amplitude was reported.

Regarding the influence of physicochemical properties of the food on HPU effectiveness, Cameron et al. (2008) applied an inactivation process of *S. cerevisiae* assisted by HPU (750 W, 20 kHz, 25 °C; Table 3) in sterile saline solution and UHT milk. After 10 min, HPU achieved 2.1 and 3.5 $\log_{10}$ reduction of *S. cerevisiae* in saline solution and UHT milk, respectively. Recently, Bevilacqua et al. (2013) evaluated the HPU application as a processing treatment to reduce *S. cerevisiae* levels in different fruit juices (apple, orange, pineapple, strawberry, and red fruit). For all juices tested, the highest *S. cerevisiae* reduction (2.00–2.25 $\log_{10}$ CFU/mL) was achieved under the maximum processing conditions (power level 54.6 W, treatment time 6 min with 6 s pulses, Table 3). *S. cerevisiae* showed the same sensitivity to ultrasonic energy in orange, strawberry, and red fruit juices. In contrast, the effects were lower in apple and pineapple juice. This trend could be attributed to the composition of each juice which may provide some protective effect on yeast cells to the cavitation phenomenon, especially thanks to its bioactive compound content (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Sánchez-Rubio et al. 2016).

High-Power Ultrasound and Other Technologies

The use of HPU for *S. cerevisiae* inactivation has been compared to the use of other technologies. Oyane et al. (2009)

compared the effects induced by HPU (20 kHz; 200 W; 0–90 min) and γ-rays (0–8 kGy) on DNA or RNA formation and membrane permeability of *S. cerevisiae* using flow cytometry. *S. cerevisiae* cells were inactivated by hydrogen peroxide formed at high ultrasonic frequencies (200 kHz; hydrogen peroxide concentration of 79.6 μM), and the authors claimed that an acoustic exposure of 18 min was long enough to provoke the death of yeast cells. The results obtained by flow cytometry showed that HPU damages yeast cells and inhibits DNA or RNA formation. However, cells exposed to acoustic energy for shorter periods (< 30 min) can recover during incubation. As for γ-rays, this technology was more effective than HPU due to its ionizing energy potential. Recently, Alighourchi et al. (2014) compared the effects of HPU (amplitude levels 24.4–61 μm, 20 kHz, 25 °C; Table 3) and low dose γ-rays (0.5–3.0 kGy) on the inactivation of *S. cerevisiae* in pomegranate juice. The application of HPU at low amplitude levels (50 and 75%) did not have significant effects on the inactivation of this yeast (~ 1 $\log_{10}$ reduction). However, when HPU was applied at 100% amplitude level for 15 min, the population of *S. cerevisiae* was reduced by 1.84–1.88 $\log_{10}$ CFU/mL. On the other hand, γ-rays treatment (3 kGy) achieved a count reduction of 5.06–5.09 $\log_{10}$ CFU/mL.

Char et al. (2010) studied the effectiveness of the single and/or combined effects of HPU (95 μm-wave amplitude, 20 kHz, 40 °C; Table 3) and UV-C light in three different media with continuous flow. Regarding experimental results reported by these authors, HPU application had a lower effect on *S. cerevisiae* inactivation than UV-C light in the three media tested. In contrast, UV-C light proved an effective *S. cerevisiae* inactivation method in peptone water and clarified apple juice. But, its effects diminished when UV-C light was applied to orange juice.

Some investigations have demonstrated the use of HPU by itself is not very effective in achieving high *S. cerevisiae* inactivation in liquid systems since extended treatment times are required (in some cases, higher than 30 min). Consequently, several works have studied the combination of HPU with other traditional preserving alternatives like the addition of natural antimicrobial compounds (Guerrero et al. 2005; Sánchez-Rubio et al. 2016) and thermal processing (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Marx et al. 2011; Wordon et al. 2012).

Guerrero et al. (2005) demonstrated that pre-incubating *S. cerevisiae* for 30 min with 1000 ppm low-weight chitosan prior to HPU treatment yielded over 3 $\log_{10}$ reductions after 25 min of acoustic energy exposure in comparison to ~ 1 $\log_{10}$ reductions reached by HPU treatment without chitosan pre-incubation. However, prolonged times of pre-incubation (90 min) with chitosan did not improve the inactivation of *S. cerevisiae*.

On the other hand, the combination of HPU and thermal processes (called thermo-sonication) has been a subject of numerous papers. Marx et al. (2011) compared the effect of thermo-sonication on *S. cerevisiae* inactivation in apple juice with other non-thermal technologies (HPP and PEF). Under the conditions tested in this work, both HPP (600 MPa at room temperature for 7 min) and the thermo-sonication treatment (120 μm, 60 °C, 30 min) achieved total inactivation of *S. cerevisiae* in apple juice. Bermúdez-Aguirre and Barbosa-Cánovas (2012) evaluated the thermo-sonication effects (continuous and pulsed mode) on the inactivation of *S. cerevisiae* at 40, 50, and 60 °C using three different types of juices (pineapple, grape, and cranberry). The results showed that low temperatures (40 °C) had no effect on cell inactivation regardless of HPU treatment conditions (400 W, 24 kHz, 10 min, pulse duration of 5 s on and 5 s off; Table 3) or the type of juice. However, when the temperature rose, the effects were more evident. In the three analyzed juices, more than 5 $\log_{10}$ reductions were observed at 60 °C using continuous treatment. Similar results were found by Wordon et al. (2012) who confirmed that a sequential process consisting in short HPU exposure (20 kHz, 124 μm, 1 min) followed by mild heat treatment (5 min, 55–60 °C) contributed to the enhancement of *S. cerevisiae* inactivation, showing decimal reduction time values from 3.53 min (60 °C) to 0.73 min (HPU + 60 °C). Recently, the combination of thermo-sonication (24 kHz; 33.31 W/mL, 50 °C) and cinnamon leaf essential oil have been used to inactivate *S. cerevisiae* in natural orange and pomegranate juice, yielding positive results both in the quality and sensory product properties (Sánchez-Rubio et al. 2016).

Furthermore, other non-thermal technologies, such as pulsed light, microwave, and pulsed electric fields, have also been studied in combination with HPU to improve *S. cerevisiae* inactivation in liquid media. The hurdle effect of HPU with these technologies has shown promising results that may potentially be adopted by the food industry.

Ferrario et al. (2015) analyzed the effect of HPU and pulsed light on the inactivation of *S. cerevisiae* inoculated in commercial and natural squeezed apple juices. HPU treatment (30 min) induced 2.5 $\log_{10}$ reduction in *S. cerevisiae* cells at 30 °C for both juices whereas 2.8 $\log_{10}$ reduction was obtained in natural apple juice treated at 44 °C. In single pulsed light treatment (47.76 J/cm^2, 44 °C, 20 s), the highest reduction achieved for *S. cerevisiae* was 3.91 $\log_{10}$ in commercial juice. The combination of both technologies (30 min HPU followed by 60 s pulsed light at 44 °C) allowed the maximum reduction of the yeast of 6.4 and 5.8 $\log_{10}$ in commercial and natural apple juices, respectively. The storage analysis conducted in this research revealed that the application of HPU and pulsed light prevented the treated *S. cerevisiae* cells from recovering after 7 days of storage at 5 ± 1 °C. After this time, treated cells began to grow at the same rate as the control samples.

On the other hand, Samani et al. (2015) developed a combined ultrasonic-microwave system and evaluated its effectiveness on *S. cerevisiae* inactivation in orange juice. The quadratic

model used in this research confirmed that ultrasonic power was more effective than microwave power in reducing *S. cerevisiae* in orange juice. The optimum process conditions for the combined method (ultrasonic-microwave system) were 350 W of microwave power, 35 °C of temperature, 778 W of ultrasonic power, and 11 min of exposure. The sensorial analysis indicated that the appearance of orange juice treated by combining HPU-microwave was better than conventionally pasteurized orange juices. However, the combined method produced a metal taste in the treated orange juice. This taste was attributed to possible corrosion of the ultrasonic probe during the cavitation process and the interaction of corroded particles with the orange juice compounds.

Finally, Lyu et al. (2016) studied the combination of thermo-sonication (35 °C, 750 W, 120 min) and pulsed electric fields treatment (12 kV/cm, 120 μs) during *S. cerevisiae* inactivation process in Chinese rice wine. The sequential application of both technologies was more effective than the application of either technology on its own (thermo-sonication 0.76 log CFU/mL; pulsed electric field 2.88 log CFU/mL; combination of both technologies 3.48–3.72 log CFU/mL). Moreover, the results of the combined technology were more pronounced when thermo-sonication was applied before pulsed electric fields (3.72 log CFU/mL) than vice-versa (3.48 log CFU/mL).

As seen in this section, HPU is a technology with high potential to be employed in enhancing the *S. cerevisiae* inactivation process. HPU can also be used in combination with other technologies in order to intensify the inhibitory effect on microorganisms. In recent years, the coupling of HPU with supercritical carbon dioxide has shown to have a greater combined effect on inactivating *S. cerevisiae* than other non-thermal technologies (Ferrentino and Spilimbergo 2016; Ortuño et al. 2014b; Paniagua-Martínez et al. 2016). In the "Supercritical Carbon Dioxide" section, the principles of supercritical carbon dioxide and its synergistic effects with HPU will be addressed. In the following "Pulsed Electric Fields" section (2.4), the effects of pulsed electric fields on *S. cerevisiae* are discussed.

In conclusion, the use of HPU as a *S. cerevisiae* inactivation technique has been studied for several decades and used on a variety of fruit juices and cultivation media; however, a lot of research is still needed in the area. Despite the fact that the existing studies revised in this paper shed light on the effect of HPU process parameters and cellular changes in the inactivation of *S. cerevisiae* in liquid media, precise molecular mechanisms of *S. cerevisiae* inactivation by means of HPU are yet to be clarified, along with the biochemical changes that may take place during these processes. These, in turn, could help improve the effectiveness of HPU application. Therefore, a lot of research is needed on the molecular level and the field could benefit from the interest of molecular biologists, chemists, and physicists.

Importantly, the industrial implementation of HPU remains a huge challenge. This is mostly because of the elevated price of high-power ultrasonic devices and the scarceness of continuous systems for HPU application in liquid media. Future applications should focus on lowering the price of this technology, as well as developing HPU systems with continuous flow.

This section has been made evident that the main drawback in using HPU as a *S. cerevisiae* inactivation technique on its own is its relatively low efficiency when compared to other technologies. Crucially, HPU have been seen to work much more efficiently in combination with other treatments, especially when applied at the same time. These results point to a possible area of opportunity for future research, since the precise mechanisms of such synergism are yet not clear.

Pulsed Electric Fields

Pulsed electric field treatment (PEF) is another emerging technology that has been applied to control the presence of yeasts in liquid foods (Table 4). Due to the high sensitivity of *S. cerevisiae* towards PEF, many researchers have devoted a particular interest to its inactivation by means of this treatment (Table 4). In a PEF treatment, a short high-energy pulse (among 1–40 kV/cm and 4 μs-13 ms) is applied to the food matrix causing the alteration of the lipid bilayers of the cell membrane and the subsequent inactivation of the yeast cell is induced. However, the main mechanism of the cell inactivation has not been fully elucidated. In this sense, several processes involved in cell inactivation have been proposed, all concerning the cell membrane. These include dielectric breakdown (Kinosita and Tsong 1977), threshold transmembrane potential, compression and viscoelastic properties of the membrane (Dimitrov 1984), fluid mosaic arrangement of lipids and proteins (Coster and Zimmermann 1975), structural defects (Pothakamury et al. 1995), and colloidal osmotic swelling (Serpersu et al. 1985).

The complexity of the PEF treatment mechanisms may be due to the relationship between several parameters. These include food property parameters (water activity, electrical conductivity, electrical resistivity, and physical state of foods), biological parameters (cell size, cell shape, growth phase, strain, and biological form), process parameters (electric field strength, treatment time, pulse shape, processing temperature, and pulse polarity), electric transfer parameters (cluster formation, cell orientation, and cell concentration), parameters involved in the design of the device for PEF treatment (electrode material, formation of gas bubbles, arching, and design of treatment chambers), and hydrodynamic parameters (density of the cell suspension, dynamic viscosity, mean flow velocity, and liquid film gap between electrodes). For example, the initial cell concentration may affect the electrical properties of the medium (Huang et al. 2014) as well as the cell

Table 4 Pulsed electric fields (PEF) inactivation of *Saccharomyces cerevisiae* in different liquid media

Media		PEF Conditions				Maximal $\log_{10}$ reduction	Reference
		EF (kV/cm)	*T* (°C)	*t* (μs)	Type of pulses		
Apple juice		40	15	48	Values not reported	6.0	(Zhang et al. 1994)
Apple juice		13, 22, 35, 50	22	5–25	SWP	6.0	(Qin et al. 1995)
Commercially sterile apple juice		40	45–80	256	SWP	4.3	(Harrison et al. 1997)
Model medium		25, 30 or 35	30	13,000	SWP	6.0	(Aronsson et al. 2001)
Model medium		25	30	13,000	SWF	3.8	(Aronsson and Rönner 2001)
Sterilized apple juice	T1	10, 15, 20, 25, 28	–	100	Bipolar square wave form	3.4	(Cserhalmi et al. 2002)
	T2	20	–	100	Bipolar square		
Model medium		12.5	30	750–6000	EDP	3.9	(Geveke and Kozempel 2003)
Commercially pasteurized orange juice	T1	8	12–15	6000	EDP	5.5	(Molinari et al. 2004)
	T2	12.5	12–15	800	EDP		
Orange juice		35	39	1000	BM	5.1	(Elez-Martínez et al. 2004)
Model medium		5, 10, 20, 25, 30	30	13,000	SWP	6.8	(Aronsson et al. 2005)
Tap water		8–48	35	72	MEDP	4.1	(Schrive et al. 2006)
Grape juice		35	40	1000	BM	4.4	(Garde-Cerdán et al. 2007)
Model medium		1–16	45–80	60,000	SWP	5	(Toepfl et al. 2007)
Grape juice		20, 27.5, 35	35	500–1000	BM	3.9	(Marsellés-Fontanet et al. 2009)
Grape juice		12, 18, 24	40	30–180	MSP	6.01	(Huang et al. 2014)
Apple juice, orange juice, watermelon juice		20	56	3.2–26	MP	7 (Orange juice)	(Timmermans et al. 2014)
Model medium		12	35	Values not reported	Exponential waveform pulse	1 $\log_{10}$ cycles were killed; 1.5 $\log_{10}$ cycles were sublethally injured	(Somolinos et al. 2008)
Model medium		20	30	0–500	Values not reported	4.1 $\log_{10}$ cycles were killed; 90% were injured	(Zhao et al. 2014)
Model medium		0, 10, 20, 30, 40, and 50	30	0, 1200, 2400, 3600, and 4800	Values not reported	3.56 $\log_{10}$ cycles were killed	(Wang et al. 2015)

EF electrical field, *T* temperature, *t* time, *SWP* square waved pulse, *SWF* square wave form, *MSP* monopolar square pulse, *BM* bipolar mode, *EDP* exponential decay pulse, *MP* monopolar pulse, *MEDP* monopolar exponential decaying pulse

orientation (Toepfl et al. 2007) and cluster formation (Heinz et al. 2001; Molinari et al. 2004). When it comes to biological parameters, it is the large cell size which explains the higher sensitivity of *S. cerevisiae* to the PEF treatment compared to other non-thermal treatments. In fact, Maxwell's equation (which has been used to describe the PEF effect), states that the critical field strength decreases when the characteristic dimension of the cell is shifted to higher values (Heinz et al. 2001). Other studies have focused on the effect of the microbial growth phase and the ionic concentration of the suspension liquid on the inactivation of yeast cells by PEF treatment (Zhang et al. 1994). As in the case of high pressure processing (HPP), it used to be generally accepted that PEF inactivation is a function of the growth phase, designating cells in the stationary phase as more resistant (Aronsson and Rönner 2001; Cserhalmi et al. 2002; Zhang et al. 1994). However, Molinari et al. (2004) showed that yeast inactivation by PEF depends on the inoculum size, regardless of the growth phase. In fact, the authors postulated that a high yeast concentration could increase the probability of the formation of clusters that may propagate the electric pulses in a better way and thus, these clusters may amplify the PEF effects. This is consistent with Heinz et al. (2001) and Molinari et al. (2004), who mention that the cell orientation affects the propagation of pulses through the media.

Most researchers have reported that the electric field intensity, the number of pulses, and the duration of the pulse are the most important processing parameters involved in PEF inactivation of yeast cells. Marsellés-Fontanet et al. (2009) reported that instead of the field intensity, the treatment time is the main parameter affecting the inactivation of *S. cerevisiae* in inoculated apple juice. In fact, the treatment time value depends on the value of the number of pulses, average pulse width, frequency, residence time, volumetric capacity of treatment chamber, and average flow rate (Heinz et al. 2001; Marsellés-Fontanet et al. 2009; Toepfl et al. 2007). Table 4 shows that the highest inactivation values are close to 7 $\log_{10}$ reduction. In accordance with the general results regarding process parameters, in both studies, the treatment time is of considerable length. Huang et al. (2014) hypothesized that the

inconsistencies regarding the treatment time effect on *S. cerevisiae* inactivation may be explained by differences between the PEF devices used (such as the number of treatment chambers), the mode of operation (batch or continuous), and the PEF treatment conditions. Differences in the results of the inactivation of *S. cerevisiae* by PEF treatment may also be explained by the viscosity of the treated liquids. Thus, more viscous liquids will show less inactivation values compared to lighter liquids (Schrive et al. 2006). This is particularly relevant as several works on PEF inactivation have been carried out in growth media that are generally more viscous than juices (Table 4).

The type of pulse is also a relevant parameter in *S. cerevisiae* inactivation (Table 4). For example, square-wave pulses resulted in higher inactivation than the exponential decay pulse waveshape, and bipolar pulses seem to be more effective than monopolar pulses (Qin et al. 1994).

As mentioned above, the configuration of the device is crucial for high inactivation of yeast cells. Even though few studies mention the material of which the electrodes are made, Toepfl et al. (2007) claim that stainless steel is commonly used in PEF equipment. However, several problems have been reported when this material is used, namely, formation of deposits, electrode corrosion, and transfer of particles into the treated food. Formation of gas bubbles and an arching in the electric field have been also reported (Toepfl et al. 2007). All of these defects in the configuration of the device make the inactivation of *S. cerevisiae* less effective.

In recent years, the study of the survival state of *S. cerevisiae* after PEF treatment has gained more attention since an incomplete sterilization may lead to the presence of sublethally injured cells. However, the main objective of PEF treatment is to produce lethally injured cells and thus achieve *S. cerevisiae* inactivation. In general, researchers agree that sublethally injured cells are produced at mild electrical field conditions (0–5 kV/cm) because the effect of PEF is reversible until critical values are achieved (Aronsson and Rönner 2001; Cserhalmi et al. 2002; Elez-Martínez et al. 2004; Marsellés-Fontanet et al. 2009; Qin et al. 1995). However, Wang et al. (2015) found that even when the PEF intensity was 5–10 kV/cm, large numbers of *S. cerevisiae* cells were in the sublethal state. This result suggests that electric fields strengths above 10 kV/cm should be employed in order to increase the number of lethally injured cells by the PEF treatment.

Pulsed Electric Fields in Combination with Other Technologies

PEF technologies have been previously combined with other technologies (such as mild thermal processing, antimicrobials, ultrasound, high hydrostatic pressure, high-pressure carbon dioxide, moderate temperature, pH reduction, and ultra violet irradiation) (García-García et al. 2015; Martín-Belloso and Sobrino-López 2011). However, few studies have been conducted on the specific effect of these combinations on the inactivation of *S. cerevisiae*, where the PEF treatment has been combined with other hurdles such as the adjustment in the value of pH of treated media (Aronsson and Rönner 2001), the pre-heating of the treated media (Aronsson and Rönner 2001; Timmermans et al. 2014), and the addition of antimicrobials (Zhang and Mittal 2005).

However, the effects of pH on PEF inactivation is not yet clear. Inactivation of *S. cerevisiae* has been reported to increase at pH other than 5 (Aronsson and Rönner 2001; García-García et al. 2015; Timmermans et al. 2014). At pH values of 3–4, the inactivation of *S. cerevisiae* cells reached 5 $\log_{10}$ reduction (Aronsson and Rönner 2001; Timmermans et al. 2014). However, at pH value of 5, the inactivation decreased. Accordingly, García-García et al. (2015) observed that in a prickly pear beverage the *S. cerevisiae* count could be only reduced down to 3.4 $\log_{10}$ at pH values of 4.2–4.6, lowered by combining sodium benzoate with potassium sorbate. For pH values of 5–7, an increase in inactivation was reported towards higher pH values by Aronsson and Rönner (2001). The low inactivation of *S. cerevisiae* near pH 5 may be explained by the fact that this value is near the optimum pH for the growth of *S. cerevisiae*. These results suggest that pH other than 5 may become an interesting factor for a hurdle technology (pH combined with PEF) in order to create a growth barrier for *S. cerevisiae* and thus help its inactivation.

As mentioned above, the processing temperature affects the inactivation of *S. cerevisiae*. Particularly, an important increase in the inactivation of *S. cerevisiae* cells is observed when the inlet temperature increases, especially when the food or medium is heated above 30–36 °C prior to the PEF treatment (Aronsson and Rönner 2001; Timmermans et al. 2014).

Zhang and Mittal (2005) observed the inactivation of spoilage microbes in mango juice. Even though this study does not assess the inactivation of *S. cerevisiae* specifically, the presence of this yeast is common in juices (Rivas et al. 2006). By combining PEF treatment, mild heat, and antimicrobials in a batch PEF system, Zhang and Mittal (2005) achieved a reduction of 4.4 $\log_{10}$ with 52 °C, 87 kV/cm, using a mixture of lysozyme and nisin (23.75% of pure lysozyme and 1.69% of pure nisin). Rzoska et al. (2015) studied the simultaneous impact of high pressures and PEF treatment of *S. cerevisiae* cells in YPG broth (yeast extract, peptone, glucose). The combination of PEF treatment (5 kV/cm, 5 kHz, 15 pulses) and high pressure processing (200 MPa, 5 min) resulted in a 5.8 $\log_{10}$ reduction.

As seen in this section, PEF have been used on a variety of fruit juices and model substrates. However, the variability of devices and treatment conditions used in these studies does not allow a direct comparison between the results, and thus, contributes to the fact that the precise mechanisms that underlie *S. cerevisiae* inactivation by means of PEF are still not

completely clear. On the other hand, what is very clear from the results of these studies is the effectiveness of PEF treatment, especially in combination with other methods. A similar conclusion has been drawn in the "High Pressure Processing" section about the use of HPU in combination with other treatments.

As for future research concerning PEF inactivation of *S. cerevisiae* in liquid food media, there is a need for experimental designs which would manipulate variables of interest while paying particular attention to controlling all the other variables that are now known to affect *S. cerevisiae* behavior in liquid media. In the following section (Supercritical Carbon Dioxide), the effects of supercritical carbon dioxide on *S. cerevisiae* are discussed.

Supercritical Carbon Dioxide

The supercritical carbon dioxide (SC-CO_2) processing is a non-thermal process capable of inactivating microorganisms, including *S. cerevisiae*, at relatively moderate pressures (7.3–50 MPa) and at temperatures low enough to avoid the thermal effects of traditional methods (Benedito et al. 2015; Damar and Balaban 2006; Kincal et al. 2005). Beyond the critical point of CO_2, the differences between liquid and gaseous CO_2 no longer exist in the newly formed supercritical fluid phase, in which its viscosity is lower than in the liquid state and its density and dissolving power are higher than in the gaseous state. Therefore, the use of SC-CO_2 for sterilization is considered to be more effective than the use of CO_2 in its subcritical state (Balaban and Duong 2014; Ferrentino et al. 2009; Ortuño et al. 2014b). Moreover, CO_2 can be used in the food industry because of its nontoxic, nonflammable, inexpensive, and GRAS status (Balaban and Duong 2014; Calvo and Torres 2010).

According to Garcia-Gonzalez et al. (2007), the fundamental step in the microbial inactivation by means of SC-CO_2 is its contact with the cell membrane and the consequent physicochemical modifications. The mechanisms involved in the microbial inactivation using SC-CO_2 include solubilization of CO_2 into the medium where the cells are suspended, intracellular pH decrease, key enzyme inactivation/cellular metabolism inhibition due to intracellular pH lowering, direct inhibitory effect of molecular CO_2 and HCO_3^- on the microbial metabolism, disordering of the intracellular electrolyte balance, and removal of vital constituents from cells and cell membranes.

As for the inactivation rate, it has been seen to increase with temperature, pressure, and exposure time, where the temperature and pressure tend to synergistically act on each other (Erkmen 2003; Lin et al. 1992). The inactivation rate is also dependent on the initial number of cells, the type of bacterial species, and the kind of suspended materials. On the other hand, organic compounds (such as carbohydrates, fats, and others) present in the media may increase the resistance of bacteria to SC-CO_2 treatment (Balaban and Duong 2014; Benedito et al. 2015). The initial pH of the treatment medium is also important in affecting the microbial inactivation rate. Low pH facilitates penetration of carbonic acid and other carboxylic acids (Lindsay 1976) through the cell membrane and thus, more inactivation is achieved.

The effect of SC-CO_2 treatment on microorganisms can be influenced by their initial number. Under the same conditions, the highest degree of microbial inactivation using SC-CO_2 can be obtained with lower initial microbial numbers (Erkmen 2003). Thus, at higher initial microbial numbers, longer exposure times to SC-CO_2 treatment are needed to achieve the same $\log_{10}$ reduction that can be achieved at low initial number. Parton et al. (2003) indicated that 4 $\log_{10}$ of *S. cerevisiae* were completely inactivated after 40 min exposure to SC-CO_2 (at 9 MPa and 38 °C), while more than 60 min were needed to achieve complete inactivation of 7 $\log_{10}$ of yeast cells at the same conditions (Table 5). The effect of the initial number of microbial cells on their inactivation can be caused by the cellular components (proteins, lipids, carbohydrates, etc.) which are released from lysed cells and can, in turn, protect other microbial cells from the effects of SC-CO_2. Therefore, microbial cells are more exposed to a pressurized CO_2 when there is a low initial microbial number and are hence more sensitive to inactivation.

As observed by Lin et al. (1992), the treatment temperature has a complex role in SC-CO_2 inactivation of *S. cerevisiae*. Higher temperatures can increase the diffusivity of CO_2 and the fluidity of cell membrane which both facilitate the penetration of CO_2 into the cells. On the other hand, increasing temperature decreases the solubility of CO_2 in the medium which can lower the inactivation rate. Another important effect of temperature is the change of CO_2 from subcritical to supercritical phase (Tc = 31.1 °C) where the penetration power of CO_2 is much higher. At the near-critical region, even small changes in temperatures can provoke significant changes in solubility and density of CO_2.

Microbial inactivation can depend on the type of system which determines the CO_2 concentration introduced into the aqueous phase of the suspension. In this sense, (semi-)continuous systems require much shorter inactivation times compared to batch ones, due to the improvement in the CO_2 mass transfer produced by the flow-related agitation. This permits both a quick saturation of the medium by CO_2 and the acceleration of the inactivation mechanisms (Damar and Balaban 2006; Lin et al. 1992).

Ishikawa et al. (1995) obtained more than four times higher $\log_{10}$ reduction of *S. cerevisiae* by using a micro-bubbling filter system for the SC-CO_2 process in comparison to a batch one. The higher efficiency in the inactivation of the microorganisms in this semi-continuous system was due to the CO_2 concentration increase in the micro-bubble reactor. Similarly, Spilimbergo and Bertucco (2003) demonstrated that a semi-continuous system is more efficient than a batch system. Under the pressure of 8.0 MPa and temperatures of 38–

Table 5 Inactivation of *Saccharomyces cerevisiae* by SC-CO_2 in liquid media

Media	Process Parameters				Reduction	References
	P (MPa)	*T* (°C)	*T* (min)	Others		
Growth medium	13.8	35	10		7.0 $\log_{10}$	(Lin et al. 1992)
Physiological saline (PS)	25	35	15		6.0 $\log_{10}$	(Ishikawa et al. 1995)
Growth media	6	35	15		9.0 $\log_{10}$	(Shimoda et al. 1998)
Potato dextrose broth	7.5	40	70		6.0 $\log_{10}$.	(Erkmen 2003)
Model media	8	40	7.5		7.0 $\log_{10}$.	(Spilimbergo and Bertucco 2003)
Grape juice	48.3	35	5		6.5 $\log_{10}$.	(Gunes et al. 2005)
Saboraud dextrose agar	9	38	18		7.0 $\log_{10}$.	(Parton et al. 2003)
Buffer solution	8, 11, and 14	35	3–30	EFS 6, 9, 12 kV/cm Energy 10, 20, 40 J/mL	3.13 $\log_{10}$.	(Pataro et al. 2010)
Peach and kiwi juices	10	35	15		10^5 cfu/mL	(Spilimbergo and Ciola 2010)
Hibiscus beverage	10	35	15		10^5 cfu/mL	(Spilimbergo and Ciola 2010)
YPD broth	10–35	31–41	140		6.7 $\log_{10}$.	(Ortuño et al. 2013)
YPD broth	10–35	31–41	140	HPU > 1 W/cm^2	6.7 $\log_{10}$.	(Ortuño et al. 2013)
Apple juice	20	36	3.1		4.3 $\log_{10}$.	(Paniagua-Martínez et al. 2016)
Apple Juice	20	36	3.1	HPU > 1 W/cm^2	6.8 $\log_{10}$.	(Paniagua-Martínez et al. 2016)

P pressure, *T* temperature, *t* time, *HPU* high-power ultrasound, *EFS* electric field strength

40 °C, a treatment time of 40–60 min was necessary for a complete inactivation of a *S. cerevisiae* in a batch system, whereas 7.5 min were sufficient to inactivate the yeast completely using a semi-continuous system (Table 5). Using a semi-continuous system, Spilimbergo and Ciola (2010) studied the effects of pressure and treatment time on the *S. cerevisiae* inactivation in peach and kiwi juices treated at 35 °C with SC-CO_2 and supercritical nitrous oxide. The total inactivation of *S. cerevisiae* strain (10^5 CFU/mL) was obtained for both juices after 15 min of the combined treatment at 10 MPa (Table 5). Lastly, Shimoda et al. (1998) compared a continuous and a batch system for applying pressurized CO_2 on *S. cerevisiae*. The continuous system effectively inactivated the microorganism in less time than the batch system. In this way, Gunes et al. (2005) studied effects of SC-CO_2 processing parameters, including temperature (35 °C), CO_2 concentration (0, 85, and 170 g/kg) and pressure (48.3 MPa), on *S. cerevisiae* inactivation. The results showed that the process resulted in more than a 6 $\log_{10}$ reduction in the *S. cerevisiae* population. With increasing CO_2–juice ratio, temperature, and pressure, the inactivation rate increased as well.

Supercritical Carbon Dioxide in Combination with Other Technologies

Although the SC-CO_2 technology represents a promising non-thermal processing method, high pressures, high temperatures, and long treatment times are required to guarantee the safety and stability of the food, especially in batch systems. In these systems, coupling with other technologies might be necessary in order to obtain the required lethality at shorter processing times and lower treatment intensities (the hurdle approach). Combinations of batch SC-CO_2 systems with other non-thermal technologies, such as HPU and PEF, have been studied to improve *S. cerevisiae* inactivation in liquid media.

Ortuño et al. (2013, 2014a) analyzed the advantages of coupling a batch SC-CO_2 system with ultrasound (SC-CO_2-HPU) to inactivate *S. cerevisiae* in broth, apple juice, and orange juice at pressures from 10 to 35 MPa and temperatures from 31 to 41 °C. Using SC-CO_2 without ultrasound, 6.7 $\log_{10}$ reduction was obtained at 35 MPa, 36 °C, and 140 min. At the same conditions but applying SC-CO_2-HPU, the same inactivation level was reached in 2 min (Table 5). This reduction in treatment time is thought to be due to the vigorous stirring produced by the ultrasonic field, which increases the solubilization rate of SC-CO_2 in the liquid, as well as its mass transfer into the microbial cells.

Additionally, the medium characteristics were reported to affect the rate of inactivation. In particular, the solid content in the medium, which determines its viscosity, could affect the HPU-related cavitation. Ortuño et al. (2014a) reported the kinetics of *S. cerevisiae* inactivation by SC-CO_2-HPU and found that although a rise in temperature and pressure increased the inactivation rate of *S. cerevisiae*, the type of medium also influenced these effects.

In order to improve the efficiency of batch SC-CO_2-HPU treatments, continuous systems have been developed by

Paniagua-Martínez et al. (2016). The optimization results showed that a continuous system could achieve a 6.8 $\log_{10}$ reduction in 3.1 min of residence time when HPU is applied (200 bar, 36 °C) in comparison to 4.3 $\log_{10}$ reduction under the same conditions and same residence time but without HPU. The effect of HPU may be attributed to the enhancement of SC-CO_2 mass transfer into the medium that accelerates the pH decrease in the liquid phase and the extraction of components from *S. cerevisiae* cells. Another possible mechanism is the cavitation produced by HPU in the liquid phase (as described in more detail in the "High Pressure Processing" section) since cavitation has been proven to cause cracked or damaged cell walls, thus enhancing the penetration of SC-CO_2 into the cells. This, in turn, changes the cellular equilibrium and facilitates the extraction of intracellular components, thus accelerating the death of the *S. cerevisiae* cells.

Furthermore, Pataro et al. (2010) applied the sequential treatment of PEF (6, 9, and 12 kV/cm electric field strength and 10, 20, and 40 J/mL) and SC-CO_2 (8, 11, and 14 MPa, for 3–30 min) on *S. cerevisiae*, and compared it with samples treated with either PEF or SC-CO_2 alone (Table 5). For PEF treatment without SC-CO_2, the maximum inactivation was achieved at 12 kV/cm and 20 J/mL with 0.35 $\log_{10}$ reduction, while for SC-CO_2 without PEF it was at 8 MPa, 35 °C, 3 min (3.13 $\log_{10}$ reduction). Further increasing the pressure and time did not affect the maximum yeast cell reduction. However, the sequential treatment of PEF and SC-CO_2 (8 MPa, 25 °C, 5 min) resulted in total inactivation, regardless of the electric field level of the PEF treatment. In this case, increasing the electric field strength and PEF energy input significantly increased the inactivation effect of SC-CO_2 on *S. cerevisiae*. These results encourage future research on combined technologies for the inactivation of *S. cerevisiae* in food systems.

As has been made evident in this section, the inactivation of *S. cerevisiae* in liquid foods by the means of SC-CO_2 seems to be a promising and feasible alternative to thermal treatments, especially thanks to the mild conditions which help preserve the quality of the treated liquid foods. Importantly, SC-CO_2 achieves better results when coupled with other technologies. Interestingly, (semi-)continuous SC-CO_2 systems achieved better results than batch systems, due to the specificity of this technology. This is a great advantage for the escalation of SC-CO_2 to industrial levels, where continuous systems are preferred to the batch ones, and also when compared to other technologies revised in this work, where the batch systems have been studied more than the continuous ones.

In the following section (Other Non-thermal Technologies for S. cerevisiae Inactivation in Liquid Media), other non-thermal technologies (namely short-wavelength ultraviolet light and atmospheric pressure plasma) and their effects on *S. cerevisiae* are discussed.

Other Non-thermal Technologies for *S. cerevisiae* Inactivation in Liquid Media

Short-Wavelength Ultraviolet Light Treatment

Among other non-thermal technologies, short-wavelength ultraviolet (UV-C) light treatment is a relatively inexpensive method (Geveke and Torres 2012) that can be used to kill several microorganisms, including *S. cerevisiae*. The UV-C light treatment has been applied to liquid foods and water supplies in general (Kaya and Unluturk 2016). The inactivation of *S. cerevisiae* using this technology has been carried out in fruit juices (Franz et al. 2009; Fredericks et al. 2011; Geveke and Torres 2012; Guerrero-Beltrán and Barbosa-Cánovas 2005; Guerrero-Beltrán et al. 2009; Kaya and Unluturk 2016), fruit nectar (Guerrero-Beltrán and Barbosa-Cánovas 2006), and wine (Fredericks et al. 2011).

The main effect of UV-C treatment in cells is the DNA damage, which affects its transcription and translation mechanisms (Franz et al. 2009). Even though UV-C has an inactivation effect on microorganisms over the range of 200–280 nm, UV-C light emitted at 254 nm is the most efficient (Fredericks et al. 2011; Guerrero-Beltrán and Barbosa-Cánovas 2005; Kaya and Unluturk 2016).

A particular feature of UV-C treatment is that the penetration ability of UV light into liquids is poor (approximately 1 mm) (Geveke and Torres 2012; Guerrero-Beltrán and Barbosa-Cánovas 2005). Furthermore, parameters such as the type of liquid, its color, its UV-C absorptivity, the presence of soluble solids and suspended matter, the type of microorganism or species, and the growth stage of the culture may limit the penetration of UV light even further (Guerrero-Beltrán and Barbosa-Cánovas 2005; Guerrero-Beltrán et al. 2009; Koutchma 2009). Due to this limitation, some authors have suggested the generation of a turbulent flow during liquid food processing, as well as the exposure of the entire fluid to a minimum of 400 J/m^2 of UV light at 254 nm (Guerrero-Beltrán and Barbosa-Cánovas 2005). In order to overcome the limitation of the penetration by UV-C light, devices have been designed to generate thin films for UV processing of liquid foods (Geveke and Torres 2012). Turbulent flow during treatment as well as the type of lamp used to generate UV light are the key parameters in the enhancement of UV-C light penetration (Guerrero-Beltrán and Barbosa-Cánovas 2005; Koutchma 2009).

The effect of the parameters involved in UV-C treatment has been observed in the results reported by several authors. Inactivation of *S. cerevisiae* mainly depends in the degree of penetration of UV-C light which, in turn, may depend on the different characteristics of the product. For example, product color might considerably affect the inactivation of *S. cerevisiae*. Kaya and Unluturk (2016) obtained a higher inactivation in pasteurized white grape juice at a 24.27-min exposure (ranging from 3.39 to 5.47 $\log_{10}$ reduction) compared to

Guerrero-Beltrán et al. (2009), who obtained a 2.42 $\log_{10}$ reduction after 30 min at a flow rate of 1.02 L/min in red grape juice (Table 6). This difference was attributed to the color difference of the types of grape used. Similarly, Guerrero-Beltrán and Barbosa-Cánovas (2006) reported that the highest microbial reduction in mango nectar was 2.71 $\log_{10}$ after 30 min of UV light processing at 0.45 L/min, which is in accordance with the intensity of the food color, since mango nectar is darker than white grape juice but clearer than red grape juice. In the case of wine, Fredericks et al. (2011) observed a lower effect of UV light in Pinotage wine compared to Chardonnay wine (1.75 $\log_{10}$ reduction at a dosage of 1377 J/L compared to 5.39 $\log_{10}$ reduction at a dosage of 918 J/L) due to its darker color. In this study, the authors also observed that when the color of liquid foods is similar, the turbidity and the initial microbial load are the factors that make a difference in the inactivation of *S. cerevisiae*.

Interestingly, the inactivation of *S. cerevisiae* by means of UV-C light may also be influenced by the presence of other microorganisms in the media. Guerrero-Beltrán and Barbosa-Cánovas (2005) observed that the maximum inactivation of *S. cerevisiae* in apple juice was of 1.34 $\log_{10}$ reduction when cells were treated at 0.548 L/min. However, when a mixture of three microorganisms (*S. cerevisiae, L. innocua*, and *E. coli*) was treated together, the inactivation of *S. cerevisiae* was much higher, approximately 7 $\log_{10}$ reduction. On the other hand, Franz et al. (2009) also worked with fresh non-pasteurized and bottled pasteurized apple juice treated with UV-C. Some of the treated samples were inoculated with a mixture of *S. cerevisiae*, *L. brevis*, and *E. coli*. The authors observed higher counts of yeasts (including *S. cerevisiae*) in non-pasteurized apple juice samples than pasteurized and bottled juice (3.5 $\log_{10}$ reduction). They hypothesized that the difference on the count of *S. cerevisiae* between the non-pasteurized and pasteurized samples might be explained by the fact that yeasts form clumps which could protect yeasts from the UV light during the inactivation. Guerrero-Beltrán and Barbosa-Cánovas (2005) reported that in their experimental conditions for apple juice, *E. coli* was more resistant than *S. cerevisiae*. However, Franz et al. (2009) found that *E. coli* was less resistant than *S. cerevisiae* (Table 6). These contradictory results may be due to the UV-C light device used, the UV-C light treatment processing conditions, and the type of apple juice used in the experiments (fresh non-pasteurized compared to bottled and pasteurized).

Atmospheric Pressure Plasma

Another non-thermal technology that has been used in the inactivation of *S. cerevisiae* cells is atmospheric pressure plasma, also

Table 6 Summary of UV-C light and atmospheric pressure plasma treatment used for the inactivation of *Saccharomyces cerevisiae* in liquid media

Technology	Media	Treatment conditions	Maximal $\log_{10}$ reduction	Reference
UV-C light treatment	Clear and turbid grape juice	UV doses 0–282.24 mJ/cm^2; flow rate 820 mL/min; treatment time values not reported	3.39	(Kaya and Unluturk 2016)
	Model medium	UV doses 4.8, 9.6, 14, 19, 24 mJ/cm^2; flow rate 300 mL/min; treatment time 3.2 s	6	(Geveke and Torres 2012)
	Cloudy apple juice	UV doses: values not reported; flow rate 300, 600, 1200, 2400 mL/min; treatment time 5, 11, 21, 41 s	2	(Franz et al. 2009)
	Fresh mango nectar	UV doses 75 to 450 kJ/m^2; flow rate 73 to 451 mL/min; treatment time 30 min	2.94	(Guerrero-Beltrán and Barbosa-Cánovas 2006)
	Apple juice	UV doses 75 to 450 kJ/m^2; flow rate 73 to 548 mL/min; treatment time 30 min	1.34	(Guerrero-Beltrán and Barbosa-Cánovas 2005)
	Grape, cranberry, and grapefruit juices	UV doses 75 to 450 kJ/m^2; flow rate 73 to 1020 mL/min; treatment time 30 min	0.53, 2.51, and 2.42 in grape, cranberry, and grapefruit juices, respectively.	(Guerrero-Beltrán et al. 2009)
	Chenin blanc and Shiraz juice; Chardonnay and Pinotage wines	UV doses 459 to 3672 J/L; flow rate 4000 L/h; treatment time values not reported	4.97 and 4.98 in Chardonnay and Pinotage wines, respectively; 4.48 and 4.25 in Chenin blanc and Shiraz, respectively.	(Fredericks et al. 2011)
Atmospheric pressure plasma treatment	Plasma	Plasma breakdown 4 kV (voltage), 13 mA (current), 22 kHz repetition rate, and 0.4 L/min	Values not reported	(Ryu et al. 2013)

known as cold atmospheric plasma or non-thermal plasma due to the low temperatures involved in its generation. In principle, plasma is produced by the application of heat or electromagnetic fields to gas (such as PEF). The high levels of energy generated by these methods lead to the formation of ions. The parameters involved in this non-thermal treatment are the device set-up, presence of ions (salts), the electric field applied to the plasma, the gases fed (gas pressure, type, flow, frequency), the time of exposure, and the surrounding media (Lee et al. 2006; Misra et al. 2011; Ryu et al. 2013).

Ryu et al. (2013) studied the effects of the surrounding media on the inactivation of *S. cerevisiae* with atmospheric pressure plasma treatment. *S. cerevisiae* cells were inoculated in water, saline solution, and YPD media (yeast extract, peptone, dextrose) and put into wells. Each well was set in an argon (Ar) plasma jet and treated for 30 s, 1 min, and 3 min periods (Table 6). Plasma breakdown was achieved at 4 kV, 13 mA, 22 kHz, and 0.4 L/min of Ar gas flow. These tests revealed that nearly all yeast cells were inactivated when they were treated with water after 120 s (near 7 $\log_{10}$ reductions). However, *S. cerevisiae* cells did not inactivate to the same extent in the other media (saline solution and YPD). Accordingly, the majority of cells observed by scanning electron microscopy in water were crushed and shrunken, unlike in saline solution and YPD. The authors attributed the inactivation of *S. cerevisiae* to the severe lipid peroxidation that the cells underwent in water. Similarly to Kamgang-Youbi et al. (2008) who inactivated *Hafnia alvei* by plasma-activated water (PAW), Ryu et al. (2013) attributed the damage suffered by *S. cerevisiae* cells to the acidification of the medium since atmospheric pressure plasma treatment seems to lower the pH values of aqueous solutions (Kamgang-Youbi et al. 2008; Burlica et al. 2006). Another major factor in *S. cerevisiae* inactivation the authors considered was the presence of reactive species, particularly the OH radical (water>saline>YPD).

Although there are several research papers on the effects of atmospheric pressure plasma on other microorganisms, evidence of its effects on the *S. cerevisiae* inactivation is scarce. Both Naïtali et al. (2010) and Kamgang-Youbi et al. (2008) mention that PAW is not very effective in the inactivation of *S. cerevisiae* but neither paper reported the relevant data. Thus, more research is needed in order to evaluate the effects of atmospheric pressure plasma on *S. cerevisiae* inactivation in liquid media. In line with this, the following section discusses future research needs in the area of non-thermal *S. cerevisiae* inactivation technology.

Future Research and Conclusions

One of the main goals of food processing is to ensure the innocuity of foodstuffs. However, it should also develop high-quality products, preserving their natural bioactive compound content and fresh-like organoleptic characteristics. In this sense, *S. cerevisiae* does not represent a health risk for the consumers but may adversely affect the quality parameters of products and cause great economic losses to the food industry due to product spoilage. Inactivation of microorganisms while preserving the product quality might prove difficult when using traditional thermal processes. In fact, worldwide food experts working in the academia, industry, and governmental agencies foresee that non-thermal and emerging technologies, such as those reviewed in this work, will be among the most impactful novel food processing technologies for the next decade in terms of product commercialization. Despite the current and envisioned commercial success of HPP and the undeniable potential of the other non-thermal technologies to follow its lead, there is a great need for thorough investigation, especially in applying these technologies to a wide range of foodstuffs under different experimental conditions. It should also be considered that most studies on the effectiveness of non-thermal technologies in the inactivation of *S. cerevisiae* have been carried out at a laboratory level. Therefore, particular attention should be paid to testing the scaling-up of these technologies to industrial levels. For example, few research papers investigate technologies that have been adapted to continuous systems which are crucial for their industrial implementation. In the same way, very few investigations focus on the cost of the energy required for the use of these non-thermal technologies. Similarly, their effects on the physicochemical parameters of liquid foods and bioactive compounds present therein should be thoroughly investigated, since both are key parameters for industrial technology applications, either as individually implemented technologies or in combination with others. Moreover, additional studies that would validate *S. cerevisiae* inactivation kinetic models are needed before governmental regulations can be implemented. In this sense, creation of standardized databases like those available for thermal food processing would be desirable.

One of the main contributions of the present review is the fact that, in the revised literature, the use of non-thermal technologies for *S. cerevisiae* inactivation is consistently more effective in combination with other technologies than on its own. Among these, the use of natural antimicrobial agents, such as essential oils, is on a rise in the food industry. Therefore, future research in the area should focus on a systematic study of *S. cerevisiae* inactivation by means of non-thermal technology combinations, always seeking the most efficient and cost-effective combinations and bearing in mind the quality of the final liquid product.

The present review has also made evident the need for interdisciplinary research in the field of *S. cerevisiae* inactivation. For instance, industrial implementation of non-thermal technologies and their combinations would benefit from collaborations between natural scientists and experts from the fields of engineering and industrial technology, in order to

develop devices and pieces of equipment that would be suitable for cost-effective, big-scale *S. cerevisiae* inactivation in liquid media.

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