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Research article

Ultrasound prolongs the liquid state of honey and stabilizes its antibacterial activity

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Abstract: Honey crystallization is a natural process that limits its handling and processing while reducing consumer acceptance. Conventional thermal treatments used to prevent crystallization can negatively impact honey's bioactive properties, particularly its antibacterial activity. This study investigated ultrasound (US) as a non-thermal alternative to thermal processing for liquefying crystallized honey and preventing crystallization in liquid honey samples. Two crystallized honey samples were subjected to US treatment at frequencies of 20, 25, 28, 33, and 40 kHz with 20% and 30% power to evaluate liquefaction efficiency. Five fresh liquid honey samples were treated with US at optimal frequencies (28 and 40 kHz) or conventional heat treatment (70 °C for 10 min) and monitored for crystallization over 20 weeks of storage at 4 °C. Antibacterial activity against *Staphylococcus aureus* was assessed using minimum inhibitory concentration (MIC) assays. US treatment effectively liquefied crystallized honey, with optimal frequencies varying by botanical origin. US treatment at 40 kHz was most effective in preventing crystallization during storage, with none of the treated samples fully crystallizing after 20 weeks, compared to complete crystallization in heat-treated samples. Antibacterial activity remained stable in most US-treated samples, with multifloral honeys showing particular resistance to activity changes, likely due to protective polyphenol content. These findings demonstrate that US processing represents a promising non-thermal alternative to conventional heat treatment, effectively maintaining honey in liquid state while generally preserving its antibacterial properties. The botanical origin of honey significantly influences optimal US treatment parameters, suggesting the need for customized processing approaches.

Keywords: honey liquefaction; crystallization; biological function; honey processing; antibacterial activity

1. Introduction

Honey is a valuable functional food and traditional remedy due to its health-promoting properties [1]. It is a supersaturated sugar solution in which the formation of monohydrate glucose crystals of different sizes leads to solidification [2]. Crystallization occurs naturally due to the presence of glucose, which precipitates out of the supersaturated solution and forms crystals, leading to solidification. Honey undergoes natural crystallization, and this process can be enhanced through seed crystal-induced crystallization to improve honey performance characteristics. Multiple factors influence the crystallization rate, including sugar composition, water content, presence of nucleation seeds, degree of supersaturation, and viscosity [3]. The latter two factors are temperature-dependent. The presence of crystallization centers in honey, primarily pollen grains, facilitates the crystallization process. Although crystallization is influenced by multiple factors, Gregrova et al. demonstrated that absolute pollen count (a quantitative parameter) positively correlates with crystallization degree [4]. Crystal size is determined by the number of crystallization centers present in the honey [4].

Overall, the speed of crystallization and crystal size mainly depend on the botanical origin of the honey [5–7]. The fructose/glucose ratio of sunflower, rape, and lime honeys is lower, so they crystallize faster than chestnut, eucalyptus, heather, acacia, and honeydew honeys, which have a higher ratio (>1.4) [7]. During natural crystallization, honey exhibits pseudoplastic fluid behavior, characterized by increased L-values, firmness, and cohesiveness. Optimal crystallization conditions occur at 14 °C with a water content of 16% [6]. Maximum crystallization is typically observed at temperatures between 10 and 15 °C [8]. However, lower storage temperatures (4–7 °C) initiate crystallization nuclei formation, resulting in faster and more uniform crystallization throughout the honey volume [9].

Crystallization limits the handling and processing of honey. The top liquid layer that forms contains more water, which makes it vulnerable to yeast growth and fermentation [2]. Additionally, crystallized honey is undesired by consumers, who prefer liquid honey that is easier to dispense [10]. Therefore, various thermal and non-thermal processing approaches have been examined to either liquify crystallized honeys or to delay crystallization. Thermal liquefaction is a conventional approach to liquify/delay crystallization; temperatures of 45, 55, and 65 °C do not affect overall antibacterial activity [11]. The main consequences of overheating honey processing include a higher concentration of 5-hydroxymethylfurfural (HMF) and reduced diastase (DN) activity [12,13]. Interestingly, a mild thermal treatment of crystallized honey can significantly increase its glucose oxidase (GOX) activity [14]. Moreover, heating honey at 70 °C is more effective than heating it to 50 and 60 °C regarding its total antioxidant activity and brown pigment formation [15]. Even though the thermal processing of honey has shown some advantages in delaying crystallization and decreasing viscosity [16], the formation of heat-generated toxicants, such as HMF, and inactivation of DN are undesirable side effects [17].

In recent years, there have been significant efforts to develop and optimize effective techniques for liquifying crystallized honey and/or to prolong the liquid state of honey. However, most emerging technologies such as high-pressure processing [18,19], cold plasma discharge [20], and ultraviolet (UV)

irradiation [21] have been applied to examine the elimination of microorganisms in honey samples. There is little to no information about the effect of these alternative processing treatments on honey's biological activities.

Ultrasound (US) represents a suitable alternative to thermal processing because its chemical and physical effects can eliminate pathogens and degrade spores without the need of high temperatures [22]. US employs sound waves with a frequency above the human audible range, typically above 20 kHz, and has been used in various industries, including the food sector [23]. US waves generate cavitations, which refer to the formation, growth, and collapse of microscopic bubbles in the liquid [24]. This process plays a vital role in honey liquefaction through mechanical disruption and breakdown of microscopic glucose crystals, facilitating the reformation of a homogeneous liquid phase without overheating [25]. Most studies on US treatment of honey have used a US water bath with a frequency of 25, 40, or 42 kHz or a US probe with a frequency of 20 or 24 kHz to de-crystallize various types of honey and to characterize HMF formation and DN and antioxidant activities. US at 40 kHz has been applied to rosemary [26] and monofloral honeys (lime, rapeseed, and honeydew) [25,27], and US at 42 kHz has been used to treat monofloral honeys (acacia, raspberry, linden, rapeseed, honeydew, and grassland) [28] and multifloral and honeydew honeys from Mexico [29,30]. There has been limited investigation of how US treatment impacts the antibacterial activity of honey, with some studies suggesting it does not have an effect [25,27].

The aim of this study was to characterize the effect of US treatment as a non-thermal alternative to conventional thermal processing for liquefying crystallized honey and preventing crystallization in liquid honey samples, as well as to evaluate its impact on honey's antibacterial activity against the model bacterium *Staphylococcus aureus*. Specifically, US and heat treatments were compared regarding their ability to liquify honey and to prevent honey crystallization over a 20-week storage period.

2. Materials and methods

2.1. Honey samples

Two raw crystallized honey samples (C1 and C2) from *Apis mellifera* harvested in 2021 were obtained from local beekeepers from Slovakia. Upon arrival, they were immediately stored in plastic containers at 4 °C in the dark. Samples were subjected to US-assisted liquefaction or heat treatment (control). Five fresh raw liquid honey samples (L1–L5), harvested in 2022 and obtained from local beekeepers, were treated to assess the ability of US or heat treatment to prevent crystallization (Table 1). They were processed upon arrival. The dominant floral source of the honey was identified by the beekeepers based on the availability of flora for nectar foraging, the location of the apiary, and organoleptic characteristics of the honey. The sampling set included honeydew honeys [electrical conductivity (EC) > 0.8 mS/cm] and blossom/mixed honeys (EC < 0.8 mS/cm) [31]. All honey samples were equilibrated to room temperature (20–25 °C) before US or heat processing.

Table 1. Honey samples used in the study.

Sample no.	Botanical origin	Harvest year	State
C1	Honeydew	2021	Crystallized
C2	Fir-heather	2021	Crystallized
L1	Floral*	2022	Liquid
L2	Floral*	2022	Liquid
L3	Mixed**	2022	Liquid
L4	Acacia	2022	Liquid
L5	Floral*	2022	Liquid

*Floral honey: multifloral. **Mixed honey: honeydew and floral.

2.2. Bacteria

The antibacterial activity of the honey samples was assessed against *S. aureus* CCM4223, a model bacterium for testing the antibacterial potential of honey. This strain was obtained from the Department of Medical Microbiology, Slovak Medical University (Bratislava, Slovakia).

2.3. US treatment

Each crystallized honey sample was weighed (50 g) in a Petri dish and subjected to US. The treatment was applied using a US prototype machine (Notus Powersonic, Vrable, Slovakia), which enables the user to change probes and to treat samples with a frequency of 20, 25, 28, 33, or 40 kHz. This device also has a thermostat for temperature control. Two powers were tested: 20% and 30%. US was applied in two cycles: the first cycle was 5 min, followed by a 5 min pause; the second cycle was 10 min, followed by a 10 min pause. Pause times were included in the experimental design to avoid high temperatures (above 40 °C). The probes and power with the highest efficiency were selected to test US-assisted prevention of honey crystallization using five fresh liquid honey samples. In this case, untreated and US-treated liquid honey samples were stored at 4 °C (due to stabilization of antibacterial activity) for 20 weeks, and the number of crystals was determined before storage and after 1, 2, 5, 9, 13, and 20 weeks of storage. US probes were inserted directly into honey samples.

2.4. Heat treatment

Heat treatment is a standard procedure for honey processing; it eliminates microorganisms and prolongs the liquid state. Each liquid honey sample (L1–L5) was heated by standard commercial treatment, as described by Chen et al. [32], with a minor modification. Thermal processing involved heating the honey to 70 °C for 10 min in a water bath incubator pre-set to 70 °C and subsequent cooling to room temperature.

2.5. Determination of honey antibacterial activity

Honey's antibacterial activity was evaluated with a broth microdilution assay to determine the minimum inhibitory concentration (MIC), according to Bucekova et al. [33], based on the broth microdilution method described by the Clinical and Laboratory Standards Institute [34]. Briefly, 4 mL

of phosphate-buffered saline (PBS) (pH 7.2) was inoculated with 100 μL of an overnight liquid bacterial culture. The turbidity of the suspension was adjusted to 1×10^8 colony-forming units (CFU)/mL and diluted with Mueller–Hinton broth (MHB) medium to a final concentration of 1×10^6 CFU/mL. Each well of a sterile 96-well polystyrene plate (Sarstedt, Germany) was inoculated with a 10 μL aliquot of the suspension. The final volume in each well was 100 μL, consisting of 90 μL of sterile medium (as a positive control) or diluted honey and 10 μL of the bacterial suspension. Serial dilutions of each honey sample were prepared from 50% (w/v) honey solution (w/w in MHB medium) by further dilution with MHB medium, resulting in final concentrations of 45%, 35%, 30%, 25%, 20%, 18%, 16%, 14%, 12%, 10%, 8%, 6%, and 4%. Wells containing 100 μL of sterile MHB medium was considered as a negative control. After incubation for 18 h at 37 °C and 1250 rpm, the inhibition of bacterial growth was determined visually as the lowest concentration of honey that completely inhibited bacterial growth, resulting in an optically clear well (lack of visual turbidity). The MIC was defined as the lowest concentration of honey inhibiting 99% bacterial growth. All tests were performed in triplicate and repeated three times.

2.6. Crystal analysis

For the observation of crystal content, the presence or absence of crystals in ultrasound-treated and untreated samples was observed with an IVM-401 microscope (Bresser, Germany) at a magnification of 10×. A 1 g aliquot of the honey sample was placed on a lamella and fixed with a sampler holder. Ten images were taken of each sample; the number of crystals was counted from each image, and the average number was calculated. Honey samples with an uncountable number of crystals was rated as ">100 crystals" per image, indicating a state of complete or near-complete crystallization.

2.7. Statistical analysis

The Shapiro–Wilk test was used to determine whether the data followed a normal distribution. Two-way analysis of variance followed by Tukey's multiple comparison test was used to analyze the number of crystals in the samples and the antibacterial activity of samples stored over time. A p-value < 0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. The efficacy of US on crystal reduction in honey samples

Two crystallized honeys, honeydew (C1) and fir-heather (C2), were used to determine the efficiency of US at frequencies of 20, 25, 28, 33, and 40 kHz to reduce the number of crystals with 20% and 30% total power, respectively. There was a radical reduction in the number of crystals in C1 after 15 min of US at all frequencies. However, only US treatment at 40 kHz could completely reduce the number of crystals after 45 min of treatment (Figure 1A). On the contrary, US treatment at 40 kHz was the least effective on C2 (Figure 1B). US treatment at 28 or 33 kHz for 15 min markedly reduced the number of crystals. There was a complete elimination of crystals after US treatment at 20 or 28 kHz for 25 min. The maximum temperature of both samples remained below 40 °C immediately after ultrasound treatment.

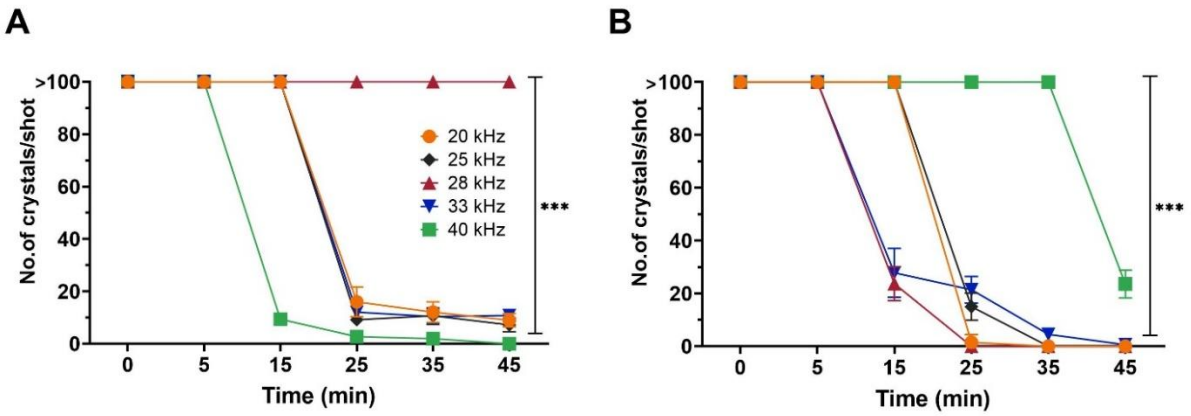

Figure 1. Ultrasound (US)-assisted liquefaction of crystallized honey samples. Two honey samples, (A) honeydew and (B) fir-heather, were subjected to US at various frequencies. Data represent the mean of the number of crystals. *** $p \leq 0.001$.

3.2. The effect of US-assisted liquefaction on honey antibacterial activity

The antibacterial activity of both crystallized honey samples was compared with the antibacterial activity of the US-treated samples. The MIC against *S. aureus* was 6% for both C1 and C2 prior to treatment (Figure 2). Interestingly, US treatment of C1 at 20 kHz reduced the MIC to 4%, while US treatment at 25, 28, 33, or 40 kHz did not change the MIC (Figure 2A). On the contrary, the US frequency had different effects on the antibacterial activity of C2. US treatment at 25 or 40 kHz reduced the MIC to 4%, while US treatment at 20 or 28 kHz increased the MIC to 14% (Figure 2B). Therefore, for the subsequent experiments that aimed to prevent honey crystallization, US at 28 and 40 kHz, which liquefied honey in the shortest amount of time, was used.

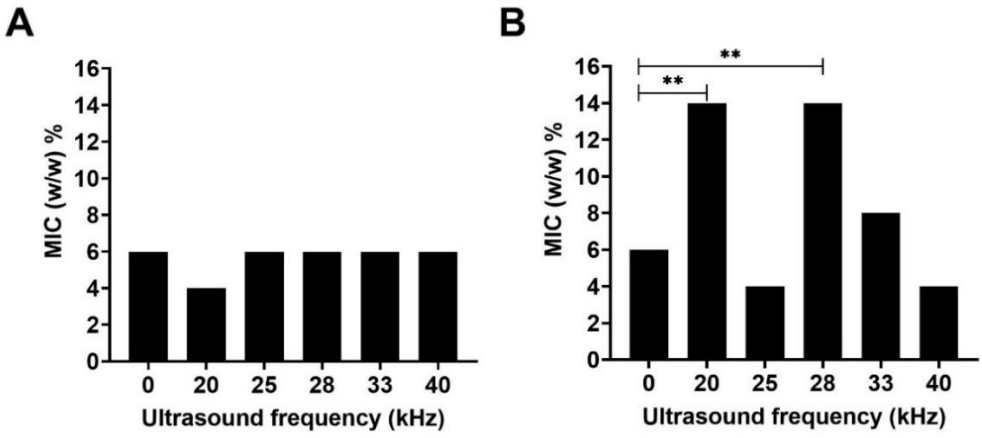

Figure 2. Antibacterial activity of ultrasound (US)-mediated liquified honey samples against *Staphylococcus aureus*. Two honey samples, (A) honeydew and (B) fir-heather, were subjected to US at various frequencies. Antibacterial activity was determined by a minimum inhibitory concentration (MIC) assay. Data are expressed as mean MIC values. ** $p \leq 0.01$.

3.3. Efficacy of US and heat treatment on delaying honey crystallization

US or heat treatment was applied to liquid blossom and mixed honey samples (L1–L5, Table 1). Figure 3 shows the average number of crystals per image for the untreated and US-treated (at 28 or 40 kHz) honey samples. Although all tested honey samples before treatment were in the liquid state, L1 and L3 contained 23.7 ± 4.5 and 40.1 ± 10.4 crystals per image, respectively. Overall, the untreated samples had significantly ($p < 0.001$) more crystals than the US-treated honey samples. L1 contained significantly ($p < 0.01$) fewer crystals after US treatment at both frequencies compared with heat-treated L1 after storage for 13 weeks. US treatment at 28 kHz was the most effective in delaying crystallization in L1, L2, and L4 (Figure 3). The maximum temperature of all samples remained below 40 °C immediately after ultrasound treatment. L3, which initially contained the highest number of crystals among all liquid honey samples, crystallized the fastest after heat treatment and reached full crystallization by week 13. Additionally, the number of crystals after initial US treatment at 40 kHz was significantly ($p < 0.001$) lower compared with the other treatments after 20 weeks of storage. Heat-treated L4 reached full crystallization by week 20. Interestingly, none of the honey samples subjected to US at 40 kHz crystallized completely by the end of the 20-week storage period. Thus, this treatment represents the most effective approach in delaying the crystallization process.

3.4. The effect of US and conventional heat treatment on antibacterial activity of liquid honey samples

For most liquid honey samples, the US and heat treatments did not change the antibacterial activity. The exception was L4: US treatment at 40 kHz significantly ($p < 0.01$) reduced the antibacterial activity by 40% (Figure 4). After 20 weeks of storage at 4 °C, only treated L4 showed a significant ($p < 0.01$) change in the antibacterial activity compared with untreated L4. Specifically, heat- and US-treated L4 showed a 50% and 100% increase, respectively, in the MIC (Figure 4D). Interestingly, there were no changes in antibacterial activity after US or heat treatment and after 20 weeks of storage for the three multifloral honey samples (L1, L2, and L5). This finding suggests that certain honey phytochemicals could stabilize the antibacterial activity after processing and storage.

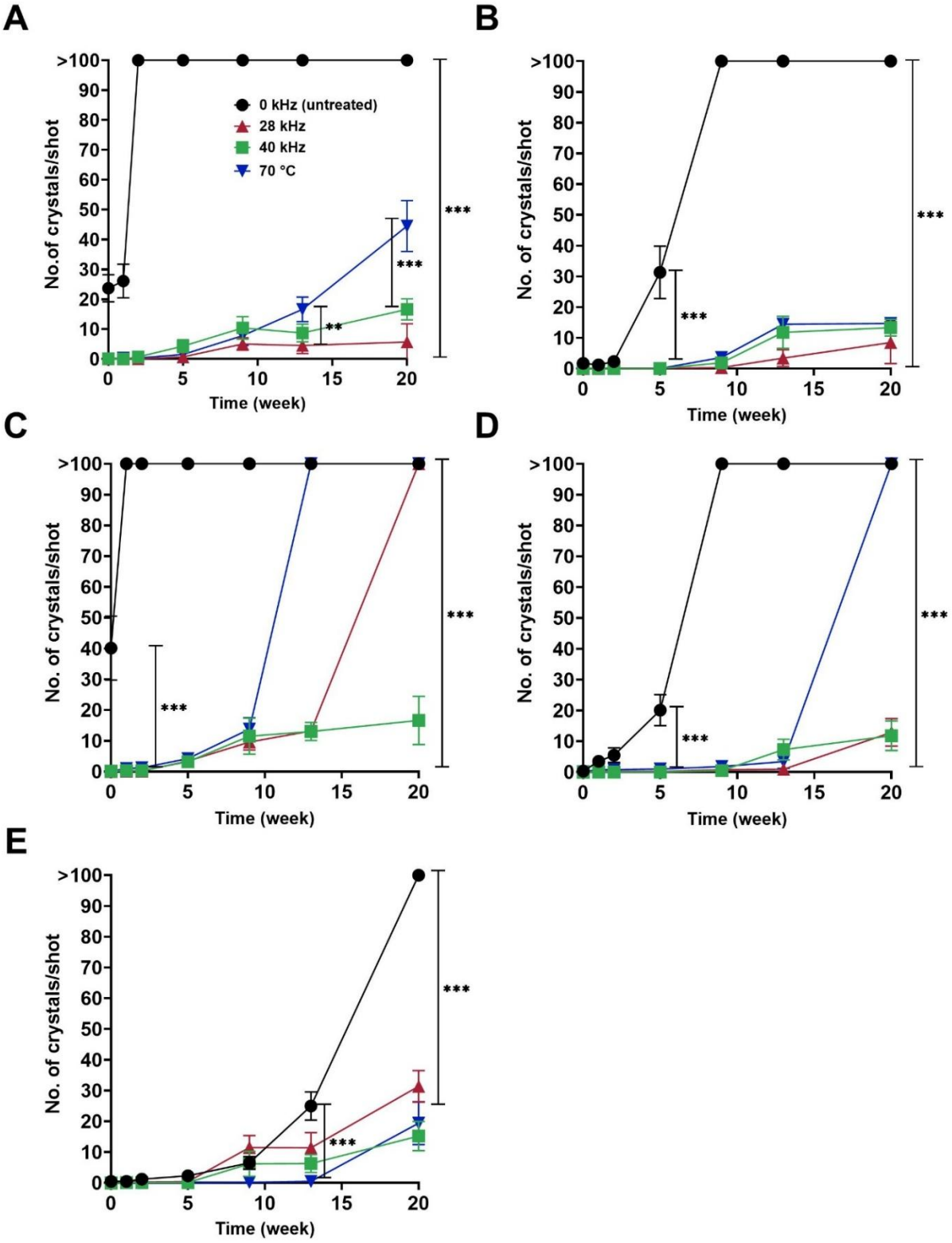

Figure 3. Ultrasound (US)-mediated processing and thermal processing of fresh liquid honey samples. Five honey samples, (A) floral honey L1, (B) floral honey L2, (C) mixed honey L3, (D) acacia sample L4, and (E) floral honey L5, were subjected to US at 28 and 40 kHz or to heat treatment at 70 °C. Data represent the mean number of crystals. *** $p \leq 0.001$.

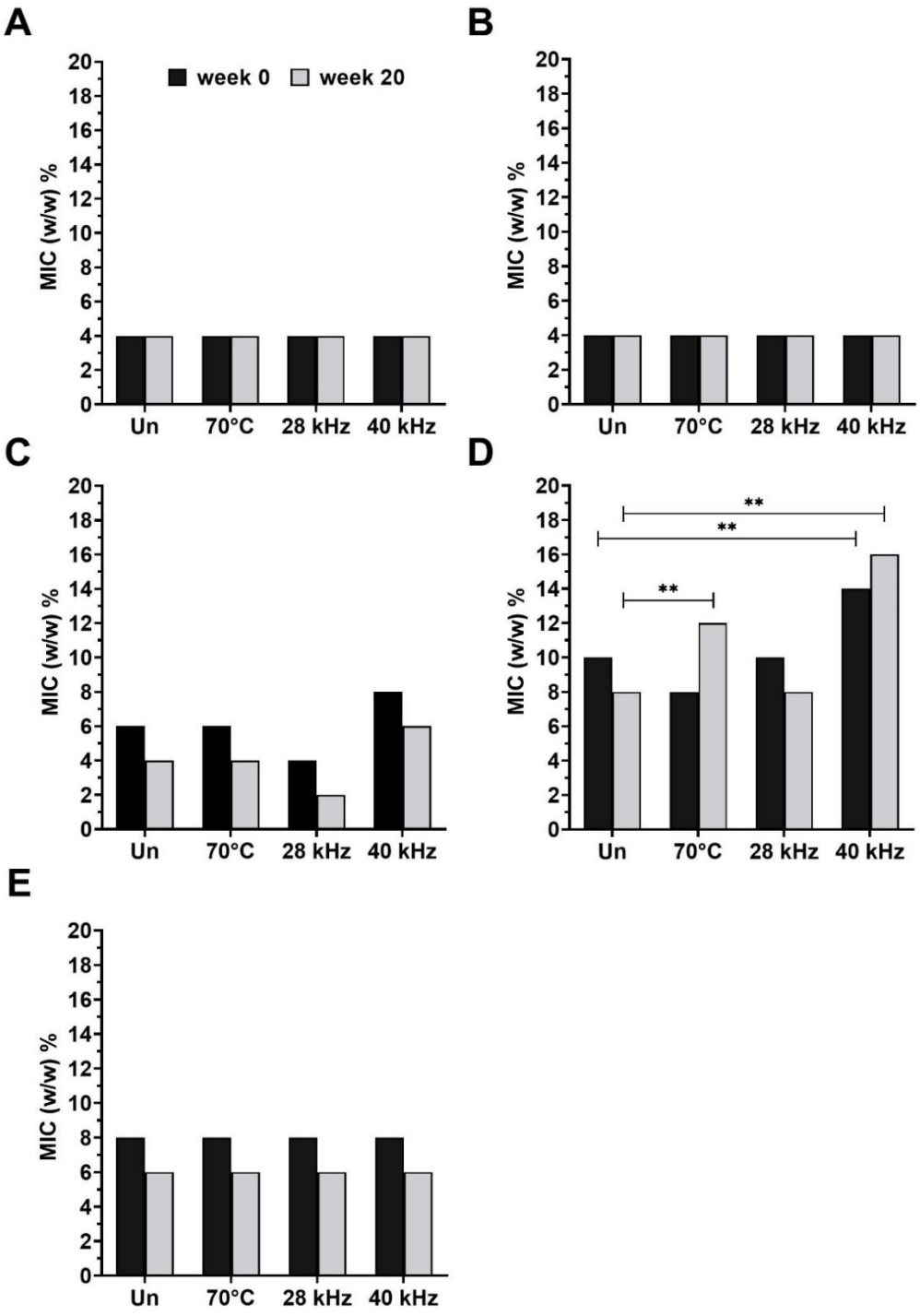

Figure 4. Antibacterial activity of ultrasound (US)-mediated processing and thermal processing of fresh liquid honey samples against *Staphylococcus aureus*. Five honey samples, (A) floral honey L1, (B) floral honey L2, (C) mixed honey L3, (D) acacia sample L4, and (E) floral honey L5, were subjected to US at 28 and 40 kHz or to heat treatment at 70 °C. Antibacterial activity was determined by a minimum inhibitory concentration (MIC) assay. Data are expressed as mean MIC values. ** $p \leq 0.01$.

4. Discussion

Industrial processes, including thermal treatment, are essential for ensuring honey quality and microbial safety. However, evidence from industrial-scale thermal processing demonstrates that exposure to either high temperatures for brief periods or moderate temperatures for extended durations can compromise the health-promoting properties of honey, particularly its antibacterial activity. Consequently, growing consumer preference for minimally processed or raw honey has driven research into non-thermal processing alternatives. Ultrasound technology represents one of the most promising non-thermal methods for honey processing [35].

The therapeutic properties of honey may be significantly affected by its industrial processing; therefore, each technology, including US treatment, should exhibit minimal or no negative effects on therapeutic potential. Only a few studies have characterized the effect of US on the antibacterial activity of honey samples [25,27,30]. Stojkovic et al. [27] reported increased antibacterial activity of honeydew honey against *S. aureus*: compared with a MIC of 12.5% for the untreated control, US treatment at 40 kHz for 1 min at 60 °C decreased the MIC to 6.25%, and US treatment at 40 kHz for 10 min at 30 °C decreased the MIC to <3.125%. These findings are not consistent with our results. The antibacterial activity of all US-treated liquid honey samples was not increased significantly. Furthermore, US treatment at 40 kHz negatively affected the antibacterial activity of acacia honey, with an increase in the MIC from 10% to 14%. Interestingly, there were no changes in the antibacterial activity of US-treated multifloral and mixed honeys, suggesting that the polyphenol content contributes to overall stabilization of honey antibacterial activity. US treatment at a low or high frequency can inactivate GOX, an enzyme responsible for the generation of hydrogen peroxide (H_2O_2) [36]. Certain plant polyphenols, including flavonoids, may effectively protect enzymes from inactivation [37]. In fact, the total polyphenol and flavonoid contents in acacia honeys are significantly lower compared to other monofloral (e.g., linden, chestnut, and spruce) and multifloral honeys [38,39]. Hence, the negative changes in antibacterial activity of the US-treated acacia honey sample (L4) could be the result, at least in part, of a low polyphenol content and thus a limited protective effect against GOX inactivation. Furthermore, US treatment may enhance the concentration of bioactive compounds, including polyphenols and flavonoids [40,41]. Quintero-Lira et al. observed increased levels of various flavonoids, such as rutin, quercetin, apigenin, and kaempferol, with extended sonication duration [29]. Similarly, a very recent study showed that low-temperature US preserved the polyphenolic richness and diversity of Jordanian honey samples with different botanical and geographical origins [42]. Surprisingly, the authors also observed an enhancement of the levels of some phenolic compounds. Conversely, a recent direct comparison of thermal liquefaction with microwave and US liquefaction revealed a significant loss of phenolic compounds (approximately 50%) in US-treated honey, although the maximum honey temperature did not exceed 45 °C [43].

The observed decrease in total phenolic content is most likely attributable to the disproportionately long treatment duration required to achieve complete liquefaction of the honey sample. Therefore, besides temperature, time of treatment needs to be monitored to minimize destruction of labile honey bioactive molecules.

In the present study, US effectively prolonged the liquid state of the tested fresh honey samples by destroying the initial crystals in untreated samples. Subsequent storage of untreated and US/thermal-treated honey samples at 4 °C for 20 weeks initiated the crystallization process, and all untreated samples were fully crystallized by the end of the storage period. On the other hand, storage

of the three multifloral honey samples (L1, L2, and L5) treated with US at 28 kHz led to the formation of only a few crystals, and the samples remained in liquid state. Based on our results, it is likely that blossom and mixed honeys react differently to US treatment. Honey composition affects the overall efficacy of US treatment. A recent study showed that US treatment at 20 kHz is superior in dissolving the initial crystals of liquid blossom honeys than US treatment at 5 kHz [44]. The multifloral honey sample L5 showed the most preserved crystallization properties within the 20-week storage period. Interestingly, acacia honey L4 was fully crystallized at week 9. This observation is not consistent with findings reported by Scripca and Amariei [28], where US-treated acacia honey was the slowest to crystallize among several blossom and honeydew honey samples. Prolongation of the liquid state of honey is highly appreciated by both beekeepers and consumers. A very recent consumer study revealed that consumers prefer the liquid consistency of honey; consumers consider liquid honey, find it easier to handle, and/or have a low preference for crystallized honey [45].

The vast majority of published studies that characterized the US-assisted liquefaction of crystallized honeys have focused mainly on basic qualitative parameters such as moisture and HMF content and DN activity (reviewed in [35]). Overall, the HMF content and DN activity are less affected by US treatment than by thermal treatment. However, it is important to note that the HMF content and DN activity depend on the botanical origin of honey [46] as well as the experimental conditions of the US treatment. Currently, the optimal conditions (e.g. temperature, time, and amplitude) for US treatment for honey liquefaction have not been determined. In the present study, five US frequencies were employed to liquify two crystallized honey samples. Surprisingly, 40 kHz, the most commonly used frequency for honey liquefaction, was the most suitable for sample C1 but the least suitable for sample C2, where 28 kHz produced the best results in crystal dissolution. Taken together, these results indicate that the botanical origin of processed honey samples may be highly determinant to the efficacy of US treatment.

Beyond US treatment, high hydrostatic pressure processing (HHP) has also been explored as an alternative method for honey liquefaction and crystallization delay. However, a recent study demonstrated the superiority of US over HHP in eliminating crystals and prolonging the liquid state of honey during storage [47]. This difference may be attributed to increased air bubble formation in HPP-treated samples, which accelerates crystallization compared to ultrasound-treated honey. While ultrasound treatment also generates air bubbles, these are fewer and larger than the numerous small bubbles produced by HPP, resulting in less pronounced effects on crystallization kinetics.

US treatment enhances both the quality and microbial safety of honey. Major sources of microbial contamination of honey include nectar, pollen, dust, air, honeybees, and post-harvest handling practices. US processing substantially reduces aerobic mesophilic bacteria and mold counts [48,49] and has demonstrated superior efficacy compared to conventional thermal treatment for microbial inactivation [49]. The antimicrobial mechanism involves micromechanical shockwaves generated during sonication, which disrupt cellular structures and induce cell lysis.

Several limitations must be acknowledged in the present study. First, melissopalynological analysis was not performed on the honey samples, which represents a significant methodological gap since pollen content substantially influences crystallization kinetics and could explain observed variations in crystallization rates among different honey samples. Pollen grains function as heterogeneous nucleation sites for glucose crystallization, directly affecting crystallization behavior. Second, the storage temperature of 4 °C was not optimal for honey crystallization, as the literature indicates that 13–15 °C provides ideal conditions for crystallization nuclei formation. Third, the

absence of data on defensin-1 content, glucose oxidase (GOX) activity, and hydrogen peroxide (H_2O_2) levels precludes a comprehensive evaluation of ultrasound treatment effects on individual antimicrobial compounds in honey.

5. Conclusions

US processing of crystallized or liquid honey samples is a suitable alternative to thermal honey processing. The experimental conditions for US treatment and its overall efficacy are highly dependent on the botanical origin of the honey. The antibacterial activity of US-treated crystallized or liquid honey samples remained unchanged in some honey samples but significantly decreased in one crystallized honey sample and one liquid honey sample. Further research with more honey samples is needed. Moreover, the therapeutic effect of honey is partially mediated through the action of proteins and peptides. Thus, it is important to determine the effect of US on their functions, stability, and structures.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Author contributions

Marcela Bucekova: Conceptualization, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; Jana Godocikova: Conceptualization, investigation, writing—review and editing; Stefan Gal: Conceptualization, methodology, validation, writing—review and editing; Ludovit Morocz: Conceptualization, data curation, methodology, validation, writing—original draft; Daniel Mikuska: Conceptualization, supervision, validation, writing—review and editing; Lubomir Svec: Conceptualization, supervision, validation, writing—review and editing; Juraj Majtan: Conceptualization, formal analysis, funding acquisition, project administration, supervision, writing—original draft, writing—review and editing.

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