INFLUENCE OF ULTRASONIC WAVES ON INACTIVATION OF SPORES OF ATCC 14579 CEPA OF *Bacillus cereus*

ABSTRACT

This study aimed to assess the high intensity ultrasound (HIUS) effect on spores of *Bacillus cereus* (ATCC 14579). The experiments were carried out at 500W for five minutes. The results showed that the treatment provided reductions between 0.78 and 0.94 on spores.

Despite reducing the microbial spore population, the effectiveness of HIUS was low. Therefore, this treatment needs to be combined with other inactivation techniques.

INTRODUCTION

Emerging technologies, such as the treatment of products for the inactivation of microorganisms, have been increasing in recent years (SCUDINO et al., 2020). The current scenario seeks alternatives to promote microbial inactivation that ensures lower impacts on food's nutritional and sensorial quality (GUIMARÃES et al., 2021). Despite the traditional heat treatments being efficient for microbial inactivation foods, they can trigger significant nutritional losses and undesirable sensory changes (CHAROUX et al., 2019; CHEN, ZHANG & YANG, 2020; GAO et al., 2014). Emerging non-thermal treatments promote bacterial inactivation and show minor nutritional quality impact, like as ultrasound (SCUDINO et al., 2020; GUIMARÃES et al., 2021).

Ultrasound is classified into low power (> 100 kHz) and high intensity (from 20 to 100 kHz). Its mechanism of inactivation of enzymes and microorganisms is through the phenomenon of cavitation. Inactivation results from cavitation, which is related to bubbles' formation, growth, and collapse, which generate localized mechanical and chemical energy. Ultrasound treatment can be applied to some microorganisms such as gram-negative bacteria, yeasts, spores, and protozoa, but its effectiveness will depend on the type of microorganisms (VERRUCK and SILVANI, 2018). Microbial spores are much more challenging to inactivate as they are resistant to extreme conditions, but when combined with heat, the inactivation rate increases.*Bacillus cereus* are aerobic spore-forming bacteria found in soil, vegetables, and raw and processed foods (FDA, 2021). *Bacillus cereus* is among the spores of great importance for the industry, especially the dairy industry, due to its ability to spore-forming after heat treatment as a form of defense, allowing its permanence in pasteurized milk. (LIMA, 2019). This bacterium has significant public health significance due to its excellent resistance to various conditions, biofilm formation, and ability to produce toxins, such as hemolysin, non-hemolytic enterotoxin, and cytotoxin are responsible for causing the diarrheal syndrome, causing acute diarrhea. , abdominal pain and nausea (LIMA, 2019; FDA, 2021). In addition, the presence of these bacteria also damages the economy of the dairy and dairy industries, for example, as they produce deteriorating enzymes such as proteases, lipases, lecithinases and phospholipases that generate changes in taste, rancidity and a reduction in industrial yield (LIMA, 2019). Thus, this study assessed the effect of HIUS on *B. cereus* spore inactivation as an alternative for thermal treatments.

OBJECTIVE

This study aimed to assess the high intensity ultrasound (HIUS) effect on spores of *B acillus cereus* (ATCC 14579).). The experiments were carried out at 500W for five minutes.

RESULT AND DISCUSSION

The logarithmic reduction ranged from 0.78 to 0.94 to HUIS treatment. The microbial population was assessed for spores (Heat shock 75°C for 15 min) and vegetative cells (without heat shock) These logarithmic reductions were slightly higher than the results found by Sagong et al. (2013), where the use of ultrasound alone promoted a 0.7 log reduction in *B.cereus* spores in carrots and lettuce a frequency of 40 kHz, power of 30 W/L and duration of 5 min.

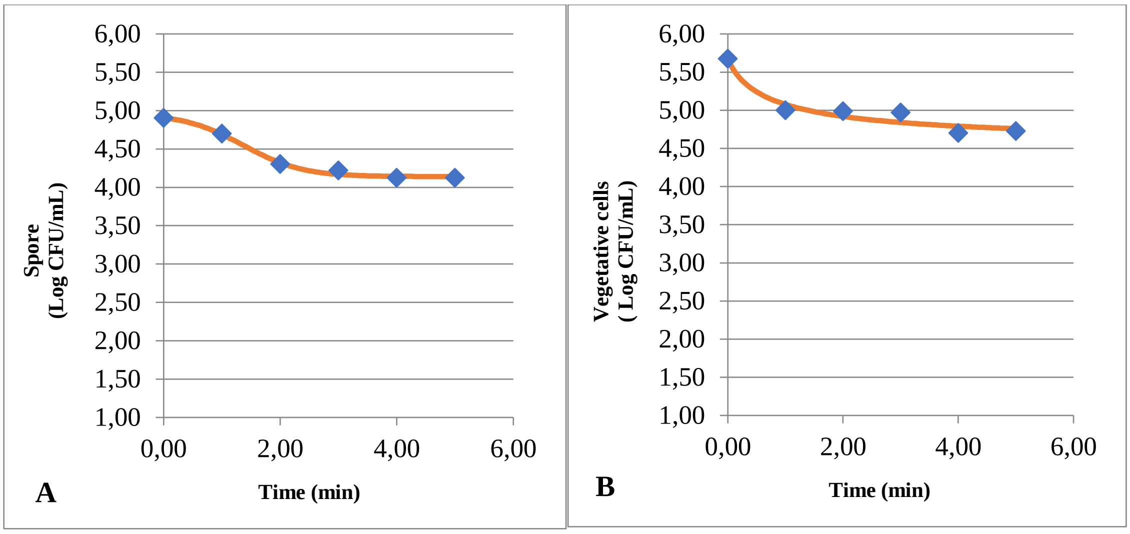

Figure 1. Logarithmic reductions of ATCC 14579 strain after 500W probe ultrasound treatment for five minutes. (A) – With thermal shock (70ºC for 15 minutes); (A) – No thermal shock.

Bacterial spores have more complex structures when compared to vegetative cells. It is including various different protective layers, such as the cortex, core, and exospore. These structures, give it additional protection against physical and chemical agents such as heat, pressure, radiation, and chemicals (SETLOW, 2003). The inactivation mechanism promoted by ultrasound is based on wave formation that generates bubbles in the treated spore suspension, as shown in Figure 2 (ONYEAKA et al., 2021).

Inactivation parameters were estimated using Ginafit (2000) and are shown in Table 1. Maximum inactivation rates were the same for vegetative cells as for spores. In both conditions, the treatment showed a residual population greater than 4 log10. For spores, the HUIS treatment exhibited a shoulder. In addition, the treatment provided less than one logarithmic reduction, with the maximum found being 85% reduction for vegetative cells and 82% for spores.

Table 1.: Estimated $\log_{10}$ reduction time for *Bacillus cereus* ATCC 14579 spores.

Cells	Shoulder length (minutes)	k_{max} (min^{-1})	N res (spores or cells/mL)
Spore	0,98	2,11	4,14
Vegetative cells	-	2,12	4,82

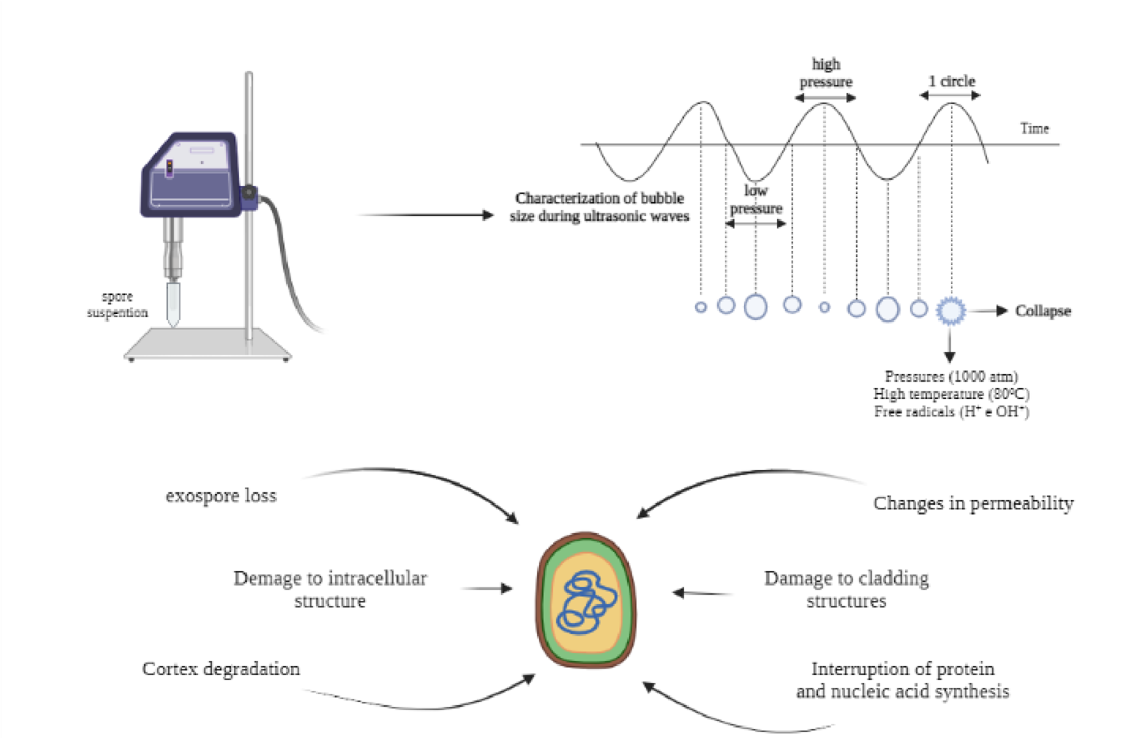

Figure 2: Effects of acoustic cavitation and its possible impacts on spore structure

Throughout the cycle, the bubbles undergo structural changes, at the points of low and high pressure. This phenomenon promotes the compression and expansion of these bubbles. After the first cycle, the bubble collapses and generates micro points of high pressure, high temperatures, bubbles formation and, free radicals (H^+ and OH^+) (ONYEAKA et al., 2021). This phenomenon is called cavitation, providing physical and chemical damage to the spore structures. However, these generated damages are sublethal, and in more complex systems, inactivation will not be significant. Therefore, the use of other technologies combined with ultrasound, such as temperature, radiation, or pressure, can promote more substantial damage to sporulating cells (ONYEAKA, 2021).

Another critical factor in assessing the ultrasound effectiveness treatment is the energy density provided during sonification, which will modulate acoustic cavitation. As mentioned earlier, during cavitation, in the collapse of the bubbles, high energy is released, promoting an increase in temperature in the treated medium, and therefore, monitoring the total thermal energy supplied by the system is important to assess its real impact on the sample (URANGO et al., 2022). In Figure 3 shows the temperature monitoring during the sonication times samples.

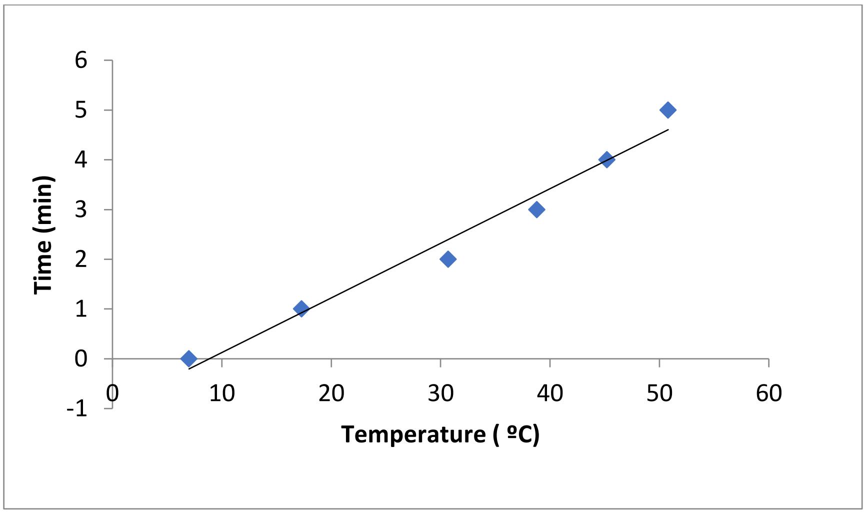

Figure 3. Temperature variation during the sonication period.

It is possible to observe that at the end of the treatment, the maximum temperature reached by the process was 50.8ºC, which is insufficient to promote the inactivation of these resistant structures (RUIS, 2022). Therefore, the temperature provided during sonication has no significant influence on the treated.

CONCLUSION

The use of ultrasound caused less than one logarithmic reduction in *B.cereus* spores. Therefore, the combination with other treatments can improve spore inactivation. The use of thermal shocks after treatment allows a more reliable assessment of sporulating cell inactivation. Furthermore, monitoring becomes necessary to dimension the real effect of the ultrasound under the sample.

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