The influence of ultrasonic treatment on the growth of the strains of *Salmonella enterica* subs. *typhimurium*

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Abstract This study proposes the destruction of pathogenic bacteria with the use of ultrasound waves because the more commonly used thermal processing methods often result in lowering the nutritional value of food. The study presents the impact of ultrasound of 20, 40 and 100 kHz frequencies and the power of 10.5 W/cm² on the growth of the strain of *Salmonella enterica* subs. *typhimurium*. The tests were carried out both in chilled and non-chilled treatment mediums, with an average bacterial population >10⁵ and >10⁸ CFU/cm³. The total inactivation of *Salmonella* spp. was observed in the tests in the low-population non-chilled treatment medium after sonication at 20 and 40 kHz for 30 min, and in high bacterial population at 20 kHz for 30 min. A reduction in the average number of bacteria was reported in the low-population non-chilled medium after 15 min of sonication at 20, 40 and 100 kHz; after 15 min of

sonication at 20 and 100 kHz of the material of high bacterial population; and in the low-population chilled treatment mediums after 15 and 30 min at 20 kHz. The samples with inactivated bacteria and those with reduced bacterial counts maintained the same levels when stored at 4 °C for 24 and 48 h. Bacteria inactivation obtained after sonication lasted for up to 48 h in storage at 21 °C. For the samples with reduced bacterial counts stored at 21 °C, a rise in the average number of bacteria was recorded.

Keywords Microbiological inactivation · *Salmonella enterica* subs. *typhimurium* · Ultrasound

Introduction

One of the foodborne pathogens is bacteria of the *Salmonella* type (EFSA and ECDC 2014). The *Salmonella enterica* serovar Typhimurium strain, characterised by resistance to many antibiotics, is one of the main causes of salmonellosis in humans in Europe, America, Africa, Asia and Oceania (Helms et al. 2005; Mukhopadhyay and Ramaswamy 2012). Products such as meat, poultry, milk and fish (Heinz et al. 2000), unpasteurized juices (Buxton et al. 1999), as well as peanut products, can be contaminated with these pathogenic bacteria. An additional danger is the fact that the bacterial population able to cause infection does not give evidence of food rot. That is why early elimination of pathogenic microflora from food is crucial. This is done by various methods including the chemical processes with the use of chlorine, chlorine dioxide, ozone, trisodium phosphate, peracetic acid, etc. (Hugas and Tsigarida 2008; Mukhopadhyay and Ramaswamy 2012). However, due to the high risk connected with chemical decontamination of foods of animal origin, the

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use of these methods must be preceded by the risk assessment approved by the European Food Safety Authority (EFSA) and the European Commission (EC) (EFSA 2009; Hugas and Tsigarida 2008). On the other hand, applying physical methods to inactivate microflora which threatens food safety, mainly thermal methods such as pasteurisation and sterilisation, all too often leads to a lowered nutritional value in food (Knorr et al. 2002); moreover, the process is highly energy-consuming. That is why it is justified to apply alternative non-destructive methods of food decontamination, ensuring its safety and high quality (Alarcon-Rojo et al. 2015; Jabbar et al. 2014; Seymour et al. 2002). One such method is ultrasound treatment. There are many reports on using ultrasound waves for inactivation of pathogens contained in foods (Álvarez et al. 2006; Turantaş et al. 2015), including those found on poultry skin (Haughton et al. 2012; Kordowska-Wiater and Stasiak 2011; Lillard 1993; Morild et al. 2011; Musavian et al. 2014), meat and meat products (Dolatowski and Stasiak 2002), eggs (Wirgley and Llorca 1992), milk (Herceg et al. 2012; Wirgley and Llorca 1992), in preserving fruit juices (Jabbar et al. 2014; Seymour et al. 2002; Valero et al. 2007; Wong et al. 2012) as well as in fruit and vegetable processing (Alexandre et al. 2012, 2013; Cao et al. 2010; Elizaquivel et al. 2012; São José and Vanetti 2012).

Research on using ultrasound waves in food technology focuses on using the phenomena accompanying production and propagation of mechanical vibrations of the frequency of ultrasound, i.e. in excess of 16 kHz. Applying ultrasound to food technology develops in two directions. Ultrasound with power below 1 W/cm² is used in non-destructive tests, mainly for diagnostic purposes, while that of higher power is used in invasive methods whose aim is to damage or even destroy cellular structures (Gao et al. 2014a, b). Such action of ultrasound is enabled *inter alia* by cavitation, which involves creation and consecutive implosions of gas bubbles. This is accompanied by momentary rises in the pressure (up to 50 MPa) and the temperature (up to 5500 °C). The resultant massive hydraulic shock wave may cause perforation of microbial cell membranes. This is why during the sonication processes, which involve disintegration of particles or cells contained in a suspension with the use of ultrasound (Ashokkumar 2015; Gao et al. 2014a, b), microflora can be eliminated; however, one of the inhibitors of this process is the increased temperature of the medium caused by cavitation. There are no studies showing the differences in inhibition or inactivation of microorganisms with the use of ultrasound without changing the temperature of the medium or with chilling the medium. Such tests could show how efficient the impact of sonication itself is on inhibition or inactivation of unwanted bacteria.

The aim of this study is to compare the impact of ultrasound of various parameters on the growth of the pathogenic bacteria strain of *S. enterica* subs. *typhimurium* type. One of the aspects of this study is the comparison of ultrasound action with and without chilling the medium during the ultrasonic treatment, comparison of the impact of ultrasound of the same parameters on nutrient broth immediately after its inoculation with the inoculum of the bacteria (low bacterial population) and on 24-h *Salmonella* spp. culture (high bacterial population).

Materials and methods

Bacterial strains

The tests were conducted on a strain of *S. enterica* subs. *typhimurium* obtained from the ATCC 14028 (Polaura, Dywity, Poland). The frozen strain was stored in the Biocorp Microbank® (Warsaw, Poland), at the temperature of −80 °C. It was multiplied on Biocorp PS110 liquid nutrient broth at the temperature of 37 °C.

Test site

Sonication was conducted with the use of a device composed of a 750 ml acid-proof steel treatment chamber with ultrasound transducers of 20, 40 and 100 kHz (Ultron, Polska). The transducers were switched and steered by an external generator of adjustable power. Its maximum power was 10.5 W/cm². A special construction of the cover equipped with coils enabled the control and adjustment of the temperature of the treatment liquid (broth).

Sample inoculation

Salmonella spp. were sonicated in two variants. The first one involved 400 ml of sterile nutrient broth with an addition of 100 µl of 18–20 h *Salmonella* spp. culture, which gave a population of 10⁵–10⁶ CFU/cm³. The other variant involved 400 ml 24-h culture of the population >10⁸ CFU/cm³.

Ultrasound treatment

The impact of ultrasound on the growth of *Salmonella* spp. strain was tested with the use of various ultrasound parameters: the frequencies of 20, 40 and 100 kHz, the power of 10.5 W/cm², the time of 15 and 30 min, without chilling the treatment medium and in a chilled treatment medium. Prior to sonication, after 15 min and again after 30 min of the process, the temperature of the treatment medium was measured with the FlukeFoodPro pyrometer (Everett, WA, USA).

The chilling of the treatment medium was conducted with the use of an especially designed cover to secure the treatment chamber. There was an acid-proof steel coil fixed in its cover. Thanks to the possibility to regulate the volume of tap water flowing through the coil, the rough adjustment of temperature was conducted. The aim was not to let the temperature of treatment medium rise above 40 °C.

Storage tests after sonication

Storage tests were conducted on all samples—after 15 and 30 min of sonication, the samples were collected and stored for up to 48 h at 4 °C in a refrigerator, and in an incubator at room temperature of 21 °C. Bacteria were counted after 24 and 48 h of storage.

Microbial analysis

Salmonella spp. were marked with the use of the surface method on Petri plates with the use of our own nutrient-enriched broth PS110 (Biocorp, Warsaw, Poland) with 1.3% agar added (Merck, Warsaw, Poland). Spreading (inoculation) was performed with a spiral inoculation instrument Eddy Jet (IUL, Barcelona, Spain), and Linear Mode 18, 100, 200 or 400 µl programmes. Spreading (inoculation) was done three times. Inoculated plates were incubated in an incubator at 37 °C for 24 h.

The colonies grown were counted with the use of an Automatic Colony Counter (IUL, Barcelona, Spain), whose software is compatible with the spiral inoculation instrument Eddy Jet. The numbers of colonies from the three repetitions of the same solution were summed up and then the average number of colonies was calculated.

Altogether there were four experiments conducted with three main factors (frequencies), each of which were repeated 3 times. Figures 1a–d show the average results of the three trials of the experiments at different frequencies and times.

Statistical analysis

The hypothesis that the average number of *S. enterica* subs. *typhimurium* before and after sonication is the same was analysed statistically, independently of the ultrasound frequency. The results obtained did not satisfy the requirements to use parametric tests, therefore the above hypothesis was verified with the use of the Kruskal–Wallis nonparametric test. The analyses were conducted at the significance level $\alpha = 0.05$. The statistical calculations were conducted with the use of Statistica ver. 10.

Results and discussion

In many overview papers the preferred ultrasound frequency for food testing was reported to be 20–100 kHz (Alarcon-Rojo et al. 2015; Herceg et al. 2012). No information on the effect of use of 100 kHz on bacteria count is available. Therefore, we arbitrarily chose the frequencies of 20, 40 and 100 kHz.

There are also many reports on ultrasound treatment in combination with other physical or chemical factors, with just a few studies describing ultrasound treatment alone. The effective ultrasound treatment was reported by Cameron et al. (2009), who achieved a positive impact of ultrasound treatment on the reduction of endospores and pathogens (*Escherichia coli*, *Pseudomonas fluorescens* and *Listeria monocytogenes*) which can be found in fresh and pasteurized milk. The researchers achieved positive results between 6 and 10 min of ultrasound treatments at 20 kHz.

The effect of ultrasound on the growth of *Salmonella* spp. immediately after sonication

In our research, the first stage was the observation of the effects of sonication on the growth of the *S. enterica* subs. *typhimurium* strain (Fig. 1). The 15-min ultrasound treatment of medium with low bacterial population and without chilling, a statistically significant reduction *Salmonella* spp. cells were obtained. This result was observed for all frequencies applied (Fig. 1a). Sonication of 20 kHz reduced the average bacterial count from the initial value of 5.35 ± 0.08 to 3.97 ± 1.03 log CFU/cm³ after 15 min, for the frequency of 40 kHz from the initial value of 5.54 ± 0.20 to 1.55 ± 2.40 log CFU/cm³ and for the frequency of 100 kHz from 5.52 ± 0.04 to 4.48 ± 0.01 log CFU/cm³ respectively.

Prolonged exposure time to 30 min resulted in complete inactivation of bacteria at 20 and 40 kHz. As for the frequency of 100 kHz, the average bacterial population fell to 1.51 ± 0.07 log CFU/cm³. The maximum temperature of medium after a 15-min sonication reached 57.4 °C (40 kHz) and 60 °C (20 kHz) after 30 min.

Sonication conducted in chilled medium with low bacterial population showed no evidence of statistically significant decrease in the average microbial counts (Fig. 1b). The temperature of the treatment medium reached a peak of 34.5 °C (40 kHz).

A 15-min ultrasound treatment of the bacterial culture with a population $>10^8$ CFU/cm³ without chilling significantly reduced the average bacteria levels at 20 and 100 kHz (Fig. 1c). The initial average number of 8.58 ± 0.5 decreased to 1.51 ± 2.62 log CFU/cm³ for the frequency of 20 kHz and from 9.20 ± 0.05 to 7.47 ± 0.09 log CFU/cm³ for the frequency of 100 kHz. Treatment at 40 kHz showed

Fig. 1 The effect of ultrasound on the growth of *Salmonella* spp. immediately after sonication. **Key:** Lo—the average bacteria number before sonication (the average of 9 trials), 15/20—after 15 min of sonication at 20 kHz frequency ($n = 3$), 15/40—after 15 min of sonication at 40 kHz frequency ($n = 3$), 15/100—after 15 min of sonication at 100 kHz frequency ($n = 3$), 30/20—after 30 min of sonication at 20 kHz frequency (the same as 15/20), 30/40—after 30 min of sonication at 40 kHz frequency (the same as 15/40),

30/100—after 30 min of sonication at 100 kHz frequency (the same as 15/100). **a** Measurements conducted without chilling of the treatment medium. Low initial population; **b** measurements conducted in chilling treatment medium. Low initial population; **c** measurements conducted without chilling of the treatment medium. High initial population; **d** measurements conducted in chilling treatment medium. High initial population

no statistically significant reduction in the average bacteria number.

Prolonged sonication to 30 min resulted in complete inactivation of bacteria at 20 kHz and a statistically significant reduction in the average count of *Salmonella* spp. at 100 kHz. The average bacterial count in this case fell to 3.09 ± 0.1 log CFU/cm³. The maximum temperature of the medium after 15-min sonication reached 58 °C (20 kHz), and 60.8 °C (100 kHz) after 30 min of sonication.

The samples from chilled culture of high bacterial population had significantly reduced average numbers only after

sonication at 20 kHz (Fig. 1d). The 15-min ultrasound treatment, decreased the numbers from the average initial value of 8.28 ± 0.12 to 7.11 ± 0.21 log CFU/cm³. Sonication prolonged to 30 min caused a further reduction of the average bacteria levels to 6.81 ± 0.27 log CFU/cm³. Treatment of the samples with a frequency of 40 and 100 kHz showed no statistically significant reduction in the average bacterial count either after 15 or 30 min of the experiment. The maximum temperature of the treatment medium was 31 °C (100 kHz).

These results, showing that microbial sensitivity to ultrasonic treatment increases at the temperature above

50 °C, are in keeping with the results presented by many researchers (Herceg et al. 2012; Valero et al. 2007; Wirgley and Llorca 1992).

Storage tests after sonication

There is little research where authors stored samples treated with ultrasound. Valero et al. (2007) conducted sonication of orange juice for 15 min at high frequency of 500 kHz and 240 W and the power of 6 W/cm² with the final juice temperature of 51 °C and at low frequency of 23 kHz 600 W at the pasteurisation temperature of 88 °C. After the reduction of bacteria achieved in the process of ultrasonic treatment by 1.08 and 1.7 log CFU/ml respectively, the authors observed the growth of aerobic mesophilic bacteria in both samples after 14 days of storing the juice at 5 and 12 °C.

The effect of ultrasound treatment on the growth of *Salmonella* spp. without chilling of the treatment medium, low bacterial population

In our research, storage of the material sonicated for 15 min at 20 and 40 kHz for up to 48 h at 4 °C (Fig. 2a) did not demonstrate statistically significant changes in average bacteria levels (the values were given in Fig. 2a) enumerated after sonication. The average numbers of bacteria after sonication at 100 kHz significantly increased from the average value of 4.48 ± 0.01 log CFU/cm³, which was enumerated immediately after the sonication, up to 5.18 ± 0.3 log CFU/cm³ after 48 h of storage.

In the samples sonicated for 30 min at 20 and 40 kHz, a total inactivation of bacteria was obtained (Fig. 2b). During 48 h of storage at 4 °C, the treated samples did not display

Fig. 2 The effect of ultrasound treatment on the growth of *Salmonella* spp. during storage (without chilling of the treatment medium, low bacterial population). **a** The impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 4 °C, sonication time Lo = 15 min; **b** the impact of sonication on the growth of *Salmonella*

spp. strain, storage temp. 4 °C, sonication time Lo = 30 min; **c** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 15 min; **d** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 30 min

any signs of bacterial growth. As for the samples sonicated at 100 kHz, the average number of bacteria did not change significantly during the whole storage period and remained at an average level of 1.23 ± 0.44 log CFU/cm³.

In the material stored for 24 h at 21 °C after 15 min of sonication, a statistically significant increase was observed in the average number of bacteria at all frequencies applied (Fig. 2c). For 20 kHz a rise was obtained from an average of 3.97 ± 1.03 to 8.45 ± 0.9 log CFU/cm³, for 40 kHz from 1.55 ± 0.4 to 7.75 ± 0.35 log CFU/cm³ and from 4.48 ± 0.01 to 9.17 ± 0.05 log CFU/cm³ for 100 kHz. 48 h of storage showed no statistically significant differences recorded between average numbers of bacteria between the 24 and the 48 h of storage.

In the samples sonicated for 30 min at 100 kHz, and then stored at 21 °C, after 24 h a dramatic increase was

observed in the average bacteria levels (Fig. 2d), from 1.52 ± 0.07 to 8.84 ± 0.03 log CFU/cm³. After 48 h there was a further slight rise in the average number of bacteria, however, the difference was statistically negligible. In the samples sonicated for 30 min at 20 and 40 kHz, the lack of *Salmonella* spp. growth recorded immediately after sonication lasted for up to 48 h of storage in 21 °C.

The effect of ultrasound treatment on the growth of Salmonella spp. without chilling of the treatment medium, high bacterial population

Storage of the samples sonicated for 15 min for up to 48 h at 4 °C demonstrated no statistically significant changes in the average numbers of bacteria (Fig. 3a). In the samples sonicated for 30 min at 20 kHz frequency and then stored

Fig. 3 The effect of ultrasound treatment on the growth of *Salmonella* spp. during storage (without chilling of the treatment medium, high bacterial population). **a** The impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 4 °C, sonication time Lo = 15 min; **b** the impact of sonication on the growth of *Salmonella*

spp. strain, storage temp. 4 °C, sonication time Lo = 30 min; **c** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 15 min; **d** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 30 min

at 4 °C for up to 48 h there was no evidence of *Salmonella* (Fig. 3b) immediately after sonication and during the whole storage period of the tested material. The samples sonicated at 100 kHz and then stored for up to 48 h had significantly reduced average bacterial count from 3.09 ± 0.1 to 1.78 ± 0.01 log CFU/cm³. The samples sonicated at 40 kHz frequency demonstrated no statistically significant changes in the average number of bacteria during the whole period of storage.

Storage at 21 °C of the samples after 15 min of sonication revealed that the average bacterial population before sonication and after 48 h of storage did not differ significantly for all frequencies applied (Fig. 3c). A similar result was obtained for the samples sonicated for 30 min at 40 and 100 kHz (Fig. 3d). After 48 h of storage the average bacterial count in these samples returned to the levels

before sonication; whereas in the samples sonicated at 20 kHz there was no evidence of *Salmonella* spp. immediately after sonication and for up to 48 h of storage.

The effect of ultrasound treatment on the growth of Salmonella spp. in a chilled treatment medium, low bacterial population

For up to 48 h of storage at 4 °C the samples sonicated at all applied frequencies showed no evidence of statistically significant differences in average bacteria levels (Fig. 4a). In the case of a 15-min sonication at 20 kHz, the average numbers of bacteria stayed at 4.76 ± 0.1 log CFU/cm³, at 40 kHz they equalled 5.09 ± 0.1 and 6.03 ± 0.1 log CFU/cm³ at 100 kHz. After 30 min of sonication the results were not significantly different (Fig. 4b). In this case, the average

Fig. 4 The effect of ultrasound treatment on the growth of *Salmonella* spp. during storage (chilled treatment medium, low bacterial population). **a** The impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 4 °C, sonication time Lo = 15 min; **b** the impact of sonication on the growth of *Salmonella*

spp. strain, storage temp. 4 °C, sonication time Lo = 30 min; **c** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 15 min; **d** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 30 min

bacterial population in the samples sonicated at 20 kHz was 4.04 ± 0.03 log CFU/cm³, at 40 kHz 4.84 ± 0.1 log CFU/cm³ and at 100 kHz 5.91 ± 0.08 log CFU/cm³.

In the samples stored at 21 °C after sonication at the three applied frequencies for 15 min, a statistically significant dramatic rise in the number of bacteria was observable after 24 h (Fig. 4c). In the material treated at 20 kHz, the average bacterial count increased from 4.85 ± 0.45 to 8.52 ± 0.05 log CFU/cm³ after 24 h of storage, followed by 9.36 ± 0.25 log CFU/cm³ after 48 h of storage. As for sonication at 40 kHz, the rise was from 5.12 ± 0.21 to 8.75 ± 0.05 log CFU/cm³ after 24 h, followed by 9.22 ± 0.29 log CFU/cm³ after 48 h of storage. For the material sonicated at 100 kHz, the situation was analogous. An increase in the average bacteria concentration was

reported from 6 ± 0.00 to 9.4 ± 0.4 log CFU/cm³ after 24 h of storage and to 9.9 ± 0.1 log CFU/cm³ after 48 h.

For the samples sonicated for 30 min the situation was analogous (Fig. 4d). After 24 h of storing the material sonicated at 20 kHz, there was a rise in the average number of bacteria from 4.07 ± 1.15 to 8.51 ± 0.12 log CFU/cm³, and after 48 h the average bacterial population went up to 9.23 ± 0.20 log CFU/cm³. In the material sonicated at 40 kHz there was an increase from the average bacteria count of 4.93 ± 0.30 to 8.69 ± 0.09 log CFU/cm³ after 24 h of storage and to 9.20 ± 0.35 log CFU/cm³ after 48 h. In the samples sonicated at 100 kHz there was a rise in the average bacteria levels from 5.86 ± 0.17 to 9.50 ± 0.47 log CFU/cm³ after 24 h and to 9.70 ± 0.32 log CFU/cm³ after 48 h of storage.

Fig. 5 The effect of ultrasound treatment on the growth of *Salmonella* spp. during storage (chilled treatment medium, high bacterial population). **a** The impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 4 °C, sonication time Lo = 15 min; **b** the impact of sonication on the growth of *Salmonella*

spp. strain, storage temp. 4 °C, sonication time Lo = 30 min; **c** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 15 min; **d** the impact of sonication on the growth of *Salmonella* spp. strain, storage temp. 21 °C, sonication time Lo = 30 min

The effect of ultrasound treatment on the concentration of Salmonella spp. in chilled treatment medium, high bacterial population

Storage at 4 °C of the samples sonicated for 15 min at three different frequencies showed no statistically significant differences between the average bacteria levels recorded after 24 and 48 h of storage (Fig. 5a). After sonication at 20 kHz, the average number of bacteria during the whole period of storage remained $7.36 \pm 0.41 \log \text{CFU/cm}^3$, after sonication at 40 kHz at $8.35 \pm 0.07 \log \text{CFU/cm}^3$ and after sonication at 100 kHz at the level of $8.29 \pm 0.11 \log \text{CFU/cm}^3$. The result after 30 min of sonication was analogous (Fig. 5b). However, the average bacteria count during the whole period of storage in the samples treated at 20 kHz remained $6.70 \pm 0.41 \log \text{CFU/cm}^3$, at 40 kHz at $8.28 \pm 0.07 \log \text{CFU/cm}^3$. In the case of sonication at 100 kHz, a statistically significant difference was recorded between the average number of bacteria enumerated after 24 h of storage, which was $8.88 \pm 0.06 \log \text{CFU/cm}^3$, and the average number of bacteria after 48 h of storage, which was $8.96 \pm 0.01 \log \text{CFU/cm}^3$.

In the material stored at 21 °C after 15 min of sonication at 20 kHz, a statistically significant difference between the average numbers of bacteria enumerated immediately after sonication and after 48 h of storage was achieved (Fig. 5c). A practically linear growth of was obtained from the average of 7.51 ± 0.21 to $9.71 \pm 0.25 \log \text{CFU/cm}^3$. The samples sonicated at 40 and 100 kHz showed no evidence of statistically significant differences between the average numbers of bacteria after 24 and 48 h of storage. In the material sonicated for 15 min at 40 kHz, the average bacteria levels stayed at $8.97 \pm 0.45 \log \text{CFU/cm}^3$, and after sonication at 100 kHz at $8.76 \pm 0.39 \log \text{CFU/cm}^3$. The situation was analogous for the samples sonicated for 30 min (Fig. 5d). In the sample stored at 21 °C sonicated at 20 kHz, a statistically significant difference was observed between the average numbers of bacteria immediately after sonication and after 48 h of storage. There was evidence of the growth of *Salmonella* spp. from the average concentration of 6.81 ± 0.27 to $9.66 \pm 0.27 \log \text{CFU/cm}^3$.

In the samples sonicated at 40 and 100 kHz, no statistically significant differences were observed between average bacteria counts after 24 and 48 h of storage. The average bacteria levels sonicated at 40 kHz remained $9.07 \pm 0.61 \log \text{CFU/cm}^3$, while for those sonicated at 100 kHz at $8.80 \pm 0.32 \log \text{CFU/cm}^3$.

Conclusion

The research showed that the best effect, which is inactivation of the *S. enterica* subs. *typhimurium*, lasted for up to 48 h with both high- and low-level bacterial populations in non-cooled mediums at 20 kHz for 30 min, and in low-level bacterial population in non-cooled medium at 40 kHz for 30 min. The results obtained suggest that further research should be conducted where ultrasonic waves must be accompanied with other physical factors, including changed water activity or pH of the medium used in the research. The aim of such research would be to confirm the efficiency of ultrasound treatment in model liquid imitating various foods.

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