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Ultrasonic disruption of yeast cells: Underlying mechanism and effects of processing parameters

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ABSTRACT

During the ultrasonic treatment of yeast cells, the damage to the cell walls and membranes was monitored by assessing the release of cell wall polysaccharides and intracellular proteins, respectively. At a low acoustic intensity (10 W/cm^2), the polysaccharides were released faster than the proteins, whereas at higher acoustic intensities (24 and 39 W/cm^2), this trend was reversed. At elevated temperatures, additional cell wall polysaccharides were released, whereas fewer intracellular proteins were released. Increasing processed volumes, initial cell concentrations, and salt concentrations led to the decrease of both fractions. However, the total releases per ultrasound treatment remained constant regardless of the processed volumes, and increased with the increase of initial cell concentrations. The results suggest that the ultrasonic disruption of yeast cells begins with the breakdown of the cell wall before continuing to the cell membrane. These findings may offer new avenues for exploring more efficient cell disruption or microbial inactivation processes.

Industrial relevance: Ultrasonic technology has been intensively studied for microbial inactivation or cell disruption. It is believed that the inner cell membrane is the target of the ultrasonic damage. The mechanism of microbial inactivation via ultrasound involves the thinning of the cell membranes.

In this study, we monitored the ultrasonic damage to yeast cell walls and membranes by monitoring the release of cell wall polysaccharides and intracellular proteins, respectively. Our results demonstrate that the ultrasonic disruption of yeast cells begins with the breakdown of the cell wall before continuing to the cell membrane. Increasing the temperature weakens the cell wall and thermally coagulates the intracellular proteins. Increasing the processed volumes, initial cell concentrations, and salt concentrations reduces the releases of cell wall polysaccharides and intracellular proteins from the viewpoint of yeast cells. However, the total releases of each fraction per ultrasound treatment remain constant regardless of the processed volumes, and increase with increasing initial cell concentrations. Our results may offer new insights towards the exploration of more efficient industrial processes for microbial inactivation or cell disruption, and in elucidating the effects of processing parameters.

1. Introduction

Recent trends in health and wellness have favored the application of non-thermal processing technologies, such as high-pressure, high-intensity ultrasound or pulsed electric fields, to minimize losses in the nutrition and quality of products associated with conventional thermal processing (Rawson et al., 2013; Tiwari, O'Donnell, & Cullen, 2009). High-intensity ultrasound refers to sound waves with intensity above 1–2 W/cm^2 and frequencies between 20 and 100 kHz (Wu, Zivanovic, Hayes, & Weiss, 2008). Using ultrasound in the food industry has numerous advantages over conventional processing technologies, such as higher processing throughput and less energy consumption (Rosello-Soto et al., 2015). The microbial inactivation activity of ultrasound was documented in 1929 (Harvey & Loomis, 1929). Thereafter numerous studies have tested the effectiveness of ultrasound against many microorganisms as a food preservation technology (Chemat, Zille, & Khan, 2011; Demirdoven & Baysal, 2009; Earnshaw, Appleyard, & Hurst, 1995). Additional works have used ultrasound as a cell disruption tool to harvest intracellular proteins, which is very effective in laboratory conditions (Balasundaram, Harrison, & Bracewell, 2009; Gogate & Kabadi, 2009; Liu, Zeng, Sun, & Han, 2013).

The microbial inactivation activity caused by ultrasound arises from the interactions between the cavitation bubbles and cells (Gao, Lewis, Ashokkumar, & Hemar, 2014a,b). Many factors modulate the effectiveness of ultrasound. Usually, the effectiveness increases exponentially with an increase in the acoustic intensity until a maximum is reached (Pagan, Manas, Raso, & Condon, 1999; Raso, Pagan, Condon, & Sala, 1998). Further increases do not improve the effectiveness due to acoustic shielding (van Iersel, Benes, & Keurentjes, 2008). Within a certain temperature range, a combination of heat treatment with ultrasound is more effective than heat treatment alone, and an additive or

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synergistic inactivation effect is observed depending on the types of microorganisms (Raso, Pagan, Condon, & Sala, 1998). Beyond this temperature range, these effects disappear (Raso, Pagan, Condon, & Sala, 1998). Consequently, the performance of ultrasonic pasteurization at 50 °C has been proposed as a replacement for conventional thermal pasteurization techniques (Patist & Bates, 2008). A similar effect occurs when the static pressure over the sonicator chamber is increased. The effectiveness of ultrasound increases markedly with an increase in the static pressure. However, increasing the pressure beyond the optimum value reduces the effectiveness (Pagan et al., 1999). The influences of temperature, pressure and other process parameters on the effectiveness of ultrasound may be explained by their influences on the behavior of cavitation bubbles (Jamshidi, Pohl, Peuker, & Brenner, 2012). At elevated temperatures, the threshold for cavitation formation decreases, thereby increasing the number of cavitation bubbles and decreasing the collapsing intensity for each bubble due to the "cushioning" effect (Wu et al., 2008). With an increase in the pressure, the threshold for cavitation formation increases and the number of cavitation bubbles decreases. However, the collapsing intensity of the individual bubbles increases (Lee, Zhou, Liang, Feng, & Martin, 2009). Therefore, the overall effects of temperature and pressure are balanced between the number of cavitation bubbles and the collapsing intensity of the individual bubbles. Consequently, a combination of pressure, heat and ultrasound, which is a technique called manothermosonication, has been proposed as a more efficient microbial inactivation technique (Lee et al., 2009; Pagan et al., 1999; Raso, Pagan, Condon, & Sala, 1998; Raso, Palop, Pagan, & Condon, 1998).

Although consensus has been achieved regarding the influence of acoustic intensity, temperature and pressure, conflicting results have been observed for other factors, particularly in the context of microorganisms. For example, some studies have reported that Gram-negative bacteria are more sensitive to ultrasonic inactivation (Cameron, McMaster, & Britz, 2008; Drakopoulou, Terzakis, Fountoulakis, Mantzavinos, & Manios, 2009), whereas others have reported that the Gram status exerts no influence (Gao et al., 2014a; Scherba, Weigel, & Obrien, 1991). Earlier studies indicated that the disruption of subcellular particles by intracellular cavitation was crucial for microbial inactivation (Cameron et al., 2008; Hughes & Nyborg, 1962). Later studies believed that the inner cell membrane was the target of ultrasonic damage because the inactivation process remained independent of the cell wall structure but changed relative to the cell membrane fluidity (Ben-Hur & Green, 1982; Scherba et al., 1991). A recent study proposed intramembrane cavitation as the unifying mechanism for ultrasound-induced bioeffects (Krasovitski, Frenkel, Shoham, & Kimmel, 2011). This model predicted that the cellular membrane was intrinsically capable of absorbing mechanical energy from the ultrasonic field and of transforming this energy into expansions and contractions in the intramembrane space without the preexistence of air voids. However, a more recent study revealed that the thickness of the cell wall capsule was responsible for the resistance of bacteria to ultrasonic deactivation (Gao et al., 2014a). In literatures, contradicted results have also been found regarding the effects of processed volumes (Al Bsoul et al., 2010; Piyasena, Mohareb, & McKellar, 2003) and initial cell concentrations (Al Bsoul et al., 2010; Apar & Ozbek, 2008; Gao et al., 2014b; Iida, Tuziuti, Yasui, Kozuka, & Towata, 2008).

To date, most studies have used colony counting to evaluate the microbial inactivation activity of ultrasound. However, the effects of ultrasound on the cell structure cannot be assessed because colony counting relies on the number of surviving cells after treatment. Although damage to the cell structure can be observed via transmission electron microscopy (TEM), this analysis is specific toward individual cells, and a quantitative analysis of the cell structure damage is almost impossible. In this study, the damage to the cell wall and membrane during ultrasonic treatments and the influences of processing parameters were quantitatively evaluated and assessed by chemically measuring the releases of cell wall polysaccharides and intracellular proteins. The findings herein may offer new avenues for the exploration of more efficient cell disruption or microbial inactivation processes.

2. Experimental details

2.1. Materials

Sugar tolerate baker's yeast (*Saccharomyces cerevisiae*) was obtained from the Angel Yeast Corporation (Yichang, China) as a compressed cake, stored at 4 °C and used before the expiration date. The BCA (bicinchoninic acid assay) reagent was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All of the other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). In addition, 18-megOhm D.I. (de-ionized) water was used in this study.

2.2. High-intensity ultrasound treatment

The yeast cells were weighed, dispersed with D.I. water, and stirred until a homogeneous state was reached in a double-walled, jacketed beaker under controlled temperatures (the temperature was controlled by a circulating water bath). The sonication treatments were performed using a 20-kHz horn-type sonicator (Sonics and Materials VC-750, Newton, CT) equipped with a 1/2-in titanium probe in a continuous mode. When we studied the effect of one of the ultrasound parameters, all other parameters were fixed as that in a typical experimental set involved the following conditions: 4.0 g of yeast, 40 ml of D.I. water, ultrasound amplitude of 40%, temperature of 5 °C, sonication time of 5 min, and a probe depth of 1 cm. One-milliliter aliquots of the yeast suspension were collected immediately after each treatment, diluted with 9.0 ml of water, and centrifuged at 3000 *g* for 10 min before analyzing the proteins and polysaccharides' contents in the supernatant. The processing parameters, including ultrasound amplitudes at 20%, 40% and 60% of the maximum amplitudes that can be delivered by the sonicator, which correspond to ultrasound intensities of 10, 24 and W/cm^2, respectively, as determined by calorimetrical analysis (Wu et al., 2008), sonication time (5 to 30 min), temperature (5 to 85 °C), processed volumes (40, 100 and 200 ml), yeast concentrations (1.25% to 20% w/v), and presence of sodium chloride (0.25 and 0.5 M) were tested to assess their influence on the release behaviors of the proteins and polysaccharides. Control samples subjected to mechanical stirring (Fisher Scientific, Isotemp, speed of 350 rpm) instead of ultrasound were also tested.

2.3. BCA protein assay

The first 0.1 ml of the supernatant was mixed with 2.0 ml of BCA reagent, and the mixture was incubated at 60 °C for 15 min. The absorbance at 562 nm was then measured with a spectrophotometer (LabTech, UV Bluestar A, Beijing). The amount of released proteins was calculated based on the yeast cell dry weight and the total protein content using bovine serum albumin as the calibration standard. Yeast cells were treated with 1 N NaOH at 60 °C for 30 min, passed through a 0.45-μm PVDF (polyvinylidene fluoride) filter (Sinopharm, Shanghai, China) and analyzed to determine the total protein content through the BCA protein assay.

2.4. Anthrone assay for polysaccharide determination

A 0.5 ml sample of the supernatant was mixed with 4.5 ml of the anthrone reagent, and the mixture was incubated at 90 °C for 10 min and then cooled to room temperature in an ice bath before its absorbance at 620 nm was measured. The amount of released polysaccharides was calculated based on the yeast cell dry weight and total polysaccharide content using glucose as the calibration standard. The total polysaccharide content of the yeast cells was determined by hydrating 0.005 g of dry yeast cells with 0.5 ml of water for 10 min and subjecting the mixture to the anthrone assay.