U. S. Food and Drug Administration
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Kinetics of Microbial Inactivation for Alternative Food Processing Technologies

Ultrasound

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Scope of Deliverables

This section describes the uses of ultrasound in the food industry. A general theory about the mechanism of microbial inactivation is presented. Data from inactivation of food microorganisms are scarce, and most applications of ultrasound involve its use in combination with other preservation methods. This review points to the need for more research on microbial inactivation in food systems when ultrasonication is used with other methods.

1. Introduction

1.1. Definition, Description and Applications

The textbook definition of ultrasound is energy generated by sound waves of 20,000 or more vibrations per second. Presently, most developments of ultrasonics (sonication) for food applications are nonmicrobial in nature (Hoover 1997). High frequencies in the range of 0.1 to 20 MHz, pulsed operation and low power levels (100 mW) are used for nondestructive testing (Gunasekaran and Chiyung1994). Ultrasonic excitation is being examined for nondestructive evaluation of the internal quality and latent defects of whole fruits and vegetables in a manner similar to the use of ultrasound for viewing the developing fetus in a mother's womb (Mizrach and others 1994). Floros and Liang (1994) noted the use of low intensity high-frequency ultrasound for improvement of food product/process monitoring due to the acceleration of diffusion. These industrial applications include texture, viscosity and concentration measurements of many solid or fluid foods; composition determination of eggs, meats, fruits and vegetables, dairy and other products; thickness, flow level and temperature measurements for monitoring and control of several processes; and nondestructive inspection of egg shells and food packages. Floros and Liang (1994) also listed direct process improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, disruption of cells, degassing of liquids, acceleration of heat transfer and extraction processes and enhancement of any process dependent upon

diffusion. It is evident that ultrasound technology has a wide range of current and future applications in the food industry.

1.2. Mechanisms of Microbial Inactivation

The bactericidal effect of ultrasound is generally attributed to intracellular cavitation (Hughes and Nyborg 1962). It is proposed that micro-mechanical shocks are created by making and breaking microscopic bubbles induced by fluctuating pressures under the ultrasonication process. These shocks disrupt cellular structural and functional components up to the point of cell lysis.

1.3. Summary of Microbial Inactivation Kinetics

The use of ultrasound alone to lyse microbial cells is a well-established laboratory method to extract intracellular components (Skauen 1976). Stumpf and others (1946) published an improved ultrasonic method to disintegrate a broader range of bacteria to obtain cell-free bacterial enzyme extracts efficiently and aseptically, acknowledging some difficulties with bacteria from genera *Sarcina, Micrococcus, Acetobacter* and the yeast, *Saccharomyces cerevisiae*.

In the laboratory, the efficiencies of lysis approach 100%; however, such cellular disruption is conducted as a small-scale batch operation, with temperature controlled by placement of the sample and its container in an ice bucket, and with immersion of the sonicator probe into a small volume of cells suspended in buffer. In an industrial food processing setting, less control of critical factors would prevent lysis efficiencies from reaching such high levels. In addition, the protective nature of foods to the ultrasonic inactivation of bacterial cells or spores is quite evident when comparing results from microorganisms suspended in buffer to those present in a food system (see Section 2). The heterogeneous nature of food with the inclusion of particulates and other interfering substances severely curtails the singular use of ultrasound as a preservation method. Although these limitations make the current probability of commercial development low, combination of ultrasound with other preservation processes (for example, heat and mild pressure) appears to have the greatest potential for industrial applications. Review of the literature indicates that no published information on microbial inactivation rates in foods is available. Such information is necessary to generate kinetic data.

1.4. Summary of Critical Process Factors

Critical processing factors are assumed to be the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, the type of microorganism, the volume of food to be processed, the composition of the food and the temperature of treatment. When ultrasound is used in combination with other processes, then the critical process factors of these methods must be taken into account.

2. Pathogens of Public Health Concern Most Resistant to the Technology

The limited current literature on process effectiveness indicates that all pathogens should be considered resistant to ultrasound, especially when ultrasound is the lone preservation treatment. Particular attention should be given when combining ultrasound with other methods with greater antimicrobial potency, because assumed optimal conditions can result in higher or lower inactivation rates of target microorganisms than expected.

2.1. Critical Process Factors When Used in Combination with Other Treatments

Survey of the literature shows that enteric gram-negative pathogens have been targeted with ultrasound in perishable animal-derived foods, such as poultry and milk (Lilliard 1994). In these instances, the application has most often been in a liquid environment. For example, Lee and others (1989) reduced populations of salmonellae by approximately 4-log cycles in peptone water using a 10-min treatment; however, in chocolate milk, a 30-min ultrasonic treatment only lowered the number of salmonellae by 0.8-log cycle, suggesting that the chocolate milk offered significant protection against microbial inactivation.

Attempts have been made to reduce salmonellae attached to poultry skin. Lilliard (1993) studied the effects of sonicating poultry in chilled baths containing chlorinated water. Reductions of *Salmonella* in the range of 2.5 to 4-log cycles were obtained with a combination of immersion in chlorinated water and sonication compared to reductions of less than 1-log when using immersion in chlorinated water alone. Sonication alone reduced the counts by only 1-1.5-log. Results were similar in the examination of pre- and post-chill broiler drumsticks treated with ultrasonic energy in 1% lactic acid at pH 2.0 and 4.0 for 0.5, 2 and 3.5 min. After 0, 7 and 15 d, aerobic plate counts showed no significant effect of ultrasonification (Sams and Feria 1991).

Ordoñez and others (1984) combined ultrasound of 20 kHz/160 W using a cell disrupter with heating over a range of 5 to 62 ° C for the inactivation of *Streptoccocus faecium* and *Streptoccocus durans*. They found that the combination of ultrasound and heat applied together was significantly more effective in inactivating these bacteria than the 2 methods used alone. Compared to a singular heat treatment, the simultaneous use of ultrasound and heat reduced the populations of thermoduric enterococci approximately 1 additional log cycle. Data presented in this paper evaluated the application using cell suspensions in 0.1 M dimethylglutaric acid buffer (pH 6.6). Although no food system was tested, the authors suggested that a milk pasteurization process incorporating ultrasonic treatment would allow for reduction of processing temperature and time.

A subsequent work by Ordoñez and others (1987) examined a similar application (referred to as thermoultrasonication) against survival of a strain of *Staphylococcus aureus* suspended in 0.05 M phosphate buffer (pH 6.8) and UHT milk. In this work, the combined process reduced D-values by 63% in the buffer as compared to the *D*- values of the heat treatment alone, and by 43% when tested in UHT milk. (These percentages represent less than a 10-fold reduction in the time in min to reduce *S. aureus* 1-log cfu/ml since a 1-log reduction represents a 90% reduction.)

Spore suspensions of *Bacillus subtilis* were targeted by Garcia and others (1989) employing thermoultrasonication in the temperature range of 70 to 95 ° C. Distilled water, glycerol and milk were used as the treatment media. Ultrasound alone had no effect, but thermoultra-sonication reduced the spore population by 63 to 73% (<1-log cycle cfu/ml) in glycerol and by 40 to 79% in milk. In distilled water the reductions ranged from 70 to 99.9% (3-log cycle cfu/ml). The effect of thermoultrasonication was dramatically diminished as the temperature of the treatment approached 100 :C. The optimum temperature for maximum inactivation of spores of *B. subtilis* under the experimental conditions was 70 :C. The mechanism for this phenomenon was unclear.

Raso and coworkers examined the response of *Yersinia enterocolitica* and spores of *B. subtilis* to a combination of heat, pressure and ultrasound. For the *Y. enterocolitica* study, cells were suspended in citrate-phosphate buffer (pH 7.0) (Raso and others 1998a). They used combined ultrasound and static pressures [manosonication (MS)] as well as heat/ultrasound and pressure [manothermosonication (MTS)] against *Y. enterocolitica*. At ambient temperature and pressure, the effect of ultrasound on *Y. enterocolitica* was insignificant. Moderate pressures of 600 kPa did not affect the survival of *Y. enterocolitica* to heat. Heat and ultrasound under pressure functioned independently. It appeared that the individual contributions of heat and ultrasound under pressure to the total effect of MTS depended primarily upon the temperature. Above 58 :C, any added inactivation caused by pressure disappeared. These results suggest that inactivation is not a simple additive reaction of the 3 treatment types. Optimal inactivation using these 3 methods requires a trial-and-error approach until the mechanisms of inactivation are resolved. It was noted that MS treatment resulted in cellular disruption. D-values recorded for *Y. enterocolitica* ATCC 9610 were 1.39 min at 59 :C, 1.5 min for the highest ultrasound setting (an amplitude of 150 μm at 20 kHz) and 0.28 min for an MS treatment of 300 kPa and 150 μm at 30 ˚C. The latter treatment was similar to an MTS treatment at 63 ˚C. The same authors (Raso and others 1998b) found that a 12-min treatment of 500 kPa and 117 μm at 20 kHz killed approximately 99% (2-log reduction) of a spore suspension of *B. subtilis* ATCC 9372 in McIlvaine citrate-phosphate buffer (pH 7.0). The sporicidal effect of MS treatments depended upon the static pressure, amplitude of ultrasonic waves and the treatment temperature. Above 500 kPa, additional increments of pressure magnitude did not increase the amount of spore inactivation. In the range of 70 to 90 ˚C, combination with 20 kHz, 300 kPa and 117˚ m for 6 min had a synergistic effect on spore inactivation. Although the authors point out the possible application of MT and MTS as a preservation system for highly heat-sensitive liquids, no food system was investigated as a test medium in either of these studies.

A focused 1-MHz ultrasound transducer, capable of generating a spatial peak pulse average intensity of 500 W/cm^2 was used to treat culture broths of *Escherichia coli* containing microbubbles by Vollmer and others (1998). It was found that stress response was induced in *E. coli* and, under some conditions, caused death. They also reported that stationary-phase cells were more resistant to sonication than those in exponential-phase growth stage. The intent of the work was not the development of ultrasound technology as a food preservation method, but rather to study stress in bacteria and perhaps to develop the technology to treat drinking water.

The articles overviewed here demonstrate that ultrasound lacks the power and versatility to inactivate microorganisms reliably for purposes of food preservation; however, ultrasound may be used in combination with other preservation processes primarily to enhance microbial inactivation in foods. Such applications will require further exploration (for example, validation studies) of important synergistic effects that are relevant for industrial use. In conclusion, ultrasound technology has the potential for future use as a preservation process; however, food systems present a very challenging environment for ultrasound to achieve the degree of microbial inactivation necessary for practical use. At the present and probably for the next several years, its applications in this area are not commercially feasible. Also, an important component of research that has received little notice with this technology is the possible effects of ultrasound (developed for food preservation purposes) on the sensory quality of the food.

3. Mechanisms of Inactivation

In general, a relatively low number of studies employ ultrasound for microbial inactivation. As stated above in Section 1.3, the mechanism of inactivation of vegetative bacteria appears to be intracellular cavitation. Maximum effectiveness results in cellular lysis. For spores, the mechanism is not clear. Cavitation must play a role, but it is an auxiliary one since ultrasound alone has no effect on spores. The other co-treatments have a main effect in any spore inactivation. Inactivation mechanisms of ultrasound used in combination with other treatments are not understood. Also unknown are ways to determine the occurrence of ultrasound-induced injury and repair, and to predict the effects of process variables and post-treatment storage of food products treated with ultrasound in combination with other inactivation methods. In the literature, conventional plating methods specific for the organism under examination have been used to enumerate microorganisms in studies involving ultrasound.

At the current state of commercial development for purposes of food preservation, ultrasound has potential to enhance the effectiveness of other processing methods. No mathematical model has been formulated for the inactivation of microorganisms by ultrasonic methods.

4. Validation/Critical Process Factors

Such is the current state of literature regarding the application of ultrasound as a preservation process that, qualifying, prioritizing and quantifying its critical process factors is by assumption or implication. Factors that appear to substantially affect the destruction of microorganisms by ultrasound are the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms, the type of microorganism, the volume of food to be processed, the composition of the food and the temperature of treatment. When ultrasound is used in combination with other processes, the critical process factors of these methods must be taken into account. For example, the presence of disinfectants or preservative compounds and levels of static pressure, irradiation or electrical energy are critical process factors when hurdle treatment is the processing

approach. Further maturity of ultrasonic processing in the food industry will define its critical process factors.

5. Process Deviations

As shown in the review of the literature and due to the limited extent of research in the area, critical process factors and, therefore, possible process deviations are not well understood.

6. Research Needs

It is evident from this review that further research is needed to determine the feasibility and usefulness of ultrasound as a food preservation method or supplementary treatment. The main areas to be addressed are:

- Determination of the effect of ultrasound on microbial inactivation efficiency when used with other processing technologies (high pressure, heat, or others).
- Identification of mechanisms of microbial inactivation when used in combination with other technologies.
- Identification of critical process factors when ultrasound is used in hurdle technology.
- Evaluation of the influence of the food properties, such as viscosity and size of particulates, on microbial inactivation.

References

Floros, J. D. and Liang, H. 1994. Acoustically assisted diffusion through membranes and biomaterials. Food Technol. 48(12):79-84

Garcia, M. L., Burgos, J., Sanz, B. and Ordonez, J. A. 1989. Effect of heat and ultrasonic waves on the survival of 2 strains of Bacillus subtilis. J Appl Bacteriol. 67:619-628

Gunasekaran, S. and Chiyung, A. 1994. Evaluating milk coagulation with ultrasonics. Food Technol. 48(12):74-78

Hoover, D. G. 1997. Minimally processed fruits and vegetables: Reducing microbial load by nonthermal physical treatments. Food Technol. 51(6):66-69, 71

Hughes, D. E. and Nyborg, W. L. 1962. Cell disruption by ultrasound. Science. 38:108-114s

Lee, B. H., Kermasha, S. and Baker, B. E. 1989. Thermal, ultrasonic and ultraviolet inactivation of Salmonella in thin films of aqueous media and chocolate. Food Microbiol. 6:143-152

Lillard, H. S. 1993. Bactericidal effect of chlorine on attached salmonellae with and without sonification. J Food Protect. 56(8):716-717

Lillard, H. S. 1994. Decontamination of poultry skin by sonication. Food Technol. 48(12):72-73

Mizrach, A., Galilli, N. and Rosenhouse, G. 1994. Determining quality of fresh products by ultrasonic excitation. Food Technol. 48(12):68-71

Ordoñez, J. A., Sanz, B., Hernandez, P. E. and Lopez-Lorenzo, P. 1984. A note on the effect of combined ultrasonic and heat treatments on the survival of thermoduric streptococci. J Appl Bacteriol. 56:175-177

Ordoñez, J. A., Aguilera, M. A., Garcia, M. L. and Sanz, B. 1987. Effect of combined ultrasonic and heat treatment (thermoultrasonication) on the survival of a strain of Staphylococcus aureus. J Dairy Res. 54:61-67

Raso, J., Pagan, R., Condon, S. and Sala, F. J. 1998a. Influence of temperature and pressure on the lethality of ultrasound. Appl Environ Microbiol. 64(2):465-471

Raso, J., Palop, A., Pagan, R. and Condon, S. 1998b. Inactivation of Bacillus subtilis spores by combining ultrasonic waves under pressure and mild heat treatment. J Appl Microbiol. 85:849-854

Sams, A. R. and Feria, R. 1991. Microbial effects of ultrasonication of broiler drumstick skin. J Food Sci. 56(1):247-248

Skauen, D. 1976. A comparison of heat production and cavitation intensity in several ultrasonic cell disrupters. Ultrasonics. 14:173-176

Stumpf, P. K., Green, D. E. and Smith, F. W. J. 1946. Ultrasonic disintegration as a method of extracting bacterial enzymes. J Bacteriol. 51:487-493

Vollmer, A. C., Everbach, E. C., Halpern, M. and Kwakye, S. 1998. Bacterial stress responses to 1-megahertz pulsed ultrasound in the presence of microbubbles. Appl Environl Microbiol. 64(10):3927-3931

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