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Effect of ultrasonic treatment on physical and biological properties of egg products

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A doktori iskola megnevezése: Élelmiszertudományi Doktori Iskola

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Az iskolavezető jóváhagyása	A témavezető(k) jóváhagyása

1. Introduction and objectives

The increasing consumer demands pose growing challenges for the food industry. Current processing technologies often force manufacturers to make compromises, as the adverse effects of food processing can significantly influence consumers perceptions of the products. To avoid such undesirable changes, considerable time and resources are devoted to developing new, milder technologies.

As a result, in recent years, there has been an increasing emphasis on gentle processing methods that offer alternatives alongside existing technologies. According to numerous studies, these gentle processing technologies, such as high-pressure treatment, irradiation, cold plasma technology, ultrasonic and ultraviolet treatments (non-thermal methods), and thermal methods like sous-vide, as well as various packaging techniques (modified atmosphere packaging, active or edible packaging) can provide effective solutions for extending the shelf life of food products.

Among these, ultrasound stands out as an excellent alternative to conventional food processing methods due to its versatile applications. The applicability of ultrasound stems from the phenomenon of acoustic cavitation, during which microscopic bubbles, just a few hundred micrometers in diameter, are formed. When these cavitation bubbles collapse, the resulting pressure and temperature can break down certain cellular components, disrupt cell function, and ultimately destroy the cells.

In my research, my aim is to investigate the effects of ultrasound on the properties of various liquid egg products and, through the obtained results, demonstrate the technology’s suitability for future developments in the industry.

Objectives

The foundation of my objectives is the extensive applicability of ultrasound treatment, which offers a gentle method for processing various egg products while enabling advantageous changes to their characteristics. In the literature, this technology is most often regarded as a complementary method that facilitates another primary treatment process. My goal is to successfully use ultrasound as the main treatment method for egg products.

In my studies, I sought answers to the following questions:

1. Can ultrasound alone significantly reduce the microbiological contamination of egg liquids artificially infected with *E. coli*?

2. Can the effects of ultrasound on egg liquids be detected using near-infrared (NIR) technology?

3. Can ultrasound, when combined with mild heat treatment, reduce the *E. coli* count in egg liquids below the detection limit?

4. What changes does standalone ultrasound treatment and combined treatment cause in the near-infrared spectra of egg products?

5. What impact does the ultrasonic process have on the foaming capacity and foam stability of egg white?

2. Materials and methods

2.1 Egg liquid samples

During the experiments, I used three types of egg products (whole egg liquid, egg white, and yolk) provided by Capriovus Kft. The egg products were made from fresh, homogenized, Class "A" chicken eggs.

The samples were stored in 1-liter beverage cartons at 0–4°C in a refrigerator until use. Before measurements, the contents of the containers were shaken and mixed in a large storage vessel to ensure homogeneous samples.

2.2 Ultrasonic treatment

The ultrasonic treatments were conducted using a 16-liter ultrasonic device manufactured by HBM Machines (MJ Mooedrecht, Netherlands). The equipment operates at frequencies of 20 kHz and 40 kHz and generates nominal ultrasonic powers of 180 W and 300 W. The effective power was determined calorimetrically, with values of 3.7 W at 180 W nominal power and 6.9 W at 300 W nominal power. For evaluations, the effective power was used, and the energy dose of the treatments - calculated as the product of effective power and treatment time—was also examined.

All three types of egg liquids were treated with every combination of ultrasonic frequency, power, and duration to identify the parameters most significantly influencing the measured properties.

During standalone ultrasonic treatments, it was crucial to prevent the medium from heating up to avoid heat-induced changes. To achieve this, I implemented a circulation system with an external buffer. A tank filled with ice water was placed next to the ultrasonic device and circulated through the system using a submersible pump to offset the heat generated by the equipment. This cooling system maintained the medium and samples at 18±2°C throughout the treatment.

Based on the results of the standalone ultrasonic treatments, I initiated a new series of experiments incorporating a combined treatment that combined ultrasound with mild heat treatment (55°C).

The duration of the treatments was 30, 45, and 60 minutes for standalone ultrasonic treatments, and 30 and 60 minutes for combined treatments.

2.3 Artificial Contamination of Samples

For microbiological analysis, 180 ml of each sample was artificially inoculated with *Escherichia coli* (ATCC 25922). The initial concentration of *E. coli* in the samples was 5 log CFU/ml. A selective and differential medium (ChromoBio COLIFORM, BioLab) was prepared using pour plate techniques to determine *E. coli* colony-forming units (CFU) and distinguish them from other microorganisms. This agar indicates the presence of *E. coli* through blue discoloration.

2.4 Near-Infrared Spectral Measurements

To monitor the effects on egg liquids, I used near-infrared (NIR) measurement technology as a non-destructive analysis method. The spectral analysis was performed with a benchtop spectrometer (MetriNIR Research, Development and Service Co., Budapest, Hungary), which records transflection spectra in the wavelength range of 740–1700 nm with a resolution of 2 nm. The spectra were recorded using METRINIR software v0.9.0.394 (Metrika Inc., Budapest, Hungary).

To reduce noise inherent to the equipment, the wavelength range of 950–1650 nm was considered for analysis.

2.5 Egg White Foaming and Foam Stability Analysis

In the case of standalone ultrasonic treatments, the foaming and foam stability properties of egg whites were also examined. I prepared egg foam at room temperature (24°C) by whisking 30 ml of egg white in a beaker with a hand mixer for 5 minutes. After whisking, the propeller was immediately removed, and the foam height was measured, followed by calculating its volume.

To assess foam stability, an inverted glass funnel was attached to the beaker to separate static moisture. After one hour, the weight of the reverted egg white was measured to compare the effects of different treatments on foam stability.

3. Results

3.1 Ultrasound as a Standalone Treatment

3.1.1 Microbiological Analysis

The analysis of ultrasonic treatment parameters revealed that treatment frequency, effective power, and duration had significant effects on the microbial count of egg products. A reduction in *E. coli* colony-forming cells was observed in treated groups.

The greatest reduction was achieved with a 60 minute treatment at 6.9 W effective power and 40 kHz frequency. The reductions were 0.5 log CFU/ml for whole egg liquid and egg white, and 0.7 log CFU/ml for egg yolk. The data showed that the control groups differed significantly from the treated samples, indicating that the ultrasonic treatment (with the applied settings) had a mild but significant effect on the survival of *E. coli* in egg products.

3.1.2 Near-Infrared Spectral Analysis Results

Using principal component analysis (PCA) and linear discriminant analysis (LDA), significant differences were observed between certain treatment settings, indicating that ultrasonic treatment affected the spectral characteristics of egg products.

Based on PCA loadings, molecular groups affected by ultrasonic treatment were indirectly identified. Further analysis of the 1300–1550 nm wavelength range revealed changes in water absorption patterns using Aquaphotomics. Systematic variations in the water absorbance matrix coordinates (WAMACS) were observed, reflecting treatment time, ultrasonic frequency, and effective power.

3.2 Ultrasound and Mild Heat Treatment

3.2.1 Microbiological Analysis

Combined treatment significantly affected the *E. coli* count in all three egg products, regardless of ultrasonic frequency, effective power, or duration. The highest dose of ultrasonic treatment (28.84 kJ) at 40 kHz reduced *E. coli* counts by five orders of magnitude in all products, lowering the microbial count below the detection limit (<0 log CFU/ml).

Treatments at 20 kHz achieved similar bactericidal effects only in egg yolk. Mild heat treatment alone at 55°C had no effect on the *E. coli* content of egg liquids.

3.2.2 Near-Infrared Spectral Analysis Results

PCA loadings indicated that combined treatment affected molecular groups such as C-C, C-N, -OH, and N-H in all three egg liquids. Changes in water absorbance patterns were also detected in the 1300–1550 nm wavelength range, attributed to both combined and mild heat treatments.

LDA revealed that treatment duration was the most influential parameter in altering the spectral characteristics of egg products.

3.3 Comparison of Standalone and Combined Treatments

3.3.1 Microbiological Analysis

Figures 1., 2. and 3. illustrate the bactericidal effects of different treatments on egg white, yolk, and whole egg liquid. The results clearly show the superiority of combined treatments over standalone ultrasonic treatments in all three products.

Notably, the mild heat-treated groups at 55°C showed no effect on *E. coli* counts, regardless of treatment duration, for any of the egg products.

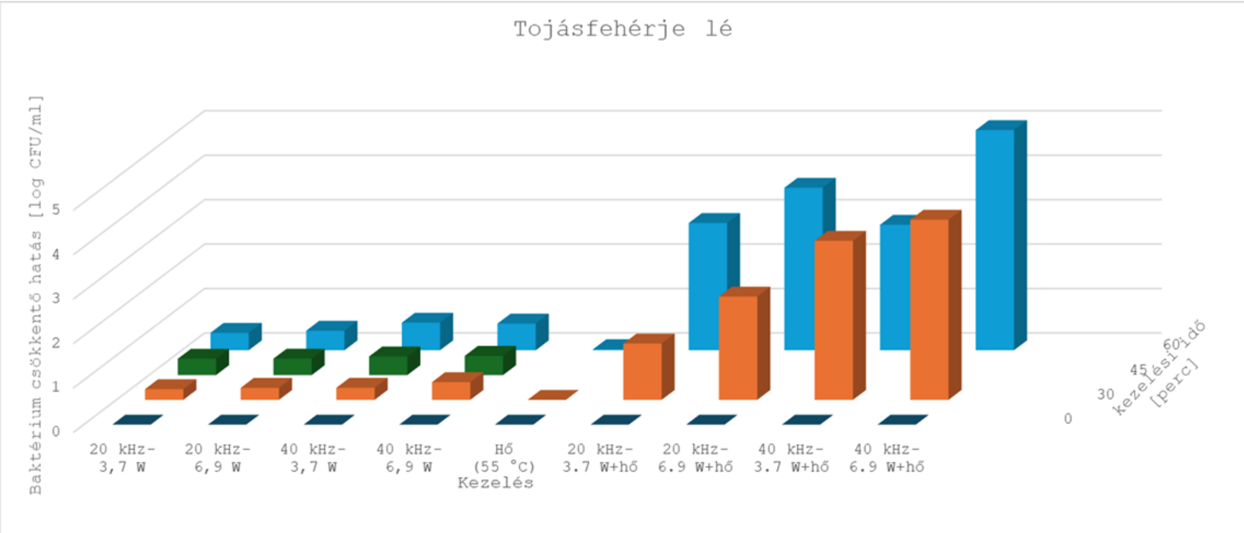

Figure 1.: Bactericidal effect of standalone ultrasound, combined ultrasound and mild heat treatments on egg white

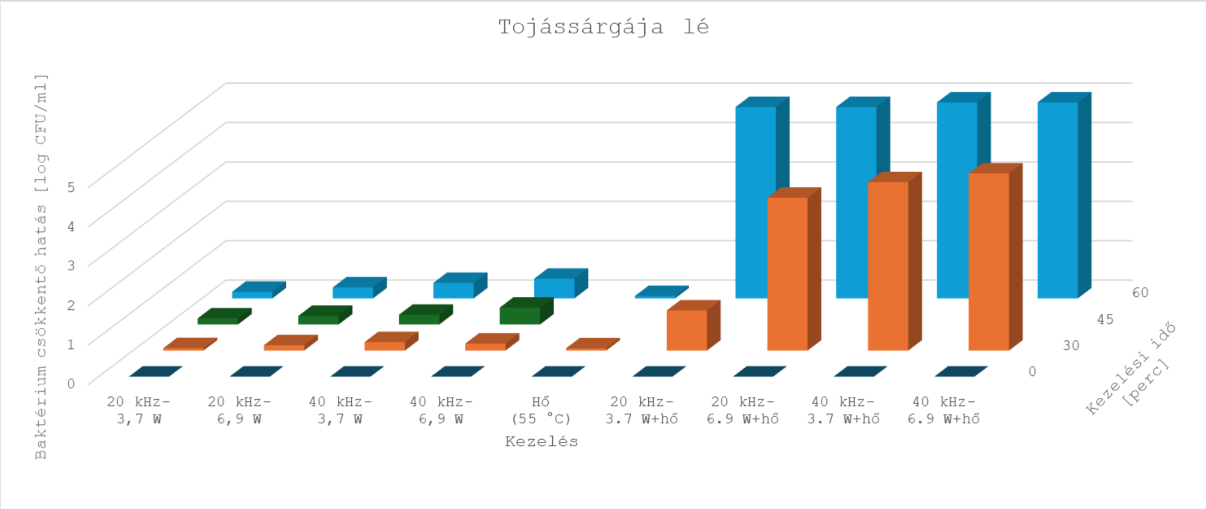

Figure 2.: Bactericidal effect of single ultrasound, combined ultrasound and mild heat treatments on egg yolk

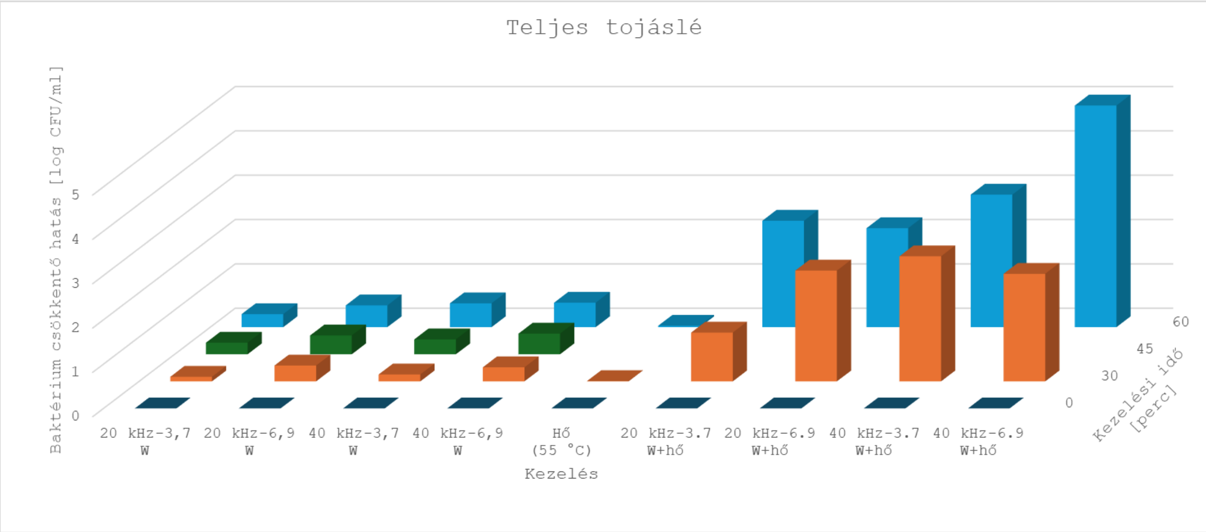

Figure 3.: Bactericidal effect of single ultrasound, combined ultrasound and mild heat treatments on whole egg liquid

3.3.2 Results of Near-Infrared Spectral Analysis

The principal component analysis and linear discriminant analysis of the spectra revealed that samples exposed to mild heat treatment (Heat, combined treatment) are distinct, whereas those subjected only to ultrasonic treatment overlap with the control samples. This indicates that, in the case of combined treatments, most of the spectral changes are primarily due to mild heat exposure across all egg products. These differences are likely the result of heat-induced protein denaturation, coagulation, and alterations in water structure, as supported by aquaphotomics findings.

3.4 Results of foam capacity and stability of egg white

With ultrasound treatment the observed highest foam volumne of 149.23ml was achieved with egg white treated at 40 kHz, 6.9 W, for 60 minutes. However, a similar effect was observed after just 30 minutes of treatment. Compared to the control groups, even a 30-minute ultrasonic treatment increased foam-forming ability by approximately 15% on average.

The stability of the egg white foam decreased as a result of the treatments. Statistical analysis of the amount of reformed liquid egg white indicated that ultrasound treatment's frequency, effective power, treatment duration, and dose significantly affected foam stability.

The experimental series demonstrated that ultrasound treatment significantly influences both the foam-forming properties and the stability of the resulting foam. Foam capacity was significantly impacted by the effective power and dose of the treatment. While no monotonic change in foam formation was observed with increasing ultrasonic doses, a significant effect was evident between effective powers of 3.7 W and 6.9 W.

4. Conclusions and Recommendations

In my research, I successfully applied standalone ultrasound treatment to significantly reduce the microbial count of *E. coli* in liquid egg products. Although the treatments did not achieve the desired threshold of microbial reduction, this effect could be enhanced with higher-dose treatments. Further exploration of the method's effectiveness under different treatment parameters is recommended.

Beyond microbial reductions, I examined the impact of ultrasound on the near-infrared spectra of egg products, utilizing aquaphotomics—a relatively new field. The analysis of near-infrared spectra effectively identified changes caused by treatments, providing indirect insights into molecular groups affected. Further studies are needed for chemometric determination of molecular groups and for elucidating the relationship between spectral data and molecular groups in liquid eggs.

The combined application of ultrasound and mild heat treatment achieved a 5 log CFU/ml reduction in *E. coli* microbial count. This reduction was sufficient to reach 0 log CFU/ml; however, additional research is needed to evaluate the combined treatment's impact on higher initial microbial concentrations.

Results from the near-infrared spectral analysis showed more pronounced changes in the spectral properties of samples subjected to heat treatment (combined or standalone). It would be valuable to investigate further the extent of spectral changes resulting from combined treatments with lower heat levels and whether such treatments can achieve comparable microbial reductions. By understanding the relationships between wavelength absorbance, and molecular groups, the method could potentially be applied as a rapid, non-invasive measurement technique for liquid egg products in the industry

Standalone ultrasound treatments also had a detectable effect on the foam-forming and foam stability properties of liquid egg white. A significant effect on foam formation was observed above a specific effective power threshold. Further research is required to accurately determine this threshold value.

5. New Scientific Findings

1. I demonstrated that ultrasound, as a standalone treatment method, can significantly reduce *E. coli* bacteria levels in liquid egg white (from 6.66 kJ, 30 min, 3.7 W), liquid egg yolk (from 13.32 kJ, 60 min, 3.7 W), and whole egg liquid (from 9.99 kJ, 45 min, 3.7 W), regardless of frequency.

2. I established that the combined application of ultrasound (3.7/6.9 W, 20/40 Hz, 30/60 min) and mild heat treatment (55 °C, 30/60 min) effectively reduced *E. coli* microbial count in all three studied egg products by 5 log CFU/ml. Heat treatment alone showed no detectable impact on microbial count reduction. For egg white and whole egg liquid, a 5 log CFU/ml reduction was achieved after 60 minutes at 6.9 W effective power (24.84 kJ) and 40 kHz ultrasound, whereas for egg yolk, a similar reduction was observed after 30 minutes at 6.9 W effective power (12.42 kJ) and 40 kHz ultrasound.

3. Near-infrared (NIR) analyses of untreated and treated egg products (egg white, egg yolk, whole egg liquid) revealed changes in spectra caused by treatments. Multivariate statistical analyses (discriminant analysis) confirmed that while ultrasound effects were minimal, heat treatment caused significant spectral changes in all egg products (100% DA separation), indicating alterations in molecular and bonding structures.

4. Using aquaphotomics, I demonstrated changes in the water structure of egg products due to treatments. Analysis of changes in water matrix coordinates (WAMACS) supported that ultrasound treatments had less impact on the developed water absorption spectral pattern (WASP) than heat treatment in all egg products, suggesting changes in water molecule and bonding structures.

5. Foam formation studies indicated that ultrasound effects emerge only above a certain effective power threshold (between 3.7 W and 6.9 W in this case), regardless of treatment duration and frequency. This could explain the contradictory results in the literature (where effective power is often unknown) and highlights the need for practical application sizing based solely on effective power.

6. The foam capacity increased by an average of 15% compared to untreated samples after a 30-minute treatment at 6.9 W effective power (40 kHz), and this effect did not increase further with time, showing saturation.

7. Foam stability studies revealed that ultrasound treatment (6.9 W, 20/40 Hz, 30/60 min) increased the amount of reformed liquid egg white. Statistical analysis indicated that both frequency and duration of the treatment significantly influenced foam stability.

8. List of publications in the field of studies

Nagy, D.; Felfoldi, J.; Taczmanne Bruckner, A.; Mohacsi-Farkas, C.; Bodor, Z.; Kertesz, I.; Nemeth, C.; Zsom-Muha, V. „Determining Sonication Effect on *E. coli* in Liquid Egg, Egg Yolk and Albumen and Inspecting Structural Property Changes by Near-Infrared Spectra” Sensors 2021, 21, 398. https://doi.org/10.3390/s21020398

Nagy, D.; Baranyai, L.; Nguyen, L.L.P.; Taczman Brückner, A.; Zsom, T.; Németh, C.; Felföldi, J.; Zsom-Muha, V. Combined Effect of Ultrasound and Low-Heat Treatments on *E. coli* in Liquid Egg Products and Analysis of the Inducted Structural Alterations by NIR Spectroscopy. Sensors 2022, 22, 9941. https://doi.org/10.3390/s22249941

Nagy, D.; Zsom, T.; Taczman-Brückner, A.; Somogyi, T.; Zsom-Muha, V.; Felföldi, J. Comparison of the Bactericidal Effect of Ultrasonic and Heat Combined with Ultrasonic Treatments on Egg Liquids and Additional Analysis of Their Effect by NIR Spectral Analysis. Sensors 2024, 24, 4547. https://doi.org/10.3390/s24144547

Nagy, D.; Zsom-Muha, V.; Németh, C.; Felföldi, J. Sonication effect on foam properties of egg white. Prog. Agric. Eng. Sci. 2021, 17, 1–8.

Nagy, D., Lambertné, M. A., Zsom, T., & Zsomné, M. V. (2018). Review on applications of ultrasonic treatments in meat industry. Animal Welfare, Ethology and Housing Systems, 14(1), 45-52.