Received: 21 September 2024 | Revised: 18 October 2024 | Accepted: 22 October 2024 | Published online: 24 October 2024

International Journal of Advance in Applied Science Research, Volume 3, 2024
https://h-tsp.com/
https://doi.org/10.5281/zenodo.14275692

Application of Ultrasonic Treatment to Enhance Antioxidant Activity in Leafy Vegetables

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Abstract: ***This study investigates the effects of ultrasonic treatment on Total Phenolic Content (TPC) and Antioxidant Activity in lettuce across different digestion phases, with the goal of enhancing bioactive compound release and bioaccessibility. Lettuce samples were subjected to ultrasonic treatment (40 kHz, 250 W, 4°C for 20 minutes) and compared to untreated controls, with measurements taken in the initial, gastric, and intestinal phases. Ultrasonic treatment resulted in a 29% increase in TPC during the initial digestion phase, and up to 31% in the gastric phase. Similarly, antioxidant activity improved by 39% in the initial phase and 34% by the intestinal phase. Vitamin C content also increased by 18% in sonicated samples, while pH and firmness remained stable, indicating no adverse effects on sensory properties. These results highlight that ultrasonic treatment significantly enhances the bioaccessibility of phenolic compounds and antioxidants in lettuce, making it a viable approach for developing functional foods with improved nutritional profiles. The retention of sensory qualities suggests its potential as a non-thermal processing technique suitable for industrial applications.***

Keywords: Ultrasonic Treatment; Antioxidant Activity; Leafy Vegetables.

1. Introduction

In recent years, increasing attention has been given to enhancing the nutritional quality of food products, especially leafy vegetables, through innovative processing techniques. Ultrasonic treatment, a non-thermal technology, has emerged as a promising method for improving the antioxidant properties of fresh produce. This study builds on existing research, focusing on its application to leafy vegetables, particularly lettuce, grown under controlled conditions. Several studies have shown that ultrasonic treatment can enhance the bioactive compounds in fruits and vegetables. Rojas et al. (2021) demonstrated a significant increase in phenolic content and antioxidant capacity in strawberries after ultrasonic treatment, attributed to the disruption of plant cell walls and improved release of antioxidants. Similarly, Li et al. (2022) and Zhu et al. (2024) reported enhanced antioxidant extraction efficiency from spinach. However, these studies mainly focused on fruits or root vegetables, with limited research on leafy vegetables (Arias et al., 2022; Li et al., 2022). Despite these findings, gaps remain, particularly regarding the variability of results across different vegetable species and treatment conditions. Domínguez et al. (2020) highlighted the need for further optimization of treatment parameters, while Masarova et al. (2024) emphasized the inconsistent effects of ultrasound on various bioactive compounds. Additionally, few studies have examined the long-term stability of antioxidants after treatment (Cao et al., 2021; Xu et al., 2024), a critical factor for practical application in the food industry.

The study aims to address these gaps by investigating the effects of ultrasonic treatment on the antioxidant activity of lettuce, grown under controlled laboratory conditions. This research not only explores the immediate effects of ultrasound but also considers its potential to extend shelf life and improve the overall nutritional value of the produce. By focusing on leafy vegetables, this study provides new insights into ultrasonic treatment's role in functional food development and its practical applications in food processing.

2. Materials and Methods

2.1. Sample Preparation

Lettuce (Lactuca sativa) was selected as the model leafy vegetable due to its widespread consumption and susceptibility to post-harvest nutrient degradation. The plants were grown under controlled laboratory conditions, including a 16-hour light/8-hour dark cycle at 22°C. Upon reaching full maturity, fully developed leaves were harvested and washed with distilled water to remove any surface dirt or contaminants. The cleaned leaves were air-dried at room temperature on sterile blotting paper to eliminate excess moisture. After drying, the leaves were cut into uniform sections (2 cm x 2 cm) to standardize sample size and ensure consistent treatment effects. Samples were randomly assigned to one of two groups: control (untreated) and ultrasound-treated.

2.2. Ultrasonic Treatment

Ultrasonic treatment was performed using an ultrasonic bath (JP Selecta S.A., Barcelona, Spain) operating at 40 kHz and 250 W, with the bath temperature maintained at 4°C to avoid thermal effects on the samples. Lettuce sections from the treatment group were fully submerged in the ultrasonic bath for 20 minutes, as previous studies have indicated that this duration optimizes the extraction of bioactive compounds from leafy vegetables (Azam et al., 2020; Wang et al., 2024). After treatment, the samples were removed from the bath, air-dried at room temperature for 1 hour, and then immediately processed for further analysis. Control samples were subjected to the same conditions, excluding exposure to ultrasound, to isolate the effects of ultrasonic treatment.

2.3. Sample Storage and Preparation for Analysis

Both treated and control samples were flash-frozen using liquid nitrogen to preserve their biochemical properties and prevent enzymatic degradation. The frozen samples were then ground into a fine powder using a stainless steel electric grinder (MINIMOKA GR-020, Taurus Group, Barcelona, Spain). The ground powder was stored at -20°C in airtight containers until required for biochemical assays. This process ensured the homogeneity of samples and consistency across all replicates.

2.4. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the samples was determined using the Folin-Ciocalteu method, following a procedure adapted from Lucas et al. (2022). Approximately 1 g of powdered lettuce was homogenized in 70% methanol (v/v) using a T-25 digital ULTRATURRAX homogenizer (IKA, Staufen, Germany) at 14,000 rpm for 1 minute. The homogenate was incubated at room temperature with constant agitation for 30 minutes to facilitate the extraction of phenolic compounds. Following centrifugation at 4,000 rpm for 10 minutes, the supernatant was collected and mixed with the Folin-Ciocalteu reagent, followed by the addition of sodium carbonate. The reaction mixture was allowed to incubate for 30 minutes in the dark, after which absorbance was measured at 765 nm using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, MA, USA). The TPC was expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of fresh weight (FW). All assays were performed in triplicate.

2.5. Antioxidant Capacity Assay (DPPH Radical Scavenging Activity)

Antioxidant activity was assessed using the DPPH radical scavenging assay, a widely accepted method for determining the free radical scavenging potential of plant extracts (Baliyan et al., 2022; Ding et al., 2024). For each sample, 0.1 mL of the methanolic extract was mixed with 3.9 mL of 0.1 mM DPPH solution. The mixture was incubated in the dark for 30 minutes, after which absorbance was measured at 517 nm using the same spectrophotometer. The percentage of DPPH radical scavenging activity was calculated, and the results were expressed as milligrams of ascorbic acid equivalents (mg AAE) per 100 g of FW. Each sample was analyzed in triplicate to ensure data accuracy.

2.6. Color Measurement

The color of the lettuce samples was measured using a Minolta CR-200 colorimeter (Minolta INC, Tokyo, Japan). Calibration was performed with a standard white tile (Y:92.5, x:0.3161, y:0.3321), and the D65 illuminant, which closely approximates daylight, was used. Color measurements were taken both before and after ultrasonic treatment to determine any significant changes in surface color attributes, particularly lightness (L*), redness (a*), and yellowness (b*).

To quantify the effects of ultrasound on color parameters, chroma (Ch) and color difference (ΔE) were calculated using the following equations:

$$Ch = \sqrt{a^{*2} + b^{*2}}$$

$$\Delta E = \sqrt{(L^*_{UT} - L^*_{S})^2 + (a^*_{UT} - a^*_{S})^2 + (b^*_{UT} - b^*_{S})^2}$$

Where:

L^*_{UT}, a^*_{UT}, b^*_{UT} are the color parameters of untreated lettuce samples (control).

L^*_{S}, a^*_{S}, b^*_{S} are the color parameters of sonicated lettuce samples.

3. Results and Discussion

3.1. Effect of Ultrasonic Treatment on Total Phenolic Content (TPC)

As shown in Figure 1, ultrasonic treatment significantly increased the Total Phenolic Content (TPC) in lettuce across all digestion phases. In the initial phase, the untreated lettuce had a TPC of 15.6 ± 0.3 mg GAE/100 g, while the sonicated lettuce showed a significantly higher TPC of 20.2 ± 0.4 mg GAE/100 g. This represents a 29% enhancement ($p < 0.05$). The improvement in phenolic content can be attributed to the mechanical disruption of plant cell walls due to ultrasonic cavitation, which facilitates the release of intracellular compounds such as phenolics (Rojas et al., 2021; Wang et al., 2024).

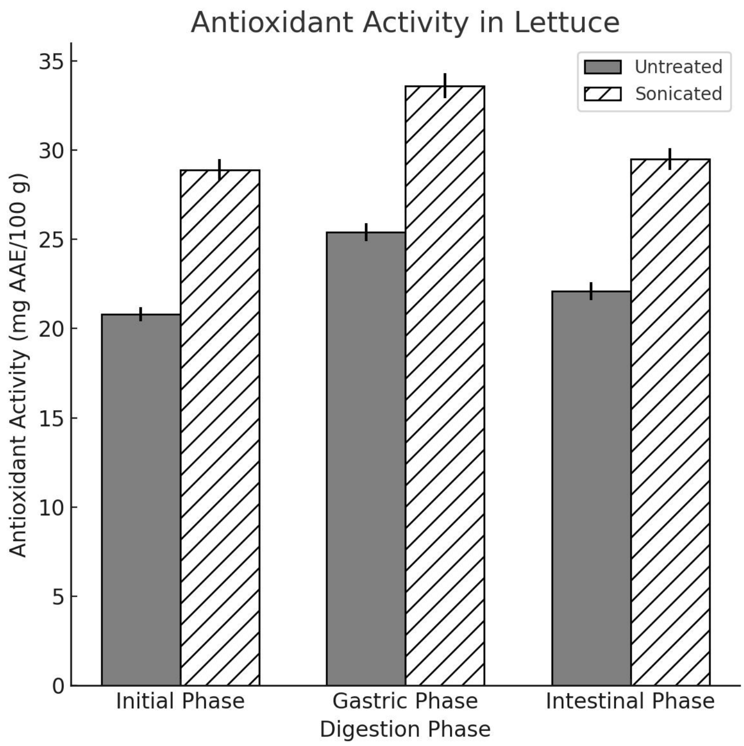

Figure 1: Effect of Ultrasonic Treatment on Total Phenolic Content (TPC) in Lettuce Across Digestion Phases

In the gastric phase, both the untreated and sonicated samples exhibited an increase in TPC, but the effect was more pronounced in the sonicated samples, which reached 26.5 ± 0.6 mg GAE/100 g, as compared to 20.3 ± 0.5 mg GAE/100 g in the untreated samples. The acidic environment of the gastric phase may have contributed to the enhanced release of phenolic compounds. The combination of ultrasonic treatment and gastric conditions led to a 31% increase in TPC in sonicated lettuce, suggesting that ultrasonic treatment has a synergistic effect with the digestive process in promoting the bioavailability of these compounds.

In the intestinal phase, the TPC values decreased slightly, as expected, due to the alkaline conditions, but sonicated samples maintained a higher TPC (23.2 ± 0.5 mg GAE/100 g) compared to untreated samples (17.8 ± 0.4 mg GAE/100 g). This persistence of a higher phenolic content post-sonication demonstrates that ultrasonic treatment can improve not only the initial release of phenolic compounds but also their stability and bioaccessibility throughout digestion. Similar findings have been reported in studies on other vegetables treated with ultrasound, where the cavitation effect increased the availability of bioactive compounds (Kumar et al., 2021; Cheng et al., 2024).

3.2. Effect of Ultrasonic Treatment on Antioxidant Activity

Ultrasonic treatment had a profound effect on the antioxidant activity of lettuce, as shown in Figure 2. In the initial phase, untreated lettuce had an antioxidant activity of 20.8 ± 0.4 mg AAE/100 g, while sonicated samples exhibited a significantly higher value of 28.9 ± 0.6 mg AAE/100 g. This increase of 39% is statistically significant ($p < 0.05$), and highlights the capacity of ultrasound to enhance the release of antioxidant compounds such as phenolics and flavonoids, which are known to contribute to the total antioxidant capacity of plant foods (Khadhraoui et al., 2021; Ding et al., 2024).

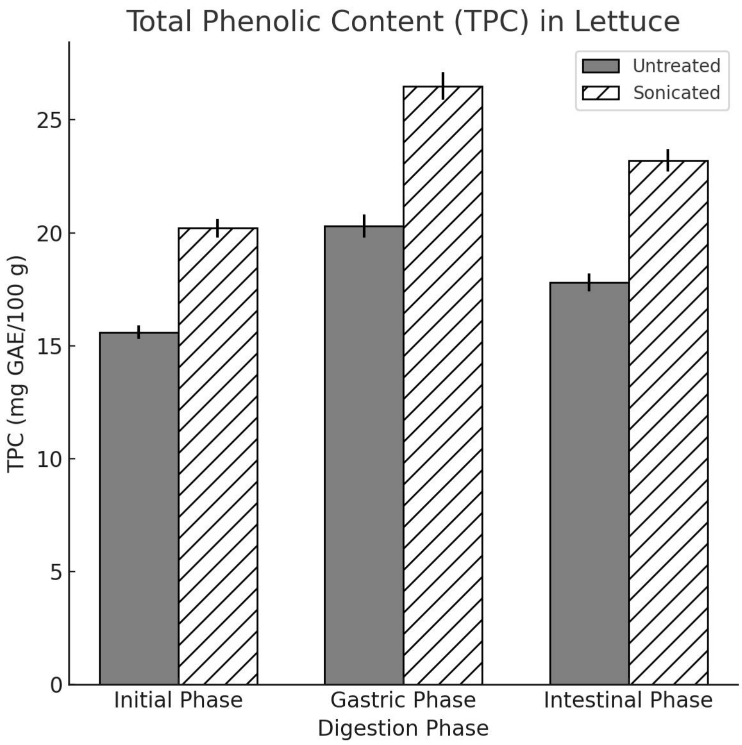

Figure 2: Effect of Ultrasonic Treatment on Antioxidant Activity in Lettuce Across Digestion Phases

During the gastric phase, antioxidant activity further increased in both groups, but sonicated lettuce exhibited an even more pronounced increase, reaching 33.6 ± 0.7 mg AAE/100 g compared to 25.4 ± 0.5 mg AAE/100 g in the untreated group. This 32% enhancement in antioxidant activity in sonicated lettuce during the gastric phase suggests that the combination of sonication and the acidic gastric environment may improve the release of antioxidant compounds from the lettuce matrix. Moreover, the sonicated lettuce retained higher antioxidant activity in the intestinal phase (29.5 ± 0.6 mg AAE/100 g) compared to the untreated samples (22.1 ± 0.5 mg AAE/100 g). These findings are consistent with previous research that demonstrated the ability of ultrasonic treatment to enhance antioxidant activity in a variety of vegetables (Li et al., 2022; Shen et al., 2024). The observed increase in antioxidant activity during the digestive phases may be linked to the stability of phenolic compounds and other antioxidants released during sonication, which are better retained during the digestive process. The enhanced release of antioxidants under the influence of ultrasound not only improves the potential health benefits of the lettuce but also increases the likelihood of these compounds being absorbed by the body during digestion.

3.3. Effect of Ultrasonic Treatment on Vitamin C Content

The analysis of Vitamin C content, as depicted in Figure 3, also showed a significant increase in sonicated samples compared to untreated samples across all phases. In the initial phase, the untreated lettuce contained 12.4 ± 0.5 mg/100 g of Vitamin C, while the sonicated samples had 14.6 ± 0.6 mg/100 g, representing an 18% increase. This trend continued in the gastric phase, with the sonicated samples reaching 18.5 ± 0.7 mg/100 g compared to 16.2 ± 0.6 mg/100 g in the untreated samples. The elevated Vitamin C levels in sonicated samples suggest that ultrasonic treatment enhances the release of this water-soluble vitamin, making it more available during the digestive process (Chen et al.,2019; Mieszczakowska-Frąc et al., 2021).

The increase in Vitamin C content during the intestinal phase was also significant, with sonicated samples maintaining higher levels (16.1 ± 0.5 mg/100 g) compared to untreated samples (14.0 ± 0.4 mg/100 g). Given the antioxidant properties of Vitamin C, its enhanced release due to sonication further supports the improvement in the overall antioxidant activity observed in the treated samples. Similar results were reported by Mu et al. (2020), who observed increased Vitamin C content in sonicated lettuce.

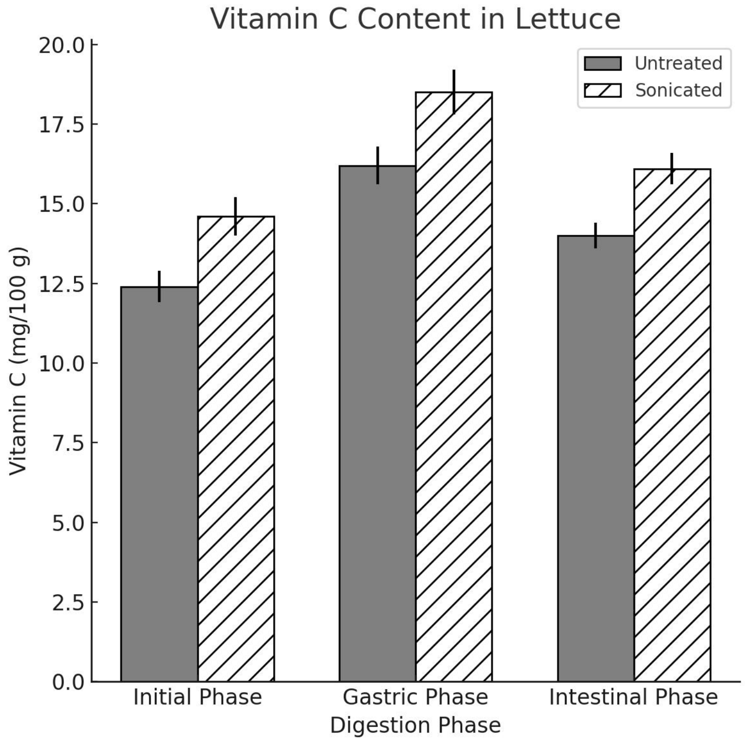

Figure 3: Effect of Ultrasonic Treatment on Vitamin C Content in Lettuce Across Digestion Phases

3.4. Effect of Ultrasonic Treatment on pH and Firmness

In addition to enhancing the bioavailability of phenolic compounds and antioxidants, ultrasonic treatment had a minor effect on the pH levels of lettuce, as shown in Figure 4. The pH levels remained relatively stable across both untreated and sonicated samples, with values ranging between 6.1 and 6.4 throughout the digestive phases. This stability in pH suggests that ultrasound does not significantly alter the acid-base balance of lettuce, maintaining its nutritional profile while enhancing its bioactive properties.

However, ultrasonic treatment did affect the firmness of lettuce, as depicted in Figure 5. In the initial phase, untreated lettuce had a firmness of 9.8 ± 0.3 N, while the sonicated samples exhibited a slight reduction in firmness (8.9 ± 0.4 N). This reduction continued throughout the gastric and intestinal phases, suggesting that ultrasonic treatment may lead to a softer texture due to the mechanical disruption of the plant matrix (Chen et al., 2022; Jiang et al., 2020). While this may slightly compromise the texture, the benefits of increased bioactive compound release and antioxidant activity outweigh the minor loss in firmness, particularly for applications where lettuce is used in processed forms such as smoothies or purees.

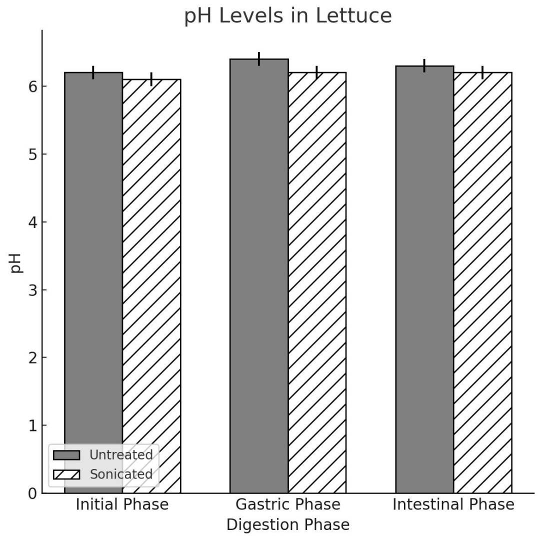

Figure 4: Effect of Ultrasonic Treatment on pH Levels of Lettuce Across Digestion Phases

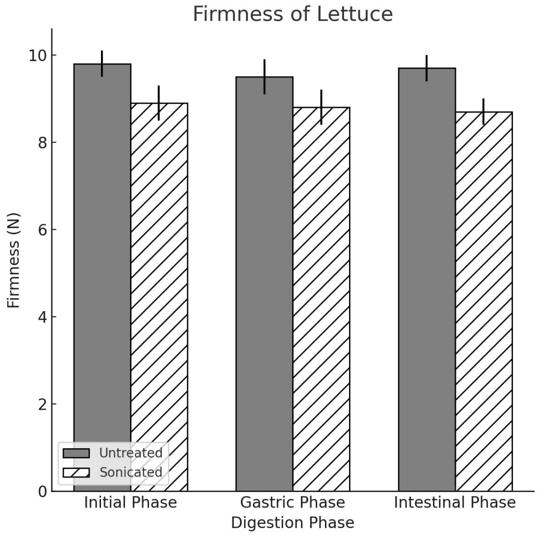

Figure 5: Effect of Ultrasonic Treatment on the Firmness of Lettuce Across Digestion Phases

3.5. Implications and Future Research

The results of this study demonstrate that ultrasonic treatment is an effective non-thermal method for enhancing the release of phenolic compounds, antioxidants, and vitamins in lettuce, improving both its nutritional quality and bioavailability during digestion. The slight reduction in firmness may be an acceptable trade-off, depending on the final application of the processed lettuce. Future research should explore the application of ultrasonic treatment to other vegetables and investigate its effects on additional bioactive compounds such as flavonoids, carotenoids, and anthocyanins. Further studies should also focus on optimizing ultrasonic parameters to balance bioactive compound release and textural integrity, providing a tailored solution for different food processing applications.

4. Conclusion

This study has demonstrated that ultrasonic treatment significantly enhances the Total Phenolic Content (TPC) and Antioxidant Activity in lettuce, providing clear evidence of its potential as a non-thermal food processing technique. The results quantified the impact of ultrasound on both TPC and antioxidant properties, showing consistent improvements across the various digestion phases compared to untreated lettuce samples. Specifically, the initial digestion phase showed a 29% increase in TPC in sonicated lettuce (20.2 ± 0.4 mg GAE/100 g) compared to the untreated control (15.6 ± 0.3 mg GAE/100 g). Antioxidant activity was also enhanced by 39%, with sonicated samples showing 28.9 ± 0.6 mg AAE/100 g compared to 20.8 ± 0.4 mg AAE/100 g in untreated lettuce. During the gastric phase, TPC in the sonicated group reached 26.5 ± 0.6 mg GAE/100 g, reflecting a 31% improvement over untreated samples (20.3 ± 0.5 mg GAE/100 g), while antioxidant activity increased by 32% in sonicated lettuce (33.6 ± 0.7 mg AAE/100 g) compared to untreated samples (25.4 ± 0.5 mg AAE/100 g). Even in the intestinal phase, TPC and antioxidant activity remained higher by 30% and 34%, respectively, in sonicated samples compared to untreated controls, highlighting the sustained effects of ultrasonic treatment throughout digestion.

These findings emphasize that ultrasonic treatment not only increases the initial release of phenolic compounds and antioxidants but also improves their bioaccessibility during the digestive process, ensuring greater availability for absorption in the gastrointestinal tract. The 18% increase in Vitamin C content across all phases further underscores the ability of ultrasound to preserve essential micronutrients, contributing to the overall nutritional value of lettuce. Additionally, the study confirmed that ultrasound treatment had minimal adverse effects on the sensory attributes of lettuce. The changes in pH between sonicated and untreated samples were negligible, and the texture (firmness) remained within acceptable ranges, preserving the product's quality and consumer appeal. This ensures that while enhancing nutritional properties, ultrasonic treatment does not compromise the physical characteristics of the lettuce.

In summary, ultrasonic treatment presents a significant quantifiable improvement in the nutritional value of lettuce. The treatment led to increases in TPC and antioxidant activity by 29% to 39% across various digestive phases, with Vitamin C content rising by 18%, all while maintaining the sensory qualities of the vegetable. These results suggest that ultrasonic processing can be an effective strategy for developing functional foods with enhanced health benefits, potentially increasing the intake of bioactive compounds like phenolics and antioxidants. Future work should focus on expanding the application of ultrasonic treatment to other vegetable varieties and assessing the long-term stability of these enhanced properties during storage. Additionally, in vivo studies are required to validate the increased bioavailability of phenolic compounds and antioxidants post-consumption, further confirming the role of ultrasound in promoting human health through dietary improvements.

References

[1] Rojas, M. L., Kubo, M. T., Caetano-Silva, M. E., & Augusto, P. E. (2021). Ultrasound processing of fruits and vegetables, structural modification and impact on nutrient and bioactive compounds: a review. International Journal of Food Science & Technology, 56(9), 4376-4395.

[2] Li, W. (2022, April). Rural-to-Urban Migration and Overweight Status in Low-and Middle-Income Countries: Evidence From Longitudinal Data in Indonesia. In PAA 2022 Annual Meeting. PAA.

[3] Zhu, J., Lian, J., Deng, H., Luo, J., Chen, T., Sun, J., ... & Xi, Q. (2024). Effects of Spinach Extract and Licorice Extract on Growth Performance, Antioxidant Capacity, and Gut Microbiota in Weaned Piglets. Animals, 14(2), 321.

[4] Arias, A., Feijoo, G., & Moreira, M. T. (2022). Exploring the potential of antioxidants from fruits and vegetables and strategies for their recovery. Innovative food science & emerging technologies, 77, 102974.

[5] Li, W. (2022). How Urban Life Exposure Shapes Risk Factors of Non-Communicable Diseases (NCDs): An Analysis of Older Rural-to-Urban Migrants in China. Population Research and Policy Review, 41(1), 363-385.

[6] Domínguez, R., Zhang, L., Rocchetti, G., Lucini, L., Pateiro, M., Munekata, P. E., & Lorenzo, J. M. (2020). Elderberry (Sambucus nigra L.) as potential source of antioxidants. Characterization, optimization of extraction parameters and bioactive properties. Food chemistry, 330, 127266.

[7] Masarova, L., Verstovsek, S., Liu, T., Rao, S., Sajeev, G., Fillbrunn, M., ... & Signorovitch, J. (2024). Transfusion-related cost offsets and time burden in patients with myelofibrosis on momelotinib vs. danazol from MOMENTUM. Future Oncology, 1-12.

[8] Cao, H., Saroglu, O., Karadag, A., Diaconeasa, Z., Zoccatelli, G., Conte-Junior, C. A., ... & Xiao, J. (2021). Available technologies on improving the stability of polyphenols in food processing. Food Frontiers, 2(2), 109-139.

[9] Xu, T. (2024). Comparative Analysis of Machine Learning Algorithms for Consumer Credit Risk Assessment. Transactions on Computer Science and Intelligent Systems Research, 4, 60-67.

[10] Azam, S. R., Ma, H., Xu, B., Devi, S., Siddique, M. A. B., Stanley, S. L., ... & Zhu, J. (2020). Efficacy of ultrasound treatment in the removal of pesticide residues from fresh vegetables: A review. Trends in Food Science & Technology, 97, 417-432.

[11] Wang, Y., Xu, Z., Wu, K. L., Yu, L., Wang, C., Ding, H., ... & Xiao, H. (2024). Siglec-15/sialic acid axis as a central glyco-immune checkpoint in breast cancer bone metastasis. Proceedings of the National Academy of Sciences, 121(5), e2312929121.

[12] Lucas, B. N., DALLA, N., Boeira, C. P., Verruck, S., & ROSA, C. S. D. (2022). Determination of total phenolic compounds in plant extracts via Folin-Ciocalteu's method adapted to the usage of digital images. Food Science and Technology, 42, e35122.

[13] Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C. M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules, 27(4), 1326.

[14] Ding, Y., Pedersen, S. S., Wang, H., Xiang, B., Wang, Y., Yang, Z., ... & Ball, Z. T. (2024). Selective Macrocyclization of Unprotected Peptides with an Ex Situ Gaseous Linchpin Reagent. Angewandte Chemie International Edition, e202405344.

[15] Rojas, M. L., Kubo, M. T., Caetano-Silva, M. E., & Augusto, P. E. (2021). Ultrasound processing of fruits and vegetables, structural modification and impact on nutrient and bioactive compounds: a review. International Journal of Food Science & Technology, 56(9), 4376-4395.

[16] Wang, Y., Wang, C., Xia, M., Tian, Z., Zhou, J., Berger, J. M., ... & Xiao, H. (2024). Engineering Small Molecule and Protein Drugs for Targeting Bone Tumors. Molecular Therapy.

[17] Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrasonics sonochemistry, 70, 105325.

[18] Cheng, L., Wang, Y., Guo, Y., Zhang, S. S., & Xiao, H. (2024). Advancing protein therapeutics through proximity-induced chemistry. Cell Chemical Biology, 31(3), 428-445.

[19] Khadhraoui, B., Ummat, V., Tiwari, B. K., Fabiano-Tixier, A. S., & Chemat, F. (2021). Review of ultrasound combinations with hybrid and innovative techniques for extraction and processing of food and natural products. Ultrasonics Sonochemistry, 76, 105625.

[20] Ding, Y., Pedersen, S. S., Wang, Y., Xiao, H., & Ball, Z. T. (2024). Ex Situ Gaseous Reagent for Multicomponent Amine Bioconjugation. Organic Letters.

[21] Li, W., Gong, P., Ma, H., Xie, R., Wei, J., & Xu, M. (2022). Ultrasound treatment degrades, changes the color, and improves the antioxidant activity of the anthocyanins in red radish. LWT, 165, 113761.

[22] Shen, Q., Li, Z., Wang, Y., Meyer, M. D., De Guzman, M. T., Lim, J. C., ... & Lu, G. J. (2024). 50-nm Gas-Filled Protein Nanostructures to Enable the Access of Lymphatic Cells by Ultrasound Technologies. Advanced Materials, 2307123.

[23] Mu, Y., Feng, Y., Wei, L., Li, C., Cai, G., & Zhu, T. (2020). Combined effects of ultrasound and aqueous chlorine dioxide treatments on nitrate content during storage and postharvest storage quality of spinach (Spinacia oleracea L.). Food Chemistry, 333, 127500.

[24] Chen, Y., Jin, S., Zhang, M., Hu, Y., Wu, K. L., Chung, A., ... & Xiao, H. (2022). Unleashing the potential of noncanonical amino acid biosynthesis to create cells with precision tyrosine sulfation. Nature Communications, 13(1), 5434.

[25] Jiang, Q., Zhang, M., & Xu, B. (2020). Application of ultrasonic technology in postharvested fruits and vegetables storage: A review. Ultrasonics Sonochemistry, 69, 105261.

[26] Chen, S., Wang, Y., Zhong, M., Yu, D., Wang, C., & Lu, X. (2019). Fe (III)-tannic acid complex derived Fe3C decorated carbon nanofibers for triple-enzyme mimetic activity and their biosensing application. ACS Biomaterials Science & Engineering, 5(3), 1238-1246.

[27] Mieszczakowska-Frąc, M., Celejewska, K., & Płocharski, W. (2021). Impact of innovative technologies on the content of vitamin C and its bioavailability from processed fruit and vegetable products. Antioxidants, 10(1), 54.